JOURNAL OF Evolutionary Biology

doi:10.1111/j.1420-9101.2010.02043.x

Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*

L. J. MICHIE*, F. MALLARD†, M. E. N. MAJERUS* & F. M. JIGGINS*

*Department of Genetics, University of Cambridge, Cambridge, UK
†CNRS\ENS UMR 7625, Ecologie & Evolution, Ecole Normale Supérieure, Paris, France

Keywords:

Harmonia axyridis;
phenotypic plasticity;
polymorphism;
thermal melanism.

Abstract

Individuals can adapt to heterogeneity in their environment through either local adaptation or phenotypic plasticity. Colour forms of the ladybird *Harmonia axyridis* are a classic example of local adaptation, in which the frequency of melanic forms varies greatly between populations. In some populations, there are also large seasonal changes in allele frequency, with melanism being costly in summer and beneficial in winter. We report that the non-melanic morph of *H. axyridis* dramatically increases its degree of melanization at cold temperatures. Furthermore, there is genetic variation in reaction norms, with different families responding to temperature in different ways. Variation at different spatial and temporal scales appears to have selected for either genetic or phenotypically plastic adaptations, which may be important in thermoregulation. As melanism is known to have a large effect on fitness in *H. axyridis*, this plasticity of melanization may have hastened its spread as an invasive species.

Introduction

Faced with a variable environment, organisms frequently evolve local adaptations that confer a fitness advantage in their local environment. However, a population may also adapt to a variable environment, not through genetic change, but through phenotypic plasticity. Phenotypic plasticity is a change in an individual's behaviour, morphology or physiology induced by the environment (Price *et al.*, 2003). For example, a cooler climate might promote the survival of individuals which have on average thicker coats (genetic differentiation), or it might induce individuals to produce thicker coats (phenotypic plasticity).

What determines whether a population evolves fixed local adaptations or a plastic phenotypic response to the environment? This question has been studied closely in plants, which can only respond to environmental change in their lifetime through plasticity, as they cannot move between environments (Alpert & Simms, 2002). The results suggest that the key to an advantage in plasticity is the scale of spatial and temporal heterogeneity in an environment. If the environment is too 'fine grained', with conditions varying over a very small area, there will be no advantage to plasticity as an individual will experience a variety of conditions and should adopt a fixed, intermediate phenotype. Likewise, when the scale of the variation is very large, greater than the dispersal range of a species, a population will only experience one set of conditions and should adapt to this (Banta *et al.*, 2007). A similar argument of scales can be made for temporal heterogeneity: plasticity should only be advantageous if the trait in question can respond quickly relative to the duration of a change in environmental states (Levins, 1968; DeWitt *et al.*, 1998). Otherwise, an individual may be constantly one step behind the environment and would do better to adopt a fixed phenotype with the greatest fitness on average.

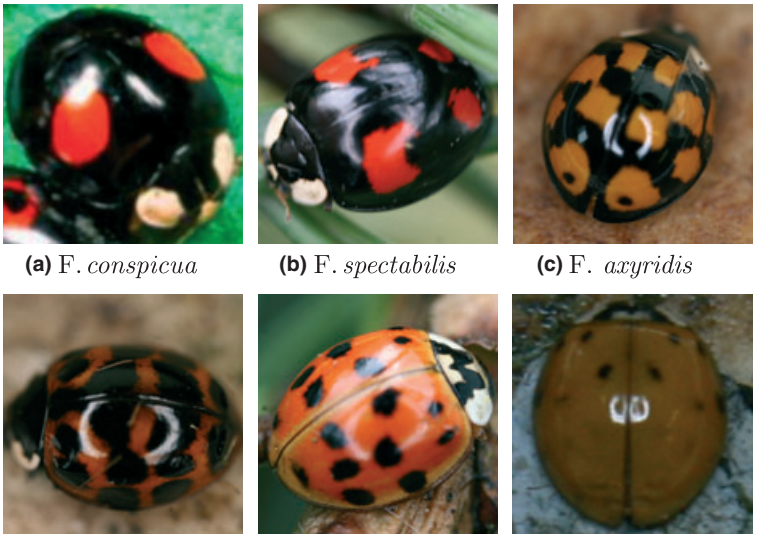
Plasticity will also be more advantageous when environmental change is predictable (Levins, 1968; Via *et al.*, 1995). If the conditions experienced by an individual are reliably indicative of the future environment, it pays to adapt in readiness. For this reason, a plastic response that occurs later in development may be more advantageous than one which occurs earlier in development, as this means that an individual need not predict the

Correspondence: Laura Jane Michie, University of Cambridge, Department of Genetics, Downing Street, Cambridge, CB2 3EH, UK. Tel.: +44 1223 333999; fax: +44 1223 333992; e-mail: ljm56@cam.ac.uk

environment as far into the future. A similar argument can be made for plasticity being more advantageous in labile rather than non-labile traits. If an event occurs once and cannot be undone, such as metamorphosis in insects, plasticity will only be advantageous if an individual can predict circumstances accurately from that point onwards. However, labile traits like behaviour can change rapidly in response to unpredictable changes in the environment.

Melanism is a classic example of adaptation, most famously in the case of the peppered moth (Tutt, 1896; Kettlewell, 1955, 1956), where melanic forms of the peppered moth rose to dominance as a result of the denuding and blackening of trees caused by pollutants during the industrial revolution. The case of the peppered moth is one of melanism being used in crypsis (dark moths were harder for birds to find against soot-blackened trees), but there are many other reasons for being melanic (Majerus, 1998). One of these is that black individuals may absorb heat more readily than light ones, which is known as thermal melanism. This could be an advantage in cold environments, as 'melanistic ectothermic individuals should heat faster and reach higher equilibrium temperatures than light ones' (Trullas *et al.*, 2007). This could result in a fitness advantage for a melanic individual in terms of feeding, finding mates, defending territories and escaping from predators. Evidence of melanic morphs being more fit, and hence more frequent, in colder climates has been demonstrated, particularly from amongst the Lepidoptera (Roland, 1982; Lewis, 1985; Guppy, 1986a,b; Kingsolver, 1987; Solensky & Larkin, 2003). This has been strengthened by laboratory experiments in both vertebrates and insects, which show that melanic morphs reach higher temperatures than non-melanics (Brakefield & Willmer, 1985; DeJong *et al.*, 1996; Soares *et al.*, 2003; Clusella-Trullas *et al.*, 2008, 2009; van Rensburg *et al.*, 2009). In these examples, the degree of melanization is genetically determined, but in Lepidoptera there are cases of phenotypic plasticity, where cold temperatures can induce melanism (Lewis, 1985; Lindstedt *et al.*, 2009).

Harmonia axyridis (the harlequin ladybird) is endemic to central and eastern Asia and has been introduced elsewhere as a biocontrol agent of aphids. It is highly polymorphic in colour pattern and the major forms are controlled by 15 alleles at one multiallelic locus (Tan & Li, 1934; Hosino, 1940; Tan, 1946; Komai, 1956). All but four of these alleles are rare, with a combined frequency of less than one per cent in populations. The four major alleles are *conspicua*, *spectabilis*, *axyridis* and *succinea* (Fig. 1), all but the last of these being melanic forms (*sensu* Kettlewell, 1973). In some of the earliest research in population genetics, the frequencies of these alleles were found to vary hugely across the native range of the harlequin (Dobzhansky, 1924, 1933). The geographical variation appeared to be linked to climate, with non-melanic forms being found most often in hot, arid regions and melanic forms being more frequent in cooler, more humid ones (Dobzhansky, 1924, 1933). However, a different pattern is found in Japan, where the non-melanic *succinea* form decreases in frequency from north-east to south-west without any significant correlation to temperature or other climatic factors (Komai *et al.*, 1950). In addition to this geographic variation, large seasonal changes in allele frequencies have been reported in some native populations (Yuan *et al.*, 1994; Jing & Zhang, 2001; Jiang *et al.*, 2008). For example, in Beijing over half the population is melanic in the spring, but this



(a) F. *conspicua* **(b)** F. *spectabilis* **(c)** F. *axyridis*

(d) F. *succinea* 14°C **(e)** F. *succinea* **(f)** F. *succinea* 28°C

Fig. 1 Four major forms of *H. axyridis*. Figures 1(d) and 1(f) show the effects of cold or hot temperatures during development on f. *succinea*.

drops to less than one-fifth by the autumn. Therefore, the melanic individuals presumably have a large fitness advantage in the winter and a disadvantage in the summer. This has been attributed to the effects of thermal melanism, possibly mediated by mate choice (Wang *et al.*, 2009).

In areas where *H. axyridis* has been introduced, such as much of Europe and North America, it has proved to be a highly invasive species and has had a detrimental impact on native species (Brown & Miller, 1998; Colunga-Garcia & Gage, 1998; Adriaens *et al.*, 2003; Koch, 2003; Nault & Kennedy, 2003; Koch & Galvan, 2008; van Lenteren *et al.*, 2008). As it has colonized a wide range of habitats, it will have had to adapt to the new conditions. It shows a great deal of plasticity in its life history traits (Sakurai *et al.*, 1992; Osawa, 2000), and this may have contributed to its invasion success. One aspect of this plasticity which has not been investigated is that of colour pattern. In late October and November 2004, atypically heavily spotted non-melanic forms of *H. axyridis* (f. *succinea*) eclosed in the UK, and Majerus *et al.* (2006) speculated that this may have been a consequence of development at low temperature. In this study, we have investigated the relative roles of genetic variation and phenotypic plasticity in controlling melanism in *H. axyridis*. As selection on melanism fluctuates seasonally in some populations, we would predict a selective advantage to phenotypic plasticity despite this being a nonlabile trait. Such phenotypic plasticity could also contribute to the spread of *H. axyridis* as an invasive species.

Materials and methods

Experimental procedure

Crosses were set up between pairs of the four common morphs of *H. axyridis* (Table 1). f. *succinea* parents were additionally categorized according to spot number as high (H – more than 16 spots), middle (M – 8–15 spots) and low (L – 0–7 spots), to ensure we captured any genetic variation present in the population. All the harlequins used in these crosses were either wild-caught British specimens or their descendants (F1 or F2), with the exception of f. *axyridis* individuals. This form is not found in the UK, so we used specimens from Siberia (Altai Republic, part of the native range of *H. axyridis*), where the species is monomorphic for this form, to give the full range of common phenotypes in this species. However, crosses involving f. *axyridis* individuals from this Siberian population were naturally analysed separately to avoid any confounding effects of the genetic background differing in the two populations. As we were using wild-collected beetles, only f. *axyridis* and f. *succinea*, which is controlled by a recessive allele, were homozygous for these alleles. Many of the f. *conspicua* and f. *spectabilis* beetles will have been heterozygous for f. *succinea* and these forms segregated in the offspring. Heterozygous offspring of f. *axyridis* can be identified phenotypically, and these were analysed separately.

The parental crosses were kept at 21 °C and fed on excess pea aphids (*Acyrthosiphon pisum*). Eggs were collected on the day they were laid and transferred to 14 °C, 21 °C or 28 ° C. For every two clutches of eggs placed at 21 °C, three were placed at 28 °C or 14 °C, where it was expected that fewer larvae would survive. Once the larvae had hatched, they were separated into groups of five in 9-cm petri dishes, to minimise cannibalism, and fed on excess *A. pisum* until pupation. The larvae remained at these temperatures until they eclosed as adults; pupae were checked daily and eclosed adults removed to 21 °C.

Spot size was measured using a graticule by taking a length (parallel to the split in the elytra) measurement and a breadth measurement (perpendicular to the split in the elytra) for each spot measured (Fig. 2). When available, three spots were measured in f. *succinea* (two, five and seven in Fig. 2). In f. *axyridis* individuals, two spots (checks) were measured (one and six in Fig. 2). For f. *spectabilis*, both of the available spots were measured. This allowed us to see any changes in pattern over the whole elytra. The adults from the first run of the experiment (21 °C and 28 °C) were scored for spot size at 24–48 h, 7–8 days and 28–29 days after eclosion. As spot size changed a negligible amount from 24 h to

Table 1 Crosses used in the study on the effects of temperature on colour pattern. Numbers are used to show where offspring from multiple crosses have been analyzed together and are shown on one figure.

Within forms	Between forms
1: *succinea* H × *succinea* H	5: *conspicua* ♀ × *axyridis* ♂
1: *succinea* M × *succinea* M	5: *axyridis* ♀ × *conspicua* ♂
1: *succinea* L × *succinea* L	6: *spectabilis* ♀ × *axyridis* ♂
2: *axyridis* × *axyridis*	6: *axyridis* ♀ × *spectabilis* ♂
3: *conspicua* × *conspicua*	7: *succinea* ♀ × *axyridis* ♂
4: *spectabilis* × *spectabilis*	7: *axyridis* ♀ × *succinea* ♂

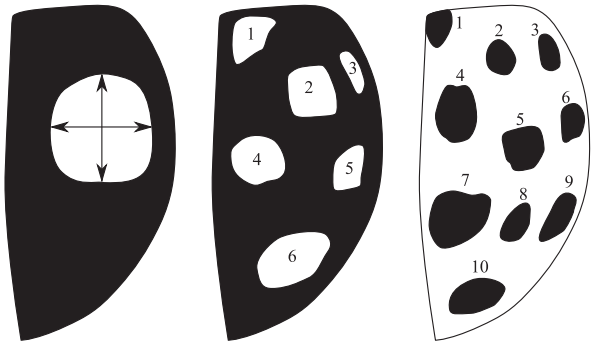


Fig. 2 Method used for measuring spots and spots measured in *H. axyridis* f. *axyridis* and f. *succinea*. After Dobzhansky 1933.

28 days, individuals from the second (21 °C and 14 °C) run were only scored at 7–8 days after eclosion.

Statistical analysis

The number of spots on an individual of *H. axyridis* f. *succinea* can range from 0 to a maximum of 19. As the distribution of this trait is thus bounded by 0 and 19, we expressed our measurements as a ratio of spots present (spot number) to spots absent (19 minus spot number) to more closely approximate a binomial distribution. This allowed us to analyse the data using a generalized linear model with a binomial error using the *lmer* package in *R*. The spot ratio, $n_{i,j,k}$, of a ladybird raised under temperature treatment i from cross j was modelled as follows:

$$n_{i,j,k} = t_i + f_j + (tf)_{i,j} + \epsilon_{i,j,k}, \quad (1)$$

where t_i is a fixed effect of temperature treatment i, f_j is a random effect of cross j and $\epsilon_{i,j,k}$ is a random effect representing the deviation for observation k from temperature treatment i and cross j. The model also allowed an interaction between temperature treatment and family, $(tf)_{i,j}$. The significances of the random effects and the interaction were assessed by comparing models with and without these terms using a likelihood ratio test. Hence, to determine the significance of the interaction, model 1 was compared to the following:

$$n_{i,j,k} = t_i + f_j + \epsilon_{i,j,k}, \quad (2)$$

where all terms are as previously defined. The significance of the random effect itself was determined by comparing model 1 to a model without any random effect. To test whether temperature affects the number of spots, the mean number of spots of each family at each temperature was calculated, and these paired observations were compared using a Wilcoxon signed-rank test. This approach was used as the correct number of degrees of freedom of fixed effects in generalized linear models is difficult to determine, which prevented us from estimating *P*-values from the *lmer* model. A Bonferroni correction was applied and the α value reduced to 0.025, as this approach also meant data from families raised at 21 °C was used twice.

Spot size was calculated as the product of length and breadth, which gave a synthetic measure of spot size. This measure was distributed normally, as seen by quantile–quantile plots of the model residuals against the normal distribution lying approximately on a straight line, $y = x$. We analyzed this data using linear mixed effect models using the *lme* package in *R* (Pinheiro & Bates, 2000). The size of each measured spot, $s_{i,j,k}$, on a ladybird raised under temperature treatment i from cross j was modelled as follows:

$$s_{i,j,k} = t_i + f_j + (tf)_{i,j} + \epsilon_{i,j,k}, \quad (3)$$

where t_i is a fixed effect of temperature treatment i, f_j is a random effect of cross j and $\epsilon_{i,j,k}$ is a random effect representing the deviation for observation k from temperature treatment i and cross j. The model also allowed an interaction between temperature treatment and cross. Separate models were fitted to the data from the different colour pattern forms. The significances of the random effects and the interaction were assessed by comparing models with and without these terms using a likelihood ratio test in a similar manner to that used for spot number. Another model, which checked for unequal variances in the three treatments by assigning separate residuals to each, was also produced and compared to the initial model of spot size (model 3). In some cases residuals did differ between treatments and, although this had a negligible effect on the significance of the test results, it did affect estimates of correlations in the random effects. We have therefore used models which assigned separate residuals in all these analyses.

Results

Effects of temperature on colour pattern

In the non-melanic f. *succinea* individuals, there is a dramatic decrease in the number of spots at higher temperatures (Fig. 3; 21 °C vs. 28 °C: Wilcox signed-rank test $V = 91$, $P < 0.001$; 21 °C vs. 14 °C: Wilcox signed-rank test $V = 0$, $P < 0.01$). Particularly striking is that at 14 °C every individual, regardless of family, had a full complement of 19 spots, compared to an average spot number of 8 at 28 °C. This decrease in spot number was mirrored by a corresponding decrease in spot size at higher temperatures (Fig. 4). All three of the spots measured were at least four-fold smaller at 28 °C than

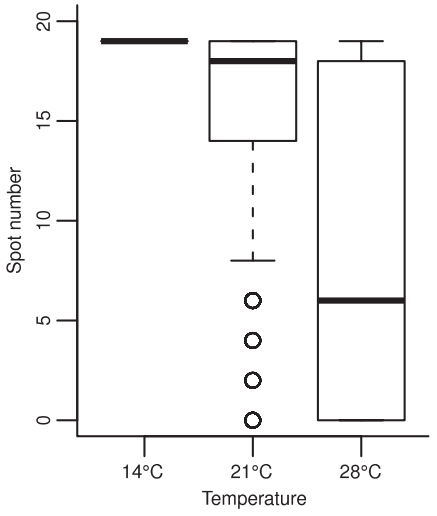


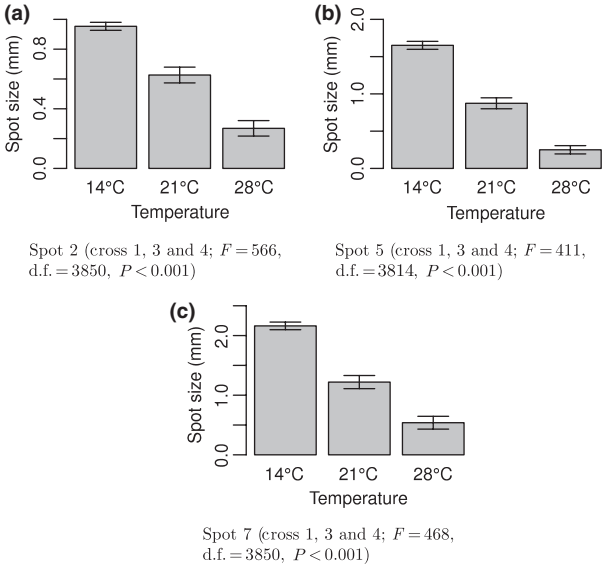
Fig. 3 The number of spots in nonmelanic f. *succinea* beetles. The horizontal bar is the median and the box the interquartile range. The open circles are outliers.

at 14 °C. The melanic area of the elytra in f. *succinea* thus increases significantly as temperature decreases (Fig. 1).

Spot size in the melanic forms also altered with temperature, but this effect is much smaller than that seen in f. *succinea*, with spots being reduced in size at most by approximately 60% (Fig. 5 and , Fig. S1). In the melanic individuals, spot size is nearly always greatest at the intermediate temperature of 21 °C (Fig. 5 and Fig. S1). A slightly different trend was observed for spot 2 in f. *spectabilis*, which declines in size with increased temperature (Fig. 5d). It should be noted that a decrease in spot size means that the melanic area of the elytra increased in size, as in the melanic forms the spots measured are the unmelanized areas of the elytra. The results from crosses involving f. *axyridis* were analysed separately (Fig. S1), as the presence of mosaic patterning in these resulted in the spots differing significantly in size compared to homozygous f. *spectabilis* or f. *conspicua* individuals. The numbers of spots in the melanic forms do not vary.

Effects of genetics on colour pattern

The genetics of the major colour forms in *H. axyridis* has long been well understood (Tan & Li, 1934; Hosino, 1940; Tan, 1946; Komai, 1956). Each form is thought to be controlled by a different allele of a single gene with a full dominance hierarchy (dominant–recessive: *conspicua* → *spectabilis* → *axyridis* → *succinea*). We found nothing to contradict this, and thus conclude that the underlying genetics of the major colour morphs is not affected by temperature. Consequently, it was possible to concentrate on the effect of the different temperatures on the variation within each colour pattern.



Spot 2 (cross 1, 3 and 4; $F = 566$, d.f. $= 3850$, $P < 0.001$)

Spot 5 (cross 1, 3 and 4; $F = 411$, d.f. $= 3814$, $P < 0.001$)

Spot 7 (cross 1, 3 and 4; $F = 468$, d.f. $= 3850$, $P < 0.001$)

Fig. 4 Spot size in f. *succinea* at different temperatures. Means and standard errors are general linear model estimates. These beetles are the offspring of the crosses in brackets, which refer to Table 1.

To investigate whether there is genetic variation affecting colour pattern within the forms, we tested whether there were differences among families of full siblings in spot size or number. In f. *succinea* crosses, there were significant differences between families in the size of all three spots (spot 2: $\chi^2 = 240$, d.f. $= 6$, $P < 0.001$; spot 5: $\chi^2 = 198$, d.f. $= 6$, $P < 0.001$; spot 7: $\chi^2 = 200$, d.f. $= 6$, $P < 0.001$). The same was true of spot number ($\chi^2 = 2884$, d.f. $= 3$, $P < 0.001$).

Next, we investigated whether there was an interaction between the effects of temperature and genetics. Do the families all respond in the same way to a change in temperature, or do they also vary in the plasticity of that pattern? We found that f. *succinea* families responded differently to changes in temperature, both in terms of the number of spots present ($\chi^2 = 56.4$, d.f. $= 7$, $P < 0.001$) and the size of spots (spot 2: $\chi^2 = 41.7$, d.f. $= 5$, $P < 0.001$; spot 5: $\chi^2 = 16.3$, d.f. $= 5$, $P < 0.01$; spot 7: $\chi^2 = 12.1$, d.f. $= 5$, $P < 0.05$).

The results of our tests for genetic variation in f. *succinea* could be contrasted with those for the melanic forms. We found no genetic variation in spot size in most of the crosses involving the melanic forms (Table 2). The two exceptions were spot 6 in the cross between f. *axyridis* and f. *succinea* and spot 1 in the cross between f. *axyridis* and f. *spectabilis* (Table 2). It is probable that this variation in spot size is because of the effect of mosaic dominance, where the f. *succinea* spots are visible in the non-melanic areas of the other forms (Tan, 1946). As f. *succinea* shows genetic variation, any non-melanic area of *axyridis* × *succinea* heterozygotes which shows *succinea* spots will also vary between families. The variation observed between *axyridis* × *spectabilis* families may also be related to mosaic effects. Although the spots of f. *axyridis* individuals do not vary in size between families, they may well vary in their position on the elytra. In neither of these cases was there an interaction between genetic variation and temperature (*axyridis* × *spectabilis* spot 1: $\chi^2 = 0.143$, d.f. $= 5$, $P = 1$; *axyridis* × *succinea* spot 6: $\chi^2 = 2.54$, d.f. $= 5$, $P = 0.770$). There is, therefore, no evidence for genetic variation in the phenotypic plasticity of melanic individuals.

Discussion

We have found that *H. axyridis* has evolved two different ways of being melanic. First, there is a genetic polymorphism that is controlled by a major-effect gene, which switches development between the spotted *succinea* form and the melanic forms (Fig. 1a–c and e), as has been shown previously. Second, there is a phenotypically plastic response to cold temperatures that causes the non-melanic f. *succinea* individuals to greatly increase their level of melanization (Fig. 1d–f). Many ladybird

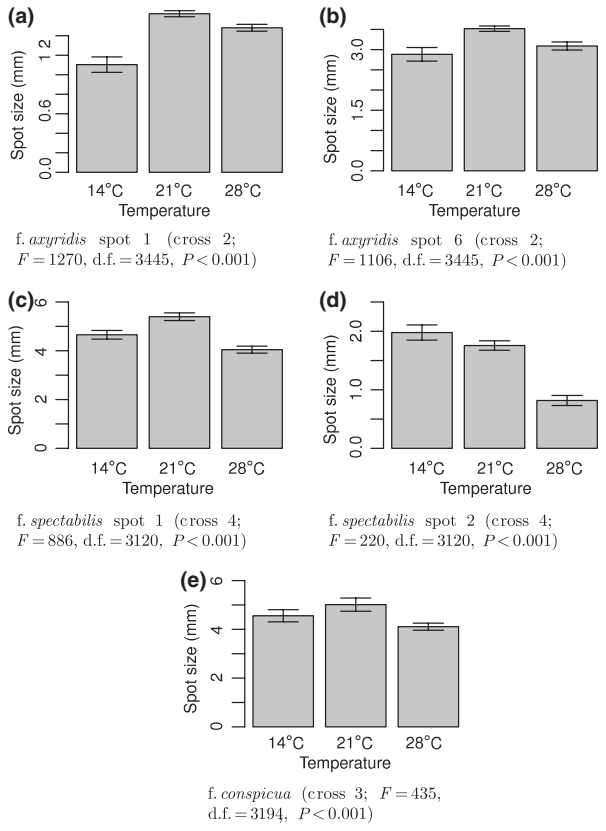


Fig. 5 Spot size in melanic beetles at different temperatures. Means and standard errors are general linear model estimates. These beetles are the offspring of the crosses in brackets, which refer to Table 1.

species have melanic polymorphisms, and the selective advantage of melanism has long been debated (Majerus, 1994, 1998). The plasticity we observed in f. *succinea* supports the hypothesis that melanism plays a thermal role. When temperature is high during development, f. *succinea* decreases the number and size of its spots, thus reducing the overall melanic area of its elytra, which may reduce its chances of overheating. Conversely, if temperature is low during development, melanism increases, which may allow the beetles to absorb heat more rapidly. It has previously been shown that the melanic morphs of both *H. axyridis* and another ladybird species, *Adalia bipunctata*, warm up faster than non-melanic morphs (Brakefield & Willmer, 1985; Soares *et al.*, 2003) and that this means they are more active at low temperatures (Benham *et al.*, 1974). Changes in the level of melanism within a morph may have a similar effect.

Plasticity can have advantages over local adaptation when an environment is temporally or spatially heterogeneous on an intermediate scale (see Introduction). In some places, such as the Siberian population included in this study, a melanic allele of the colour pattern locus (f. *axyridis*) has been fixed. It is likely that these populations experience a homogeneous and cold thermal environment, so there is no need for plasticity in melanization, and the melanic form has become genetically fixed. Further south, however, there may well be more heterogeneity through the seasons, which could make plasticity the better adaptation. In particular, more southerly populations have multiple generations each year, which will experience different thermal environments. In China, there are dramatic seasonal changes in the frequency of melanic alleles that provide strong evidence for melanics having a selective advantage in winter, which is then lost in summer (Wang *et al.*, 2009). In Britain, as in China, *H. axyridis* is bivoltine (Brown *et al.*, 2008b), so it is possible that there may be similar seasonal changes in the relative fitness of melanics. Under these circumstances, there appears to be a clear advantage to phenotypic plasticity, as individuals that altered their degree of melanization depending on the season would maximise their fitness. Melanization is not a labile trait, and it is set 24–48 hours after eclosion, so phenotypic plasticity will only be advantageous if the thermal environment varies on a time scale similar to the lifespan of an adult ladybird. This would appear to be the case here, with one generation experiencing mainly summer temperatures, and the second, more melanic, generation autumn, winter and spring temperatures. Indeed, the seasonal changes in the frequency of melanic alleles in China demonstrate that on average it is advantageous to be melanic in the winter generation, but not in the summer generation. Therefore, the conditions necessary for phenotypic plasticity to be favoured have been met.

A reaction norm describes the pattern of phenotypic expression of a single genotype across a range of environments – the response of the genotype to a change in environment (Woltereck, 1909). We found that in different families, temperature has different effects on the size and number of spots in f. *succinea*. Therefore, there is genetic variation in reaction norms, and the reaction norm itself will be able to evolve in response to selection (Scheiner & Lyman, 1989; Engelmann & Schlichting, 2005). In addition to genetic variation in reaction norms in f. *succinea*, we also found that there is genetic variation between families of full siblings in spot size and number. This may also contribute to the huge variation which is observed in this form, and such variation could also come under selection. However, in this study the genetic variation between families in terms of colour pattern had a relatively small effect compared to that of temperature.

The three melanic forms also show some plasticity when exposed to different temperatures, but the effect is much smaller. Whereas cold temperatures have a dramatic effect on f. *succinea*, transforming red beetles into largely melanic ones, the effects of temperature on the melanic forms are subtle – it is impossible to tell a *conspicua* individual raised under cold conditions from any other with the naked eye. These small changes are

Table 2 Test statistics for genetic variation in spot size in the melanic forms of *H. axyridis*.

Cross	Spot	χ^2	d.f.	*P*
conspicua × *conspicua*	N/A	7.58	6	0.271
spectabilis × *spectabilis*	1	5.42×10^{-5}	6	1
	2	3.04	6	0.804
axyridis × *axyridis*	1	7.87	6	0.247
	6	6.79	6	0.341
axyridis × *conspicua*	N/A	7.17	6	0.306
axyridis × *spectabilis*	1	29.5	6	< 0.001
	2	3.44	6	0.752
axyridis × *succinea*	1	1.54	6	0.957
	6	28.3	6	1×10^{-4}

not consistent with any thermal principle, and it is unclear whether they have any adaptive advantage. It may be that the plasticity seen with temperature in the melanic forms is simply a developmental response to the stress of an extreme temperature. This difference between the melanic and non-melanic forms holds even when only British ladybirds are examined (f. *succinea*, f. *conspicua* and f. *spectabilis*), so it cannot be explained as an artefact of including some Siberian beetles in our experiments (f. *axyridis*). The genetic variation in spot size between families which is seen in f. *succinea* is also absent from the melanic forms.

Harmonia axyridis is an invasive species and this invasiveness may partly stem from its ability to adapt to new environments. Thermal properties are important for ectotherms (see Trullas *et al.* (2007) for review) and in *H. axyridis* the dramatic seasonal changes in the frequency of melanic alleles suggest that melanism has a large impact on fitness. Therefore, adaptation to different thermal environments through thermal melanism may have allowed *H. axyridis* to colonise new areas. Genetic adaptation could potentially be rapid, as the alleles controlling melanism were already present at an intermediate frequency in the founding *H. axyridis* populations that arrived in Britain (Majerus & Roy, 2005). Phenotypic plasticity will allow an even faster change in response to the environment, which could be advantageous for the first generation of invaders. Both of these processes may have been important during the British invasion. There was a decline in the frequency of melanic alleles during the first two years of the spread of *H. axyridis*, suggesting that there was genetic adaptation to the new environment (Brown *et al.*, 2008a; M.E.N. Majerus, unpublished data). This is consistent with the rest of the invasive range of *H. axyridis*, where the highly plastic f. *succinea* is the dominant form across a variety of climatic conditions in Europe and North America (Majerus *et al.*, 2006). In contrast, within the native range of *H. axyridis* there are a variety of form frequencies, which seem to be local adaptations. The invasive populations therefore have greater phenotypic plasticity in melanism in response to temperature than the native ones, which may have facilitated the invasion.

In conclusion, our results indicate that the plasticity of colour pattern observed in *H. axyridis* f. *succinea* is an adaptation to increase the melanic area of the elytra, and hence activity level, when the insect is exposed to cold temperatures. This strengthens the thermal melanism hypothesis in the evolution of ectotherms. Theoretical models have demonstrated an advantage to a more plastic population in a variable environment (Thompson, 1991), and the benefits of being melanic are known to change through the seasons. This may be why the plastic f. *succinea* dominates in Great Britain where different generations experience different temperatures. Furthermore, this phenotypic plasticity may have been important in the invasion of *H. axyridis* in Europe and North America (LaMana & Miller, 1996; Majerus *et al.*, 2006). Perhaps, a species as plastic as *H. axyridis* was an unfortunate choice for a biocontrol agent, as the very plasticity that has made one particular morph so successful may also have contributed to its invasive success.

Acknowledgments

We thank Remy Ware, Emma Rhule and Ian Wright for assistance with practical work, and Anna Masson for kindly permitting the use of her photograph in Fig. 1f. Francis Jiggins is funded by a Royal Society University Research Fellowship.

References

Adriaens, T., Branquart, E. & Maes, D. 2003. The multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? *Belg. J. Zool.* **133**: 195–196.

Alpert, P. & Simms, E.L. 2002. The relative advantages of plasticity and fixity in different environments when is it good for a plant to adjust? *Evol. Ecol.* **16**: 285–297.

Banta, J.A., Dole, J., Cruzan, M.B. & Pigliucci, M. 2007. Evidence of local adaptation to coarse-grained environmental variation in *Arabidopsis thaliana*. *Evolution* **61**: 2419–2432.

Benham, B.R., Lonsdale, D. & Muggleton, J. 1974. Is polymorphism in 2-spot ladybird and example of non-industrial melanism? *Nature* **249**: 179–180.

Brakefield, P.M. & Willmer, P.G. 1985. The basis of thermal melanism in the ladybird *Adalia bipunctata* – differences in reflectance and thermal-properties between the morphs. *Heredity* **54**: 9–14.

Brown, M.W. & Miller, S.S. 1998. Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*. *Entomol. News* **109**: 143–151.

Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hagg, T., Kenis, M., Klausnitzer, B.E.M., Kovar, I., Loomans, A.J.M., Majerus, M.E.N., Nedved, O., Pedersen, J., Rabitsch, W., Roy, H.E., Ternois, V., Zakharov, I.A. & Roy,

D.B. 2008a. *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. *Biocontrol* **53**: 5–21.

Brown, P.M.J., Roy, H.E., Rothery, P., Roy, D.B., Ware, R.L. & Majerus, M.E.N. 2008b. *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid. *Biocontrol* **53**: 55–67.

Clusella-Trullas, S., Terblanche, J.S., Blackburn, T.M. & Chown, S.L. 2008. Testing the thermal melanism hypothesis: a macrophysiological approach. *Funct. Ecol.* **22**: 232–238.

Clusella-Trullas, S., van Wyk, J.H. & Spotila, J.R. 2009. Thermal benefits of melanism in cordylid lizards: a theoretical and field test. *Ecology* **90**: 2297–2312.

Colunga-Garcia, M. & Gage, S.H. 1998. Arrival, establishment, and habitat use of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Michigan landscape. *Environ. Entomol.* **27**: 1574–1580.

DeJong, P.W., Gussekloo, S.W.S. & Brakefield, P.M. 1996. Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spot ladybird beetles (*Adalia bipunctata*) under controlled conditions. *J. Exp. Biol.* **199**: 2655–2666.

DeWitt, T.J., Sih, A. & Wilson, D.S. 1998. Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**: 77–81.

Dobzhansky, T. 1924. Die geographische und individuelle Variabilität von *Harmonia axyridis* Pall in ihren Wechselbeziehungen. *Biol. Zentralbl.* **44**: 401–421.

Dobzhansky, T. 1933. Geographical variation in lady-beetles. *Am. Nat.* **67**: 97–126.

Engelmann, K.E. & Schlichting, C.D. 2005. Coarse- versus fine-grained water stress in *Arabidopsis thaliana* (Brassicaceae). *Am. J. Bot.* **92**: 101–106.

Guppy, C.S. 1986a. The adaptive significance of alpine melanism in the butterfly *Parnassius phoebus* f (Lepidoptera, Papilionidae). *Oecologia* **70**: 205–213.

Guppy, C.S. 1986b. Geographic variation in wing melanism of the butterfly *Parnassius phoebus* f (Lepidoptera, Papilionidae). *Can. J. Zool.* **64**: 956–962.

Hosino, Y. 1940. Genetical studies on the pattern types the lady-bird beetle, *Harmonia axyridis* Pallas. *J. Genet.* **40**: 215–228.

Jiang, W., Pan, X., Liu, J. & Liu, L. 2008. Studies on the type of spot on the wing of *Harmonia axyridis* (Pallas) in Baoding. *Hebei. J. For. Orchard Res.* **22**: 198–202.

Jing, Y. & Zhang, Y. 2001. Studies on the type of spot on the wing of *Harmonia axyridis* (Pallas) in Shanxi Province. *Journal of Shanxi Agricultural University* **4**: 42–45.

Kettlewell, H. 1955. Selection experiments on industrial melanism in the Lepidoptera. *Heredity* **9**: 323–342.

Kettlewell, H. 1956. Further selection experiments on industrial melanism in the Lepidoptera. *Heredity* **10**: 287–301.

Kettlewell, H. 1973. *The Evolution of Melanism*. Clarendon Press, Oxford.

Kingsolver, J.G. 1987. Evolution and coadaptation of thermoregulatory behavior and wing pigmentation pattern in Pierid butterflies. *Evolution* **41**: 472–490.

Koch, R.L. 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**: 32–48.

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

Komai, T. 1956. Genetics of ladybeetles. *Adv. Genet.* **8**: 155–188.

Komai, T., Chino, M. & Hosino, Y. 1950. Contributions to the evolutionary genetics of the lady-beetle, *Harmonia* .1. Geographic and temporal variations in the relative frequencies of the elytral pattern types and in the frequency of elytral ridge. *Genetics* **35**: 589–601.

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

van Lenteren, J.C., Loomans, A.J.M., Babendreier, D. & Bigler, F. 2008. *Harmonia axyridis* an environmental risk assessment for Northwest Europe. *Biocontrol* **53**: 37–54.

Levins, R., 1968. *Evolution in Changing Environments*. Princeton University Press, Princeton.

Lewis, J.E. 1985. Temperature induced seasonal melanism in the wings of *Copaeodes minima* (Lepidoptera, Hesperiidae). *Fla. Entomol.* **68**: 667–671.

Lindstedt, C., Lindstrom, L., Mappes & J. 2009. Thermoregulation constrains effective warning signal expression. *Evolution* **63**: 469–478.

Majerus, M.E.N. 1994. *Ladybirds*. The New Naturalist Library, Harper Collins, London.

Majerus, M.E.N. 1998. *Melanism: Evolution in Action*. Oxford University Press, Oxford.

Majerus, M.E.N. & Roy, H.E. 2005. Scientific opportunities presented by the arrival of the harlequin ladybird, *Harmonia axyridis*, in Britain. *Antenna* **29**: 196–208.

Majerus, M., 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.

Nault, B.A. & Kennedy, G.G. 2003. Establishment of multicolored Asian lady beetle in eastern North Carolina: seasonal abundance and crop exploitation within an agricultural landscape. *Biocontrol* **48**: 363–378.

Osawa, N. 2000. Population field studies on the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): resource tracking and population characteristics. *Popul. Ecol.* **42**: 115–127.

Pinheiro, J. & Bates, D. 2000. *Mixed-effects Models in S and S-PLUS*. Springer, New York.

Price, T.D., Qvarnstrom, A. & Irwin, D.E. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proc. R. Soc. Lond. B Biol. Sci.* **270**: 1433–1440.

van Rensburg, D.A.J., Mouton, P.L.N. & van Niekerk, A. 2009. Why cordylid lizards are black at the south-western tip of Africa. *J. Zool.* **278**: 333–341.

Roland, J. 1982. Melanism and diel activity of alpine Colias (Lepidoptera, Pieridae). *Oecologia* **53**: 214–221.

Sakurai, H., Kawai, T. & Takeda, S. 1992. Physiological-changes related to diapause of the lady beetle, *Harmonia axyridis* (Coleoptera, Coccinellidae). *Appl. Entomol. Zool.* **27**: 479–487.

Scheiner, S.M. & Lyman, R.F. 1989. The genetics of phenotypic plasticity. 1. Heritability. *J. Evol. Biol.* **2**: 95–107.

Soares, A.O., Coderre, D. & Schanderl, H. 2003. Effect of temperature and intraspecific allometry on predation by two phenotypes of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). *Environ. Entomol.* **32**: 939–944.

Solensky, M.J. & Larkin, E. 2003. Temperature-induced variation in larval coloration in *Danaus plexippus* (Lepidoptera: Nymphalidae). *Ann. Entomol. Soc. Am.* **96**: 211–216.

Tan, C.C. 1946. Mosaic dominance in the inheritance of color patterns in the lady-bird beetle, *Harmonia axyridis*. *Genetics* **31**: 195–210.
Tan, C.C. & Li, J.C. 1934. Inheritance of the elytral color patterns of the lady-bird beetle, *Harmonia axyridis* Pallas. *Am. Nat.* **68**: 252–265.
Thompson, J.D. 1991. Phenotypic plasticity as a component of evolutionary change. *Trends Ecol. Evol.* **6**: 246–249.
Trullas, S.C., van Wyk, J.H. & Spotila, J.R. 2007. Thermal melanism in ectotherms. *J. Therm. Biol.* **32**: 235–245.
Tutt, J. 1896. *British Moths*. George Routledge, London.
Via, S., Gomulkiewicz, R., deJong, G., Scheiner, S.M., Schlichting, C.D. & Vantienderen P.H. 1995. Adaptive phenotypic plasticity – consensus and controversy. *Trends Ecol. Evol.* **10**: 212–217.
Wang, S., Michaud, J.P., Zhang, R.Z., Zhang, F. & Liu, S. 2009. Seasonal cycles of assortative mating and reproductive behaviour in polymorphic populations of *Harmonia axyridis* in China. *Ecol. Entomol.* **34**: 483–494.
Woltereck, R. 1909. Weitere experimentelle Untersuchungen über Artverandenmg, speziell über das Wesen quantitativer Artunterschiede bei Daphniden. *Verh. Deut. Z.* **19**: 110–172.
Yuan, R., Zhang, F. & Wen, G. 1994. Color pattern of *Harmonia axyridis* in Changbai Mountains. *Jilin Agricul. Sci.* **4**: 45–54.

Supporting information

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

Figure S1 Spot size in melanic beetles at different temperatures. These individuals are all heterozygotes carrying the *axyridis* allele and one of the other alleles. Means and standard errors are general linear model estimates. These beetles are the offspring of the crosses in brackets, which refer to Table 1.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be reorganized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Received 9 March 2010; revised 22 April 2010; accepted 11 May 2010