

Genetic variation in growth and development time under two selection regimes in *Leptinotarsa decemlineata*

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Abstract

It is possible to predict the potential range of a species on the basis of its ecological characteristics and those of the invaded ecosystem. The existence of genetic variation indicates a species' potential to respond to new environmental conditions, thus facilitating its success as an invader. Accordingly, evolutionary and ecological approaches are needed to identify the factors explaining both species' range and their potential to invade new areas. We combined these two approaches and studied whether genetic variation in life-history traits under abiotic (temperature) and biotic (host plant) selection pressures contributes to the potential range expansion of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). We reared full-sib families of *L. decemlineata* from the current northernmost European population at 15 °C (temperature below that in the current range) and 25 °C (optimal temperature) and on three potato varieties. We monitored development time, adult weight and larval-to-adult survival, and estimated the amount of heritable variance. The development time and adult weight of progenies were more variable between than within families. Thus, there was genetic variability in traits relevant to the ability to adapt to a colder environment (i.e., accelerate development and become heavier) allowing range expansion further north in Europe, even though low temperature increased beetle mortality. Temperature strongly affected all traits measured. Potato variety, in turn, did not strongly affect beetles' performance. Beetle ability to adapt to a cool environment was further enhanced by the fact that size was not constrained by fast development. The results showed that beetle populations possess genetic variation allowing a response to temperature and thus have the evolutionary potential to adapt and spread beyond their current range.

Introduction

During the past 200 years, the number of species that have spread to new areas has increased dramatically (di Castri, 1989) and the rate of introduction is predicted to further increase because of international travel and trade (Levine & D'Antonio, 2003). Only a tiny fraction (1%) of all invasion events is, however, successful (Williamson & Fitter, 1996) but global climate warming may increase the success rate (e.g., Jeffree & Jeffree, 1996; Crozier, 2004; Crozier & Dwyer, 2006). This raises the question of why so few species finally manage to establish themselves in a previously unoccupied area and what we can do to predict future success in the

face of ecological change. The issue is of interest to applied biologists, as invaders can severely impact invaded ecosystems (reviewed by Mack et al., 2000; Mooney & Cleland, 2001). To mitigate the impact, eradication programs are needed. The eradication of alien species is more likely to be successful if invaders are detected at the earliest possible stage of an invasion (Genovesi, 2005) when the population size is small. Early detection and rapid response require that we have tools to predict the probability of a biotic invasion.

We can take an ecological and/or evolutionary approach to identify the factors explaining both species borders and invasion success. An ecological approach investigates the requirements of a species and compares them to the environmental conditions in a new habitat (Heger & Treppl, 2003; Peterson et al., 2007). If environmental conditions

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are suitable, invasion success increases. It is difficult to make general rules that would predict the invasion outcome for all species and, therefore, we have to treat each potential invader as a special case (Lodge, 1993) although Arim et al. (2006) established general rules for the invasion process. Climate is one of the most important abiotic factors affecting insects' success and, consequently, determining their geographic range (Hoffmann & Blows, 1994; Hochachka & Somero, 2002; Gaston, 2003). Non-optimal temperatures can kill individuals or affect the duration of a certain stage of their life cycle (Hochachka & Somero, 2002), thus impacting fitness. Along with temperature, the characteristics of host and food plants, such as secondary plant products (see, for example, Brower et al., 1972; Hare, 1987) and nutrients (e.g., Hare, 1987; Hunt et al., 1992), also affect insect fitness. Furthermore, the ability to use a specific plant species as host might vary with temperature (Hilbeck & Kennedy, 1998). Thus, performance differences may be influenced by both the characteristics of plant species and ambient temperature.

An ecological approach does not take into account that a population can respond to new environmental challenges through evolutionary adaptation, resulting in increased mean fitness. The prerequisites for evolutionary change are genetic variation and selection. For example, time available for development varies with latitude and, thus, this time stress selects for fast growth rates at high latitudes (e.g., Conover & Schultz, 1995; Gotthard et al., 1999; Margraf et al., 2003). If there is genetic variability, the evolution of traits to adapt to a new environment can be fast (Reznick & Ghalambor, 2001). For example, a new fruit fly (*Drosophila subobscura*) was introduced to the USA from Europe and within 20 years of the invasion, the species had evolved a cline where wing length increased with latitude (Huey et al., 2000). Blows & Hoffmann (1993) determined that evolutionary constraints (e.g., lack of heritable genetic variation for desiccation resistance) can limit expansion in *Drosophila serrata*. A genetic approach apprehends that the successful colonisation of a new environment may require genetic variability in traits relevant to adaptation to new conditions (Ellstrand & Schierenbeck, 2000; Lee, 2002).

Previous studies on invasive species have mainly focused on species that had already established themselves in novel areas (Kolar & Lodge, 2001). *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) is a species that is currently expanding its range (Plant Production Inspection Centre, Helsinki, Finland), providing us with a unique opportunity to study the invasion process before establishment has occurred. We can proactively assess whether the species is able to expand its range by estimating its potential for evolutionary response to novel environmental conditions. Studying *L. decemlineata* also offers an opportunity to

observe evolutionary changes as an invasive species enters or tries to enter novel areas. This might lead to a broader understanding of factors explaining invasion success or failure. Our series of studies on this species also provide needed science-based information for managers to plan methods to prevent and eradicate pest species.

Leptinotarsa decemlineata spread from the USA to Europe in the 1920s and currently occurs throughout Europe except for Britain, Ireland and Scandinavia, having its northern range limit in Russia (60°N) (EPPO, 2006). *Leptinotarsa decemlineata* seems to have arrived in Europe in a single successful invasion. The population was subjected to a founder effect during its introduction, causing a decrease in the level of mitochondrial and nuclear variability in the beetles (Grapputo et al., 2005). Thus, we might expect the beetles to also show low genetic variation in fitness-related traits. However, a small inoculating population size does not always result in reduced genetic diversity in fitness traits (Zeisset & Beebe, 2003; Zenger et al., 2003). Invaders have often undergone bottlenecks during range expansion that might have decreased the success, and consequently impeded the likelihood of further range expansion (Gaston, 2003) but did not necessarily do so (Tsutsui et al., 2000).

The focus of our work was to investigate whether genetic variability exists in a marginal population, even though the original European invading population of *L. decemlineata* was small. To estimate the amount of genetic variability (i.e., family effect), we reared full-sib *L. decemlineata* larvae that were descendants of beetles collected from range margins (St. Petersburg Region, Russia) under two temperature regimes and on three potato varieties to determine reaction norms for development time and adult weight. Second, we addressed the correlated ecological questions of whether the environmental conditions of the target area fit the beetles' requirements or whether it is the cooler climate that prevents *L. decemlineata* from spreading. Thus, the experimental temperature regimes were chosen so that we could study the performance of the beetles under conditions that match those beyond the current borders of the species. This allowed us to assess whether conditions in a novel environment are suitable for these beetles. The ambient temperature and the length of the growing season are the most probable abiotic explanations of the northern borders of the beetles' current range, as they have few natural enemies (Hough-Goldstein et al., 1993b). Their aposematic coloration, combined with their distastefulness (Hough-Goldstein et al., 1993a), creates an enemy-free habitat for the beetles. The absence of natural enemies would facilitate expansion (Wolfe, 2002; Torchin et al., 2003). Third, we determined whether developmental times differ between individuals reared on different diets at

various temperatures. The various potato varieties on which beetles fed, differed in foliar glycoalkaloid (plant secondary metabolite) content (Lyytinen et al., 2007) that might reduce the herbivore's performance. According to a previous experiment, beetle performance was not affected by larval host although beetles showed hierarchies of preference between them (Lyytinen et al., 2007). However, in that experiment, all beetles were reared under one temperature regime. The result might not be applicable to all thermal conditions, as insect herbivore performance can be affected by interactions between a specific allochemical and the thermal environment where it inhabits (e.g., Stamp & Yang, 1996). Therefore, in this experiment, larvae were reared on three diets at two temperatures. The fitness indices were larval-to-adult survival, development time, and adult size as measured by body mass. By assessing both ecological attributes (phenotypic plasticity) and the evolvability of an invasive species, we were able to predict its potential for range expansion (Lee, 2002; Gaston, 2003).

Materials and methods

Colorado potato beetles

Adult Colorado potato beetles (16 females) were collected in 2001 from the region of St. Petersburg (59°57'N), Russia, which represents a peripheral population and a likely source of beetles that are able to invade Finland. They formed the parental generation of our study. The parental generation overwintered in a laboratory, after which females were allowed to lay eggs. Thus, dams experienced the same environmental conditions prior to (i.e., during diapause), and during egg laying. Randomly paired beetles were maintained in a greenhouse in individual Petri dishes on moistened filter paper and fresh potato foliage (varieties were the same as in the experiment, see below) that was replenished daily. We could not confirm that females were unmated before overwintering. We could, however, assume that most of the larvae were sired by sperm from the last mating: in *L. decemlineata*, the last male accounts for the greatest proportion of offspring (Roderick et al., 2003) and spring matings have sperm precedence over stored sperm (Baker et al., 2005). Newly laid eggs from adult beetles were collected daily for 5 days and egg masses were placed in Petri dishes on filter paper saturated with water. Eggs were kept in a greenhouse having a natural photoperiod until they hatched. Newly hatched larvae were randomly assigned to one of the treatments described below.

Host plants

Three host plants, varieties of potato, *Solanum tuberosum* L. (Solanaceae), were used as treatments: Nevsky (the Russian variety), Hankkijan Timo (an early season variety

in Finland) and Van Gogh (an intermediate season variety in Finland). They differ in foliar glycoalkaloid content (α -chaconine and α -solanine) [$\mu\text{g}/\text{mg}$ (mean \pm SE) dry weight, Nevsky: 11.154 ± 1.114 ; Timo: 9.125 ± 1.013 ; Van Gogh: 14.821 ± 1.054] but contain equal amounts of nitrogen (Lyytinen et al., 2007). Tuber seeds were planted in pots (3 l) containing fertilised soil (N:P:K, 8:4:14%; Kemira, Helsinki, Finland), and were grown in a greenhouse under natural light conditions (23 °C). To minimise the effect of leaf senescence, new tuber seeds were planted regularly.

Larval performance

Newly hatched larvae were collected over 1–2 days to achieve sufficient individuals to assign to each treatment. The first instars from each dam (maximum 30 larvae from each female) were paired by weight (within the family) and half of the brood ($n = 16$) was assigned to the lower and half to the higher temperature regime. Larvae were reared at a constant temperature of either 15 °C or 25 °C in growth chambers and under a photoperiod of L18:D6. These two thermal regimes represent the average and an unusually warm Finnish summer, respectively (Drebs et al., 2002). Thus, we reared beetles under conditions (15 °C) that match those beyond their current borders. In St. Petersburg, the average temperature in June is 15 °C and in July 18 °C (www.eurometeo.com).

The larvae were raised singly in a Petri dish in growth chambers. For both temperature treatments, the larvae of each female were evenly divided among the three host plants (maximum five larvae from each female per treatment). Thus, we created six groups of larvae according to the two temperatures and three host plants. If a female did not produce an adequate number of offspring to divide evenly between treatments, its larvae were assigned to the 15 °C treatment, where survival was expected to be the lowest.

Larvae were fed on detached potato foliage from one of the three potato varieties, Van Gogh (15 °C: $n = 86$; 25 °C: $n = 61$), Timo (15 °C: $n = 85$; 25 °C: $n = 60$), or Nevsky (15 °C: $n = 58$; 25 °C: $n = 59$), that were harvested every day from greenhouse-grown potato plants so that they always had enough food. Larvae were subjected to this regime until the end of the fourth instar, when they were transferred individually to jars (10 cm high \times 8.5 cm in diameter), covered with a net and filled with peat for pupation. The pots were checked daily, at the same time of day, for emergence. The young adult beetles were weighed on the day they emerged. Survival and the number of days required for larvae to complete their development (from first instar to emergence) were recorded at both temperatures.

Data analysis

Factors affecting survival were analysed using a binary logistic regression model with survival (alive/dead) as a dependent variable and temperature and host plant as covariates. Two-way interactions between temperature and host were not significant (binary logistic regression: $P \geq 0.202$), indicating that temperature affected survival similarly on all three potato varieties. Therefore, these interactions were not included in the model. The effect of the weight of first instars was tested with a Mann–Whitney U-test. As it is not possible to distinguish between female and male larvae, the survival is not sex-specific.

We assessed whether development times for beetles reared at different temperatures and on different potato varieties were significantly different with a three-way ANOVA (the data were also analysed with the maximum likelihood method which confirmed the results). To control for variation in the timing of the experiment (the experimental day), we calculated unstandardised residuals for the development times corrected by the experimental day from a linear regression. Because the structure of the data on development times did not meet the assumptions of parametric statistics, we transformed the data to ranked data, where cases with the same value as the original variable got the average of their ranks. We then applied a three-way ANOVA where the fixed factors were temperature and host and the random factor was family. If interactions were not statistically significant, they were omitted stepwise from the final models, starting with the highest P-value. Sexes were ranked and analysed separately, as females completed their development faster than males. The difference was significant at 25 °C (Mann–Whitney U-test: $Z = -2.941$, $P = 0.003$) although both sexes emerged within 1 day on average (mean \pm SE: 20.43 \pm 0.12 for females vs. 20.91 \pm 0.14 for males). At 15 °C, there was no difference in male (62.29 \pm 1.33) and female (62.02 \pm 0.88) development times (Mann–Whitney U-test: $Z = -0.124$, $P = 0.901$). In cases where the interaction was significant, main effects were analysed with univariate tests. In cases where the interaction was not significant, we used post-hoc tests for pairwise comparisons.

Because adult weight affects fitness, we recorded the weight of newly emerged adults and tested this against temperature and potato variety. As the structure of the data did not meet assumptions of parametric statistics, we transformed the data to ranked data and then applied a two-way ANOVA with temperature and host as fixed factors and family as the random factor (we also performed the maximum likelihood method which gave similar results). If interactions were not statistically significant, they were omitted stepwise from the final models, beginning with the highest P-value. Larval

weight on emergence was not included in the analysis, as it did not correlate with adult weight (Spearman correlation: $P \geq 0.299$). Females and males were analysed separately because there was a significant difference in adult weight between the sexes at both temperatures, with females being larger (one-way ANOVA: $F_{1,72} = 7.011$, $P = 0.010$ at 15 °C; $F_{1,136} = 22.213$, $P < 0.001$ at 25 °C). Pairwise comparisons were performed with post-hoc tests.

Because the host plant affected both adult size and development time only slightly (see Results), data from the potato varieties was pooled in order to test whether there was a correlation between these traits. A Spearman correlation was performed for the pooled data. The means from each family within the experimental day were used as variables.

In order to predict the ability of beetles to respond to natural selection, three estimates were calculated: heritability (h^2), the additive genetic coefficient of variance (CV_A) and I_A -evolvability. Narrow sense heritabilities for larval-to-adult development time and adult mass were estimated by the formula given in Roff (1997) for the full-sib design: $h^2 = 2(MS_{AF} - MS_{AP}) [MS_{AF} + (k-1)MS_{AP}]^{-1}$ where MS_{AF} is the among-families variance, and MS_{AP} is the within-family variance. As family size was unequal, k is the weighted estimate. Only families with more than one offspring surviving to adulthood were included in the estimation of heritability. Because heritability can vary with physical environment (Hoffmann & Merilä, 1999), the estimates were calculated within temperature regime (the sexes were calculated separately). Individuals reared on different host plants were pooled because variety did not greatly affect the life history traits of the beetles (see Results), and by doing this the number of offspring per family was higher, producing a more reliable heritability estimate. In a full-sib design, V_G includes dominance variance as well as maternal effect, which was minimised by keeping dams under equal environmental conditions prior to (i.e., during diapause) and during egg laying (Fox, 2000). However, we were not able to completely eliminate maternal effects and therefore h^2 should be interpreted as an upper bound. The additive genetic coefficient of variance (CV_A) was calculated with a formula $CV_A = 100 \times^{-1} \sqrt{V_A}$ where \times is the sample mean and $V_A = h^2 \times V_P$ with V_P calculated as the sample variance (Houle, 1992). When h^2 was negative, we did not calculate CV_A . As CV_A lacks a biological interpretation, we further calculated I_A -evolvability which is $(CV_A/100)^2$ (Houle, 1992). A value of $100 \times I_A$ can be interpreted as a percentage trait change per generation (Hansen et al., 2003). All analyses were performed using SPSS version 10.1 (SPSS Inc., Chicago, IL, USA). All P-values are two-tailed.

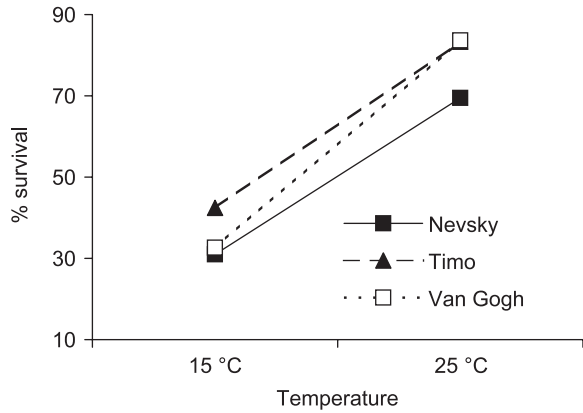


Figure 1 Survival of *Leptinotarsa decemlineata* larvae fed on three potato diets [filled squares: Nevsky (Russian variety); filled triangles: Hankkijan Timo; open squares: Van Gogh] at 15 °C and 25 °C. Survival was determined by dividing the total number of adults emerged by the total number of larvae reared per potato variety.

Results

Temperature- and host-dependent survival

As expected, temperature affected larval-to-adult survival, being, in general, higher at 25 °C (78.8%) than at 15 °C (35.8%) (binary logistic regression, overall model: $x^2 = 84.307$, Wald = 70.618, d.f. = 1, $P < 0.001$; Figure 1). The host plant tended to affect larval survival to some extent (binary logistic regression: Wald = 5.249, d.f. = 2, $P = 0.072$). Survival

for larvae fed on Nevsky was lower than for those fed on Timo (Wald = 5.248, d.f. = 1, $P = 0.022$), but similar to those fed on Van Gogh (Wald = 1.736, d.f. = 1, $P = 0.188$). The survival rate was independent of the weight of first instars [Mann–Whitney U test, at 15 °C: $Z = -0.899$, $P = 0.369$, mean (mg) \pm SE = 0.58 ± 0.02 ; at 25 °C: $Z = -0.945$, $P = 0.345$, mean (mg) \pm SE = 0.59 ± 0.02].

Temperature- and host-dependent development times

Female development time. The significant interaction between host and temperature indicates that the effect of the host on development time differed with temperature (Table 1). Given this significant interaction, we performed univariate tests, which revealed that development time was indeed slightly dependent on the host at 15 °C ($F_{2,80} = 3.523$, $P = 0.034$; Table 2), but not at 25 °C ($F_{2,80} = 0.335$, $P = 0.716$). At the lower temperature, beetles tended to reach adulthood slowest when fed on Timo (the least significant difference (LSD), Timo vs. Nevsky: $P = 0.021$; Timo vs. Van Gogh: $P = 0.035$; Nevsky vs. Van Gogh: $P = 0.457$). Temperature accelerated larval development time on all diets (univariate test, Nevsky: $F_{1,80} = 91.682$, $P < 0.001$; Timo: $F_{1,80} = 308.739$, $P < 0.001$; Van Gogh: $F_{1,80} = 200.248$, $P < 0.001$). On average, it took 20.43 days to develop from first instar to adult at 25 °C, whereas at 15 °C it took 62.02 days. The interaction between temperature and family was also highly significant for development time, indicating that larvae of different dams reacted differently to temperature (Figure 2). Family affected development time at both

Table 1 ANOVA tests for development time and emergence weight in female and male *Leptinotarsa decemlineata*

Sex	Source	Development time				Weight			
		d.f.	MS	F	P	d.f.	MS	F	P
Female	F	15	577.841	1.332	0.31	15	1606.218	1.766	0.09
	T	1	61717.495	150.603	<0.01	1	5125.209	8.737	<0.01
	H	2	184.789	1.537	0.22	2	2729.651	3.026	0.06
	F*T	12	446.689	3.716	<0.01	¹			
	F*H	¹				25	952.503	1.624	0.06
	T*H	2	391.626	3.258	0.04	¹			
	Error	80	120.198			67	586.598		
Male	F	14	996.639	3.225	0.04	14	1192.645	1.852	0.04
	T	1	50643.698	163.399	<0.01	1	13378.940	20.773	<0.01
	H	2	98.535	0.564	0.57	2	1965.489	3.052	0.05
	F*T	9	314.757	1.801	0.08	¹			
	F*H	¹				¹			
	T*H	¹				¹			
	Error	76	174.790			84	644.067		

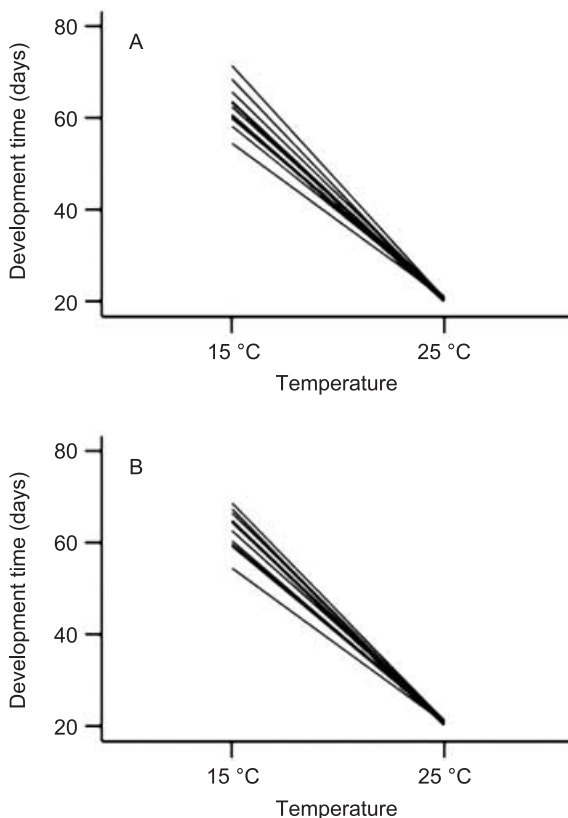
Univariate tests further revealed that female development time differed among hosts at 15 °C and among families at both temperatures. F, family (random factor); T, temperature (fixed factor); H, host plant (fixed factor); MS, mean square; d.f., degrees of freedom.

¹Not included in the final model.

Table 2 The number of days required for *Leptinotarsa decemlineata* to complete development and the weight (mg) of newly emerged adult beetles at 15 °C and 25 °C on three potato varieties

Temperature	Variety	Sex	Development time (days)			Weight (mg)		
			No.	Mean	SE	No.	Mean	SE
15 °C	Nevsky	♀	9	59.11	1.882	8	122.15	4.895
		♂	6	63.33	2.765	6	102.52	6.778
	Timo	♀	22	64.05	1.157	21	103.70	3.529
		♂	13	61.92	2.466	13	91.76	4.427
	Van Gogh	♀	17	60.94	1.578	17	102.09	4.667
		♂	9	62.11	1.467	9	95.50	5.198
25 °C	Nevsky	♀	22	20.59	0.284	22	129.50	4.672
		♂	19	21.00	0.324	18	115.93	3.344
	Timo	♀	20	20.30	0.128	20	120.00	3.287
		♂	29	20.79	0.188	29	110.87	3.387
	Van Gogh	♀	23	20.39	0.163	23	126.33	4.020
		♂	27	20.96	0.247	26	108.79	2.797

temperatures (univariate test, 25 °C: $F_{12,80} = 11.874$, $P < 0.001$; 15 °C: $F_{15,80} = 1.869$, $P = 0.039$). However, there was more variation in development times among families at 15 °C.

**Figure 2** Reaction norms for *Leptinotarsa decemlineata* family development times (days) in response to two temperatures, 15 °C and 25 °C (A: females, B: males). Data from all potato varieties was pooled. Each line represents a family mean.

Male development time. Both two- ($P \geq 0.259$) and three-way ($P = 0.339$) interactions were non-significant and were therefore excluded from the final model (Table 1). The interaction between temperature and family, which was marginally non-significant, was included in the final model. The larval host plant did not affect larval development time. Temperature had a significant effect on larval development time, with a three-fold longer development time at 15 °C (mean \pm SE = 62.29 ± 1.33 days) than at 25 °C (20.91 ± 0.14 days) (Table 2). Family had a slight effect on larval development time, indicating that progeny from different mothers developed at different speeds (Figure 2, Table 1).

Adult weight at different temperatures and on different potato varieties

Female adult weight. Two-way interactions between host and temperature ($P = 0.856$) and family and temperature ($P = 0.866$) were non-significant for females in the full model and were therefore excluded from the final model. There was a nearly significant interaction between host and family (Table 1). The effect of temperature on the weight of the newly emerged female adult beetles was significant (Tables 1 and 2). Adults attained a larger body size when the temperature was high. Furthermore, female size was slightly dependent on potato variety, with females attaining a larger body size when larvae fed on Nevsky rather than on Timo (LSD: $P = 0.004$) or Van Gogh (LSD: $P = 0.005$) at 15 °C. Other pairwise comparisons were non-significant at both temperatures (LSD: $P \geq 0.078$). There was also a nearly significant family effect (Figure 3). As there was a nearly significant interaction between host and family, we also analysed the main effect of family with

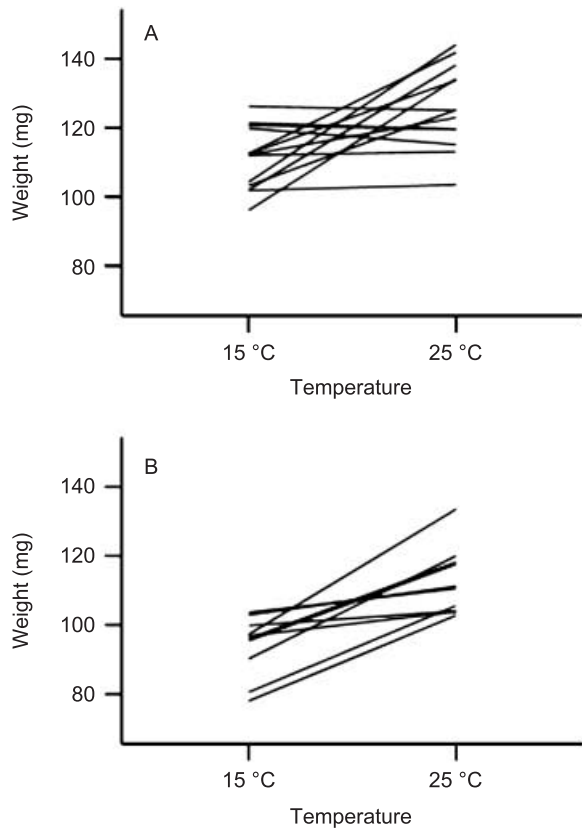


Figure 3 Reaction norms for the weight (mg) of newly emerged adult *Leptinotarsa decemlineata* (A: females, B: males) across two temperatures of 15 °C and 25 °C. Data from potato varieties was pooled.

univariate tests. These tests revealed that individuals from different dams reached significantly different adult weights when reared on Van Gogh ($F_{15,67} = 2.407$, $P = 0.007$) but not when reared on Nevsky ($F_{11,67} = 1.855$, $P = 0.062$) or Timo ($F_{14,67} = 1.428$, $P = 0.165$).

Male adult weight. Two-way interactions between host and temperature ($P = 0.830$), host and family ($P = 0.768$) and family and temperature ($P = 0.913$) were non-significant for male weight in the full model and were therefore excluded from the final model. Like females, males reared at the higher temperature attained a larger body size (Tables 1 and 2). Potato variety had some effect on male weight. Post-hoc tests revealed that males reared on Nevsky tended to be slightly, but not significantly, larger than those individuals that fed on Van Gogh ($P = 0.056$) or Timo at 25 °C ($P = 0.088$). Other pairwise comparisons were highly non-significant at both temperatures ($P \geq 0.132$). Family affected male weight slightly (Figure 3).

Correlation between adult size and development time

There was a significant negative correlation between larval development time and adult weight within sexes at 25 °C (Spearman, females: $r = -0.865$, $P < 0.001$; family $n = 13$; males: $r = -0.703$, $P = 0.011$; family $n = 12$; Figure 4). This indicates that fast-developing individuals became heavier than individuals that grew more slowly. At 15 °C, the correlation between development time and adult weight was also negative, but non-significant (females: $r = -0.234$, $P = 0.383$, $n = 16$; males: $r = -0.456$, $P = 0.117$, $n = 13$). The low number of offspring per dam precluded exploration of correlations between families.

Heritability estimates

Heritability estimates were relatively low, ranging from -0.125 to 0.033 for development time and from -0.041 to 0.448 for adult mass (Table 3). Evolvabilities, which indicate the rate of change per generation as a response to selection, were in the range 0.01 – 0.09 for development and 0.92 – 0.98 for adult mass. Evolvabilities were calculated

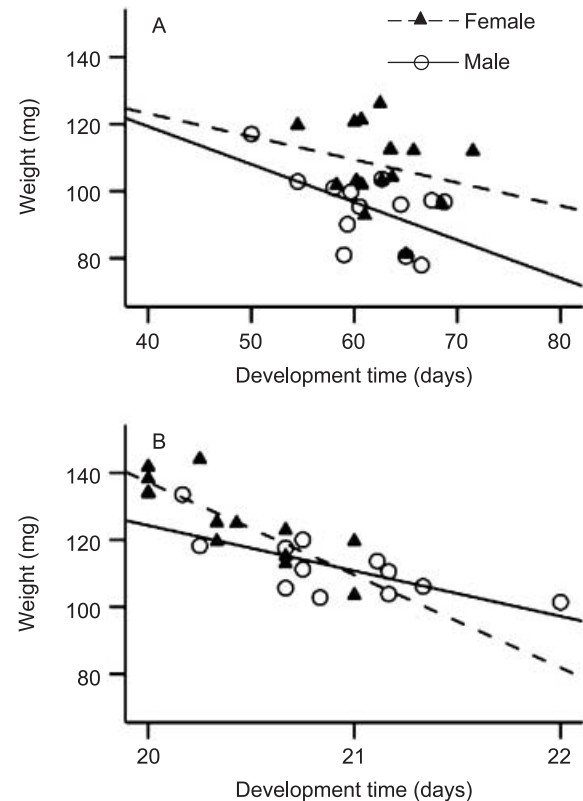


Figure 4 The relationship between development time (days) and adult weight (mg) at two temperatures (A: 15 °C, B: 25 °C) in *Leptinotarsa decemlineata* reared on potato. The symbols represent family means for females (filled triangles, broken line) and males (open circles, solid line).

Table 3 Heritability estimates ($h^2 \pm SE$), the additive genetic coefficient of variance (CV_A), and I_A -evolvability (%) at two temperatures of development time (days) and adult weight (mg) in *Leptinotarsa decemlineata*

Temperature	Sex	Development time				Weight			
		No.	$h^2 \pm SE$	CV_A	I_A	No.	$h^2 \pm SE$	CV_A	I_A
15 °C	♀	16	0.093 ± 0.31	2.98	0.09	15	-0.041 ± 0.27	–	–
15 °C	♂	13	0.033 ± 0.45	1.98	0.04	10	-0.448 ± 0.36	–	–
25 °C	♀	13	-0.125 ± 0.15	–	–	13	0.425 ± 0.26	9.92	0.98
25 °C	♂	12	0.020 ± 0.16	0.82	0.01	12	0.448 ± 0.25	9.60	0.92

only for beetles reared at the higher temperature because of negative heritabilities at 15 °C.

Discussion

We set out to assess the potential of *L. decemlineata* to invade north of its current range in Europe. This potential for northward invasion can be detected when we compare the performance of beetles from different dams. Development time and adult size (we found differences for females only when the host was Van Gogh) for progeny from different dams was variable among families, suggesting the presence of heritable variability for these traits. A significant interaction between family and temperature in development time for females suggested the same conclusion. The presence of adaptive genetic variation is surprising because beetles have successfully invaded Europe only once and have a reduced level of mitochondrial and nuclear variability compared to North American populations (i.e., the source of the invasion) (Grapputo et al., 2005). Our results and those of Boman et al. (2008) suggest that beetles from a marginal population possess the evolutionary capacity to improve their performance in response to changes in, for example, temperature, facilitating range expansion (see Endler, 1986; Lee, 2002; Gaston, 2003). Genetic diversity in traits relevant for adaptation to the conditions in new habitats has been suggested to be more important than a species' broad physiological tolerance (i.e., if an organism is able to withstand, for example, a broad range of temperatures) or plasticity (i.e., the same genotype produces different phenotypes depending on environmental conditions) in determining success in range expansion (Lee, 2002). The evolvabilities in this study, however, predict that development time can change only a fraction of a percent per generation. This means that it would take 18 and 40 generations for female and male beetles, respectively, to accelerate development by 1 day at 15 °C. This, together with the fact that heritabilities were low, as is common for life history traits (Mousseau & Roff, 1987), suggests that the shortness of growing season might slow their northward

spread. Adult emergence weight, in turn, possesses a higher, albeit low, capacity to change (1% per generation) but only when beetles were reared at a high temperature.

The rate of adaptive changes depends not only on the strength of selection and the amount of heritable variance for a trait but also on trade-offs acting against adaptive change. Often, life-history studies find a trade-off between an individual's final size and its development time (Klingenberg & Spence, 1997; but see Rodrigues & Moreira, 2002; Chown & Nicolson, 2004), but this was not the case in *L. decemlineata*. In fact, the beetles that reached adulthood the fastest were also the heaviest (Figure 4). As both traits (rapid development and large body size) are crucial contributors to insect fitness (e.g., Roff, 1992; Zhou et al., 1995; Gotthard et al., 1999), the lack of trade-off between these traits may partly explain the beetles' successful range expansion and would allow further northward invasion.

Although evolutionary processes play a crucial role in invasion success, the beetles' range expansion does not necessarily require an evolutionary response from them. Beetles seemed to tolerate colder conditions than in their current range. They were able to complete their development at a temperature of 15 °C in a time-frame comparable to the length of the growing season beyond their current range. For example, the mean temperatures for the summer seasons (June–August) 1971–2000 in southern Finland ranged between 14.6 and 15.9 °C (Drebs et al., 2002). Therefore, even though only 36% of larvae survived to adulthood when reared under the cool conditions this would slow (as population size decreases; Memmott et al., 2005) rather than prevent invasion. This is because mortality rate would have only a relatively moderate impact on population size due to the high fecundity of female beetles. Furthermore, high fecundity increases the probability of successful invasion (Kolar & Lodge, 2001; Sakai et al., 2001) as rapid population growth can ameliorate reductions in genetic diversity (Zenger et al., 2003). We did not, however, keep eggs at 15 °C but eggs are able to hatch at 17 °C (A Lyytinen, S Boman, L Lindström & J Mappes unpubl.). Thus, we assume they could also do so at a

temperature 2 °C lower, as shown in an experiment conducted in North America (Ferro et al., 1985). Alternatively, beetles may expand their range in the event of climate change if the temperature in the north increases and new, ecologically suitable, habitats become available (Jeffree & Jeffree, 1996).

Although beetle survival differed slightly with larval host, the impact was not as pronounced as for temperature. Nevertheless, as mortality due to food plant was additive, not compensatory, it cannot be seen as a negligible mortality agent but a substantial contributor to total mortality. However, there were no significant effects of host plant on male development time, whereas larval host was found to be marginally significant in explaining differences observed in female development time at 15 °C. Females tended to develop slowest when their larval host was Timo. Furthermore, the effect of host was dependent on temperature and hence development time and consequently success is dependent, at least slightly, on the joint effect of the composition of cultivated potato varieties and temperature. This might be due to the interactive effects of glycoalkaloids (or other plant characteristics) and temperature on beetle performance (see Stamp & Yang, 1996). Interactive effects might explain the discrepancy between the results of this and a previous experiment (Lyytinen et al., 2007) that did not find any effects of potato variety when beetles were reared at 23 °C. The contrasting results demonstrate the importance of conducting experiments that aim to determine the dependence of insect performance on host plant under at least two temperature regimes. As beetle performance varies with potato variety, along with temperature, the composition of cultivated potato varieties might affect the northward expansion of *L. decemlineata*. Under extreme conditions, where temperature strongly retards the beetle performance, these minor differences might additionally impair beetle performance and thus affect the outcome of the invasion attempt.

In this paper, we have focused on the role of temperature, host plant and genetic variation in invasion success but other factors also contribute to the outcome of an invasion. One hindrance to successful colonisation is natural enemies (Shea & Chesson, 2002) as they can be important mortality factors and thus reduce population growth rate. *Leptinotarsa decemlineata* has, however, relatively few natural enemies (Hough-Goldstein et al., 1993b) and, in addition, some of its enemies have a higher temperature threshold for development than do the beetles and, consequently, have not accompanied the beetle to new areas. For example, its egg parasitoid *Edovum puttleri* does not survive at low temperatures (Obrycki et al., 1985; Lashomb et al., 1987) and is unable to overwinter in temperate regions (Obrycki et al., 1985). Furthermore, beetles

have aposematic coloration (sensu Poulton, 1890) and are distasteful (Hough-Goldstein et al., 1993a) which makes them an undesirable prey for potential predators. These attributes have created an enemy-free habitat for beetles that might have facilitated their spread in Europe (see Torchin et al., 2003). The impact of predation and parasitism on beetle density is further reduced by the beetle's high fecundity, ranging from one to 1300 eggs per female in the laboratory (A Lyytinen, pers. obs.). Thus, it is unlikely that predation would considerably hinder beetles' expansion.

Although there are ecologically suitable conditions for *L. decemlineata* in unoccupied areas and adaptive response is possible for new abiotic factors, we cannot predict with certainty whether beetles will actually establish themselves in a new area. There are at least two reasons for uncertainty: human activities and winter conditions. Pest management may retard the beetles' spread (see Genovesi, 2005) as, in Finland, eradication has so far been successful. On the other hand, the use of insecticides has resulted in the development of resistance to a wide variety of chemicals that have been used against this beetle (e.g., Casagrande, 1987; Hawthorne, 2001). Additionally, distribution might be limited by the ability of a given species to overwinter (Valosaari et al., 2008). Beetles must acquire enough lipid reserves (which they use as their principal energy source during diapause) in the limited time available to survive the long northern winter (see Pullin, 1987; Zhou et al., 1995). According to our unpublished results, beetles are able to store enough lipid reserves in their body fat to enable them to spend a long time in diapause. Thus, the geographic range of *L. decemlineata* is the outcome of different selection pressures. Ambient temperature alone can retard but might not totally prevent the spread of beetles further north because beetles are rather tolerant of low temperature and, more importantly still, possess evolutionary capacity to respond to selection by temperature.

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