Evolutionary Applications

Open Access

Evolutionary Applications ISSN 1752-4571

ORIGINAL ARTICLE

Evolution in biocontrol strains: insight from the harlequin ladybird *Harmonia axyridis*

Ashraf Tayeh,[1] Arnaud Estoup,[1] Guillaume Laugier,[1] Anne Loiseau,[1] Julie Turgeon,[2] Stefan Toepfer[3] and Benoit Facon[1*]

[1] Inra, Cbgp (Inra/Ird/Cirad/Montpellier SupAgro) Montpellier, France
[2] Département de biologie, Université Laval Québec, QC, Canada
[3] CABI Europe Switzerland, Plant Protection & Soil Conservation Directorate Hodmezovasarhely, Hungary

Keywords
biological control, biological invasion, fungal entomopathogen, genetic drift, *Harmonia axyridis*, inadvertent selection, laboratory adaptation, life-history traits.

***Correspondence**
Benoit Facon, Inra, Cbgp (Inra/Ird/Cirad/Montpellier SupAgro), Montpellier, France.
Tel.: (33) 499 62 33 22;
fax: (33) 499 62 33 45;
e-mail: facon@supagro.inra.fr

Received: 7 November 2011
Accepted: 1 May 2012
First published online: 7 June 2012

doi:10.1111/j.1752-4571.2012.00274.x

Abstract

After being used as a biocontrol agent against aphids for decades without harmful consequences, the Asian harlequin ladybird *Harmonia axyridis* has suddenly become an invasive pest on a worldwide scale. We investigate the impact of captive breeding on several traits of this ladybird such as genetic diversity, fecundity, survival and pathogen resistance. We conducted an experiment in the laboratory to compare the fecundity and the susceptibility to the entomopathogenic fungus *Beauveria bassiana* of wild and biocontrol adults of *H. axyridis*. We compiled these new findings with already published data. Altogether, our findings suggest that mass rearing of biological control agents may strongly impact genetic diversity and life-history traits. We discuss how such changes may subsequently affect the fitness of biological control strains in natural environments.

Introduction

Biological control agents can be viewed as a type of domesticated species (Diamond 2002; Savolainen et al. 2002; Ross-Ibarra et al. 2007), particularly when they are reared in captivity prior to release. As such, they may experience the same types of phenotypic and genetic changes experienced by other domesticated species (Burke et al. 2007; O'Neill et al. 2010; Rubin et al. 2010).

As such, it is important to document and understand the evolutionary changes that occur in captive populations of biological control agents. Three major mechanisms may cause genetic changes under laboratory rearing conditions: random drift, inbreeding and selection (Hopper et al. 1993). The first two mechanisms can be related to small population size. Indeed, drift arises from taking a finite sample from a population: by chance, some individuals, and thus genotypes, contribute more and some less in each generation. This process can be substantial and induces a high initial loss of genetic variability when only few founders are used to start laboratory populations and/or when such populations include a low number of effective parents at each generation (Wajnberg 1991; Fiumera et al. 2000). Inbreeding, the mating of close relatives, increases the frequency of homozygotes and can lead to changes in gene frequencies by exposing deleterious recessive alleles to selection. Deleterious effects of inbreeding could deter the successful field colonization of exotic species in classical biological control programs. For instance, Kuriwada et al. (2011) reported higher inbreeding depression in a mass-reared strain of *Cylas formicarius* than in a wild strain, suggesting that mass-reared weevils suffer serious inbreeding depression.

Because captive environments differ from wild ones, selection can favour some peculiar genetic variants. In agreement with this, genetic adaptations to captivity have been documented in various species (see for instance, Zouros et al. 1982; Allard 1988; Levin et al. 2001; Lewis and Thomas 2001; Heath et al. 2003). It is worth stressing that selection can be intentional or not. On the one hand, humans can alter deliberately the genetic composition of a set of individuals to suit their needs, a process called genetic improvement (Hopper et al. 1993). Such intentional selection has allowed improving the efficiency of

several biological control agents, especially in relation to host acceptance/suitability, temperature tolerance, diapause induction and insecticide resistance (Hoy 1985). For instance, two greenhouse populations of *Amblyseius fallacis* have shown a 64-fold increase in resistance against permethrin insecticide after 12 rounds of selection. On the other hand, continued laboratory breeding may result in relaxed selection for some life-history characteristics (Sgro and Partridge 2000; Mack et al. 2001). Such characteristics selected under captivity conditions can sometimes be disadvantageous in the natural environment (Waples 1999; McGinnity et al. 2003; Kraaijeveld-Smit et al. 2006; Araki et al. 2007; Frankham 2008). For instance, a hatchery stock of chinook salmon (*Oncorhynchus tshawytscha*) in Canada evolved smaller eggs and supplementation of wild populations using this stock reduced egg size in introgressed wild populations, resulting in reduced fitness in nature (Heath et al. 2003). Such inadvertent selection may also occur for biological control agents during laboratory culture and maintenance of captive populations, favouring laboratory-adapted genotypes that are maladapted to the field (Hopper et al. 1993).

Given its use for biological control and invasive history (reviewed in Brown et al. 2011), the Asian harlequin ladybird *Harmonia axyridis* is a good biological model to examine the evolutionary impact of conditions in captivity and its potential consequences in the field. Native to Asia, the coccinellid *H. axyridis* has been introduced repeatedly in North America as a biocontrol agent against aphids since 1916 (Tedders and Schaefer 1994; Krafsur et al. 1997) and in Europe and South America since 1980s (Ongagna et al. 1993; Poutsma et al. 2008). Despite recurrent intentional releases, the species did not establish for decades. However, it suddenly became invasive in eastern and western North America in 1988 and 1991 (Chapin and Brou 1991; LaMana and Miller 1996), in Europe in 2001 (Belgium, Adriaens et al. 2003), South America in 2001 (Argentina, Saini 2004) and in Africa in 2004 (South Africa, Stals and 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 and Galvan 2008). Based on the analysis of neutral genetic variation, Lombaert et al. (2010, 2011) recently retraced the routes of all five worldwide *H. axyridis* 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, Lombaert et al. (2010) found evidence for genetic admixture between eastern North American founders and individuals from the laboratory European biocontrol population used locally to manage aphid populations (with a contribution of biocontrol genes estimated around 43%).

In the present study, we took advantage of this context to investigate the impact of captive breeding on several characteristics (such as fecundity, larval survival, resistance to a pathogen or genetic diversity) by comparing different sources of *H. axyridis*, i.e. the laboratory mass-reared population used for biological control in Europe, as well as wild populations (invasive alien and native ones). To this end, we first conducted an experiment in the laboratory to compare the fecundity and the susceptibility to the entomopathogenic fungus *Beauveria bassiana* of wild and biocontrol adults of *H. axyridis*. We then compiled these new findings with already published data by our research group and close collaborators to test for several predictions regarding the impact of captive breeding practices on *H. axyridis*.

First, we expect a lower genetic diversity in European biocontrol population compared to wild ones owing to genetic drift. It is likely that only a few founders would have been used to start laboratory population and/or the number of effective parents at each generation was most likely lower than in a wild population.

Second, the laboratory environment strongly differs from the wild ones. Notably, lab-rearing conditions do not display seasonal variations of temperatures and cultures are maintained as free of pathogen as possible. Owing to the absence of these selective pressures in captivity, we could hypothesize that biocontrol individuals should exhibit a lower survival rate at low temperatures and a lower resistance to a pathogen such as *B. bassiana*, the latter being present on both the native and introduced ranges of *H. axyridis*. It has to be noted that a decrease of both traits upon relaxation of selection requires the additional assumption of a cost, either a quantitative cost or a trade-off with some other trait (Hufbauer 2002).

Third, similar selection in different environments might not be equally effective in leading to adaptation (Wilson et al. 2006). Notably, lab-rearing conditions being less multifaceted and more constant than field conditions, directional selection on particular traits maximizing fitness in captivity may be more effective in biocontrol population. For instance, we might predict that biocontrol individuals would display higher larval survival, higher male reproductive success and an earlier and higher fecundity. Obviously, these four traits may also boost invasive success. By specifically comparing biocontrol population, European invasive and American invasive ones, we could assess whether the traits with higher values in the biocontrol population have been preserved or not in the field (i.e. in the European invasive population, the latter being the result of an admixture between American population and biocontrol one).

Materials and methods

Description of published data

Our research group and close collaborators have previously produced data comparing biocontrol-type *H. axyridis* with field collected ones for several characteristics. Lombaert et al. (2011) estimated genetic diversity at 18 microsatellites in the European biocontrol populations and in several native and invasive populations. Three other studies have compared, using experiments in controlled conditions, the performances of European biocontrol individuals and field collected ones. First, Lombaert et al. (2008) measured the survival rate during quiescence at low temperatures for biocontrol and invasive European adults. Second, Facon et al. (2011) estimated the male reproductive success of biocontrol and invasive European adults. Third, Turgeon et al. (2011) assessed larval survival, age at first reproduction and fecundity for individuals coming from biocontrol, American and European invasive populations. We compiled these published data with those obtained in our new study (see above), to test for predictions described in Introduction regarding the impact of captive breeding practices on *H. axyridis*.

New experiments: susceptibility to *B. bassiana* and fecundity

Population sampling and rearing conditions

Five *H. axyridis* populations of three different types were used in this study, i.e. a laboratory-reared type commonly used for biological control purposes as well as invasive and native types. Two populations were collected from the native range of *H. axyridis* in 2009 (Beijing in China and Fuchuk in Japan). Two invasive populations were collected in North America and Europe between 2008 and 2009 (Quebec City in Canada and Bataszek in Hungary). One population was obtained from a laboratory population of a biocontrol agent producer (winged strain of *H. axyridis*; Biotop company, Valbonne, France) in 2007. This biocontrol population has been reared in laboratory conditions since 1982; this is for a minimum of 70 generations (assuming 2.5 generations per year; Koch 2003; Koch et al. 2006). This population was commercialized and used for the biocontrol of aphids between 1995 and 1999 throughout Europe (Ferran et al. 1997; Tourniaire et al. 2000). We reared the five populations for two generations under controlled conditions (24°C; L: D 14:10; 60% relative humidity) to minimize potential biases owing to maternal effects until the third generation was used for experiments. All populations were fed ad libitum with irradiated eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) for these generations as well as for the following experiment.

Experimental procedures

A total of 40 females and 40 males of the third laboratory-standardized generation of each of the five test populations were placed into a large box during 10 days to allow copulations. After this period, females were transferred individually into 9-cm-diameter Petri dishes and kept at 24°C; L: D 14:10; 60% relative humidity. Each female was presented to a single male for a period of 24 h. This was repeated three times with three different males per week. This procedure minimized density effects (e.g., delayed growth or reduced fecundity in paired individuals owing to competition) whilst allowing multiple copulations.

For each fertilized female, the number of eggs laid during seven consecutive days following the first clutch were counted and averaged to estimate the mean daily fecundity prior treatment. Females of each population were then infected with *B. bassiana* or remained untreated. It is known to be a natural mortality agent among overwintering adult coccinellids (Steenberg and Harding 2009). This result has led some authors to suggest that *B. bassiana* could be a potential candidate for the biological control of invasive populations of *H. axyridis* (Shah and Pell 2003; Kenis et al. 2008; Roy et al. 2011). The strain of *B. bassiana* used in this study corresponded to a commercial preparation (BotaniGardES®, strain GHA 2.2×10^{13} conidia per kg; Laverlam International Corporation, Butte, USA) that was already used in an experiment with *H. axyridis* (Roy et al. 2008). The concentration of viable conidia was 6.4×10^8 per g. Infected females were singly held in a *B. bassiana* solution in plastic cups (2 cm×3 cm) for 5 s (2.2×10^8 conidia per ml). The exposures have been made during four consecutive days with an equal representation of each population each day. It has to be noted that no significant differences were found between exposure days. The control females were held singly in sterile distilled water for the same duration. A total of 89 females were infected with the fungus and 48 females were used in controls. After exposures to fungus or sterile distilled water, all females were transferred individually into 9-cm-diameter Petri dishes and kept at 24°C; L: D 14:10; 80% relative humidity. Subsequently, eggs were counted per female and removed on a daily basis during 15 days to estimate the mean daily fecundity after treatment. Mortality was recorded daily until 15 days after treatment. Dead individuals were transferred to 1.5-mL Eppendorf tubes with moistened cotton to observe external fungal growth and hence to confirm *B. bassiana* as likely cause of death. External fungal growth has been recorded for around 60% (with no differences between populations) of treated females, as it is classically observed with this kind of bioassay.

Statistical analyses

All data were analysed using the software JMP 8.01 (SAS Institute Inc., Cary, NC, USA). First, we calculated the proportional reduction in daily mean egg production for each female as follows: $Reduction_{Fecundity}$ = (*Daily fecundity prior treatment* − *Daily fecundity after treatment*)/*Daily fecundity prior treatment*. This trait was then analysed using ANOVA with *population type* (biocontrol, native or invasive), *treatment* (infected or control) and *population* nested in *population type*, as well as the interaction between population type and *treatment* as fixed factors. Second, we analysed the mortality using GLM (binomial distribution) with *population type* (biocontrol, native or invasive) and *population* nested in *population type* as fixed factors. We excluded the control females from this analysis because none of them died during the experiment. Therefore, the factor treatment was removed from the full model. For the dead infected females, the date of death (i.e. survival time) was analysed using ANOVA with *population type* (biocontrol, native or invasive) and *population* nested in *population type* as fixed factors. Finally, we used control females to analyse the fecundity apart from the effect of the fungus using ANOVA with *population type* (biocontrol, native or invasive) and *population* nested in *population type* as fixed factors.

For the response variables analysed using ANOVA, we performed two analyses, one with the original data and one with the traditional transformations (square root for fecundity, log for survival time and Box-Cox for $Reduction_{Fecundity}$; Sokal and Rohlf 1995). As similar results were obtained, only results from untransformed data will be reported.

Results and discussion

This study supports the predictions that laboratory conditions have significantly impacted several characteristics of *H. axyridis* (Table 1). We detailed these results in three parts: neutral genetic diversity, traits counter-selected in the laboratory and traits under positive selection in the laboratory.

Neutral genetic diversity

Genetic drift is the most important of the random processes that influence the gene frequencies in a laboratory colony (Joslyn 1984). To avoid a substantial loss of genetic diversity, Wajnberg (1991) recommended that laboratory cultures should be started with the maximal effective population size, and maintaining a maximum number, within the limitations imposed, during the entire rearing process.

In the case of *H. axyridis*, the European biocontrol population exhibits evidence of substantial genetic drift, as it harbours significantly lower genetic diversity (2.4 alleles per locus) than native (5.3–6.6 alleles per locus) or invasive (4.1–6.5 alleles per locus; Lombaert et al. 2011) populations. Because the European invasive population originated from a genetic admixture between eastern North American founders and individuals from the laboratory European biocontrol population (Lombaert et al. 2010), the level of genetic diversity is also significantly lower in the European biocontrol population than in the European invasive population (6.0 alleles per locus).

Traits counter-selected in the laboratory

In agreement with our predictions, the European biocontrol population exhibits lower performance than natural populations (Table 1). It has a significantly lower survival rate (41%) during quiescence at low temperatures compared to invasive European population (86%). Similarly, European biocontrol individuals are significantly more susceptible to *B. bassiana* infection than individuals collected from native or invasive populations. In the biocontrol

Table 1. Predictions and compilations of results regarding the impact of captive breeding practices in *H. axyridis* on various traits (for details, see Introduction and Results). Symbols + and − indicate relatively high and low values of these traits, respectively, and NA indicates that the data are not available.

Trait considered	Predictions for the European biocontrol population	European biocontrol population	European invasive population	American invasive population	Native populations	Sources
Genetic diversity	−	−	−	−	+	Lombaert et al. 2011;
Survival in quiescence	−	−	+	NA	NA	Lombaert et al. 2008;
Resistance to pathogen	−	−	+	+	+	This study
Larval survival	+	+	+	−	NA	Turgeon et al. 2011;
Delay before reproduction	−	−	−	+	NA	Turgeon et al. 2011;
Fecundity	+	+	−	−	−	This study & Turgeon et al. 2011;
Male reproductive success	+	+	−	NA	NA	Facon et al. 2011

Table 2. Results of statistical analyses for reduction of fecundity, mortality and survival time owing to fungus infection and for fecundity without fungus infection.

Traits	Test statistic	*P*-value
(A) Fecundity reduction	*F* (df)	
Population type	2.09 (2)	0.12
Treatment	41.11 (1)	0.0001
Population (population type)	1.01 (2)	0.36
Population type × treatment	1.38 (2)	0.25
(B) Mortality	L-R chi-square (df)	
Population type	14.68 (2)	0.0007
Population (population type)	0.17 (2)	0.92
(C) Survival time	F (df)	
Population type	0.14 (2)	0.87
Population (population type)	0.19 (2)	0.82
(D) Fecundity	F (df)	
Population type	6.12 (2)	0.005
Population (population type)	2.49 (2)	0.10

population, there are twice as many deaths as in the natural native and invasive populations (93% vs 38% and 46%, respectively; $P = 0.0007$) owing to *B. bassiana* infection, whereas no significant differences in mortality are found between invasive and native females (Table 2 and Fig. 1). On the contrary, the decrease of fecundity and the survival time of the infected females owing to fungus infection are not influenced by the population type (i.e. biocontrol, native or invasive; Table 2 and Fig. 2).

We suggest that both mortality during quiescence and mortality because of fungal infection can be viewed as examples of adverse genetic changes as a result of captive rearing. An important issue raised by such adverse genetic changes is how selection for laboratory-adapted genotypes may induce maladaptation to the field when laboratory-reared individuals are introduced into the wild and reproduce among wild individuals. According to the literature, characteristics selected under captive conditions are often disadvantageous in natural environments (see for instance Frankham et al. 1986; Fleming and Gross 1993; Reisenbichler and Rubin 1999; Chilcote 2003; Heath et al. 2003; Kraaijeveld-Smit et al. 2006). The most emblematic examples come from fishes where selection in hatcheries causes highly deleterious effects. For instance, lifetime reproductive success of hatchery fish stocks, once returned to the field, was found to be only 5–15% of that for the wild fish populations (Leider et al. 1990). In the case of *H. axyridis*, the decrease of survival during quiescence and pathogen resistance upon relaxation of selection suggests that there exists a cost for both traits, either a quantitative cost or a

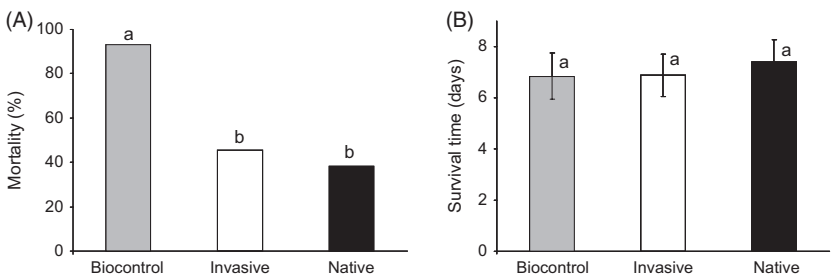

Figure 1 Mean values for (A) mortality and (B) survival time owing to fungus infection, depending on the population type, i.e. biocontrol, invasive and native.

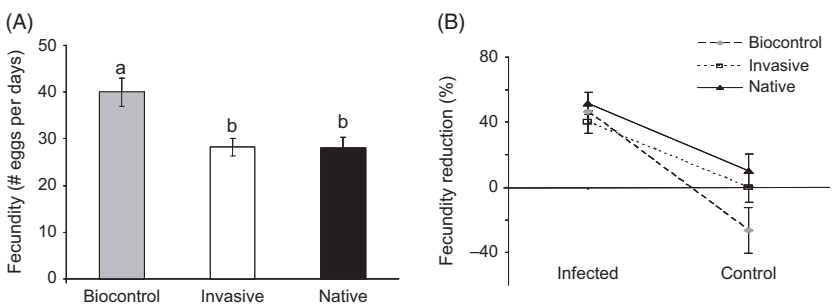

Figure 2 Mean values for (A) fecundity without fungus infection and (B) fecundity reduction owing to fungus infection, depending on the population type, i.e. biocontrol, invasive and native.

trade-off with some other trait that has been selected in laboratory conditions. The very poor survival rate at low temperatures of the biocontrol strain seems to be counter-selected in the field as the invasive European populations show a much higher survival rate. Regarding the susceptibility to *B. bassiana*, the invasive European population displays the same mortality owing to this fungus as the invasive American population, which is twofold lower than the biocontrol population. This result indicates that this higher susceptibility of biocontrol population seems also to be counter-selected in the field. Both results are, at least intuitively, not surprising as survival during quiescence and resistance to pathogens are certainly important parts of the fitness of *H. axyridis* individuals during the invasion process of natural habitats.

Traits under positive selection in the laboratory

The European biocontrol population displays higher values for several traits suggesting a higher efficiency of directional selection in the less multifaceted laboratory environment (Table 1). Indeed, it has a significant higher male reproductive success than the European invasive population (Facon et al. 2011). When competing with European invasive males, biocontrol males sire around 75% of offspring produced by females. Biocontrol females display also higher fecundity (average = 40.0 eggs per day) than native (average = 28.6 eggs per day) and invasive ones (28.2 eggs per day; Fig. 2 and Table 2). This trend is confirmed by Turgeon et al. (2011), who showed that biocontrol females lay significantly more eggs (38.9 eggs per day) than females from the American (25.4 eggs per day) and European (29.5 eggs per day) invasive populations. For two other traits, biocontrol individuals performed better than American invasive individuals but not compared to European invasive ones. Biocontrol individuals survive at significantly higher rates in the larval period (91%) and reproduce significantly earlier (11.1 days after emergence on average) than the American invasive population (73% and 13.3 days, respectively) but not compared to European invasive population (92% and 12.1 days).

A number of genetic adaptations to captivity have been previously suggested in a variety of taxa (Frankham 2008). Most of these cases also correspond to an increase of fecundity and a reduction of development time (Hopper et al. 1993; Frankham 2008). For instance, a threefold increase in reproductive fitness has been reported over 84 generations in the dipteran fly *Drosophila* (Gilligan and Frankham 2003). The butterfly *Pieris brassicae* laid many more eggs in cage experiments and had a higher ovary mass when laboratory reared for 100-150 generations than females from a stock recently obtained from the wild (Lewis and Thomas 2001). For this kind of traits, we could envisage that adaptation to laboratory conditions may also inadvertently select for values favouring establishment and range expansion in the field. With respect to *H. axyridis*, it seems to be the case for larval survival and age at first clutch as European invasive population has retained the biocontrol genetic background associated to higher fitness. On the other hand, European invasive population displays lower fecundity than biocontrol individuals. It is more difficult to envision how lower fecundity may be advantageous for the invasive European beetles. This result suggests that it may exist a trade-off between fecundity and unknown trait(s) not considered in the study that could be selected in the field.

Conclusions

A weakness of our study lies in the absence of replicate biocontrol populations. Unfortunately, to our knowledge, there remain only two biocontrol strains of *H. axyridis* (Biotop and Biobest; Turgeon et al. 2011). Furthermore, the Biobest strain was derived from the Biotop strain used in this study and thus even was it available to us, it would be somewhat problematic as a replicate. Given this limitation, we cannot rule out with certainty that the differences of life-history trait values observed in the biocontrol-type *H. axyridis* only stem from genetic drift, more especially as we demonstrated that the European biocontrol population exhibits evidence of substantial genetic drift. However, this would imply that only individuals with these trait values were sampled in the creation of this laboratory mass-reared biocontrol population. It seems unlikely that genetic drift alone can explain all the changes of trait values along our predictions on the impact of captive breeding and the action of selection seems more parsimonious. Ruling out this alternative hypothesis more definitively would require experimental selection, which would provide a better understanding of the genetic architecture of the traits, and would give us information about the relative likelihood of drift versus selection.

This study hence supports the predictions that biological control agents can undergo drastic genetic and phenotypic changes as a consequence of laboratory conditions. However, we are still at an early stage in understanding how mass rearing of biological control agents can induce genetic changes of life-history traits important for the success of field releases or for evolutionary trajectories of wild populations. We hope that our study will stimulate new research on this topic.

Acknowledgements

We thank D. Bourguet and N. Ris for comments and discussions. This work was supported by grants from the

French Agropolis Foundation (RTRA – Montpellier, BIOFIS project number 1001-001) and from the INRA department SPE.

Data archiving statement

Raw data used to generate the main results of the paper are available as Online Supplementary Material.

Literature cited

Adriaens, T., E. Branquart, and D. Maes. 2003. The Multicoloured Asian Ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? Belgian Journal of Zoology **133**:195–196.

Allard, R. W. 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. Journal of Heredity **79**:225–238.

Araki, H., B. Cooper, and M. S. Blouin. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science **318**:100–103.

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

Burke, J. M., J. C. Burger, and M. A. Chapman. 2007. Crop evolution: from genetics to genomics. Current Opinion in Genetics and Development **17**:525–532.

Chapin, J. B., and V. A. Brou. 1991. *Harmonia axyridis* Pallas, the 3rd species of the genus to be found in the United-States (Coleoptera, Coccinellidae). Proceedings of the Entomological Society of Washington **93**:630–635.

Chilcote, M. W. 2003. Relationship between natural productivity and the frequency of wild fish in mixed spawning populations of wild and hatchery steelhead (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences **60**:1057–1067.

Diamond, J. 2002. Evolution, consequences and future of plant and animal domestication. Nature **418**:700–707.

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

Ferran, A., L. Giuge, J. Brun, J. Gambier, and F. Kabiri. 1997. Coccinelle *Harmonia axyridis* pallas: mise au point sur son introduction et son utilisation en lutte biologique. Adalia **36**:21–24.

Fiumera, A. C., P. G. Parker, and P. A. Fuerst. 2000. Effective population size and maintenance of genetic diversity in captive-bred populations of a Lake Victoria Cichlid. Conservation Biology **14**:886–892.

Fleming, I. A., and M. R. Gross. 1993. Breeding success of hatchery and wild coho salmon *Oncorhynchus kisutch* in competition. Ecological Applications **3**:230–245.

Frankham, R. 2008. Genetic adaptation to captivity in species conservation programs. Molecular Ecology **17**:325–333.

Frankham, R., H. Hemmer, O. A. Ryder, E. G. Cothran, M. E. Soule, N. D. Murray, and M. Snyder. 1986. Selection in captive populations. Zoo Biology **5**:127–138.

Gilligan, D. M., and R. Frankham. 2003. Dynamics of genetic adaptation to captivity. Conservation Genetics **4**:189–197.

Heath, D. D., J. W. Heath, C. A. Bryden, R. M. Johnson, and C. W. Fox. 2003. Rapid evolution of egg size in captive salmon. Science **299**:1738–1740.

Hopper, K. R., R. T. Roush, and W. Powell. 1993. Management of genetics of biological-control introductions. Annual Review of Entomology **38**:27–51.

Hoy, M. A. 1985. Recent advances in genetics and genetic improvement of the Phytoseiidae. Annual Review of Entomology **30**:345–370.

Hufbauer, R. A. 2002. Evidence for nonadaptive evolution in parasitoid virulence following a biological control introduction. Ecological Applications **12**:66–78.

Joslyn, D. J. 1984. Maintenance of genetic variability in reared insects. In E. G. King, and N. C. Leppla, eds. Advances and Challenges in Insect Rearing, pp. 20–29. USA Agricultural Research Service, Southern Region, New Orleans, Louisiana.

Kenis, M., H. E. Roy, R. Zindel, and M. E. N. Majerus. 2008. Current and potential management strategies against *Harmonia axyridis*. BioControl **53**:235–252.

Koch, R. L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. Journal of Insect Science **3**:1–16.

Koch, R. L., and T. L. Galvan. 2008. Bad side of a good beetle: the North American experience with *Harmonia axyridis*. BioControl **53**:23–35.

Koch, R. L., R. C. Venette, and W. D. Hutchison. 2006. Invasions by *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in the Western Hemisphere: Implications for South America. Neotropical Entomology **35**:421–434.

Kraaijeveld-Smit, F. J. L., R. A. Griffiths, R. D. Moore, and T. J. C. Beebee. 2006. Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. Journal of Applied Ecology **43**:360–365.

Krafsur, E. S., T. J. Kring, J. C. Miller, P. Nariboli, J. J. Obrycki, J. R. Ruberson, and P. W. Schaefer. 1997. Gene flow in the exotic colonizing ladybeetle *Harmonia axyridis* in North America. Biological Control **8**:207–214.

Kuriwada, T., N. Kumano, K. Shiromoto, and D. Haraguchi. 2011. Inbreeding avoidance or tolerance? Comparison of mating behaviour between mass-reared and wild strains of the sweet potato weevil. Behavioural Ecology and Sociobiology **65**:1483–1489.

LaMana, M. L., and J. C. Miller. 1996. Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in Oregon. Biological Control **6**:232–237.

Leider, S. A., P. L. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture **88**:239–252.

Levin, P. S., R. W. Zabel, and J. G. Williams. 2001. The road to extinction is paved with good intentions: negative association of fish hatcheries with threatened salmon. Proceedings of the Royal Society of London Series B-Biological Sciences **268**:1153–1158.

Lewis, O. T., and C. D. Thomas. 2001. Adaptations to captivity in the butterfly *Pieris brassicae* (L.) and the implications for ex situ conservation. Journal of Insect Conservation **5**:55–63.

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

Lombaert, E., T. Guillemaud, J.-M. Cornuet, T. Malausa, B. Facon, and A. Estoup. 2010. Bridgehead Effect in the Worldwide Invasion of the Biocontrol Harlequin Ladybird. PLoS One **5**:e9743.

Lombaert, E., T. Guillemaud, C. Thomas, L.-J. Lawson Handley, J. Li, S. Wang, H. Pang et al. 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.

Mack, P. D., V. K. Lester, and D. E. L. Promislow. 2001. Age–specific effects of novel mutations in *Drosophila melanogaster*: II. Fecundity and male mating ability. Genetica **110**:31–41.

McGinnity, P., P. Prodohl, K. Ferguson, R. Hynes, N. O'Maoileidigh, N. Baker, D. Cotter, B. O'Hea, D. Cooke, G. Rogan, J. Taggart, and T. Cross. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London Series B – Biological Sciences **270**:2443–2450.

O'Neill, C. J., D. L. Swain, and H. N. Kadarmideen. 2010. Evolutionary process of *Bos taurus* cattle in favourable versus unfavourable environments and its implications for genetic selection. Evolutionary Applications **3**:422–433.

Ongagna, P., L. Giuge, G. Iperti, and A. Ferran. 1993. Life-cycle of *Harmonia-axyridis* (Col, Coccinellidae) in its area of introduction - South-Eastern France. Entomophaga **38**:125–128.

Poutsma, J., A. J. M. Loomans, B. Aukema, and T. Heijerman. 2008. Predicting the potential geographical distribution of the harlequin ladybird, *Harmonia axyridis*, using the CLIMEX model. BioControl **53**:103–125.

Reisenbichler, R. R., and S. P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. Ices Journal of Marine Science **56**:459–466.

Ross-Ibarra, J., P. L. Morrell, and B. S. Gaut. 2007. Plant domestication, a unique opportunity to identify the genetic basis of adaptation. Proceedings of the National Academy of Sciences of the United States of America **104**:8641–8648.

Roy, H. E., P. M. J. Brown, P. Rothery, R. L. Ware, and M. E. N. Majerus. 2008. Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. BioControl **53**:265–276.

Roy, H. E., E. Rhule, S. Harding, L.-J. Lawson Handley, R. L. Poland, E. W. Riddick, and T. Steenberg. 2011. Living with the enemy: parasites and pathogens of the ladybird *Harmonia axyridis*. BioControl **56**:663–679.

Rubin, C. J., M. C. Zody, J. Eriksson, J. R. S. Meadows, E. Sherwood, M. T. Webster, L. Jiang et al. 2010. Whole-genome resequencing reveals loci under selection during chicken domestication. Nature **464**:587–591.

Saini, E. D. 2004. Presence of *Harmonia axyridis* Pallas (Coleoptera: Coccinelidae) in BS.AS. Province, Argentina. Biological and morphological aspects. RIA, Revista de Investigaciones Agropecuarias **33**:151–160.

Savolainen, P., Y. P. Zhang, J. Luo, J. Lundeberg, and T. Leitner. 2002. Genetic evidence for an East Asian origin of domestic dogs. Science **298**:1610–1613.

Sgro, C. M., and L. Partridge. 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. American Naturalist **156**: –353.

Shah, P. A., and J. K. Pell. 2003. Entomopathogenic fungi as biological control agents. Applied microbiology and biotechnology **61**:413–423.

Sokal, R. R., and F. J. Rohlf 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. W. H. Freeman and Co., New York. 887 pp.

Stals, R., and G. Prinsloo. 2007. Discovery of an alien invasive, predatory insect in South Africa: the multicoloured Asian ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). South African Journal of Science **103**:123–126.

Steenberg, T., and S. Harding. 2009. Entomopathogenic fungi recorded from the harlequin ladybird, *Harmonia axyridis*. Journal of Invertebrate Pathology **102**:88–89.

Tedders, W. L., and P. W. Schaefer. 1994. Release and establishment of *Harmonia axyridis* (Coleoptera, Coccinellidae) in the Southeastern United-States. Entomological News **105**:228–243.

Tourniaire, R., A. Ferran, L. Giuge, C. Piotte, and J. Gambier. 2000. A natural flightless mutation in the ladybird, *Harmonia axyridis*. Entomologia Experimentalis et Applicata **96**:33–38.

Turgeon, J., A. Tayeh, B. Facon, E. Lombaert, P. De Clercq, N. Berkvens, J. G. Lundgren, and A. Estoup. 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.

Wajnberg, E. 1991. Quality control of mass-reared arthropods: a genetical and statistical approach. Fifth Workshop of the IOBC Global Working Group on Quality Control of Mass Reared Arthropods : 15–25.

Waples, R. S. 1999. Dispelling some myths about hatcheries. Fisheries **24**:12–21.

Wilson, A. J., J. M. Pemberton, J. G. Pilkington, D. W. Coltman, D. V. Mifsud et al. 2006. Environmental coupling of selection and heritability limits evolution. PLoS Biology **4**:e216. DOI: 10.1371/journal.pbio.0040216.

Zouros, E., M. Loukas, A. Economopoulos, and B. Mazomenos. 1982. Selection at the alcohol-dehydrogenase locus of the olive fruit-fly dacus-oleae under artificial rearing. Heredity **48**:169–185.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Raw data concerning the experiment of susceptibility to *Beauveria bassiana*.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.