

Quantitative genetic variation in an island population of the speckled wood butterfly (*Pararge aegeria*)

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Evidence of changes in levels of genetic variation in the field is scarce. Theoretically, selection and a bottleneck may lead to the depletion of additive genetic variance (V_A) but not of nonadditive, dominance variance (V_D), although a bottleneck may converse V_D to V_A . Here we analyse quantitative genetic variation for the Speckled Wood butterfly *Pararge aegeria* on the island of Madeira about 120 generations after first colonisation. Colonisation of the island involved both a bottleneck and strong natural selection, changing the average value of traits. Several life history and morphological traits with varying levels of change since colonisation were analysed. In accordance with expectations, all traits except

one showed relatively low levels of V_A , with an average heritability (h^2) of 0.078. Levels of V_D for these traits were relatively high, 20–94% of total variance and on average 80% of V_G . The exception was a morphological trait that probably had not experienced strong natural selection after colonisation, for which a h^2 of 0.27 was found. Another interesting observation is that the population seems resistant to inbreeding effects, which may be the result of purging of deleterious alleles.

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Introduction

Genetic variation varies over traits, species and populations. Understanding the sources of this variation is one of the main areas of research in evolutionary genetics. Theoretically, variation in population size and natural selection are, apart from mutation, the most important factors that may change amounts of genetic variation. Quantitative genetic variance can be split into variance caused by the direct inheritance of genes from the parents (additive genetic variance, V_A) and variance caused by the specific combination of genes in the offspring (nonadditive variance, mostly dominance variance V_D) (Falconer, 1989; Lynch and Walsh, 1998). Consistent (natural) selection should result in the fixation of the most favourable genotype and consequent elimination of additive genetic variation (V_A) (Fisher, 1930; Bulmer, 1971; Roff, 1997). Selection, however, does not reduce V_D directly. Reduction in population size will also eliminate genotypes and hence reduce V_A (Roff, 1997). However, V_D may be converted to V_A after a severe bottleneck (Willis and Orr, 1993; Wang *et al*, 1998), thus increasing V_A after a bottleneck, although this depends on the type and number of genes involved in the nonadditive variance (Lopez-Fanjul *et al*, 2000; Naciri-Graven and Goudet, 2003).

In laboratory experiments, some evidence for these processes has been found. After selection for many generations, a decrease in response has been found frequently, but depletion of V_A is generally not the case (overview in Roff, 1997, Chapter 4.7). Bottleneck experiments have both resulted in reductions of V_A and increases in V_A at the expense of V_D , although the latter occurs less frequently (Bryant and Meffert, 1996; Whitlock and Fowler, 1999; Saccheri *et al*, 2001). Comparison of levels of V_A and V_D in life history traits, thought to be under consistent natural selection, with morphological traits points in the same direction. Heritabilities tend to be lower in life history traits than in morphological traits (Mousseau and Roff, 1987) although V_A is not depleted in life history traits (Mousseau and Roff, 1987; Houle, 1992). Moreover, life history traits tend to harbour relatively higher amounts of dominance variance relative to other traits (Crnokrak and Roff, 1995).

Information on these processes in the field and how large a role they play is scarce. Careful analysis in populations of the collared flycatcher (*Ficedula albicollis*) of genetic variation and the response to natural selection has shown in one case (body condition) that there was indeed a reduction in V_A after natural selection (Merila *et al*, 2001). However, in another case (tarsus length), no change in V_A could be demonstrated despite natural selection (Kruuk *et al*, 2001).

During colonisation of a new area by a species, genetic variation is expected to change. Both strong selection and an initial reduction in population size (a bottleneck) will generally occur. These processes would cause a reduction of V_A and a relative increase in V_D (see above). In

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this study, we test this prediction by examining levels of additive and dominance variation for life history and morphological traits in an island population of the Speckled Wood Butterfly *Pararge aegeria*. This island was relatively recently colonised (Owen *et al*, 1986) and life history theory and comparison with mainland populations indicate that selection must have played a substantial role after colonisation (Nylin *et al*, 1993).

Material and methods

Study species and population

The butterflies in this study were sampled from the Atlantic island of Madeira (Portugal, 33°N, 17°W). This island was relatively recently (1970s) colonised by *Pararge aegeria* (Owen *et al*, 1986). Despite close attention of lepidopterologists due to the presence of the unique, endemic *P. xiphia*, *P. aegeria* was never previously observed on Madeira before. Initially after colonisation only a few individuals were observed, but now *P. aegeria* is abundant, and more common than the endemic *P. xiphia*. Because of the mild climate *P. aegeria* can breed continuously on Madeira, unlike the rest of its range, and has approximately five (overlapping) generations per year. This suggests that the population at the time of study had gone through about 120 generations since colonisation. Although the exact source of Madeiran *P. aegeria* is as yet unknown, comparisons with mainland populations suggest that during and since colonisation the population has adapted to the nonseasonal climate, with considerable changes in life history (Nylin *et al*, 1993; Gotthard *et al*, 1994). Protandry (males emerging before females) has been lost, growth rate has decreased and starvation resistance has increased, and *P. aegeria* has thereby shifted in the direction of *P. xiphia*. These changes are in accordance with predictions from life history theory (Nylin *et al*, 1993). It is thus likely that natural selection has changed these traits.

Breeding

In all, 24 wild caught females were caught and allowed to oviposit on grass leaves in small containers at the end of October 1997. Females of the speckled wood butterfly have never been found to pair more than once (based on spermatophore counts), so their offspring can be considered full sibs (Wiklund and Forsberg, 1991). Upon emergence, the offspring were transferred to grass tufts standing in water. Individuals were raised solitarily at a constant temperature of 23°C and a day length of 14 h. In the second laboratory generation, offspring of 24 pairs of unrelated, first-generation individuals were raised. Each of these full sib families consisted of 12 individuals.

Traits

Two larval traits, three pupal traits and two adult traits were measured. Both life history traits and morphological traits were included. Two juvenile periods were measured: larval development time, days from egg laying until pupation and pupal development time, days from pupation until adult emergence. Both traits were log transformed prior to analysis to obtain normally distributed data. They have probably been under intense natural selection after colonisation because their means diverge from means in other populations (Nylin *et al*,

1993). Pupal weight, measured 2 days after pupation, probably has not been under such intense selection since it appears not to have changed much since colonisation. Larval growth rate was calculated as the quotient of pupal weight and larval time (assuming negligible variation in egg weight). Adult size was measured as the total area of hind wings in mm², determined with an image analyser. Details of measurement procedures can be found in Windig and Nylin (2000).

Two morphological traits measured were pupal spot and adult colour. The pupal spot was a small brown spot on the segment of the pupa that covers the fore leg. Its width was measured with a microscope fitted with a micrometer at a magnification of × 50 (details in Windig and Nylin, 2002). Its function is unknown, although it might help to disrupt the shape of the pupa, making it less easy to detect for predators. Natural selection on its width has probably been small or absent. Adult colour was measured as the average darkness of the base of the forewings. The darkness was determined with an image analyser on a grey scale from 0 (=black) to 255 (=white). The colour plays a role in thermoregulation and, because of that, also in mate-searching behaviour in males (Van Dyck and Matthysen, 1998). Pale males more often adopt territorial behaviour and dark males patrolling behaviour. Since climatic conditions are different on Madeira from anywhere else in the range of *P. aegeria*, some natural selection since colonisation seems likely.

Genetic analysis

Breeding produced a mixture of parents, partly being full sibs, and offspring consisting of full sibs and half cousins. A residual maximum likelihood (REML) analysis was used to analyse this data set. Such a procedure takes into account all relationships in the data set and, given an appropriate data set, can be used to estimate additive as well as dominance variance components (Lynch and Walsh, 1998). It proceeds by calculating a likelihood for a particular set of (co-)variances, and repeating the calculation in an iterative procedure until the maximum likelihood (ML) is found.

Analyses were carried out with the software program ASREML (Gilmour *et al*, 2000). Generation and sex were added as fixed effects to the model to eliminate their effects on genetic variation. Both additive and dominance variance components were estimated. For the estimation of the dominance variance components, the inverse of the matrix of dominance coefficients of relationship was computed and used as input for ASREML. These coefficients are easily derived from the coancestries of the parents of two individuals (Falconer 1989, equation (9.12)). Coancestries were computed following Meuwissen and Luo (1992). Analyses were also performed for sexes separately to determine if levels of genetic variation differ between the sexes. The relationship coefficients for the sex-specific analyses included relationships of the other sex so that they remain accurate.

All analyses were run unconstrained, so that negative variance components were possible. This is important because, due to sampling errors, negative variance components are expected. Calculating averages over traits of, for example, heritabilities with constrained variances may bias results if only downward error,

resulting in negative variance components, is eliminated. Significance testing of variance components is best performed by comparing the ML of an unconstrained analysis to the ML in an analysis where the variance component is constrained to 0 (Shaw, 1987; Lynch and Walsh, 1998). Significance tests were performed for V_A and V_D separately and for V_A and V_D combined. If V_A (V_D) is constrained separately, most of its variance will end up in V_D (V_A), since its covariance structure is more similar to it than the covariance structure of the residual variance. This test thus indicates whether a significant portion of the genetic variance is due to additive (dominance) effects. When both V_A and V_D are constrained at the same time, the analysis provides a test of whether a significant portion of the variance is genetic. Covariances and genetic correlations could not be reliably estimated for this data set due to the limited sample size.

Results

Phenotypic level

Average phenotypic values for traits are similar to those found in previous research on Madeiran *P. aegeria* (Nylin *et al*, 1993). For all traits, differences between males and females are significant (Table 1). Larval time was longer for females than for males, but pupal time was shorter in females. In a previous research (Nylin *et al*, 1993), this difference was not significant, probably due to smaller sample size in their study. Larval growth rate was higher in females. There is no significant difference between males and females in the total length of juvenile period (*t*-test -0.596 , $P = 0.551$), contrary to Spanish mainland butterflies (Nylin *et al*, 1993). Pupal weight is higher in females as is adult size. Females have a relatively smaller pupal spot and males have a darker adult colour.

Genetic level

Additive variance components did not differ significantly from 0, except for pupal spot (Table 2). Dominance variance components, on the other hand, were significantly different from 0 for two traits (larval time and adult colour), and approached significance for a third (pupal weight). For all traits, total genetic variance ($V_A + V_D$) was significantly different from 0 (Table 2). For all traits except PUPAL SPOT, V_D was larger than V_A .

Heritabilities (V_A/V_P) were rather low (average 0.078 and less than 0.115, except for pupal spot which was 0.272, Table 3). Dominance variances as a fraction of total

variance (V_D/V_P), on the other hand, are comparable to what is usually found. Values range from 0.101 (pupal spot) to 0.659 (adult colour) and are on average 0.313 (Table 3). V_D as a fraction of total genetic variation is rather high; values range from 0.699 to 1.162, except for pupal spot (0.271).

Table 2 Estimated variance components and their significance (P , in brackets) as estimated by REML analysis

	V_A	V_D	V_G	V_R
Larval time	-0.055 (ns)	0.608 (0.050)	0.553 (0.001)	0.871
Pupal time	0.071 (0.222)	0.197 (0.161)	0.268 (0.019)	0.702
Growth rate	-0.200 (ns)	2.384 (0.156)	2.184 (0.021)	6.390
Pupal weight	1.662 (0.239)	7.755 (0.061)	9.417 (***)	10.861
Adult size	0.394 (0.154)	0.914 (0.129)	1.308 (0.005)	2.119
Pupal spot	0.533 (0.019)	0.198 (0.301)	0.732 (0.004)	1.225
Adult colour	0.162 (0.451)	1.512 (0.046)	1.674 (***)	0.622

ns = not significant (negative variance component); *** $P < 0.001$. Bold signifies $P < 0.05$.

V_A = additive variance component, p indicates whether the full model is significantly different from a model without V_A , but including V_D . V_D = dominance variance component, P indicates whether the full model is significantly different from a model without V_D , but including V_A . V_G , P indicates significant difference between the full model and the model without V_A and V_D . V_R = residual variance component.

Table 3 Relative size of variance components estimated by REML analysis

	h^2 (V_A/V_P)	SE	V_D/V_P	SE	V_D/V_G
Larval time	-0.039	0.080	0.427	0.257	1.101
Male	0.035	0.370	0.527	0.444	0.938
Female	Not converged				
Pupal time	0.073	0.095	0.203	0.214	0.736
Male	Not converged				
Female	-0.081	0.168	0.580	0.427	1.162
Growth rate	-0.022	0.065	0.278	0.261	1.085
Male	Not converged				
Female	0.070	0.196	0.626	0.454	0.900
Pupal weight	0.082	0.115	0.382	0.247	0.823
Male	-0.009	0.167	0.723	0.458	1.012
Female	0.096	0.151	0.243	0.336	0.717
Adult size	0.115	0.119	0.267	0.234	0.699
Male	0.075	0.164	0.443	0.414	0.855
Female	0.096	0.190	0.349	0.309	0.785
Pupal spot	0.272	0.121	0.101	0.195	0.271
Male	0.298	0.222	0.285	0.486	0.489
Female	0.200	0.200	0.395	0.310	0.664
Adult colour	0.070	0.174	0.659	0.404	0.903
Male	0.036	0.257	0.936	0.641	0.963
Female	-0.006	0.176	0.379	0.506	1.016
Average	0.078		0.331		0.803
Male	0.086		0.582		0.851
Female	0.062		0.514		0.874

h^2 (V_A/V_P) = narrow sense heritability, V_D/V_P = dominance variance as fraction of total variance, V_D/V_G = dominance variance as a fraction of genetic variance.

Table 1 Average value for the traits measured, and the significance of the difference between males and females

	Male	Female	<i>t</i> -test	<i>P</i>
<i>N</i>	200	194		
Larval time (days)	23.97	25.07	-2.834	0.0048
Pupal time (days)	10.13	9.66	3.516	0.0005
Growth rate (g/day)	4.9	5.4	-4.980	****
Pupal weight (g)	1.166	1.343	-12.474	****
Adult size (cm ²)	6.58	7.09	-7.530	****
Pupal spot	7.24	5.11	19.720	****
Adult colour	68.1	73.8	9.643	****

**** $P < 0.0001$.

The analysis of the sexes separately does not change the overall picture. With the exception of pupal spot, h^2 's are low (<0.1), V_D/V_P 's are intermediate (>0.25) and V_D/V_G 's are high (>0.7). The largest difference between males and females is found between V_D/V_P for adult colour, which is 0.94 for males and 0.38 for females. Due to the relatively large standard errors, none of the differences are significantly different between the sexes. Moreover, not all analyses converged for both sexes. The average of V_D/V_P was somewhat higher for the sexes analysed separately than for the analysis of all butterflies together (0.58 and 0.51 *vs* 0.31).

Discussion

The island population of *P. aegeria* on Madeira shows low levels of V_A relative to levels of V_D . This is in accordance with predictions from theory and laboratory experiments that V_A , but not V_D , decreases after a bottleneck and strong selection. Unfortunately, no estimates of V_A and V_D in mainland populations of *P. aegeria* are available for direct comparison. Only in a Belgian population have h^2 's been estimated (Van Dyck *et al*, 1998). These are, however, broad sense heritabilities (h^2_{bs}) and cannot be compared directly with strict sense heritabilities (h^2_{ss}), as estimated in this paper. The minimum value for h^2_{ss} given a h^2_{bs} can be found assuming all variation is genetic ($h^2_{ss} + V_D/V_P = 1.00$). Given that $h^2_{bs} = h^2_{ss} + 1/2V_D/V_P$, the h^2_{ss} is then $2h^2_{bs} - 1.00$. In the Belgian population, a h^2_{bs} of 0.63 for adult colour and 0.51 for adult size was found. This implies that for both traits total genetic variation is smaller in Madeira than in the Belgian population. Moreover, for adult colour, h^2_{ss} is necessarily smaller in Madeira than in Belgium (minimum estimate in Belgium = 0.26 *vs* 0.07 for Madeira).

In other butterfly species, h^2_{bs} has been estimated for the same or similar traits as in this study. For example, for adult colour, h^2_{bs} 's have been estimated in *Bicyclus anynana* (Windig, 1994a), *Pieris occidentalis* (Kingsolver and Wiernasz, 1991), *Araschnia levana* (Windig and Lammar, 1999) and *Inachis io* (Windig, 1999). These estimates range from 0.599 to 0.885, all substantially higher than the h^2_{bs} of 0.40 found in this study and implying a larger h^2_{ss} . The same conclusion holds for larval time (estimates for the same species range from 0.511 to 0.671). For pupal time, growth rate and adult size, h^2_{bs} range from 0.303 to 0.525, all larger than those found in the Madeiran population, but whether h^2_{ss} is larger cannot be determined for these traits. Paulsen (1996) estimated h^2_{ss} for a number of traits related to ADULT SIZE in *Precis coenia* and *P. evarete*. She found an average h^2_{ss} of 0.349 and 0.440, respectively, both substantially larger than what is found in the Madeiran population (0.085). Thus, in other butterfly species, h^2_{bs} is clearly larger for all traits discussed above and h^2_{ss} is larger for at least some traits, but possibly for all traits. There is one exception to this pattern. In the peacock butterfly (*Inachis io*), the h^2_{bs} was estimated for pupal spot as 0.264 (Windig, 1998), somewhat smaller than the value of 0.322 found in this study. This exception may be explained, since it is likely that there has been no substantial natural selection on this trait.

Roff and co-workers have studied the overall pattern of relative amounts of V_A and V_D , as reported in literature. Both the average h^2 of 0.26 for life history

traits and of 0.46 for morphological traits (Mousseau and Roff, 1987) are larger than the average of 0.078 found in this study. The picture for V_D/V_P is more complicated. Average values of 0.31 for life history traits and 0.13 for morphological traits (Crnokrak and Roff, 1995) for V_D/V_P are smaller than the values found in this study if females and males are analysed separately. But when the sexes are analysed jointly, the average of 0.331 is comparable to the overall value for life history traits. Thus, the value of V_D/V_P seems to be elevated, but not as clearly as the h^2 . Average values in literature for V_D/V_G are 0.54 (life history traits) and 0.17 (morphological traits), while in this study an average value of 0.808 was found. Overall, the conclusion is that the relative amount of V_A in the Madeiran population is remarkably lower than what is usually found, while it is larger for V_D at least relative to V_G .

Nylin *et al* (1993) showed that Madeiran butterflies develop more slowly than Spanish butterflies at 17°. Gotthard *et al* (1994) showed that this coincides with a greater resistance to starvation, and proposed a physiological trade-off between growth rate and starvation resistance. Stronger resistance to starvation and a slower development are even more strongly accentuated in the Madeiran endemic *P. xiphia*. It thus seems likely that at least larval time and growth rate have been under strong natural selection since colonisation.

For the other traits, with the exception of pupal spot, we know that they are related to fitness, but it is not clear to what extent they have been under the influence of natural selection since colonisation. For example, adult females in Madeira are larger and colour is paler in females as in other populations of *P. aegeria* (Van Dyck and Matthysen, 1998), and indeed other satyrine butterflies such as *Bicyclus anynana* (Windig, 1994b). We know that adult colour plays an important role in thermo-regulation and that it is related to territorial behaviour in males in Belgium. Thus, it is likely that natural selection has played a role since colonisation, at least in males, but strong evidence for it is lacking.

V_A seems to have been reduced since colonisation, whereas V_D seems not. To what extent this difference is due to a bottleneck in population size and to what extent to natural selection after colonisation cannot be determined in this study. An increase in V_A due to conversion of V_D does not seem to have played an important role. Another possible effect of the bottleneck and subsequent selection may have been the purging of recessive deleterious alleles. In the laboratory, it was noted that there were no detectable effects of inbreeding (unpublished results). Full sib matings and several generations of strong selection did not affect the fitness of the descendants of the butterflies used in this study. This result is in contrast with the situation in the related butterfly *Bicyclus anynana*, where inbreeding effects are pronounced (Saccheri *et al*, 1996). Wang *et al*. (1999) have investigated under which situations purging of inbreeding depression might occur. They concluded that it would only be important during a rapid increase after a population bottleneck. Miller and Hedrick (2001) found evidence for such an effect in laboratory populations of *Drosophila*. The population of *P. aegeria* in this study has likewise experienced a rapid increase of population size after introduction of one or a few individuals (Owen *et al*, 1986). Consequently, this population may be another

example of purging of deleterious alleles in the field, as was found for a specific population of cattle in England (Visscher *et al*, 2001). Finding the original source of the Madeiran population and careful analysis of the genes lost since colonisation could help verify this interpretation.

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