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Current Biology *21*, 1–4, 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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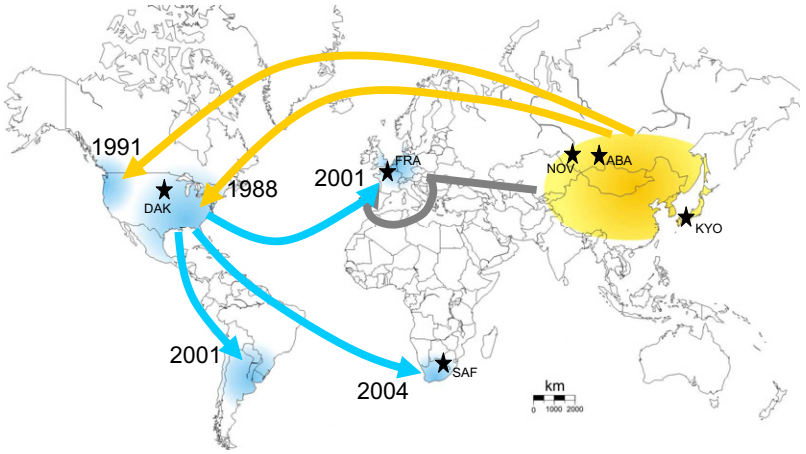

Figure 1. Worldwide Routes of Invasion of *Harmonia axyridis*

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

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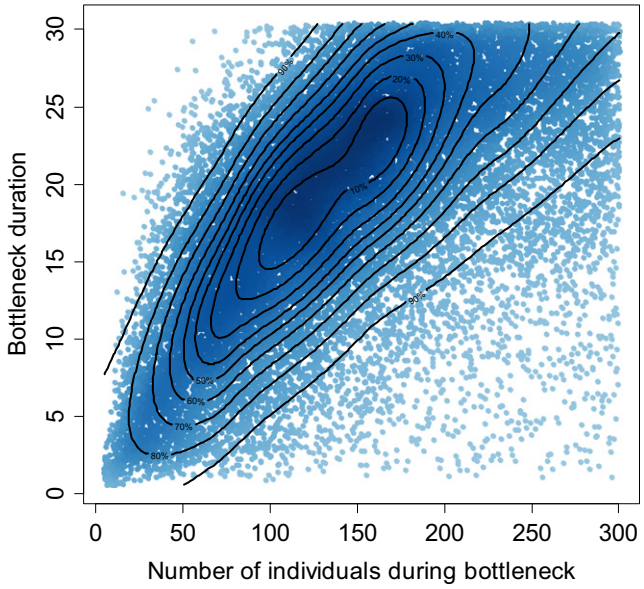

Figure 2. Intensity of the Bottleneck Event Following the Introduction of *Harmonia axyridis* in Eastern North America from Its Native Area

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.

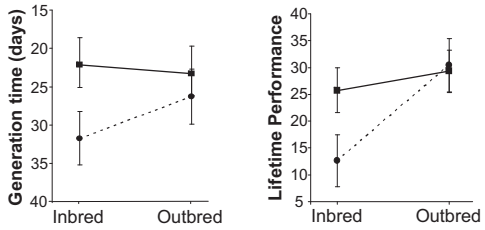

Figure 3. Generation Time and Lifetime Performance of Native versus Invasive Populations and Consanguinity of Inbred versus Outbred Populations

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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Supplemental Information

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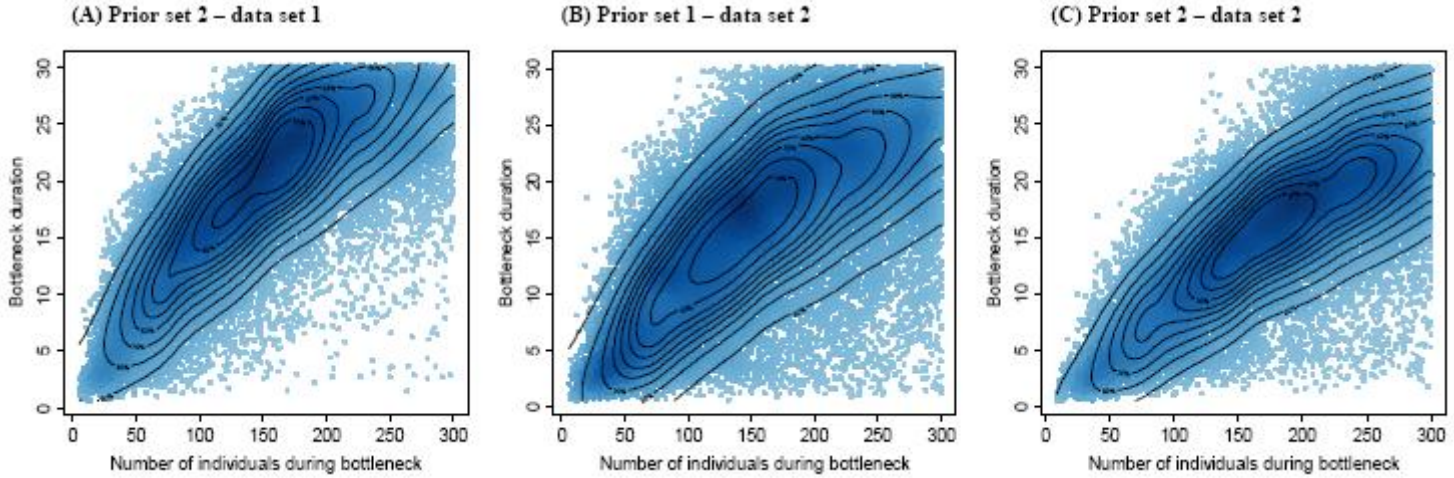
Table S1 (Related to Figure 3). Results of statistical analyses for lifetime performance and generation time. Population-level means for generation time are added due to the significance of the population effect for this trait.

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}

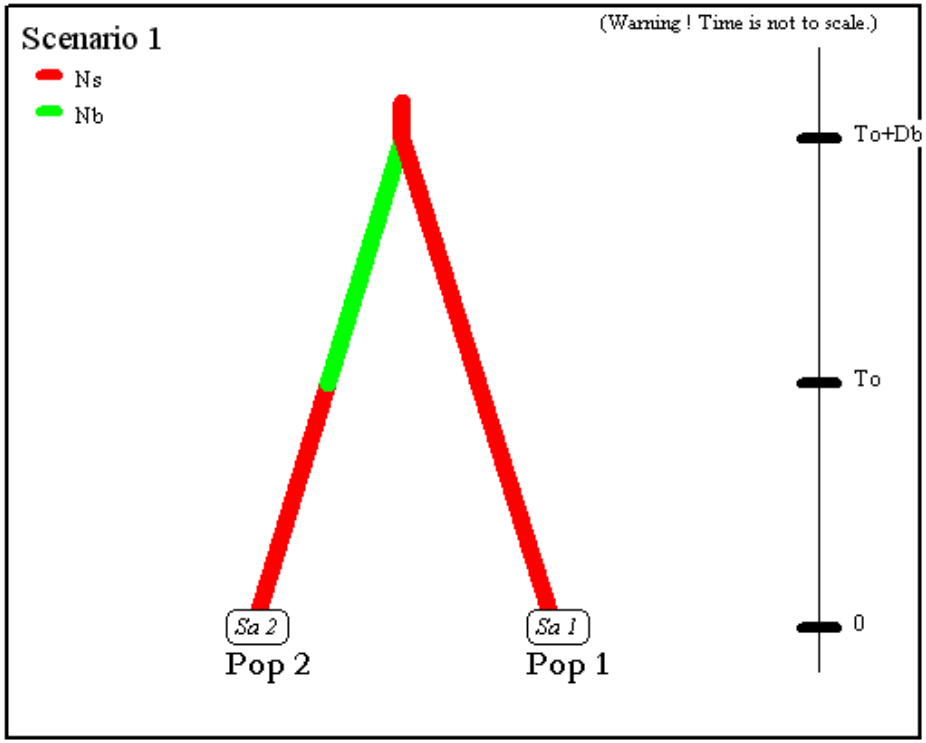
Table S3. Summary statistics of microsatellite data used for ABC analysis of bottleneck event with the corresponding observed values in the two sets of analyzed *H. axyridis* population samples.

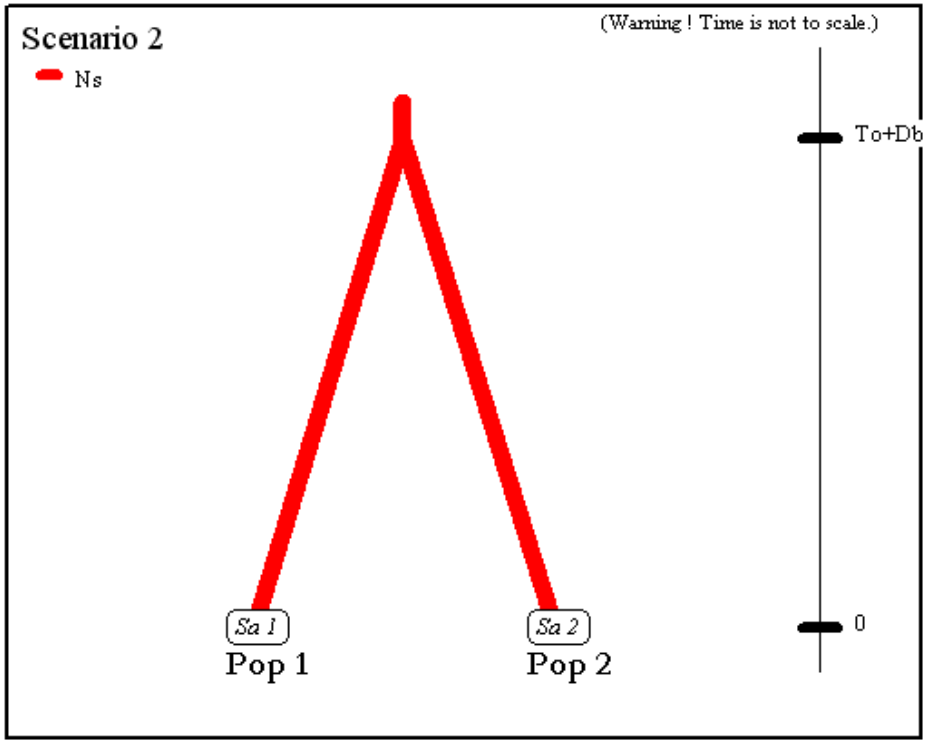
Note: *N* = native population sample; *I* = introduced population sample. Data set 1 = we used population samples from Kyoto (Japan) and Brookings (South Dakota, USA) as representative of the native and introduced areas, respectively. Data set 2 = we used a pool of individuals collected in eastern Asia (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.

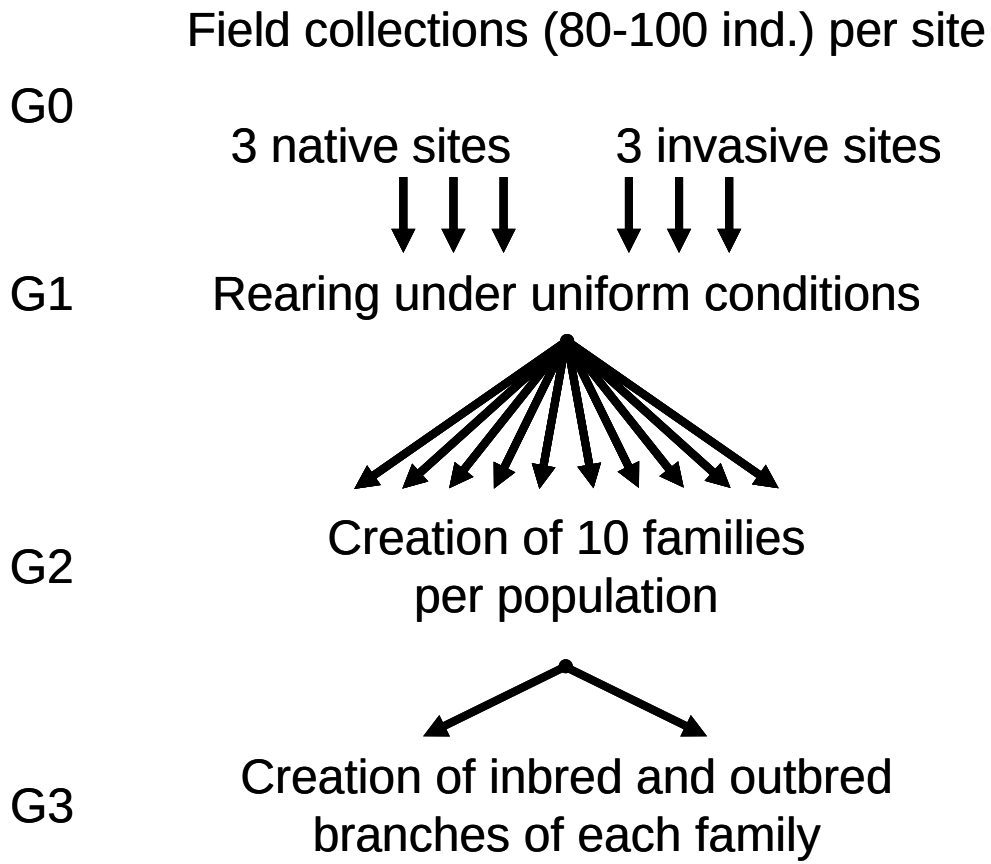
The experiment started with the creation of the third generation. Mature G_2 adults were used to create two types of crosses: inbred and outbred. Inbred crosses were between pairs of siblings, and outbred were between unrelated individuals of the same population. At the end of the experiment, we genotyped 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

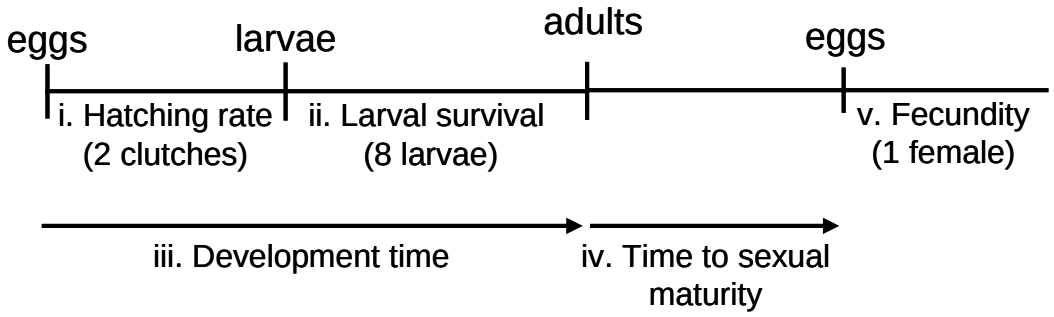
crosses, respectively, and 0.18 and -0.02 for invasive inbred and outbred crosses, respectively). We employed this protocol for 10 families per population, where each family was initiated with two sisters: one mated with a brother and one mated with an unrelated male. We collected and isolated two clutches 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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Feature

The benefits of bottlenecks

Population bottlenecks are commonly thought to be disadvantageous because they deplete genetic variation. But they can be advantageous too, in particular for biological invaders like the harlequin ladybird. **Florian Maderspacher** reports.

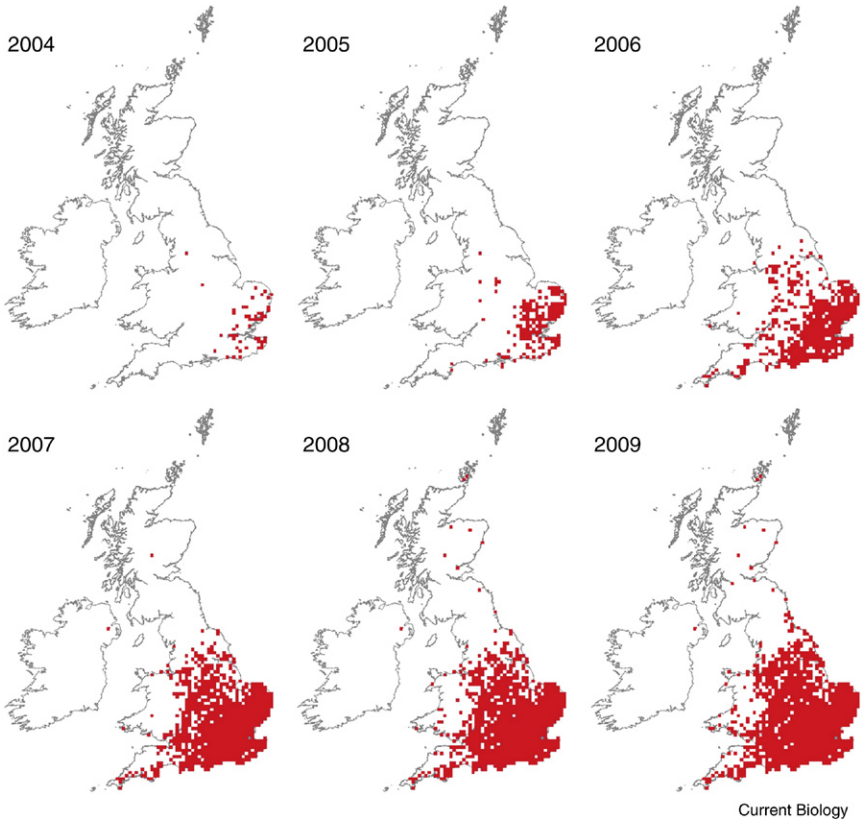
The last successful invasion of Britain began in the summer of 2004. Like all evils, it stemmed from the continent, and quickly the invaders were rumoured to be smelly, to squat in houses by the thousands and to damage the livelihood of their autochthonous relatives. The invaders go by the harmless-sounding name of *Harmonia axyridis* or harlequin ladybird, but there is nothing harmonious or lady-like about them. These beetles spread at a fierce pace and are more ferocious eaters and breeders than other ladybird species. In autumn, they can form spectacular mass aggregates, which may have contributed to their notoriety. Like other famous examples of biological invasions — grey squirrels in Britain, rabbits in Australia or giant hogweed in central Europe — their success is staggering, which for biologists begs the question why this should be so.

Biological invaders do suffer one obvious disadvantage, namely that at the beginning of the invasion their numbers are low. Such so-called population bottlenecks mean that the invader populations can go extinct quite easily, and that, compared with larger native populations, their genetic variability will be lower. But this need not always be a disadvantage, as a paper by Benoit Facon and colleagues on harlequin ladybirds in this issue of *Current Biology* illustrates. In fact, a bottleneck of the right size can have quite the opposite effect — it can purge a population of deleterious alleles and thus increase their fitness rather than reduce it though inbreeding depression.

Harlequin ladybirds stem naturally from Central and Eastern Asia. Unlike many other species of the ladybird family (Coccinellidae) that can be identified by the number of spots on their hardened wings, these beetles' colour patterns are highly variable. Harlequin ladybirds are also bigger than most of their relatives and are voracious aphid eaters. Their appetite and their prolificacy make them a potential threat to resident plant and animal species. Although the actual impact is as yet unclear, Peter Brown from the UK ladybird survey notes that "there is growing evidence that native ladybirds — and some other insects — are being negatively affected, probably due to direct predation, but also due to competition for shared resources. Most at risk are species with a high niche overlap with the harlequin, in particular, the two-spot ladybird (*Adalia bipunctata*)."

Ironically, their big appetite was precisely the reason they were introduced to the US as early as 1916 to protect crops against aphids and other pests. However, it was not until 1988 that a stable population had established itself in Louisiana. Within the next 20 years harlequin ladybirds spread rapidly within the US and Canada, but also into Europe and South America — where there had been previous small-scale introductions — and even to South Africa. The long lag phase between initial colonisation and full-fledged invasion is quite typical for biological invasions. Recent genetic analyses of several invasive ladybird populations across the world indicated that they all are derived from the American population. In the militaristic language used by students of biological invasions, such a population is called a 'bridgehead'. But what could have happened to the bridgehead that made the ladybird invasion all of a sudden so virulent?

Purged: Invasive harlequin ladybirds showing their characteristic extreme variability in colour patterns. (Photograph: William Mettey.)



Beetle of Britain: Spread of the harlequin ladybird in Britain between 2004 and 2009. Images courtesy of CEH/UK Ladybird Survey.

Intuitively, new invaders are almost always at a disadvantage. They are usually low in numbers and generally less well adapted to the new environment than the autochthons. But of course, organisms can evolve and adapt to new conditions. Yet, as invader numbers are low, they also bring with them fewer genetic variants than the source population. Such a genetic bottleneck means that there is less genetic raw material for selection to act upon. And thus, invaders might not be able to adapt quickly to their new environment. In addition, the genetic bottleneck can lead to inbreeding depression — the reduction of the population's fitness due to an increased likelihood of breeding between relatives that share deleterious genetic variants. This is a somewhat paradoxical situation: population genetic theory predicts that genetics should act against invaders, yet sometimes they flourish nonetheless.

One possible way out of the bottleneck conundrum is that, in terms of genetic diversity, there is actually no bottleneck. Indeed, studies on several invasive species have shown invaders can have quite high genetic variability. Brown anole lizards, for instance, came to Florida first in the late 19th century and began to spread — after the usual lag phase — in the second half of the 20th century. An analysis of the invaders' population genetic make-up, published in 2004 by Jason Kolbe, Jonathan Losos and others (Nature *431*, 177–181), indicated that variation was unexpectedly high. As Jason Kolbe says, they were "quite surprised at the overall pattern of high genetic variation; conventional wisdom would have suggested one or a few native-range populations as the source of the invasion." Instead, there must have been at least eight different introduction events, from different source populations. That way, the invader population becomes actually highly diverse — a sort of nutshell version of the variation of several original populations. This highly concentrated genetic variation might be one foundation of their high invasive potential. Jason Kolbe notes: "If the elevated level of variation I found for neutral markers is indicative of adaptive variation, then rates of adaptive evolution may be enhanced." Notably, in line with the bridgehead scenario, anole lizards of Florida then went on to invade places further afar, such as Taiwan and Hawaii.

That increased genetic variance accrued through multiple invasions can be beneficial for the invader was substantiated through a 2007 study by Benoit Facon and colleagues (Curr. Biol. *18*, 363–367) on the freshwater snail *Melanoides tuberculata*, which has invaded the Caribbean island of Martinique. (Biological invaders, or those who study them, do seem to have a penchant for scenic locales.) *M. tuberculata* invaders exhibit a much higher genetic variability than any of their multiple source populations. But, the invaders also show extremely high variation in important fitness-related traits such as fecundity and size. So again, rather than a bottleneck that depletes variation, invasive populations can be like a funnel that bundles and concentrates variation.

But for the harlequin ladybirds, this does not seem to apply. For their new study, Benoit Facon and his colleagues chose the harlequin ladybird as a model, as it "has invaded several different geographical areas, which represents replicate introductions, and is a good example of invasion after a substantial lag time", Facon explains. And indeed, when they compared invasive and native populations, Facon and his colleagues found that there is indeed evidence for a genetic bottleneck with reduced genetic variation.

Population genetic theory had long suggested that a bottleneck need not be entirely bad. Because of the smaller number of breeding individuals, after some generations, the likelihood of siblings mating will increase during the bottleneck. This means inevitably that the overall level of homozygosity in the population will increase. When an allele is recessive and has a deleterious effect, or is even lethal, the presence of more homozygotes will mean that this allele declines in frequency. In other words, the bottleneck population will be effectively purged of such deleterious alleles.

Theoretical studies have shown that the effectiveness of purging depends on several variables, such as the size of the population, the strength of the allele's negative effects, the strength of selection and the duration of inbreeding. And indeed, the duration and size Facon and colleagues modelled for the invasive ladybirds' bottleneck — 20 generations and <10% of what would be considered a healthy

population size — seemed to fit well the predictions for purging to occur.

But did purging actually take place? In the next step, the authors measured the extent of inbreeding depression in native and invasive species. Beetles from three native and three invasive populations were mated either with their siblings (inbreeding) or with unrelated individuals (outbreeding), and fitness-related traits were compared. Both in terms of generation time and reproductive output, the invasive populations performed significantly better than the native populations.

"Inbred individuals of invasive populations are clearly fitter than inbred individuals of native populations and as fit as outbred individuals from both types of populations," explains Benoit Facon, and "this means that this decrease of inbreeding depression in invasive populations is due to a loss of deleterious mutations, namely purging."

Even though the notion of purging in small populations has been studied intensely on theoretical grounds, empirical evidence had been scarce. So far, the effect had mainly been shown in laboratory animals, like fruit flies. "The potential effects of population bottlenecks during invasion are mixed, in some cases enhancing additive genetic variance and in other cases decreasing variation, so it's great to see such clear-cut empirical evidence for their role", says Jason Kolbe, and "the combination of molecular markers, simulations and breeding experiments to measure fitness-related differences between introduced and native populations makes this study unique."

Of course, it is as yet not clear if the observed purging effect is really responsible for the ladybird invasions. And it is also not entirely certain that purging has not occurred before invasion in one of the founding populations. But it is certainly tempting to speculate that such purging effects might contribute to the striking success of the harlequin ladybirds and possibly other invasive species. In the words of Jason Kolbe: "population bottlenecks may be a case of 'damned if you do, damned if you don't' for invasion success." And who knows, perhaps native species that might suffer from the invasive harlequin ladybirds can one day bounce back having slipped through their very own purging bottlenecks.

Quick guide

Drosophila embryonic hemocytes

Iwan Robert Evans and Will Wood*

What are they? *Drosophila* embryonic hemocytes are the highly motile macrophages that represent the main cellular arm of the innate immune system in this organism. These cells are specified during embryonic development and persist through larval stages to adulthood.

Any pseudonyms? Also known as haemocytes, plasmatocytes (this is more specific since strictly speaking the hemocyte lineage includes lamellocytes and crystal cells as well as plasmatocytes), or *Drosophila* macrophages, phagocytes or blood cells.

Where do they come from? There are two waves of hemocyte production; the first occurs in the head mesoderm, while the second occurs in a stem cell niche in the lymph gland. Early hemocytes are speculated to be equivalent to primitive embryonic blood cells and disperse to cover the entire embryo, whereas lymph gland hemocytes are released during late larval stages and in response to parasitisation. Homologs of the GATA (Serpent) and Runx (Lozenge) families of transcription factors involved in vertebrate hematopoiesis play important roles in hemocyte specification, whilst hemocyte plasma membranes are packed with molecules related to those found on vertebrate macrophages (e.g. Croquemort, a CD36 homolog, and Draper and Nimrod, scavenger receptors that resemble CED-1-like proteins such as MEGF10 and Jedi in vertebrates).

Where do they go? Hemocytes migrate out from the head along two main pathways to disperse over the entire embryo: dorsal migration, involving penetration of an epithelial barrier to enter the extended germband, which carries them posteriorly during germband retraction, and ventral migration along the ventral nerve cord (Figure 1A). The two populations meet on the ventral nerve cord and then migrate laterally, ensuring an even spread over the embryo.

And what do they do? Hemocytes are important in both development and immunity. Without hemocyte function embryos fail to develop correctly, with defects in the ventral nerve cord due to the roles of hemocytes in uptake of apoptotic corpses and possibly also secretion of matrix, since these cells are responsible for the secretion of much of the extracellular matrix and express numerous matrix-remodelling enzymes. Hemocyte-derived matrix also potentiates bone morphogenetic protein (BMP) signalling in the developing renal tubules and hemocytes are therefore required for the correct morphogenesis of these structures. Hemocytes are also able to recognise and respond to pathogens and epithelial wounds at both embryonic and larval stages. Although hemocytes are dispensable for wound closure, they are necessary for protection against infection. In fact, although hemocytes are essential to complete embryogenesis due to their developmental roles, during larval stages their primary role appears to be the phagocytosis of pathogens because larvae that lack hemocytes can only survive through to adulthood if reared under sterile conditions.

What gets them going? Hemocytes appear to disperse primarily in response to the expression of platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF)-related ligands (Pvfs) that are expressed along their route ways in the embryo, but restriction of space also plays a role in constraining where they can migrate. Other ligands controlling these migrations remain obscure, although cell–cell repulsion, a process that requires the microtubule-binding protein Orbit/CLASP, may contribute to their dispersal and/or maintenance of their even distribution in the embryo. The open circulation system in *Drosophila* larvae and adults means that hemocytes are passively pumped around the hemolymph by the dorsal vessel (the heart equivalent); this difference means that, unlike in