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Title: Northward range expansion requires synchronization of both overwintering behaviour and physiology with photoperiod in the invasive Colorado potato beetle (Leptinotarsa decemlineata)

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1 **Northward range expansion requires synchronization of both**

2 **overwintering behaviour and physiology with photoperiod in the invasive**

3 **Colorado potato beetle (*Leptinotarsa decemlineata*)**

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Abstract

Photoperiodic phenological adaptations are prevalent in many organisms living in seasonal environments. As both photoperiod and growth season length changes with latitude, species undergoing latitudinal range expansion often need to synchronize their life-cycle with a changing photoperiod and growth season length. Since adaptive synchronization often involves a large number of time-consuming genetic changes, behavioural plasticity might be a faster way to adjust to novel conditions. We compared behavioural and physiological traits in overwintering (diapause) preparation in three latitudinally different European Colorado potato beetle (*Leptinotarsa decemlineata*) populations reared under two photoperiods. Our aim was to study whether behavioural plasticity could play a role in rapid range expansion into seasonal environments. Our results show that while burrowing into the soil occurred in the southernmost studied population also under a non-diapause inducing long photoperiod, the storage lipid content of these beetles was very low compared to the northern populations. Similar behavioural plasticity was however not found in the northern populations. Furthermore, the strongest suppression of energy metabolism was seen in pre-diapause beetles from the northernmost population. These results could indicate accelerated diapause preparation and possibly energetic adjustments due to temporal constraints imposed by a shorter, northern, growth season. Our results indicate that behavioural plasticity in burrowing may have facilitated initial range expansion of *L. decemlineata* in Europe. However, long term persistence at high latitudes has required synchronization of burrowing behaviour with physiological traits. The results underline that eco-physiological life-history traits of insects, such as diapause, should be included in studies on range expansion.

Key words: Coleoptera, diapause, latitude, metabolic rate, behavioural plasticity

Introduction

Many organisms use the photoperiod as the main environmental cue for phenological synchronization, since it is a reliable indicator of time of year (Nelson et al. 2010) and it allows organisms to predict coming seasonal shifts accurately. This has led to various photoperiodic adaptations in physiological, behavioural and life history traits (Nelson et al. 2010). Species undergoing latitudinal range expansion face a change in the photoperiod, which can lead to a mismatch between the timing of seasonal adjustments in physiological and life history traits with seasonal environmental changes (Bradshaw and Holzapfel 2007). This mismatch can lead to lowered survival, for example, due to unsynchronized preparation for overwintering or timing of migration (Rankin and Burchsted 1992). Consequently, latitudinal range expansion may fail unless evolutionary changes in critical photoperiod take place (Gaston 2003; Bradshaw and Holzapfel 2007; Urbanski et al. 2012) and species are able to adjust their behavioural and physiological traits with the abiotic and biotic requirements of the new environment (Elton 1958).

Range expansion to and persistence in novel environments may be possible also without any genetic changes (i.e. adaptive evolution) if invading individuals possess phenotypic plasticity in their abilities to avoid stressful conditions (Sakai et al. 2001; West-Eberhard 2003; Duckworth 2009, Sunday et al. 2014). Phenotypic plasticity can manifest itself on many organismal levels, including in physiological and behavioural traits (West-Eberhard 2003). Through behavioural plasticity maladapted individuals might persist, thus allowing time for physiological adaptation to occur (Sakai et al. 2001; Phillips and Suarez 2012) (Fig. 1). The potential roles of behavioural plasticity in facilitating the establishment of species, especially invasive species, have been increasingly appreciated (Sagata and Lester 2009; Wright et al. 2010; Gabel et al. 2011; Hanshew and Garcia 2012; Phillips and Suarez 2012). Therefore, in order to better predict species invasions into novel environments with

62 seasonally occurring periods of stress (Fig. 1) and to be able to better assess the capacity of

63 species to respond to climate change (Bradshaw and Holzapfel 2001; Lee 2002; Saikkonen et

64 al. 2012; Sunday et al. 2014), it is important to consider the relative contribution of

65 behavioural plasticity vs. adaptation for stress avoidance.

66   One such important stress avoidance mechanism by which temperate insects survive

67 harsh winter conditions and which can show between-population differences as a result of

68 plastic or genetic change is diapause (Tauber et al. 1986). Correct timing and physiological

69 synchronization with photoperiod is often vital for diapause preparation since overwintering

70 mortality usually is very high in unprepared individuals (Danks 1987; Hahn and Denlinger

71 2007, see Fig. 1 path 1). Diapause is usually triggered by a shortening of the photoperiod

72 (preceding autumn and winter), but can be modulated by for instance, low temperatures or the

73 quality or abundance of food (Danilevskij 1965; Tauber et al. 1986; Danks 1987). Diapause

74 itself is usually characterized by reproductive arrest, suppressed metabolism and high

75 tolerance to many environmental stressors (Tauber et al. 1986; Kostal 2006). In many species,

76 preparation for diapause is also accompanied by changes in behaviour (Beck and Hanec 1960;

77 Beekman et al. 1998) including, active foraging for building energy stores, and seeking out

78 overwintering locations (Tauber et al. 1986). Individuals generally choose overwintering

79 microhabitats where they are buffered against exposure to adverse climatic conditions such as

80 extreme temperatures, large temperature fluctuations and desiccation (Danks 1987). This

81 opens the possibility that individuals can survive winters in novel environments without

82 physiological adaptation (Fig. 1, path 2).

83   To study whether rapid range expansion into seasonal environments is driven by

84 adaptation or behavioural plasticity, we used the very successful pest invader, the Colorado

85 potato beetle, *Leptinotarsa decemlineata* (Say) which has undergone rapid latitudinal range

86 expansion. *L. decemlineata* is native to Mexico and the south-western United States of

America (Casagrande 1987; Alyokhin 2009), and currently occupies an area covering 16 million km^2 in North America, Europe and Asia (EPPO 2006). The beetles that invaded Europe, Bordeaux, France, in the 1920s have been suggested to come from lower latitudes in North America (Johnson 1967). There was a clear genetic bottleneck during the initial introduction and establishment in Europe, resulting in reduced genetic variation (Grapputo et al. 2005; Piiroinen et al. 2013). Despite this reduction, the subsequent range expansion of *L. decemlineata* has been very rapid (Piiroinen et al. 2013) and the annual expansion distance has been estimated to be between 80 and 130 km (Johnson 1967; EPPO 2006). Reasons for the successful invasion include the beetles' versatile life-cycle and capability to overwinter in diapause (Casagrande 1987; Hsiao 1985; de Kort 1990; Mahdjoub and Menu 2008; Alyokhin 2009) where cold-tolerance is significantly increased (Boiteau and Coleman 1996; Costanzo et al. 1998).

In *L. decemlineata*, diapause initiation occurs at around 15 days after adult eclosion under short photoperiods (Lefevere and de Kort 1989). During the pre-diapause period storage lipid content increases dramatically (Lehmann et al. 2012) and metabolic rate shows a bell-shaped pattern, which peaks at 3-5 days post eclosion (May 1989; Piiroinen et al. 2010). In addition to physiological changes, pre-diapause behaviour is characterized by adults becoming negatively phototactic and by burrowing into the soil (Alyokhin 2009). Burrowing into the soil is commonly associated with pre-diapause not only in *L. decemlineata* (de Kort 1990; Piiroinen et al. 2011; Izzo et al. 2013) but also in other coleopterans (Rasa 1999; Kuang et al. 2011) and in insects in general (Roubik and Michener 1980; Beekman et al. 1998; Solbreck and Windefalk 2012). Adult *L. decemlineata* beetles can also burrow into the soil even though reproductively active (Hsiao 1985; Tauber et al. 1988; Alyokhin 2009). Burrowing is probably a means to avoid exposure to stressful climatic conditions and harmful biotic interactions (Tauber et al. 1986; Danks 1987). Since burrowing can be decoupled from

112 diapause and still be advantageous, plasticity in burrowing behaviour may allow individuals

113 to survive winters at higher latitudes without associated photoperiodically induced

114 physiological changes, which often require local adaptation (Fig. 1). Behavioural plasticity in

115 burrowing could therefore be among the reasons of the invasion success and rapid range

116 expansion of *L. decemlineata* (Casagrande 1987; de Kort 1990; Alyokhin 2009).

117   Our aim in this study is to investigate whether behavioural plasticity could have

118 played a role in the rapid range expansion of *L. decemlineata* in Europe. This can be

119 indirectly studied by comparing the burrowing behaviour of individuals reared under two

120 photoperiods from three populations of different latitudinal origin: northern (Petroskoi,

121 Russia), middle (Belchow, Poland) and southern (Padua, Italy) Europe. We take advantage of

122 prior knowledge on differences in their photoperiodic responses: the northernmost population

123 uses a longer photoperiod as cue for diapause initiation than the southernmost population

124 (Piiroinen et al. 2011; Lehmann et al. 2012; 2014). If range expansion is mainly driven by

125 photoperiodic adaptation, we expect populations to burrow for diapause only when reared

126 under photoperiods which match their local late summer conditions (Piiroinen et al. 2011) and

127 that diapause-related physiological traits are strongly correlated with burrowing behaviour

128 (Fig. 1, path 1). However, if behavioural plasticity is maintained in all populations, we expect

129 that burrowing behaviour is observed also when beetles are reared under non-diapause

130 inducing conditions and not necessarily correlated with diapause-related physiological traits

131 (increased lipid content and decreased metabolic rate) (Fig. 1, path 2).

132   We frame our study with three specific hypotheses. 1) In case range expansion has

133 been accompanied by photoperiodic phenological synchronization, we predict that beetles

134 from all populations burrow only under diapause-inducing photoperiods (Fig. 1, path 1).

135 Furthermore, we predict a strong association between burrowing and high lipid content as

136 well as low metabolic rate in all burrowed beetles (Fig. 1, path 1). As energy sequestering and

137 economic utilization of limited energy reserves during overwintering are vital for successful

138 overwintering, accumulation of lipids and suppression of resting metabolic rate (RMR) are

139 commonly used as physiological measures of preparation for diapause (Coleman and Boiteau

140 1996; Guppy and Withers 1999; Kostal 2006; Hahn and Denlinger 2007; Lehmann et al.

141 2012). 2) In case burrowing behaviour is plastic, we predict that beetles burrow also under

142 non-diapause inducing photoperiods (Fig. 1, path 2). If burrowing on its own has been

143 sufficient for beetles to survive winters faced, we predict a weak association between

144 burrowing and high lipid content as well as low metabolic rate in beetles burrowed under non-

145 diapause inducing conditions (Fig. 1, path 2). 3) In parallel with pre-diapause behaviour, we

146 study whether populations differ in resting metabolic rate (RMR) trajectories when reared

147 under diapause inducing and non-inducing conditions. We predict that the steepness of the

148 RMR trajectory slope increases with latitude in beetles preparing for diapause, which could

149 relate to more effective use of limited energy reserves (May 1989; Guppy and Withers 1999.

150

151 **Materials and methods**

152 *Study animals*

153 Three *L. decemlineata* populations were used in this study. They were the descendants of

154 beetles collected from potato fields near Petroskoi in northern Russia (61°49'N, 34°10'E) (N

155 = 917) in the summer of 2006, from potato fields near Belchow in Poland (52°01'N, 20°34'E)

156 (N = 400) in 2010 and from potato fields near Padua in Italy (45°48'N, 12°07'E) (N = 249) in

157 the summer of 2010. The Petroskoi population represents the northernmost current range limit

158 (EPPO 2006). The parental generations were reared in the laboratory under a long day

159 photoperiod (18 h light, 23°C, 60% relative humidity) on potato (*Solanum tuberosum* of the

160 *van Gogh* variety). The next generation beetles were then overwintered at 5°C in climate

161 chambers according to the protocol described previously (Lehmann et al. 2012).

162

Rearing conditions and experimental setup

The experiment was conducted in 2010 and 2013. In 2010, overwintered Petroskoi (24 families, fourth laboratory generation) and Paduan beetles (12 families) were mated (1 male to 1 female) within population. The Paduan beetles were first acclimatized to the laboratory conditions (18 h light, 23°C, 60% relative humidity) for two weeks. In 2013, overwintered Petroskoi (26 families, seventh laboratory generation), Belchow (21 families, third laboratory generation) and Padua beetles (32 families, third laboratory generation) were mated (1 male to 1 female) within population. Mated pairs were checked for deposited eggs daily. Larvae (10-15 per family) were reared on whole potato plants (which were covered in a plastic housing) in a greenhouse till adulthood at 23°C degrees, 60% relative humidity and 18 hours light. After larvae burrowed into the soil for pupation, pots were checked daily for emerged adults. Adults were sexed and weighed on the day of emergence (± 0.1mg, AM100; Mettler).

Based on emergence mass, beetles from each family were divided into two photoperiod (long and short day) and age (5 and 10 days) groups so that each family was represented in both photoperiods and ages and the emergence mass was similar among groups (two-way ANOVA: $P > 0.05$ for both main factors and interactions, sexes were analysed separately). The two photoperiods were: long day (LD, 18 h light): 16 hours full light, 6 of darkness with two 1-hour medium-light periods imitating dawn and dusk, and short day (SD, 12 h light): 10 hours full light, 12 hours dark with two 1-hour medium-light period imitating dawn and dusk. The day length change during the end of summer (15th of July to 15th of August) is from: 18:40 (hours:minutes) to 15:56 in Petroskoi, 16:15 to 14:34 in Belchow and 15:22 to 14:08 in Padua. The temperature was set to 23°C during lights on and 21°C during lights off, with 60% air relative humidity. Beetles were kept individually on petri dishes (diameter of 9 cm) containing a water moistened filter paper and fed daily with fresh potato leaves (*Solanum*

187 *tuberosum* of the *van Gogh* variety). To investigate burrowing behaviour in response to

188 photoperiod, 7-day-old beetles were moved from the petri dishes into plastic jars (120 ml)

189 containing peat soil (40 ml). The jars had clear lids to allow full penetration of light and holes

190 for air exchange. The soil was kept moistened and adults were fed daily with fresh potato

191 leaves. Beetles were fed *ad libitum* to avoid burrowing as a response to lack of food (Hsiao

192 1985; Alyokhin 2009). Burrowing into the soil (burrowed into the soil or still on the soil

193 surface) was recorded when beetles were 10 days old.

194

195 *Resting metabolic rate*

196 CO_2 production of individual 5- and 10-day-old beetles (Table 1) was used as a measure of

197 resting metabolic rate (RMR). RMR represents the metabolic activity of a post-absorptive

198 animal at rest kept at a neutral temperature (Randall et al. 2002). To avoid CO_2 emissions

199 resulting from the assimilation of nutrients, all beetles were starved for 24 h prior to CO_2

200 measurements. CO_2 production was measured approximately at the same time during the

201 synthetic photoperiod, between 2 and 4 hours after lights on. This was to avoid any possible

202 circadian rhythms in behavioural or physiological traits interfering with the RMR. Each beetle

203 was measured only at one time point: day 5 or 10. RMR of the 10-day-old beetles was

204 measured after their burrowing behaviour was recorded. CO_2 production was measured with a

205 Li-6252 CO_2 analyser (LiCor, Lincoln, NE, USA) connected to a flow through respirometry

206 system. We performed a weekly calibration of the CO_2 analyser with 470 ppm CO_2 in

207 nitrogen. Ambient air from which water vapour and CO_2 were removed (with Drierite, WA

208 Hammond Drierite, USA and Ascarite II, Acros Organics, USA, respectively) was pumped

209 through the system (SS-2 pump; Sable Systems, Henderson, NV, USA). The flow rate was set

210 to 150 ml min^{-1} with a mass flow controller (840 Series; Sierra Instruments Inc., California,

211 USA). Eight cylindrical glass respirometry chambers (volume 1.7 ml) (of which one empty

212   chamber served as the baseline) were connected to a multiplexer (Sable Systems). The

213   chambers were located inside a dark constant temperature cabinet (PTC-1; Sable Systems) in

214   which the temperature was set to 23°C and controlled with a temperature controller (Pelt-5;

215   Sable Systems). Preliminary tests were performed to ensure that the incurrent air temperature

216   that flowed through the respirometry chamber was stabilized with the ambient temperature in

217   the cabinet. The multiplexer was programmed so that the series of seven beetle chambers was

218   measured twice. The recording of each beetle lasted 5 min during which the moving activity

219   of the beetle was recorded with an infra-red light scattering based activity detector (AD-1;

220   Sable Systems). The sampling interval was 1 s. Beetles were weighed ($\pm$0.1 mg, AM100;

221   Mettler) after the measurement. Respirometry data was baseline corrected and converted to ml

222   CO_2 h^{-1} by the acquisition and analysis software Expedata version 1.1.15 (Sable Systems).

223   The metabolic value was the mean value of the respirometry recordings of two 5 min periods.

224   Between the first and second measurement 60 minutes passed. From the activity data an

225   absolute difference sum was calculated and a linear regression was taken. The steepness of the

226   slope (arbitrary number) describes the level of activity. Preliminary experiments showed that

227   beetles generally remained inactive during the measurements. Nine individuals were active

228   throughout the measurement period and excluded from the later analyses, as their CO_2

229   emissions likely did not reflect RMR.

230

231   *Total lipid content*

232   To investigate the size of energy reserves, abdominal total lipid content was measured from

233   10-day-old beetles. After the measurement of RMR, beetles were weighed, anesthetized on

234   ice, the elytrae removed and the whole abdomen cut away and stored at -80°C until analysis.

235   Total lipid content of the abdomen was measured with Chloroform:Methanol (Chl:Meth)

236   extraction (Folch et al. 1957) as described previously (Lehmann et al. 2012). Abdomen were

237   dried for 72 h at 55 °C, after which lipids were extracted by placing the abdomen in 10 ml of

238   2:1 Chl:Meth for 72 h. Lipid content was then assessed gravimetrically after drying the

239   samples for 72 h at 55 °C. Around 85% of abdominal total lipids extracted in this way are

240   neutral lipids, which mainly are storage triacylglycerids (Lehmann et al. 2012). During the

241   first 10 days of adult life, *L. decemlineata* does not accumulate glycerol (P. Lehmann,

242   unpublished), which could interfere with the Chl:Meth extraction method (Williams et al.

243   2011). We used absolute lipid content (mg) as well as relative lipid content (%) for the

244   analyses (see below). Absolute lipid content was calculated by subtracting lean mass (post-

245   extraction dry mass) from dry mass, while total relative lipid content (w/w) was calculated by

246   dividing absolute lipid content by dry mass.

247

248   *Statistical analyses*

249   The potential difference between the years (2010 and 2013) was studied by adding year as a

250   random (block) factor in all tested models. Since year was non-significant in all the analyses,

251   the data for both years were pooled for Petroskoi and Padua. All statistical tests were

252   performed with the IBM SPSS statistics 20.0 (IBM SPSS Inc., Chicago, IL, USA) statistical

253   software package. To study whether burrowing differed between photoperiods or among

254   populations and among populations within photoperiods, we used a binary logistic

255   generalized linear model (GZLM) with population (Petroskoi, Belchow and Padua) and

256   photoperiod (short day and long day) as factors. The interaction between photoperiod and

257   population was removed from the final model since it was non-significant (Sokal and Rohlf

258   2003). To study the association between population, rearing photoperiod and burrowing

259   behaviour on CO_2 production of 10-day-old beetles (RMR, ml CO_2 h^{-1}), we used a general

260   linear model (GLM). RMR was log-transformed to improve normality and linearity.

261   Burrowing behaviour (burrowed, surface) was included as a fixed factor in addition to

photoperiod, population and sex. Activity during the RMR measurement and body mass were added as covariates. The non-significant main factor sex, as well as interactions which were non-significant, was removed from the final model (Sokal and Rohlf 2003). If the interaction terms were significant, the main level effects were post-hoc tested with univariate F-tests and pair-wise tests done on group estimated marginal means with Bonferroni multiple-comparison (BMC) corrections. The results were consistent when the above model was built using mass-specific RMR (ml CO_2 g^{-1} h^{-1}). To study the association between population and burrowing behaviour on lipid content, the abdominal total lipid content (mg) of 10-day-old beetles were analysed with a factorial GLM with sex, population and burrowing behaviour entered as fixed factors and abdomen lean mass (post extraction dry mass) as a covariate. The data were split by photoperiod to improve the power of the test, due to uneven group sizes. The main factor sex was removed from the final model (Sokal and Rohlf 2003). If the interaction was significant, the main level effects were post-hoc tested with univariate tests as described above. We furthermore investigated the strength of association between energy sequestering and utilization as the relationship between log-transformed RMR (ml CO_2 h^{-1}) and total relative lipid content (% of abdominal dry mass). This was done using a GZLM with an identity link function where log-RMR was the dependent variable and lipid content as well as body mass were added as covariates. The data was split by population and photoperiod. Finally, to investigate the association between age, population and rearing photoperiod on RMR, the log-transformed CO_2 (ml CO_2 h^{-1}) was analysed with a factorial GLM. Age (5 and 10 days), sex, population and photoperiod were added as fixed factors and activity as well as body mass was added as covariates. The main factor sex was removed from the final model (Sokal and Rohlf 2003). If the interaction was significant, the main level effects were post-hoc tested with univariate tests as described above.

Results

Burrowing behaviour

While there was no difference in burrowing propensity between the short and long photoperiod in any of the populations (Wald χ^2 = 0.006, df = 1, P = 0.936) (Fig. 2), a difference was detected when burrowing was compared among the populations (Wald χ^2 = 10.300, df = 2, P = 0.006). A significantly lower proportion of the beetles from the Paduan population were burrowed than in the Petroskoi (Bonferroni Multiple Comparison, BMC: P = 0.004) and Belchow (BMC: P = 0.045) populations, which in turn did not differ from each other (BMC: P = 1.000).

Burrowing and RMR

In 10-day-old beetles, the main effects of burrowing behaviour and population were significant (Table 2a). Beetles which had burrowed into the soil had lower RMR than beetles still on the soil surface (Fig. 3a, 3b). While the beetles from Petroskoi had significantly lower RMR than those from Belchow (BMC: P = 0.004), they did not differ from beetles from Padua (BMC: P = 0.282). Similarly, no burrowing related difference in RMR of beetles from Padua and Belchow was detected (BMC: P = 0.252). Photoperiod did not have a significant effect on RMR.

Burrowing and total lipid content

In the SD photoperiod, the beetles from all populations accumulated similar amounts of lipids which differed depending on burrowing behaviour (Table 3). Beetles which had burrowed into the soil had on average 6.5 mg more lipids than those still on the soil surface (Fig. 3c). In the LD photoperiod, however, the populations differed in total lipid content depending on whether beetles were on the surface or were burrowed into the soil (Fig. 3d). While beetles on

13

312   the surface from all populations had similar amounts of lipids (BMC: $P > 0.679$), the

313   burrowed beetles from Padua had significantly lower lipid content than those from Petroskoi

314   and Belchow (BMC: $P < 0.001$ and $P = 0.006$ respectively) and the latter populations did not

315   differ significantly from each other (BMC: $P = 1.000$). The burrowed beetles from Petroskoi

316   and Belchow had significantly higher lipid content than those on the surface (Univariate test:

317    $F_{1, 126} = 19.129$, $P < 0.001$ and Univariate test: $F_{1, 126} = 19.963$, $P < 0.001$ respectively) while

318   the burrowed beetles from Padua had as low lipid content as those on the surface (Univariate

319   test: $F_{1, 126} = 0.354$, $P = 0.553$).

320

321   *Relationship between total lipid content and RMR*

322   A negative relationship between RMR and lipid content was seen in beetles from all

323   populations when reared under SD conditions (Fig. 4). The relationship was significant for the

324   beetles from Padua and Petroskoi (Wald $\chi^2 = 7.330$, $df = 1$, $P = 0.007$ and Wald $\chi^2 = 31.676$,

325    $df = 1$, $P < 0.001$, respectively), while a marginally significant trend was seen for the beetles

326   from Belchow (Wald $\chi^2 = 3.508$, $df = 1$, $P = 0.061$). When reared in the LD photoperiod, the

327   beetles from Petroskoi showed the strongest negative relationship between RMR and lipid

328   content, which however was non-significant (Wald $\chi^2 = 3.113$, $df = 1$, $P = 0.078$). Also in

329   beetles from Padua or Belchow was the relationship negative but non-significant (Wald $\chi^2 =$

330   0.169, $df = 1$, $P = 0.681$ or Wald $\chi^2 = 0.999$, $df = 1$, $P = 0.318$, respectively).

331

332   *RMR trajectory with age*

333   RMR differed with age in a population and photoperiod dependent manner (Table 2b, Fig. 5).

334   At 5 days after emergence, no difference was detected in RMR among the populations in

335   either photoperiod (Univariate test: $F_{2, 502} = 1.522$, $P = 0.219$). RMR was higher under the SD

336   than under the LD photoperiod in all populations at 5 days after emergence (Univariate test:

14

337 $F_{1, 502} = 15.379$, $P < 0.001$). By day 10, the difference in RMR between photoperiods had

338 disappeared (Univariate test: $F_{1, 502} = 1.483$, $P = 0.224$) but there were differences among

339 populations (Univariate test: $F_{2, 502} = 7.660$, $P = 0.001$). The beetles from Petroskoi had

340 significantly lower RMR than the beetles from Belchow or Padua (BMC): $P < 0.001$ and $P =$

341 0.017, respectively) which, in turn, did not differ significantly from each other (BMC: $P =$

342 0.840).

343

344 **Discussion**

345 Successful expansion to a novel environment often requires that an animal either is able to

346 match its life-history with the biotic and abiotic requirements of the novel environment (Fig.

347 1, path 1; Elton 1958; Sakai et al. 2001; Lee 2002) or is capable of avoiding adverse

348 conditions through behaviour (Fig. 1, path 2; Sakai et al. 2001; Duckworth 2009; Wright et al.

349 2010; Phillips and Suarez 2012; Sunday et al. 2014). In the present study we investigated

350 whether the rapid latitudinal range expansion of *L. decemlineata* into novel, high latitude

351 environments in Europe has been associated with behavioural plasticity in overwintering

352 behaviour.

353   Our study shows that burrowing behaviour was plastic, as burrowing into the soil

354 occurred under both photoperiods in all populations and not only when the photoperiod

355 matched the one in their native area (Fig. 2). This was especially evident in the southern

356 population from Padua which, in case burrowing would be strictly related to photoperiodic

357 adaptation, was expected to burrow into the soil only under the short photoperiod (Lehmann

358 et al. 2012). This behavioural plasticity may have been one major factor that assisted in the

359 initial spread of *L. decemlineata* from native areas in Mexico to unpredictable agricultural

360 habitats in the southern United States of America (Hsiao 1985; Alyokhin 2009). Later, harsh

361 seasonal conditions, experienced when *L. decemlineata* invaded higher latitudes, selected for

362 overwintering in diapause and consequently diapause has become prevalent especially at high

363 latitudes (Danilevskij 1965; Hsiao 1985). Similar results are seen in the mosquito *Aedes*

364 *albopictus* where invasion and initial range expansion in North America took place without

365 significant phenological (photoperiodic) adaptation (Urbanski et al. 2012). Populations have

366 instead secondarily become adapted to local seasonal conditions (Urbanski et al. 2012). There

367 is a growing number of studies on both plants and animals which suggest that phenotypic

368 plasticity can play a major role in buffering initial responses to novel environmental

369 conditions, be this during range expansion or in response to climate change (Sexton et al.

370 2002, Merilä and Hendry 2014; Sunday et al. 2014).

371   Our results have implications for the use of burrowing as an indicator of diapause.

372 Since burrowed beetles from Padua show high overwintering mortality when reared under a

373 long day photoperiod, but not when reared under a short day photoperiod (Lehmann et al.

374 2012), burrowing behaviour on its own is not a sufficient, general, biomarker of diapause

375 initiation. Instead, it seems that beetles can burrow for other reasons than diapause (Hsiao

376 1985). In fact, several studies suggest that *L. decemlineata* does not overwinter in diapause in

377 its native range (northern Mexico) (Hsiao 1985; Alyokhin 2009; Izzo et al. 2013), and is

378 known to burrow into the soil for other reasons, such as avoidance of parasites, drought, high

379 temperatures or lack of food (Hsiao 1985; Tauber et al. 1986; Danks 1987). The capacity of

380 animals to use behaviour to choose optimal microclimates and avoid stress, and the

381 implications behavioural phenotypic plasticity has in mitigating deleterious effects associated

382 with stress due to for instance climate change, range expansion or anthropogenic factors has

383 been increasingly appreciated (Wright et al. 2010; Phillips and Suarez 2012; Sunday et al.

384 2014).

385   Even though beetles from all populations burrowed under both photoperiods,

386 indicating behavioural plasticity (Fig. 2), they differed in how burrowing behaviour was

387 linked to diapause-related physiological traits. In the beetles from Padua, burrowing was

388 accompanied with high lipid content only when reared under short day conditions (Fig. 3b).

389 Since lipid accumulation is an inherent part of diapause preparation (Hahn and Denlinger

390 2007; Lehmann et al. 2012), the results suggest that the burrowed Paduan beetles reared under

391 long day conditions had not prepared for diapause. Indeed the Paduan population suffer from

392 high winter mortality when reared under a long day (18 hours light) photoperiod (Lehmann et

393 al. 2012). Unlike the Paduan beetles, the beetles from Petroskoi and Belchow seem to prepare

394 for diapause also under a long photoperiod, as indicated by their high lipid content in both

395 photoperiods (Lehmann et al. 2012; see also Lehmann et al. 2014). Taken together these

396 results suggest that physiological adaptation has been important for successful establishment

397 at higher latitudes (Fig. 1, path 1; Piiroinen et al. 2011; Lehmann et al. 2012). Because

398 adaptive changes take time, the shift from behavioural-plasticity-driven to photoperiodic-

399 adaptation-driven range expansion could partly explain why the latitudinal range expansion of

400 *L. decemlineata* in Europe has slowed down during the last 30 years (Hsiao 1985; EPPO

401 2006). Other reasons can include temporal constraints due to a short summer (but see Boman

402 et al. 2008) or thermal constraints during a longer and harsher winter (Valosaari et al. 2008).

403 Furthermore, changes in anthropogenic factors (such as pesticide usage) should not be

404 overlooked when assessing range expansion of an agricultural pest (Alyokhin 2009; Piiroinen

405 et al. 2013).

406   The other physiological trait, RMR, was strongly linked to burrowing behaviour in all

407 populations (Fig. 3a), irrespective of whether or not beetles had prepared for diapause (based

408 on lipid content). The results suggest that lowered RMR of burrowed beetles is not

409 necessarily related to diapause initiation in *L. decemlineata*, as reported in several other

410 insects (Guppy and Withers 1999). Burrowed individuals might simply get immediate

411 energetic benefits by lowering RMR (Chown and Nicolson 2004). Lower CO_2 production

could also result from a metabolic shift from burning carbohydrates to lipids, not due to an active suppression of metabolic rate itself (Sinclair et al. 2011). Unfortunately we cannot separate these alternatives in the present study since we did not measure O_2 consumption (Lighton 2008). Diapause-related RMR suppression is also often driven by ambient temperature (Guppy and Withers 1999) which was not manipulated in the present study. However diapause initiation at higher latitudes generally occurs under relatively benign conditions in nature (Tauber et al. 1986) and thus the results of the present study likely reflect natural conditions when beetles initiate and burrow for diapause. Therefore the diapause-related suppression of RMR reported in previous studies likely reflects either metabolic responses to changes in ambient temperature, which generally decreases as diapause progresses (Guppy and Withers 1999), or a general shutdown of metabolic functions during later phases of diapause (Boiteau and Coleman 1996; Kostal 2006; Hahn and Denlinger 2007; but see Hodkova and Hodek 2004).

While RMR did not seem to be associated with the decision to enter diapause, it was associated with age, population and photoperiod. Beetles from all populations reared under short day conditions had higher RMR than beetles reared under long day conditions when 5 days old (Fig. 5). Taken together with the lipid data of 10-day-old beetles this result suggest that while beetles from Petroskoi and Belchow prepare for overwintering under both photoperiods, but they might do so faster when the photoperiod is shorter (de Kort 1990; Piiroinen et al. 2011). Between days 5 and 10, RMR decreased in all populations, and the photoperiodic effect disappeared. The general age-dependent decrease in RMR observed in all populations is probably not related to diapause initiation but is more likely due to developmental changes part of the maturation process (Hack 1997; Guppy and Withers 1999; Roberts and Rosenberg 2006). The 10-day-old adults might, after feeding intensively, lessen feeding and thus have lower gut metabolic activity (Guppy and Withers 1999), regardless of

437   whether they are undergoing reproductive maturation or pre-diapause development. However,

438   the northernmost population had lower RMR at age 10 days than the other populations (Fig.

439   5) even when the proportion of burrowed beetles is taken into account (data not shown). Since

440   the populations did not differ at the age of 5 days, this result suggests that RMR in beetles

441   from Petroskoi has a steeper negative age-dependent slope than southern beetles. These

442   results could suggest that the northernmost beetles prepare for diapause faster than the other

443   populations, which could relate to temporal constraints imposed by a shorter growth season

444   (and time to prepare for diapause) at high latitudes. Alternatively, since energy consumption

445   decreases with metabolic rate, a lower absolute RMR could be linked to stronger conservation

446   of limited energy reserves during the winter (Guppy and Withers 1999; Hahn and Denlinger

447   2007), which becomes progressively longer at higher latitude. Clines in RMR are known in

448   many ectothermic species with large ranges, and therefore RMR has been suggested to be

449   linked to adaptation to season length and range expansion propensity (Chown and Gaston

450   1999). Both negative (Clarke 1993) and positive (Block and Young 1978) within-species

451   correlations between latitude and RMR have been documented (Chown and Gaston 1999).

452   While we cannot separate whether the differences between the populations in RMR in the

453   present study relate to slope or absolute value, the results suggest that RMR varies with

454   latitude in *L. decemlineata* and that this variation could have contributed to the expansion

455   process, as suggested also in other species (Block and Young 1978; Clarke 1993; Chown and

456   Gaston 1999; Guppy and Withers 1999).

457

458   *Conclusions*

459   In this study we found behavioural plasticity in overwintering behaviour in European *L.*

460   *decemlineata*, since Paduan beetles burrowed into the soil when reared under a non-diapause

461   inducing long photoperiod. Even though RMR was lower in burrowed beetles regardless of

photoperiod, lipid content was higher in burrowed beetles only when they were reared under diapause inducing photoperiods. Since low lipid content in burrowed beetles is associated with low winter survival (Lehmann et al. 2012), these data suggest that neither RMR at the age when beetles burrow nor burrowing behaviour necessarily are good indicators of diapause initiation. This study suggests that burrowing behaviour on its own has not been sufficient for successful range expansion into environments with strong seasonality (Fig. 1, path 2) even though it might have assisted in the initial invasion of more benign environments in southern Europe. Instead synchronization of burrowing behaviour and relevant diapause related physiological traits with photoperiod have been needed for survival of *L. decemlineata* during the long and harsh winters in northern Europe (Fig. 1, path 1). Lastly, a steeper slope in age-dependent RMR trajectory of northern *L. decemlineata* suggests that diapause preparation becomes faster as latitude increases (Lehmann et al. 2014) which could be due to temporal constraints. Our results underline that eco-physiological and life-history traits, such as diapause, should be included in studies on range expansion (including applied pest-management modelling). Only then can we make more reliable range expansion predictions, in particular in the face of climate change (Bradshaw and Holzapfel 2001; Gomi 2007; Mahdjoub and Menu 2008; Bean et al. 2012; Saikkonen et al. 2012; Urbanski et al. 2012, Jönsson et al. 2013, Sunday et al. 2014).

487   Biological Interactions Research). *L. decemlineata* is a quarantine species in Finland and

488   therefore this experiment was carried out under permission (Evira 3861/541/2007). The

489   authors declare that they have no conflict of interest.

490

491 **References**

492   Alyokhin AV (2009) Colorado potato beetle management on potatoes: current challenges and

493   future prospects. Fruit Veg Cereal Sci Biotech 3:10-19

494   Arrese EL, Soulages JL (2010) Insect fat body: energy, metabolism, and regulation. Annu

495   Rev Entomol 55:207-225

496   Bean DW, Dalin P, Dudley TL (2012) Evolution of critical day length for diapause induction

497   enables range expansion of *Diorhabda carinulata*, a biological control agent against

498   tamarisk (*Tamarix* spp.). Evol App 5:511-523

499   Beck SD, Hanec W (1960) Diapause in the European corn borer, *Pyrausta nubialis* (Hübn.). J

500   Insect Physiol 4:304-318

501   Beekman M, van Stratum P, Lingeman R (1998) Diapause survival and post-diapause

502   performance in Bumblebee queens (*Bombus terrestris*). Entomol Exp Appl 89:207-214

503   Block W, Young SR (1978) Metabolic adaptations of Antarctic terrestrial micro arthropods.

504   Comp Biochem Physiol 61A:363-368

505   Boiteau G, Coleman W (1996) Cold tolerance in the Colorado potato beetle, *Leptinotarsa*

506   *decemlineata* (Say) (Coleoptera: Chrysomelidae). Can Entomol 128:1087-1099

507   Bradshaw WE, Holzapfel CM (2001) Genetic shift in photoperiodic response correlated with

508   global warming. Proc Nat Acad Sci USA 98:14509-14511

509   Bradshaw WE, Holzapfel CM (2007) Evolution of animal photoperiodism. Ann Rev Ecol

510   Evol Syst 3:1-25

511   Casagrande RA (1987) The Colorado potato beetle: 125 years of mismanagement. Bull

512   Entomol Soc Am 33:142–150

513   Chown SL, Gaston K (1999) Exploring links between physiology and ecology at macro-

514   scales: the role of respiratory metabolism in insects. Biol Rev 74:87-120

515   Chown SL, Nicolson SW (2004) Insect physiological ecology - mechanisms and patterns.

516   Oxford University Press, New York

517   Clarke A (1993) Seasonal acclimatization and latitudinal compensation in metabolism: do

518   they exist? Funct Ecol 7:139-149

519   Danilevski AS (1965) Photoperiodism and seasonal development of insects. Oliver and Boyd,

520   London

521   Danks HV (1987) Insect dormancy: an ecological perspective. Biological Survey of Canada

522   (Terrestrial Arthropods), Ottawa. Biological Survey of Canada Monograph Series No. 1

523   Duckworth RA (2009) The role of behavior in evolution: a search for mechanism. Evol Ecol

524   23:513-531

525   Elton CS (1958) The ecology of invasions by animals and plants. Melthuen & Co, London

526   EPPO (2006) Distribution maps of quarantine pests for Europe, *Leptinotarsa decemlineata*.

527   ADAS, Nottingham, UK. Available at: http://pqr.eppo.org/datas/LPTNDE/LPTNDE.pdf

528   Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and

529   purification of total lipids from animal tissue. J Biol Chem 226:497-509

530   Gabel F, Pusch MT, Breyer P, Burmester V, Walz N, Garcia X (2011) Differential effect of

531   wave stress on the physiology and behaviour of native versus non-native benthic

532   invertebrates. Biol Invasions 13:1843-1853

533   Gaston K (2003) The structure and dynamics of geographic ranges. Oxford Series in Ecology

534   and Evolution, Oxford University Press, New York

535 Gomi T (2007) Seasonal adaptations of the fall webworm Hypantria cunea (Drury)

536   (Lepidoptera:Arctiidae) following its invasion of Japan. Ecol Res 22:855-861

537 Grapputo A, Boman S, Lindström L, Lyytinen A, Mappes J (2005) The voyage of an invasive

538   species across continents: genetic diversity of North American and European Colorado

539   potato beetle populations. Mol Ecol 14:4207-4219

540 Guppy M, Withers P (1999) Metabolic depression in animals: physiological perspectives and

541   biochemical generalizations. Biol Rev 74:1-40

542 Hack MA (1997) The effects of mass and age on standard metabolic rate in house crickets.

543   Physiol Entomol 22:325-331

544 Hahn DA, Denlinger DL (2007) Meeting the energetic demands of insect diapause: Nutrient

545   storage and utilization. J Insect Physiol 53:760-773

546 Hanshew BA, Garcia TS (2012) Invasion of the shelter snatchers: behavioural plasticity in

547   invasive red swamp crayfish, *Procambarus clarkii*. Freshwater Biol 57:2285-2296

548 Hodkova M, Hodek I (2004) Photoperiod, diapause and cold-hardiness. Eur J Entomol

549   101:445-458

550 Hsiao TH (1985) Eco-physiological and genetic aspects of geographic variations of the

551   Colorado potato beetle. Research bulletin - Massachusetts Agricultural Experiment

552   Station 704:63-77

553 Izzo VM, Hawthorne DJ, Chen YH (2013) Geographic variation in winter hardiness of a

554   common agricultural pest, Leptinotarsa decemlineata, the Colorado potato beetle. Evol

555   Ecol, Accepted Online Version DOI: 10.1007/s10682-013-9681-8

556 Johnson CG (1967) International dispersal of insects and insect-borne viruses. Eur J Plant

557   Pathol 73:21-43

558 Jönsson AM, Pulatov B, Linderson M-J, Hall K (2013) Modelling as a tool for analysing the

559   temperature-dependent future of the Colorado potato beetle in Europe. Glob Change Biol

560   19:1043-1055

561 Kenneth P, Case TJ (1996) An experimental demonstration of exploitation competition in an

562   ongoing invasion. Ecology 77:118-132

563 de Kort CAD (1990) Thirty-five years of diapause research with the Colorado potato beetle.

564   Entomol Exp Appl 56:1-13

565 Kostal V (2006) Eco-physiological phases of insect diapause. J Insect Physiol 52:113-127

566 Kuang X, Xu J, Xia Q, He H, Xue F (2011) Inheritance of the photoperiodic response

567   controlling imaginal summer diapause in the Cabbage beetle, *Colaphellus bowringii.* J

568   Insect Physiol 57:614-619

569 Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386-391

570 Lefevere KS, de Kort CAD. (1989) Adult diapause in the Colorado potato beetle,

571   *Leptinotarsa decemlineata*: effects of external factors on maintenance, termination and

572   post-diapause development. Physiol Entomol 14:299-308

573 Lehmann P, Lyytinen A, Sinisalo T, Lindström L (2012) Population dependent effects of

574   photoperiod on diapause related physiological traits in an invasive beetle (Leptinotarsa

575   decemlineata). J Insect Physiol 58:1146-1158

576 Lehmann P, Piiroinen S, Kankare M, Lyytinen A, Paljakka M, Lindström L (2014)

577   Photoperiodic effects on diapause-associated gene expression trajectories in European

578   Leptinotarsa decemlineata populations. Insect Mol Biol (in press)

579 Lyytinen A, Mappes J, Lindström L (2012) Variation in Hsp70 levels after cold shock: signs

580   of evolutionary responses to thermal selection among *Leptinotarsa decemlineata*

581   populations. PLoS ONE 7:e31446

582 Mahdjoub T, Menu F (2008) Prolonged diapause: A trait increasing invasion speed? J Theor

583   Biol 251:317-330

584 May ML (1989) Oxygen consumption by adult Colorado potato beetles, *Leptinotarsa*

585   *decemlineata* (Say) (Coleoptera: Chrysomelidae). J Insect Physiol 35:797-804

586 Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the

587   problem and the evidence. Evol Appl 7:1-14

588 Nelson RJ, Denlinger DL, Somers DE (eds) (2010) Photoperiodism, the biological calendar.

589   Oxford University Press, Oxford

590 Phillips BL, Suarez AV (2012) The role of behavioural variation in the invasion of new areas.

591   in Candolin U, Wong BM. (eds): Behavioural responses to a changing world:

592   mechanisms and consequences. Oxford University Press, Oxford

593 Pigliucci M, Murren CJ (2003) Genetic assimilation and a possible evolutionary paradox: can

594   macroevolution sometimes be so fast as to pass us by? Evolution 57:1455-1464

595 Piiroinen S, Lindström L, Lyytinen A (2010) Resting metabolic rate can vary with age

596   independently from body mass changes in the Colorado potato beetle, *Leptinotarsa*

597   *decemlineata*. J Insect Physiol 56:277-282

598 Piiroinen S, Ketola T, Lyytinen A, Lindström L (2011) Energy use, diapause behaviour and

599   northern range expansion potential in the invasive Colorado potato beetle. Funct Ecol

600   25:527-536

601 Piiroinen S, Lindström L, Lyytinen A, Mappes J, Chen YH, Izzo V, Grapputo A (2013) Pre-

602   invasion history and demography shape the genetic variation in the insecticide resistance-

603   related acetylcholinesterase 2 gene in the invasive Colorado potato beetle. BMC Evol

604   Biol 13:13

605 Randall D, Burggren W, French K (eds) (2002) Animal physiology. Freeman and Company,

606   New York

607 Rasa OAE (1999) Division of labour and extended parenting in a desert tenebrioid beetle.

608   Ethology 105:37-56

609 Rankin MA, Burchsted JCA (1992) The cost of migration in insects. Annu Rev Entomol

610   37:533-559

611 Roubik DW, Michener CD (1980) The seasonal cycle and nests of *Epicharis zonata*, a bee

612   whose cells are below the wet-season water table (Hymenoptera, Anthophoridae). Biotrop

613   12:56-60

614 Roberts SB, Rosenberg I (2006) Nutrition and aging: changes in the regulation of energy

615   metabolism with aging. Physiol Rev 86:651-667

616 Sagata K, Lester PJ (2009) Behavioural plasticity associated with propagule size, resources,

617   and the invasion success of the Argentine ant *Linepithema humile*. J App Ecol 46:19-27

618 Saikkonen K, Taulavuori K, Hyvönen T, Gundel PE, Hamilton CE, Vänninen I, Nissinen A,

619   Helander M (2012) Climate change-driven species' range shifts filtered by

620   photoperiodism. Nature Clim Change 2:239-242

621 Sakai AK, Allendorf FW, Holt JS, Lodge DM, Mologsky J, With KA, Baughman S, Cabin

622   RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thomspon JN, Weller

623   SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305-332

624 Sexton JP, McKay JK, Sala A (2002) Plasticity and genetic diversity may allow saltcedar to

625   invade cold climate in North America. Ecol Appl 12:1652-1660

626 Sinclair BJ, Bretman A, Tregenza T, Tomkins JL, Hosken DJ (2011) Metabolic rate does not

627   decrease with starvation in Gryllus bimaculatus when changing fuel use is taken into

628   accound. Physiol Entomol 36:84-89

629 Sokal RR, Rohlf FJ (2003) Biometry, the principles and practice of statistics in biological

630   research. W.H. Freeman and Company, New York

631 Solbreck C, Windefalk O (2012) Very long diapause and extreme resistance to population

632   disturbance in a galling insect. Ecol Entomol 37:51-55

633 Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB (2014)

634   Thermal-safety margins and the necessity of thermoregulatory behavior across latitude

635   and elevation. Proc Nat Acad Sci USA 111:5610-5615

636 Tauber MJ, Tauber CA, Masaki S (1986) Seasonal adaptations of insects. Oxford University

637   Press, New York

638 Tauber MJ, Tauber CA, Obrycki JJ, Gollands B, Wright RJ (1988) Voltinism and the

639   induction of aestival diapause in the Colorado potato beetle *Leptinotarsa decemlineata*

640   (Coleoptera: Chrysomelidae). University of Nebraska, Faculty Publications: Department

641   of Entomology, Paper 109

642 Urbanski J, Mogi M, O'Donnell D, DeCotiis M, Toma T, Armbruster P (2012) Rapid

643   adaptive evolution of photoperiodic response during invasion and range expansion across

644   a climatic gradient. Am Nat 179:490-500

645 West-Eberhard M-J (2003) Developmental plasticity and evolution. Oxford University Press,

646   New York

647 Williams CM, Thomas RH, MacMillan HA, Marshall KE, Sinclair BJ (2011)

648   Triacylglyceride measurement in small quantities of homogenised insect tissue:

649   comparisons and caveats. J Insect Physiol 57:1602-1613

650 Williams JB, Lee RE Jr (2008) Differences in cold tolerance, desiccation resistance, and

651   cryoprotectant production between three populations of *Eurosta solidaginis* collected

652   from different latitudes. J Comp Physiol B 178:365-375

653 Wright TF, Eberhard JR, Hobson EA, Avery ML, Russello MA (2010) Behavioral flexibility

654   and species invasions: the adaptive flexibility hypothesis. Ethol Ecol Evol 22:393-404

Tables

Table 1 The number of *Leptinotarsa decemlineata* adults used in the lipid and metabolic (shown in brackets) study from the two age groups and three populations (sexes are pooled). For the 5-day-old beetles, no lipid quantification was performed and for the 10-day-old beetles the number burrowed beetles is also shown. The mean weight of beetles (mg ± SE) is also shown.

	Short Day			Long Day		
	Petroskoi	Belchow	Padua	Petroskoi	Belchow	Padua
	N (N in RMR)	N (N in RMR)	N (N in RMR)	N (N in RMR)	N (N in RMR)	N (N in RMR)
5 days old	-- (46)	-- (36)	-- (43)	-- (43)	-- (35)	-- (37)
Weight (mg)	141 ± 3.6	125 ± 3.3	130 ± 3.1	140 ± 3.5	128 ± 2.9	132 ± 3.4
10 days old	50 (47)	40 (41)	43 (42)	53 (55)	39 (39)	42 (43)
Weight (mg)	146 ± 3.0	136 ± 2.9	134 ± 3.0	143 ± 3.1	139 ± 3.4	130 ± 2.9
Burrowed	29 (28)	19 (20)	12 (12)	27 (28)	20 (20)	14 (15)

662 Table 2 GLM (ANCOVAs) testing differences in RMR of adult *L. decemlineata* from three

663 populations reared under two photoperiods in a) 10-day-old beetles either burrowed or on soil

664 surface and b) 5- and 10-day-old beetles.

Effect	df	MS	F	P
a)				
Intercept	1	10.490	483.290	<0.001
Activity (covariate)	1	0.417	19.193	<0.001
Mass (covariate)	1	0.182	8.405	0.004
Population	2	0.116	5.323	0.005
Photoperiod	1	0.029	1.339	0.248
Burrowing behaviour	1	0.358	16.479	<0.001
Error	265	0.022		
b)				
Intercept	1	20.380	1088.209	<0.001
Activity (covariate)	1	1.043	55.690	<0.001
Mass (covariate)	1	0.461	24.640	<0.001
Age	1	3.645	194.617	<0.001
Population	2	0.050	2.695	0.068
Photoperiod	1	0.255	13.638	<0.001
Age*Population	2	0.114	6.086	0.002
Age*Photoperiod	1	0.019	4.096	0.044
Error	502	0.019		

665

666 Table 3 GLMs (ANCOVAs) of total abdominal lipid content (mg) of 10-day-old adult *L.*

667 *decemlineata* originating from three populations. Photoperiods were analysed in separate

668 models. Abdomen lean (lipid-free) mass was used as covariate.

	Effect	df	MS	F	P
Short day	Intercept	1	0.216	0.006	0.937
	Abdomen mass (covariate)	1	1001.071	29.449	<0.001
	Population	2	55.222	1.624	0.201
	Burrowing behaviour	1	1241.258	36.514	<0.001
	Population*Burrowing behaviour	2	2.297	0.068	0.935
	Error	126	33.994		
Long day	Intercept	1	194.747	6.742	0.011
	Abdomen mass (covariate)	1	1762.929	61.030	<0.001
	Population	2	179.939	6.210	0.003
	Burrowing behaviour	1	804.683	27.857	<0.001
	Population*Burrowing behaviour	2	122.615	4.245	0.016
	Error	127	28.886		

669

670 **Figure captions**

671 **Fig. 1** Conceptual path diagram summarizing the potential role of behavioural plasticity

672 during range expansion into novel stressful environments. Path 1 represents behaviour

673 initially buffering against stressors, but ultimately leading to local adaptations through genetic

674 assimilation (Lee, 2002; Pigliucci and Murren 2003; Duckworth 2009). Path 2 represents

675 behaviour buffering against environmental stressors to such a degree that adaptation is

676 hindered or significantly slowed down (Duckworth 2009; Wright et al. 2010).

677 **Fig. 2** The percentage of *L. decemlineata* beetles burrowed into the soil at age of 10 days after

678 eclosion when reared under a short and long photoperiod. Numbers at base of pillars show

679 sample size.

680 **Fig. 3** Difference in (a/b) mass-specific resting metabolic rate and (c/d) total abdominal lipid

681 content of *L. decemlineata* beetles on the soil surface and burrowed into the soil when reared

682 under a (a/c) short or (b/d) long photoperiod. Although mass-specific values are plotted,

683 analyses were conducted with mass as covariate.

684 **Fig. 4** Mass-specific resting metabolic rate as a function of lipid content (% of abdomen dry

685 mass) for 10-day-old *L. decemlineata* originating from Petroskoi (Pet.), Belchow (Bel.) and

686 Padua (Pad.) reared under a short (SD) or long (LD) photoperiod. Lines are drawn when the

687 relationship was significant. See text for statistical details. Although mass-specific values are

688 plotted, analyses were conducted with beetle total mass (including lipids) as covariate.

689 **Fig. 5** Mass-specific resting metabolic rate of 5- and 10-day-old *L. decemlineata* from three

690 populations reared under either a long (LD) or short (SD) photoperiod. Although mass-

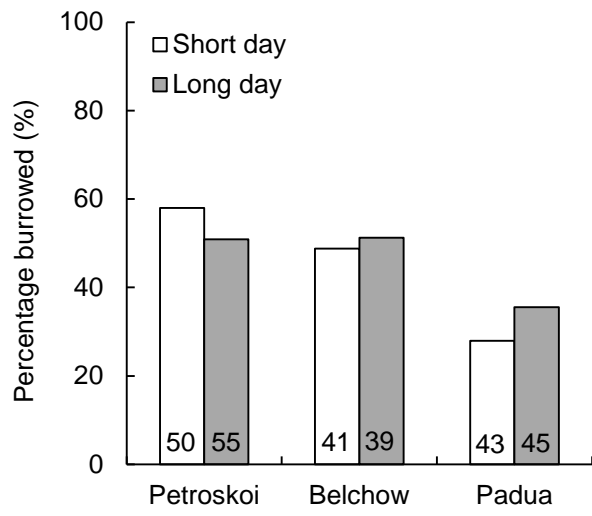691 specific values are plotted, analyses were conducted with mass as covariate.
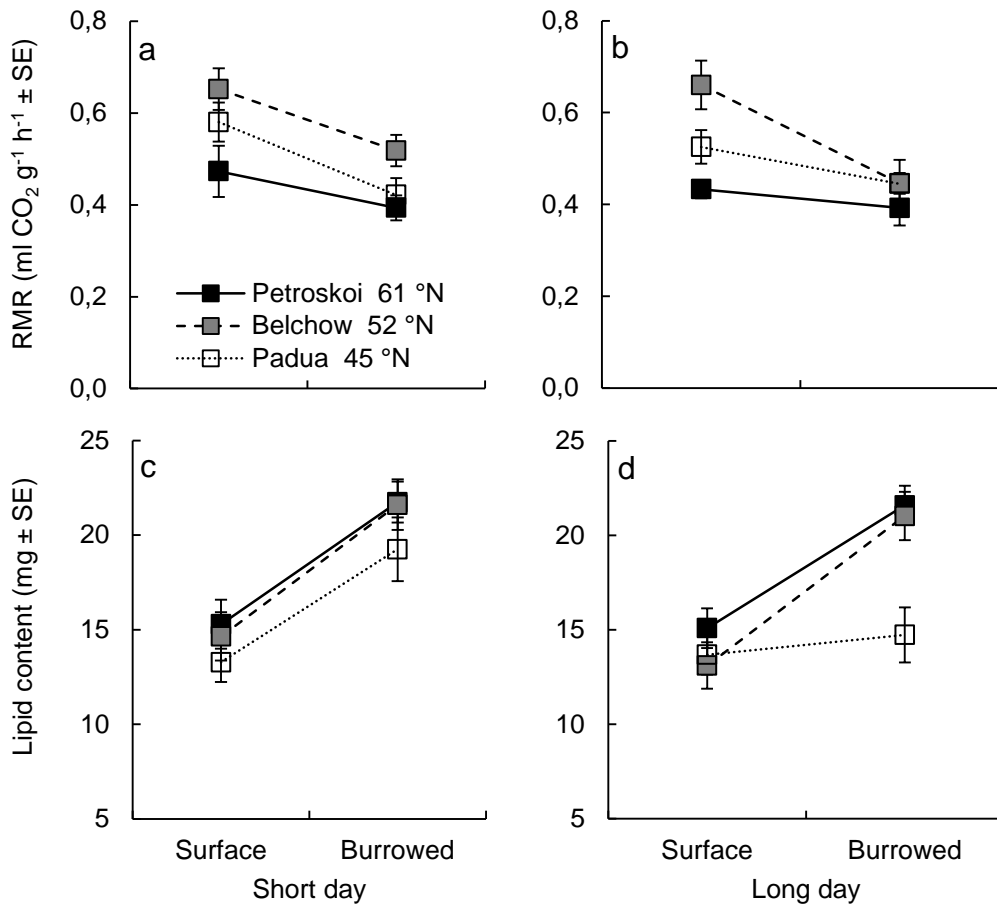
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692 **Figures**

693 Figure 1
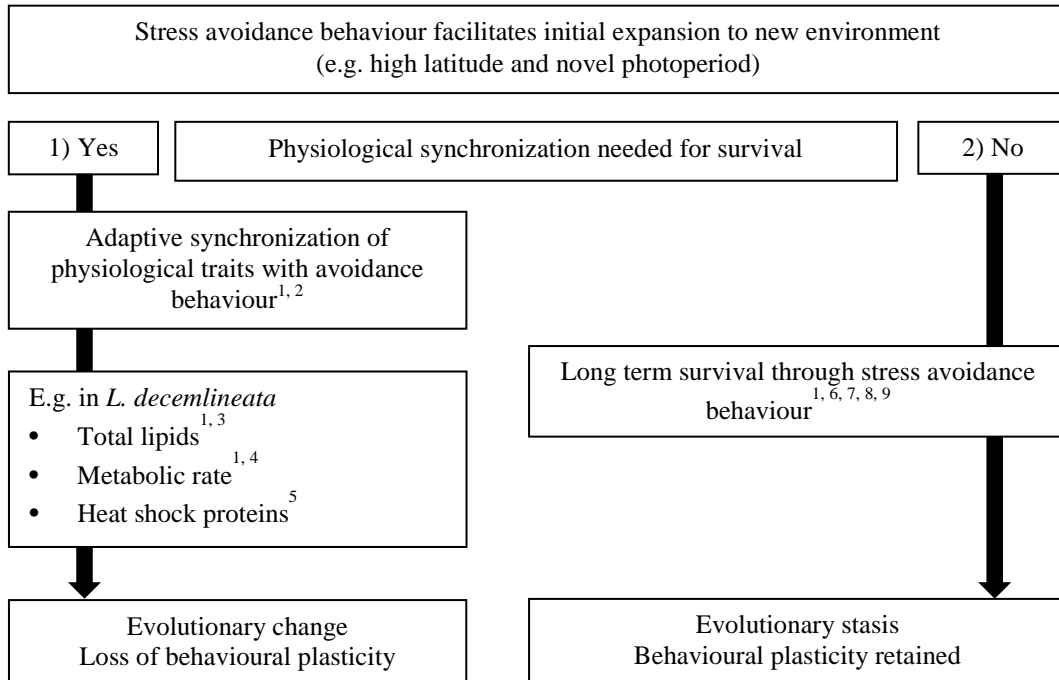
Studies cited in this figure: The present study[1], Williams et al, 2008[2],Lehmann et al. 2012[3], Piiroinen et al. 2011[4], Lyytinen et al. 2012[5], Kenneth and Case 1996[6], Wright et al. 2010[7], Hanshew and Garcia 2012[8], Gabel et al. 2011[9]
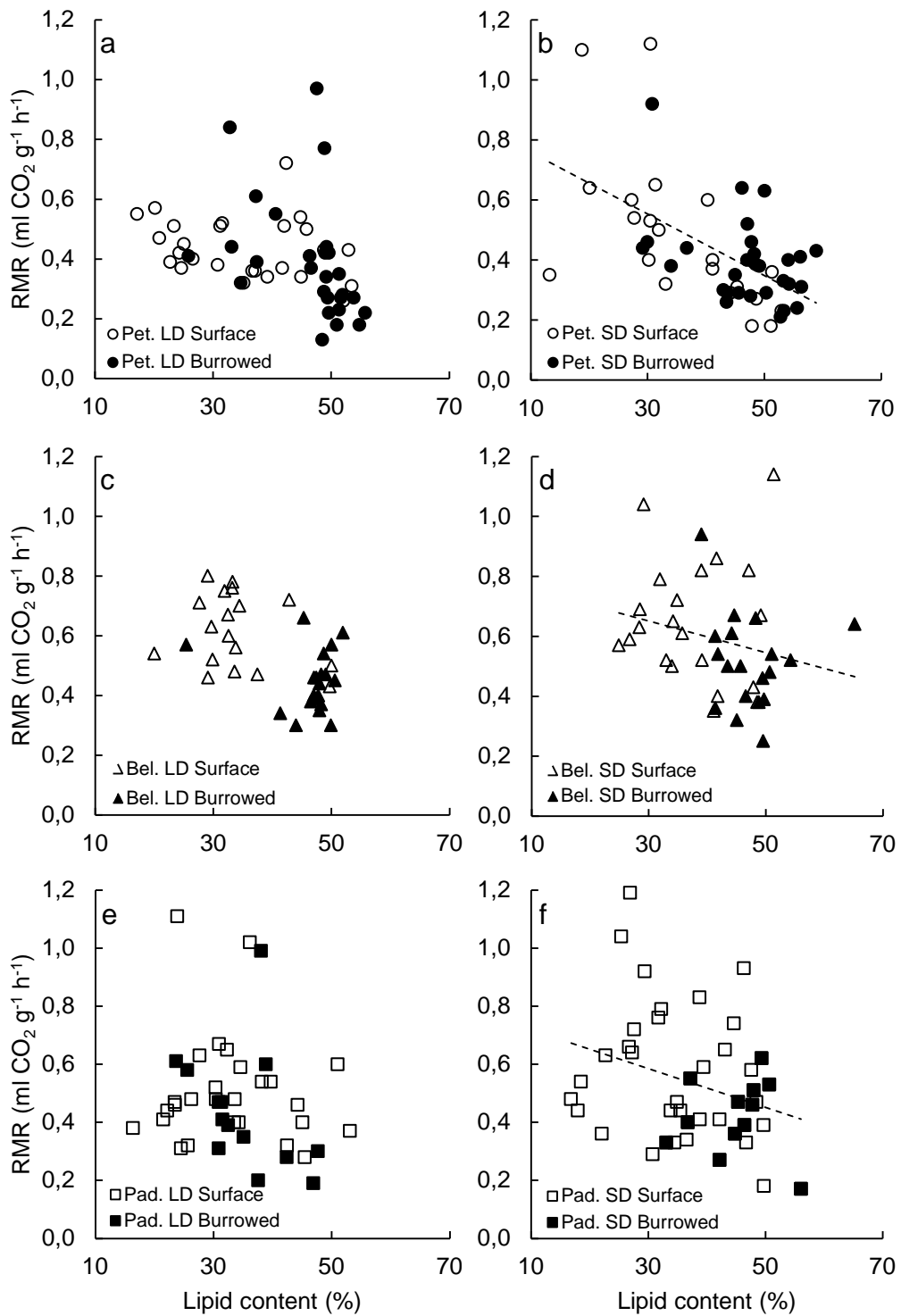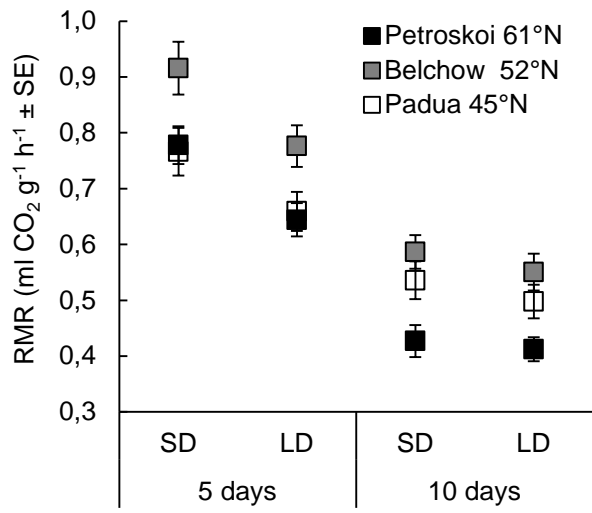
694

695   Figure 2

696

697   Figure 3

701   Figure 5

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