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Inheritance patterns of photoperiodic diapause induction in *Leptinotarsa decemlineata*

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Running title: Diapause inheritance in an invasive beetle

Abstract

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

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

Introduction

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

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

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

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

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

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

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

Materials and methods

Study animals and rearing conditions

The descendants of *Leptinotarsa decemlineata* collected from potato fields from two populations, a northern and a southern European population, were used in this experiment. The northern beetles were collected from fields near Petroskoi in northern Russia (61°49'N, 34°10'E and 59°95'N, 30°30'E) in 2006, and the southern beetles from fields near Padua in Italy (45°48'N, 12°07'E) in 2008 and 2010. Field collected beetles were used to establish laboratory populations. In each generation, unrelated parental beetles (overwintered generation) were mated within populations under a photoperiodic cycle of LD 18:6 h, and their offspring (summer generation) were reared in family groups until adulthood under a photoperiodic cycle of LD 18:6 h at the constant temperature of 23°C in a controlled environmental chamber (Type B1300; Weiss Technik, Reiskirchen-Lindenstruth, Germany). After eclosion adult beetles in each generation were maintained individually in petri dishes (diameter of 9 cm) containing a water moistened filter paper and fed daily with fresh potato leaves and stems (*Solanum tuberosum* Linnaeus, van Gogh variety) under a diapause inducing photoperiodic cycle of LD 12:12 h (at 23°C, ~60% relative humidity) (Lehmann *et al.*, 2015). Adult beetles were overwintered in plastic jars (volume 80 ml) containing peat and a layer of plasticine for moisture retention. When beetles had been burrowed for 10 days they were moved to a dark environmental chamber at 15°C for 2 weeks, then to a dark chamber at 10°C for another 2 weeks and finally to a dark chamber at 5°C, which was the diapause holding temperature. At least 50 families were overwintered per population and year.

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

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

Statistical analyses

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

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

Results

Burrowing behaviour

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

Oviposition

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

Discussion

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

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

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

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

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

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

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

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

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

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

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