doi:10.1111/j.1420-9101.2004.00855.x

The evolutionary genetics of egg size plasticity in a butterfly

M. J. STEIGENGA,* B. J. ZWAAN,† P. M. BRAKEFIELD† & K. FISCHER*

*Department of Animal Ecology I, University of Bayreuth, Bayreuth, Germany
†Institute of Biology, Leiden University, Leiden, the Netherlands

Keywords:

Bicyclus anynana;
genotype–environment interaction;
half-sib analysis;
offspring size;
phenotypic plasticity;
pupal mass;
temperature.

Abstract

The evolution of phenotypic plasticity requires that it is adaptive, genetically determined, and exhibits sufficient genetic variation. For the tropical butterfly *Bicyclus anynana* there is evidence that temperature-mediated plasticity in egg size is an adaptation to predictable seasonal change. Here we set out to investigate heritability in egg size and genetic variation in the plastic response to temperature in this species, using a half-sib breeding design. Egg size of individual females was first measured at a high temperature 4 days after eclosion. Females were then transferred to a low temperature and egg size was measured after acclimation periods of 6 and 12 days respectively. Overall, additive genetic variance explained only 3–11% of the total phenotypic variance, whereas maternal effects were more pronounced. Genotype–environment interactions and cross-environmental correlations of less than unity suggest that there is potential for short-term evolutionary change. Our findings strengthen the support for the adaptive nature of temperature-mediated plasticity in egg size.

Introduction

One of the central goals of evolutionary biology is to understand the processes by which organisms adapt to complex environments. To detect such processes, the causes and the effects of variation in traits that influence fitness need to be known. Two sources of phenotypic variation in life-history traits have long been recognized, namely genetic differentiation and effects of different environments on the expression of the phenotype (Schmalhausen, 1949; Endler, 1986). The latter source of variation, called phenotypic plasticity, may be merely a biochemical or physiological interaction of the organism with its environment, or it may be an adaptation to spatially heterogeneous or temporarily varying environments (Levins, 1963; Bradshaw, 1965). An adaptive hypothesis requires that phenotypic changes enhance the performance of an individual organism in the environment in which those changes were induced (Schmalhausen, 1949; Stearns, 1992). Recently, advances in evolutionary physiology have stimulated a renewed and increasing interest in the magnitude and nature of nongenetic effects on the expression of an organism's phenotype (e.g. Nylin & Gotthard, 1998; Feder *et al.*, 2000; Pigliucci, 2003).

As for any other biological feature, the evolution of phenotypic plasticity requires that (i) it is adaptive, (ii) it is at least in part genetically determined, and (iii) it exhibits sufficient standing genetic variation in the population concerned (cf. Ernande *et al.*, 2004). The adaptive nature of phenotypic plasticity is usually assessed by its fitness consequences in different environments. The genetic basis is evaluated by means of heritability estimates and the proportion of phenotypic variance explained by genetic factors. Genetic variation in phenotypic plasticity, finally, is explored by the genotype–environment interaction, through quantifying the variation in the plastic response to environmental change among genotypes (Scheiner & Lyman, 1991; Stearns, 1992; Falconer & Mackay, 1996). In summary, the study of phenotypic plasticity is quite a challenging enterprise, and consequently there are only a few studies testing experimentally for all three conditions in animals

Correspondence: Marc Steigenga, Department of Animal Ecology I, Bayreuth University, PO Box 101 251, D-95440 Bayreuth, Germany.
Tel.: +49 921 553079; fax: +49 921 552784;
e-mail: marc.steigenga@uni-bayreuth.de

(e.g. Czesak & Fox, 2003; Ernande *et al.*, 2004). Currently, the most serious lack in our understanding of phenotypic plasticity is experimental studies on its genetic basis (Scheiner & Lyman, 1991; Scheiner, 1993).

Here, we set out to test for the existence of genetic variation in the plastic response of egg size to temperature in the tropical butterfly *Bicyclus anynana* (Butler, 1879), employing a half-sib breeding design (e.g. Falconer & Mackay, 1996). For several reasons we believe that our study system is highly appropriate for studying the genetics of phenotypic plasticity. First, egg size is generally believed to be closely related to fitness (Fox & Czesak, 2000). Secondly, egg size is known to readily respond to differences in developmental and adult temperature in *B. anynana*, with lower temperatures causing larger egg sizes and vice versa (Fischer *et al.*, 2003a, b, c, 2004). This pattern is also observed in many other arthropods (e.g. Azevedo *et al.*, 1996; Blanckenhorn, 2000; Atkinson *et al.*, 2001). Thirdly, temperature is considered one of the most important mediators of phenotypic variation in ectothermic animals, resulting in not only predictable changes in egg size, but also in body size (temperature-size rule; e.g. Atkinson, 1994; Partridge & French, 1996; Chown & Gaston, 1999). Finally and perhaps most importantly, there is evidence that temperature-related egg size plasticity in *B. anynana* is adaptive (Fischer *et al.*, 2003a, c).

Recent studies show that *B. anynana* females kept at a lower oviposition temperature laid larger but fewer eggs than those kept at a higher temperature. Based on differential survival probabilities among temperatures, these data suggest that it does pay to produce fewer but larger offspring (with increased fitness) at a lower temperature, but more and smaller offspring at a higher temperature where offspring survival is generally high in this tropical butterfly (Fischer *et al.*, 2003a, c). Consequently, females should be able to maximize their reproductive success by adjusting the size of eggs to the temperature experienced during oviposition, which could provide a predictable cue for the environmental conditions experienced by the offspring in early life. These suppositions closely match environmental demands, as *B. anynana* lives in a seasonal environment with a beneficial wet season of high temperatures, and a rather adverse dry season when average temperature is low (Brakefield, 1997). Thus, those results can be readily interpreted within an ecologically realistic context.

If this scenario about the adaptive matching of variation in egg size with seasonal temperature environments holds, genetic variation in the plastic response to temperature for natural selection to act upon is expected (as long as the variation has not been completely eroded; Via & Lande, 1985; Scheiner, 1993). The existence of a genotype–environment interaction would be indicative of the potential for short-term evolutionary change, and thus that natural selection should be able to reshape the plastic response to temperature, for example in response to a changing relationship between temperature and ecological seasons because of climate change (see Roskam & Brakefield, 1999). However, an earlier study failed to find evidence of genotype–environment interaction when focussing on temperature environments during preadult development (Fischer *et al.*, 2004). Here, in contrast, we deal with plasticity in the adult stage that is probably more relevant to environments in the wild (Fischer *et al.*, 2003a, b, c, 2004), and we also employ an experimental design with more power.

Materials and methods

Study organism

Bicyclus anynana is a tropical, fruit-feeding butterfly with a distribution ranging from Southern Africa to Ethiopia (Larsen, 1991). The species exhibits striking phenotypic plasticity (two seasonal morphs) that is thought to function as an adaptation to alternative wet-dry seasonal environments and the associated changes in resting background and predation (Brakefield, 1997; Lyytinen *et al.*, 2004). A laboratory stock population of *B. anynana* was established at Leiden University in 1988 from about 80 gravid females caught at a single locality in Malawi. Several hundred adults are reared in each generation, maintaining high levels of heterozygosity (Saccheri & Bruford, 1993). For this study, butterflies from the stock population were used.

Experimental design

A half-sib breeding design was used to estimate additive genetic variation in egg size and to explore genetic variation in temperature reaction norms (Falconer & Mackay, 1996). All butterflies were reared in a climate room at 27 °C, high humidity, and a photoperiod of L12:D12. Parental generation larvae were fed on young maize plants in population cages (50 × 50 × 80 cm). The resulting pupae were collected from the plants and transferred to cylindrical hanging cages. Following adult eclosion, males and females were individually marked and kept separated by sex until mating. Mating trials started as soon as there were sufficient females (they eclose a few days later than males because of protandry; Zijlstra *et al.*, 2002; Fischer *et al.*, 2003a). To keep males in fresh condition for later matings, they were kept in another climate room at 20 °C (high humidity and L12:D12 throughout; male larval or adult temperature does not affect female egg size; Fischer *et al.*, 2003b).

For mating, equal numbers of males and females were put together in hanging cages for 2–3 h, where they were continuously observed. Mating pairs were transferred individually to 1-L plastic containers with a small egg-laying plant. After mating males (sires) were removed from the containers and were allowed to recover for 1 day, after which they were set up for mating again.

Sires that failed to mate in the first round were discarded, but unmated females were re-used. This lead to a total of 34 sires mated successfully to an average of 2.3 females (dams) (range 2–4), creating a total of 78 full-sib families. These numbers exclude all sires having mated with less than two females as well as all dams producing less than three female offspring. To create full-sib families, dams were allowed to oviposit individually for 1 week, after which the eggs were transferred to elongated, sleeve-like cages containing a young maize plant for further development. Afterwards, an additional group of eggs from each dam was collected to measure egg size (see below).

Female F_1 pupae were weighed on the day following pupation to the nearest 0.01 mg and afterwards kept individually until adult eclosion, while all male pupae were pooled in a single hanging cage. After adult eclosion, females were marked individually and kept separated from males for 1 day. Then, females were given 2 days for mating with random males, after which they were set up individually for egg laying in 1-L plastic containers with a small oviposition plant. Eggs were collected and measured the next day (i.e. on day 4 of adult life). These eggs were the first ones laid within the females' adult lifespan, thus effectively controlling for any confounding effects of female age (e.g. Karlsson & Wiklund, 1984; Brakefield *et al.*, 1994; Braby & Jones, 1995).

Having collected the initial eggs, individual females were transferred from 27 to 20 °C to induce a plastic response in egg size. Further eggs were collected and measured after acclimation periods of 6 and 12 days, respectively (i.e. on days 10 and 16 of adult life). In *B. anynana* a clear plastic response to temperature can be expected to occur within 6–10 days (Fischer *et al.*, 2003a, b, c). We have chosen to transfer females from high to low temperature (and not vice versa) because the plastic response appears to be more pronounced in the cold as compared with the warmth (Fischer *et al.*, 2003a, c), and more importantly because the pattern of reduced egg size at warmer temperatures is potentially confounded by effects of female age. As in most other arthropods, egg size normally declines with female age in *B. anynana* (Wiklund & Karlsson, 1984; Fox & Czesak, 2000; for *B. anynana* see Brakefield *et al.*, 1994; Fischer *et al.*, 2003a,b). In total, 545 females (involving on average seven female offspring per dam) laid eggs for analysis. Throughout all experiments, butterflies had access to moist banana (replaced every other day) for adult feeding.

Egg measurements

As the eggs of *B. anynana* are nearly perfect spheres, egg size was measured as cross-sectional area (mm^2) using a digital camera (Leica DC200; Leica Microsystems, Wetzlar, Germany) connected to a binocular microscope. The resulting images were analysed using Scion Image public software (Scion Corporation, 2000). Tight correlations between egg area (applying image analysis) and egg mass as well as hatchling size confirm that this method provides a highly reliable measurement of egg size in *B. anynana* (Fischer *et al.*, 2002). To calculate egg size for individual females, the mean of about 10 eggs was used. Previous experiments showed that the mean values did not change substantially above a critical minimum number of seven to eight eggs (data not shown).

Statistical analyses

In a first step, effects of sire, dam (nested within sire) and time (i.e. comparing the measurements from days 4, 10 and 16 of female adult life) were analysed. As there are three measurements per individual female, a repeated measurements analysis of variance (ANOVA) was used. In this analysis a significant time effect indicates a change in egg size over time, and interactions between sire respectively dam and time would indicate genotype–environment interactions (note that repeated measures ANOVAS do not support random factors; thus sire and dam are treated as fixed effects). Thereafter, we applied ANOVAS with sire and dam (nested within sire) as random factors separately to the different egg measurement days and to pupal mass, using estimated mean squares. Additive genetic variances (V_A) in egg size (separately for the different measurement days) and pupal mass were calculated from variance components using the restricted error maximum likelihood method (REML). Additive genetic variances were estimated according to Falconer & Mackay (1996) as $4V_s$ (V_s is the among sire component).

As in other studies, maternal effects variance (V_M) was calculated by assuming that dominance variance and epistatic interactions were zero (such that $V_M = V_d - V_A/4$, where V_d is the dam variance; Fox *et al.*, 1999; Czesak & Fox, 2003). This does not mean that dominance and epistatic interactions do not occur, but rather that these are included in the maternal effects variance, as a traditional half-sib design does not allow for a distinction between these sources of variation. Thus, these estimates set an upper limit to the maternal effects. Sire and dam component heritabilities were computed following Falconer & Mackay (1996) as $4V_s/V_p$ and $4V_d/V_p$ (V_p is the total phenotypic variance) respectively. Additionally, heritability of egg size was calculated by means of parent–offspring regression (i.e. by regressing the mean egg size of all full-sib sisters from the first measurement made at high temperature on day 4, weighted by the number of sisters in each full-sib family, on the mean egg size of the mother, also at high temperature).

To investigate the evolutionary potential of phenotypic plasticity in egg size we calculated genetic cross-environmental correlations of half-sib family mean values (Via & Lande, 1985; Via, 1993), using Pearson's product–moment correlation. As this estimate is potentially confounded by within-family variance in the (co)variance terms, we additionally applied a mixed-model ANOVA (Fry, 1992) as was done previously for this system (Wijngaarden *et al.*, 2002). In short, a mixed-model ANOVA (with half-sib family and temperature as random and fixed effects respectively) was used with the variance component for the family effect providing an estimate of the family covariance across temperatures. Additionally, the variance components of the half-sib family effect within each temperature were estimated. The REML method was used to estimate variance components. The cross-environment genetic correlation was then estimated as the family covariance across temperatures divided by the geometric mean family variance in each temperature environment. This method provides reliable and robust estimates (Windig, 1997). Confidence intervals for correlation coefficients were obtained using the *z*-transformation as described in Sokal & Rohlf (1995). Throughout, mean values are given ±1 standard error. All statistical tests were performed using JMP version 4.02 (SAS Institute, Cary, NC, USA).

Results

Egg size plasticity in relation to temperature

Egg size showed a clear plastic response to oviposition temperature, increasing after the transfer from 27 to 20 °C from initially 0.649 ± 0.002 mm^2 on day 4 to 0.665 ± 0.002 and 0.705 ± 0.002 mm^2 on days 10 and 16 of female adult life respectively. Those increases in area are equivalent to increases of 5.3% (day 10) and 15.4% (day 16) in egg volume. A repeated measurements ANOVA on days 4, 10, 16 confirmed significant effects of sire ($F_{23,388} = 2.1$, $P < 0.01$), dam (nested within sire; $F_{45,388} = 2.1$, $P < 0.0001$) and time ($F_{2,387} = 235.7$, $P < 0.0001$). The interaction between time and dam was also significant ($F_{90,774} = 1.7$, $P < 0.001$; Fig. 1a); however, the one between time and sire was marginally nonsignificant ($F_{46,774} = 1.3$, $P = 0.068$; Fig. 1b). By including all females measured (i.e. including those from groups with small sample sizes; see Materials and methods), the time–sire interaction reaches significance ($F_{66,854} = 1.6$, $P < 0.01$). These interactions suggest the existence of genetic variation in reaction norms. Cross-environmental genetic correlations were positive and significant, explaining 35–52% of variance (Table 1). However, the 95% confidence intervals consistently excluded 1, suggesting that the correlations differ significantly from unity. Both methods, product–moment correlations and mixed-model ANOVAs, gave comparable results with overlapping confidence intervals throughout (Table 1).

Heritability of egg size

In spite of the prevalence of a significant sire effect when the data from all three measurement days were jointly

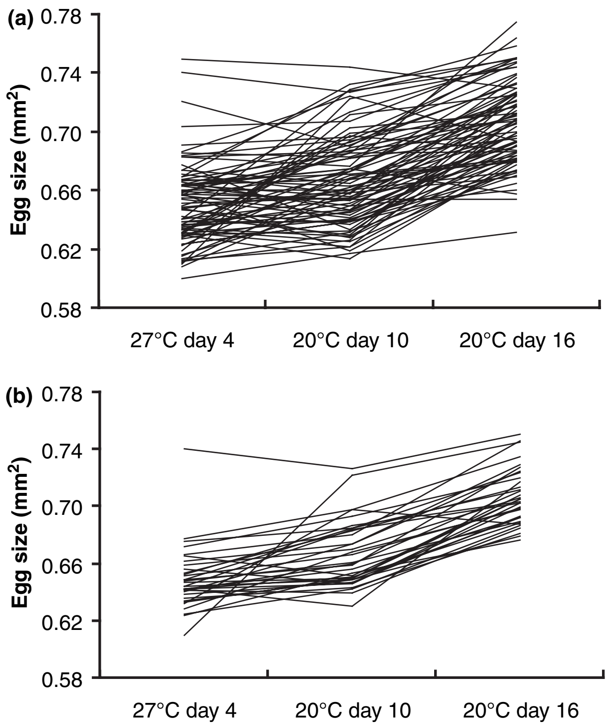

Fig. 1 Reaction norms for egg size in relation to adult temperature in the butterfly *Bicyclus anynana*. Each line represents the mean values of a full-sib (a) or half-sib family (b) respectively. Measurements took place on days 4, 10 and 16 of the females' adult lifespan. Females were transferred from 27 to 20 °C on day 4.

Table 1 Genetic cross-environmental correlations for egg size at different temperatures. Measurements took place on days 4, 10 and 16 of the females' adult lifespan. Females were transferred from 27 to 20 °C on day 4. The correlations between measurement on days 10 and 16 was included because females had only begun to respond to the temperature change by day 10. CI: 95% confidence interval. (a) Calculations based on product–moment correlations of half-sib family mean values weighted by the number of females. (b) Calculations based on mixed-model ANOVAS (see Materials and methods).

	r	CI	*t*	*P*	*n*
(a)					
Day 4 (27 °C)–Day 10 (20 °C)	0.676	0.608–0.744	21.08	<0.0001	573
Day 4 (27 °C)–Day 16 (20 °C)	0.591	0.516–0.666	17.56	<0.0001	522
Day 10 (20 °C)–Day 16 (20 °C)	0.687	0.616–0.758	21.66	<0.0001	515
(b)					
Day 4 (27 °C)–Day 10 (20 °C)	0.680	0.613–0.747			
Day 4 (27 °C)–Day 16 (20 °C)	0.654	0.585–0.722			
Day 10 (20 °C)–Day 16 (20 °C)	0.722	0.653–0.790			

analysed (see above), separate analyses for each of those days failed to reveal significant sire effects (Table 2). This difference results from the fact that in the latter analysis the dams-within-sire mean square is the denominator for the *F*-ratio of the sire effect. Overall, additive genetic effects on egg size were weak, as judged by the low estimates for additive genetic variance (V_A), explaining 3–11% of the total phenotypic variance (Tables 2 and 3). Dam effects, in contrast, were highly significant throughout and maternal effect variance (V_M) explained 10–14% of the total phenotypic variance (Table 2). Accordingly, the dam component heritability was relatively high (h^2: 0.45–0.62; Table 3). These results suggest the existence of either nonadditive genetic variances or rather strong effects of the female's environment, phenotype or genotype on egg size.

Because a female's egg size may covary with her body mass (for butterflies e.g. Garcia-Barros, 2000), genetic or maternal effects on egg size could be largely because of variation in body size. However, in our study egg size was only weakly related to pupal mass on egg measurement days 4 and 16, and not at all on day 10 (day 4: $r = 0.21$, $P < 0.0001$, $n = 600$; day 10: $r = 0.05$, n.s., $n = 576$; day 16: $r = 0.11$, $P < 0.01$, $n = 527$). Repeating all analyses presented in Tables 2 and 3, with the inclusion of pupal mass as a covariate to the model, does not change any of the results qualitatively (results not shown). Additive genetic variance was then estimated to explain 6–8% of the total phenotypic variance, with maternal effect variance accounting for 8–14%. The estimate for heritability of egg size derived from the parent–offspring regression was $h^2 = 0.20 \pm 0.04$ ($r = 0.20$, slope $= 0.10$, $P < 0.0001$, $n_{\text{families}} = 78$, $n_{\text{individuals}} = 517$).

Heritability of pupal mass

Pupal mass was significantly affected by both sire and dam effects (Table 2). Additive genetic variance explained 25% of the total phenotypic variance in pupal mass (Tables 2 and 3). Maternal effects variance, in contrast, explained very little of the phenotypic variation in pupal mass (Table 2). Thus, the mother's environment, phenotype or genotype did not substantially influence pupal mass. Consequently, sire and dam component heritabilities were rather similar and can be regarded as equally reliable, and therefore their combination based on the resemblance between full sibs may be taken as the best estimate ($h^2 = 0.27$; sire + dam in Table 3; see Falconer & Mackay, 1996).

Table 2 Nested analysis of variance (using estimated mean squares) and variance component analysis (using REML) for egg size (at different temperatures) and pupal mass in the butterfly *Bicyclus anynana*.

	Analysis of variance				Observational	Genetic	
Source	d.f.	MS ($\times10^{-3}$)	F	P	variance component ($\times10^{-4}$)	Variance component ($\times10^{-3}$)	Proportion of V_p explained ± SE (%)
Day 4 (27 °C)							
Sire	23	3.30	0.82	0.6944	$V_s = 0.178$	$V_A = 0.0712$	2.9 ± 11.1
Dam (sire)	45	4.08	1.89	0.0007	$V_d = 2.711$	$V_M = 0.253$	10.4 ± 2.0
Error	471	2.17			$V_e = 21.458$	$V_E = 2.11$	86.7
$r^2 = 0.23$					$V_p = 24.347$		
Day 10 (20 °C)							
Sire	23	5.21	1.12	0.3642	$V_s = 0.734$	$V_A = 0.294$	10.8 ± 16.0
Dam (sire)	45	4.68	2.10	0.0001	$V_d = 3.862$	$V_M = 0.313$	11.5 ± 2.1
Error	449	2.27			$V_e = 22.646$	$V_E = 2.12$	77.8
$r^2 = 0.28$					$V_p = 27.241$		
Day 16 (20 °C)							
Sire	23	3.28	0.68	0.8361	$V_s = 0.381$	$V_A = 0.152$	5.7 ± 14.6
Dam (sire)	45	4.83	2.18	<0.0001	$V_d = 4.159$	$V_M = 0.378$	14.2 ± 2.5
Error	403	8.94			$V_e = 22.121$	$V_E = 2.14$	80.3
$r^2 = 0.29$					$V_p = 26.661$		
Pupal mass							
Sire	23	166371	1.90	0.0318	$V_s = 340564.81$	$V_A = 136226$	25.1 ± 17.0
Dam (sire)	45	890247	1.96	0.0003	$V_d = 400050.84$	$V_M = 5949$	1.1 ± 0.5
Error	474	454400			$V_e = 4689420.40$	$V_E = 400829$	73.8
$r^2 = 0.25$					$V_p = 5430036.05$		

Egg measurements took place on days 4, 10 and 16 of the females' adult lifespan. Females were transferred from 27 to 20 °C on day 4.
REML, restricted error maximum likelihood; V_s, among-sire variance component; V_d, among-dam variance component; V_e, error variance; V_p, total phenotypic variance; V_A, additive genetic variance; V_M, maternal effects variance; V_E, environmental variance.

Table 3 Estimates for sire and dam component heritability as well as for the heritability of both components combined (±1SE) of egg size at different measurement days and pupal mass.

	Egg size			
	Day 4 (27 °C)	Day 10 (20 °C)	Day 16 (20 °C)	Pupal mass
Sire	0.029 (0.11)	0.108 (0.16)	0.057 (0.15)	0.251 (0.17)
Dam	0.112 (0.19)	0.567 (0.24)	0.624 (0.24)	0.291 (0.19)
Sire + dam	0.239 (0.02)	0.337 (0.20)	0.341 (0.19)	0.273 (0.18)

Discussion

Plasticity in egg size

In agreement with earlier studies using *B. anynana* as a model organism (Fischer *et al.*, 2003a, b, c, 2004), temperature clearly induced a plastic response in egg size. As all females were reared in a common environment, the differences found cannot be attributed either to temperature-mediated effects on body size or to physiology during development, but are a direct consequence of differences in the oviposition environment. Note that, in agreement with most other arthropods, butterfly egg size usually declines with female age (there is not a single exception known for Lepidoptera; Fox & Czesak, 2000), making the increase in egg size even more striking than is apparent from the values given above. Such temperature-related plasticity, resulting in larger offspring at lower temperatures, seems to be a near universal rule in ectotherms (Yampolski & Scheiner, 1996; Fox & Czesak, 2000; Atkinson *et al.*, 2003). The interactions between time (i.e. acclimation period) and sire and dam suggest the existence of genetic variation in the plastic response to temperature, and thus the potential for short-term evolutionary change. This genotype–environment interaction is graphically evident in crossing of the temperature reaction norms in Fig. 1. While most of the full-sib families follow the expected pattern of an increase in egg size with time, some families behave differently in showing virtually no response (between −2 and +2% change in egg volume between first and last egg measurement only, $n = 7$) or even, in a single case, a decrease in egg size (>−2%). However, it is important to note that the time–sire interaction is of borderline significance and caused by only a small proportion of the families implying only rather weak evidence for the existence of a genotype–environment interaction (but see also below).

A similar pattern was found in an earlier study, although in that case genotype–environment interactions were overall not significant (Fischer *et al.*, 2004). The lack of statistical support there was probably because of insufficient statistical power. In addition to the genetic variation in reaction norms, cross-environmental correlations, although being positive and significant, were less than unity suggesting that any constraints on evolutionary potential because of genetic correlations are unlikely to prevent evolution to new phenotypic values in different environments, although they may reduce the rate of such evolution (Beldade *et al.*, 2002; Zijlstra *et al.*, 2004). The correlations explain about 40–50% of the total variance, thus leaving scope for evolutionary change in different directions and strengthening the case for genetic variation in plasticity.

Heritability of egg size

In spite of a significant sire effect when the data from all three measurement days were jointly analysed using a repeated measurements ANOVA, separate analyses for each of these days failed to reveal significant sire effects. These results suggest that, overall, additive genetic effects on egg size were weak (estimates for V_A range between 3 and 11%). These results differ quite substantially from other estimates of egg size heritability in *B. anynana*. Parent–offspring regressions gave estimates of 0.20 (this study) and *ca* 0.4 (Fischer *et al.*, 2004), and a full-sib design showed a broad-sense heritability of *ca* 0.2 (Fischer *et al.*, 2004). Realized heritability, as revealed by selection experiments, was also about 0.4 (Fischer, Bot, Brakefield & Zwaan, in preparation). Thus, compared with those other results the current estimate for additive genetic variance based on half-sibs is rather low. Although we cannot explain this difference (note e.g. the rather low broad-sense heritability), some of the variation may arise from selecting on maternal effects and/or specific (rare) genotypes during selection experiments thus subsequently increasing realized heritability. In addition, the parent–offspring regressions include maternal effects, with the data based on the half-sib analysis giving the only unbiased estimate of additive genetic variance. At this stage, we conclude that heritability of egg size in *B. anynana* is low to moderate, as expected for a typical life-history trait (Roff, 1997).

Generally, there are surprisingly few data available on genetic variation in egg size within insect populations (Fox & Czesak, 2000). In two seed beetles, egg size was found to be highly heritable and estimates ranged between 0.22 and 0.91, varying between populations and host plants (Fox *et al.*, 1999). For one of those species recent data from a half-sib breeding design and a selection experiment confirm the earlier ones with estimates for additive genetic variance ranging between 0.36 and 0.67 (Czesak & Fox, 2003).

In contrast to sire effects, dam effects were highly significant throughout the present study, resulting in a relatively high dam component heritability (h^2: 0.45–0.62). In contrast, Czesak & Fox (2003) found that egg size in the seed beetle *Stator limbatus* was only marginally affected by maternal effects variance (h^2: 0.0–0.4). Our results suggest that either nonadditive genetic variance exists or that egg size is rather strongly affected by the female's environment, phenotype or genotype. The upper limit of maternal effect variance is estimated to explain 10–14% of the total phenotypic variance. The question of what pathways are involved in introducing such maternal effects on egg size remains unanswered, although we can largely exclude maternal environmental effects (see below). Thus, the most parsimonious explanation is that the maternal effects are due to female genotype. It is well known from other animals that even within single clones reared in a common environment, maternal identity effects can have a profound influence on offspring size (e.g. in *Daphnia*; Sakwinska, 2004), and also the data from parent–offspring regressions suggest that maternal effects are important (see above). An alternative explanation would be the occurrence of nonadditive genetic variance resulting in an overestimation of maternal effects variance.

Maternal environmental effects may evolve for cross-generational phenotypic plasticity, with mothers passing on their experience to the offspring to increase offspring fitness in predictable environments (Mousseau & Dingle, 1991; Rossiter, 1996; Fox & Mousseau, 1998; Mousseau & Fox, 1998). Although cross-generational maternal effects could potentially occur in *B. anynana* as this butterfly lives in a highly predictable environment with distinct seasons (Brakefield, 1997), such a mechanism is unlikely to account for our results as all butterflies were reared for many generations under identical conditions.

Alternatively, maternal environmental effects could be mediated through differences in the quantity or quality of resources available to the mothers, and by maternal size or maternal age. None of those potential causes are likely to explain our results. First, all animals were reared under similar conditions within a single climate cell and were provided greenhouse-reared maize plants in ample supply. If differences in food quality or quantity among full-sib families (e.g. due to chance effects), potentially affecting the amount of resources available to reproduction, had been important, then similar maternal effects on pupal mass would be expected (for example periods of starvation would cause a decrease of pupal mass for whole full-sib families). However, this was not observed (see below). Secondly, maternal size is generally a poor predictor of egg size in *B. anynana* (see above; Fischer *et al.*, 2002). Thirdly, egg measurements were standardized for maternal age, as all egg measurements took place on days 4, 10 and 16 of adult life.

Heritability of pupal mass

Pupal mass was affected by both sire and dam effects. Both sources of variation influenced pupal mass to a similar extent, as is indicated by comparable variance components and heritability estimates. Additive genetic variance explained 25% of the total phenotypic variance, well within the range found in other animals (e.g. 21–25%, Kause *et al.*, 1999; 25%, Ueno, 2003; for a review see Mousseau & Roff, 1987). In contrast the mother's environment or phenotype had little influence on pupal mass.

Conclusions

In summary, our results demonstrate that in the butterfly, *B. anynana*, egg size responds in a plastic manner to oviposition temperature, that egg size is heritable and that there seems to be genetic variation in the plastic response to temperature. These findings, together with earlier ones suggesting that temperature-mediated egg size plasticity in this particular species is adaptive (Fischer *et al.*, 2003a, c), suggest that the conditions necessary for the evolution of phenotypic plasticity to occur are fulfilled. It is particularly noteworthy that the supposed existence of genetic variation in temperature reaction norms challenges the widely held notion that temperature-mediated plasticity (in egg and body size) might be purely a physiological constraint (e.g. Van Voorhies, 1996; Van der Have & De Jong, 1996). This notion mainly arises because of a lack of evidence for the adaptive significance of such plasticity (Partridge & French, 1996; Ernsting & Isaaks, 1997; Chown & Gaston, 1999; Blanckenhorn, 2000; Fox & Czesak, 2000). A more comprehensive understanding of the adaptive significance of temperature-mediated plasticity as well as of genetic variation in temperature reaction norms is highly desirable.

Acknowledgments

We thank Casper Breuker for writing a macro to automate the egg measurements, Niels Wurzer and Mariël Lavrijzen for the supply of maize plants for the always-hungry caterpillars, and two anonymous reviewers for constructive criticism that helped to improve the quality of this manuscript. This study was supported by a grant from the German Research Council (DFG grant no. Fi 846/1–2 to KF).

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Received 16 September 2004; accepted 30 September 2004