The diapause decision as a cascade switch for adaptive developmental plasticity in body mass in a butterfly

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Abstract

Switch-induced developmental plasticity, such as the diapause decision in insects, is a major form of adaptation to variable environments. As individuals that follow alternative developmental pathways will experience different selective environments the diapause decision may evolve to a cascade switch that induces additional adaptive developmental differences downstream of the diapause decision. Here, we show that individuals following alternative developmental pathways in a Swedish population of the butterfly, *Pararge aegeria*, display differential optimization of adult body mass as a likely response to predictable differences in thermal conditions during reproduction. In a more northern population where this type of selection is absent no similar difference in adult mass among pathways was found. We conclude that the diapause decision in the southern population appears to act as a cascade switch, coordinating development downstream of the diapause decision, to produce adult phenotypes adapted to the typical thermal conditions of their expected reproductive period.

Introduction

Developmental plasticity is one of the major forms of adaptation to variable environments and it plays a central role in the diversification of life (West-Eberhard, 2003). During ontogeny, the phenotype is highly responsive to both environmental and genetic inputs that have the potential to alter the course of development. Developmental switches that integrate genetic and environmental effects on phenotypes are important for the organization of this process and natural selection on these switches may favour the expression of alternative phenotypes in response to environmental information about differences in future selective conditions (Hazel et al., 1990; Moran, 1992; Gotthard & Nylin, 1995; Nijhout, 2002, 2003; West-Eberhard, 2003; Leimar et al., 2006; Gotthard, 2008). In many organisms, developmental switches have major effects on phenotypic expression and produce discontinuous variation in particular phenotypic traits (polyphenisms) that is often

adaptive (Shapiro, 1976; Moran, 1992; Gotthard & Nylin, 1995; Roff, 1996; Brakefield et al., 2003; Moczek, 2003; Nijhout, 2003; West-Eberhard, 2003; Leimar et al., 2006). As individuals that differ in polyphenic traits will typically be distributed differently in time or in space, it is likely that individuals following alternative developmental pathways will experience consistently different selection on an array of traits (Moran, 1992; Brakefield et al., 2003; West-Eberhard, 2003). Hence, the developmental pathway followed by a given individual may provide high quality information about the future selective conditions that this individual is likely to meet. Given that there is some developmental independence among the alternative developmental pathways that determine the polyphenism we may expect natural selection to favour a differential optimization of other traits downstream of the major switch (Moran, 1992). This has been referred to as a switch cascade where one initial switch coordinates a series of additional developmental events that lead to individual phenotypes that differ in a suite of traits (West-Eberhard, 2003).

Insect diapause is a classic and well-known example of switch controlled adaptive developmental plasticity inducing direct or diapause development in response to

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cues of seasonal change that predict the oncoming adverse conditions (e.g. photoperiod and temperature) (Danilevskii, 1965; Tauber *et al.*, 1986). These alternative developmental pathways invariably produce adults that reproduce during different parts of the year when there may be substantial differences in a range of selective conditions (Teder *et al.*, 2010). Indeed, there are several studies reporting potentially adaptive phenotypic differences among direct and diapause pathways in insects indicating a very general pattern (Shapiro, 1976; Tauber *et al.*, 1986; Greene, 1989; Kingsolver, 1995; Fric *et al.*, 2006; Friberg & Wiklund, 2007; Karlsson & Johansson, 2008; Karlsson *et al.*, 2008; Teder *et al.*, 2010). However, there are few studies that have employed direct fitness assays testing these alternative phenotypes in the respective environments. Furthermore, we know of no examples where it has been possible to link the presence/absence of a specific selection pressure to the presence/absence of phenotypic differences among these alternative seasonal pathways. We suggest that because the developmental pathway followed by a given individual typically predicts the selective conditions that this individual will experience during reproduction, natural selection is expected to favour the evolution of a switch cascade where the developmental decision to enter a given pathway coordinates a series of other developmental events. This may then lead to differential optimization of life history traits in relation to the expected differences in selective conditions. Here, we test this hypothesis by examining divergent populations of the speckled wood butterfly, *Pararge aegeria*, and testing if natural selection favours differential optimization of adult body mass among alternative developmental pathways, in response to predictable variation in selective regime during reproduction.

Swedish populations of *P. aegeria* differ in colonization history and seasonal adaptations and provide an ideal situation for testing if the diapause decision may evolve to an adaptive cascade switch. In central Sweden, the species has been present for as long as there are records while southern Sweden was colonized from Denmark as late as in the 1930' (Nordström, 1955; Wiklund *et al.*, 1983), and these two populations are still geographically separated (Eliasson *et al.*, 2005). The seasonal adaptations in the south of Sweden include three distinct alternative developmental pathways because of developmental plasticity in relation to photoperiod and temperature: (i) direct development, (ii) development through pupal diapause during winter, (iii) development through larval diapause during winter (Nylin *et al.*, 1989; Van Dyck & Wiklund, 2002). In this study, we have focused on the two alternative diapause strategies (in the pupal or the larval stage) that are present in southern Sweden every year and produce adults that fly in two nonoverlapping periods in April/May and June, respectively (C. Wiklund, personal communication). As thermal conditions during these two periods differ substantially (Fig. 1) life history

models predicts that optimal female mass is significantly smaller in May conditions than in conditions typical of June (Gotthard *et al.*, 2007; Berger *et al.*, 2008). The reason for this is that the cold conditions early in spring limit large females from realizing their high potential fecundities, whereas June conditions give a higher pay-off for being large because thermal conditions allow more time for oviposition. Interestingly, differences in body mass between the two diapause strategies in the south Swedish population support these predictions (Van Dyck & Wiklund, 2002). However, the predicted adaptive value of this difference remains to be empirically demonstrated.
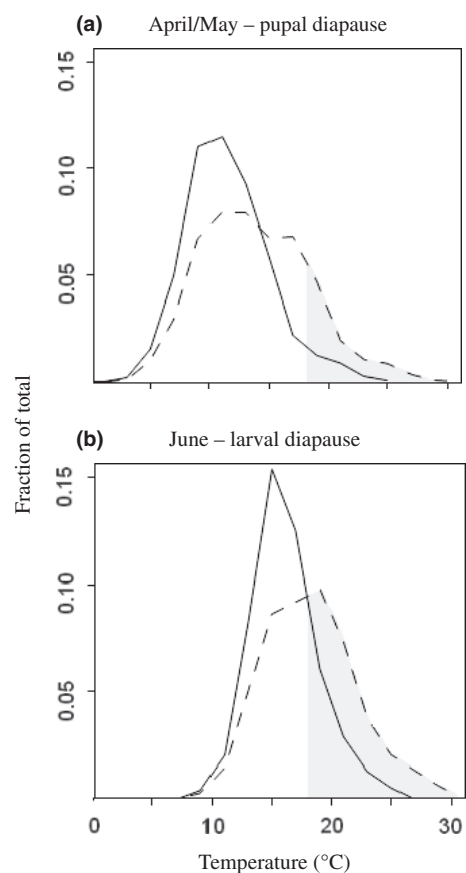

Fig. 1 A description of the different thermal conditions experienced by adults of (a) the pupal pathway diapause pathway (April 20–May 20) and (b) the larval diapause in southern Sweden (June 1–30). The full drawn lines show the distributions of ambient temperatures experienced during daytime (8.00 and 18.00) when the butterflies may be active. The dashed lines show the same set of data when the butterflies are assumed to be able to raise their body temperatures 4 °C by basking whenever the sun is out. The shaded areas show the portion of the time when temperatures are high enough for females to oviposit (Gotthard *et al.*, 2007). The figure is based on data on temperature and sun hours for the period 1996–2007 from the weather station closest to the field site in southern Sweden.

In contrast to the situation in the south, the population in central Sweden (Stockholm area) typically flies in one generation in June and all individuals follow the same developmental pathway through pupal diapause (Wiklund *et al.*, 1983). The other two alternative pathways (larval diapause and direct development) are, therefore, hardly ever exposed to selection for maintaining an adaptive cascade switch where the development decision to enter a given diapausing pathway subsequently influences the development of body size.

If the size difference between pupal and larval diapause in the south Swedish population is because of selection for a cascade switch to produce adult phenotypes that are differentially optimized in relation to thermal conditions during reproduction we predict the following:

(1) In the southern population, the relatively larger size of females that have followed the larval diapause pathway should result in higher realized fecundity in thermal conditions typical of June but not in thermal conditions typical of April/May.

(2) Individuals of the south Swedish population that have followed the larval diapause pathway should grow larger than individuals that have followed the pupal diapause pathway, while there should be no such difference in the central Swedish population where the larval diapause pathway has not been exposed to natural selection.

Hence, our research rationale has two steps: first, we test if natural selection is likely to favour the size difference seen in the southern population; second, we test if the absence of selection in the central population means that the difference in body size is not present.

Materials and methods

The speckled wood butterfly, *P. aegeria*, is distributed throughout Northern Africa, Europe and Asia, where it is most typically found in forests. The adults are often seen flying in clearings and along small roads and forest paths. Females typically mate only once soon after emergence and spend the rest of their lives searching for oviposition and feeding sites. The eggs are laid singly on a variety of grasses, preferable in moist and shady locations (Wickman & Wiklund, 1983). Field estimates on life expectancy in England and in Sweden indicate that the males have a mean life span of 6 days (Davies, 1978; Wickman & Wiklund, 1983). There are no similar field estimates for females but laboratory experiments suggest that they typically live twice as long as the males in laboratory conditions (Gotthard *et al.*, 2000), and a given female may survive and oviposit for up to 40 days if temperatures are kept cool (Gotthard *et al.*, 2007).

Oviposition experiment

The aim of this experiment was to estimate the relationship between size and realized fecundity of females of the two diapause strategies in the southern population of *P. aegeria* under thermal conditions of spring (when the pupal diapause cohort oviposits) and of early summer (larval diapause cohort oviposits). Hence, the logic of the experiment was very similar to classic 'reciprocal transplant' experiments only that the phenotypes were transplanted in a temporal rather than a spatial dimension. Individuals of the southern Swedish population were reared outdoors in semi-natural conditions to enter winter in either pupal or larval diapause (see description of the Population comparison experiment below for details of the rearing protocol of these animals). Adults of both diapausing pathways hatched in spring after diapause and were given the opportunity to mate in cages during 3 days in the laboratory. At hatching, all adults were marked individually so it was possible to keep between 5 and 10 pairs in each mating cage. After 3 days in the mating cages, all females were put in 1.5 L plastic cups, covered by a fine net, where a tuft of the plant *Dactylus glomerata* was cultured as oviposition host. Females of both diapausing classes were randomly assigned to one of two thermal treatments mimicking either May or June conditions, respectively (see below). Fecundity was censored after 2 days, and then every third day until the female died. At each census, females were given a new fresh host for oviposition. All individuals had constant access to sugar water through a piece of wet cotton put on top of the covering net.

The two thermal treatments were designed to mimic average thermal conditions of May and June in southern Sweden. Data on temperature (hourly measurements between 1996 and 2007) and sunshine (proportion per hour between 1996 and 2007) from the weather station closest to the field site in southern Sweden were obtained from the Swedish meteorological and hydrological institute (SMHI). We calculated mean nightly temperatures, mean daily temperatures and the mean time available for oviposition behaviour [time with temperatures above 18 °C (Gotthard *et al.*, 2007; Berger *et al.*, 2008)] for May and June, assuming that females may raise their body temperatures 4 °C above ambient when there is sun (Van Dyck & Matthysen, 1998; Berwaerts *et al.*, 2001; Gotthard *et al.*, 2007). Figure 1 shows the frequency distribution of temperatures experienced during the active part of day (08.00–18.00) by butterflies flying in April/May and June, with and without thermoregulation. The 'May' – treatment had a daily thermal rhythm with a night temperature (20.00–07.00) of 10 °C and a daytime temperature of 14 °C (07.00–12.00, 15.00–20.00) interrupted by a 3-h period of 24 °C that allowed oviposition (12.00–15.00). The equivalent daily thermal regime in the 'June' – treatment was a night and day temperature of 14 °C (16.30–10.00) interrupted by a 6.5-h period with 24 °C for oviposition (10.00–16.30). The average temperatures of the two treatments were for May ≈ 13.4 °C and June ≈ 16.7 °C (c.f. mean

temperatures at the field site excluding the effects of sunshine during the period 1961–1990: May = 11.2 °C and June = 15.2 °C).

Population comparison

The aim of this experiment was to induce either pupal or larval diapause in individuals from both the southern and the central Swedish population of *P. aegeria* and then estimate adult mass of these individuals after diapause in spring. To provide realistic environmental conditions for diapause development, all individuals were reared outdoors until entering diapause and they also spent the winter outdoors. To do this in semi-natural conditions, cohorts of larvae had to be started at different times during late summer and autumn so that the earlier cohorts enter pupal diapause while the later cohorts enter larval diapause.

For the central population, we started with ten field-collected females that were allowed to oviposit in the laboratory in early July. Approximately, 60 randomly chosen larvae from these females were reared in long day conditions in the laboratory (22L:2D, 23 °C) to produce a directly developing generation whose offspring would start larval development in August and September. In addition, approximately 120 larvae from the field-collected females were reared in a mass culture outdoors (start in the middle of July) to produce diapausing pupae. The directly developing generation mated and oviposited in the laboratory during the month of August. Some of these eggs were kept in room temperature and hatched after approximately a week, whereas another batch of eggs was kept in a cold room (10 °C) to delay hatching. These eggs were then brought out to room temperature at two different periods to produce two cohorts of individuals that differed in the start of larval development. This procedure in combination with the fact that females in the laboratory oviposited during approximately 3 weeks allowed us to produce three different cohorts of larvae that were started in outdoors conditions at August 6, August 27 and September 14. For the south Swedish population, we reared the offspring of eight mated females that were collected in the field in southern Sweden during late July/August [these females originated from the same population investigated by Van Dyck & Wiklund (2002)]. These females oviposited in the laboratory, and the eggs were treated in a similar way as the northern population. This allowed us to start one cohort of larvae August 7 and one cohort of larvae September 14.

All larvae were reared in mass cultures in large plastic containers or in hanging net cages where potted host plants (the grass species *D. glomerata*) were renewed at regular intervals. As we did not want to disturb newly hatched larvae that had just started to feed on their host plants we only roughly estimated the number of larvae

that were started in each cohort. In total, we started approximately 500 larvae from the central population and about 400 larvae from the southern population. From September to November, we continuously monitored the larval rearings and at regular intervals we removed all pupae and placed them in plastic cups covered by a net. In the cohorts started on September 14, we expected larvae of both populations to enter diapause when they reached the third larval instar (Nylin *et al.*, 1989; Van Dyck & Wiklund, 2002), and visual inspection of the outdoor rearings in the end of November verified that there were large numbers of third instar larvae alive on the grass at this time. To avoid disturbing the small larvae and causing additional mortality by handling, we did not quantify the number of individuals at this time but we roughly estimated that there were more than 100 larvae per population. During the rest of the winter, larvae were allowed to stay on the grass tufts in covered rearing containers outdoors next to the diapausing pupae.

On April 2, the following spring, the containers expected to hold diapausing larvae were brought into the laboratory. To break the diapause they were supplied with fresh host plants (*D. glomerata*) in a photoperiod of 17L:7D and a temperature of 17–19 °C. Surviving larvae of both populations that started feeding were monitored daily and were supplied with fresh hosts at all times until pupation. The resulting pupae were removed from the rearing containers and were sexed and stored individually until hatching. Adults were weighed after having released their meconium and were subsequently fed 20% sugar solution on a piece of cotton before being stored in a cold room (10 °C) until mating experiments started. The diapausing pupae were kept outdoors and from the end of April they were monitored daily for hatching adults. These adults were treated identically as the larval diapausing adults.

Direct development

To test if differences in body mass between the two diapause strategies of the southern population may be partly because of genetic correlations with winter survival (e.g. large genotypes survive larval diapause better than small genotypes), we performed an additional rearing experiment where the offspring of the diapausing individuals were reared under directly developing conditions (19L:5D, 17 °C). We used the offspring of nine females of the pupal diapausing cohort and of 10 females of the larval diapausing cohort (all females were mated with a male that had followed the same diapausing pathway as herself). From each family, we started ten larvae that were reared individually in 0.5 L cups where a tuft of *D. glomerata* was cultured in fertilized water through a hole in the bottom of the cup. We noted development time, pupal and adult weight as well as sex of all surviving individuals.

Statistical methods

For the analysis of the oviposition experiment, we fitted linear models to life-time fecundity as well as cumulative fecundity data at days 8 and 14 as these are realistic life spans of females in the field. In all models, we included adult weight and treatment (May or June thermal conditions) and their interaction; the interaction being the direct test of our hypothesis. In these models, we also initially included the stage of diapause to test if there were any effects of diapause strategy other than the expected mass difference. In the analysis of life-time fecundity, we also included female life span in the model as we wanted to control statistically for the amount of time females had available for oviposition. To make sure that our results did not depend on a few individuals that for some reason did not do well in the experiment we re-ran all analyses excluding all females that laid < 10 eggs during their life. In all analyses, we sequentially removed interactions where $P > 0.1$. We used Cox proportional hazards regression to investigate potential effects of female weight and treatment on mortality rates.

In the population comparison, we fitted linear models to the data on adult weight. In the models, we first included population, diapause stage and sex as well as all interactions but continued by stepwise removing all interactions where $P > 0.1$ to produce the final model.

Finally, in the analysis of adult weights of directly developing offspring of the two diapause strategies we fitted linear models including sex, parental diapause and family nested within parental diapause. Again, we first fitted a model including all interactions but sequentially dropped interactions where $P > 0.1$. We also investigated if we could detect a genetic correlation among sexes in body mass by regressing sex-specific family means of females on males. All analyses were performed with R 2.8.1 (R Development Core Team, 2005) and STATA 9.2 (StataCorp, 2005).

Results

Oviposition experiment

After 8 days of oviposition, there were significant effects of female weight, oviposition treatment and their interaction on cumulative fecundity [General linear model (GLM), weight: $F_{1,105} = 11.19$, $P = 0.0011$; treatment: $F_{1,105} = 8.24$, $P = 0.0049$; weight × treat.: $F_{1,105} = 7.11$, $P = 0.009$, $r^2_{adj} = 0.17$, Fig. 2a]. There was no additional effect of diapause strategy ($F_{1,104} = 0.87$, $P = 0.35$), which was consequently dropped from the final model. As some females laid a low number of eggs we repeated the analysis and included only females that laid 10 or more eggs during their life but this analysis gave a qualitatively
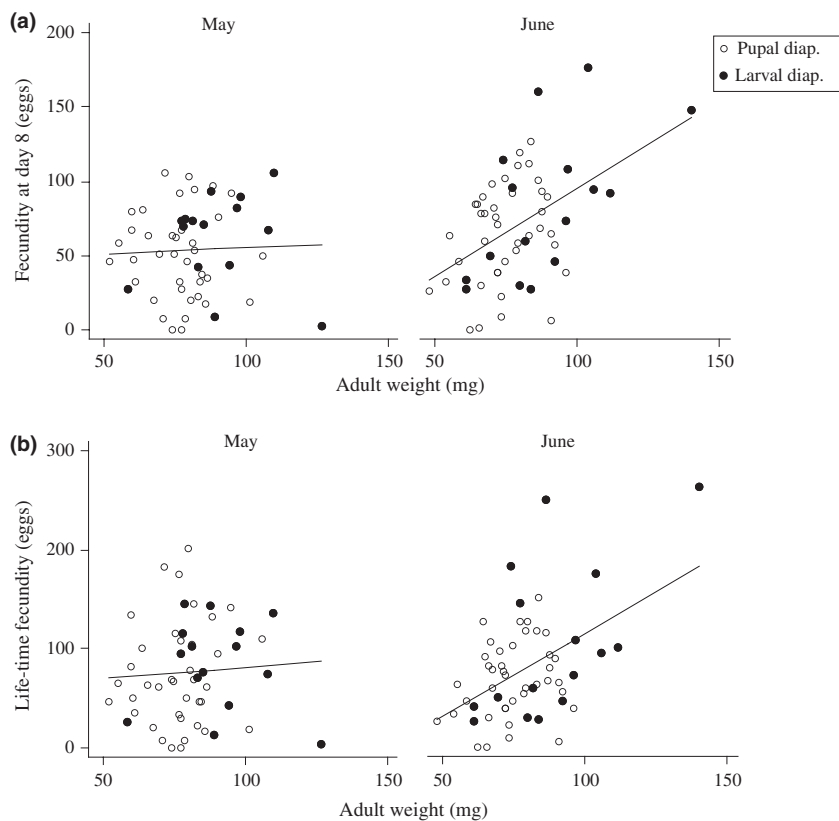

Fig. 2 Results of the oviposition experiment on the southern population showing the relationship between female weight and (a) cumulative fecundity after 8 days of oviposition, and (b) life-time fecundity. Left panels show results from the May treatment while the right panels show the June treatments.

similar result (GLM, weight: $F_{1,96} = 15.44$, $P = 0.00016$; treatment: $F_{1,96} = 8.79$, $P = 0.0038$; weight × treat.: $F_{1,96} = 4.47$, $P = 0.037$, $r^2_{adj} = 0.20$). The significant effect of the interaction between weight and treatment indicates that large females can only realize their high potential fecundities in the warmer conditions typical of June (Fig. 2a). This pattern was consistent over the reproductive lives of females as also fecundity after 14 days of oviposition was significantly affected by female weight, oviposition treatment and their interaction (GLM on fecundity at day 14 including only females that survived to this day, weight: $F_{1,41} = 8.94$, $P = 0.0047$; treatment: $F_{1,41} = 7.74$, $P = 0.0081$, weight × treat.: $F_{1,41} = 4.37$, $P = 0.043$, $r^2_{adj} = 0.29$). Also, life-time fecundity was influenced by the weight × treatment interaction when controlling for variation in age at death of the females (GLM, age: $F_{1,104} = 108.46$, $P < 0.0001$; weight: $F_{1,104} = 23.71$, $P < 0.0001$; treatment $F_{1,104} = 14.74$, $P = 0.0001$; weight × treat.: $F_{1,104} = 8.35$, $P_{w \times t} = 0.0047$, $r^2_{adj} = 0.56$, Fig. 2b). Female survival in the oviposition experiment differed significantly between treatments but was not influenced by female weight (Cox regression, weight: $z = -0.5$, $P = 0.61$; treatment: $z = 3.73$, $P = 0.0002$, $N = 109$, Model LR$\chi^2 = 14.32$, d.f. = 2, $P_{model} = 0.0008$, median$_{May}$ = 14 days, median$_{June}$ = 11 days).

Population comparison

Outdoor rearing of the southern population of *P. aegeria* during autumn and winter was successful in producing adults of both diapause strategies the next spring. In the central population, the naturally occurring pupal diapause strategy, also led to a high winter survival with a large number of adults emerging, whereas survival of larval diapausing individuals was low. In the southern population, 200 adults hatched successfully after pupal diapause while there were 85 adults that hatched after larval diapause; the same numbers for the central population were 292 pupal diapause and only six from larval diapause. Survival of diapausing pupae during winter was significantly higher for the southern population ($\chi^2 = 4.99$, d.f. = 1, $P = 0.025$) but the difference was relatively small (Southern = 0.79, $N = 252$, and Central = 0.72, $N = 408$). As methodological concerns kept us from estimating abundance of diapausing larvae before winter, we could not calculate survival of these groups. Nevertheless, visual inspections of the rearing containers before winter showed that the abundance of larvae was approximately similar for both populations, which strongly suggests that survival during larval diapause was lower in the central population than in the southern population.

The low success of the larval diapause strategy in the northern population created an unbalanced dataset for the analysis of how the diapause strategy influences adult body mass in the two populations, but the statistical methods used are able to handle unbalanced designs

(StataCorp, 2005). There were significant differences between populations, sexes and diapause pathways, as well as a significant effect of the interaction between diapause pathway and population (GLM: population: $F_{1,576} = 97.10$ $P < 0.0001$; diapause $F_{1,576} = 50.35$, $P < 0.0001$; sex $F_{1,576} = 342.19$, $P < 0.0001$; pop. × diap.: $F_{1,576} = 7.91$ $P = 0.0051$; pop. × sex $F_{1,576} = 3.16$, $P = 0.078$, all other interactions dropped from the model as $P > 0.1$, $r^2_{adj} = 0.48$). Individuals of the southern population that followed the larval diapause pathway grew significantly larger compared to individuals that entered pupal diapause; while the individuals of the central population that survived the winter as larvae did not differ in body mass compared to the pupal diapausing individuals (Fig. 3).

Direct development

To test if differences in body mass between the two diapause strategies of the southern population may be partly because of genetic correlations with winter survival, we reared offspring of both diapause strategies of the southern population (nine females from pupal diapause and 10 females from larval diapause). The two subsets of larval and pupal diapausers (LD and PD
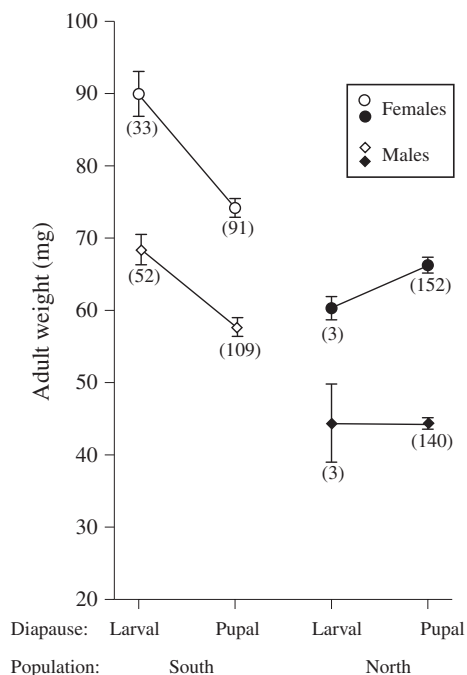

Fig. 3 Results of the population comparison showing mean adult weight ± 1SE of individuals of both populations that followed either the pupal diapause pathway or the larval diapause pathway. Open symbols represent the southern Swedish population, whereas the filled symbols denote the central Swedish population. Sample sizes for each group are given in parenthesis.

respectively) that sired this next generation differed markedly in female mass but not in male mass (mean ± 1SE in mg, $PD_{Female} = 75.6 \pm 4.9$, $PD_{Male} = 70.5 \pm 4.9$, $LD_{Female} = 92.3 \pm 6.8$, $LD_{Male} = 69.3 \pm 4.0$). As expected, there was a strong sexual mass dimorphism also in the directly developing generation but even though there was a significant effect of family there was no effect of the diapause strategy of the parental generation [ANOVA, sex: $F_{1,17} = 88.27$, $P < 0.0001$, Parental diap: $F_{1,17} = 0.049$, $P = 0.82$, Family (nested within Parental diap.): $F_{17,115} = 6.44$, $P_{family} < 0.0001$, interactions dropped from the model as $P > 0.1$, $r^2_{adj} = 0.57$; Mean adult weight ± 1SE in mg, by parental diapause and sex: $PD_{Female} = 71.2 \pm 3.5$, $PD_{Male} = 59.8 \pm 2.0$, $LD_{Female} = 73.9 \pm 1.8$, $LD_{Male} = 56.3 \pm 1.6$]. In addition, there was a strong genetic correlation between sexes in adult mass in this directly developing generation [Regression of family means of female weight on male weight; estimates (1SE): FemW = 1.07 (0.20) × MaleW + 12.0 (11.5); $F_{1,16} = 28.32$, $P < 0.0001$, $r^2_{adj} = 0.62$].

Discussion

In line with theoretical predictions (Gotthard et al., 2007; Berger et al., 2008) and the earlier study of Van Dyck & Wiklund (2002), the present results show that the two diapause strategies of *P. aegeria* in southern Sweden produce adults of substantially different weight. Moreover, the results of our 'reciprocal transplant' oviposition experiment suggest that this mass difference is likely to be favoured by natural selection. The large weight reached by females of the larval diapause pathway led to a higher realized fecundity in the typical thermal conditions of June when the LDs naturally appear as adults, but not in the thermal conditions of April/May when the PDs are flying in the field (Fig. 2). The main explanation for this pattern is that the typical thermal condition of April/May limits oviposition rates so that large females cannot realize their high potential fecundities (Gotthard et al., 2007; Berger et al., 2008). Therefore, when an individual has been switched to follow the pupal diapause pathway, fecundity selection for reaching the weight of the LDs is likely to be weak and outweighed by costs because of juvenile mortality. The results are in line with predictions from life history models of optimal size and provide further support for the idea that thermally imposed time limitation for oviposition is important for the evolution of optimal size in *P. aegeria* and other insects (Gotthard et al., 2007).

It is noteworthy that unlike the study of Van Dyck & Wiklund (2002), in this study also male mass differed between the two developmental pathways in the southern population (Fig. 3). The relationship between male fitness and weight in *P. aegeria* is less straightforward than for females (Kemp et al., 2006; Bergman et al., 2007), but it is possible that also this relationship may change with thermal conditions (Van Dyck & Wiklund, 2002). However, in the direct development experiment, we found a strong genetic correlation in body size between sexes, and it is possible that selection for gene expression patterns inducing larger females has pleiotropic effects also on gene expression in males following the same developmental pathway. This could then potentially explain why also the males grew larger when following the larval diapause pathway.

The comparison between our two Swedish populations of *P. aegeria* indicates that the mass difference between developmental pathways in the southern population is maintained by natural selection. This is because the central population that does not experience selection on the larval diapause pathway showed no sign of developing a weight difference between pathways (Fig. 3). A general conclusion concerning the typical consequences of this type of difference in selective regimes will nevertheless have to await investigations into further populations. Moreover, as larval diapause is not used naturally in the central population winter survival was low, and the lack of size difference may potentially reflect developmental constraints in this population. It is therefore of interest to scrutinize possible alternative non-adaptive explanations for the weight difference in the southern population. Although growth conditions in autumn and spring may differ in natural conditions because of variation in food availability and temperature our experimental setup largely controlled for this possibility. All individuals in our experiment were given the same host plant species (*D. glomerata*) in similar and adequate quantities, and there were no differences between treatments in thermal conditions that could explain the mass difference between developmental pathways as a consequence of the temperature-size rule (Atkinson, 1994). Finally, in addition to the present study, the mass difference between diapause strategies in the south Swedish population has been documented in field collected as well as in strict laboratory reared butterflies (Van Dyck & Wiklund, 2002), demonstrating that this difference develops consistently under a variety of conditions. In combination, this suggests that the difference in weight between the diapausing pathways is likely to be a result of different developmental regulation and not because of some unavoidable physiological consequence of spending the winter in the larval stage, or to differences in growth conditions between autumn and spring. Although the result of the central Swedish population should be interpreted with some caution, it still indicate that because the larval diapause pathway is rarely, if ever, expressed in the central population there is no selection that favours a similar developmental regulation of size as seen in the southern population.

The thermal conditions of April/May, when the pupal diapause cohort is active in southern Sweden, are typically less favourable for butterfly activity and oviposition than thermal conditions of June when the LDs are

on the wing (Figs 1 and 3). Nevertheless, pupal diapause is likely to be favoured by natural selection as it leads to an earlier flight period, which increases the chances of a successful second, and occasionally third generation, the same year. The larval diapause strategy may be present because individuals are doing the best of a bad situation when conditions in autumn no longer allow larval development all the way to the pupal stage. The beauty of the situation of *P. aegeria* in southern Sweden as well as other populations further south in Europe is that both these options as well as direct development seem to be present within any typical genotype and is regulated by developmental decisions in response to seasonal cues (Shreeve, 1986; Nylin *et al.*, 1989; Van Dyck & Wiklund, 2002). The two diapause strategies in autumn channels individuals of the south Swedish population towards divergent conditions during the adult stage in spring/ early summer. Hence, an individual's stage of diapause provides high quality information to the developmental system about future selective conditions. Our results suggest that this information is used to differentially optimize adult weight. Moreover, as there were no effects of parental diapause on the mass of directly developing offspring we conclude that the weight difference between the two diapausing strategies could not be explained by genetic correlations between the capacity to survive larval diapause and adult mass. In combination with the earlier study of Van Dyck & Wiklund (2002) that also demonstrated potential adaptive differences in wing melanization and resource allocation among pathways it seems likely that the diapause decision may act as a cascade switch.

The main hypothesis tested in this study is whether the presence of a developmental switch between alternative pathways allows natural selection to favour a semi-independent optimization of adult traits downstream of the switch. Our results indicate that this is indeed possible. It is important to note that the evolution of the developmental switch studied here most likely is because of selection for high winter survival and an efficient use of the limited part of the year that is available for growth and reproduction (Shreeve, 1986; Nylin *et al.*, 1989). However, once these alternative developmental pathways have evolved, the resulting modular organization of development may allow natural selection to further fine-tune the developmental control of these pathways (Snell-Rood *et al.*, 2010). This is possible if the switch between pathways influences gene expression pattern downstream of the switch (Denlinger, 2002; West-Eberhard, 2003).

Developmental switches can be seen as mechanisms that allow integration of genetic and environmental inputs on the development of phenotypes. The relative influence of genes and environment may vary between different switches and situations where the determination of alternative morphs is almost completely because of genetic factors (e.g. genetic polymorphisms) can be treated as an endpoint in this continuum (Leimar *et al.*, 2006). If one considers developmental switches from this perspective, it becomes clear that differential developmental regulation downstream of a major switch is a very common phenomenon (West-Eberhard, 2003). The perhaps most obvious example is the alternative developmental pathways of males and females (Fisher, 1958; Lande, 1980). In most species, the developmental switch between sexes is primarily because of genetic factors (although there are notable exceptions (Bull, 1983; West-Eberhard, 2003; Janzen & Phillips, 2006)), and it is obvious that natural selection typically favours differential development of males and females to produce the wide array of fascinating sexual dimorphisms seen in the living world (Andersson, 1994). The main difference to most examples of sex determination is then that the developmental switch determining diapause is more strongly influenced by environmental inputs. We suggest that diapause often has such profound and predicable influence on the expected lives of insects that natural selection may be expected to favour the evolution of partly independent developmental regulation subsequent to the diapause decision. This independence may then allow differential optimization of traits in response to consistent differences in selective conditions of alternative developmental pathways. We suggest that this is an important mechanism for the evolution developmental plasticity and its role in the generation of biodiversity, as it may explain how a syndrome of traits may show concerted adaptive phenotypic plasticity.

Acknowledgments

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