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

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

Eric Lombaert · Thibaut Malausa · Rémi Devred · Arnaud Estoup

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

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

E. Lombaert (✉)
Centre de Recherches de Sophia Antipolis, Unité de Lutte Biologique, INRA, 400 Route des Chappes, BP 167, 06903 Sophia Antipolis Cedex, France
e-mail: lombaert@sophia.inra.fr

T. Malausa · R. Devred
INRA, UMR ROSE (INRA/UNSA), 06903 Sophia-Antipolis Cedex, France
e-mails: tmalausa@sophia.inra.fr; devred@sophia.inra.fr

A. Estoup
INRA, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus international de Baillarguet, CS 30016, 34988 Montferrier-sur-Lez Cedex, France
e-mail: estoup@supagro.inra.fr

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

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

Introduction

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

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

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

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

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

Material and methods

Population sampling and rearing

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

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

Experiment 1: life history traits and phenotypic plasticity

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

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

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

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

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

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

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

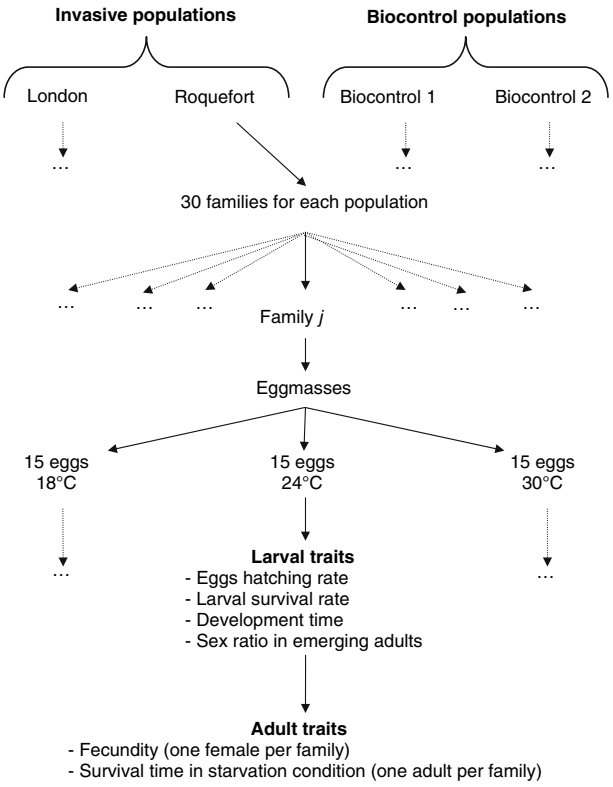

Fig. 1 Protocol design of experiment 1

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

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

Experiment 2: quiescence

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

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

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

Experiment 3: cannibalism

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

Data analysis

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

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

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

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

Results

Phenotypic plasticity of life history traits and fitness

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

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

Quiescence

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

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

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

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

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

Cannibalism

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

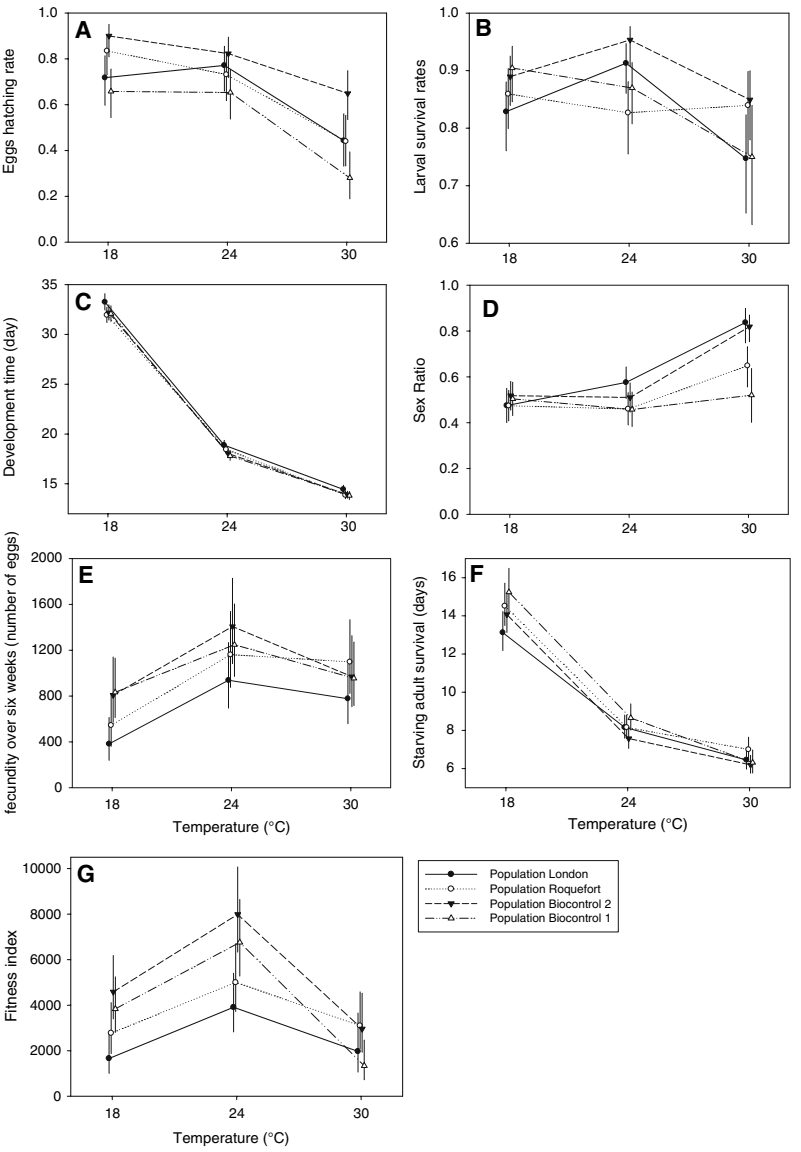

Fig. 2 Reaction norms to temperature (18, 24 and 30°C) of traits measured in experiment 1: (**a**) eggs hatching rates, (**b**) larval survival (from eggs until adult emergence), (**c**) family mean development time (until adult emergence), (**d**) sex ratio (proportion of males) of emerging adults,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

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

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

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

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

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

P-values are obtained from χ^2 test

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

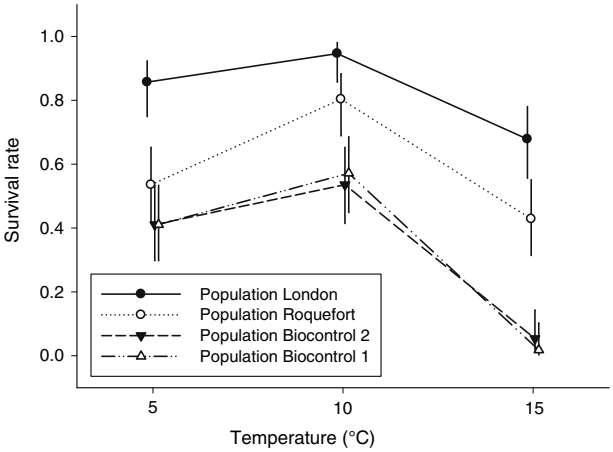

Fig. 3 Survival of adults after 5 weeks at low temperature (5, 10 and 15°C) and without food. Vertical lines correspond to 95% confidence interval

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

Discussion

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

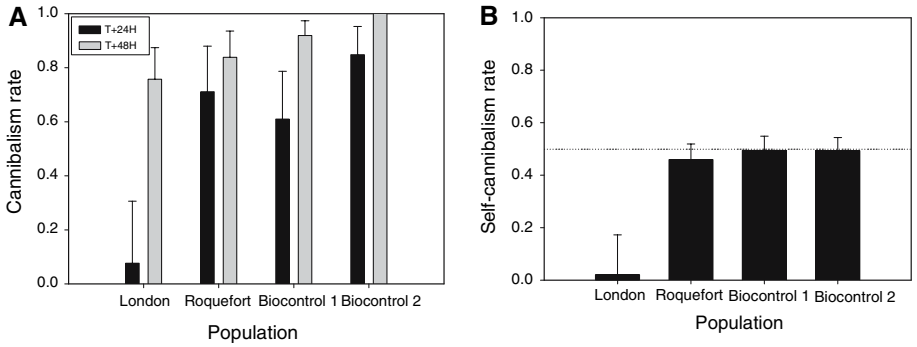

Fig. 4 Cannibalism in *H. axyridis*. (**a**) mean proportion of eggs eaten after 24 and 48 h (T + 24 h and T + 48 h, respectively) for each population. (**b**) Self-cannibalism = mean proportion of self-eaten eggs among eaten eggs after 24 h. Vertical lines correspond to 95% confidence interval

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

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

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

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

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

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

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

References

Agarwala BK, Dixon AFG (1993) Kin recognition: egg and larval cannibalism in *Adalia bipunctata* (Coleoptera: Coccinellidae). Eur J Entomol 90:45–50

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326

Berkvens N, Bonte J, Berkvens D, Deforce K, Tirry L, De Clercq P (2007) Pollen as an alternative food for *Harmonia axyridis*. BioControl (this issue). doi:10.1007/s10526-007-9128-7

Brown PMJ, Adriaens T, Bathon H, Cuppen J, Goldarazena A, Hägg T, Kenis M, Klausnitzer BEM, Kovář I, Loomans AJM, Majerus MEN, Nedved O, Pedersen J, Rabitsch W, Roy HE, Ternois V, Zakharov IA, Roy DB (2007a) *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. BioControl (this issue). doi:10.1007/s10526-007-9132-y

Brown PMJ, Roy HE, Rothery P, Roy DB, Ware RL, Majerus MEN (2007b) *Harmonia axyridis* in the Great Britain: analysis of the spread and distribution of a non-native coccinellid. BioControl (this issue). doi:10.1007/s10526-007-9124-y

Coutanceau J-P (2006) *Harmonia axyridis* (Pallas, 1773): une Coccinelle asiatique introduite, acclimatée et en extension en France. Bulletin de la société entomologique de France 111:395–401

Debat V, David P (2001) Mapping phenotypes: canalization, plasticity and developmental stability. Trends Ecol Evol 16:555–561

Geng YP, Pan XY, Xu CY, Zhang WJ, Li B, Chen JK, Lu BR, Song ZP (2007) Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. Biol Invasions 9:245–256

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol 21:394–407

Grill CP, Moore AJ, Brodie ED (1997) The genetics of phenotypic plasticity in a colonizing population of the ladybird beetle, *Harmonia axyridis*. Heredity 78:261–269

Kaufman SR, Smouse PE (2001) Comparing indigenous and introduced populations of *Melaleuca quinquenervia* (Cav.) Blake: response of seedlings to water and pH levels. Oecologia 127:487–494

Koch RL (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. J Insect Sci 3:1–16

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204

Lambrinos JG (2004) How interactions between ecology and evolution influence contemporary invasion dynamics. Ecology 85:2061–2070

Lee CE, Remfert JL, Chang YM (2007) Response to selection and evolvability of invasive populations. Genetica 129:179–192

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689–710

Majerus MEN, Strawson V, Roy H (2006) The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain. Ecol Entomol 31:207–215

Malausa T, Guillemaud T, Lapchin L (2005) Combining genetic variation and phenotypic plasticity in tradeoff modelling. Oikos 110:330–338

Masel J, King OD, Maughan H (2007) The loss of adaptive plasticity during long periods of environmental stasis. Am Nat 169:38–46

Osawa N (1992) Sibling cannibalism in the ladybird beetle *Harmonia axyridis*: fitness consequences for mother and offspring. Res Popul Ecol 34:45–55

Pervez A, Gupta AK, Omkar (2005) Kin recognition and avoidance of kin cannibalism by the larvae of co-occurring ladybirds: a laboratory study. Eur J Entomol 102:513–518

Pervez A, Gupta AK, Omkar (2006) Larval cannibalism in aphidophagous ladybirds: influencing factors, benefits and costs. Biol Control 38:307–313

Polis GA (1981) The evolution and dynamics of intraspecific predation. Annu Rev Ecol Syst 12:225–251

Preziosi RF, Snyder WE, Grill CP, Moore AJ (1999) The fitness of manipulating phenotypes: implications for studies of fluctuating asymmetry and multivariate selection. Evolution 53:1312–1318

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecol Lett 9:981–993

Roy HE, Baverstock J, Ware RL, Clark SJ, Majerus MEN, Baverstock KE, Pell JK (2008) Intraguild predation of the aphid pathogenic fungus *Pandora neoaphidis* by the invasive coccinellid *Harmonia axyridis*. Ecol Entomol (in press)

SAS Institute Inc. (1999) SAS/Stat user's guide, version 8. SAS Institute, Cary

Schanderl H, Ferran A, Garcia V (1988) Rearing two coccinellids *Harmonia axyridis* and *Semiadalia undecimnotata* on eggs of *Anagasta kuehniella* killed with ultraviolet radiation. Entomol Exp Appl 49:235–244

Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. Annu Rev Ecol Syst 24:35–68

Specty O, Febvay G, Grenier S, Delobel B, Piotte C, Pageaux JF, Ferran A, Guillaud J (2003) Nutritional plasticity of the predatory ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae): comparison between natural and substitution prey. Arch Insect Biochem Physiol 52:81–91

Stillwell R, Wallin W, Hitchcock L, Fox C (2007) Phenotypic plasticity in a complex world: interactive effects of food and temperature on fitness components of a seed beetle. Oecologia 153:309–321

Stockwell CA, Hendry AP, Kinnison MT (2003) Contemporary evolution meets conservation biology. Trends Ecol Evol 18:94–101

Tourniaire R, Ferran A, Gambier J, Giuge L, Bouffault F (2000a) Locomotory behavior of flightless *Harmonia axyridis* pallas (Col., Coccinellidae). J Insect Physiol 46:721–726

Tourniaire R, Ferran A, Giuge L, Piotte C, Gambier J (2000b) A natural flightless mutation in the ladybird, *Harmonia axyridis*. Entomol Exp Appl 96:33–38

Valladares F, Sanchez Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94:1103–1116

van Lenteren JC, Loomans AJM, Babendreier D, Bigler F (2007) *Harmonia axyridis*: an environmental risk assessment for Northwest Europe. BioControl (this issue). doi:10.1007/s10526-007-9120-2

Williams T, Hernandez O (2006) Costs of cannibalism in the presence of an iridovirus pathogen of *Spodoptera frugiperda*. Ecol Entomol 31:106–113

Williamson M, Fitter A (1996) The varying success of invaders. Ecology 77:1661–1666

Yeh PJ, Price TD (2004) Adaptive phenotypic plasticity and the successful colonization of a novel environment. Am Nat 164:531–542