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Genetics of development time in a butterfly: predictions from optimality and a test by subspecies crossing

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SUMMARY

Earlier studies on adaptive plasticity in development time and diapause regulation in the speckled wood butterfly, *Pararge aegeria* L., have been based on optimality models and have not considered genetics. Nevertheless, they have been successful in predicting patterns observed. From the results of these studies we predicted the genetics of larval and pupal development time, as well as of diapause control, to be polygenic and sex linked. We show that this is the case by crossing a population of the northern subspecies *P. a. tircis* from southern Sweden, which shows a diapause in some daylengths and is protandrous, with a population of the southern subspecies *P. a. aegeria* from Madeira. The latter inhabits a much less seasonal environment, develops directly at all daylengths, and is not protandrous. Offspring showed variable and intermediate larval and pupal development times when reared at daylengths inducing diapause in Swedish pure stock. Female offspring were more similar to their mothers in the reciprocal crosses, whereas development time in male offspring was not sensitive to the direction of the cross. This suggests the presence of a sex-linked modifying factor. The results show that the outcome of tests of optimality models can be used to predict genetic systems.

1. INTRODUCTION

Insects in seasonal environments are convenient model systems for the study of plasticity, in particular because seasonal variation in environmental conditions is relatively predictable. Plastic responses to such variation should in principle be predictable as well, from mathematical or verbal optimality models, and the predictions can be tested in experiments using controlled environments (Nylin 1994). Such studies on butterfly life-history plasticity have been successful (Nylin *et al.* 1989, 1993; Wiklund *et al.* 1991, 1992; Nylin 1992), without any consideration of genetics. This is a special case of the 'conflict' between optimality and genetic approaches to the study of evolution (Roff 1990, 1992; Moore & Boake 1994). The optimality approach may not always work because genetic constraints may prevent populations from reaching the predicted optimum. Without consideration of genetics, this cannot be distinguished from a failure to use the relevant optimality model (Moore & Boake 1994). With an increasing degree of plasticity in a trait, it seems likely that the optimum will be reached more easily, and that the optimality approach will work. However, there is no guarantee that this will be true, even for plastic traits such as behaviour.

Roff (1990, 1992) and Moore & Boake (1994) argue convincingly that the genetic and optimality approaches are not conflicting, but complementary, approaches to evolutionary study. An increased knowledge of the genetic control of life-cycle 'decisions' and life-history plasticity in insects is desirable for several reasons. Plasticity and genetic differentiation are not separate phenomena, because genetic variation in plasticity (reaction norms) exists, and studying this variation is vital for the understanding of the evolution of plasticity and of life-history adaptation (Gupta & Lewontin 1982; Stearns 1989; Stearns *et al.* 1991; Via 1993). Plasticity and life-history theory should both gain from a study of genetic variation in life-history plasticity.

A fact which has received much less attention is that the converse may also be true. As stated above, the existence of life-history plasticity in a system means that an experimental approach is possible, where predictions are made on the basis of what would be the optimal or evolutionarily stable life history to 'choose' in each environment. Thus, predictions and tests are possible that are independent of a knowledge of the genetics of the system, in the sense that no genetic constraints on life-history plasticity are assumed beforehand. Can the study of genetics gain from the study of life-history plasticity? This may be the case if the results of experiments of the type just described can be used to predict the nature of genetic control in a system.

In the study reported here, we investigated the genetic control of life-cycle decisions and life-history plasticity in the speckled wood butterfly, *Pararge aegeria* L. We have previously reported that larval and pupal

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development time show a high degree of plasticity in response to photoperiod in this species (Nylin *et al.* 1989). The responses were at two levels, corresponding to variation between and within four major developmental pathways (life cycles), namely, direct development through both the larval and pupal stages, pupal winter diapause with or without a preceding larval summer diapause, or larval winter diapause. Of relevance to the present study is the observation that none of the three distinct classes of diapause were of the 'all-or-none' type. Rather, the mean development time of individuals increased or decreased progressively going from photoperiod to photoperiod in the range 22 h to 9 h light. Also, larval and pupal development time showed much individual variation at some photoperiods, probably corresponding to photoperiods giving ambiguous information about the date in the season or to photoperiods which induced different development times in individuals following different developmental pathways (Nylin *et al.* 1989; S. Nylin, K. Gotthard, P.-O. Wickman & C. Wiklund, unpublished results). From the observation of quantitative variation among individuals within photoperiods, we predict that the control of both larval and pupal development time in *P. aegeria* will prove to be polygenic (additive). This would probably also facilitate the evolution of quantitative plasticity among photoperiods seen in *P. aegeria*. If the genetic control of development time was of a more mendelian type, it seems likely that plasticity in this trait would also be of a more qualitative type. The genetics and evolution of reaction norms are, however, controversial (Scheiner 1993; Schlichting & Pigliucci 1993; Via 1993).

Furthermore, it can be predicted from considerations of optimality theory on protandry (the earlier seasonal entry of males into populations (Wiklund & Fagerström 1977; Iwasa *et al.* 1983)) that the sexes should differ in the propensity to enter diapause or direct development (Wiklund *et al.* 1992). A higher propensity for diapause should be seen in males at conditions where some, but not all, individuals enter diapause development, e.g. at photoperiods near the 'critical daylength' (defined as the daylength when 50% enter diapause development (Danilevskii 1965)). In brief, the rationale behind this prediction is that male individuals that occur late in the season and thus are not able to achieve protandry after direct development should gain by instead entering a diapause pathway and achieve protandry by breaking diapause early next season. The prediction was upheld in several species of butterflies, including *P. aegeria* (Wiklund *et al.* 1992). Consequently, we predict that the genetic control of diapause in *P. aegeria* should be sex linked.

In fact, the genetic control of plasticity in development time in general, as well as the differences in development time between populations of *P. aegeria*, should be sex linked. This prediction is based on two observations. Sexual differences in development time, related to protandry, are conditional. Males display shorter development time than females only in daylengths corresponding to field situations when protandry is advantageous (Nylin *et al.* 1993). Also shorter male development time (protandry) is seen in populations inhabiting strongly seasonal environments, but not in populations inhabiting less seasonal environments, such as the island of Madeira (Nylin *et al.* 1993).

To test the above predictions regarding the genetic control of diapause regulation and plasticity in development time in *P. aegeria*, we performed a cross between two extreme populations representing different subspecies. A population of the northern subspecies *P. a. tircis* from southern Sweden was crossed with a population of the southern subspecies *P. a. aegeria* from Madeira. The former population inhabits a strongly seasonal environment, is protandrous under direct development, and displays the complex pattern of diapause described above (Nylin *et al.* 1989, 1993). The latter population inhabits a much less seasonal environment, is not protandrous, shows no evidence of diapause, and in general shows very weak responses to photoperiod (Nylin *et al.* 1993; S. Nylin, K. Gotthard, P.-O. Wickman & C. Wiklund, unpublished results). Crosses between populations are useful not only because they may reveal genetic differences between populations, and explain the lower fitness of hybrids that may contribute to reproductive isolation, but also because they may reveal something of the genetic architecture within populations (cf. references in Tauber & Tauber (1981)).

2. MATERIALS AND METHODS

The stock of *P. aegeria* originated from ten gravid females caught in Ransvik, southern Sweden (56° N), and ten caught on Madeira (33° N). Four crosses were made between a male from Sweden and a female from Madeira (by placing them together in flight cages), and four of the reciprocal crosses were also made. Ten larvae from each of the eight crosses were reared until the adult stage in an environmental chamber at 17 °C and at a photoperiod of 14L:10D. Larvae were reared individually in plastic jars in which the host plant *Poa annua* was cultured in ample supply.

Since the Madeiran population develops directly through both the larval and pupal stages at all daylengths, it can function as a baseline when a population from a more seasonal area is crossed with it. A daylength of 14 h was chosen because pupal winter diapause peaks at this daylength in the Swedish population, and larval development times are also longer than under direct development (Nylin *et al.* 1989). Rearing the hybrid offspring at 14 h daylength should therefore reveal variation present in the Swedish population, and whether inheritance is additive (intermediate in hybrids) or mendelian (segregation in hybrids). Larval and pupal development time, as well as pupal mass, were noted for each individual. Individuals were sexed in the pupal stage. An F_2 generation was also reared, but systematic crossings could not be made because of asynchronous emergence of adults.

3. RESULTS

Of the offspring, 82% (33 out of 40) completed development and emerged from the pupa, i.e. hybrid offspring was viable. The results from the reciprocal crosses, concerning larval and pupal development time, are shown in figures 1 and 2, together with a representation of the normal range of variation at this daylength, in lines of the stock populations. Individuals of the Swedish population typically enter pupal winter diapause at this daylength, and this is preceded by

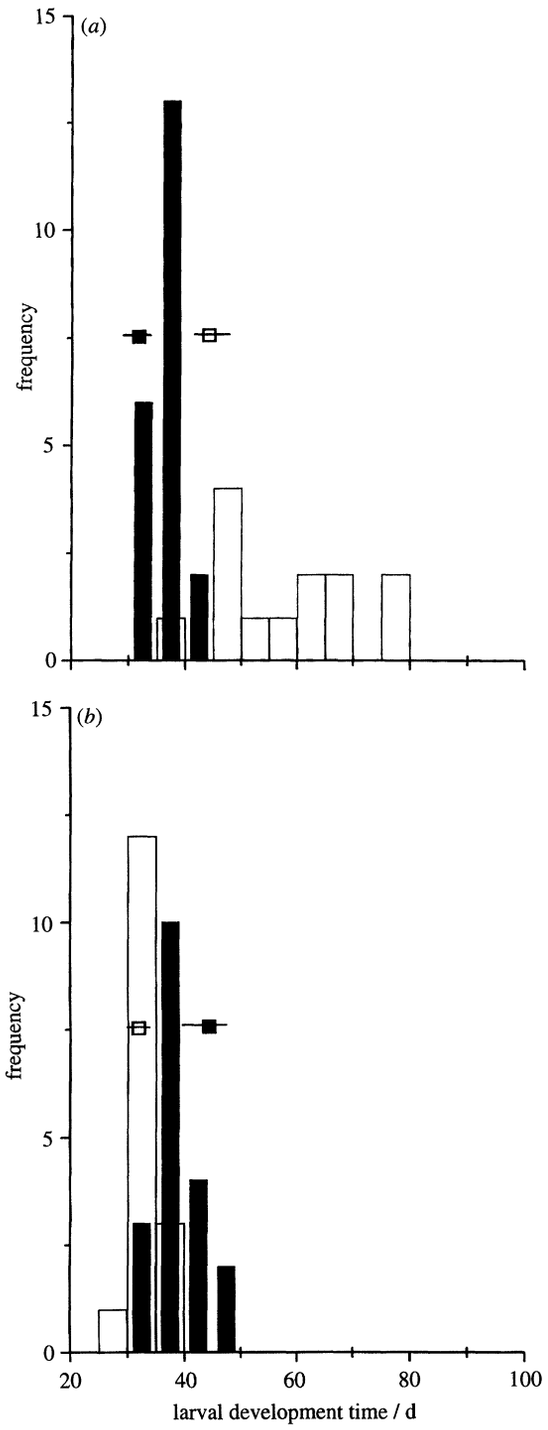

Figure 1. Larval development time at 14 h daylength of male (black, thin bars) and female (white, wide bars) offspring of crosses between (*a*) males of the Madeiran population and females of the Swedish population of *P. aegeria*, and (*b*) the reciprocal cross. Squares show mean larval development time (at this daylength) in the source populations for the sex involved in each cross (males filled, females open squares) with horizontal bars showing the range.

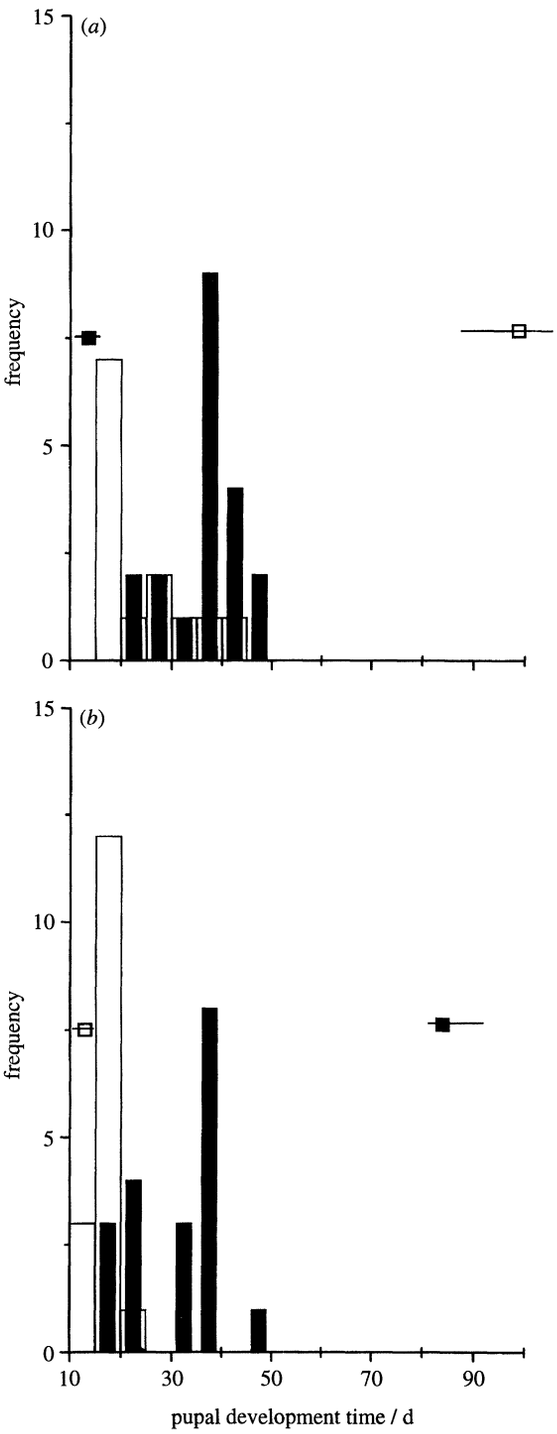

Figure 2. Pupal development time in offspring of crosses between (*a*) Madeiran males and Swedish females, and (*b*) Swedish males and Madeiran females. Otherwise as in figure 1.

relatively slow development in the larval stage. This can be seen from the long larval and pupal development times of both males and females, compared with Madeiran stock, in figures 1 and 2. There is no evidence of a diapause in the Madeiran population at any daylength (S. Nylin, K. Gotthard, P.-O. Wickman & C. Wiklund, unpublished results).

The results of the crosses show several interesting patterns. A polygenic control of development time is suggested by the result that hybrid development time was in most cases intermediate between that displayed by the source populations (figures 1 and 2). The hybrids were more variable in development time than either of the pure lines, probably because the polygenic system is broken down by hybridization as the coadapted genomes of the source populations are split apart and combined in new ways. In one case (figure 1*a*), larval development time of female hybrids was

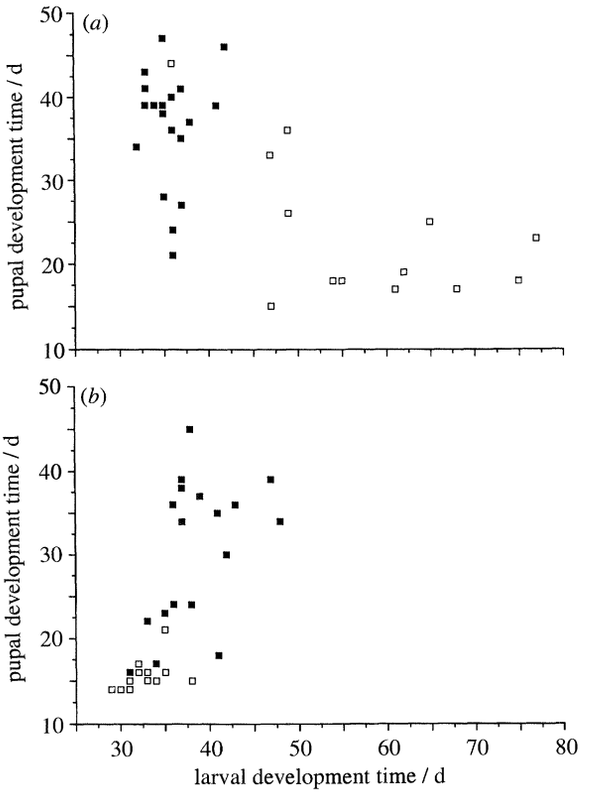

Figure 3. Pupal development time plotted against larval development time for each individual (males filled, females open squares). Offspring of crosses between (*a*) males of the Madeiran population and females of the Swedish population, and (*b*) the reciprocal cross.

very variable, and the mean duration of the larval stage far exceeded that of any of the source populations.

It is obvious from the differences between males and females in the crosses, and from the differences between the reciprocal crosses, that the genetic control of development time and diapause in *P. aegeria* is also linked to sex. When Madeiran males were crossed to Swedish females, mean larval development time was much higher in female than in male offspring, and also much more variable (figure 1*a*). In the reciprocal cross, males took longer to develop as larvae (figure 1*b*). However, these differences were almost entirely due to differences between the reciprocal crosses in the larval development of females, as is obvious from figure 3, where individual larval and pupal development times have been plotted against each other. In other words, female offspring displayed long larval development times when their mother was of the Swedish population, and short durations of the larval stage when their mother was of the Madeiran population.

Concerning pupal development time, the results of the reciprocal crosses were more similar (figure 2). In both crosses, females had shorter pupal durations than males, i.e. males showed a higher propensity to enter diapause development. Male hybrids of both crosses displayed variable development times somewhat intermediate between the direct development of the Madeiran source population and the pupal winter diapause of the Swedish source population (figures 2 and 3). Again, females differed more between crosses, and the female hybrids showed longer mean pupal development times when their mother was of the Swedish, diapausing, population (figures 2 and 3).

4. DISCUSSION

As could be predicted from previous studies on life-cycle regulation and life-history plasticity in *P. aegeria* (Nylin *et al.* 1989, 1993; Wiklund *et al.* 1992), the results of the present study suggest that the genetic regulation of development time in this species is polygenic, but also sex linked. We do not discriminate strongly between genetics of diapause regulation and genetics of plasticity in larval and pupal development time because diapause may occur in both of these two developmental stages, and previous results have suggested that diapause and plasticity in development time may not be qualitatively different phenomena in *P. aegeria* (Nylin *et al.* 1989).

The development times of females were more similar to that of their mother than to that of their father in the reciprocal crosses, including the case of pupal diapause in the mother's source population. Male hybrids did not differ much between crosses. Since the female is the heterogametic sex in Lepidoptera (we will refer to it as XY), these results suggest that both larval and pupal development time, and pupal diapause, are strongly modified by one or several genes on the Y chromosome. They would be carried over to all female offspring, but not to any male offspring (see Tauber & Tauber (1981) for references to other insect systems with sex-linked control of life cycles). This modifying effect of sex can be predicted from the observations of sexual differences in the propensity to diapause (Wiklund *et al.* 1992) and conditional protandry (shorter male development time in either the larval or the pupal stage, depending on developmental pathway (Nylin *et al.* 1993)). These observations, in turn, can be predicted from optimality models of the choice between pathways and the choice of development time and growth rate. It is not clear whether the sex-linked control of diapause and development time should be viewed as a 'pre-adaptation' of the genetic architecture that makes an optimal response possible, or as a consequence of sexual selection on these traits (Wiklund *et al.* 1992; Nylin *et al.* 1993). Sexual selection for protandry may have favoured the expression of modifying factors on the sex chromosomes.

The intermediate and variable development times displayed by hybrids may contribute to selection against them in the field, and towards maintaining the two subspecies, but this depends on whether the same result would be obtained with a crossing between less extreme populations. We saw no evidence for reproductive isolation in the form of mating barriers, since the pairs mated willingly and matings resulted in fertile offspring.

The results of the present study help demonstrate the complementary nature of the genetic and optimality approaches to the study of evolution. Knowledge of the genetic architecture of a system (degree and type of inheritance, and patterns of genetic correlation) can be used to predict responses to selection and deviations from an optimal response (see, for example, Roff

1990). Negative results (deviations from patterns predicted) of studies based on optimality models can suggest the presence of genetic constraints (lack of genetic variation, strong genetic correlations, and gene flow from other environments (Roff 1990; Moore & Boeke 1994)). In addition, however, positive results from tests of optimality models may be used to predict the genetic architecture, as another part of the effort to bridge the gap between the different approaches. The optimality approach is based on the (often implicit) assumption that there are no genetic constraints on selection in a given case, which is unlikely. However, the predictive abilities of the genetic approach rest on the equally unlikely assumption that genetic constraints (e.g. genetic correlations between sexes preventing sexual differences) do not change during selection. This is why the approaches are complementary. Researchers in the optimality tradition need to consider genetics. Conversely, it may prove fruitful to use more frequently the results of the optimality approach to answer a question which is important to both geneticists and ecologists: to what degree should features of genetic architectures be seen as starting points or even prerequisites for selection towards an optimal state, and to what degree should they themselves be seen as the results of selection towards optimality?

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