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The evolution of alternative developmental pathways: footprints of selection on life-history traits in a butterfly

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Abstract

Developmental pathways may evolve to optimize alternative phenotypes across environments. However, the maintenance of such adaptive plasticity under relaxed selection has received little study. We compare the expression of life-history traits across two developmental pathways in two populations of the butterfly *Pararge aegeria* where both populations express a diapause pathway but one never expresses direct development in nature. In the population with ongoing selection on both pathways, the difference between pathways in development time and growth rate was larger, whereas the difference in body size was smaller compared with the population experiencing relaxed selection on one pathway. This indicates that relaxed selection on the direct pathway has allowed life-history traits to drift towards values associated with lower fitness when following this pathway. Relaxed selection on direct development was also associated with a higher degree of genetic variation for protandry expressed as within-family sexual dimorphism in growth rate. Genetic correlations for larval growth rate across sexes and pathways were generally positive, with the notable exception of correlation estimates that involved directly developing males of the population that experienced relaxed selection on this pathway. We conclude that relaxed selection on one developmental pathway appears to have partly disrupted the developmental regulation of life-history trait expression. This in turn suggests that ongoing selection may be responsible for maintaining adaptive developmental regulation along alternative developmental pathways in these populations.

Introduction

Natural selection is expected to favour the expression of alternative phenotypes in response to environmental information about differences in future selective conditions (West-Eberhard, 2003). Such adaptive plasticity is often due to developmental switches that integrate genetic and environmental effects on phenotypes (Moran, 1992; West-Eberhard, 2003). Examples of switch-induced developmental plasticity are cellular differentiation and organization in multicellular organisms (Schlichting, 2003; West-Eberhard, 2003), sex determination (Graves, 2008; Salz, 2011; Uller & Helantera, 2011) and caste determination in social insects (Wheeler, 1986; Nijhout, 1999; Evans & Wheeler, 2001). The presence of adaptive developmental plasticity implies that there are genes with environmentally specific expression that may be carried and transmitted by all individuals in a population but only expressed and exposed to selection in a fraction of these. Mutation–selection–drift balance predicts the amount of standing genetic variance to be strongly dependent on how often encoded phenotypes are exposed to selection through time and space. Conditionally expressed traits are therefore expected to harbour more genetic variance and be less evolutionary robust due to the reduced purifying and eroding effects of selection (Van Dyken & Wade, 2010).

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However, often pathway-specific expression by gene regulation is only partial, and many genes have strong pleiotropic effects and influence expressed phenotypes similarly across alternative pathways (Moran, 1992; West-Eberhard, 2003; Snell-Rood *et al.*, 2010; Van Dyken & Wade, 2010). Thus, selection on one phenotype in one generation may, through correlated evolution, also influence the expression of the alternative phenotype in subsequent generations. Pleiotropy is therefore considered instrumental in maintaining trait function under relaxed selection (Lande, 1980; Jones *et al.*, 2003; Turelli & Barton, 2004; Maughan *et al.*, 2007; Lahti *et al.*, 2009). Few studies, however, have examined the consequences of relaxed selection on trait expression along alternative developmental pathways and its potential for influencing evolutionary processes (Pfennig *et al.*, 2010; Snell-Rood *et al.*, 2010).

Insect diapause is a classic example of switch-induced adaptive developmental plasticity that leads to the expression of either direct development (i.e. continued development into the reproductive stage) or diapause (i.e. postponing maturation by entering a dormant stage) in response to cues of seasonal change (Clark & Platt, 1969; Wiklund *et al.*, 1983; Tauber *et al.*, 1986; Nylin *et al.*, 1995; Gotthard & Berger, 2010). In temperate latitudes, diapause is a crucial adaptation that allows insects to survive the unfavourable conditions during winter (Tauber *et al.*, 1986; Kivelä *et al.*, 2009), as well as synchronize important life-history events over the season (i.e. reproduction and growth) (Tauber & Tauber, 1976; Nylin & Gotthard, 1998; Hahn & Denlinger, 2011). Individuals that have followed different developmental pathways (diapause or direct development) often show consistent phenotypic differences, that is, seasonal polyphenism (Shapiro, 1976 and references therein). Although polyphenisms in morphological traits such as wing coloration have received much attention and are commonly linked to diapause, relatively little is known about potential life-history differences (Van Dyck & Wiklund, 2002; Gotthard & Berger, 2010; Teder *et al.*, 2010).

Explanations for any trait differences between the developmental pathways connected to diapause decision fall into two classes: they are indirect effects due to allocation or acquisition trade-offs with the diapause/direct decision itself (Danks, 2006) (e.g. entering diapause may necessitate the accumulation of additional resources which might lead to a larger body size and longer development time compared to direct development), or they are direct effects due to differences in the selective regimes that meet the two alternative phenotypes (Moran, 1992; Gotthard & Berger, 2010; Teder *et al.*, 2010) (e.g. selection on body size and reproductive investment may differ between different parts of the year due to differences in thermal conditions, or abundance of predators). The first class would represent an effect of selection on the diapause/direct development ability (Danks, 2006; Friberg & Karlsson, 2010; Friberg *et al.*, 2011). The second class, however, would be due to natural selection for semi-independent regulation of development that allows adaptive expression of alternative phenotypes along different developmental pathways (Van Dyck & Wiklund, 2002; Karlsson & Wiklund, 2005; Karlsson & Johansson, 2008; Gotthard & Berger, 2010). Some studies have demonstrated potentially adaptive differences in life-history traits among individuals that have followed either direct or diapause development in bivoltine populations where both developmental pathways are exposed to natural selection (Gotthard & Berger, 2010; Larsdotter Mellström *et al.*, 2010; Teder *et al.*, 2010). Such alternative developmental regulation for 'fine-tuning' of phenotypes along diapausing and directly developing pathways is likely to be present only in situations where both developmental pathways are regularly expressed and subjected to diversifying selection (Gotthard & Berger, 2010; Snell-Rood *et al.*, 2010). In many species, however, there is a shift to univoltinism (one diapausing generation per year) at higher latitudes (Danilevskii, 1965; Roff, 1980), and in these populations, there is no selection on the direct development pathway (Gotthard & Berger, 2010). In such a situation, genes with pathway-specific expression patterns will be subjected to genetic drift and mutation accumulation, which is likely to erode adaptive developmental plasticity and may reduce trait differences among developmental pathways.

Testing the effects of differential selection on conditional expression of developmental pathways in the *Pararge aegeria* study system

Here, we study alternative expression of life-history traits among diapausing and directly developing individuals of two different Swedish populations of the speckled wood butterfly, *Pararge aegeria*. This study system is rare in that central and northern populations in Sweden are univoltine in nature but can be made to express direct development under laboratory conditions, whereas the species is naturally bivoltine in southern Sweden. Thus, by comparing a naturally univoltine central population to a naturally bivoltine southern population, we aim to test whether ongoing selection favours differential expression of life-history traits along the direct and diapause pathways, and investigate the effects of relaxed selection on trait expression in direct developers of the central population. This study system and experimental design allows us to explore to what degree trait differences between pathways are due to the two classes of explanation suggested above: that is, indirect effects of trade-offs versus selection for pathway-specific expression. Any difference between populations in pathway-specific expression will suggest that the degree of life-history differences between pathways may be influenced by selection independently of potential trade-offs

with the diapause/direct development decision. Such variation among populations is likely to be due primarily to relaxed selection on the direct pathway in the central Swedish univoltine population that will allow trait values associated with low fitness under direct development to be maintained. Hence, we predicted that the central Swedish population under direct development should have relatively longer development times, lower pupal weights and lower larval growth rates compared to their southern conspecifics. Such an accumulation of low-fitness genes with specific effects on the directly developing pathway can also be expected to lead to increased phenotypic and genetic variation in the central Swedish population expressed under direct development (Van Dyken & Wade, 2010). However, it is important to note that another potential mechanism for population differences in pathway-specific expression is that there are differences in local selection pressures on the diapause pathway that is under selection at both locations. Such selection differences may, for example, be due to latitudinal variation in seasonal time constraints (Roff, 1980; Blanckenhorn & Demont, 2004; Stillwell, 2010).

Finally, as the expression of life-history traits along alternative developmental pathways is unlikely to be entirely independent, we also aimed to explore the effect of relaxed selection on genetic correlations between development pathways. We further compared these interpathway correlations to intersexual genetic correlations because life-history differences between males and females, such as protandry, are likely to depend on what seasonal developmental pathway is followed (Wiklund *et al.*, 1991; Brisson & Nuzhdin, 2008; Larsdotter Mellström *et al.*, 2010). Protandry is the tendency for males to emerge as adults before females, and it is a very common pattern in insect populations with nonoverlapping generations (Blanckenhorn *et al.*, 2007). It has been explained as the result of sexual selection on males to maximize the expected number of matings (Wiklund & Fagerström, 1977) and/or on females to minimize the period between adult emergence and mating (Fagerström & Wiklund, 1982). Strong pleiotropy is predicted to maintain function in traits under relaxed selection (Turelli, 1985). However, reciprocal predictions for how genetic correlations will evolve when selection is relaxed on one developmental pathway are not as straightforward (Jones *et al.*, 2003; Sgrò & Hoffmann, 2004; Arnold *et al.*, 2008) but should depend on the relative difference in the number of loci and their mutual effects contributing to common versus pathway-specific phenotype variance (Turelli, 1985; Hansen, 2003). With regard to life-history traits, it seems likely that a large proportion of the genetic background is shared between pathways and that any differences in phenotypic expression among pathways are due to a limited number of genes with pathway-specific effects. When selection on one of these pathways is relaxed, we expect an accumulation of mutations in genes with specific expression in this pathway, which is likely to reduce adaptive phenotypic differences between pathways. Furthermore, this accumulation of random genetic variation may override the effects of pleiotropic genetic variation expressed in both pathways, and result in a weaker genetic correlation under relaxed selection compared to when selection is acting on both pathways.

Materials and methods

The speckled wood butterfly, *P. aegeria*, has a disjunct distribution on the Scandinavian Peninsula. In central Sweden, the species has existed for as long as there are records, whereas southern Sweden was unoccupied until 1939 when it was first colonized by migrants from Denmark (Nordström, 1955). In central Sweden, it is typically univoltine with pupal diapause but direct development can be induced in individuals of this distribution if given long day lengths in laboratory conditions (Nylin *et al.*, 1989). Observations over the last 10 years show that individuals representing a second generation are only occasionally seen in the field (ArtDatabanken (2011) http://www.artdata.slu.se/. SLU, Uppsala). The south Swedish distribution has two and sometimes three generations during the favourable season and occurs naturally in several distinct cohorts (Van Dyck & Wiklund, 2002). Although this newly established south Swedish distribution is spreading further north, the two distributions are still geographically separated (Eliasson *et al.*, 2005).

The experiment included individuals from one population from the south Swedish distribution and one population from central Sweden, and was conducted with a split-brood design in two blocks. Gravid field-collected females were allowed to oviposit on the host plant *Poa annua* in 1-L plastic containers, and eggs were allowed to hatch at room temperature. Newly hatched larvae were placed in individual transparent plastic containers (0.5 L) with *ad libitum* access to *P. annua*, and offspring of each female were divided equally between two different experimental conditions (a diapause-inducing short-day treatment: 15:9-h light/dark; 17 °C and a direct development-inducing long-day treatment: 21:3-h light/dark; 17 °C). The treatments and the corresponding developmental pathways will henceforth be referred to as 'diapause' and 'direct'. For each individual, the identity of the maternal female, the start date (defined as the date the larvae were introduced to the experimental treatment shortly after hatching), date at pupation and pupal weight (weighed at a Kern 410 microscale) were recorded. The sex was determined by examining the number of genital slits on the underside of the abdomen of the pupae (Friberg *et al.*, 2011). Individuals that eclosed as adults within 25 days of pupation were noted as having developed directly, whereas all individuals having spent more than 25 days in the pupal stage were considered to have entered pupal diapause (Friberg *et al.*, 2011).

Due to the diverse phenology and variation in flight period of *P. aegeria* in Sweden, wild-caught females from both populations are not always available at the same time. The first block of the experiment was started before the central Swedish population's natural flight period, and we therefore used experimentally reared and overwintered F1-females from the Stockholm area. In the first block, 160 offspring of 8 gravid females of the south Swedish distribution collected at Ransvik, Skåne (56° 16′ 57″ N, 12° 29′ 38″ E), in April 2009 were included in the experiment alongside 140 larvae of 7 laboratory-reared females originating from Riala (100 km north of Stockholm, 59° 37′ 50″ N, 18° 31′ 12″ E). The 7 laboratory-reared females were first-generation offspring of wild-caught females collected the previous summer, having been reared to pupation in the laboratory, allowed to overwinter outdoors by the laboratory buildings at Stockholm University alongside male pupae and subsequently induced to eclose and mate in laboratory. The larvae were evenly distributed between the two experimental treatments (see above) in four climate chambers (Termaks Series KB8400L; Termaks, Bergen, Norway).

In the second block, another 312 larvae from 13 females collected at Riala, Stockholm, in early June 2009 and 75 larvae from four females collected at Ransvik, Skåne, in late June 2009 were included in the experiment. For the remainder of this paper, the individuals originating from Riala, Stockholm, and the population these individuals represent will be referred to as the 'central Swedish' population. Individuals collected at Ransvik, Skåne, and the population these individuals belong to will be referred to as the 'south Swedish' population. In total, 687 individual larvae were included in the experiment, 452 from central Sweden and 235 from south Sweden. Larval mortality was 17.9%, and of the remaining 564 individuals, eight died early in the pupal stage and were excluded from the analyses, as were 17 individuals that did not choose the 'correct' developmental pathway with regard to treatment (i.e. developed directly under diapause-inducing treatment or entered diapause under direct development-inducing treatment). For the statistical analyses, 276 individuals were included in the direct treatment (170 from central Sweden and 106 from south Sweden) and 263 in the diapause treatment (157 from central Sweden and 106 from south Sweden).

Three major life-history traits were analysed: pupal weight (mg), larval development time (days) and growth rate. Pupal weights were log-transformed before analysis. A measure of growth rate was calculated for each individual according to the equation: Growth rate = [log10 (pupal weight)]/larval development time.

Statistics

Analyses were carried out using R version 2.13.2 for Windows (R Development Core Team, 2011). All life-history traits (pupal weight, larval development time and growth rate) were analysed by restricted maximum likelihood (REML) in mixed models using the lme4-package implemented in R. Main effects of population, pathway, sex and block as well as their interactions were tested. We used a variance–covariance structure with family as a random effect crossed by treatment and sex, ensuring appropriate evaluation of associated model likelihoods used in significance testing of fixed effects. For the selection of appropriate statistical models, we calculated *P*-values from likelihood ratio tests based on the profiled deviance of each compared model and removed nonsignificant interactions serially unless $P < 0.1$, to produce the final models. For the analysis of within-family protandry, we fitted a linear model including population, pathway and their interaction, and tested the significance of effects using type II SS. This was followed by *t*-tests for exploring to what degree group means differed from 1.

Growth rate is arguably the trait for which we have the best understanding of how differential selection is acting on the sexes and developmental pathways (Wiklund *et al.*, 1991; Gotthard *et al.*, 1999; Berger & Gotthard, 2008; Gotthard, 2008; Larsdotter Mellström *et al.*, 2010; Berger *et al.*, 2011). Therefore, we focused on the growth rate and continued by estimating phenotypic and genetic variance specific to each combination of population/pathway/sex, that is, in eight groups, and corresponding genetic covariance and resulting genetic correlations between these groups. With the growth rate data, we also explored the genetic background of protandry (the earlier emergence of males into the reproductive population) by calculating the within-family sexual dimorphism in growth rate of the four combinations of pathway and population. The independent observation in this analysis was the ratio of the mean male to mean female larval growth rate per family, in all combinations of pathway and population. Large-scale experiments involving butterfly rearing is a difficult task. We managed to include 12 and 20 sampled families of the south and central Swedish population, respectively. However, for analysing genetic (co)variance matrixes, these family numbers are small, and these complementary analyses are necessarily associated with low statistical power. Nevertheless, they offer interesting information about the genetic architecture of conditional expression of traits. Due to the unbalanced sample sizes across groups and within families for these analyses, we complemented the REML estimates from our main analysis (above) with Bayesian approximations available through the MCMCglmm package implemented in R (Hadfield, 2010). MCMCglmm allows the estimation of parameter values for both fixed effects and variance components with confidence limits and handles unbalanced designs efficiently. Starting values for the Bayesian simulations were based on the REML estimates but the priors were set weak (nu = 0.1 for the 4×4 (co)variance

matrix of random effects) to minimize the impact on the posterior probability distributions as recommended by Hadfield (2010). We ran 1 500 000 iterations for each population separately with a burn-in of 500 000 iterations and a thinning interval of 1000, resulting in 1000 stored uncorrelated parameter estimates of each effect. Bayesian estimation of the genetic (co)variance matrix allowed the calculation of trait heritabilities within population, pathway and sex, and genetic correlations between groups with confidence limits. The broad-sense heritability in our case using a full-sib design equals: $H^2 = 2 \times V_{fam}/(V_{fam} + V_{error})$. Estimated phenotypic and genetic variance, trait heritabilities as well as mean scaled phenotypic variation ($I = V_P/mean^2$) can be found in Table 2. The scaled estimate of variance allows more unbiased comparisons of phenotypic variation between groups that differ in mean values. The genetic correlation between groups (i.e. both intersexual and interpathway correlations) equals: $cov(A_1, A_2)/\sqrt{(V_{A1} \times V_{A2})}$, where A_1 and A_2 are the estimated genetic effects (breeding values) for the focal trait in environments (group) 1 and 2 respectively, and V_{A1} and V_{A2} their variances. For comparison, we also calculated genetic correlations based on partial Pearson's correlations of family means across the groups. In all analyses, we blocked the main effect of block by adding it as a fixed effect in the models. Estimates of intersexual and interpathway genetic correlations can be found in Table 3 and Fig. 3.

Table 1 Results of the mixed model analyses of 539 individuals based on 32 families for (a) pupal weight, (b) larval development time and (c) larval growth rate showing main effects of population (pop), block, pathway (path), sex and interactions. Significant effects are highlighted in boldface.

	Source of variation	χ^2	*P*
(a)			
Response variable:	Pop	152.4392	**< 0.0001**
Pupal weight	Block	0.7661	0.3814
	Path	30.3882	**< 0.0001**
	Sex	229.9780	**< 0.0001**
	Pop × block	17.0701	**< 0.0001**
	Pop × path	4.1321	**0.04208**
	Pop × sex	0.2906	0.58981
	Block × path	1.2635	0.26100
	Block × sex	0.0067	0.93489
	Path × sex	0.2830	0.59475
	Pop × block × path	3.1853	0.07430
	Pop × path × sex	3.7739	0.05206
	Block × path × sex	2.9486	0.08595
(b)			
Response variable:	Pop	0.0292	0.86433
Larval development time	Block	3.2392	0.07190
	Path	695.0966	**< 0.0001**
	Sex	9.8980	**0.00166**
	Pop × block	2.2456	0.13400
	Pop × path	109.3276	**< 0.0001**
	Pop × sex	4.2512	**0.03922**
	Block × path	0.0734	0.78646
	Path × sex	3.6674	0.05549
	Pop × block × path	5.6545	**0.01741**
(c)			
Response variable:	Pop	14.9512	**0.00011**
Growth rate	Block	1.5732	0.20975
	Path	692.7520	**< 0.0001**
	Sex	3.1454	0.07614
	Pop × block	8.3975	**0.00395**
	Pop × path	104.5038	**< 0.0001**
	Pop × sex	4.9090	**0.02672**
	Block × path	0.7353	0.39117
	Path × sex	11.3970	**< 0.0001**
	Pop × block × path	3.8735	**0.04905**

Results

Analyses revealed a significant difference in body size (i.e. pupal weight) between the two populations (see Table 1a), with individuals from the south Swedish population in general being larger (see Fig. 1a,b). The analyses further showed a significant difference in size between the two developmental pathways as well as between the sexes; in all cases, females were larger than males and the diapause pathway produced individuals that were significantly larger than the direct pathway in both populations and in both sexes. Most importantly, the two populations responded differently to the experimental treatments with the size difference between developmental pathways being smaller in the south Swedish population. There was also an effect of the interaction between population and block mainly because the south Swedish population in the second block grew to a somewhat smaller size, whereas the central Swedish population became larger.

The analyses showed a significant difference in larval development time between the two pathways as well as between the sexes (see Table 1b); overall, the diapause pathway had longer development times than the direct pathway, and females had slightly longer development times than males. Again there was a significant interaction between population and pathway, with the south Swedish population having both a shorter development time in the direct pathway and a longer development time in the diapause pathway than the central Swedish population (see Fig. 1c,d). Although this interaction differs between the two blocks – more pronounced in the second block – the general patterns are congruent between blocks. Furthermore, there was an interaction between population and sex, with the difference in development times between males and females being larger in the south Swedish population than in the central Swedish population. Moreover, a near-significant interaction between pathway and sex hints to males overall having a relatively short development time in the direct pathway compared to females.

Finally, the two populations differ in growth rate (Table 1c). In addition, there is a difference in growth

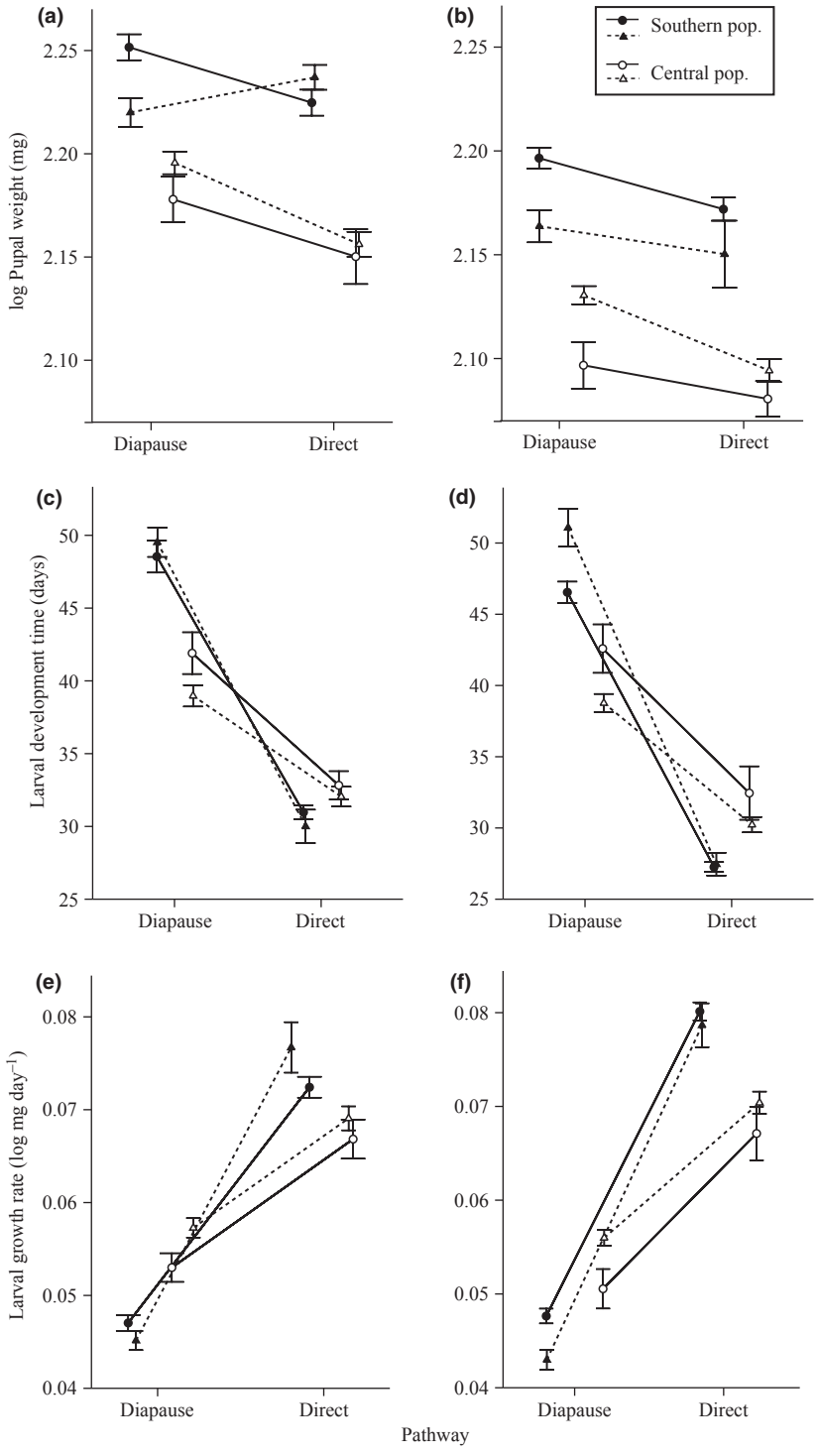

Fig. 1 Interpopulation comparisons for 'diapause' and 'direct' pathways for all three life-history traits (mean ± 1 SE) with filled symbols representing the south Swedish population and open symbols representing the central Swedish population. Circle symbols represent the first block of the experiment, and triangle symbols represent the second block. Left column (a, c, e) shows results for females, whereas the right column (b, d, f) shows results for males. (a, b) depict values for pupal size; (c, d) show larval development times, whereas (e, f) display results for larval growth rate. For the south Swedish population males in the second block (b, d and f, filled triangles), the 'diapause' and 'direct' pathway groups contain 11 and 12 individuals, respectively. For the remainder, each group contains between 17 and 65 individuals.

rate between pathways: the direct pathway having higher growth rates than the diapause pathway (Fig. 1e,f). The populations responded differently to block with the central Swedish population having somewhat higher growth rates in the second block of the experiment, whereas the south Swedish population, with the exception of directly developing females, had somewhat lower growth rates in the second block. Once more the two populations responded differently to pathway, with the south Swedish population having both the lowest growth

rate in the diapause pathway and the highest growth rate in the direct pathway. This interaction was somewhat more pronounced in the second block. As for larval development time, there is an interaction between population and sex with the difference in growth rate between males and females being larger in the south Swedish population. However, for growth rate, as opposed to larval development time, the interaction between pathway and sex is significant with males having a higher growth rate than females in the direct pathway.

Phenotypic and genetic variation

In the analysis of phenotypic and genetic variance among pathways, sexes and populations, we concentrated on growth rate as it is a composite measure of size and development time for which selection pressures are relatively well understood. In general, the estimates of heritability for growth rate were reasonably high. Moreover, the central population expressed more phenotypic variation compared to the southern population (Table 2). However, in contrast to our expectations, there was no general increase in either phenotypic or genetic variance in the direct developing individuals of the central population that are under relaxed selection. The most striking pattern was the relatively low degree of phenotypic variation in the directly developing males of the southern population compared to that of males from the central population. To explore this potential interaction between developmental pathway, sex and population, we continued to examine within-family sexual dimorphism in growth rate as a measure of the genetic background of protandry of the four combinations of pathways and population (Fig. 2). This analysis first of all showed that, in accordance with theoretical predictions, protandry was more apparent under direct development than under diapause development in both populations (Fig. 2, linear model; Pop: $F_{1,56} = 3.25$, $P = 0.077$, Pathw: $F_{1,56} = 12.60$, $P < 0.001$). The index of protandry was significantly larger than one in the direct developing individuals from the southern population ($t_{1,10} = 6.86$, $P < 0.0001$) and significantly smaller than one in the diapausing individuals from the central population ($t_{1,18} = -2.34$, $P = 0.031$). More interestingly, the degree of between-family variation for this protandry was significantly larger in the central population than in the southern population under direct development, whereas there was no such significant difference between populations under diapause development (Fig. 2, variance ratio f test; direct dev.: $F_{17,10} = 9.19$, $P = 0.0011$; diapause dev.: $F_{18,11} = 0.92$, $P = 0.84$).

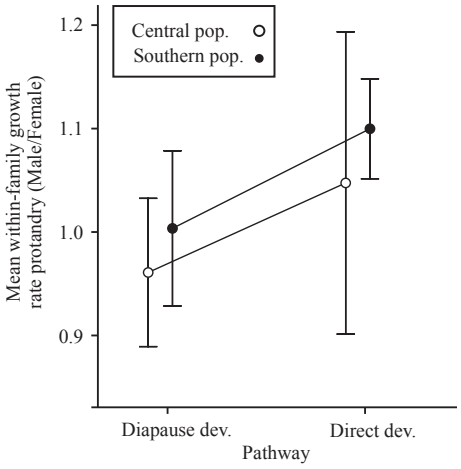

Fig. 2 The degree of protandry measured as the ratio of female to male growth rates within families. Results are divided by population and developmental pathway (Mean ± 1 SD). Filled symbols represent the south Swedish population, and open circles represent the central Swedish population.

Genetic correlations

The estimates of genetic correlations were congruent between the Bayesian and partial Pearson's correlation approach with two of the estimates being significant. Although sample sizes are too low to allow quantitative

Table 2 Bayesian estimations (posterior modal values) of phenotypic variance (V_P), broad-sense heritabilities (H^2) and their 95% confidence limits (*CI*), mean scaled phenotypic variance (I) (V_P/mean^2), for larval growth rate in all combinations of population, pathway and sex. The number of individuals (ind) and the number of families (fam) are also given. *P*-values for family effects are reported for restricted maximum likelihood estimates performed on each group separately.

Population	Pathway	Sex	Fam	Ind	$V_p \times 10^5$ (CI)	I	H^2	CI	P
Southern	Diapause	Females	12	58	2.8 (1.6–3.5)	0.013	0.24	0.10–0.94	0.151
		Males	12	48	2.5 (1.4–3.3)	0.012	0.99	0.39–1.59	**0.0007**
	Direct	Females	12	55	7.7 (6.0–13)	0.014	0.34	0.05–1.00	**0.020**
		Males	11	51	4.3 (2.8–6.1)	0.007	0.42	0.08–1.16	**0.033**
Central	Diapause	Females	20	71	5.4 (3.7–7.5)	0.017	0.16	0.05–0.74	0.461
		Males	19	86	5.4 (4.0–7.2)	0.019	0.16	0.04–0.63	0.457
	Direct	Females	18	85	11 (7.2–14)	0.024	0.20	0.03–0.79	**0.029**
		Males	20	85	10 (8.0–15)	0.021	0.31	0.04–0.90	**0.007**

comparisons of these correlations, several interesting qualitative differences emerge. The estimates of intersexual genetic correlations for growth rate were relatively high and positive for all categories except for the directly developing individuals from the central population, which is the one group that experience relaxed selection (Table 3, Fig 3a,b). The estimates of correlations between developmental pathways were also positive and relatively strong for females of both populations. For the males, however, the interpathway correlation was generally weaker and this was most pronounced in the males of the central population (Table 3, Fig. 3c). In fact, both estimates involving the directly developing males of the central population returned relatively low correlations (Fig. 3a,d).

Discussion

The switch between direct and diapause development in insects gives information to the developmental system about future selective conditions, and downstream regulation of development might be expected. Such fine-tuning of development is likely to be maintained by stabilizing selection and thus may deteriorate under relaxed selection. In this study, we found significant interactions, for all three life-history traits, between population and developmental pathway, which show that the pattern of pathway-specific expression of life-history traits differs between populations. These effects were significant even when controlling for the relatively minor effect of block, which except for one case gave qualitatively similar results (Fig. 1). Interestingly, whereas the difference between pathways in pupal weight is smaller in the south Swedish population than in the central Swedish population, the differences in larval development time and growth rate are instead larger in the south Swedish population. These patterns indicate that differences in pathway-specific selection among the populations have influenced the regulation of development downstream of the diapause/direct development decision. An obvious limitation of our study is that we were only able to explore these patterns in two populations (albeit sampled at different time points). Although this precludes us from making general

Table 3 Results of the analysis of genetic correlations of larval growth rate between sexes and developmental pathways. Partial Pearson's correlations with associated *P*-values and Bayesian estimates as posterior modal values (means in parentheses) with associated confidence intervals.

Type	Population	Group	Pearsons	*P*	Bayesian	95% CI
Intersexual	Southern	Diapause	0.49	0.12	0.79 (0.53)	−0.13 to 0.98
		Direct	0.64	**0.048**	0.94 (0.72)	0.13 to 0.99
	Central	Diapause	0.24	0.34	0.64 (0.43)	−0.28 to 0.91
		Direct	−0.20	0.44	0.03 (0.02)	−0.80 to 0.80
Interpathway	Southern	Females	0.55	0.080	0.80 (0.54)	−0.12 to 0.98
		Males	0.14	0.71	0.73 (0.42)	−0.33 to 0.97
	Central	Females	0.53	**0.030**	0.76 (0.60)	0.07 to 0.96
		Males	−0.07	0.78	−0.30 (0.16)	−0.58 to 0.89

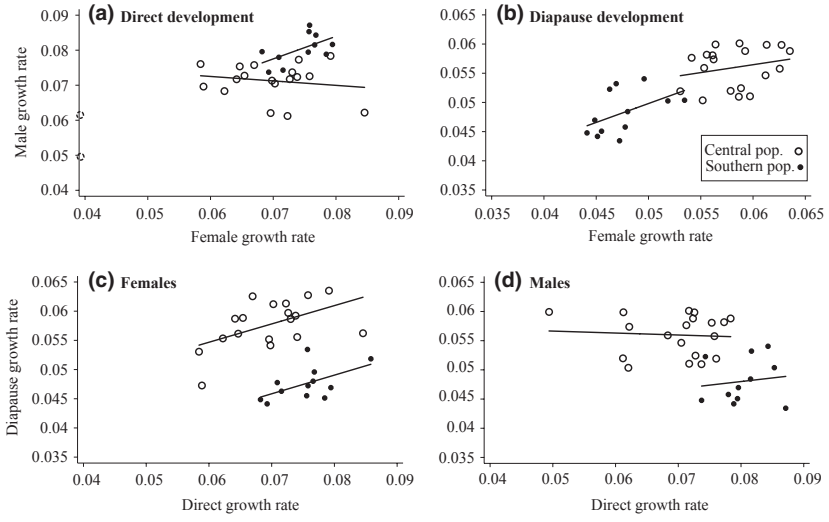

Fig. 3 Genetic correlations based on family means of growth rate standardized for block effects. Panels display the intersexual correlation for individuals following (a) the direct pathway and (b) the diapause pathway. The same results concerning interpathways correlations for (c) females and (d) males. Filled symbols represent the south Swedish population, and open circles represent the central Swedish population. The two dashed circles on the *y*-axis in figure a) represent two families that did not have values for within-family growth rate for females. Lines show Pearson's correlations. The southern population contains 11–12 families, and the central population contains 18–20 families (see Table 2).

inferences, we argue that the results reveal novel and interesting patterns, and highlight a promising methodology for investigating the evolutionary dynamics of conditionally expressed traits. With this caveat in mind, we discuss how differential selection may have shaped pathway-specific expression in body size, development time, growth rate and protandry, respectively. We summarize by discussing how relaxed selection and pleiotropy may act to shape the patterns of standing genetic variation and evolutionary dynamics of conditionally expressed phenotypes among developmental pathways.

Body size

It appears likely that the general difference in body size between the two study populations is due to the isolation in evolutionary time of the two distributions of *P. aegeria* in Scandinavia (see Nordström, 1955). It can be expected that the south Swedish population that has recently colonized the southernmost parts of Sweden from the south will be characterized by life-history adaptations to a more southern climate (Roff, 1980; Mousseau & Roff, 1989). Among intraspecific changes in body size over a latitudinal gradient [e.g. 'Bergmann's clines' (Van Voorhies, 1996; Blanckenhorn & Demont, 2004; Nygren *et al.*, 2008) and 'Converse Bergmann's clines (Roff, 1980; Nygren *et al.*, 2008)], the pattern found here, a decrease in size at higher latitudes (Converse Bergmann's cline), appears to be the most common in ectotherms (Mousseau, 1997; Nygren *et al.*, 2008). This effect is typically explained by the latitudinal decrease in season length that would lead to the selection for a shorter development time and a smaller final size (Blanckenhorn & Demont, 2004). Our results support this idea. Another possible explanation for the observed difference in size between the two distributions is oviposition time limitation reducing the fecundity benefit of a larger size (Gotthard *et al.*, 2007). Under cooler thermal conditions, it can be expected that females experience greater limitations on the amount of time for egg-laying activities and limitations on thermal conditions for egg maturation that both are likely to result in realized fecundities being a product of temperature constraints and less so a product of female reproductive reserves (Gotthard *et al.*, 2007; Berger *et al.*, 2008; Gotthard & Berger, 2010).

In both populations, diapausing individuals typically grow larger than direct developing individuals (one block of females in the southern population showed a different pattern). This indicates that the observed size difference between the pathways is a general pattern for this species. The fact that this pattern is also present in the central Swedish population that hardly experiences any selection on the direct development pathway suggests that the size difference between pathways is at least partly due to some direct trade-off with the diapause/direct development decision itself. For example, entering and surviving diapause in the pupal stage entails particular physiological processes that may necessitate the acquisition of additional resources during larval growth compared to individuals that develop directly through the pupal stage (Danilevskii, 1965; Friberg *et al.*, 2011). The difference in pupal weight between the alternative pathways was somewhat larger in the central Swedish population, where there is relaxed selection on the direct pathway, than in the south Swedish population, where the direct pathway is subject to selection. This indicates that relaxed selection on the direct pathway in the central Swedish population may have allowed an accumulation of pathway-specific genetic effects that lead to lower pupal size in the directly developing individuals. In the south Swedish population, such pathway-specific genetic effects that entail a small size, and thus a lower fecundity of directly developing females, are likely to be constantly removed by natural selection (Gotthard *et al.*, 2007).

Development time and growth rate

Contrary to the results on pupal size, it is interesting to note that for larval development time and growth rate, the significant interactions between pathway and population are due to larger difference between pathways in the southern population than in the central population. This is a joint effect of a shorter larval development time during direct development and a longer development time of the diapause pathway in the south Swedish population as compared to the central Swedish population. It seems very likely that the short development time in the direct pathway in the southern population is due to the selection for a short generation time, allowing the offspring of the direct developers (i.e. the diapause generation) to reach the diapause stage prior to the onset of winter. That is, developing quickly through this generation may facilitate the survival of the subsequent diapausing generation that will face more stochastic variation in growth conditions that is typical towards the end of the favourable season. An alternative but not mutually exclusive hypothesis that has also been proposed is that differences in time stress between generations of temperate multivoltine populations might be connected to seasonal variation in predation risk in the juvenile stage (Teder *et al.*, 2010). Either way, the high growth rate of direct developing individuals in the south Swedish population suggests that selection in southern Sweden favours a short larval development time and a large body size which can only be achieved by a higher larval growth rate.

In contrast, when following the diapause pathway, the central population had considerably shorter larval development times and higher growth rates compared to the southern population. As both populations experience selection on this pathway, it seems likely this difference reflects the fact that the two populations interpret the

experimental photoperiod differently. The constant day length of 15-h light/dark used in the diapause pathway corresponds to approximately 3 to 5 days difference in the two populations (17/18 August in south Sweden and 21/22 August in central Sweden), indicating a somewhat longer time left before the onset of winter in the south Swedish population. This difference in the interpretation of time of season may be further increased due to latitudinal differences in season length (Roff, 1980; Mousseau & Roff, 1989; Blanckenhorn & Demont, 2004). It is known that *P. aegeria* may adjust developmental rate in response to such cues of seasonal progression (Nylin *et al.*, 1989). However, the difference in voltinism between these two populations makes it difficult to estimate the exact time available for each pathway. It is possible that the lack of a directly developing generation in central Sweden leads to less intense selection for an ability to interpret and regulate the development in relation to late season cues, compared to the situation further south.

Protandry

In contrast to expectations, we found no general increase in either phenotypic or genetic variance in growth rate in the directly developing individuals of the central population as compared to all other categories. The most divergent observation was the comparatively low degree of phenotypic variation seen in the south Swedish males under direct development. The ratio of female to male growth rate reflects the degree of protandry that is expressed in the larval stage. When comparing the degree of protandry across families, we found that direct developers of the central population showed a significantly larger degree of genetic variation compared to any of the other categories (Fig. 2). That this ratio is not greater than one in any of the diapausing cohorts can be expected because these individuals spend the entire winter in the pupal stage, and therefore, protandry in this pathway will, for the most part, only be influenced by differential pupal development in spring (Wiklund *et al.*, 1991; Larsdotter Mellström *et al.*, 2010). However, the high amount of variation in this trait in the directly developing individuals of the central population as compared to their southern conspecifics indicates that relaxed selection on this pathway has allowed the accumulation of genes that disrupt protandry. In line with this argument, the direct developers of the central Swedish population were also the only group that did not show a positive intersexual genetic correlation for growth rate. One may speculate that the regulation of this type of sexual fine-tuning within one developmental pathway requires some developmental mechanisms in addition to the regulation of growth between developmental pathways. This could render the regulation of protandry during direct development particularly sensitive to relaxed selection and accumulation of genetic variation.

Conditional expression and life-history evolution across developmental pathways

In line with previous work, the present study demonstrates that important life-history traits show a pathway-specific expression (Van Dyck & Wiklund, 2002; Karlsson & Wiklund, 2005; Karlsson & Johansson, 2008; Gotthard & Berger, 2010; Teder *et al.*, 2010). However, here we could also show that variation in the strength of selection on alternative developmental pathways may influence the pattern of differential expression. We found that relaxed selection on direct development was associated with a relatively larger decrease in pupal size (compared to diapause development), long developmental times and low growth rates. These are changes that would have been associated with a decrease in fitness in a bivoltine population that experiences time stress during direct development. We interpret these patterns as footprints of relaxed selection in the central population or, alternatively expressed, as footprints of ongoing selection in response to time stress in the southern population.

Theory predicts that pleiotropic effects can maintain function in traits under relaxed selection (Jones *et al.*, 2003; Turelli & Barton, 2004; Van Dyken & Wade, 2010). This suggests that that the outcome of relaxed selection may well be the result of the underlying genetic architecture of the focal traits. The principal prediction for our preliminary exploration of the genetic correlations for growth rate was to find positive correlations both between sexes and between pathways. This was also typically the case even if only a few of these correlations attained statistical significance (Fig. 3, Table 3). Interestingly, the correlation estimates that deviated most from this expectation involved directly developing individuals from the central population, which is the one category that is under relaxed selection. In particular, it appears that our estimates involving males of this category are mainly responsible for this deviant pattern. This is despite the fact that the sample of families from the southern population was considerably smaller and our power to detect genetic correlations should be lower there. This pattern is in line with our expectation of a weaker genetic correlation in the central population due to predicted mutation accumulation in genes specific to the pathway under relaxed selection. Reciprocally, this result suggests that at least in males ongoing selection for alternative developmental regulation in the southern population (underlying protandry in this case) may act to fix pathway-specific genes that allow differential phenotypic expression, and leave only genetic variation with pleiotropic effects across pathways to determine the genetic correlation.

Insect diapause is a classic example of switch-induced developmental plasticity that channels individuals towards substantially different selective conditions (Gotthard & Berger, 2010). It seems obvious that the capacity

to 'choose' between these two alternatives is primarily an adaptation for allowing insects to survive harsh conditions while still allowing the production of several generations during the favourable period of the year (Tauber *et al.*, 1986; Kivelä *et al.*, 2009). Nevertheless, once these two developmental pathways exist, it can be expected that natural selection will favour alternative developmental regulation in response to differences in selective conditions experienced by individuals following the two pathways (Moran, 1992; Gotthard & Berger, 2010; Teder *et al.*, 2010). In effect, when the pathway decision is made it contains information of potential differences in future selective conditions that is available for the developmental system. Our results suggest that ongoing selection for fast growth and protandry under direct development is necessary for upholding the expression of alternative phenotypes along these developmental pathways. A more general conclusion regarding these phenomena must, however, await further studies including additional populations that display the same natural variation in voltinism. Nevertheless, the present results show that the direct and diapause developmental pathways express different life-history phenotypes and that the research strategy of exploring footprints of relaxed selection on one of the pathways as analogous to a knock-out experiment (Lahti *et al.*, 2009) may be a way to examine the evolutionary dynamics of differential selection on alternative developmental pathways.

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