

Energy use, diapause behaviour and northern range expansion potential in the invasive Colorado potato beetle

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Summary

1. As organisms expand their range towards northern latitudes they will encounter selective factors like harsh winter conditions. The ability to cope with and adapt to harsh winters may depend on the variability and evolutionary potential of relevant traits.

2. One adaptation in insects is winter diapause. It is characterized by changes in physiology, behaviour or in both. Physiological changes include lowered metabolic rate that enhances survival by saving limited energy reserves during overwintering. Active behavioural changes like burrowing into the soil allow individuals to escape harsh conditions.

3. We examined variation in overwintering body mass, resting metabolic rate (CO₂ production) and diapause behaviour (burrowing into the soil), and their effects on overwintering success in the adult Colorado potato beetles (*Leptinotarsa decemlineata*). We conducted a full-sib/half-sib rearing experiment to estimate the evolutionary potential (heritability) of these traits.

4. High overwintering body mass and low metabolic rate were phenotypically associated with high diapause propensity (i.e. burrowing), which was linked to high overwintering survival. We found that once beetles had entered the soil, only large body mass of males was associated with high overwintering survival. However, the heritability estimates in all traits examined were low.

5. Our results show that winter conditions impose selection on diapause behaviour, which was linked to lower metabolic rate and larger body mass. If range expansion to higher latitudes requires adaptive genetic changes in diapause behaviour, metabolism, or body mass, the insufficient genetic variation in these traits suggest that the Colorado potato beetle's future potential to respond to selection due to harsher winters could be limited and thus, its range expansion could be hindered. Both physiological and behavioural adaptations are important to consider when assessing range expansion potential.

Key-words: distribution, evolutionary potential, invasive species, survival, temperate species

Introduction

Understanding factors that influence a species' range is a central issue in ecology and evolutionary biology (Gaston 2003; Sexton *et al.* 2009). Species distributions are often shaped by biotic (e.g. predation) or abiotic (e.g. temperature) stress factors (Blows & Hoffmann 1993; Hoffmann *et al.* 2003; Kellermann *et al.* 2009; Lee *et al.* 2009). For instance, at higher latitudes, prolonged and often harsh winter periods expose insects to a high amount of stress (e.g. cold, starvation and desiccation) that can directly limit

northern range expansion. Many insects survive winter through behavioural and physiological adaptations, including diapause (Tauber, Tauber & Masaki 1986; de Kort 1990). Therefore, the extension of distribution ranges may depend on the variability and evolutionary potential in traits associated with diapause.

Successful diapause is a dynamic manifold physiological process (Kostál 2006) that often requires the completion of certain physiological changes (Noronha & Cloutier 2006; Hahn & Denlinger 2007) and correct behavioural responses to environmental cues. For example, insects that diapause as adults must accumulate sufficient fat reserves before entering diapause since these reserves are the main source of energy

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during long winter periods (Hahn & Denlinger 2007). In addition to energy reserves, diapause involves physiological changes, such as increased cold tolerance (Hahn & Denlinger 2007) and a greatly lowered energy metabolism compared to the active state (Denlinger, Willis & Fraenkel 1972; Wipking, Viebahn & Neumann 1995).

Lower energy metabolism has been assumed to relate to higher overwintering survival by saving limited energy reserves (Han & Bauce 1998; Eilers & Van Alphen 2002; Irwin & Lee 2003) and to influence post-diapause performance such as fecundity (Irwin & Lee 2000). On the other hand, higher metabolic rate of ectothermic species or populations in colder environments than those in warmer environments may be beneficial by enabling the individual to develop and grow faster in relatively short and cool growing seasons (i.e. metabolic cold adaptation hypothesis) (Fox 1939; Chown & Gaston 1999; Addo-Bediako, Chown & Gaston 2002; Lardies, Bacigalupe & Bozinovic 2004). These contrasting views reflect the general discussion around the metabolic rate. Although it is commonly assumed that metabolic rate plays a strong role in life history traits (Chown & Gaston 1999) and stress resistance (Parsons 2005), it is less certain whether the high metabolic rate increases or decreases individual's fitness (Nilsson 2002; Artacho & Nespolo 2009; Ketola & Kotiaho 2009, 2010).

Selection experiments have shown that metabolic rate can change in response to direct selection (Ksiazek, Konarzewski & Lapo 2004) or as a result of indirect selection for resistance to other stressors (Hoffmann & Parsons 1989a,b). However, to date, only a few studies have estimated the heritability of metabolic rate in insects (Rantala & Roff 2006; Nespolo, Castañeda & Roff 2007; Ketola & Kotiaho 2009; Klemme & Hanski 2009), and these have found relatively low and non-significant heritabilities, typical to fitness related traits (Mousseau & Roff 1987; DeRose & Roff 1999).

Predicting species range changes has become especially important in regard to the recent increase in alien species invasions (Sakai *et al.* 2001; Suarez & Tsutsui 2008). Predictions should incorporate potential of the species to respond to selection exerted by new stressful conditions. The Colorado potato beetle (*Leptinotarsa decemlineata*) (Fig. 1) is an invasive pest of potato that has expanded its distribution over a wide range of latitudes and environmental conditions during the last century and a half (Casagrande 1987; EPPO 2006). It originates in Mexico where its original host plant is buffalo burr (*Solanum rostratum*), but it adapted to use the potato as a host in the 1850s. In Europe, the beetle's range expansion has slowed down at the northernmost latitudes of its distribution. Currently, the northernmost permanent populations are in Russia, near 62°N (EPPO 2006). Previous studies have shown that the northern range of this species is not limited by summer conditions such as summer frosts or low average summer temperatures (Boman *et al.* 2008; Lyytinen, Lindström & Mappes 2008; Lyytinen *et al.* 2009), but more likely by high mortality during winter diapause (Tauber & Tauber 2002; Hiisaar *et al.* 2006; Valosaari, Aikio & Kaitala 2008).



Fig. 1. An adult Colorado potato beetle (*Leptinotarsa decemlineata*). Photograph taken by Jurian Törnroth.

We examined the influence of body mass and metabolic rate on diapause behaviour (burrowing into the soil for diapause) and overwintering success in the European Colorado potato beetle adults, using a population originating from the northern range limit. We hypothesized that high body mass, indicative of energy reserves and low metabolic rate increase overwintering survival. We also studied the adaptive potential of these traits. A full-sib/half-sib experiment allowed us to examine whether there is additive genetic variation in physiological and life-history traits (metabolic rate, body mass, diapause behaviour and overwintering survival) that would allow the beetles to respond to the selection exerted by harsh winter conditions and potentially expand their range further north.

Materials and methods

STUDY SPECIES AND REARING

The Colorado potato beetles used in the study were the third generation descendants of beetles collected in Republic of Karelia in the Northern Russia (61°49'N, 34°10'E) in 2006 ($N = 917$). To maintain genetic variability in the laboratory stock at least 50 families (over 1000 individuals) were reared in each generation. Adult beetles were individually reared at a constant temperature of 23 °C under a fluctuating light regime of L : D 18 : 6 (16 h light, 4 h dark, 2 h dim light imitating sunset and sunrise) in a controlled environmental chamber (Type B1300; Weiss technic, Reiskirchen-Lindenstruth, Germany). For the purpose of estimating trait heritabilities, each sire ($N = 26$) was mated to three unrelated females. However, not all females produced offspring and hence 16 males had only 1–2 females (in total 55 full-sib families). Eggs were collected daily. Within 5 days of hatching, larvae that belonged to the same egg clutch were divided equally among three to five potato plants (variety Van Gogh) planted in pots so that each potato plant had a maximum of 15 larvae. A maximum of 45 larvae per family were reared. Larvae were reared at 20 °C (± 1 °C) in a greenhouse under natural light conditions (Agrifood Research Finland, Jokioinen, Finland, 60°48'N, 23°29'E, early August L : D 18 : 6). Larvae were allowed to pupate and pots were checked daily for newly emerged adults. Immediately after emergence, adults were weighed (± 0.1 mg, AM100; Mettler, Columbus, OH, USA) (hereafter referred to as emergence body mass) and sexed.

Adults were then placed individually on Petri dishes lined with a moisturized filter paper and fed daily with fresh potato leaves (variety Van Gogh). Adults were maintained in the controlled environmental chambers under the same conditions as described above.

METABOLIC MEASUREMENTS

CO₂ production of 6-day-old beetles was used as a measure of resting metabolic rate. Age was controlled because metabolic rate can vary with age (Piiroinen, Lindström & Lyytinen 2010). To avoid CO₂ emissions resulting from the assimilation of nutrients, beetles were starved for 24 h. CO₂ production at 23 °C was measured by using a CO₂ analyzer Li-6252 (LiCor, Lincoln, NE, USA) connected to a flow-through respirometry system. The CO₂ analyser was calibrated weekly with a calibration gas (470 ppm CO₂ in nitrogen). Ambient air that was filtered for water vapour (drierite) and CO₂ (soda lime) was pulled through the system with a pump (SS-2; Sable Systems, Henderson, NV, USA). The flow rate was controlled to constant 150 mL min⁻¹. A cylindrical glass respirometry chamber (volume ≈ 1.7 mL) was located inside a Peltier effect constant temperature cabinet (PTC-1; Sable Systems) in which the temperature was set and controlled with a temperature controller (Pelt-5; Sable Systems). Preliminary tests were performed to ensure that the incurrent air temperature that flowed through the respirometry chamber was stabilized with the ambient temperature in the cabinet.

The recording of the CO₂ production started when the CO₂ level had stabilized. Each recording lasted a minimum of 5 min during which the moving activity of the beetle was recorded with an infra-red light scattering based activity detector (AD-1; Sable Systems). The sampling interval was 0.2 s. Baselines (empty chamber without the beetle) were recorded before and after each measurement. Beetles were weighed (± 0.1 mg, AM100; Mettler) after the measurements and then maintained in environmental chambers until the diapause experiment (see below).

Respirometry data was baseline corrected and converted to mL CO₂ h⁻¹ by the acquisition and analysis software Expedata (Sable Systems). The metabolic value was the mean value of the respirometry recording of a period of 5 min. From the activity data an absolute difference sum was calculated and a linear regression was taken. The steepness of the slope (arbitrary number) describes the level of activity.

DIAPAUSE BEHAVIOUR AND OVERWINTERING SURVIVAL

Digging behaviour (dug or not dug into the soil for overwintering) was used as an indicator of the onset of diapause. First, 10-day-old beetles were weighed (hereafter referred to as overwintering body mass) and put into plastic containers (volume 80 mL) filled with soil (food supplied) to allow digging into the soil. Diapause behaviour was observed every day for 19 days. Temperature was lowered stepwise at 7-day-intervals from constant 23 to 17 °C and from 17 to 10 °C and finally to overwintering conditions at 5 °C after maintaining beetles at 10 °C for 5 days. Stepwise procedure was used to allow acclimation to cold. The diapausing beetles in the wild reside under the snow cover 20–60 cm deep in the soil, in which conditions the soil temperatures generally remain above 0 °C (Lefevere & De Kort 1989; Milner *et al.* 1992). In the southern part of Finland (62°N), the average winter soil temperature at the depth of 50 cm is 3.2 °C [range -0.1 to +13 °C, between September and May (Geological Survey of Finland; Heikinheimo & Fougstedt 1992)]. As the difference between the labora-

tory overwintering temperature and the average winter temperature is small, we assume that the relative differences in survival among individuals would remain intact. Overwintering survival was recorded from August – September onwards every 2 months (October, December, February, April) until next June. In total, survival was recorded for 9–10 months. Beetle movements to the soil surface or back into the soil were also recorded. Soil in the pots was moistened once in 2 months to maintain adequate moisture.

Winter diapause behaviour was rather dynamic. 88.7% of the beetles (1203 out of 1356 that were put into overwintering) dug into the soil within the 19 days of diapause observation. Few beetles burrowed into the soil until in December ($N = 15$) and 123 beetles stayed on the surface during the whole winter. Surprisingly not all beetles stayed in the soil for the whole overwintering period (surfaced in October $N = 134$, December $N = 24$, February $N = 25$, April $N = 31$) and some moved several times between the surface and the soil ($N = 24$). In the final analysis, the beetles that moved to the soil surface in October ($N = 134$) were pooled with the beetles that stayed on the surface during the whole observation period ($N = 123$). This is because there were no differences between these groups (stayed on the surface vs. surfaced in October) in overwintering body mass or metabolic rate (data not shown). In the analysis of diapause behaviour and overwintering survival, we had two groups of beetles: beetles that entered the soil for diapause ($N = 980$) and those that stayed on the surface of the soil ($N = 257$) for overwintering.

STATISTICS

We used binary logistic regression implemented in *SPSS* (v16; SPSS Inc., Chicago, IL, USA) to test the statistical significance of the association between overwintering body mass and metabolic rate on diapause behaviour (digging into the soil or not) and overwintering survival (dead or alive). In the analysis of diapause behaviour, we included overwintering body mass and residual metabolic rate as independent variables. Residual metabolic rate refers to residuals from the regression of metabolic rate on 6-day body mass, activity and the time of day of the metabolic measurement. The results were consistent when we analysed diapause behaviour and winter survival using metabolic rate which was not corrected for 6-day body mass. In the analysis of overwintering survival, we included diapause behaviour, in addition to the above independent covariates, as a categorical covariate. As diapause behaviour affected overwintering survival, we ran a further analysis of overwintering survival for only those beetles that resided under the soil during the whole overwintering period. The analysis was principally similar as above, but in addition to overwintering body mass and residual metabolic rate, we included the time required to enter diapause (number of days between the beetle's introduction into the overwintering pot and digging into the soil) as an independent variable. In the analyses, we first entered all interactions between categorical and/or independent variables. Non-significant interactions ($P > 0.05$) were omitted from the final models (Sokal & Rohlf 2003). As sexes differ in body mass (Independent samples T -test: $t = 19.687$, d.f. = 1235, $P < 0.001$) we analysed them separately.

As digging behaviour and survival were binary variables, we used multiple logistic regression to estimate selection gradients. All independent variables (body mass, time required to enter diapause, residual metabolic rate) were standardized to mean 0 and standard deviation 1 prior to analysis. Coefficients derived from multiple logistic regression models were transformed into selection gradients according to Janzen & Stern (1998). As absolute fitness was used as

the response variable, the transformed selection coefficients (β_{avggrad}) were converted to relative fitness scale according to Janzen & Stern (1998). This procedure results in selection coefficients that are comparable to estimates obtained from multiple linear regression (Janzen & Stern 1998). We also tested for disruptive/stabilizing selection in the above regression models by including quadratic terms of the continuous traits. However, all quadratic terms were non-significant (all $P > 0.1$) and therefore were omitted from the final models.

As the experiment was based on full-sib/half-sib experiment we were able to examine whether there was additive genetic variation in measured traits. We used general linear model with restricted maximum-likelihood (REML) for the estimation of the variance components as our dataset was not balanced (Lynch & Walsh 1998). The analysis was performed by ASReml 2.0 software (Gilmour *et al.* 2006). Random effects in all models were sire, dam and larval rearing environment (i.e. potato plant). Sex was entered as a fixed factor when sexes were analysed together. Emergence rank (the order of the adults emerging from each potato) was included as a covariate in the analysis of emergence body mass. The phenotypic variance (V_P) was partitioned into causal components of additive genetic variance ($V_A = 4 \times \text{sire variance}$), maternal variance ($V_M = \text{dam-sire}$), larval rearing environment (V_{Pot} , i.e. potato plant) and residual variance ($V_R = \text{residual-2} \times \text{sire}$), calculated following Falconer & Mackay (1996, p. 170). The narrow sense heritability (h^2) was calculated as V_A/V_P and maternal genetic effect (m^2) as V_M/V_P , larval rearing environment effect (p^2) as V_{Potato}/V_P , and phenotypic variation explained by random effects (r^2) as V_R/V_P . The significance of estimates was tested with a one-tailed z -test. The test was one-tailed since theoretically variance component cannot be negative. In the case of diapause behaviour and overwintering survival, the estimation of heritability at underlying scale was performed as a binary trait analysis with log link function.

Results

DIAPAUSE BEHAVIOUR

79.2% of the beetles entered diapause and remained under the soil during the whole overwintering period. In females, there was a significant interaction between overwintering body mass and residual metabolic rate (Table 1) indicating that the effect of metabolic rate on diapause behaviour differed in relation to body mass. When females had a low body mass, low metabolic rate was associated with high probability to enter diapause, but when females had a high body mass the probability to enter diapause was high irrespective of the level

of metabolic rate (Fig. 2a). In males, only the main effects were significant. Large body mass and low metabolic rate were related to high probability of diapause propensity (Table 1, Fig. 2b).

OVERWINTERING SURVIVAL

Total overwintering survival was 57.4% (710 out of 1237, Table 2). Females had higher survival (62.8%) than males (51.5%) ($\chi^2 = 16.0$, d.f. = 1, $P < 0.001$). Beetles that burrowed into the soil had higher probability to survive the overwintering period than beetles that stayed on the surface (Table 3, Fig. 3). Also, the probability to survive increased with body mass (Fig. 3) but was not affected by metabolic rate (Table 3).

The analysis of overwintering survival of the beetles in diapause (that resided under the soil during the whole overwintering period) revealed that in females, neither body mass or metabolic rate predicted survival (Table 4). In males, the probability of overwintering survival increased with body mass but was not affected by metabolic rate (Table 4). The time of entering diapause affected survival in both sexes. Beetles that entered diapause earlier had higher probability to survive the overwintering period than those that dug into the soil later (Table 4, Fig. 4). To investigate more closely the potential reasons for this difference in survival, we compared body mass and metabolic rate of the beetles that entered diapause latest (dug within 9–19 days) to those beetles that entered diapause the earliest (dug within 1–2 days). Our analysis revealed no significant differences in these traits between the groups in either sexes (independent samples t -test, females: metabolic rate: $t = -1.630$, d.f. = 371, $P = 0.104$; body mass: $t = 0.844$, d.f. = 384, $P = 0.399$. Males: metabolic rate: $t = -0.216$, d.f. = 240, $P = 0.829$; body mass: $t = 0.357$, d.f. = 252, $P = 0.722$). Thus, the low probability of overwintering survival for the beetles that entered diapause latest was not explained by metabolic rate or overwintering body mass.

HERITABILITY

A full-sib/half-sib experimental design allowed us to estimate whether there is additive genetic variation in body

Table 1. Binary logistic regression analysis for diapause behaviour in relation to overwintering body mass and residual metabolic rate. Binary logistic coefficients were transformed to selection gradients β_{avggrad} that are comparable to those obtained from multiple linear regression approach according to Janzen & Stern (1998)

Sex	Variables	Coefficient	SE	Wald	d.f.	P	β_{avggrad}
Female	Body mass	21.193	5.61	14.270	1	< 0.001	0.074
	Residual metabolic rate	-0.303	0.113	7.183	1	0.007	-0.051
	Body mass \times Residual metabolic rate	14.975	6.089	6.048	1	0.014	0.052
	Constant	1.614	0.114	201.872	1	< 0.001	0.271
Male	Body mass	38.279	6.097	39.421	1	< 0.001	0.157
	Residual metabolic rate	-0.262	0.111	5.577	1	0.018	-0.056
	Body mass \times Residual metabolic rate	10.298	6.477	2.528	1	0.112	0.042
	Constant	1.311	0.113	135.488	1	< 0.001	0.278

R^2 (Nagelkerke) 0.093 and 0.150 for females and males respectively.

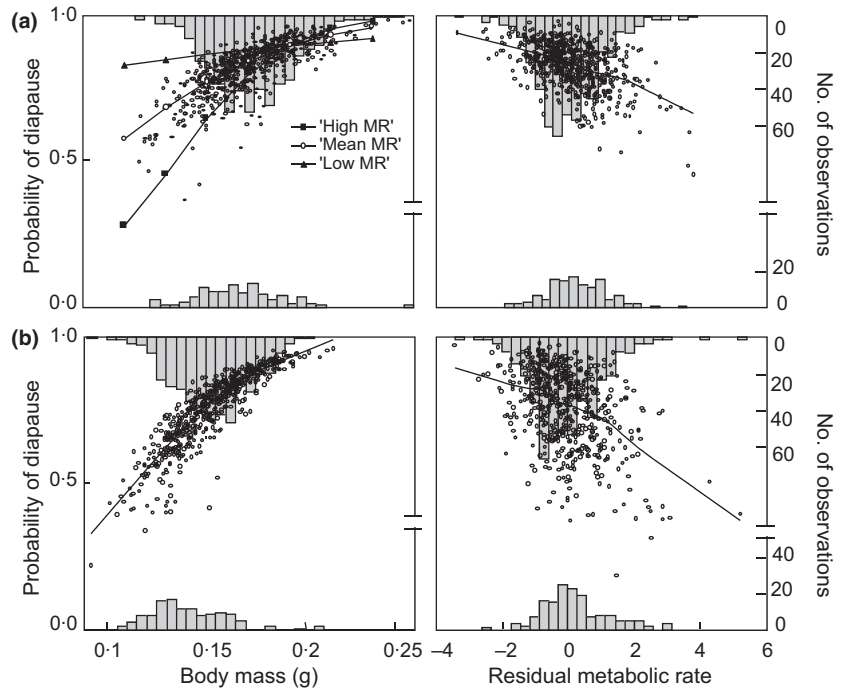


Fig. 2. Predicted probability of diapause behaviour (dug 1, not dug into the soil 0) in relation to overwintering body mass and residual metabolic rate for (a) female and (b) male Colorado potato beetles. Lines in the right panel (a) denote predicted probability function of diapause behaviour of individuals with average metabolic rate, or metabolic rate 1 SD above or below the average (Mean MR, High MR and Low MR respectively). Circles show the individual data points for the predicted probabilities. Histograms show the observed data.

Table 2. Overwintering survival (%) of the beetles that remained on the soil surface and within the soil during the whole overwintering period

	Survival on the soil surface (%) (n/n surface)	Survival in the soil (%) (n/n soil)	Total survival (%) (n/n total)
Female	14.4 (17/118)	73.6 (388/527)	62.8 (405/645)
Male	6.5 (9/139)	65.3 (296/453)	51.5 (305/592)
All	10.1 (26/257)	69.8 (684/980)	57.4 (710/1237)

The number of surviving and the total number of beetles are given in parentheses.

mass, metabolic rate, diapause behaviour and overwintering survival. Descriptive statistics of the traits are shown in Table 5. All examined traits had small heritability estimates (Table 6). Most of the phenotypic variance in all traits was explained by variation due to the common rearing environment (p^2 , i.e. potato plant) or residual variation (r^2). We also calculated genetic correlations between sexes in the studied traits (Spearman's rho of sire breeding values, $N = 26$), of which all that were statistically significant were positive correlations (emergence body mass = 0.488, $P = 0.011$, overwintering body mass = 0.707, $P < 0.001$, metabolic rate = 0.612, $P = 0.001$, mass independent metabolic rate = 0.502, $P = 0.009$, and days until entered diapause = 0.649, $P < 0.001$). Diapause behaviour was tentatively positively correlated between sexes (0.344, $P = 0.086$), whereas no genetic correlation between sexes was found in overwintering survival (-0.116 , $P = 0.573$). Moreover, we found positive genetic correlations between emergence body mass and overwintering body mass (females: 0.506, $P = 0.008$, males 0.738, $P < 0.001$), as

well as between metabolic rate and mass independent metabolic rate (females: 0.642, $P < 0.001$, males: 0.856, $P < 0.001$). Body mass (at age of 6 days) was positively genetically correlated with metabolic rate (females: 0.465, $P = 0.017$, males: 0.417, $P = 0.034$), but not with mass independent metabolic rate (females: -0.231 , $P = 0.256$, males: 0.006, $P = 0.978$). None of the genetic correlations were significantly negative.

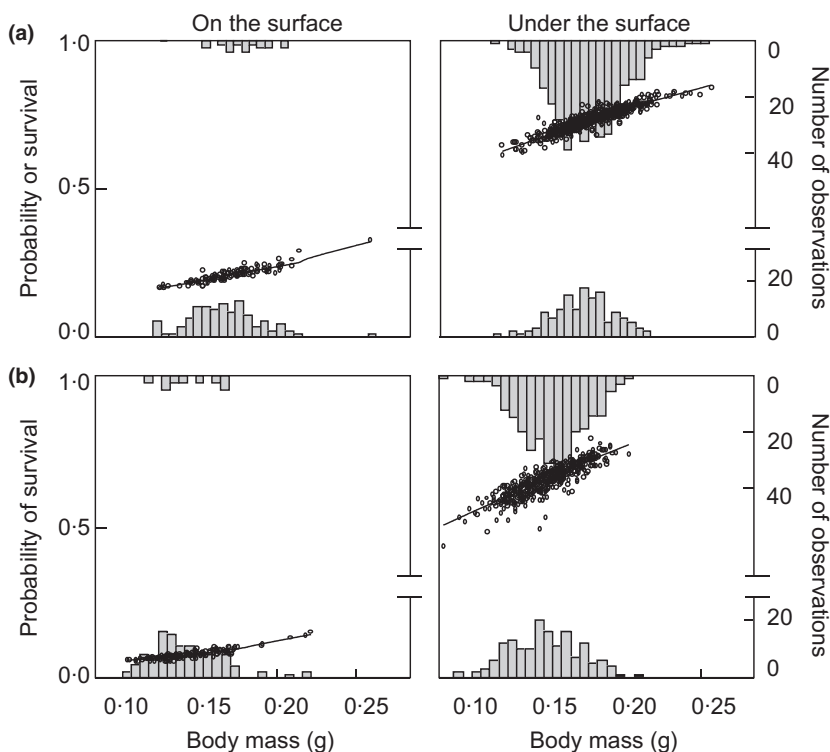
Discussion

A species' ability to successfully expand its range to novel conditions may require several changes that range from physiology to behaviour (Blows & Hoffmann 1993; Hoffmann *et al.* 2003; Gilchrist & Lee 2007; Kellermann *et al.* 2009; Lee *et al.* 2009). We studied the influence of metabolic rate and body mass on diapause behaviour and overwintering success in the European Colorado potato beetles originating from a population at the northern range limit. We also estimated the amount of heritable variation in these traits to evaluate the ability of the beetle to expand its range further north (i.e. its adaptive potential). Our results show that winter conditions impose selection on diapause behaviour (burrowing into the soil), which was associated with lower metabolic rate and larger body mass (Figs 2 and 3). These physiological traits were less influential once beetles had entered the soil for diapause, in which case only the large body mass of males was associated with high overwintering survival. Furthermore, the timing of the winter diapause was important to survival as the earlier a beetle entered diapause, the higher the overwintering survival (Fig. 4). Although measured traits affected survival, all heritability estimates were low and non-significant. Together, these results reveal

Table 3. Binary logistic regression analysis for overwintering survival in relation to diapause behaviour (dug, not dug into the soil), overwintering body mass and residual metabolic rate. Binary logistic coefficients were transformed to selection gradients β_{avggrad} that are comparable to those obtained from multiple linear regression approach according to Janzen & Stern (1998)

Sex	Variables	Coefficient	SE	Wald	d.f.	<i>P</i>	β_{avggrad}
Female	Diapause behaviour	-2.691	0.287	87.820	1	< 0.001	-0.774
	Body mass	9.071	4.680	3.757	1	0.053	0.054
	Residual metabolic rate	0.085	0.097	0.768	1	0.381	0.024
	Constant	-0.542	0.814	0.444	1	0.505	0.295
Male	Diapause behaviour	-3.249	0.382	72.155	1	< 0.001	-1.148
	Body mass	11.938	5.353	4.974	1	0.026	0.082
	Residual metabolic rate	-0.124	0.101	1.517	1	0.218	-0.044
	Constant	-1.171	0.821	2.038	1	0.153	0.220

R^2 (Nagelkerke) 0.272 and 0.355 for females and males respectively.

**Fig. 3.** Predicted probability of overwintering survival (dead 0, survived 1) for (a) female and (b) male Colorado potato beetles that stayed on the surface or entered the soil for diapause in relation to overwintering body mass. Lines denote the predicted probability function. Circles show the individual data points for the predicted probabilities. Histograms show the observed data.**Table 4.** Binary logistic regression analysis for overwintering survival for beetles residing under the soil during the overwintering period in relation to overwintering body mass, residual metabolic rate and days until a beetle entered diapause (i.e. dug into the soil). Binary logistic coefficients were transformed to selection gradients β_{avggrad} that are comparable to those obtained from multiple linear regression approach according to Janzen & Stern (1998)

Sex	Variables	Coefficient	SE	Wald	d.f.	<i>P</i>	β_{avggrad}
Female	Body mass	6.299	5.138	1.503	1	0.220	0.033
	Residual metabolic rate	0.155	0.108	2.048	1	0.152	0.039
	Days until entered diapause	-0.131	0.029	19.830	1	< 0.001	-0.109
	Constant	1.684	0.989	2.897	1	0.089	0.267
Male	Body mass	12.049	5.689	4.486	1	0.034	0.077
	Residual metabolic rate	-0.125	0.106	1.402	1	0.236	-0.041
	Days until entered diapause	-0.091	0.031	8.375	1	0.004	-0.096
	Constant	0.054	0.977	0.003	1	0.956	0.208

R^2 (Nagelkerke) 0.066 and 0.048 for females and males respectively.

possible factors that may set limits for the range expansion potential (see Hoffmann *et al.* 2003; Kellermann *et al.* 2009) of the Colorado potato beetle.

The relationship between metabolic rate and overwintering survival has not been assessed extensively in temperate ectotherms. Earlier studies on insects have, mostly indirectly,

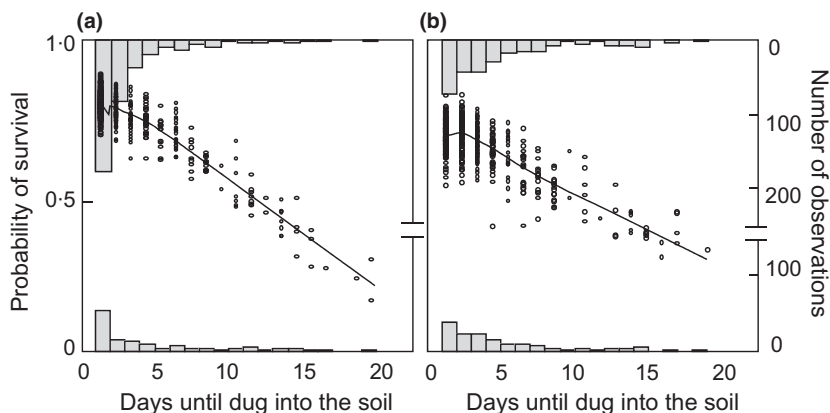


Fig. 4. Predicted probability of overwintering survival (dead 0, survived 1) of (a) females and (b) males in relation to the number of days until beetles entered diapause (dug into the soil). Lines denote the predicted probability function. Circles show the individual data points for the predicted probabilities. Histograms show the observed data.

Table 5. Descriptive statistics of the traits in the genetic analysis, (a) continuous traits, (b) days until entered the diapause and bivariate traits

Trait	<i>n</i>	Mean	Raw σ^2_p	SD	Min	Max
			($\times 10^3$)			
(a)						
Emergence body mass (g)						
Female	744	0.138	0.308	0.017	0.079	0.193
Male	714	0.119	0.214	0.015	0.068	0.173
Both	1458	0.129	0.354	0.019	0.068	0.193
Overwintering body mass (g)						
Female	703	0.173	0.430	0.021	0.113	0.253
Male	674	0.149	0.392	0.020	0.086	0.210
Both	1377	0.536	0.265	0.023	0.086	0.253
Metabolic rate (mL CO ₂ h ⁻¹)						
Female	691	0.107	0.919	0.030	0.017	0.228
Male	667	0.093	0.606	0.025	0.019	0.221
Both	1358	0.100	0.815	0.028	0.017	0.228
(b)						
Diapause behaviour						
Female	645	0.817	0.149			
Male	592	0.765	0.179			
Both	1237	0.792	0.165			
Overwintering survival						
Female	527	0.736	0.195			
Male	453	0.653	0.227			
Both	980	0.698	0.211			
Days until entered diapause						
Female	527	3.002	10.816	3.289	1	19
Male	453	3.678	11.024	3.320	1	18
Both	980	3.314	11.015	3.319	1	19

n is the number of individuals included in the heritability analysis. The raw phenotypic variances (σ^2_p) may differ from the total phenotypic variances estimated in the general linear model with REML. This is because the variance components calculated by the REML method is affected by the fixed effects included in the model (Lynch & Walsh 1998; Wilson 2008).

indicated that energy metabolism could be associated with winter survival by affecting the depletion of energy reserves (Han & Bauce 1998; Ellers & Van Alphen 2002). We found that lower metabolic rate was associated with higher overwintering survival indirectly through the diapause behaviour. Selection on diapause behaviour was twofold. Selection acted strongly against those individuals who did not burrow (Table 3), and favoured entering diapause earlier for those

individuals that did burrow (Table 4). This underlines that behavioural adaptations are important to consider when studying diapause. High mortality of the beetles on the surface may reflect that they were physiologically unprepared to overwinter. It is unclear why the probability of overwintering survival decreased the later the beetles entered diapause as we did not find any differences in the physiological traits (metabolic rate, body mass) measured. Overall, the fact that heavier individuals were more likely to enter diapause (Fig. 2) is in line with previous studies that suggest insects must achieve certain thresholds for body mass (Munyiri, Shintani & Ishikawa 2004; Hiiesaar *et al.* 2006; Noronha & Cloutier 2006) in addition to lowered metabolic rate (see Piironen, Lindström & Lyytinen 2010) to successfully enter diapause. A threshold for large body mass likely reflects the accumulation of sufficient amount of energy reserves whereas a threshold for metabolic rate may reflect shutting down of energetically expensive functions (Hahn & Denlinger 2007) e.g. degeneration of flight muscles (Stegwee *et al.* 1963). Once beetles had entered the soil for diapause, only large body mass of males was associated with high overwintering survival.

Range expansion into new environments may require adaptation and thus genetic variation in relevant traits. The northern range expansion potential of many temperate insect species may especially be influenced by evolutionary potential in diapause (Schmidt *et al.* 2005). In line with previous studies (e.g. Nespolo, Castañeda & Roff 2007), we found low and non-significant narrow sense heritability estimates of metabolic rate. We also found low heritabilities of diapause behaviour (Table 6). Low heritabilities of diapause behaviour and physiological traits associated with diapause (body mass, metabolic rate) may suggest that the beetle's evolutionary potential to expand its current range further north may be limited (see Roff 1997). Since we estimated the evolutionary potential of a marginal population at the range limit, the low additive genetic variance could be explained by its erosion by strong selection in new environments (Hoffmann & Parsons 1993) or by founder effects (Hoffmann & Blows 1994; Dlugosch & Parker 2008; but see Hard, Bradshaw & Holzapfel 1993). For instance, van Heerwaarden *et al.* (2010) found that selection for increased desiccation resistance may have limited the southern range expansion of *Drosophila birchii* towards

Table 6. Quantitative genetic parameters for measured traits from the full-sib/half-sib experiment with 26 sires and 54–55 full-sib families, (a) continuous traits, (b) days until entered diapause and bivariate traits, diapause behaviour and overwintering survival

Trait	V_{Sire}	V_{Dam}	V_{Potato}	V_{Resid}	h^2	SE	p	m^2	SE	p	p^2	SE	P	r^2	SE	P
(a)																
Emergence body mass (g)*	($\times 10^3$)	($\times 10^3$)	($\times 10^3$)	($\times 10^3$)												
Female	0.0089	0.0375	0.0814	0.1718	0.119	0.215	0.290	0.096	0.111	0.195	0.272	0.052	<0.001	0.514	0.122	<0.001
Male	0.0078	0.0020	0.0711	0.1291	0.148	0.160	0.177	–	–	–	0.339	0.060	<0.001	0.541	0.098	<0.001
Both	0.0034	0.0243	0.0637	0.1621	0.054	0.151	0.360	0.082	0.082	0.160	0.251	0.040	<0.001	0.612	0.087	<0.001
Overwintering body mass (g)																
Female	0.0209	0.0304	0.0239	0.3549	0.194	0.174	0.133	0.022	0.079	0.390	0.056	0.033	0.044	0.728	0.111	<0.001
Male	0.0034	0.0497	0.0365	0.3022	0.035	0.161	0.414	0.118	0.089	0.092	0.093	0.040	0.010	0.754	0.096	<0.001
Both	0.0121	0.0451	0.0267	0.3317	0.116	0.149	0.218	0.079	0.072	0.136	0.064	0.021	0.001	0.740	0.091	<0.001
Metabolic rate (mL CO ₂ h ⁻¹ †)																
Female	<0.001	0.0070	0.0504	0.8085	–	–	–	0.008	0.023	0.364	0.058	0.037	0.060	0.934	0.032	<0.001
Male	0.0145	<0.001	0.0425	0.5156	0.102	0.084	0.113	–	–	–	0.074	0.036	0.021	0.850	0.063	<0.001
Both	0.0117	0.0090	0.0293	0.6744	0.064	0.064	0.158	–	–	–	0.041	0.021	0.026	0.899	0.043	<0.001
Mass independent metabolic rate‡																
Female	0.0020	<0.001	0.0465	0.7335	0.010	0.055	0.428	–	–	–	0.059	0.034	0.040	0.933	0.044	<0.001
Male	0.0123	<0.001	0.0294	0.4760	0.095	0.080	0.118	–	–	–	0.057	0.035	0.054	0.872	0.061	<0.001
Both	0.0129	<0.001	0.0296	0.6137	0.079	0.055	0.075	–	–	–	0.045	0.020	0.010	0.896	0.041	<0.001
(b)																
Diapause behaviour																
Female	0.0309	0.1798	<0.001	3.2890	0.035	0.161	0.413	0.043	0.085	0.309	–	–	–	0.922	0.096	<0.001
Male	0.0516	0.0217	0.2630	3.2890	0.057	0.126	0.326	–	–	–	0.073	0.062	0.119	0.879	0.084	<0.001
Both	0.0202	0.1792	0.0885	3.2890	0.023	0.098	0.409	0.045	0.053	0.202	0.025	0.032	0.220	0.908	0.062	<0.001
Overwintering survival																
Female	0.1498	<0.001	0.1003	3.2890	0.169	0.140	0.114	–	–	–	0.028	0.050	0.287	0.845	0.100	<0.001
Male	0.0230	<0.001	0.1160	3.2890	0.027	0.103	0.397	–	–	–	0.034	0.050	0.251	0.946	0.074	<0.001
Both	0.0423	<0.001	0.0577	3.2890	0.050	0.064	0.218	–	–	–	0.017	0.028	0.272	0.946	0.047	<0.001
Days until entered diapause																
Female	0.2750	0.7105	0.3936	9.6970	0.099	0.138	0.236	0.039	0.069	0.285	0.036	0.043	0.206	0.826	0.091	<0.001
Male	0.4437	0.9595	0.0087	9.5340	0.162	0.194	0.202	0.047	0.092	0.305	0.001	0.047	0.493	0.790	0.123	<0.001
Both	0.4230	0.6351	0.3596	9.8600	0.155	0.123	0.104	0.019	0.053	0.358	0.003	0.021	0.439	0.823	0.081	<0.001

Observed variance components (V_{Sire} , V_{Dam} , V_{Potato} and V_{Resid}) were estimated by fitting sire, dam and potato (larval rearing environment) as random effects in the general linear model with REML estimation. The proportions of phenotypic variation explained by each effect (h^2 , m^2 , p^2 and r^2) were calculated according to Falconer & Mackay (1996, p. 170). Sex was as entered as a fixed factor when sexes were analysed together. Diapause behaviour and survival estimates at underlying scale.

*Emergence rank as a covariate in the model.

†Activity corrected metabolic rate.

‡Body mass and activity corrected metabolic rate.

drier environments due to reduced genetic diversity in desiccation resistance. Founder effects may be common when population sizes decrease towards the margins (Dlugosch & Parker 2008). However, founder effects are unlikely to explain the lack of heritable genetic variation in the beetles. Previous study on nuclear (AFLPs) genetic variation among European beetle populations showed no reduction of genetic variation in the northern populations compared to southern ones (Grapputo *et al.* 2005). Alternatively, low heritability estimates may result either from low repeatability of traits or from a low power in heritability estimation (Lynch & Walsh 1998) due to the small number of families. These explanations are unlikely as others (e.g. Nespolo *et al.* 2005) have also found moderate and significant heritabilities with similar sample sizes. Moreover, body mass can be measured with high accuracy. In this study, however, its heritability estimates were non-significant. Thus, the low heritability estimates are likely to be real.

Evolutionary change, however, is not the only prerequisite or always necessary for successful range expansions (Sakai *et al.* 2001; Sexton *et al.* 2009). Ecological factors such as high physiological tolerance and/or high reproductive capacity may allow species to spread to new environments (Lee *et al.* 2009). High fecundity and growth rate can also ameliorate reduction in genetic variation (Zenger, Richardson & Vachot-Griffin 2003). In our study, 57.4% of the beetles overwintered successfully under conditions that mimicked benign winter conditions in the wild (Heikinheimo & Fougstedt 1992). Even if winters would be harsher and mortalities higher (Hiisaar *et al.* 2006), the high fecundity of the female beetles (Weber & Ferro 1996) could buffer the effect of mortality on population sizes.

In conclusion, we found that winter conditions impose selection through the diapause behaviour on lower metabolic rate and larger body mass. Low evolutionary potential in these traits might suggest that the beetle's future ability to expand its current distribution via evolutionary change may be limited (see Roff 1997; Kellermann *et al.* 2009; van Heerwaarden *et al.* 2010). However, as overwintering survival can be over 50% under optimal conditions, benign winters may facilitate the range expansion of the beetle by allowing high overwintering survival. Our study shows that both physiological and behavioural adaptations should be taken into consideration when predicting species range changes which have become especially important in regard to recent increases in alien species invasions (Sakai *et al.* 2001; Suarez & Tsutsui 2008).

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