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Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*

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Abstract

The evolutionary trajectories associated with demographic, genetic and spatial disequilibrium have become an issue of growing interest in population biology. Invasive species provide unique opportunities to explore the impact of recent range expansion on life-history traits, making it possible to test for a spatial arrangement of dispersal abilities along the expanding range, in particular. We carried out controlled experiments in laboratory conditions to test the hypothesis of an increase in dispersal capacity with range expansion in *Harmonia axyridis*, a ladybird that has been invading Europe since 2001. We found a marked increase in the flight speed of the insects from the core to the front of the invasion range in two independent sampling transects. By contrast, we found that two other traits associated with dispersal (endurance and motivation to fly off) did not follow the same spatial gradient. Our results provide a striking illustration of the way in which predictable directional genetic changes may occur rapidly for some traits associated with dispersal during biological invasions. We discuss the consequences of our results for invasion dynamics and the evolutionary outcomes of spatially expanding populations.

Introduction

Some invasive species have detrimental impacts on human health, economy or environment (Ruiz *et al.*, 2000; Pimentel *et al.*, 2001; Olden *et al.*, 2004), whereas others are regarded as beneficial organisms because they act as a source of food or help to regulate other damaging invasive species (Guillemaud *et al.*, 2011). Regardless, studies of all these organisms provide useful information about fundamental issues in ecology and evolutionary biology (e.g. Lodge, 1993; Huey *et al.*, 2005). The course of a biological invasion can be divided into three steps (Elton, 1958): (i) the introduction of a group of individuals into a remote area, (ii) their establishment and (iii) their spatial expansion into the new area. Each of these steps provides us with an opportunity to test theoretical predictions. In particular, spatial expansion can be used to explore the eco-evolutionary processes at work in populations in 'spatial disequilibrium' (sensu Phillips *et al.*, 2010), in which space plays a crucial role in shaping demographic, genetic and life-history trait patterns.

How do we expect genetic variation to be spatially distributed in a newly expanding population? We know that expanding populations are confronted by evolutionary forces, which vary spatially from the core to the front of the expansion. Genetic drift, for example, is thought to be an important factor during spatial expansion: at the front, fewer individuals are responsible for recurrent founder events, resulting in potentially large effects on genetic structure in this zone through the random loss of alleles, the stochastic variation of allele

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frequencies and spatial differentiation (Excoffier *et al.*, 2009). Various selective factors may also act during expansion, leading to spatially differential adaptation. One of these factors is population density, which may vary considerably along the expansion gradient, being high at the core of the expanding population and low at its edges. Consequently, density-dependent traits may evolve in opposite ways in different parts of the geographical range (Phillips, 2009; Phillips *et al.*, 2010; Kelehear *et al.*, 2012; Kilkenny & Galloway, 2013). Finally, dispersal capacity is probably heterogeneous over space, simply because the best dispersers are likely to be the first to found new populations and are therefore likely to be at the vanguard of the expanding front (Van Valen, 1971). Heritable traits associated with dispersal would therefore be expected to display deterministic sorting with range expansion (Shine *et al.*, 2011). The combination of this mechanism and the spatial heterogeneity of selective pressures may have major evolutionary consequences, modifying the overall frequencies of these traits in the population.

Higher levels of dispersal at the edge of a spatially growing population have been predicted by a number of recently developed theoretical models, with and without the assumption of a variation of selective pressures with density and/or space (Travis & Dytham, 2002; Hughes *et al.*, 2007; Phillips *et al.*, 2008; Travis *et al.*, 2009, 2010; Burton *et al.*, 2010; Shine *et al.*, 2011; Benichou *et al.*, 2012). By analysing proxies of dispersal abilities, this prediction has been confirmed in a number of species displaying range expansion around their native areas in response to climate change (e.g. Cwynar & Macdonald, 1987; Hill *et al.*, 1999; Thomas *et al.*, 2001; Simmons & Thomas, 2004; Darling *et al.*, 2008; Leotard *et al.*, 2009), but in only a few introduced invasive species (but see Phillips *et al.*, 2006; Monty & Mahy, 2010; Berthouly-Salazar *et al.*, 2012). Invasions constitute natural situations in which this question can be addressed appropriately for several reasons: (i) in some cases, a precise recent history of introduction and spread is available; (ii) many invasions are still underway, resulting in a maintenance of the spatial disequilibrium and (iii) progressive changes to the environment (e.g. climate change) are not a prerequisite (Hill *et al.*, 2011). Moreover, the question of the evolution of dispersal capacity in invasive populations is highly relevant, because it may accelerate spread, with serious applied and theoretical consequences (Travis & Dytham, 2002; Phillips *et al.*, 2007, 2010; Travis *et al.*, 2009; Benichou *et al.*, 2012; Lehe *et al.*, 2012). Two obstacles may hinder the emergence of a spatial gradient of dispersal traits with range expansion: (i) founder events during introduction may greatly decrease the genetic variability of the traits associated with dispersal and (ii) introduction may have occurred too recently for the observation of measurable changes in the spatial distribution of genetic variation. However, recent studies have indicated that strong genetic bottlenecks are less frequent than previously thought in successful invasive populations (Bossdorf *et al.*, 2005; Wares *et al.*, 2005; Dlugosch & Parker, 2008), and rapid evolutionary changes have repeatedly been observed in invasive species (e.g. Huey *et al.*, 2005; Whitney & Gabler, 2008).

In this study, we investigated the spatial variation of dispersal abilities during the spread of the harlequin ladybird *Harmonia axyridis* in Europe. This insect species of Asian origin combines a number of characteristics making it a good model for testing the prediction that dispersal levels are higher at the edge of the range of an introduced population that is expanding spatially. First, its worldwide invasion is very well documented (Lombaert *et al.*, 2010, 2011; Brown *et al.*, 2011). In particular, *H. axyridis* was first observed in 2001 in Belgium (Adriaens *et al.*, 2003), and its spread in Europe has been well monitored ever since (Brown *et al.*, 2011). Second, this species is known to have passed through only moderate bottlenecks during its introduction into Europe (Lombaert *et al.*, 2010; Facon *et al.*, 2011). Indeed, the genetic diversity of the European invasive population is almost as broad as that of the native populations. This high degree of genetic diversity can be explained at least partly by an admixture event between a wild eastern North American invasive population and a laboratory strain formerly used for biocontrol purposes in Europe (Lombaert *et al.*, 2010, 2011; Turgeon *et al.*, 2011). Third, heritable variation for dispersal capacity is known to exist in *H. axyridis*: differential flight capacities have been selected independently in the laboratory on at least two occasions: once in a native population (Seko *et al.*, 2008) and once in a European biocontrol strain (Tourniaire *et al.*, 2000).

We characterized the spatial variation of *H. axyridis* dispersal abilities during the expansion of its range in Europe. We collected ladybirds from eight sites, along two transects, extending from the core of the population to two different edges of the invaded area in Europe. We then used selectively neutral genetic markers (microsatellites) to check that all the insects were derived from the expansion of the same population unit. Finally, we studied three traits associated with flight, which is known to be the major process involved in dispersal of ladybirds (Hodek & Honek, 1996; Brown *et al.*, 2011), in controlled laboratory experiments: flying speed, flying endurance and motivation to fly off.

Materials and methods

Sampling and rearing

The invasion of Europe by *H. axyridis* probably began in 2001, as indicated by the first records of feral populations near Ghent and Brussels in Belgium, followed by a rapid demographic and spatial expansion over much of Europe (Adriaens *et al.*, 2008; Brown *et al.*, 2011).

We thus considered the centre of Belgium to be the invasion core. Data on the spatial and temporal progress of the invasion front in France (date of first observation in each French administrative area (*département*)) were obtained from the French national *H. axyridis* survey (Fig. 1, Ternois, 2010). Based on our knowledge of the expansion history of *H. axyridis*, we collected, between October and November 2010, eight *H. axyridis* population samples along two transects (four samples per transect) extending from the core region of the European invasion (Brussels area) to the invasion fronts in Southern and Western France (Table S1, Fig. 1). All population samples consisted of at least 90 live adult individuals collected from an area of less than 10 km². Each population sample was characterized by (i) the transect to which it belonged (transect ID), (ii) its latitude, (iii) the geographical distance, in kilometres, to the core sample, measured with Google Earth V4.3 (Google, 2008) and (iv) the date on which the species was first observed at the sampling site concerned (Ternois, 2010) (Table S1, Fig. 1). As expected, these last two variables were highly correlated (Pearson's correlation coefficient = 0.95; $P < 10^{-4}$). In subsequent statistical analyses, we decided to use the date of the first observation to account for the distance to the core, because it was less sensitive to environmental variation (which may artificially slow down or accelerate spread) than geographical distance in kilometres.

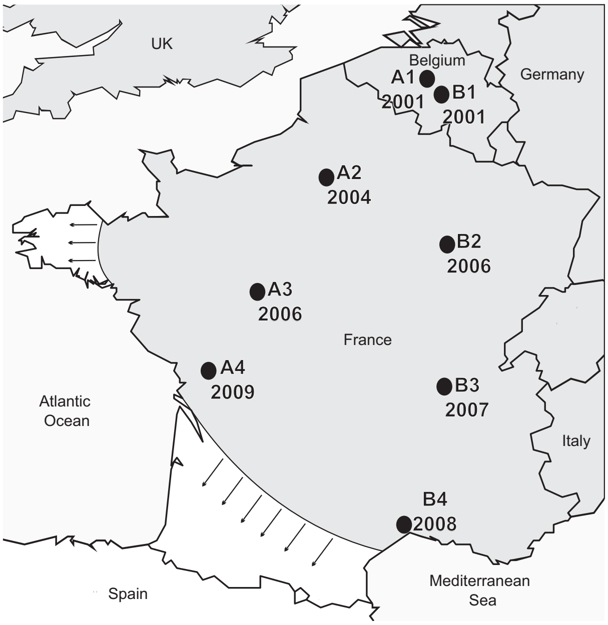

Fig. 1 Geographical origins of the *Harmonia axyridis* samples collected along two transects in Western Europe. The core area of the invasive outbreak is located in Belgium. The grey area roughly corresponds to the distribution of the species in 2010. Arrows indicate the direction of expansion. Each black spot corresponds to a population sample. Letters (A or B) indicate the transect, and the associated numbers give the sample codes (see Table S1). The years indicated below the sample codes are the dates on which the species was first observed at the geographical site concerned (see Table S1).

Before beginning the controlled laboratory experiments, we reared all eight population samples collected *in natura* (G_0) in the laboratory for two generations (G_1 and G_2), under strictly controlled conditions, to minimize biases due to maternal and environmental effects (but see Braendle *et al.*, 2006). During these two generations, individuals were fed exclusively on ionized *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs and reared in constant environmental conditions (23 °C; 50% HR; L : D 16 : 8). For each population, G_1 was initiated from 90 G_0 individuals (45 males and 45 females) and G_2 was initiated from 100 G_1 individuals (50 males and 50 females). The rearing protocol design is described in Fig. S1. Generation G_2 contained a large number of individuals (more than 1600 individuals per population), and males and females were separated immediately after emergence to prevent mating, which is not a prerequisite for dispersal in this species (Brown *et al.*, 2011). G_2 individuals were then maintained in the same environmental conditions for 2 weeks, to ensure that they were sexually mature at the start of the experiment.

Genetic differentiation between populations

Many studies have suggested that multiple introductions of invasive species might be a common phenomenon (Bossdorf *et al.*, 2005; Estoup & Guillemaud, 2010). We therefore began by checking that the eight population samples used in this study were part of the same spatial expansion of a single invasive outbreak. We genotyped 31 G_0 individuals from each of the eight population samples at 18 microsatellite loci, as described by Loiseau *et al.* (2009). The degree of genetic differentiation between population samples was assessed by calculating pairwise F_{ST} estimates (Weir & Cockerham, 1984). Fisher's exact tests, based on contingency tables of genotypic frequencies, were also carried out for all pairs of populations, with Genepop software (Raymond & Rousset, 1995; Rousset, 2008). Finally, we used the Bayesian clustering approach implemented in STRUCTURE v2.3.3 (Pritchard *et al.*, 2000) to infer the number of population units within the area studied (see Fig. S2 for a description of the analysis).

Flight speed and endurance experiment

We used an automated flight mill system (Movie S1), which made it possible to assess the flying speed and endurance of 10 individuals simultaneously. Each of the ten mills was isolated in a box and consisted of a horizontal steel rod bent by 90° at both ends: the first end was inserted into the hollow needle of a vertical syringe head, which served as a rotation axis. The second end was used as the attachment point for the insect. The horizontal part of the steel rod was 15.915 cm long. Thus, when an insect completed one complete rotation, it was considered to have covered a distance of 1 m. An infrared ray emitted by a photogate was interrupted by a flag attached to the middle of the steel rod. This photogate was connected to a computer, which recorded the time co-ordinates for each rotation. The whole flight mill system was located in a chamber maintained at 23 °C, with controlled lighting and air movement.

Before each trial, ladybirds were isolated and starved for one hour. Body weight, sex and elytral colour patterns (hereafter referred to as 'morphs', corresponding to four of the five classes proposed by Seo *et al.*, 2008) were also recorded. For each mill, a ladybird randomly chosen from the eight population samples and the two sexes was fixed, by the pronotum, to the attachment point. Recording began as soon as the insect began to fly, which was generally immediately, and lasted for one hour. In total, 752 individuals were tested (see Table S2), corresponding to a mean of 47 replicates per population and per sex (minimum = 45; maximum = 49).

We analysed the following response variables: (i) the total distance travelled over the entire 60 min (referred to hereafter as 'flight speed') and (ii) the $[2^*D_{30}/D_{60}]$ ratio (hereafter referred to as 'endurance'), where D_{30} is the distance travelled during the first 30 min and D_{60} is the total flight mill distance. For both response variables, we used mixed general linear models ('lmer' function from the 'lme4' R package) with the following factors included as fixed effects: the date of first observation and the latitude of each collected sample, the day of the experiment (day 1 is the first day of the experiment, which lasted 17 days, this variable being highly correlated with the age of the ladybirds), body weight, sex and transect ID (A or B, Fig. 1). Three factors were also included as random effects: flight mill ID number (10 levels), morph (four levels) and population sample (eight levels) acting on the transect fixed effect. We used the Akaike information criterion (AIC) to select the best model, with the following model comparison procedure (Zuur *et al.*, 2009). We first selected the best random effect structure, by comparing models with different random effects but with the same fixed structure (referred to hereafter as the full fixed structure), which included all simple fixed effects and simple interactions between the date of first observation and either sex or transect. Once the best random effect structure had been identified (lowest AIC value), we compared all models with various degrees of complexity of the fixed effect structure but the same random effect structure. We thus compared the full range of models, extending from a model with no fixed effects to the full fixed structure, and we retained the model with the lowest AIC.

Experiment assessing the motivation to fly off

We assessed the motivation of *H. axyridis* individuals to fly off, by a method similar to that described by Li *et al.* (2010). The flight stands consisted of an inverted white plastic funnel (h: 180 mm, dia.: 110 mm at base). The base of the funnel was surrounded by water, to prevent the individuals from walking off the stand. An individual that had been isolated and starved in a 2-mL opaque microtube for 3–6 h was installed on the base of the funnel. The experiment began with the opening of the tube. The occurrence of flight and the time from the tube being opened to take-off were then recorded with Observer 5.0 software (Noldus Information Technology, Wageningen, The Netherlands). The trial ended when the adults flew off the stand or when 300 s had elapsed. Four individuals were tested in parallel during a single trial, with a space of 10 cm between adjacent funnels. Individuals were chosen randomly from the eight population samples, but all the insects in any given trial were of the same sex, to prevent mating pheromone interactions. The sex and morph of the insects tested were recorded. The experiment took place in a growth chamber maintained at 23.5 °C, with controlled lighting and air movement. Temperature and humidity were recorded at the start of each trial. In total, we tested 760 individuals, corresponding to a mean of 47.5 replicates per population and per sex (minimum = 45; maximum = 48).

The following response variables were considered: (i) take-off behaviour (the proportion of 'flyers') and (ii) the time from release of the individual (opening of the tube) to take-off (referred to hereafter as 'take-off time'). We analysed the proportion of flyers with mixed generalized linear models with a binomial probability distribution and a logit link function (the 'lmer' function from the 'lme4' R package). For the take-off time variable, we used a Cox's proportional hazards model for survival data with random effects (the 'coxph' function in the R 'survival' package). For both response variables, we considered the following factors as fixed effects: the date of first observation, the latitude of each sample collected, the day of the experiment (day 1 is the first day of the experiment, which lasted 12 days, this variable being strongly correlated with the age of the ladybirds), the isolation time (i.e. the time spent by each individual in the microtube before the trial), sex and transect ID (A or B). Three factors were also included as random effects: funnel ID number, morph and population sample (eight levels), nested within transect. The best model was selected as that with the smallest AIC, according to the procedure described in the flight mill experiment section. All statistical analyses were performed with R software V2.13.0 (R_Development_Core_Team, 2011).

Results

Genetic differentiation between populations

Pairwise F_{ST} estimates were low, with a mean value of −0.001 and no value exceeding 0.007. None of the 28 exact tests of genotypic differentiation gave a significant P-value at the 5% threshold (smallest P-value = 0.058), even without correction for multiple comparisons. STRUCTURE clustering analysis provided consistent results over the 20 runs tested for each K value. The mean natural logarithm of the likelihood of the data (lnP(X|K)) was maximal for $K = 1$. Mean lnP(X|K) decreased (the ΔK method was thus not applicable, Evanno *et al.*, 2005), and its variance increased with increasing values of K (Fig. S2). In addition, individual Q-values for $K = 2$ revealed that all individuals were similarly admixed (coefficient of variation of Q = 7.6%), as expected when no genetic structure is supported by the data (Pritchard *et al.*, 2003). Overall, the microsatellite data provide strong evidence that all the samples were derived from the expansion of a single population unit.

Flight speed and endurance

For the response variable 'flight speed', the final model included the date of first observation, latitude, day of the experiment, body weight, sex, transect, sex x date of first observation and transect x date of first observation interactions as fixed effects and mill ID number as a random effect. The statistical results obtained with this model are reported in Table 1. A highly significant fixed effect was found for the date of first observation ($P < 10^{-3}$). Flight speed was higher for individuals collected from sites with more recent first observation dates (i.e. closer to the front) (Fig. 2). Mean flight speed increased from about 2500 to 2800 metres per hour from the core to the front. The only other significant fixed effect was the day of the experiment ($P < 10^{-3}$): beetles travelled further in the last few days of the experiment than in the first few days. This probably reflects differences in the ages of the ladybirds, which were all born at about the same time (the maximum age difference between the oldest and youngest insects was 4 days). Randomization within our experimental design (Fig. S3) ensures us an absence of correlation between both significant fixed effects, date of first observation and day of the experiment (Pearson correlation coefficient = 0.035, $P = 0.333$). Body weight was not significant at the 5% threshold ($P = 0.055$), but a slight trend towards highest flight speed in the largest ladybirds was observed (mean weight of ladybirds per population sample can be found in Table S2). None of the other variables or interactions included in the model selected were significant: sex ($P = 0.225$) and transect ($P = 0.824$) had no significant effect, as well as latitude ($P = 0.072$) despite a strong correlation (Pearson correlation coefficient = −0.912, $P < 10^{-3}$) with the highly significant date of first observation variable (see above).

For the response variable 'flight endurance', the best model, with the lowest AIC criterion, included no fixed effect and the variable 'mill' as a random effect, indicating that endurance was similar between samples. The overall mean value of endurance ratio was greater than one (1.16), indicating that ladybirds covered greater distances during the first half of the flight (first 30 min) than during the second half of the flight.

The response variables of the two Belgian population samples corresponding to the core of the two transects did not differ significantly, and analyses in which the two core populations were inversed (each

Table 1 Results obtained with the best model selected from the various linear mixed models run for the trait 'flight speed'.

	Total flight mill distance			
Parameter				
Fixed effect	Estimate	Std error	t-value (df = 735)	P
Date of first observation	93.01	26.91	3.457	$<10^{-3}$
Latitude	65.10	36.08	1.804	0.072
Day of the experiment	42.35	5.40	7.843	$<10^{-3}$
Body weight	10.55	5.48	1.923	0.055
Sex	123.53	131.46	0.940	0.348
Transect	144.07	113.34	1.271	0.204
Sex × date of first observation	−22.54	18.57	−1.213	0.225
Transect × date of first observation	−4.65	21.15	−0.220	0.826
Random effect			−2 Log-likelihood ratio (df = 1)	P
Mill			5.476	0.019

used in the other transect) gave similar results (results not shown).

Motivation to fly off

For both studied response variables, that is, the proportion of flyers and take-off time, the final selected model included funnel ID number as a random effect, and transect and isolation time as fixed effects (Table 2). Thus, for these two response variables, the date of first observation had no significant effect. We found a significant ($P < 10^{-3}$) effect of isolation time on both the proportion of flyers and take-off time, with the motivation to fly off being greater for longer isolation times. In both bases, the transect effect was significant ($P < 0.05$), with a larger propensity to fly in transect B (e.g. $P_{FLY}\_transectA = 0.45$ and $P_{FLY}\_transectB = 0.53$). Despite the absence of significant differences between the two Belgian population samples, the inversion of these two samples eliminated the transect effect for both response variables (results not shown).

Discussion

Considerable interest has recently focused on the evolution of nonequilibrium populations as a result of spatial spread during range expansion (e.g. Excoffier & Ray, 2008; Excoffier *et al.*, 2009; Sexton *et al.*, 2009; Phillips *et al.*, 2010; Hill *et al.*, 2011; Shine *et al.*, 2011). Changes in range margin due to modern-day climate

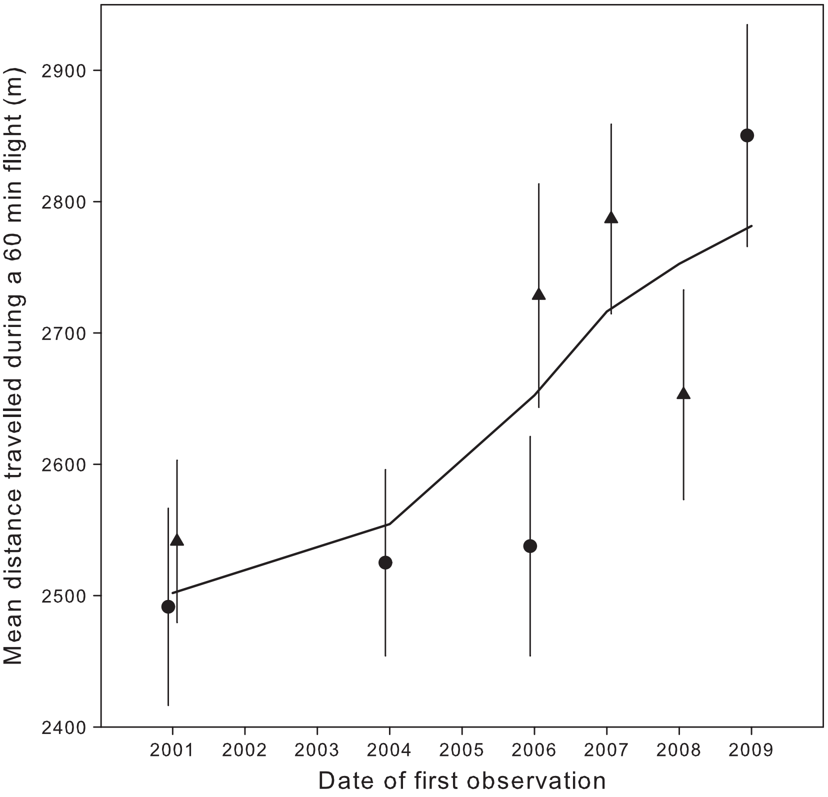

Fig. 2 Mean distance travelled in 1 h by *Harmonia axyridis* in flight mills, as a function of dates of first observation of the species during range expansion. Triangles and circles indicate mean population values for transects A and B, respectively. The vertical bars are standard errors. The line corresponds to the mean values predicted by the selected statistical model (see Table 1).

Table 2 Results obtained with the best model selected from the various statistical models run for the traits assessing the motivation to fly off (i.e. proportion of flyers and time to take-off).

	Proportion of flyers				Time to take-off			
	Selected random effect = funnel				Selected random effect = funnel			
Fixed effects	Estimate	Std error	*z*-value (df=739)	*P*	Estimate	Std error	Chi²	*P*
Isolation time	0.005	0.001	3.323	$<10^{-3}$	0.004	0.001	12.66 (df = 1)	$<10^{-3}$
Transect	0.301	0.147	2.046	0.041	0.241	0.104	5.33 (df = 1)	0.021

change and the current explosion in the number of biological invasions have led to many recent studies on this topic (Phillips *et al.*, 2010; Hill *et al.*, 2011). It has been predicted that dispersal capacity is greater at the edge of expanding populations than at their core (Travis & Dytham, 2002; Phillips *et al.*, 2010; Shine *et al.*, 2011). We tested this hypothesis for a well-known invasive insect, through controlled laboratory experiments and the use of an appropriate sampling design.

Evidence for the evolution of flying speed

We found clear evidence of a strong, rapid increase in flight speed with range expansion of *H. axyridis* in Western Europe. We found that individuals with grandparents sampled close to the front covered larger distances (~ +10%) during a one-hour trial in a flight mill than individuals with grandparents sampled close to the core of the invasion. The front populations used here were first observed in 2008 and 2009, depending on the transect, whereas the core population samples were first observed in 2001. The cline of dispersal capacity observed in this study was thus remarkably rapid, as it occurred in less than a decade (i.e. in less than 20 generations, assuming two generations per year, Koch, 2003). Population genetic structure analyses confirmed that all samples originated from the expansion of a single population unit. The observed variation of dispersal ability over space is, therefore, not due to several spatially independent introductions of genetically differentiated groups of *H. axyridis* ladybirds. Instead, it likely corresponds to the post-introduction organization of genetic variation within a single geographically continuous population. The absence of a significant effect of transect variable (alone or in interaction) suggests that the observed spatial gradient for flight speed was reproducible over space and was therefore unlikely to have occurred by chance, through genetic drift or mutation surfing (Hallatschek *et al.*, 2007). Finally, the absence of a significant effect of latitude suggests that the observed spatial gradients were not due to adaptation to a geoclimatic cline.

By contrast, we detected no spatial variation of flight endurance and, therefore, no trade-off between flight speed and endurance in *H. axyridis*, although such a trade-off might be expected (e.g. Wilson & James, 2004; Stephens & Wiens, 2008; Oufiero *et al.*, 2011). Longer flight mill trials may be required to detect differences in endurance between insects from the core and front populations of this invasive species. However, although not significant, the observed trend towards greater endurance at the front than in the core is not consistent with a trade-off.

We also observed no significant variation in motivation to fly off over the expansion range. However, the moderate effect of transect does suggest that variations in motivation to fly off may have a genetic basis, which would make it possible for evolutionary responses to occur. Unlike flight speed, the motivation to fly off is a complex behavioural trait that may be subject to several different selection pressures associated with resource foraging, mating and escaping from predators. The effect of the spatial sorting of dispersal may thus be attenuated by these complex patterns of selective pressure in traits of this type.

Evolutionary consequences of the spatial sorting of dispersal traits

The spatial sorting of genetically controlled traits underlying dispersal abilities over the expansion range is likely to have important evolutionary consequences. For example, a rare allele or a new mutation that can surf on the expansion wave, reaching high frequencies in newly colonized areas (Edmonds *et al.*, 2004; Klopfstein *et al.*, 2006; Excoffier & Ray, 2008; Hallatschek & Nelson, 2008; Lehe *et al.*, 2012), will end up being associated with the more dispersive genotypes of the population (Travis *et al.*, 2010). Moreover, the spatial variation of selective pressures in expanding populations is likely to lead to the negative or positive selection of genotypes with strong dispersal abilities at the level of the overall population (Phillips *et al.*, 2010). For example, Allee effects may constitute a disadvantage for the good migrants, due to the low population densities encountered at the invasion front (e.g. Travis & Dytham, 2002). However, this issue may not be particularly important in *H. axyridis,* as it has recently been shown that inbreeding depression (one of the mechanisms underlying Allee effects, Courchamp *et al.*, 1999) has been purged from invasive populations of this species, including the European population (Facon *et al.*, 2011). On the other hand, selection for dispersal is possible, as in the case of classical negative density dependence: low levels of intraspecific competition at the invasion front result in higher growth rates (e.g. Travis & Dytham, 2002; Burton *et al.*, 2010), conferring a selective advantage on the best dispersers.

Further studies are required to determine the importance of the observed spatial sorting of flight speed over the expansion of the range of *H. axyridis* in the evolution of this species and its current invasion of Europe. Indeed, it is not possible to conclude definitively from our results that there has been selection for the flight speed of *H. axyridis* during expansion in Europe, as the spatial cline observed here may result solely from the spatial rearrangement of individuals according to their dispersal capacity (Shine *et al.*, 2011). This issue could be resolved by analysing the overall dispersal polymorphism of the population over time, to assess potential changes to genetically determined dispersal trait frequencies. However, based on current knowledge of the biology of *H. axyridis*, including its highly generalist nature in food and habitat use (Koch, 2003; Brown *et al.*, 2011), and the purging of deleterious mutations during invasion (Facon *et al.*, 2011), we suggest that the benefits of being in a new environment with lower intraspecific competition are likely to outweigh the costs in this species. This has not yet been demonstrated unequivocally for *H. axyridis*, but the rapid spread of invasive *H. axyridis* populations over various continents suggests that this is probably the case (Brown *et al.*, 2011).

Conclusion and perspectives

Harmonia axyridis can now be found on almost all continents (Africa, South America, North America, Europe, Brown *et al.*, 2011). The expansion of the range of this species in continents other than Europe may provide more robust, independent, experimental replicates for confirming and extending our results. However, we should first properly explore the heritability of dispersal abilities, which is highly suggested but not formally demonstrated in our experiment. In general, it would be interesting to explore the genomic basis of the observed trait variation, to improve our understanding of the genetic and selective mechanisms at work and to facilitate explorations of the fate of dispersal polymorphism during the entire worldwide invasion process.

This study is one of the first to provide strong evidence for rapid spatial sorting of a trait directly associated with dispersal with spatial expansion of the range of an invading species. The observed shift towards a higher flying speed at the invasion front of European *H. axyridis* populations was remarkably rapid, as it was demonstrated after only 8 years of expansion, corresponding to about 16 generations (Koch, 2003), highlighting a rather high rate of evolution (~ 0.07 haldanes, Haldane, 1949; Hendry & Kinnison, 1999; Gingerich, 2001), even in the case of invasive species (Prentis *et al.*, 2008; Whitney & Gabler, 2008). These results have important implications at both applied and fundamental levels. We expect there to be a rapid acceleration in the rate of spread of this harmful species (Travis & Dytham, 2002; Phillips *et al.*, 2007), and a rapid evolution of life-history traits positively and negatively linked to dispersal. Future studies on invasive species, including *H. axyridis*, should consider the evolution of other important traits, such as competitiveness and rates of reproduction and cannibalism during spatial expansion (Phillips *et al.*, 2010; Rudolf *et al.*, 2010). Information about the evolution of these additional key life-history traits, together with findings for dispersal, would provide useful insight into the dynamics of invasive species.

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Supporting information

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

Figure S1 Rearing protocol design.

Figure S2 Likelihood of the multilocus genetic data as a function of the number of genetic clusters among European samples of *Harmonia axyridis*.

Figure S3 Number of ladybirds used every day during the flight mill experiment.

Table S1 European Harmonia axyridis samples collected between October and November 2010 along two transects extending from the outbreak core to zones close to the invasion front (see Fig. 1).

Table S2 Number (n) and mean weight in milligrams (with standard deviation) of male and female ladybirds tested in the flight speed and endurance experiment.

Movie S1 The flight mill system.

Data deposited at Dryad: doi:10.5061/dryad.v1s3s

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