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

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Abstract

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

Introduction

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

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

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

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

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

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

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

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

Materials and methods

Experimental procedures

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

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

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

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

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

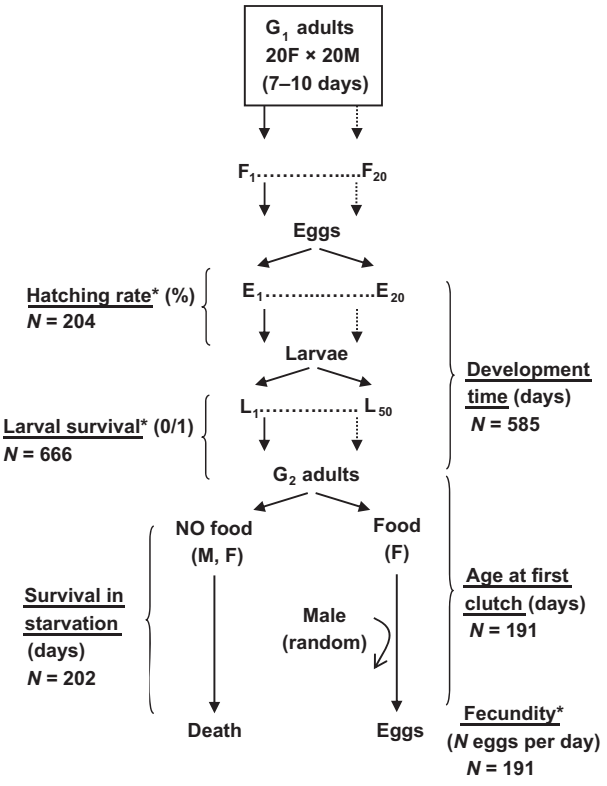

Fig. 1 *Harmonia axyridis* life history traits estimated for each experimental cross (see Table 1), along with total sample sizes. G_2-larvae and G_2-adults were kept individually in separate Petri dishes. Variables followed by a star were used to calculate the composite fitness index for 147 G_1-females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

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

females were isolated in Petri dish and data collection started.

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

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

Statistical analyses

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

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

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

Results

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

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

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

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

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

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

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

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

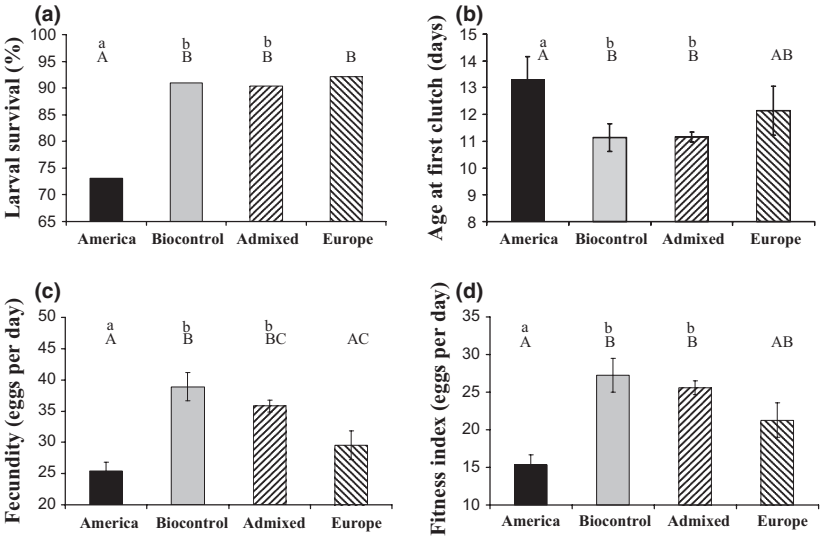

Fig. 2 Mean *Harmonia axyridis* trait values by *Type* (*American, Biocontrol, Admixed* and *Europe*) for variables showing *Type* effect (see Table 3). Error bars indicate SEM. Types with same letter are not significantly different. Small letters refer to tests for differences between *Admixed* and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

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

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Discussion

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

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

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

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

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

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

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

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

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

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