UNIVERSITE MONTPELLIER II

Ecole doctorale : Systèmes Intégrés en Biologie, Agronomie, Géosciences, Hydrosciences, Environnement (SIBAGHE)

THESE
Pour obtenir le grade de
Docteur de l'Université Montpellier II

Spécialité : Evolution, Ecologie, Ressources Génétiques Paléontologie

Présentée et soutenue par
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Biologie évolutive d'une espèce envahissante, la coccinelle asiatique *Harmonia axyridis*

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Soutenue le 24 novembre 2011

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Résumé

Les invasions biologiques constituent aujourd'hui une source d'inquiétude du fait de leur nette augmentation et des conséquences écologiques, économiques et sanitaires dont elles sont à l'origine. Pour qu'une population devienne envahissante, il faut (i) qu'elle soit introduite, (ii) qu'elle s'établisse et (iii) qu'elle prolifère. Chacune des ces trois étapes constitue un défi difficile à relever, et les processus en jeu sont encore mal connus. Cette thèse décrit un ensemble de recherches visant à comprendre l'invasion mondiale particulièrement réussie de la coccinelle asiatique *Harmonia axyridis*.

Nous avons dans un premier temps étudié l'étape d'introduction en retraçant les routes d'invasion d'*H. axyridis* à l'aide de marqueurs microsatellites et de la méthode ABC (Approximate Bayesian Computation). Nous avons montré que la population envahissante la plus ancienne dans le nord-est américain avait été la tête de pont de l'invasion mondiale en devenant la source des foyers européen, sud américain et africain. En Europe, on constate également une hybridation avec une souche de lutte biologique.

Dans un deuxième temps, nous avons exploré l'étape d'établissement de l'espèce. Nous avons montré que les populations envahissantes d'*H. axyridis* avait subit une purge génétique réduisant considérablement les effets associés à la dépression de consanguinité. Par ailleurs, l'événement d'hybridation en Europe apporte des avantages phénotypiques probables à cette population envahissante.

Nous avons ensuite étudié plusieurs aspects de l'étape de prolifération. Nous avons montré que les populations européennes avaient évolué vers de plus fortes capacités de dispersion sur le front d'invasion. Par ailleurs, nos résultats montrent que la commercialisation en France d'une souche non-volante de lutte biologique a pu avoir des conséquences positives sur l'expansion de la population envahissante par des phénomènes d'hétérosis ou d'augmentation de variance génétique.

Enfin, nous discutons de l'importance d'étudier de manière approfondie une espèce modèle telle qu'*H. axyridis* pour améliorer nos connaissances générales sur les mécanismes éco-évolutifs impliqués lors des invasions biologiques.

Mots-clés : Génétique des populations, routes d'invasion, scénario « tête de pont », Approximate Bayesian Computation, microsatellites, hybridation intraspécifique, génétique quantitative, traits d'histoire de vie, hybridation, adaptation, purge génétique, dispersion, homogamie spatiale.

Abstract

Evolutionary biology of an invasive species, the Asian ladybird *Harmonia axyridis*

Biological invasions are a concern because of their increase and their environmental, economic and human health consequences. To become invasive, a population must (i) be introduced, (ii) established itself and (iii) proliferates. Each of these three steps constitutes a challenge, and the processes involved are still poorly understood. This thesis describes a set of research actions which aims at understanding the worldwide successful invasion of the Asian ladybird *Harmonia axyridis*.

We first investigated the introduction step by retracing invasion routes of *H. axyridis* using microsatellite markers and the ABC (Approximate Bayesian Computation) method. We have shown that the oldest invasive population in the eastern North America acted as a bridgehead of the worldwide invasion by becoming the source of the European, the South American and the African outbreaks. We also found evidence for a genetic admixture event in Europe with a biological control strain.

Second, we explored the establishment step. We have shown that invasive populations of *H. axyridis* endured a genetic purge which significantly reduced adverse effects associated with inbreeding depression. In addition, the admixture event in Europe likely brought phenotypic benefits to this invasive population.

We then studied several aspects of the proliferation step. We found that European populations had evolved towards higher dispersal abilities on the invasion front. Moreover, our results show that the use of a flightless biocontrol strain which is still sold in France may have a positive impact on the expansion of the invasive population through heterosis or increased of genetic variance.

Finally, we discuss the importance of studying in detail a model species such as *H. axyridis* to improve our general understanding of the eco-evolutionary mechanisms involved in biological invasions.

Keywords: Population genetics, invasion routes, bridgehead scenario, Approximate Bayesian Computation, microsatellites, hybridization, quantitative genetics, life history traits, genetic purging, dispersal, spatial sorting.

Remerciements

Un grand merci à Arnaud et Thomas pour avoir accepté d'encadrer un vieux thésard comme moi. Et merci surtout d'avoir fait de ces quelques années une partie de plaisir (j'exagère à peine).

Restons centrés sur la thèse elle-même. Merci à tous les membres de mon comité de thèse qui m'ont évité des voies tortueuses : Sylvain Gandon, Benoît Facon, Thomas Lenormand, Yannis Michalakis et Nicolas Ris.

Merci à toute l'équipe BPI (la « dream team » disent certains) présente et passée. Dans le désordre le plus absolu : Alexandra, Aurélie, Xavier, Isabelle, Thibaut, Elodie, Flavie, Bertrand, Pascal, Marc, Chloé, Laurent, re-Thomas, Géraldine, Gérald… Et j'en oublie sans doute…

Je souhaite également remercier le CBGP qui m'a toujours accueilli de manière fort sympathique chaque fois que j'essayais de réduire la distance géographique qui me séparait de mon directeur de thèse. En particulier (dans un désordre toujours aussi anarchique) Anne, re-Benoît, Ashraf, re-Arnaud, re-Flavie, Jean-Marie… J'ai encore peur d'en oublier…

Merci à toutes les personnes qui ont travaillé de manière plus temporaire avec moi. En particulier les stagiaires : Rémi, Anthony, Priscilla, Caroline, Laura et Benjamin. Au passage, salutations à tous les non-permanents qui sont passés dans l'équipe pendant cette période.

Merci à tous les échantillonneurs de coccinelles et les collègues nationaux et internationaux passionnés d'insectes à points. Pour le coup, ils sont tellement nombreux qu'il me faudrait doubler le volume de ce manuscrit pour pouvoir tous les citer. Donc simplement : merci.

En dehors du labo, il y a bien sûr du monde à remercier. Merci donc aux amis du Nord ou du Sud. Un merci spécial à Didier, re-Thomas, Henry et Pouaz''rlk, Chris, Matthieu et mueros, Joëlle, Philippe et Talweg pour les belles aventures musicales. En parlant de musique, mes oreilles auraient beaucoup de monde à remercier de Bach à Sunn O))). Et merci à la famille : Maman, Fanny, Valérie, Stéphane, Yohann, Alex, Dominique et Christian.

Et bien sûr une infinité de milliards de merci à Mélanie pour son soutien et tout le reste ! Et même si Zélie n'a pas vraiment allégé mon emploi du temps, je la remercie pour sa bonne humeur communicative, et son super caractère qui a largement simplifié la vie de sa mère pendant la rédaction de ce manuscrit ! Love you girls…

Avant Propos

Une thèse est sensée être le compte rendu d'un travail personnel effectué dans un laboratoire d'accueil et sous l'encadrement d'une ou deux personnes. Mais la recherche ne fonctionne pas ainsi : elle est faite d'interactions, de collaborations, de coups de main et d'échanges. Ainsi, si mon travail a été principalement centré sur la génétique des populations et les routes d'invasion, j'ai pris le parti dans ce manuscrit de décrire de manière beaucoup plus large les connaissances acquises sur le modèle biologique étudié pendant la durée de ma thèse. Le lecteur trouvera donc un certain nombre d'articles dont je suis co-auteur et qui ne sont pas issus du cœur de mon activité. Et si les hypothèses testées sont parfois directement tirées de mes propres résultats, je ne peux prétendre avoir eu un rôle prépondérant dans tous ces travaux. Il m'a toutefois semblé que l'ensemble de ces éléments (les articles en premier auteur et les articles en co-auteur) mis bout à bout généraient une histoire, pas encore complète, mais cohérente sur l'invasion mondiale de la coccinelle asiatique. J'espère que l'effort de synthèse portera ses fruits aux yeux du lecteur.

Par conséquent, pour éviter tout malentendu, j'ai indiqué en page de garde (dans les annexes) de chaque article dont je suis co-auteur des précisions sur le rôle précis que j'ai exercé dans le travail de recherche considéré.

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CHAPITRE I
Introduction générale : vers une meilleure compréhension des invasions biologiques

I.1. Colonisation et répartition des espèces dans le monde.

I.1.1. La colonisation d'une nouvelle aire géographique

Le temps a permis aux organismes d'évoluer en réponse aux forces évolutives telles que la dérive génétique ou la sélection naturelle. Mais c'est l'espace, et plus précisément le déplacement des individus en son sein, qui a permis à l'évolution de générer la biodiversité actuelle. La répartition des espèces dans le monde est le fruit de la colonisation historique de différents milieux par des groupes d'individus. La limitation des flux de gènes entre populations du fait par exemple de barrières naturelles (vicariance), ainsi que l'hétérogénéité environnementale ont été les principaux moteurs de la spéciation à l'origine de la biodiversité actuelle.

Le mouvement des individus dans l'espace est donc un moteur primordial de l'évolution. Mais la colonisation d'un nouveau milieu nécessite que celui-ci soit accessible. Pour cela, il est possible de formaliser trois types de phénomènes.

a) Elargissement local d'aire de répartition

Premièrement, l'élargissement local de l'aire de répartition est permis par la modification de l'environnement attenant. De nombreux facteurs écologiques limitent l'aire de répartition des espèces, mais si les conditions deviennent progressivement moins défavorables en bordure de l'aire de répartition d'une espèce, celle-ci pourra théoriquement

coloniser le nouveau milieu. Le réchauffement climatique à la fin d'une période de glaciation peut par exemple permettre aux espèces de s'étendre sur de nouvelles latitudes. Dans ce cas, le chalenge adaptatif est par nature limité, mais des processus évolutifs importants sont toutefois à l'œuvre, principalement du fait des déséquilibres spatiaux engendrés pouvant générer des réductions de diversité génétique, des pressions de sélection sur des traits densité dépendants ou de l'homogamie spatiale (cf. section I.5, Hewitt 2000; Hewitt 1996; Hill *et al.* 2011; Shine *et al.* 2011a). L'expansion vers le Nord du criquet *Chorthippus parallelus* en Europe à la fin de la dernière glaciation (il y a environ 10 000 ans) a été inférée à partir de variations de séquences d'ADN (Cooper *et al.* 1995; Lunt *et al.* 1998; Taberlet *et al.* 1998). Trois principales zones refuges (la péninsule Ibérique, l'Italie et les Balkans, constituant donc l'aire de répartition du criquet au cours de la glaciation) ont été mises en évidence. Une expansion vers le Nord a eu lieu pour chacune d'entre elles, mais seuls les individus issus de la zone refuge des Balkans sont parvenus à coloniser avec un grand succès l'ensemble de l'Europe jusqu'à la Scandinavie, et des zones hybrides entre cette population et les deux autres ont été détectées dans les Pyrénées et dans les Alpes.

b) Suppression des barrières physiques

Le second processus permettant la colonisation d'un nouveau milieu est la suppression d'une barrière physique qui isolait auparavant deux zones l'une de l'autre. Ce processus est historiquement directement lié à la tectonique des plaques : le mouvement des plaques lithosphériques a, au fil des temps géologiques, séparé ou mis en contact des terres émergées, créant ainsi des couloirs entre des océans ou mettant en contact deux continents. L'opportunité qui s'offre alors de coloniser un nouveau milieu est grande, mais des contraintes adaptatives fortes peuvent être présentes : le nouvel environnement biotique a de forte chance d'être très différent (selon la durée de l'isolement ancestral), et l'environnement abiotique l'est potentiellement également, notamment si de nouvelles latitudes deviennent accessibles. La création de l'isthme de Panama il y a environ 3 millions d'années, est un exemple bien documenté de ce type de phénomène. Cet événement géologique a permis aux espèces d'Amérique du Sud de coloniser l'Amérique du Nord et vice-versa (on parle du « grand échange interaméricain », Webb 1991; Woodburne 2010). Son impact sur la biodiversité actuelle des mammifères terrestres a été particulièrement étudié et a permis de mettre en évidence des échanges dans les deux directions avec des succès toutefois très variables. Les mammifères d'Amérique du Nord en particulier ont colonisé avec beaucoup

plus de succès le nouveau continent qui s'offrait à eux que leurs homologues sud-Américains. La question de la raison de ces différences de succès reste posée, mais deux hypothèses non exclusives sont souvent mises en avant : (i) les conditions climatiques en Amérique du Nord moins hospitalières (plus froides et sèches) qu'en Amérique du Sud et (ii) la surface disponible pour les espèces nord-américaines (alors en contact avec l'Eurasie et l'Afrique) six fois supérieure à celle disponible pour les espèces sud-Américaines. Ces deux hypothèses suggèrent que les espèces d'Amérique du Nord disposaient des potentiels adaptatifs plus grands.

c) Dispersion à longue distance

Enfin, le troisième processus à l'origine des colonisations anciennes est la dispersion à longue distance. Celle-ci permet à un individu ou un groupe d'individus de traverser une barrière physique et d'atteindre ainsi un nouveau milieu potentiellement favorable. Ce type de déplacement peut se faire activement lorsque les capacités migratoires sont élevées (certains oiseaux par exemple), mais elle se fait majoritairement passivement par l'utilisation de courants marins ou aériens, ou par l'utilisation de « véhicules » (e.g. une autre espèce ou un débris flottant). Contrairement au deux premiers types de processus, celui-ci est beaucoup moins fréquent, même si son importance n'est pas négligeable, notamment dans la colonisation des îles. Par ailleurs, il est le processus qui est le plus limitant en terme évolutif : le nombre d'individus transportés est par nature souvent faible, et le nouveau milieu est éventuellement écologiquement très différent de celui d'origine, ce qui pose des problèmes adaptatifs d'autant plus grands que la variabilité génétique disponible est faible. L'origine de la faune et de la flore particulières de Nouvelle-Zélande par exemple a longtemps été attribuée à d'anciennes spéciations dues à un phénomène de vicariance au moment de sa séparation du supercontinent Gondwana il y a 80 millions d'années. Toutefois, des études plus récentes ont montré, notamment à l'aide de marqueurs moléculaires, qu'une grande partie de la biodiversité pré-anthropique de la Nouvelle-Zélande était issue de colonisations beaucoup plus récentes via la dispersion à longue distance (Cooper & Millener 1993; Goldberg *et al.* 2008; Trewick *et al.* 2007). Celle-ci est notamment mise en évidence par des niveaux de variation génétique parfois faible suggérant des nombres de colonisateurs faibles.

I.1.2. Rôle de l'homme dans le déplacement des espèces

Les exemples ci-dessus illustrent les mouvements naturels des espèces au cours des temps géologiques. Mais l'homme a récemment profondément accéléré ces phénomènes en étendant lui-même son aire de répartition et en modifiant son environnement. Ainsi, chacun des processus permettant aux espèces de coloniser de nouveaux milieux peut actuellement trouver des causes anthropiques.

Le réchauffement climatique contemporain du à la production de gaz à effet de serre (Cox *et al.* 2000; Hughes 2000), est une illustration bien documentée de modification environnementale liée aux activités humaines permettant l'accroissement local d'aire de répartition. Les exemples d'espèces ayant étendu leur aire de répartition vers les pôles (et/ou en altitude) au cours du dernier siècle s'accumulent très rapidement. Parmesan *et al.* (1999) ont montré que, parmi 35 espèces de papillons Européens étudiées, 63% ont étendue leur aire de répartition de 35 à 240 km vers le nord au cours du XX^{ième} siècle. Deux tiers de ces espèces n'ont pas eu de modification de leur limite géographique sud indiquant clairement un accroissement de leur territoire.

En modifiant drastiquement son environnement, l'homme a supprimé brutalement un grand nombre de barrières physiques. En 1869, le Canal de Suez est inauguré. Celui-ci permet à la mer Rouge et à la mer Méditerranée d'entrer en contact pour la première fois depuis 20 millions d'années. Cette ouverture a permis à de nombreuses espèces d'avoir accès à une nouvelle mer. Ainsi, plus de 60 espèces de poissons natifs de la mer Rouge ont colonisé la Méditerranée, représentant environ 10% du nombre total d'espèces dans cette mer (Mavruk & Avsar 2008). Parmi ceux-ci, le poisson lapin *Siganus luridus* s'est très largement répandu dans le bassin oriental, et il a récemment atteint les côtes Siciliennes et Tunisiennes (Azzurro & Andaloro 2004; Hassan *et al.* 2003).

Enfin, l'homme, en colonisant lui-même l'ensemble de la planète dès 40 000-60 000 ans avant notre ère (Mellars 2006) puis en développant le transport de marchandises et de personnes depuis le 15^{ième} siècle, a également servi de « véhicule » pour de nombreuses autres espèces (Wilson *et al.* 2009). Ce nouveau type de dispersion à longue distance s'est fait en partie de manière volontaire. L'exemple le plus frappant est celui des espèces utilisées pour l'alimentation humaine : certaines d'entre elles ont été très largement répandues sur la plupart des continents par l'homme, et actuellement, seulement 15 espèces de plantes parmi les 250 000 connues constituent 90% de l'alimentation humaine mondiale (Pimentel 2002).

Beaucoup d'espèces sont également transportées de manière accidentelle d'un endroit à un autre. Un exemple ancien et bien connu est celui de trois espèces asiatiques de rats (Rattus rattus, R. norvegicus et R. exulans, Harris 2009) qui ont suivi clandestinement les mouvements humains très tôt dans l'histoire (il y a environ 6000 ans pour *R. rattus*). Les liens entre les déplacements de l'homme et ceux des rats sont si étroits que Matisoo-Smith *et al.* (1998) sont parvenus à retracer l'histoire de la colonisation de la Polynésie par l'homme en utilisant des marqueurs mitochondriaux de *R. exulans*.

Globalement, le rôle de l'homme dans la colonisation des espèces a pris un tournant décisif au cours des deux derniers siècles en devenant beaucoup plus important que celui des processus naturels (décrits en I.1.1.). La prise de conscience (i) de certains effets néfastes de ces échanges biotiques dès le XIX^{ième} siècle et (ii) de l'intérêt scientifique que ces colonisations récentes et souvent rapides constituent a largement contribué à la popularisation, surtout depuis la seconde moitié du XX^{ième} siècle, d'un terme dont la définition reste toutefois assez floue, celui d'invasion biologique.

I.2. Qu'est-ce qu'une invasion biologique ?

I.2.1. Définition d'une notion vague

Toute utilisation de la notion d'invasion biologique fait référence à la colonisation par une espèce (dite envahissante) d'une zone géographique dont elle était précédemment absente. Toutefois, lorsque l'on entre dans le détail, on se rend compte que la littérature regorge de définitions plus ou moins précises de ce terme. Deux principaux éléments varient d'une définition à l'autre.

Tout d'abord, l'idée d'impact. En effet, certains auteurs ou organisations considèrent qu'une espèce n'est dite envahissante que si elle a un impact négatif sur l'environnement, l'économie ou la santé humaine (e.g. IUCN SSC Invasive Species Specialist Group (ISSG) 2009). Ce type de définition se rattache directement à l'utilisation du terme « invasion » qui a clairement une connotation anthropocentrique négative dans le langage usuel. Toutefois, la principale limitation de ce type de définition est la notion même d'impact. Celui-ci est en effet

très subjectif, comme l'illustre par exemple la notion de seuil de nuisibilité utilisée en agriculture, ou encore la difficulté à quantifier l'impact d'une nouvelle espèce sur un écosystème par nature complexe. J'ai donc pris le choix d'utiliser une définition neutre dans ce manuscrit (voir plus bas). Ceci n'est pas un problème en soi car, s'il est plus utile pour la société d'étudier et d'acquérir des connaissances sur des espèces ayant des impacts négatifs, les mécanismes éco-évolutifs à l'œuvre au cours d'une invasion n'ont pas de raisons d'être différents selon qu'on la considère bénéfique, néfaste ou neutre (Colautti & MacIsaac 2004).

Ensuite, certaines définitions se différencient les unes des autres par ce qui est entendu par « nouvelle zone géographique ». Ainsi, une définition très large consiste à considérer qu'une espèce envahissante est une espèce qui accroît son aire de répartition (e.g. Williamson & Fitter 1996a). Tout les processus de colonisation décrits dans la partie I.1 sont inclus dans cette description. Nous préférerons toutefois une définition plus restrictive qui considère que la zone envahie est originellement déconnectée de la zone native. Ainsi, les élargissements locaux d'aires de répartition ne seront pas considérés dans le reste du manuscrit. Les bordures d'aires de répartition sont en effet par nature instables (notion de cycle de taxon, e.g. Ricklefs & Bermingham 2002) et il est souvent difficile de définir avec précision les limites d'une aire native.

Les invasions biologiques seront donc définies comme suit dans le reste de ce manuscrit : il y a invasion biologique lorsque des organismes sont introduits dans une nouvelle aire géographique, souvent distante de l'aire d'origine, y persistent puis y prolifèrent démographiquement et spatialement. Cette définition, également proposée par Elton (1958) et Mack *et al.* (2000), est neutre vis-à-vis des impacts potentiels et inclut clairement une étape d'introduction. Notons également que, d'après cette définition, il ne suffit pas d'être introduit et de survivre pour être considéré comme envahissant, mais il faut également croître en nombre et s'étendre spatialement à partir de la zone d'introduction.

Après avoir défini la notion d'invasion biologique, voyons maintenant pour quelle raison il est important de les étudier et de les comprendre.

I.2.2. Un nombre d'invasions biologiques en forte augmentation

Le rôle de l'homme dans le mouvement des espèces au niveau mondial est devenu bien supérieur à celui des processus naturels (voir partie I.1.2.). Par ailleurs, le taux d'introduction d'espèces exotiques croît constamment, en particulier depuis deux siècles avec

l'augmentation exponentielle des migrations de personnes et de biens ainsi que l'amélioration des moyens de transport permettant des déplacements de plus en plus rapides, plus fréquents et sur de plus grandes distances (e.g. Lockwood *et al.* 2007; Tatem & Hay 2007). Le National Research Council Américain a, en se basant sur des données bibliographiques, estimé que la flore Hawaïenne s'enrichissait d'une espèce tous les 100 000 ans avant l'arrivée de l'homme, d'une espèce tous les 50 ans après l'arrivée des Polynésiens, et d'une espèce tous les 22 ans après l'arrivée des Européens (Lockwood *et al.* 2007; NRC 2002). Sur une échelle de temps plus courte, Cohen & Carlton (1998) ont pu montrer une nette augmentation du taux d'invasion au cours du temps dans la baie de San Fransisco entre 1850 et 1995 avec notamment la moitié des espèces envahissantes arrivée après 1960.

Notons toutefois que des études montrent dans certaines zones géographiques une stabilisation, voire un ralentissement des taux d'invasion pour certains groupes taxonomiques. C'est le cas par exemple en Europe depuis les années 90 des plantes vasculaires ou de certains vertébrés pour lesquels la majorité des introductions liées à l'homme ont historiquement été volontaires. La meilleure régulation de ce type d'introduction intentionnelle explique cette tendance (DAISIE 2009; Lockwood *et al.* 2007). Néanmoins, ces mêmes études confirment l'augmentation exponentielle des invasions d'origine accidentelle en particulier dans le cas des invertébrés tels que les insectes (DAISIE 2009). Les taux actuels d'invasions sont donc globalement anormalement élevés (i.e. en comparaison aux taux non-anthropiques) et, étant donnés les nombreux impacts dont les invasions sont responsables, il est important de mieux décrire et comprendre les invasions biologiques.

I.2.3. Coûts, bénéfices et fantasmes liés aux invasions biologiques

L'intérêt suscité par les invasions biologiques est né de la constatation d'un certain nombre d'impacts négatifs liés à des espèces envahissantes et à une augmentation sans précédent du nombre d'invasions dans le monde. Les bénéfices liés aux invasions ne sont toutefois pas à négliger, en particulier dans un contexte où certains fantasmes, liés à la terminologie ou aux situations géopolitiques, sont profondément enracinés dans l'imaginaire collectif. Qu'ils soient positifs ou négatifs, les impacts des invasions biologiques sont souvent classés en 3 catégories : les impacts sur les écosystèmes, les impacts sur l'économie et les impacts sur la santé humaine.

a) Impacts sur les écosystèmes

Il s'agit de la seule catégorie d'impact qui soit antérieure à l'homme et pour laquelle la neutralité n'existe pas. En effet, l'arrivée d'une espèce dans un écosystème dont elle était absente et son expansion a nécessairement un impact puisque l'espèce occupera de l'espace, consommera des ressources et, éventuellement, se fera elle-même consommer.

Ainsi, les espèces envahissantes sont connues pour perturber les écosystèmes, et, via des interactions directes avec les espèces locales, elles entraînent des disparitions (ou d'importantes réductions d'effectif) qui en font la seconde cause d'érosion de la biodiversité au niveau mondial après la destruction et la fragmentation des écosystèmes (Vitousek *et al.* 1997). Les causes écologiques de ces impacts peuvent être directes en cas de prédation ou de compétition pour une ressource commune. La couleuvre *Boiga irregularis* a entraîné l'appauvrissement de l'avifaune de l'île de Guam par prédation directe (Fritts & Rodda 1998). De même, la célèbre perche du Nil (*Lates niloticus*) serait en partie responsable (avec l'eutrophisation) de la disparition d'environ 50% des 500 espèces de cichlidés du Lac Victoria en Afrique (Kaufman *et al.* 1997; Witte *et al.* 2000). D'autres causes écologiques plus indirectes peuvent également entrer en jeu, telle que la compétition apparente (Courchamp *et al.* 2000; Holt 1977). Ainsi, trois sous-espèces du renard gris insulaire (*Urocyon litoralis*) vivant sur des îles au large de la Californie auraient presque disparu sous la pression de prédation d'un aigle natif (*Aquila chrysaetos*) dont les densités de populations ont largement augmenté grâce à la présence de cochons sauvages envahissants (Roemer *et al.* 2001).

Historiquement, les invasions biologiques sont aussi le principal moteur de la spéciation allopatrique en favorisant les isolements géographiques entre populations. Lors du grand échange interaméricain via l'isthme de Panama, certaines espèces de mammifères disparurent (principalement des marsupiaux sud-américains, mais le rôle des échanges biotiques dans ces disparitions est controversé), mais la colonisation de nouveaux milieux a surtout permis une radiation évolutive formidable à l'origine d'un grand nombre de nouvelles espèces sur tout le continent (Webb 1991). Actuellement, les invasions biologiques sont clairement à l'origine d'augmentation de diversité spécifique à l'échelle régionale (i.e. localement, le nombre d'espèces introduites est en moyenne supérieur au nombre d'espèces qui s'éteignent, Sax & Gaines 2003; Wardle *et al.* 2011). Par ailleurs, certaines espèces envahissantes peuvent avoir un effet positif sur le nouvel écosystème, tandis que des espèces natives sont parfois particulièrement nuisibles (Davis *et al.* 2011). Toutefois, à l'échelle mondiale, il est évident que le rythme actuel des invasions biologiques entraîne une réduction

majeure de biodiversité sans que les phénomènes de spéciation et de radiation n'aient le temps d'opérer (Sax & Gaines 2003). De plus, les extinctions sont précédées d'un temps de latence, et les conséquences écologiques de nombreuses invasions récentes ne sont par conséquent pas encore visibles (Sax & Gaines 2008). Certains auteurs parlent d'homogénéisation biotique à l'échelle mondiale, faisant référence à la réduction de la biodiversité, mais également au grand succès planétaire d'un nombre limité d'espèces (McKinney & Lockwood 1999; Olden *et al.* 2004; Ricciardi 2007).

b) Impacts économiques

De nombreux auteurs ont tenté de quantifier les coûts associés aux espèces envahissantes. Ce travail s'avère particulièrement difficile (e.g. Born *et al.* 2005), mais toutes les études indiquent que les conséquences économiques des invasions biologiques sont considérables. Pimentel *et al.* (2001) ont estimé le coût des invasions biologiques dans 6 pays (USA, Royaume Uni, Australie, Afrique du Sud, Inde et Brésil) et ont extrapolé les résultats obtenus à l'ensemble de la planète. Ils estiment ainsi qu'environ 480 000 espèces sont envahissantes dans le monde, dont 20 à 30% coûteraient chaque année 1 400 milliards US$.

L'agriculture et la pêche sont particulièrement affectées négativement par les invasions biologiques. Ainsi, les pertes de production et la mise en place de méthodes de lutte liées à la chrysomèle du maïs *Diabrotica virgifera virgifera* (originaire d'Amérique Centrale) aux Etats-Unis auraient un coût d'environ 1,17 milliard US$ par an (Sappington *et al.* 2006). D'autres coûts peuvent également être liés à de la dégradation de matériel : le taret *Teredo navalis* engendre un coût d'environ 205 millions US$ par an en détériorant les quais et les coques de bateaux dans la baie de San Fransisco où il est envahissant (Pimentel *et al.* 2001). Enfin, les coûts liés à la perturbation des écosystèmes et à la réduction de biodiversité sont difficile à quantifier, mais probablement importants (Balmford *et al.* 2002; Costanza *et al.* 1997).

Les espèces envahissantes engendrent également des bénéfices économiques. Les plantes cultivées et les animaux d'élevage en sont un exemple évident (Pimentel 2002). De même, le contrôle de ravageurs par la lutte biologique classique permet à des prédateurs ou des parasites de réduire durablement des pertes de production agricole dans une région donnée en devenant eux-mêmes envahissants (Eilenberg *et al.* 2001). La pêche commerciale profite parfois également de certaines espèces envahissantes comme ce fut le cas, au moins jusqu'au milieu des années 90, avec la perche du Nil dans le Lac Victoria (Witte *et al.* 2000). Certains

bénéfices économiques sont également visibles dans le domaine du tourisme et du loisir, notamment dans les domaines liés à la chasse ou à la pêche sportive (e.g. Loomis 1995). Toutefois, l'ensemble de ces bénéfices, qui sont d'ailleurs en partie pris en compte dans l'analyse de Pimentel *et al.* (2001), ne suffit pas à compenser les coûts importants occasionnés par les espèces envahissantes.

c) Impacts sur la santé humaine

Enfin, les impacts des invasions sur la santé humaine sont réels et inquiétants. Il a par exemple été démontré que les ballasts des bateaux étaient capables de transporter sur de grandes distances de nombreux microorganismes potentiellement pathogènes (e.g. Ruiz *et al.* 2000). Bien sûr, un des meilleurs moyens de transport pour un pathogène de l'homme est l'homme lui-même, et beaucoup de virus, bactéries ou protozoaires se sont disséminés en suivant les déplacements de l'homme (Smith *et al.* 2007; Tatem *et al.* 2006). Des espèces vectrices, elles-mêmes envahissantes, sont également à l'origine de l'introduction de maladies humaines. L'exemple du rat noir (*Rattus rattus*) qui a entraîné en Europe une série d'épidémies de peste bubonique est bien connu (e.g. Monecke *et al.* 2009; Panagiotakopulu 2004). Plus récemment, la présence depuis quelques années du moustique tigre asiatique *Aedes albopictus* en Afrique, en Europe et en Amérique inquiète les autorités sanitaires car l'espèce est un vecteur efficace d'une trentaine de virus dangereux pour l'Homme, dont les virus du chikungunya, de la dengue ou de la fièvre jaune (Eritja *et al.* 2005; Juliano & Lounibos 2005; Lounibos 2002). Certaines espèces envahissantes peuvent également présenter un fort potentiel allergène. C'est le cas de l'ambroisie (*Ambrosia artemisiifolia*), une plante herbacée nord-américaine qui a envahi l'Europe au XX^{ième} siècle (Genton *et al.* 2005), et dont le pollen provoque de fortes rhinites allergiques (D'Amato *et al.* 2007). Enfin, les impacts des espèces envahissantes sur l'alimentation humaine ont également d'importantes conséquences sanitaires. L'introduction en Europe autour de 1843 de l'oomycète d'origine américaine *Phytophthora infestans*, responsable du mildiou de la pomme de terre, fut à l'origine de la Grande Famine en Irlande qui entraîna la mort de plus d'un million de personnes (Andrivon 1996).

Les effets bénéfiques des invasions biologiques sur la santé humaine passent principalement par la domestication et la dissémination d'espèces faciles à élever et à reproduire pour l'alimentation humaine. D'autres conséquences positives sont moins évidentes. Ainsi, le remplacement en Europe du rat noir (*Rattus rattus*) par le rat brun (*R.*

norvegicus) au cours du XVIII^{ième} siècle pourrait avoir joué un rôle dans la fin des épidémies de peste car la seconde espèce est plus résistante à la bactérie responsable de la maladie (Monecke *et al.* 2009). Enfin, tout comme dans le domaine économique, l'introduction d'auxiliaires de lutte biologique peut avoir un impact positif sur la santé humaine. C'est le cas pour le guppy sauvage (*Gambusia affinis*), poisson américain largement introduit dans le monde, qui est un efficace prédateur des larves de moustique (Pyke 2008). Toutefois ce dernier exemple illustre dans le même temps les impacts négatifs liés aux invasions biologiques car le guppy sauvage est également reconnu comme nuisible vis-à-vis de l'intégrité des écosystèmes (Pyke 2008).

d) Lutte contre les invasions biologiques

Les précédentes parties mettent en évidence les nombreux impacts négatifs associés aux espèces envahissantes. Dans un contexte où le nombre d'invasions biologiques est en forte augmentation, la nécessité de réduire les flux d'espèces liés aux activités humaines et de contrôler les espèces déjà envahissantes est évidente. Les méthodes de prévention et de lutte sont nombreuses, et une large littérature leur est consacrée (e.g. Veitch & Clout 2003). Je ne développerai toutefois pas ce large sujet ici. Néanmoins, notons que l'efficacité de ces méthodes est très largement liée à de bonnes connaissances sur la biologie des espèces ainsi que sur le processus même d'invasion biologique (Mack *et al.* 2000; Simberloff *et al.* 2005). Les espèces envahissantes constituent ainsi une formidable source de connaissances à des fins appliquées, mais également plus académiques.

I.2.4. Ecologie et biologie évolutive des invasions biologiques

On a l'habitude de parler d'espèces envahissantes, mais il serait plus correct de parler de populations envahissantes (Colautti & MacIsaac 2004). Par exemple, plusieurs populations d'une seule et même espèce n'auront pas forcément le même potentiel pour envahir. Ainsi, toutes les disciplines reliées à la biologie des populations sont susceptibles d'aider à mieux comprendre les invasions biologiques. Inversement, les espèces envahissantes peuvent être considérées comme des expériences grandeur nature permettant d'aborder des questions fondamentales liées à la colonisation, aux interactions interspécifiques ou aux variations démographiques (e.g. Cox 2004; Lockwood *et al.* 2007; Sax *et al.* 2005). Ces questions peuvent également avoir un intérêt pour d'autres disciplines très appliquées comme la

biologie de la conservation ou la lutte biologique. On distingue deux principales catégories de questions liées aux invasions : (i) quelles sont les raisons du succès d'une invasion et (ii) quelles sont les conséquences éco-évolutives d'une invasion réussie ?

a) Raisons du succès d'une invasion

Dans la définition indiquée dans la partie I.2.1 (« il y a invasion biologique lorsque des organismes sont introduits dans une nouvelle aire géographique, souvent distante de l'aire d'origine, y persistent puis y prolifèrent démographiquement et spatialement »), on constate qu'une invasion biologique est constituée de trois principales étapes (Figure 1) : (i) l'introduction, (ii) l'établissement et (iii) la prolifération. La plupart des auteurs s'accordent sur cette description, même si chaque étape peut elle-même être décomposée en sous étapes (e.g. Blackburn *et al.* 2011; Colautti & MacIsaac 2004; Duncan *et al.* 2003; Kolar & Lodge 2001; Lockwood *et al.* 2007; Richardson *et al.* 2000; Sakai *et al.* 2001). Au niveau spatial et/ou démographique, les trois principales étapes d'une invasion sont particulièrement bien définies (Figure 1) : (i) un nombre limité d'individus est introduit dans un espace donné du nouveau milieu, (ii) s'ensuit une relative stabilité spatiale et démographique (cette étape peut éventuellement être très courte, e.g. Daehler 2009) et enfin (iii) la population grossit et s'étend. Si elle parvient à passer sans encombre chacune de ces étapes, la population est dite envahissante. Toutefois, un tel succès est rare.

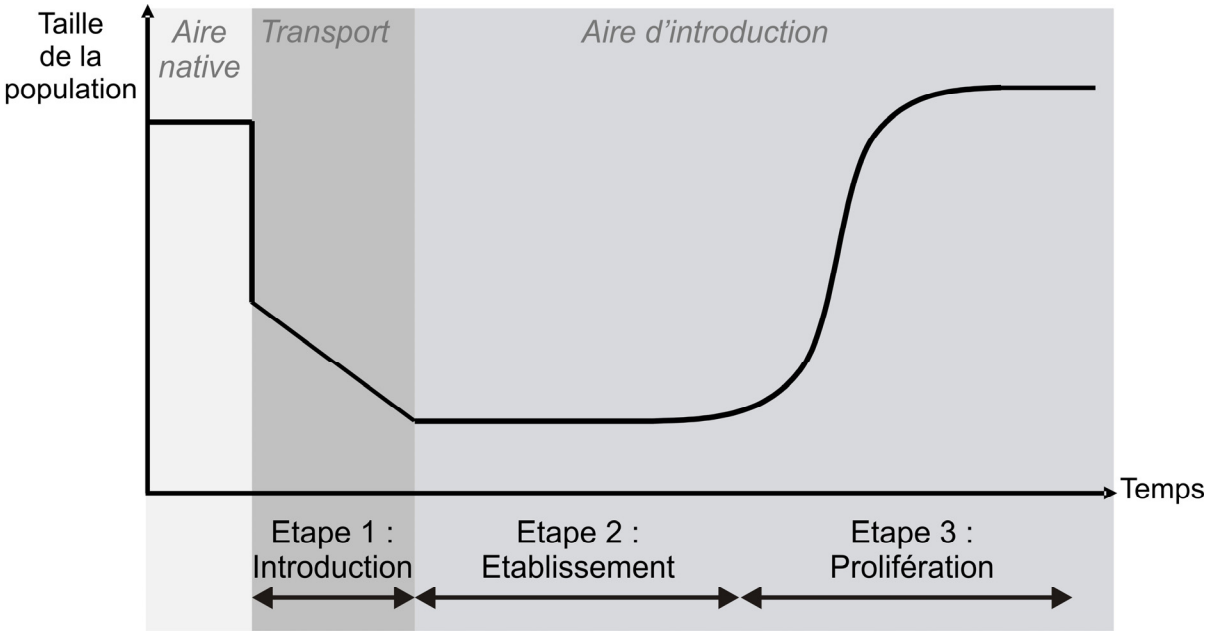


Figure 1 : Représentation schématique de la démographie d'une invasion biologique. Les durées de chacune des trois étapes sont arbitraires.

En effet, malgré le nombre important d'espèces envahissantes dans le monde, différentes études ont montré que la majorité des introductions d'individus dans une aire donnée se solde par un échec : les individus ne s'établissent pas (extinction de la population), ou les individus s'établissent mais ne parviennent pas à étendre leur aire de répartition. Ainsi, Suarez *et al.* (2005) ont recensé 232 espèces de fourmis interceptées dans les marchandises importées aux USA entre 1927 et 1985 par le USDA. Parmi ces espèces, 12 % sont établies et seulement 1% sont envahissantes aux USA. D'autres études ont également mis en évidence la forte proportion d'échecs à chacune de ces étapes sur d'autres groupes d'espèces (e.g. Bomford *et al.* 2009; Jeschke & Strayer 2005; Vall-Ilosera & Sol 2009). Williamson & Fitter (1996a) ont tenté de formaliser le risque d'échouer à chacune des étapes par la « règle des 10 » (« Tens rule » : 10% de succès à chacune des étapes). Cette règle est succincte et ne doit pas être prise au pied de la lettre (Williamson 2006), mais elle a eu le mérite d'illustrer très tôt un fait maintenant clairement établi : il est beaucoup plus fréquent de rater que de réussir une invasion.

Dès lors, cette constatation a amené les scientifiques et les gestionnaires à se poser la question suivante : quels sont les facteurs-clés du succès d'une invasion ? Cette question est centrale dans le domaine de la biologie de l'invasion (e.g. Facon *et al.* 2006; Lee 2002; Lockwood *et al.* 2007; Sax *et al.* 2007; Yoshida *et al.* 2007) qui consiste à décrire et comprendre comment une espèce parvient à franchir les barrières géographiques, écologiques et génétiques auxquelles elle est confrontée.

b) Conséquences éco-évolutives d'une invasion réussie

Une invasion biologique réussie aura de nombreuses conséquences appliquées du fait des impacts écologiques, économiques et/ou sanitaires dont elle peut être responsable (voir partie I.2.3). Mais les conséquences éco-évolutives sont également nombreuses. En effet, une espèce envahissante colonise un nouveau milieu dans lequel elle entre en interaction avec des écosystèmes constitués de nombreuses espèces, de gradients environnementaux, de milieux plus ou moins anthropisés, etc. De nombreux concepts en écologie des communautés (e.g. Bruno *et al.* 2005) ou en biologie évolutive (e.g. Huey *et al.* 2005) par exemple peuvent être abordés et testés en observant les espèces envahissantes. Celles-ci présentent en effet certaines caractéristiques qui en font de bonnes candidates pour aborder diverses questions fondamentales.

De nombreuses invasions sont récentes, et il est alors possible d'observer la naissance de nouvelles interactions interspécifiques et d'étudier des phénomènes évolutifs rapides. Par ailleurs, certaines espèces sont envahissantes dans différentes zones géographiquement distinctes (e.g. Ciosi *et al.* 2008), ce qui permet de disposer de répétitions indépendantes permettant d'étudier des processus de colonisation et des trajectoires évolutives. Enfin, les populations envahissantes sont en déséquilibre spatial pendant l'étape d'expansion géographique, et les processus évolutifs impliqués peuvent être précisément étudiés (e.g. Phillips *et al.* 2010a).

c) Décrire et comprendre les trois étapes clés d'une invasion biologique

Décrire et comprendre chacune des trois étapes clés d'une invasion biologique (i.e. introduction, établissement et prolifération) permet d'aborder les questions liées au succès des invasions (en particulier lors des étapes d'introduction et d'établissement) et à l'écologie évolutive des espèces (en particulier lors des étapes d'établissement et de prolifération). Les connaissances acquises peuvent ensuite avoir de réelles implications appliquées dans les domaines de la gestion des espèces envahissantes, la lutte biologique ou la biologie de la conservation. Dans les parties suivantes, je vais résumer une partie des questions et concepts en écologie et surtout en biologie évolutive associés à chacune de ces étapes.

I.3. L'introduction : démographie, dérive et routes d'invasion.

Au cours d'une invasion biologique, l'étape d'introduction correspond au franchissement d'une barrière géographique limitant l'aire de répartition d'une espèce (Richardson *et al.* 2000). Elle consiste en une phase de prélèvement dans le milieu d'origine, d'une phase de transport et d'une phase de relâchement dans le nouveau milieu.

I.3.1. Quel moyen de transport ?

a) Introductions naturelles

Comme décrit dans la section I.1, les organismes ont depuis toujours colonisé de nouvelles régions grâce notamment à l'apparition de contact(s) entre des zones précédemment isolées, ou par différents modes de dispersions actives ou passives. Encore aujourd'hui, de tels types d'invasions « naturelles » sont observables. Surtsey est une île d'1,4 km² au large des côtes Islandaises qui est née d'une éruption volcanique entre 1963 et 1967. Les oiseaux ont été les premiers à coloniser l'île (premières nidifications de goélands observées en 1970) illustrant le rôle de la dispersion active dans une étape d'introduction. Les plantes, quant à elles, ont parfaitement illustré la palette des différents modes de transport passif : parmi les espèces de plantes vasculaires recensées sur l'île en 1986, Fridriksson (1987) estime que 9% ont été transportés par les airs, 27% par les courants marins et 64% par les oiseaux. Tous ces différents modes de transport sont liés à diverses adaptations permettant de se déplacer sur de longues distances ou de saisir le bon « véhicule ».

Mais l'homme a considérablement accéléré le rythme des invasions biologiques en devenant le principal vecteur d'introduction (e.g. Ricciardi 2007). Les introductions liées à l'homme peuvent être très simplement classées en deux catégories : les introductions intentionnelles et les introductions accidentelles.

b) Introductions intentionnelles

Le transport intentionnel d'espèces ou de groupes d'espèces d'un endroit à un autre est très ancien, comme l'illustre l'introduction en Chine du tamarinier (*Tamarindus indica*) originaire d'Afrique il y a 2400 ans (Xie *et al.* 2001). La première cause des introductions intentionnelles est bien sûr l'alimentation humaine, mais d'autres raisons motivent également le transport délibéré d'espèces. La chasse et la pêche de loisir sont par exemple à l'origine de la très grande répartition géographique de certaines espèces telles que la truite arc-en-ciel (*Oncorhynchus mykiss*), originaire d'Amérique du Nord et maintenant présente sur tous les continents et dans plus de 100 pays (Lockwood *et al.* 2007). Une autre cause est l'importation des espèces pour leur intérêt esthétique (c'est le cas pour de nombreuses plantes) ou culturel. L'exemple de l'introduction de l'étourneau sansonnet (*Sturnus vulgaris*) en 1890 à New York par un fanatique de Shakespeare qui voulait introduire à Central Park toutes les espèces citées dans l'œuvre de l'auteur est fameux (Cabe 1998), mais il est symptomatique de ce besoin

culturel qui explique par exemple que la Nouvelle-Zélande soit presque devenue une réplique biologique de la Grande-Bretagne. Enfin, la lutte contre les organismes nuisibles est également à l'origine de nombreuses introductions. La lutte biologique est en effet très ancienne puisqu'on retrouve des récits d'utilisation de chats pour lutter contre certains rongeur il y a plus de 2000 ans. Mais la lutte biologique s'est vraiment développée au cours du 20^{ième} siècle avec l'utilisation d'espèces très différentes comme des mammifères (e.g. la petite mangouste indienne, Simberloff *et al.* 2000), des batraciens (e.g. le crapaud buffle, Easteal 1981), des poissons (e.g. le guppy sauvage, Pyke 2005), des microorganismes (e.g. le virus de la myxomatose, Best & Kerr 2000) mais aussi et surtout des arthropodes dans le domaine de la lutte contre les mauvaises herbes ou les ravageurs des plantes cultivées (e.g. les trichogrammes, Smith 1996).

c) Introductions accidentelles

De nombreuses espèces sont transportées et relâchées par l'homme de manière accidentelle. Ce type d'événement est lui aussi ancien (e.g. le cas des rats dans les bateaux, Harris 2009), mais il a pris un essor fulgurant au cours des deux derniers siècles avec l'augmentation des échanges commerciaux et l'amélioration des modes de transport (Tatem & Hay 2007; Wilson *et al.* 2009). Parmi les moyens de transport fréquemment empruntés par des organismes clandestins, on trouve par exemple les ballasts des bateaux (Carlton & Geller 1993; Drake & Lodge 2004) et les soutes des avions (Tatem & Hay 2007). On pense par exemple que la célèbre moule zébrée (*Dreissena polymorpha*) originaire de la Mer Noire et de la Mer Caspienne a été introduite dans les Grands Lacs Américains via des ballasts de bateaux (Griffiths *et al.* 1991). La mouche du fruit *Ceratitis capitata* à l'origine de lourdes conséquences économiques a probablement été importée aux USA dans des marchandises transportées dans des avions comme le suggèrent les fréquentes observations de cette espèce dans ce type de véhicule (Liebhold *et al.* 2006). Dans certains cas, le transport d'individus d'une espèce donnée se fait de manière intentionnelle, mais pas le relâchement dans le milieu. Ainsi, des évasions des établissements zoologiques ou botaniques sont à l'origine d'invasions biologiques remarquables, comme l'illustre l'expansion de l'algue *Caulerpa taxifolia* en Mer Méditerranée après son relâchement accidentel depuis le Musée Océanographique de Monaco en 1984 (Jousson *et al.* 1998; Meinesz & Hesse 1991). De même, le commerce des animaux de compagnies est à l'origine de nombreuses introductions involontaires ayant mené à des succès d'invasions. Entre 1989 et 1997, plus de 52 millions de tortues de Floride (*Trachemys*

scripta elegans) ont été commercialisés dans le monde, et l'espèce est maintenant envahissante dans une trentaine de pays probablement suite à l'abandon d'individus dans la nature par des particuliers (Cadi & Joly 2004; Telecky 2000).

I.3.2. L'introduction : une étape importante du processus d'invasion.

De nombreux auteurs considèrent que l'étape d'introduction est la plus importante car elle est celle pour laquelle les mesures de gestion auront le plus d'efficacité (Mack *et al.* 2000), mais aussi parce qu'il s'agirait de la plus difficile à franchir dans le processus d'invasion. Ainsi, Jeschke *et al.* (2005) ont montré que, pour trois groupes de vertébrés (poisson, mammifères et oiseaux), l'étape d'introduction était de loin la plus difficile à franchir avec environ 5% de succès contre environ 50% pour chacune des deux autres étapes. En fait, malgré la grande variété des modes de transport, toutes les espèces ne seront pas adaptées à un déplacement sur de longues distances dans des conditions souvent spartiates. Les organismes planctoniques semblent par exemple bien mieux supporter les longs trajets dans les ballasts des bateaux que les poissons (Carlton & Geller 1993). Pour ces derniers, les espèces se nourrissant dans le noir seront favorisées (Kolar & Lodge 2001). Par ailleurs, la probabilité d'être mis en contact avec un moyen de transport varie selon l'espèce. Le fait d'être adapté aux milieux anthropisés, d'avoir une aire de répartition native de grande taille (e.g. Goodwin *et al.* 1999) et/ou d'être proche d'une plaque tournante de transport (e.g. Tatem & Hay 2007) sera avantageux. Bien entendu, pour mettre toutes les chances de son côté, le mieux est de se rendre indispensable à l'homme et cette étape sera alors rendue beaucoup plus simple (i.e. introductions intentionnelles).

On observe aussi quelques cas d'adaptation permettant une meilleure dissémination par les transports anthropiques. L'évolution d'agroécotypes en est un bon exemple. La mauvaise herbe « Panic des marais » (*Echinochloa crus-galli*) a ainsi évolué dans les cultures de riz (*Oryza* sp.) afin d'imiter la phénologie et la morphologie du riz lui-même. Une telle adaptation aux pratiques culturales a permis aux agroécotypes de cette espèce d'être disséminés dans tous les pays où le riz est cultivé (Barrett 1983). Toutefois, à l'exception de l'exemple des agroécotypes de mauvaises herbes, l'importance réelle des phénomènes adaptatifs dans le succès de l'étape d'introduction est inconnue. Par contre, les conséquences de l'étape d'introduction sur le potentiel adaptatif nécessaire au succès de l'invasion ont été

beaucoup plus étudiées. Il s'agit principalement du rôle des effets de fondation dont les répercussions en terme de variabilité génétique et de dérive sont potentiellement fortes.

I.3.3. Effet de fondation et composition génétique

Il est bien entendu impossible de prélever tous les individus d'une espèce donnée dans une aire d'origine pour les relâcher ensuite dans un nouveau milieu (que ce soit de manière accidentelle ou intentionnelle). Par nature, un nombre limité d'individus sera prélevé, et ce nombre peut diminuer parfois fortement avant la libération dans le milieu à cause de la mortalité lors du transport. De même, la zone de prélèvement dans l'aire d'origine sera restreinte. On appelle « effets de fondation » les conséquences génétiques liées à ces phénomènes.

a) Conséquences génétiques des effets de fondation

Le nombre de fondateurs (i.e. le nombre d'individus vivants finalement introduits dans le nouveau milieu) est souvent faible, en particulier dans les cas d'introduction accidentelle. On parle alors de « goulots d'étranglement » : la diversité génétique sera réduite par rapport à celle de l'aire d'origine (Nei *et al.* 1975). Et si la population introduite ne s'éteint pas mais reste de petite taille pendant plusieurs générations, la forte dérive induite réduira d'autant plus la diversité génétique (Nei *et al.* 1975). Par conséquent, ont peut s'attendre à ce que, par rapport à la population d'origine, une population introduite soit caractérisée par (i) une diversité allélique plus faible, (ii) une hétérozygotie plus faible, (iii) des fréquences alléliques différentes et (iv) une variance génétique additive plus faible (Wares *et al.* 2005). Ce dernier point est particulièrement important car il pourrait avoir une implication directe dans la capacité de la population à devenir envahissante en limitant le potentiel adaptatif permettant l'établissement dans le nouveau milieu (Lee 2002).

b) Effets de fondation et effet Allee

L'introduction d'un nombre limité d'individus aura d'autres conséquences. Les petites populations sont par nature soumises à des contraintes qui peuvent compromettre leur survie. Une corrélation positive entre le taux d'accroissement et la taille d'une population constitue ce que l'on appelle un effet Allee (Courchamp *et al.* 1999). Cette relation qui désavantage les petites populations peut être due (i) à la dépression de consanguinité, (ii) à la stochasticité

démographique et (iii) la réduction des interactions de coopération (e.g. difficulté à trouver un partenaire sexuel, avantage du groupe face à un prédateur, etc.). Cette relation entre accroissement et nombre d'individus a été observée de manière empirique à de nombreuses reprises (Beirne 1975; Green 1997; Memmott *et al.* 2005; Simberloff 2009; Veltman *et al.* 1996). Ainsi, Hopper & Roush (1993) ont montré à partir de données bibliographiques que le succès d'invasion des insectes parasitoïdes intentionnellement introduits lors d'opérations de lutte biologique est d'autant plus probable que le nombre d'individus initialement introduits est grand.

c) Invasion = paradoxe évolutif ?

Certains auteurs ont suggéré l'idée que les invasions biologiques réussies constituaient de véritables paradoxes évolutifs du fait de succès visiblement incompatibles avec les goulots d'étranglement ayant lieu lors de l'étape d'introduction (e.g. Allendorf & Lundquist 2003). L'accumulation de données génétiques sur de nombreuses espèces envahissantes a en grande partie levé ce paradoxe car la réduction de diversité génétique observée est bien souvent faible ou nulle, et dans certains cas, les populations envahissantes sont même plus diversifiées que les populations natives (Bossdorf *et al.* 2005; Dlugosch & Parker 2008; Wilson *et al.* 2009). Roman et Darling (2007) ont ainsi constaté que seulement 37% des 43 introductions qu'ils ont répertoriées montraient une réduction de diversité génétique. Les explications peuvent être de plusieurs natures : (i) le nombre d'individus introduits est souvent grand dans les cas de succès, (ii) l'étape d'établissement dure peu de temps, (iii) l'aire envahie est en contact avec l'aire d'origine ou (iv) il y a des introductions multiples qui peuvent notamment aboutir à des mélanges génétiques (« hybridations ») entre des individus issus de populations génétiquement différenciées. Le dernier point semble particulièrement important, et la notion de « pression de propagule » qui associe le nombre d'introductions et le nombre d'individus à chaque introduction est souvent utilisée (Lockwood *et al.* 2005). Notons toutefois que les analyses de différenciation génétique entre aires natives et aires envahies peuvent pâtir d'une mauvaise identification de la vraie population source d'une population envahissante donnée, en particulier lorsque l'aire native est génétiquement très structurée (et donc fatalement échantillonnée de manière incomplète) et/ou lorsqu'il existe d'autres populations envahissantes. La description des routes d'invasion constitue une étape clé dans l'étude de l'étape d'introduction (e.g. Wares *et al.* 2005).

I.3.4. Décrire les routes d'invasion

a) Pourquoi s'intéresser aux routes d'invasion ?

On appelle route d'invasion les voies géographiques empruntées par des individus entre leur population source et la population envahissante dont ils sont à l'origine (Estoup & Guillemaud 2010). Connaître précisément l'origine des populations envahissantes a un intérêt appliqué indéniable. En effet, la prévention des introductions est considérée comme la méthode la plus efficace pour limiter les invasions biologiques (Mack *et al.* 2000). Si une zone géographique ou un moyen de transport à l'origine d'introductions récurrentes est identifié, des mesures de surveillance accrue ou de quarantaine peuvent être mises en place. De même, la lutte biologique classique contre un ravageur envahissant peut-être facilitée par l'identification de l'ère d'origine précise de celui-ci, en permettant le choix d'un auxiliaire spécifique ayant co-évolué avec le ravageur (Roderick & Navajas 2003). Retracer les routes d'invasion a également un intérêt fondamental important.

L'étude des invasions biologiques passe bien souvent par la comparaison des populations envahissantes avec les populations natives, notamment afin d'identifier d'éventuels changements évolutifs importants (Hierro *et al.* 2005; Keller & Taylor 2008). Il est alors primordial de pouvoir comparer une population envahissante avec sa vraie source (Dlugosch & Parker 2008; Estoup & Guillemaud 2010; Keller & Taylor 2008; Richards *et al.* 2006; Wares *et al.* 2005). Ainsi, l'observation de divergences phénotypiques entre une population envahissante et une population native qui n'est pas sa source peut être considérée à tort comme de l'adaptation dans le contexte de l'invasion alors qu'il peut s'agir d'adaptation locale dans l'aire native (Keller & Taylor 2008). De même, la connaissance du nombre d'introductions et de l'occurrence de phénomènes d'hybridation est indispensable à toute étude exhaustive d'un processus d'invasion.

b) Comment retracer les routes d'invasion : les méthodes directes

Il existe deux types de méthodes permettant de retracer les routes d'invasion (Estoup & Guillemaud 2010). Tout d'abord, les méthodes directes sont basées sur l'utilisation d'informations géographiques, historiques et climatiques : dates et sites de première observation, proximité d'un site de transport (aéroport, gare ferroviaire, port, etc.), enregistrements par les services de quarantaine, etc. L'origine des populations envahissantes de lapins Européens (*Oryctolagus cuniculus*) en Australie est par exemple particulièrement

bien documentée, et les 13 lapins importés de Grande-Bretagne par un riche amateur de chasse en 1859 sont considérés comme l'origine des 600 millions d'individus recensés 80 ans plus tard (Zenger *et al.* 2003). Tatem *et al.* (2006) ont, quant à eux, retracé les routes d'invasion du moustique tigre *Aedes albopictus* à partir de données de trafics aériens et de données climatiques. Toutefois, les méthodes directes sont très limitées pour plusieurs raisons. Tout d'abord, les informations documentaires associées à une invasion donnée sont souvent incomplètes ou même absentes. En particulier, les densités souvent faibles d'individus pendant les étapes d'introduction et d'établissement rendent la détection précoce difficile. De plus, les scénarios d'invasions peuvent être très complexes avec par exemple des phénomènes d'introductions multiples (Bossdorf *et al.* 2005; Roman & Darling 2007).

c) Comment retracer les routes d'invasion : les méthodes indirectes

Les méthodes indirectes sont basées sur l'utilisation de marqueurs moléculaires. A partir de divergences de séquences ou de fréquences alléliques, il est possible d'identifier des patrons génétiques intra et inter-populationnel permettant d'inférer des liens de parenté entre populations. Par exemple, des statistiques résumées de la diversité génétique inter-populationnelles, telles que les F_{ST} (Weir & Cockerham 1984) ou les vraisemblances d'assignation (Rannala & Mountain 1997), peuvent être utilisées afin de retracer les routes d'invasion (Genton *et al.* 2005; Tepolt *et al.* 2009; Thibault *et al.* 2009). Ciosi *et al.* (2008) ont ainsi mis en évidence au moins cinq introductions indépendantes en Europe depuis l'Amérique du Nord de la chrysomèle du maïs *Diabrotica virgifera virgifera*. D'autres méthodes plus récentes de regroupement d'individus ou de populations basées sur des modèles et le calcul de vraisemblances tels que STRUCTURE (Pritchard *et al.* 2000), BAPS (Corander *et al.* 2003) ou GENELAND (Guillot *et al.* 2005) sont également utilisées pour inférer des populations sources (e.g. Darling *et al.* 2008; Marrs *et al.* 2008; Rollins *et al.* 2009).

Toutefois, toutes ces méthodes ont également leurs limites. Estoup & Guillemaud (2010) ont ainsi montré à l'aide de données simulées que lorsque les temps de divergence entre populations sont faibles (comme c'est le cas pour beaucoup d'invasions récentes), jusqu'à 40% des topologies de dendrogrammes (ici des arbres de voisinage construits sur des marqueurs génétiques neutres de type microsatellite) donnaient une image incorrecte des routes d'invasion. De même, il a été montré que les routes d'invasion inférées à partir des F_{ST} et les vraisemblances d'assignation étaient soumises à des taux d'erreur parfois élevés

(Guillemaud *et al.* 2010). De manière générale, toutes ces méthodes présentent trois limites importantes. Premièrement, elles ne prennent pas en compte la stochasticité démographique et génétique potentiellement très forte au cours d'une invasion biologique. En particulier, la dérive génétique liée aux effets de fondation va augmenter la différentiation génétique entre une population envahissante et sa source en modifiant les fréquences alléliques aléatoirement (Knowles 2009). Une seule population source peut ainsi donner naissance à une infinité de populations très différenciées au hasard des échantillonnages et des goulots d'étranglement, et brouiller ainsi considérablement le signal génétique (Estoup & Guillemaud 2010). Deuxièmement, elles considèrent que l'échantillonnage est exhaustif, or parmi les échantillons à disposition d'un généticien des populations, la source réelle peut par exemple être absente (Guillemaud *et al.* 2010). Finalement, ces méthodes indirectes (tout comme les méthodes directes) ont le désavantage de ne pas fournir de supports statistiques permettant de mesurer quantitativement la confiance que l'on peut accorder à un résultat plutôt qu'à un autre.

L'Approximate Bayesian Computation (ABC, Beaumont *et al.* 2002) est une méthode récemment développée qui se révèle être un outil puissant pour aborder de nombreuses questions en génétique des populations. L'ABC est une méthode d'inférence fondée sur des modèles dans un cadre Bayésien et qui consiste notamment à approximer des vraisemblances de modèles complexes (généralement très difficiles à calculer) par le biais de simulations de grands nombres de jeux de données (voir Encadré 1, Beaumont 2010; Bertorelle *et al.* 2010; Csillery *et al.* 2010; Lopes & Beaumont 2010). L'utilisation de la méthode ABC permet d'inclure à l'analyse des données historiques (e.g. temps depuis une divergence) et biologiques (e.g. modèle mutationnel de marqueurs) en plus des données génétiques. Dans le cadre de l'inférence de routes d'invasion, l'ABC s'est révélée très puissante : elle permet de prendre en compte l'existence de goulots d'étranglement (e.g. Pascual *et al.* 2007), d'hybridations (e.g. Verdu *et al.* 2009) ou de populations non échantillonnées (e.g. Miller *et al.* 2005). Par ailleurs, il est possible de quantifier la probabilité d'un scénario d'introduction donné par rapport à un autre et d'évaluer les erreurs de type I et de type II associées au résultat obtenu. Les résultats de routes d'invasion inférés avec la méthode ABC s'accumulent (e.g. Ascunce *et al.* 2011; Miller *et al.* 2005; Pascual *et al.* 2007; Zepeda-Paulo *et al.* 2010). Il est intéressant de noter que si les observations historiques sont parfois confirmées par les analyses (e.g. Pascual *et al.* 2007), des résultats parfois très différents et moins intuitifs ont également été obtenus (e.g. Miller *et al.* 2005).

Notons toutefois que la méthode ABC comporte également quelques limites. Par exemple, la question du choix du nombre et de la nature des statistiques résumées (cf. Encadré 1) à utiliser pour répondre à une question donnée reste posée, même si des propositions de solutions qu'il reste à évaluer sont apparues ces dernières années (Blum & Francois 2010; Joyce & Marjoram 2008; Nunes & Balding 2010; Sousa *et al.* 2009; Wegmann *et al.* 2009). L'ABC subit également des critiques propres à sa nature Bayésienne qui oblige l'utilisateur à fixer des distributions de paramètre *a priori* (cf. Encadré 1) et notamment à choisir un nombre limité de modèle (Templeton 2009; Templeton 2010). Cependant, il en est de même pour les autres méthodes fondées sur des modèles, dès lors que l'on s'attaque à des situations complexes (Beaumont *et al.* 2010). Il est en fait nécessaire d'être prudent et d'avancer pas à pas au cours d'une analyse ABC en testant des scénarios compatibles avec la biologie de l'espèce considérée et en vérifiant la confiance que l'on peut accorder à nos résultats (e.g. calcul des erreurs de type I et II et « model checking », Beaumont 2010; Beaumont *et al.* 2010; Bertorelle *et al.* 2010; Csillery *et al.* 2010). En fait, couplée avec des méthodes directes et indirectes plus classiques, l'ABC peut se révéler d'autant plus efficace (Estoup & Guillemaud 2010).

Encadré 1 : Inférence des routes d'invasion à l'aide de l'Appoximate Bayesian Computation (ABC).

Principe général

A partir de données observées (par exemple des génotypes multilocus individuels), il est possible dans un contexte Bayésien de choisir des modèles et/ou d'estimer des paramètres sous un modèle donné (Beaumont & Rannala 2004; Shoemaker *et al.* 1999). Ainsi, on pourra rechercher la distribution *a posteriori* d'un paramètre (ou d'un modèle) θ connaissant les données observées D :

$$P(\theta|D) \propto P(D|\theta)P(\theta)$$

Où $P(\theta)$ est la distribution *a priori* du paramètre (ou du modèle). La probabilité $P(D|\theta)$ d'observée les données D étant donnée le paramètre (ou le modèle) θ correspond à la vraisemblance de θ.
Il est possible de calculer ou d'estimer cette vraisemblance (par exemple à l'aide de chaîne de Markov de Monte-Carlo), mais si les modèles sont très complexes, cela peut être très difficile, voir impossible. Des méthodes sans calcul implicite de la vraisemblance (dite « likelihood free ») permettent de calculer une distribution *a posteriori* en remplaçant l'estimation de la vraisemblance par une approximation via l'utilisation de statistiques résumés et de simulations. L'« Approximate Bayesian Computation » (ABC, Beaumont *et al.* 2002) est une de ces méthodes particulièrement bien adaptée à l'analyse de données génétiques (Beaumont 2010; Bertorelle *et al.* 2010; Csillery *et al.* 2010; Lopes & Beaumont 2010).

Déroulement d'une analyse ABC pour l'inférence de routes d'invasion

Le déroulement d'une analyse ABC peut se décomposer en 5 étapes :

- Etape 1 : Définition des modèles. Il s'agit d'établir des scénarios d'introduction (e.g. Figure E1) et d'associer des valeurs ou des distributions *a priori* à chacun des paramètres génétiques (e.g. taux de mutation des marqueurs), démographiques (e.g. tailles efficaces des populations à l'équilibre et lors des goulots d'étranglement), historiques (e.g. dates de première observation de chacune des deux populations envahissantes) et à la fréquence des modèles. Il s'agit de l'étape de définition des « priors ».

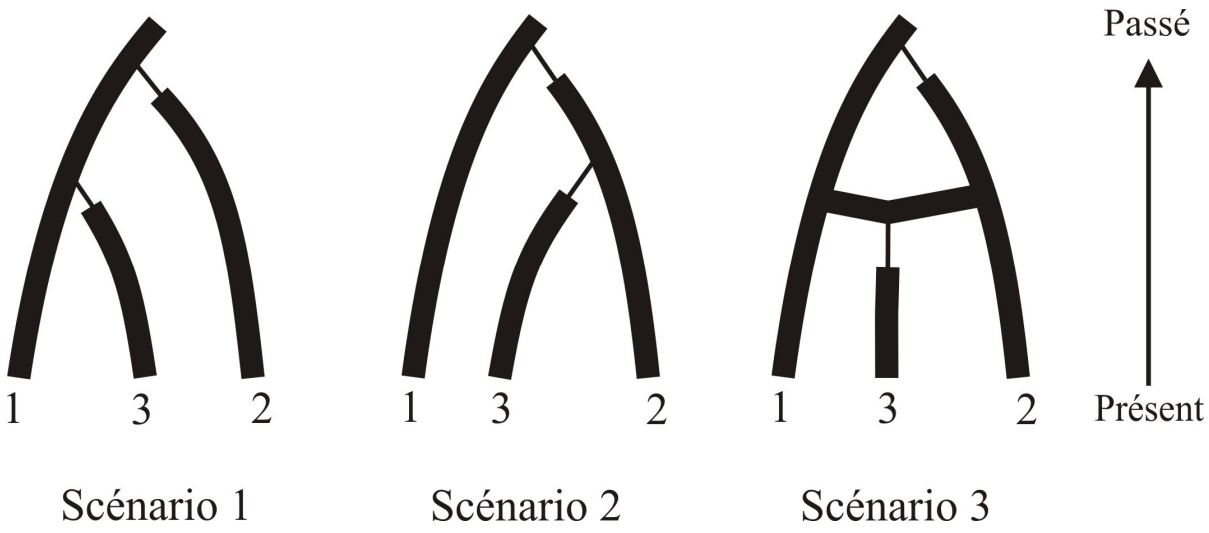


Figure E1 : exemple simple de scénarios d'introduction définis afin de retracer les routes d'invasion de la population 3. L'épaisseur des traits est proportionnelle à la taille efficace de la population. La population 1 est une population de l'aire native. Les populations 2 et 3 sont envahissantes, ce qui est notamment illustré ici par une réduction de taille efficace au moment de l'introduction (goulot d'étranglement). Notons que dans cet exemple, les informations historiques nous permettent de ne pas considérer les cas où la population 2 est issue de la population 3.

- Etape 2 : Choix des statistiques résumées et simulations de données génétiques. Il s'agit de simuler des données génétiques selon chacun des scénarios à partir d'un modèle stochastiques de mutation et de dérive liant démographie et génétique (modèle de coalescence). Les paramètres sont tirés dans les priors. On simule par exemple 1 million de jeux de données pour chacun des scénarios que nous avons définis lors de l'étape 1. Chacun des jeux de données est ensuite résumé à l'aide de statistiques précédemment choisies pour décrire les variations génétiques intra et interpopulationnelles (e.g. nombre moyen d'allèles par locus, hétérozygotie attendue, F_{ST} par paire de population, etc.).

- Etape 3 : Rejet des jeux de données les moins informatifs. Pour cela, on calcule des distances euclidiennes entre les statistiques simulées et observées, puis on rejette les simulations éloignées des observations au-delà d'un certain seuil.

- Etape 4 : Sélection du scénario le plus probable. Calcul de la probabilité *a posteriori* de chaque scénario par une régression logistique sur les jeux de données simulés conservés lors de l'étape 3. Il est ensuite possible d'estimer des distributions *a posteriori* des paramètres du scénario gagnant.

- Etape 5 : Evaluation de la puissance d'analyse et de la pertinence des inférences. Il est possible (et important) (i) de vérifier la puissance de l'analyse effectuée en calculant les erreurs de type I et les erreurs de type II à l'aide de données simulées (on répond à la question : notre analyse nous permet-elle de bien distinguer les différents scénarios ?) et (ii) de contrôler la concordance entre le scénario sélectionné et les données observées en simulant des données à partir de valeurs de paramètres tirées dans les distributions *a posteriori* (i.e. « model checking » ; on répond à la question : le scénario finalement sélectionné et les distributions *a posteriori* des paramètres inférées reproduisent-ils convenablement les données observées ?). En cas de problème, il peut être nécessaire de repasser à l'étape 1.

I.4. L'établissement : dérive, adaptation et facteurs-clés du succès de l'invasion.

Suite à l'introduction, l'étape d'établissement, également appelée phase de latence, est l'étape de l'invasion pendant laquelle la population nouvellement arrivée doit franchir de nouvelles barrières cette fois-ci écologiques et reproductrices (Richardson *et al.* 2000). La répartition spatiale et la densité de la population sont par nature limitées durant cette phase. Les caractéristiques du milieu et le potentiel adaptatif de la population introduite y auront un rôle primordial dans le succès ou l'échec final de l'invasion biologique.

I.4.1. Une étape importante… mais pas indispensable

Si l'étape d'établissement est un succès, cela signifie que la population introduite pourra se maintenir durablement dans le nouveau milieu. Elle pourra ensuite croître démographiquement et spatialement pour enfin devenir envahissante *stricto sensu*. L'exemple précédemment présenté du faible taux d'établissement des fourmis introduites aux USA (12%, Suarez *et al.* 2005) illustre clairement qu'il ne suffit pas de franchir une barrière géographique pour devenir envahissant (e.g. Bomford *et al.* 2009; Jeschke & Strayer 2005; Vall-Ilosera & Sol 2009). Le franchissement de la barrière écologique constituée par un environnement biotique et abiotique différent de celui d'origine peut en effet se révéler délicat.

Il n'est pas facile d'étudier cette étape pendant laquelle les individus sont peu nombreux et souvent difficiles à détecter. Généralement, les recherches visant à comprendre les facteurs clés du succès de l'établissement s'effectuent sur des populations déjà clairement envahissantes et qui se sont donc étendues sur un large territoire. Certaines méthodes indirectes permettent alors d'entrevoir ce qui s'est passé lors de la phase d'établissement, et on constate qu'elle peut parfois être pratiquement inexistante. Ainsi, la diversité génétique de populations de lapins Australiens mesurée à 7 marqueurs microsatellites n'est pas inférieure à celle des populations sources en Europe malgré un nombre d'individus introduits particulièrement faible (Zenger *et al.* 2003). Ce résultat suggère que la population introduite n'a pas connu un grand nombre de générations avec un petit effectif, et s'est au contraire développée démographiquement et spatialement très rapidement après l'introduction, ce qui lui a permis de limiter la dérive (Austerlitz *et al.* 1997; Nei *et al.* 1975). Le lapin, et d'autres

espèces envahissantes présentant le même type de patron génétique (e.g. Zeisset & Beebee 2003), n'a probablement eu aucune difficulté à franchir la barrière écologique, et l'espèce est manifestement passée de l'étape d'introduction à l'étape d'expansion sans passer par l'étape d'établissement proprement dite. Toutefois, le temps de latence lié à l'établissement peut au contraire être long pour certaines espèces (Caley *et al.* 2008). Dès lors, il est important de comprendre pourquoi certaines populations échouent lors de l'étape d'établissement, pourquoi d'autres réussissent après un certain temps et pourquoi d'autres réussissent immédiatement.

I.4.2. Les caractéristiques du nouveau milieu

a) Pré-adaptation, régime de migration et modification du milieu

Il est intuitivement évident que la plupart des populations et espèces introduites ne parviennent pas à s'établir car les conditions rencontrées dans le nouveau milieu sont beaucoup trop défavorables. Toutefois, de nombreux environnements éloignés spatialement possèdent des conditions de vie similaires (biomes). Une espèce peut donc être pré-adaptée à un milieu auquel elle n'a pas eu accès jusque là. Dans ce cas, il suffit d'un changement du régime de migration pour que l'espèce en question franchisse la barrière géographique la séparant du milieu auquel elle est pré-adaptée et devienne envahissante (Facon *et al.* 2006). Le serpent brun arboricole *Boiga irregularis* n'avait jamais eu accès à l'île de Guam perdue dans l'Océan Pacifique à plus de 1800 km des côtes de la Nouvelle-Guinée dont il est natif. Pourtant, les conditions climatiques y sont extrêmement similaires. La seconde guerre mondiale fut à l'origine de la création d'un couloir aérien entre la Nouvelle-Guinée et l'île de Guam destiné au transport de matériel militaire américain. C'est probablement dans des conteneurs ou des trains d'atterrissage que des individus de *B. irregularis* parvinrent clandestinement sur l'île entre 1945 et 1950, où ils établirent une population particulièrement abondante et nuisible pour la faune locale (Fritts & Rodda 1998).

Dans certains cas, le régime de migration en place permet à une espèce d'atteindre régulièrement une zone géographique donnée, mais les conditions locales dans le nouvel environnement ne permettent pas la survie. Si les conditions changent soudainement dans cet environnement, alors l'établissement devient possible (Facon *et al.* 2006). Les modifications des écosystèmes par l'homme sont un bon exemple de ces modifications rapides de l'environnement qui permettent à certaines espèces d'envahir des zones géographiques dans lesquelles elles ne parvenaient pas à s'établir auparavant. L'invasion de l'Amérique du Nord

par la chrysomèle des racines du maïs (*Diabrotica virgifera virgifera*) aurait par exemple été rendue possible par le développement, après 1950, de l'irrigation dans les cultures de maïs et de l'arrêt des rotations culturales dans la corn belt (Gray *et al.* 2009).

b) Quels milieux seront envahis ?

L'invasion d'un environnement par une espèce pré-adaptée conduit parfois à l'étonnante constatation que la population envahissante est (i) plus performante que les espèces locales de l'environnement envahi, et (ii) plus performante que ses conspécifiques dans son aire d'origine. Dès lors, des questions se posent. En particulier, quels sont les avantages pour une espèce donnée d'être introduite dans un biotope avec lequel elle n'a pas co-évolué ? Nous aborderons cette question un peu plus loin. Une autre question, liée au nouvel environnement, se pose également : existe-t-il des caractéristiques d'un écosystème qui le rendent plus ou moins sensible aux invasions ? Cette dernière question a fait l'objet de nombreuses études en écologie de l'invasion (Lockwood *et al.* 2007). Très tôt, Elton (1958) nota d'importantes différences de nombre d'espèces envahissantes selon la « nature » du milieu. Ainsi, les environnements insulaires seraient plus faciles à envahir que les milieux continentaux (e.g. Cassey 2003). De même, les écosystèmes tempérés ont davantage d'espèces envahissantes que les écosystèmes tropicaux (e.g. Sax 2001). Ou encore, le nouveau monde serait beaucoup plus facilement colonisé par des espèces exogènes que l'ancien monde (di Castri 1989). De nombreux mécanismes ont été proposés pour expliquer ces tendances. Certains sont liés aux activités humaines. Par exemple, les introductions intentionnelles ont historiquement été moins nombreuses dans les écosystèmes tropicaux que dans les écosystèmes tempérés (Sax 2001). De même, les espèces de l'ancien monde seraient mieux adaptées et résistantes aux perturbations du fait d'une beaucoup plus longue association avec l'homme et les milieux anthropisés que les espèces du nouveau monde (di Castri 1989). Mais une cause purement écologique est souvent exposée : plus la biodiversité d'un écosystème est importante, plus il sera résistant à l'invasion par des espèces exogènes (Kennedy *et al.* 2002; Stachowicz & Tilman 2005). Cela serait principalement dû au phénomène de saturation des niches et à la sélection pour de fortes capacités compétitrices lorsque le nombre d'espèces est élevé (Stachowicz & Tilman 2005). Cette hypothèse est corroborée par de nombreuses observations, mais elle est difficile à tester et constitue encore aujourd'hui un sujet de recherche florissant.

I.4.3. Effet de fondation, génétique des petites populations : échec de l'établissement ?

a) Effet de fondation et pré-adaptation

Le nombre d'individus transportés et survivants au cours de l'étape d'introduction est bien souvent faible. Nous avons vu que les effets de fondation auront un impact très important sur le succès de l'invasion : plus la population introduite sera de petite taille, plus elle aura de risque de s'éteindre (cf. I.3.3, e.g. Hopper & Roush 1993). Mais il existe bien sûr des contre-exemples dont l'étude peut également apporter des informations très importantes quant aux raisons du succès de l'invasion. La pré-adaptation à un milieu peut très probablement favoriser l'établissement d'une population envahissante à partir d'un nombre réduit d'individus fondateurs. Les risques d'extinction liés aux effets Allee sont toujours présents, mais l'arrivée dans un milieu favorable permet probablement de réduire certains effets néfastes, par exemple en réduisant les besoins en variance génétique additive nécessaire à l'adaptation à un nouveau milieu. Plusieurs introductions à partir de l'Amérique du Nord de la chrysomèle des racines du maïs (*Diabrotica virgifera virgifera*) en Europe dans différentes régions spatialement disjointes ont été mises en évidence, et les populations envahissantes issues de ces introductions sont presque toutes caractérisées par une forte réduction de diversité génétique par rapport à leurs sources (Ciosi *et al.* 2008). Parmi les hypothèses expliquant cette faible diversité génétique en Europe, il est possible que les conditions environnementales abiotiques (climat tempéré) et biotiques (grande surface de monoculture de maïs) similaires sur les deux continents aient favorisé l'installation des populations parvenant à être introduites, même si celles-ci sont de petite taille.

b) Effet de fondation et système de reproduction

Les risques de s'éteindre lorsque le nombre d'individus est faible sont en partie liés au système de reproduction sexué (e.g. dépression de consanguinité et difficulté à trouver un partenaire sexuel). Par conséquent, on peut supposer que les organismes asexués auront un avantage par rapport aux organismes sexués lorsque le nombre de fondateurs est réduit (Reichard & Hamilton 1997; Sakai *et al.* 2001). Roman et Darling (2007) ont constaté au cours de leur revue bibliographique de 43 espèces envahissantes que 63% des 16 ayant subit un effet fondateur significatif sont capables de reproduction asexuée. A l'inverse, seulement 19% des 27 espèces ne présentant pas de baisse de diversité en sont capables. Il est possible

que les espèces asexuées perdent plus facilement de la diversité génétique lors d'un effet fondateur, mais le régime de reproduction semble réellement important comme le montre l'observation d'évolution vers l'asexualité chez certaines plantes lors de l'établissement (Barrett *et al.* 2008).

c) Avantage des effets de fondation

De manière moins intuitive, l'introduction d'un nombre limité d'individus peut, dans certaines circonstances, devenir le facteur même qui va favoriser l'établissement puis l'invasion. La fourmi d'Argentine (*Linepithema humile*) a été introduite en Amérique du Nord dans l'état du New Orleans à la fin du 19^{ième} siècle. L'espèce est rapidement devenue envahissante et a atteint la Californie où elle est devenue une menace pour la biodiversité locale en atteignant des densités très importantes. Holway *et al.* (1998) démontrèrent que les fourmis envahissantes étaient beaucoup moins agressives entre elles qu'elles ne le sont dans l'aire native où les densités sont beaucoup plus faibles. Les populations de *L. humile* ont en fait subi un fort goulot d'étranglement génétique responsable d'une variabilité génétique très réduite en Californie (60% de diminution de l'hétérozygotie attendue par rapport à l'aire native, Tsutsui *et al.* 2000) et d'un probable manque de variation aux loci responsables de la reconnaissance intra-colonie, conduisant à la formation d'une « supercolonie » très dense (Suarez *et al.* 1999; Tsutsui *et al.* 2003; Tsutsui *et al.* 2000). Dans cet exemple, la perte de diversité génétique probablement liée à l'introduction d'un nombre limité d'individus a permis un établissement particulièrement réussi.

D'autres mécanismes génétiques, moins spécifiques au cas des espèces sociales, sont susceptibles d'accroître la probabilité d'établissement grâce aux goulots d'étranglement génétique (Bouzat 2010). On peut par exemple s'attendre à ce qu'une partie de la variance génétique épistatique soit transformée en variance génétique additive sous l'effet d'un goulot d'étranglement (Goodnight 1987; Goodnight 1988). Toutefois, les démonstrations de ce phénomène sont rares en conditions de laboratoire (Neiman & Linksvayer 2006), et presque inexistantes en conditions naturelles (mais voir Knopp *et al.* 2007). Il est également possible qu'un goulot d'étranglement génétique, s'il n'est pas trop fort, permette la purge des allèles délétères par dérive (Glemin 2003) et donc la diminution, voire la disparition, de la dépression de consanguinité. Un tel mécanisme serait particulièrement avantageux dans le cadre d'une invasion biologique où les effets fondateurs sont susceptibles de se répéter régulièrement lors de l'expansion spatiale. Toutefois, malgré quelques démonstrations empiriques en conditions

artificielles (e.g. Avila *et al.* 2010; Boakes *et al.* 2007; Crnokrak & Barrett 2002) ou indirectement sur le terrain (e.g. Pujol *et al.* 2009), l'importance et le rôle de la purge génétique dans les processus naturels, et en particulier au cours des invasions biologiques, restent inconnus.

I.4.4. Introductions multiples et hybridation

Comme nous venons de le voir, les effets fondateurs n'ont pas inévitablement un effet négatif sur l'établissement d'une population introduite. Toutefois, en espérance, plus une population est petite, moins elle aura de chances de devenir envahissante. Parfois, le nombre d'individus introduits peut être très élevé, limitant ainsi les risques associés aux petites populations. C'est en particulier le cas dans beaucoup d'introductions intentionnelles : lors d'opérations de lutte biologique classique, il n'est pas rare que le nombre d'individus introduits dans le nouveau milieu soit supérieur à 10 000 (Hopper & Roush 1993). Mais l'effectif des populations introduites accidentellement est bien souvent beaucoup plus faible. Toutefois, le nombre d'introductions lui-même aura une importance toute aussi grande que le nombre d'individus introduits à chaque fois sur le succès de l'invasion (notion de pression de propagule, Lockwood *et al.* 2005). En effet, les introductions multiples, qui semblent constituer la règle plutôt que l'exception lors des invasions biologiques (Bossdorf *et al.* 2005; Dlugosch & Parker 2008; Novak 2007; Roman & Darling 2007; Wilson *et al.* 2009), peuvent avoir plusieurs conséquences. De manière générale, elles peuvent potentiellement avoir un effet positif sur le succès d'une invasion, mais cela dépendra énormément des patrons spatiaux et temporels impliqués (Dlugosch & Parker 2008) et certaines introductions multiples ne sont pas responsables du succès de l'invasion (e.g. Ciosi *et al.* 2008; Grosholz & Ruiz 1996; Parker *et al.* 2003).

D'un point de vue démographique, les introductions multiples peuvent permettre de limiter les effets Allee par un phénomène de rescousse (« rescue effect », Gotelli 1991). Le canal de Suez laissant constamment en contact la Mer Rouge et la Mer Méditerranée, il est probable que beaucoup d'invasions fructueuses ait été permises par le maintien de ce couloir à l'origine d'introductions récurrentes (Mavruk & Avsar 2008). Notons que des introductions multiples n'ayant pas lieu exactement au même endroit peuvent également être entretenues par un effet rescousse à la manière de métapopulations (e.g. Fauvergue *et al.* 2007). Au niveau génétique, les introductions multiples peuvent théoriquement permettre de rétablir la

variabilité génétique d'origine (mais les fréquences alléliques pourront par contre rester très différentes), et d'offrir ainsi une meilleure réponse à la sélection naturelle. En outre, l'introduction récurrente d'allèles échantillonnés aléatoirement dans la population d'origine peut permettre d'obtenir par hasard les allèles ou les combinaisons alléliques les mieux adaptés au nouveau milieu.

Dans certains cas, les introductions multiples impliquent plusieurs populations sources génétiquement différenciées. Dans ce cas là, il peut y avoir des mélanges génétiques (on parlera d'hybridation intraspécifique) au sein de l'aire envahie à l'origine de nouvelles combinaisons génétiques absentes de l'aire d'origine. Ce phénomène explique également l'observation de populations envahissantes plus diversifiées génétiquement que leurs sources. Kolbe *et al.* (2004) ont comparé la variabilité génétique de populations du lézard Cubain (*Anolis sagrei*) dans son aire native (Caraïbes) et dans son aire d'introduction en Floride. Ils constatèrent que l'hybridation issue de la rencontre d'au moins 8 introductions indépendantes avait permis de transformer la diversité génétique interpopulationnelle en diversité génétique intrapopulationnelle. Des résultats similaires ont été obtenus sur d'autres espèces envahissantes (Facon *et al.* 2005; Facon *et al.* 2008; Lavergne & Molofsky 2007). Si les événements d'hybridation peuvent dans certains cas être préjudiciables (Arnold & Hodges 1995; Burke & Arnold 2001), ils représentent une force évolutive essentielle pouvant être importante au cours des invasions biologiques grâce au phénomène d'hétérosis et à la génération de nouveaux génotypes associés à une variance génétique additive importante (Ellstrand & Schierenbeck 2000; Lee 2002; Sakai *et al.* 2001; Wares *et al.* 2005).

I.4.5. Adaptation au nouvel écosystème

a) Différences phénotypiques et adaptation

L'idée que la variance génétique additive est importante au cours de l'établissement sous-entend que le nouvel environnement rencontré présente des différences biotiques et/ou abiotiques notables avec l'environnement d'origine. Ainsi, sauf en cas de pré-adaptation, la sélection naturelle aura un rôle très important dans le succès ou l'échec d'une invasion biologique (Lee 2002; Sakai *et al.* 2001). Beaucoup d'études ont mis en évidence des différences phénotypiques entre des populations envahissantes et leurs sources qui illustrent bien souvent des taux d'évolution très rapides (e.g. Bossdorf *et al.* 2005; Cox 2004; Garcia-Ramos & Rodriguez 2002; Mooney & Cleland 2001; Whitney & Gabler 2008). Mais la

question de la nature instrumentale de telles différences quantitatives dans le succès de l'invasion se pose. En effet, les phénomènes liés à la dérive et/ou à l'hybridation intraspécifique peuvent également mener à des différences phénotypiques importantes qui peuvent n'avoir aucun lien avec l'établissement d'une population introduite (Keller & Taylor 2008), et la difficulté pour un biologiste de l'évolution sera d'éviter lorsqu'il le faut le réflexe adaptationiste (Gould & Lewontin 1979). C'est pourquoi il est important de disposer d'hypothèses testables quant aux traits potentiellement soumis à sélection au cours d'une invasion biologique.

b) Réponse aux pressions de sélection du nouveau milieu

Dans certains cas, il est relativement aisé d'identifier un facteur environnemental sélectif fort susceptible d'avoir été une barrière importante pour le succès de l'établissement. Ainsi, le copépode d'origine marine *Eurytemora affinis* s'est adapté plusieurs fois indépendamment à l'eau douce, ce qui lui a été indispensable pour devenir envahissant dans différents lacs et rivières (Lee 1999). De manière plus générale, les tentatives récurrentes visant à identifier des facteurs clés et prédictifs du succès d'une invasion (Mack *et al.* 2000) ont mené au développement d'un certain nombre d'hypothèses évolutives testables. Certaines de ces hypothèses sont basées sur l'idée qu'il existe des avantages à être introduit par rapport aux espèces locales (Alpert 2006). Parmi celles-ci, le relâchement de la pression de bioagresseurs (i.e. les bioagresseurs parasites et prédateurs d'une population ne sont pas, ou pas tous, introduits avec elle dans le nouvel environnement, « enemy release hypothesis », Keane & Crawley 2002) pourrait permettre à la population introduite de réallouer les ressources précédemment utilisées pour la défense vers les capacités compétitrices (« Evolution of Increased Competitive Ability », EICA, Blossey & Notzold 1995). Ainsi, par rapport aux populations natives, les populations envahissantes de l'herbe *Silene latifolia* présentent une plus grande sensibilité à ses parasites d'origine tout en possédant un meilleur potentiel reproductif (floraison plus précoce et plus longue, Wolfe *et al.* 2004). Toutefois, l'EICA reste une hypothèse controversée car (i) d'autres traits que ceux liés à la compétition peuvent être concernés par la réallocation de ressources (e.g. la résistance aux bioagresseurs généralistes, Joshi & Vrieling 2005), (ii) beaucoup d'études sont incomplètes (i.e. la compétition et la résistance aux bioagresseurs sont rarement considérées au sein de la même espèce, Bossdorf *et al.* 2005) et (iii) de nombreux contre-exemples ont été mis en évidence (e.g. van Kleunen & Schmid 2003).

c) Hybridation interspécifique

Il peut arriver dans certains cas que l'évolution de caractères adaptés à un nouvel environnement d'introduction se fasse par le biais d'une hybridation interspécifique (Abbott *et al.* 2003; Ellstrand & Schierenbeck 2000). Il peut s'agir d'une hybridation entre une espèce introduite et une espèce locale (e.g. Ayres *et al.* 1999; Baumel *et al.* 2001; Blair & Hufbauer 2009). Dans ce cas, cette dernière étant déjà adaptée au milieu d'introduction, elle peut fournir une base génétique grâce à laquelle certains gènes de l'espèce introduite vont pouvoir s'exprimer plus facilement (introgression de gènes, Ellstrand & Schierenbeck 2000; Lee 2002; Wares *et al.* 2005). Dans certains cas, l'augmentation de la ploïdie pourrait également être un facteur important du succès d'une invasion (Lee 2002; Soltis & Soltis 2000). Les singularités de l'hybride peuvent être si grandes qu'on parle parfois de nouvelle espèce. Ainsi, la plante envahissante en Europe *Spartina anglica* est issue de l'hybridation entre *S. alterniflora*, une plante exogène américaine, et *S. maritima*, une plante native du milieu d'introduction en Europe (Baumel *et al.* 2001).

d) Rôle de la plasticité phénotypique

Enfin, notons que les adaptations au nouvel environnement d'introduction ne passent pas forcément par des facteurs purement génétiques. La plasticité phénotypique (i.e. la capacité d'un génotype à développer des phénotypes différents en fonction des conditions environnementales) est en effet considérée comme un mécanisme pouvant favoriser l'établissement (Agrawal 2001; Ghalambor *et al.* 2007; Lee 2002; Richards *et al.* 2006; Sakai *et al.* 2001). Les individus introduits peuvent être plastiques dès avant l'introduction, et la plasticité sera alors considérée comme une caractéristique importante d'une espèce pour devenir envahissante (e.g. Geng *et al.* 2007; Williams *et al.* 1995; Yeh & Price 2004). Mais la plasticité peut également être sélectionnée au sein de la population introduite au cours de l'établissement (Richards *et al.* 2006). Cette plasticité phénotypique adaptative peut théoriquement conduire à une valeur sélective homogène sur un gradient environnemental (i.e. « homéostasie de la valeur sélective », Richards *et al.* 2006; Scheiner 1993) et pourra ensuite favoriser l'étape de prolifération dans un milieu hétérogène. Cette plasticité pourra ensuite devenir la matière première d'adaptations locales (Sexton *et al.* 2002; West-Eberhard 2003). Pour correctement identifier l'évolution de la plasticité phénotypique comme facteur instrumental du succès de l'invasion, il est nécessaire de comparer des populations envahissantes avec leur(s) source(s) native(s) (Keller & Taylor 2008; Richards *et al.* 2006).

Kaufman & Smouse (2001) ont montré que les populations envahissantes américaines de l'arbre *Melaleuca quinquenervia* étaient plus plastiques dans leur croissance en réponse à différents pH que les populations natives australiennes. Toutefois, ce type d'étude reste rare (Keller & Taylor 2008). En outre, le choix des populations natives n'est pas toujours basé sur une bonne connaissance des routes d'invasion, ce qui peut, comme pour tous les autres tests d'hypothèse de changement adaptatif, amener à des conclusions erronées (Estoup & Guillemaud 2010).

I.5. La prolifération : adaptation, dérive et évolution post-introduction

Une fois la population établie (on dit qu'elle est naturalisée, Richardson *et al.* 2000), elle aura potentiellement les ressources pour croître démographiquement et s'étendre spatialement, et devenir envahissante *stricto sensu*. Pour cela, certaines caractéristiques indispensables à la prolifération devront être présentes ou sélectionnées directement dans l'aire envahie. Mais, indépendamment du succès de l'invasion, cette étape donne aussi l'occasion d'observer de nombreux phénomènes évolutifs propres aux populations en déséquilibre spatial.

I.5.1. Hétérogénéité spatiale et adaptation

Au cours de l'étape de prolifération, l'espèce envahissante va croître démographiquement et se disperser afin d'établir des populations viables au-delà du seul foyer d'invasion. Les capacités et modes de dispersion auront bien entendu un rôle prépondérant dans le succès de cette étape (Sakai *et al.* 2001; Shigesada *et al.* 1995). Toutefois, même si les capacités de dispersion sont suffisantes, l'adaptation (ou la pré-adaptation) à l'environnement biotique et abiotique du foyer ne garantit pas un succès de la phase d'expansion si l'environnement est spatialement très hétérogène. Le rôle de l'homme sera à nouveau prépondérant au cours de cette étape. Dans le cas des introductions intentionnelles, l'homme modifiera bien souvent l'environnement afin de réduire

volontairement l'hétérogénéité spatiale. L'irrigation a par exemple permis d'accroître considérablement l'aire de répartition de nombreuses plantes cultivées. Les perturbations d'origine anthropique ont de manière générale tendance à homogénéiser les conditions environnementales, et cela expliquerait (en plus des flux migratoires plus élevés) pourquoi les zones urbaines et agricoles contiennent un nombre plus élevé d'espèces envahissantes que les milieux naturels ou semi-naturels (e.g. Deutschewitz *et al.* 2003; di Castri 1989; McIntyre & Lavorel 1994; McKinney 2002; Pysek *et al.* 2010).

Lorsque l'environnement n'est pas homogène, l'espèce envahissante doit être capable de s'adapter rapidement à la gamme de milieux rencontrés. La plasticité phénotypique aurait alors un rôle important lors de la prolifération (Parker *et al.* 2003; Richards *et al.* 2006). Certains auteurs pensent que la plasticité expliquerait en partie pourquoi les espèces dont l'aire d'origine est très étendue ont de meilleures prédispositions pour devenir envahissantes (McKinney & Lockwood 1999; Williamson & Fitter 1996b). Mais, outre la plasticité, il est également possible, et même fréquent, d'observer des adaptations locales rapides ayant une base génétique (Huey *et al.* 2005; Novak 2007; Prentis *et al.* 2008; Whitney & Gabler 2008). La drosophile *Drosophila subobscura* originaire d'Europe a envahi l'Amérique du Sud à la fin des années 70 et l'Amérique du Nord dans les années 80 (Huey *et al.* 2005). Ces deux invasions, bien que non indépendantes (Pascual *et al.* 2007), sont spatialement déconnectées. Il a alors été possible d'observer l'évolution rapide et indépendante d'un gradient géographique des tailles d'ailes en fonction de la latitude (identique à celui de l'aire native) dans les deux zones d'introduction (Gilchrist *et al.* 2001; Huey *et al.* 2000; Huey *et al.* 2005). Il est intéressant de noter que ce rétablissement d'un patron évolutif natif continu est d'autant plus étonnant que les populations envahissantes ont subi un fort goulot d'étranglement génétique (Pascual *et al.* 2007). D'autres exemples existent dans la littérature (e.g. Leger & Rice 2007; Maron *et al.* 2004) et le rôle conjoint de l'évolution génétique et de la plasticité phénotypique semble souvent important (e.g. Hendry *et al.* 2000; Williams & Moore 1989). Notons qu'il est parfois difficile d'identifier le rôle exact des patrons évolutifs observés dans le succès de l'étape de prolifération et donc de l'invasion (e.g. évolution de la taille des ailes chez D. subobscura, Huey *et al.* 2000). L'étude de l'étape d'expansion offre néanmoins une opportunité unique d'étudier les phénomènes de microévolution et d'adaptation locale (Cox 2004; Sax *et al.* 2005).

I.5.2. Déséquilibre spatial et adaptation

Lors de l'étape de prolifération, une population envahissante est confrontée à un fort déséquilibre spatial caractérisé par de fortes variations de densité de population et par la succession d'effets de fondation précédant des croissances démographiques locales importantes (Figure 2). Quoique temporaire (l'expansion s'arrêtera nécessairement à cause de barrières physiques et/ou écologiques), il est particulièrement intéressant d'étudier les conséquences évolutives de ce phénomène qui sont nombreuses. Ces conséquences auront par ailleurs potentiellement une influence sur le succès de l'expansion et sur la structuration génétique et phénotypique finale de la population.

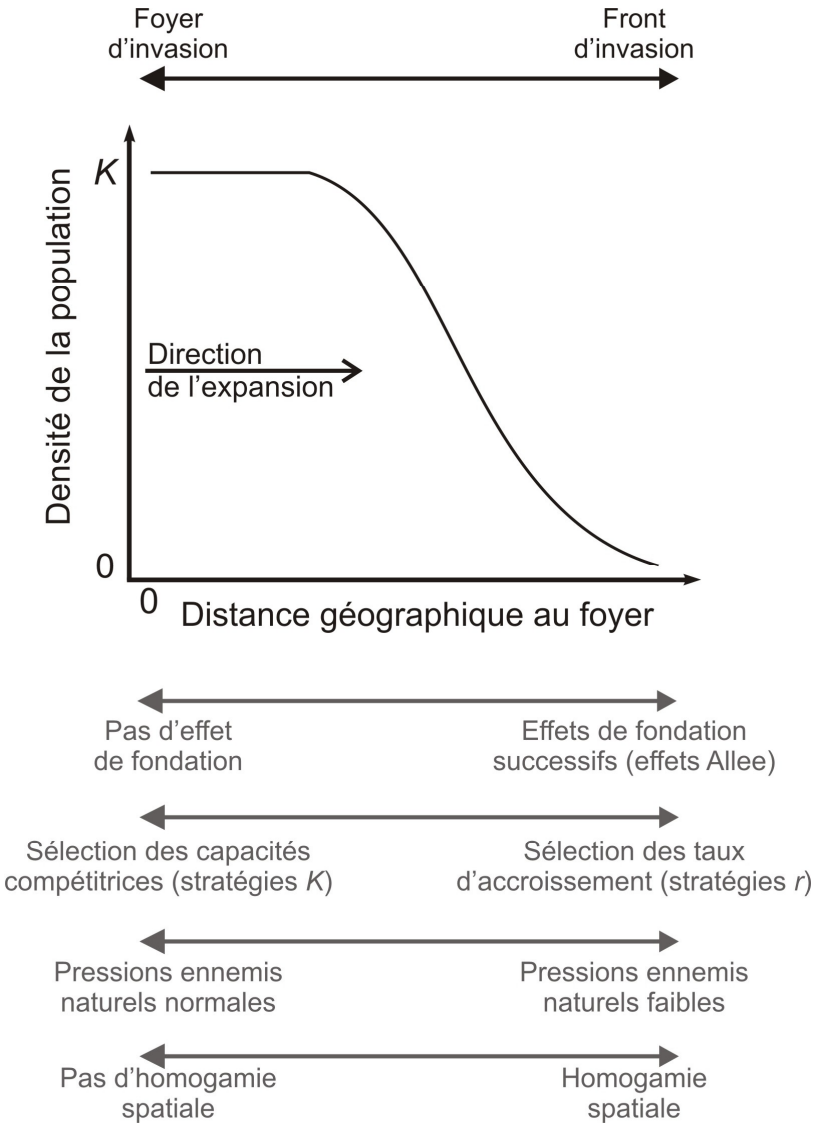


Figure 2 : Illustration du déséquilibre spatial et de ses conséquences évolutives lors de l'étape de prolifération. *K* est la capacité de soutien du milieu. On constate que le front d'invasion, du fait de densité de population faible (et donc contrairement au foyer) expérimentera des effets de fondation, une sélection pour des taux d'accroissement élevé, une baisse des pressions liées aux ennemis naturels les plus spécialisés et de l'homogamie spatiale (voir section I.5.4).

a) Expansion spatiale et effet Allee

Tout d'abord, le front d'expansion sera soumis à des effets fondateurs importants qui peuvent résulter en effets Allee locaux pouvant particulièrement ralentir l'expansion d'une population envahissante (Figure 2, Lewis & Kareiva 1993; Tobin *et al.* 2007). Ainsi, la plante *Spartina alterniflora*, envahissante sur la côte ouest américaine, souffre d'effets Allee liés à la dépression de consanguinité (Daehler 1999) et à des problèmes de reproduction à faible densité (Davis *et al.* 2004a; Davis *et al.* 2004b). En conséquence, le taux d'expansion de l'espèce dans l'aire envahie est significativement réduit (Davis *et al.* 2004b; Taylor *et al.* 2004). Toutefois, notons que l'expansion de populations envahissantes est souvent particulièrement rapide, ce qui pourrait être notamment expliqué par le passage réussi de l'étape d'établissement pendant laquelle les problèmes liés aux effets Allee ont déjà été rencontrés et potentiellement atténués.

b) Pression de sélection et compromis évolutifs au cours de l'expansion spatiale

Le déséquilibre spatial a la particularité de soumettre la population à des pressions de sélection très variables du foyer au front d'invasion. La densité d'individus elle-même constitue une pression de sélection qui sera très variable sur l'ensemble de l'aire de répartition de la population envahissante. Tous les traits associés à de la densité-dépendance peuvent possiblement évoluer dans des directions très différentes selon la localisation des individus concernés. Par exemple, des fortes capacités compétitrices seront sélectionnées au niveau du foyer (i.e. les stratégies *K* seront favorisées) où les densités sont élevées, et de forts taux d'accroissement seront favorisés sur le front (i.e. les stratégies *r* seront favorisées) où les densités sont faibles (Figure 2, Phillips *et al.* 2010a). L'arbre d'origine asiatique *Sapium sebiferum* est devenu envahissant en Amérique du nord, en Georgie, à la fin du 18^{ième} siècle, puis au Texas et en Louisiane au début du XX^{ième} siècle. Siemann & Rogers (2001) ont montré que les populations envahissantes les plus récentes de *S. sebiferum* produisaient plus de graines, mais étaient moins résistantes aux bioagresseurs que les populations envahissantes les plus anciennes. Malgré quelques autres exemples dans un contexte d'invasion (e.g. Phillips 2009) ou pas (e.g. Hanski & Saccheri 2006; Saastamoinen 2007), cette hypothèse a été très peu testée jusqu'à maintenant (Phillips *et al.* 2010a). Pourtant, si ce phénomène s'avère fréquent, il serait très important de le prendre en compte dans les études de biologie évolutive et d'écologie qui comparent des populations envahissantes avec leurs sources natives.

En outre, la nature dynamique du processus d'expansion peut également provoquer la réduction de certaines pressions de sélection sur le front d'invasion, et des changements phénotypiques génétiquement déterminés peuvent avoir lieu si des compromis évolutifs forts existent. Par exemple, on peut s'attendre à ce que les populations en expansion perdent leurs ennemis naturels spécialistes sur le front du fait d'effets fondateurs (Figure 2), et elles pourront ainsi moins investir de ressources dans les défenses au profit d'autres traits (Moorcroft *et al.* 2006; Phillips *et al.* 2010a; Phillips *et al.* 2010b). Cette hypothèse a encore été peu testée, mais elle a été confirmée chez le papillon *Aricia agestis* (Menendez *et al.* 2008) et le crapaud buffle *Bufo marinus* (Phillips *et al.* 2010b).

I.5.3. Déséquilibre spatial et dérive génétique

Comme précisé précédemment, une expansion spatiale se caractérise notamment par une succession d'effets de fondation sur son front. Les conséquences en terme de structuration génétique intéressent les généticiens depuis longtemps (Baker & Stebbins 1965). En tout premier lieu, on peut s'attendre à ce que la dérive génétique engendrée par les effets de fondation entraîne une diminution de la diversité génétique sur le front d'expansion (Austerlitz *et al.* 1997; Excoffier *et al.* 2009; Sexton *et al.* 2009). Un tel patron génétique a été observé à diverses reprises, aussi bien au cours d'invasions biologiques, que lors d'élargissements locaux d'aire de répartition (e.g. Cooper *et al.* 1995; Estoup *et al.* 2004; Garroway *et al.* 2011; Leotard *et al.* 2009; Rollins *et al.* 2009; Santucci *et al.* 1998). Toutefois, si l'expansion est rapide, la diversité génétique pourra au contraire être stabilisée à l'échelle de la totalité de la population (e.g. Estoup *et al.* 2004; Zenger *et al.* 2003).

Une conséquence moins évidente des effets de fondation sur le front est le « surf » de gène (« gene surfing », Edmonds *et al.* 2004; Klopfstein *et al.* 2006). Ainsi, des allèles rares ou des mutations apparaissant sur le front d'expansion peuvent y augmenter fortement en fréquence sous l'effet de la dérive et « surfer » sur la vague d'avance, se propageant ainsi rapidement (Edmonds *et al.* 2004; Excoffier *et al.* 2009; Hallatschek & Nelson 2008; Klopfstein *et al.* 2006). Ce phénomène entraînera une différentiation génétique entre le foyer et le front, mais également entre différents secteurs angulaires de la zone envahie (Excoffier & Ray 2008; Hallatschek *et al.* 2007). Il est par ailleurs intéressant de noter que le « surf » de gène ne concerne pas uniquement les allèles neutres, mais également les allèles sélectionnés, qu'ils soient avantageux ou non (Excoffier *et al.* 2009; Klopfstein *et al.* 2006; Travis *et al.*

2007). Ainsi, une mutation délétère peut devenir très fréquente dans une population en expansion du fait de ce phénomène (Travis *et al.* 2007). Les démonstrations expérimentales du « surf » de gène sont encore rares. Hallatschek *et al.* (2007) ont suivi la croissance des deux souches de bactéries marquées par fluorescence et mises en concentration égale au centre d'une plaque de gélose. Après une journée de croissance, ils ont observé la formation de secteurs stables constitués chacun d'une souche unique rayonnant à partir du centre de la plaque, illustrant un processus analogue au « surf » de gène. Il serait toutefois intéressant de tester *in natura* l'hypothèse du « surf » de gène de manière systématique dans les études de génétique des populations visant à décrire les invasions biologiques. Par ailleurs, il serait également important de pouvoir analyser d'anciens jeux de données à la lumière de ce mécanisme (Klopfstein *et al.* 2006). Cela permettrait d'évaluer le rôle évolutif réel de ce phénomène dont on sait que la portée peut être réduite sous l'effet d'autres phénomènes telle que la dispersion longue distance (Excoffier *et al.* 2009; Fayard *et al.* 2009).

I.5.4. Déséquilibre spatial et homogamie

Une troisième force évolutive sera à l'œuvre sous l'effet d'un déséquilibre spatial. Il s'agit de « l'homogamie spatiale » (Shine *et al.* 2011a) qui n'agit pas sous l'effet d'un avantage sélectif (contrairement à l'adaptation) mais qui reste toutefois un phénomène déterministe et prédictible (contrairement à la dérive génétique). Ce phénomène concerne spécifiquement les capacités de dispersion (la dispersion étant définie comme le déplacement d'un individu de son lieu de naissance à son lieu de reproduction, Ronce 2007) et tous les traits associés au syndrome de dispersion. Lors d'une expansion spatiale, le front sera en effet formé par les meilleurs dispersants qui n'auront d'autre choix que de s'accoupler entre eux. Si les capacités de dispersion sont liées à un polymorphisme génétique, cette homogamie sur le front entraînera mécaniquement la production d'une descendance ayant en moyenne des capacités de dispersion plus élevées que les individus plus proches du foyer (Figure 2, Phillips *et al.* 2010a; Shine *et al.* 2011a). Les conséquences appliquées sont importantes car il peut y avoir une nette augmentation de la vitesse d'expansion (Phillips *et al.* 2007; Phillips *et al.* 2011; Travis & Dytham 2002; Travis *et al.* 2009). Au niveau macroévolutif, les répercussions de ce mécanisme à long terme (i.e. lorsque l'équilibre spatial est retrouvé) sont encore mal connues (e.g. Lee 2011; Shine *et al.* 2011b).

L'évolution vers de plus grandes capacités de dispersion sur le front d'expansion a été prédit dans des modèles théoriques à plusieurs reprises (Burton *et al.* 2010; Hughes *et al.* 2007; Phillips *et al.* 2008; Shine *et al.* 2011a; Travis & Dytham 2002; Travis *et al.* 2010; Travis *et al.* 2009). Toutefois, différents paramètres peuvent minimiser les effets de l'homogamie spatiale. Ainsi, les effets Allee et/ou la fragmentation de l'habitat peuvent fortement limiter l'évolution de la dispersion sur les fronts en réduisant la survie des meilleurs dispersants (Hughes *et al.* 2007; Travis & Dytham 2002). De même, la présence de compromis évolutifs forts entre les capacités de dispersion et d'autres traits peuvent perturber les trajectoires évolutives sur les fronts d'expansion. Par exemple, des compromis évolutifs entre dispersion et taux d'accroissement sont parfois observés (Roff 2002). Or, comme pour les capacités de dispersion, la théorie prédit une augmentation des taux d'accroissement sur le front (Phillips *et al.* 2010a), et les deux ensembles de traits devraient entrer en conflit. Toutefois, la réalité est souvent beaucoup plus complexe, et si une troisième catégorie de traits telle que la capacité compétitrice est associée par des compromis évolutifs à la dispersion et au taux d'accroissement, alors ces derniers pourront évoluer parallèlement au détriment du troisième (Burton *et al.* 2010). Ainsi, chez les insectes par exemple, si les capacités de dispersion sont parfois négativement corrélées à la fécondité comme chez le grillon *Gryllus firmus* (Roff & Fairbairn 2007), la situation totalement inverse est également rencontrée chez d'autres espèces comme chez le papillon *Melitaea cinxia* (Hanski *et al.* 2006).

In natura, de plus grandes capacités de dispersion sur les fronts d'expansion ont été mises en évidence lors d'accroissements locaux d'aire de répartition à la fois anciens (post-glaciation, e.g. Cwynar & Macdonald 1987; Leotard *et al.* 2009) et récents (réchauffement climatique contemporain, e.g. Hill *et al.* 1999; Hughes *et al.* 2003; Simmons & Thomas 2004; Thomas *et al.* 2001), mais les études lors d'invasions biologiques sont encore rares (Monty & Mahy 2010; Phillips *et al.* 2006). Pourtant, par rapport aux accroissements locaux des aires de répartition, les invasions présentent de nombreux avantages pour l'étude de ce phénomène car (i) l'historique complet est plus souvent disponible, (ii) beaucoup d'invasions sont en cours (avec parfois des replicats spatiaux) et (iii) l'expansion est presque exclusivement due à la dispersion et n'est généralement pas conditionnée par une modification progressive de l'environnement, ce qui lui permet d'être particulièrement rapide (Hill *et al.* 2011). Ainsi, Phillips *et al.* (2006) ont montré que le front d'invasion du crapaud buffle (*Bufo marinus*) en Australie était constitué d'individus ayant de plus longs tibias que ceux situés proche du foyer, et que la longueur des tibias était corrélée avec de plus grandes capacités à se disperser

sur de longues distances. En conséquence, chez cette espèce qui a été particulièrement bien étudiée, une nette augmentation de la vitesse d'expansion a été mise en évidence (Phillips *et al.* 2011).

I.6. Principales études réalisées au cours de cette thèse.

Comment se déroule une invasion biologique ? Pourquoi certaines populations parviennent-elles à devenir envahissantes ? Pourquoi la majorité des introductions se soldent-elles par un échec ? Pourquoi les populations envahissantes sont-elles parfois plus performantes que les populations natives ? Pourquoi l'étape de prolifération est-elle si explosive ? Comme souligné précédemment, toutes ces questions sont liées entre elles, et être capable d'y répondre est devenu aujourd'hui un défi important car la place des invasions biologiques dans le contexte du changement global actuel est majeure (e.g. Vitousek *et al.* 1997). Toutefois, jusqu'à présent, très peu d'invasions biologiques ont été étudiées de manière approfondie de l'étape d'introduction à l'étape de prolifération. L'accumulation de telles connaissances sur des espèces modèles permettrait pourtant de mieux comprendre les processus écologiques et évolutifs réellement à l'œuvre lors des invasions biologiques.

Au cours de ma thèse, j'ai tenté d'apporter des éléments de réponse pour chacune des étapes clés de l'invasion biologique de la coccinelle asiatique *Harmonia axyridis*. Cette espèce emblématique présente un certain nombre de caractéristiques qui en font un modèle particulièrement attractif pour des approches intégratives sur les invasions biologiques : (i) une longue période d'échec d'envahissement est documenté, (ii) sa répartition géographique mondiale est relativement bien connue, (iii) elle est envahissante sur différents continents, (iv) elle est facile à échantillonner, et enfin (v) elle est facile à élever et à reproduire en laboratoire avec des temps de générations courts. Dans le chapitre suivant (i.e. Chapitre II : Histoire évolutive d'une invasion mondiale : le cas de la coccinelle asiatique *Harmonia axyridis*), je m'efforcerai, après avoir décrit l'espèce *H. axyridis*, de présenter les connaissances acquises au cours de ma thèse sur chacune des étapes clés de l'invasion de cette coccinelle : son introduction, son établissement et sa prolifération. L'objectif global de ce travail est de fournir

une étude relativement transversale et détaillée d'une espèce envahissante et de tenter d'en extraire des règles ou des pistes de recherche générales.

Enfin, dans une troisième partie (chapitre III : Discussion et perspectives), je reviendrai sur mes principaux résultats avec un œil critique et discuterai leurs implications générales dans notre compréhension des invasions biologiques.

CHAPITRE II
Histoire évolutive d'une invasion mondiale : le cas de la coccinelle asiatique *Harmonia axyridis*.

II.1. La coccinelle asiatique *Harmonia axyridis* : une invasion biologique et médiatique.

II.1.1. Généralités sur *H. axyridis*

Harmonia axyridis Pallas est un coléoptère de la famille des Coccinellidae dont l'aire de répartition naturelle s'étend sur une large partie de l'Asie (d'où son nom commun en Français : « coccinelle Asiatique »). Elle est ainsi présente en Chine (du nord jusqu'à des provinces très méridionales comme le Yunnan et le Guangxi), au Japon, en Corée, en Mongolie, au Kazakhstan et dans le Sud de la Sibérie (Poutsma *et al.* 2008).

Principalement arboricole, *H. axyridis* est une espèce entomophage qui se nourrit principalement de pucerons, mais qui s'attaque également volontiers à d'autres hémiptères tels que les cochenilles ou les psylles (Koch 2003). Lorsque ses proies habituelles viennent à manquer, l'espèce peut reporter son appétit sur des acariens, des lépidoptères, des névroptères, d'autres espèces de coccinelles ou des congénères. Le cannibalisme est en fait relativement fréquent chez *H. axyridis* : ce comportement est lié à la disponibilité en ressources, mais également aux densités de population (Hironori & Katsuhiro 1997; Michaud & Grant 2004; Osawa 1989; Yasuda & Ohnuma 1999). En outre, le cannibalisme a probablement un rôle adaptatif important au cours des toutes premières heures après

l'éclosion pendant lesquelles il est moins lié aux conditions environnementales locales (Osawa 1989).

D'une taille adulte d'environ 8 mm, *H. axyridis* est une espèce holométabole qui passe par quatre stades larvaires mobiles suivis d'un stade nymphal fixe (Figure 3). Le cycle de développement complet de l'œuf à l'adulte dure de 15 à 25 jours selon les conditions environnementales (Pervez & Omkar 2006). La durée de vie de l'adulte est ensuite relativement longue (généralement plusieurs mois). L'espèce est considérée comme étant bivoltine, mais il est possible d'observer jusqu'à 5 générations au cours d'une année (Katsoyannos *et al.* 1997; Wang 1986). Le nombre d'œufs pondus par une seule femelle peut être très important (jusqu'à 3800 avec une moyenne de 25 œufs par jour, Koch 2003). *H. axyridis* se regroupe en grand nombre à l'automne sur des sites d'hivernation où elle va entrer en diapause au stade adulte jusqu'au printemps.

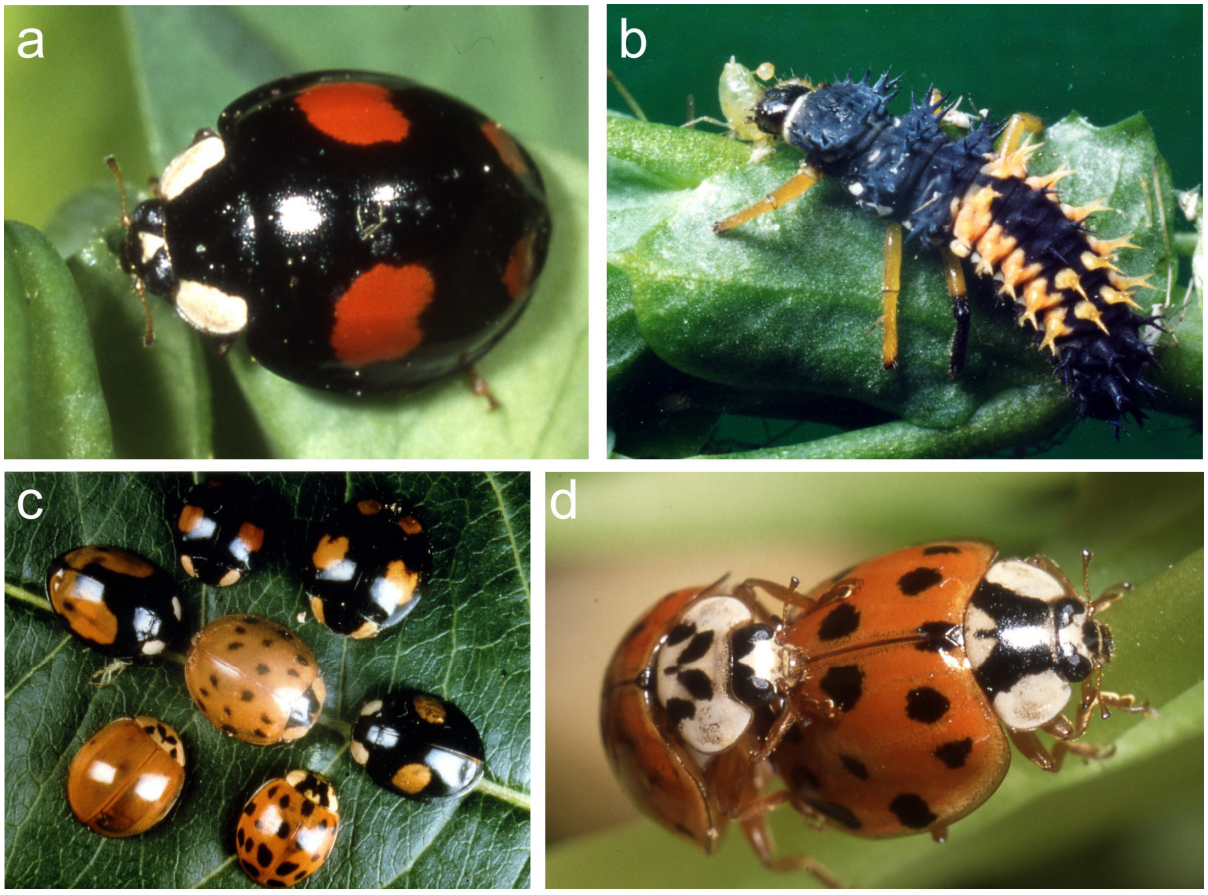


Figure 3 : La coccinelle Asiatique *Harmonia axyridis*. a) Femelle adulte ; b) larve de 4^{ième} stade attaquant un puceron ; c) quelques morphes ; d) couple.

H. axyridis est caractérisée par un polymorphisme de couleur remarquable (Figure 3). Cette particularité, qui lui vaut le nom commun anglais de « multicoloured Asian lady

beetle », explique en partie son utilisation très précoce comme modèle de génétique des populations (Dobzhansky 1933). Ainsi, jusqu'à 120 formes différentes de coloration des élytres auraient été recensées. Le déterminisme de ce polymorphisme a une base génétique (e.g. Tan 1946) mais est également fortement influencé par les conditions environnementales (alimentation aux stades larvaires et température, Michie *et al.* 2010; Pervez & Omkar 2006). Dans l'aire native, plusieurs morphes peuvent être observés sur un site donné, mais des variations géographiques de fréquences existent (Dobzhansky 1933). Le rôle adaptatif de ce polymorphisme est encore mal connu, mais les variations de température sont souvent évoquées comme étant importantes (Michie *et al.* 2010). D'autres causes non adaptatives sont également proposées (e.g. Wang *et al.* 2009).

II.1.2. Entre lutte biologique et invasion biologique : l'histoire complexe d'*H. axyridis*

a) **H. axyridis*, l'auxiliaire de lutte biologique : échec des tentatives d'acclimatation***

H. axyridis est une espèce particulièrement vorace puisqu'un seul adulte consomme entre 15 et 65 pucerons par jour (Hu *et al.* 1989; Lucas *et al.* 1997; Seko & Miura 2008). Elle fut donc très tôt choisie comme auxiliaire de lutte biologique contre les pucerons. De premières tentatives d'acclimatation furent tentées aux Etats-Unis dès 1916 (Figure 4). L'efficacité prédatrice de l'espèce fut confirmée, mais l'espèce ne s'établit pas. De nombreuses introductions furent répétées en Amérique du Nord dans la deuxième moitié du 20^{ième} siècle (surtout entre 1965 et 1982) à partir de nombreux prélèvements indépendants dans l'Est de l'aire native (Japon, Est de la Chine, Est de la Sibérie, Krafsur *et al.* 1997; Tedders & Schaefer 1994). Aucune population acclimatée ne fut toutefois constatée avant 1988 (Chapin & Brou 1991).

En Europe, une population fut échantillonnée en 1982 en Chine et ramenée dans un laboratoire de l'INRA (Figure 4, Ongagna *et al.* 1993). Jusqu'à 1990, la souche fut testée en laboratoire (efficacité prédatrice, possibilité d'élevage, etc.), puis des essais en culture sous serre et en plein air commencèrent. En 1995, l'espèce commença à être commercialisée par des entreprises françaises, hollandaises et belges. Une souche non volante fut sélectionnée en laboratoire à la fin des années 90 (Tourniaire *et al.* 2000a; Tourniaire *et al.* 2000b), et commercialisée par une entreprise française à partir de 2000. A l'exception de la souche non volante, la commercialisation d'*H. axyridis* en Europe stoppa en 2003. En 1997, Ferran *et al.*

(1997) soulignaient l'incapacité de l'espèce à s'acclimater en Europe malgré les nombreux lâchers. Enfin, notons que cette souche européenne de lutte biologique fut également utilisée en Amérique du Sud (Argentine, Brésil et Chili) en 1986 et à la fin des années 90 (Poutsma *et al.* 2008), ainsi qu'en Afrique du Nord (Egypte) dans les années 2000.

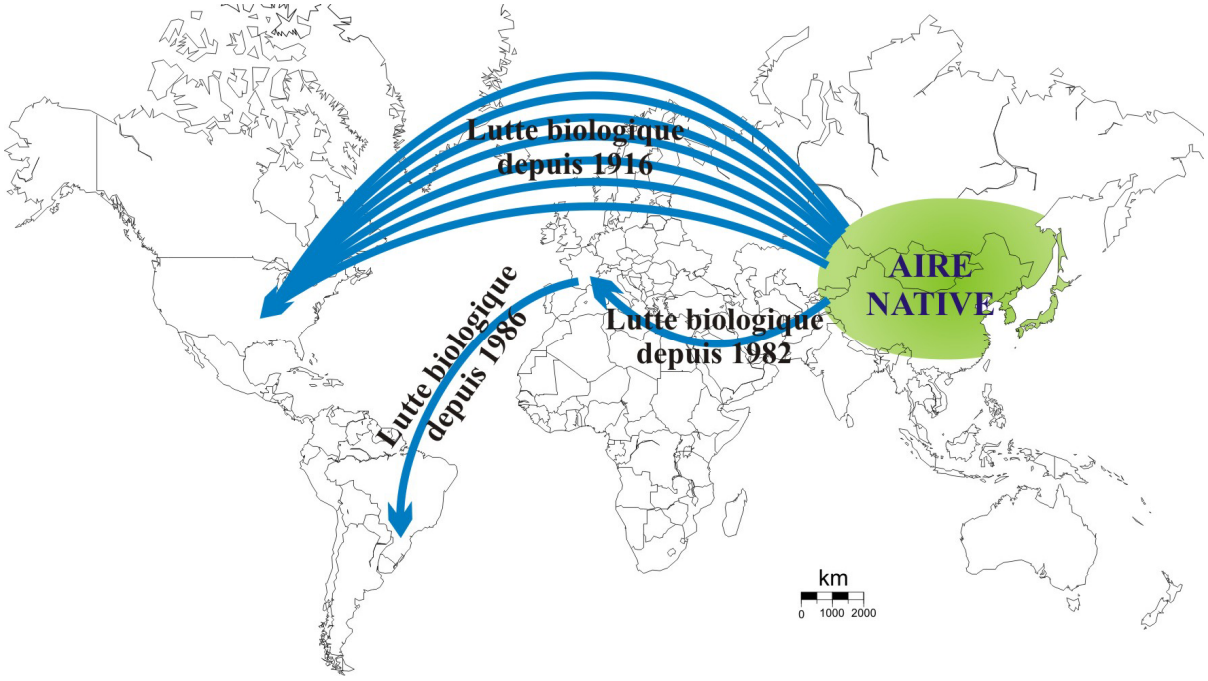


Figure 4 : Représentation schématique des principales introductions d'*H. axyridis* liées à la lutte biologique dans le monde. En Amérique du Nord, de nombreuses populations natives différentes ont été utilisées. A l'inverse, en Europe, une seule population, introduite en laboratoire en 1982, fut utilisée.

b) H. axyridis*, l'espèce envahissante : un succès mondial fulgurant*

La première population établie fut observée en Amérique du Nord en Louisiane en 1988 (Chapin & Brou 1991), puis un autre foyer fut identifié dans l'Ouest américain dans l'Oregon en 1991 (LaMana & Miller 1996) (Figure 5). L'espèce s'est rapidement étendue sur le continent nord américain puisqu'en 1994 elle était présente dans 24 états (Koch *et al.* 2006). En 2010, les seuls états américains où *H. axyridis* n'a pas encore été observée sont le Wyoming et l'Alaska. De même, on la trouve au Canada depuis 1994, en Terre Neuve depuis 2009 (Hicks *et al.* 2010) et au Mexique depuis au moins 2006 (Koch *et al.* 2006).

En Europe, plusieurs populations furent signalées en France, en Allemagne et en Grèce avant 2000 (Brown *et al.* 2008), mais le début de l'invasion (i.e. population établie qui s'accroît démographiquement et spatialement) commença en Belgique avec une première observation en 2001 (Adriaens *et al.* 2003). Depuis, l'espèce s'est rapidement répandue, et on la trouve en 2010 dans 26 pays Européens différents (Figure 5).

En Amérique du Sud, la première observation d'*H. axyridis* provient de Buenos Aires en Argentine en 2001 (Saini 2004). L'historique de l'invasion en Amérique du Sud est globalement mal connu (Koch *et al.* 2006). On sait toutefois que l'espèce est au moins présente dans 7 pays couvrant une très large partie du continent (Argentine, Brésil, Chili, Pérou, Paraguay, Uruguay et Colombie ; Figure 5).

Enfin, en Afrique, *H. axyridis* est présente en Afrique du Sud depuis 2004 de manière certaine, et peut-être même dès 2001 (Stals & Prinsloo 2007). Après avoir été observée dans la région du Cap, elle s'est rapidement étendue vers le nord, envahissant le Lesotho à partir de 2008 (Figure 5). Très récemment, l'espèce a également été observée dans le sud-est du Kenya (Nedvěd *et al.* 2011). *H. axyridis* est parfois signalée en Egypte, mais elle est toujours utilisée en lutte biologique sur place et il est donc difficile de savoir s'il s'agit de populations établies.

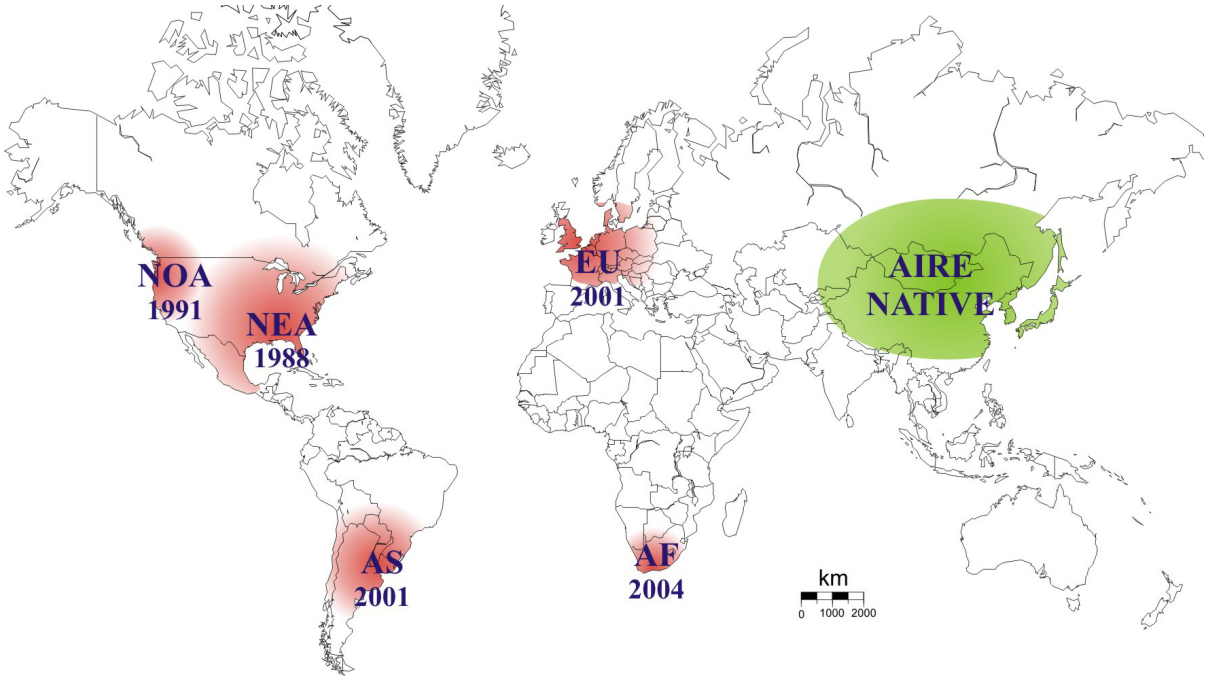


Figure 5 : Représentation approximative de l'aire de répartition actuelle d'*H. axyridis* dans le monde. En vert l'aire native et en rouge les zones envahies (NEA = Nord-Est Américain ; NOA = Nord-Ouest Américain ; EU = Europe ; AS = Amérique du Sud ; AF = Afrique du Sud). Les dates de première observation de chaque foyer envahissant sont indiquées.

La coccinelle *H. axyridis* est donc devenue envahissante dans une quarantaine de pays sur presque tous les continents en moins de 25 ans (Figure 5). Sa répartition géographique est en constante augmentation, et de récentes études de modélisation de niche (Bidinger *et al.* 2011; Poutsma *et al.* 2008) prédisent que de nombreuses autres régions du globe sont susceptibles d'être colonisées par l'espèce. Les conditions abiotiques en Australie sont par exemple optimales sur une large portion du Sud du pays (Poutsma *et al.* 2008), et

l'interception récente par les douanes australiennes de quelques individus vivants en provenance des Etats-Unis (Carvan 2009) laisse penser qu'il ne s'agit plus que d'une question de temps.

II.1.3. Impacts d'*H. axyridis*

Au milieu des années 1930, le crapaud buffle *Bufo marinus* fut introduit en Australie pour lutter contre des coléoptères (*Dermolepida albohirtum* et *Lepidiota frenchi*) qui ravageaient les plantations de canne à sucre (Easteal 1981). Comme prévu, l'espèce s'est acclimatée et est devenue envahissante. Toutefois, les ravageurs de canne à sucre ont à peine remarqué la présence du prédateur, et, à l'inverse, de nombreuses espèces d'oiseaux endémiques telle que le guêpier arc-en-ciel (*Merops ornatus*) constituent des proies fréquente du batracien (Boland 2004). Le crapaud buffle fait partie de ces quelques exemples d'auxiliaires de lutte biologique ayant « mal tourné ». *H. axyridis* est également considérée comme un échec de la lutte biologique en illustrant un certain nombre d'effets non-intentionnels (Louda *et al.* 2003). La coccinelle asiatique pose en effet des problèmes dans les aires envahies (Koch & Galvan 2008; Vilà *et al.* 2009) à tel point que des méthodes de lutte sont envisagées (Kenis *et al.* 2008).

a) Premier impact négatif : perturbation des écosystèmes

La prédation intraguilde par *H. axyridis* a été l'objet de nombreuses études en laboratoire. En effet, la coccinelle asiatique est capable de s'attaquer et de se nourrir d'espèces qui utilisent les mêmes ressources qu'elle (Pell *et al.* 2008). Il s'agit la plupart du temps d'autres espèces de coccinelles aphidiphages (i.e. se nourrissant de pucerons). Ainsi, *H. axyridis* peut avoir un fort impact de prédation sur la coccinelle *Adalia bipunctata*, alors que l'inverse n'est pas vrai (e.g. Kajita *et al.* 2000; Sato & Dixon 2004; Ware *et al.* 2009). Toutefois, l'importance de la prédation intraguilde sur le terrain est encore mal connue, même si des techniques indirectes permettent maintenant d'étudier ce phénomène de manière plus approfondie (e.g. Aebi *et al.* 2011; Hautier *et al.* 2008). Néanmoins, les suivis temporels de la guilde des coccinelles en Amérique du Nord, en Amérique du Sud et en Europe ont mis en évidence une nette diminution de la proportion des autres espèces de coccinelles au profit d'*H. axyridis* (Adriaens *et al.* 2008; Brown *et al.* 2011b; Colunga-Garcia & Gage 1998; Martins *et al.* 2009; Michaud 2002). La compétition interspécifique pourrait toutefois suffire à

expliquer ces tendances. Outre les autres Coccinellidae, *H. axyridis* peut également avoir des impacts sur des espèces non cibles d'autres guildes comme par exemple des lépidoptères non ravageurs de culture ou certains auxiliaires de lutte biologique contre les mauvaises herbes (Koch & Galvan 2008).

b) Second impact négatif : un consommateur occasionnel des productions fruitières

L'extrême polyphagie d'*H. axyridis* lui permet d'utiliser des ressources alimentaires d'origine végétale lorsque ses proies habituelles viennent à manquer. Outre le pollen (Berkvens *et al.* 2008a), elle est parfois retrouvée s'alimentant sur des fruits à destination commerciale comme le raisin, les pommes, les pêches, les prunes, les poires ou les framboises (Koch & Galvan 2008). *H. axyridis* reste toutefois une espèce essentiellement carnivore, et les dommages directs infligés sont peu nombreux. Mais, dans le cas du raisin, de réels impacts économiques ont été rencontrés en Amérique du Nord dans la production de vin. Par exemple, des mesures ont montré qu'il suffisait d'1,9 individus dans un kilogramme de raisin pour que 10% des consommateurs de vins détectent la contamination (Galvan *et al.* 2007). Ainsi, la présence de quelques individus fixés à des grappes au moment des vendanges peut suffire à dégrader fortement la qualité gustative du vin produit du fait de la présence d'alcaloïdes en grande quantité dans le corps d'un seul individu (Sloggett *et al.* 2011). *H. axyridis* est ainsi à l'origine de réelles conséquences économiques en Amérique du Nord qui se chiffrent en millions de dollars (Hutchison *et al.* 2010).

c) Troisième impact négatif : gêne sociale et risques allergènes.

Comme beaucoup d'espèces de coccinelles, *H. axyridis* hiverne en s'agrégeant en grand nombre au stade adulte. Toutefois, elle semble avoir une tendance plus forte que les autres espèces à choisir les zones les plus anthropisées comme sites d'hivernation, y compris dans son aire native (Wang *et al.* 2011). L'agrégation en grand nombre (plusieurs centaines ou plusieurs milliers d'individus) sur ou dans des bâtiments habités à l'automne occasionne diverses gênes sociales (nombre important, odeurs désagréables, vols répétés autour des lampes en fin de journée et parfois morsures). Ce comportement d'agrégation est vraisemblablement la principale cause de la médiatisation importante de l'espèce dans beaucoup de pays dans lesquels elle est envahissante. Par ailleurs, des cas de réactions allergiques ont été décrits, s'exprimant principalement sous la forme de rhinite et plus

rarement d'asthme, d'urticaire ou d'angio-œdème (e.g. Dutau 2008; Goetz 2007; Nakazawa *et al.* 2007; Yarbrough *et al.* 1999).

d) Quatrième impact négatif : une image négative pour la lutte biologiques

Un dernier impact négatif d'*H. axyridis*, qui n'est que rarement évoqué, concerne l'image de la lutte biologique en tant que méthode de lutte « écologique ». En effet, la lutte biologique a longtemps eu une image de méthode respectueuse de l'environnement, non polluante, etc. Si les effets non-intentionnels liés à cette méthode sont pris en compte par les chercheurs travaillant sur la recherche d'auxiliaires depuis longtemps, ils étaient souvent inconnus du grand public. La médiatisation d'un « échec de la lutte biologique » lié, qui plus est, à une espèce porte-étendard de cette méthode de lutte aux yeux du grand public (i.e. une coccinelle) n'est probablement pas bénéfique. Il est toutefois difficile à l'heure actuelle de quantifier un tel impact.

e) Quelques aspects positifs pour équilibrer le débat

Malgré ces impacts négatifs, *H. axyridis* reste une excellente prédatrice très vorace de pucerons. En Amérique du Nord par exemple, elle est devenue un auxiliaire naturel important dans la gestion des populations du puceron envahissant du soja *Aphis glycines* (Ragsdale *et al.* 2011). Sa polyphagie lui permet en effet d'être parmi les principales prédatrices de certaines espèces envahissantes nouvellement arrivées.

II.1.4. Principales actions de recherches réalisées sur *H. axyridis* au cours de la thèse

Après de très nombreuses tentatives d'acclimatation infructueuses, *H. axyridis* est soudainement devenue une espèce envahissante en colonisant presque la totalité de la planète en moins de 25 ans. Cette constatation pose de nombreuses questions quant aux raisons du succès de l'invasion de l'espèce. Dans un premier temps, nous avons tenté de retracer les routes d'invasion pour identifier les populations sources les plus importantes dans l'histoire de l'invasion et caractériser les événements démographiques potentiellement importants d'un point de vue évolutif (goulots d'étranglement génétique et événements d'hybridation). A partir de ces résultats, nous avons testé des hypothèses évolutives concernant les facteurs clés du succès de l'établissement. Enfin, nous avons étudié des phénomènes évolutifs

potentiellement à l'œuvre lors de l'étape de prolifération de l'espèce, en nous focalisant sur l'Europe.

II.2. Introduction d'*H. axyridis* : les routes d'invasion

- Article 1 (Annexe I) :

  Lombaert E, Guillemaud T, Cornuet JM, Malausa T, Facon B, Estoup A (2010) Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. *Plos One* **5**, e9743.

- Article 2 (Annexe II) :

  Lombaert E, Guillemaud T, Thomas C, Lawson Handley L-J, Li J, Wang S, Pang H, Goryacheva II, Zakharov IA, Jousselin E, Poland R, Migeon A, van Lenteren JC, De Clercq P, Berkvens N, Jones W, Estoup A (2011) Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: case study of the invasive ladybird *Harmonia axyridis*. *Molecular Ecology* **20**, 4654-4670.

- Article 3 (Annexe III) :

  Brown P, Thomas C, **Lombaert E**, Jeffries D, Estoup A, Lawson Handley L-J (2011) The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. *BioControl* **56**, 623-641.

II.2.1. Contexte, intérêts et questions posées

Retracer les routes d'invasion est une étape nécessaire (i) pour optimiser les mesures de quarantaine et (ii) pour générer des hypothèses concernant les causes éco-évolutives à l'origine des succès et des échecs des invasions biologiques. Les données historiques permettant d'inférer les routes d'invasion d'une espèce donnée étant souvent incomplètes voire inexistantes, l'utilisation de méthodes indirectes basées sur la variabilité génétique mesurée sur des marqueurs nucléaires ou mitochondriaux se révèle nécessaire (Estoup & Guillemaud 2010; Wares *et al.* 2005). D'autre part, l'utilisation de marqueurs génétiques permet souvent la mise en évidence d'histoires inattendues, même dans les cas d'invasions particulièrement bien suivies (e.g. Kolbe *et al.* 2004; Miller *et al.* 2005).

Dans le cas de la coccinelle asiatique *H. axyridis*, les informations historiques sont relativement nombreuses et de deux types : (i) les introductions intentionnelles dans le cadre d'opérations de lutte biologique, et (ii) les dates de première observation des populations

envahissantes. En ce qui concerne les lâchers intentionnels, on sait que de nombreuses introductions indépendantes ont eu lieu en Amérique du Nord depuis l'Est de l'aire native (e.g. Koch 2003; Krafsur *et al.* 1997; Tedders & Schaefer 1994), et qu'officiellement, une seule souche fut utilisée en Europe, en Amérique du Sud et en Afrique du Nord issue d'une unique population échantillonnée en Chine, vraisemblablement à Pékin, en 1982 (Ferran *et al.* 1997; Ongagna *et al.* 1993). Les dates de première observation sont globalement considérées comme relativement fiables, et en particulier en Amérique du Nord et en Europe (e.g. Brown *et al.* 2008; Krafsur *et al.* 1997). L'espèce est en effet de grande taille, facile à repérer, s'agrège en grand nombre à certaines périodes de l'année et elle intéresse à la fois les agronomes et les entomologistes amateurs. Toutefois, toutes ces informations sont insuffisantes pour retracer les routes d'invasion avec un niveau de fiabilité élevé.

Avant notre étude, il n'existait que quelques rares travaux de génétique des populations sur *H. axyridis*. Dans l'aire native, des études de variations géographiques de divers traits morphologiques (couleur et forme des élytres) suggèrent l'existence de deux groupes populationnels géographiquement bien distincts : un à l'Ouest et un à l'Est, la limite entre les deux se trouvant probablement le long du lac Baïkal (Blekhman 2008; Dobzhansky 1933). Ces résultats sont corroborés par l'étude récente du polymorphisme à un marqueur mitochondrial en Sibérie (Blekhman *et al.* 2010). En Amérique du Nord, Krasfur *et al.* (1997) fournirent la première étude de génétique des populations d'*H. axyridis* dans une zone envahie. Leur étude, basée sur le polymorphisme protéique mesuré à 52 allozymes, couvre 8 états Américains principalement situés dans la moitié Est du pays, mais également à l'Ouest (Oregon). Les résultats suggèrent une introduction unique dans ce pays, mais les auteurs ne disposent malheureusement pas d'échantillons de l'aire native pour tester cette hypothèse.

Au final, les données historiques et les premières données de génétique des populations amènent à se poser différentes questions. Par exemple, la zone Est de l'aire native est-elle bien à l'origine de toutes les populations envahissantes ? Y a-t-il eu une seule introduction en Amérique du Nord ou deux indépendantes (une à l'est et une à l'ouest) ? Les populations envahissantes européennes et sud américaines sont-elles bien issues de la même souche de lutte biologique Européenne. Quant à l'Afrique du Sud, il s'agit probablement d'une introduction accidentelle, mais provenant de quel continent ? Nous avons voulu répondre à ces différentes questions en utilisant principalement une méthode récente d'inférence en génétique des populations : l'Approximate Bayesian Computation (ABC, Encadré 1, Beaumont *et al.* 2002). L'utilisation de jeux de données simulées a également permis d'apporter de nouveaux éléments quant à l'évaluation de cette méthode.

II.2.2. Méthodes utilisées

a) Echantillons, génotypage et variabilité génétique

Pour retracer les routes d'invasion d'*H. axyridis*, nous avons développé 18 marqueurs microsatellites (Annexe XI, Loiseau *et al.* 2009) et génotypé des populations de l'aire d'origine, des populations des continents envahis ainsi que des échantillons issus d'élevages de lutte biologique. Chaque zone envahie a été représentée dans nos analyses par un seul échantillon géographiquement proche du site de première observation de l'espèce (i.e. Louisiane pour l'Amérique du Nord-Est ; Washington pour l'Amérique du Nord-Ouest ; Belgique pour l'Europe ; Sud du Brésil pour l'Amérique du Sud ; région du Cap pour l'Afrique du Sud) (Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011). La variabilité génétique intra et interpopulationnelle a été décrite à l'aide de différentes statistiques classiques de génétique des populations (nombre d'allèles, hétérozygotie attendue, F_{ST} par paire de population, etc.) (Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011). La structure de l'aire native a été inférée à l'aide de méthodes Bayésiennes de regroupement d'individus implémentées dans différents logiciels (Article 2, Lombaert *et al.* 2011; Corander *et al.* 2003; Pritchard *et al.* 2000).

*b) Inférence des routes d'invasion d'***H. axyridis** *par une approche ABC*

Les routes d'invasion ont été retracées par une approche ABC (à l'aide du logiciel DIYABC ; Cornuet *et al.* 2010; Cornuet *et al.* 2008) en procédant de manière hiérarchique en cinq analyses successives s'appuyant sur les informations historiques (Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011). Chaque analyse consiste à comparer un ensemble de scénarios d'invasion comprenant une population cible (la population envahissante dont on veut inférer l'origine) et un ensemble de populations sources potentielles (natives, de lutte biologique et éventuellement envahissantes plus anciennes). En plus des scénarios d'introduction simple (e.g. A est issu de B), tous les scénarios avec hybridation entre deux populations sources ont été testés (e.g. A est issu d'une hybridation entre B et C). La première analyse a consisté à comparer des scénarios d'invasion pour inférer les routes d'invasion du Nord-Est Américain (NEA) avec uniquement les populations natives et de lutte biologique comme sources potentielles puisqu'il s'agit de la population envahissante la plus ancienne (i.e. 1988, Chapin & Brou 1991). Lors de la seconde analyse, les routes d'invasion du Nord-Ouest Américain (NOA, seconde population la plus ancienne avec une date de

première observation en 1991, LaMana & Miller 1996) ont été inférées avec les mêmes populations sources potentielles, mais en ajoutant la population envahissante NEA. Et ainsi de suite pour les trois analyses suivantes (Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011).

Les résultats obtenus par ABC ont ensuite été évalués de deux manières. Tout d'abord, la confiance que l'on peut accorder aux analyses et leur réel pouvoir résolutif ont été examinés en calculant pour chaque analyse les erreurs de type I (i.e. le risque que le scénario *i* ne soit pas sélectionné lorsqu'il est vrai) et les erreurs de type II (i.e. le risque que le scénario *i* soit sélectionné alors qu'il est faux). Pour cela, nous simulons pour chaque scénario 100 jeux de données que nous analysons en ABC comme s'il s'agissait de données réellement observées (Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011). Enfin, une étape de vérification de modèle (« model checking ») propre aux analyses bayésiennes (Gelman *et al.* 1995) a été entreprise (Article 2, Lombaert *et al.* 2011). En effet, le scénario sélectionné, même s'il est le « meilleur » des scénarios en compétition, ne s'ajuste pas forcément bien aux données car il n'est peut-être pas le vrai scénario (qui lui ne ferait simplement pas partie des scénarios mis en compétition). Nous avons effectué la vérification de modèle sur le scénario sélectionné de la cinquième analyse qui inclut toutes les populations. Nous avons ainsi simulé $2x10^6$ jeux de données selon ce scénario final, puis sélectionné les $2x10^4$ jeux de données les plus proches des données observées sur la base de distances euclidiennes et d'une étape de régression linéaire (Beaumont *et al.* 2002). Enfin, nous avons simulé 10^4 jeux de données en tirant les paramètres du modèle dans les distributions *a posteriori* de l'étape précédente. On s'attend à ce que les statistiques résumées issues de cette dernière étape de simulation soient proches des données observées (Gelman *et al.* 1995). Une probabilité peut être calculée pour la valeur observée de chaque statistique à partir de la distribution des statistiques simulées.

c) Evaluation de la méthode ABC : hybridation et échantillonnage incomplet

L'intérêt de l'utilisation de la méthode ABC par rapport à des méthodes plus classiques (F_{ST} par paire de populations et vraisemblance d'assignation) pour étudier certains cas d'hybridation a été évalué par simulation (Article 1, Lombaert *et al.* 2010). Par ailleurs, nous avons évalué (i) les risques associés à un échantillonnage incomplet d'une aire native structurée sur les inférences de routes d'invasion en ABC et (ii) la possibilité de limiter ce risque en incluant explicitement dans les scénarios mis en compétition des populations non échantillonnées (Article 2, Lombaert *et al.* 2011).

II.2.3. Principaux résultats et bilan

*a) Routes d'invasion d'***H. axyridis** *: tête de pont et hybridation*

Nous avons montré par des méthodes classiques de génétique des populations et par des méthodes Bayésiennes de regroupement populationnel que l'aire native d'*H. axyridis* était constituée de deux principaux groupes génétiques (Figure 6, Articles 2 et 3, Lombaert *et al.* 2011; Brown *et al.* 2011a), confirmant l'existence d'un découpage biogéographique Ouest/Est (Blekhman 2008; Blekhman *et al.* 2010; Dobzhansky 1933). Les analyses ABC nous indiquent que les deux zones envahies en Amérique du Nord (NEA et NOA) sont indépendamment issues de l'air native (Figure 6, Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011). Le foyer envahissant NEA est issu d'une hybridation entre les deux groupes natifs Est et Ouest tandis que le foyer envahissant NOA est issu de la zone Est de l'aire native (Figure 6, Article 2, Lombaert *et al.* 2011). Les foyers d'Amérique du Sud et d'Afrique du Sud sont tous les deux issus du foyer envahissant NEA. Il en est de même pour l'Europe, mais avec un événement d'hybridation avec la souche de lutte biologique Européenne (Figure 6, Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011). Notons que dans chaque foyer envahissant, les goulots d'étranglement génétiques mesurés sont d'intensité moyenne (Article 1, Lombaert *et al.* 2010). Les évaluations de la qualité de nos analyses (erreurs de type I et II et « model checking ») suggèrent qu'elles sont robustes (Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011).

Nos résultats désignent une population particulièrement importante dans l'histoire de l'invasion mondiale : il s'agit de la population envahissante NEA qui est à l'origine de toutes les autres invasions à l'exception de NOA (Figure 6). La population NEA a donc agi comme une tête de pont (« bridgehead ») de l'invasion mondiale (Articles 1, 2 et 3, Lombaert *et al.* 2010; Lombaert *et al.* 2011; Brown *et al.* 2011a). Ce résultat est assez inattendu étant donnée l'histoire de la lutte biologique détaillée précédemment. Les introductions accidentelles semblent avoir un rôle important dans la dissémination d'*H. axyridis* dans le monde (Articles 1 et 3, Lombaert *et al.* 2010; Brown *et al.* 2011a;). Nous avons d'ailleurs illustré l'existence de telles introductions en assignant un échantillon retrouvé sur du bois importé de Pennsylvanie en Norvège (Article 1, Lombaert *et al.* 2010). Mais l'existence de cette tête de pont pose surtout la question de la raison du succès fulgurant de cette population NEA précise après des décennies d'échec des tentatives d'acclimatation (Articles 1 et 3, Lombaert *et al.* 2010; Brown *et al.* 2011a). Nous aborderons cette question dans la section II.3.

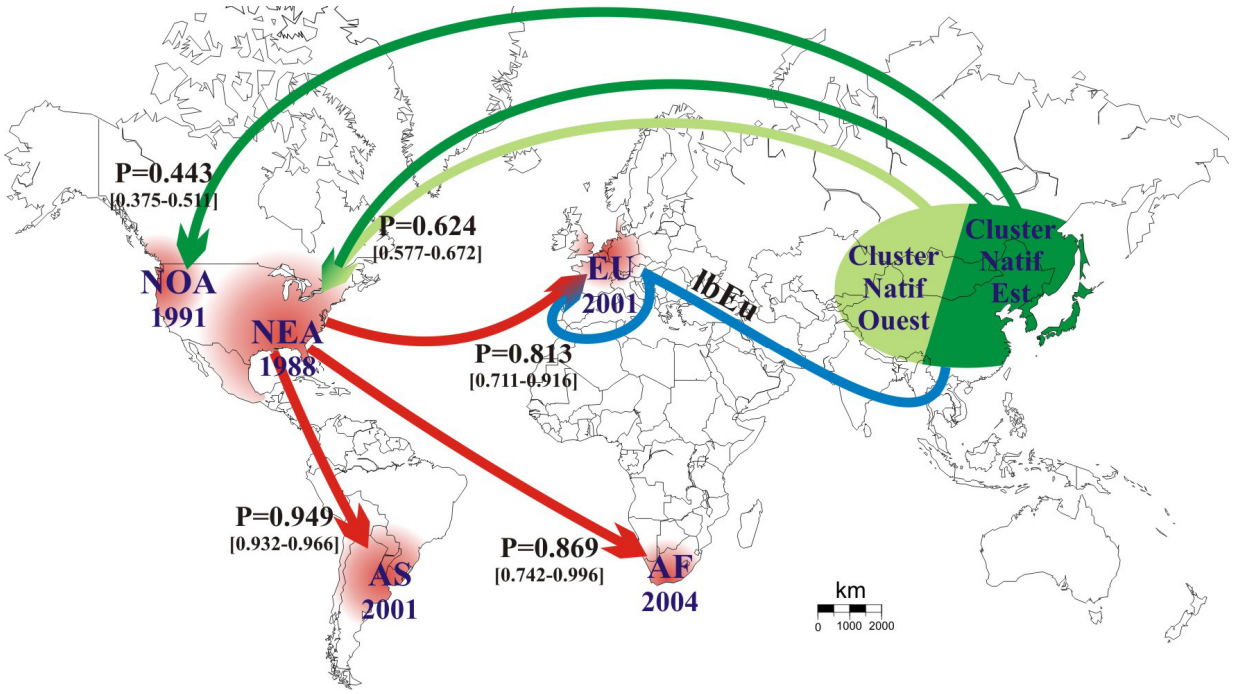


Figure 6 : Scénario le plus probable de l'invasion mondiale d'*H. axyridis* obtenu par ABC. En vert, l'aire native et en rouge les zones envahies (NEA = Nord-Est Américain ; NOA = Nord-Ouest Américain ; EU = Europe ; AS = Amérique du Sud ; AF = Afrique du Sud). Chaque flèche représente la route d'invasion la plus probable avec la probabilité *a posteriori* associée (P) et les intervalles de confiance à 95% entre crochet. Les dates de première observation de chaque foyer envahissant sont indiquées. Initialement échantillonnée dans l'aire native en 1982, la population de lutte biologique Européenne (lbEu) est représentée par une flèche bleue. Notons que la population envahissante NEA peut n'être constituée que d'une seule introduction issue d'une population naturellement hybride dans l'aire native.

Au moins deux phénomènes d'hybridation ont également eu lieu au cours de cette invasion mondiale (Figure 6). Le premier en NEA implique les deux groupes populationnels natifs Est et Ouest (avec un taux d'hybridation estimé par ABC à 57% de gènes issus de l'Est natif) (Article 2, Lombaert *et al.* 2011). Il est cependant encore impossible de dire si l'hybridation a eu lieu avant ou après l'introduction. Il existe en effet probablement une zone de contact naturelle dans l'aire native entre les deux groupes populationnels Est et Ouest localisée autour du lac Baïkal (Blekhman 2008; Blekhman *et al.* 2010; Dobzhansky 1933). Nous ne disposons cependant pas à l'heure actuelle d'échantillons collectés dans cette zone géographique. Dans tous les cas, ce résultat est particulièrement intéressant puisqu'il concerne directement la population tête de pont. Le second phénomène d'hybridation identifié a eu lieu en Europe et implique la population NEA et la souche de lutte biologique importée en Europe en 1982 (avec un taux d'hybridation estimé par ABC à 57% de gènes issus de NEA) (Article 1, Lombaert *et al.* 2010). Le rôle de l'apport génétique de la souche de lutte biologique dans l'invasion Européenne se pose. En effet, la population NEA semble capable d'envahir seule,

tandis que la souche de lutte biologique ne semble pas avoir été capable de s'acclimater pendant les années où elle a été testée sur le terrain et commercialisée (Article 1, Lombaert *et al.* 2010). Nous aborderons également ce point dans la section II.3.

b) Méthodologie : intérêts et écueils possibles de la méthode ABC

Comme nous l'avons vu précédemment, la méthode ABC présente un certain nombre d'avantages. Parmi ceux-ci, il est par exemple possible de tester explicitement la possibilité d'hybridation entre plusieurs sources dans un scénario d'invasion. De manière générale, les introductions multiples ne sont pas des phénomènes rares (Bossdorf *et al.* 2005; Dlugosch & Parker 2008; Novak 2007; Roman & Darling 2007; Wilson *et al.* 2009), et les hybridations au niveau intra-spécifique entre des sources génétiquement différenciées ne sont probablement pas des phénomènes exceptionnels au cours des invasions (e.g. Facon *et al.* 2005; Kolbe *et al.* 2004). En outre, il est particulièrement pertinent d'identifier ce phénomène lorsqu'il a lieu car il est potentiellement important d'un point de vue évolutif. Nos résultats sur des données simulées indiquent que la nature hybride d'une population envahissante peut mener à des résultats très incorrects lorsqu'on tente d'inférer sa source à l'aide de statistiques plus classiques que l'ABC tels que les F_{ST} ou les maximums de vraisemblance. Par exemple, si la population A est issue de l'hybridation entre les populations B et C, les statistiques classiques désigneront dans certains cas à tort une population D, génétiquement plus diversifiée (e.g. une population native), comme étant la source (Article 1, Lombaert *et al.* 2010).

Toutefois, nous avons également montré que la méthode ABC pouvait surestimer les cas d'hybridation lorsque les échantillons utilisés dans l'analyse ne correspondent pas (génétiquement) aux populations parentales. De manière générale, l'échantillonnage est un problème récurant en génétique des populations (e.g. Muirhead *et al.* 2008; Waples & Gaggiotti 2006). Dans le cas des espèces envahissantes, il n'est pas rare que les aires natives soient génétiquement structurées, de grande taille et mal identifiées géographiquement. Il est alors difficile d'échantillonner de manière exhaustive une aire de répartition, et les vraies populations sources peuvent ne pas être à notre disposition. Dans le cas d'*H. axyridis*, l'aire native est très grande et mal connue dans sa totalité (Article 3, Brown *et al.* 2011a), et nos analyses montrent qu'une différentiation génétique légère mais significative existe au sein même de chacun des deux groupes populationnels Est/Ouest (Article 2, Lombaert *et al.* 2011). Nos simulations montrent qu'un échantillonnage erroné, s'il n'est pas pris en compte dans les analyses ABC, peut mener à conclure à une hybridation entre les deux groupes natifs lorsque

la population envahissante ne provient en réalité que d'un seul groupe (mais d'une population non échantillonnée au sein de ce groupe, Article 2, Lombaert *et al.* 2011). Toutefois, l'utilisation de scénarios incluant explicitement des populations non échantillonnées dans les analyses ABC permet de réduire grandement ce risque (e.g. en diminuant dans nos simulations les erreurs de type I de 0.35 à 0.16 et les erreurs de type II de 0.17 à 0.08). C'est pourquoi nous avons appliqué ce type de design dans nos analyses sur les vraies données d'*H. axyridis* (Article 2, Lombaert *et al.* 2011). La conclusion d'une origine hybride de la population NEA semble ainsi d'autant plus robuste.

II.3. Etablissement d'*H. axyridis* : plasticité phénotypique, purge et hybridation

- Article 4 (Annexe IV) :

  Lombaert E, Malausa T, Devred R, Estoup A (2008) Phenotypic variation in invasive and biocontrol populations of the harlequin ladybird, *Harmonia axyridis*. *Biocontrol* **53**, 89-102.

- Article 5 (Annexe V) :

  Facon B, Hufbauer RA, Tayeh A, Loiseau A, **Lombaert E**, Vitalis R, Guillemaud T, Lundgren JG, Estoup A (2011) Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. *Current Biology* **21**, 424-427.

- Article 6 (Annexe VI) :

  Turgeon J, Tayeh A, Facon B, **Lombaert E**, De Clercq P, Berkvens N, Lundgren JG, Estoup A (2011) Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion. *Journal of Evolutionary Biology* **24**, 1044-1052.

II.3.1. Contexte, intérêts et questions posées

Comprendre comment une population envahissante est parvenue à s'établir dans un nouveau milieu après son introduction peut nous aider à mieux comprendre les facteurs éco-évolutifs à l'origine du succès des invasions biologiques. L'espèce peut dans certains cas être pré-adaptée au nouveau milieu, et les processus évolutifs en jeu seront alors peu importants (Facon *et al.* 2006). Mais dans d'autres cas, des phénomènes adaptatifs seront nécessaires (Lee 2002; Sakai *et al.* 2001; Wares *et al.* 2005) : ceux-ci devront permettre l'adaptation au

nouveau milieu et/ou la diminution des risques d'extinction liés aux petites tailles de population initiales.

L'inférence des routes d'invasion d'*H. axyridis* nous a permis d'éloigner (sans l'éliminer totalement car l'aire native est de grande taille) l'hypothèse d'une pré-adaptation chez *H. axyridis*. En effet, l'existence d'une population tête de pont (Figure 6, Article 1, Lombaert *et al.* 2010), associée au long historique d'échecs d'acclimatation de l'espèce, suggère qu'un changement évolutif majeur a eu lieu précisément au sein de cette population. Parmi les hypothèses testables, une augmentation de la plasticité phénotypique adaptative (e.g. Richards *et al.* 2006) est potentiellement importante pour cette population présente maintenant dans des biomes très variés dans les aires envahies (Bidinger *et al.* 2011; Poutsma *et al.* 2008). Par ailleurs des études antérieures focalisées sur des échantillons issus de la population envahissante NEA suggèrent (i) que la plasticité phénotypique est importante pour certains traits chez cette population et (ii) que cette plasticité à une base génétique permettant donc à la sélection naturelle d'agir (Grill *et al.* 1997; Preziosi *et al.* 1999).

De même, nos résultats semblent montrer que les conditions d'introduction d'*H. axyridis* ont impliqué un goulot d'étranglement génétique d'intensité moyenne (Article 1, Lombaert *et al.* 2010), ce qui pourrait, au moins théoriquement, rendre possible une purge d'allèles délétères (Glemin 2003). Une telle purge pourrait permettre une réduction de la différence de performance entre des individus consanguins et des individus dont les parents sont non-apparentés.

Enfin, plusieurs événements d'hybridation ont été détectés : le premier en Amérique du Nord-Est (cf. population NEA issue d'une hybridation entre deux populations natives, Article 2, Lombaert *et al.* 2011) et le second en Europe (cf. hybridation entre la population NEA et la souche de lutte biologique Européenne, Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011). Pour l'instant, nous n'avons étudié que le second qui est un cas particulièrement intéressant car il implique une population dont le succès dans l'invasion mondiale est évident (NEA, Article 1, Lombaert *et al.* 2010) et une souche de lutte biologique qui semble n'avoir jamais pu devenir envahissante malgré de nombreuses introductions (Ferran *et al.* 1997). Or, l'hybridation peut aussi bien avoir des effets négatifs sur la valeur sélective (Arnold & Hodges 1995; Burke & Arnold 2001) que des effets positifs particulièrement intéressants dans le cadre d'une invasion biologique (Ellstrand & Schierenbeck 2000; Lee 2002; Sakai *et al.* 2001; Wares *et al.* 2005).

II.3.2. Méthodes utilisées

Pour chaque hypothèse testée (i.e. rôle de la plasticité phénotypique, de la purge génétique et de l'hybridation intraspécifique), nous avons échantillonné des populations vivantes (généralement entre 50 et 100 individus) que nous avons ensuite élevées pendant 2 générations en conditions contrôlées de laboratoire afin de réduire au maximum les éventuels effets maternels. Différents traits d'histoire de vie, généralement considérés comme étant de bons indicateurs de la valeur sélective (e.g. taux d'éclosion, survie larvaire, durée de développement, fécondité, etc.), ont ensuite été mesurés. Les analyses statistiques ont majoritairement été effectuées à l'aide de modèles linéaires généralisés, incluant ou non des effets aléatoires.

a) Rôle de la plasticité phénotypique : échantillonnage et traits mesurés

Lorsque nous avons abordé la question de la plasticité phénotypique (Article 4, Lombaert *et al.* 2008), nous ne disposions malheureusement pas de populations natives vivantes. Nous avons utilisé à la place des populations de lutte biologique qui peuvent théoriquement constituer un cas particulièrement intéressant à étudier. En effet, alors qu'on peut théoriquement s'attendre à une plasticité phénotypique importante chez les populations envahissantes de *H. axyridis*, les populations de lutte biologique au contraire devraient être très peu plastiques. Cette hypothèse repose sur la faible variabilité des conditions d'élevage qui devrait conduire à la perte de plasticité adaptative si celle-ci est coûteuse (DeWitt *et al.* 1998; Masel *et al.* 2007).

Quatre populations ont été utilisées lors de cette expérience. Les deux populations de lutte biologique proviennent d'élevages Européens indépendants depuis 50 à 100 générations. La première population envahissante provient de Londres en Angleterre, et la seconde provient de Roquefort-les-Pins dans le Sud-Est de la France. La plasticité d'un trait peut être mesurée en déterminant son expression phénotypique dans différents environnements (on parle de norme de réaction). Nous avons donc mesuré six traits phénotypiques (taux d'éclosion, taux de survie larvaire, durée de développement, sex-ratio, fécondité pendant six semaines et survie adulte sans nourriture) à trois températures différentes (18, 24 et 30°C). De même, nous avons mesuré la survie de groupes d'individus sans nourriture à trois températures basses (5, 10 et 15°C) pendant 5 semaines.

b) Purge génétique : échantillonnage, goulot d'étranglement et traits mesurés

Afin de tester l'hypothèse d'une purge génétique chez les populations envahissantes d'*H. axyridis* (Article 5, Facon *et al.* 2011b), nous avons commencé par quantifier l'intensité du goulot d'étranglement génétique ayant eu lieu en NEA pour savoir si le taux de dérive associé était compatible avec ceux préconisés (à l'équilibre au moins) par les études théoriques reliant purge et dérive (Boakes *et al.* 2007; Glemin 2003). Pour cela, nous avons génotypé à 18 marqueurs microsatellites (Annexe XI, Loiseau *et al.* 2009) deux des populations utilisées pour les expérimentations (voir paragraphe suivant) : la population Nord-Est américaine et la population japonaise. Nous avons ensuite estimé les paramètres du goulot d'étranglement (effectif efficace de la population pendant le goulot d'étranglement et durée, en nombre de générations, de celui-ci) par Approximate Bayesian Computation (ABC, Beaumont *et al.* 2002) sur 1% de $2x10^6$ jeux de données simulés d'un scénario simple d'introduction de la population Américaine à partir de la Japonaise avec réduction de taille efficace pendant une durée donnée (paramètres tirés dans des distributions *a priori*). Notons qu'au moment de cette étude, l'éventualité d'une hybridation native en Amérique du Nord Est (Article 2, Lombaert *et al.* 2011) n'était pas encore connue. J'ai donc récemment refait l'estimation des paramètres du goulot d'étranglement en suivant la même procédure, mais en considérant que la population Américaine était cette fois-ci issue de l'hybridation entre la population Japonaise et une des populations Sibériennes (Abakan, voir Article 5, Facon *et al.* 2011b) elle aussi génotypée. Notons que le goulot d'étranglement simulé a lieu après l'hybridation.

Pour les croisements et les mesures de traits d'histoire de vie, nous avons échantillonné trois populations dans l'aire native (une au Japon et deux en Sibérie) et trois populations dans les aires envahies (une aux USA, une en Afrique du Sud et une en Europe). Deux types de croisements ont été effectués : (i) des croisement consanguins entre frères et sœurs et (ii) des croisements entre non-apparentés issus de la même population. Cinq traits d'histoire de vie ont ensuite été mesurés sur les descendants de ces croisements (taux d'éclosion, taux de survie larvaire, durée de développement, age à la maturité sexuelle et fécondité pendant huit jours).

c) Hybridation en Europe : échantillonnage et traits mesurés

Pour étudier le rôle de l'hybridation dans l'invasion Européenne (Article 6, Turgeon *et al.* 2011), nous avons comparé des traits d'histoire de vie entre des populations parentales

pures (i.e. Américaines et de lutte biologique), une population hybride naturelle (i.e. population Européenne) et des populations hybrides produites en laboratoire. Nous avons pour cela échantillonné deux populations nord-Américaine (Dakota du Sud et Québec), deux populations de lutte biologique Européenne (historiquement issues toutes les deux de la population originellement échantillonnée en Chine en 1982) et une population Européenne (Belgique). Au total, 13 croisements ont été réalisés, dont 8 croisements hybrides. Six traits d'histoire de vie ont été mesurés (taux d'éclosion, taux de survie larvaire, durée de développement, age à la maturité sexuelle, fécondité pendant six semaines et survie adulte sans nourriture).

II.3.3. Principaux résultats et bilan

a) Plasticité phénotypique

Les traits mesurés au cours de la première expérience (évaluation des normes de réaction à 18, 24 et 30°C) nous ont permis de calculer un indice composite de la valeur sélective (Article 4, Lombaert *et al.* 2008). Pour cet indice, une plasticité phénotypique adaptative forte doit théoriquement amener à une norme de réaction plate (cf. « homéostasie de la valeur sélective », Richards *et al.* 2006; Scheiner 1993). Nous avons observé une interaction significative ($P < 0,05$) entre le statut des populations (i.e. envahissante ou de lutte biologique) et la température suggérant une différence de plasticité (Figure 7). Toutefois, cet effet est faible, et cette tendance n'est retrouvée sur aucun des traits traités individuellement. De plus les analyses effectuées sur un indice de mesure de la plasticité (RDPI, Valladares *et al.* 2006) n'indiquent aucune différence significative. Indépendamment de la plasticité, on trouve toutefois des différences phénotypiques entre populations, les populations de lutte biologique étant globalement plus performantes. Celles-ci ont probablement été favorisées par les conditions expérimentales proches des conditions d'élevage.

En revanche, la seconde expérience (survie à des températures de 5, 10 et 15°C) montre un très net avantage et une plasticité adaptative plus importante pour les populations envahissantes (Figure 7, Article 4, Lombaert *et al.* 2008). Notons que la population « envahissante » de Roquefort-les-Pins a (comme dans l'expérience précédente) une position intermédiaire entre les populations de lutte biologique et la population de Londres. Des résultats de génétique des populations récents, non publiés (et inconnus à l'époque), suggèrent en fait que cette population n'est pas issue du même foyer envahissant que la population de

Londres, et qu'il s'agit même d'une population non-envahissante dans le sens ou elle est établie, mais ne prolifère pas. Son origine initiale serait la population de lutte biologique Européenne seule et elle a donc un lien de parenté fort avec les deux populations de lutte biologique utilisées dans cette étude.

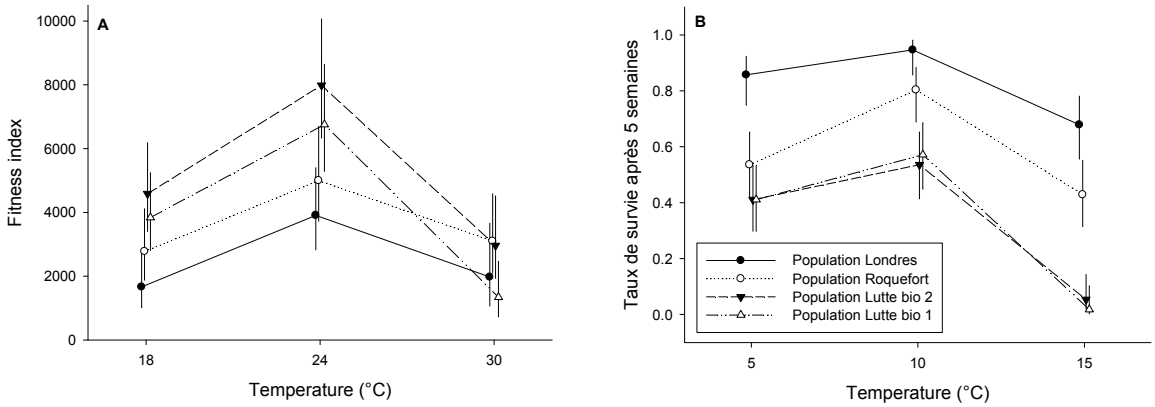


Figure 7 : Normes de réactions à la température de : (a) un indice composite de valeur sélective (voir Article 4, Lombaert *et al.* 2008) et (b) le taux de survie dans un groupe d'individus après 5 semaines sans nourriture à des températures réduites. Les barres verticales représentent les intervalles de confiances à 95%.

En conclusion, ce travail préliminaire n'encourage pas à tester des populations natives sur le même type de protocole car on ne s'attend pas à ce qu'elles soient moins plastiques que les populations de lutte biologique. En fait, le paramètre environnemental manipulé (la température) n'est probablement pas optimal pour mettre en évidence une éventuelle évolution de la plasticité phénotypique (excepté peut-être pour les températures faibles). La nourriture pourrait par exemple constituer un meilleur candidat car elle est, au moins potentiellement, assez différente dans l'aire native et les aires envahies (Berkvens *et al.* 2008b; Preziosi *et al.* 1999; Specty *et al.* 2003).

b) Purge génétique

Notre estimation des paramètres du goulot d'étranglement génétique lors de l'invasion en Amérique du Nord-Est nous confirme que son intensité a été moyenne avec une taille efficace d'environ 140 individus pendant une vingtaine de générations (Figure 8a, Article 5, Facon *et al.* 2011b). Une nouvelle estimation de ce goulot prenant en compte l'hybridation entre deux populations natives Est et Ouest asiatiques (Article 2, Lombaert *et al.* 2011) ne modifie pas de manière importante ce résultat. Dans ce cas, la taille efficace inférée est d'environ 160 individus pendant une vingtaine de générations (Figure 8b). L'ensemble de ces

valeurs restent tout à fait compatibles avec la possibilité, au moins théorique, de purge génétique (Glemin 2003).

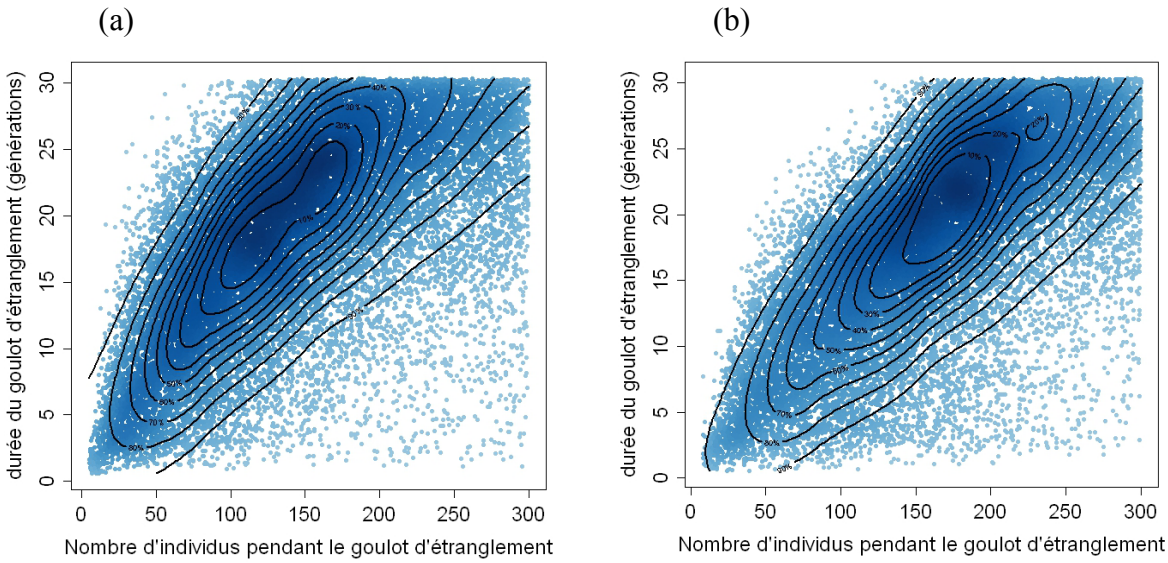


Figure 8 : Intensité du goulot d'étranglement suite à l'introduction d'*H. axyridis* dans le Nord-Est Américain. Les densités conjointes des distributions *a posteriori* des deux paramètres démographiques décrivant le goulot d'étranglement ont été obtenues par ABC. Les courbes noires indiquent les contours de densité de 10 à 90%. (a) Résultats obtenus à partir d'un scénario simple d'introduction décrivant la population du Nord-Est américain (représenté par un échantillon du Dakota du Sud) comme étant issue de la population Est asiatique (représentée par un échantillon de Kyoto) (résultats présentés dans l'Article 5, Facon *et al.* 2011b). (b) Résultats obtenus à partir d'un scénario d'introduction décrivant la population du Nord-Est américain (échantillon du Dakota) comme étant issue d'une hybridation entre la population native Est asiatique (Kyoto) et la population native Ouest asiatique (échantillon d'Abakan). Ces analyses non publiées sont inspirées des résultats de l'Article 2 (Lombaert *et al.* 2011).

A partir des cinq traits d'histoire de vie mesurés, deux traits composites ont été calculés (le temps de génération et la performance globale) (Article 5, Facon *et al.* 2011b). Pour chacun des deux traits composites, nous avons trouvé un effet significatif de l'interaction entre le statut (envahissant ou natif) et le niveau de consanguinité ($P = 0.047$ pour le temps de génération ; $P = 0.001$ pour la performance globale). Pour les deux traits composites, les populations natives présentent une dépression de consanguinité significative contrairement aux populations envahissantes (Figure 9). Dans les croisements non apparentés, les populations natives et envahissantes atteignent les mêmes valeurs pour chacun des traits. En revanche, dans les croisements consanguins, ces valeurs sont maintenues pour les populations envahissantes, mais chutent pour les populations natives (dépression de consanguinité).

Ainsi, les populations envahissantes, contrairement aux natives, ne sont pas soumises à la dépression de consanguinité sur les traits que nous avons mesurés. Notre travail suggère fortement que le goulot d'étranglement lié au phénomène d'introduction d'*H. axyridis* a

permis de purger au moins une partie des allèles délétères présents dans l'aire native. En outre, toutes les populations envahissantes testées sont liées dans leur histoire à la première population envahissante du Nord-Est américain NEA (Articles 1 et 2, Lombaert *et al.* 2010; Lombaert *et al.* 2011), et il est donc fort probable que la purge ayant permis de réduire la dépression de consanguinité ne soit survenue qu'une fois lors de cette première introduction.

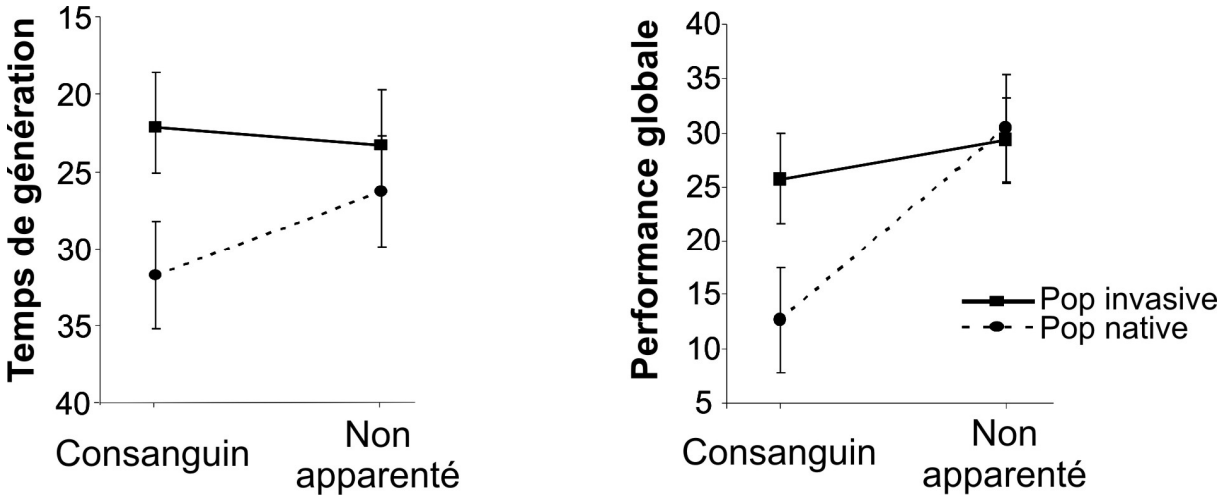


Figure 9 : Temps de génération moyen et performance globale moyenne de populations envahissantes et natives en situation de croisements consanguins ou non apparentés. Les barres verticales représentent les intervalles de confiances à 95%.

c) Hybridation en Europe

Quel que soit le trait mesuré, les populations de lutte biologique semblent être plus performantes que les populations sauvages (Figure 10, Article 6, Turgeon *et al.* 2011). Ce résultat confirme ceux obtenus lors de l'étude sur la plasticité phénotypique (Article 4, Lombaert *et al.* 2008) et est très probablement lié aux conditions d'expérimentation qui sont très proches des conditions d'élevage habituelles pour la lutte biologique.

On constate que les hybrides ont toujours des performances non significativement différentes de celles des populations de lutte biologique (Figure 10, Article 6, Turgeon *et al.* 2011). Ils présentent donc toujours des performances meilleures que celles de leurs parents envahissants américains (population NEA). Ce résultat suggère qu'une population introduite issue de la population NEA peut bénéficier de ce phénomène d'hybridation. Notons qu'il est fort probable que les populations de lutte biologique soient, en revanche, beaucoup moins performantes sur d'autres traits non étudiés ici, ce qui expliquerait leur absence de succès d'invasion (Ferran *et al.* 1997). C'est vraisemblablement le cas pour la résistance au froid comme nous l'avons montré précédemment (Article 4, Lombaert *et al.* 2008).

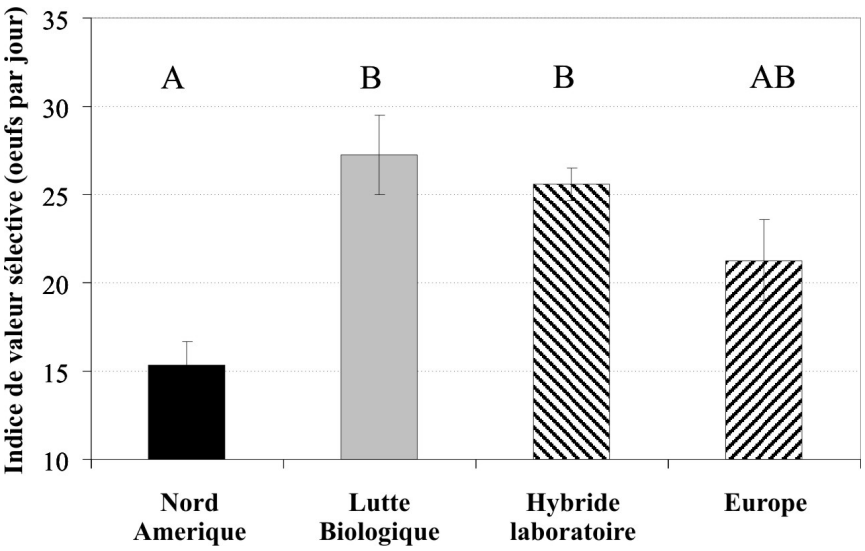


Figure 10 : Illustration des variations phénotypiques selon le type (Nord Amérique, Lutte Biologique, Hybride ou Europe) avec l'indice composite de valeur sélective (voir Article 6, Turgeon *et al.* 2011). Des lettres identiques indiquent qu'il n'y a pas de différence significative.

Les résultats obtenus sur la population Européenne envahissante (i.e. l'hybride *in natura*) sont globalement proches de ceux obtenus avec les hybrides de laboratoire. Cela suggère que les performances observées ne sont pas uniquement importantes en conditions de laboratoire. En outre, les bénéfices de l'hybridation semblent être maintenus sur un nombre important de générations.

*d) Etablissement d'***H. axyridis** *: que faut-il retenir ?*

Nos résultats suggèrent que la plasticité phénotypique n'est pas un facteur clé du succès de l'établissement d'*H. axyridis* (Article 4, Lombaert *et al.* 2008). En fait, étant donnée la très grande taille de l'aire native de l'espèce, ainsi que la faible structuration génétique qui suggère des flux de gènes importants (Article 2, Lombaert *et al.* 2011), il est fort probable que la plasticité adaptative soit déjà importante dans les populations natives (e.g. Michie *et al.* 2010). Même si cette plasticité adaptative est utile à l'établissement, elle ne semble pas suffisante pour assurer le succès de l'invasion comme l'illustre la longue période d'échec d'acclimatation. Il serait toutefois nécessaire de tester des populations natives pour s'en assurer.

En revanche, la purge génétique mise en évidence (Article 5, Facon *et al.* 2011b) pourrait constituer un le facteur favorisant le succès de l'établissement de l'espèce. Ce résultat est la première démonstration de ce type dans un contexte d'invasion biologique. Ce phénomène pourrait tout à fait être répandu car il expliquerait un certain nombre de caractéristiques communes à de nombreux processus d'invasion. Premièrement, la purge initiale expliquerait pourquoi les populations envahissantes prolifèrent souvent si rapidement.

En effet, la diminution de la dépression de consanguinité pourrait permettre aux populations sur le front d'invasion de croître rapidement malgré les faibles densités. Deuxièmement, le temps de latence souvent observé pendant la phase d'établissement pourrait être lié au temps nécessaire à la réalisation d'une purge efficace. Troisièmement, la purge pourrait être à l'origine de l'augmentation de performance souvent observée au cours des invasions et qui est généralement attribuée à la conséquence d'une réponse à un challenge adaptatif. Quatrièmement, la sélection de l'autofécondation fréquemment rencontrée chez des plantes envahissantes pourrait être permise par la purge génétique. Cinquièmement, la purge génétique pourrait être un facteur clé à l'origine des populations tête de pont. En effet, l'absence de dépression de consanguinité pourrait permettre à des nombres limités d'individus d'être, plus facilement, à l'origine de nouvelles populations.

Enfin, même si la population tête de pont NEA possède des caractéristiques lui permettant de devenir envahissante sur d'autres continents, nous avons montré que l'hybridation en Europe entre des individus de cette population NEA avec ceux issus d'une souche de lutte biologique pouvait avoir entraîné des conséquences positives sur sa valeur sélective (Article 6, Turgeon *et al.* 2011). Le succès de l'invasion Européenne (Article 3, Brown *et al.* 2011a) suggère que les traits limitant l'invasion de la population de lutte biologique ont été rapidement contre-sélectionnés dans la population hybride européenne.

II.4. Prolifération d'*H. axyridis* : évolution post-introduction

- Article 7 (Annexe VII) :

  Lombaert E, Estoup A, Joubard B, Grégoire J-C, Jannin A, Facon B, Guillemaud T (In prep.) Rapid evolution of dispersal abilities during the expansion of the invasive ladybird *Harmonia axyridis* in Europe.

- Article 8 (Annexe VIII) :

  Facon B, Crespin L, Loiseau A, **Lombaert E**, Magro A, Estoup A (2011) Can things get worse when an invasive species hybridizes? The harlequin ladybird *Harmonia axyridis* in France as a case study. *Evolutionary Applications* **4**, 71-88.

II.4.1. Contexte, intérêts et questions posées

Lorsqu'une population est établie dans un nouveau milieu, elle va pouvoir proliférer (i.e. croître démographiquement et spatialement) et devenir ainsi envahissante *stricto sensu*. Toutefois, certains facteurs, et en particulier l'hétérogénéité environnementale, peuvent faire échouer cette dernière étape (Sakai *et al.* 2001). De même, le déséquilibre spatial engendré par l'expansion peut perturber le succès final de l'invasion si les effets Allee sont trop forts sur le front d'invasion (Lewis & Kareiva 1993; Tobin *et al.* 2007).

Dans le cas de la coccinelle asiatique *H. axyridis*, la réponse à l'hétérogénéité environnementale n'est probablement pas le facteur le plus limitant car l'espèce est généraliste et dispose probablement déjà d'une grande plasticité phénotypique dans son aire native très étendue (Articles 2, 3 et 4, Lombaert *et al.* 2011; Brown *et al.* 2011a; Lombaert *et al.* 2008). Les facteurs à l'origine du succès de l'étape d'établissement ont probablement eu un rôle important dans le succès observable aujourd'hui de l'étape de prolifération. En particulier, la purge génétique que nous avons mise en évidence (Article 5, Facon *et al.* 2011b) est clairement un facteur avantageux au cours de l'expansion spatiale qui se caractérise notamment par une succession d'effets de fondation sur le front d'invasion. Les problèmes d'ordres démographiques et comportementaux (stochasticité démographique et difficulté à trouver un partenaire sexuel) sont potentiellement toujours présents, mais l'absence de dépression de consanguinité réduit considérablement les problèmes liés aux petits effectifs.

Par ailleurs, différents processus évolutifs sont à l'œuvre sous l'effet d'un déséquilibre spatial des densités de population : la sélection naturelle, la dérive génétique et l'homogamie spatiale (Phillips *et al.* 2010a). L'homogamie spatiale, qui doit entraîner une augmentation de la fréquence des individus bons dispersants sur le front d'invasion, a été très peu étudiée dans le cadre des invasions biologiques (Shine *et al.* 2011a). *H. axyridis* est un bon modèle biologique pour étudier ce processus évolutif. Une condition préalable à l'existence de ce type de phénomène est qu'un polymorphisme génétique des capacités de dispersion existe au sein de la population envahissante. Or, il a été facilement possible de sélectionner artificiellement à deux reprises (indépendamment) les capacités de dispersion chez cette espèce, une fois sur une population native (Seko *et al.* 2008) et une fois sur une souche de lutte biologique Européenne (Tourniaire *et al.* 2000b). Nous avons donc décidé de tester cette hypothèse

d'homogamie spatiale dans les populations Ouest européennes d'*H. axyridis* pour lesquelles l'historique de l'expansion est bien connu (Article 3, Brown *et al.* 2011a).

En outre, l'expansion spatiale d'*H. axyridis* en Europe peut également être affectée par une situation assez spécifique. Une souche de lutte biologique est en effet toujours commercialisée par une entreprise Française. Il s'agit d'une souche incapable de voler qui a été sélectionnée à la fin des années 90 à partir de la population originellement importée par l'INRA en 1982 (Tourniaire *et al.* 2000b). Le phénotype « non-volant » permet un contrôle local plus efficace des populations de pucerons, mais il est vraisemblablement peu viable à long terme (e.g. impossibilité de migrer vers les sites d'hivernation). Toutefois, il n'est lié qu'à une mutation récéssive à un seul locus, et la question des conséquences évolutives de l'hybridation de cette souche avec la population envahissante Européenne se pose. Nous avons décidé d'aborder ce point lors d'une expérience de génétique quantitative.

II.4.2. Méthodes utilisées

Tout comme pour les expérimentations présentées dans la partie II.3, toutes les populations échantillonnées sur le terrain ont tout d'abord été élevées pendant 2 générations en conditions contrôlées de laboratoire afin de réduire au maximum les éventuels effets maternels. Différents traits (traits d'histoire de vie « classiques », capacités de dispersion, motivation à voler, etc.) ont ensuite été mesurés et les analyses statistiques ont majoritairement été effectuées à l'aide de modèles linéaires généralisés mixtes.

a) Homogamie spatiale : échantillonnage, génotypage et traits mesurés

Pour tester l'hypothèse d'une augmentation des capacités de dispersion d'*H. axyridis* du foyer au front d'invasion (Article 7, Lombaert *et al.* In prep.), nous avons échantillonné à l'automne 2010 un total de 8 populations le long de deux transects joignant chacun le foyer de l'invasion (en Belgique) à deux zones spatialement déconnectées mais toutes deux proches du front en France (Figure 11). Dans un premier temps, nous avons voulu nous assurer que les 8 échantillons populationnels étaient bien issus de la même introduction ayant eu lieu en Belgique vers 2001. Pour cela, nous avons génotypé une trentaine d'individus de la génération 0 de chaque population à 18 marqueurs microsatellites (Annexe XI, Loiseau *et al.* 2009) puis effectué un certain nombre d'analyses de différentiation génétique et de regroupement d'individus.

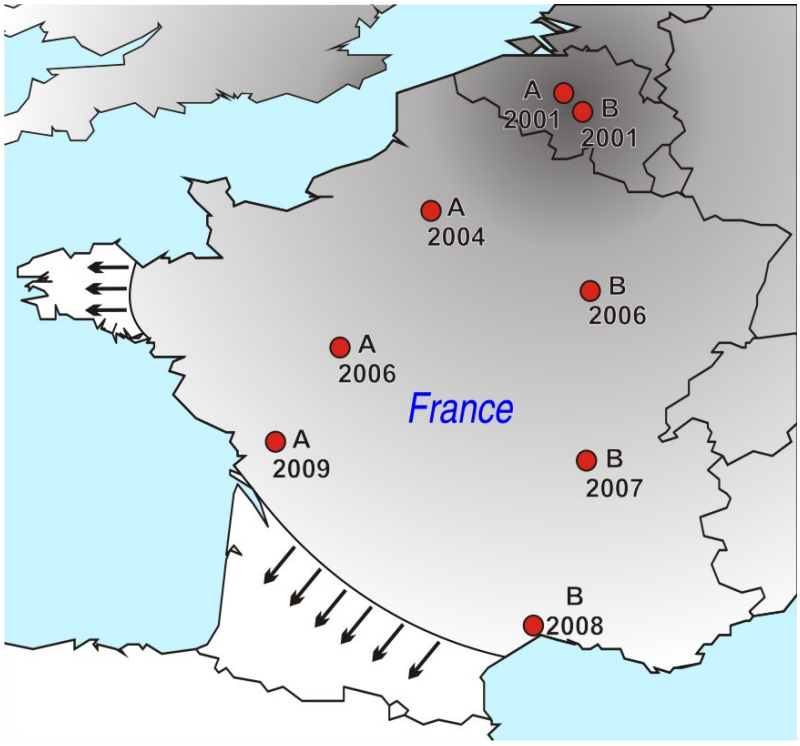


Figure 11 : Sites d'échantillonnage des populations utilisées pour tester l'évolution des capacités de dispersion chez *H. axyridis* au cours de l'expansion spatiale. Les zones grisées sont les zones envahies par l'espèce. Les flèches représentent les directions de l'expansion. Chaque point rouge correspond à un site d'échantillonnage d'une population (i.e. au moins 90 individus). Les lettres (A ou B) correspondent aux noms arbitrairement donnés aux transects. Les années sous les lettres correspondent aux dates de première observation de l'espèce sur le site géographique considéré.

Nous avons ensuite mesuré les capacités de vol à l'aide d'instruments de mesure permettant d'enregistrer pour un individu une distance parcourue en vol pendant une durée de temps donnée. Ces instruments, appelés « moulins de vol », nous ont permis d'enregistrer une heure de vol pour une centaine d'individus par population. Outre les capacités de vol, nous avons également pu mesurer l'endurance des individus en comparant les distances parcourues pendant la première et la seconde demi-heure. Enfin, nous avons évalué la motivation à voler d'une centaine d'individus par population en mesurant le temps nécessaire à un individu (à jeun depuis plusieurs heures) pour décoller.

b) Hybridation post-invasion : échantillonnage et traits mesurés

Une population échantillonnée dans le nord de la France a été utilisée comme population représentative de l'invasion Européenne (Article 8, Facon *et al.* 2011a). La souche non-volante de lutte biologique nous a été fournie par l'entreprise qui la commercialise. Après avoir génotypé à 18 marqueurs microsatellites (Annexe XI, Loiseau *et al.* 2009) les deux populations et mesuré leur niveau de différentiation génétique, nous avons voulu savoir si des barrières pré ou postzygotiques existaient entre ces deux populations. Pour cela, nous avons créé des trios constitués d'une femelle issue d'une population ou de l'autre et de deux mâles, un de chaque population. Nous avons ensuite compté à deux reprises le nombre d'œufs pondu en une journée, puis nous avons mesuré la survie larvaire sur cinq jours. Enfin, nous avons

utilisé 7 marqueurs microsatellites très polymorphes pour assigner chaque larve à un des pères à l'aide du logiciel PROBMAX (Danzmann 1997).

Nous avons ensuite comparé des traits d'histoire de vie entre les deux populations et leurs hybrides produits en laboratoire (femelle/mâle et mâle/femelle). En tout, 6 traits ont été mesurés (taux de survie larvaire, durée de développement, survie adulte sans nourriture, survie en condition de quiescence, longueur du corps et nombre d'ovarioles).

II.4.3. Principaux résultats et bilan

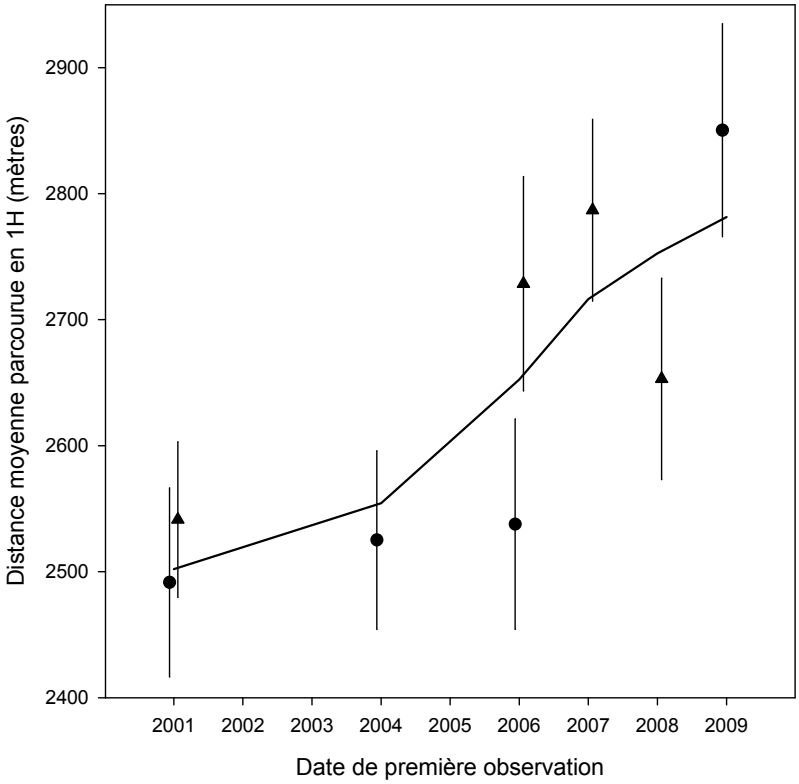
a) Homogamie spatiale

Toutes les analyses de génétique des populations confirment que les 8 populations échantillonnées le long de deux transects en Europe proviennent d'un seul et unique foyer (Article 7, Lombaert *et al.* In prep.). Par exemple, la plus grande valeur de F_{ST} entre deux sites échantillonnés n'excède pas 0.007. De même, aucun des 28 tests exacts de différentiation génotypique par paire de populations (Raymond & Rousset 1995) n'est significatif ($P > 0.05$). Ces résultats nous permettent donc de tester l'évolution de traits phénotypiques uniquement liée au phénomène d'expansion spatiale.

Nos analyses montrent que l'éloignement par rapport au foyer (ici représenté par les dates de première observation de l'espèce sur chacun des sites d'échantillonnage ; Figure 11) a un effet hautement significatif sur les distances parcourues en une heure en moulin de vol ($P < 10^{-3}$; Figure 12) (Article 7, Lombaert *et al.* In prep.). Les facteurs « sexe » et « transect » ne sont pas significatifs ($P = 0.304$ et $P = 0.205$ respectivement), et aucune interaction n'est significative. Nos mesures en moulins de vol montrent que les individus issus des populations les plus récentes (proches du front d'invasion) parcourent des distances plus grandes que les populations les plus anciennes (proches du foyer) (Figure 12). En revanche, aucun effet significatif de l'éloignement par rapport au foyer sur l'endurance ainsi que sur la motivation à voler n'a été détecté lors de nos expérimentations ($P > 0.05$).

Nos résultats montrent donc clairement une évolution des capacités de dispersion chez *H. axyridis* en Europe (Article 7, Lombaert *et al.* In prep.). L'absence d'effet du transect et d'interaction avec l'éloignement du foyer suggère que cette évolution n'est pas aléatoire (i.e. par dérive génétique) et concorde donc avec l'hypothèse d'une homogamie spatiale. Le processus évolutif observé a eu lieu en moins de 10 ans (soit une vingtaine de générations), ce qui est une durée particulièrement courte (Prentis *et al.* 2008; Whitney & Gabler 2008). Notre

étude montre l'intérêt d'utiliser des populations envahissantes pour étudier précisément des phénomènes évolutifs. C'est en particulier vrai lorsque nous disposons, comme c'est le cas pour *H. axyridis*, d'informations détaillées concernant (i) les routes d'invasion, (ii) la variabilité génétique neutre et (iii) l'historique spatio-temporel de l'expansion.



Figures 12 : Distance moyenne parcourue en une heure en moulins de vol en fonction de la date de première observation de l'espèce. L'année 2001 correspond au foyer initial d'invasion. Les triangles et les cercles correspondent à deux transects d'échantillonnage indépendants (Figure 11). Les barres verticales représentent les erreurs types des moyennes. La courbe correspond aux valeurs prédites par le modèle statistique retenu.

b) Hybridation post-invasion

Nos résultats montrent clairement qu'il n'existe aucune barrière reproductive entre la population envahissante Européenne et la souche non-volante de lutte biologique puisque tous les croisements donnent naissance à des larves viables (Article 8, Facon *et al.* 2011a). On constate en outre que les mâles de la souche de lutte biologique engendrent davantage de descendants que les mâles de la population envahissante, quelle que soit l'origine de la femelle.

Quel que soit le trait d'histoire de vie mesuré, les hybrides ne sont jamais significativement moins performants que les individus issus de la population envahissante. Dans ce contexte, la présence d'individus de lutte biologique sur la zone d'expansion de la population envahissante pourrait avoir un effet démographique positif en limitant les effets Allee sur les zones de front sans amoindrir les performances des descendants.

Nous avons pu mettre en évidence un phénomène d'hétérosis pour deux traits. Ainsi, les hybrides se développent plus rapidement et sont plus gros que les envahissants (Figure 13), ce qui peut avoir un avantage à la fois sur le taux d'accroissement de la population, mais également sur ses capacités compétitrices vis-à-vis des espèces locales. Enfin, une augmentation de la variance génétique est détectable pour un trait : les coefficients de variation mesurés pour la survie sans nourriture sont significativement plus importants chez les hybrides (Figure 13). On observe en particulier la présence d'individus caractérisés par un phénotype transgressif largement meilleur que ceux des parents. Une sélection sur ce trait peut donc être facilitée au cours d'une invasion dans un environnement par nature imprédictible.

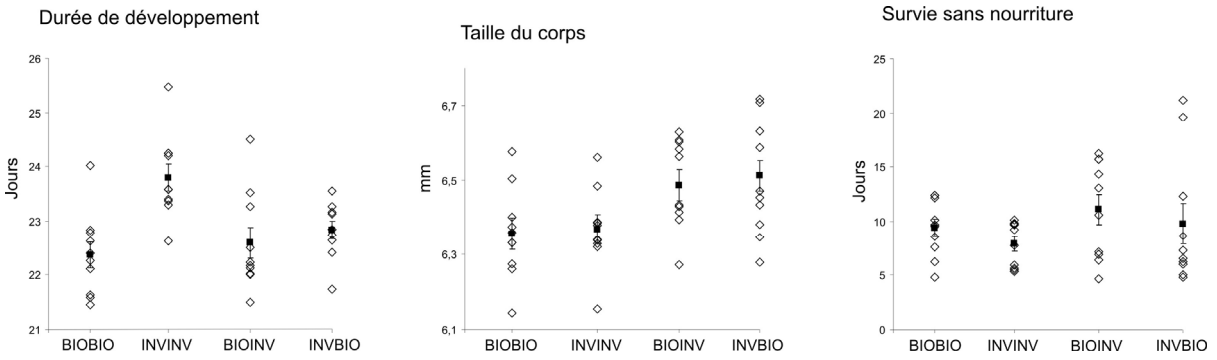


Figure 13 : Valeurs de trois traits d'histoire de vie pour chaque croisement. Les carrés noirs représentent les moyennes par croisement, et les barres verticales les erreurs types. Les diamants blancs représentent les moyennes par famille.

*c) Prolifération d'*H. axyridis *: que faut-il retenir ?*

Nous n'avons pas exploré les causes générales du succès de la phase de prolifération chez *H. axyridis*. Toutefois, il est fort probable que la purge génétique que nous avons mise en évidence précédemment ait facilité l'expansion spatiale de l'espèce dans les différentes zones géographiques où elle est envahissante (Article 5, Facon *et al.* 2011b). De plus en Europe, la commercialisation et l'utilisation d'une souche non-volante de lutte biologique peuvent également avoir eu un rôle positif dans la prolifération rapide de l'espèce sur ce continent (Article 8, Facon *et al.* 2011a).

Le succès de l'invasion d'*H. axyridis* est évident, et l'espèce s'étend très rapidement sur tous les continents où elle est présente (Article 3, Brown *et al.* 2011a). Nous avons montré que cette expansion était, au moins en Europe, à l'origine d'une homogamie spatiale entraînant des différences phénotypiques significatives entre le foyer et le front d'invasion (Article 7, Lombaert *et al.* In prep.). L'augmentation des capacités de dispersion sur le front

mise en évidence est susceptible d'avoir des implications importantes sur la dynamique même de l'invasion en accroissant la vitesse de colonisation (Phillips *et al.* 2007; Phillips *et al.* 2011; Travis & Dytham 2002; Travis *et al.* 2009) et/ou en intensifiant la fréquence de certains phénotypes défavorables liés aux capacités de dispersion élevées (Brown *et al.* 2007; Lee 2011). Enfin, notre étude montre qu'une évolution quantifiable de traits écologiquement importants peut avoir lieu en une période de temps très courte (e.g. Hairston *et al.* 2005; Hendry & Kinnison 1999; Thompson 1998) et confirme l'intérêt d'utiliser les espèces envahissantes pour étudier les processus microévolutifs contemporains (Huey *et al.* 2000; Prentis *et al.* 2008; Whitney & Gabler 2008).

CHAPITRE III
Discussion et perspectives

- Article 9 (Annexe IX) :

  Guillemaud T, Ciosi M, **Lombaert E**, Estoup A (2011) Biological invasions in agricultural settings: Insights from evolutionary biology and population genetics. *Comptes Rendus Biologies* **334**, 237-246.

- Article 10 (Annexe X) :

  Lawson Handley L-J, Estoup A, Evans DM, Thomas CE, **Lombaert E**, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. *Biocontrol* **56**, 409-428.

III.1. Invasion mondiale de la coccinelle asiatique *H. axyridis* : bilan et perspectives

III.1.1. Introduction, établissement et prolifération *d'H. axyridis* : principaux résultats

L'ensemble de ce travail centré sur la coccinelle asiatique *H. axyridis* nous a permis d'explorer chacune des étapes clés d'une invasion biologique. La Figure 14 illustre l'état des connaissances et les principales hypothèses quant aux raisons du succès de l'invasion d'*H. axyridis* dans le monde.

Au cours de cette thèse, nous avons dans un premier temps retracé les routes d'invasion de l'espèce dans le monde (Articles 1, 2 et 3, Lombaert *et al.* 2010; Lombaert *et al.* 2011; Brown *et al.* 2011a). Ce travail s'est révélé indispensable pour poser les bases des expérimentations suivantes. En particulier, nous avons mis en évidence l'existence d'une population envahissante dans l'est de l'Amérique du Nord qui est à l'origine de presque tous les autres foyers envahissants (Europe, Amérique du Sud et Afrique du Sud). Nous avons baptisé ce phénomène le scénario d'invasion « tête de pont ». Ce point précis sera discuté en

détail dans la deuxième partie de ce chapitre. De plus, ce travail a permis de montrer que l'invasion mondiale d'*H. axyridis* est caractérisée par des événements d'hybridation intraspécifique (i) en Amérique du Nord-Est entre deux groupes populationnels natifs et (ii) en Europe entre la population tête de pont et une souche de lutte biologique commercialisée en Europe pendant des années.

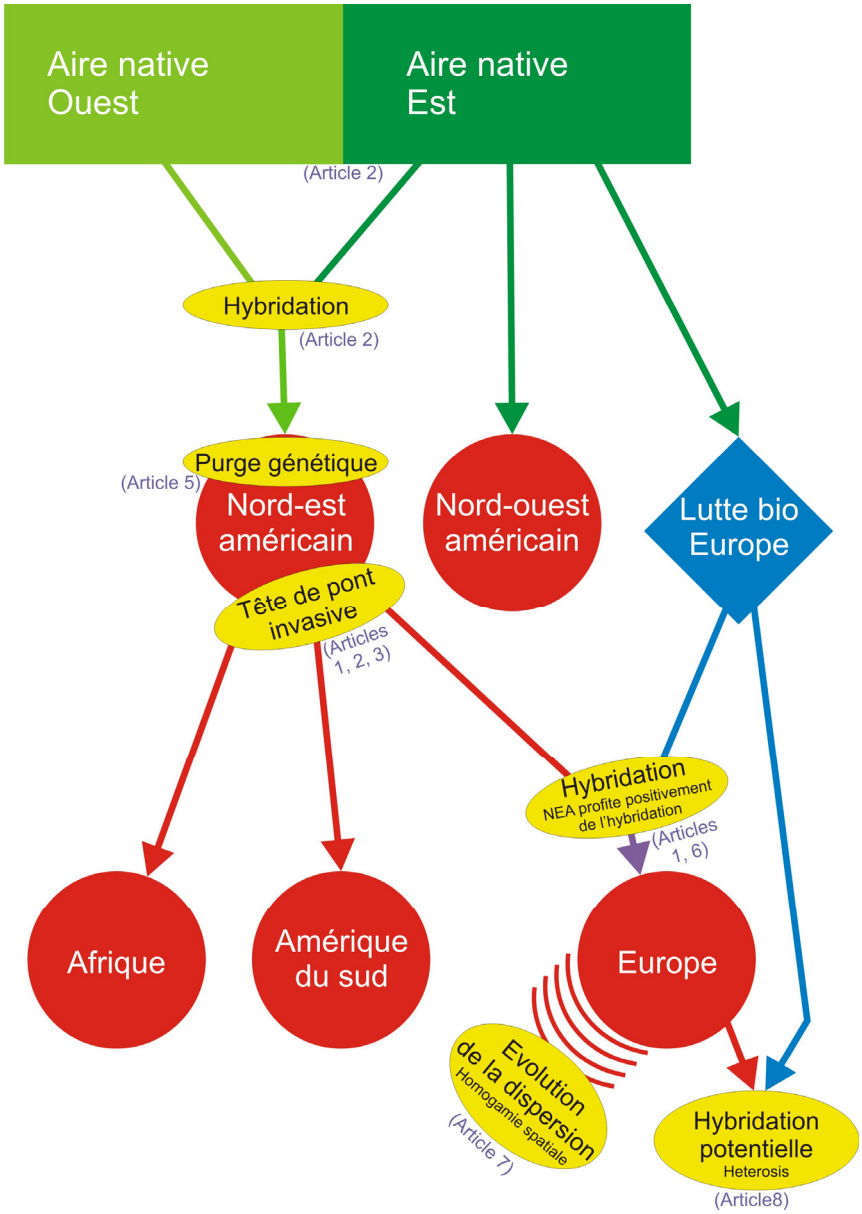


Figure 14 : Bilan graphique des principaux résultats obtenus au cours de cette thèse sur l'invasion mondiale d'*H. axyridis*. Les routes d'invasion globales sont issues des articles 1, 2 et 3. Une flèche correspond à une introduction. Chacune des deux couleurs de l'aire native correspond à un groupe populationnel. Les cercles rouges correspondent aux populations envahissantes. Le diamant bleu correspond à la souche de lutte biologique Européenne (lbEu).

Nous avons donc testé dans un second temps le rôle de l'hybridation, ainsi que d'autres facteurs potentiellement importants, i.e. la purge génétique et la plasticité phénotypique, sur le succès de l'établissement de l'espèce dans les zones envahies (Articles 4, 5 et 6, Lombaert *et al.* 2008; Facon *et al.* 2011b; Turgeon *et al.* 2011). Nous avons mis en évidence une nette diminution de la dépression de consanguinité dans les populations

envahissantes d'*H. axyridis* en comparaison avec les populations natives, ainsi qu'un rôle potentiellement favorable de l'hybridation pour l'invasion en Europe.

Dans un troisième temps, nous avons montré que des processus évolutifs étaient à l'œuvre chez *H. axyridis* au cours de son expansion spatiale en zone d'introduction, au moins en Europe (Articles 7 et 8, Lombaert *et al.* In prep.; Facon *et al.* 2011a). Nous avons ainsi mis en évidence une très probable homogamie spatiale sur les fronts successifs d'invasion conduisant à une augmentation significative des capacités de dispersion des populations les plus éloignées du foyer original. Par ailleurs, nous avons montré que la présence sur le terrain d'une souche non-volante de lutte biologique toujours commercialisée pouvait, via l'hybridation, entraîner de l'hétérosis et de l'augmentation de variance génétique potentiellement favorables pour le succès de l'étape de prolifération.

L'ensemble de ces résultats nous permet de mieux comprendre la dynamique de l'invasion de cette espèce et d'entrevoir les raisons de son succès mondial. Toutefois, de nombreuses questions restent en suspens, auxquelles il serait intéressant de tenter de répondre dans le futur via différentes actions de recherche afin de compléter au mieux le « puzzle de l'invasion d'*Harmonia axyridis* ».

III.1.2. Introduction, établissement et prolifération *d'H. axyridis* : perspectives

En ce qui concerne les perspectives de recherches sur l'étape d'introduction, il est important de noter que seules les grandes lignes des routes d'invasion d'*H. axyridis* ont été retracées pour le moment (Articles 1, 2 et 3, Lombaert *et al.* 2010; Lombaert *et al.* 2011; Brown *et al.* 2011a). En effet, nous avons choisi un nombre limité d'échantillons dans les zones envahies en nous basant (i) sur l'historique connu (date et lieu de première observation de l'espèce) et (ii) sur des résultats de structuration génétique sur un nombre d'échantillons plus large (résultats non publiés qui confirment que chaque population étudiée a proliféré en s'étendant spatialement sur de grandes superficies). Toutefois, pour ce deuxième point, une étude plus précise de la structuration génétique des populations dans chacune des aires envahies permettra de mettre en évidence d'éventuelles introductions indépendantes supplémentaires. Cette hypothèse est tout à fait réaliste chez *H. axyridis* dont le transport accidentel est documenté et probablement fréquent (Articles 1 et 3, Lombaert *et al.* 2010; Brown *et al.* 2011a). Des résultats préliminaires semblent d'ailleurs confirmer l'existence de

plusieurs foyers envahissants, au moins en Europe et en Amérique du Sud. Il sera important de tenter d'inférer l'origine de ces foyers, tout comme l'origine de nouveaux foyers récemment mis en évidence (par exemple au Kenya, Nedvěd *et al.* 2011). Ces compléments d'analyse permettront notamment de mieux mesurer l'ampleur de l'effet tête de pont de la population Nord-Est Américaine. Pour y parvenir au mieux, il sera nécessaire de poursuivre l'évaluation et l'amélioration des méthodes indirectes d'inférence des routes d'invasion tel que l'Approximate Bayesian Computation (Article 2, Lombaert *et al.* 2011), afin notamment de pouvoir traiter des scénarios incluant un nombre encore plus élevé de populations sources potentielles.

Outre une description plus approfondie de aires envahies, il serait également intéressant de préciser l'origine native des deux populations envahissantes américaines. En effet, la faible structure génétique mesurée dans l'aire native (Article 2, Lombaert *et al.* 2011) ne nous permet pas de localiser avec précision les zones géographiques à l'origine de ces deux introductions indépendantes. La population du Nord-Ouest Américain serait issue de la zone Est de l'aire native, mais celle-ci est de très grande taille puisqu'elle inclut le Japon, la Corée, l'Est de la Sibérie et la majeure partie de la Chine. Une localisation plus fine nous permettrait de mieux identifier les traits phénotypiques ayant évolué au cours de l'invasion lors des comparaisons entre populations natives et populations envahissantes (Estoup & Guillemaud 2010; Keller & Taylor 2008). Il en est de même pour la population tête de pont du Nord-Est Américain car celle-ci est probablement issue d'une hybridation entre les deux groupes populationnels natifs. Il serait intéressant de déterminé si l'hybridation a eu lieu avant (i.e. introduction d'une population native située sur la zone hybride naturelle) ou après (i.e. introduction de deux populations s'hybridant en Amérique du Nord). Pour préciser l'ensemble de ces questions, il sera nécessaire (i) de disposer d'échantillons natifs supplémentaires (notamment de la probable zone de contacte naturelle), et (ii) d'augmenter le nombre de marqueurs génétiques (e.g. Thomas *et al.* 2010).

En ce qui concerne l'étape d'établissement d'*H. axyridis*, nous avons vu que la purge génétique mise en évidence chez les populations envahissantes a probablement facilité cette étape (Article 5, Facon *et al.* 2011b). Toutefois, il n'est pas encore possible d'affirmer qu'il s'agit là du principal facteur clé du succès de l'invasion, après plusieurs décennies d'échec des tentatives d'acclimatation malgré, parfois, des nombres importants d'individus lâchés. Pour répondre à cette question, il serait tout d'abord nécessaire d'identifier plus précisément les populations sources. D'ailleurs, l'hybridation native qui a conduit à la population tête de

pont du Nord-Est américain (Article 2, Lombaert *et al.* 2011) pourrait également représenter un facteur important dans le succès de l'invasion (Ellstrand & Schierenbeck 2000; Lee 2002; Sakai *et al.* 2001; Wares *et al.* 2005). Ce dernier point n'a pas encore été abordé via des expériences de génétique quantitative sur traits d'histoire de vie en milieu contrôlé, ceci malgré la mise en évidence d'effets phénotypiques forts chez *H. axyridis* lors d'autres événements d'hybridation (Articles 6 et 8, Turgeon *et al.* 2011; Facon *et al.* 2011a). De manière générale d'autres traits que ceux étudiés jusqu'à maintenant (e.g. capacités d'apprentissage, détection de partenaires sexuels, résistance aux parasites, adaptation aux milieux anthropiques, etc.) mériteraient d'être étudiés dans des analyses comparatives entre populations natives et envahissantes.

Un autre point important à souligner est que la population du Nord-Ouest américain n'a pour l'instant pas été étudiée du point de vue des ses traits d'histoire de vie. Il s'agit pourtant d'une introduction indépendante de celle de l'est (Article 1, Lombaert *et al.* 2010), et son étude pourrait apporter d'importantes informations concernant les facteurs-clés du succès de l'établissement d'*H. axyridis*. Il s'agit également d'une population ayant réussi l'invasion. Ainsi, a-t-elle également subi une purge génétique ? Si la réponse est oui, cela préciserait le rôle instrumental important de la purge chez cette espèce. En revanche, cette population du Nord-Ouest américain semble moins performante que la population du Nord-Est américain. En effet, elle s'est beaucoup moins étendue (Koch *et al.* 2006) et elle ne semble pas pour l'instant avoir servi de tête de pont d'invasion. Comprendre pourquoi est primordial. Enfin, notons qu'une zone de contact entre les deux populations Nord américaine (Est et Ouest) a été détectée à l'aide des marqueurs microsatellites autour de l'Utah et du Colorado (résultats non publiés). Il serait intéressant d'étudier les conséquences phénotypiques d'un tel événement d'hybridation post-introduction et post-expansion.

Mieux comprendre l'étape d'établissement discutée précédemment permettra très probablement de mieux comprendre les raisons du succès de l'étape de prolifération chez *H. axyridis*. Par ailleurs, la mise en évidence d'homogamie spatiale en Europe (Article 7, Lombaert *et al.* In prep.) suggère que des processus évolutifs peuvent avoir lieu extrêmement rapidement lors de la phase d'expansion spatiale d'une espèce envahissante. D'autres traits ont-ils également évolué sous l'effet du déséquilibre spatial ? On s'attend par exemple à une augmentation des taux d'accroissement proche du front (e.g. Phillips 2009). Dans le cas d'*H. axyridis*, on pourrait également avoir une sélection différentielle entre le front et le foyer des comportements de cannibalisme qui sont généralement très densité-dépendants (Fox 1975).

L'évolution des capacités de dispersion par homogamie spatiale n'est pas liée à de la sélection naturelle *stricto sensu* (Shine *et al.* 2011a), mais les traits liés à la dispersion sont généralement fortement adaptatifs et soumis à la sélection. La question des conséquences à long terme des patrons évolutifs observés sur la valeur sélective de la population Européenne étudiée se pose donc.

L'invasion d'*H. axyridis* étant caractérisée par une série d'introductions sur des continents spatialement déconnectés, il serait intéressant de profiter de ces « réplicats naturels d'invasion » afin de tester la répétabilité des processus évolutifs observés au cours de l'étape de prolifération (Huey *et al.* 2005). Enfin, l'obtention de patrons spatiaux à des marqueurs moléculaires de gènes dont le polymorphisme est lié aux capacités de dispersion serait particulièrement intéressante dans ce but. Il existe des candidats tel que le gène *for* (Osborne *et al.* 1997) ou la protéine *pgi* dont, par exemple, certaines formes alléliques confèrent chez le papillon *Melitaea cinxia* de meilleures capacités de vol (Haag *et al.* 2005; Hanski 2011).

III.2. Scénario « tête de pont » d'invasion.

III.2.1. Généralisation

La reconstruction des routes d'invasion est une étape indispensable pour pouvoir proposer des hypothèses éco-évolutives permettant de mieux comprendre les facteurs-clés du succès d'invasion (Estoup & Guillemaud 2010). Nous avons mis en évidence chez *H. axyridis* un scénario d'invasion que nous avons baptisé « tête de pont » (Article 1, Lombaert *et al.* 2010) : dans ce scénario, une première population envahissante (e.g. la population du Nord-Est américain chez *H. axyridis*) devient la source de plusieurs invasions secondaires sur des sites géographiques distants (e.g. les populations Sud Américaine, Africaine et Européenne chez *H. axyridis*). Mais quelle est la généralité d'un tel scénario ? En explorant la littérature liée aux routes d'invasion, on constate que ce scénario, bien que non formalisé par les auteurs et non discuté jusqu'à récemment, est compatible avec de nombreuses autres invasions (e.g. Ascunce *et al.* 2011; Downie 2002; Grapputo *et al.* 2005; Hanfling *et al.* 2011; Heinicke *et al.* 2011; Hirsch *et al.* 2011; Kim *et al.* 2009; Kolbe *et al.* 2004; Miller *et al.* 2005). Par exemple, Ascunce *et al.* (2011) ont montré que la fourmi de feu d'origine Sud Américaine *Solenopsis*

invicta avait tout d'abord envahi le sud des Etats-Unis, et que cette première population envahissante était devenue indépendamment la source d'au moins huit autres foyers envahissants dans l'Ouest des Etats-Unis, en Chine et en Australie. Les auteurs font explicitement référence au scénario « tête de pont » dans la discussion de leur résultats.

III.2.2. Un scénario parcimonieux

Nous proposons que le scénario « tête de pont » est fréquent parce qu'il est parcimonieux (Article 9, Guillemaud *et al.* 2011). Imaginons une espèce devenue envahissante dans plusieurs zones géographiques déconnectées et pour laquelle le succès de l'invasion passe par un changement évolutif génétiquement déterminé (i.e. il n'y a pas de pré-adaptation). Sans population tête de pont, le changement évolutif devra avoir lieu autant de fois qu'il y aura de populations envahissantes. Au contraire, dans un scénario « tête de pont », un seul changement évolutif sera nécessaire au sein de la population tête de pont (Figure 15 A et B). Par exemple, on suppose que l'hybridation intraspécifique chez le lézard Cubain *Anolis sagrei* en Floride a été un facteur évolutif important du succès de l'invasion en Floride, or les populations envahissantes de Floride sont ensuite devenues la source d'autres foyers à Hawaï et à Taiwan (Kolbe *et al.* 2004). De même, la purge génétique chez *H. axyridis* n'a peut-être eu lieu qu'une seule fois dans la population du Nord-Est américain permettant à celle-ci de devenir la tête de pont de l'invasion mondiale (Articles 1 et 5, Lombaert *et al.* 2010; Facon *et al.* 2011b).

Toutefois, il est important de souligner que le scénario tête de pont d'invasion peut également fortement dépendre du régime de migration. En effet, une population introduite une première fois dans une zone géographique où se trouve une importante plaque tournante de transport peut devenir une tête de pont sans qu'un changement évolutif n'ait été nécessaire si celle-ci est pré-adaptée (Figure 15 C et D). Floerl *et al.* (2009) ont montré à l'aide d'un modèle que les espèces marines introduites dans les ports avaient une probabilité d'autant plus grande de devenir des têtes de pont que le trafic dans le port est important. En ce qui concerne *H. axyridis*, le Nord-Est américain où se trouve la population tête de pont est un nœud de transport mondial important. Toutefois, notons que l'aire native asiatique de l'espèce est de grande taille et comporte également des zones très actives en terme de transport (en particulier à l'Est, e.g. Tatem & Hay 2007).

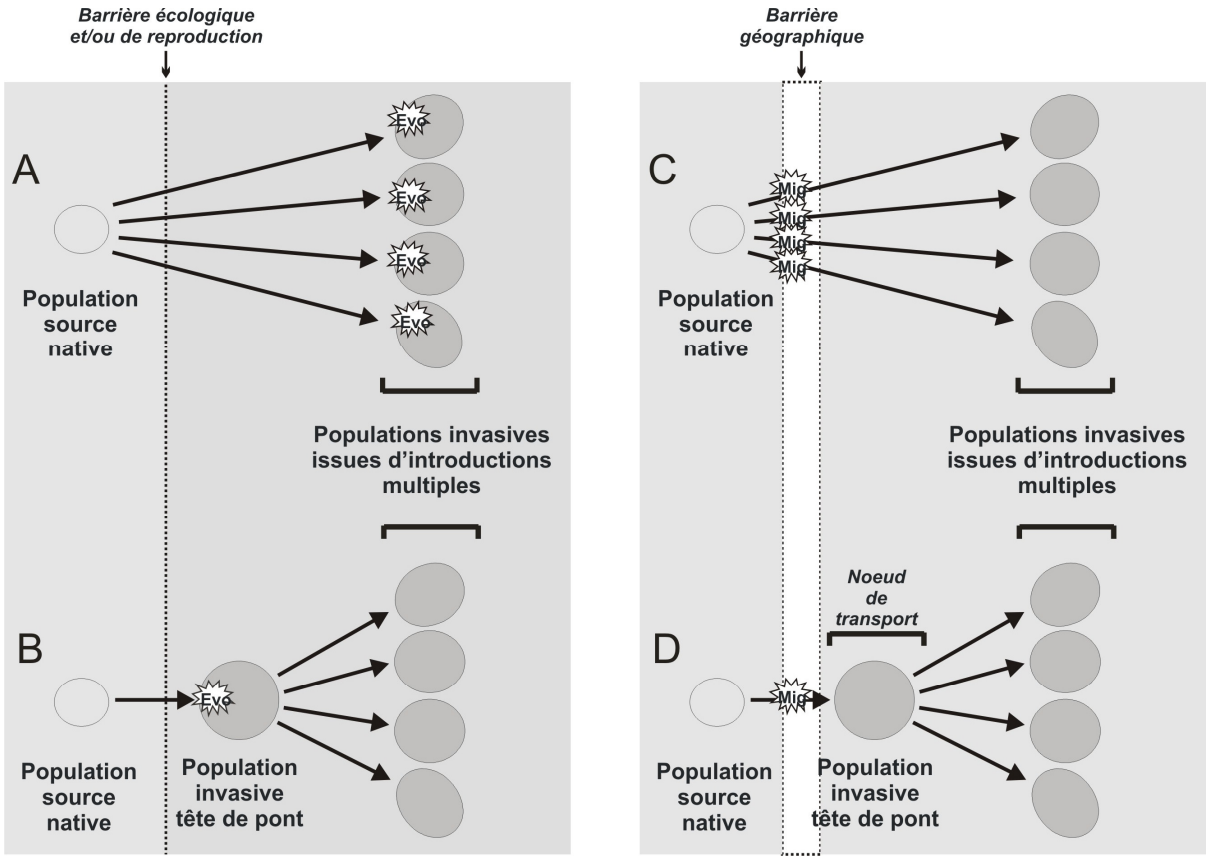


Figure 15 : Illustration schématique du scénario « tête de pont ». Dans les cas A et B, il y a une barrière écologique et/ou de reproduction nécessitant l'évolution de caractères adaptatifs (représentée par l'étoile « Evo ») pour la réussite de l'invasion. Dans les cas C et D, il n'y a pas de challenge adaptatif (il y a pré-adaptation), mais l'invasion n'est possible que s'il y a franchissement d'une principale barrière géographique (représentée par l'étoile « Mig »). A et C : scénarios classiques d'introductions multiples nécessitant des événements évolutifs (scénario A) ou de migration (scénario C) multiples. B et D : scénarios « tête de pont » dans lesquels un seul événement adaptatif (scénario B) ou un seul franchissement de la principale barrière géographique (scénario D) est nécessaire. Notons que la présence conjointe d'une barrière écologique et d'une barrière géographique est possible, voire fréquente.

III.2.3. Implications pratiques

Les deux aspects précédemment cités sont vraisemblablement importants dans la formation de nombreuses têtes de pont d'invasion. Un changement évolutif peut avoir lieu une fois, suivi d'un changement de mode de migration. Ce phénomène « à double lame » est probablement très fréquent dans le cas particulier, mais répandu, des espèces envahissantes des cultures agricoles (Article 9, Guillemaud *et al.* 2011). En effet, beaucoup d'insectes phytophages sont devenus ravageurs de cultures suite à un changement évolutif majeur qui a consisté, par exemple, à changer de plante hôte et à se spécialiser sur une plante cultivée. Ce changement est accompagné plus ou moins directement d'une modification du régime de migration lié au caractère hautement anthropisé des milieux agricoles : les plantes cultivées sont souvent répandues et transportées pour le commerce. La chrysomèle des racines du maïs

Diabrotica virgifera virgifera est originaire de l'Amérique centrale et a commencé à envahir l'Amérique du Nord à la fin du 19ième siècle (Gray *et al.* 2009). Un changement évolutif permettant une forte spécialisation sur le maïs cultivé a probablement permis cette première invasion. L'espèce a proliféré et s'est spatialement étendue, surtout à partir des années 1950 suite à différents changements de techniques culturales (fin des rotations, irrigation, etc.). Dans les années 1990, l'espèce atteint le Nord-Est des Etats-Unis qui constitue un important nœud de transport international (e.g. Tatem & Hay 2007). C'est justement à partir de 1992 que de nombreuses introductions indépendantes à partir de cette tête de pont américaine furent découvertes en Europe (Ciosi *et al.* 2008; Miller *et al.* 2005). Nous avons pu trouver de nombreux exemples très similaires dans la littérature (e.g. Downie 2002; Gladieux *et al.* 2008; Goodwin *et al.* 1994; Grapputo *et al.* 2005; Kim *et al.* 2009; Puillandre *et al.* 2008) qui suggèrent que ce scénario est fréquent.

Par ailleurs, la mise en évidence d'un tel scénario n'est possible que dans les cas d'invasions biologiques bien documentées. Il est donc probable que nous sous-estimions son importance réelle. Ainsi, le scénario tête de pont d'invasion est peut-être très répandu et son identification peut permettre de mieux comprendre les invasions biologiques en focalisant prioritairement les recherches sur de possibles changements évolutifs et/ou de régime de migration dans la population tête de pont. D'un point de vue appliqué, les mesures de gestion et de quarantaine devraient par exemple surtout se focaliser sur les zones où ces populations « faites pour envahir » se trouvent plutôt que sur les aires natives (Articles 9 et 10, Guillemaud *et al.* 2011; Lawson Handley *et al.* 2011).

III.3. Conclusion générale

Les travaux présentés dans cette thèse sur *H. axyridis* explorent chacune des étapes de l'invasion : l'introduction, l'établissement et la prolifération. Les résultats obtenus apportent des informations importantes d'un point de vue fondamental (concept de « tête de pont », mise en évidence de purge génétique, effet de l'hybridation intraspécifique, illustration d'une évolution très rapide liée à l'homogamie spatiale, etc.) et d'un point de vue appliqué (identification de la population « tête de pont », conséquence de la commercialisation d'une population non-volante de lutte biologique, mise en évidence d'homogamie spatiale dont les

conséquences seront une probable augmentation de la vitesse d'expansion, etc.). Ils illustrent également de manière plus générale (i) le rôle indispensable de la description des routes d'invasion, souvent complexes, pour la compréhension du processus d'invasion dans son ensemble, et (ii) l'intérêt de combiner différentes approches telles que la génétique des population et la mesure de traits d'histoire de vie en laboratoire.

Les espèces envahissantes constituent des modèles particulièrement intéressants dans des domaines aussi variés que la génétique évolutive, l'évolution des traits d'histoire de vie et l'écologie (Article 10, Lawson Handley *et al.* 2011). Il n'est donc pas étonnant que l'intégration de plusieurs disciplines s'avère particulièrement fructueuse pour l'étude des invasions biologiques et permet, en retour, d'aborder des questions très larges. Si cette thèse illustre comment il est possible d'explorer d'importantes questions et hypothèses sur l'évolution contemporaine des populations et sur la génétique de traits écologiquement importants, soulignons également que les invasions biologiques constituent de formidables laboratoires *in natura* pour étudier les interactions interspécifiques et entre les organismes et leur environnement (Article 10, Lawson Handley *et al.* 2011). Les méthodes modernes de biologie moléculaire offrent par ailleurs d'encourageantes perspectives pour l'étude de ces interactions complexes (e.g. Aebi *et al.* 2011; Lopezaraiza-Mikel *et al.* 2007; Meyer *et al.* 2008; Tylianakis *et al.* 2007).

Nous sommes encore loin d'une théorie générale de la biologie de l'invasion qui nous permettrait de prédire l'échec et/ou le succès de l'invasion d'une espèce donnée, malgré une dense littérature sur le sujet (e.g. Enserink 1999; Jeschke & Strayer 2005; Kolar & Lodge 2001; Romanuk *et al.* 2009; Vall-Ilosera & Sol 2009; Williamson & Fitter 1996a; Wilson *et al.* 2009). Il est probable qu'une telle théorie n'existera jamais et que seule la formalisation de cadres de réflexion soit possible et réaliste (e.g. Facon *et al.* 2006). Néanmoins, la multiplication des études complètes incluant chacune des trois étapes clés d'invasions biologiques en cours semble indispensable pour tendre vers une meilleure compréhension des invasions biologiques. L'étude approfondie d'une espèce envahissante telle qu'*H. axyridis* (qui peut ainsi devenir une espèce modèle en biologie de l'invasion) présente de nombreux avantages si on parvient à extraire des règles potentiellement générales que l'on peut tester ensuite sur d'autres espèces. Le cas du scénario « tête de pont » est un bon exemple : formalisé chez *H. axyridis*, ce scénario s'est révélé particulièrement répandu parmi les espèces envahissantes dont les routes d'invasion étaient connues. Qu'en est-il, par exemple, de la purge génétique ou de l'évolution des capacités de dispersion ? Affaire à suivre…

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ANNEXES

ANNEXE I

Article 1 (2010)

Lombaert E, Guillemaud T, Cornuet JM, Malausa T, Facon B, Estoup A (2010) Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. *Plos One* **5**, e9743.

OPEN ACCESS Freely available online

PLoS one

Bridgehead Effect in the Worldwide Invasion of the Biocontrol Harlequin Ladybird

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Abstract

Recent studies of the routes of worldwide introductions of alien organisms suggest that many widespread invasions could have stemmed not from the native range, but from a particularly successful invasive population, which serves as the source of colonists for remote new territories. We call here this phenomenon the invasive bridgehead effect. Evaluating the likelihood of such a scenario is heuristically challenging. We solved this problem by using approximate Bayesian computation methods to quantitatively compare complex invasion scenarios based on the analysis of population genetics (microsatellite variation) and historical (first observation dates) data. We applied this approach to the Harlequin ladybird *Harmonia axyridis* (HA), a coccinellid native to Asia that was repeatedly introduced as a biocontrol agent without becoming established for decades. We show that the recent burst of worldwide invasions of HA followed a bridgehead scenario, in which an invasive population in eastern North America acted as the source of the colonists that invaded the European, South American and African continents, with some admixture with a biocontrol strain in Europe. This demonstration of a mechanism of invasion via a bridgehead has important implications both for invasion theory (i.e., a single evolutionary shift in the bridgehead population versus multiple changes in case of introduced populations becoming invasive independently) and for ongoing efforts to manage invasions by alien organisms (i.e., heightened vigilance against invasive bridgeheads).

Citation: Lombaert E, Guillemaud T, Cornuet J-M, Malausa T, Facon B, et al. (2010) Bridgehead Effect in the Worldwide Invasion of the Biocontrol Harlequin Ladybird. PLoS ONE 5(3): e9743. doi:10.1371/journal.pone.0009743

Editor: Jerome Chave, Centre National de la Recherche Scientifique, France

Received February 12, 2010; **Accepted** February 23, 2010; **Published** March 17, 2010

Funding: This work was supported by grants from the Agence Nationale de la Recherche (ANR-06-BDIV-008-01 and ANR-09-BLAN-0145-01). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Elucidating the routes and modalities of introductions of undesirable organisms is crucial for managers who wish to prevent new invasions. [1,2]. It is also a prerequisite to generating and testing useful hypotheses regarding the environmental and evolutionary factors responsible for biological invasions [3]. In the large body of literature on biological invasions, a number of studies suggest that successful invasions involve a particular invasive population, which serves as the source of colonists for remote new territories [4,5,6,7,8]. We call here this phenomenon the *invasive bridgehead effect*, likening the successful population of a biological invader to a military force that establishes a foothold, historically at the far side of a bridge, prior to further incursions into hostile territories. Convincing demonstrations of such an invasive bridgehead effect are however scarce because of the lack of appropriate methods to confidently reconstruct the routes of invasion and hence formally test this scenario against alternative ones. Moreover, too few invasive populations have been studied to capture the global picture of the worldwide invasion process for most species.

The harlequin ladybird *Harmonia axyridis* (HA), or multicolored Asian lady beetle, is an appropriate biological model to test for the existence of an invasive bridgehead effect at a worldwide scale [9]. Native to Asia, HA has been introduced repeatedly as a biocontrol agent against aphids since 1916 in North America [10,11], since 1982 in Europe [12] and since 1986 in South America [13]. Despite recurrent intentional releases of ladybirds originating from various source populations in its native range for acclimation attempts, the species did not establish for decades. However, for unknown reasons it recently and suddenly became invasive on four different continents. Invasive populations were first recorded in eastern (Louisiana, USA) and western (Oregon, USA) North America in 1988 and 1991, respectively [14,15]. They were then recorded in Europe (Belgium) [16] and South America (Argentina) [17] in 2001 and in Africa (South Africa) [18] in 2004. The species has spread widely in these areas where it has become a harmful predator of non-target arthropods, a household invader, and a pest of fruit production [19].

Most of our knowledge about introduction pathways of invasive species relies on historical and observational data, which are often sparse, incomplete and sometimes misleading. Population genetics has proven to be a useful approach to reconstruct routes of introduction, highlighting how complex and counter-intuitive the real story can be [4,5]. This is especially true since the recent development of a new approach termed approximate Bayesian computation (ABC) [20,21,22,23,24]. Using molecular and historical data, this method allows performing model-based inference in a Bayesian setting for complex demographic or evolutionary scenarios such as those related to the introduction

histories of invasive species, where bottleneck, multiple introductions and/or genetic admixture events are often suspected. Here, we retraced the routes of all five worldwide invasive HA populations using a rigorous quantitative analysis of microsatellite genetic variation relying on ABC methods. We compared large sets of HA invasion scenarios covering all invaded areas, taking into account historical data (e.g. dates of first observation of the outbreaks and dates of initial collection of biocontrol strains) and all potential sources (native, older outbreaks and biocontrol), accounting for the possibility of genetic admixture among them.

Results and Discussion

Our results unambiguously point to a surprising scenario (Fig. 1). The outbreaks in eastern and western North America originated from two independent introductions from the native range (either from biocontrol or accidental introductions). The South American and African outbreaks both originated independently from eastern North America. The European outbreak also originated from eastern North America, but with substantial genetic admixture with individuals of the European biocontrol strain (estimated at 43%, 95%CI: [18%–83%]; Fig. 2a). The establishment of new invasive populations is characterized by low (South America) to minute (all other outbreaks) bottleneck severities suggesting a substantial number of founding individuals and/or a quick demographic recovery (Fig. 2b). Each choice of scenario is supported by very high posterior probabilities using two different sets of prior distributions of demographic, historic and mutation parameters (Fig. 1, Table S2 and Table S3). In all analyses the 95% CI of the most likely scenario never overlapped with those of competing scenarios. We also computed the type I and type II errors from control simulated data sets for each of the five nested analyses and found that our method selected the true scenario with high confidence and markedly low type II errors (Table 1).

For the scenarios not complicated by substantial admixture, the raw classical statistics measuring genetic variation between populations such as pairwise F_{ST} and assignment likelihood [23] agree with our conclusions based on ABC methods (Table S4). On the other hand, such classical statistics are unable to detect North America and the European biocontrol strain as the sources of the admixed European invasive population. Rather, these simpler methods suggest that the latter outbreak originated from the native area only. We show with simulated data that, in case of admixture between two populations deriving from the same source, pairwise F_{ST} and assignment likelihood values tend to select the ancestral population (here the native area) as the source of the admixed population (Fig. S2). An admixed origin of the European invasive population involving eastern North America and the European biocontrol strain as inferred from ABC is also supported by the raw allelic distributions observed in these samples (see Fig. S3 for an illustration). The ABC methods used here have hence three advantages over more standard methods based on raw measures of genetic distance: they use all the data simultaneously in inference, allow an assessment of uncertainty in all inferences and therefore provide confidence in the choice of invasion routes [23], and they avoid misleading biases such as those due to genetic admixture (if included in the set of compared scenarios), an increasingly acknowledged common feature of species invasions [3,5].

The complete panorama of the invasion history of HA strikingly points to the worldwide dissemination of a single successful invasive population (eastern North America) into remote new territories. Although ladybirds were repeatedly introduced in North America, Europe and South America for biocontrol from native Asian populations and laboratory strains, eastern North

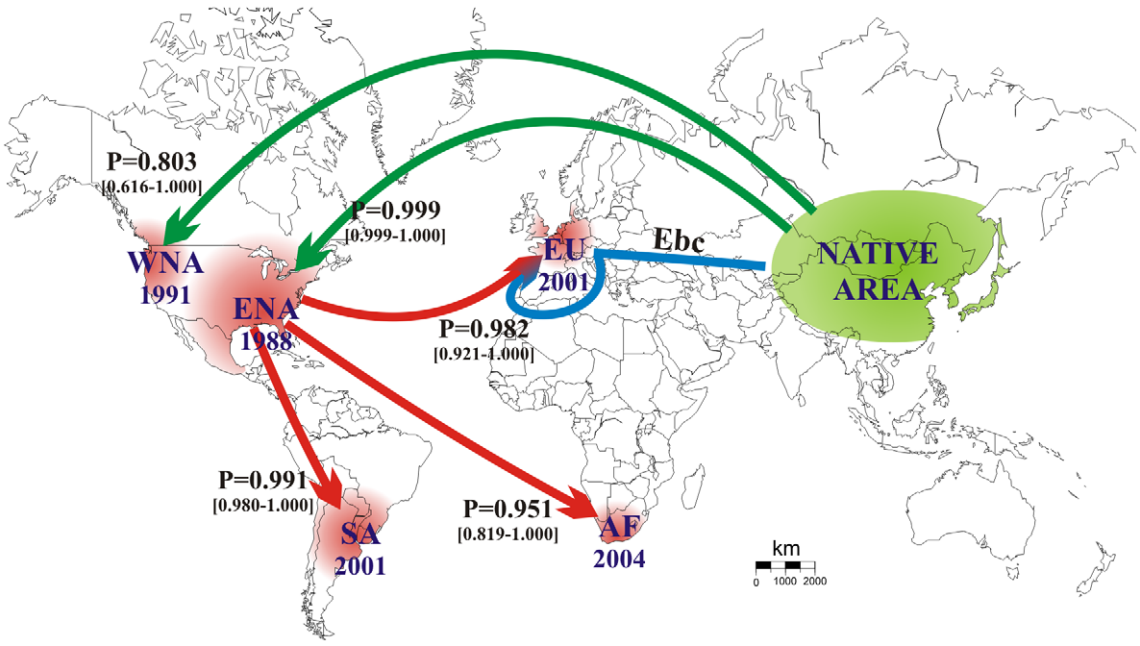

Figure 1. Worldwide routes of invasion of *Harmonia axyridis*. Most likely scenario of invasions into eastern North America (ENA), western North America (WNA), South America (SA), Europe (EU) and Africa (AF) by *Harmonia axyridis*, deduced from analyses based on approximate Bayesian computation. For each outbreak, the arrow indicates the most likely invasion pathway and the associated posterior probability value (P), with 95% confidence intervals in brackets. Years of first observation of invasive populations are indicated. Initially collected from the native area in 1982, the European biocontrol strain (Ebc; blue arrow) was used for biocontrol efforts in Europe and South America. Introductions to North America from the native area (green arrows) may have involved releases for biocontrol efforts.
doi:10.1371/journal.pone.0009743.g001

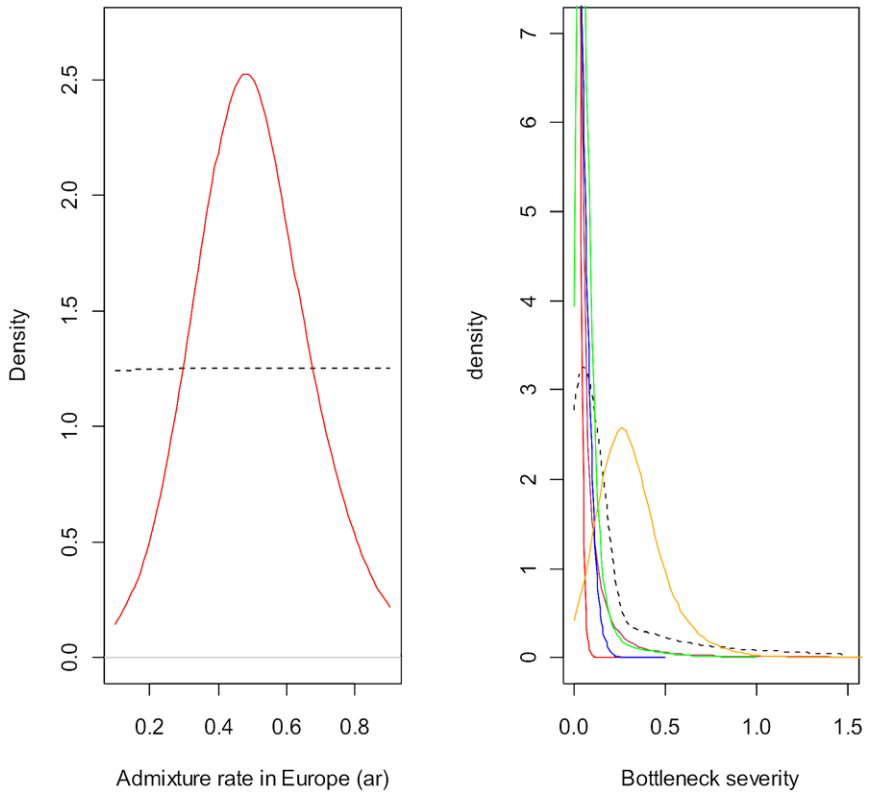

Figure 2. Posterior distributions of the genetic admixture rate in Europe (left panel) and the bottleneck severity for the five outbreaks (right panel) of *Harmonia axyridis*. The best estimates of admixture and bottleneck severity occur where the posterior probability density function peaks. Left panel: admixture in Europe involves the European biocontrol strain at a rate ar and the eastern North American population at a rate 1-ar. Y-axis: probability density of the genetic admixture ar in Europe. The dotted line is the prior distribution of admixture rate. Right panel: bottleneck severity was computed as the ratio between the duration (in number of generations) of the bottleneck following introduction and the effective number of individuals during this period [28,29]. Y-axis: probability density of bottleneck severity. Continuous lines in red, blue, maroon, green and orange are the posterior distributions of the European, eastern North American, western North American, South African and South American outbreaks, respectively. The dotted line is the prior distribution of bottleneck severity. It ranges from zero (complete absence of bottleneck) to 2.5 (strong bottleneck; i.e. 2 effective individuals during 5 generations). Posterior distributions support bottleneck severity values considerably lower than those of the prior, except for South America.
doi:10.1371/journal.pone.0009743.g002

America is the proximate origin of the worldwide invasion of HA. This pattern illustrates what we call the invasive bridgehead effect, whereby a particular invasive population serves as the source of colonists for other areas. Our results have important implications for the understanding and the management of biological invasions. The history of HA reveals that prevention of new invasions should include minimizing accidental dissemination from invasive bridgehead populations (here eastern North America) rather than focusing on the native range of invasive species. For instance, we found that live HA individuals intercepted in Europe (Norway) in 2007 on imported timber most likely originated from eastern North America based on the sample being significantly differentiated genetically from all tested populations ($p<1\times10^{-6}$) except that of eastern North America ($p=0.060$). The implication of

Table 1. Confidence in scenario selection obtained from the ABC analyses.

Invaded area (ABC analysis)	Number of competing scenarios	Selected scenario	Type I error	Type II error Mean (min – max)
Eastern North America (Analysis 1)	3	Introduction from the native area	0.12	0.080 (0.03–0.13)
Western North America (Analysis 2)	6	Introduction from the native area	0.16	0.030 (0.00–0.12)
Europe (Analysis 3)	10	Admixture between eastern North America and European biocontrol	0.16	0.010 (0.00–0.04)
South America (Analysis 4)	10	Introduction from eastern North America	0.03	0.013 (0.00–0.06)
Africa (Analysis 5)	21	Introduction from eastern North America	0.12	0.006 (0.00–0.06)

doi:10.1371/journal.pone.0009743.t001

eastern North America being an invasive bridgehead, together with the absence of remote establishment and slower local spread of the population in western North America and the long history of unsuccessful biocontrol introductions of HA from its native range, jointly suggest that an evolutionary shift triggering invasion occurred in eastern North America. It is worth pointing out that the invasive bridgehead effect is evolutionarily parsimonious: a single evolutionary shift in a single introduced population (the bridgehead) is required whereas multiple changes are required if introduced populations had become invasive independently. In Europe, the potential role of admixture with the European biocontrol strain is unknown, but the single eastern North American origin of the South American and South African outbreaks suggests that the genetic admixture observed in Europe is not required for an eastern North American propagule to establish and start an invasive population in diverse ecological contexts.

In conclusion, our results provide a convincing demonstration of an invasive bridgehead scenario in a worldwide emblematic invasive species. That invasion can proceed via a bridgehead has important implications both for invasion theory (i.e. the number and nature of evolutionary shift(s) involved in large scale invasions) and for ongoing efforts to manage invasions by alien organisms (i.e. heightened vigilance against invasive bridgehead populations). Our study highlights the interest of new model-based methods such as approximate Bayesian computation to rigorously compare complex invasion scenarios, including those with genetic admixture, a feature of species invasions that is increasingly acknowledged to be common [2,3,5]. It would be useful to revisit previously published genetic data sets on other worldwide invasive species using similar ABC approaches to examine whether bridgeheads and/or admixtures are a common mechanism driving invasion. Our ability to confidently reconstruct the routes of introduction of worldwide invaders is crucial to generating sensible hypotheses regarding the environmental and evolutionary factors responsible for biological invasions [3]. In HA, our results suggest that an evolutionary shift triggering invasion likely occurred in the bridgehead population in eastern North America. Forthcoming studies based on quantitative genetics approaches [3,25] will shade light on the evolutionary basis of HA invasiveness in eastern North America and into the potential role of genetic admixture in Europe.

Materials and Methods

Population samples and genotyping

Population samples were genotyped at 18 microsatellite markers [26]. The samples of populations from the five invaded areas (Fig. 1 and Table S1) were collected near the sites where the outbreaks were first observed. The three samples from the native range were collected in eastern Asia, where individuals historically used for biocontrol purposes had been collected [10,11,12]. The three native population samples were genetically homogeneous (mean pairwise $F_{ST} = 0.0057$), and were therefore pooled into a single sample. Using native population samples individually did not change our conclusions. We genotyped numerous additional population samples collected from all invaded areas (30 additional samples) and from the native range (5 additional samples); analysis of these samples indicated that the subset of samples used in the study provides an adequate description of the main invasive and native populations (i.e. none significant to low level of genetic differentiation within the native range and within each invaded area). The European biocontrol sample (Ebc) was from the INRA rearing stock of 1987 initially collected from the native area in 1982 and used for biocontrol in Europe and South America. Genotyping of the biocontrol strains produced in European biofactories from the late 90's to the present (4 additional samples) confirmed that they are indeed derived from the original INRA strain we genotyped.

Inferring invasion scenarios using approximate Bayesian computation

Genetic variation within and between populations was summarized using a set of statistics traditionally employed in approximate Bayesian computation analyses (ABC) [23,24]. For each population and each population pair we used the mean number of alleles per locus, the mean expected heterozygosity and the mean allelic size variance. The other statistics used were the mean ratio of the number of alleles over the range of allele sizes, pairwise F_{ST} values, mean individual assignment likelihoods of population i assigned to population j and the maximum likelihood estimate of admixture proportion.

We performed five serial nested ABC analyses of invasion scenarios involving successive HA outbreaks (Table S1). In analysis 1, we dealt with the introduction pathway for the first recorded outbreak in eastern North America in 1988, with native populations (either from biocontrol or accidental introduction) and/or the European biocontrol population as potential sources, thereby defining three competing scenarios (see Fig. S1). The second outbreak in western North America in 1991 was examined in analysis 2, taking into account the scenario selected in analysis 1. For this analysis, there were three possible sources: the first outbreak (eastern North America), the native range, and the European biological control strain (Table S1). With the potential for admixture between them, this gave 6 competing scenarios. The European and South American outbreaks in 2001 were addressed in analyses 3 and 4, respectively (10 scenarios for each outbreak), taking into account the scenario selected in analysis 1 and 2. The year of first observation in Europe and South America was the same, thus we assumed that one could not be a potential source of the other. Nevertheless, we performed a specific ABC analysis to test this assumption which confirmed the independence of these two outbreaks ($P = 0.773$ with $CI = 0.555–0.991$). Finally, the African outbreak in 2004 was considered in analysis 5 (21 scenarios), taking into account the scenarios selected in analyses 1, 2, 3 and 4.

The ABC analyses were performed using parameter values drawn from the prior distributions described in Table S2 (prior set 1), and by simulating 10^6 microsatellite data sets for each competing scenario in the first four analyses and 5×10^5 data sets in analysis 5 because of the high number of scenarios (21) and summary statistics (170) which made a larger analysis computationally unfeasible. For each of the five analyses, we estimated the posterior probabilities of the competing scenarios using a polychotomous logistic regression [24] on the 0.1% of simulated data sets closest to the observed data set. The selected scenario is that with the highest significant probability value with a non-overlapping 95% confidence interval. We estimated the posterior distributions of demographic parameters under the final HA invasion scenario presented in Fig. 1 using a local linear regression on the 1% closest of 5×10^6 simulated data sets [22,24].

Confidence in scenario selection

We evaluated the robustness of our inferences by running a second analysis with an alternative set of priors (prior set 2 in Table S2) and by estimating the posterior probabilities of scenarios using the 0.1% or 1% closest simulated data sets for both sets of priors (Table S2). For each of our five ABC analyses, we evaluated

the ability of the methodology to correctly select the true scenario by analyzing test data sets simulated from the various competing scenarios with the same number of loci and individuals as in the real data set. For each scenario, one hundred such data sets were simulated using parameter values drawn from the same probability distributions as the priors (prior set 1, Table S2). Posterior probabilities of each competing scenario were estimated for each simulated test data set using the 0.1% closest data sets. These probabilities were used to compute type I and II errors in the selection of scenarios.

Most ABC computations were processed using a modified version of the Windows package DIYABC [24]. Some of the computations for assessing confidence in scenario selection (i.e. those for analysis 5) necessitated the development of specific Linux programs and scripts running on a computer cluster (available under request from A.E.).

Additional simulation and statistical treatments

We ran computer simulations to tackle the question of a high frequency of erroneous selection of the source population in a situation of genetic admixture when considering only the raw values of genetic differentiation statistics (i.e. pairwise F_{st} and assignment likelihood values). We used the program DIYABC to simulate genetic data sets under a scenario of invasion with admixture similar to the one considered in the case of the invasion of Europe by HA. We simulated 6×10^4 data sets drawing the parameter values in the prior set 1 (Table S2), except the admixture rate (*ar*) which was drawn in a discrete {0, 0.1, …,1.0} distribution instead of a uniform [0.1;0.9] distribution. The number of sampled diploid individuals was fixed to 30 in all populations. The deduced origin of the admixed introduced population is the population with which the F_{ST}-value is the smallest or the assignment likelihood value is the highest. We then calculated the proportion of simulated data sets, as a function of the admixture rate, for which the deduced origin was the ancestral population of the two actual source populations (i.e. the native area in the case of the invasion of Europe by HA), considering either F_{ST} or assignment likelihood values (see Fig. S2).

Finally, we also genotyped 200 live HA individuals intercepted in Europe (Åndalsnes, Norway) in 2007 on timber imported from North America. We tested for the genetic differentiation, using Fisher's exact tests [27], between this interception HA sample and each population sample used in the ABC analysis to elucidate its most likely origin.

Supporting Information

Figure S1 Graphic representation of the three competing HA invasion scenarios considered in ABC analysis 1, which focused on the origin of the eastern North American outbreak ENA. Notes: Time 0 is the sampling year 2007 and time 50 is the sampling year 1987 (2.5 generations per year). Pop 1 is the HA population from the native area; Pop 2 is the European biocontrol strain (EBC); Pop 3 is the eastern North American population (ENA); Population 4 (light blue segment) corresponds to an unsampled biocontrol strain released in eastern North America. Introduction events in the wild include a period of *BD* generation(s) of potentially small population size (NF_3 for pop 3). Scenario 1 corresponds to a native origin of the ENA outbreak, possibly through an intermediate biocontrol population (population 4). Scenario 2 corresponds to a European Biocontrol origin of the ENA outbreak. In scenario 3, the ENA outbreak is the result of an admixture between individuals from the native area at a rate *ar* and from the European biocontrol strain at a rate 1-*ar*. All parameters with associated prior distributions are described in Table S2.
Found at: doi:10.1371/journal.pone.0009743.s001 (5.94 MB TIF)

Figure S2 Erroneous selection of source population in a situation of genetic admixture when using raw values of genetic differentiation statistics. Notes: (a) We used the program DIYABC [24] to simulate genetic data sets under a scenario of invasion with admixture similar to the one considered in the case of the invasion of Europe by HA. The two sources populations Pop 2 and Pop 3 of the admixed population Pop 4 derive from the same population Pop 1. Pop 1 stands for the HA population from the native area, Pop 2 for the European biocontrol strain (EBC), Pop 3 for the eastern North American population (ENA), and Pop 4 for the European population (EU). Population 5 (light blue segment) corresponds to an unsampled biocontrol strain released in eastern North America. NS_k stand for the stable effective population sizes in population k. Introduction events in the wild include a period of *BD* generation(s) of potentially small population size (NF_3 for Pop 3 and NF_4 for Pop 4). We simulated 6×10^4 data sets drawing the parameter values in the prior set 1 (Table S2), except the admixture rate (ar) which was drawn in a discrete {0, 0.1, …,1.0} distribution instead of a uniform [0.1;0.9] distribution. (b) Pairwise F_{st} and assignment likelihood values were computed for each simulated data set between the (admixed) Pop 4 and other populations. The deduced origin of Pop 4 is the population with which its F_{st}-value is the smallest or its assignment likelihood value is the highest. We have represented here the proportion of simulated data sets, as a function of the admixture rate, for which the deduced origin was the ancestral population of the two actual source populations (i.e., Pop 1), considering either F_{st} or assignment likelihood values.
Found at: doi:10.1371/journal.pone.0009743.s002 (7.30 MB TIF)

Figure S3 Raw signatures of admixture in the HA invasive population in Europe. Notes: We present here the histograms of allele frequencies at two of the 18 microsatellite loci genotyped in the invasive populations from Europe (EU) and Eastern North America (ENA), and from the European biocontrol strain (Ebc). Only a mixture of the Eastern North American and biocontrol gene pools makes it possible to generate all alleles observed in the European invasive population.
Found at: doi:10.1371/journal.pone.0009743.s003 (9.97 MB TIF)

Table S1 Native, invasive and biocontrol populations of *Harmonia axyridis* (HA), with the possible sources of each population considered for each nested ABC analysis. Notes: The definition of potential sources is based on the year of first observation of invasive populations. The EBC population was considered a potential source in all analyses even though historical records indicate that it was used for biocontrol purposes only in Europe and South America [13]. Admixtures between all pairs of potential sources also were considered in specific scenarios. In analyses 1 and 2, when the native population was involved we considered that there may have been a period of laboratory rearing in preparation for release for biocontrol purposes resulting in a lower effective population size, instead of a direct introduction from the native area (see Table S2 and Figure S1). For each ABC analysis, the number of competing scenarios is given in parentheses. Population code names as in Figure 1.
Found at: doi:10.1371/journal.pone.0009743.s004 (0.06 MB DOC)

Table S2 Two sets of prior distributions of demographic, historic and mutation parameters used in ABC analyses. Notes: Populations *i* are wild populations (invasive and native) and

populations k are biocontrol strains (i.e., laboratory reared populations). Times were translated into numbers of generations running back in time from 2007 by assuming 2.5 generations per year in prior set 1, and 3 generations per year in prior set 2 [19]. *NS* = stable effective population size (number of diploid individuals); *NF* = effective number of founders during an introduction step lasting *BD* generation(s); *ar* = admixture rate (only for scenarios with admixture); t_i = introduction date of invasive populations i with bounds x_i or y_i fixed from dates of first observation, assuming 2.5 or 3 generations per year, respectively; *tbc* = creation date of unsampled biocontrol strain for ENA and WNA populations (with condition $t_i <$ or = tbc_i) bounded between the dates of first observation of invasive population (which would correspond to a direct introduction into the wild) and the number of generations from 1970, the start date of a period of intense HA biocontrol activity in the USA. For microsatellite marker parameters, the loci were assumed to follow a generalized stepwise mutation model [S1] with two parameters: the mean mutation rate (mean μ) and the mean parameter of the geometric distribution (mean P) of the length in number of repeats of mutation events. Each locus has a possible range of 40 contiguous allelic states and is characterized by individual μ_{loc} and P_{loc} values, with μ_{loc} drawn from a Gamma (mean = mean μ and shape = 2) distribution and P_{loc} drawn from a Gamma (mean = mean P and shape = 2) distribution [S2]. Uneven insertion/deletion events that were suspected for several of our microsatellite loci based on observed allele sizes (i.e., allele lengths were sometimes not multiple of the motif length implying that there has been insertion-deletion mutations [28]) were also simulated with a mean mutation rate μSNI (for single nucleotide instability) and $\mu\text{SNI}_{\text{loc}}$ drawn for each locus from a Gamma (mean = mean μSNI and shape = 2). Boundaries of distributions are in brackets. Parameters of Normal and Gamma distributions are in parentheses. In prior set 2, Normal, Lognormal and Gamma distributions are truncated between the same boundaries as in prior set 1. All prior quantities presented were computed from 100,000 values. NA = not applicable; DV = can take different values. Supporting references: S1. Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Molecular Ecology 11: 1591–1604. S2. Verdu P, Austerlitz F, Estoup A, Vitalis R, Georges M, et al. (2009) Origins and Genetic Diversity of Pygmy Hunter-Gatherers from Western Central Africa. Current Biology 19: 1–7.
Found at: doi:10.1371/journal.pone.0009743.s005 (0.07 MB DOC)

Table S3 Posterior probabilities (P) of the selected (most likely) scenarios in each ABC analysis at two different thresholds of smallest Euclidian distances (0.1% and 1%) and two different sets of priors. Notes: Prior sets are detailed in Table S2. 95% confidence intervals (CI) are in brackets. The 95% CI of the selected scenarios never overlapped those of competing scenarios. The values presented in Figure 1 of the main text are those obtained using the 0.1% threshold and prior set 1.
Found at: doi:10.1371/journal.pone.0009743.s006 (0.05 MB DOC)

Table S4 Classical population genetics statistics of the studied HA populations and inferred source populations of the five HA invasive outbreaks. Notes: Mean corrected number of alleles per locus (*Na*), expected heterozygosity (*He*) [S3], pairwise F_{ST} matrix and mean individual assignment log-likelihood of invasive populations to putative source populations (in parentheses) [28]. Dashes refer to the pairs of invasive and source populations that are chronologically incompatible. The deduced origin of each outbreak is the sample for which the F_{ST}-value is the smallest and the assignment likelihood is maximized (values in bold). Ebc = European biocontrol strain; ENA = Eastern North America; WNA = Western North America; SA = South America; EU = Europe; AF = Africa. Supporting reference: S3. Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.
Found at: doi:10.1371/journal.pone.0009743.s007 (0.06 MB DOC)

Acknowledgments

This study would not have been possible without generous help from colleagues from around the world who provided us with some of the HA samples used in this study: N. Berkvens, C. Borges, R. Koch, L. Lawson Handley, J. Li, J. Lundgren, M. Majerus, A. Migeon, R. Staals, A. Staverlokk, W. Su, C. Thomas, and R. Ware. We also thank L. Bernatchez, D. Bourguet, R. Hufbauer, T. Sappington, J. Turgeon, R. Vitalis for comments and discussions.

Author Contributions

Conceived and designed the experiments: EL AE. Performed the experiments: EL. Analyzed the data: EL TG AE. Contributed reagents/materials/analysis tools: EL TG JMC TM BF AE. Wrote the paper: EL AE.

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Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird

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Supplementary information:

Table S1: Native, invasive and biocontrol populations of Harmonia axyridis (HA), with the possible sources of each population considered for each nested ABC analysis.

Table S2: Two sets of prior distributions of demographic, historic and mutation parameters used in ABC analyses.

Table S3: Posterior probabilities (P) of the selected (most likely) scenarios in each ABC analysis at two different thresholds of smallest Euclidian distances (0.1% and 1%) and two different sets of priors.

Table S4: Classical population genetics statistics of the studied HA populations and inferred source populations of the five HA invasive outbreaks.

Figure S1: Graphic representation of the three competing HA invasion scenarios considered in ABC analysis 1, which focused on the origin of the eastern North American outbreak ENA.

Figure S2: Erroneous selection of source population in a situation of genetic admixture when using raw values of genetic differentiation statistics.

Figure S3: Raw signatures of admixture in the HA invasive population in Europe.

Population (code name)	Date of 1st observation	Sampling location	Geographic coordinates	sampling date	Number of genotyped individuals	Potential sources	ABC Analyses
Native 1	-	Beijing, China	40.24°N 116.23°E	May 2007	28	-	-
Native 2	-	Shilin city, Yunnan, China	24.90°N 103.35°E	August 2007	35	-	-
Native 3	-	Fuchu, Japan	34.57°N 133.24°E	September 2005	36	-	-
European Biocontrol (EBC)	1982 [S1] In laboratory	Rearing stock, INRA laboratory	-	April 1987	18	Native	-
Eastern North-America (ENA)	1988 [S2]	Joyce, Louisiana, USA	31.94°N 92.60°W	November 2007	34	Native (possibly through a biocontrol release); EBC	Analysis 1 (3 scenarios)
Western North-America (WNA)	1991 [S3]	Sunnyside, Washington, USA	46.32°N 120.01°W	September 2007	42	Native (possibly through a biocontrol release); EBC; ENA; admixture between all pairs of potential sources	Analysis 2 (6 scenarios)
Europe (EU)	2001 [S4]	Gent, Belgium	51.05°N 3.71°E	October 2007	32	Native; EBC; ENA; WNA; admixtures between all pairs of potential sources	Analysis 3 (10 scenarios)
South America (SA)	2001 [S5]	Curitiba, Brazil	25.45°S 49.24°W	February 2008	30	Native; EBC; ENA; WNA; admixtures between all pairs of potential sources	Analysis 4 (10 scenarios)
Africa (AF)	2004 [S6]	Somerset West, South Africa	34.03°S 18.83°E	May 2008	31	Native; EBC; ENA; WNA; EU; SA; admixtures between all pairs of potential sources	Analysis 5 (21 scenarios)

Table S1: Native, invasive and biocontrol populations of *Harmonia axyridis* (HA), with the possible sources of each population considered for each nested ABC analysis.

parameters	Prior Set 1 Distribution	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%	Prior Set 2 Distribution	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%
NS_i	Uniform [100 – 20,000]	10,056	10,040	NA	640	19,490	Normal (10,000 ; 5,000)	9,993	9,990	9,980	1,640	18,340
NS_k	Uniform [10 – 1,000]	506	508	NA	35	975	Normal (500 ; 250)	502	501	498	86	922
NF_i	Loguniform [2 – 1,000]	162	45	2	2	862	Lognormal (30 ; 30)	136	39	44	2	797
ar	Uniform [0.1 – 0.9]	0.5	0.5	NA	0.12	0.88	Normal (0.5 ; 0.25)	0.5	0.5	0.5	0.15	0.86
t_i	Uniform [x_i – x_i+5]	DV	DV	NA	DV	DV	Uniform [y_i – y_i+5]	DV	DV	NA	DV	DV
tbc_i	Loguniform [x_i – 93]	DV	DV	DV	DV	DV	Loguniform [y_i – 111]	DV	DV	DV	DV	DV
BD_i	Uniform [0 – 5]	2.5	2.5	NA	0	5	Uniform [0 – 5]	2.5	2.5	NA	0	5
mean μ	Uniform [10^{-5} – 10^{-3}]	$5.0x10^{-4}$	$5.0x10^{-4}$	NA	$3.5x10^{-5}$	$9.8x10^{-4}$	Loguniform [10^{-5} – 10^{-3}]	$2.1x10^{-4}$	$1.0x10^{-4}$	$1.0x10^{-5}$	$1.1x10^{-5}$	$8.9x10^{-4}$
mean P	Uniform [0.1 – 0.3]	0.2	0.2	NA	0.10	0.29	Gamma (30 ; 136)	0.22	0.22	0.21	0.15	0.29
mean μSNI	Uniform [10^{-8} – 10^{-4}]	$5.0x10^{-5}$	$5.0x10^{-5}$	NA	$2.5x10^{-6}$	$9.7x10^{-5}$	Loguniform [10^{-8} – 10^{-4}]	$1.1x10^{-5}$	$1.0x10^{-6}$	$1.0x10^{-8}$	$1.3x10^{-8}$	$7.9x10^{-5}$

Table S2: Two sets of prior distributions of demographic, historic and mutation parameters used in ABC analyses.

Invaded area	Prior set 1 Selected scenario	P 0.1%	P 1%	Prior set 2 Selected scenario	P 0.1%	P 1%
East North America (Analysis 1)	Introduction from the native area	0.999 [0.999 – 1.000]	0.996 [0.994 – 0.998]	Introduction from the native area	0.999 [0.999 – 1.000]	0.999 [0.999 – 1.000]
West North America (Analysis 2)	Introduction from the native area	0.803 [0.616 – 0.989]	0.727 [0.653 – 0.800]	Introduction from the native area	0.953 [0.893 – 1.000]	0.848 [0.794 – 0.903]
Europe (Analysis 3)	Admixture between eastern North America and European biocontrol	0.982 [0.921 – 1.000]	0.951 [0.915 – 0.987]	Admixture between eastern North America and European biocontrol	0.844 [0.710 – 0.953]	0.892 [0.813 – 0.972]
South America (Analysis 4)	Introduction from eastern North America	0.991 [0.980 – 1.000]	0.979 [0.969 – 0.989]	Introduction from eastern North America	0.980 [0.944 – 1.000]	0.977 [0.964 – 0.991]
Africa (Analysis 5)	Introduction from eastern North America	0.951 [0.819 – 1.000]	0.844 [0.750 – 0.938]	Introduction from eastern North America	0.973 [0.849 – 1.000]	0.754 [0.603 – 0.904]

Table S3: Posterior probabilities (P) of the selected (most likely) scenarios in each ABC analysis at two different thresholds of smallest Euclidian distances (0.1% and 1%) and two different sets of priors.

			Invasive outbreaks				
Possible source populations	N_a	H_E	ENA	WNA	EU	SA	AF
Native area	5.33	0.601	**0.017 (-18.8)**	**0.011 (-19.9)**	**0.048 (-24.2)**	0.094 (-24.3)	0.031 (-20.5)
Ebc	2.94	0.431	0.188 (-33.2)	0.184 (-34.4)	0.111 (-28.9)	0.279 (-39.4)	0.188 (-34.1)
ENA	4.55	0.553		0.023 (-22.0)	0.059 (-27.1)	**0.064 (-18.5)**	**0.023 (-17.6)**
WNA	4.79	0.566	-		0.064 (-27.8)	0.107 (-23.8)	0.037 (-21.0)
EU	4.68	0.614	-	-		0.109 (-23.7)	0.066 (-22.4)
SA	3.47	0.496	-	-	-		0.089 (-23.2)
AF	4.13	0.549	-	-	-	-	
Inferred source based on raw values of F_{ST} and assignment statistics			Native area	Native area	Native area	ENA	ENA
Inferred source based on ABC analyses			Native area	Native area	admixture EBC + ENA	ENA	ENA

Table S4: Classical population genetics statistics of the studied HA populations and inferred source populations of the five HA invasive outbreaks.

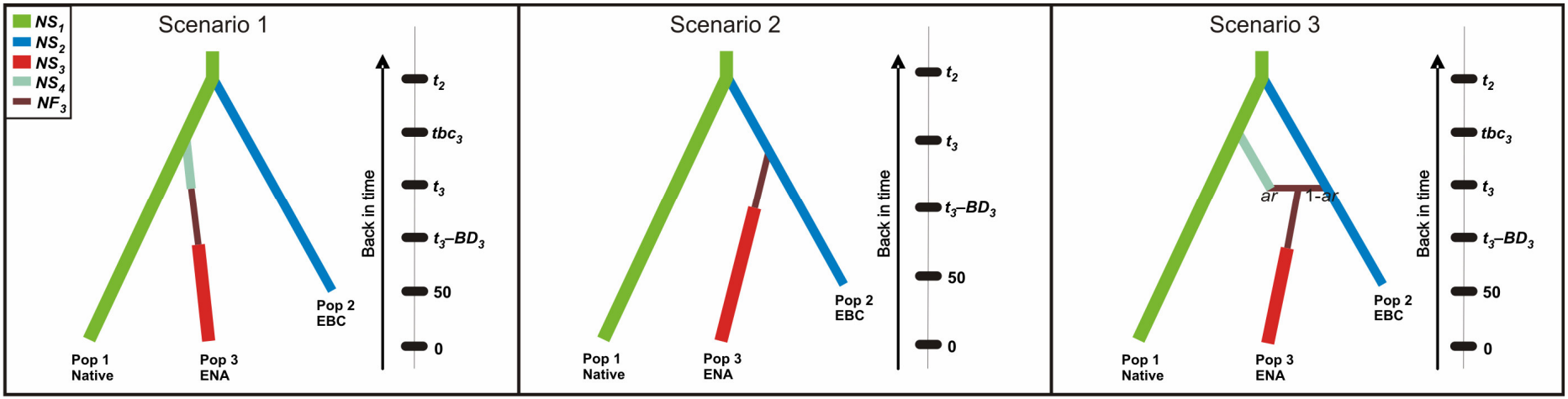
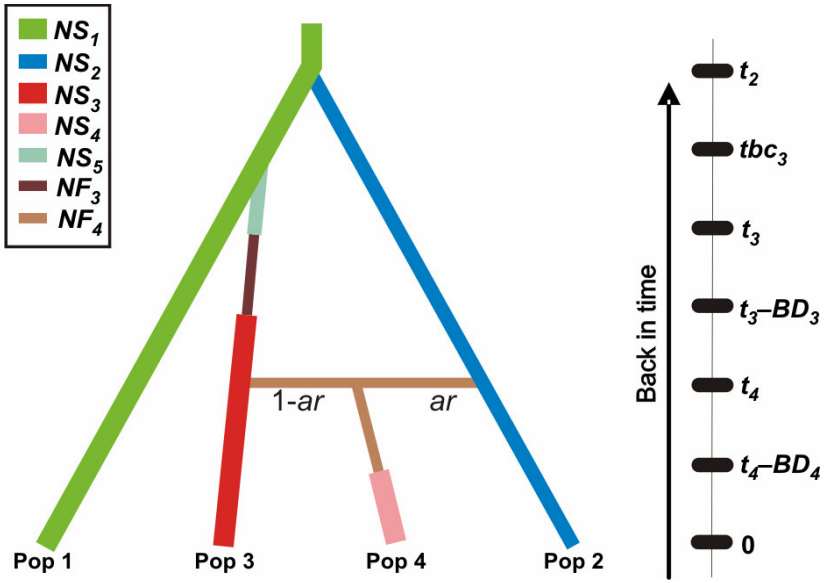


Figure S1: Graphic representation of the three competing HA invasion scenarios considered in ABC analysis 1, which focused on the origin of the eastern North American outbreak ENA.

(a) Graphic representation of the European-HA-like scenario of introduction with admixture.

(b) Relationship between the admixture rate and the erroneous selection of Pop 1 as source population of the admixed Pop 4



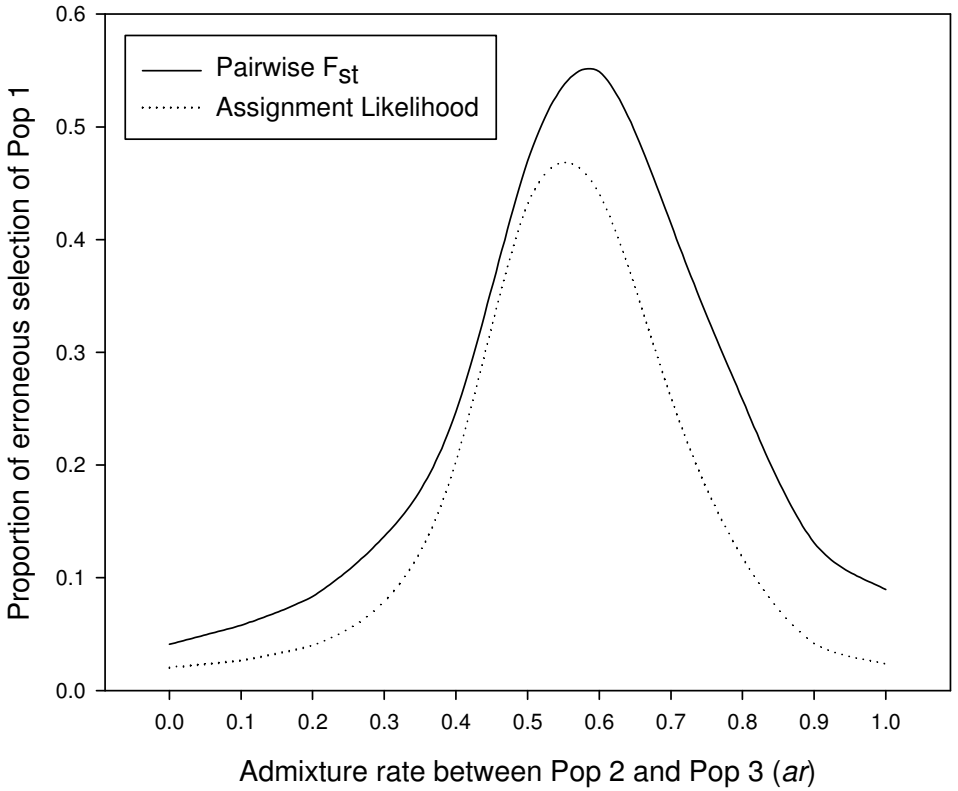


Figure S2: **Erroneous selection of source population in a situation of genetic admixture when using raw values of genetic differentiation statistics.**

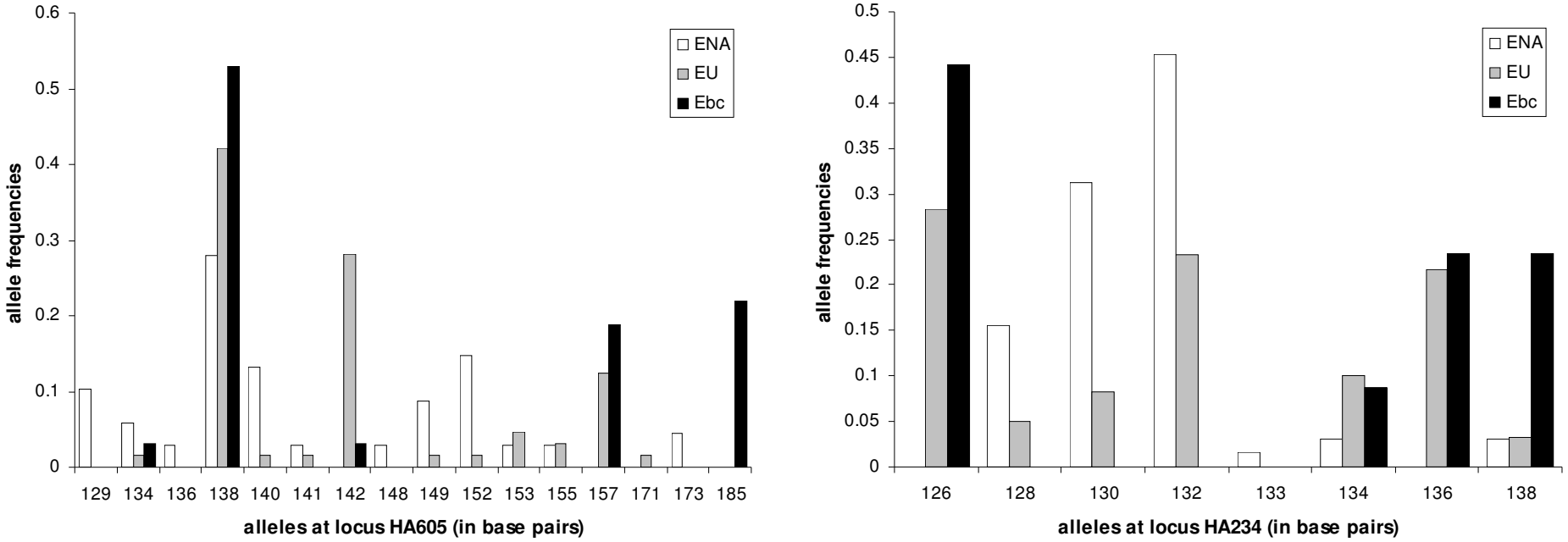


Figure S3: Raw signatures of admixture in the HA invasive population in Europe.

ANNEXE II

Article 2 (2011)

Lombaert E, Guillemaud T, Thomas C, Lawson Handley L-J, Li J, Wang S, Pang H, Goryacheva II, Zakharov IA, Jousselin E, Poland R, Migeon A, van Lenteren JC, De Clercq P, Berkvens N, Jones W, Estoup A (2011) Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: case study of the invasive ladybird *Harmonia axyridis*. *Molecular Ecology* **20**, 4654-4670.

MOLECULAR ECOLOGY

Molecular Ecology (2011) doi: 10.1111/j.1365-294X.2011.05322.x

Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: case study of the invasive ladybird *Harmonia axyridis*

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Abstract

Correct identification of the source population of an invasive species is a prerequisite for testing hypotheses concerning the factors responsible for biological invasions. The native area of invasive species may be large, poorly known and/or genetically structured. Because the actual source population may not have been sampled, studies based on molecular markers may generate incorrect conclusions about the origin of introduced populations. In this study, we characterized the genetic structure of the invasive ladybird *Harmonia axyridis* in its native area using various population genetic statistics and methods. We found that native area of *H. axyridis* most probably consisted of two geographically distinct genetic clusters located in eastern and western Asia. We then performed approximate Bayesian computation (ABC) analyses on controlled simulated microsatellite data sets to evaluate (i) the risk of selecting incorrect introduction scenarios, including admixture between sources, when the populations of the native area are genetically structured and sampling is incomplete and (ii) the ability of ABC analysis to minimize such risks by explicitly including unsampled populations in the scenarios compared. Finally, we performed additional ABC analyses on real microsatellite data sets to retrace the origin of biocontrol and invasive populations of *H. axyridis*, taking into account the possibility that the structured native area may have been incompletely sampled. We found that the invasive population in eastern North America, which has served as the bridgehead for worldwide invasion by *H. axyridis*, was probably formed by an admixture between the eastern and western native clusters. This admixture may have facilitated adaptation of the bridgehead population.

Keywords: biocontrol, biological invasion, harlequin ladybird, invasive species, microsatellite, source population

Received 18 March 2011; revision received 15 September 2011; accepted 16 September 2011

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Introduction

Historical and observational data for invasive species are often sparse and incomplete, so molecular genetic markers are increasingly used and have proved to be efficient tools for the inference of invasion routes (Estoup & Guillemaud 2010). However, such inference remains a major challenge, because of two specific features of invasions. First, invasion history is often marked by stochastic genetic and demographic events, which may make it difficult to interpret the observed genetic patterns. In particular, introduction is often characterized by a loss of genetic diversity relative to the source population (a founder event) and may be followed by a demographic bottleneck, resulting in strong genetic drift and substantial genetic differentiation between the introduced population and all other populations, including the source population. Moreover, multiple introductions may give rise to genetic admixtures between several differentiated populations in the invasive range, thus generating unique genetic combinations that are not found together in the native range. Second, sampling issues may compromise inference. An invasive population may be derived from different types of source population: (i) populations from the native area that may be large, poorly known and/or genetically structured or (ii) other invasive outbreak(s), which serve as a source of colonists for other areas, the existence of which may be unknown because they occur in unexpected or unexplored areas. The actual geographical range of a target species may be large and difficult to explore exhaustively and, in many cases, the actual source population may not have been sampled.

The use of approximate Bayesian computation (ABC, Beaumont *et al.* 2002; Bertorelle *et al.* 2010; Csillery *et al.* 2010) on molecular data makes it possible to generate model-based inferences for complex scenarios, such as those related to the introduction histories of invasive species (Estoup & Guillemaud 2010). This method has recently been successfully used to retrace the invasion routes of various invasive species, explicitly taking into account demographic and genetic stochasticity resulting from bottlenecks, multiple introductions and/or genetic admixture events (Miller *et al.* 2005; Pascual *et al.* 2007; Lombaert *et al.* 2010). Studies of simulated data have shown that, in most cases, ABC is more powerful in this context than other more traditional methods for population genetics studies, such as neighbour-joining trees or *F*-statistics (Estoup & Guillemaud 2010; Guillemaud *et al.* 2010; Lombaert *et al.* 2010).

Another advantage of the ABC method is that it allows the explicit inclusion of unsampled populations in the evolutionary scenarios compared, although the power of ABC to deal with unsampled populations has been little investigated (but see Guillemaud *et al.* 2010). The native range of a species is characterized by a long evolutionary history shaped by mutation, drift, migration and selection operating in a spatially and temporally heterogeneous environment. A strong geographical genetic structure is therefore often found in the native range of invasive species (e.g. Kolbe *et al.* 2004; Ciosi *et al.* 2008). Exhaustive sampling is difficult in native areas that are often large and may be poorly known. It is therefore important to evaluate the effects of unsampled native source populations on the inference of introduction routes in the presence of genetic structure within the native area. We addressed this question with controlled simulated microsatellite data sets and real data sets obtained from wild and biocontrol populations of the harlequin ladybird *Harmonia axyridis*.

The native area of *H. axyridis* covers a large part of Asia (Kazakhstan, southern Siberia, Mongolia, eastern China, Korea and Japan, reviewed in Poutsma *et al.* 2008). *H. axyridis* has been repeatedly introduced into North America since 1916 as a biocontrol agent for aphids. Several source populations are known to have contributed to American biocontrol stocks, including, in particular, the populations of Eastern Siberia, China, South Korea and Japan (Tedders & Schaefer 1994; Krafsur *et al.* 1997). In Europe, biocontrol with *H. axyridis* began in the early 1990s, with individuals derived from a single population brought from China in 1982 by an INRA laboratory (Ongagna *et al.* 1993), which was subsequently reared in research laboratories and several biofactories. This same European biocontrol population was also used repeatedly in South America from 1986 (Argentina and Brazil Poutsma *et al.* 2008). Despite the recurrent intentional releases of ladybirds for acclimation attempts in Europe and South America, the species took decades to establish itself (Koch 2003). However, for unknown reasons, it recently suddenly became highly invasive on four continents. Invasive populations were first recorded in eastern (Louisiana, USA, Chapin & Brou 1991) and western (Oregon, USA, LaMana & Miller 1996) North America in 1988 and 1991, respectively. They were then recorded in Europe (Belgium, Adriaens *et al.* 2003) and South America (Argentina, Saini 2004) in 2001 and in Africa (South Africa, Stals & Prinsloo 2007) in 2004. The species has widely spread in these areas and has become a major predator of nontarget arthropods, a household invader, and a pest in fruit crops (Koch 2003). Using ABC methods on microsatellite and historical data, Lombaert *et al.* (2010) showed that the two North American outbreaks originated from two independent introductions from the native area, but the exact geographical origins of the source

populations were not investigated. They also found that the eastern North American (ENA) population acted as a bridgehead for worldwide invasion, acting as the source population of the European, South American and African outbreaks, with some admixture with the biocontrol population in Europe.

In this study, we characterized the genetic structure of *H. axyridis* in its native area by Bayesian clustering methods and more classical population genetic statistics and methods (e.g. F_{ST} and neighbour-joining trees). We then performed ABC analyses on controlled simulated microsatellite data sets to evaluate (i) the risk of selecting incorrect scenarios when using an incomplete sampling strategy in a genetically structured native area and (ii) the ability of ABC analysis to minimize such risks by explicitly including unsampled populations in the scenarios compared. Finally, we performed additional ABC analyses to retrace the origin of biocontrol and invasive populations of *H. axyridis*, taking into account the possibility that the structured native area may have been incompletely sampled.

Methods

Sampling and genotyping

Harmonia axyridis samples were collected within the native area, at nine sites, covering a substantial part of the natural distribution of this species (Kazakhstan, Russia, China, South Korea and Japan; Fig. 1; Table S1, Supporting information). Three of these samples were previously used by Lombaert *et al.* (2010). We also collected five European biocontrol samples believed to be derived from the original 1982 INRA sample. Three of these samples were obtained from different commercial biofactories, and two were obtained from INRA rearing stocks from 1987 and 2006 (Table S1, Supporting information). The oldest INRA sample, EB-INRA87, corresponds to that used by Lombaert *et al.* (2010). A large number of native populations have been used for biocontrol in North America (Tedders & Schaefer 1994; Krafsur *et al.* 1997; Koch 2003), but only one sample, collected in 1980, could be obtained and analysed (http://www.ars-grin.gov/cgi-bin/nigrp/robo/f941spl?50902) (Table S1, Supporting information). The samples representative of the five invaded areas described by Lombaert *et al.* (2010) were also used in the present study (Table S1, Supporting information). The sample size for each population ranged from 18 to 42 individuals (mean 29.3; Table S1, Supporting information). Samples were genotyped at 18 microsatellite markers, as described by Loiseau *et al.* (2009). Four biocontrol populations were obtained from insect collections and had been stored dry at room temperature for a long period of time, greater than 20 years in some cases (Table S1, Supporting information). The DNA extracted from these samples was highly degraded, necessitating several modifications to the protocols described by Loiseau *et al.* (2009): (i) DNA was extracted from entire bodies (rather than just the pronotum and head), (ii) annealing temperature for PCR was set at 55 °C (rather than 57 °C) and (iii) the number of PCR cycles was set at 35 (rather than 25).

Genetic variation within and between populations

Genetic variation within samples was quantified by calculating the mean expected heterozygosity H_e (Nei 1987) and the mean allelic richness (AR) with the rarefaction method of Leberg (2002) in FSTAT (version 2.9.3.2 Goudet 2002). Amplification was difficult at eight of the 18 microsatellite loci in the four biocontrol samples that had been stored dry (i.e. allelic PCR profiles could be safely interpreted for only a small number of individuals for these eight loci). We therefore calculated two indices of allelic richness: AR_{10} was calculated for 10 microsatellite loci for all 20 populations, whereas AR_{18} was calculated for the entire set of 18 microsatellite data for a subset of 16 populations.

Genetic variation between populations was summarized by calculating pairwise F_{ST} estimates as described by Weir & Cockerham (1984), with Genepop (Raymond & Rousset 1995b). Exact tests for population genotypic differentiation (Raymond & Rousset 1995a) were carried out for all pairs of populations within the native area, with the same software. Because these tests involve nonorthogonal and multiple comparisons, we corrected significance levels by the false discovery rate procedure (Benjamini & Hochberg 1995). We plotted a neighbour-joining (NJ) tree (Saitou & Nei 1987), using the pairwise genetic distances described by Cavalli-Sforza and Edwards (Cavalli-Sforza & Edwards 1967), in Populations 1.2.30 software (http://bioinformatics.org/~tryphon/populations/). The robustness of tree topology was evaluated by carrying out 1000 bootstrap replicates over loci.

Population structure and isolation by distance within the native area

The clustering approach implemented in STRUCTURE v2.3.3 (Pritchard *et al.* 2000) was used to infer the number of potential population units within the native area of *H. axyridis*. We chose the admixture model with correlated allele frequencies and, because our sampling scheme involved the collection of many individuals from a few discrete distant locations (Schwartz & McKelvey 2009), we used the sampling location as prior

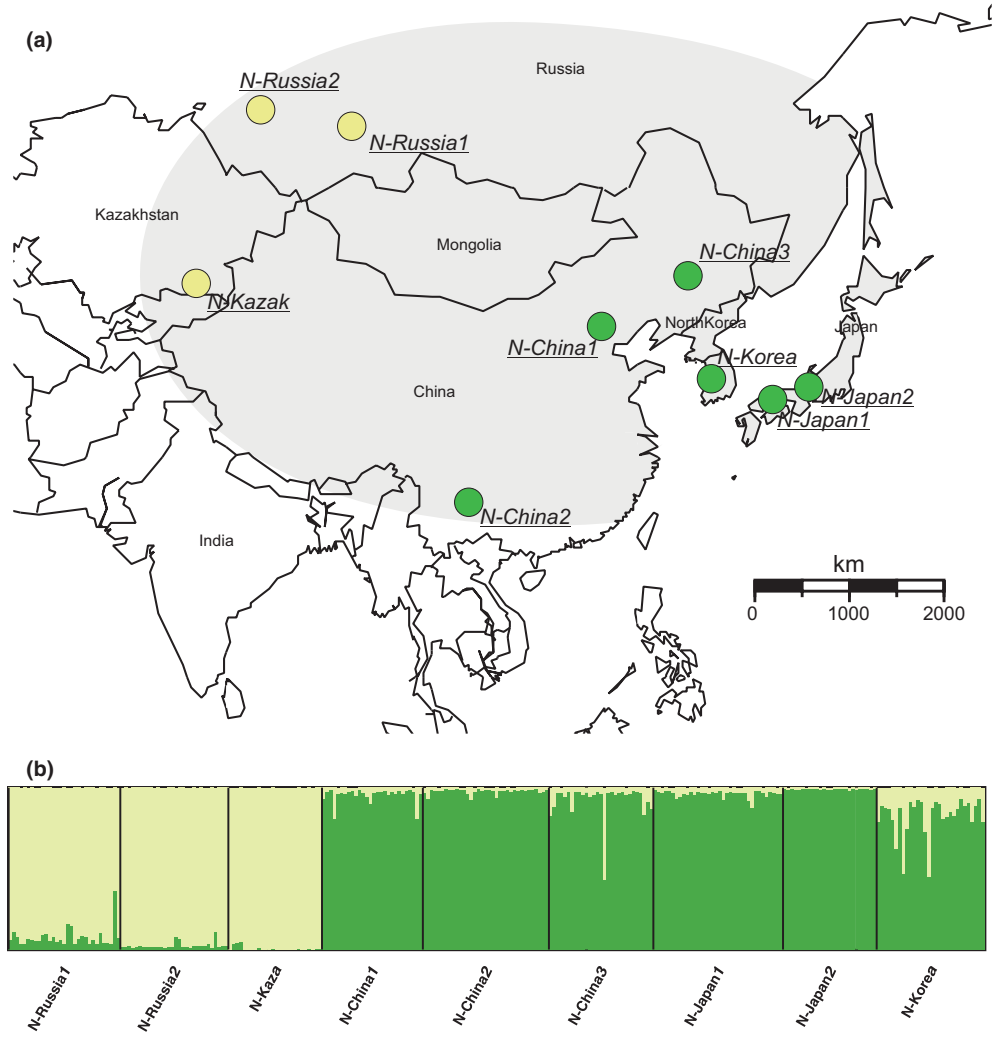

Fig. 1 Geographical origins and genetic clustering of sampled native populations of the Asian ladybird *Harmonia axyridis*. (a) Locality codes are underlined and in italics (see Table S1, Supporting information for details about the sites sampled). The shaded area approximately corresponds to the known native distribution of the species. Sampled sites with similar colours belong to the same genetic cluster, as assessed by the spatial group clustering method of Corander *et al.* (2004) implemented in BAPS software. (b) Ancestry estimation based on the Bayesian clustering method STRUCTURE in the native *Harmonia axyridis* samples, assuming two population clusters ($K = 2$). Each vertical line represents an individual, and each colour represents a cluster. Individuals are grouped by sampling location (at the bottom).

information (Hubisz *et al.* 2009). We used default values for all other parameters of the software. Each run consisted of a burn-in period of 10^5 Markov chain Monte Carlo (MCMC) iterations, followed by 10^6 MCMC iterations. We carried out 20 replicate runs for each value of the number (K) of clusters, set between 1 and 9 (i.e. the number of samples). The natural logarithm of the likelihood of the data $\ln(P(X|K))$ was calculated: it is expected to be high with a low variance for the true K (Pritchard *et al.* 2000).

We also used the clustering approach based on groups of individuals (i.e. population samples) implemented in BAPS 5.2 software (Corander *et al.* 2004), with the spatial coordinates of the samples as prior information. We conducted a series of 20 replicate runs, with the upper limit for the number of clusters set at 9 (the actual number of sampled native sites) for each run.

Finally we tested for isolation by distance patterns within the native range. The model of isolation by distance predicts that the genetic distances between populations, as measured by pairwise $F_{ST}/(1-F_{ST})$, increase approximately linearly with logarithm of spatial distances (Rousset 1997). We conducted this method for (i) the whole set of samples throughout the native area and (ii) for the genetic clusters inferred by the

aforementioned clustering approaches when the number of sites sampled within a cluster was sufficient. All the correlations between the natural logarithmic distances and the pairwise $F_{ST}/(1-F_{ST})$ were tested using Mantel tests with 10 000 permutations on the Spearman's rank correlation coefficient as implemented in SPAGeDI (Hardy & Vekemans 2002).

ABC analyses on controlled simulated data sets

We ran computer simulations to investigate the impact of genetic structure in the native range on the ability to determine the origin of an introduced population by ABC. All simulations of data sets and ABC analyses were performed with DIYABC v.1 (Cornuet *et al.* 2010). We focused on the simple case of a native range structured into two main population clusters, cluster A and cluster B (the situation identified for *H. axyridis*, see the Results section), within which substructure could exist. We considered that one population was sampled from each of the two native clusters (native sample A and native sample B) and one invasive population, whose origin depends on the scenario considered. Following our sampling design for real *H. axyridis* samples, the number of diploid individuals was fixed at 30 in each of the three population samples, and the data set consisted of genotypes for 18 statistically independent microsatellite loci. No migration between any pair of populations was assumed. Two sets of scenarios were devised, with three competing scenarios in each set:

The sampled origin scenario set (SO): the invasive population originated directly from one of the two sampled native populations (scenarios SA and SB for clusters A and B, respectively; Fig. 2) or from an admixture of these two populations (scenario SAB; Fig. 2).
The unsampled origin scenario set (UO): we simulated substructuring within each native cluster. The introduced population originated from an unsampled population belonging to cluster A or B (scenarios UA and UB for clusters A and B, respectively; Fig. 2) or from an admixture of the two unsampled populations (scenario UAB; Fig. 2).

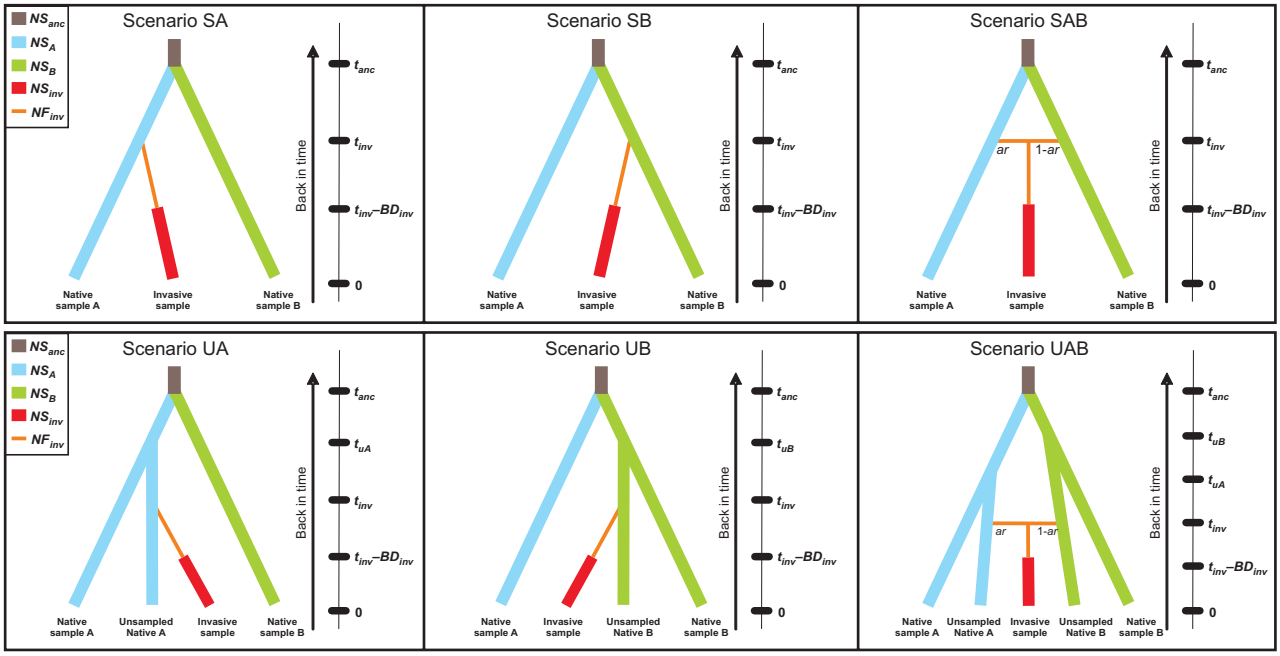

Fig. 2 Graphical representation of the two sets of competing scenarios used for the ABC analyses on controlled simulated data sets. Scenarios SA, SB and SAB correspond to the sampled origin scenario set (SO), and scenarios UA, UB and UAB correspond to the unsampled origin scenario set (UO). Historical and demographic parameters were the same for all introduction models. Time 0 is the sampling date. The invasive population was founded t_{inv} generations ago, had an effective number of founders, NF_{inv}, remaining constant for a few generations (bottleneck duration BD_{inv}) and then reached a larger stable effective population size, NS_{inv}. The two native clusters, A and B, merged in an ancestral unsampled population t_{anc} generations ago. Effective population sizes were stable over time and equal to NS_A, NS_B and NS_{anc} in the populations of clusters A, B and the ancestral population, respectively. When admixture occurs, the admixture rate *ar* is the genetic contribution of the native population from cluster A. In the unsampled origin scenario set (scenarios UA, UB and UAB), each unsampled native population merges into the sampled native population at time t_{uA} and t_{uB} (for clusters A and B, respectively), with t_{uA} and $t_{uB} \geq t_{inv}$ and $\leq t_{anc}$. For all models, populations were assumed to be isolated from each other, with no exchange of migrants. All parameters with associated distributions are described in Table 1.

ABC analysis was performed with historical, demographic and mutational parameter values drawn from the prior distributions described in Table 1 ('broad parameter distribution set') and by simulating two reference tables (i.e. set of summary statistics computed from data simulated according to each model, with parameters drawn from the prior distributions), one based on the three sampled origin (SO) scenarios and the other on the three unsampled origin (UO) scenarios. Each reference table contains 10^6 simulated microsatellite data sets per scenario. We summarized the genetic variation within and between populations, using a set of statistics that we successfully employed in previous ABC analyses (Cornuet *et al.* 2008; Guillemaud *et al.* 2010; Lombaert *et al.* 2010). For each population and each population pair, we used the mean number of alleles per locus, the mean expected heterozygosity (Nei 1987) and the mean allelic size variance. The other statistics used were the mean ratio of the number of alleles over the range of allele sizes (Garza & Williamson 2001), the pairwise F_{ST} values (Weir & Cockerham 1984), the mean individual assignment likelihoods of population *i* being assigned to population *j* and the maximum likelihood estimate of admixture proportion (Pascual *et al.* 2007). Overall, a total number of 31 summary statistics was used. All these statistics are thought to be informative in this study. Both lack and excess of summary statistics can be troublesome for model selection. Unfortunately, there is still no general rule or method as to which and how many summary statistics should be used in an ABC analysis. Recent improvements of ABC get round this problem using dimension reduction techniques, including a nonlinear feed-forward neural network (Blum & Francois 2010) and partial least squares regression (Wegmann *et al.* 2009). These types of algorithms have not been implemented yet in the DIYABC package. The added value of such algorithms in the context of complex models and large data sets remains, however, to be thoroughly tested (Bertorelle *et al.* 2010; But see Hamilton *et al.* 2005; Joyce & Marjoram 2008; Nunes & Balding 2010). Most importantly, it is worth stressing that the aforementioned dimension reduction techniques have been developed mostly for the estimation of posterior distribution of demographic parameters under a given scenario and not for the discrimination among a set of competing scenarios. Our set of statistics may not be optimal, which may reduce the ability of finding the true scenario, but we believe that we will still be able to properly compare the power of the UO and SO scenario sets.

For each of the six scenarios described previously, we simulated pseudo-observed genetic data sets (referred to hereafter as 'pods') with parameters drawn either from the same distributions as the large prior distributions (Table 1, 'broad parameter distribution set') or from an alternative narrower set of distributions mimicking the low level of differentiation and high level of diversity found within the native area of *H. axyridis* (see Results, Table 1, 'HA-like parameter distribution set', Table S2 and Fig. S1, Supporting information). For each of the two reference tables (the first based on the three SO scenarios and the second based on the three UO scenarios), we performed ABC analyses on 500 pods per scenario and per prior distribution set (total of 12 000 pods analysed). For each pod, we estimated the posterior probabilities of each of the three competing scenario by polychotomous logistic regression (Cornuet *et al.* 2008) on the 1% of data sets of the reference table closest to the pod. The selected scenario was that with the highest posterior probability value.

It should be stressed that the two competing scenario sets (SO and UO reference tables) are qualitatively equivalent, differing only in terms of the direct use (SO) or nonuse (UO) of the native samples as sources. Thus, when a pod is simulated according to a scenario absent from the reference table, we still have an expected result. For example, if the pod is simulated according to scenario SB (the invasive population originates from the sampled cluster B of the native area; Fig. 2) and the reference table is the UO table (which includes scenarios with unsampled origin UA, UB and UAB; Fig. 2), we would still expect the scenario selected to be UB, as both scenarios SB and UB indicate introduction from a population belonging to the native cluster B. This made it possible to determine which of the competing scenario sets, between SO and UO, was the most prone to error, in choices between introduction from cluster A, cluster B and an admixture of clusters A and B.

ABC analyses on real data sets

Origin of biocontrol strains. In a first set of ABC analyses, we independently considered each of the six biocontrol samples (five European and one American), using the two native population clusters inferred from the population structure analyses and an admixture between them (see Results section) as potential source populations. For each of the six analyses (one for each biocontrol sample) and for each inferred native cluster, we used the native samples displaying the lowest mean pairwise F_{ST} with nonnative populations (i.e. biocontrol and invasive populations; see Results section). The use of other native population samples did not change our conclusions (results not shown). Each ABC analysis was carried out twice: once with an SO scenario design and once with a UO scenario design. Parameter priors were

Table 1 Two sets of parameter distributions for the demographic, historical and mutation parameters used in ABC analyses on controlled simulated data sets

	Broad parameter distribution set						HA-like parameter distribution set					
Parameters	Distribution or value	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%	Distribution or value	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%
NS_A	Uniform [100–20 000]	10 023	10 010	NA	590	19 520	10 000	NA	NA	NA	NA	NA
NS_B	Uniform [100–20 000]	10 023	10 010	NA	590	19 520	15 000	NA	NA	NA	NA	NA
NS_{anc}	Uniform [100–20 000]	10 023	10 010	NA	590	19 520	10 000	NA	NA	NA	NA	NA
NS_{inv}	Uniform [100–20 000]	10 023	10 010	NA	590	19 520	Uniform [10 000–15 000]	12 520	12 521	NA	10 121	14 884
NF_{inv}	Loguniform [2–1000]	162	45	2	2	862	Loguniform [2–200]	41	17	2	2	178
BD_{inv}	Uniform [0–5]	2.5	2.5	NA	0	5	Uniform [1–5]	3	3	NA	1	5
t_{anc}	Uniform [100–3000]	1858	1940	NA	380	2,960	800	NA	NA	NA	NA	NA
t_{inv}	50	NA	NA	NA	NA	NA	50	NA	NA	NA	NA	NA
t_{uA} and t_{uB}	Loguniform [50–3000]	475	260	50	50	1980	Loguniform [50–800]	265	190	50	50	750
ar	Uniform [0.1–0.9]	0.5	0.5	NA	0.12	0.88	Uniform [0.1–0.9]	0.5	0.5	NA	0.12	0.88
Mean μ	Uniform [10^{-5}–10^{-3}]	5.0×10^{-4}	5.0×10^{-4}	NA	3.5×10^{-5}	9.8×10^{-4}	5.10^{-5}	NA	NA	NA	NA	NA
Mean *P*	Uniform [0.1–0.3]	0.2	0.2	NA	0.10	0.29	0.22	NA	NA	NA	NA	NA
Mean μSNI	Uniform [10^{-8}–10^{-4}]	5.0×10^{-5}	5.0×10^{-5}	NA	2.5×10^{-6}	9.7×10^{-5}	2.10^{-5}	NA	NA	NA	NA	NA

Historical and demographic parameters are detailed in Fig. 2. The microsatellite loci were assumed to follow a generalized stepwise mutation model (Estoup *et al.* 2002) with three parameters (Pascual *et al.* 2007; Verdu *et al.* 2009; Lombaert *et al.* 2010): the mean mutation rate (mean μ), the mean parameter of the geometric distribution (mean *P*) of length in terms of the number of repeats of mutation events and the mean mutation rate for single nucleotide instability (mean μSNI). Each locus has a possible range of 40 contiguous allelic states and is characterized by individual μ_{loc} drawn from a gamma (mean = mean μ and shape = 2), P_{loc} drawn from a gamma (mean = mean *P* and shape = 2) and μSNI_{loc} drawn from a gamma (mean = mean μSNI and shape = 2) distribution. Note that for a loguniform[*x*;y] distribution, log(*x*) and log(*y*) are the bounds of a uniform distribution. The two sets of parameters were used either to simulate a reference table (broad parameter distribution set) or to simulate pseudo-observed genetic data sets (broad parameter distribution set or HA-like parameter distribution set). The broad parameter distribution set aimed at considering a large set of possible levels of genetic structure and diversity. The HA-like parameter distribution set aimed at mimicking the low level of differentiation, and the high level of diversity found within the native area of *Harmonia axyridis* (see Fig. S1, Supporting information).

identical to those for the 'broad parameter distribution set' used in the simulation analyses (Table 1), assuming 2.5 generations per year for historical parameters and with a few exceptions because of the particular nature of biocontrol populations, which differ from invasive populations: biocontrol populations were assumed to maintain a low effective size remaining constant over time since their collection (i.e. NS_{inv} = log uniform distribution [10–1000]). The steps of the ABC were as described in the previous section.

Worldwide routes of invasion. As suggested by our ABC analyses on controlled simulated data sets (see Results section), geographical genetic structure in the native area of *H. axyridis* may have had an impact on the worldwide invasion routes inferred by Lombaert *et al.* (2010). We therefore performed ABC treatments on the worldwide *H. axyridis* data set, taking into account the possibility that the structured native area may have been incompletely sampled (see the Results section for more details). Because ABC methods for scenario comparison provide relative posterior probabilities with no information on the goodness of fit, we then used the model checking option of DIYABC 1.0 on the final worldwide invasion scenario inferred (as described by Cornuet *et al.* 2010) to determine whether this scenario matches well with the observed genetic data for *H. axyridis*. Briefly, if a model (here, an invasion scenario) fits the observed data correctly, then data simulated under this model with parameters drawn from their posterior distribution should be close to the observed data (Gelman *et al.* 1995) (pp. 159–163). The lack of fit of the model to the data with respect to the posterior predictive distribution can be measured by determining the frequency at which the observed summary statistics are extreme with respect to the test statistic (here, our simulated summary statistics) distribution (hence defining a tail-area probability or *P*-value, for each summary statistic). We simulated 2×10^6 data sets under the final *H. axyridis* invasion scenarios inferred in this study. We then obtained a 'posterior sample' of 2×10^4 values of the posterior distributions of parameters through a rejection step based on Euclidian distances and a linear regression post-treatment (Beaumont *et al.* 2002). We simulated 10^4 data sets with parameter values drawn, with replacement from this 'posterior sample'. Our set of test statistics included the summary statistics used for ABC analysis and two previously unused statistics: the shared allele distances (Chakraborty & Jin 1993) and $(\delta\mu)^2$ distances (Goldstein *et al.* 1995) between each population pair. We did this to reduce the conservative bias associated with the use of summary statistics previously selected for ABC analysis as test statistics (Cornuet *et al.* 2010). Each observed test statistic was compared with 10^4 simulated test statistics, and its p-value was calculated.

Results

Genetic variation within populations

We genotyped a total of 271 individuals originating from nine sites sampled within the native range (Table S1, Supporting information). The level of polymorphism estimated over all native sites was substantial, with a mean number of alleles per locus of 12.9. Allelic richness at 18 microsatellite loci, corrected for 20 individuals per sample (AR_{18}), ranged from 5.26 alleles per locus for the Kazakhstan sample (N-Kazak) to 6.59 for one of the Japanese samples (N-Japan1) (Fig. S2, Supporting information). The two European biocontrol samples for which AR_{18} could be calculated (EB-INRA06 and EB-Biotop) displayed much lower levels of diversity, with <2.4 alleles per locus. Other European biocontrol populations also displayed substantially lower diversities: allelic richness at 10 microsatellite loci corrected for 13 individuals per sample (AR_{10}) was at least 30% lower than that for the least diverse native sample. By contrast, the American biocontrol sample had an AR_{10} very similar to that of native populations. All invasive populations displayed high genetic diversities (Fig. S2, Supporting information). However, slightly lower diversity values were obtained for the African population (I-AF), and markedly lower diversity values were obtained for the South American population (I-SA).

Genetic variation between populations

Most pairwise comparisons between populations collected within the native area showed significant genotypic differentiation (Table S2, Supporting information). However, despite the large geographical distances between our sample sites (mean spatial distance = 2700 km), pairwise F_{ST} estimates were low, with a mean of 0.013 and values ranging from −0.006 to 0.035 (Table S2, Supporting information). By contrast, the level of genetic differentiation between European biocontrol samples was high, with a mean F_{ST} of 0.231. European biocontrol samples systematically yielded their lowest F_{ST} values with the Yunnan Chinese sample (N-China2) in the native range (mean F_{ST} between EB samples and N-China2 = 0.206). The American biocontrol sample had low F_{ST} values with native samples, the lowest being 0.017 with the Jilin Chinese sample (N-China3). Genetic differentiation within the invasive range was moderate (mean F_{ST} = 0.064), and the lowest F_{ST} values with populations from the native range were those for the N-China2 or N-China3 sample.

Native samples grouped together in the NJ tree (Fig. 3), with two subclusters, one including the three western samples (N-Russia1, N-Russia2 and N-Kaza) and the other the six eastern samples (N-China1, N-China2, N-China3, N-Japan1, N-Japan2 and N-Korea). Despite the long branches, all European biocontrol samples grouped together, tending to confirm a common origin of these samples.

STRUCTURE analyses (Pritchard *et al.* 2000) of *H. axyridis* individuals sampled within the native area provided consistent results over the 20 runs tested for each *K*. The natural logarithm of the likelihood of the data $\ln(P(X|K))$ increased from $K = 1$ to $K = 2$, for which it was maximal (Fig. S3, Supporting information). The proportion of ancestry from each of the two clusters of each native sample defined two geographical areas identical to those suggested by the NJ tree: a 'western cluster' and an 'eastern cluster' (Fig. 1). The use of other STRUCTURE models [with or without (i) admixture, (ii) correlated allele frequencies or (iii) sampling location information] gave similar results. $K = 1$ had the highest likelihood in a few cases, but this is not surprising given the low level of differentiation between populations. BAPS spatial clustering analysis (Corander *et al.* 2004) confirmed the existence of these two geographical clusters (Fig. 1). Mean pairwise F_{ST} was 0.000 and 0.007 within the western and eastern clusters, respectively, whereas the mean F_{ST} between populations from different clusters was 0.021 (Table S2, Supporting information). The level of differentiation within clusters was thus low, but still significant for many pairwise comparisons (Table S2, Supporting information), revealing slight structuring of the populations within the western and eastern clusters.

A significant correlation between the measures of genetic differentiation and geographical distance was found within the native area ($r^2 = 0.304$; $P < 10^{-2}$; slope = 0.008). However, this correlation most probably reflected the presence of two populational groups separated by large geographical distances rather than a continuous pattern of isolation by distance. In agreement with this, we did not found any significant correlation when considering only samples from Eastern Asia despite large geographical distances between the sampled sites (six samples: $r^2 = 0.009$; $P = 0.534$).

ABC in a structured native range: simulation-based study

We first considered the pseudo-observed data sets (pods) simulated with the 'broad parameter distribution set' (Table 1). When the true scenarios were those for

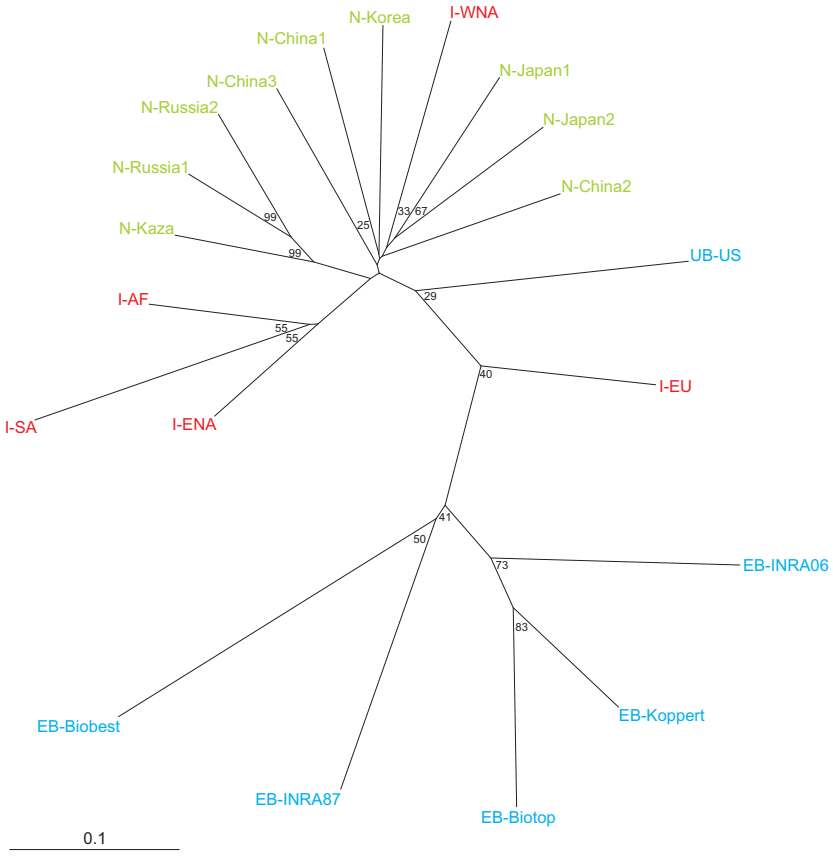

Fig. 3 Neighbour-joining tree for *Harmonia axyridis* population samples based on the chord distance of Cavalli-Sforza & Edwards (1967). Population code names are as in Table S1 (Supporting information). Native population samples are shown in green, invasive population samples in red and biocontrol samples in blue. Bootstrap values calculated over 1000 replications are given as percentages (only values >20% are shown).

which the actual source population of the invasive population had been sampled (SA, SB or SAB), the proportion of error (i.e. inference of an incorrect population cluster as the source) was very low, whatever the reference table used (SO or UO reference table, Table 2). By contrast, when the true scenarios were those in which the actual source of the invasive population had not been sampled (but a genetically different population from the same cluster, i.e. UA, UB or UAB), large error rates were obtained when using the reference table assuming that the source population has been sampled (the SO reference table; Table 2). These errors corresponded principally to incorrect selection of the admixed scenario SAB in 42.4% of cases when the true scenario was a single nonadmixed introduction (scenario UA or scenario UB). Error rates were markedly lower if it was assumed that the actual source population had not been sampled (the UO reference table; Table 2). In particular, the frequency of incorrect selection of the admixture scenario decreased to 8.4%.

We then considered the pods simulated with the 'HA-like parameter distribution set', chosen because they fitted the real *H. axyridis* situation more closely (Table S2 and Fig. S1, Supporting information). As for the 'broad parameter distribution set', results were generally better when the reference table was simulated assuming that the actual source had not been sampled (UO reference table; Table 2). Overall, error rates shown in Table 2 were remarkably high, especially when considering the 'HA-like parameter distribution set'. Most errors actually corresponded to pods obtained with *ar* (admixture rates) values close to the upper and lower limits of this parameter distribution (i.e. close to 0.1 or 0.9) and/or with small t_{anc} (splitting time between the two native population clusters from an ancestral population) values (see Table S3, Supporting information for type I and type II error rates when *ar* is intermediate and t_{anc} is large).

In conclusion, the UO reference table gave globally better inferences about invasion pathways, generating lower type I and type II error rates than the SO reference table (Table 2). In particular, we found that use of the UO reference table substantially reduced the risk of finding admixture between native source population clusters when there was none and only slightly increased the risk of selecting simple scenarios without admixture when there was admixture.

Origin of biocontrol strains

The native population samples displaying the lowest pairwise F_{ST} with all nonnative populations were the N-Kaza sample from the western cluster (mean F_{ST} = 0.139) and the N-China2 sample from the eastern cluster (mean F_{ST} = 0.114). We thus used these two native samples as representative of the western and

Table 2 Confidence in scenario selection based on ABC analyses on pseudo-observed data sets

Pods' parameter distribution set	Scenario considered	Competing scenario set (reference table)			
		Sampled origin (SO)		Unsampled origin (UO)	
		Type I error	Type II error	Type I error	Type II error
Broad	SA	0.032	0.052	0.024	0.052
	SB	0.024	0.036	0.016	0.060
	SAB	0.176	0.028	0.224	0.020
	S mean	0.077	0.039	0.088	0.044
	UA	0.440	0.056	0.096	0.100
	UB	0.424	0.040	0.096	0.064
	UAB	0.176	0.424	0.304	0.084
	U mean	0.347	0.173	0.165	0.083
HA-like	SA	0.120	0.146	0.024	0.196
	SB	0.140	0.128	0.056	0.164
	SAB	0.520	0.116	0.688	0.024
	S mean	0.260	0.130	0.256	0.128
	UA	0.392	0.112	0.208	0.200
	UB	0.320	0.092	0.192	0.200
	UAB	0.376	0.340	0.616	0.108
	U mean	0.363	0.181	0.339	0.169

The compared scenarios are detailed in Fig. 2, and parameter distributions are given in Table 1. Type I error: proportion of cases in which the scenario considered is excluded but is actually the true one. Type II error: proportion of cases in which the scenario considered is selected but is not the true one.

eastern native clusters, respectively, in all ABC analyses of real data sets. Using other samples gave qualitatively similar results (data not shown).

With the UO reference table, all ABC analyses performed on the separate biocontrol strains gave the highest posterior probability for the eastern native cluster being the origin (Table S4, Supporting information). Interestingly, when the SO reference table was used with the EB-INRA87 sample, the confidence interval for the probability of an eastern native cluster origin almost entirely overlapped with that for the admixed scenario. This made it impossible to distinguish between these two scenarios and highlights the advantages of using the UO reference table, as previously demonstrated in the simulations. Taking into account the inferred eastern native cluster origin of all biocontrol strains, we then showed, by ABC, that all European biocontrol strains were actually derived from the same ancestral population (see Table S5, Supporting information). This result confirmed that the main biofactories in Europe had been rearing *H. axyridis* samples originating from the same population collected by INRA in the eastern part of the native area in 1982.

Worldwide routes of invasion

As described by Lombaert *et al.* (2010), we performed five serial nested ABC analyses of invasion scenarios involving successive *H. axyridis* outbreaks (eastern North America, western North America, Europe, South America and then South Africa). Each analysis was thus carried out by simulating a new reference table taking into account the previous result. For example, the most likely origin of the ENA outbreak inferred in the first ABC analysis was included in the second analysis when this population became a potential source of the western North American outbreak. As for the parameters of the scenarios, the same prior were used at every steps (i.e. the posterior distributions of parameters from an analysis were not used as prior in the next analysis). Samples, priors and scenarios were as described by Lombaert *et al.* (2010), with a few exceptions: (i) we used the western and eastern native clusters as potential sources (with N-Kaza and N-China2 as representative samples); (ii) the competing scenarios involving a native sample were drawn from the UO scenario set design, that is taking into account the possibility that the actual native source population might not have been sampled and (iii) we added the American biocontrol sample (UB-US sample) as a potential source for the eastern and western North American outbreaks only. As reported previously, all biocontrol populations used in the analyses were derived from the eastern native cluster. Information about the set of scenarios considered and prior distributions are given in Table 3 and Table S6 (Supporting information), respectively. The worldwide routes of invasion of *H. axyridis* inferred by Lombaert *et al.* (2010) were confirmed by this new ABC analysis (Table 3). The main new findings were that the ENA outbreak was the result of an admixture between the eastern and the western native clusters (the use of other native population samples did not change our conclusions; Table S7, Supporting information), with each cluster making an approximately equal contribution (admixture rate estimated at 57% for the eastern native cluster, 95% CI: [16%–86%]). By contrast, the western North American outbreak originated exclusively from the eastern native cluster. The relationships of the samples in the NJ tree analysis were consistent with our ABC-based conclusions (Fig. 3).

To better evaluate to what extend the admixed native origin of ENA (referred to hereafter as 'scenario5' of analysis 1) could be trusted, we computed the type I and type II errors of this scenario in analysis 1. To do so, we simulated 100 pods per scenario. As expected from our previous simulation study, type I error rate was substantial with a value of 0.45. More importantly, however, type II errors were very low: the mean value was equal to 0.02 with values ranging from 0 to 0.07. It is worth pointing out that type I and type II errors do not take into account the posterior probability that was actually found with the real dataset. Following Fagundes *et al*. (2007), we used our estimations of type I and type II error rates to compute the probability that scenario5 was the correct scenario given our observation that $P_{\text{scenario5}} = 0.6242$ as $\Pr(P_{\text{scenario5}} \geq 0.6242 \mid \text{scenario5 is true}) / \sum_{i=1}^{10} \Pr(P_{\text{scenario5} \geq 0.6242} \mid \text{scenario}i \text{ is true}) = 0.8649$. These results reinforce the overall conclusion of our study, specially the admixed origin of ENA.

Model checking was carried out for the final selected worldwide invasion scenario that includes the five *H. axyridis* invasive outbreaks (see Fig. S4, Supporting information). We found that the observed values of only six summary statistics (none of those not used for ABC inferences) of a total of 279 (i.e. 2.2%) lay in the tail of the probability distribution of statistics calculated from the posterior simulation (i.e. $P < 0.05$ or $P > 0.95$). Because this analysis may suffer from nonindependence between the summary statistics, we also performed a principal component analysis (PCA). Figure S5 (Supporting information) illustrates the result of a PCA in the space of the summary statistics. It shows that PCA points simulated from the posterior predictive distribution nicely grouped and relatively well centred on the target point corresponding to the real data set. Altogether, these results indicate that the final selected worldwide invasion scenario provides a satisfying description of our real *H. axyridis* data set.

Table 3 Description of the five ABC analyses attempting to retrace step by step the worldwide invasion routes of *Harmonia axyridis* and posterior probabilities of the selected (most likely) scenarios in each ABC analysis

Analysis and target outbreak	Potential source populations (+admixture between all source pairs)	Number of scenarios	Selected scenario	Posterior probability of selected scenario
Analysis 1: Eastern North America	(1) Western native cluster; (2) Eastern native cluster; (3) American biocontrol; (4) European biocontrol	10	Admixture: western native cluster + eastern native cluster	0.6242 [0.5767,0.6717]
Analysis 2: Western North America	(1) Western native cluster; (2) Eastern native cluster; (3) American biocontrol; (4) European biocontrol; (5) Eastern North America	15	Eastern native cluster	0.4425 [0.3746,0.5105]
Analysis 3: Europe	(1) Western native cluster; (2) Eastern native cluster; (3) European biocontrol; (4) Eastern North America; (5) Western North America	15	Admixture: European biocontrol + eastern North America	0.8134 [0.7107,0.9160]
Analysis 4: South America	(1) Western native cluster; (2) Eastern native cluster; (3) European biocontrol; (4) Eastern North America; (5) Western North America	15	Eastern North America	0.9489 [0.9315,0.9663]
Analysis 5: Africa	(1) Western native cluster; (2) Eastern native cluster; (3) European biocontrol; (4) Eastern North America; (5) Western North America; (6) Europe; (7) South America	28	Eastern North America	0.8692 [0.7422,0.9961]

In analyses 1–4, posterior probabilities were calculated by polychotomous logistic regression on the simulations corresponding to the 1% smallest Euclidean distances. In analysis 5, because of computational issues owing to the large number of scenarios compared and summary statistics, posterior probabilities were calculated in two steps: (i) we calculated posterior probabilities on the 0.01% smallest Euclidean distances by the direct approach (Cornuet *et al.* 2008) and then removed 11 of the 28 compared scenarios for which the direct posterior probability was lower than 10^{-3} and (ii) we re-estimated the posterior probabilities of each of the 19 remaining competing scenarios by polychotomous logistic regression on the 0.5% smallest Euclidean distances. 95% confidence intervals (CI) are shown in square brackets. The 95% CI of the selected scenarios never overlapped with those of competing scenarios. The samples used in the analyses were as follows (see Table S1, Supporting information): western native cluster = N-Kazak; eastern native cluster = N-China2; American biocontrol = UB-US; European biocontrol = EB-INRA87; eastern North America = I-ENA; western North America = I-WNA; Europe = I-EU; South America = I-SA; Africa = I-AF.

Discussion

Sampling effort, genetic structure within the native range and false admixture

Comprehensive sampling of a species distribution area is often impossible for practical reasons (e.g. difficulties reaching some locations and/or poor knowledge of the exact range). This is the case for *H. axyridis*, which has a large and imperfectly known native area (e.g. Poutsma *et al.* 2008). Sampling effort and design are recurrent issues in population genetics, and several studies have shown that incomplete sampling may introduce bias into inferences relating to genetic structure and connectivity between populations (Waples & Gaggiotti 2006; Muirhead *et al.* 2008). Bayesian clustering methods have been improved to incorporate sampling scheme or space in models (Corander *et al.* 2004; Guillot *et al.* 2005; Hubisz *et al.* 2009), but they still cannot fully compensate for the absence of samples from a number of locations in the native range, for genetically structured populations.

Our results based on the analyses of controlled simulated data sets show that when an invasive species is genetically structured in its native area, the ability of ABC analyses to infer invasion routes correctly may be

jeopardized by incomplete sampling of the native area. In particular, in the simplest case when genetic structure exists within each of two main genetic clusters (as found for *H. axyridis*), ABC analyses often erroneously select a scenario of admixture between the two clusters when the true scenario is a simple origin, without admixture, from an unsampled population from one of the two clusters. Fortunately, an ABC package, such as DIYABC, can incorporate the possibility that the native samples used in the analysed data set are not the direct source populations, by modelling unsampled populations genetically differentiated from those samples to some extent. This approach led to a halving of type I and type II errors with the broader parameter distribution set used in our analyses. This was because of a large decrease in the frequency of erroneous selection of admixture scenarios. In addition, it is worth stressing that the simulation of unsampled populations may also make it easier to deal with too large numbers of slightly differentiated samples, which would make the already cumbersome ABC analyses impossible if they were all used as potential source populations.

The robust identification of admixture between two or more native population clusters as the origin of an invasive outbreak is crucial in the field of invasion biology. Admixture can produce new recombinant genotypes and compensate for the loss of diversity and additive genetic variance potentially following founder events. Admixture has therefore been identified as one of the key factors underlying invasion success, through its effects on the process of adaptation following establishment (Wares *et al.* 2005; Facon *et al.* 2006; Keller & Taylor 2008). It is therefore important to include admixture events between native potential sources as competing invasion route scenarios. This is particularly true given that classical population genetic statistics usually provide little information about this phenomenon and may be misleading in some cases (e.g. Lombaert *et al.* 2010). However, it is also essential to avoid the selection of false admixture scenarios in ABC analysis, to prevent erroneous interpretations of the evolutionary factors instrumental to the success of an invasion.

Genetic structure within the native range of H. axyridis

Our genetic analyses inferred a clear genetic structure of *H. axyridis* in its native area, consisting of two distinct geographical clusters with (i) Kazakhstan and central Siberia in the west and (ii) China, Korea and Japan in the east. Consistent with this pattern, an analysis of phenotypic traits, such as elytral patterns, indicated that *H. axyridis* could be divided into two geographical groups, with a dividing line between them located in the zone of the Baikal fracture (Dobzhansky 1933; Blekhman 2008; Blekhman *et al.* 2010). The observed genetic structure could be due to the occurrence of a natural barrier, such as the dry central Asia plateau and the Baikal rift zone, which may limit gene flow between the two parts of the native area of *H. axyridis*. Furthermore, as suggested by Blekhman *et al.* (2010), natural populations of *H. axyridis* may have split into two separate geographical groups during the last Pleistocene glaciation, subsequently merging during the Holocene warming, leading to hybridization around the Baikal fracture. Bayesian clustering methods such as STRUCTURE (Pritchard *et al.* 2000) tends to overestimate genetic structure when analysing a data set characterized by genetic isolation by geographical distances (IBD, e.g. Frantz *et al.* 2009). In the case of *H. axyridis*, however, the absence of significant correlation between genetic and geographical distances within the eastern cluster (where six of nine samples were collected) suggests that the significant correlation that was found considering all nine native samples most probably reflected the presence of two populational groups separated by large geographical distances rather than a continuous pattern of isolation by distance. In agreement with this, no cline was found on morphological traits within both groups despite strong differences between groups (Blekhman *et al.* 2010). Additional genetic data, particularly for samples collected from the intermediate area between the Russian administrative regions of Irkutsk and Amur and Mongolia, are required to shed light on the evolutionary factors involved in the genetic structure of *H. axyridis* in its native range.

As predicted, the ABC analyses performed to elucidate the native origin of the *H. axyridis* biocontrol samples were enhanced by the simulation of unsampled native populations. We found that all biocontrol samples originated from the eastern cluster of the native area of *H. axyridis*, and the validity of this result was further supported by subsequent ABC analysis, which confirmed, with a high posterior probability, that all of our European biocontrol samples were derived from a single ancestral population sampled from the native area of *H. axyridis* by INRA in 1982. This finding was also supported by the monophyletic relationship of these samples in the NJ tree. Finally, the inferred eastern origin of the biocontrol samples analysed here is consistent with the available historical information: the original European biocontrol population was sampled in China (Beijing, Ongagna *et al.* 1993) and the American biocontrol sample used in this study originated from the far east of Russia (Ussuriysk), according to the USDA database (http://www.ars-grin.gov/cgi-bin/nigrp/robo/f941s.pl?50902).

Worldwide invasion routes of H. axyridis*: what's new?*

The overall history of *H. axyridis* introduction inferred by Lombaert *et al.* (2010) was largely supported by these ABC analyses. We confirmed that the recent burst of worldwide invasion by *H. axyridis* has followed a bridgehead scenario, in which an invasive population in eastern North America acted as the source of the colonists invading the European, South American and African continents, with some admixture with a biocontrol strain in Europe. The two North American outbreaks were confirmed to have originated independently from the native area. The single American biocontrol sample included in our ABC analyses was not involved in any of the American outbreaks. However, although an accidental origin has been suggested before (Koch 2003), many different native populations have been imported and used for biocontrol purposes in North America, so a biocontrol origin cannot be excluded.

Posterior model checking for the final worldwide scenario of *H. axyridis* invasion gave good results. This suggests that the simulation of an incompletely sampled, but structured, native area in the analysis of *H. axyridis* invasion routes provides a good fit with the real dataset. In addition, these ABC analyses made it possible to make further inferences about the origin of the North American invasive populations. The source of the western North American (WNA) outbreak was the eastern cluster of native area of *H. axyridis*, whereas the ENA outbreak resulted from an admixture between the two native clusters, with each cluster making an approximately equal genetic contribution. This admixed origin of the ENA outbreak is of particular interest. First, this result was, to some extent, unexpected, given the known history of *H. axyridis* biocontrol (Tedders & Schaefer 1994; Krafsur *et al.* 1997) and current airline transportation networks (e.g. Tatem & Hay 2007), both of which identified eastern Asia as the most likely origin of the American outbreaks, as confirmed for the WNA outbreak. Second, the ENA population has served as a bridgehead for worldwide invasion by *H. axyridis*, and the finding that it is probably a genetically admixed population has important implications for our understanding of the key factors involved in the invasion success of this ladybird. Indeed, after decades of unsuccessful acclimation of biocontrol strains, genetic admixture in the ENA population may have facilitated adaptation by allowing the appearance of new gene combinations. However, it remains unknown whether admixture occurred before or after the introduction. The sampling and genotyping of populations from the contact zone between the two native clusters might provide us with some answers to this question. Finally, Facon *et al.* (2011) recently found that deleterious mutations at life history traits important for invasion success have been purged in the ENA bridgehead population, probably due to bottleneck event(s) of appropriate intensity. Additional studies are required to assess the relative and/or complementary roles of admixture, bottlenecks and purging in the success of this key *H. axyridis* outbreak.

Acknowledgements

We thank the SDAR service of Sophia-Antipolis, Nicole Bareste, Paulette Flacchi, Aurélie Blin and Alexandra Auguste for technical and administrative assistance. We also thank our colleagues T. Malausa, C. Borges, R. Koch, J. Lundgren, M. Majerus and R. Staals for additional *H. axyridis* samples. This work was funded by the French ANR Biodiversité #ANR-06-BDIV-008-01 Grant.

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E.L., T.G. and A.E.'s research focuses on the evolutionary biology of invading species, and are currently interested in both the application of methods for historical inferences using molecular markers and the evolution of life history traits in the context of biological invasions. C.E.T. is a PhD student investigating the evolutionary genetics of the *Harmonia axyridis* invasion. L.-J.L.H. is a lecturer in Evolutionary Biology, and is particularly interested in the evolutionary biology of invasions. J.L. is an entomologist, and his research focuses on taxonomy and phylogeny. S.W.'s interest is the ecological manipulation of predacious ladybird application in biological control. H.P.'s research interests are phylogeny and evolution of ladybird beetles (Coleoptera: Coccinellidae) Mass rearing and genetics of *Harmonia axrydis*, *Cryptolaemus montrouzieri*. I.G.'s research interests are in the field of molecular ecology, insects phylogenetics and genetics of insect-bacteria symbiosis. I.A.Z.'s main research interests are population genetics of *Adalia bipunctata* and *Harmonia axyridis* and interaction of insects with symbiotic bacteria. E.J.'s main research interests are phylogeny and evolution of interspecific interactions. R.L.P. is a secondary school teacher (biology) at Clifton College. She has extensive research expertise in intraguild interactions. A.M. is interested in the systematics and biology of mites. He works, among other things, on introduction and invasion pathways of alien spider mites. He is also interested in distribution patterns and modelling of potential distribution of these invasive species. J.L. works since 1970 on behavioural ecology and population dynamics of parasitoids, theoretical and practical aspects of biological control, IPM and sustainable crop production, and anatomy and sensory physiology of parasitoid ovipositors. P.C.'s research group focuses on the integrated management of arthropod pests, with emphasis on the potential of predatory insects and mites for augmentative biological control. His special attention goes out to risk assessment for biological control agents and to the development of rearing systems for predatory arthropods. N.B. is interested in ecology and life history traits of invasive ladybirds. W.J. is currently Director of the USDA, ARS, National Biological Control Laboratory in Mississippi. He has conducted research for over 30 years, focused on biological control of invasive insects and weeds, with special interest in the ecology and behavior of egg parasitoids of pest Heteroptera.

Data accessibility

Sample locations and microsatellite data: DRYAD entry doi:10.5061/dryad.7m0b37bn.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Simulated controlled data sets and corresponding levels of genetic diversity and levels of genetic differentiation between population clusters A and B (continuous circles) and between 'unsampled' populations within each cluster (dashed circles).

Fig. S2 Genetic diversity in the native, biocontrol and invasive population samples of *Harmonia axyridis*.

Fig. S3 Estimated number of population clusters in the native *Harmonia axyridis* samples according to the Bayesian clustering method STRUCTURE.

Fig. S4 Final selected worldwide invasion scenario which includes the five *H. axyridis* invasive outbreaks.

Fig. S5 Graphical representation of the result of a principal component analysis (PCA) in the space of the summary statistics performed on the final selected worldwide invasion scenario.

Table S1 Native, biocontrol and invasive population samples of *Harmonia axyridis* used in this study.

Table S2 Pairwise estimates of F_{ST} between all *Harmonia axyridis* population sample pairs.

Table S3 Confidence in scenario selection based on ABC analyses on pseudo-observed data sets.

Table S4 ABC posterior probabilities of the three competing scenarios modeling the genetic origin of each biocontrol sample within the native area of *Harmonia axyridis* (western native cluster, eastern native cluster or admixture of the western and eastern native clusters).

Table S5 ABC analyses to assess the relationship between the five European Biocontrol populations.

Table S6 Prior distributions of demographic, historic and mutation parameters used in ABC analyses attempting to retrace the worldwide routes of invasion of *Harmonia axyridis*.

Table S7 Inferred origin of the 'Eastern North American' (ENA) outbreak with various combinations of native samples representative of the Western and Eastern native clusters.

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Supplementary information

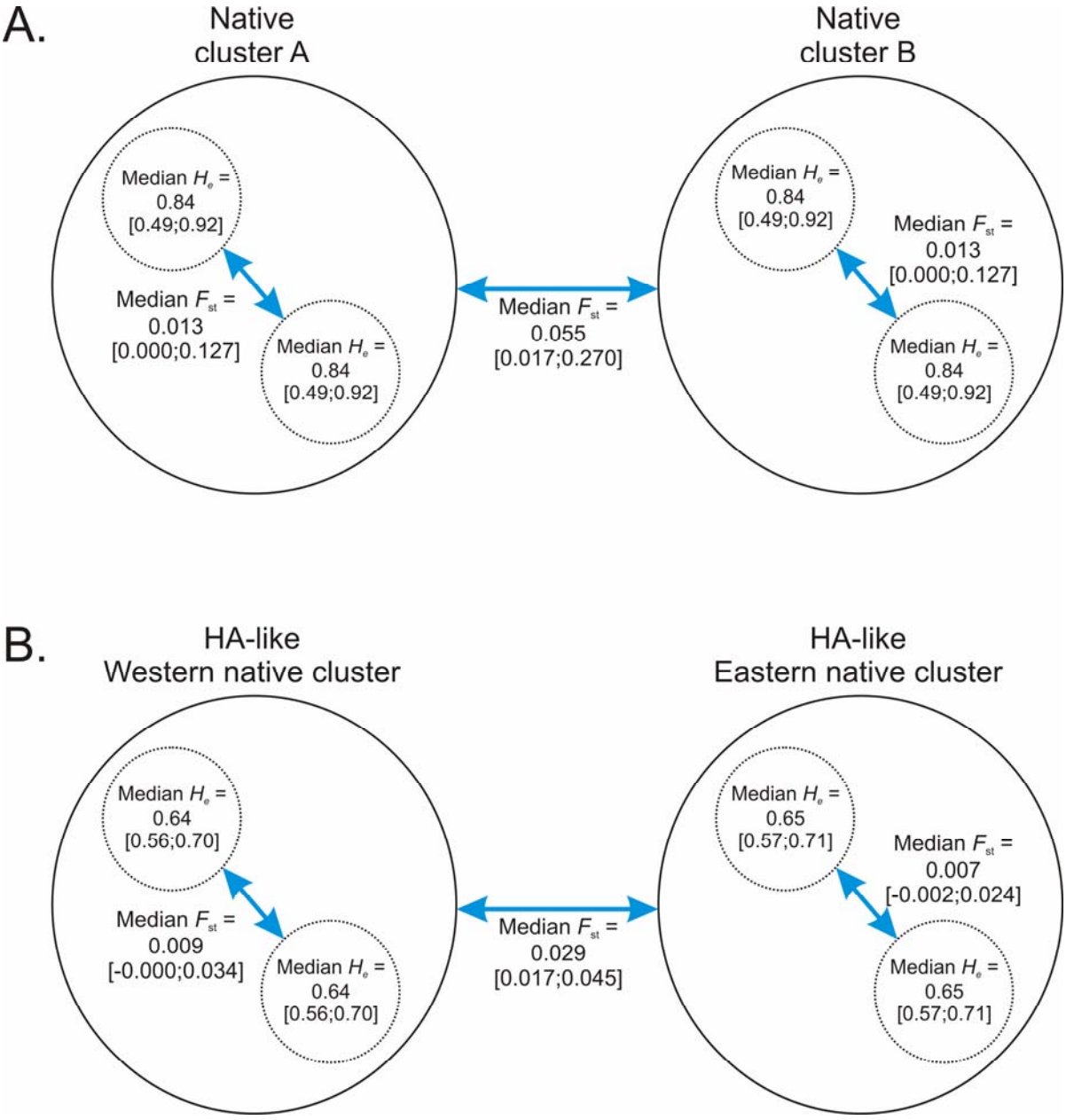

Figure S1: Simulated controlled data sets and corresponding levels of genetic diversity and levels of genetic differentiation between population clusters A and B (continuous circles) and between "unsampled" populations within each cluster (dashed circles).
(A) Pseudo-observed data sets were simulated with the "broad parameter distribution set" (Table 1); (B) pseudo-observed data sets were simulated with the "HA-like parameter distribution set" (Table 1). Median H_e, median F_{ST} and 95% confidence interval (in brackets) were calculated from 10,000 simulated data sets.

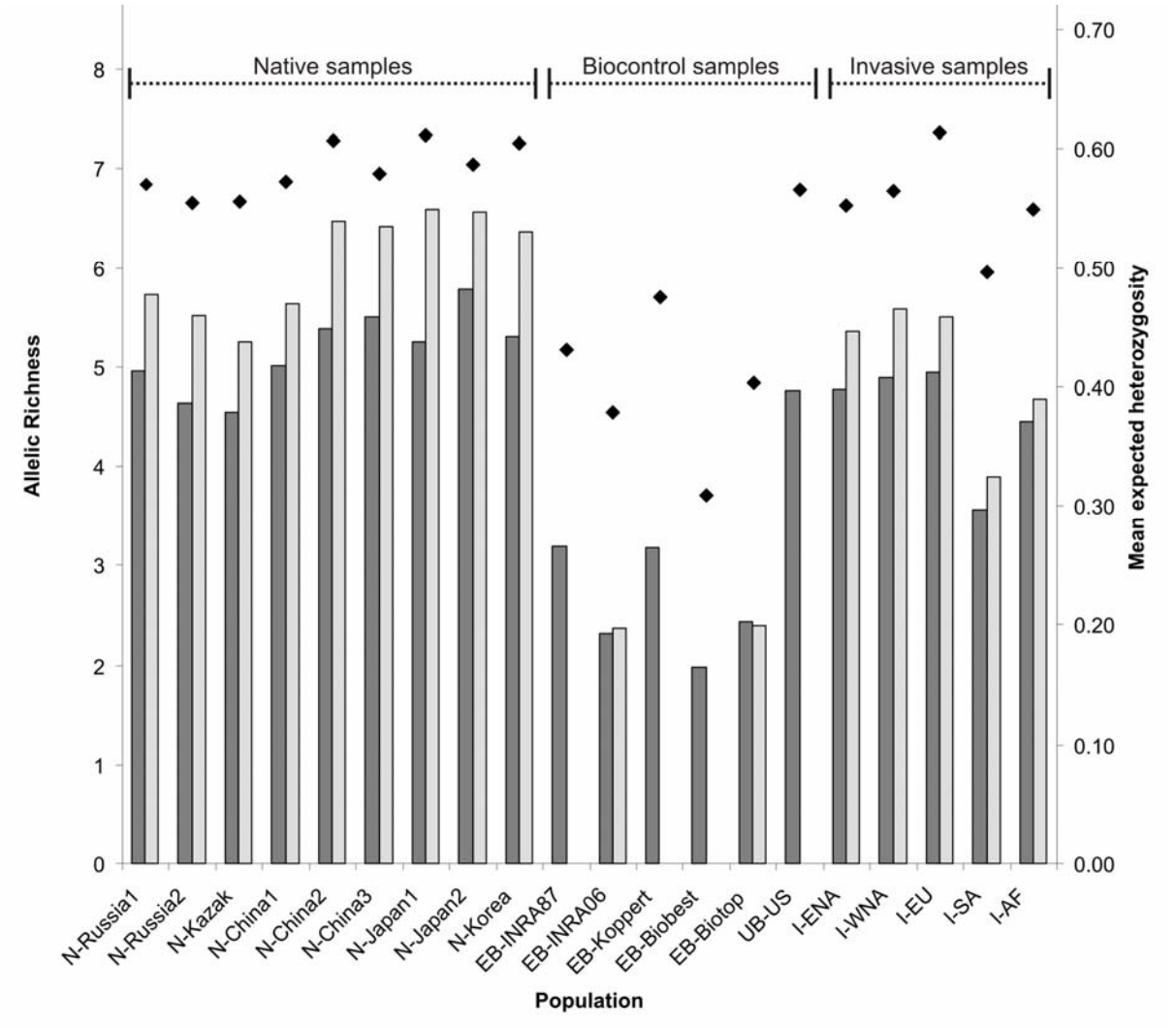

Figure S2: Genetic diversity in the native, biocontrol and invasive population samples of *Harmonia axyridis*.
Several measurements are displayed: expected heterozygosity (H_e; black diamond) and average genetic diversity estimated as allelic richness at either 18 microsatellite loci (AR_{18}; light gray bars) or a subset of 10 microsatellite loci (AR_{10}; dark gray bars). H_e are very similar for 10 and 18 loci, thus only the values for 18 loci are shown.

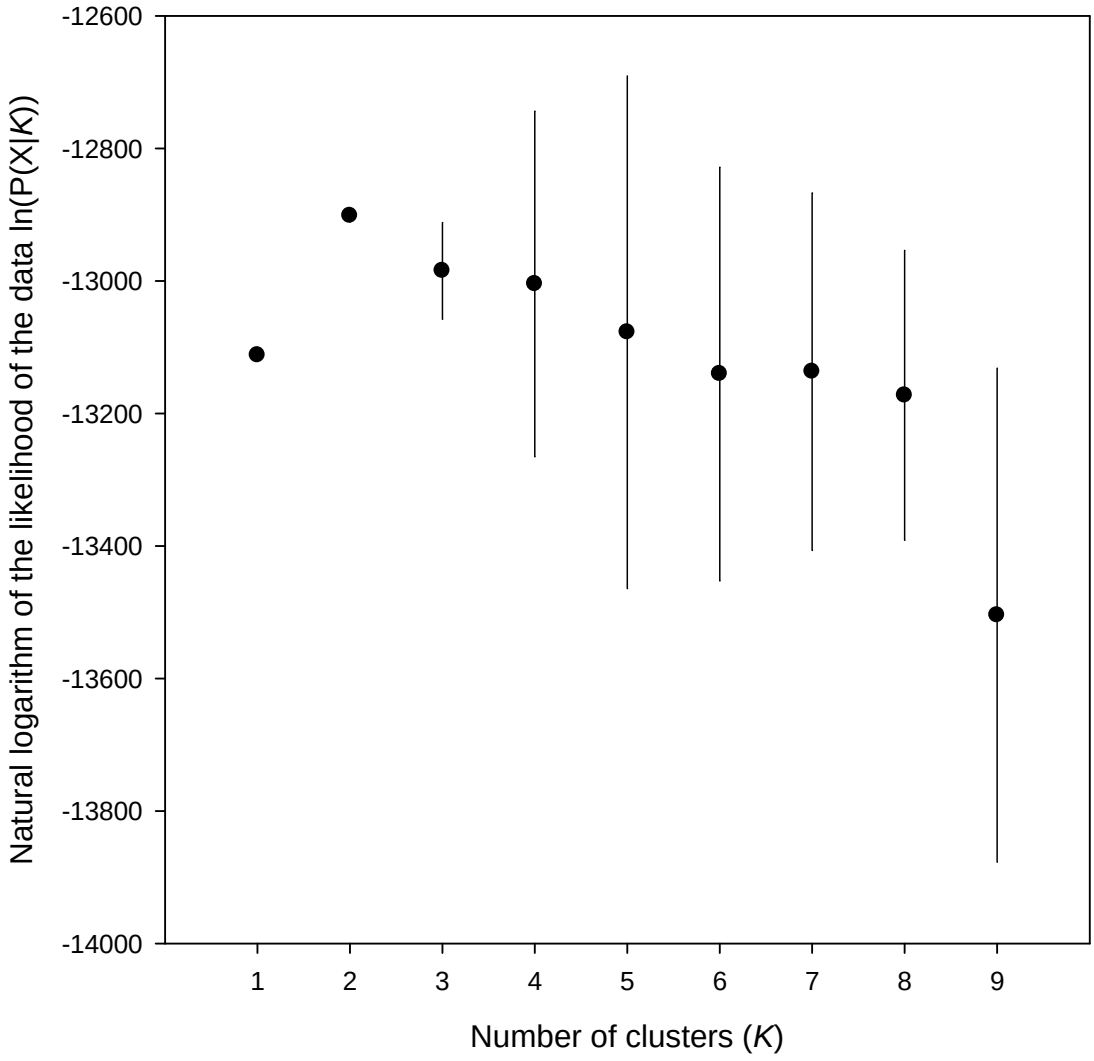

Figure S3: Estimated number of population clusters in the native *Harmonia axyridis* samples according to the Bayesian clustering method STRUCTURE.
The mean (±SD) natural logarithm of the likelihood of the data (LnP(X|*K*)) calculated over 20 STRUCTURE replicated runs is given for each value of the putative number of clusters (*K*). We used the admixture model with correlated allele frequencies and sampling location as prior information. The maximum value of LnP(X|*K*) is obtained for *K*=2. Note that the *ΔK* method (Evanno et al., 2005, *Molecular Ecology* 14:2611-2620) provides the same result (*K*=2).

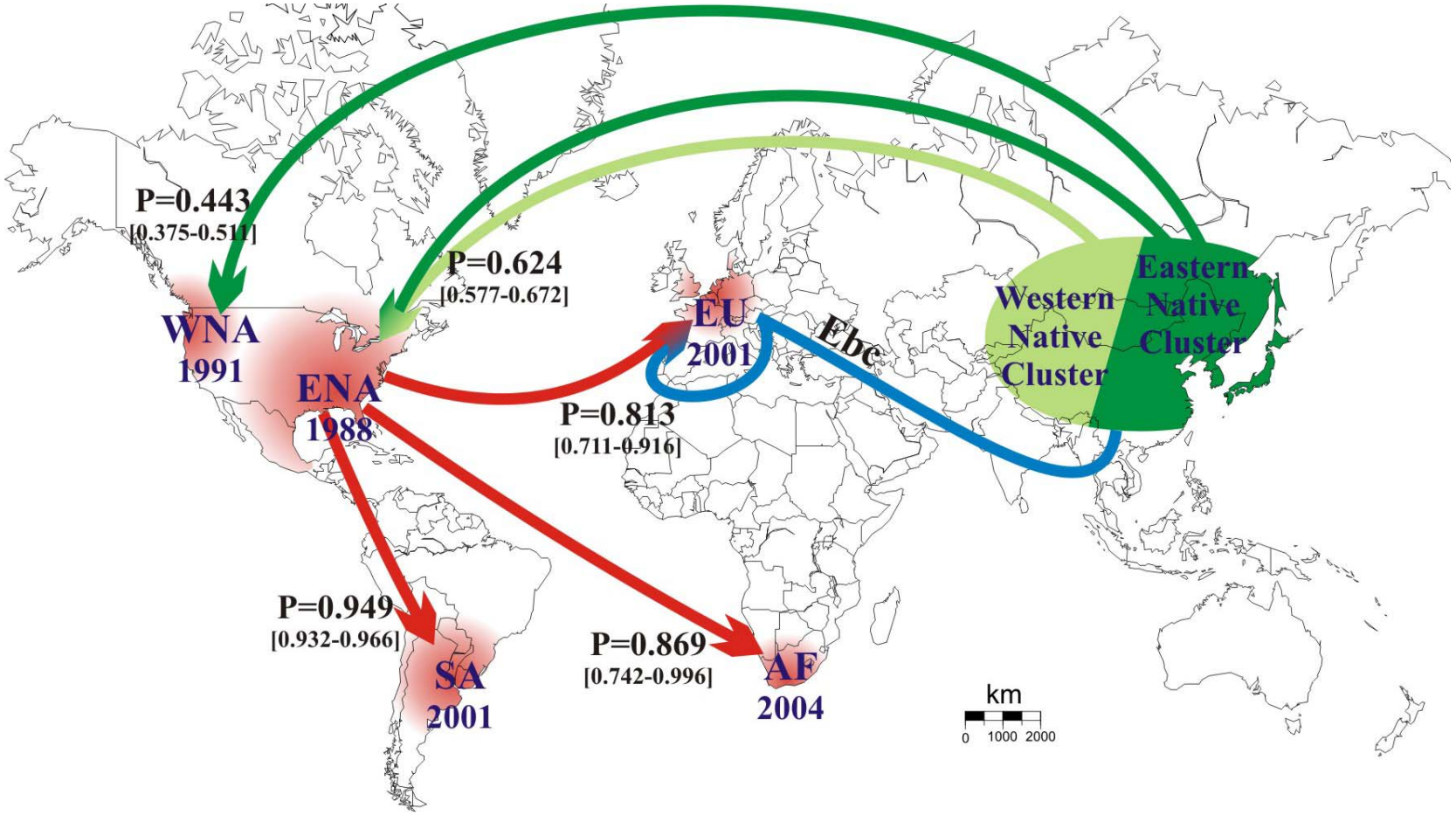

Figure S4: Final selected worldwide invasion scenario which includes the five *H. axyridis* invasive outbreaks.
Most likely scenario of invasions into eastern North America (ENA), western North America (WNA), South America (SA), Europe (EU) and Africa (AF) by *Harmonia axyridis*, deduced from analyses based on approximate Bayesian computation (see Table 3). For each outbreak, the arrow indicates the most likely invasion pathway and the associated posterior probability value (P), with 95% confidence intervals in brackets. Years of first observation of invasive populations are indicated. Initially collected from the native area in 1982, the European biocontrol strain is represented by a blue arrow (Ebc). The ranges of the two native clusters are drawn very roughly. Note that the ENA introduction may involve only one introduction from a naturally admixed native population.

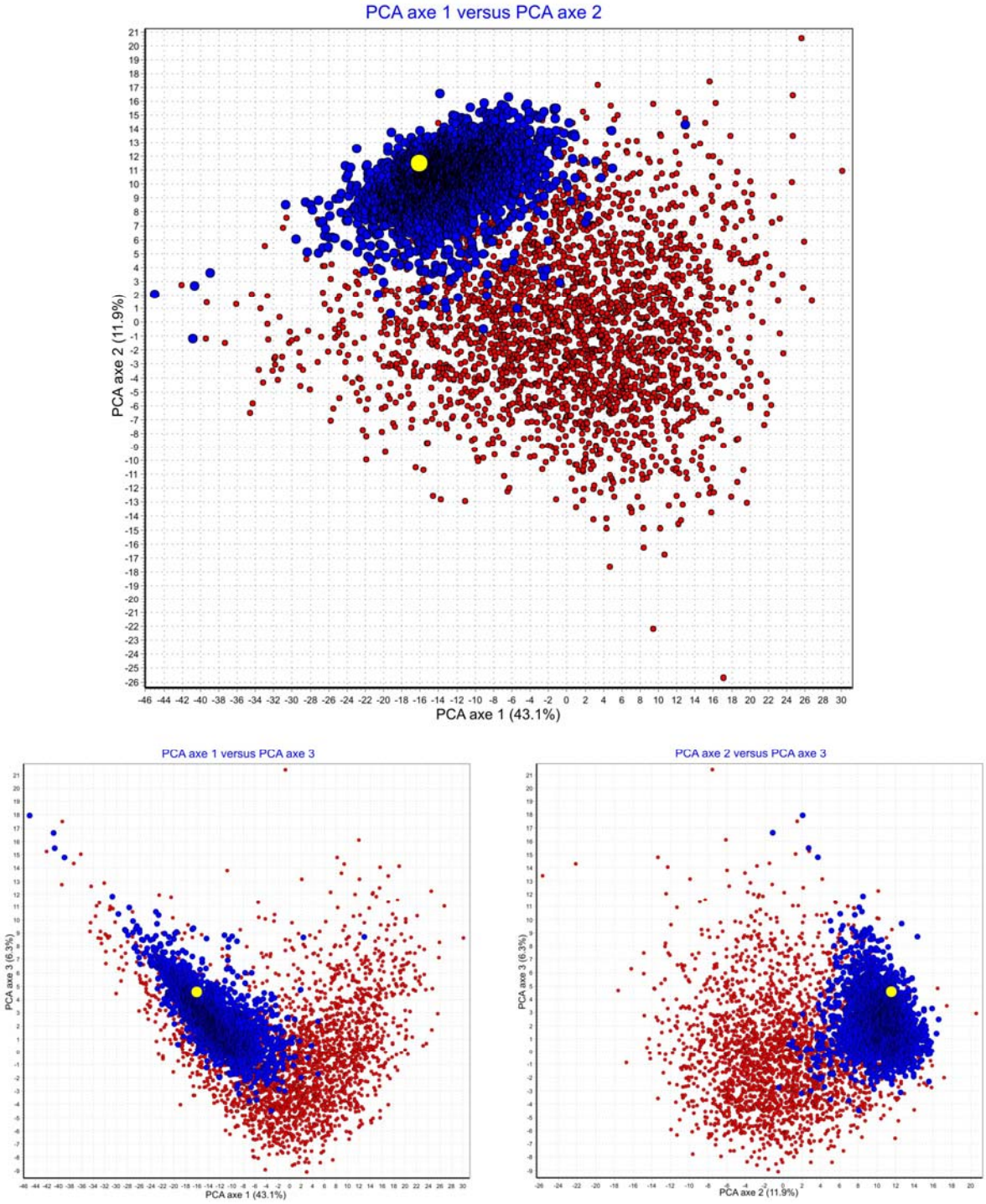

Figure S5: Graphical representation of the result of a principal component analysis (PCA) in the space of the summary statistics performed on the final selected worldwide invasion scenario. In this PCA, the observations are the simulated data sets and the variables are the summary statistics. Each blue dot corresponds to a dataset simulated with parameters drawn from the posterior distributions (2500 dataset are randomly shown here). The yellow dot corresponds to the real *H. axyridis* dataset. Each red dot corresponds to a dataset simulated with parameters drawn from the prior distributions (2500 dataset are randomly shown here).

Population code name	Sampling location and historical information	Geographic coordinates	Sampling date (month-year)	Number of genotyped individuals
N-Russia1	Native area: Abakan, Khakassia, Russia	53.73°N 91.46°E	10-2007	31
N-Russia2	Native area: Novosibirsk, Novosibirsk Oblast, Russia	55.04°N 82.93°E	10-2007	30
N-Kazak	Native area: Almaty, Oblys d'Almaty, Kazakhstan	43.24°N 76.95°E	10-2007	26
N-China1 (§)	Native area: Beijing, China	40.24°N 116.23°E	05-2007	28
N-China2 (§)	Native area: Shilin City, Yunnan Province, China	24.90°N 103.35°E	08-2007	35
N-China3	Native area: Changchun City, Jilin Province, China	43.88°N 125.31°E	11-2006	29
N-Japan1 (§)	Native area: Fuchu, Japan	34.57°N 133.24°E	09-2005	36
N-Japan2	Native area: Kyoto, Japan	35.01°N 135.77°E	08-2008	26
N-Korea	Native area: Daejeon, South Korea	36.37°N 127.35°E	11-1998	30
EB-INRA87 (§ *)	European Biocontrol: Rearing stock, INRA laboratory History: descendent from a population sampled in China in 1982	-	04-1987	18
EB-INRA06	European Biocontrol: Rearing stock, INRA laboratory History: descendant of EB-INRA87	-	11-2006	27
EB-Biotop	European Biocontrol: Rearing stock, Biotop biofactory History: strain obtained by Biotop from EB-INRA87 in 1995	-	11-2007	29
EB-Koppert (*)	European Biocontrol: Rearing stock, Koppert biofactory History: strain obtained by Koppert from EB-Biotop in 1997	-	07-2003	20
EB-Biobest (*)	European Biocontrol: Rearing stock, Ghent University laboratory (obtained from Biobest biofactory in 2003) History: strain obtained by Biobest from EB-Biotop in 1997	-	04-2007	27
UB-US (*)	North American Biocontrol: Insect collection USDA (http://www.ars-grin.gov/cgi-bin/nigrp/robo/f941s.pl?50902)	-	10-1980	25
I-ENA (§)	Invasive area: Joyce, Louisiana, USA	31.94°N 92.60°W	11-2007	34
I-WNA (§)	Invasive area: Sunnyside, Washington, USA	46.32°N 120.01°W	09-2007	42
I-EU (§)	Invasive area: Ghent, Belgium	51.05°N 3.71°E	10-2007	32
I-SA (§)	Invasive area: Curitiba, Brazil	25.45°S 49.24°W	02-2008	30
I-AF (§)	Invasive area: Somerset West, South Africa	34.03°S 18.83°E	05-2008	31

Table S1: Native, biocontrol and invasive population samples of *Harmonia axyridis* used in this study.

In the "Population code name" column, "§" indicates that the corresponding sample was previously used by Lombaert *et al.* (2010), and "*" indicates that the corresponding sample was stored dry and was thus difficult to genotype at some loci (see main text for details).

pop	N-China1	N-China2	N-China3	N-Japan1	N-Japan2	N-Korea	N-Russia1	N-Russia2	N-Kazak	EB-INRA87	EB-INRA06	EB-Koppert	EB-Biobest	EB-Biotop	UB-US	I-ENA	I-WNA	I-EU	I-SA
N-China2	0.010																		
N-China3	0.009	0.002																	
N-Japan1	0.007	0.000	0.005																
N-Japan2	0.007	0.006	**0.004**	**0.002**															
N-Korea	0.018	0.008	0.012	0.005	0.008														
N-Russia1	0.033	0.021	0.021	0.020	0.019	0.015													
N-Russia2	0.035	0.027	0.027	0.026	0.023	0.022	**-0.006**												
N-Kazak	0.024	0.015	0.013	0.015	0.012	0.014	**0.004**	**0.003**											
EB-INRA87	0.179	0.149	0.165	0.164	0.161	0.157	0.178	0.174	0.182										
EB-INRA06	0.261	0.216	0.240	0.225	0.231	0.230	0.262	0.277	0.271	0.231									
EB-Koppert	0.211	0.168	0.182	0.176	0.182	0.181	0.202	0.213	0.208	0.140	0.174								
EB-Biobest	0.319	0.305	0.309	0.308	0.318	0.319	0.313	0.312	0.325	0.243	0.381	0.312							
EB-Biotop	0.233	0.190	0.196	0.203	0.214	0.205	0.226	0.235	0.224	0.165	0.218	0.062	0.332						
UB-US	0.029	0.031	0.017	0.031	0.033	0.050	0.063	0.067	0.053	0.179	0.301	0.205	0.294	0.204					
I-ENA	0.029	0.017	0.012	0.016	0.022	0.034	0.036	0.041	0.028	0.188	0.255	0.207	0.335	0.224	0.048				
I-WNA	0.026	0.008	0.009	0.011	0.016	0.019	0.030	0.038	0.019	0.184	0.239	0.197	0.325	0.206	0.047	0.023			
I-EU	0.061	0.043	0.046	0.048	0.049	0.055	0.069	0.080	0.069	0.111	0.158	0.070	0.231	0.120	0.068	0.059	0.064		
I-SA	0.114	0.096	0.097	0.097	0.101	0.103	0.122	0.143	0.116	0.279	0.312	0.248	0.418	0.281	0.170	0.064	0.107	0.109	
I-AF	0.039	0.032	0.030	0.034	0.042	0.030	0.031	0.041	0.034	0.188	0.286	0.209	0.337	0.221	0.065	0.023	0.037	0.066	0.089

Table S2: Pairwise estimates of F_{ST} between all *Harmonia axyridis* population sample pairs.
F_{ST} in bold typeface indicates non significant pair-wise differentiation, as assessed in Fisher's exact test with correction for multiple comparisons.

		Competing scenario set (reference table)			
		Sampled origin (*SO*)		Unsampled origin (*UO*)	
Pods' Parameter distribution set	Scenario considered	Type I error	Type II error	Type I error	Type II error
Broad with	SA	0.030	0.000	0.000	0.010
ar = 0.5 and	SB	0.020	0.005	0.010	0.005
t_{anc} = 3000	SAB	0.010	0.025	0.030	0.005
	S mean	**0.020**	**0.010**	**0.013**	**0.007**
	UA	0.400	0.010	0.120	0.030
	UB	0.400	0.015	0.100	0.050
	UAB	0.050	0.400	0.130	0.095
	U mean	**0.283**	**0.142**	**0.117**	**0.058**

Table S3: Confidence in scenario selection based on ABC analyses on pseudo-observed data sets. Results obtained with (i) intermediate admixture rate and (ii) high splitting time between the two native population clusters.

The compared scenarios are detailed in Figure 2. Parameter distributions are given in Table 1, except for *ar* and t_{anc} which are here fixed to 0.5 and 3000 respectively. Type I error: proportion of cases in which the scenario considered is excluded but is actually the true one. Type II error: proportion of cases in which the scenario considered is selected but is not the true one.

		Competing scenario set used	
Biocontrol sample	Scenarios	Sampled origin (*SO*)	Unsampled origin (*UO*)
EB-INRA87	Western cluster	0.0191 [0.0117,0.0266]	0.2441 [0.1940,0.2943]
	Eastern cluster	***0.4924 [0.4347,0.5501]***	**0.4946 [0.4426,0.5465]**
	Admixture West/East	***0.4884 [0.4317,0.5452]***	0.2613 [0.2228,0.2998]
EB-INRA06	Western cluster	0.0081 [0.0040,0.0121]	0.0861 [0.0587,0.1135]
	Eastern cluster	**0.7527 [0.6974,0.8079]**	**0.6950 [0.6397,0.7503]**
	Admixture West/East	0.2392 [0.1852,0.2933]	0.2189 [0.1731,0.2647]
EB-Biotop	Western cluster	0.0022 [0.0010,0.0035]	0.0435 [0.0275,0.0596]
	Eastern cluster	**0.8852 [0.8574,0.9129]**	**0.7878 [0.7471,0.8284]**
	Admixture West/East	0.1126 [0.0852,0.1400]	0.1687 [0.1333,0.2041]
EB-Koppert	Western cluster	0.0012 [0.0006,0.0018]	0.0751 [0.0547,0.0954]
	Eastern cluster	**0.7888 [0.7521,0.8255]**	**0.6987 [0.6580,0.7394]**
	Admixture West/East	0.2100 [0.1734,0.2466]	0.2263 [0.1914,0.2611]
EB-Biobest	Western cluster	0.0328 [0.0226,0.0431]	0.1947 [0.1589,0.2305]
	Eastern cluster	**0.6047 [0.5620,0.6474]**	**0.5024 [0.4603,0.5444]**
	Admixture West/East	0.3625 [0.3215,0.4035]	0.3029 [0.2670,0.3389]
UB-US	Western cluster	0.0004 [0.0002,0.0006]	0.1047 [0.0816,0.1279]
	Eastern cluster	**0.7557 [0.7177,0.7938]**	**0.7470 [0.7163,0.7776]**
	Admixture West/East	0.2439 [0.2058,0.2819]	0.1483 [0.1287,0.1679]

Table S4: ABC posterior probabilities of the three competing scenarios modeling the genetic origin of each biocontrol sample within the native area of *Harmonia axyridis* (western native cluster, eastern native cluster or admixture of the western and eastern native clusters).
95% confidence intervals (CI) are shown in square brackets. Results are given for a sampled origin scenario design (*SO*) or an unsampled origin scenario design (*UO*); see main text for details. Posterior probabilities, with CI, of the selected scenarios are shown in bold typeface (and in bold/italic typeface when the CIs of several scenarios overlap).

	Scenarios	Posterior probabilities
S1	Native ➔ (EB-INRA87); EB-INRA87 ➔ (EB-INRA06, EB-Biotop, EB-Koppert, EB-Biobest)	0.999 [0.995,1.000]
S2	Native ➔ (EB-INRA87, EB-Koppert); EB-INRA87 ➔ (EB-INRA06, EB-Biotop, EB-Biobest)	0.000 [0.000,0.000]
S3	Native ➔ (EB-INRA87, EB-Biobest); EB-INRA87➔ (EB-INRA06, EB-Biotop, EB-Koppert)	0.001 [0.000,0.005]
S4	Native ➔ (EB-INRA87, EB-INRA06); EB-INRA87➔ (EB-Biotop, EB-Koppert, EB-Biobest)	0.000 [0.000,0.000]
S5	Native ➔ (EB-INRA87, EB-Biotop); EB-INRA87 ➔ (EB-INRA06, EB-Koppert, EB-Biobest)	0.000 [0.000,0.000]
S6	Native ➔ (EB-INRA87, EB-Koppert, EB-Biobest); EB-INRA87 ➔ (EB-INRA06, EB-Biotop)	0.000 [0.000,0.000]
S7	Native ➔ (EB-INRA87, EB-INRA06, EB-Koppert); EB-INRA87 ➔ (EB-Biotop, EB-Biobest)	0.000 [0.000,0.000]
S8	Native ➔ (EB-INRA87, EB-Biotop, EB-Koppert); EB-INRA87 ➔ (EB-INRA06, EB-Biobest)	0.000 [0.000,0.000]
S9	Native ➔ (EB-INRA87, EB-INRA06, EB-Biobest); EB-INRA87 ➔ (EB-Biotop, EB-Koppert)	0.000 [0.000,0.000]
S10	Native ➔ (EB-INRA87, EB-Biotop, EB-Biobest); EB-INRA87 ➔ (EB-INRA06, EB-Koppert)	0.000 [0.000,0.000]
S11	Native ➔ (EB-INRA87, EB-INRA06, EB-Biotop); EB-INRA87 ➔ (EB-Koppert, EB-Biobest)	0.000 [0.000,0.000]
S12	Native ➔ (EB-INRA87, EB-INRA06, EB-Koppert, EB-Biobest); EB-INRA87 ➔ (EB-Biotop)	0.000 [0.000,0.000]
S13	Native ➔ (EB-INRA87, EB-Biotop, EB-Koppert, EB-Biobest); EB-INRA87 ➔ (EB-INRA06)	0.000 [0.000,0.000]
S14	Native ➔ (EB-INRA87, EB-INRA06, EB-Biotop, EB-Koppert); EB-INRA87 ➔ (EB-Biobest)	0.000 [0.000,0.000]
S15	Native ➔ (EB-INRA87, EB-INRA06, EB-Biotop, EB-Biobest); EB-INRA87 ➔ (EB-Koppert)	0.000 [0.000,0.000]
S16	Native ➔ (EB-INRA87, unsampled pop); EB-INRA87 ➔ (EB-INRA06, EB-Biotop); unsampled pop ➔ (EB-Koppert, EB-Biobest)	0.000 [0.000,0.000]
S17	Native ➔ (EB-INRA87, unsampled pop); EB-INRA87 ➔ (EB-Koppert, EB-Biobest); unsampled pop ➔ (EB-INRA06, EB-Biotop)	0.000 [0.000,0.000]
S18	Native ➔ (EB-INRA87, EB-INRA06, EB-Biotop, EB-Koppert, EB-Biobest)	0.000 [0.000,0.000]

Table S5: ABC analyses to assess the relationship between the five European Biocontrol populations.
For formal assessment of the relationship between the European biocontrol populations, we used ABC to compare 18 competing scenarios representing of a gradient from a scenario of full dependence (i.e. all samples derived from the 1982 INRA population: S1) to a scenario of full independence (i.e. all samples independently collected within the native area: S18). For instance, in scenario S3, the biocontrol populations EB-INRA87 and EB-Koppert were independently collected from the native area and all three biocontrol populations EB-INRA06, EB-Biotop and EB-Koppert originate from the same biocontrol population, EB-INRA87. Building on the results we obtained considering each biocontrol population separately (Table S4), we used the eastern native population cluster as the native source population for all the scenarios compared (we used N-China2 as the native sample) and the *UO* scenario set design.

Parameter priors were those used in the "broad parameter distribution set" used in simulation analyses (Table 1), assuming 2.5 generations per year and with biocontrol populations assumed to maintain a low effective size that has remained constant over time since their collection (i.e. log uniform distribution [10;1000]). $5x10^5$ microsatellite data sets per scenario were simulated. The steps of the ABC were as described in the main text, section "*ABC analyses on controlled simulated data sets*". Posterior probability of each scenario is given with 95% confidence interval between brackets.
We found, with a very high posterior probability (P = 0.999; 95% CI = 0.995 – 1.000) that all European biocontrol strains were derived from the same population (i.e. S1 = full dependence scenario). We also performed a model checking analysis under the selected scenario (S1). To do so, 10^6 data sets were simulated. A "posterior sample" of 10^4 values of the posterior distributions was obtained. We then simulated 10^4 data sets with parameter values drawn, with replacement, from this "posterior sample". Our set of test statistics were the one described in the main text. We found that only 8 statistics out of 144 were in the tail of the distributions of the statistics simulated from the posterior predictive distributions. Altogether, these results confirmed that the main biofactories in Europe had been rearing *H. axyridis* samples originating from the same population collected by INRA in the eastern part of the native area in 1982.

Parameter	Distribution	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%
NS_i and NS_j	Uniform [100 – 20,000]	10,056	10,040	NA	640	19,490
NS_k	Loguniform [10 – 1,000]	506	508	NA	35	975
NF_i	Loguniform [2 – 1,000]	162	45	2	2	862
BD_i	Uniform [0 – 5]	2.5	2.5	NA	0	5
ar	Uniform [0.1 – 0.9]	0.5	0.5	NA	0.12	0.88
t_i	Uniform [x_i – x_i+5]	DV	DV	NA	DV	DV
tbc_i	Loguniform [t_i – 93]	DV	DV	DV	DV	DV
tu_j	Loguniform [tbc_i – 3000]	DV	DV	DV	DV	DV
t_{anc}	Uniform [100 – 3000]	1,858	1,940	NA	380	2,960
mean μ	Uniform [10^{-5} – 10^{-3}]	$5.0\text{x}10^{-4}$	$5.0\text{x}10^{-4}$	NA	$3.5\text{x}10^{-5}$	$9.8\text{x}10^{-4}$
mean *P*	Uniform [0.1 – 0.3]	0.2	0.2	NA	0.10	0.29
mean μSNI	Uniform [10^{-8} – 10^{-4}]	$5.0\text{x}10^{-5}$	$5.0\text{x}10^{-5}$	NA	$2.5\text{x}10^{-6}$	$9.7\text{x}10^{-5}$

Table S6: Prior distributions of demographic, historic and mutation parameters used in ABC analyses attempting to retrace the worldwide routes of invasion of *Harmonia axyridis*.
Notes: Populations *i* are invasive populations, clusters *j* are native clusters (either western or eastern cluster) and populations *k* correspond to biocontrol strains (i.e. laboratory reared populations). Times were translated into numbers of generations running back in time and assuming 2.5 generations per year. *NS* = stable effective population size (number of diploid individuals); *NF* = effective number of founders during an introduction step lasting *BD* generation(s); *ar* = admixture rate (only for scenarios with admixture); t_i = introduction date of invasive populations *i* with limits x_i fixed from dates of first observation, assuming 2.5 generations per year; *tbc* = creation date of unsampled biocontrol strain for eastern and western North American populations bounded by the dates of the first observation of the invasive population (corresponding to a direct introduction into the wild) and the number of generations from 1970, the start date of a period of intense *H. axyridis* biocontrol activity in the USA; tu_j = in native cluster j, date of merging of the source unsampled native population with the sampled native population (this parameter is included only in the model in which the scenario contains one or both native populations as possible source(s)); t_{anc} = date of the merging of the two native populations into an ancestral unsampled population (with condition $tu_j \leq t_{anc}$). For microsatellite marker parameters, parameters were as in Table 1. All prior quantities presented were calculated from 100,000 values. NA = not applicable; DV = may take different values.

Western native sample	Eastern native sample	Selected scenario	Posterior probability of selected scenario
N-Kazak	N-China2	Admixture: western + eastern native clusters	0.6242 (see Table 3)
N-Kazak	N-China1	Admixture: western + eastern native clusters	0.6279
N-Russia2	N-China2	Admixture: western + eastern native clusters	0.7068
N-Russia2	N-China1	Admixture: western + eastern native clusters	0.6937

Table S7: Inferred origin of the "Eastern North American" (ENA) outbreak with various combinations of native samples representative of the Western and Eastern native clusters. Posterior probabilities are obtained from ABC analyses with 10 competing scenarios as described in the main text.

Notes: N-Kazak and N-China2 are the samples which have the mean smallest F_{ST} values with invasive and biocontrol populations. N-Russia2 and N-China1 are the samples which have the mean highest F_{ST} values with invasive and biocontrol populations (see Table S2).

ANNEXE III

Article 3 (2011)

Brown P, Thomas C, **Lombaert E**, Jeffries D, Estoup A, Lawson Handley L-J (2011) The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. *BioControl* **56**, 623-641.

Précisions sur le rôle de co-auteur :

- ➔ Co-rédaction avec C.E. Thomas et A. Estoup de la section « Investigating the invasion routes of *H. axyridis* using genetic analyses ».
- ➔ Co-rédaction avec P.M.J. Brown de la sous-section « The role of anthropogenic dispersal » dans la section « Dispersal of *H. axyridis* »
- ➔ Participation globale à la rédaction du manuscrit.

BioControl (2011) 56:623–641
DOI 10.1007/s10526-011-9379-1

The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion

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Received: 22 January 2011 / Accepted: 19 May 2011

Abstract Released as a biological control agent of aphids and coccids, *Harmonia axyridis* (Coleoptera: Coccinellidae) has spread from Asia to four additional continents. Since 1988 *H. axyridis* has established in at least 38 countries in its introduced range: three countries in North America, six in South America, 26 in Europe and three in Africa. In different continents the species has spread at rates estimated between 100 and 500 km year^{-1}. Here, the global spread of *H. axyridis* is thoroughly reviewed. Mechanisms of short- and long-distance dispersal in coccinellids are discussed, as are the reasons for them, with particular emphasis on *H. axyridis*. Dispersal via anthropogenic means has been particularly important in the case of *H. axyridis*. Preliminary studies investigating the invasion routes of *H. axyridis* using genetic analyses (involving both microsatellite and mitochondrial DNA) are outlined.

Keywords Alien species · Biological control · Coccinellidae · Coleoptera · Dispersal mechanism · Harlequin ladybird · Invasive species · Multicolored Asian lady beetle · Range expansion

Handling Editor: Patrick De Clercq

This research was conducted as part of a collaboration of scientists working on *Harmonia axyridis*.

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Introduction

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) is an organism that has induced hundreds of studies over many decades (Sloggett 2005). This beetle, with many vernacular names, including harlequin ladybird, multicolored Asian lady beetle and Halloween beetle, is of interest to biologists in several fields. Firstly, it is a highly polymorphic species, with variation in colour morphs evident across its range, and has thus long been a study species for geneticists (e.g. Dobzhansky 1933; Komai et al. 1950). Secondly, it is a large, voracious and resilient coccinellid and was therefore a target for use in biological control programs. Indeed, *H. axyridis*

has a long history of use as a classical biological control agent of aphids and coccids since 1916 (Gordon 1985). It has been widely used for pest control in crops as diverse as pecans (Tedders and Schaefer 1994) and red pines (McClure 1987). As a biological control agent, *H. axyridis* has incidentally succeeded in controlling pest aphid species on other crops, including apples (Brown and Miller 1998) and citrus fruits (Michaud 2002) and has had a pest-controlling role in other crop systems, including soybean, maize, alfalfa, tobacco, winter wheat and cotton (reviewed in Koch 2003; Koch and Galvan 2008). In Europe *H. axyridis* was released in a number of augmentative biological control programs (e.g. Trouvé et al. 1997; Adriaens et al. 2003; Coutanceau 2006). Thirdly, from its native range in Asia, *H. axyridis* has spread in four further continents at a very fast rate during the last 23 years (see below). This is of great interest to biologists studying range expansions of species, and specifically, invasions by alien species. Possible negative impacts of *H. axyridis* on native species have been a particular concern (Roy and Wajnberg 2008), with many studies focussing on the role of intraguild predation (Pell et al. 2008), though cannibalism may be even more important in maintaining *H. axyridis* dominance (Osawa 2011).

For all of these reasons, *H. axyridis* can be seen as a model organism, and many of the studies are no doubt of wide general interest, and not restricted to those researchers studying the Coccinellidae. In this paper we outline the global spread of *H. axyridis* and its current known distribution, the mechanisms behind that spread, and discuss genetic evidence of its invasion routes (using analyses of both microsatellite and mitochondrial DNA).

Global distribution of *H. axyridis*

Asia

Harmonia axyridis is native to China (range extending to the far south, e.g. Yunnan and Guangxi Provinces), Japan, Korea, Mongolia, and eastern Russia (Dobzhansky 1933; Kuznetsov 1997), although its entire native range has not been clearly recorded. In Russia, the species occurs at least as far north as Krasnoyarsk (southern Siberia) (Poutsma et al. 2008), as far west as the Altai Mountains and Novosibirsk (Iablokoff-Khnzorian 1982) and the range extends to the eastern seaboard (through Amur region, Khabarovsk territory and Primorye territory) and beyond, to the islands of Sakhalin and Kuril (Kuznetsov 1997). Kuznetsov (1997) also reports the range to include northern Kazakhstan, and introductions of *H. axyridis* were made in that country, at least in the south-east (e.g. Alma-Ata) (Hodek 1973). However, studies of *H. axyridis* specimens from Kazakhstan indicate no obvious genetic differentiation from populations in the western part of the native range (E. Lombaert and A. Estoup, unpublished data; C. Thomas, unpublished data), suggesting that the species may be native to that country. Presence in Taiwan and the Himalayas was documented but is in doubt (Poutsma et al. 2008), in the former case because of possible misidentification with *Harmonia yedoensis* Takizawa.

North America

Harmonia axyridis of Japanese origin was first introduced in the USA (California and Hawaii) in 1916 (Iablokoff-Khnzorian 1982) (Table 1). However, despite many further releases, including at least fourteen in various states between 1964 and 1982 (Gordon 1985) *H. axyridis* was not reported as established in the country until 1988, in Louisiana (Chapin and Brou 1991). However, evidently the species then spread very quickly and by 1994 it was present in at least 24 states, including most states adjoining both the east and west coasts (Koch et al. 2006). *Harmonia axyridis* continued to be found in further states, and was recently recorded for the first time in Montana (2006) (Foley et al. 2009) and Arizona (2008) (Fothergill et al. 2010), leaving Wyoming and Alaska as the only states without a record of the species. *Harmonia axyridis* became established in Canada by 1994 (Coderre et al. 1995) and quickly spread across much of the southern part of the country (Majka and McCorquodale 2006). Having recently been recorded in Newfoundland (2009, although an earlier specimen from 2000 was also noted) (Hicks et al. 2010), the species has now been found in all but two jurisdictions (i.e. Labrador and Saskatchewan) (Hicks et al. 2010). *Harmonia axyridis* was introduced in Mexico as a biological control agent in the states of Chihuahua, Colima, and Yucatán (Koch et al. 2006). The species is

Table 1 The global distribution of *Harmonia axyridis*, listed by continent and country

Country	Year of first record in the wild (not necessarily established)	Deliberately introduced? (Earliest year of introduction)	Evidence of establishment?	References
Asia				
Russia (Eastern, S. Siberia), Mongolia, China, Japan and Korea	N/A (native range)	N/A	N/A	Dobzhansky (1933) and Kuznetsov (1997)
Kazakhstan	Unknown	Yes (1968)	Yes	Hodek (1973)
Georgia	Unknown	Yes (1927)	Unknown	Poutsma et al. (2008)
North America				
USA	1988	Yes (1916)	Yes	Chapin and Brou (1991)
Canada	1994	No	Yes	Coderre et al. (1995)
Mexico	Pre 2006	Yes (pre 2001)	Yes	Koch et al. (2006)
South America				
Argentina	2001	Yes (1986)	Yes	Saini (2004)
Brazil	2002	No	Yes	de Almeida and da Silva (2002)
Chile	2003	Yes (1998)	Yes	Grez et al. (2010)
Peru	2003	No	Yes	Grez et al. (2010)
Paraguay	2006	No	Yes	Silvie et al. (2007)
Uruguay	2009	No	Yes	Nedvěd and Krejčík (2010)
Colombia	2010	No	Unknown	J. Lundgren (personal communication)
Europe				
France	1991	Yes (1982)	Yes (2003)	Coutanceau (2006) and Ferran et al. (1997)
Greece	1998	Yes (1994)	Limited	Kontodimas et al. (2008)
Germany	1999	Yes (1997)	Yes	Tolasch (2002)
Belgium	2001	Yes (1997)	Yes	Adriaens et al. (2003)
Netherlands	2002	Yes (1996)	Yes	Cuppen et al. (2004)
England	2003	No	Yes	Majerus et al. (2006)
Switzerland	2004	Yes (1996)	Yes	Klausnitzer (2004)
Luxembourg	2004	No	Yes	Schneider and Loomans (2006)
Italy	2006	Yes (1990s)	Yes	Brown et al. (2008a) and Burgio et al. (2008)
Czech Republic	2006	Yes (2003)	Yes	Brown et al. (2008a)
Denmark	2006	Yes (2000s)	Yes	Brown et al. (2008a) and Steenberg and Harding (2009)

Table 1 continued

Country	Year of first record in the wild (not necessarily established)	Deliberately introduced? (Earliest year of introduction)	Evidence of establishment?	References
Austria	2006	No	Yes	Rabitsch and Schuh (2006)
Norway	2006	No	Yes	Staverloekk et al. (2007)
Poland	2006	No	Yes	Przewozny et al. (2007)
Wales	2006	No	Yes	Brown et al. (2008b)
Spain	2007	Yes (1995)	No	Goldarazena and Calvo (2007)
Liechtenstein	2007	No	Yes	Brown et al. (2008a)
N. Ireland	2007	No	No	Murchie et al. (2008)
Scotland	2007	No	Yes	Holroyd et al. (2008)
Sweden	2007	No	Yes	Brown et al. (2008a)
Croatia	2008	No	Yes	Stanković et al. (2010)
Hungary	2008	No	Yes	Merkl (2008)
Serbia	2008	No	Yes	Thalji and Stojanovic (2008)
Slovakia	2008	No	Yes	O. Nedvěd and V. Marko (personal communications)
Slovenia	2008	No	Yes	Bravničar et al. (2009)
Ukraine	2009	Yes (1964)	Yes	Marko and Pozsgai (2009)
Bulgaria	2009	No	Yes	Tomov et al. (2009)
Latvia	2009	No	Yes	Barševskis (2009)
Romania	2009	No	Yes	Marko and Pozsgai (2009)
Bosnia and Herzegovina	2010	No	No	Kulijer (2010)
Ireland	2010	No	No	http://www.invasivespeciesireland.com
Belarus	Unknown	Yes (1968)	Unknown	Sidlyarevich and Voronin (1973)
Portugal	None	Yes (1984)	No	
Africa				
South Africa	2001	No	Yes	Stals and Prinsloo (2007)
Egypt	Pre 2007	Yes (pre 2000)	Limited	Ferran et al. (2000)
Lesotho	2008	No	Yes	Stals (2010)
Kenya	2010	No	Limited	Nedvěd et al. (in press)
Tunisia	None	Yes	No	EPPO (2002)
Australia				
Australia	None, but imported specimens intercepted	No	No	Smith (2008)

widespread in Mexico. Koch et al. (2006) reported *H. axyridis* from five further states (where *H. axyridis* was not released)—Coahuila, Jalisco, Morelos, Puebla and Mexico State—and the species has since been found established in the southern-most state, Chiapas (O. Nedvěd, personal communication), which borders Guatemala.

South America

The first report of *H. axyridis* in South America came from Argentina (Saini 2004) (Table 1; Fig. 1a). The species was introduced as a biological control agent, first in Mendoza in 1986 (Poutsma et al. 2008) and was recorded in Buenos Aires (2001) (Saini 2004), Santa Fe (2004) (Montero and Vignaroli 2008) and Entre Rios (2008) (R. Stals, personal communication), so it appears to be widespread in the northern half of the country. The flightless biocontrol strain of *H. axyridis* (Tourniaire et al. 2000) was introduced in Chile in 1998 (Grez et al. 2010). There is no record of establishment at that time and the first report of the species in the wild is from 2003 (Los Andes, Valparaiso region), although with no further reports until recently (Grez et al. 2010). However, *H. axyridis* is clearly now established in central Chile, with 17 records (including larvae) in various localities and habitats in the Metropolitan and Valparaiso regions, as documented by Grez et al. (2010). The species is abundant in places; an aggregation of over 650 *H. axyridis* individuals was reported from an apartment in Santiago in May 2010 (Grez et al. 2010). As far as we know, *H. axyridis* was not deliberately released in Brazil but it has been recorded there in four regions—Paraná (2002) (de Almeida and da Silva 2002), São Paulo (2004) (Arruda Filho et al. 2009), Minas Gerais (2006) (Rezende et al. 2010) and Brasilia (2009) (Martins et al. 2009)—suggesting a northerly spread up the eastern side of the country. *Harmonia axyridis* is established in Paraguay, and appears to be widespread in the south of the country, with records from Caaguazú, Coronel Bogado and Caacupé (Silvie et al. 2007). The earliest record is from Caaguazú in 2006, and adults and larvae have been recorded in cotton crops there (Silvie et al. 2007). Present in Peru since 2003 (L. Valencia, personal communication), *H. axyridis* was reported from Lima and Tumbes (Grez et al. 2010), both of which are on the Pacific coast. The latter is very close to the border with Ecuador. Apparently the species is established in Peru and is common in places (Grez et al. 2010). *Harmonia axyridis* is also reported to be present in western Colombia (Valle de Cauca) (J. Lundgren, personal communication). The latest South American country in which *H. axyridis* has been found is Uruguay, where it was recently reported as established (Nedvěd and Krejčík 2010). Records were from Canelones (adults found in late 2009, with larvae found at the same locality in early 2010), and Montevideo (2010) (Nedvěd and Krejčík 2010). Both of these localities are in the south of the country. *Harmonia axyridis* has clearly spread quickly in South America, and further countries seem likely to be invaded.

Europe

In Europe, early introductions of *H. axyridis* occurred in the east, including to Ukraine from 1964 (for control of aphids on fruit trees) (Katsoyannos et al. 1997) and Belarus from 1968 (Sidlyarevich and Voronin 1973). In western Europe, *H. axyridis* was first used as a biological control agent in 1982 in France and first marketed in 1995 (Coutanceau 2006), with various companies making the species commercially available (Adriaens et al. 2003). It established in the late 1990s and expanded its range rapidly, especially from 2002 (Brown et al. 2008a) (Table 1; Fig. 1b). The spread and distribution of *H. axyridis* in Europe is detailed in Brown et al. (2008a), who reported establishment in at least 13 European countries. However, the species has continued to spread rapidly and is now known to be established in 13 additional countries (Table 1). In the west there are recent (2010) first records from Ireland (although establishment there is yet to be confirmed) (http://www.invasivespeciesireland.com) and in the east the established range now includes Poland (2006) (Przewozny et al. 2007), Hungary (2008) (Merkl 2008), Slovakia (2008) (O. Nedvěd and V. Marko, personal communications), Latvia (2009) (Barševskis 2009), Romania (2009) (Marko and Pozsgai 2009) and Ukraine (2009) (Marko and Pozsgai 2009). In the south, Croatia (2008) (Stanković et al. 2010), Serbia (2008) (Thalji and Stojanovic 2008), Slovenia (2008) (Bravničar et al. 2009) and Bulgaria (2009) (Tomov et al. 2009) have established populations, and the species has recently been reported in

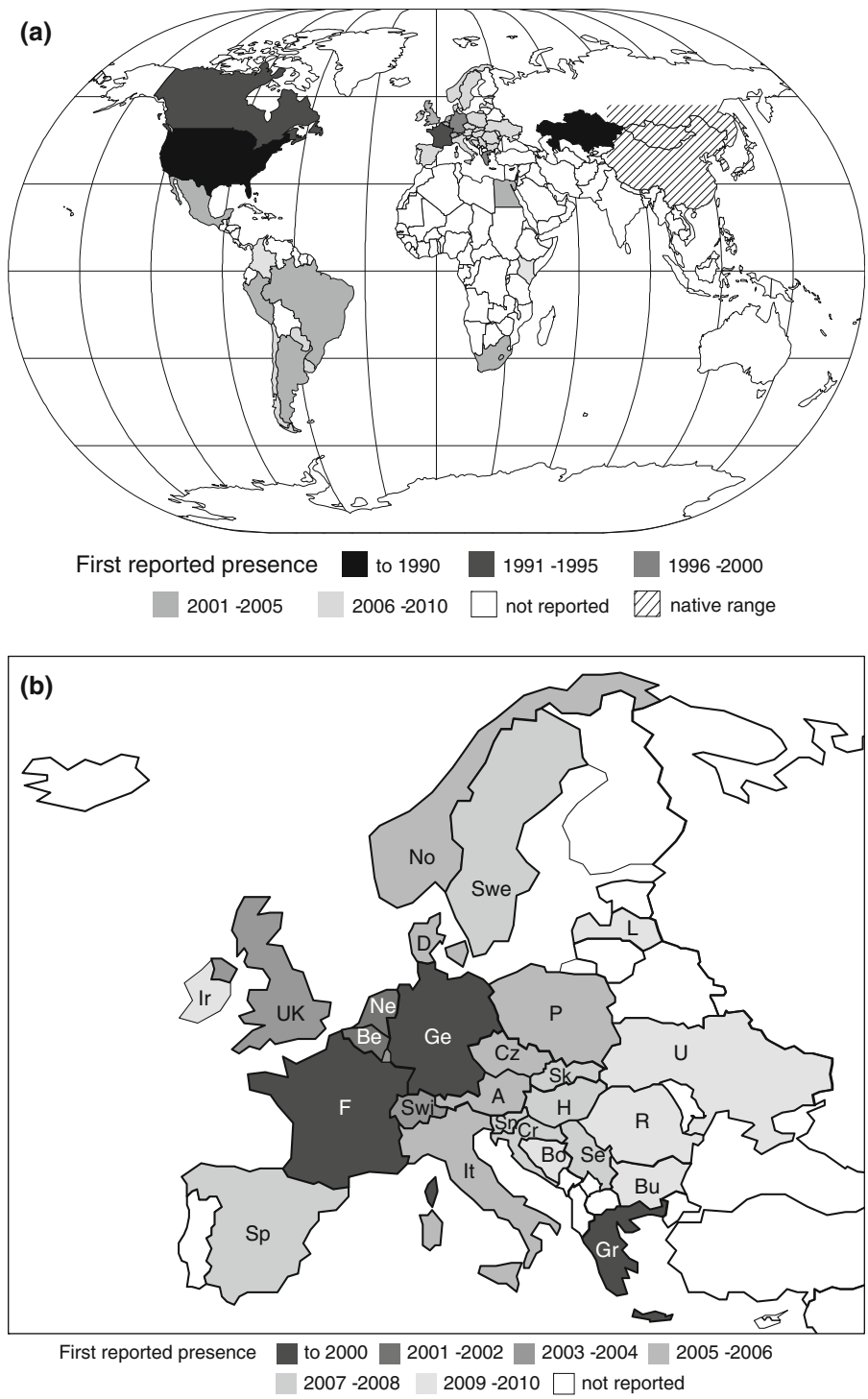

Fig. 1 The known distribution of *Harmonia axyridis* (based on confirmed reports of the species in the wild) up to and including 2010: **a** globally; **b** in Europe. *Note*: in most cases entire countries are coloured, but this does not mean that *H. axyridis* necessarily occurs throughout. *A* Austria, *Be* Belgium, *Bo* Bosnia and Herzegovina, *Bu* Bulgaria, *Cr* Croatia, *Cz* Czech Republic, *D* Denmark, *F* France, *Ge* Germany, *Gr* Greece, *H* Hungary, *Ir* Ireland, *It* Italy, *L* Latvia, *Ne* Netherlands, *No* Norway, *P* Poland, *R* Romania, *Se* Serbia, *Sk* Slovakia, *Sn* Slovenia, *Sp* Spain, *Swe* Sweden, *Swi* Switzerland, *U* Ukraine, *UK* United Kingdom

Bosnia and Herzegovina (2010) (Kulijer 2010). The northern-most location in Europe where the species has been recorded is Trondheim, Norway (Saethre et al. 2010).

Africa

Harmonia axyridis was introduced in Tunisia (EPPO 2002) and Egypt (Ferran et al. 2000) (Table 1; Fig. 1a). The status of the species in northern Africa is largely unknown to us. However, *H. axyridis* may be established around Cairo, as reported by Brown et al. (2008a) and was recently still being released as a biological control agent in Egypt. In South Africa *H. axyridis* has been established since 2001, when it was recorded in Cape Town and Stellenbosch (Western Cape Province), with larvae and pupae present (Stals 2010). At first this went largely unnoticed, but since late 2006, when *H. axyridis* was found again in Western Cape Province, Riaan Stals has gradually been piecing together the spread of the species in South Africa (Stals and Prinsloo 2007; Stals 2010). Thus *H. axyridis* has now been recorded in all nine provinces, as follows: Western Cape (2001), Eastern Cape (2006), KwaZulu-Natal, Free State and Gauteng (all 2007–2008), Mpumalanga, Northern Cape, North-West Province and Limpopo (all 2009). The species is not thought to have been deliberately introduced in South Africa, and Stals (2010) hypothesises its arrival first to Western Cape Province via human transport (sea, air or road). Lesotho (a mountainous country entirely surrounded by South Africa) is the second country in southern Africa with confirmed reports of *H. axyridis*. The species is established there, having been discovered in June 2008, and was recorded at altitudes of up to 2500 m (Stals 2010). In 2010 *H. axyridis* was found on the east coast of Kenya (Kikambala) and may be established there (Nedvěd et al. in press), indicating tolerance to a tropical climate. *Harmonia axyridis* may well be present, but unreported, in other regions of Africa.

Australia

Harmonia axyridis is not known to be established in the continent of Australia. However, there are documented examples of the species arriving in Australia. It was inadvertently imported with excavation equipment to Bunbury (Western Australia) in 2008, but all specimens were dead (Smith 2008), and about 20 *H. axyridis* (some live and pupating) were intercepted at Darwin (Northern Territory), having arrived by air from the USA in 2009 (Carvan 2009).

Predicted global distribution

Poutsma et al. (2008) provided very useful predictions of the potential distribution of *H. axyridis* based on climate modelling. The subsequent observed spread of the species so far conforms to these predictions. We propose that the eastern edge of the European range of *H. axyridis* will continue to expand and may eventually meet the western edge of the Asian range, thus giving a more-or-less continuous distribution across the Palearctic, from Ireland in the west to Japan in the east. There is little scope for further range expansion in North America, as Alaska and northern Canada are climatically unsuitable for the species and almost all other regions have already been invaded. In South America, Venezuela, Ecuador and Bolivia are suitable for the species from a climatic viewpoint—and all of these share borders with countries that already have *H. axyridis*. Whilst Amazonia may be unsuitable for *H. axyridis*, much of the remainder of South America is suitable for invasion. Very large areas of southern and eastern Africa (including Madagascar) and the coastal belt of northern Africa are also climatically suitable for the species. Whilst invasion risk is likely to be lessened by a combination of geographic isolation and rigorous import procedures, New Zealand and coastal Australia (excluding northern regions) are climatically suitable for *H. axyridis* (Poutsma et al. 2008). In summary, there are clearly further vulnerable ecosystems beyond the current known distribution that are potentially under threat of invasion by *H. axyridis*. In the long term on a global scale, it may only be northerly and southerly latitudes, high altitudes, desert regions, and some tropical regions, that escape invasion.

Habitat use by *H. axyridis*

Harmonia axyridis is generally stated in the literature to be semi-arboreal (Hodek 1973), but it occupies many habitats, and in parts of both its native and introduced ranges has been recorded in meadows,

heathlands and riparian zones (Adriaens et al. 2008), reed beds (Ware et al. 2005) and crop systems (Colunga-Garcia and Gage 1998; Jansen and Hautier 2008). *Harmonia axyridis* has been recorded on fruit trees in many countries. Indeed, some of the earliest records of the species in several countries were from fruit trees, e.g. Italy (peach and citrus) (Burgio et al. 2008), Denmark (apple and *Prunus* sp.) (Steenberg and Harding 2009), Hungary (apple and pear) (Marko and Pozsgai 2009), Latvia (orchard trees) (Barševskis 2009) and São Paulo, Brazil (citrus) (Arruda Filho et al. 2009). In the review by Koch et al. (2006), *H. axyridis* in North America is reported from 40 plant species, including three *Malus* spp. (apple), two *Prunus* spp. (peach and plum) and *Citrus* spp. Thus it is evident that fruit trees are to some extent targeted by *H. axyridis* as a feeding habitat, either because of the aphid and coccid prey that the trees harbour, or because of the fruit itself, which *H. axyridis* may feed on (Kovach 2004). Feeding on grapes has been a particular focus of attention, as if harvested with *H. axyridis*, about one beetle per kg of fruit is enough to taint the wine (Linder et al. 2009). This has caused concern in North America (Galvan et al. 2008), Europe (Linder et al. 2009) and South Africa (Stals 2008).

In Great Britain, verified records of *H. axyridis* collected via a public engagement survey (http://www.harlequin-survey.org) were analysed in terms of broad habitat categories. Taking 2008 as an example, the species was found in the following habitats: 1. built up areas and gardens (53.4% of the 1298 × 1 km^2 with records of *H. axyridis*); 2. grassland (20.4%); 3. arable and horticultural land (19.6%); 4. woodland (5.5%) (P. Brown, unpublished data). These data, presented at coarse summary resolution here, are biased in terms of both the density of human population (affecting record submission) and the relative land areas of each habitat in Great Britain, but illustrate the generalist nature of *H. axyridis* habitat use. Deciduous trees and shrubs dominated in terms of plant use by *H. axyridis* in this study, with the majority of such records coming from built up areas and gardens, rather than from woodland. *Harmonia axyridis* was recorded as occurring on at least 75 families of flowering plant, with 50 families recorded with larvae present, suggesting a very broad range of suitable plants for *H. axyridis* in Great Britain (P. Brown, unpublished data). Adriaens et al. (2008) reported similar results from Belgium.

Six of the seven biomes of South Africa (Grassland, Savanna, Fynbos, Forest, Thicket and Nama Karoo) have records of *H. axyridis*, which has been recorded from sea level up to altitudes of at least 1800 m. The exception is the very arid Succulent Karoo biome that dominates in the north-west of South Africa. Although many reports were from urban locations, the species was also found in natural habitats, including unspoilt fynbos (Cape maquis) and pristine grassland, and large populations have been recorded in important wine production areas of Western Cape Province (Stals 2010). In Brazil, *H. axyridis* was recently reported from tropical semi-deciduous forest (Milleo et al. 2008). Also in Brazil, 38 plant species (from 18 families) were recorded with the presence of *H. axyridis* (Martins et al. 2009), further illustrating the versatility of the species. *Harmonia axyridis* forms large overwintering aggregations, and in its invaded range these tend to be in or on buildings, e.g. North America (Koch and Galvan 2008) and Europe (Brown et al. 2008a). The resulting nuisance factor of the beetles indoors is also a problem in at least parts of the native range of *H. axyridis*, e.g. Northeast China (Wang et al. 2011).

In summary, whilst it is evident in all invaded continents that *H. axyridis* has a tendency to thrive in anthropogenic habitats (both urban and agricultural), it is also able to establish in a wide range of natural and semi-natural habitats. Continued negative impacts of *H. axyridis* on native species through intraguild predation and competition (e.g. Brown et al. 2011a) are predicted. Thus it is likely that biodiversity in important ecosystems, especially in Africa and South America, may suffer negative effects, if, as seems likely, *H. axyridis* continues to thrive and expand in its invaded range.

Investigating the invasion routes of *H. axyridis* using genetic analyses

Inferring invasion routes is an essential step towards implementing control measures and understanding the success of an invasive species (Estoup and Guillemaud 2010). As discussed above, observational records have proved useful for mapping the spread of an invasive species (Brown et al. 2008b), but molecular genetic approaches provide additional, unique insights into invasion dynamics, and allow

specific hypotheses to be tested. For example, were there several independent introductions of individuals from the native area into the invasive range? Or was there a stepping stone colonisation process, involving a single introduction from the native range into the invasive range, followed by subsequent invasions occurring from within the invasive range (e.g. Estoup and Guillemaud 2010)? It is generally expected that an introduced population will have low genetic diversity, and therefore reduced potential for adaptation to a new environment. However, multiple introductions are common during invasions by many taxa, e.g. plants (Kang et al. 2007), reptiles (Kolbe et al. 2004), molluscs (Facon et al. 2003), crustaceans (Kelly et al. 2006), and insects (Fonseca et al. 2000; Miller et al. 2005; Ciosi et al. 2008) (and see Lawson Handley et al. (2011), for a review). This can lead to combinations of novel genotypes that could increase genetic diversity in the invasive relative to native ranges, particularly if source populations are highly differentiated (e.g. Kolbe et al. 2004; Dlugosch and Parker 2008). This is particularly the case for species with wide geographical ranges, which are more likely to be successful invaders (Theoharides and Dukes 2007), and could be pertinent to *H. axyridis*. In addition to source populations and invasion routes, data generated in molecular studies can be used to infer the number of founders, and the genetic characteristics of the founding population, giving insights into the mechanisms underlying a successful invasion.

We recently investigated the global invasion routes of *H. axyridis* using neutral molecular markers (18 microsatellites) in populations from across the native Asian range (eight populations from Japan, China, South Korea, Kazakhstan and Russia), from biocontrol companies (Biobest and Biotop) and from the invasive range (five populations from North America, Europe, South America and South Africa) (Lombaert et al. 2010). The invasive populations represent the initial invasive population found in each invaded area. We performed traditional genetic analyses, and a modern Approximate Bayesian Computation (ABC) method implemented in DIYABC (Cornuet et al. 2008, 2010); see also Lawson Handley et al. (2011) for a description of ABC methods. The DIYABC software allows probabilistic estimates of competing introduction scenarios, using prior historical, biological and genetic information about the system to formally test hypotheses of invasion scenarios.

The data strongly suggest that the invasive populations in the east and west of North America originated from two independent introductions from the native range. The eastern North American population then acted as a bridgehead for the invasion, with separate introductions from this population into South America, Africa and Europe (though the European populations were admixed with individuals from biocontrol populations) (Lombaert et al. 2010). These invasion scenarios are supported with very high posterior probabilities (Lombaert et al. 2010). The findings also suggest that for *H. axyridis*, a still unknown evolutionary shift towards characteristics allowing invasion success occurred in eastern North America. Unfortunately, microsatellite data showed that there is very low genetic differentiation in the native Asian range, making inferences of the specific source population difficult. However, preliminary results from mitochondrial DNA (mtDNA) (Thomas et al. 2010) suggest greater population structure in the native range, which should help to pinpoint source populations more specifically.

In addition to the mtDNA analyses, further work is ongoing to address: (i) the genetic relationships between European biocontrol strains of *H. axyridis*; (ii) the genetic structure of *H. axyridis* within its native range and potential effects of such structure on inferences about invasion routes; (iii) the detailed genetic structure within each introduced area, to investigate the number of founders of invasive populations and the likelihood of multiple introduction events; (iv) the possibility that distribution of bacterial endosymbionts could shed additional light on invasion routes. Progress so far is outlined below:

(i) In agreement with historical information, the common genetic origins of all the European strains used for biocontrol in Europe and South America were confirmed using microsatellite data, i.e. they are all derived from a single population of *H. axyridis* sampled from the native area by Institut National de la Recherche Agronomique (INRA) in 1982 (E. Lombaert and A. Estoup, unpublished data).

(ii) The microsatellite and mtDNA data suggest that the genetic structure of *H. axyridis* within its

native Asian range consists of two groups: one located in the east of the range and one located in the west. Historical information about biocontrol practices (Tedders and Schaefer 1994; Krafsur et al. 1997), and current airline transportation networks (e.g. Tatem and Hay 2007), suggest eastern Asia is the more likely source of the American outbreaks, which is why only *H. axyridis* populations from eastern Asia were considered as potential source populations in Lombaert et al. (2010). Interestingly, new DIYABC analyses of microsatellite data, taking into account the above genetic structure within the native range, indicated that populations from western Asia also contributed in a significant manner to the invasive bridgehead population located in eastern North America (E. Lombaert and A. Estoup, unpublished data) and indeed Gordon (1985) lists the Former USSR as a source of introduced *H. axyridis* biocontrol stock to eastern North America. For other invaded areas, the inferred invasion scenarios remained the same as those described in Lombaert et al. (2010), without significant contribution of populations from western Asia. If confirmed, this new finding may alter our understanding of key factors that could have enhanced the invasion potential of this ladybird. Indeed, after decades of unsuccessful acclimatization of biocontrol strains in North America, it is possible that admixture in eastern North America between genetically differentiated populations from the native range may have facilitated adaptation, by allowing the appearance of new genomic combinations. However, additional studies are needed in order to confirm this admixture event (e.g. using mtDNA, which shows greater differentiation in the native range) and assess the role of admixture in the success of this particular invasive bridgehead population (e.g. using quantitative genetics approaches).

(iii) A detailed study of genetic structure at microsatellite loci within different introduced areas confirms the genetic homogeneity of invasive populations within each area, except Europe, and to a lesser extent, South America. For example, the populations sampled all over Europe clustered in several genetically differentiated groups, with one major group located in western Europe. This group includes the invasive population from Ghent, Belgium analysed in Lombaert et al. (2010). Such preliminary results may indicate multiple introductions in Europe. Additional studies based on DIYABC analyses are needed in order to reconstruct the historical genetic relationships between these European populations, as well as between them and other invasive and native populations. Further data collection of mtDNA, to include additional populations from across each invasive continent, is ongoing. The complete dataset will be analysed in DIYABC, and by more traditional methods, to address whether high genetic diversity is related to multiple introductions within invasive populations, and to investigate the numbers of maternal founders of invasive populations.

(iv) Several bacterial endosymbionts have been identified in global populations of *H. axyridis* (Aebi and Zindel 2010; L. Lawson Handley and C. Thomas, personal observation). Since endosymbionts are typically transmitted vertically (from mother to offspring), their distributions could potentially shed additional light on invasion routes. This, together with the transmission dynamics of endosymbionts in *H. axyridis,* and their impact on host mitochondrial genomes, is currently under investigation.

Dispersal of *H. axyridis*

Dispersal is critical to the establishment and persistence of invasive populations, and understanding dispersal mechanisms is essential for predicting the spread of invasive species. However, dispersal can be complex and difficult to measure, particularly in highly mobile insects that are difficult to study using traditional mark-release-recapture experiments. Determining the probability of long-distance dispersal (LDD) by wind or anthropogenic means is particularly important for predicting spread of an invasive species, as this can accelerate the rate of range expansion (Urban et al. 2008; Lawson Handley et al. 2011). However, short-distance dispersal (SDD) is important for local population dynamics, hence both SDD and LDD should be considered in predictive models. Indeed, a combination of SDD and LDD

(i.e. "stratified dispersal") may be a common feature of invasions, and has already been described in several invasive insects, e.g. *Phyllonorycter leucographella* (Zeller) (Lepidoptera: Gracillariidae) (Nash et al. 1995), *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Sharov and Liebhold 1998), *Dendroctonus micans* (Kug.) (Coleoptera: Scolytidae) (Gilbert et al. 2003), *Cameraria ohridella* Deschka and Dimič (Lepidoptera: Gracillariidae) (Gilbert et al. 2004) and see Lawson Handley et al. (2011) for a review. This is likely to be the case in *H. axyridis,* which is an active flyer and disperses locally in response to prey density to and from overwintering sites, but is also capable of passive LDD by both wind and anthropogenic means (see below). In order to fully understand the dispersal capability of species such as *H. axyridis*, we consider below: (i) the mechanism(s) of dispersal; (ii) the reasons for dispersal; (iii) the influence of abiotic factors such as geography or climate on dispersal, and; (iv) the role of anthropogenic dispersal.

Mechanisms of dispersal

Short-distance dispersal (SDD)

Ladybirds are generally active fliers, and flight is considered to be the most important process determining distribution (Van der Werf et al. 2000). Most studies of dispersal have however focused on walking rather than flight, and therefore underestimate the true dispersal ability. *Harmonia axyridis* is often considered to be "a good flyer" (e.g. Obata 1986; Hodek et al. 1993; Tourniaire et al. 2000) with high dispersal capacity (Nalepa et al. 1996; Osawa 2000; With et al. 2002; Berkvens et al. 2009), and to be able to actively disperse over long distances to overwintering sites (Hodek and Honek 1996; Nalepa et al. 1996; Osawa 2000; With et al. 2002), but field and experimental data is scant. In field trials in Japan, control strains of *H. axyridis* had a median flight distance of 431 m for males and 396 m for females (Seko et al. 2008). In another field experiment, however, mean distance travelled by individuals (in a 3.3–7.6 day period) was considerably lower (9.71 ± 1.29 m), with females moving further than males in this case, and males moving slightly further in spring than in summer (Osawa 2000). This illustrates the difficulty of assessing dispersal distance in the field. It is also difficult to evaluate the dispersal ability of *H. axyridis* without comparative data from other coccinellids, and flight experiments are needed in order to test this. A mark-recapture study of native coccinellids in Great Britain reported a maximum dispersal distance of 1.5 km (Zhou et al. 1994). So far, the only experimental data on *H. axyridis* flight ability comes from comparisons of selected flightless strains and non-selected laboratory strains (Tourniaire et al. 2000; Seko et al. 2008), which are unlikely to be representative of natural populations.

Long-distance dispersal (LDD)

An estimated spread rate of 442 km year^{-1} by *H. axyridis* in North America was calculated by McCorquodale (1998). Taking Europe as a whole, we have calculated that *H. axyridis* has spread at a maximum rate of approximately 200 km year^{-1}. This calculation is based on a south-easterly spread from Belgium, the Netherlands or northern France to Bulgaria (approximately 1600 km) in an eight year period. More detailed calculations for Great Britain reveal a northerly spread rate of 105 km year^{-1} (data from 2004 to 2008) and a rather faster westerly spread rate of 145 km year^{-1}. In South Africa, the species spread at a rate of approximately 500 km year^{-1} (Stals 2010). The European and North American calculations include the effects of multiple intentional biological control releases of *H. axyridis*. Whilst this does not apply to the British and South African calculations (where the species is not known to have been deliberately introduced), the dispersal rates in all regions were influenced by further anthropogenic factors (such as inadvertent dispersal with produce) which presumably played an important, but unquantifiable, part. The American figures were used to estimate a likely dispersal of >10 km per release for >25% of released beetles, giving an Environmental Risk Index (ERI) of 80 for *H. axyridis*. This is above the recommended threshold, indicating that the species should not be intentionally released (van Lenteren et al. 2008). Although these calculations are unquestionably useful, they were obtained indirectly from historical data which may be incomplete, and more direct, quantitative estimates of dispersal are needed. As yet, the mechanisms driving this spread, and the comparative roles of active flight, passive wind dispersal, and

anthropogenic spread are poorly understood (Hodek et al. 1993).

Reasons for dispersal

It is generally accepted that coccinellids perform four main types of active dispersal, classified according to the motivations behind them: 1. trivial appetitive flight (i.e. short-distance flight, e.g. to find prey); 2. hectic appetitive flight (i.e. long-distance flight initiated by overpopulation and the resultant prey shortage); 3. migration to overwintering sites and; 4. non directional dispersal from overwintering sites (Hodek et al. 1993). Non directional dispersal refers to that which is either passive (e.g. wind-induced) or somewhat random in nature.

Coccinellid flights of less than 2 m undoubtedly correspond to trivial appetitive flight (Elliott et al. 2000), but the cut off distance between trivial and hectic appetitive flight is difficult to quantify. Osawa (2000) inferred a mean dispersal distance of *H. axyridis* of 9.7 m (obtained from April to July) that likely includes both trivial and hectic appetitive flight. Aphids are arguably the biggest ecological predictor of dispersal in coccinellids such as *H. axyridis*. Appetitive flight is associated with foraging and ovipositioning behaviour, and is linked intricately with aphid population dynamics. This type of dispersal is essential in *H. axyridis* and other aphidophagous coccinellids, due to the ephemeral nature of their prey. Density of adult ladybirds is often positively correlated with aphid density (e.g. Turchin and Kareiva 1989; Hodek and Honek 1996; Osawa 2000; Evans and Toler 2007), and there is strong evidence that emigration rate decreases with increasing number of prey (see Evans (2003) for a review). Short-distance prey searching has been studied in many coccinellids (Banks 1956; Carter and Dixon 1982; Nakamuta 1982, 1984, 1985; Osawa 2000), and *H. axyridis* is highly effective at resource tracking, which enables it to maintain stable populations in temporally and spatially heterogeneous habitats and in pursuit of its prey (Osawa 2000). Therefore SDD is possibly determined by patterns of resource distribution within a habitat (Osawa 2000). Although some authors have suggested that visual and olfactory cues are important in coccinellids migrating to a particular habitat (e.g. Carter and Dixon 1982; Obata 1986; van der Werf et al. 2000), others have inferred that specific signals, such as honeydew odour, are not reliable cues for SDD (Osawa 2000). Trivial appetitive flight is also important for coccinellid reproduction. Since larvae are limited in their dispersal ability, parents must locate developing aphid colonies before oviposition and optimise timing with prey availability. Such flight is non directional (Hodek and Honek 1996). Dispersal is also an important mechanism for avoiding inbreeding, and could explain why inbreeding is low in wild coccinellid populations, e.g. *Adalia bipunctata* (L.) (Hurst et al. 1996).

While active dispersal for foraging and mate finding will usually occur at small spatial scales (within the same habitat), dispersal towards overwintering sites can occur at larger scales if suitable shelter is not available locally (Hodek et al. 1993; Grez et al. 2005). *Harmonia axyridis* is, however, commonly associated with urban areas, and sheltered sites such as man-made structures can often be found close to their foraging and ovipositioning locations. Thus some overwintering dispersal takes place at small spatial scales. Dispersal from overwintering sites is thought to consist of a series of short, exploratory flights in search of aphids.

Finally, hectic appetitive flight is an extension of trivial appetitive flight, performed at times of low food abundance. It is during these longer-distance dispersal events that coccinellids are carried to high altitudes on thermal currents and then passively transported via wind (Hodek et al. 1993). Occasionally, this can result in large groups of coccinellids being simultaneously deposited in the same location, and can explain huge aggregations (millions of beetles) such as those of *Coccinella septempunctata* L. seen in Great Britain in the summers of 1976 and 2009.

The influence of abiotic factors on dispersal

Even when aphids are abundant in a given habitat, a significant proportion of coccinellid adults disperse every day (Krivan 2008). There must therefore be other factors that are important in driving coccinellid dispersal. Effects such as habitat fragmentation are likely to strongly influence active dispersal across a landscape. Habitat fragmentation has been shown to affect dispersal in *Eriopis connexa* Mulsant (Grez et al. 2005) and *C. septempunctata* (Kareiva 1987).

It has been argued that temperature is the single most important predictor of insect flight (Taylor 1963). In *H. axyridis,* temperature acts as a cue for dispersal, with movement from overwintering sites commencing on the first day over 18°C after a period of colder weather (Heulsman et al. 2002). There are upper and lower temperature thresholds between which take-off can occur, and between which aerial densities (and hence dispersal) are maximal (Taylor 1963). The wider the thresholds, the greater is the probability that an alien species will adapt to a wide range of climatic conditions. Given the climatic conditions encountered in the native range, from tropical southern Japan to Siberia, it is likely that *H. axyridis* has a very broad temperature threshold. There is likely to be considerable local adaptation to regional climate regimes across the geographic range. Nevertheless, it may be that the heterogeneous nature of its native range has equipped *H. axyridis* with valuable flight adaptations which have helped in its colonisation of a wide range of novel environments.

Wind is also important in insect dispersal and generally increases with altitude, with wind speeds being either facilitative or inhibitory, depending on their magnitude. The "flight boundary layer" (FBL), a term coined by Taylor (1974), is the theoretical altitude at which the wind speed exceeds the maximum flight speed of an insect. For directional SDD (e.g. for foraging), insects must remain below their FBL so that they are free to perform active flight. By contrast, LDD for colonisation is facilitated by high wind speeds above the FBL. Although the height of the FBL is unknown for *H. axyridis,* there is evidence from Vertical Looking Radar (VLR) (Riley and Reynolds 1997; Chapman et al. 2003) of large coccinellids flying at high altitudes (D. Jeffries, unpublished observation). This suggests that LDD of *H. axyridis* could be facilitated by passive transport at high altitudes. Indeed, since the first observations of *H. axyridis* in Great Britain were concentrated in the south-east, one plausible route of entry to Britain is via passive dispersal on winds across the English Channel (Brown et al. 2008b). However, it is unlikely that passive wind dispersal alone can explain the spread of *H. axyridis* in Britain, since observational records strongly indicate a north-westerly spread (Brown et al. 2008b) in contrast to the prevailing south-westerly wind. The spread of *H. axyridis*, at least in Britain, presumably therefore involved a considerable component of active flight.

Determining what constitutes a barrier to dispersal is essential for predictive modelling of the spread of invasive species. Both temperature and wind are likely to be important, since lower temperatures may inhibit dispersal and higher wind speeds mean less control over directed flight. These conditions are affected by landscape topography, so it seems fair to assume that mountain ranges may act as dispersal barriers, even for active-flying insects. Whilst this could explain why the main British distribution of *H. axyridis* is bordered by the Pennine and Cambrian Mountains, the role of mountain ranges as dispersal barriers warrants formal investigation.

The role of anthropogenic dispersal

Anthropogenic dispersal has been an important factor in the spread of alien coccinellids (Evans et al. 2011). In the case of *H. axyridis*, anthropogenic dispersal has taken various forms, both deliberate (i.e. use in biological control) and inadvertent, and there is no doubt that collectively these have been key to the very rapid spread of the species. Transport of *H. axyridis* with produce such as fruit, vegetables and flowers was documented, with clear examples of range expansion evident, e.g. first records from Norway (Staverloekk et al. 2007), northern England (Brown et al. 2008b), Northern Ireland (Murchie et al. 2008) and Orkney, northern Scotland (Ribbands et al. 2009). Inadvertent dispersal with people in motor vehicles and trains is a further mechanism. For example, the first record of *H. axyridis* in Scotland arose from the beetle being transported in a suitcase (Holroyd et al. 2008). Intercontinental anthropogenic dispersal of *H. axyridis* has also been observed. For example, over 2000 adult *H. axyridis* were accidentally transported on timber from the USA to Norway (Saethre et al. 2010), and the species was found in Great Britain on packing cases from Canada (Majerus et al. 2006). Further examples of the potential for large numbers of a coccinellid to be transported anthropogenically were provided by Minchin (2010) and Brown et al. (2011b), who each reported several thousand *C. septempunctata* being transported on ships, from South America to northern Africa in the former case and from Denmark to England in the latter. *Harmonia axyridis* has reached Australia by anthropogenic means at least twice (once from the USA and once from an unknown source country),

although in each case the beetles were either dead or intercepted during quarantine inspection (Smith 2008; Carvan 2009). Whilst these examples do not all provide subsequent evidence of establishment of the alien species in the new region (which is dependent on many factors, including the number of beetles transported and climatic and habitat suitability in the new area), they clearly illustrate that accidental anthropogenic transport can be an important mechanism for the spread of invasive alien species such as *H. axyridis*. Nedvěd et al. (2011) outlined similar mechanisms involved in the spread of arachnids in Europe and North America. Some of the examples also indicate that *H. axyridis* is a species that is robust enough to survive very long journeys, and that its aggregative behaviour at certain times of the year is potentially an important feature in terms of dispersal.

Avenues for future research on dispersal

We are a long way from fully understanding the mechanisms and reasons for dispersal in *H. axyridis,* but technological developments could provide valuable insights. Physiological flight ability can be tested in flight mills (Hocking 1953), a method recently incorporated in assessments of *H. axyridis* residence periods in open fields (Seko et al. 2008). Harmonic radar, using diode transponders and tagged insects, can be used to study insect activity at ground level (Riley and Smith 2002). This technique has provided unique insights into the foraging behaviour of the honeybee *Apis mellifera* L. (Hymenoptera: Apidae) (Capaldi et al. 2000), and the dispersal of the Glanville fritillary *Melitaea cinxia* (L.) (Lepidoptera: Nymphalidae) (Niitepold et al. 2009), and could be particularly useful for studying SDD in *H. axyridis* and other coccinellids. VLR technology provides an opportunity to study long-distance wind-borne dispersal, vertical distribution and insect layering, and temporal variation in dispersal (Chapman et al. 2002, 2003, 2010). One VLR study showed that a large migration from the Netherlands of the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) occurred in May 2000, and that this migration route was responsible for the re-establishment of the species in Great Britain (Chapman et al. 2002). Recent studies have used meteorological and VLR data to backtrack dispersal trajectories of noctuid moths, showing that they can compensate for cross-wind-drift to maximise their long-distance migration efficiency (Chapman et al. 2010). Similar techniques could be used to investigate the dispersal and spread of *H. axyridis*, and provide a more concrete answer to the question of whether regular dispersal occurs, e.g. from the European mainland to Great Britain. Aside from radar technologies, genetic studies are already providing insights into the global spread of *H. axyridis* (Lombaert et al. 2010 and see above), and a landscape genetics approach is currently being used to indirectly investigate dispersal barriers in the native range (Lawson Handley et al. 2011). Additional studies could provide insights into the genes underlying dispersal, and in combination with harmonic radar, be used to investigate the relationship between dispersal, genotype, physiology and environment, as recently demonstrated in *M. cinxia* (Niitepold et al. 2009).

Acknowledgments We thank Nick McWilliam (Anglia Ruskin University) for GIS work for Fig. 1. For help and advice we thank John Day, Irina Goyacheva, Satoshi Koyama, Yoshiaki Obara, Helen Roy, Toshiyuki Satoh, Ilya Zakharov and the University of Hull Evolutionary Biology Group. We thank colleagues from around the world for providing some of the *H. axyridis* samples used in genetic analyses: N. Berkvens, C. Borges, R. Koch, J. Li, J. Lundgren, A. Migeon, R. Poland, R. Stals, A. Staverlokk and W. Su. We are grateful to members of the UK public for providing records and samples of *H. axyridis* via the Harlequin Ladybird Survey and UK Ladybird Survey. C.E. Thomas was funded by the Natural Environment Research Council. E. Lombaert and A. Estoup were supported by grants from the Agence Nationale de la Recherche (ANR-06-BDIV-008-01) and from the Agropolis Fondation (RTRA—Montpellier, BIOFIS project).

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ANNEXE IV

Article 4 (2008)

Lombaert E, Malausa T, Devred R, Estoup A (2008) Phenotypic variation in invasive and biocontrol populations of the harlequin ladybird, *Harmonia axyridis*. *Biocontrol* **53**, 89-102.

BioControl (2008) 53:89–102
DOI 10.1007/s10526-007-9131-z

Phenotypic variation in invasive and biocontrol populations of the harlequin ladybird, *Harmonia axyridis*

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Received: 13 August 2007 / Accepted: 21 October 2007 / Published online: 7 November 2007

Abstract Despite numerous releases for biological control purposes during more than 20 years in Europe, *Harmonia axyridis* failed to become established until the beginning of the 21st century. Its status as invasive alien species is now widely recognised. Theory suggests that invasive populations should evolve toward greater phenotypic plasticity because they encounter differing environments during the invasion process. On the contrary, populations used for biological control have been maintained under artificial rearing conditions for many generations; they are hence expected to become specialised on a narrow range of environments and show lower phenotypic plasticity. Here we compared phenotypic traits and the extent of adaptive phenotypic plasticity in two invasive populations and two populations commercialized for biological control by (i) measuring six phenotypic traits related to fitness (eggs hatching rate, larval survival rate, development time, sex ratio, fecundity over 6 weeks and survival time of starving adults) at three temperatures (18, 24 and 30°C), (ii) recording the survival rate and quiescence aggregation behaviour when exposed to low temperatures (5, 10 and 15°C), and (iii) studying the cannibalistic behaviour of populations in the absence of food. Invasive and biocontrol populations displayed significantly different responses to temperature variation for a composite fitness index computed from the traits measured at 18, 24 and 30°C, but not for any of those traits considered independently. The plasticity measured on the same fitness index was higher in the two invasive populations, but this difference was not statistically significant. On the other hand, invasive populations displayed significantly higher survival

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and higher phenotypic plasticity when entering into quiescence at low temperatures. In addition, one invasive population displayed a singular cannibalistic behaviour. Our results hence only partly support the expectation of increased adaptive phenotypic plasticity of European invasive populations of *H. axyridis*, and stress the importance of the choice of the environmental parameters to be manipulated for assessing phenotypic plasticity variation among populations.

Keywords Adaptive phenotypic plasticity · Alien species · Biological control · *Harmonia axyridis* · Biological invasion

Introduction

The Asian ladybird beetle *Harmonia axyridis* (Pallas) was first brought into Europe in 1982 (Coutanceau 2006). The species was studied in southern France in the laboratory and in experimental greenhouses during the eighties with a view to using this coccinellid as a biological control agent of pest aphids and scale insects. Large experimental as well as commercial releases *in natura* were then performed until 2003 in many European countries (Coutanceau 2006). Despite those numerous releases during more than 20 years, the species failed to become established until 2000–2001 when it started to be observed and subsequently spread into Germany and Belgium. It is now present in many European countries from southern France to Denmark (Brown et al. 2007a). Its status as invasive species is now widely recognised for a number of reasons including its impact on functional biodiversity (van Lenteren et al. 2007). Despite some differences, the European situation parallels the North American experience where *H. axyridis* was first released in 1916 but first established populations were not observed until 1988 after which there was a very rapid spread across the continent (Koch 2003). In both cases, whether the invasive populations resulted from intentional introductions, accidental migrants or both remains unknown. Therefore, the chronology of *H. axyridis* invasions is symptomatic of a general recurrent question around invasion biology: why now and not before?

Understanding the factors driving biological invasions has become of major interest within the past few decades. This is because the recent rise of human activities has greatly accelerated the invasion rate of non-native species and some of these invasions have dramatic economical, ecological or human-health consequences (Mack et al. 2000). However, among the species which arrive in a new location, only few persist and even less spread (Williamson and Fitter 1996). The main reason for that is the unsuitability of the site and/or the environmental stochasticity which promote local extinction of non-adapted populations. Therefore, of particular interest are (i) whether key-characteristics which predispose a species to successful establishment exist and (ii) whether those characteristics evolve during the geographical spread following the establishment phase (Kolar and Lodge 2001; Lee et al. 2007). One mechanism that is frequently suggested in this context is adaptive phenotypic plasticity (Agrawal 2001; Kaufman and Smouse 2001; Yeh and Price 2004; Richards et al. 2006; Geng et al. 2007; Ghalambor et al. 2007).

Adaptive phenotypic plasticity can be defined as a set of processes historically selected to produce the highest fitness among different environments by means of various plastic traits (Debat and David 2001). The plasticity of a trait can be assessed by determining the pattern of its phenotypic expression in different environments (called a reaction norm). Absolute adaptive phenotypic plasticity should lead to a flat fitness reaction norm (i.e. fitness homeostasis, Scheiner 1993; Richards et al. 2006). Theory suggests that invasive

populations are expected to evolve toward greater phenotypic plasticity because of the wide range of environments encountered during the invasion process. However, genetic assimilation, the evolutionary loss of plasticity after successful colonization of a novel environment, should be taken into account as an alternative scenario (West-Eberhard 2003). Some studies that have previously addressed the role of plasticity in invasions have reported increased levels of plasticity in invasive species or populations. The overall evidence remains however limited so that it is premature to draw any firm and general conclusions from these results (reviewed in Richards et al. 2006). Moreover, there is likely to be a bias towards publishing positive results. *Harmonia axyridis* is a suitable biological model to test such predictions because its invasion has been far from instantaneous, despite the numerous intentional releases, and variation in level of plasticity has already been described in this species (Grill et al. 1997; Preziosi et al. 1999). While invasive populations of *H. axyridis* are expected to show high adaptive phenotypic plasticity, biocontrol populations which have long failed to invade are expected to display low phenotypic plasticity. This hypothesis rests on the low variability of the artificial rearing conditions which should lead to the loss of adaptive plasticity (Masel et al. 2007).

In this paper, we compare the adaptive phenotypic plasticity displayed by two invasive (from England and southern France) and two biocontrol populations of *H. axyridis*. In a first experiment, we measured six phenotypic traits related to fitness (eggs hatching rate, larval survival rate, development time, sex ratio, fecundity over 6 weeks and survival time of starving adults) at three temperatures (18, 24 and 30°C). In a second experiment, we recorded the survival rate and quiescence aggregation behaviour when exposed to low temperatures (5, 10 and 15°C). Finally, we studied the cannibalistic behaviour of populations in the absence of food. The implications of our results in relation to the choice of the environmental parameters to be manipulated for assessing phenotypic plasticity variation among *H. axyridis* populations are discussed.

Material and methods

Population sampling and rearing

Four populations were used in this study. Two strains maintained in the laboratory for several years and used as biological control agents were provided by the firm BIOTOP (Valbonne, France): the biocontrol strain, commercialized between the years 1995 and 1999 all over Europe (hereafter referred to as population Biocontrol 1) and the so called flightless strain, selected in the late 1990's from the Biocontrol 1 strain for its incapacity to fly and disperse (Tourniaire et al. 2000a, b) and commercialised since 2000 in Europe (hereafter referred to as population Biocontrol 2). Although the biocontrol strains 1 and 2 evolved separately for 50–100 generations and phenotypic traits are supposed to evolve quickly, they cannot be considered as strictly independent evolutionary replicates of biocontrol populations. Two other samples were collected in the wild from two invasive populations in Europe. The first one, referred to as population London, was collected on September 2006 in London, England (51°28′44″ North; 00°09′02″ West) where *H. axyridis* has been reported since 2004 (Majerus et al. 2006; Brown et al. 2007b). The second one, referred to as population Roquefort, was collected on October 2006 in Roquefort-les-Pins, Southern France (43°40′44″ North; 07°02′26″ East), where it has been observed for at least 3 years including 2007 (Christine Delclos, Pers. Com. and Pers. Observation).

Before the experiment started, we maintained all four populations in the lab for two generations, under strict control conditions, in order to avoid bias due to maternal effects. During these two generations, populations were exclusively fed with ionized *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs and reared at constant environmental conditions (20°C; 60% HR; L:D 16:8). At generation F2, males and females were separated immediately after emergence to avoid any mating event. They were then maintained in the same environmental conditions for 2 weeks in order to insure their reproductive maturity at the beginning of the experiment. Fifty families of each population were then randomly created by pooling one male and one female in a cylindrical box (height = 3 cm; diameter = 8.5 cm) and temperature was increased to 24°C. Eggs produced by these families were then used to start the experiments. We further used 30 randomly chosen families from the 50 initially created.

Experiment 1: life history traits and phenotypic plasticity

The protocol used for this experiment is summarized in Fig. 1. At the beginning of the experiment, 45 eggs (3 × 15) of each family were equally distributed in three different rectangular boxes (length = 25 cm; width = 12 cm; height = 8 cm). The three boxes were placed in three separate rooms differing by their temperature: 18, 24 and 30°C. Relative humidity was maintained at ~60% in all rooms. After hatching, larvae were fed to excess every 2 days with fresh ionized eggs of *E. kuehniella* until adulthood. Several traits were measured for each box: (i) the number of hatched eggs among the 15 initially placed in each box (egg survival), (ii) the number of individual reaching adulthood (larval survival), (iii) the total development time (from egg laying to adult emergence) of each individual, and (iv) the sex of each adult. The boxes were then discarded after individuals were picked for subsequent experiments.

One adult female was picked from each box to measure fecundity. Each female was placed in a cylindrical box (see above) with one male from another box of the same population and the same temperature treatment. Eggs were counted and removed every two days during 6 weeks (42 days).

We also measured the lifespan of starving adults (male or female) at each temperature (one individual per family for each temperature) by placing each individual just after emergence in a small cylindrical box (height = 2 cm; diameter = 5 cm) with a damp piece of cotton wool. These small boxes were monitored daily and the date of death of each individual was recorded.

A global fitness index (w) for each family in each environment was calculated from four of the above traits using the following equation:

$$w = P_h * N_l * (1 - S_r) * F_{tot} \quad (1)$$

where P_h is the proportion of hatched eggs, N_l the number of individuals reaching adulthood, S_r the sex ratio (expressed as the proportion of males) and F_{tot} the total fecundity of the female after 6 weeks of adulthood.

For this fitness index, the adaptive phenotypic plasticity was quantified by computing the relative distance plasticity index (RDPI) proposed by Valladares et al. (2006). For each population, the RDPI was computed using the following equation:

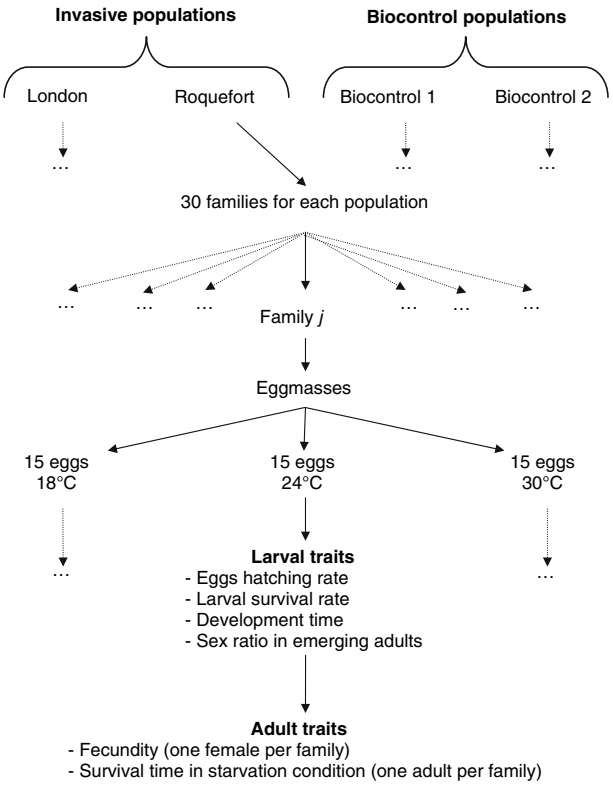

Fig. 1 Protocol design of experiment 1

$$RDPI_w = \frac{1}{n}\sum_{j}\sum_{i,i'}\frac{\left|w_{ij} - w_{i'j}\right|}{w_{ij} + w_{i'j}} \quad (2)$$

where j is the family index, i and i' are temperature indexes ($i \neq i'$) and n is the total number of relative distances. The $RDPI_w$ ranges from 0 to 1, and a value close to 0 means that the fitness is well canalised among environments, and thus that adaptive phenotypic plasticity is potentially high.

Experiment 2: quiescence

About 15 new-laid eggs of the 30 couples of each F2 population were randomly pooled in five rectangular boxes (length = 25 cm; width = 25 cm; height = 8 cm). Indeed, contrary to experiment 1, we could not use family structure for practical reasons (low number of individuals per family available at this stage of the experiment and reduced space available in environmental test chambers). Individuals were raised until adulthood in constant abiotic conditions (24°C; 60% HR; L:D 16:8) and fed with fresh ionized eggs of *E. kuehniella*. Temperature was then lowered to 18°C for 1 month. Twelve groups of 14 individuals (seven males and seven females) of each population were then put into

rectangular boxes (length = 25 cm; width = 12 cm; height = 8 cm) with a damp piece of cotton wool, but no food. The bottom of the box was covered with a piece of corrugated cardboard (length = 25 cm; width = 12 cm). For each population, four boxes were then placed in a climatic chamber. Three climatic chambers were used in order to test three temperatures (5, 10 and 15°C; 60% HR; L:D 12:12). After 5 weeks, we measured at each temperature (i) the number of live individuals in each box and (ii) the proportion of live individuals that aggregated under the cardboard, revealing quiescence aggregation-like behaviour.

Because we did not use a family structure here as in experiment 1, we could not calculate a RDPI parameter. Hence we calculated a coefficient of variation (CV) for each population with the mean number of survivors at each temperature to roughly evaluate fitness canalisation from our quiescence data. In this case, low CV indicates strong adaptive phenotypic plasticity.

Experiment 3: cannibalism

Depending on the population, from 13 to 18 females were randomly collected from fecundity measures of experiment 1 at 24°C. Each female was put alone in a small cylindrical box (height = 3 cm; diameter = 8.5 cm) with no food except 20 of its own eggs and 20 eggs laid by a randomly chosen female from one of the three other populations. Eggs were all laid in the preceding 12 h on small pieces of drawing paper which were marked in order to discriminate between the origin of the different egg patches. Monitoring was performed after 24 h and 48 h by counting eggs eaten and identifying their origin.

Data analysis

In experiment 1, we used generalized linear models to assess the effect on each of the six studied phenotypic traits and on the fitness index of the temperature, the population status (either invasive or biocontrol), the population nested within status and the two interactions involving the temperature. A binomial probability distribution and a logit link function were used for rate data (i.e. hatching rate of eggs, larval survival rate and sex-ratio). A Gamma probability distribution and an inverse link function were used for temporal data (i.e. family mean development time and lifespan of starving adult). Finally, a Poisson probability distribution and a log link function were used for count data (i.e. 6 weeks total fecundity of each female and fitness index as the latter was expressed as an indirect count of descendants). The effect of the population on the RDPI was tested with a non-parametric Kruskal–Wallis test using family scores as replicate units within population.

In experiment 2, a generalized linear model with a binomial probability distribution and logit link function was used to test the effect of the temperature, the population status, the population nested within status and the two interactions involving the temperature on the survival rate of individuals. For each population at each temperature, we investigated the aggregation behaviour by testing deviation from the null hypothesis of a 1:1 ratio of individuals under and over the cardboard using a χ^2 test. Because in standard rearing conditions, individuals are generally active and patrol all over the cardboard surface available and the surface under and over the cardboard was the same, we have considered that a 1:1 ratio corresponded the null hypothesis of random (i.e. non-aggregative) distribution of the beetles in the box.

In experiment 3, generalized linear models with a binomial probability distribution and logit link function were used to test the effect of the population on (i) the proportion of eggs eaten among the 40 after 24 h and after 48 h (cannibalism rates at T + 24 h and T + 48 h, respectively) and (ii) the proportion of own-laid eaten eggs among the total number of eaten eggs after 24 h (self-cannibalism rate). Using observation records at T + 24 h and T + 48 h, we also assessed for each population which type of egg patch (own-laid eggs versus eggs laid by a randomly chosen female from one of the three other populations) was consumed first by using a sign test.

All statistical analyses were performed with SAS software version 8.1 (SAS Institute Inc. 1999).

Results

Phenotypic plasticity of life history traits and fitness

We found a significant effect of the temperature for every trait (Table 1; Fig. 2). The population status (invasive or biocontrol) had a significant effect for three traits (larval survival rate, development time, and fecundity over 6 weeks) and for the fitness index. Most importantly, the interaction between the population status and the temperature, which reflects a potentially different response to temperature of invasive and biocontrol populations, was significant for the composite fitness index computed from the traits measured at 18, 24 and 30°C ($\chi^2 = 7.58$; df = 2; $P < 0.05$), but not for any of those traits considered independently. The population nested within status had a significant effect on all traits including fitness index (excepted on the fecundity over 6 weeks). No significant effect of the interaction between the latter factor and the temperature was detected for most traits (except for larval survival rate and sex ratio). Therefore, populations of same status displayed different traits values in each treatment but responded to variation of temperature in approximately the same way. Fitness was higher at 24°C for all populations, and biocontrol populations were globally more efficient in our experimental conditions (Fig. 2g). This trend was observed for most traits, including the total development time (not incorporated in the fitness index) for which the population London took slightly longer time to reach adulthood than the three others (Fig. 2c). This feature was less clear for the starving adult survival time (not incorporated in the fitness index) for which invasive populations lived in some cases longer than biocontrol populations (Fig. 2f).

The RDPI of fitness index, which is inversely proportional to the extent of adaptive phenotypic plasticity, was on average higher for the biocontrol populations ($RDPI_w$_Biocontrol 1 = 0.56; $RDPI_w$_Biocontrol 2 = 0.51) than for the invasive populations ($RDPI_w$_London = 0.42; $RDPI_w$_Roquefort = 0.5), but this difference was not statistically significant ($\chi^2 = 2.42$; df = 3; $P = 0.49$).

Quiescence

In experiment 2, the number of survivors after 5 weeks without any food was strongly explained by the temperature ($\chi^2 = 126.7$; df = 2; $P < 10^{-4}$), the population status ($\chi^2 = 148.29$; df = 1; $P < 10^{-4}$) and the interaction between both factors ($\chi^2 = 18.72$; df = 2; $P < 10^{-4}$) (Fig. 3). Population nested within status had a significant effect as well ($\chi^2 = 28.22$; df = 2; $P < 10^{-4}$), but the effect of the interaction between the latter factor

Table 1 Summary of statistical results using the generalized linear models for each traits of experiment 1, and the composite fitness index computed from these traits

	Effects				
	Temperature	Status	Temperature × status	Population (status)	Temperature × population (status)
Trait A: egg hatching rate	$\chi^2 = 83.31$	$\chi^2 = 0.17$	$\chi^2 = 0.18$	$\chi^2 = 36.6$	$\chi^2 = 4.51$
	df = 2	df = 1	df = 2	df = 2	df = 4
	$\mathbf{P < 10^{-4}}$	$P = 0.68$	$P = 0.91$	$\mathbf{P < 10^{-4}}$	$P = 0.34$
Trait B: larval survival rate	$\chi^2 = 20.82$	$\chi^2 = 4.83$	$\chi^2 = 1.44$	$\chi^2 = 8.37$	$\chi^2 = 14.23$
	df = 2	df = 1	df = 2	df = 2	df = 4
	$\mathbf{P < 10^{-4}}$	$\mathbf{P < 0.05}$	$P = 0.49$	$\mathbf{P < 0.05}$	$\mathbf{P < 0.01}$
Trait C: development time	$\chi^2 = 9183.92$	$\chi^2 = 10.91$	$\chi^2 = 5.51$	$\chi^2 = 10.11$	$\chi^2 = 1.6$
	df = 2	df = 1	df = 2	df = 2	df = 4
	$\mathbf{P < 10^{-4}}$	$\mathbf{P < 10^{-3}}$	$P = 0.06$	$\mathbf{P < 0.01}$	$P = 0.81$
Trait D: sex ratio	$\chi^2 = 63.48$	$\chi^2 = 1.08$	$\chi^2 = 3.74$	$\chi^2 = 27.57$	$\chi^2 = 21.74$
	df = 2	df = 1	df = 2	df = 2	df = 4
	$\mathbf{P < 10^{-4}}$	$P = 0.3$	$P = 0.15$	$\mathbf{P < 10^{-4}}$	$\mathbf{P < 10^{-3}}$
Trait E: fecundity over 6 weeks	$\chi^2 = 31.21$	$\chi^2 = 9.16$	$\chi^2 = 4.76$	$\chi^2 = 4.37$	$\chi^2 = 0.52$
	df = 2	df = 1	df = 2	df = 2	df = 4
	$\mathbf{P < 10^{-4}}$	$\mathbf{P < 0.01}$	$P = 0.09$	$P = 0.11$	$P = 0.97$
Trait F: starving adult survival	$\chi^2 = 841.58$	$\chi^2 = 0.89$	$\chi^2 = 4.91$	$\chi^2 = 6.72$	$\chi^2 = 3.72$
	df = 2	df = 1	df = 2	df = 2	df = 4
	$\mathbf{P < 10^{-4}}$	$P = 0.35$	$P = 0.86$	$\mathbf{P < 0.05}$	$P = 0.45$
Trait G: fitness index	$\chi^2 = 55.22$	$\chi^2 = 7.16$	$\chi^2 = 7.58$	$\chi^2 = 11.29$	$\chi^2 = 3.05$
	df = 2	df = 1	df = 2	df = 2	df = 4
	$\mathbf{P < 10^{-4}}$	$\mathbf{P < 0.01}$	$\mathbf{P < 0.05}$	$\mathbf{P < 0.01}$	$P = 0.55$

Significant *P*-values at the 5% threshold level are in bold characters. Status = invasive or biocontrol population

and temperature was not significant ($\chi^2 = 2.65$; df = 4; $P = 0.62$). Therefore, invasive populations always had higher survival rates than the biocontrol populations, with the population London showing the lowest mortality. At 5°C, 86% of the population London survived versus 54% for the population Roquefort and 41% for both biocontrol populations. The coefficients of variation (CV) calculated from the mean number of survivors at each temperature were substantially higher for the biocontrol populations (CV_Biocontrol 1 = 0.85; CV_Biocontrol 2 = 0.75) than for the invasive populations (CV_London = 0.16; CV_Roquefort = 0.33). Whereas at 15°C no population displayed a significant trend for aggregation under the cardboard, significant aggregation behaviour was observed for all populations at 5°C. At 10°C, the population London was the only one to display significant aggregation behaviour (Table 2).

Cannibalism

The factor population significantly explained the cannibalism at T + 24 h ($\chi^2 = 29.37$; df = 3; $P < 10^{-4}$) and T + 48 h ($\chi^2 = 12.61$; df = 3; $P < 0.01$) (Fig. 4a), as well as

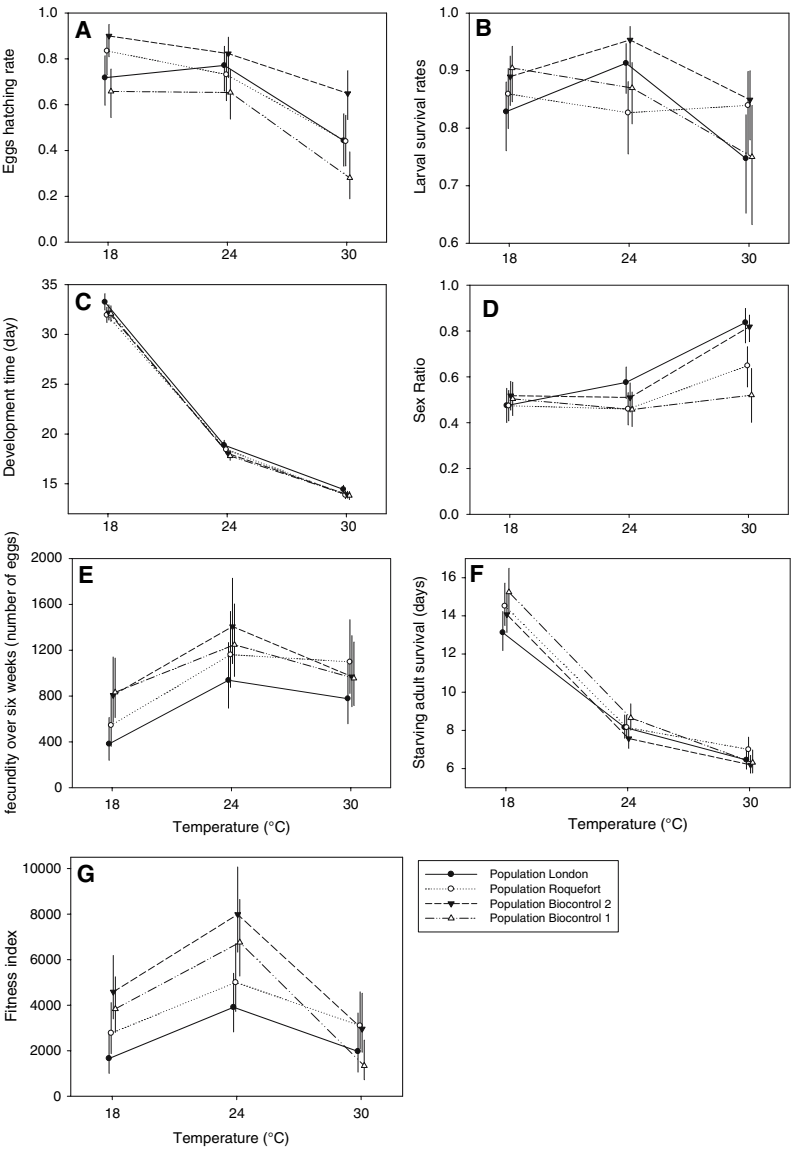

Fig. 2 Reaction norms to temperature (18, 24 and 30°C) of traits measured in experiment 1: (**a**) eggs hatching rates, (**b**) larval survival (from eggs until adult emergence), (**c**) family mean development time (until adult emergence), (**d**) sex ratio (proportion of males) of emerging adults, (**e**) fecundity over 6 weeks, (**f**) survival time in starvation conditions and (**g**) composite fitness index (see "Materials and methods" section for details). For each population and each temperature, vertical lines correspond to 95% confidence interval. The *P*-values associated to the effects of "temperature", "status", "temperature × status", "population" (nested within status) and "temperature × population" of the generalized linear models are given for each trait in Table 1. Status = invasive or biocontrol population

differences in self-cannibalism rates ($\chi^2 = 40.57$; df = 3; $P < 10^{-4}$) (Fig. 4b). The population London was mostly responsible for this effect. At T + 24 h, *H. axyridis* from population London had eaten only 8% of the total number of eggs versus 61–85% for the

Table 2 Proportion of individuals which aggregated under the cardboard (P_u) in experiment 2

Population	5°C			10°C			15°C		
	n	P_u	*P*-value	*n*	P_u	*P*-value	n	P_u	*P*-value
London	48	**0.96**	**$<10^{-4}$**	53	**0.79**	**<0.01**	38	0.42	0.49
Roquefort	30	**0.90**	**$<10^{-3}$**	45	0.58	0.46	24	0.29	0.14
Biocontrol 1	23	**1.00**	**$<10^{-4}$**	32	0.53	0.80	1	NC	0.41
Biocontrol 2	23	**1.00**	**$<10^{-4}$**	30	0.53	0.79	3	NC	0.68

Significant *P*-values at the 5% threshold level are in bold characters

n is the total number of surviving individuals at the time of the P_u measurement

P-values are obtained from χ^2 test

NC = χ^2 test not computed due to low sample size ($n < 5$)

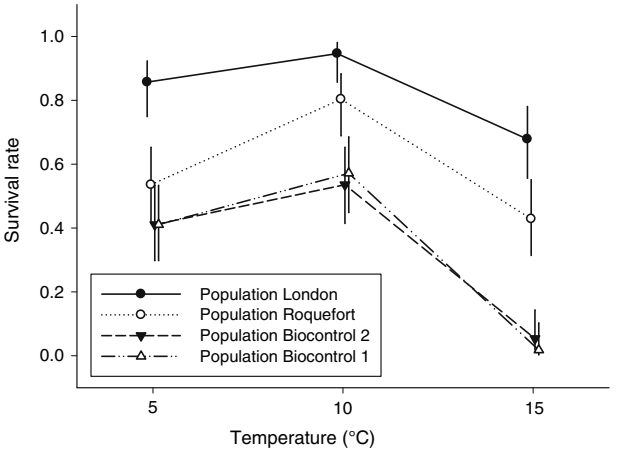

Fig. 3 Survival of adults after 5 weeks at low temperature (5, 10 and 15°C) and without food. Vertical lines correspond to 95% confidence interval

three other populations. After 48 h, more than 75% of all the eggs were eaten in all populations. The population London was the only one for which the eggs originating from other populations were eaten first (signed test; $M = 4$; $P = 0.0386$). Indeed, only 2% of the eggs eaten after 24 h by females from the population London were their own eggs (versus $\sim$50% for the three other populations).

Discussion

Results from experiment 1 show that invasive and biocontrol populations display significantly different responses to temperature variation for the composite fitness index computed from the traits measured at 18, 24 and 30°C, but not for any of those traits considered independently. The adaptive plasticity measured from the RPDI of the fitness index was higher in the two invasive populations than in the two biocontrol populations. However, this difference was far from being statistically significant. Thus, the results of experiment 1 suggest only minor differences in adaptive phenotypic plasticity between

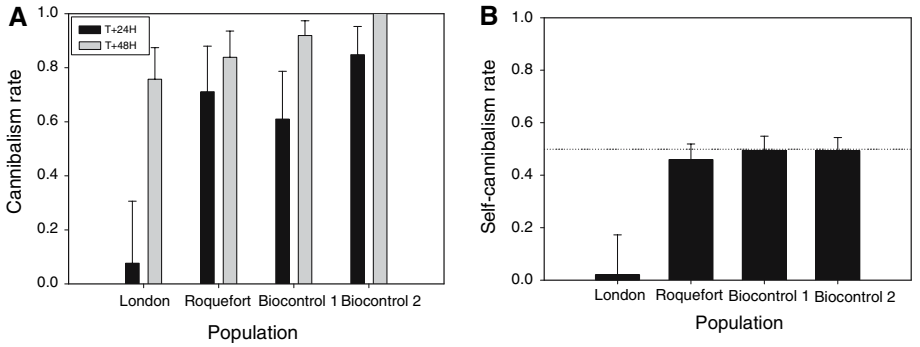

Fig. 4 Cannibalism in *H. axyridis*. (**a**) mean proportion of eggs eaten after 24 and 48 h (T + 24 h and T + 48 h, respectively) for each population. (**b**) Self-cannibalism = mean proportion of self-eaten eggs among eaten eggs after 24 h. Vertical lines correspond to 95% confidence interval

populations and hence do not strongly support the expectation of increased phenotypic plasticity in invasive populations of *H. axyridis*.

This conclusion should, however, be mitigated for at least three reasons. First, some other environmental parameters may be more suitable than temperature to detect phenotypic plasticity (Stillwell et al. 2007). For example, food may be a better environmental parameter to test for phenotypic plasticity in *H. axyridis* which is known to be polyphagous (e.g. Preziosi et al. 1999; Specty et al. 2003; Berkvens et al. 2007) and encounter a taxonomically diverse range of phytophagous insects associated with various vegetation communities. Second, our estimation of fitness is likely to be a poor representation of fitness in the wild. In particular, the "flightless" population Biocontrol 2 had the highest fitness index in our experiment, but probably suffers from low or null fitness in the wild because of its incapacity to disperse and migrate to aggregation sites (Tourniaire et al. 2000a). Third, the use of *E. kuehniella* eggs in our experiments may have distorted at least some of our results, as this food is likely to favour the biocontrol populations that have been fed this way for at least 20 years (Schanderl et al. 1988). This probably increased artificially the extent of adaptive phenotypic plasticity in biocontrol populations and may have prevented us from detecting differences between invasive and biocontrol populations. Indeed, high resource availability and high resource acquisition capacity are expected to mask resource allocation strategies in response to environmental variations (e.g. Malausa et al. 2005). In other words, the fact that biocontrol populations consumed more food than the other populations probably allowed them to canalize their fitness better as they could allocate increased resources to the expression of every phenotypic trait whatever the environmental conditions.

In contrast to the estimation of reaction norms to temperature ranging from 18 to 30°C (experiment 1), measures of survival during quiescence (experiment 2) clearly showed higher fitness and adaptive plasticity of invasive over biocontrol populations. Invasive populations and especially the population London suffered far lower mortality than biocontrol populations, the latter showing a poor ability to enter into quiescence. The response to low temperature variations assessed from the three tested temperatures was significantly different between invasive and biocontrol populations and CV were substantially lower for invasive populations. Such low coefficients of variation indicate fitness homeostasis through adaptive phenotypic plasticity. The problem of entering into quiescence experienced by the biocontrol populations may explain, at least partly, why the species failed to become established for around 10 years despite numerous intentional releases in the 1990's

of individuals originating from such populations. It is worth noting here that this result should not be taken as an argument that the present invasive populations in Europe do not originate from those biocontrol populations. Adaptive evolutionary change can indeed be very rapid, and this might be particularly important in biological invasions, which often involve drastic changes in selective regimes (e.g. Stockwell et al. 2003; Lambrinos 2004; Roy et al. 2008). Yet the origin of genetic variance at quantitative traits in invasive populations largely remains a mystery. In particular, the respective roles of ancestral genetic variation and in situ creation of new genotypes by mutation or recombination or hybridization events due to multiple introductions of individuals originating from genetically differentiated populations remain unclear. To tackle this question, we have started research actions based on genetic markers to elucidate pathways of introduction of invasive *H. axyridis* populations as well as their level of genetic variation relatively to native and biocontrol populations both in Europe and in America.

Cannibalism may also be an important trait in an invasion context. Our results highlight strong differences in cannibalistic behaviour of the invasive population London compared to the three others. First, the population London displayed a significantly lower degree of cannibalism after 1 day of starvation. Cannibalism can either be globally beneficial or costly depending on the ecological context (Polis 1981; Osawa 1992; Pervez et al. 2006; Williams and Hernandez 2006). The potential benefit of delaying cannibalistic behaviour during invasion remains unknown. Second, the population London clearly avoided self-cannibalism whereas the three other populations did not discriminate. This feature parallels cannibalism results obtained previously in a more standard kin selection context in other coccinellid species (Agarwala and Dixon 1993; Pervez et al. 2005). Selective cannibalism might be a determinant trait in an invasion context as it could be linked to associated behaviours such as inter-specific predation.

In conclusion, our results indicate that, despite globally weak differences in responses to temperature variation between invasive and biocontrol populations, phenotypic plasticity and its evolution may still play a role in determining the success of invasive populations in some extreme and/or ecologically relevant environmental conditions. Our results also highlight the fact that the traits to be measured and environments to be tested must be chosen carefully when attempting to detect variation of adaptive phenotypic plasticity among populations. In the case of *H. axyridis*, traits relative to activity regulation (ability to enter into quiescence during periods of low resource availability) and ability to forage for a variety of different food sources (including through cannibalism) appear to be of particular interest. In a more general perspective, a comparison based on those traits of invasive populations with populations from the native range would be of great interest to assess the evolution of phenotypic plasticity in *H. axyridis* during the invasion process.

Acknowledgments We thank Michael Majerus, the firm Biotop and Christine and Aurélia Delclos who kindly provided the population London, the biocontrol populations and the population Roquefort, respectively. We also thank André Ferran and Ludovic Giuge for advice about the *Harmonia axyridis* nursery stock, Helen Roy for comments and help in correcting our English writing, Thomas Guillemaud for helpful discussions, and three anonymous reviewers for useful comments. This work was supported by a grant from the French "Agence Nationale de la Recherche", project BioInv-4I.

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ANNEXE V

Article 5 (2011)

Facon B, Hufbauer RA, Tayeh A, Loiseau A, **Lombaert E**, Vitalis R, Guillemaud T, Lundgren JG, Estoup A (2011) Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. *Current Biology* **21**, 424-427.

Précisions sur le rôle de co-auteur :

- ➔ Gestion des élevages d'*H. axyridis* (maintien puis multiplication en prévision des expérimentations).
- ➔ Génotypage microsatellites.
- ➔ Discussions.
- ➔ Participation globale à la rédaction du manuscrit.

Current Biology 21, 424–427, March 8, 2011 ©2011 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2011.01.068

Report

Inbreeding Depression Is Purged in the Invasive Insect *Harmonia axyridis*

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Summary

Bottlenecks in population size reduce genetic diversity and increase inbreeding, which can lead to inbreeding depression [1]. It is thus puzzling how introduced species, which typically pass through bottlenecks, become such successful invaders [2]. However, under certain theoretical conditions, bottlenecks of intermediate size can actually purge the alleles that cause inbreeding depression [3]. Although this process has been confirmed in model laboratory systems [4], it has yet to be observed in natural invasive populations. We evaluate whether such purging could facilitate biological invasions by using the world-wide invasion of the ladybird (or ladybug) *Harmonia axyridis*. We first show that invasive populations endured a bottleneck of intermediate intensity. We then demonstrate that replicate introduced populations experience almost none of the inbreeding depression suffered by native populations. Thus, rather than posing a barrier to invasion as often assumed, bottlenecks, by purging deleterious alleles, can enable the evolution of invaders that maintain high fitness even when inbred.

Results and Discussion

Reductions in population size, or bottlenecks, decrease genetic variation and lead to inbreeding, which can cause inbreeding depression within introduced populations [1]. However, there is growing recognition that the consequences of bottlenecks are varied and that, under some circumstances, they can actually lead to increased individual and population performance [5, 6]. We lack the ability to say whether positive effects of bottlenecks are theoretical curiosities or whether they truly influence the dynamics of natural populations [7]. One mechanism by which bottlenecks can have positive effects is through the purging of deleterious mutations that lead to inbreeding depression [8]. Theory states that for purging to occur, the reduction in population size should be of intermediate intensity (i.e., ranging from 40–300 individuals, depending upon intensity of selection) and the mutations leading to inbreeding depression should be strongly deleterious and highly recessive [3, 7]. Such purging of deleterious mutations has been demonstrated empirically in artificially bottlenecked populations [4, 9, 10], but given the conditions imposed, high rates of extinction have been observed. This makes it difficult to directly extrapolate to natural populations [7]. To date, studies documenting a purge of deleterious mutations during bottlenecks are scarce and rely on indirect evidence [5].

The ability of invasive species to dominate novel ecosystems has been considered puzzling given that they typically pass through bottleneck in population size during introductions ([2], although see [11]. Such bottlenecks have been seen as detrimental to invasion success; the implicit assumption is that they reduce genetic variation, and thereby inhibit the ability of introduced species to adapt to their new environments, and that they increase inbreeding and associated inbreeding depression [2]. However, it may be that rather than increasing inbreeding depression, bottlenecks that occur during invasions tend to be of the intensity that could enhance invasion ability via the purging of the deleterious alleles underlying inbreeding depression.

Here, we use a world-wide invader, the harlequin ladybird *Harmonia axyridis* (HA), as a model system to examine whether bottlenecks might have led to reduced inbreeding depression in invasive populations relative to native ones. Native to Asia, HA was repeatedly introduced as a biological control agent into North America and Europe, but for decades it failed to establish itself. However, by 1988, it had not only established itself in North America but had also rapidly become an invasive pest on a world-wide scale. A recent study showed that invasions of HA followed a bridgehead scenario [12], in which the initial invasive population in eastern North America acted as the source of the invasions into the European, South American, and African continents (Figure 1). This result suggests that an evolutionary shift that triggered invasion probably occurred in the bridgehead population in eastern North America. With this background knowledge, we first use data from neutral genetic markers to test the hypothesis that the introduction of HA in eastern North America was associated with a population bottleneck, and we evaluate whether the size of this bottleneck was of the appropriate level for purging to occur. Then, we experimentally test the hypothesis that invasive populations have evolved reduced inbreeding depression with respect to life-history traits important for invasion success.

We investigated evidence for a bottleneck of an appropriate intensity for purging to occur by using data from 18 microsatellite loci that we analyzed with approximate Bayesian computation [13, 14]. Specifically, we evaluated whether a bottleneck occurred during the introduction of HA from the native area

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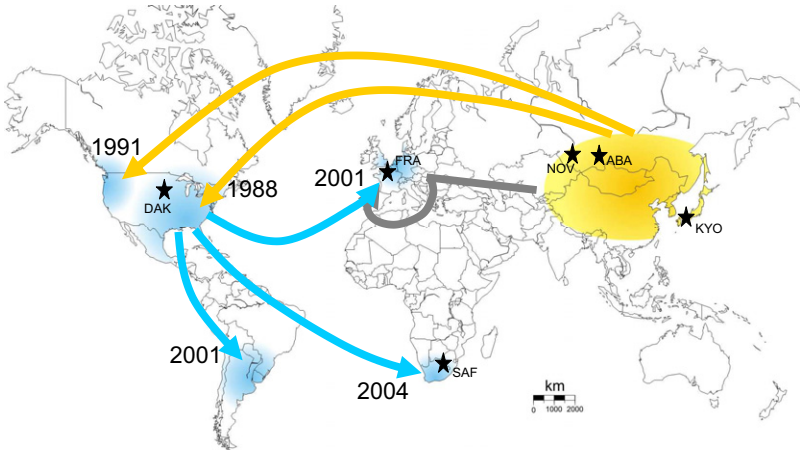

Figure 1. Worldwide Routes of Invasion of *Harmonia axyridis*

For each outbreak, the arrow indicates the most likely invasion pathway [12]. Yellow and blue indicate native and invasive areas, respectively. Years of first observation of invasive populations are indicated. Abbreviations correspond to the samples used in the experimental study (see Experimental Procedures for further explanations).

into the bridgehead population from eastern North America [12], and we estimated its intensity (see Supplemental Experimental Procedures). Two sets of population samples were considered as representative of the native and introduced areas. In one, we used the same populations as those used for the present quantitative genetics studies, and in the other, we used the same populations as those analyzed in [12] to make inferences about introduction routes in *H. axyridis* (see Supplemental Information). We found that a scenario including a bottleneck during the introduction was supported by very high probabilities in comparison to a scenario without a bottleneck (see Supplemental Experimental Procedures). The highest joint posterior densities of the size and duration of the bottleneck corresponded to values around 150 individuals and 20 generations compared to an estimated stable effective population size of several thousand individuals in the native area (Figure 2). Similar results were obtained when we considered other sets of priors and data sets (Figure S1). These results are well within the theoretical range that can lead to the purging of deleterious alleles [3]. However, it has to be noted that theoretical work is still needed to assess the range of magnitudes and durations of bottlenecks that make purging likely after introduction from a large equilibrium population. Indeed, up to now, theoretical studies investigating the purging of recessive mutations have mainly focused on mutation-selection-drift equilibrium populations.

To test the premise that purging might have occurred during the invasion of HA, we brought six natural field populations into the laboratory and compared their fitness (Figure 1 and Supplemental Experimental Procedures) under two contrasting levels of consanguinity (inbred versus outbred). By using three replicate populations from both the native and the invasive ranges, we could evaluate differences between the ranges robustly, providing a potent test of how the response to inbreeding is affected by population status (native versus invasive; see Supplemental Experimental Procedures). We measured two traits clearly linked to fitness: generation time and lifetime performance. Generation time is an important trait to examine with respect to invasions because a shorter generation time leads to faster population growth [15]. Our measure of lifetime performance accounts for both survival through the life stages and subsequent reproduction. It thus represents individual fitness well, and it is independent of generation time (Supplemental Experimental Procedures).

We found that the generation time of invasive populations is on average 6.3 days shorter than that of native populations ($p = 0.0005$). Invasion status and level of consanguinity interact ($p = 0.047$; Figure 3 and Table S1) such that the difference is most apparent in inbred individuals. Native populations suffer strong inbreeding depression with respect to generation time (coefficient of inbreeding depression, $\delta = 0.21$, $p = 0.03$), whereas invasive populations suffer none ($\delta = -0.05$, $p = 0.57$) and are thus able to maintain the outbred phenotype. A significant population effect nested within the origin effect (Table S1) reveals that one native population (Abakan, Russia) exhibits a longer generation time in outbred treatment than the other native populations, implying no significant inbreeding depression for this trait in this population. As for generation time, invasive populations have higher average lifetime performance than native ones ($p = 0.02$), and there is a strong interaction between invasion status and level of consanguinity ($p = 0.001$; Figure 3 and Table S1). In general, native populations suffer intense inbreeding depression ($\delta = 0.59$, $p < 0.001$), whereas invasive populations do not ($\delta = 0.12$, $p = 0.16$).

For both traits, invasive individuals exhibit a decline in inbreeding depression and are thus able to maintain the high performance of the outbred phenotype. Inbred invasive individuals developed more quickly and attained a higher lifetime performance than native ones ($p = 0.0005$ and 0.0057, respectively), indicating that inbreeding depression decreased within invasive populations, which is consistent with the predicted purging of recessive deleterious mutations. Moreover, inbred lines from invasive populations developed just as quickly and attained just as high lifetime performance as outbred lines from both invasive and native populations (Figure 3). Purging leads to an overall increase in performance of the invasive populations for these two traits closely linked to fitness, and it might thus have boosted the invasiveness of HA. Indeed, by shortening average generation time and increasing average lifetime performance, the drop in inbreeding depression might increase the population growth rate of invasive populations. Our two main results, evidence of a type of bottleneck consistent with the purging of alleles that lead to inbreeding depressions (i.e., a bottleneck of intermediate intensity) and evidence of such purging in two fitness-related traits, together match the theoretical expectations well. Moreover, theory [3, 5] illustrates that the greatest purging occurs when inbreeding depression is mainly due to mutations that are both strongly deleterious and highly recessive, suggesting that inbreeding depression in native populations of HA probably stems from highly recessive and strongly deleterious mutations.

Several theoretical [3, 16] and empirical [8, 17, 18] studies establish that consanguineous mating increases the efficiency of purging. Geographical spread during the invasion process

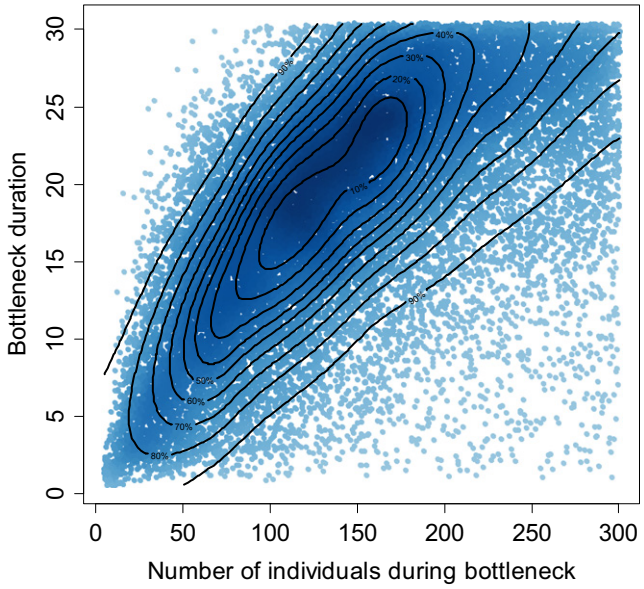

Figure 2. Intensity of the Bottleneck Event Following the Introduction of *Harmonia axyridis* in Eastern North America from Its Native Area

The joint densities of posterior distributions for the correlated pair of demographic parameters number of individuals during bottleneck and bottleneck duration (in number of generations) were obtained via ABC analysis of microsatellite data under the introduction scenario 1 (Figure S1); prior set 1 was assumed (Table S1), and population samples were from Kyoto (Japan) and Brookings (South Dakota, USA), taken as representative of the native and introduced areas, respectively (i.e., the samples were from the same populations as those used for the experiment). See Experimental Procedures for justification of population sampling and Figure S2 for complementary results obtained with different priors and sampling combinations. The black lines represent the 10%–90% highest density contours of the plot of joint densities. Median value of the stable effective population size before and after the bottleneck period was estimated at 2940 individuals (95% confidence interval: 1220 – 8930). See also Figure S1.

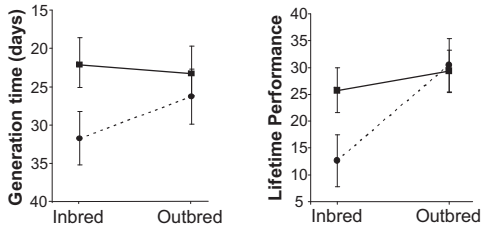

Figure 3. Generation Time and Lifetime Performance of Native versus Invasive Populations and Consanguinity of Inbred versus Outbred Populations

Circles represent native populations, and squares represent invasive ones. Note that the *y* axis shows low values of generation time, which correspond to high fitness, at the top, and high values of generation time (low fitness) at the bottom. Mean values are ±1.96 standard error. See also Table S1.

can promote consanguineous mating in the invasion front. Density in the front can be very low [19], setting up a situation in which individuals from the same clutch have only each other to mate with. In this scenario, purging could be further facilitated in invasive populations and could occur for a broader range of population sizes and in populations with less strongly recessive deleterious mutations.

The invasive populations used in our study are connected by their recent history [12]: the eastern North American invasive population is the main source of the South African and European invasive populations. It is hence probable that the reduction in inbreeding depression evolved only once, in eastern North America, and was subsequently transmitted to the other invasive populations. This mechanism could be responsible for the North American's status as an invasive bridgehead. Because we obviously could not sample all locations within the native range of HA, we cannot completely reject the hypothesis that purging of deleterious alleles occurred within the native range in an unknown way. The hypothesis that purging occurred in the introduced range during the bottleneck period is nevertheless far more parsimonious.

Our results shed new light on four patterns commonly observed in biological invasions. First, they help explain how non-native species spread so rapidly when they become invasive. Even small populations on the invasion front, in which consanguineous matings are probable, can grow quickly without being slowed by inbreeding depression if recessive deleterious alleles have been purged. Second, our findings might explain the "lag time" of invasions: the period of time that is often observed between initial introductions and subsequent invasions [20]. This lag time could be due to negative population growth and initially high rates of local extinction associated with the purging of the deleterious alleles. Once the recessive deleterious alleles are purged, explosive population growth would follow. In HA, it could be that a high rate of extinction of inbred populations contributed to the repeated failures of efforts to establish populations for biological control. Third, our results might explain the finding that invasive populations often have higher performance than native ones even when reared in a common environment. This has been attributed mainly to adaptation to the new range [21–23]. However, a purging of inbreeding depression could explain, at least partly, the increase in performance without invoking local adaptation. This mechanism could be particularly appropriate when there is no obvious adaptive challenge associated with the new introduced environment, as suspected for HA. Finally, a shift toward selfing has been observed in some invasive plants [24, 25]. Inbreeding depression is considered to be one of the main forces opposing the evolution of self-fertilization [26]. A reduction in genetic load during invasions could thus promote a shift from outcrossing toward selfing in invasive plant populations.

Our results link, for the first time in natural populations, bottlenecks of intermediate size during invasion with purging of deleterious mutations. This purging results in the evolution of populations that experience no inbreeding depression in important fitness traits and leads to higher mean fitness relative to native populations. Thus, not only might bottlenecks not pose the problems previously assumed for invasive species [27], but they might actually facilitate invasion. This kind of purging should be particularly important during the first stages of the invasion (when there is a small effective population size) and during the spatial expansion (at the front of invasion), i.e., when mating between relatives is likely to occur most frequently. After this stage, when invasive populations reach a large, stable effective population size, it might be that new deleterious mutations start to accumulate, and thus inbreeding depression might return to invasive populations.

Experimental Procedures

Biological Material

Three native populations (Kyoto in Japan [KYO], Novosibirsk [NOV], and Abakan [ABA] in Russia) and three invasive populations (Croix [FRA] in

France, Brookings [DAK] in South Dakota-USA, and Bethlehem [SAF] in South Africa) were sampled in the wild between 2007 and 2008. The locations were chosen because they cover major parts of the current native and introduced distribution of *H. axyridis* (Figure 1), and the native range populations are within the region likely to have been the source of the invasion [12]. In each population, 80–100 adults were collected. See Supplemental Information for further details on sampled populations.

Inferences about the Bottleneck Event

Two field-collected samples, Kyoto and Brookings, representing the native and bridgehead invasive populations, respectively, were genotyped at 18 microsatellite markers. Using approximate Bayesian computation (ABC), we analyzed two competing introduction scenarios that differed by the presence or absence of a bottleneck event after introduction. We assessed the robustness of our ABC inferences by considering two different sets of prior distributions and by processing our analyses on a second microsatellite data set that included other representative population samples (see Supplemental Experimental Procedures).

Quantitative Genetic Experiment

For each of the six populations sampled, 100 field-collected (G_0) individuals initiated populations in the laboratory. We maintained these populations for two generations under strictly controlled conditions to minimize potential biases due to maternal effects (see Supplemental Information). We then created two types of crosses: inbred (between pairs of siblings) and outbred (between unrelated individuals of the same population). For the two types of G_3 individuals produced, we measured hatching rate, larval survival, development time, time to sexual maturity, and fecundity. Finally, we analyzed two combined traits linked to fitness: generation time and lifetime performance (see Supplemental Experimental Procedure). To calculate generation time, we added egg-to-adult development time and time to reach sexual maturity into a single cumulative measure. We obtained a measure of lifetime performance by multiplying hatching rate by larval survival by subsequent fecundity for each family and cross. We analyzed these data by using mixed-model ANOVAs. Origin (invasive versus native), treatment (inbred versus outbred), population nested in origin, and their interactions were entered as fixed effects. Family nested within population was treated as a random effect.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, one figure, and one table and can be found with this article online at doi:10.1016/j.cub.2011.01.068.

Acknowledgments

We thank L. Wolfe and D. Bourguet for comments and discussion on an earlier draft. We thank I. Goryacheva, I. Zakharov, N. Osawa, R. Stals, and G. Prinsloo, who provided us with some of the HA samples used in this study. This work was supported by grants from the Agence Nationale de la Recherche (ANR-06-BDIV-008-01) and from the Agropolis Fondation (RTRA, Montpellier, BIOFIS project). R.A.H. received support from Fulbright-France, INRA, NSF DEB-0541673 and the Colorado Agricultural Experiment Station during this work.

Received: November 22, 2010
Revised: January 25, 2011
Accepted: January 26, 2011
Published online: February 17, 2011

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Current Biology volume *21*

Supplemental Information

Inbreeding Depression Is Purged

in the Invasive Insect *Harmonia axyridis*

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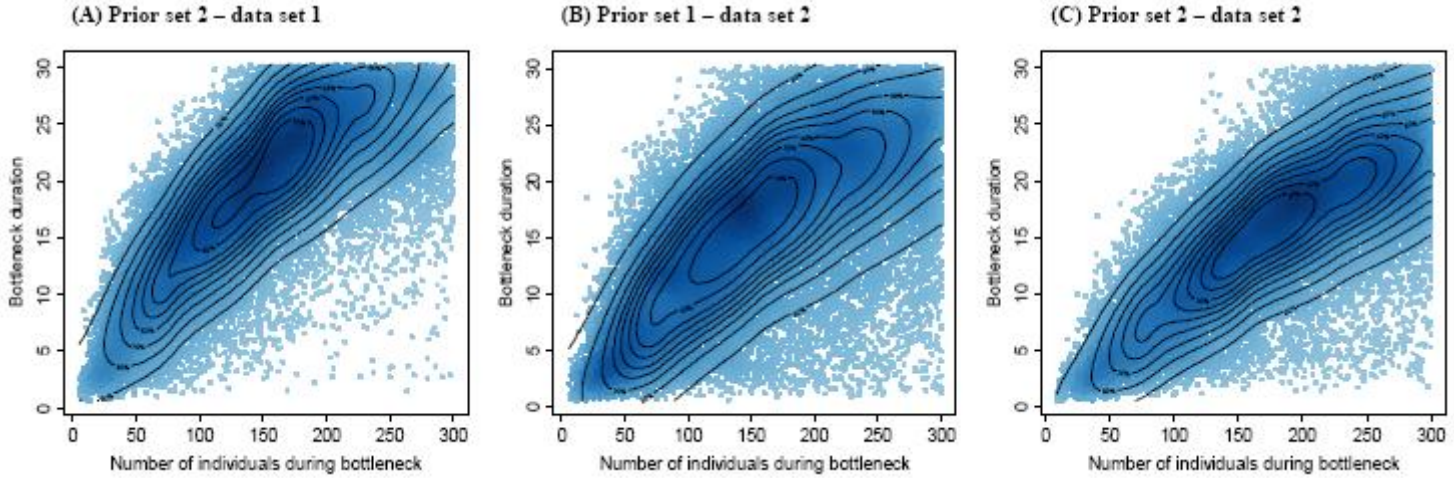
Table S1 (Related to Figure 3). Results of statistical analyses for lifetime performance and generation time. Population-level means for generation time are added due to the significance of the population effect for this trait.

Sources	Test statistic	P			
(A) Lifetime Performance					
Fixed effects	F (df)				
treatment	26,91 (1;45,3)	<0,0001			
origin	5,77 (1;47,4)	0,0203			
population (origin)	0,24 (4;46,4)	0,9152			
origin × treatment	11,97 (1;45,3)	0,0012			
pop (origin) × treatment	1,91 (4;43,8)	0,1251			
Random effect	Wald test				
fam(pop)	1.41	0.0789			
(B) Generation time				Population-level means	
Fixed effects	F (df)			Inbred	Outbred
			Invasive		
treatment	1,72 (1;45,7)	0,1962	populations		
origin	13,90 (1;46,1)	0,0005	CRO	20.55	23.76
population (origin)	2,60 (4;45)	0,0486	DAK	22.77	21.33
origin × treatment	4,15 (1;45,7)	0,0474	SAF	23.76	24.66
			Native		
pop (origin) × treatment	0,56 (4;44,4)	0,6956	populations		
Random effect	Wald test		ABA	34.01	32.28
fam(pop)	0.30	0.3817	KYO	26.91	21.53
			NOV	34.18	25.07

Figure S1 (Related to Figure 2). Intensity of bottleneck event following the introduction of *H. axyridis* in eastern North America from its native area: robustness of results. The presented joint posterior densities of number of individuals during bottleneck and bottleneck duration (in number of generations) were all obtained using ABC analysis of microsatellite data under the introduction scenario 1 (Figure S2). In (A), the analysis was achieved using the prior set 2 (Table S2) and data set 1 (Table S3). In (B) and (C), we used the prior set 1 and 2 (Table S2), respectively, and the data set 2 (Table S3). The black lines represent the 10 to 90% highest density contours of the plot of joint densities. Median values of the stable effective population size before and after the bottleneck period were 6090 individuals (95% confidence interval: 1220 – 8930) for (A), 7890 (95% CI: 4250 – 14460) for (B) and 11200 (95% CI: 5280 – 17600) for (C).



Supplemental Experimental Procedures

Biological material

Harmonia axyridis is native to Asia and invasive in North and South America, Europe, and Africa. It originally was introduced into North America and Europe as a biological control agent against aphids [28]. Despite repeated introductions, initiated in 1916 in North America and in 1982 in Europe, it did not establish readily. Suddenly, it not only established, but became invasive in four different continents. The invasion of North America started first, in 1988 [29], Europe and South America were invaded in 2001 [30, 31] and Africa in 2004 [32]. It is now considered to be a pest, and a harmful predator of non-target arthropods, a household invader, and a pest of fruit production [28]. Lombaert et al. [12] used Approximate Bayesian Computation on microsatellite data to demonstrate that the invasion followed what has been called a bridgehead scenario, with the oldest invasive population in eastern North America acting as the source, or bridgehead, for the colonists that invaded Europe, South America and Africa with some admixture with a biological control strain in the case of Europe (Figure 1).

Population sampling

Three live native populations and three live invasive populations were sampled in the wild between 2007 and 2008. The locations were chosen to cover major parts of the current distribution of *H. axyridis* (Figure 1) and to encompass the native regions used for biological control sampling [12]. The native range samples were from Kyoto (Japan, KYO), Novosibirsk and Abakan (Russia, NOV and ABA respectively). The

invaded range samples included Croix (France, FRA), Brookings (South Dakota, USA, DAK) and Bethlehem (South Africa, SAF) (Figure 1). In each population around 100 adults were collected (with ~1:1 sex ratio). It could be argued that only representative native and introduced populations have been sampled, and not necessarily the actual source and introduced HA populations. It is worth stressing, however, that we genotyped at 18 microsatellite markers [33] a large number of HA population samples collected within the eastern and western parts of the native range (9 locations) as well as within all invaded areas (more than 50 locations). Such analysis processed at selectively neutral markers confirms the genetic homogeneity of invasive populations within each area, except parts of Europe and to a lesser extent, South America (EL and AE, unpublished results). Therefore, the population samples used in this study, either for life history trait analysis or bottleneck analysis (see below), are likely to provide an appropriate representation of the main native and invasive populations over large geographic areas. Our sample size of three live populations per range (native and introduced) for life history trait analysis is, as required by logistical constraints, relatively low, While this lessens our statistical power to discern differences between the native and introduced range populations, differences we do find are likely to be ecologically significant.

Approximate Bayesian computation (ABC) to make inferences about the bottleneck event during introduction

Native populations of *Harmonia axyridis* in eastern Asia were genetically homogeneous over large geographic distances and introduced populations in eastern North America were homogeneous over large geographic distances (unpublished results, see also [12]). The bridgehead invasion scenario illustrated in Figure 1 brought us to focus our ABC estimations of the demographic parameters on the bottleneck event that

occurred during the introduction from the native range in eastern Asia into eastern North America. Two sets of population samples were considered. In a first sampling set, we used field-collected samples from the same populations than those used for the present quantitative genetics studies, i.e. Kyoto (Japan; n = 26) and Brookings (South Dakota, USA; n = 30), as representative of the native and introduced areas, respectively. In a second sampling set, we used field collected population samples similar to those analysed in [12] to make inferences about introduction routes in *H. axyridis*. More precisely, we used a pool of individuals collected in eastern Asia (Beijing - China, Shilin city - China and Fuchu – Japan; n = 99) and individuals collected in the first *H. axyridis* invasive foci observed in eastern North America (Joyce – Louisiana - USA; n = 34) as representative of the native and introduced areas, respectively. Details on this second set of population samples can be found in the Table S2 of [12]. We genotyped the two sets of population samples at the same 18 microsatellite markers [33].

Genetic variation within and between populations was summarized using a set of statistics traditionally employed in ABC [13, 34] (Table S3). We considered two competing introduction scenarios that differed by the presence or absence of a bottleneck event following introduction (Figure S2). The ABC analyses were performed using parameter values drawn from the prior distributions described in Table S2 and by simulating 2×10^6 microsatellite data sets for each competing scenario. We estimated the posterior probabilities of the competing scenarios using a polychotomous logistic regression on the 1% of simulated data sets closest to the observed data set, as defined by Euclidian distances [14]. The selected scenario is that with the highest probability value with no overlapping of the 95% confidence interval. We then estimated the posterior distributions of demographic parameters under the selected scenario (i.e. the introduction scenario with a bottleneck event; see Figure S2) using a local linear regression on the 1% closest of 2×10^6 simulated data sets [13]. The joint posterior densities of the demographic parameters of

interest, i.e. the effective number of individuals during bottleneck and the bottleneck duration (in number of generations), were estimated using the geneplotter R package [35]. The 10 to 90% highest density contours obtained using a personal R function based on the library locfit were then superimposed on the plot. The robustness of our ABC inferences were assessed considering two different sets of prior distributions and by processing our analyses on the two different data sets described above and in Table S2.

Table S2. Two sets of prior distributions of demographic, historical and mutation parameters used in ABC analyses

Notes: The time of first observation (T_o) was translated into generation numbers running back in time from sampling time in 2007 to first observation in eastern North America in year 1988 by assuming 2.5 generations per year in prior set 1, and 3 generations per year in prior set 2. N_s = stable effective population size (number of diploid individuals); N_b = effective number of individuals during the post-introduction bottleneck period lasting D_b generation(s). For microsatellite marker parameters, the loci were assumed to follow a generalized stepwise mutation model [39] with two parameters: the mean mutation rate ($\overline{\mu}$) and the mean parameter of the geometric distribution ($\overline{P}$) of the length in number of repeats of mutation events. Each locus has a possible range of 40 contiguous allelic states and is characterized by individual μloc and $Ploc$ values, with μloc and $Ploc$ drawn from a Gamma (mean = $\overline{\mu}$ and shape = 2) and a Gamma (mean = $\overline{P}$ and shape = 2) distribution, respectively [40]. Uneven insertion/deletion events that were detected for several of our microsatellite loci based on observed allele sizes (i.e. allele lengths were sometimes not multiple of the motif length implying that there has been insertion-deletion mutations [39, 41]) were also simulated with a mean mutation rate $\overline{\mu}SNI$ (for single nucleotide instability) and $\overline{\mu}SNIloc$ drawn for each locus from a Gamma (mean = $\overline{\mu}SNI$ and shape = 2).

Boundaries of distributions are in brackets. Parameters of Normal and Gamma distributions are in parentheses. In prior set 2, Normal, Loguniform and Gamma distributions are truncated between the same boundaries as in prior set 1. All prior quantities presented were computed from 100,000 values. NA = not applicable.

parameters	Prior Set 1 Distribution	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%	Prior Set 2 Distribution	Mean	Median	Mode	Quantile 2.5%	Quantile 97.5%
N_s	Uniform [100 – 20,000]	10,056	10,040	NA	640	19,490	Normal (10,000 ; 5,000)	9,993	9,990	9,980	1,640	18,340
N_b	Uniform [1 – 300]	151	151	NA	8	292	Uniform [1 – 300]	151	508	NA	8	292
D_b	Uniform [1 – 30]	15	15	NA	1	29	Uniform [1 – 30]	15	15	NA	1	29
T_0	Fixed at 47 generations	NA	NA	NA	NA	NA	Fixed at 58 generations	NA	NA	NA	NA	NA
$\overline{\mu}$	Uniform [10^{-5} – 10^{-3}]	5.0×10^{-4}	5.0×10^{-4}	NA	3.5×10^{-5}	9.8×10^{-4}	Loguniform [10^{-5} – 10^{-3}]	2.1×10^{-4}	1.0×10^{-4}	1.0×10^{-5}	1.1×10^{-5}	8.9×10^{-4}
$\overline{P}$	Uniform [0.1 – 0.3]	0.2	0.2	NA	0.10	0.29	Gamma (30 ; 136)	0.22	0.22	0.21	0.15	0.29
$\overline{\mu SNI}$	Uniform [10^{-8} – 10^{-4}]	5.0×10^{-5}	5.0×10^{-5}	NA	2.5×10^{-6}	9.7×10^{-5}	Loguniform [10^{-8} – 10^{-4}]	1.1×10^{-5}	1.0×10^{-6}	1.0×10^{-8}	1.3×10^{-8}	7.9×10^{-5}

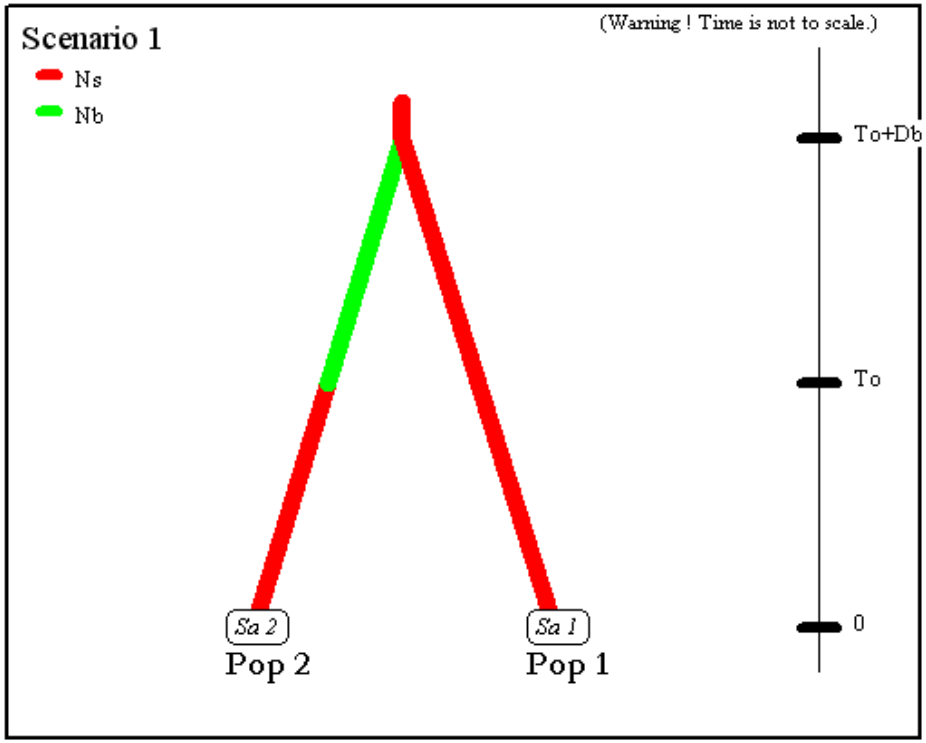
Table S3. Summary statistics of microsatellite data used for ABC analysis of bottleneck event with the corresponding observed values in the two sets of analyzed *H. axyridis* population samples.

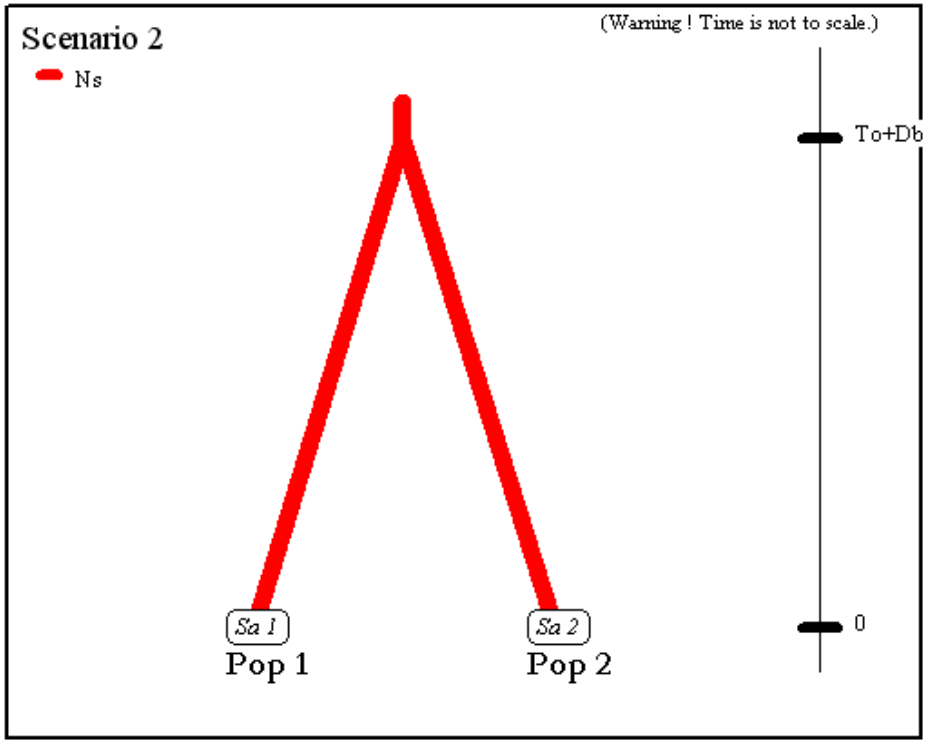
Note: *N* = native population sample; *I* = introduced population sample. Data set 1 = we used population samples from Kyoto (Japan) and Brookings (South Dakota, USA) as representative of the native and introduced areas, respectively. Data set 2 = we used a pool of individuals collected in eastern Asia (Beijing - China, Shilin city - China and Fuchu - Japan) and individuals collected in the first *H. axyridis* invasive foci observed in USA (Louisiana) as representative of the native and introduced areas, respectively. NAL_*i* = mean number of alleles in the native (*i* = *N*) or introduced (*i* = *I*) population, HET_*i* = mean expected heterozygosity [42], VAR_*i* = mean allelic size variance, MGW_*i* = mean ratio of the number of alleles over the range of allele sizes [43], F_{st} = F_{st} value between the native and introduced populations [44], LIK_*i*_*j* = mean individual assignment likelihoods of population *i* assigned to population *j* [41], H2P = mean expected heterozygosity pooling samples from the native and introduced populations, V2P = mean expected heterozygosity pooling samples the native and introduced populations. Populations *N* and *I* correspond to the populations 1 and 2 in Figure S2 respectively.

Summary statistics	Observed value	
	Data set 1 ($n_N = 26$; $n_I = 30$)	Data set 2 ($n_N = 99$; $n_I = 34$)
NAL_*N*	6.9444	10.3333
NAL_*I*	5.4444	5.8889
HET_*N*	0.5865	0.6007
HET_*I*	0.5674	0.5530
VAR_*N*	2.8448	2.7893
VAR_*I*	2.7469	2.5955
MGW_*N*	1.0176	0.9015
MGW_*I*	0.9577	0.9394
N2P	8.0556	10.8333
H2P	0.5810	0.5922
V2P	2.8085	2.7668
FST	0.0333	0.0170
LIK_*N*_*I*	1.3392	1.2088
LIK_*I*_*N*	1.2234	1.0408

Figure S2. Graphic representation of the two competing scenarios considered in ABC analysis of microsatellite data focusing on the introduction of *H. axyridis* in eastern North America (Pop 2) from its native area (Pop 1).

Notes: T_o and Time 0 are the first observation year in eastern North America (1988) and the sampling year (2007), respectively. The scenarios 1 and 2 correspond to an introduction with and without a bottleneck event with N_b individuals during D_b generations, respectively. All parameters with associated prior distributions are described in Table S2. Ns stands for the stable effective population size. The green line corresponds to the relative position of the bottleneck.


Scenario 1
(Warning ! Time is not to scale.)
Ns
Nb
To+Db
To
0
Sa 2
Sa 1
Pop 2
Pop 1


Scenario 2
(Warning ! Time is not to scale.)
Ns
To+Db
0
Sa 1
Sa 2
Pop 1
Pop 2

Quantitative genetic experiment

For each of the six populations sampled, we created families with inbred and outbred branches, and then compared their performance to evaluate whether introduced populations exhibit a different level of inbreeding depression than native populations (Figure S3). First, field sampled individuals (G_0) were used to initiate populations in the laboratory that were maintained for two generations under strictly controlled conditions to minimize potential biases due to maternal effects (Figure S3). For these first generations and the rest of the experiment, individuals were fed with ionized *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs and maintained at 23 °C, 65% relative humidity, with a photoperiod of L:D 14:10. From the G_0 individuals, we created around 50 pairs to produce the following generation by keeping one new male and female from each pair. We then randomly created 50 pairs of G_1 individuals to produce the G_2 individuals in the same way. During this step, males and females were separated immediately after emergence to prevent mating. They were then maintained in the same environmental conditions for two weeks to ensure that all individuals had reached reproductive maturity. All this procedure allowed us to minimize the risk of purging or fixation of deleterious alleles.

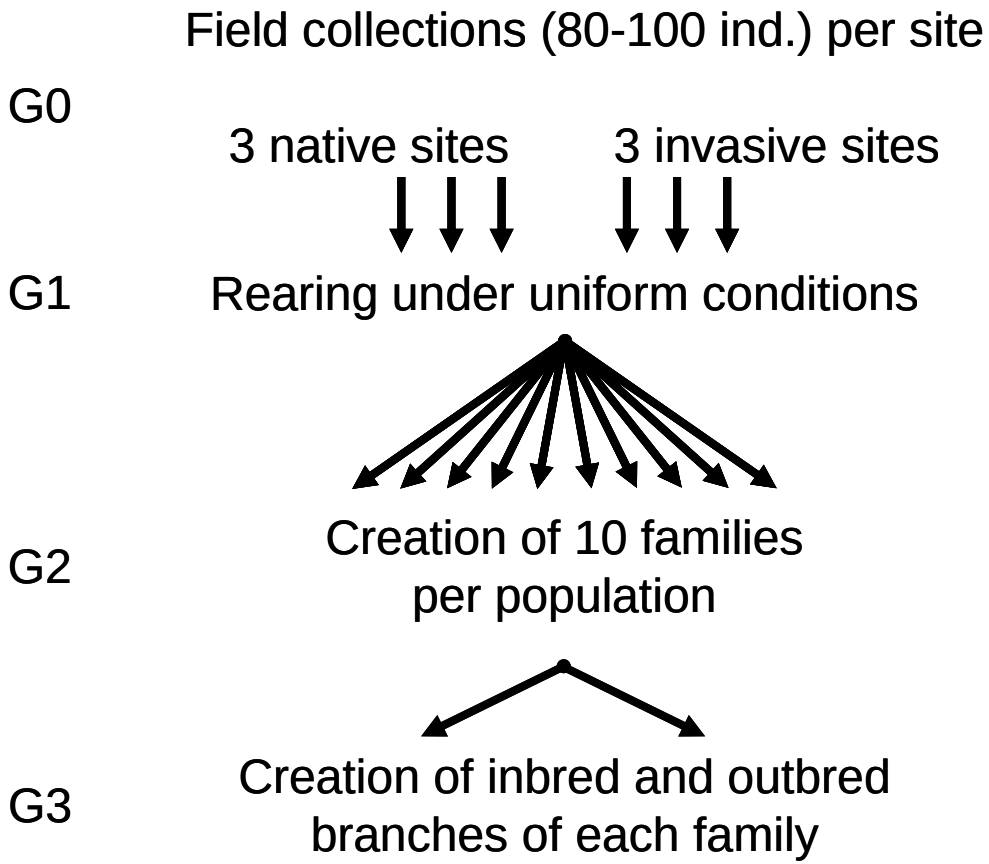
The experiment started with the creation of the third generation. Mature G_2 adults were used to create two types of crosses: inbred and outbred. Inbred crosses were between pairs of siblings, and outbred were between unrelated individuals of the same population. At the end of the experiment, we genotyped all the parents at eighteen microsatellites [33] and confirmed the difference of kinship between the two types of crosses for both native and invasive populations using the software SPAGeDi [36] (average kinship: 0.21 and -0.08 for native inbred and outbred

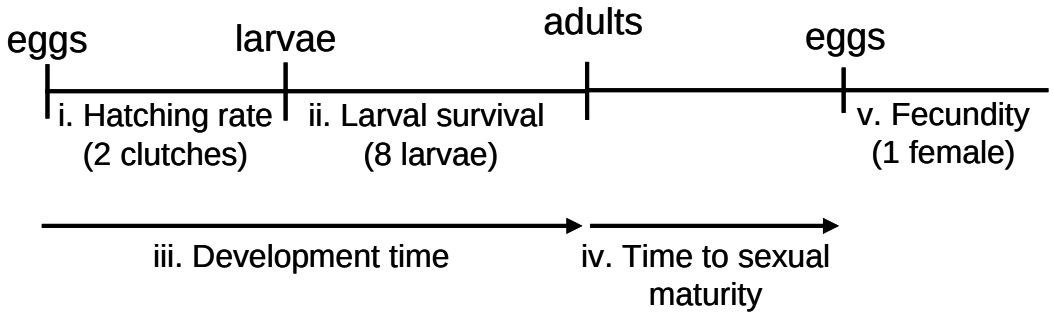
crosses, respectively, and 0.18 and -0.02 for invasive inbred and outbred crosses, respectively). We employed this protocol for 10 families per population, where each family was initiated with two sisters: one mated with a brother and one mated with an unrelated male. We collected and isolated two clutches of G_3 eggs with at least 20 eggs per clutch from each couple. At the day of hatching (the fourth day), 8 larvae were randomly chosen and each isolated for individual monitoring in a small cylindrical box (height = 2 cm; diameter = 5 cm).

The following traits were measured on the eggs and larvae (Figure S3). (i) Hatching rate was determined by counting eggs from all clutches and recording the number of living larvae after four days divided by the number of eggs in the clutch. (ii) Larval survival was scored daily. (iii) Development time was recorded as the period it took for individuals to develop from an egg into an adult.

A subset of individuals reaching adulthood was used for two additional measurements (iv – v). Ten days after emergence, one female per family and per cross was presented with potential mates. Each female was presented with a single male for a period of 24 hrs, and this was repeated three times with three different males during the course of a week. This procedure minimized density effects (e.g., delayed growth or reduced fecundity in paired individuals due to competition) while leaving time for multiple copulations to occur. Males were randomly chosen from the stock colony obtained with different mixing of individuals from the six populations to minimize bias due to male identity. (iv) Time to sexual maturity was estimated for the mated females by scoring the day when each first laid a clutch of eggs. At a maximum, we followed females for 60 days, after which we noted a failure to reproduce at all. (v) Fecundity was estimated as the number of eggs laid during the first eight days after the start of oviposition.

Figure S3. The rearing of *H. axyridis*, creation of inbred and outbred lines, and performance measurements taken. G_0 individuals were collected from six field sites, and used to initiate G_1 colonies reared in uniform conditions. Ten G_2 families from each site were used to create inbred and outbred lineages of each family. Numbering of measured traits follows the description in Supplemental Experimental Procedures.





Data analysis

We analyzed two combined traits linked to fitness for the experimental G_3 individuals: generation time and lifetime performance. To calculate generation time, we added egg-to-adult development time and time to reach sexual maturity into a single cumulative measure. Lifetime performance was obtained by multiplying hatching rate by larval survival by subsequent fecundity for each family and cross.

These data were analyzed using mixed-model ANOVAs (PROC MIXED, [37]). Origin (invasive vs. native), treatment (inbred vs. outbred), population nested in origin and their interactions were entered as fixed effects. Family nested within population was treated as random effect. A difference in the strength of inbreeding depression between native and invasive populations is revealed by the interaction term origin ×

treatment. We used linear estimates (ESTIMATE statement) to evaluate the direction and significance of differences between inbred and outbred within origins. Finally, for each trait, we calculated the proportional reduction in fitness due to inbreeding depression (δ) following Fox [38]. For generation time, $\delta = (Mean_{inbred} - Mean_{outbred})/Mean_{outbred}$ (the proportional increase of generation time), and for lifetime performance $\delta = (Mean_{outbred} - Mean_{inbred})/Mean_{outbred}$. Results are given in Table S1.

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ANNEXE VI

Article 6 (2011)

Turgeon J, Tayeh A, Facon B, **Lombaert E**, De Clercq P, Berkvens N, Lundgren JG, Estoup A (2011) Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion. *Journal of Evolutionary Biology* **24**, 1044-1052.

Précisions sur le rôle de co-auteur :

- ➔ Gestion des élevages d'*H. axyridis* (maintien puis multiplication en prévision des expérimentations).
- ➔ Discussions
- ➔ Participation globale à la rédaction du manuscrit.

JOURNAL OF Evolutionary Biology

doi: 10.1111/j.1420-9101.2011.02234.x

Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion

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Keywords:

adaptive evolution;
admixture;
biocontrol;
biological invasion;
Europe;
harlequin ladybird;
Harmonia axyridis;
Hybridization;
life history;
phenotype.

Abstract

Hybridization can fuel evolutionary processes during biological invasions. The harlequin ladybird *Harmonia axyridis* has long been used as a biocontrol agent before the species became invasive worldwide. Previous analysis based on microsatellite data has shown that European invasive populations bear traces of admixture between an eastern North American source, which is at the origin of the worldwide invasion, and biocontrol strains used in Europe. In this study, we tested the hypothesis that this early admixture event may have fostered the European invasion by impacting on the phenotypes of wild European populations. Mean life history traits of experimental F_1 hybrids are compared with pure parental sources and wild European crosses. Our results reveal a biased impact whereby North American beetles benefitted from being admixed with European biocontrol strains. Resemblance between experimental hybrids and wild European invasive crosses further suggests a long-lasting effect of admixture that may still be at work and fostering invasiveness.

Introduction

Biological invasions offer prime examples of rapid, contemporary adaptive evolution (e.g. Reznick & Ghalambor, 2001; Lee, 2002; Facon *et al.*, 2006; Carroll *et al.*, 2007; Dlugosch & Parker, 2008; Prentis *et al.*, 2008; Suarez & Tsutsui, 2008). In the introduced range, new selective regimes can cause genetically based shifts in phenotypes that provide a greater fitness in the new environment. Examples are quickly accumulating and include changes in tolerance to the abiotic environment and/or in major life history traits (e.g. Lee *et al.*, 2003; Bohn *et al.*, 2004; Bossdorf *et al.*, 2005; Xu *et al.*, 2009).

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Hybridization is one way to foster such adaptive evolution during invasion (Ellstrand & Schierenbeck, 2000; Rieseberg *et al.*, 2007; Schierenbeck & Ellstrand, 2009). Interspecific hybridization leads to new allelic composition, and evolutionary novelties may become fixed in allopolyploids or clonally reproducing lineages (e.g. Thompson, 1991; Abbott *et al.*, 2003). At the intraspecific level, multiple introductions and admixture of genetically differentiated source populations increase genetic diversity and often result in novel genotypes in invasive populations (e.g. Kolbe *et al.*, 2004; Darling *et al.*, 2008). Hybrid vigour may favour heterozygotes in early generations (Lynch & Walsh, 1998) and change mean population phenotypes. Importantly, admixture may also increase evolutionary potential when higher genetic variance, involving novel, recombinant and potentially fitter phenotypes, translate in heritable phenotypic variation, hence facilitating prolonged response

to selection. Through these processes, invasive hybrid populations may outperform parental sources, strongly indicating that admixture can promote invasiveness (Facon *et al.*, 2005; Lavergne & Molofsky, 2007; Kolbe *et al.*, 2007; Facon *et al.*, 2008; Keller & Taylor, 2010; but see Wolfe *et al.*, 2007). Certainly, hybridization and admixture may also have negative effects by disrupting coadapted gene complexes and weakening local adaptations (e.g. Barton & Hewitt, 1985; Keller *et al.*, 2000, Burke & Arnold, 2001; Bailey & McCauley, 2006).

Demonstrating that admixture resulted in adaptive evolution and so enabled a species to become invasive is not an easy task. It first requires that the identity of the ancestral populations at the origin of admixed invasive population be known (Keller & Taylor, 2008; Estoup & Guillemaud, 2010). Second, differences between derived and parental populations must confer higher fitness to admixed individuals and should not be because of chance events (Wolfe *et al.*, 2007; Xu *et al.*, 2009). Ideally, the fitness advantages should be matched with the new selective challenge imposed by the new environment in the introduced range and/or their impact on population growth, survival and expansion should be quantified.

Native to Asia, the coccinellid *Harmonia axyridis* (Pallas) (HA) has been introduced repeatedly in North America as a biocontrol agent against aphids since 1916 (Tedders & Schaefer, 1994; Krafsur *et al.*, 1997) and in Europe and South America since 1980s (Ongagna *et al.*, 1993; Poutsma *et al.*, 2008). These biocontrol strains were developed from small samples originating from various regions of the vast native area. Despite recurrent intentional releases, the species did not establish for decades. However, for unknown reasons, it recently and suddenly became invasive in eastern and western North America in 1988 and 1991 (USA, Chapin & Brou, 1991; LaMana & Miller, 1996), Europe in 2001 (Belgium, Adriaens *et al.*, 2003), South America in 2001 (Argentina, Saini, 2004) and Africa in 2004 (South Africa, Stals & Prinsloo, 2007). The species has spread widely in these areas where it consumes nontarget arthropods, invades households and is a pest of fruit production (Koch, 2003; Koch & Galvan, 2008).

On the basis of analysis of neutral genetic variation, Lombaert *et al.* (2010) recently retraced the routes of all five worldwide HA invasions. Eastern and western North American invasive populations originate from two independent introductions from the native Asian range. Surprisingly, eastern North America is the source of colonists for all other successfully invaded areas. In South America and South Africa, invasive populations bear no trace of genetic admixture with other sources. In Europe, however, there is clear evidence of admixture between eastern North American and the local biocontrol strain (with a contribution of biocontrol genes estimated at 43%, 95% CI: 18–83%; Lombaert *et al.*, 2010). The admixture scenario in Europe is strongly supported by quantitative comparisons with alternative invasion scenarios not involving admixture. Moreover, the microsatellite allele distribution in the European invasive population taken as reference sample (Gent, Belgium) is better explained by invoking contributions from both eastern North American populations and biocontrol strain; at several loci, the few European biocontrol strain alleles, of which some are not observed in America, are overrepresented and co-occur with alleles common in America.

Given the success of the colonists from eastern North America at invading several remote areas, parsimony suggests that the most important evolutionary shift enabling HA invasion has occurred in eastern North America following the introduction from the native range. The nature of this shift remains unknown. Moreover, it appears that admixture may not be necessary for invasiveness to develop in other areas colonized by eastern North American propagules. Indeed, there are no traces of admixture in South America, and HA was never used for biocontrol in South Africa prior to the recent invasion. Nevertheless, the admixture between eastern North American HA and the European biocontrol strain evidenced in Lombaert *et al.* (2010) may have played a role in impeding or facilitating the first outburst in Europe. The positive influence of admixture is classically associated with heterosis, i.e. admixed individuals display higher fitness than the mean of parental sources. In the context of the European invasion by HA, however, we are especially interested by the positive or negative consequences of admixture on the fitness of American propagules.

This study hence follows from our previous knowledge of the global *H. axyridis* invasion routes indicating that invasive European HA derive from the admixture between wild invasive populations from eastern North America and the European biocontrol strain (Lombaert *et al.*, 2010). We specifically tested the hypothesis that this admixture event affected HA life history traits early during the European invasion. Experimental crosses between biocontrol and American harlequin ladybird were performed to obtain admixed individuals. The impact on mean life history trait values was examined in the first hybrid generation and reveal that American HA benefited from admixture. Also, phenotypic resemblance between experimentally admixed and wild invasive European HA offers support for a long-term impact of admixture in the invasion process in Europe.

Materials and methods

Experimental procedures

We used invasive populations from eastern North America and European biocontrol strains as the two parental types (*American, Biocontrol*). One American population was sampled in the late summer of 2009 in Quebec City, Quebec, Canada (hereafter 'Q'). The other was sampled

in October 2007 in Brookings, South Dakota, USA (hereafter 'D') and was kept in the laboratory for two generations before this experiment started in the fall of 2009. South Dakota is located in central North America, but close monitoring of the spatial expansion of the invasion as well as microsatellite data (E. Lombaert & A. Estoup, unpublished data) indicate that *H. axyridis* from this state derived from the eastern North American invasion originating from Louisiana (Koch *et al.*, 2006). In Europe, three commercial *H. axyridis* strains have been used for biocontrol, all being derived from the INRA strain (Institut National de Recherche Agronomique, France) first released in France in 1982. Microsatellite genotyping of HA samples collected in these biocontrol strains confirmed that they are derived from the original INRA strain (unpublished results). Here, we used two strains that were in use in Europe when the first invasive population was reported in 2001 (Adriaens *et al.*, 2003); the third strain no longer exists. The first strain originates from the company Biobest NV (hereafter 'B') and was maintained in a laboratory at Ghent University (Belgium) at low population size for over 60 generations. The second strain was commercialized by the firm Biotop SA (hereafter 'T') until 2000 and was also maintained in the laboratory for many generations at INRA and then at Biotop rearing facilities. It is worth noting that this is not the Biotop flightless strain (Tourniaire *et al.*, 2000a,b) first released in France in 2000 and which is the only biocontrol strain used in Europe since 2002. Finally, we used a wild invasive European population sampled in 2009 in Belgium (Ghent, hereafter 'G'), the area where the European invasion began in the early 2000s (Adriaens *et al.*, 2003, 2008; Brown *et al.*, 2008). Based on data at 18 microsatellite loci, genetic diversity and levels of differentiation vary sharply among these populations and strains (unpubl. data). The two biocontrol strains have relatively low genetic diversity (expected heterozygosity: 0.31 and 0.38 in B and T, respectively), and they are strongly differentiated from one another (F_{st} = 0.38) as well as from every other wild invasive population (F_{st} = 0.16–0.33, mean: 0.26). In contrast, wild populations are genetically more diverse (expected heterozygosity: 0.57, 0.58 and 0.61 in D, Q and G, respectively), with the American populations not differentiated from one another (F_{st} = 0) and only moderately differentiated from Ghent (F_{st} = 0.034–0.046).

To reduce maternal effect, 50–60 individuals from each available population or strain (experimental G_0) were kept separately in the laboratory for one generation prior to the experimental crosses. For each population or strain, 20 couples were bred separately to produce the next generation (G_1). Upon emergence, adult males and females (G_1) were kept separated until all individuals were at least 1 week old. Rearing conditions remained constant for the entire experiment (23 °C; 65% RH; L : D 14 : 10). Individuals were fed *ad libitum* with irradiated eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae).

Table 1 Description of *Harmonia axyridis* experimental crosses. Admixed crosses (QB, QT, DB, DT, underlined) involve reciprocal crosses between sexes (e.g. QB = $Q_F \times B_M$ and $Q_M \times B_F$).

			Crosses				
Status	Provenance	Pop/Strain	Q	D	B	T	G
Invasive	North America	Quebec (Q)	QQ	–	QB	QT	–
		S. Dakota (D)		DD	DB	DT	–
Biocontrol	European biocontrol	Biobest (B)			BB	–	–
		Biotop (T)				TT	–
Invasive	Europe	Ghent (G)					GG

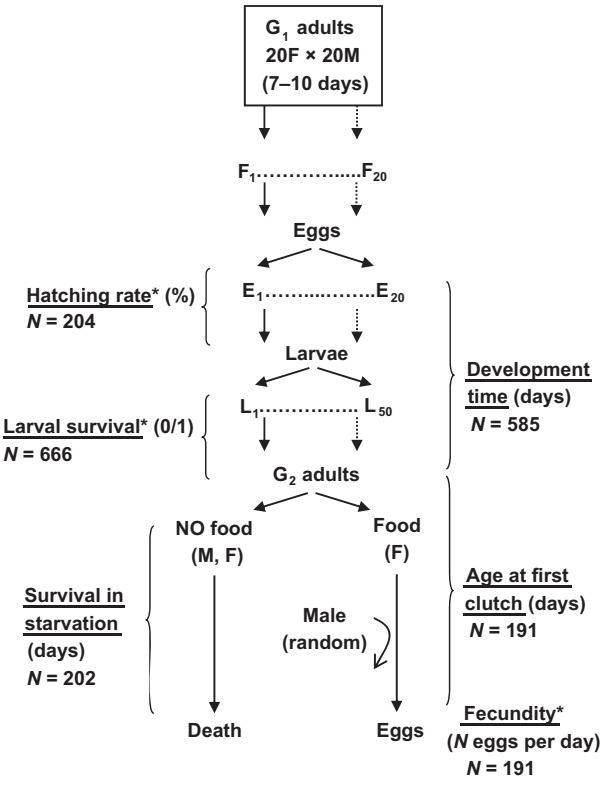

Fig. 1 *Harmonia axyridis* life history traits estimated for each experimental cross (see Table 1), along with total sample sizes. G_2-larvae and G_2-adults were kept individually in separate Petri dishes. Variables followed by a star were used to calculate the composite fitness index for 147 G_1-females (Fitness Index = Hatching Rate × Larval Survival × Fecundity).

Each American population (Q and D) was crossed with each biocontrol strain (B and T), including reciprocal crosses between the sexes, totalling eight crosses; intra-population/strain crosses were also performed including that of the wild invasive European population (G), totalling five additional crosses (Table 1). These 13 crosses are grouped into four *Types*, namely *American*, *Biocontrol*, *Admixed* and *Europe*. For each cross, 20 G_1-females were placed with 20 G_1-males in a large box and allowed to mate (Fig. 1). Seven to ten days elapsed before

females were isolated in Petri dish and data collection started.

For each cross, several life history traits were recorded for the G_2-individuals (Fig. 1). Hatching rate (*HatchRate*) was estimated as the proportion of eggs that developed into larvae. This was estimated as the mean hatching rate of two clutches from each of 11–20 females that laid eggs per cross. Larvae were then kept individually, and larval survival (*LarvSurv*) to successful pupation was recorded (0 or 1) for a mean of 50 individuals per cross (mean of 2.90 larvae per female for 12–20 females per cross). Development time (*DevoTime*, days) from egg to pupation was recorded for a mean of 45 individuals per cross (mean of 3.26 individuals per female for 12–20 females per cross).

G_2-adults were then separated in two groups (see Fig. 1). A subset of females were kept individually and fed upon emergence. These were used to record the age at laying of the first egg clutch (*AgeClutch*), fecundity (*Fecund*) and a composite fitness estimate (*FitIndex*). At age 7–10 days, each female was presented with a male for 24 h. Males were of similar age and randomly selected from a pool of males containing a balanced mix of males from each cross. The next day, they were offered another such male, again for 24 h. In cases where the females did not lay eggs within the next week, this procedure was repeated. *AgeClutch* was estimated as the number of days elapsed from pupation to the first clutch. The number of eggs laid over eight consecutive days following the first clutch were counted and averaged to estimate *Fecund*. The composite fitness estimate (*FitIndex*) was calculated for 147 G_2-females by multiplying *HatchRate*, *LarvSurv* (expressed in %) and *Fecund*. Individuals (both males and females) not used for *AgeClutch* and *Fecund* were kept individually without any food upon emergence. The number of days they survived was recorded as the starvation survival period (*StarvSurv*).

Statistical analyses

For each variable, conformance to Normal and Poisson distributions was appraised with Shapiro Wilk W and Kolmogorov's D tests, respectively. Larval survival coded as 0 (death) and 1 (survival) was treated as binomial. All time variables (estimated in days) were Poisson-distributed, whereas *Fecund* and *FitIndex* were normally distributed. *HatchRate* was arcsin-transformed to approach normality. For each variable, differences between reciprocal crosses (e.g. cross DB: $D_F \times B_M$ vs. $D_M \times B_F$) were assessed by means of Tukey–Kramer HSD tests as well as hierarchical ANOVAS with female origin nested within female *Type* (B or T within *Biocontrol*; D or Q within *American*). The vast majority of reciprocal crosses displayed no significant differences (results not shown). The only significant difference was between *DevoTime* for $D_M \times B_F$ vs. $D_F \times B_M$ ($P = 0.013$) when using HSD tests. Therefore, all subsequent analyses were performed with pooled reciprocal crosses (DB, DT, QB, QT).

First, we tested the hypothesis that admixed individuals are different from parental source(s). To do so, we tested for differences among the three corresponding *Types* of crosses, i.e. *Admixed*, *Biocontrol* and *American*. We used hierarchical general linear models with *Cross* nested within *Type* while specifying the appropriate distribution (normal, Poisson or binomial). When a *Type* effect was detected, we performed pairwise contrasts between *Types* to determine which *Type* differed and whether differences indicated lower or higher fitness. Higher values for *HatchRate*, *LarvSurv*, *Fecund* and *StarvSurv*, as well as lower values for *AgeClutch* and *DevoTime*, were considered indicative of higher fitness. For these variables, we also tested whether there was evidence of heterosis, i.e. if hybrids had mean trait values suggesting higher fitness than the mean traits of parents. This was performed by testing whether the mean of each admixed cross was higher (*LarvSurv*, *Fecund*, *FitIndex*) or lower (*AgeClutch*) than the mean of pure parental crosses [e.g. DB vs. mean (DD, BB)] using one-sided Tukey–Kramer HSD tests for *Fecund* and *FitIndex*, Wilcoxon rank-sum test for *AgeClutch* and logistical regression (modelling error variance with binomial distribution) for *LarvSurv*.

Second, we compared wild invasive European *H. axyridis* (*Europe*, represented by cross GG) with the other three *Types* to determine whether European invasive were different from *Admixed* and/or most similar to either alleged parental source (*Biocontrol* and *American*). To do so, we performed GLM analysis as above, using only the *Type* effect, followed by contrasts between *Europe* and the other three *Types*. Tukey–Kramer HSD tests provided highly similar results (not shown). All analyses were performed with JMP 8.01 (SAS Institute 2009, JMP, release 8.01: SAS Institute, Cary, NC, USA).

Results

For each variable, Table 2 summarizes results for *Types* and *Crosses* within *Types*. Variables were not correlated globally, within *Type* or within *Cross*. While a few odd correlations were detected, they were generally only slightly significant ($0.01 < P < 0.09$), and the same variables were not correlated in more than two *Cross* comparisons.

GLM statistical results are reported in Table 3. For *HatchRate*, *StarvSurv* and *DevoTime*, there were no significant *Type* effect detected among *Admixed*, *American* and *Biocontrol* ($P > 0.39$). For *LarvSurv*, *AgeClutch*, *Fecund* and *FitIndex*, highly significant statistical *Type* effects were detected ($P < 0.01$). In these cases, pairwise contrasts indicated that *American* was significantly different from *Admixed* and *Biocontrol*, but the two latter types were not different (Fig. 2a–d). *American* crosses had lower larval survival and laid their first clutch when they were older than *Admixed* and *Biocontrol* crosses. Fecundity was also lower in *American* than in *Admixed* and *Biocontrol* crosses.

Table 2 Mean values (and SEM) for life history traits estimated in *Harmonia axyridis* experimental crosses. Values for reciprocal admixed crosses (QB = $Q_F \times B_M$ and $Q_M \times B_F$) are pooled. See Table 1 for codes and cross description.

	American			Biocontrol			Admixed					Europe	
Type cross	DD	QQ	Total	BB	TT	Total	DB	DT	QB	QT	Total	GG	Grand total
Hatching rate													
Mean	73.83	73.88	73.85	66.41	78.87	73.14	75.43	75.44	76.21	76.97	76.02	77.28	75.29
SEM	4.35	4.24	2.99	3.56	2.45	2.32	3.03	2.54	2.17	2.79	1.33	3.09	1.02
N	15	14	29	17	20	37	34	34	30	33	131	16	213
Larval survival													
%	67.9	78.8	73.1	88.0	94.0	91.0	91.0	91.1	88.5	91.2	90.4	92.2	87.84
N	56	52	108	50	50	100	100	101	104	102	407	51	666
Development time (days)													
Mean	20.47	19.20	19.81	20.14	20.09	20.11	20.15	19.52	19.49	19.22	19.59	19.43	19.69
SEM	0.14	0.17	0.13	0.13	0.07	0.07	0.12	0.08	0.12	0.10	0.06	0.14	0.04
N	38	41	79	44	47	91	91	92	92	93	368	47	585
Age first clutch (days)													
Mean	12.79	13.92	13.31	11.47	10.80	11.13	10.69	11.68	10.68	11.57	11.16	12.14	11.52
SEM	0.99	1.48	0.86	0.69	0.78	0.52	0.26	0.48	0.28	0.43	0.19	0.91	0.20
N	14	12	26	15	15	30	29	31	31	30	121	14	191
Fecundity (egg/day)													
Mean	22.91	28.33	25.41	31.26	46.48	38.87	35.96	34.80	39.00	33.48	35.82	29.54	34.43
SEM	2.09	1.48	1.40	3.25	1.51	2.26	1.57	1.88	2.09	1.82	0.94	2.30	0.79
N	14	12	26	15	15	30	29	31	31	30	121	14	191
Composite fitness index (egg/day)													
Mean	14.26	16.47	15.30	20.13	33.86	27.23	26.36	22.83	27.49	25.91	25.59	21.28	24.15
SEM	2.54	2.01	1.62	2.62	2.16	2.10	2.46	1.63	2.37	2.04	1.06	2.35	0.85
N	10	9	19	14	15	29	20	23	21	22	86	13	147
Survival in starvation (days)													
Mean	8.21	8.07	8.14	8.41	7.53	7.97	7.37	7.39	7.94	8.03	7.69	8.00	7.82
SEM	0.61	0.38	0.36	0.26	0.24	0.19	0.26	0.34	0.30	0.28	0.15	0.33	0.11
N	14	14	28	17	17	34	30	31	32	31	124	16	202

Table 3 Results of nested GLM analyses (and pairwise contrasts) testing for differences in *H. axyridis* life history variables among parental (*American*: AME and *Biocontrol*: BIO) and *Admixed* (ADM) cross types. Significant *P*-values are shown in bold.

	Type (d.f. = 2)		Cross (*Type*) (d.f. = 5)		*Type* Contrasts			
Larval survival	χ^2	*P*-value	χ^2	*P*-value	AME-BIO	AME-ADM	BIO-ADM	Pattern
Hatching rate	1.84	0.39	6.28	0.27	–	–	–	–
Larval survival	19.21	**< 0.001**	3.39	0.64	**< 0.001**	**< 0.001**	0.77	AME↓
Development time	0.30	0.59	1.46	0.57	–	–	–	–
Age at first clutch	8.94	**0.011**	3.33	0.65	**0.018**	**0.003**	0.98	AME↑
Fecundity	28.49	**< 0.001**	2.09	**< 0.001**	**< 0.001**	**< 0.001**	0.11	AME↓
Composite fitness	21.07	**< 0.001**	18.61	**0.002**	**< 0.001**	**< 0.001**	0.49	AME↓
Survival (no food)	0.77	0.68	2.35	0.79	–	–	–	–

The two *Biocontrol* crosses were variable, but nevertheless averaged higher than *American* crosses (Table 2). *FitIndex* varied as fecundity, one of its component variables.

Heterosis was apparent, but affected crosses differently for different life history traits. There was no evidence for heterosis for *AgeClutch* (for DB vs. (DD, BB), DT vs. (DD, TT), QB vs. (QQ, BB) and QT vs. (QQ, TT); $Z = 1.37, 1.33, -0.76, -0.49$ and one-sided $P = 0.084, 0.091, 0.222, 0.310$, respectively). There was heterosis for *LarvSurv* only when the Dakota population was involved, but not when Quebec served as one parent (for DB vs. (DD, BB); DT vs. (DD, TT); QB vs. (QQ, BB) and QT vs. (QQ, TT): $\chi^2_1 = 7.36, 1.12, 5.09, 1.23$ and one-sided $P = 0.003, 0.010, 0.133, 0.144$, respectively). Evidence for heterosis was also found for *Fecund*; mean parental values were lower than those of the admixed cross only when the Biobest strain served as one parent (DB < mean (DD, BB) and QB < mean (QQ, BB); HSD one-sided $P = 0.002$). This same pattern was detected for *FitIndex*, which involves fecundity (HSD one-sided $P = 0.002$).

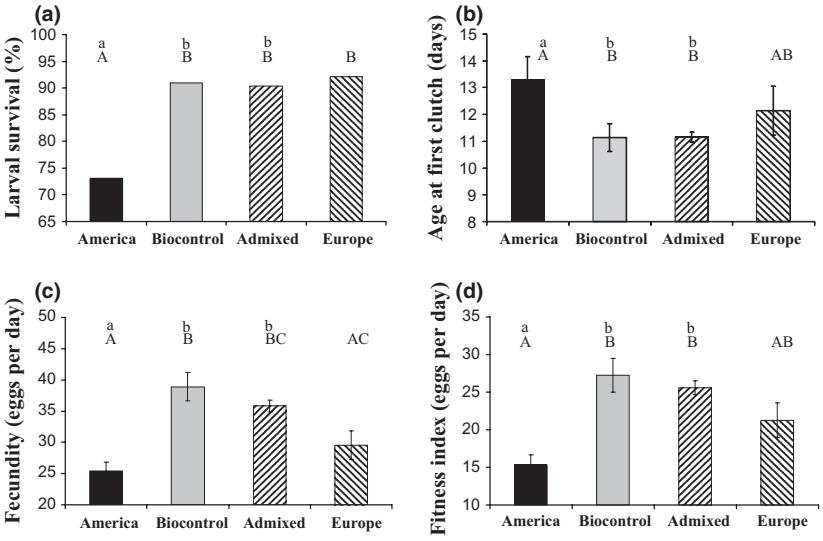

Fig. 2 Mean *Harmonia axyridis* trait values by *Type* (*American, Biocontrol, Admixed* and *Europe*) for variables showing *Type* effect (see Table 3). Error bars indicate SEM. Types with same letter are not significantly different. Small letters refer to tests for differences between *Admixed* and parental types (*American* and *Biocontrol*). Capital letters refer to tests comparing all four cross *Types* (*American, Biocontrol, Admixed* and *Europe*).

Comparisons among all four cross types indicated a strong *Type* effect (*LarvSurv*: $\chi^2_3 = 21.75$, $P < 0.001$; *AgeClutch*: $\chi^2_3 = 9.12$, $P = 0.0277$; *Fecund*: $\chi^2_3 = 29.71$, $P < 0.001$; *FitIndex*: $\chi^2_3 = 20.63$, $P = 0.001$). Comparisons between European invasive and the three other *Types* revealed that *Europe* was never different from *Admixed* ($P > 0.13$, Fig. 2a–d). For *LarvSurv*, *Europe* was similar to *Admixed* and *Biocontrol* and clearly significantly higher than *American* ($P = 0.003$, Fig. 2a). For *AgeClutch*, *Fecund* and *FitIndex*, *Europe* was intermediate between *American* and the other two *Types*. *American* was not different from *Europe* for these traits (Fig. 2b–d; $P > 0.32$), but nevertheless remained significantly different from *Biocontrol* and *Admixed* ($P < 0.02$, Fig. 2b; $P < 0.001$, Fig. 2c; $P < 0.003$, Fig. 2d).

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Discussion

Our precise knowledge of invasion routes of *H. axyridis* (Lombaert *et al.*, 2010) allowed generating the hypothesis that hybridization between eastern North American populations and the European biocontrol strain might have had an impact early during the European outburst. On one hand, the eastern North American propagules, having spread worldwide, were probably already invasive when they reached Europe. On the other hand, the European biocontrol strains seem to have been unable to establish sustained populations (Ferran *et al.*, 1997) and are thus likely to have low overall fitness in the wild. It was hence hypothesized that such an admixture could either enhance or restrict the invasive process in Europe.

Our experimental results show that admixed HA were often different from at least one parental type. The strongest trend was that admixed individuals possessed mean trait values different from American HA. Admixed individuals survived better in the larval period, females laid more eggs and at an earlier age. Changes in larval survival and fecundity coincided with egg hatching rate and so resulted in a higher composite fitness estimate of admixed relative to American individuals. Moreover, differences between admixed and American HA were all biased towards values contributing to faster population increase rate in the admixed crosses. In contrast, admixed individuals were similar to the biocontrol type for these traits and never possessed values suggesting lower fitness. This biased impact suggests that American HA can benefit from admixture. Despite recurrent use in agriculture, historical and genetic data indicate that, at least in Europe, the biocontrol strains never established and spread *in natura*. The biocontrol strains used in Europe seemingly possess unfavourable characteristics at some other important fitness-related traits not investigated in this study. For example, the Biotop strain has been shown to have lower hatching rate and very poor survival rate at low temperature (5–15 °C) compared with invasive European populations (Lombaert *et al.*, 2008); these debilitating traits may have prevented its establishment in Europe. Nevertheless, both biocontrol strains can, via admixture, enhance fitness-related trait value of American populations.

It could be argued that the observed bias in the effect of admixture, which mostly changed traits of the wild American type, simply reflects the acquisition of alleles conferring trait values favourable in the laboratory environment, for which biocontrol strains have long been indirectly selected for. However, the wild invasive European beetles were generally similar to the admixed

and biocontrol individuals, indicating that the laboratory environment is not, *per se*, the sole factor at work to explain the observed phenotypic effects. Likewise, heterosis does not appear to be a common cause for the change in life history traits of the admixed individuals. For fecundity (and the composite fitness index including fecundity), heterosis was observed only when crosses involved the biocontrol strain Biobest. This strain is characterized by a low fecundity relative to the biocontrol strain Biotop, as well as a suite of trait values suggesting lower general fitness (lower egg hatching and larval survival rate, greater age at first clutch, Table 2) We suspect that the Biobest laboratory strain we used was subjected to long-term low effective population size, and our experimental admixture per se may indeed have been beneficial for this strain. In any cases, crosses between both American sources and either biocontrol strains resulted in similar fecundity levels in Admixed individuals (GLM: $\chi^2 = 4.94$, d.f. = 3, $P = 0.17$), suggesting that crossing these two specific HA types does cause increased fitness in admixed individuals relative to the American parents.

General resemblance between experimentally admixed and wild invasive European HA bring support to the hypothesis that the admixture process affected important phenotypic characteristics of the resulting invasive populations. For larval survival and the age at first clutch, admixed and European crosses were comparable with the biocontrol strains, possessing higher mean trait values compatible with higher fitness. For these traits, it thus appears that admixed European invasive populations have retained the biocontrol genetic background associated to higher fitness. In contrast, the European cross displayed lower fecundity than the biocontrol strains. Higher fecundity in these two biocontrol strains relative to other invasive European populations has already been documented (Lombaert *et al.*, 2008), suggesting that our results are representative of a real difference. It is difficult to envision how lower fecundity may be advantageous for the invasive European beetles. The intermediate fecundity of European beetles may simply reflect their intermediate (i.e. hybrid) ancestry and segregation of additive genetic effects. However, when fecundity is combined with larval survival and hatching rate into the composite fitness index, invasive European HA resemble both admixed and biocontrol types. Given that fecundity is often related to fitness, there may also exist a trade-off between fecundity and unknown trait(s) not considered in that study.

Differences in values between experimentally admixed and wild invasive European HA relative to the parental sources may partly results from the fact that this comparison involves two types of hybrids. In our experiment, we measured phenotypic traits in F_1 hybrids raised in the laboratory. The invasive European population used for comparison is likely not composed of F_1 hybrids. The invasion was detected in 2001, and our Ghent sample is from 2009. Given that HA can produce 2–3 generations per year (Koch *et al.*, 2006), *ca.* 20 generations of evolution in natural settings might have elapsed. This time lag between experimentally admixed individuals (F_1) and wild invasive European HA (F_n) may, in fact, reveal the action of natural selection in nature on F_1 hybrids. In this case, our results showing that F_1 admixed HA are not significantly different from wild invasive European HA, while differing from the American parental source, would strongly suggest that phenotypic changes operating in the early admixture stage are, to a large extent, maintained in further generations. Alternatively, the intermediate values of European invasive relative to representative of American and biocontrol parental types may only result from additive effects. Nonetheless, these values would confer higher population increase rate (fitness) to the admixed individuals.

Our experimental design was inspired from the inferred invasion route indicating that European invasive genotypes are admixed HA between biocontrol and American sources at neutral genetic loci. Here, we show that life history traits of experimentally admixed individuals were also affected. Having used only two populations for the American parental type, and a single population to represent invasive European HA, it obviously cannot be strictly affirmed that our results are fully representative of what happened in the wild early during the European invasion. Nevertheless, despite the variation present between American populations (e.g. *Larv-Surv*, *Fecund*, Table 2), the latter were clearly affected by admixture. Also, the extant Ghent population was the best choice for comparison with F1 hybrids probably formed in this area where the European invasion began in the early 2000s. Overall, our experiment may not be an exact reproduction of the admixture event, but our results show quite clearly that biocontrol strains can favourably affect wild population via admixture. It is worth noting that genetic differentiation at neutral loci was not always related to phenotypic resemblance. For example, American populations were phenotypically very different from wild European HA, yet they were only slightly genetically differentiated ($F_{st} = 0.034$–0.046, unpublished data). In contrast, the strong genetic differentiation of each biocontrol strain with wild populations ($F_{st} = 0.26$–0.42, unpublished data) was paralleled by either strong (with American) or generally weak (with Europe) phenotypic differences. As per other studies (Dlugosch & Parker, 2008; Keller & Taylor, 2008), these comparisons stress the fact that neutral genetic characteristics, while crucial for reconstructing invasion routes, are not sufficient to inform on the adaptive processes at work during invasions.

Experimental evidence is accumulating that admixture can effectively fuel both early and late HA invasion stages in Europe. In this study, we reproduced the initial European admixture event previously evidenced by

neutral genetic markers. We show that American propagules benefit from contacts with biocontrol strain, leading to phenotypes resembling established invasive European populations. Recently, Facon *et al.* (2011) showed that admixture with another biocontrol strain still used in Europe (i.e. the flightless strain, also derived from the initial INRA biocontrol strain; Tourniaire *et al.*, 2000a,b) can further affect the phenotypic characteristics of contemporary invasive European populations. These two genetically differentiated HA types breed readily in the laboratory, and admixed offspring differ from parental types in terms of development time and their ability to withstand starvation periods. Moreover, mate choice experiments revealed that males of the biocontrol flightless strain sired more offspring, suggesting that admixture may be fostered by invasive female preferences and/or biocontrol male superiority. Altogether, it thus appears that both the initial propagule and the ensuing admixed wild invasive HA can benefit from genetic introgression with biocontrol individuals. Given the invasive success of propagules from eastern North America in South America and South Africa (Lombaert *et al.*, 2010), admixture may not have been necessary for the spread of HA in Europe. Nevertheless, biocontrol strains can effectively contribute to phenotypic changes compatible with higher invasion potential. A simple precautionary principle calls for ceasing to release HA strains for biocontrol control in Europe, irrespective of their flying ability.

Acknowledgments

This work was supported by grants from the *Agence Nationale de la Recherche* (ANR-06-BDIV-008-01) and from the Agropolis Fondation (RTRA – Montpellier, BIOFIS Project). We are grateful to A. Loiseau for help in the laboratory during rush periods.

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Received 26 October 2010; revised 16 December 2010; accepted 10 January 2011

ANNEXE VII

Article 7 (en préparation)

Lombaert E, Estoup A, Joubard B, Grégoire J-C, Jannin A, Facon B, Guillemaud T (In prep.) Rapid evolution of dispersal abilities during the expansion of the invasive ladybird *Harmonia axyridis* in Europe.

Rapid evolution of dispersal abilities during the expansion of the invasive ladybird *Harmonia axyridis* in Europe

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Abstract

The development of evolutionary theory has been mainly based on the concept of equilibrium; however, many populations experience demographic and genetic disequilibria during their history, including their range expansion or contraction. Understanding the evolutionary trajectories associated with disequilibrium has recently became an issue of growing interest. Invasive species offer a unique opportunity to study in detail the fate of genetic variation in rapidly spatially growing population and in particular the spatial sorting by which a trait linked to dispersal rate increases in frequency along the expanding range in a deterministic way. Here, we test the hypothesis of an increase of dispersal abilities during the range expansion of the Asian ladybird *Harmonia axyridis* that is invading Europe since 2001. We found a marked heritable increase of flight speeds from the core to the front of the invasion range. On the contrary, neither endurance nor behavioural propensity to fly was found to evolve on the same spatial gradient. Our results provide a striking illustration of how fast predictable directional evolution under spatial disequilibrium can occur. We discuss the consequences of our results on invasion dynamics and on global evolution of expanding populations.

Introduction

Some invasive species have detrimental impacts on human health, economy or environment (Olden *et al.*, 2004; Pimentel *et al.*, 2001; Ruiz *et al.*, 2000), others are regarded as beneficial organisms because they provide us with food supplies or help to regulate other damaging invasive species (Guillemaud *et al.*, 2011), but all can provide a fruitful source of knowledge about fundamental issues in evolutionary biology (e.g. Huey *et al.*, 2005). The course of a biological invasion can be partitioned into three steps: the introduction of a group of individuals into a remote area (introduction step), the establishment (acclimation step) and the demographic and geographic growth (expansion step). Each of these steps represents an opportunity to approach theoretical predictions and questions about evolution. In particular, the study of drift and neutral evolution associated with bottlenecks and/or hybridization during the introduction step has been significantly rising during the past 10 years (e.g. Estoup & Guillemaud, 2010; Simberloff, 2009; Wares *et al.*, 2005). Also, evidences of evolutionary shift allowing the adaptation to the new selective pressures encountered during the acclimation and the expansion steps are increasing and have highlighted how rapid natural selection can act when sufficient additive genetic variance is available in the introduced population (Huey *et al.*, 2005; Lee, 2002; Prentis *et al.*, 2008; Whitney & Gabler, 2008). However an important feature of biological invasions has only been barely studied so far: geographic expansions provide a singular opportunity to explore the evolutionary processes at work in populations that display strong genetic and demographic disequilibria where space plays a crucial role (hereafter referred to as "spatial disequilibrium").

Expanding populations are facing many evolutionary forces which vary in space from the core to the front. First, new selective pressures due to spatial disequilibrium may be encountered during expansion and can lead to local adaptation. One predictable selective pressure that varies with space along the expansion direction is the population density itself: high in the core and lower than the mean on the expansion edge. As a consequence, every density-dependent associated trait may evolve differently in various parts of the geographical range spectrum. In particular, high competitive ability will be favoured in the core of the population while high reproductive rates will be favoured in the front (Phillips, 2009; Phillips *et al.*,

2010). The second important evolutionary force which is at work in expanding population is drift. Recurrent founder events and a lower number of individuals are encountered in the front and can strongly shape the genetic structure in this area (loss of alleles, random variation of allele frequencies, spatial differentiation… Excoffier *et al.*, 2009). As a consequence, and because of the dynamic aspect of the process, a rare allele or a new mutation (either neutral, advantageous or deleterious) can theoretically surf on the expansion wave and reach high frequencies in newly colonized areas (Edmonds *et al.*, 2004; Excoffier & Ray, 2008; Hallatschek & Nelson, 2008; Klopfstein *et al.*, 2006). Such evolution generates peculiar allele frequency gradients and may lead to genetic differentiation between sectors of the expanding population. Finally, the third evolutionary force is what was called by Shine et al. (2011) the "spatial sorting". Spatial sorting allows the increase of a trait frequency along the expanding range in a deterministic way (unlike drift) but without conferring any direct fitness advantage (unlike adaptation). This process concerns specifically the evolution of dispersal rates and thus of any trait that improves an organism's dispersal aptitude: the edges of an expansion front will be formed by the best-dispersing individuals who will thus breed with each other. If the heritability of the dispersal ability is not null, such assortative breeding will mechanically produce offsprings with larger mean dispersal rates near the front than near the core of the expanding population (Shine *et al.*, 2011).

Evolution of increased dispersal at an expanding edge of a spatially growing population has been predicted by a number of recent theoretical models (e.g. Burton *et al.*, 2010; Hughes *et al.*, 2007; Phillips *et al.*, 2008; Shine *et al.*, 2011; Travis & Dytham, 2002; Travis *et al.*, 2010; Travis *et al.*, 2009). This prediction has been tested and confirmed in a number of species which experience range expansions around their native area in response to climate change (e.g. Cwynar & Macdonald, 1987; Darling *et al.*, 2008; Hill *et al.*, 1999; Leotard *et al.*, 2009; Simmons & Thomas, 2004; Thomas *et al.*, 2001) but only in a few invasive species (e.g. Monty & Mahy, 2010; Phillips *et al.*, 2006). Invasions provide nevertheless a good *in natura* laboratory to address this question for several reasons: (i) precise recent history of introduction and spread may be available, (ii) many invasions are in course and spatial disequilibrium is thus still at work and (iii) spread is mostly, at least during a period of time, not promoted by progressive modifications of the environment (e.g. climate change), thus reducing the weight of selective pressures in the process (Hill *et al.*, 2011). Moreover, applied issues regarding undesirable invasive organisms make the question very relevant as evolution of dispersal can accelerate the rate of spread (Phillips *et al.*, 2007; Phillips *et al.*, 2010; Travis *et al.*, 2009). The other side of the coin however is that sufficient genetic variability of traits associated with dispersal may not be available in invasive populations that experienced founder events during their introduction. In addition the time interval since introduction may be insufficient to observe measurable life-history traits evolution in the case of recent invasions. However the recent literature shows that genetic bottlenecks are less frequent than previously though in successful invasive populations (Bossdorf *et al.*, 2005; Dlugosch & Parker, 2008; Wares *et al.*, 2005) and rapid short-term evolution has been observed repeatedly (Huey *et al.*, 2005; Whitney & Gabler, 2008). On the other hand, multiple introductions are also known to be frequent in biological invasions (Bossdorf *et al.*, 2005; Estoup & Guillemaud, 2010), and it is thus important to ensure that the observed spread is corresponding to the spatial expansion of a single population.

The purpose of this study was to investigate the evolution of dispersal ability during the geographical spread of the harlequin ladybird *Harmonia axyridis* in Europe. This Asian species gathers a variety of characteristics which makes it a good model to test such a prediction. First, the worldwide invasion is very well documented (Brown *et al.*, 2011; Lombaert *et al.*, 2010; Lombaert *et al.*, In press). *H. axyridis* was first observed in 2001 in Belgium (Adriaens *et al.*, 2003) and since then its spread in Europe has been pretty well monitored (Brown *et al.*, 2011). Second, the species is known to have passed through rather moderate bottlenecks during the invasion steps (Facon *et al.*, 2011; Lombaert *et al.*, 2010). In addition, in the case of the European invasive population, genetic diversity has been increased by an admixture event between individuals from a wild eastern North American invasive population and a laboratory strain used for biocontrol purposes (Lombaert *et al.*, 2010; Turgeon *et al.*, 2011). Third, heritable variation for dispersal ability is known in *H. axyridis*. Differential flight capacities could be artificially selected in the laboratory at least twice independently: once on a native population (Seko *et al.*, 2008) and once on the European biocontrol strain (Tourniaire *et al.*, 2000).

We studied ladybirds collected along two transects going from the centre to the edge of the invaded area in Europe. We then used neutral genetic markers (microsatellites) to verify that all insects derived from the same introduction event. Finally, we tested three traits directly linked to dispersal ability: (i) the flying speed and (ii) flying endurance were measured in a flight mill experiment and (iii) the propensity to fly was measured by evaluating the motivation to fly after a short period of starvation.

Methods

Sampling and rearing

In Europe, the invasion of *H. axyridis* is believed to have started in 2001, with first observations of feral populations near Ghent and Brussels in Belgium, followed by a rapid demographic and spatial expansion to a large part of Europe (Adriaens *et al.*, 2008; Brown *et al.*, 2011). We thus considered the centre of Belgium as the invasion core. Data on the spatial and temporal progress of the invasion front in France (date of first observation in each French administrative department) were obtained from the French national *H. axyridis* survey (Fig. 1, Observatoire permanent pour le suivi de la coccinelle asiatique en France, 2010). Based on our knowledge of the expansion history of *H. axyridis*, we collected between October and November 2010 a total of eight *H. axyridis* population samples along two transects (four samples per transect) located from the invasion core of the European outbreak (Brussels area) to the invasion front in Southern France (Table 1, Fig. 1). All population samples consisted of at least 90 live adult individuals sampled in an area of less than 10km². Each population sample was characterized by (i) the transect to which it belongs, (ii) the geographical distance in kilometres to the core sample measured with Google Earth V4.3 (Google, 2008) and (iii) the date of first observation of the species in the considered sampling site (Observatoire permanent pour le suivi de la coccinelle asiatique en France, 2010) (Table 1, Fig. 1).

Before the experiments started, we reared all eight samples in the lab for two generations (G_0 to G_2), under strictly controlled conditions, in order to avoid bias due to maternal effects. During these two generations, individuals were exclusively fed with ionized *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs and reared at constant environmental conditions (23°C; 50% HR; L:D 16:8). For each population, G_1 was initiated from 90 G_0 individuals (45 males and 45 females), and G_2 was initiated from 100 G_1 individuals (50 males and 50 females). Generation G_2 consisted in a large number of individuals (more than 1600 individuals per population), and males and females were separated immediately after emergence to avoid any mating event. They were then maintained in the same environmental conditions for 2 weeks in order to insure their maturity at the beginning of any experiment.

Genetic differentiation between populations

Many studies suggest that multiple introductions of invasive species might be a common phenomenon (Bossdorf *et al.*, 2005; Estoup & Guillemaud, 2010). We thus first verified that the eight population samples used in this study are part of the same geographic expansion of an outbreak originating from a single introduction event. To do so, we genotyped 31 G_0 individuals per population sample at 18 microsatellite loci following Loiseau *et al.* (2009). The level of genetic variation between populations was summarized by computing pairwise F_{ST} estimates (Weir & Cockerham, 1984). Exact tests for population genotypic differentiation (Raymond & Rousset, 1995a) were carried out for all pairs of populations. All computations were processed using the software Genepop (Raymond & Rousset, 1995b; Rousset, 2008).

In addition, we used the clustering approach implemented in STRUCTURE v2.3.3 (Pritchard *et al.*, 2000) to infer the number of population units within the studied area. We chose the admixture model with correlated allele frequencies and, because our sampling scheme involved the collection of many individuals from a few discrete distant locations (Schwartz & McKelvey, 2009), we used the sampling location as prior information (Hubisz *et al.*, 2009). We used default values for all other parameters of the software. Each run consisted of a burn-in period of 10^5 Markov chain Monte Carlo (MCMC) iterations, followed by 10^6 MCMC iterations. We carried out 20 replicate runs for each prior value of the number (K) of clusters, set between 1 and 8 (i.e. the number of samples). The natural logarithm of the likelihood of the data $\ln(P(X|K))$ was calculated and was expected to be large with a low variance for the most probable K (Pritchard *et al.*, 2000).

Flight speed and endurance experiment

We used an automated flight mill system allowing the simultaneous measure of the flying speed and endurance of 10 individuals in parallel. Each of the 10 mills was isolated in a box and consisted of a horizontal steel rod bent 90° at both ends: the first end was inserted into the hollow needle of a vertical syringe head that serves as rotation axis, and the second end was used as the attachment point of the insect. The horizontal part of the steel rod was 15.91 cm length, so that when an insect accomplishes a complete rotation, the corresponding distance is 1 meter. An infrared ray emitted by a photogate was interrupted by a flag attached to the middle of the steel rod. This photogate was connected to a computer which recorded the time coordinates of each rotation. The whole flight mill system was located in a climatically-controlled chamber maintained at 23°C and controlled for light and air movement. Temperature and humidity were recorded at the beginning of each trial. They were very stable during the whole experiment and were thus ignored in the statistical analyses.

Before each trial, ladybirds were isolated and starved during one hour. Sex and elytral color patterns (hereafter named "morphs" which consists of five classes as proposed by Seo *et al.*, 2008) were recorded as well. For each mill, a ladybird randomly chosen among the 8 population samples and the two sexes was then fixed by the pronotum to the attachment point. The record started as soon as the insect began to fly (usually instantly) and lasted one hour. A total of 752 individuals were tested, so that a mean number of 47 replicates per population and sex was used (minimum = 45; maximum = 49).

We analyzed the following response variables: (i) the total distance traveled during the whole 60 minutes (hereafter named "flight speed") and (ii) the ratio $[2*D_{30} / D_{60}]$ (hereafter named "endurance ratio") where D_{30} is the distance traveled during the first 30 minutes and D_{60} is the total flight mill distance. For both response variables, we used mixed general linear models ("lmer" function from the "lme4" R package) with the day of the experiment (day 1 is the first day of the experiment which lasted 17 days), the sex, the transect (A or B, Fig. 1) and the distance to the core (either the geographical distance in kilometers or the date of first observation) as fixed effects. Three random effects were included: the flight mill that was used for the record (10 levels), the morph {4 levels) and the population sample (8 levels). We used the Akaike Information Criterion (AIC) to select for the best model. First, we selected the best random effect structure by comparing models with different random effects but with the same fixed structure (called full fixed structure hereafter): all simple fixed effects and the double interactions between the distance to the core and either the sex or the transect. Once the best random effect structure was found (lowest AIC value), we compared all models with varying complexity of the fixed effect structure and with the same random effect structure. We thus compared fixed effect structure models varying from the model without fixed effect to the full fixed structure and retained the most parsimonious of them (lowest AIC). AIC selection method was also used to select the best parameter accounting for the distance to the core (i.e. geographical distance in kilometers or date of first observation).

Flying propensity experiment

The flying propensity of *H. axyridis* individuals was evaluated with a method close to the one described by Li *et al.* {Li, 2010 #867}. The flight stands consisted of an inverted white plastic funnel (h: 180 mm, dia.: 110 mm at base). The base of the funnel was surrounded with water to prevent the individuals from walking off of the stand. An individual previously isolated and starved in a 2 ml opaque micro tube during 3 to 6 hours was installed onto the base of the funnel. The experience started at the opening of the tube. The occurrence of flight and the time from the tube opening to takeoff were then recorded with the software Observer 5.0 (Noldus Information Technology, Wageningen, The Nederlands). The trial ended when the adults flew off the stand or when 300 seconds had elapsed. Four individuals were tested in parallel during a single trial, the space between two funnels being 10 cm. Individuals were chosen randomly among the eight population samples, but only one sex was tested per trial to avoid any mating pheromonal interaction. Sex and morphs were recorded. The experiment took place in a controlled climatic chamber maintained at 23.5°C and controlled for light and air movement. Temperature and humidity were recorded at the beginning of each trial. A total of 760 individuals were tested, so that mean number of 47.5 replicates per population and sex was used (minimum = 45; maximum = 48).

The following response variables were considered: (i) the proportion of individuals taking off (hereafter named "flyers proportion") and (ii) the mean time from individual release (tube opened) to takeoff (hereafter named "takeoff time"). We used mixed generalized linear models with a binomial probability distribution and a logit link function ("lmer" function from the "lme4" R package) and Cox proportion hazard models for survival data with random effects ("coxph" function from the "survival" R package) for the analyses of the flyers proportion variable and the takeoff time variable respectively. We used the day of the experiment (day 1 is the first day of the experiment which lasted 12 days), the isolation time (i.e. the time spent by each individual in the micro tube before the trial), the sex, the transect (A or B) and the distance to the core (either the geographical distance in kilometers or the date of first observation) as fixed effects. The three random effects were the funnel number (4 levels), the morph (4 levels) and the population sample (8 levels). The best model was selected on the basis of the smallest AIC as described in the flight mill experiment section. All statistical analyses were performed with R software V2.13.0 (R_Development_Core_Team, 2011).

Results

Genetic differentiation between populations

Pairwise F_{ST} estimates were low with a mean of -0.001 and values never exceeding 0.007. None of the 28 exact tests of genotypic differentiation were significant at a 5% threshold (smallest *P-value* equal to 0.058) even without performing any correction for multiple comparisons. The STRUCTURE clustering analysis (Pritchard *et al.*,

2000) provided consistent results over the 20 runs tested for each *K*. The mean natural logarithm of the likelihood of the data (lnP(X|*K*)) was maximum for $K = 1$. Mean lnP(X|*K*) decreased and its variance increased for increasing values of *K* (Fig. 2). Alltogether, the results provides firm evidence that all samples derived from a single population unit and thus from a single introduction.

Flight speed and endurance experiment

As expected, the variables "date of first observation" and "geographical distance in kilometres" accounting for the distance of each population to the core of the invasion were highly correlated (Pearson correlation coefficient = 0.95). However, AIC values were always lower when "date of first observation" was used as an explanatory variable instead of "geographical distance in kilometres" (cf supp material ???). The final selected model included the day of the experiment, sex, transect, date of first observation, sex*date of first observation, and transect*date of first observation as fixed effect and the mill as a random effect. Statistical results obtained with this model are reported in Table 2. The day of the experiment had a strong significant effect ($P < 10^{-3}$): beetles travelled more distance the last days of the experiment than during the first days. This effect could be due to the slight increase in humidity during the experiment (Pearson correlation coefficient between day of the experiment and humidity = 0.57), but it was more likely due to the age of the ladybirds which were all born at the same time (the largest age difference was 4 days). The only other significant fixed effect was the date of first observation ($P < 10^{-3}$). The more recent is the date of first observation (i.e. the closer is the population to the front), the larger is the flight speed (Fig. 3). All other variables or interactions that were kept in the selected model were not significant. In particular, no effect of sex ($P = 0.304$) and transect ($P = 0.205$) were found.

In the case of the endurance ratio variable, the best model according to the AIC criterion included no fixed effect and the variable "mill" as a random effect, denoting a similar endurance among samples. The overall mean value of the endurance ratio was larger than one (1.16), indicating that ladybirds travelled more distances during the first half of the flight (30 first minutes) than during the second half.

Note that in both analyses, the two Belgian population samples (i.e. core of each transect) were not significantly different, and inverting both population samples did not change the results.

Flying propensity experiment

For both response variables (flyers proportion and take off time), the funnel was selected as a random effect and the transect as well as the isolation time were retained as fixed effect. In addition the geographical distance in kilometres was selected for the Flyers proportion response variable (Table 3). We found a significant ($P<10^{-3}$) effect of the time of isolation both on the flyers proportion and on the takeoff time with an overall larger motivation to fly when the isolation time was longer. The transect variable was in both case slightly significant ($P<0.05$), with a larger propensity to fly in transect B (e.g. $P_{FLY\_transectA} = 0.45$ and $P_{FLY\_transectB} = 0.53$). Despite no significant differences between the two Belgian population samples, inverting both samples eliminated the transect effect for both response variables (results not shown). Contrary to the previous experiment, the distance to the invasion core had no significant effect on the response variables (not selected for the take off time and $P=0.445$ for the flyers proportion).

Discussion

There has been substantial recent interest in the evolution of non-equilibrium populations as a result of spatial spread (e.g. Excoffier *et al.*, 2009; Excoffier & Ray, 2008; Hill *et al.*, 2011; Phillips *et al.*, 2010; Sexton *et al.*, 2009; Shine *et al.*, 2011). Modification of range margin due to modern climate change as well as the current burst in biological invasions has motivated a number of recent studies on this topic (Hill *et al.*, 2011; Phillips *et al.*, 2010). In particular larger dispersal abilities are predicted to evolve at the edge of expanding populations by mean of spatial sorting (Phillips *et al.*, 2010; Shine *et al.*, 2011). This hypothesis has been barely tested on invasive species (but see Monty & Mahy, 2010; Phillips *et al.*, 2006).

Evidence for evolution of flying ability

Our study clearly demonstrates a measurable evolution of dispersal abilities in less than a decade in the invasive ladybird *H. axyridis* in Europe. Individuals whose grandparents were sampled close to the front were able to travel a larger distance during a one hour trial in a flight mill than individuals whose grandparents were sampled close to the core of the invasion. Population genetic structure analysis confirmed that all samples originated from a single introduction in Europe. The observed variation in dispersal ability through space is thus not due to various independent introductions of groups of *H. axyridis* ladybeetles displaying various abilities of dispersion. On the contrary, the observation results from a post-

introduction evolutionary phenomenon at play on a single population. The absence of significant effect of the transect variable (alone or in interaction) also suggests that the observed evolution was repeatable through space and has not been random as in case of genetic drift or mutation surfing for example (Hallatschek *et al.*, 2007).

No significant effect of the distance to the core on the endurance of the ladybirds was measured. We thus did not detect any evolution on this trait (e.g. better endurance on the front than on the core), but it also means that no trade-off between speed and endurance was identified. Such trade-off was expected (e.g. Oufiero *et al.*, 2011; Stephens & Wiens, 2008; Wilson & James, 2004) and would have lowered the impact of the observed evolution of flight activity. It can be argued that longer trials may have allowed better detection of endurance variation between the core and the front of the invasion outbreak. However, the non significant trend that we observed tended toward a larger endurance on the front compared to the core and thus does not suggest that a trade-off exists.

As for flight propensity, no evolution of the measured traits could be detected along the expansion range. The observed moderate transect effect suggests however a genetic basis for flight propensity variations, allowing responses to evolutionary forces. Contrary to flight speed, flight propensity is a complex behavioral trait which may be under strong selective pressures associated with resource foraging and escape from predators. The effect of spatial sorting may be minored by these strong selective pressures. The difference of flight propensity between the two transects may be due to the local adaptation of *H. axyridis* to its environment.

How could dispersal evolve?

Evolution via spatial sorting necessitates (i) the presence of underlying heritable variation in dispersal ability, (ii) the absence of strong trade-offs between dispersal ability and other traits evolving at the edge of expanding populations, and (iii) the absence of strong costs associated with larger dispersal ability. According to our results, European invasive populations of *H. axyridis* display high heritability in dispersal traits allowing them to quickly respond to evolutionary forces. The Belgian invasive outbreak of *H. axyridis* derived from the admixture of an older invasive population established in North Eastern America since 1988 and a laboratory strain brought from Beijing in Europe in 1982 (Lombaert *et al.*, 2010). Genetic diversity stemming from this admixture was found to be rather high when measured at microsatellite markers (Lombaert *et al.*, In press). Although neutral molecular markers are often considered poor indicators of heritable variation in quantitative traits (McKay & Latta, 2002; Merila & Crnokrak, 2001; Reed & Frankham, 2001), this high neutral genetic variation may at least partially reflect the presence of strong additive genetic variance in the European invasive *H. axyridis* including traits like dispersal ability. Selection on polymorphic dispersal abilities in *H. axyridis* has previously been documented (Seko *et al.*, 2008; Tourniaire *et al.*, 2000) highlighting, together with our present results, that evolutionary forces maintain dispersal polymorphism (likely associated to strong costs and benefits, Bowler & Benton, 2005; Harrison, 1980; Ronce, 2007) in this species.

The low population densities encountered in the front of an invasion expansion is likely to exert strong selective pressures on other important traits. Some of these traits may trade off and thus act negatively on dispersal ability. For example, in insects, trade-off may exist between dispersal abilities and reproductive rate (Roff & Fairbairn, 2007). As dispersal, reproductive rate is expected to evolve higher on the front (Phillips *et al.*, 2010), and a conflict between both traits may appear during the expansion (e.g. Hughes *et al.*, 2003). However, trade-offs are often more complex: they often involve several other traits such as competition ability which is expected to evolve lower on the front. In such cases, dispersal and fecundity can theoretically evolve in the same direction even though they trade off against each other (Burton *et al.*, 2010). Moreover, given the complexity of dispersal and reproductive rate it seems unlikely that a strong and linear trade-off exist between them, and positive correlation between both traits has even been found on a butterfly species (Hanski & Saccheri, 2006). Also, in the case of *H. axyridis*, evolution of dispersal abilities was very rapid, and other complex traits may not be able to evolve that quickly.

Finally, costs associated with larger dispersal ability are probably low in European *H. axyridis*. Indeed, Allee effects may be encountered on the front and may limit dispersal evolution during range expansion if they are strong (Travis & Dytham, 2002). In particular, low population density is responsible for higher inbreeding rates. However, in the case of *H. axyridis*, it may not be an issue: it has recently been shown that inbreeding depression was purged in the invasive populations (Facon *et al.*, 2011). This may be an important factor which authorized the dispersal abilities of *H. axyridis* to evolve so quickly along the invasion range.

Conclusion and perspective

The study of European *H. axyridis* benefits from an extremely good knowledge of invasion routes and range expansion based on neutral genetic variation and historical records. This made it possible to

eliminate mechanisms such as multiple introductions to explain the observed variability of dispersal abilities. In addition, the identical phenotypic response along two different transects and the likely low advantages of being a good disperser on the edge of a population range (e.g. Gros *et al.*, 2006) suggest a rapid evolution of *H. axyridis* due to spatial sorting (Shine *et al.*, 2011). The importance of this mechanism in the current invasion of Europe by *H. axyridis* should be explored. In particular, an acceleration of the rate of spread of the invasive population is expected to result from the spatial sorting of dispersal ability (e.g. Phillips *et al.*, 2007; Phillips *et al.*, 2006).

H. axyridis is a worldwide invasive species which can now be found in almost all continents (Africa, South America, North America, Europe, Brown *et al.*, 2011). Studying the evolution of dispersal during the range expansion of this species in other continents than Europe may provide a valuable source of knowledge by supplying robust replicates (similar spatial spread of the same species in different environments) to confirm our results. However, whether heritable polymorphism of dispersal abilities is available or not in these other invasive outbreaks is still unknown. Indeed, the admixed origin of the European outbreak (Lombaert *et al.*, 2010) is known to have had important implications on various phenotypic traits of this population (Turgeon *et al.*, 2011), and the European biocontrol genetic contribution in particular has likely provided a substancial source of variation of dispersal abilities (Tourniaire *et al.*, 2000).

Our results provide a striking illustration of how fast predictable directional evolution can occur in nature. Rates of evolution have been the focus of many studies (e.g. Hairston *et al.*, 2005; Hendry & Kinnison, 1999; Thompson, 1998) and invasive species provide a good opportunity to test many evolutionary predictions (Huey *et al.*, 2000). In our case, the dispersal abilities of *H. axyridis* have clearly and measurably evolved in only 9 years which probably corresponds to about 18 generations in Europe (Koch, 2003). This timescale is notably low, even in the case of invasive species (Prentis *et al.*, 2008; Whitney & Gabler, 2008).

In general evolution of life history traits may or may not always be necessary to ensure the success of the acclimation step of an invasion process (e.g. Facon *et al.*, 2006). On the contrary, it is very likely to happen, either in a deterministic (through adaptation or spatial sorting) or contingent (drift, founder events) way, during the expansion steps. We have shown that, considering dispersal, *H. axyridis* displays a high evolutionary potential. Future studies should focus on the evolution of other traits important through space and time such as competitive abilities, reproductive rates or cannibalism rates. The evolution of this bench of traits together with dispersal ability may have a dramatic consequence on the dynamics of the invasive populations.

Acknowledgments

Many thanks to Adrien Adelsri, Violaine Chatre-Belle, Samuel Courtaut, Bertrand Gauffre, Louis Hautier, Sylvain Lanziliere, Guillaume Laugier, Bernard Lemesle and Ashraf Tayeh for providing us with *Harmonia axyridis* live samples. We also thank Xavier Fauvergue for helping us with data summarizing and mueros for friendly support in Brussels. This work was funded by the French ANR Biodiversité #ANR-06-BDIV-008-01 grant.

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Figures

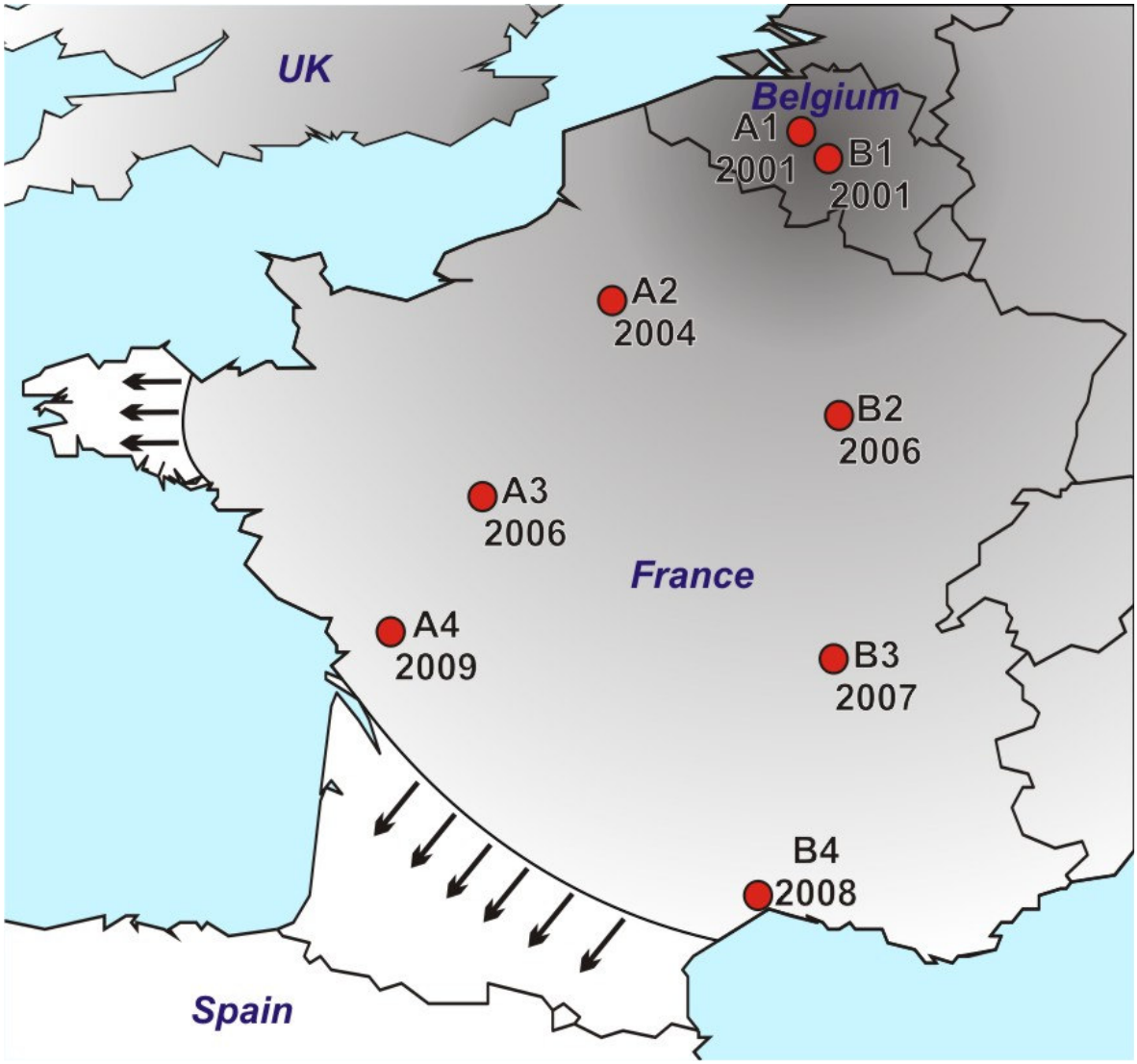

Figure 1: Geographical origins of sampled populations of *Harmonia axyridis*.
The shaded area approximately corresponds to the distribution of the species in 2010. Arrows indicate the direction of the expansion. Each red spot corresponds to a population sample. Letters (A or B) correspond to the transect names, and the associated numbers give the sample codes (see Table 1). Years below the sample codes are the dates of first observation of the species in the given geographic localities (see Table 1).

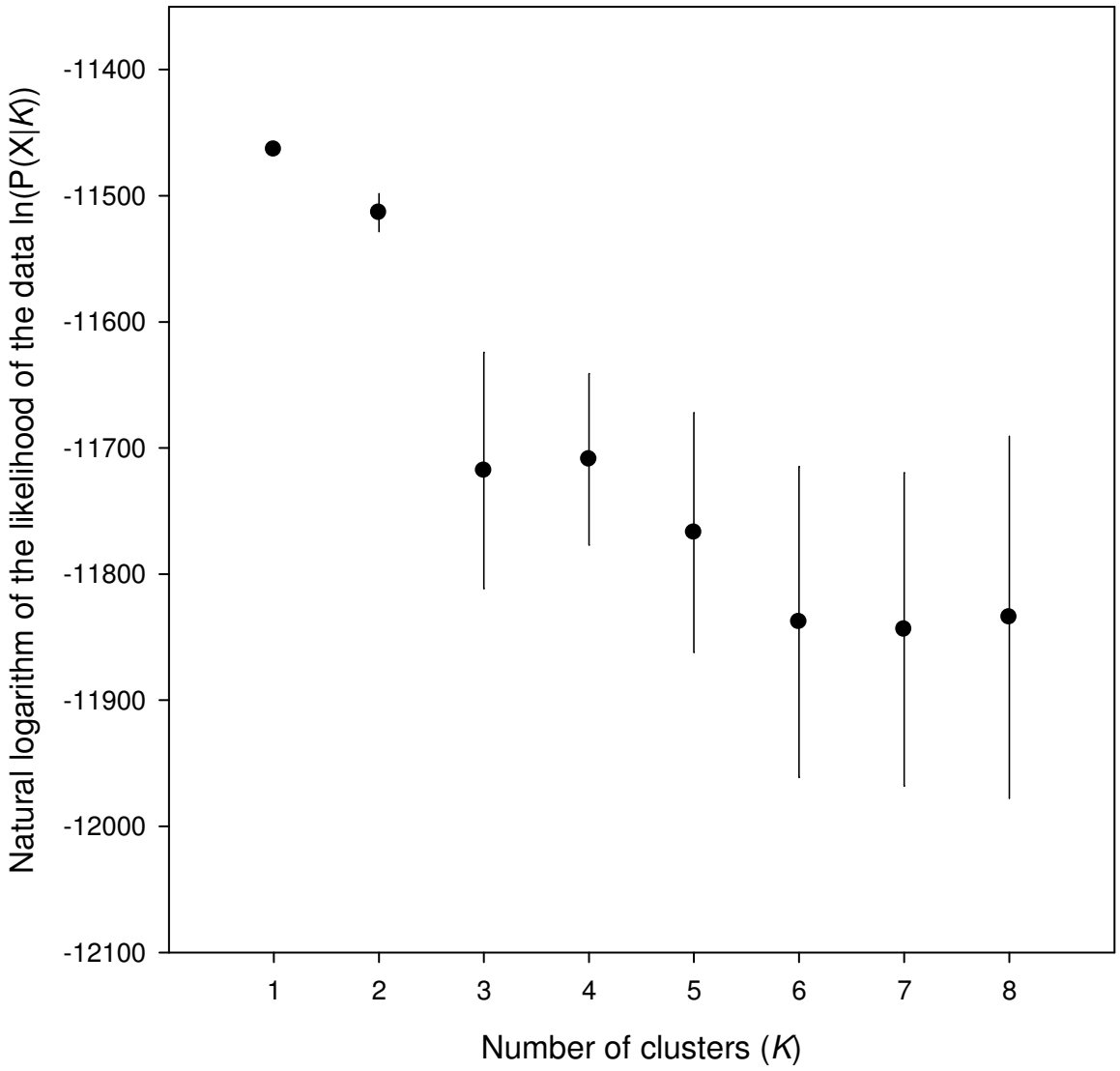

Figure 2: Estimated number of population clusters in the *Harmonia axyridis* samples according to the Bayesian clustering method STRUCTURE.
The mean (±SD) natural logarithm of the likelihood of the data (LnP(X|*K*)) calculated over 20 STRUCTURE replicated runs is given for each value of the putative number of clusters (*K*). We used the admixture model with correlated allele frequencies and sampling location as prior information. The maximum value of LnP(X|*K*) is obtained for *K*=1.

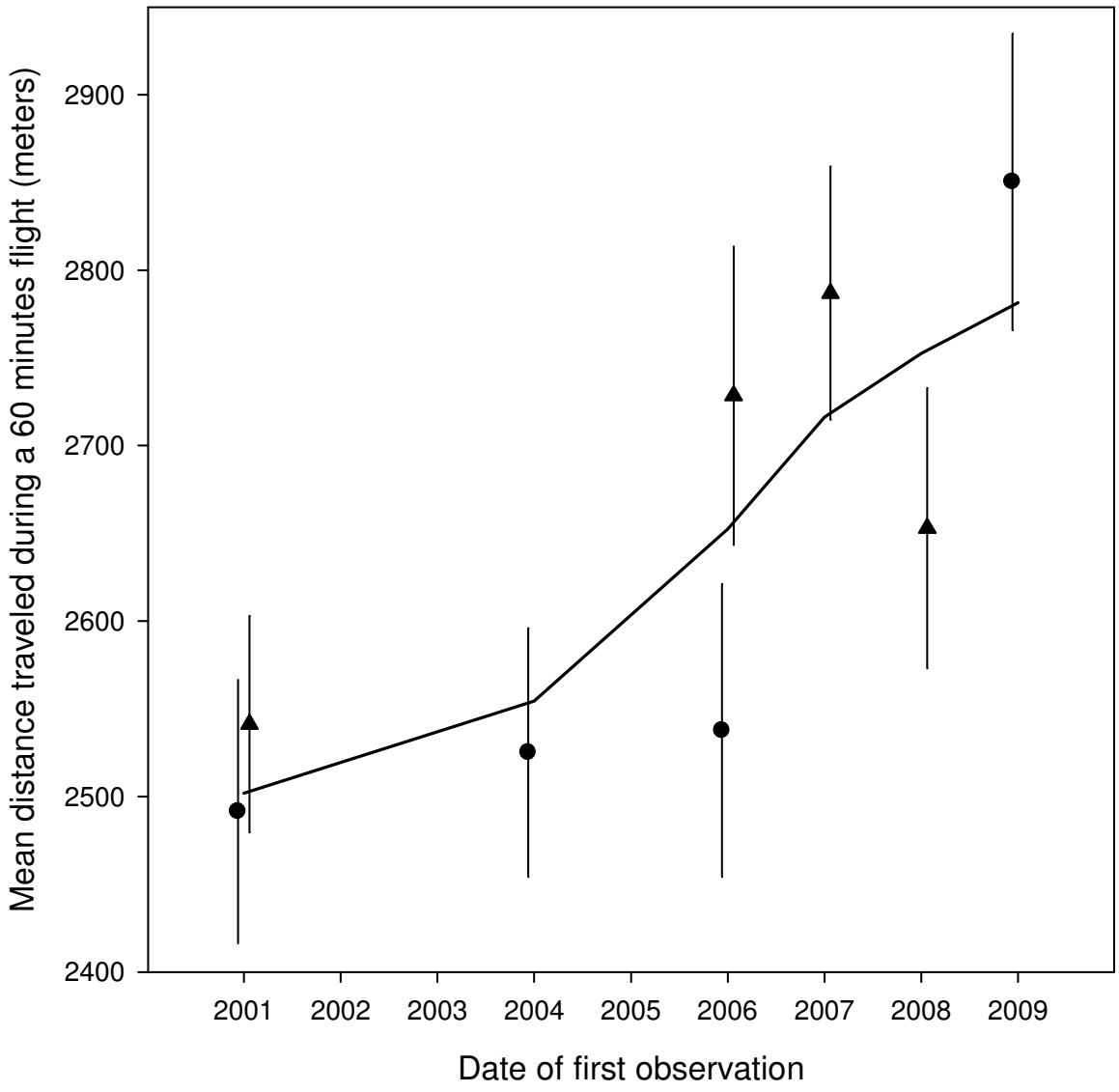

Figure 3: Mean distance travelled in 1 hour in flight mills as a function of dates of first observation. Triangle and Circle correspond to mean population values in transect A and B respectively. Vertical bars are standard errors. The line corresponds to the mean predicted values obtain from the selected statistical model (see Table 2).

Tables

Sample code	Sampling site (town, country)	Coordinates	Distance from outbreak core (km)	Date of first observation	Sampling medium
A1	Brussels, Belgium	50.839°N 4.368°E	0	2001	*Tilia sp.*
A2	Laboissiére-en-Thelle, France	49.288°N 2.158°E	233	2004	House
A3	Fondettes, France	47.402°N 0.637°E	469	2006	House
A4	Chizé, France	46.148°N 0.424°W	629	2009	House
B1	Walhain, Belgium	50.612°N 4.668°E	0	2001	*Tilia sp.*
B2	Clairvaux, France	48.170°N 4.784°E	270	2006	House
B3	Quincieux, France	45.909°N 4.758°E	522	2007	House
B4	Prades-le-Lez, France	43.698°N 3.863°E	770	2008	*Albizia sp.*

Table 1: Population samples information.

	Total flight mill distance	
Selected model	*t*-value (df = 736)	*P*
Selected random effect = Mill		
Day of the experiment	7.633	**$< 10^{-3}$**
Sex	1.028	0.304
Transect	1.269	0.205
Date of first observation	3.405	**$< 10^{-3}$**
Sex*Date of first observation	-1.032	0.303
Transect*Date of first observation	-1.267	0.206

Table 2: Results from the best model after model selection among the different linear mixed models run for the trait "flight speed".

	Flyers proportion		Takeoff time	
	Selected random effect = Funnel		Selected random effect = Funnel	
Fixed effects	*z*-value (df = 739)	*P*	Chi²	*P*
Isolation time	3.381	**$< 10^{-3}$**	12.66 (df = 1)	**$< 10^{-3}$**
Transect	1.969	**0.049**	5.33 (df = 1)	**0.021**
Distance in kilometers	0.764	0.445	Not selected	

Table 3: Results from the best model after model selection among the different linear mixed models run for the traits "flyers proportion" and "takeoff time" of the Flying propensity experiment.

ANNEXE VIII

Article 8 (2011)

Facon B, Crespin L, Loiseau A, **Lombaert E**, Magro A, Estoup A (2011) Can things get worse when an invasive species hybridizes? The harlequin ladybird *Harmonia axyridis* in France as a case study. *Evolutionary Applications* **4**, 71-88.

Précisions sur le rôle de co-auteur :

- ➔ Echantillonnage de la population INV.
- ➔ Gestion des élevages d'*H. axyridis* (maintien puis multiplication en prévision des expérimentations).
- ➔ Génotypage microsatellites et analyse des données pour la section « are INV and BIO genetically distinct at microsatellite loci? »
- ➔ Discussions.
- ➔ Participation globale à la rédaction du manuscrit.

Evolutionary Applications www.evolutionaryapplications.org

Evolutionary Applications ISSN 1752-4571

ORIGINAL ARTICLE

Can things get worse when an invasive species hybridizes? The harlequin ladybird *Harmonia axyridis* in France as a case study

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Keywords
admixture, biological invasion, *Harmonia axyridis*, intraspecific hybridization, life-history traits, quantitative genetics.

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Received: 31 March 2010
Accepted: 26 April 2010

doi:10.1111/j.1752-4571.2010.00134.x

Abstract

So far, only a few studies have explicitly investigated the consequences of admixture for the adaptative potential of invasive populations. We addressed this question in the invasive ladybird *Harmonia axyridis*. After decades of use as a biological control agent against aphids in Europe and North America, *H. axyridis* recently became invasive in four continents and has now spread widely in Europe. Despite this invasion, a flightless strain is still sold as a biological control agent in Europe. However, crosses between flightless and invasive individuals yield individuals able to fly, as the flightless phenotype is caused by a single recessive mutation. We investigated the potential consequences of admixture between invasive and flightless biological control individuals on the invasion in France. We used three complementary approaches: (i) population genetics, (ii) a mate-choice experiment, and (iii) a quantitative genetics experiment. The invasive French population and the biological control strain showed substantial genetic differentiation, but there are no reproductive barriers between the two. Hybrids displayed a shorter development time, a larger size and a higher genetic variance for survival in starvation conditions than invasive individuals. We discuss the potential consequences of our results with respect to the invasion of *H. axyridis* in Europe.

Introduction

Hybridization (interbreeding between genetically differentiated lineages) takes place in a very wide range of organisms (Barton and Hewitt 1985, Dowling & Secor 1997, Mallet 2005) and may play an active role in a variety of evolutionary processes ranging from local adaptation to speciation (Stebbins 1959; Arnold 1992; Barton 2001; Rieseberg et al. 2003). In the field of invasion biology, hybridization is now seen as a potential stimulus for the evolution of invasiveness (Ellstrand and Schierenbeck 2000; Lavergne and Molofsky 2007; Ryan et al. 2009; Blair and Hufbauer 2010).

Traditionally, hybridization involves interspecific or intergeneric crosses as exemplified by the invasive plant *Spartina anglica* that mixes with native and other alien *Spartina* species (Gray et al. 1991; Baumel et al. 2002). However, crosses between individuals from genetically differentiated populations of the same species (i.e. admixture, Ellstrand and Schierenbeck 2000; Culley and Hardiman 2009) are also considered hybridization (Wolfe et al. 2007; Culley and Hardiman 2009). Admixture seems to be frequent in biological invasions. An increasing number of studies document biological invasions resulting from multiple introductions from distinct populations that bring together genetically differentiated individuals into a

common introduced area (Facon et al. 2003; Kolbe et al. 2004; Bossdorf et al. 2005, Wares et al. 2005; Lavergne and Molofsky 2007). To date, most studies dealing with admixture have aimed at detecting multiple source populations in biological invasions from selectively neutral markers (e.g. Kolbe et al. 2004). Only a few studies have explicitly investigated the consequences of intraspecific hybridization for the evolution of life-history traits and thus for the adaptative potential of introduced populations (Lavergne and Molofsky 2007; Wolfe et al. 2007; Facon et al. 2008).

Hybridization may lead to very different outcomes ranging from detrimental to beneficial (Arnold and Hodges 1995; Burke and Arnold 2001). On the one hand, hybridization may reduce the fitness of parental individuals either due to incipient reproductive isolation in the form of genetic incompatibilities that reduce the mating success of parents (prezygotic isolation) or through a decrease in the fitness of offspring due to the loss of local adaptation and/or breakdown of co-adapted gene complexes (outbreeding depression, as exemplified in tension zones; Barton and Hewitt 1985). On the other hand, hybridization has the potential to boost invasiveness through two nonexclusive mechanisms: heterosis and generation of new genotypes. Heterosis (or hybrid vigor) occurs when hybridization masks deleterious alleles (Keller and Waller 2002) or in case of overdominance and/or synergistic epistasis between alleles inherited from the parental taxa. Allopolyploidy, which sometimes accompanies hybridization, may also contribute to the heterotic effect (Ainouche et al. 2009). The generation of new genotypes occurs through recombination (Arnold et al. 1999; Ellstrand and Schierenbeck 2000; Facon et al. 2005; Schierenbeck and Ellstrand 2009), and alleviates the loss of genetic variance after founder events and hence restores or even increases the efficiency of selection (Lee 2002).

Given its invasion history, the invasive harlequin ladybird *Harmonia axyridis* provides an opportunity to examine whether individuals from genetically distinct populations interbreed freely and how admixture affects life-history traits. Native to Asia, *H. axyridis* has been introduced repeatedly as a biological control agent against aphids since 1982 in Europe (Ongagna et al. 1993). Despite recurrent intentional releases of beetles for acclimation attempts, the species did not establish for 20 years. For unknown reasons it recently and suddenly became invasive on four different continents (Poutsma et al. 2008). The species is known to be a harmful predator of nontarget arthropods, a household invader, and a pest of fruit production (Koch 2003); In Europe, invasive populations were first recorded in Belgium in 2001 (Adriaens et al. 2003). It has now spread widely in Europe with a current distribution that extends from Southern France to Denmark (Brown et al. 2008). Up to now, whether the European invasive populations result from intentional introductions, accidental migrants or both remains unknown.

In France, a flightless strain of *H. axyridis* is sold commercially for biological control (Tourniaire et al. 2000). This flightless strain, called Coccibelle® (BIOTOP, Valbonne, France) was selected in the late 1990s for its inability to fly and disperse from a traditional flying biological control stock. The flightless phenotype is caused by a single recessive mutation in a gene involved in flight muscles (Tourniaire et al. 2000); thus only individuals homozygous for the mutant allele cannot fly. The Coccibelle® strain was developed with the goal of obtaining a more localized and hence effective control of aphids by both larvae and adults. As with most coccinellids, *H. axyridis* diapauses during cooler periods. It congregates into large groups (up to thousands individuals) to overwinter and is attracted to light colored dwellings and other man-made objects as overwintering sites (Labrie et al. 2008). Thus, an additional advantage of the Coccibelle® strain is the inability of flightless individuals to reach wintering sites which minimizes both its impact as a household pest, and its ability to establish populations in the wild. However, the continued use of Coccibelle® for biological control raises the possibility that it will cross with invasive individuals in Europe, especially in France. If such crosses occurred, they would yield individuals able to fly and hence could potentially impact the invasive process.

The purpose of this study was to investigate the potential role of intraspecific hybridization (i.e. admixture) between Coccibelle® and invasive individuals on the invasion of *H. axyridis* in France. Wolfe et al. (2007) outlined three criteria that must be met for intraspecific crosses to play a role in biological invasions. First, the populations involved in the admixture process should be genetically differentiated. Second, crosses should be possible between individuals from the different populations. Third, the admixed individuals should differ from parental ones in some of their life-history traits to impact the invasion process. This last criterion may involve direct heterosis, an increase in genetic variance, or both (Ellstrand and Schierenbeck 2000; Burke and Arnold 2001; Lee 2002; Facon et al. 2005; Culley and Hardiman 2009). Here, we assessed the three above criteria for crosses between the Coccibelle® biological control strain and the invasive French population of *H. axyridis*. First, we determined the level of differentiation between Coccibelle® and the invasive French populations at 18 microsatellite markers. Second, we evaluated whether there are reproductive barriers that could prevent interbreeding between biological control and invasive populations using a mate

choice experiment. Third, we used a quantitative genetics experiment to estimate the phenotypic means and variances for several key life-history traits of offspring produced by crossing Coccibelle® with the French invasive population.

Material and methods

Population sampling and rearing conditions

Invasive individuals (hereafter referred to as INV) were collected in the wild from an invasive population in Croix, Northern France (50°40′35″N, 3°08′33″E) where *H. axyridis* has been observed since 2004 (Brown et al. 2008). It is worth stressing that we previously genotyped seven French populations covering the French repartition area (in 2007–2008) and found no genetic structure between them at 18 microsatellite loci (average F_{ST} = 0.052; Arnaud Estoup, unpublished data). This absence of genetic structure at neutral loci made it reasonable to base our quantitative genetics study on a single invasive French population sample. The corresponding experimental design, while large (2400 larvae, as described below), was feasible, while additional crosses would not have been. Individuals from the Coccibelle® biological control strain (hereafter referred to as BIO) were obtained from the firm BIOTOP (Valbonne, France), which originally commercialized it.

Approximately 70 mature individuals of both INV and BIO were obtained in September 2007. These first generation individuals (G_0) were used to initiate both INV and BIO populations in the laboratory for two generations, under strictly controlled conditions, to avoid potential biases due to maternal effects. During these two generations, populations were fed with ionized *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs and reared at constant environmental conditions (23°C; 65% RH; L:D 14:10). At generation G_2, males and females were separated immediately after emergence to prevent mating. They were then maintained in the same environmental conditions for 2 weeks to ensure that all individuals had reached reproductive maturity at the beginning of the experiments.

Are INV and BIO genetically distinct at microsatellite loci?

To answer this question, we genotyped 28 G_0 individuals per population (both INV and BIO) at 18 microsatellite loci following Loiseau et al. (2009). We estimated the genetic diversity within-population by computing both the allelic richness (R_S; ElMousadik and Petit 1996) and the expected heterozygosity (H_E; Nei 1987). The level of genetic differentiation between INV and BIO populations was estimated by computing F_{ST} (Weir and Cockerham 1984). All computations were processed using the software FSTAT (Goudet 1995). Differences in R_S and H_E values were tested using a Wilcoxon Sign Rank test and the F_{ST} value was tested for significant deviation from zero using the permutation test implemented in FSTAT (Goudet 1995).

Are there reproductive barriers between the INV and BIO populations?

We addressed this question by performing mate choice trials involving three individuals (one female and two males) in cylindrical boxes (height = 3 cm; diameter = 8.5 cm). We used virgin G_2 adults 2 weeks after emergence and created trios of one female from the focal population for an individual trial (either INV or BIO) and one male from each of the two populations (INV and BIO). We set up 23 such trios with BIO females and 26 with INV females. We left the three partners together until the female laid her first clutch. We then collected the males and preserved them in ethanol for genetic analysis. We isolated the first clutch and counted the eggs. After 5 days, we counted the number of living larvae and preserved them in ethanol. We repeated the procedure for another clutch 4 weeks later. We then preserved all females in ethanol for genetic analysis.

We extracted individual genomic DNA using the Chelex® method (Estoup et al. 1996) for each mother and the two putative fathers as well as for eight larvae from each clutch (N = 49, 98 and 784 respectively for females, males and larvae). All these individuals were genotyped following Loiseau et al. (2009) for a subset of seven microsatellite loci (Ha 005, Ha 201, Ha 215, Ha 244, Ha 267, Ha 281, Ha 605). These seven loci were selected among a total of 18 loci available, as they can unambiguously discriminate the genetic origin (INV or BIO) of individuals, using the program WHICHRUN (Banks and Eichert 2000). We assigned each offspring to their parents based on their multilocus genotypes using the program PROBMAX version 1.3 (Danzmann 1997). This program assigns progeny to a set of possible contributing parents given that the genotypes are known for both the progeny and the possible parents.

We used SAS version 9.1 (SAS Institute 2003) to analyze these data. We tested the null hypothesis that the male reproductive success is equal (1:1 ratio) for the two types of males (INV and BIO) separately for each female type (INV or BIO) using a chi-square test for proportions. We also tested the effect of the female type on the male reproductive success with an analysis of independence in two way table. Finally, we tested whether the hatching rate differed significantly according to the parents using a generalized linear model with a binomial

probability distribution and a logit link function; with female and male and the interaction as factors.

Do life-history traits differ between hybrids and their parents?

We addressed this question by creating four types of crosses (female × male) from the two parent samples BIO and INV: BIO × BIO, BIO × INV, INV × BIO and INV × INV. For each cross, we randomly set up 10 couples (all the larvae produced by a couple will be thereafter referred to as a family) by putting one male and one female in a cylindrical box (height = 3 cm; diameter = 10 cm). As a consequence of this experimental design, the factor family was actually nested within the factor cross as it was not possible to produce the four crosses from a given pair of male and female (whose offspring formed a given family). At the beginning of the experiment, we collected and isolated four clutches (more than 20 eggs per clutch) of each couple. At the day of hatching (the fourth day), 15 larvae per clutch were randomly chosen and placed in a small cylindrical box (height = 2 cm; diameter = 5 cm) with a damp piece of cotton wool. For this experiment, we thus used of 2400 larvae (4 boxes × 15 larvae × 10 couples × 4 crosses). Larvae were fed *ad libitum* every 2 days until adulthood with freeze-dried aphids (*Acyrthosiphon pisum*) for 30 larvae per family and with eggs of *Artemia salina* for the 30 remaining larvae. Individuals were maintained at constant environmental conditions (23°C; 65% HR; L:D 14:10) during the experiment. Larvae were checked every day and we recorded the number of individuals reaching adulthood (i.e. the larval survival) and the total development time from egg laying to adult emergence of each individual.

A subset of individuals reaching adulthood was used to estimate four additional traits: reproductive investment of females, the lifespan of starving adults, the survival rate in quiescent conditions and the body length. To estimate reproductive investment, two adult females from each family were dissected and the number of ovarioles was counted using a binocular microscope (Ware et al. 2008).

To estimate the lifespan of starving adults from one to three females and one to three males (depending on the size of the family) were randomly collected and placed individually in a small cylindrical box (height = 2 cm; diameter 5 cm) with no food and thereafter checked every day for 45 days.

To estimate the survival rate in quiescent conditions, from one to three females and one to three males (again, depending on the size of the family) were randomly collected and placed in a cylindrical box (height = 3 cm; diameter 10 cm) with no food in constant abiotic conditions that corresponded to conditions for diapause (5°C; 60% HR; L:D 12:12). After 5 weeks, we measured the number of individuals still alive in each box to estimate the survival rate. Finally, the body length of all the adults used to estimate survival rate in quiescent conditions was measured with a binocular stereomicroscope micrometer using the software ImageJ© (http://rsbweb.nih.gov/ij/index.html).

We analyzed data on the two juvenile traits (larval survival and development time) and the four adult traits (reproductive investment, lifespan of starving adults, survival rate in quiescent conditions and body length) using SAS version 9.1 (SAS Institute 2003). For the response variables known to deviate markedly from a normal distribution (i.e. counts and proportions), we used the traditional transformations (square root for reproductive investment and arcsin for larval survival and survival rate in quiescent conditions; Sokal and Rohlf 1995). For the remaining variables, which followed approximately normal distributions, we used the original data. This choice is justified by the fact that (i) there was no obvious transformation that improved the normality of residuals and (ii) the experimental design was almost perfectly balanced and included large sample sizes, two features known to mitigate the effects caused by a non-normal distribution and/or the heterocedasticity of variances (Ananda and Weerahandi 1997).

We used model selection following Burnham and Anderson (1998) and Shoukri and Chaudhary (2007) to determine the appropriate models on which to test the significance of effects of interest. First, including all main fixed effects (cross and food for the response variables reproductive investment, larval survival, development time, and cross, food, and sex for body length, survival rate in quiescent conditions, and survival in starvation) and their interactions, we compared models with different random effects. Models for all response variables included family nested within cross and family (cross) × food as random effects. For the variables that included sex as a fixed effect, we also considered the interactions family (cross) × food × sex, family (cross) × sex as random effects. Note that with the random effect of family (cross), we can either estimate one variance component (assuming the same variance in families over the four crosses) or four variance components (each one specific to each cross, assuming that the variances were heterogeneous).

We compared the full models with simpler nested models by removing a different variance component each time, using Restricted Maximum Likelihood (REML) to assess the significance of random effects. If this removal worsened the fit of the model significantly as evidenced by likelihood ratio tests, the variance component was kept in the model; otherwise, the variance component was

removed from the model and the model selection pursued from this simpler model (Shoukri and Chaudhary 2007; Goldman and Whelan 2000; Shapiro 1988; see Appendix A for details).

Once a covariance structure was selected, we used Maximum Likelihood (ML) to select which fixed effects improved the fit of the model. Model selection was carried out based on the Information Criterion of Akaike corrected for small sample sizes (hereafter AIC_c) following Burnham and Anderson (1998). As suggested by the same authors, we considered models with a delta AIC_c of 2 or less as undistinguishable on statistical grounds; and on the basis of parsimony, we selected the model with the lower number of parameters for inferences. Results of the models selection procedures are detailed for each variable in Appendix A.

To compare the genetic variance of the life-history traits between hybrid individuals and their parents, we used the variance components estimated for the family effect within each cross (V_G). The genetic variances of the measured traits were compared among crosses using the genetic coefficient of variation (CV_G), which is the square root of the genetic variance (V_G) divided by the trait mean (see Houle 1992). For each trait, we tested the hypothesis that admixture increases the genetic variance by comparing the CV_G of the four crosses using Likelihood Ratio Tests.

Results

Are INV and BIO genetically distinct at microsatellite loci?

The within-population variability was significantly higher in the INV sample ($R_S = 6.08$, $H_E = 0.60$) than in the BIO sample ($R_S = 2.44$, $H_E = 0.40$; $P < 0.0001$ for R_S and $P = 0.0005$ for H_E). We also found that the BIO and INV populations were genetically substantially differentiated with $F_{ST} = 0.13$ ($P < 0.0001$).

Are there reproductive barriers between the INV and BIO populations?

We observed mating and egg clutches production in all mate choice trials. All genotyped larvae could be unambiguously assigned to a male. Within clutch, eggs were sired by one or two males in variable proportion. For a given female fertilized by two males, the proportion of eggs sired by a given father could change drastically among successive clutches.

Interestingly, we found that for both type of females (BIO and INV), the BIO males sired a higher proportion of offspring than INV males (Fig. 1). BIO males sired 80.3% of BIO female offspring, and 71.8% of INV females. Both proportions are significantly higher than the expected 50% fertilization by each male type ($\chi^2 = 132.01$, $P < 0.0001$ and $\chi^2 = 81.70$, $P < 0.0001$ for BIO and INV females, respectively). A similar result was obtained when using the clutch as an independent statistical unit, (excluding in this case the clutches sired by two males): for BIO females, 81% of clutches are sired only by BIO male and 19% only by INV male; for INV females, 78% of clutches are sired only by BIO male and 22% only by INV male. In both cases, BIO males sired significantly more offspring than INV males ($P < 0.05$). It is worth noting that we rejected the null hypothesis of independence between the two variables (Female type and Male type; $P = 0.0135$, Fig. 1). This result could be interpreted as the BIO males siring more offspring when mated with BIO females than with INV females.

To test whether the hatching rate differed significantly according to the parents, we split up the male status in three categories: BIO, INV or a mixture of both types. The mean hatching rate across all the observed clutches was 73%. We did no detect any significant effect of male parent ($P = 0.58$), female parent ($P = 0.52$), or the interaction ($P = 0.96$) (see Fig. 2).

Do life-history traits differ between hybrids and their parents?

Results for models selection are detailed in the Appendix A. The results of the best models for the six studied traits are summarized in Table 1 and results of the full models in Appendix B.

We first focused our analysis on the comparison between the hybrids and their parents. We found that the type of cross had a significant effect on development time ($P = 0.0009$) and length ($P = 0.0006$). INV individuals had a significantly longer development time than the BIO individuals and both hybrid types (INV-INV vs. BIO-BIO: $P = 0.0011$, INV-INV vs. BIO-INV: $P = 0.0055$,

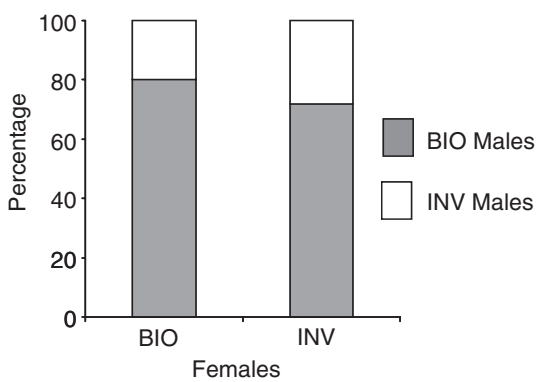

Figure 1 INV and BIO male reproductive success mated to each type of female (INV or BIO).

INV-INV vs. INV-BIO: $P = 0.0361$; Fig. 3B). BIO-INV and INV-BIO hybrids did not differ for this trait ($P = 0.98$). Individuals of both hybrid types were marginally longer than those from pure parental crosses (BIO-INV vs. INV-INV: $P = 0.09$, BIO-INV vs. BIO-BIO: $P = 0.09$, INV-BIO vs. INV-INV: $P = 0.09$, INV-BIO vs. BIO-BIO: $P = 0.08$; Fig. 3F). Individuals from pure parental crosses did not differ between each other ($P = 0.97$). The type of cross did not have any significant effect for the four remaining traits (larval survival, reproductive investment, survival in starvation, and survival in quiescent conditions; respectively Fig. 3A,C,D,E). However, for reproductive investment, Fig. 3C shows that INV females tend to invest more in reproductive structures. Although the cross effect had not been retained in the best model for reproductive investment (see Appendix A), this effect was marginally significant in the full model ($P = 0.094$). In pairwise comparisons, the only significant comparison is between pure invasive females and pure biological control females.

Regarding random effects, we found a significant family effect for all traits except for length and a significant interaction between food and family for development time, survival in starvation and length. This result means that variation for all the studied traits was, at least partly, genetically based (Table 1). Genetic coefficients of variation ranged widely among traits (Table 2). CV_G was low for development time, reproductive investment and length (less than 5%) but high for larval survival, survival in starvation and survival in quiescent conditions (between 10% and 68%; Table 2). For development time, survival in quiescent conditions and length, there was no obvious difference between the four crosses. For reproductive investment, the two crosses involving an INV mother (i.e. INV-INV and INV-BIO) had a higher CV_G than the two crosses involving a BIO mother (i.e. BIO-BIO and BIO-INV), although this trend was not significant (Table 2). For the two other traits (larval survival and survival in starvation), the observed pattern was an increase of CV_G in the hybrid crosses relative to the invasive cross. This trend was significant, however, only for survival in starvation ($P = 0.017$; Table 2). Accordingly, survival in starvation is the only trait for which taking into account four specific variance components for the family effect improves the model (Table 1). For larval survival, the CV_G of INV-INV was lower than that of the three other crosses. For survival in starvation, the two hybrid crosses had a higher CV_G than the two parental crosses. Moreover, if we consider the family mean for this later trait as an average genotype within a family, we can observe some 'genotypes' in admixed individuals (INV-BIO or BIO-INV) that consistently outperformed both parental genotypes (Fig. 3D).

We now deal with two factors, the type of food and sex, which are worth mentioning although they do not directly relate to the comparisons between hybrids and their parents. The type of food had a significant influence on development time, larval survival, survival in quiescent conditions and length (Table 1). Larvae fed with aphids had a greater larval survival and a shorter development time than larvae fed with *Artemia* eggs (SurvLarv = 80% and 65%, DvptTime = 22.01 and 24.02 days for individuals fed with aphids and *Artemia* eggs respectively). Individuals fed with *Artemia* eggs survived better in quiescent conditions than individuals fed with aphids (60% and 39% respectively), but had a smaller adult body size (6.27 and 6.56 mm for individuals fed with Artemia eggs and aphids respectively). Sex had a significant effect on survival in starvation and length (Table 1) with females having a greater survival in starvation (10.1 days) than males (8.4 days) and a larger body size (6.7 and 6.1 mm for females and males respectively). The interaction between food source and sex was only significant for length, and that no other interaction between fixed effects was significant. Finally, we did not find any significant interaction between cross and food or sex.

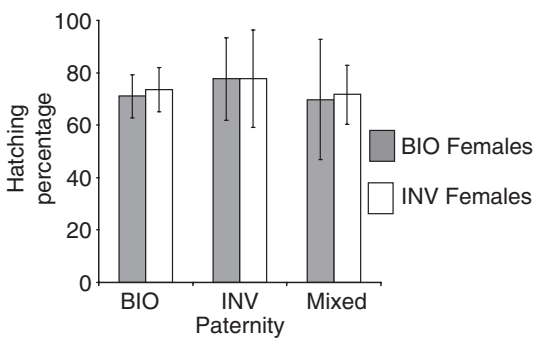

Figure 2 Mean hatching rate (±SE) according to the involved parents. We split male status into three categories: BIO, INV or a mixture of both types.

Discussion

Our study clearly demonstrates that admixture between individuals from the French invasive population and from the flightless biological control strain of the harlequin ladybird could potentially alter the invasion process.

The first criterion proposed by Wolfe et al. (2007) to evaluate the potential role of intraspecific hybridization in invasion was that populations involved in admixture should be genetically differentiated. Using 18 microsatellites, we found that the two studied populations showed substantial genetic differentiation ($F_{ST} = 0.13$). This differentiation could at least partly result from the loss of allelic diversity in the biological control population.

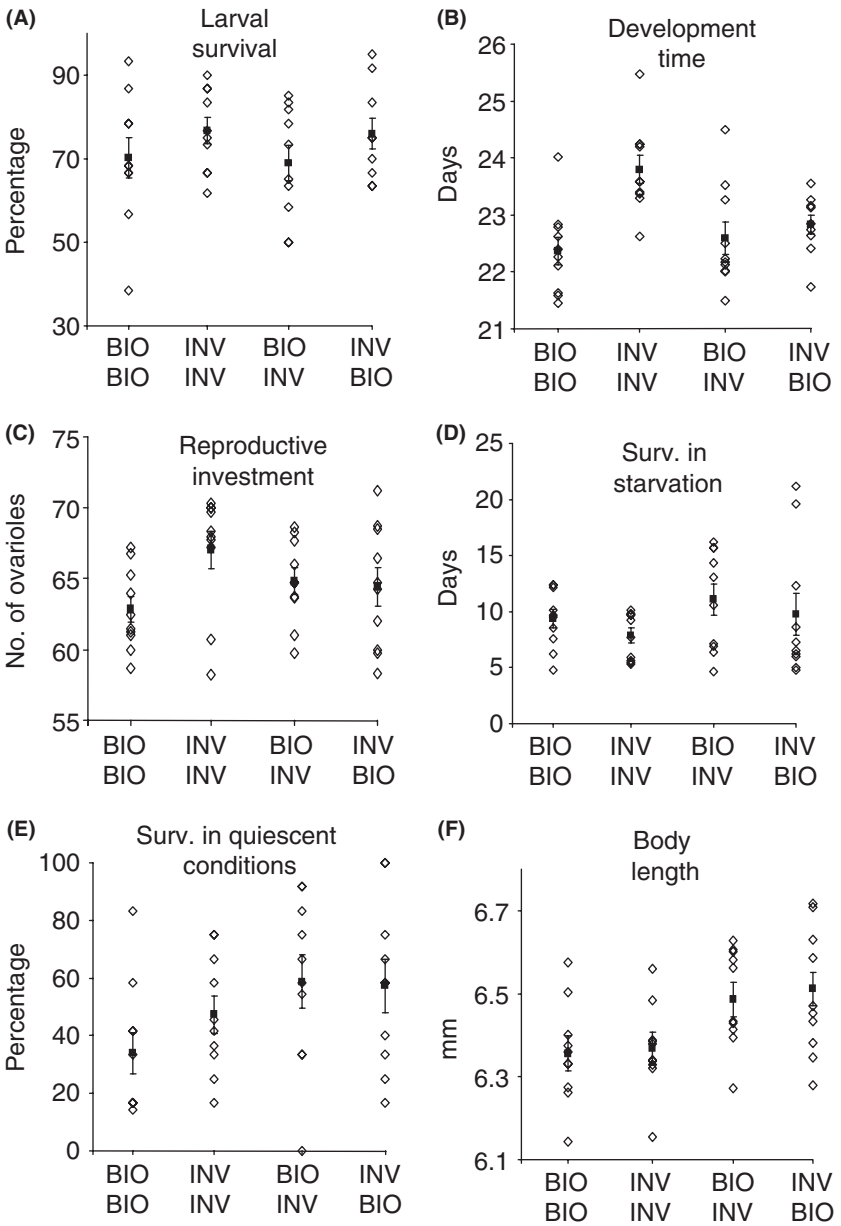

Figure 3 Life-history trait values for each cross. Black squares stand for the means for the four different crosses with associated standard errors. Diamonds represent family mean values within each cross. The six panels correspond to the six life-history traits studied: larval survival, development time, reproductive investment, lifespan of starving adults, survival rate in quiescent conditions, and body length. In each type of cross, female is indicated first and male in second. For instance, the cross named INV-BIO involved an invasive female and a biological control male.

This result can be explained by the fact that captive populations usually experience strong genetic drift due to a small number of initial founders and small effective population size during subsequent generations (Fiumera et al. 2000). With regards to the flightless biological control strain, it is worth noting that low effective size probably also occurred during selection for the flightless phenotype.

The second criterion of Wolfe et al. (2007) is that there must not be substantial barriers to crossing. Indeed, for *H. axyridis*, crosses turned out to be possible between the involved populations, at least in laboratory conditions. Our mating experiment, based on trios of one female and two males (one of each population), clearly illustrates that no reproductive barrier has evolved between these two distinct *H. axyridis* populations as every cross yielded viable offspring in similar proportions. Moreover, we found that males from the flightless biological control strain sired more offspring whatever the type of female.

Table 1. Results from the best model after model selection among the different linear mixed models run for the six traits studied.

Source	Degrees of freedom	Test statistic	*P*
(A) Larval survival			
Fixed effects		Type III *F*	
Food	1	27.27	<0.0001
Random effect		Wald test	
Fam (cross)		1.97	0.0246
(B) Development time			
Fixed effects		Type III *F*	
Cross	3	6.74	0.0009
Food	1	161.68	<0.0001
Random effect		Wald test	
Fam (cross)		2.31	0.0105
Food × Fam (cross)		3.81	<0.0001
(C) Reproductive investment			
Random effect		Wald test	
Fam (cross)		2.14	0.0162
(D) Survival in starvation			
Fixed effects		Type III *F*	
Sex	1	14.94	0.0001
Random effect		Wald test	
Fam (BIOBIO)		0.81	0.2089
Fam (BIOINV)		1.8	0.0361
Fam (INVBIO)		1.69	0.0457
Fam (INVINV)		0.24	0.4039
Food × Fam (cross)		2.14	0.0161
(E) Survival in cold conditions			
Fixed effects		Type III *F*	
Food	1	17.97	0.0001
Random effect		Wald test	
Fam (cross)		2.57	0.0051
(F) Body length			
Fixed effects		Type III *F*	
Cross	3	6.42	0.0006
Food	1	70.68	<0.0001
Sex	1	932.57	<0.0001
Food × Sex	1	10.49	0.0013
Random effect		Wald test	
Food × Fam (cross)		4.07	<0.0001

This result suggests that the cross between wild females and males from the flightless biological control strain might even be favored in nature. The advantage that males of the flightless biological control strain exhibited might be explained by selection on traits that increase male fitness in captive conditions, a feature already demonstrated in captive populations of several other invertebrates (Sgro & Partridge 2000, Lewis and Thomas 2001).

The third criterion of Wolfe et al. (2007) is that the admixed individuals should differ from the parental ones in life-history traits in a direction likely to enhance invasion. In the case of *H. axyridis*, the relevant comparison is between pure invasive individuals and admixed individuals, because individuals of the flightless biological control strain are unlikely to be able to overwinter and thus to durably settle a sustainable population *in natura* due to their flightless phenotype.

A first important point is that invasive individuals never significantly outperformed the admixed ones. This result highlights that the use of flightless individuals as biological control agents in the field could potentially enhance invasion by decreasing the Allee effect typical of dispersing individuals founding new populations (Tobin et al. 2007). Indeed, in the invasion front, population sizes are expected to be low. If recurrent releases of flightless individuals are made near the invasion front, Allee effects would be reduced. A comparison of invasive females directly with pure biological control females reveals that they tend to invest more in reproductive structures. Additional experiments should be performed to understand whether this difference translates into effective fecundity.

A second important point is that we found that admixture led to both heterosis and increased genetic variance. Admixed individuals developed more quickly and grew larger. These shifts indicate heterosis. Admixture increased genetic variance for survival in starvation, with CV_G of hybrids significantly exceeding parental ones for this trait. While there was no significant shift in the mean value for survival in starvation some hybrid genotypes consistently outperformed parental ones. Thus, admixture could boost the efficiency of selection in direction of higher survival under stressful conditions of starvation (Ellstrand and Schierenbeck 2000; Lee 2002; Facon et al. 2005).

We will now consider how changes in development time, body length and increased variability for survival in

Table 2. Genetic coefficients of variation within each cross for the six traits studied and the associated likelihood ratio tests.

	BIO-BIO	BIO-INV	INV-BIO	INV-INV	Test
Larval survival	0.140	0.103	0.113	0.044	LRT = 1.4; *P* = 0.474
Development time	0.035	0.037	0.024	0.033	LRT = 1; *P* = 0.447
Reproductive investment	0.010	0.011	0.026	0.026	LRT = 2.5; *P* = 0.295
Survival in starvation	0.227	0.384	0.684	0.174	LRT = 7.7; *P* = 0.017
Survival in quiescence	0.344	0.456	0.376	0.310	LRT = 2.5; *P* = 0.295
Body length	0.015	0.017	0.024	0.016	LRT = 1.3; *P* = 0.382

starvation could affect the ongoing invasion of *H. axyridis*. Shifts in life-history traits due to hybridization/admixture events and associated with higher invasiveness have already been reported (e.g. Facon et al. 2005; Lavergne and Molofsky 2007). Several studies have also highlighted that such recombination events often produce an increase in cell volume, body size or seed/juvenile size (see for instance Vila and D'Antonio 1998). In the case of *H. axyridis*, the observed increase of body size in admixed individuals has the potential to impact the interactions between this species and the native coccinellid species by enhancing the dominance of *H. axyridis* in interspecific competition and intraguild predation (Polis et al. 1989; Lucas et al. 1998). It is worth noting that this increase in adult size does not occur at the expense of a longer development time. On the contrary, admixed individuals grow faster than invasive ones. This shorter development time should enhance population growth rate and hence impact the invasive potential of the species. As mentioned above, *H. axyridis* diapauses during cooler periods. During the rest of the year, it can complete between two and five generations (Koch 2003), and a shorter generation could shift that range up. The third trait impacted by admixture is linked to survival in stress conditions (absence of food). Several studies have pointed out that invasiveness may be associated with a higher stress-tolerance (see for instance Milne and Abbott 2000). For *H. axyridis*, increased ability to survive periods of famine may be especially advantageous when prey populations fluctuate or in areas where preys are at low density.

The three traits for which admixture had an effect are hence likely to be advantageous in the context of invasion. Therefore, if crosses do occur in nature, selection should promote the introgression of genes from the flightless biological control strain into the invasive populations and enhance the invasive potential of *H. axyridis*.

As noted, changes in these traits fall into two different categories: (i) for development time and body length, the shift in trait means provides evidence for heterosis and (ii) for survival in starvation, the difference between hybrids and parents stems from an increase in the genetic variance in hybrids. Predicting the long-term consequences of hybridization/admixture is not an easy task as they are strongly influenced by the genetic basis of hybrid fitness (Fitzpatrick and Shaffer 2007). Indeed, heterosis effects could be transitory due for instance to increasing homozygosity in later generations. Hybrids are also known to often express phenotypic breakdown in the F_2 generation as a result of recombination disrupting co-adapted gene complexes or meiotic problems (Barton and Hewitt 1985; Burke and Arnold 2001). It is hence possible that outbreeding depression might be expressed in future generations of admixed *H. axyridis* individuals. Our results are only based on a F_1-hybrid generation. Additional studies over further generations are hence needed to forecast the long-term consequences of a possible hybridization event.

To better apprehend the evolutionary consequences of admixture between *H. axyridis* invasive and biological control individuals, both empirical and theoretical studies should be performed. For instance, it would be fruitful to simulate the introgression process through experimental evolution in the lab or in semi-natural conditions during several generations. The impact of the 'flightless' allele on the flying ability of heterozygous individuals should also be tested in experimental wind tunnel or flight mills. Moreover, it would be interesting to test how the higher male reproductive success of the flightless males translates into the admixed individuals. Another direction for future research would be to include into theoretical models the fitness consequences of admixture (with both the changes in traits we measured and the presence of the recessive 'flightless' allele), to better predict the impact of admixture with flightless biological control individuals on the invasion dynamics.

We are still at an early stage in understanding how admixture between invasive individuals and biological control ones could affect invasion. Our ongoing study of *H. axyridis* supports the view that intraspecific hybridization (admixture) potentially alters the evolutionary process by contributing novel genetic advantages to admixed individuals (Facon et al. 2005; Lavergne and Molofsky 2007, Schierenbeck and Ellstrand 2009). Finally, our study illustrates a new situation where such admixture can occur, i.e. between invasive and biological control individuals, whereas situations documented so far corresponds to biological invasions resulting from multiple introductions from distinct native range populations bringing together genetically differentiated individuals into a common introduced area (Facon et al. 2003; Wares et al. 2005; Lavergne and Molofsky 2007; Wolfe et al. 2007).

Acknowledgements

We thank J. Turgeon, V. Ravigné and T. Guillemaud for comments and discussions. We appreciated the technical assistance of J-F. Garrigues regarding the aphid's rearing. This work was supported by grants from the Agence Nationale de la Recherche (ANR-06-BDIV-008-01) and from the INRA department SPE and Scientific Direction.

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Appendix A: Procedures of models selection

Regarding model selection in the context of mixed models, Shoukri and Chaudhary (2007) recommend (i) to select only the variance components that improve significantly the fit of the model (with all fixed effects kept in the model) and (ii) to carry out the tests of significance of fixed effects (with all variance components deemed significant at the

first step). The first step allows the user to carry out a decomposition of the variance, by identifying the factors contributing much to the variance, keeping them into the model and discarding the other, less important, variance components. In the present study, we therefore started from a model with all variance components (and all fixed effects) and then built simpler nested models, by removing each time a different variance component. If this removal worsened significantly the fit of the model (as assessed by a Likelihood Ratio Test), the variance component was kept in the model; otherwise, if the removal of the variance component under investigation did not worsen significantly the fit of the model, the variance component was removed from the model and the model selection pursued from this simpler model. The same procedure was followed for the fixed effect once a reasonable covariance structure has been selected (see main text for additional details). For variables reproductive investment, larval survival, development time and survival in quiescent conditions fixed effects were cross, food and the interaction cross × food; we thus compared five models in the model selection. For variables Length and survival in starvation, we incorporated sex as a fixed effect into the models. The fixed effects were then cross, food, sex and the interactions cross × food, cross × sex, food × sex and cross × food × sex. All models run with an interaction included the main effects involved in that interaction for fixed effects. All models run with an interaction as a fixed effect included the main effects involved in that interaction for fixed effects. A total of 19 models were hence run.

In the tables presented below, we used the '+' to indicate additive relationships between effects, the '.' to indicate an interaction and the '*' to indicate the two main effects and the interaction (notations as in Lebreton et al. 1992). To spare space we used the following code for each variable in the tables (c for cross, f for food, s for sex and fam for family). The notation 'fam (4 VCs)' means that a different variance component is estimated for each cross while the notation 'fam' means that a single variance component is estimated for all the four crosses in the model (assuming thus the same variance for each cross). For all tables, the column entitled 'Description' displays the list of effects present in the model under concern, the column 'effect removed' the list of effects removed from the reference model and the column 'Ref.' the model from which is derived the model under concern (i.e. the reference model).

(1) Length

Random effects

	Variable length: random effects				
Model	Description	LRT	*P*-value	Effect removed	Ref.
M1	fam (4 VCs) s.fam f.fam s.f.fam	.	.	None	.
M2	fam (4 VCs) s.fam f.fam	0.1	0.9999	s.f.fam	M1
M21	fam (4 VCs) s.fam	18.4	0.0078	f.fam s.fam	M2 M2
M22	fam (4 VCs) f.fam	0	1		
M31	fam f.fam	1.3	0.3822	fam (4 VCs)	M22
M32	fam (4 VCs)	18.4	0.00389	f.fam	M22
M41	f.fam	1.2	0.65	fam	M31
M42	fam	18.4	0.00023	f.fam	M31

So the best model is the model with 'food.family' as random effect.

Fixed effects

The score of the best model in terms of AIC_c is displayed in bold.

	Variable length: fixed effects	
Model	Description	AIC_c
c + f + s + c.f + f.s + c.s + c.f.s	Three main effects plus three interactions plus one triple interaction	−85.2
c + f + s + c.f + f.s + c.s	Three main effects plus three interactions	−87.2
c + f + s + c.f + f.s	Three main effects plus two interactions	−91.5
c + f + s + c.f + c.s	Three main effects plus two interactions	−78.8
c + f + s + f.s + c.s	Three main effects plus two interactions	−89.3
c + f + s + c.f	Three main effects plus one interaction	−83.3
c + f + s + f.s	Three main effects plus one interaction	**−93.5**
c + f + s + c.s	Three main effects plus one interaction	−80.9
c + f + s	Three main effects	−85.3
c*f	Two main effects plus one interaction	349.3
c*s	Two main effects plus one interaction	−33.8
f*s	Two main effects plus one interaction	−82.7
c + f	Two main effects	346.1
c + s	Two main effects	−38.1
f + s	Two main effects	−74.4
c	One main effect	390.2
f	One main effect	356.8
s	One main effect	−36.1
.	Intercept	391.6

(2) SurvStarv

Random effects

	Variable SurvStarv: random effect				
Model	Description	LRT	*P*-value	Effect removed	Ref.
M1	fam (4 VCs) s.fam f.fam s.f.fam	.	.	None	.
M2	fam (4 VCs) s.fam f.fam	0	1	s.f.fam	M1
M21	fam (4 VCs) s.fam	8.5	0.247	f.fam	M2
M22	fam (4 VCs) food.fam	4.6	0.653	s.fam	M2
M23	fam s.fam f.fam	7.8	0.016	fam (4 VCs)	M2
M24	fam (4 VCs)	11.8	0.001	s.fam f.fam	M2

Regarding the model selection concerning random effects for the variable SurvStarv, one can note that the removal of one of the random effects either 'sex.family' or 'food.family' did not worsen significantly the fit of the model while the removal of both effects led to a model significantly worst (LRT = 11.8 $P = 0.01$). Thus, we were left as best covariance structure model with either the model including 'sex.family' and 'family (4 VCs)' or the model including 'food.family' and 'family (4 VCs)', both models including the four variance components for the crosses. However, the estimates of variance components between the two models were very similar with, in particular, the same ranking among crosses (results not

shown). Therefore, in the following steps of model selection we kept the model including 'food.family' and 'family (4 VCs)' (its deviance value was indeed slightly better; 2774.0 vs. 2777.9). At the end of the model selection process, the best covariance structure had the random effects 'food.family' and 'family (4 VCs)' including a different variance component for each cross.

Fixed effects

The score of the best model in terms of AIC_c is displayed in bold.

	Variable SurvStarv: fixed effects	
Model	Description	AIC_c
c + f + s + c.f + f.s + c.s + c.f.s	Three main effects plus three interactions plus one triple interaction	2855.6
c + f + s + c.f + f.s + c.s	Three main effects plus three interactions	2851.6
c + f + s + c.f + f.s	Three main effects plus two interactions	2847.4
c + f + s + c.f + c.s	Three main effects plus two interactions	2853.1
c + f + s + f.s + c.s	Three main effects plus two interactions	2848.8
c + f + s + c.f	Three main effects plus one interaction	2848.9
c + f + s + f.s	Three main effects plus one interaction	2844.8
c + f + s + c.s	Three main effects plus one interaction	2850.1
c + f + s	Three main effects	2846.1
c*f	Two main effects plus one interaction	2861.2
c*s	Two main effects plus one interaction	2848.1
f*s	Two main effects plus one interaction	2845.1
c + f	Two main effects	2858.7
c + s	Two main effects	**2844.2**
f + s	Two main effects	2846.4
c	One main effect	2856.7
f	One main effect	2859.1
s	One main effect	2844.5
.	Intercept	2857.1

The best model in terms of AIC_c is displayed in bold in the table and has cross and sex as fixed effects. However, the evidence for the inclusion of factor cross was weak (model 'c + s' vs. model 's') and thus for the sake of parsimony we used the model 's' for inferences.

(3) ReproInvest

Random effects

	Variable ReproInvest: random effects				
Model	Description	LRT	*P*-value	Effect removed	Ref.
M1	fam (4 VCs) f.fam	.	.	None	.
M2	fam (4 VCs)	0	1	f.fam	M1
M3	fam f.fam	2.5	0.2095	fam (4 VCs)	M1
M4	fam	2.5	0.295	f.fam and fam (4 VCs)	M1

So the best model is the model with 'family' as random effect.

Fixed effects

The score of the best model in terms of AIC_c is displayed in bold.

	Variable ReproInvest: fixed effects
Model	AIC_c
f*c	116.7
f + c	116.2
f	115.9
c	115.5
Intercept	**115.3**

(4) LarvSurv

Random effects

	Variable LarvSurv: random effects				
Model	Description	LRT	*P*-value	Effect removed	Ref.
M1	fam (4 VCs) f.fam	.	.	None	.
M2	fam (4 VCs)	0	1	f.fam	M1
M3	fam f.fam	1.4	0.3632	fam (4 VCs)	M1
M4	fam	1.4	0.4745	f.fam and fam (4 VCs)	M1

So the best model is the model with 'family' as random effect.

Fixed effects

The score of the best model in terms of AIC_c is displayed in bold.

	Variable LarvSurv: fixed effect
Model	AIC_c
f*c	−23.8
f + c	−30.2
f	**−34.2**
c	−11.9
Intercept	−15.8

(5) DvptTime

Random effects

	Variable DvptTime: random effects				
Model	Description	LRT	*P*-value	Effect removed	Ref.
M1	fam (4 VCs) f.fam	.	.	None	.
M2	fam (4 VCs)	146.8	0	f.fam	M1
M3	fam f.fam	1	0.4466	fam (4 VCs)	M1
M4	f.fam	7.6	0.03871	fam	M3
M5	fam	147.3	0	f.fam	M3

The random effects were kept as 'food.family' and 'family'.

Fixed effects

The score of the best model in terms of AIC_c is displayed in bold.

	Variable DvptTime: fixed effects
Model	AIC_c
f*c	5553.1
f + c	**5552.3**
f	5562.5
c	5618.9
Intercept	5624.4

(6) SurvCold

Random effects

	Variable SurvCold: random effects				
Model	Description	LRT	*P*-value	Effect removed	Ref.
M1	fam (4 VCs) f.fam	.	.	None	.
M2	fam (4 VCs)	0	1	f.fam	M1
M3	fam f.fam	2.5	0.2095	fam (4 VCs)	M1
M4	fam	2.5	0.2950	f.fam and fam (4 VCs)	M1

So the best model is the model with 'family' as random effect.

Fixed effects

The score of the best model in terms of AIC_c is displayed in bold.

	Variable SurvCold: fixed effects
Model	AIC_c
f*c	85.4
f + c	80.2
f	**77.4**
c	92.4
Intercept	90.0

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Appendix B: Results of ANOVAs with full models for the six traits studied

Source	Degrees of freedom	Test statistic	*P*
(A) Larval survival (LarvSurv)			
Fixed effects		Type III *F*	
Cross	3	1.10	0.3722
Food	1	28.26	<0.0001
Food × Cross	3	0.45	0.7160
Random effect		Wald test	
Fam (BIOBIO)		1.27	0.1016
Fam (BIOINV)		0.92	0.1786
Fam (INVBIO)		1.06	0.1452
Fam (INVINV)		0.30	0.3823
Food × Fam (cross)		*	*
(B) Development time (DvptTime)			
Fixed effects		Type III *F*	
Cross	3	5.86	0.0047
Food	1	185.03	<0.0001
Food × Cross	3	1.86	0.1529
Random effect		Wald test	
Fam (BIOBIO)		1.38	0.0844
Fam (BIOINV)		1.39	0.0822
Fam (INVBIO)		0.70	0.2407
Fam (INVINV)		1.33	0.0920
Food × Fam (cross)		3.63	0.0001
(C) Reproductive investment (ReproInvest)			
Fixed effects			
Cross	3	2.15	0.1248
Food	1	1.66	0.2009
Food × Cross	3	2.06	0.1089
Random effect		Wald test	
Fam (BIOBIO)		0.37	0.3555
Fam (BIOINV)		0.44	0.3283
Fam (INVBIO)		1.27	0.1021
Fam (INVINV)		1.35	0.0890
Food × Fam (cross)		*	*
(D) Survival in starvation (SurvStarv)			
Fixed effects		Type III *F*	
Cross	3	1.61	0.2176
Food	1	0.09	0.7616
Sex	1	10.14	0.0027
Food × Cross	3	2.19	0.1032
Cross × Sex	3	0.58	0.6341
Food × Sex	1	3.71	0.0548
Food × Cross × Sex	3	0.93	0.4261
Random effect		Wald test	
Fam (BIOBIO)		0.60	0.2747
Fam (BIOINV)		1.61	0.0542
Fam (INVBIO)		1.56	0.0593
Fam (INVINV)		*	*
Food × Fam (cross)		2.09	0.0185
Fam × Sex (cross)		1.66	0.0487
Food × Fam × Sex (cross)		*	*
(E) Survival in cold conditions (SurvCold)			
Fixed effects		Type III *F*	
Cross	3	1.34	0.2909

Source	Degrees of freedom	Test statistic	*P*
Food	1	19.29	<0.0001
Food × Cross	3	0.85	0.4736
Random effect		Wald test	
Fam (BIOBIO)		1.04	0.1501
Fam (BIOINV)		1.57	0.0579
Fam (INVBIO)		1.30	0.0960
Fam (INVINV)		0.64	0.2621
Food × Fam (cross)		*	*
(F) Body length (Lgth)			
Fixed effects		Type III *F*	
Cross	3	5.01	0.0006
Food	1	88.37	<0.0001
Sex	1	943.25	<0.0001
Food × Cross	3	1.80	0.1638
Cross × Sex	3	0.65	0.5831
Food × Sex	1	10.22	0.0015
Food × Cross × Sex	3	1.56	0.2000
Random effect		Wald test	
Fam (BIOBIO)		0.04	0.4829
Fam (BIOINV)		0.61	0.2693
Fam (INVBIO)		1.08	0.1406
Fam (INVINV)		0.31	0.3787
Food × Fam (cross)		2.40	0.0082
Fam × Sex (cross)		*	*
Food × Fam × Sex (cross)		0.33	0.3705

ANNEXE IX

Article 9 (2011)

Guillemaud T, Ciosi M, **Lombaert E**, Estoup A (2011) Biological invasions in agricultural settings: Insights from evolutionary biology and population genetics. *Comptes Rendus Biologies* **334**, 237-246.

Précisions sur le rôle de co-auteur :

- ➔ Discussions.
- ➔ Participation globale à la rédaction du manuscrit.

C. R. Biologies 334 (2011) 237–246

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Review/Revue

Biological invasions in agricultural settings: Insights from evolutionary biology and population genetics

Les invasions biologiques en agriculture : points de vue de la biologie évolutive et de la génétique des populations

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ARTICLE INFO

Article history:
Available online 1 February 2011

Keywords:
Invasive species
Alien species
Biological control
Bridgehead
Multiple introductions
Diabrotica virgifera virgifera
Harmonia axyridis

Mots clés :
Espèce envahissante
Espèce exotique
Lutte biologique
Tête de pont
Introductions multiples
Diabrotica virgifera virgifera
Harmonia axyridis

ABSTRACT

Invasion biology and agriculture are intimately related for several reasons and in particular because many agricultural pest species are recent invaders. In this article we suggest that the reconstruction of invasion routes with population genetics-based methods can address fundamental questions in ecology and practical aspects of the management of biological invasions in agricultural settings. We provide a brief description of the methods used to reconstruct invasion routes and describe their main characteristics. In particular, we focus on a scenario – the bridgehead invasion scenario –, which had been overlooked until recently. We show that this scenario, in which an invasive population is the source of other invasive populations, is evolutionarily parsimonious and may have played a crucial role in shaping the distribution of many recent agricultural pests.

RÉSUMÉ

La biologie de l'invasion et l'agriculture sont intimement liées pour plusieurs raisons et en particulier parce que de nombreuses espèces de ravageurs agricoles sont des envahisseurs récents. Nous suggérons que la reconstruction des routes d'invasion par des méthodes de génétique des populations permet d'aborder des questions écologiques fondamentales et des aspects pratiques de la gestion des invasions biologiques en agriculture. Nous fournissons une brève description des méthodes utilisées pour reconstruire les routes d'invasion et décrivons leurs principales caractéristiques. En particulier, nous nous concentrons sur un scénario – le scénario d'invasion « tête de pont » – qui n'avait pas été considéré jusqu'à présent. Nous montrons que ce scénario, dans lequel une population envahissante est la source d'autres populations envahissantes, est parcimonieux du point de vue évolutif et a probablement joué un rôle crucial dans l'élaboration de la distribution géographique de nombreux ravageurs des cultures récents.

1. Introduction

Biological invasions are major ecological phenomena that influence biodiversity by shaping the worldwide distribution of species. In recent times, they have become a

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1631-0691/$ – see front matter
doi:10.1016/j.crvi.2010.12.008

significant element in global change and have been accused of having adverse effects on public health, the economy and biodiversity [1]. The development of human trade and transport since the 15th Century, which has accelerated over the last 200 years, has increased the importance of invasions as a cause of human-induced global change [2]. Invasive species are important vectors of emerging diseases [3], agricultural pests [4] and responsible for many species extinctions and changes in biodiversity worldwide (e.g. [5]).

Many studies on biological invasions have been published since the 1990s, but the definition of an invasive species remains vague. The terminology relating to biological invasions includes a plethora of terms and a wide variety of uses. For example, terms such as "introduction", "establishment" or "invasive species" have been used in different ways in previous publications [6,7]. The vocabulary associated with biological invasions suffers from two flaws: polysemy (multiple meanings for one word, e.g. "invasive") and synonymy (several words for one meaning, e.g. "alien", "exotic", "non indigenous", "introduced"). These problems partly account for the difficulties involved in finding a definition acceptable to most biologists. In addition, one of the problems encountered when trying to define the term "invasive species" arises from the tendency of the word "invasion" to evoke anthropocentric concepts (e.g. "Barbarian Invasions", assault, attack, intrusion, incursion, raid, etc.) [7] associated with negative connotations that may not necessarily apply to ecological phenomena. Current definitions differ in the relative importance attributed to three major components: 'range expansion' [8], 'high local abundance' [9] and 'disruption of ecosystem function' [10]. We will use the following definition here: *an invasion may be considered to have occurred when a group of individuals has been introduced into a new area, in which they have established themselves, increased in number and spread geographically*. This definition does not necessarily imply a spread into new ecological conditions and does not necessarily result in negative effects on the invaded ecosystem.

Many studies on this topic have been published, but only very few biological invasions have been properly described, studied and understood, due to conceptual, methodological and experimental limitations (e.g. [8]). Consequently, most of the hypotheses formulated concerning the key factors determining the probability of success or failure of invasions, such as propagule size, genetic variability or hybridization, have never been tested. One of the key scientific questions concerning biological invasions that has yet to be answered concerns the reasons why some species become successful invaders whereas others do not [1,11]. The general characteristics of species (such as dispersal, competitiveness) may determine the probability of the species becoming invasive [12]. There is, however, some intraspecific variation for this probability as illustrated by the observation that only a small fraction of populations becomes invasive in many "invasive species" (e.g. [13–15]). There is therefore still a need to identify explanatory evolutionary and environmental factors at the population level. We argue that the precise descriptions of biological invasions, including their history, geography, demography and genetics – referred to here as invasion routes – represent a first step toward identifying these factors.

We focus here on biological invasions in agricultural settings. We briefly review the specificity of invasions in agricultural settings and explain why the reconstruction of invasion routes can be used to address fundamental questions about the determinants of invasions and practical aspects of biological invasion management. We then consider the methodological challenges associated with studies of invasion routes and describe the main evolutionary and environmental insights drawn to date from the large set of published studies dealing with the reconstruction of invasion routes.

2. Invasion in agricultural settings

The history of agriculture is intimately linked to biological invasions. The invention and development of agriculture allowed the worldwide spread of human populations [16]. It also led to the invasion, as defined above, of areas by cultivated plants and livestock animals [17]. As a result, a few animal and plant species are now found throughout the world. Pimentel et al. [18] observed that agricultural activities have led to 90% of the food of the world's human population being provided by a mere 15 plant species, and eight animal species accounting for most of the animal proteins consumed by humans. Species such as maize (*Zea mays*) and chicken (*Gallus gallus domesticus*) are found all over the world (with the exception of hot and cold deserts) and their populations are much larger than that of humans (references in Pimentel et al. [18]).

A large number of animals, plants and microbes living in agricultural ecosystems decrease the quality and/or quantity of the cultivated resource and are therefore considered to be pest species. These pests are often recently introduced organisms capable of taking advantage of the extraordinarily large amount of resources provided by cultivated crops or animals for settlement and spread. In the absence of predators and parasites, invaders often undergo explosive population increases, with severe consequences for the crop plants and domesticated animals concerned. This results in the invader being classified as a major pest species. For example, the oomycete *Phytophthora infestans*, the causal agent of potato blight, was introduced into Europe from America around 1843 [19]. It invaded large cultivated areas of Europe and was the cause of the Great Famine in Ireland in the mid-19th Century. Another famous example is grape phylloxera, *Daktulosphaira vitifoliae*, which is a worldwide pest of grapevine [20]. This insect devastated European vineyards after its introduction in the region of Bordeaux, France, and its spread across Europe in the second half of the 19th Century. Similarly, *Ceratitis capitata*, the Mediterranean fruit fly, is a famous pest of fruit crops originating from Africa. It invaded the Americas and Australia during the late 19th and 20th centuries and is now one of the world's most threatening agricultural pests, attacking over 200 different cultivated plants [21]. Ten of the 16 invasive terrestrial invertebrates present in the DAISIE (a European

consortium of researchers studying invasive species in Europe) "100 of the worst" list are crop pests [22].

The invasion of agricultural settings by pests may have tremendous economic and social consequences. Such invasions may generate costs due to production loss, decreases in the value of the product and the need for control practices (survey, containment, eradication). The estimated world cost of biological invasions in agriculture reaches the astronomical range of 50 to 250 billion US dollars per year [18]. Many social and economic activities have also developed to deal with this problem, from the chemical industry, genetic engineering development and agricultural advisory services to public and private agronomy research.

2.1. Invasion and biological control

Biological control is a promising approach to the control of pest species in agriculture, because it has few if any adverse effects on the environment and human health. Classical biological control (CBC) is a component of both integrated pest management and organic farming. It involves the introduction of an organism – often a predator or a parasite of the pest species targeted – into an area in which it was not previously found, in the hope of establishing stable populations capable of reducing the density of a specific pest [23]. CBC and invasion biology are intimately linked for at least two reasons:

- **the target of CBC is often an invading species that has recently acquired pest status**. A successful example of CBC against an invasive pest species is provided by the glassy-winged sharpshooter, *Homalodisca vitripennis*. This large leafhopper is a xylem-feeding pest that transmits *Xelella fastidiosa*, a parasitic bacterium responsible for a lethal infection in plants. This species originates from the South East USA and Northern Mexico, and has invaded a number of sites in the Pacific, including several archipelagos in French Polynesia, since 1999 [24–26]. A CBC operation was implemented in 2004, with *Gonatocerus ashmeadi*, a parasitoid wasp that parasitizes the eggs of the pest species. After the release of more than 10,000 individuals in Tahiti in 2005, invasive glassy-winged sharpshooter populations decreased in size by about 90% around the release sites [26];
- **CBC and biological invasions have similar properties**. CBC aims to establish and spread populations of beneficial species, through ecological processes resembling those occurring during unintentional invasions [27–29]. Biological invasions, which may be unintentional and detrimental (in the case of pest species) or intentional and beneficial (in the case of CBC), have enough characteristics in common to be considered as a single eco-evolutionary process. Thus, an understanding of the ecological and genetic factors underlying efficient biological control may help us to understand and to manage detrimental biological invasions. Conversely, the information obtained from descriptions of accidental biological invasions may help us to design more effective biological control. In a CBC operation, the initial demographic (number of individuals, number of release points, timing of releases) and genetic (genetic variance and adaptive traits of the introduced population) parameters of the invasion – the introduction parameters – can be controlled experimentally [30,31]. Meta-analyses of ancient CBC successes and failures have been used to address certain questions [32], but CBC can also be used *in natura,* in the design of specific experiments testing biological invasion hypotheses. This approach has seldom been used, but has recently begun to drive the use of CBC in model experiments. A recent example is provided by the work of Fauvergue et al. [33], who manipulated the demographic characteristics of a parasitoid introduced in a CBC context to test for a positive effect of the size of the population introduced on the success of establishment and, hence, of invasion. These authors introduced the North American parasitoid *Neodryinus typhlocybae* into Southern France, to control the North American invasive flatid planthopper, *Metcalfa pruinosa,* and demonstrated a total absence of the expected positive demographic effect on the success of settlement [33].

2.2. Factors promoting invasions

2.2.1. Role of humans in shaping invasion routes

Human activities are responsible for a large proportion of recent biological invasions [34] in two main ways. First, human activities serve as a vector for the introduction of propagules (canals, marine ballast, air, road and train traffic) into new, geographically disconnected areas and for geographic expansion of populations that have already been introduced (see p. 21 of [35]). The second role of human activities in promoting biological invasions involves environmental modification. The disturbance of natural habitats by human activities is thought to facilitate bioinvasions [36] and disturbances due to agriculture development may play a particular role in this respect [37,38]. Agriculture has a particular consequence in terms of habitat disturbance: it has homogenized the environment worldwide. The cultivation of domesticated plants, such as maize, has homogenized habitats, decreasing ecological differences between regions in different parts of the world, from Africa to Asia, and from North and South America to Europe [39]. When a species is introduced into a new and remote area, the expected mismatch between its phenotypic characteristics and local ecological conditions is greatly attenuated by this homogenization.

2.2.2. Adaptation

Natural selection and adaptation probably play key roles in determining the success of invasion during the establishment phase [40,41]. In the absence of strong environmental homogenization, the new geographic area into which individuals are introduced may have ecological conditions very different from those of the native area. A large additive genetic variance in the introduced population should increase adaptability, thereby increasing the probability of settlement and subsequent demographic growth and geographic spread (e.g. [8]). However, adaptation may also occur in populations with low levels of genetic variation, provided that "good" genetic combinations are present [8]. This is particularly true in

agricultural contexts, in which the selection pressure exerted by pest control strategies may be very strong (see p. 128 of [35]). For example, pesticide resistance may be the main prerequisite for the settlement and spread of introduced pests in areas commonly treated with one or more pesticides to control resident pests. In the aphid *Aphis gossypii*, a pest of many cultivated plants (cotton, melon, potato, pepper, eggplant, citrus, etc.), a few genetic clones have spread worldwide. These clones are adapted both to their host plants – they display an intimate degree of host specialization [42] – and to the most common pesticide treatments, with most clones resistant to organophosphate and pyrethroid insecticides [43]. The spread of the western corn rootworm, *Diabrotica virgifera virgifera*, in the US during the 1960s probably resulted from the appearance and spread of allelic forms conferring insecticide resistance [44]. This insect pest of maize has been present in the Great Plains since at least 1867, and by 1955, its geographic distribution remained limited to parts of Kansas, Colorado and Nebraska [45]. Cyclodiene insecticides were introduced in 1952 and were massively used, and the first reported case of resistance occurred in 1959 [44]. Resistant rootworm rapidly spread throughout the Corn Belt and reached Northwest Indiana in 1968. In 1979, it was present throughout most of the US Corn Belt, from Nebraska to Ohio and from Minnesota to Missouri [44,45]. It has not been demonstrated that insecticide resistance was responsible for accelerating the geographic spread of the western corn rootworm in the 1960s [45], but it is nonetheless clear that this spread would not have been possible if the insects had not evolved cyclodiene resistance.

2.3. Routes of invasion

2.3.1. An approach to tackling academic issues

The genetic variability of invading populations depends on the history and demography of the populations or groups of individuals, from their emigration from the source population to their introduction and spread [46]. The description of this history depicts invasion routes. It includes information about source populations (number and genetic composition), the number of introductions from the sources, the number of individuals involved in each introduction, the occurrence of admixture between independently introduced populations, the number of intermediate invasive populations between the initial introduction point and the invasive population studied and demographic dynamics at each step in the history of the invasion. In a recent review on this topic, Estoup and Guillemaud [47] argued that knowledge of invasion routes was required to decipher the factors responsible for the success of invasions. More specifically, information about sources and invasion pathways is essential if we are to avoid making erroneous conclusions when testing the hypothesis that a particular environmental or evolutionary factor affects invasion success. Keller and Taylor [48] argued that adaptive evolution could not be inferred from the simple observation of changes in the distribution of phenotypic traits between the invaded and the native area. They pointed out that the hypothesis of neutral evolution during invasion processes could only be rejected if ancestor–descendent comparisons or *Q*st-*F*st analyses are carried out. Estoup and Guillemaud [47] also argued that such analyses need to compare "comparable entities" (here, the invasive populations and their precise source(s)), which requires a basic knowledge of invasion routes.

2.3.2. An approach to tackling practical issues in agricultural settings

The reconstruction of routes of invasion can contribute to the development or optimization of measures for preventing invasions, particularly in an agricultural context, in two main ways. Firstly, invasion routes basically describe the geographic origins of invasive pests until their introduction. This geographic information can be used as the basis of management actions directed against the main steps of the invasion process: exit from the native area (emigration), vector transport or migration and entry into the invasion site (see Introduction). In the case of recurrent introductions, as demonstrated for the chrysomelid *D. virgifera* [49], identification of the precise location of the escape path in the native area (e.g. a specific airport, harbor, ecosystem, region etc.) can lead to the design of specific monitoring and quarantine measures targeting the sources [17]. The same rationale can be applied to the vectors responsible for recurrent introductions and to the entry portals for the pest: control strategies focusing on specific vectors (e.g. freight containers of a particular crop seed or a specific human mode of transportation) or entry locations (e.g. a specific airport, harbor, ecosystem, region etc. as in the case of "exit doors"). By contrast, for pests arriving in a new area through a single or a small number of introduction events, eradication or containment strategies may be efficient if applied shortly after the arrival of the pest [17].

Secondly, identification of the invasion route provides information about the original environment and the genetic properties of the source population of the invading pest. A knowledge of the biotic and abiotic environment to which the pest is adapted may make it easier to design an effective control strategy. This is particularly true when choosing pesticides, as this choice must take into account the potential resistance of the source population. This simple rationale applies to all strategies for which susceptibility varies within the source populations of the pest (e.g. parasite or predator use, crop rotation). In the context of biological control, the choice of natural enemy to be introduced may depend on what we know about the source populations of the invader. Generally, the aim is to choose species or populations of a species with the same geographic origin as the pest population [35]. The probability of an invasive pest being controlled by a natural enemy depends on the level of adaptation of the two protagonists to their environment, their adaptation to each other and their ability to evolve [29,50]. In particular, biological control agents may be more effective against the native populations with which they coevolved and to which they have adapted than against other populations (see for a complication of the simple case [15,29]).

Box 1. Methods for reconstructing invasion routes.

The methods for reconstructing invasion routes have been described in detail elsewhere [47,55] and are not affected by their application to agricultural settings. There are two types of methods: direct methods based on historical and observational data and indirect methods based on population genetics data. Direct methods have long been used and can be informative (e.g. [4]). However, they are often imprecise and depend on observations that are rare and/or difficult to obtain. Indirect methods are based on genetic data obtained in invasive and native populations, through the use of molecular markers (e.g. [13,21]). Based on comparisons of simple genetic statistics or more elaborate model-based statistical analyses, these indirect methods can be used to infer historical relationships between populations, such as ''population B is derived from population A'' or ''population A is the result of hybridization between population B and C''. A model-based Bayesian approach, the approximate Bayesian computation (ABC) approach, has recently been developed [75] and adapted for invasion route inference [55]. This new methodology has two advantages over most other indirect methods:

- it takes into account the stochasticity of the demographic and genetic history considered;
- it makes it possible to estimate confidence in invasion route inference by calculating a probability for each alternative invasion route tested [47].

2.3.3. Mistakes to avoid when retracing invasion routes

Several problems may occur during the reconstruction of invasion routes, leading to erroneous conclusions. Whatever the method used (see Box 1), inappropriate sampling schemes may be problematic. Muirhead et al. [51] reviewed published studies of invasion based on mitochondrial or chloroplast DNA markers and noticed that the introduced and native populations were not generally sampled with the same intensity. In general, fewer individuals were sampled from populations collected from the native area (60% of the studies sampled a mean of fewer than six individuals per native population), but a larger number of populations were sampled in the native area. Verbal models and simulations have shown that the sampling of too few individuals from native populations or of too few native populations probably leads to the erroneous characterization of source populations of invaders [51]. Accuracy in the determination of the source population is also strongly dependent on spatial genetic structure in the native area [51,52], with greater genetic differentiation between native populations generally ensuring more accurate source determination. As pointed out by Geller et al. [52] if there is strong local genetic differentiation, then source determination is theoretically optimal. However, in this case, the sampling efforts required to ensure that the real source population is not missed may be so great that ''genetic methods will be unable to determine any likely source at all'' (see Fig. 2 in [52]). Temporal variation in genetic structure may also lead to the misidentification of source populations. Allele frequencies for genetic probably vary significantly over time in both the native and introduced area if there is a large amount of drift (i.e. for small populations, see [52]).

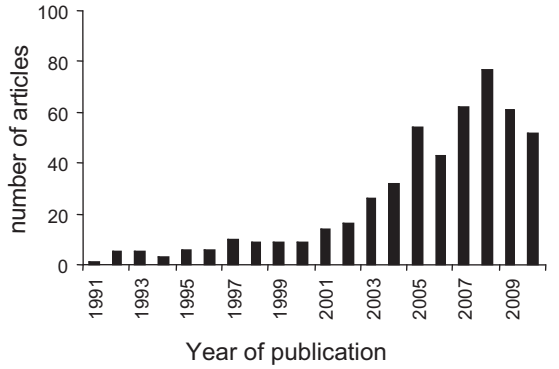

Fig. 1. Change over time in the number of scientific articles published on invasion routes. The pool of articles searched consisted of those published between 1975 and August 2010 present in the SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, CCR-EXPANDED and IC databases of ISI Web of Knowledge. The following keyword formula was used to search for target articles: Topic = ([invasi* or alien or exoti*] and [pathway* or route* or source*] and [genet* or ''molecular marker'' or microsatellite or mitochond* or choroplast*] and [ecolog* or population] not [cancer or medicin* or therap*]).

Other potential problems are direct consequences of the methods used to reconstruct invasion routes. Most methods, whether direct or indirect (Box 1), cannot resolve complex invasion routes. For example, recurrent introductions from the same source (e.g. in *D. virgifera* [49]), admixture between various introduced populations and intermediate invasive populations playing the role of source populations (e.g. in *H. axyridis* [53]) are particular features that are difficult to consider with most genetic methods of inference, particularly those based on the calculation of genetic distances only [47]. Lombaert et al. [53] simulated invasive populations originating from the admixture between two source populations and applied classical methods based on assignment likelihood and the calculation of Fst to determine their source (e.g. [54]). In this particular but not unusual case, most of the results obtained were false, with the correct source being identified only rarely. Guillemaud et al. [55] also simulated genetic data with an intermediate introduced population serving as the source of two invading populations. Classical analyses often concluded that there have been multiple introductions from the native area when all the invaders actually originated from a single introduction into the invaded area. However, the complex scenarios described above can be correctly treated by the ABC method described in Box 1, with DIYABC software, for example [56,57].

2.4. Common invasion scenarios

The retracing of invasion routes from molecular genetic data is increasingly being carried out (Fig. 1). More than 500 scientific articles have been published on this subject since 1991, with more than 40 papers per year on this theme published since 2005. The results obtained for invasion route descriptions are extremely variable, but three general trends can be observed:

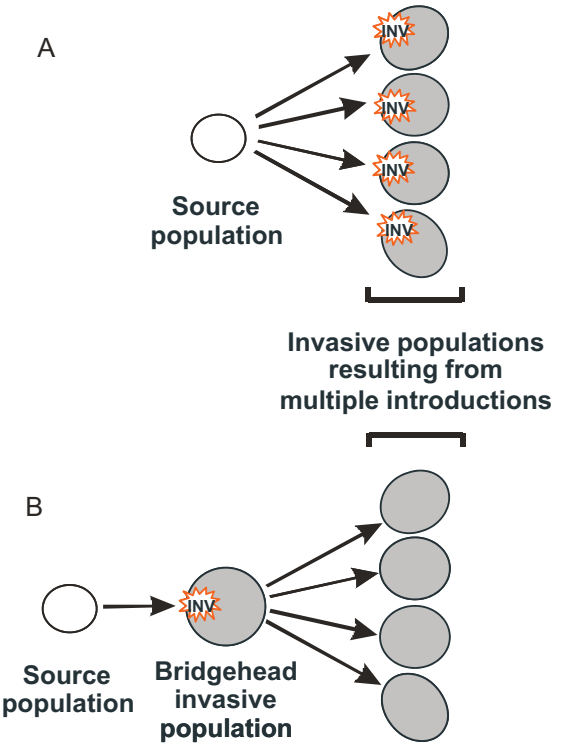

Fig. 2. Origin of the invasive characteristics of invasive populations resulting from multiple introductions. A. Standard scenario of multiple introductions requiring multiple acquisitions of invasive traits. B. "Bridgehead invasion" scenario with a single acquisition of invasive characteristics in the intermediate bridgehead invasive population. Populations in gray are invasive. Arrows indicate introduction events, "Inv" refers to the evolution of invasive traits.

2.4.1. Multiple introductions

Recent papers on invasion routes suggest that invasions are often associated with multiple introductions (i.e. several introductions from one or several sources into one or several remote areas). This scenario has been demonstrated in the cases of the maize pest *D. virgifera* [13,49], the false brome *Brachypodium sylvaticum* [58], the spotted knapweed *Centaurea stoebe micranthos* [59], the shrub Scotch broom [15], the mosquito *Culex quiquefasciatus* [60], the amphipod *Gammarus tigrinus* [14], the Cuban lizard [61], the freshwater snail *M. tuberculata* [62], and many other invasive species [63].

2.4.2. Admixture

Studies using indirect genetic methods to reconstruct invasion routes have suggested that admixtures between different source populations often occur in invasions, as demonstrated principally in plants (e.g. [58]) although this scenario has also been reported for a number of invasive animals, including the Cuban lizard [64], the freshwater snail *Melanoides tuberculata* [65], and the harlequin ladybeetle *Harmonia axyridis* [53].

2.4.3. Bridgehead effect

In a number of articles describing invasion routes, successful invasive populations appear to originate from an intermediate population, which is, itself, a successful invasive population [49,61,66–68]. In this scenario, the intermediate invasive population at the origin of the secondary invasive populations plays the role of a bridgehead. As described below, this "invasion bridgehead", is particularly important for an understanding of how and why invasions occur in agricultural settings. As suggested by Facon et al. [11], several factors are required to account for the occurrence of an invasion:

- a change in migration regime;
- an environmental change in the area into which the species is introduced;
- a genetic change in the introduced population leading to a new match between the environment and the introduced individuals.

Let us consider an invasion scenario including multiple introductions, in which an evolutionary genetic change

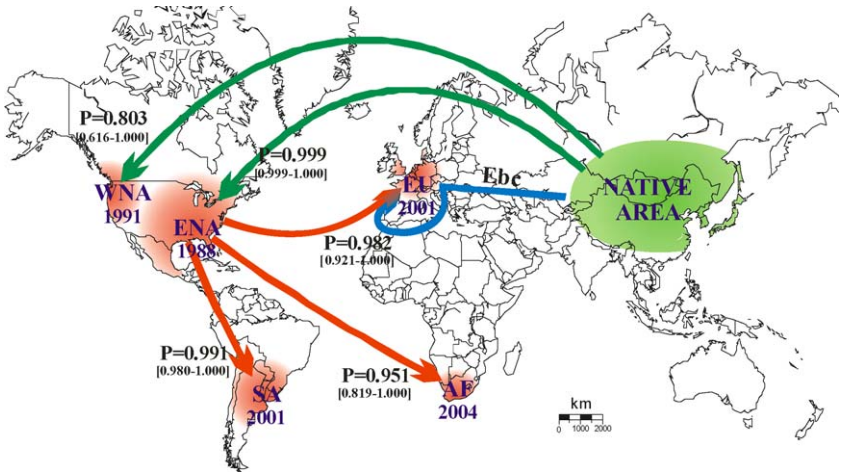

Fig. 3. Most likely invasion routes of *Harmonia axyridis*, deduced from genetic analysis based on microsatellite markers variation and approximate Bayesian computation by Lombaert et al. [53]. For each outbreak, the arrow indicates the most likely invasion pathway and the associated posterior probability value (P), with 95% confidence intervals in brackets. The years in which the invasive populations were first observed are indicated.

Box 2. Bridgehead invasion in the biocontrol agent *Harmonia axyridis*.

The Harlequin ladybeetle or multicolored Asian lady beetle, *Harmonia axyridis*, a native of Asia, has long been used as a biological control agent to control aphid populations. Despite repeated attempts at introduction since 1916, the establishment of this species was not observed until recently (references in Lombaert et al. [53]). In 1988 and 1999, the first invasive populations were recorded in Louisiana and Oregon, respectively. Invasive populations of the Asian ladybeetle were then observed in Europe (Belgium in 2001) and South America (Argentina in 2001), followed by South Africa in 2004. Using molecular markers, historical information and ABC methods, Lombaert et al. [53] showed that the invasive populations in western and eastern North America arose from two introductions from Asia, either through biological control or accidental introductions, and that the European, South American and African outbreaks all originated independently from eastern North America. In addition, evidence of an admixture between the eastern North American population and the native Asian population were found in Europe. The invasion routes summarized in Fig. 3 indicate that the eastern North American invasive population acted as a bridgehead population in the worldwide invasion of *H. axyridis*. The role of eastern North America in the sudden invasion of Europe, South America and Africa, the long history of unsuccessful introductions of the ladybeetle from its native range for biological control, and the apparent absence of invasive populations originating from western North America suggest that an evolutionary change or a change in emigration regime probably occurred in eastern North America. Additional quantitative genetics studies of key life history traits are underway.

Box 3. Bridgehead invasion of the western corn rootworm, *Diabrotica virgifera virgifera*.

This chrysomelid, a pest that attacks the root system of maize, is one of the most important pests of maize in the USA and is sometimes referred to as the ''billion dollar bug'' [76]. It originates from what we now call Mexico, was first observed in the US in 1867, in the Great Plains, and invaded North America during the second half of the 20th century [45]. It was first observed in Europe in 1992, in the former Yugoslavia, and rapidly invaded a large part of Central and South Eastern Europe. A number of isolated outbreaks have been detected almost every year since 1998, in various countries, including Italy, France, Switzerland, Belgium, the United Kingdom, the Netherlands and Germany [77]. Using molecular markers and historical information, Miller et al. [49] and Ciosi et al. [13] showed that the invasion of Europe by *D. virgifera* was very probably due to multiple introductions from North America and, more precisely, from the Northern US [13]. The invasions of North America and Europe by this agricultural pest thus form a succession of introductions and geographic expansions and correspond to a bridgehead invasion scenario: the native Mexican population gave rise to an invasive bridgehead population in the US, which acted as the source of all the other invasive populations in Europe. We propose the following hypotheses for invasion by *D. virgifera*: after its introduction from the Mexican source population into the US, invasion by the North American bridgehead population was triggered by one or more adaptive changes, such as specialization on a widespread resource (in this case, maize). A change in emigration regime in the bridgehead population when it reached the North East US was then sufficient to initiate remote invasions in Europe.

accounts for the success of invasion. Without a bridgehead population, the framework of Facon et al. [11] requires multiple genetic shifts (one in each introduced area, Fig. 2A). In a bridgehead invasion scenario, only one evolutionary shift toward invasiveness has to occur in the bridgehead population (Fig. 2B). This scenario is therefore evolutionarily more parsimonious than that without a bridgehead. Two illustrative example of a bridgehead invasion scenario are given in Boxes 2 and 3.

How general are bridgehead invasion scenarios? Apart from the studies on *D. virgifera* and *H. axyridis* (Boxes 2 and 3), other studies have shown that intermediate invasive populations may be the sources of other, often distant, invasive populations [61,66,69]. However, it is possible to demonstrate the occurrence of such scenarios only in very well documented cases of biological invasions: demonstrating the existence of a ''bridgehead'' population requires a good knowledge of the geographic distribution of the species (native and invaded areas) and of the routes of invasion of the species. Crop pests probably commonly establish bridgehead populations. We found several examples of pests or pathogens for which precise documentation of the invasion made it possible to identify a bridgehead population, or at least to show that the likely scenario involved an intermediate invasive population giving rise to several secondary invasions. A non-exhaustive list of examples is provided below:

- potato blight, *Phytophthora infestans*, was introduced into Europe in the 1840 s from America (references in [19]). Goodwin et al. [70] suggested that the European population then served as the source population for a number of other introductions, resulting in the worldwide distribution of this oomycete;
- the causal agent of apple scab, *Venturia inaequalis*, invaded the world by following its host [71]. It originated in Central Asia, and was first introduced into Europe during the Ancient history. Europe then acted as a secondary source for world colonization by the pathogen over the last 500 years;
- the European corn borer, *Ostrinia nubilalis* first invaded Europe, establishing a bridgehead from which it was subsequently introduced into North America [72,73];
- the Colorado beetle, *Leptinotarsa decemlineata* first invaded the United States. It was then introduced into Europe [69];

- the grape phylloxera, *Daktulosphaira vitifoliae*, in addition to invading the whole of Europe from France, probably initially invaded California, subsequently being introduced, from the American population, into Australia, New Zealand and Peru [66];
- the Guatemalan potato moth, *Tecia solanivora*, first invaded the southern part of Central America and, from there, was introduced into South America and the Canary Islands [74].

Most of the species mentioned above, like *D. virgifera* (see Box 2), probably achieved pest status following an evolutionary shift allowing the bridgehead population to become invasive: adaptation to a cultivated plant. *D. virgifera* and the European corn borer probably left several wild herbaceous hosts to adapt to maize. The Colorado beetle, the Guatemalan potato moth and the potato blight probably all moved onto the cultivated potato from wild tuber-bearing plants. Phylloxera may not have adapted: this aphid became a major pest on grapes because the *Vitis* species used happened to be susceptible. In this case, a change in migration regime was probably the cause of the secondary introductions worldwide from California. Once it was discovered that the American *Vitis* species could be used as a rootstock for the efficient control of phylloxera, the intensive collection and exchange of American native *Vitis* plants occurred, undoubtedly increasing the number of phylloxera introductions worldwide [66]. In the case of apple scab, the global codispersal of apple and its pathogen probably provides the best explanation of the current worldwide distribution of *Venturia inaequalis*. This scenario reflects a major change in the migration regime of the pathogen due to the colonization of the rest of the world by European settlers.

3. Conclusion

The histories of invasions and agriculture are intimately linked, with many crop and livestock pests being invasive species and vice versa. In addition, some pest management practices essentially constitute intentional and beneficial invasions. Such practices require a precise knowledge of invasion biology and, conversely, their application may provide valuable information about invasion biology. Recent methods based on analyses of genetic markers have provided tools for the retracing of invasion routes – the history of the invading populations from their geographic origin to their final spread in the invaded area. The examples for which a precise description of invasion routes at the global level is available provide new insight into invasion biology and have highlighted previously unsuspected global trends. In particular the bridgehead invasion scenario seems to apply to many cases of pest invasions and merits more thorough consideration when trying to explain the distribution of other invasive species in agricultural settings. The number of publications on the invasion routes of crop and livestock pests and pathogens is growing, and future studies in this field will undoubtedly provide valuable information challenging the generalization of this evolutionary scenario.

Conflicts of interest statement

The authors declared no conflict of interest.

Acknowledgments

This work was supported by grants from the *Agence nationale de la recherche* (ANR-06-BDIV-008-01 and ANR-09-BLAN-0145-01) and from the Agropolis Fondation (RTRA-Montpellier, BIOFIS project).

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ANNEXE X

Article 10 (2011)

Lawson Handley L-J, Estoup A, Evans DM, Thomas CE, **Lombaert E**, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. *Biocontrol* **56**, 409-428.

Précisions sur le rôle de co-auteur :

- ➔ Co-rédaction avec L.-J. Lawson Handley, A. Estoup et C.E. Thomas des sections « Inferring source populations and invasion routes » et « Appendix 1 ».
- ➔ Participation globale à la rédaction du manuscrit.

BioControl (2011) 56:409–428
DOI 10.1007/s10526-011-9386-2

Ecological genetics of invasive alien species

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Received: 21 December 2010 / Accepted: 6 June 2011

Abstract There is growing realisation that integrating genetics and ecology is critical in the context of biological invasions, since the two are explicitly linked. So far, the focus of ecological genetics of invasive alien species (IAS) has been on determining the sources and routes of invasions, and the genetic make-up of founding populations, which is critical for defining and testing ecological and evolutionary hypotheses. However an ecological genetics approach can be extended to investigate questions about invasion success and impacts on native, recipient species. Here, we discuss recent progress in the field, provide overviews of recent methodological advances, and highlight areas that we believe are of particular interest for future research. First, we discuss the main insights from studies that have inferred source populations and invasion routes using molecular genetic data, with particular focus on the role of genetic diversity, adaptation and admixture in invasion success. Second, we consider how genetic tools can lead to a better understanding of patterns of dispersal, which is critical to predicting the spread of invasive species, and how studying invasions can shed light on the evolution of dispersal. Finally, we explore the potential for combining molecular genetic data and ecological network modelling to investigate community interactions such as those between predator and prey, and host and parasite. We conclude that invasions are excellent model systems for understanding the role of natural selection in shaping phenotypes and that an ecological genetics approach offers great potential for addressing fundamental questions in invasion biology.

Keywords Invasive alien species · Ecological genetics · Molecular ecology · Invasion routes · Dispersal · Community interactions

Handling Editor: Patrick De Clercq

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Introduction

Ecological genetics, a field pioneered by EB Ford and his pivotal book of 1964 (Ford 1964), is the study of (1) evolution in modern-day populations, (2) the genetics of ecologically important traits, and (3) genetics in the context of interactions among organisms and between organisms and their environment. Although not directly discussed in Ford's book, all three of these definitions are highly relevant to the study of biological invasions, which are one of the greatest threats to biodiversity, agriculture, health and the global economy (Pimentel et al. 2001; Roy et al. 2011a). Integrating genetics with ecology in the context of biological invasions is indeed crucial, since the two are explicitly linked: ecological conditions in a new environment may be considerably different from the native range, and this can present major adaptive challenges for an invasive population (Reznick and Ghalambor 2001; Schierenbeck and Ainouche 2006; Ciosi et al. 2008). It is only in recent years that biological invasions have become regarded as "natural experiments", offering unique insights into ecological and evolutionary processes occurring in real-time (Lee 2002; Sax et al. 2007), and increasingly, understanding these processes is seen as crucial for implementing successful management policies.

Information on the demographic history and genetic make-up of an invasive founding population is critical for answering one of the most fundamental questions in invasion biology: what determines the success of invasive alien species (IAS)? In this case, an ecological genetics approach refers to the ecology of particular genotypes, and the role they play in adaptation to new environments, and ultimately invasion success. This question has already received considerable attention in the ecological and evolutionary genetics communities, and we are starting to uncover general insights (see below). In addition, genes (or more commonly, neutral molecular genetic markers) can be used as tools to study ecological processes such as colonization, dispersal or community interactions. While the field of "molecular ecology" has been established for decades, and there have been exciting new developments in both data generation (particularly from next generation sequencing, see "Appendix 3" section and Metzker 2010 for a recent review) and statistical analyses (e.g. Approximate Bayesian Computational approaches, "Appendix 1" section; and landscape genetics, "Appendix 2" section) there has so far been relatively little uptake of these applications to studying invasive populations. Initial focus has been on determining the sources and routes of invasions, and the genetic make-up of founding populations, which is critical for defining and testing ecological/evolutionary hypotheses (Estoup and Guillemaud 2010, and see below). Now that considerable progress has been made in this area, we envisage a growth in the number of ecological genetics studies applied to IAS in the near future.

The aims of this paper are to (1) function as a review of ecological genetics in the context of IAS, (2) introduce new methods in the field and discuss how they can be applied to questions on invasive species, and importantly, (3) promote dialogue between ecologists and geneticists regarding fundamental questions in invasion biology. We begin with a review of recent progress in determining source populations and invasion routes, and advances in our understanding of the role of genetic variation in invasion success. We then focus on two areas that are beginning to be investigated in the context of ecological genetics of IAS: dispersal and community interactions.

Inferring source populations and invasion routes

Inferring source populations and invasion routes is a key first stage in invasion biology, with obvious practical applications for designing and implementing quarantine strategies, identifying natural enemies as potential biological control agents (Roderick and Navajas 2003), defining ecological characteristics of introduced populations to predict their spread (Kolar and Lodge 2001), and potentially direct the focus of conservation strategies. It is also a critical step for defining and testing ecological and evolutionary hypotheses and ultimately understanding the reasons for invasion success (see below, and Estoup and Guillemaud 2010). Historical and observational data on the spread of invasive populations is often sparse, but even when there is good documentary evidence, molecular genetic data can offer unique insights into the sources, routes and mechanisms of spread (e.g. Hoos et al. 2010; Lombaert et al. 2010, and see "Appendix 1" section and Fig. 1). However,

inferring routes using molecular genetic methods should supplement observational and historical records, not attempt to replace them. Indeed, when using an Approximate Bayesian Computational (ABC) approach ("Appendix 1" section), having observational data is a necessary requirement for defining a limited set of invasion scenarios that can be tested against each other statistically (Fig. 1).

Main insights from molecular genetic studies of invasion routes

Arguably the main insight from molecular genetic studies of invasion routes is that multiple introductions are commonplace, and go some way to explaining how populations of IAS overcome founder effects associated with colonization since they can lead to similar or even greater levels of genetic diversity in the invasive compared to native ranges (see below and e.g. thiarid snails, *Melanoides tuberculata* (Muller) (Sorbeoconcha: Thiaridae), Facon et al. 2003; anole lizards, *Anolis sagrei* (Cocteau in Duméril and Bibron) (Squamata: Iguanidae), Kolbe et al. 2004; western corn rootworm, *Diabrotica virgifera* (LeConte) (Coleoptera: Chrysomelidae), Miller et al. 2005; Ciosi et al. 2008; amphipods, *Gammarus tigrinus* (Sexton) (Amphipoda: Gammaridae), Kelly et al. 2006; and scotch broom, *Cytisus scoparius* (L.) (Fabales: Fabaceae), Kang et al. 2007).

A particularly interesting case is highlighted by the western corn rootworm, *D. virgifera*, which is native to Mexico and the east coast of North America, but was first observed near Belgrade in 1992, and is expanding in central and eastern Europe at a rate of 100 km per year. The expansion is essentially continuous, but there have been several isolated outbreaks peripheral to the main invasion front, which were thought to stem from a "leapfrogging" effect from the expanding eastern European population. Molecular genetic studies however revealed that this hypothesis was incorrect, and that most of the separate outbreaks result instead from repeated trans-Atlantic introductions (Miller et al. 2005; Ciosi et al. 2008). In contrast to most studies performed so far, *D. virgifera* shows higher genetic variation between invasive populations than within (Ciosi et al. 2008).

Molecular genetic studies have also revealed that invasions can lead to rapid adaptive evolution in spite

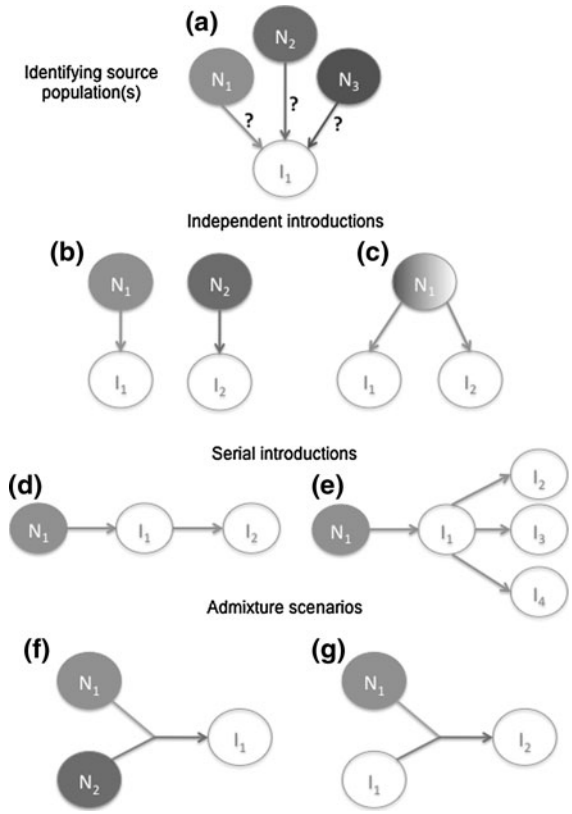

Fig. 1 Hypothetical scenarios of invasion routes that can be formally tested using DIYABC (Cornuet et al. 2008). *N* Native range populations, *I* invasive range populations, subscript numbers indicate different populations. **a** putative source populations of IAS can be identified. This is greatly facilitated if there is genetic differentiation (illustrated by different coloured shading) between source populations; **b** and **c** examples of independent introductions from the native range. In **b** native populations are genetically differentiated, whereas in **c** the native range is one panmictic population; **d** and **e** examples of serial introductions or stepping-stone colonisation events, where **e** corresponds to a "bridgehead effect" scenario, as seen in *H. axyridis* (Lombaert et al. 2010); **f** and **g** correspond to admixture scenarios between native populations, or between native and invasive populations respectively. The latter case is illustrated by *H. axyridis* in Europe, which results from a combination of European biocontrol stocks and invasive East USA individuals (Lombaert et al. 2010)

of strong bottlenecks (e.g. Amsellem et al. 2000; Dlugosch and Parker 2008), and that successful invasions may involve "bridgehead effects" in which widespread secondary invasions stem from a particularly successful invasive population (Fig. 1e) e.g. harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), Estoup and Guillemaud 2010;

Lombaert et al. 2010). In *H. axyridis,* the recent burst of worldwide invasions followed a bridgehead scenario, with the invasive population in Eastern North America acting as a source population for colonists invading Europe, South Africa and South America (Lombaert et al. 2010). Although the bridgehead effect is a new concept in invasion biology, it is potentially a common phenomenon (for example it could apply to the invasions described by Downie 2002; Hänfling et al. 2002; Kolbe et al. 2004; Miller et al. 2005). There are important practical reasons for identifying bridgehead populations (Estoup and Guillemaud 2010). Invasive populations are generally thought to experience a lag phase between colonization and expansion, during which time they evolve adaptations that determine invasion success (Keller and Taylor 2008). Unless the native population is preadapted to become invasive, these adaptations must occur independently in the case of multiple independent introductions directly from the native area. During a bridgehead scenario however, the "evolutionary shift" occurs in a single introduced population, which makes this scenario parsimonious (Estoup and Guillemaud 2010). Identifying such populations should therefore be a high priority for preventing subsequent spread (Estoup and Guillemaud 2010).

In addition to information about the demographic history of invasive populations, recent analyses have provided insights into the genetic make-up of founding populations, and the role of genetic variation in invasion success. We now outline three key questions that can be addressed with this data: (1) what is the role of genetic diversity in invasion success (i.e. successful establishment and spread of an IAS), (2) does admixture during multiple introductions increase invasion success, and (3) does invasion lead to non-neutral evolution and novel adaptation?

The role of genetic diversity in invasion success

Genetic variability determines a population's capacity to adapt to new or changing environmental conditions (Fisher 1930; Sakai et al. 2001), and should therefore play an important role in determining its potential to become invasive (Lee 2002; Kolbe et al. 2004; Drake and Lodge 2006; Facon et al. 2006; Lavergne and Molofsky 2007; Roman and Darling 2007). Populations of IAS are traditionally thought to have reduced genetic variation relative to their source populations, because of genetic founder effects linked to small population size during the introduction and establishment phases of an invasion. The low genetic variability associated with founder effects should, in theory, inhibit successful invasion, by limiting the population's ability to respond to selective pressures (but see Goodnight 1987, 1988). Moreover, small population size is predicted to increase the chance of inbreeding, which can result in exposure of deleterious recessive mutations in homozygous individuals. How invasive populations overcome the low variability associated with founder effects, and adapt to their new environments, was once regarded as a "paradox of invasion biology" (Roman and Darling 2007; Dlugosch and Parker 2008), but thanks to the considerable amount of molecular data that has now been collected to address this question, the paradox has essentially been put to rest.

Instead, data indicates that most invasions are not characterized by significant loss in neutral genetic diversity (see for instance Bossdorf et al. 2005), as typically measured using nuclear microsatellites or maternally-inherited mitochondrial DNA (mtDNA). Comparatively high neutral genetic diversity in invasive populations can be explained by multiple introductions, particularly when source populations are genetically divergent (e.g. Facon et al. 2003; Kolbe et al. 2004; Kang et al. 2007). However multiple introductions do not necessarily explain high genetic diversity (and evolutionary potential) in invasive populations (Lavergne and Molofsky 2007). For example, Eales et al. (2010) demonstrated, in an elegant study that illustrates the marriage between genetics and ecology, that high genetic diversity in an invasive population of anole lizards, *Anolis cristatellus* (Duméril and Bibron) (Squamata: Iguanidae), was a consequence of a single introduction event containing several genotypes. In this case the reproductive mechanism of the study species was also deemed important, since female anole lizards mate multiple times, and can store sperm from several males. This could have increased the number of genotypes above that of the number of founding individuals if the founding population included recently-mated females. Studies that compare levels of genetic diversity in multiple independent introductions in different locations of the same species are particularly useful, since these are equivalent to natural

biological replicates (Bossdorf et al. 2005), although note that this is tempered by difficulties of sampling introductions that fail to become invasive. In the case of *D. virgifera*, mentioned above, levels of genetic diversity differed considerably between the five independently introduced populations studied (Ciosi et al. 2008). When taken together with examples of successful invasions that are characterised by very low genetic diversity at neutral loci, this suggests that genetic variation is not an essential component of invasion success (Dlugosch and Parker 2008). The important point to make here is that there is a key difference between genetic variability at neutral molecular markers, which are irrelevant for selection and adaptation, and additive genetic variation, which is needed to respond to selection. Future studies need to focus on quantifying additive genetic variation (ideally in species characterised by multiple, independent introductions) to fully address the role of genetic diversity in invasion success.

Does admixture from multiple introductions increase invasion success?

As discussed above, multiple introductions are a common feature of biological invasions, but one major question that deserves attention is the role of intraspecific hybridization (i.e. "admixture") in invasion success. Admixture, like interspecific hybridization, can change the distribution of phenotypes in a population, and admixed individuals are able to outcompete their parental genotypes as a result of either heterosis effects or by creating new genotypes through recombination (Facon et al. 2005), or via phenotypic plasticity (e.g. Lavergne and Molofsky 2007). Both interspecific hybridization and intraspecific admixture are therefore important potential stimuli of invasion success (Lee 2002; Facon et al. 2005).

Admixture has been documented in invasive populations that stem from multiple introductions (e.g. Facon et al. 2005; Kolbe et al. 2008; Lavergne and Molofsky 2007), and may be driving invasion success in these examples. However, so far there have been few direct tests of this hypothesis. One direct and comprehensive test of the effects of admixture on invasive success was carried out in invasive parthenogenetic thiarid snails, *M. tuberculata,* using a combination of genetic analyses, laboratory experiments and field data (Facon et al. 2005, 2008). In the invasive range (Martinique) five introduced asexual morphs from Japan, Indo-Malaysia and the Philippines are found, plus two sexual morphs produced locally through sexual reproduction. Sexual morphs exhibit novel combinations of traits that differ significantly from the parents (e.g. they produce larger but fewer offspring), suggesting non-additive interaction (heterosis) between parental genotypes, which allowed them to outcompete parents in natural habitats, and increase invasiveness, strongly suggesting their novel life-history strategies provided a strong selective advantage (Facon et al. 2005, 2008). Combining molecular genetic data on source populations, with field and quantitative genetic data, provided the first direct evidence that multiple introductions are primarily responsible for accumulation of adaptive potential in key ecological traits in this species (Facon et al. 2008).

The potential for invasion success to be increased by admixture was also recently tested in laboratory crosses between individuals from flightless biocontrol stocks and invasive European populations of the harlequin ladybird, *H. axyridis* (Facon et al. 2010). The authors tested the three criteria, outlined by Wolfe et al. (2007), that must be met for admixture to play a role in biological invasions, namely: (1) parental populations should be genetically differentiated from each other, (2) crosses should be possible between individuals from different parental populations and (3) admixed individuals should differ from their parents in life-history traits crucial to invasion success (e.g. fecundity, dispersal ability, parasite resistance etc.). All three criteria were met, and admixed individuals developed more quickly and were slightly bigger than parentals, indicating possible heterosis effects. This could have serious negative consequences for the native competitors and prey with which *H. axyridis* interacts. Admixture also increased genetic variance for survival during starvation periods, which could boost the efficacy of selection and give admixed *H. axyridis* an advantage during periods of famine. Evaluating whether effects of heterosis persist over several generations, and whether admixture occurs in the wild, are important avenues for future research in this area.

Does invasion lead to non-neutral evolution and novel adaptation?

Biological invasions happen over contemporary time-scales, and so, they can be viewed as windows

to observe evolution in action. Not surprisingly therefore, there is considerable interest in using invasions as model systems to better understand the role of natural selection and adaptation in shaping phenotypes (Keller and Taylor 2008). A comprehensive knowledge of sources and invasion routes is needed in this case to successfully disentangle the effects of demographic and stochastic events from selection. So far, although many examples of evolution during biological invasions have been described (see Whitney and Gabler 2008 for a review), it is not always clear whether changes in phenotypic and life-history traits during establishment and range expansion reflect adaptive evolution during the invasion process, or neutral changes linked to genetic drift (Keller and Taylor 2008). However, even in cases of clear adaptation, it is not always clear whether adaptation allowed the invasion, coincided with the invasion, or was a consequence of the invasion (Estoup and Guillemaud 2010). Indeed, not all invasions need adaptation. Again, investigations using independent introductions from separate locations should be fruitful here, and ideally they should include a subset of populations that have been introduced but have not become invasive (Estoup and Guillemaud 2010). If the same phenotype occurs in independent successful introductions, this is strong evidence that phenotypic evolution is adaptive rather than plastic (Keller and Taylor 2008).

Another approach that has been useful for investigating adaptation during invasions is to compare population differentiation at neutral molecular markers and quantitative traits (F_{ST} and Q_{ST} respectively, Keller and Taylor 2008). If adaptation occurs in the new environment, Q_{ST} is expected to be significantly greater than F_{ST}, in line with a response to selection (e.g. Lavergne and Molofsky 2007; Keller and Taylor 2008). For example, in invasive *A. cristatellus*, in Dominica, $Q_{ST} \gg F_{ST}$, and an altitudinal cline in scalation traits similar to those for related endemic species convincingly indicated that trait divergence in the invasive population was due to directional natural selection acting in just ten generations since introduction (Eales et al. 2010). This study demonstrates the combined power of using molecular genetic, ecological and experimental studies to fully explain observed phenotypes in introduced populations (Eales et al. 2010).

On a cautionary note, recent studies (e.g. Klopfstein et al. 2006; Excoffier and Ray 2008) have shown that a neutral phenomenon occurring during a population range expansion could be interpreted as a signature of positive selection. This phenomenon, coined "gene surfing", is due to strong genetic drift taking place in populations located on the edge of the expansion. Low-frequency alleles can thus surf on the wave of advance of a population range expansion, reaching high frequencies and spreading over large areas, leading to potentially large allele frequency differences between the source and the edge of the spatial expansion**.** This can be explored using simulations, and should be taken into consideration when investigating adaptation at range margins.

Dispersal

Dispersal is a key life-history trait of fundamental importance to invasion success since it influences the genetic and demographic structure of expanding populations and their ability to adapt to new environments. Although awareness of the crucial role that dispersal plays in biological invasions is increasing (Kokko and Lopez-Sepulcre 2006; Ronce 2007), so far few studies have actually tried to measure the dispersal ability of IAS, except in the context of biological control (but see below and Heimpel and Asplen 2011). This information is crucial for understanding and predicting spread of invasive species and biological control agents (Heimpel and Asplen 2011), as well as consequences of other global environmental change (Urban et al. 2008; Niitepõld et al. 2009). Since there is a direct, causal relationship between dispersal, gene flow and population structure, detailed analyses of genetic structure can be used to quantify "effective" dispersal (i.e. dispersal with breeding) in wild populations, and this approach can be particularly useful in species that are difficult to study using traditional mark-release-recapture experiments. Traditionally, methods were based on estimating the genetic distance between populations (i.e. F_{ST}), however in recent years, great progress has been made in individual-based methods to detect migrants, and in incorporating geographically explicit information (e.g. geographic features, habitat quality) into analyses in order to detect barriers to dispersal (i.e. landscape genetics, "Appendix 2" section). Here we identify and discuss two main objectives that we believe are particularly relevant: (1) studying

dispersal patterns (i.e. mechanism, rate, dispersal barriers) of IAS to learn more about their dispersal ability and predict future spread, which is also highly relevant in a biological control context (see e.g. Heimpel and Asplen 2011), and (2) using IAS as model organisms to increase understanding of the fundamental processes of dispersal and colonization.

Patterns of dispersal

Our ability to predict the spread of IAS is still limited, and in order to successfully do so, we need to understand the mechanisms underlying range expansion (e.g. Urban et al. 2008). The simplest model of range expansion is a random-diffusion process, often referred to as Fisher's "wave of advance" model, in which the range of an invading species is predicted to increase linearly with time (Fisher 1937; Skellam 1951). Although there are examples where this model applies (e.g. muskrat, *Ondatra zibethicus* (L.) (Rodentia: Cricetidae), in Europe, Skellam 1951; coypu, *Myocaster coypus* (Molina) (Rodentia: Myocastoridae), in the UK, Reeves and Usher 1989), other cases demand more complex range expansion models that include, for example, probability of long-distance dispersal (LDD) events (either by wind or human transport), which can accelerate the rate of range expansion as the length of the invasion front increases (Shigesada et al. 1995; Ciosi et al. 2010). A combination of both short and long distance dispersal (i.e. "stratified" dispersal) may be a common feature of invasions, and has already been described in several species of invasive insect (e.g. firethorn leaf miner, *Phyllonorycter leucographella* (Zeller) (Lepidoptera: Gracillariidae), Nash et al. 1995; gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), Sharov and Liebhold 1998; horse chestnut leaf miner, *Cameraria ohridella* (Deschka and Dimic) (Lepidoptera: Gracillariidae), Gilbert et al. 2004). This has important implications for control measures, which could be improved by preventing establishment of new focal populations or eliminating new ones rather than focusing on established invasion fronts (Moody and Mack 1988; Suarez et al. 2001).

The simplest models of range expansions are based on (1) the intrinsic growth rate and (2) a diffusion coefficient that assumes normally-distributed dispersal distances (Skellam 1951; reviewed in Suarez et al. 2001), but in reality this assumption is often violated, and the utility of these models therefore limited, as a proportion of the population undergo LDD. Both rare LDD and stratified dispersal skew the distribution of dispersal distances so that distributions are often leptokurtic (i.e. normal with a narrow variance) rather than normal (Ibrahim et al. 1996; Suarez et al. 2001). LDD can increase invasion rate by an order of magnitude (Higgins and Richardson 1999) and even rare LDD events can result in conflicts between theoretical predictions and empirical data (Suarez et al. 2001).

Differentiating between different mechanisms of dispersal, and quantifying the rate and distance of LDD events is therefore essential for constructing accurate predictive models (Suarez et al. 2001). Unfortunately though, measuring LDD is not trivial because of the scarcity and unpredictability of LDD events (Gilbert et al. 2004) and so far few studies have quantitatively estimated its importance. Genetic methods offer some hope for determining dispersal mechanism and quantifying LDD since the process of expansion leaves unique genetic "signatures" in the population (Ciosi et al. 2010). A simple pattern of geographic "isolation-by-distance" (IBD), where there is strict agreement between pairwise genetic and geographic distances because gene-flow is predominantly via neighbouring populations, is expected under the wave of advance model (Slatkin 1993), whereas a weak pattern of IBD may reflect a more complex dispersal process. More sophisticated statistical frameworks are now in place to identify individual migrants ("Appendix 2" section). Of course, these methods are most powerful when used in conjunction with observational and/or historical records. A combination of these approaches was recently used to infer that the European outbreak of *D. virgifera* expanded its range via stratified dispersal (Ciosi et al. 2010).

The models of range expansion we have discussed so far assume that dispersal is through homogeneous environments or independent of the environment, which is likely to be an oversimplification. Environmental heterogeneity is expected to be an important determinant of range expansion, with invasions accelerating as individuals encounter favourable conditions, and decelerating as they reach less favourable environments (Urban et al. 2008). Heterogeneous environmental conditions can now be incorporated

into theoretical frameworks for predicting expansion (Hastings et al. 2005) and ecological and landscape variables (including spatial structure and metapopulation dynamics) can be explicitly linked to invasion rates (e.g. Facon and David 2006; Urban et al. 2008). For example, Urban et al. (2008) analysed invasion trajectories in invasive cane toads, *Bufo marinus* (L.) (Anura: Bufonidae), in Australia to determine whether range expansion accelerated, decelerated or was linear, and if certain environmental conditions influenced population growth. Cane toad invasion dynamics include both accelerating and decelerating range expansions, and sensitivity to temperature, topography, road networks and patch connectivity, indicating that environmental influences are essential for accurate theoretical predictions (Urban et al. 2008). Recently, spatially explicit models, developed using an ABC framework for investigating dynamics of invasions, have been applied to cane toads (landscape-ABC, Estoup et al. 2010). From these, it is evident that there was a small initial founder population, which was followed by a dispersal distance of 19 km generation^{-1} resulting in a spread of 50 km year^{-1}.

Understanding what constitutes a barrier or corridor to dispersal is critical for predicting and managing the spread of IAS. Recent developments in landscape genetics (see "Appendix 2" section) offer great promise for understanding how landscapes shape gene-flow, and identifying barriers and corridors to dispersal, but so far few studies of invasive species have taken advantage of them. However, in one notable exception, Zalewski et al. (2009) investigated genetic structure of invasive American mink, *Neovison vison* (Schreber) (Carnivora: Mustelidae) in Scotland, and identified genetic discontinuities consistent with the Cairngorn Mountains presenting significant barriers to dispersal. This work has important implications for mink eradication programmes. Barriers to dispersal can also take the form of more subtle landscape or environmental features, such as habitat type or temperature and/or humidity gradients. For example, in line with known ecology and habitat preferences, water and urban areas appear to act as substantial barriers to gene flow for fragmented populations of solitary bees, *Colletes floralis* (Eversmann) (Hymenoptera: Colletidae), whereas beaches, sand dunes and agricultural land facilitate gene flow (Davis et al. 2010). In another example, urban and rural developed land provided high landscape resistance for amphibians (Goldberg and Waits 2010). Information on which geo-climatic features increase population connectivity is being used to conserve fragmented populations, and to predict how species will respond to climate change, but is also useful for modelling the spread of IAS (e.g. Knowles and Alvarado-Serrano 2010; Sork et al. 2010).

Evolution of dispersal

Dispersal is not a fixed trait. Instead, it is an excellent example of a trait that can evolve in response to natural selection, and this is particularly evident during periods of range expansion (Kokko and Lopez-Sepulcre 2006; Ronce 2007), as exemplified by the evolution of longer legs in cane toads, *B. marinus*, which has facilitated rapid dispersal at the invasion front (Phillips et al. 2006). Strong selection is expected to favour increased dispersal at the expansion front since there are major fitness benefits to being among the earliest colonists of a new patch (Travis et al. 2009). This can create a positive feedback loop that can potentially accelerate the wave of expansion (Kokko and Lopez-Sepulcre 2006; Excoffier and Ray 2008). Travis et al. (2009) showed theoretically that accelerating invasion rates result from the evolution of density-dependent dispersal, even when costs associated with dispersal are moderate. Moreover, selection pressures for high dispersal must be very strong in order to overcome genetic drift and Allee effects in the small populations at the expansion front (Travis and Dytham 2002; Excoffier and Ray 2008). Understanding the evolution of dispersal (and density-dependent dispersal in particular, Travis et al. 2009) is essential for making accurate predictions about species range expansions (or contractions), particularly under current anthropogenic environmental changes (Kokko and Lopez-Sepulcre 2006). Studying dispersal evolution during biological invasions is not only necessary for predicting spread, but can also provide more general insights into the ultimate and proximate causes of dispersal.

Theoretical studies have generated clear, testable predictions about the evolution of dispersal during range expansions. For example, Travis and Dytham (2002) showed that range expansion is characterized by two distinct phases. First, populations at the invasion front should be characterized by an excess of

migratory individuals (relative to established populations) due to a selection advantage for founding new populations. Second, as more populations are established and the selective advantage to dispersal reduced, migration costs should select for lower dispersal (Travis and Dytham 2002). In a direct test of these predictions, Simmons and Thomas (2004) observed increased frequencies of dispersive, long-winged individuals in recently colonized populations of different species of bush cricket (Orthoptera: Tettigoniidae), relative to established core populations. However, within ten years after colonization, wing-morph frequencies stabilised, to resemble the core (Simmons and Thomas 2004). Such a trade-off between dispersal and fecundity has been investigated theoretically (Burton et al. 2010) and observed in several insect species (e.g. speckled wood butterflies, *Pararge aegeria* (L.) (Lepidoptera: Nymphalidae), Hughes et al. 2003; sand crickets, *Gryllus firmus* (Scudder) (Orthoptera: Gryllidae), Roff and Fairbairn 2007).

An obvious consideration is that for dispersal to evolve in response to natural selection, there must be underlying heritable variation in dispersal ability. This is illustrated beautifully in insects, where there is considerable evidence for additive genetic variance and high heritability in dispersal traits such as wing length and morphology, initiation and duration of flight, and production of enzymes linked to locomotion (e.g. several species of bush cricket (Orthoptera: Tettigoniidae), Simmons and Thomas 2004; sand crickets, *G. firmus,* Roff and Fairbairn 2007; and large milkweed bugs, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae), see Roff and Fairbairn 2007 for review). Investigating the genetics behind these particular phenotypes is essential for a more mechanistic understanding of dispersal evolution, and for increasing the likelihood of predicting its rate (Travis et al. 2009). Studies of the *Glanville fritillary*, *Melitaea cinxia* (L.) (Lepidoptera: Nymphalidae), have been particularly enlightening in this regard. Butterflies from newly formed populations in the Åland archipelago have higher flight ability (accompanied by higher metabolic rate, Haag et al. 2005) and fecundity than those in established patches (Hanski et al. 2002). Moreover, dispersal ability is highly heritable (Saastamoinen and Hanski 2008) and associated with allelic variation at a single gene for phosphoglucose isomerase (PGI), a temperature-sensitive, glycolytic enzyme (Haag et al. 2005; Niitepõld et al. 2009). Individuals heterozygous at *Pgi* move longer distances at lower temperatures than homozygous individuals (Niitepõld et al. 2009). Although several studies have focused on PGI at the functional level (e.g. Watt et al. 2003; Wheat et al. 2006), to our knowledge this locus has not yet been investigated in the context of dispersal ability other than for *M. cinxia.* Although dispersal is without doubt a complex trait, under control of many genes, investigating whether allelic variation at *Pgi*, and selection acting on this locus, generally underlies enhanced dispersal ability during invasions, will be a worthwhile starting point.

Community interactions

Understanding the interactions between invasive alien species and other species within an invaded range is challenging but essential, particularly for quantifying effects on communities (Roy et al. 2009; Hesketh et al. 2010), and developing practical approaches to the management of IAS. Much of the current knowledge on community interactions stems from conventional laboratory and field studies, but there is a need to integrate theory with a multidisciplinary empirical approach. Species identification by molecular gut-content analyses is currently labour intensive, but is revealing unique insights into predator–prey relationships in the context of biological invasions (see Aebi et al. 2011). Recent developments in second and next generation sequencing offer considerable potential for investigating both predator–prey and host-parasite interactions, without prior development of species-specific markers (see "Appendix 3" section). This data can then be input into ecological networks, which represent the biotic interactions in an ecosystem, with species (nodes) connected by pairwise interactions (links), such as the quantitative food web illustrated in Fig. 2. Characterizing the structure of ecological networks is essential in the context of invasion biology to evaluate the impact of IAS on their prey, and to determine whether the invasive species themselves are parasitized or predated on. By quantifying the interactions within entire communities, it is possible to describe network structure and complexity as well as measure the responses of ecological systems to

environmental change. Recent advances in network ecology have been used to assess the impacts of (1) biological control on the wider insect community (Henneman and Memmott 2001), (2) habitat modification on host-parasite interactions and ecosystem services (Tylianakis et al. 2007), and (3) alien plants on plant-pollinator networks (Lopezaraiza-Mikel et al. 2007).

Below, we discuss how an approach based on molecular genetic data and ecological modelling could be used to investigate predator–prey and host-parasite interactions. It is important to note though that these two interactions could be investigated simultaneously. A combined molecular-ecological network approach would be particularly illuminating in the case of invasive generalist predators such as *H. axyridis* (see Aebi et al. 2011), which predate not only on aphids and other herbivorous pests, but also on beneficial insects within the same guild (i.e. a community of species that share the same host or prey). Laboratory experiments indicate that intraguild predation (IGP) by *H. axyridis* could be devastating to native coccinellids and other beneficial insects (e.g. Ware et al. 2008; Ware and Majerus 2008). Molecular gut-content analyses of *H. axyridis* have confirmed IGP in open field plots and in the wild (Chacón et al. 2008; Aebi et al. 2011), however whether IGP happens at an appreciable frequency in the wild is still subject to debate. Moreover, assessing rates of parasitism on *H. axyridis* (and other invasive insects) by native hymenopteran parasitoids as a natural form of pest control can be laborious using traditional laboratory rearing methods, and is potentially biased (Henneman and Memmott 2001). Molecular genetic approaches ("Appendix 3" section) can overcome this problem and have the potential to provide rapid, highly-resolved data on predator–prey (e.g. gut-content analysis) and host-parasitoid interactions (e.g. host screening). They also have the advantage of being able to distinguish between morphologically indistinguishable species. However it should be noted that a molecular approach, when used alone, also has its drawbacks. For example, it may be difficult to detect encapsulated/undeveloped parasitoids, which are quite easy to detect with dissection, due to DNA degradation (Hoogendoorn and Heimpel 2002). The most powerful approach is therefore to couple molecular methods with conventional experimental and field survey methods, which together can assist in deciphering the dynamic relationships between species within ecological networks. From these networks it is then possible to assess the impacts of invasive insect infiltration on entire communities as well as exploring differences in network structure across the species range.

Predator–prey interactions

A number of studies have assessed the addition of alien species into a community using food web analysis (Henneman and Memmott 2001; Memmott and Waser 2002; Sheppard et al. 2004, and see Fig. 2). For example, Sheppard et al. (2004) examined the interactions between alien predators, introduced to Hawaii to control pest insects, and endemic

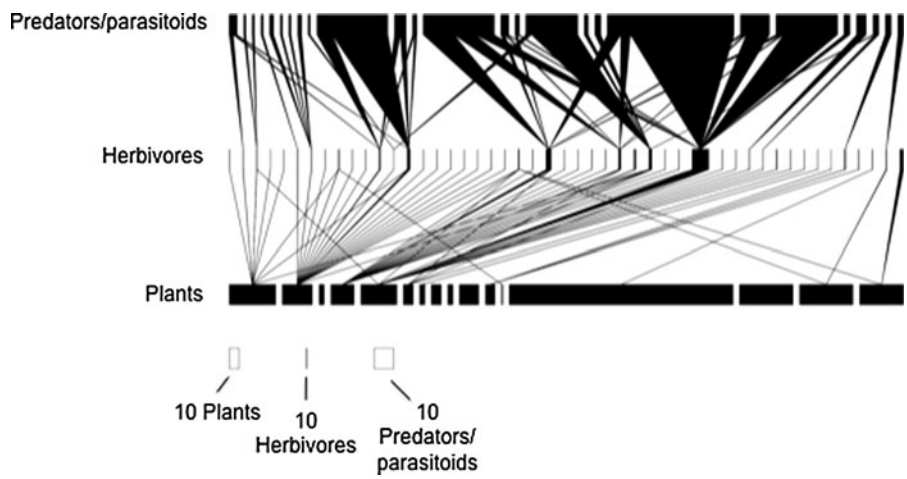

Fig. 2 Hypothetical quantitative food web showing the interactions between plants, herbivores and their predators/parasitoids. Each bar represents a species and bar width represents the species' abundance among all individuals sampled. The area of the triangles connecting trophic levels represents the relative number of higher-trophic-level species attacking the lower-level species

invertebrates (mainly Lepidoptera) within pristine upland habitats. Approximately 11% of the predators within the food web were alien to Hawaii (Sheppard et al. 2004). The findings of Henneman and Memmott (2001) were dramatic: 83% of Lepidoptera parasitoids, in a native forest on Kauai Island, were alien species introduced as biological control agents, and a further 14% were accidentally introduced adventive wasps (only 3% of the parasitoids were native). With the exception of these case studies, using networks to assess the impacts of invasive insect infiltration on communities is yet to be widely applied, partly due to problems of identifying cryptic interactions in the field. This could be overcome by employing molecular techniques more widely in network analyses, and recent advances in molecular gut-contents analysis have allowed unique insights (King et al. 2008).

A combined molecular-network approach could be particularly valuable for generating food webs to investigate the complex concept of invasional meltdown. Invasional meltdown describes the process by which an alien species facilitates invasion by another alien species by increasing the likelihood of its survival and/or the magnitude of its impact (Simberloff and Von Holle 1999). So, essentially, invasional meltdown is used to describe synergistic interactions among invasive alien species, which lead to accelerated and devastating impacts on native ecosystems. Invasional meltdown is a contentious theory (Simberloff 2006). It is a concept that is difficult to explore because, although many studies have examined individuals of one species providing a benefit to the establishment and spread of another, there is a scarcity of information on population impacts. The introduction of the yellow crazy ant, *Anoplolepis gracilipes* (Smith) (Hymenoptera: Formicidae), on Christmas Island and its interactions with native and alien scale insects (Hemiptera: Coccoidea) is considered to have led to major disruption of the community structure (O'Dowd et al. 2003). The complex set of interactions leading to invasional meltdown on Christmas Island requires understanding of the intricacies of the yellow crazy ant food web. The devastating alteration of the Christmas Island ecosystem is thought to be the only convincing example of invasional meltdown in action, however, more subtle effects through the infiltration of alien species into communities are widely reported. Interestingly, it has recently been hypothesized that extensive invasional meltdown is occurring in North America involving eleven Eurasian IAS, including *H. axyridis,* with the presence of invasive soybean aphids, *Aphis glycines* (Matsumara) (Hemiptera: Aphididae) increasing regional abundances of other IAS (Heimpel et al. 2010). Exploration of this system with a molecular-network approach would be a particularly exciting avenue for further research.

Host-parasite interactions

Molecular genetic techniques are particularly useful for examining host-parasite interactions within a community context. Molecular markers can be used to identify species of parasite when morphological characters are limited, when there are problems identifying juvenile stages of the life cycle, or when the parasite is either cryptic or covert (Bonsall et al. 2005). By using current methods, which focus on species or genus-specific primers and target a particular species, it is likely that we are underestimating parasite diversity (Hesketh et al. 2010). It has been estimated that more than 1600 parasitic fungi attack beetles (Coleoptera), but studies generally focus on a few genera (Riddick et al. 2009). 454 sequencing or similar "metagenomics" approaches ("Appendix 3" section) offer powerful opportunities to detect and quantify all parasites present in a host community. This approach was recently used to identify Israeli acute paralysis virus as a potential agent of colony collapse disorder in honeybees, *Apis mellifera* (L.) (Hymenoptera: Apidae) (Cox-Foster et al. 2007), and to characterise microbes associated with the primary pest of bees, the ectoparasitic mite *Varroa destructor* (Anderson and Trueman) (Mesostigmata: Varroidae) (Cornman et al. 2010). In principle, the same approach could be used to characterize the community of parasitic organisms living within or on an IAS, and therefore to test the prediction of the enemy release hypothesis (Roy et al. 2011b) that there should be lower infection levels in alien populations compared to native populations of the same host species.

Enemy release is considered to be one of the mechanisms by which invasive alien species gain advantage in the invaded range. However, it is also probable that an alien species is host to pathogens that have not been experienced by species occupying the invaded range. "Pathogen spillover" refers to the transmission of disease from alien to native hosts

when a parasite hitchhikes with the invading species. So far, pathogen spillover has been given little consideration in the context of biological invasions, and studies have focused on disease outbreaks in wild populations, as a consequence of spread from infected domestic animals (e.g. pathogen spillover has been implicated in the decline of wild fish populations, Morton et al. 2004; and in pathogen transmission from commercial to wild bumblebees, *Bombus terrestris* (L.) (Hymenoptera: Apidae), Colla et al. 2006). Investigation of pathogen spillover during invasions is warranted since this key process could exacerbate the effects of invasive alien species within a community, perhaps even contributing to invasional meltdown (Prenter et al. 2004; Colla et al. 2006). Such studies would benefit from the inclusion of molecular techniques, which provide the potential to rapidly screen invasive alien species for pathogens and to model the risk posed to native species.

Investigating the transmission dynamics of parasites using molecular methods is of vital importance for identifying potential biological control agents and for understanding the role of parasites in invasions. Parasites that are strictly vertically transmitted (i.e. from mother to offspring) are well suited as biological control agents, since there is low risk of host-switching to non-target species. Maternally-inherited endosymbiont bacteria, such as *Wolbachia* (Rickettsiales), are very common in insects (Hilgenboecker et al. 2008) and impose a range of consequences on their hosts' reproduction, including cytoplasmic incompatibility (CI), male-killing, and induced parthenogenesis (reviewed in Werren et al. 2008). Endosymbionts can also negatively influence other aspects of their host's biology, including life-span (McMeniman et al. 2009) and dispersal (Goodacre et al. 2009). CI-induction should facilitate *Wolbachia* invasion into wild host populations and may be a viable strategy to reduce pest populations (Zabalou et al. 2004) and pathogen transmission (McMeniman et al. 2009). For example, CI-*Wolbachia* has been proposed as a control agent against medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), (Zabalou et al. 2004), which is a major agricultural pest. In addition, life-shortening CI-*Wolbachia* was successfully introduced into *Aedes aegypti* (L.) (Diptera: Culicidae), the mosquito vector of dengue virus, and was maternally transmitted at high frequency (McMeniman et al. 2009). This offers hope for reducing the impact of dengue fever, which has grown dramatically in recent decades (World Health Organisation). However, it is important to bear in mind that by increasing the proportion of females, and in some cases conferring substantial indirect fitness benefits to their female hosts (e.g. female neonate coccinellid larvae gain an indirect fitness benefit by consuming undeveloped eggs of their brothers, Hurst and Majerus 1993) these reproductive manipulators might actually facilitate host invasion (Hatcher et al. 1999; Galbreath et al. 2004). For example it was recently demonstrated, by an elegant series of experiments, that increased fitness and female-biased sex ratio linked to *Rickettsia* spp. nr *belli* likely facilitated invasion of the notorious sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemptera: Aleyrodidae), in the USA (Himler et al. 2011). In addition, there is mounting evidence of extensive horizontal transmission of endosymbionts between different host species (reviewed in Werren et al. 2008). Investigations of parasite transmission dynamics are therefore essential in the context of biological invasions, and caution is needed before recommending reproductive parasites in biological control.

Finally, much can be learned about host-parasite interactions by comparing genetic structure in the interacting species. Host-parasite complexes can shed light on the interaction between gene-flow and the ability of natural selection to promote local adaptation (Criscione et al. 2005). It is also possible that the genetic structure of the parasite, or even just its distribution, could be used to help identify geographical origin of the host (Aebi and Zindel 2010). This approach might be particularly useful when there is low genetic structure of the host in its native range (and therefore reduced power to identify source populations). Again, for such an approach to be successful, parasite transmission must be strictly vertical.

Conclusions and perspectives

If EB Ford was alive to publish a new edition of "Ecological Genetics", biological invasions would surely constitute a significant component of his book. Studying ecological genetics of invasions is already allowing insights into the fundamental processes described in this review. We highlight two particular

areas of importance for future study. First, there is currently considerable focus in the ecological genetics community on studying adaptation in wild populations (see Stapley et al. 2010 for a recent review), and invasions are excellent model systems for understanding the role of natural selection in shaping phenotypes. Although technical challenges are still associated with next generation sequencing, rapid progress is being made. In particular, the RAD-tag method (Baird et al. 2008) allows an unprecedented number of genetic markers to be characterised and typed, offering a powerful means to identify loci contributing to adaptation during invasions (see Hohenlohe et al. 2010 for a recent application and Stapley et al. 2010 for a review). Second, the combination of molecular genetic techniques, particularly Roche/454-pyrosequencing or similar ("Appendix 3" section), and ecological network modelling offer great potential for quantifying predator–prey and host-parasite interactions between species in a community (Hesketh et al. 2010). This approach has particularly important implications for biological control—for example identifying previously uncharacterised natural enemies that could be potential biocontrol agents—and ultimately for addressing two of the most fundamental questions in invasion biology: (1) what is the impact of biological invasions on native, recipient species? and (2) does release from natural enemies increase invasion success? With the increasing potential to address these and other fundamental questions, the field of ecological genetics of invasive alien species has an exciting future ahead of it.

Acknowledgments We are grateful to the IOBC/WPRS for supporting this publication. AE, EL and BF were supported by grants from the Agence Nationale de la Recherche (ANR-06-BDIV-008-01) and from the Agropolis Fondation (RTRA—Montpellier, BIOFIS project number 1001-001). The authors would also like to thank three reviewers for their thorough and insightful suggestions on this manuscript.

Appendix 1: Introduction to molecular genetic methods for inferring source populations and invasion routes

Two types of method have been used to make inferences concerning source populations and invasion routes: direct methods based on current and historical observations of IAS and indirect methods based on patterns in molecular data.

Direct methods are based on records of the presence and absence of invasive taxa. Routine controls carried out in airports and harbours by quarantine services and monitoring by environmental or agricultural agencies are particularly informative in this respect (Work et al. 2005). However, it is rarely possible to infer the routes of invasion with a high degree of precision by these direct methods. Indeed, given the low rates of establishment and expansion recorded for introduced individuals (Williamson 2006), there is no guarantee that the individuals intercepted would have spearheaded a successful invasion.

Indirect methods are based on the genetic patterns observed within and between populations at molecular markers. Traditional statistical treatments include the construction of trees from matrices of genetic distances between populations (e.g. Lozier et al. 2009; Thibault et al. 2009), parsimony networks (e.g. Voisin et al. 2005; Hoos et al. 2010) and the calculations of assignment likelihood (e.g. Genton et al. 2005; Ciosi et al. 2008). More recently, a number of studies have used clustering methods like those implemented in STRUCTURE (Pritchard et al. 2000). If the invasive population clusters clearly with one of the potential source populations, this is considered to provide fairly conclusive information about the origin of the invasive population (e.g. Marrs et al. 2008; Rollins et al. 2009). A shared ancestry of the individuals of invading populations with various populations from the native area is sometimes interpreted as evidence for an admixture origin of the invasive population considered, although it may also reflect the presence of unsampled sources, drift, or insufficient numbers of markers (Darling et al. 2008; Rosenthal et al. 2008). It is worth stressing that, although the abovementioned indirect methods have proved useful in many cases, they are all subject to two major limitations: (1) they poorly take into account the stochasticity of the demographic and genetic history considered and (2) they do not allow probabilistic estimations of competing introduction scenarios (e.g. Knowles and Maddison 2002).

Recently, a new indirect method called approximate Bayesian computation (ABC, Beaumont et al. 2002, implemented in DIYABC, Cornuet et al. 2008) has been proposed and used to draw inferences from molecular and historical data, about the complex evolutionary scenarios typically encountered in the

introduction histories of IAS (Fig. 1). General statistical features of ABC have been reviewed in two recent papers (Bertorelle et al. 2010; Csillery et al. 2010) and some practical aspects that are important when using this method to make inferences about invasion routes can be found in Estoup and Guillemaud (2010). Briefly, ABC is a model-based Bayesian approach in which the posterior probabilities of different models and/or the posterior distributions of the demographic parameters under a given model are determined by measuring the similarity between the observed data set (i.e. the target) and a large number of simulated data sets. ABC has four main advantages over the more traditional indirect methods described above: (1) it uses all the data simultaneously in inference, (2) it can be used to estimate probabilities, with confidence intervals for each of the scenarios compared (e.g. Cornuet et al. 2008, Fig. 1), (3) it allows the evaluation of the power of a given analysis on the basis of controlled simulated datasets (Cornuet et al. 2008; Guillemaud et al. 2010), and (4) it avoids the introduction of misleading biases, such as those due to unsampled populations (Guillemaud et al. 2010) or genetic admixture between multiple sources (Lombaert et al. 2010). ABC thus constitutes a real advance for inferring source populations and invasion routes.

Appendix 2: Introduction to molecular genetic methods for investigating dispersal

The rapidly developing field of landscape genetics aims to understand how population genetic processes are affected by spatial and temporal environmental heterogeneity, by integrating population genetics with landscape ecology and spatial statistics. Landscape genetics approaches enable two major insights into dispersal: first, individuals with multilocus genotypes that are representative of a population other than the one they were sampled in can be identified. This is a powerful way of identifying immigrants and therefore quantifying dispersal (e.g. Guillot et al. 2005a, b). Second, the pattern of spatial genetic structuring can be tested for correlations with landscape or environmental features, allowing identification of genetic continuity (or connectivity) between patches, or discontinuities resulting from barriers to dispersal (see e.g. Balkenhol et al. 2009; Guillot et al. 2009; Storfer et al. 2010, for recent reviews).

Under a landscape genetics approach, the individual is the unit of study, and their exact geographic location must be recorded. Populations do not have to be identified a priori. Bayesian statistics are used to assign individuals to populations according to their multilocus genotypes, using software that employ clustering algorithms based on pre-defined population genetic models (e.g. STRUCTURE, Pritchard et al. 2000, see also "Appendix 1" section). In recent years, new technologies have greatly assisted marker development, vastly increasing the amount of data that can be collected, and decreasing the computation time required for data analysis. For example, new statistical approaches such as Discriminant Analysis of Principal Components (DAPC, Jombart et al. 2010), offer great potential for assigning individuals into clusters with minimal computing time when datasets are large, and when there is low population structure.

Isolation by distance tests (e.g. Mantel test) have long been used to identify correlations between genetic distance and environmental variables, but new statistical approaches are also being developed to model the relationship between genetic structuring and the environment, which allow inferences on the microevolutionary processes generating spatial genetic structure (see e.g. Guillot et al. 2009). Geographical information systems-based landscape analysis overlays landscape variables onto population genetic data to visualise patterns of genetic structuring (for example using ArcGIS or PATHMATRIX, Ray 2005), allowing environmental parameters likely to influence dispersal in heterogenous environments to be investigated. The spatial domain occupied by inferred clusters can be examined to identify dispersal barriers, using programmes such as GENELAND (Guillot et al. 2005b), and genetic diversity can be simulated, accounting for environmental and spatial heterogeneity, using software such as SPLATCHE (Currat et al. 2004). This latter approach has been modified to reconstruct invasion scenarios, investigating parameters such as dispersal distance and speed (Estoup et al. 2010). These types of simulations show how demographic processes interact with landscape features to determine spatial genetic structure (Epperson et al. 2010) and to

investigate how dispersal is affected not only by obvious geographical features (e.g. mountain ranges), but also by more subtle habitat characteristics (e.g. Davis et al. 2010). They therefore offer great potential for understanding dispersal ability, and ultimately, generating information that can be used to predict the spread of IAS.

Appendix 3: Introduction to molecular genetic methods for investigating community interactions

So far, molecular studies that have attempted to investigate the strength and structure of predator–prey and parasite-host interactions, within a community context, have primarily used standard PCR (e.g. Symondson 2002; Harper et al. 2005; Sheppard and Harwood 2005). The advantage of such markers is to be able to qualitatively evaluate specific interactions between a predator and its prey or a parasitoid and its host. On the other hand, developing species–specific molecular probes can be long and costly (see Aebi et al. 2011), and the development of species-specific markers to describe whole community's food web structure is impractical. Advances in second and next generation sequencing offer great promise as they do not rely on design of species-specific primers, are extremely sensitive, and could be used to create quantitative interaction networks. For example, Roche/454 massively parallel pyrosequencing offers considerable scope for investigating community interactions. By generating tags from 16S or 18S rDNA, data is generated for almost every organism in a sample to reveal previously uncharacterised aspects of the biological diversity (e.g. Dethlefsen et al. 2008). Datasets can then be compared to see how they differ in terms of composition. A particular advantage to this method is that many individual samples can be tagged, pooled, and sequenced in parallel (e.g. Meyer et al. 2008), and several populations can be investigated simultaneously (by "gasketting", i.e. splitting a 454 picotiter plate into several sections). This technique has already proven successful in assessing biological diversity in the ocean (e.g. Sogin et al. 2006; Huber et al. 2007), soil (e.g. Leininger et al. 2006), and in the human body (e.g. Dethlefsen et al. 2008). Of particular relevance, a metagenomic survey of 454 sequence data from 16S and 18S rDNA in honeybee, *A. mellifera* hives uncovered presence of bacteria, fungi, parasites, metazoa, and viruses and found strong correlation between a particular virus and colony collapse disorder (Cox-Foster et al. 2007). A major challenge is to block amplification of the host DNA, but this can be achieved with the use of "blocking primers" (Vestheim and Jarman 2008).

Another challenge with this type of analysis is dealing with the volume of data generated. However, since metagenomics is an established method, several bioinformatics pipeline options already exist. For example, MG-RAST is a fully-automated service for annotating metagenome samples including phylogenetic classification (Meyer et al. 2008). MEGAN (http://ab.inf.uni-tuebingen.de/software/megan/welcome.html) and CARMA (http://www.cebitec.uni-bielefeld.de/brf/carma/carma.html) are also specific for metagenomics analysis to analyse large data sets and group operational taxonomic units (OTUs). Homology detection can be performed by comparing 16S and 18S sequences to reference databases such as SILVA (http://www.arb-silva.de/) using (for example) BLAT (BLAST-like alignment tool, Kent 2002) and OTUs defined based on multiple sequence alignment (Dethlefsen et al. 2008).

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ANNEXE XI

Primer Note (2009)

Loiseau A, Malausa T, **Lombaert E**, Martin JF, Estoup A (2009) Isolation and characterization of microsatellites in the harlequin ladybird, *Harmonia axyridis* (Coleoptera, Coccinellidae), and cross-species amplification within the family Coccinellidae. *Molecular Ecology Resources* **9**, 934-937.

Précisions sur le rôle de co-auteur :

- ➔ Test des marqueurs sur *H. axyridis* : génotypage de la population du Kazakhstan et analyse des données.
- ➔ Test des marqueurs sur 7 autres espèces de coccinelles.
- ➔ Participation globale à la rédaction du manuscrit.

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Isolation and characterization of microsatellites in the harlequin ladybird, *Harmonia axyridis* (Coleoptera, Coccinellidae), and cross-species amplification within the family Coccinellidae

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Abstract

A total of 18 microsatellite DNA loci were isolated and characterized from the harlequin ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae). We optimized a multiplex panel consisting of two polymerase chain reactions, allowing the genotyping of all loci. The number of alleles and heterozygosity observed at each locus ranged from 1 to 12 and from 0 to 100%, respectively. After Bonferroni correction for multiple tests, none of the loci deviated significantly from Hardy–Weinberg equilibrium and there was no indication of significant linkage disequilibrium among pairs of loci. Successful cross-species amplification was obtained for only three of the seven tested species of Coccinellidae.

Keywords: Biological invasion, Insect, Invasive Species, Microsatellites, Nuclear marker

Received 11 September 2008; revision accepted 26 October 2008

The harlequin ladybird, *Harmonia axyridis*, is a coccinellid native to Asia, which has been used in numerous countries as a biocontrol agent for about a century (Koch 2003). This species has become invasive in northern America since the late 1980s and in Europe about 15 years later (Koch 2003; Coutanceau 2006). It has now spread worldwide (see DAISIE database at http://www.europe-aliens.org/ for European data) and is considered good model for the study of invasive species. It is expected to become one of the most widely distributed ladybirds in the world (Brown *et al*. 2008; Poutsma *et al*. 2008). We have developed here a large set of microsatellite markers to address various questions regarding the historical, demographical and ecological factors involved in the worldwide invasion of the harlequin ladybird.

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Harmonia axyridis genomic DNA was extracted using DNeasy Tissue Kit (QIAGEN) from ethanol-preserved larvae originating from the laboratory rearing of the biocontrol firm Biotop. A total of 2.5 μg of genomic DNA was digested using *RSA1* restriction enzyme (Promega). Fragments of digested DNA of 500–900 bp were isolated from an agarose gel, purified and ligated to *Mlu*I oligo adaptors (*RSA21* and phosphorylated *RSA25*; Edwards *et al*. 1996). Biotinylated oligo probes [$(TC)_{10}$, $(TG)_{10}$, $(ATCT)_6$, $(TGTA)_6$] were hybridized to the ligated DNA and selected using streptavidin magnetic particles (Promega). Polymerase chain reactions (PCR) were performed on the microsatellite-enriched solution using one of the oligo adaptors (*RSA21*) as primer. The PCR products were purified (QIAquick PCR Purification Kit, QIAGEN) and ligated into a plasmid vector (pGEM-T Easy Vector system, Promega), transformed

Table 1 Primer pairs used for the two PCR sets of the multiplex panel. For each of the 18 loci, the table includes the repeat motifs in the sequence used to design primers, sequence and fluorescent dye label used for each primer, number of individuals successfully genotyped, observed number of alleles, allelic size range, expected heterozygosity (Nei 1978), observed heterozygosity and *P* value (before Bonferroni correction) for the test of Hardy–Weinberg equilibrium (HWE). The loci HA-200 and HA-282 are not shown because they were found to be monomorphic in this population (details are available on request)

PCR set	Locus	Repeat motifs	GenBank Accession no.	Primers sequences 5′–3′	Fluorescent dye	*N*	Alleles	Size range (bp)	H_O	H_E	HWE
PCR 1	Ha-244	$(TG)_{10}TC(TG)_2$	FJ263403	F: TGACGGACGCACGAAGAT R: ACAGCTGACCATAGAGGATCG	FAM	26	8	81–96	0.81	0.86	0.43
	Ha-201	$(CA)_8$	FJ263399	F: CTTCGCCATCATCCACTAGG R: GTGCGGTCATTAATTCAGGC	FAM	26	6	306–319	0.73	0.77	0.33
	Ha-555	$(CA)_{10}$	FJ263408	F: GATGCGCCCTCTAGAAAAG R: CCCTATAACGCCAACAATG	VIC	26	6	75–85	0.62	0.72	0.8
	Ha-605	$(GA)_{16}$	FJ263410	F: TCCGACGCACAGATAACAGA R: GTTACGTTGACCCGTCGC	VIC	26	11	134–167	0.65	0.78	0.07
	Ha-281	$(TG)_7$	FJ263406	F: TTCGCACGTTCCATTGTTC R: GCCGTTTGCGGTATGTTC	NED	26	12	134–147	0.5	0.78	0.02
	Ha-627	$(GA)_{11}$	FJ263411	F: CGTAACTTTAACGATCACTCAGC R: GAACATTGTCTTCGCGTGG	NED	26	11	227–254	1	0.88	0.75
	Ha-565	$(GA)_{10}$	FJ263409	F: TCTGAACATTCGACCTACATAGT R: AATGCGTGATGAACGACC	NED	24	3	326–334	0.08	0.08	1
	Ha-234	$(CA)_8$	FJ263402	F: GCTAAAACCAACGTCAGG R: CTCGCGCGATTATTGGAC	PET	24	7	128–138	0.63	0.79	0.17
PCR 2	Ha-267	$(AC)_8$	FJ263405	F: AACCTGTAATTCGATTGTGGAAC R: CCGACCTGACCTTTCGTC	FAM	26	6	177–187	0.65	0.69	0.21
	Ha-005	$(GA)_5$	FJ263394	F: AGGGTGTGTATGTAGAACAGAGG R: AACCGCAATAACTCGATTGG	FAM	22	3	275–279	0.36	0.62	0.01
	Ha-253	$(CA)_7$	FJ263404	F: GATACATCGTCCTTTCAGTCCTC R: CCTGCAAACTCTTCCAGACC	VIC	26	6	182–188	0.65	0.66	0.48
	Ha-105	$(GA)_5$	FJ263396	F: CGCCTAACAAATAGGCATCAC R: AGGGTGGAGAATGGAATAACC	VIC	26	4	240–243	0.54	0.53	1
	Ha-194b	$(GCA)_4$	FJ263397	F: ACCAGATTGCTGCTTGGATT R: ACAAATTGGGCGTGAGAAAC	NED	26	2	80–83	0.35	0.34	1
	Ha-215	$(CA)_7$	FJ263400	F: CGAATCAATAACCCTAGGCG R: AGCGATCTCCTGTTCTACGG	NED	26	5	174–182	0.5	0.66	0.23
	Ha-223	$(TG)_6$	FJ263401	F: TCGTTTAACCGTGATAGGAGAG R: ACGAATTCCGAAAGATGAGG	NED	23	2	229–233	0.09	0.16	0.13
	Ha-094	$(TAGA)_5$	FJ263395	F: TTAGTCGGCGGGTCCATC R: GGGCCGATAAGTCAAACGAG	PET	24	6	350–359	0.5	0.67	0.05

into JM109 competent cells (Promega), and plated onto Luria-Bertani (LB) agar medium with ampicillin. Recombinant plasmids were identified by means of blue-white screening. Positive-transformed cells were grown on LB-agar, transferred onto Hybond-N + membranes (Amersham) and screened using digoxigenin-end-labelled $(TC)_{10}$, $(TG)_{10}$, $(ATCT)_6$, and $(TGTA)_6$ probes. Out of the 742 cell colonies detected as positive, 380 were sequenced with the standard primer *SP6*. Sequencing was performed by Macrogen Inc. using the BigDye Terminator chemistry and an ABI 3700 automatic sequencer. Finally, 284 readable sequences containing microsatellite motifs were obtained (84 contained no microsatellite motif and 16 were not readable). A set of 45 primer pairs was designed from nucleotide sequence regions flanking microsatellites using the online version of Primer3 (http://frodo.wi.mit.edu/primer3/input.htm). When possible, criteria used for designing primers were (i) 18–24 bp length (ii) G/C-3′ end, and (iii) annealing temperature around 60 °C. PCR primers were first tested in monoplex PCR, on individual DNA samples of adults originating from different populations and extracted using the DNeasy Tissue Kit (QIAGEN). Only the primers producing good quality PCR products for all samples were retained. These primers were then labelled with fluorescent dyes (Applied Biosystems) and integrated in a multiplex panel consisting of two multilocus PCR sets including eight and 10 loci, respectively (Table 1). Multiplex PCR were performed using the QIAGEN Multiplex kit and a thermocycler Mastercycler (Eppendorf). 2 µL of genomic DNA (~10 ng) were added

Locus/Species	*Harmonia quadripunctata*	*Harmonia conformis*	*Harmonia yedoensis*
Ha-244	81	81	83, 85, 88
Ha-201	—	307	308, 310, 312, 316
Ha-555	—	85, 89	77, 79, 81
Ha-605	—	122, 141	129, 137
Ha-281	—	135	136, 138, 141
Ha-627	—	—	228, 230, 232, 233, 234, 242
Ha-565	—	—	—
Ha-234	—	—	126, 128, 134, 136, 141
Ha-267	—	—	179, 181, 183
Ha-005	—	—	277, 279
Ha-200	—	—	110
Ha-253	—	—	178, 180, 181
Ha-105	235	—	239, 240
Ha-194b	—	—	80
Ha-215	—	—	174, 176
Ha-223	—	—	233
Ha-282	98	—	98
Ha-094	—	—	356

Table 2 Results of cross-amplification tests, for each locus and each species. The size of each amplified allele is given. Sample size is four diploid individuals per species. Amplification with DNA from all other tested species (i.e. *Adalia bipunctata*, *Coccinella undecimpunctata*, *Coccinella septempunctata* and *Hippodamia variegata*) did not give readable PCR product

to the 8 μL of mix consisting of 1X QIAGEN buffer, water and primers at final concentration of 0.2 μM, except for loci HA282 (0.04 μM), HA105, HA194b, HA223 (0.1 μM) and HA005, HA244, HA565 (0.3 μM). PCR conditions for both multilocus set were as followed: initial denaturation at 94 °C for 15 min; 25 cycles of denaturation (94 °C, 30 s), annealing (57 °C, 60 s) and elongation (72 °C, 2 min); final extension at 60 °C for 30 min. A total of 2 μL of diluted (1:10) PCR products was mixed with 0.25 μL of 500 LIZ Size Standard (Applied Biosystems) and 8.75 μL of formamide (Applied Biosystems). Products were then electrophoresed using an ABI PRISM 3130 sequencer (Applied Biosystems).

We have estimated the level of polymorphism of loci by genotyping 26 adult individuals of *H. axyridis* collected in Kazakhstan (Almaty, 43°14′22.2″N; 76°56′68.4″E). Genotypes were scored using Gene Marker version 1.5 (SoftGenetics). The software GenePop version 3.3 (Raymond & Rousset 1995) was used to estimate expected and observed heterozygosities and to test for genotypic linkage disequilibrium and deviation from Hardy–Weinberg equilibrium (HWE). The program Micro-Checker (Van Oosterhout *et al*. 2004) was used to detect null alleles and estimate their frequencies.

The number of alleles per locus ranged from 1 to 12. Two loci (HA200 and HA282) were found to be monomorphic but preliminary data from other populations shows that HA200 displays several alleles (unpublished). For other loci, expected heterozygosity values ranged from 0.08 to 1 (Table 1). We did not find any significant deviation from HWE after Bonferroni sequential correction for multiple comparisons. However, null alleles were detected by Micro-Checker at loci HA005 and HA281, with frequencies estimated at 0.18 and 0.17, respectively. Finally, there was no indication of significant linkage disequilibrium among pairs of loci.

The multiplex panel was also tested using the same PCR conditions on individual DNA extracts from seven coccinellid species (four individuals per species): *Adalia bipunctata*, *Coccinella undecimpunctata*, *Coccinella septempunctata brucki*, *Harmonia quadripunctata*, *Harmonia conformis*, *Harmonia yedoensis*, *Hippodamia variegata*. We obtained successful PCR amplification at three to 17 loci in the three most closely related species only: *H. yedoensis* and to a lesser extent, *H. conformis* and *H. quadripunctata* (Table 2).

Acknowledgements

We thank Pascal Maignet and Jacques Frandon from BIOTOP for providing biological material, Armelle Coeur d'Acier, Emmanuelle Jousselin, Gaël Kergoat for the collection of samples in Kazakhstan and Nathalie Leroudier (IPMC Sophia Antipolis) for handling plates of genotyping. We also thank Philippe Gauthier for intellectual support and Intermarché Prades-le-Lez for the availability of toothpicks. This work was funded by the French ANR grants ANR-06-BDIV-008-01.

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doi: 10.1111/j.1755-0998.2009.02517.x

Development and characterization of nine polymorphic microsatellite markers in the Chilean kelp *Lessonia nigrescens*

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Abstract

A total of nine microsatellite loci were isolated and characterized in the Chilean kelp *Lessonia nigrescens* Bory. Using two different enriched libraries, we observed 1–14 alleles per locus in two samples of 21 kelp individuals each. The observed heterozygosities ranged from 0.05 to 0.80 and all loci are in Hardy–Weinberg equilibrium for one or both samples. Seventeen samples collected from different sites showed high allele diversity along the species distribution. The variation detected at these markers is currently being used for the study of populations of *Lessonia nigrescens* at different geographical scales.

Keywords: genetic variability, kelp, *Lessonia nigrescens*, microsatellite markers

Received 28 August 2008; revision accepted 26 October 2008

Lessonia nigrescens Bory is an intertidal kelp inhabiting the southeast Pacific coasts, from Cape Horn to southern Peru. It is a keystone species and a bioengineer of the intertidal communities. In order to establish kelp stocks, estimate some important demographic parameters and design effective conservation plans, the study of the distribution of the genetic diversity became a main concern. However, the progress of such research was severely limited by the poor quality and reduced statistical power of the random amplified polymorphic DNA markers used so far (Martínez *et al*. 2003; Faugeron *et al*. 2005), highlighting the need for highly polymorphic, codominant and reliable molecular markers. Microsatellite markers have been developed in only one kelp species, *Laminaria digitata* (Billot *et al*. 1998), and cross-amplification in different kelp species within the order Laminariales has been unsuccessful, in particular with *L. nigrescens* (Martínez *et al*. 2005). For all these reasons, species-specific microsatellites markers were developed.

We describe here the isolation and characterization of nine polymorphic microsatellites markers for *L. nigrescens* using two enriched libraries. Genomic DNA was extracted from a bulk of immature and healthy fronds of *L. nigrescens*

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