

**This is an electronic reprint of the original article.
This reprint *may differ* from the original in pagination and typographic detail.**

Author(s): Lehmann, Philipp; Margus, Aigi; Lindström, Leena

Title: Inheritance patterns of photoperiodic diapause induction in *Leptinotarsa decemlineata*

Year: 2016

Version:

Please cite the original version:

Lehmann, P., Margus, A., & Lindström, L. (2016). Inheritance patterns of photoperiodic diapause induction in *Leptinotarsa decemlineata*. *Physiological Entomology*, 41(3), 218-223. <https://doi.org/10.1111/phen.12145>

All material supplied via JYX is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.

1 Lehmann, P., Margus, A., & Lindström, L. (2016). Inheritance patterns of photoperiodic diapause
2 induction in *Leptinotarsa decemlineata*. *Physiological Entomology*, 41 (3), 218-223.
3 doi:10.1111/phen.12145
4 <http://onlinelibrary.wiley.com/doi/10.1111/phen.12145/abstract>

5

6 **Inheritance patterns of photoperiodic diapause induction in *Leptinotarsa***
7 ***decemlineata***

8

9 Philipp Lehmann^{1,2}, Aigi Margus², Leena Lindström²

10

11 ¹Department of Zoology, 10691, University of Stockholm, Sweden

12

13 ²Centre of Excellence in Biological Interactions Research, Department of Biological and
14 Environmental Science, P.O. Box 35, 40014 University of Jyväskylä, Finland

15

16 Correspondence:

17 Philipp Lehmann, Department of Zoology, 10691, University of Stockholm, Sweden, Phone:
18 +468164089, email: philipp.lehmann@zoologi.su.se

19

20 Running title: Diapause inheritance in an invasive beetle

21 **Abstract**

22

23 Photoperiod is a reliable indicator of season and an important cue which many insects use for
24 phenological synchronization. While undergoing range expansion insects can face a change
25 in the local photoperiod to which they need to resynchronize. Rapid range expansion can be
26 associated with rapid photoperiodic adaptation which can be associated with intense selection
27 on strongly heritable polygenic traits. Alternatively it has been proposed that, in insects with
28 a XO sex-determination system, genes with large effect residing on the sex chromosome
29 could be driving photoperiodic adaptation, since the gene or genes are exposed to selection in
30 the sex carrying only a single X-chromosome. The current study seeks to understand which
31 of these alternatives is more likely in explaining rapid photoperiodic adaptation in European
32 Colorado potato beetles, *Leptinotarsa decemlineata*. Diapause induction is assessed in beetles
33 from a northern and a southern population as well as from reciprocal hybrid crosses between
34 the northern and southern population, when reared at an intermediate length photoperiod. The
35 crosses within population display expected responses, the northern showing high and the
36 southern low diapause propensity. The hybrids show intermediate responses in all studied
37 traits. No clear difference in responses in hybrids depending on latitudinal origin of their
38 father or mother are detected even though partial paternal line dominance is seen in responses
39 of male beetles in one hybrid cross. These results therefore indicate that, in *L. decemlineata*,
40 photoperiodic diapause induction is strongly heritable, and has an additive polygenic
41 autosomal background.

42

43 **Key words.** Autosomal inheritance, Coleoptera, hybridization, invasive species, sex linkage,
44 rapid adaptation

45 **Introduction**

46

47 At high latitude the year generally comprises a benign season which is favourable for
48 ontogenic development and reproduction, and a harsh season which needs to be endured until
49 the advent of the next favourable season. Many strategies exist by which life-cycles are
50 optimally synchronized with local season length in different organisms (Gaston, 2003).
51 Insects and other small ectotherms generally endure the harsh part in a resting stage called
52 diapause, a deep form of dormancy, where development is arrested, metabolism is suppressed
53 and stress-tolerance increased (Tauber *et al.*, 1986). The decision to enter diapause is often
54 regulated by external factors such as temperature, humidity or photoperiod (Nelson *et al.*,
55 2010). Since photoperiod is an inter-annually reliable estimator of time of the year, it is the
56 most commonly used signal which triggers diapause, be this in maternally controlled
57 embryonic diapause, larval, pupal or adult diapause (Tauber *et al.*, 1986).

58

59 Insects expanding their range along a latitudinal axis can face a change in local season length
60 to which they often must respond. This can be through sheer abiotic and biotic stress
61 tolerance, through phenotypic plasticity or by adaptive resynchronization of their life-cycle
62 with the changes in seasonality (Tauber *et al.*, 1986; Gaston, 2003; Bradshaw & Holzapfel,
63 2007; Lehmann *et al.*, 2014). While the diapause phenotype itself seems to be fairly
64 conserved between different species (Ragland *et al.*, 2010) the genetic background and
65 inheritance patterns of diapause induction seems to differ between species. Differences in
66 inheritance pattern could be related to which part of the induction machinery is under
67 selection (Emerson *et al.*, 2009). It is suggested that three major components are needed to
68 translate photoperiod into diapause: a photoreceptor, a photoperiodic counter and a hormonal
69 effector (Saunders, 2002; Shiga & Numata, 2007; Saunders, 2012; Goto, 2013). Different

70 modes of inheritance could relate to which parts of the diapause induction machinery that has
71 relevant adaptive variation upon which selection can act.

72

73 Some studies show that between-population differences in diapause induction depend on the
74 action of autosomal genes of varying effect sizes (Kim *et al.*, 1995; Söderlind & Nylin, 2011;
75 Chen *et al.*, 2012). Other studies suggest that between-population differences are strongly
76 controlled by the action genes residing on the sex chromosomes (Lumme & Keränen, 1978;
77 Kimura & Yoshida, 1995; Ikten *et al.*, 2011). Further, while diapause induction seems to be
78 inherited in an additive manner in some species, other species show varying degrees of
79 additivity, dominance and epistasis (Mathias *et al.*, 2007, Bradshaw *et al.*, 2012).
80 Photoperiodicity is shown to be strongly heritable in several studies (see Bradshaw &
81 Holzapfel, 2007; Urbanski *et al.*, 2012), in part likely since selection often weeds out
82 individuals with mismatched phenology (Tauber *et al.*, 1986). Indeed, rapid photoperiodic
83 adaptation are observed in a number of species, both as a response to climate change induced
84 phenological shifts (Bradshaw & Holzapfel, 2001) or as a consequence of invasion and
85 subsequent range expansion in a novel environment (Sadakiyo & Ishihara, 2011; Urbanski *et*
86 *al.*, 2012).

87

88 The present study represents a first test of mode of inheritance of photoperiodic diapause
89 induction in the invasive pest insect, the Colorado potato beetle, *Leptinotarsa decemlineata*
90 (Say). This species originates from low latitudes of around 30°N in North America and has
91 rapidly spread across areas where potato or its relatives, including paprika, tomato and
92 eggplant, are cultivated (Walsh, 1865; Alyokhin, 2009). In Europe *L. decemlineata* became
93 established around 1922 and has spread to latitudes of around 62°N in Russia in under 100
94 generations (Johnson, 1967). Winter is spent in facultative diapause, and the number of

95 annual generations depend on local summer length (Hisao, 1985; Alyokhin, 2009). Previous
96 studies show that local populations in northern and southern Europe differ in their
97 photoperiodic responses, northern populations use longer photoperiods as cue for diapause
98 than the southern populations (Lehmann *et al.*, 2012, 2015).

99

100 Here the overwintering response of *L. decemlineata* from pure northern and southern
101 European populations, along with the responses of reciprocal crosses between the populations
102 is studied. An intermediate photoperiod (16.5 h) which should induce diapause in the
103 northern but not southern population (Lehmann *et al.*, 2015) is used. Like most beetles
104 (Smith & Virkki, 1978) *L. decemlineata* has an XO sex-determination system (Hsiao &
105 Hsiao, 1983), with male beetles only inheriting one copy of the sex chromosome, from their
106 mother. This allows us to assess whether photoperiodic responses are likely inherited in an
107 additive autosomal manner, or if inheritance is linked to genetic factors residing on the sex
108 chromosome. In case the genetic factors determining the photoperiodic response are
109 autosomal, between-population hybrids should show an intermediate diapause response to the
110 treatment photoperiod, when compared to within-population beetles. This hypothesis assumes
111 that inheritance is fully additive, and not influenced by dominance or epistatic effects (Lynch
112 & Walsh, 1998; Bradshaw *et al.*, 2012). On the contrary, in case the genetic determinants
113 reside on the sex chromosome, between-population hybrids should differ in their response
114 depending on origin of their mother and father, respectively. Female hybrids should show an
115 intermediate response, while male hybrids should show a similar response as their mothers,
116 again assuming additive inheritance. Since evolution of sex chromosome-linked traits can be
117 very rapid (e.g. Singh & Petrov, 2007; Johnson & Lachance, 2012), sex-linked inheritance of
118 photoperiodic diapause induction could partly explain why *L. decemlineata* has been able to
119 spread across such a large latitudinal range so quickly.

120

121 **Materials and methods**

122

123 *Study animals and rearing conditions*

124 The descendants of *Leptinotarsa decemlineata* collected from potato fields from two
125 populations, a northern and a southern European population, were used in this experiment.

126 The northern beetles were collected from fields near Petroskoi in northern Russia (61°49'N,
127 34°10'E and 59°95'N, 30°30'E) in 2006, and the southern beetles from fields near Padua in
128 Italy (45°48'N, 12°07'E) in 2008 and 2010. Field collected beetles were used to establish

129 laboratory populations. In each generation, unrelated parental beetles (overwintered
130 generation) were mated within populations under a photoperiodic cycle of LD 18:6 h, and
131 their offspring (summer generation) were reared in family groups until adulthood under a
132 photoperiodic cycle of LD 18:6 h at the constant temperature of 23°C in a controlled
133 environmental chamber (Type B1300; Weiss Technik, Reiskirchen-Lindenstruth, Germany).

134 After eclosion adult beetles in each generation were maintained individually in petri dishes
135 (diameter of 9 cm) containing a water moistened filter paper and fed daily with fresh potato
136 leaves and stems (*Solanum tuberosum* Linnaeus, van Gogh variety) under a diapause

137 inducing photoperiodic cycle of LD 12:12 h (at 23°C, ~60% relative humidity) (Lehmann *et*
138 *al.*, 2015). Adult beetles were overwintered in plastic jars (volume 80 ml) containing peat and

139 a layer of plasticine for moisture retention. When beetles had been burrowed for 10 days they
140 were moved to a dark environmental chamber at 15°C for 2 weeks, then to a dark chamber at
141 10°C for another 2 weeks and finally to a dark chamber at 5°C, which was the diapause
142 holding temperature. At least 50 families were overwintered per population and year.

143

144 This experiment was conducted in 2014. Unrelated Russian and Italian females and males
145 were mated within and between populations in a reciprocal manner; Russian females with
146 Russian males (called RxR henceforth, 9 families), Russian females with Italian males (RxI,
147 13 families), Italian females with Russian males (IxR, 11 families) and Italian females with
148 Italian males (IxI, 9 families). The Russian population was the 8th laboratory generation,
149 while the Italian population a mix between the 5th and 3rd laboratory generation. Thus,
150 possible maternal effects due to local conditions should have been minimized (Mousseau &
151 Dingle, 1991). Larvae (10 per family) were reared on whole potato plants in a greenhouse
152 until adulthood at 23°C degrees, ~60% relative humidity and a photoperiodic cycle of LD
153 18:6 h. Potato pots were checked once daily for emerged adults, which were sexed and
154 weighed (± 0.1 mg, AM100; Mettler) on the day of emergence.

155

156 Beetles were reared singly in transparent plastic jars (120 ml) containing peat (50 ml) in the
157 bottom. The lid had a net covering, which allowed free air circulation and full penetration of
158 light. Beetles were fed *ad libitum* with fresh potato leaves and pieces of potato stem (*Solanum*
159 *tuberosum*, van Gogh variety). A photoperiodic cycle of LD 16.5:7.5 h was used (Lehmann *et*
160 *al.*, 2015), and temperature kept at 23°C degrees, ~60% relative humidity throughout the
161 experiment. Beetles were checked daily for burrowing and for oviposition, in case of which,
162 eggs were removed. At the age of 20 days after eclosion, all beetles were scored for
163 burrowing and weighed (± 0.1 mg, AM100; Mettler). Beetles generally burrow for diapause
164 between 10 and 15 days after adult eclosion (de Wilde *et al.*, 1959; Piironen *et al.*, 2011).

165

166 *Statistical analyses*

167 Generalized linear models (GZLM) were employed for all analyses due to heterogeneity of
168 error variances. Burrowing at 20 days was analysed with a binomial GZLM with a log-link

169 function. Burrowing at 20 days (yes/no) was the binary dependent variable, while sex and
170 cross were added as factorial explanatory variables. Oviposition was also analysed with a
171 binomial GZLM with a log-link function. Here oviposition during the 20 day experimental
172 period (yes/no) was the binary dependent variable, while cross was added as factorial
173 explanatory variable. In all models the Akaike Information Criterion (AIC) was used to track
174 model improvement. Non-significant terms were removed starting from the sex*cross
175 interaction, and only then any non-significant main factor (see Table 1 for model selection
176 variables). Differences between cross levels were analysed with GZLM multiple comparison
177 tests with Bonferroni corrections (Sokal & Rohlf, 2003). All tests were performed in IBM
178 SPSS Statistics 20.0 (IBM SPSS Inc., Chicago, IL, USA) statistical software package.

179

180 **Results**

181

182 *Burrowing behaviour*

183 Beetles differed in burrowing propensity depending on the cross (Table 2a). The within-
184 population crosses from Russia had the largest proportion burrowed beetles, followed by the
185 hybrid crosses, and lastly the within-population crosses from Italy (Fig. 1). While the RxR
186 and IxI crosses differed from all other crosses significantly, the hybrid crosses showed more
187 complex patterns, neither differing from one another, both being significantly higher than the
188 IxI cross, but only the RxI lower than the RxR cross (Fig. 1). The female and male beetles did
189 not differ in burrowing propensity generally (Wald $\chi^2 = 4.27$, d.f. = 1, $P = 0.513$), or based on
190 the latitudinal origin of their respective parent (sex*cross interaction Wald $\chi^2 = 3.317$, d.f. =
191 3, $P = 0.345$), contrary to what would be expected based on the X-linkage hypothesis.

192

193 *Oviposition*

194 Also oviposition differed according to the cross (Table 2b) and the pattern was opposite to
195 the one seen in burrowing, as expected. The RxR cross had the lowest proportion of
196 ovipositing females, followed by the hybrid crosses and lastly, the IxI cross where almost all
197 females oviposited (Fig. 2). While the RxR and IxR crosses differed from all other crosses,
198 the hybrid crosses did not differ from each other but did differ from both within population
199 crosses significantly (Fig. 2).

200

201 **Discussion**

202

203 The critical photoperiod for diapause (when 50% of adults enter diapause, forfeiting
204 reproduction) has been determined to be 16 h 50 min for the beetles from Petroskoi and 15 h
205 10 min for the beetles from Padua (Lehmann *et al.*, 2015). Thus, our results on the burrowing
206 propensity of *L. decemlineata* from the within-population crosses are in line with the critical
207 photoperiod (Fig. 1) and furthermore reflect values found in previous studies where the same
208 populations have been used (Piiroinen *et al.*, 2011; Lehmann *et al.*, 2012). Hybrid beetles
209 reared in the current experiment show clear intermediate responses, lying between the within-
210 population crosses both in terms of burrowing and oviposition, indicating that the
211 photoperiodic control of diapause induction is strongly heritable in this species (Danilevskii
212 1965) as has been shown for many other insect species (Tauber *et al.*, 1986; Nelson *et al.*,
213 2010). This is likely due to strong selection imposed in part by winter mortality in beetles
214 undergoing reproductive development in autumn and in part by beetles preparing too late for
215 overwintering which overwinter with too small energy stores (Piiroinen *et al.*, 2011).

216

217 The main purpose of the present study is to assess if photoperiodic control of diapause is
218 inherited in an additive autosomal manner and if there is evidence of sex-linkage in the

219 inheritance pattern. In case genes driving the evolutionary diverged photoperiodic responses
220 between these populations reside on the sex chromosome, between-population hybrids should
221 differ in their response depending on origin of their mother and father, respectively.
222 Assuming additive inheritance, female hybrids which inherit one copy of the sex
223 chromosome from each parent should show an intermediate response, while male hybrids
224 which only inherit their sex chromosome from their mother should show a similar response as
225 their mothers. Even though hybrids show intermediate responses in burrowing and
226 oviposition, indicating that inheritance of photoperiodic diapause induction has an additive
227 genetic basis, no difference can be seen between male and female hybrids, indicating that
228 inheritance is autosomal and not sex chromosome-linked. Autosomal, non-sex chromosome
229 linked inheritance of diapause photoperiodic responses is observed in several other insects,
230 such as the butterfly *Polygonia c-album* (Söderlind & Nylin, 2011), the moth *Helicoverpa*
231 *armigera* (Chen *et al.*, 2012) and the fly *Musca autumnata* (Kim *et al.*, 1995). In the fly
232 *Chlorops oryzae* photoperiodic control of summer-diapause follows a polygenic autosomal
233 inheritance pattern but which includes a single X-chromosomal gene of strong effect
234 (Takeda, 1998). In drosophilid flies from seasonal environments diapause inheritance seems
235 to follow rather straightforward inheritance patterns which include strong X-chromosomal
236 control or influence (Lumme & Keränen, 1978; Kimura & Yoshida, 1995). Also in the
237 mosquito *Wyeomyia smithii* is X-chromosomal control of diapause photoperiodicity seen, but
238 here inheritance follows a more complex pattern than in the drosophilid flies, being under
239 control by multiple loci (Mathias *et al.*, 2007). Further sampling and QTL-mapping reveals
240 that the mode of photoperiodic inheritance varies between replicated *W. smithii* populations
241 (Bradshaw *et al.*, 2012).

242

243 While no general sex-chromosomal control of diapause induction is detected in *L.*
244 *decemlineata*, hybrid male beetles from the IxR cross show responses significantly closer to
245 the parental paternal line than the maternal line. This is not due to mating incompatibilities,
246 since hybrid crosses do not differ statistically in mating success (86% in the within
247 population crosses and 96% in the hybrid crosses, $P = 0.171$, Fisher's exact test) or larva to
248 adult survival (33% in the within population crosses and 42% in the hybrid crosses, t-test: df
249 = 45, $t = -1.516$, $P = 0.136$) from the within population crosses. Therefore, the northern
250 paternal line might be exerting stronger influence on diapause induction of hybrids than the
251 northern maternal line or either southern parent. Similar strong paternal effects on diapause
252 decisions have been found in other insects (Raina *et al.*, 1988; Ikten *et al.*, 2011; Söderlind &
253 Nylin, 2011; Chen *et al.*, 2012; Xia *et al.*, 2012) which all, intriguingly, are members of
254 Lepidoptera, indicating a potential phylogenetic effect, as suggested previously by Fu and co-
255 workers (Fu *et al.*, 2015). In the beetle *Colaphellus bowringi*, adult summer diapause, or
256 aestivation, was found to be sex-linked and responses of hybrid offspring strongly influenced
257 by maternal genotype (Kuang *et al.*, 2011). Thus, it seems as if beetles might have evolved
258 different modes of inheritance of diapause induction, which involve both maternal and
259 paternal drive, and could be less constrained than members of Lepidoptera.

260

261 Together the published literature, which spans several insect groups, highlights the complex
262 nature of diapause induction evolution, ranging from relatively simple Mendelian inheritance
263 (Han & Denlinger 2009), to polygenic and varying in degree of additivity, dominance and
264 epistasis (Bradshaw *et al.*, 2012), with strong or weak sex-linkage. Since diapause likely has
265 evolved multiple times independently in diverse insect groups (Hoy, 1978; Ragland *et al.*,
266 2010), this variation is not surprising. Thus, while the data in the current study suggests that
267 the evolutionary diverged photoperiodic response observed among the studied populations

268 (Lehmann *et al.*, 2015) is inherited in an additive autosomal manner, crosses of more
269 populations, and backcrosses of hybrids (e.g. Chen *et al.*, 2012) would be needed to definitely
270 establish that sex-linkage is not involved.

271

272 **Acknowledgements**

273

274 This work was financed by the Academy of Finland (project number 250248) and the Centre
275 of Excellence in Biological Interactions Research (252411). The Colorado potato beetle is a
276 quarantine species in Finland and therefore this experiment was carried out under permission
277 (Evira 3861/541/2007).

278

279 **References**

280

281 Alyokhin, A. (2009) Colorado potato beetle management on potatoes: current challenges and
282 future prospects. *Fruit, Vegetable and Cereal Science and Biotechnology*, **3**, 10-19.

283 Bradshaw, W.E. & Holzapfel, C.M. (2001) Genetic shift in photoperiodic response correlated
284 with global warming. *Proceedings of the National Academy of Sciences of the United*
285 *States of America*, **98**, 14509-14511.

286 Bradshaw, W.E. & Holzapfel, C.M. (2007) Evolution of animal photoperiodism. *Annual*
287 *Review of Ecology, Evolution and Systematics*, **3**, 1-25.

288 Bradshaw, W.E., Emerson, K.J., Catchen, J.M. *et al.* (2012) Footprints in time: comparative
289 quantitative trait loci mapping of the pitcher-plant mosquito, *Wyeomyia smithii*.
290 *Proceedings of the Royal Society B*, **279**, 4551-4558.

291 Chen, C., Xia, Q., Chen, Y. *et al.* (2012) Inheritance of photoperiodic control of pupal
292 diapause in the cotton bollworm, *Helicoverpa armigera* (Hübner). *Journal of Insect*
293 *Physiology*, **58**, 1582-1588.

294 Danilevskii, A.S. (1965) *Photoperiodism and Seasonal Development of Insects*. Oliver and
295 Boyd, London.

296 Emerson, K.J., Bradshaw, W.E. & Holzapfel, C.M. (2009) Complications of complexity:
297 integrating environmental, genetic and hormonal control of insect diapause. *Trends in*
298 *Genetics*, **25**, 217-225.

299 Fu, S., Chen, C., Xiao, L. *et al.* (2015) Inheritance of diapause in crosses between the
300 northernmost and the southernmost strains of the Asian corn borer *Ostrinia furnacalis*.
301 *PLoS ONE*, **10**, e0118186.

302 Gaston, K. (2003) *The Structure and Dynamics of Geographic Ranges*. Oxford Series in
303 Ecology and Evolution, Oxford University Press, New York

304 Goto, S.G. (2013) Roles of circadian clock genes in insect photoperiodism. *Entomological*
305 *Science*, **16**, 1-16.

306 Han, B. & Denlinger, D.L. (2009) Mendelian inheritance of pupal diapause in the flesh fly,
307 *Sarcophaga bullata*. *Journal of Heredity*, **100**, 251-255.

308 Hoy, M.A. (1978) Variability in Diapause Attributes of Insects and Mites: Some
309 Evolutionary and Practical Implications. *Evolution of Insect Migration and Diapause*
310 (ed. by H. Dingle), pp. 101-127. Springer-Verlag, Berlin.

311 Hsiao, T.H. & Hsiao, C. (1983) Chromosomal analysis of *Leptinotarsa* and *Labidomera*
312 species (Coleoptera: Chrysomelidae). *Genetica*, **60**, 139-150.

313 Hsiao, T.H. (1985) Eco-physiological and genetic aspects of geographic variations of the
314 Colorado potato beetle. *Research Bulletin - Massachusetts Agricultural Experiment*
315 *Station*, **704**, 63-77.

316 Ikten, I., Skoda, S.R., Hunt, T.E. *et al.* (2011) Genetic variation and inheritance of diapause
317 induction in two distinct voltine ecotypes of *Ostrinia nubilalis* (Lepidoptera:
318 Crambidae). *Faculty Publications: Department of Entomology*, Paper 268.

319 Johnson, C.G. (1967) International dispersal of insects and insect-borne viruses. *Netherlands*
320 *Journal of Plant Pathology*, **73**, 21-43.

321 Johnson, A. & Lachance, J. (2012) The genetics of sex chromosomes: evolution and
322 implications for hybrid incompatibility. *Annals of the New York Academy of Sciences*,
323 **1256**, E1-22.

324 Kim, Y., Krafur, E.S., Bailey, T.B. & Zhao, S. (1995) Mode of inheritance of fly diapause
325 and its correlation with other developmental traits. *Ecological Entomology*, **20**, 359-
326 366.

327 Kimura, M.T. & Yoshida, T. (1995) A genetic analysis of photoperiodic reproductive
328 diapause in *Drosophila triauraria*. *Physiological Entomology*, **20**, 253-256.

329 Kuang, X., Xu, J., Xia, Q. *et al.* (2011) Inheritance of the photoperiodic response controlling
330 imaginal summer diapause in the cabbage beetle, *Colaphellus bowringi*. *Journal of*
331 *Insect Physiology*, **57**, 614-619.

332 Lehmann, P., Lyytinen, A., Sinisalo, T. & Lindström, L. (2012) Population dependent effects
333 of photoperiod on diapause related physiological traits in an invasive beetle
334 (*Leptinotarsa decemlineata*). *Journal of Insect Physiology*, **58**, 1146-1158.

335 Lehmann, P., Lyytinen, A., Piironen, S., & Lindström, L. (2014) Northward range expansion
336 requires synchronization of both overwintering behaviour and physiology with
337 photoperiod in the invasive Colorado potato beetle (*Leptinotarsa decemlineata*).
338 *Oecologia*, **176**, 57-68.

339 Lehmann, P., Lyytinen, A., Piironen, S. & Lindström, L. (2015) Latitudinal differences in
340 diapause related photoperiodic responses of European Colorado potato beetles
341 (*Leptinotarsa decemlineata*). *Evolutionary Ecology*, **29**, 269-282.

342 Lumme, J. & Keränen, L. (1978) Photoperiodic diapause in *Drosophila lummei* Hackman is
343 controlled by an X-chromosomal factor. *Hereditas*, **89**, 261-262.

344 Lynch, M. & Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer
345 Associates, Sunderland.

346 Mathias, D., Jacky, L., Bradshaw, W.E. & Holzapfel, C.M. (2007) Quantitative trait loci
347 associated with photoperiodic response and stage of diapause in the Pitcher-plant
348 mosquito, *Wyeomyia smithii*. *Genetics*, **176**, 391-402.

349 Mousseau, T.A., Dingle, H. (1991) Maternal effects in insect life histories. *Annual Review in*
350 *Entomology*, **36**, 511-534.

351 Nelson, R.J., Denlinger, D.L. & Somers, D.E. (2010) *Photoperiodism, the Biological*
352 *Calendar*. Oxford University Press, New York.

353 Piironen, S., Ketola, T., Lyytinen, A. & Lindström, L. (2011) Energy use, diapause
354 behaviour and northern range expansion potential in the invasive Colorado potato
355 beetle. *Functional Ecology* **25**, 527-536.

356 Ragland, G.J., Denlinger, D.L. & Hahn, D.A. (2010) Mechanisms of suspended animation are
357 revealed by transcript profiling of diapause in the flesh fly. *Proceedings of the National*
358 *Academy of Sciences of the United States of America*, **107**, 14909-14914.

359 Raina, A.K., Bell, R.A. & Klassen, W. (1988). Diapause in the pink bollworm: Preliminary
360 genetic analysis. *Insect Science and Application*, **1**, 231-235.

361 Sadakiyo, S. & Ishihara, M. (2011) Rapid seasonal adaptation of an alien bruchid after
362 introduction: geographic variation in life cycle synchronization and critical photoperiod
363 for diapause induction. *Entomologia Experimentalis et Applicata*, **140**, 69-76.

364 Saunders, D.S. (2002) *Insect Clocks*. Elsevier Science, Amsterdam.

365 Saunders, D.S. (2012) Insect photoperiodism: seeing the light. *Physiological Entomology*, **37**,
366 207-218.

367 Shiga, S. & Numata, H. (2007) Neuroanatomical approaches to the study of insect
368 photoperiodism. *Photochemistry and Photobiology*, **83**, 76-86.

369 Singh, N.D. & Petrov, D.A. (2007) Evolution of gene function on the X chromosome versus
370 the autosomes. *Gene and Protein Evolution* (ed. by J Volf), pp. 101-118. Karger, Basel.

371 Sokal, R.R. & Rohlf, F.J. (2003) *Biometry, the Principles and Practice of Statistics in*
372 *Biological Research*. W.H. Freeman and Company, New York.

373 Smith, S.G. & Virkki, N. (1978) *Animal cytogenetics 3, Insecta 5, Coleoptera*. Bontraeger,
374 Berlin.

375 Söderlind, L. & Nylin, S. (2011) Genetics of diapause in the comma butterfly *Polygonia c-*
376 *album*. *Physiological Entomology*, **36**, 8-13.

377 Takeda, M. (1998) Genetic basis of photoperiodic control of summer and winter diapause in
378 geographic ecotypes of the rice stem maggot, *Chlorops oryzae*. *Entomologia*
379 *Experimentalis et Applicata*, **86**, 59-70.

380 Tauber, M.J., Tauber, C.A. & Masaki, S. (1986) *Seasonal Adaptations of Insects*. Oxford
381 University Press, New York.

382 Urbanski, J., Mogi, M., O'Donnell, D. *et al.* (2012) Rapid adaptive evolution of
383 photoperiodic response during invasion and range expansion across a climatic gradient.
384 *American Naturalist*, **179**, 490-500.

385 Walsh, B.D. (1865) The new potato-bug, and its natural history. *The Practical Entomologist*,
386 **1**, 1-4.

- 387 de Wilde, J., Duintjer, C.S. & Mook, L. (1959) Physiology of diapause in the adult Colorado
388 beetle (*Leptinotarsa decemlineata*) I. The photoperiod as a controlling factor. *Journal*
389 *of Insect Physiology*, **3**, 75-85.
- 390 Xia, Q., Chen, C., Tu, X. *et al.* (2012) Inheritance of photoperiodic induction of larval
391 diapause in the Asian corn borer *Ostrinia furnacalis*. *Physiological Entomology*, **37**,
392 185-191.

393 **Table 1.** Model selection for the GZLM testing proportion of within- and between-population
 394 hybrid crosses of European *Leptinotarsa decemlineata* beetles burrowed at 20 days after
 395 eclosion was done using an information technology approach. The model only containing
 396 cross was the best in explaining the data.

| Test | Model ¹ | AIC ² |
|-----------------------------|--------------------|------------------|
| Full model with interaction | ~ Cross*Sex | 45.212 |
| Both main levels | ~ Cross+Sex | 42.606 |
| Only cross | ~ Cross | 25.432 |

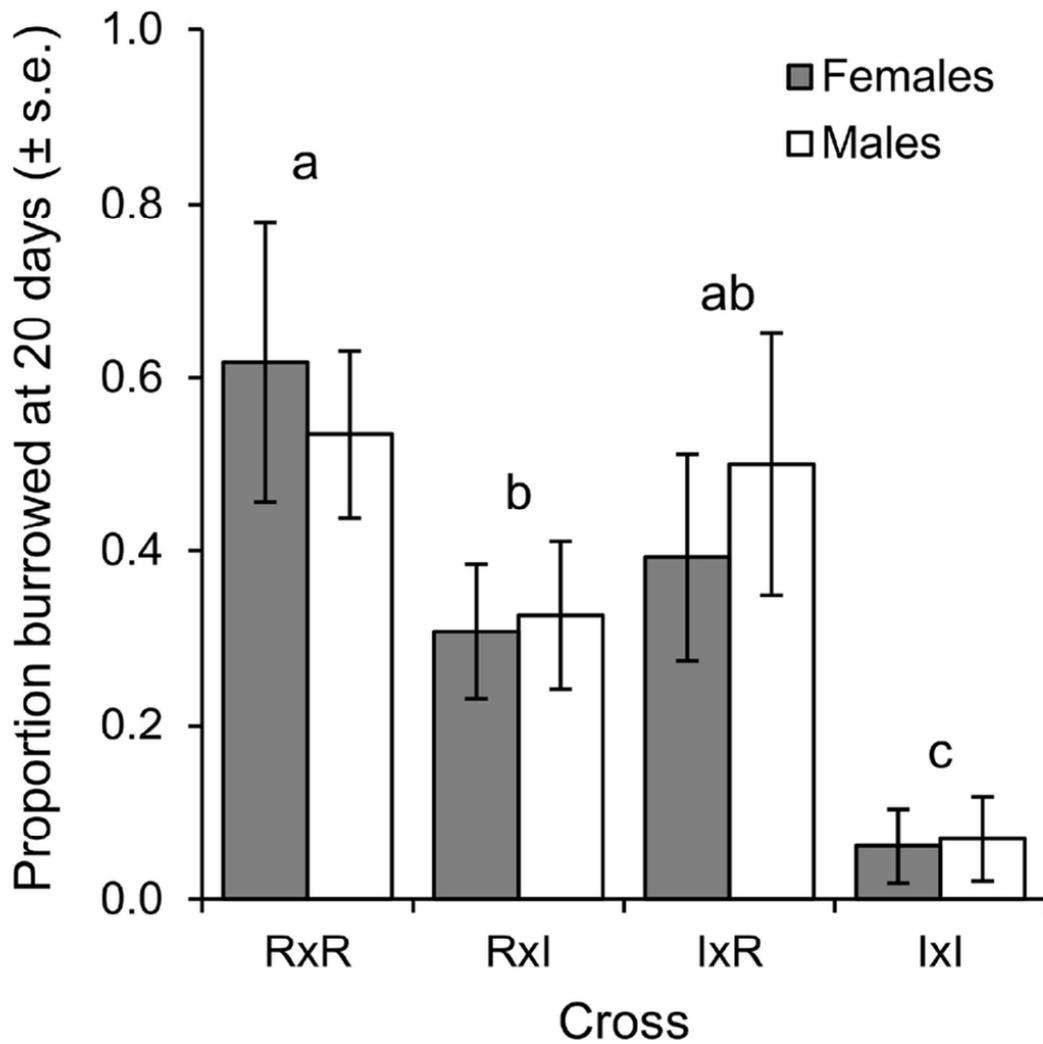
397 ¹Model explanatory variables. A '*' denotes a full factorial comparison (main levels and
 398 interaction) while a '+' denotes only main level tests. ²AIC (Akaike Information Criterion),
 399 the best model has been bolded.

400 **Table 2.** Final GZLM models explaining differences between within- and between-
 401 population hybrid crosses of European *Leptinotarsa decemlineata* in (a) burrowing for
 402 diapause at 20 days after eclosion and (b) oviposition when reared at a photoperiodic cycle of
 403 LD 16.5:7.5 h.

| Effect | Wald χ^2 | df | <i>P</i> |
|--------------------------|---------------|----|----------|
| (a) Burrowing at 20 days | | | |
| Intercept | 23.089 | 1 | < 0.001 |
| Cross | 33.967 | 3 | < 0.001 |
| (b) Oviposition | | | |
| Intercept | 14.903 | 1 | < 0.001 |
| Cross | 20.366 | 3 | < 0.001 |

404

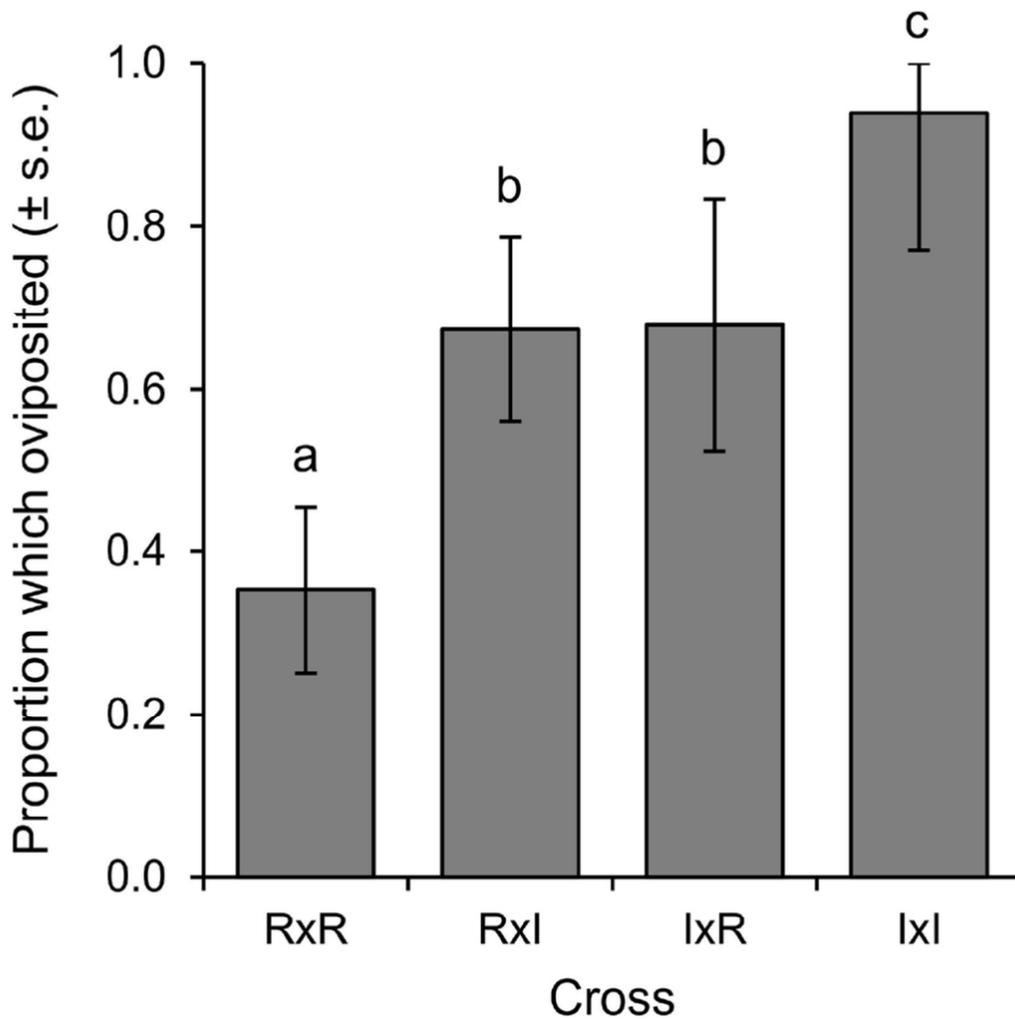
405



406

407 **Fig. 1.** The proportion adult female (filled columns) and male (closed columns) *Leptinotarsa*
 408 *decemlineata* burrowed for diapause at 20 days after adult eclosion. Beetles from a northern
 409 and a southern European population were crossed within and between populations. The x-
 410 axis shows crosses, female first, male second (R: Russia, I: Italy). Different letters above
 411 pillars denote significant (Bonferroni corrected multiple comparison: $P < 0.05$) pair-wise
 412 differences between crosses. Sexes did not differ significantly (see text for details). Error bars
 413 were calculated using the formula $s.e. = \text{square root}(pq/n)$, where pq is the mean proportion
 414 and n is the group sample size.

415



416

417 **Fig. 2.** The proportion adult female *Leptinotarsa decemlineata* which oviposited during the
 418 experiment. Beetles from a northern and a southern European population were crossed within
 419 and between populations. The x-axis shows parental crosses, female first, male second (R:
 420 Russia, I: Italy). Different letters above pillars denote significant (Bonferroni corrected
 421 multiple comparison $P < 0.05$) pair-wise differences between crosses. Error bars were
 422 calculated using the formula $s.e. = \text{square root}(pq/n)$, where pq is the mean proportion and n
 423 is the group sample size.