Evolutionary Ecology Research, 2005, **7**: 203–217

Heat-induced maternal effects in *Drosophila mercatorum* and its evolutionary consequences

Cino Pertoldi,[1,2,3]* Anni Røgilds,[1] Ditte Holm Andersen[1,4] and Volker Loeschcke[1]

[1]*Department of Ecology and Genetics, Institute of Biological Sciences, University of Aarhus, Aarhus, Denmark,* [2]*Department of Wildlife Ecology and Biodiversity, National Environmental Research Institute, Rønde, Denmark,* [3]*Department of Applied Biology, Estación Biológica Doñana, CSIC, Seville, Spain and* [4]*Dipartimento di Biologia Evoluzionistica Sperimentale, Bologna, Italy*

ABSTRACT

The impact of temperature-induced maternal effects on offspring phenotypic variability (σ^2_p), developmental instability, the interaction between developmental instability and the environmental variability (σ^2_e), here called the environmental covariance effect (ECV), and the size of ten wing traits were investigated in a parthenogenetic strain of *Drosophila mercatorum*. The maternal flies were treated in water-baths at different high temperatures (25°C [control], 36°C, 37°C, 37.5°C and 38°C). The results showed an overall increase in offspring wing traits' σ^2_p and ECV with increasing maternal temperature up to 37°C. Above this temperature σ^2_p and ECV decreased, and in some cases dropped below the values found for the control temperature. Developmental instability and σ^2_e did not show a clear pattern with increasing temperature stress, even within the same trait. The mean size of the offspring wing traits increased with maternal temperature up to 37.5°C, where it reached a plateau. By using a parthenogenetic strain we were able to exclude any genetic contribution to the variation among individuals. The results show that the effect of temperature stress can be maternally transmitted and that it has an impact on offspring size and σ^2_p. This study is the first to provide a precise quantitative estimate of the maternal effect and its components (σ^2_p, developmental instability, ECV, σ^2_e and mean trait size). The evolutionary consequences of the changes on these components are discussed.

Keywords: developmental instability, environmental variability, maternal effect, phenotypic variability, thermal stress.

INTRODUCTION

Much effort has been expended to separate the impact of environmental from genetic components in the expression of a phenotype (see Mousseau and Fox, 1998a, and references therein). To distinguish transmitted environmental effects from inherited genetic effects is a great

* Address all correspondence to Cino Pertoldi, Department of Applied Biology, Estación Biológica Doñana, CSIC, Pabellón del Perú, Avda. Maria Luisa, s/n 41023 Seville, Spain. e-mail: cino@ebd.csic.es

challenge (Cheverud and Moore, 1994; Bernardo, 1996). It has been shown that a mother or father can influence the phenotype of its offspring by transmitted environmental effects, also termed maternal or paternal effects (Rossiter, 1996). Maternal effects appear to be ubiquitous across a diversity of taxa and are often responsible for a considerable proportion of the variance in many characters, especially those expressed early in development (Wolf *et al.*, 2000a). The importance of maternal effects is not questioned, with some experiments indicating such effects can account for as much as 50–80% of the offspring variation in clutch size, individual size, survival and behavioural differences (Cheverud and Moore, 1994; McAdam *et al.*, 2002; Pakkasmaa *et al.*, 2003).

Maternal effects may play an important role in conservation issues, but are often not included (Rossiter, 1996; Mousseau and Fox, 1998b). Small populations may not be able to respond to environmental changes in an evolutionary way, as they are mainly governed by a drift–mutation equilibrium and not by selective forces (Lynch, 1996). The incapacity of evolutionary adaptation means that if small populations encounter perturbations in their environment, they are left with few options. One strategy is to migrate, but if the landscape is fragmented, migration may not be possible. The only way to adapt to environmental changes is therefore by plastic responses or maternal transmission (Réale *et al.*, 2003). Hence, the future survival of populations with no or very little genetic variation may rely on maternal effects and/or phenotypic plasticity. The term 'plasticity' is here defined as the property of a genotype to produce different phenotypes, when exposed to different environments, without any maternal contribution from the parental generation.

The impact of the maternal environment on offspring phenotype depends on the nature of the contribution and the ecological context in which the offspring exist (Rossiter, 1996). When offspring resemble their mother, due to maternal effects, which results in a decreased phenotypic variability (σ_p^2) around the mean trait size of the mothers, the maternal effect is said to be positive. In cases where the offspring's phenotype is diverse and different from that of the mother (increased σ_p^2 around the mean trait size of the mother), it is called a negative maternal effect (Boonstra and Hochachka, 1997).

The advantage and disadvantage of a negative or positive maternal effect is determined by the variability of the environment in which the organism lives. A changing environment will in many cases produce stress on the organisms, which has been shown to affect the production of a specific phenotype under a given set of environmental conditions (Zakharov, 1992). Developmental instability results when developmental noise or stress affects the buffering capacity of the processes that provide stability (Palmer, 1996).

One commonly used estimator of developmental instability is σ_p^2, which can be analysed when variation caused by environmental variability (σ_e^2) and genetic variability (σ_g^2) is negligible (Lajus *et al.*, 2003). By the term σ_e^2 we refer to the environmental variation among distinct environments, which can alter σ_p^2 and the mean size of the trait measured. Several studies have revealed that the σ_p^2 of quantitative traits increases in populations experiencing environmental stress (Imasheva *et al.*, 1997; Loeschcke *et al.*, 1999; Kristensen *et al.*, 2003). The problem with estimating σ_p^2 even in a monoclonal strain ($\sigma_g^2 = 0$) is that the estimate in general will be strongly affected by σ_e^2 (Pertoldi *et al.*, 2001a; Kristensen *et al.*, 2003). To use σ_p^2 of a quantitative character as a measure of developmental instability (DI), there must be no σ_g^2, no σ_e^2 among individuals ($\sigma_p^2 = \mathrm{DI}$), and no interaction between DI and σ_e^2, called the environmental covariance effect (ECV), which can affect σ_p^2 and the mean trait size (Pertoldi *et al.*, 2001b). Otherwise, developmental instability and ECV will affect σ_p^2 (Pertoldi *et al.*, 2001b):

$$\sigma_p^2 = \mathrm{ECV} + \mathrm{DI}$$

The aim of this study was to determine whether maternal effects influence σ_p^2 in offspring whose mothers have been exposed to different temperature treatments and, if present, to quantify its effects. A cross-generational effect of temperature has been shown to influence thermal tolerance, the rate and timing of development, body size at maturity and the ability to produce persistent effects on the adult phenotype (Lau *et al.*, 2001). Surprisingly, despite the potential importance of temperature as a stressor, relatively few examples of temperature-induced maternal effects have been investigated in detail. One reason could be the difficulties associated with the identification and quantification of the different components, which determine the individual phenotypes and the selective forces acting on it (Pertoldi *et al.*, 2001b). The lack of genetic variability excludes the possible effects produced by selective pressures, additive and residual genetic variance (Wolf *et al.*, 2000b). Therefore, in this study we avoided such difficulties by using a parthenogenetic strain of *Drosophila mercatorum*. This species reproduces by pronuclear duplication and is totally homozygous ($\sigma_g^2 = 0$) (Templeton *et al.*, 1976).

As the strain of *Drosophila mercatorum* used reproduces by parthenogenesis, maternal effects can be studied in a very straightforward way. To measure maternal effects, one can rear mothers of one clone under various environmental conditions and measure traits of interest in their parthenogenetically produced offspring under identical environmental conditions. Any systematic differences among the offspring produced in this way must be due to the environmental factors acting on the maternal phenotype.

The exposure of maternal flies to different regimes of stress and σ_e^2 caused by exposure to a high stressful temperature may cause qualitative and quantitative differences in the maternal effects transmitted to the offspring. To estimate the maternal effects, we used the mean size of traits σ_p^2, DI, σ_e^2 and ECV. Estimates of these parameters were compared with estimates from progeny whose mothers had not been treated with high stressful temperatures (control group). Given that σ_e^2 and its interaction with developmental instability (ECV) can play an important role in the expression of σ_p^2 in clonal populations, we have to bear in mind that offspring can be affected both by the σ_e^2 experienced by the mother and by the σ_e^2 directly encountered by the offspring during development. As offspring were all kept under identical environmental conditions, it is assumed that σ_e^2 encountered by the offspring during development was of the same magnitude as the σ_e^2 encountered by the control group ($\sigma_e^2 = k$). Hence, differences in mean trait size, σ_p^2, DI, σ_e^2 and ECV among the offspring are ascribed to maternal effects.

MATERIALS AND METHODS

Experimental design

The experiment was conducted using a parthenogenetic strain of *Drosophila mercatorum*, originating from a single individual belonging to stock Iv-23-0-Im (Kramer and Templeton, 2001). The flies used in this experiment were kept at 25°C on instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC) using a 12:12 hour light:dark cycle. Two-day-old parental flies were transferred to 200 new vials, each containing 15 flies. The parental flies used for the experiment were all of the same age, as σ_p^2 in the progeny has been shown to increase with maternal age in *Drosophila* (Parsons, 1962; Røgilds *et al.*, in press). These flies were stressed in water-baths (40 vials for each temperature) at 36°C, 37°C, 37.5°C, 38°C or at a control temperature at 25°C (accuracy of temperature was ±0.1°C) for 30 min twice a day

(at the same times of day), 12 hours apart, for a total of 4 days. Before each stress exposure, flies were transferred to empty vials with moistened stoppers to avoid desiccation stress during heat exposure. One hour after stress exposure, the flies were returned to vials with medium. On day 6, one hour after the last water-bath treatment, the flies were transferred to vials for egg laying, with each vial containing 15 flies. The new vials ensured that all eggs were laid after the mothers had been exposed to the full stress period. Those vials in which some of the flies had died because they remained attached to the medium were discarded from further analysis. However, the number of discarded vials (16 vials) represented less than 8% of the total number of vials and this number was not correlated with the temperature treatments (three vials at 25°C, five vials at 36°C, four vials at 37°C, one vial at 37.5°C, three vials at 38°C), indicating that temperature treatment did not have an effect on the survival of the parental flies. A pilot experiment showed that the flies' survival began to become affected by temperature treatments of 39°C (A. Røgilds *et al.*, unpublished data). Adults began to emerge after about 14 days. In each vial and for all temperatures, the first 10 flies to emerge from each vial were used in the analysis. The number of vials used for the experiment was reduced to 25 for each temperature. The temperatures at which the eggs were treated neither affected development time nor the number of emerging flies in the vials, according to Kristensen *et al.* (2003).

Only flies that hatched from these vials within the first 3 days were used for phenotypic measurements. The reason for choosing early emerging flies only was that these will have experienced a low degree of chemical stress caused by waste products (ammonia, etc.) and crowding, compared with flies which hatched later. This is important because chemical stress may effect σ_p^2 (Lazebny *et al.*, 1996). Neither chemical stress nor the crowding effect are relevant for *Drosophila mercatorum*, as the hatching rate for this fly is very low (1–2%) (Templeton *et al.*, 1976). Furthermore, the results from a pilot experiment showed no significant difference among the number of larvae and the size of the offspring among the different temperature stress groups (A. Røgilds, unpublished data).

For each temperature, 250 flies (10 flies per vial) were collected and measured. Both the right (r) and the left (l) wing of each fly were removed and placed in a droplet of lactic acid on a microscope slide and covered with a cover slip. They were examined using a camera attached to a dissecting microscope. By using five landmarks, we measured ten traits on each wing (see Fig. 1), using the software package Object Image 1.62p (Vischer, 2000).

To quantify possible measurement errors, we chose a sub-sample of 30 parthenogenetic flies at random. For each of these flies, all the wing traits were measured twice. The second set of measurements was made within 24 h without reference to the first set. The within-individual coefficient of variation for each mean was then taken as an estimate of the measurement error, adding Haldane's (1955) correction for small sample size. Measurement errors were low for all the wing measurements, ranging from 0.08% to 0.2%, with a mean value of 0.12% (median = 0.14%). Therefore, all traits measured were included in our investigation.

Offspring σ_p^2, DI, ECV, σ_e^2 and mean trait size affected by maternal stress temperature

As a measure of σ_p^2 we use the variance of (r + l), $\sigma_p^2 = \sigma^2$ (r + l). The use of the homozygous strain of *Drosophila mercatorum* in this experiment made it possible to detect whether the environmental covariance effect (ECV) was present and also to quantify its effect on σ_p^2. A correlation among sides in clonal or total homozygous organisms can only be due to environmental factors. The ECV has been estimated to be equal to four times the covariance

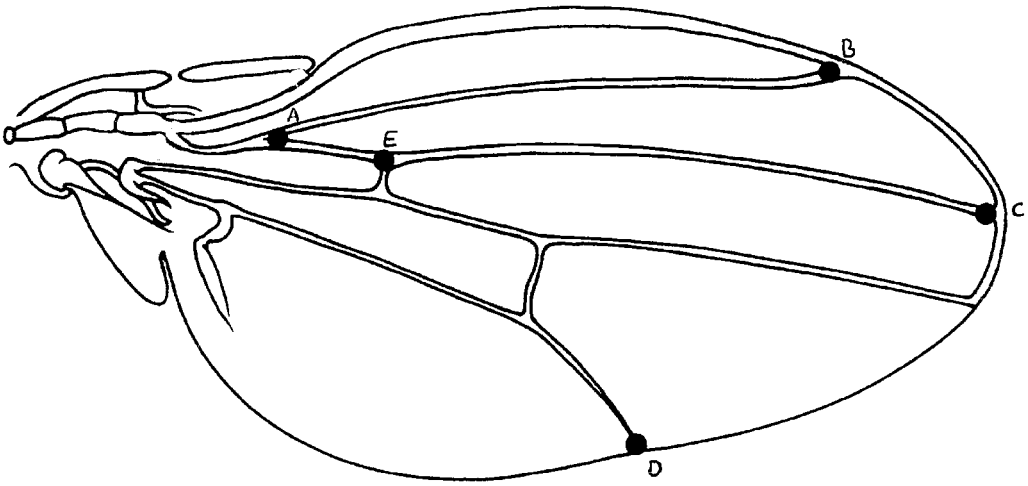

Fig. 1. Wing landmarks used for measurements of the ten wing traits. Position of landmark A: intersection between the second and third longitudinal vein. Position of landmark B: end of the second longitudinal vein. Position of landmark C: end of the third longitudinal vein. Position of landmark D: end of the fifth longitudinal vein. Position of landmark E: intersection between the anterior cross vein and the third longitudinal vein.

between the left and right side of a bilateral trait (4cov(r, l)) (for a description of the method used, see Pertoldi *et al.*, 2001b). Furthermore, an estimate of developmental instability (DI) unaffected by ECV has been calculated by subtracting 4cov(r, l) from σ_p^2:

$$DI = \sigma_p^2 - 4cov(r, l)$$

A Levene's test (1000 permutations) was performed to test for significant differences in σ_p^2 and DI between each maternal stress temperature group and the control group. For each temperature and each trait, the significance of the ECV was examined using a Pearson product–moment correlation test, correlating right and left wing trait size (Zar, 1999).

The correlation test was also used to establish if there was a correlation between the square root of DI and the mean trait size of the wing traits at the different temperatures. As ECV is the interaction of σ_e^2 and DI, we standardized ECV to obtain the estimate of σ_e^2:

$$\sigma_e^2 = ECV/DI$$

where ECV = 4cov(r, l) and

$$DI = \sigma_p^2 - 4cov(r, l)$$

Substituting

$$\sigma_e^2 = 4cov(r, l)/[\sigma_p^2 - 4cov(r, l)]$$

σ_e^2 for each temperature was quantified. To determine if these were significantly different from the σ_e^2 of the control temperature, a z-transformation of all correlation coefficients between the right and left of each trait for each temperature was performed (Zar, 1999). The

z-values of the control temperature and the other temperatures were tested for differences with an unpaired *t*-test, the null hypothesis being that the *z*-transformed correlation coefficients should not be significantly different from the *z*-transformed correlation coefficients of the control temperature.

A sequential Bonferroni test (Rice, 1989) was applied to avoid significant results arising as a consequence of a large number of related tests. Following Miller's (1981) suggestion, we made a separate probability statement for each trait, group and test.

To test for significant changes in mean length between the offspring from different maternal temperature treatments, we performed a one-way analysis of variance. Pairwise comparisons were made using Scheffé's *F*-test (Zar, 1999).

RESULTS

Levene's test revealed significant differences in σ_p^2 (37.5% of the tests showed significant differences), DI (55%), ECV (27.5%) and σ_e^2 (37.5%) between the offspring from mothers exposed to the high stressful temperatures and those from the control group (Table 1). Phenotypic variability (σ_p^2), ECV and σ_e^2 showed the same general pattern, even though this was not significant in all cases. This pattern consisted in the highest values for 37°C and a decrease towards higher and lower temperatures (including control) (Table 1), with the exception of traits AE and BD, which showed the highest σ_e^2 at 37.5°C, and trait CE, which showed the highest σ_e^2 at 36°C (Table 1). Some wing traits appeared to be relatively unaffected by the maternal effect, with no significant differences in σ_p^2 or ECV when compared with the control group (see Table 1).

Developmental instability did not show a clear pattern with increasing temperature. However, at the higher temperatures (37.5°C and 38°C), there was a tendency towards smaller DI when compared with the control group, even though this was not significant in all cases (see Table 1).

Significant differences in mean length were found for all ten wing traits among temperatures (ANOVA: $23.792 \leq F_{4,1245} \leq 514.513$) (Table 2). Pairwise Scheffé's tests revealed several significant differences between the different temperatures for all traits (Table 2). In all cases, the trait sizes became larger in flies exposed to a higher maternal stress temperature, with a plateau being reached for most traits at 37.5°C (Table 2). All the correlations between the square-root of DI and the mean trait size were negative with the exception of trait CE ($0.23 \leq R \leq 0.94$, $0.018 \leq P \leq 0.68$) (see Table 1).

DISCUSSION

This experiment allowed the estimation of maternal effects without biases caused by genetic variation. The results provide evidence that there is transfer of a non-genetic effect across generations. Furthermore, we have shown differences in the response of phenotypic variability (σ_p^2) among the offspring from the maternal control and heat-stressed groups. This is due to the complex interaction between environmental variability (σ_e^2) and developmental instability (DI).

The increased σ_p^2 at 36°C and 37°C, compared with the control group, seems to be due to an increased environmental susceptibility of the offspring whose mothers have experienced higher stress. An increase in maternal stress temperature (36°C or 37°C) did not lead to an increase in developmental instability. Instead, there was an overall tendency for a decrease

or no change in developmental instability with increasing temperatures compared with the control group. This means that σ_e^2 played a major role in the environmental covariance effect (ECV), whereas the observed decrease in σ_p^2 at 37.5°C and 38°C seems to be mainly due to the decrease in developmental instability, which counteracted the effect produced by the general tendency for an increase in σ_e^2.

There was clear evidence for an increase in offspring wing trait size with higher maternal stress temperature. The flies displayed an increasing negative maternal effect with increasing temperature treatment of the mothers. The maternal effect passed on from mothers that were stressed at increasing temperatures could have led to the observed increase in wing size in the offspring as a result of the desiccation stress experienced by the mothers.

The significant reduction in developmental instability and the simultaneous increase in size of the wing traits with increasing temperature stress could lead us to conclude that the offspring have increased their fitness in comparison with the parental generation, and that this fitness increase is the greater the higher the temperature stress experienced by the mothers. The relatively high correlation coefficients (R) found for the negative relationship between the square-root of DI with the mean trait size could therefore support the hypothesis that developmental instability is negatively correlated with fitness (Clarke, 1995).

There is some evidence that large wing size may increase *Drosophila* fitness at low temperatures (McCabe and Partridge, 1997; Reeve *et al.*, 2000). However, 'bigger is not necessarily always better', meaning that environmental influences are capable of altering the associations between size and fitness (Bochdanovits and De Jong, 2003).

Thorax and wing lengths show both a genetic and phenotypic positive correlation (e.g. Robertson and Reeve, 1952; Wilkinson *et al.*, 1990); therefore, we assume that the observed increase in wing size with increasing temperature treatment is also accompanied by an increase in body size. In *Drosophila*, a number of associations of size with adult fitness components has typically been described at only one, rather optimal, temperature (but see Zamudio *et al.*, 1995). There is often a positive correlation between body size and longevity in natural populations of insects (e.g. Aspi and Hoikkala, 1995; Norry and Colombo, 1999; Rodriguez *et al.*, 1999, and references therein). However, the physiological properties responsible for such trait associations might respond to temperature in different and complex ways (Partridge and French, 1996), suggesting that the evolution of body size in *Drosophila* is constrained by a trade-off between adult reproductive fitness and the fitness cost of increasing larval growth rates (Partridge and Fowler, 1993). Neither body size nor wing size is a fitness component, but increased size often appears to confer an important advantage for fecundity and survival in adult *Drosophila* (for references, see Partridge and Fowler, 1993). Both longevity and body size vary with temperature (e.g. Partridge *et al.*, 1995; Nunney and Cheung, 1997).

Additionally, the heterogeneity of the R-values indicates that the relationship between developmental instability and fitness, if it exists, is trait specific. The increase in the size of wings with higher temperatures could be due to a greater amount of resources that the mothers allocate to their eggs when experiencing stress. The general lack of a linear relationship between developmental instability and stress could be due to the varying strength of phenotypic selection, which is expected to be more intensive at the highest levels of stress (Kristensen *et al.*, 2003). This tendency has been observed, as developmental instability was significantly reduced at 37.5°C and 38°C in several traits. Therefore, we suspect that phenotypic selection acts primarily on groups stressed at temperatures higher than 37°C. As shown by Campbell *et al.* (1998), this may cause selective mortality of the most developmentally unstable individuals during development.

Table 1. Phenotypic variability: $\sigma_p^2 = \sigma^2 (r + 1)$, environmental covariance effect (ECV), developmental instability (DI) and environmental variability (σ_e^2) values are shown for offspring from each temperature group for ten wing traits

	Temp. (°C)	$\sigma_p^2 = \sigma^2 (r + 1)$	*P*	ECV (4cov)	*P*	DI ($\sigma_p^2 - 4cov$)	*P*	σ_e^2 (ECV/DI)	*P*
Wing AB	25.0	0.058		0.044		0.014		3.143	
	36.0	0.073	N.S.	0.060	N.S.	0.013	N.S.	4.615	(+)***
	37.0	0.103	(+)***	0.092	(+)**	0.011	N.S.	8.364	(+)***
	37.5	0.060	N.S.	0.052	N.S.	0.008	(−)***	6.5	(+)***
	38.0	0.041	(−)*	0.032	(−)**	0.009	(−)**	3.556	(+)*
Wing AC	25.0	0.073		0.064		0.009		7.111	
	36.0	0.082	N.S.	0.072	N.S.	0.010	N.S.	7.2	N.S.
	37.0	0.124	(+)***	0.116	(+)**	0.008	N.S.	14.5	(+)***
	37.5	0.071	N.S.	0.064	N.S.	0.007	N.S.	9.143	(+)**
	38.0	0.046	(−)**	0.040	(−)***	0.006	(−)**	6.667	N.S.
Wing AD	25.0	0.042		0.028		0.014		2.0	
	36.0	0.042	N.S.	0.032	N.S.	0.010	N.S.	3.2	(+)***
	37.0	0.061	(+)**	0.052	N.S.	0.009	(−)**	5.778	(+)***
	37.5	0.032	N.S.	0.024	N.S.	0.008	(−)***	3.0	(+)***
	38.0	0.024	(−)***	0.016	(−)***	0.008	(−)***	2.0	N.S.
Wing AE	25.0	0.010		0.004		0.006		0.667	
	36.0	0.009	N.S.	0.004	N.S.	0.005	N.S.	0.8	(+)*
	37.0	0.011	N.S.	0.004	N.S.	0.007	N.S.	0.571	N.S.
	37.5	0.008	N.S.	0.004	N.S.	0.004	(−)*	1.0	(+)**
	38.0	0.007	N.S.	0.002	(−)**	0.005	N.S.	0.4	(−)*
Wing BC	25.0	0.007		0.004		0.003		1.333	
	36.0	0.009	N.S.	0.004	N.S.	0.005	(+)***	0.8	(−)**
	37.0	0.010	N.S.	0.008	N.S.	0.002	(−)*	4.0	(+)***
	37.5	0.006	N.S.	0.004	N.S.	0.002	(−)*	2.0	(+)***
	38.0	0.006	N.S.	0.004	N.S.	0.002	(−)*	2.0	(+)***

Wing BD	25.0	0.037		0.028		0.009		3.111	
	36.0	0.027	N.S.	0.020	N.S.	0.007	N.S.	2.857	(−)*
	37.0	0.037	N.S.	0.028	N.S.	0.009	N.S.	3.111	N.S.
	37.5	0.021	(−)***	0.016	(−)***	0.005	(−)***	3.2	N.S.
	38.0	0.025	(−)**	0.016	N.S.	0.009	N.S.	1.778	(−)***
Wing BE	25.0	0.037		0.032		0.005		6.4	
	36.0	0.054	(+)**	0.044	N.S.	0.010	(+)**	4.4	(−)***
	37.0	0.066	(+)***	0.060	(+)***	0.006	N.S.	10.0	(+)***
	37.5	0.041	N.S.	0.036	N.S.	0.005	N.S.	7.2	(+)**
	38.0	0.028	N.S.	0.024	N.S.	0.004	N.S.	6.0	(−)*
Wing CD	25.0	0.035		0.024		0.011		2.182	
	36.0	0.026	N.S.	0.020	N.S.	0.006	(−)***	3.333	(+)***
	37.0	0.035	N.S.	0.028	N.S.	0.007	(−)**	4.0	(+)***
	37.5	0.023	(−)**	0.016	N.S.	0.007	(−)**	2.286	(+)*
	38.0	0.023	(−)**	0.012	N.S.	0.011	N.S.	1.091	(−)***
Wing CE	25.0	0.049		0.044		0.005		8.8	
	36.0	0.060	N.S.	0.056	N.S.	0.004	N.S.	14.0	(+)***
	37.0	0.084	(+)***	0.076	(+)***	0.008	(+)***	9.5	(+)*
	37.5	0.051	N.S.	0.044	N.S.	0.007	N.S.	6.286	(−)*
	38.0	0.033	(−)**	0.028	(−)**	0.005	N.S.	5.6	(−)**
Wing DE	25.0	0.028		0.020		0.008		2.5	
	36.0	0.029	N.S.	0.024	N.S.	0.005	(−)***	4.8	(+)***
	37.0	0.037	N.S.	0.032	N.S.	0.005	(−)***	6.4	(+)***
	37.5	0.020	N.S.	0.016	N.S.	0.004	(−)***	4.0	(+)**
	38.0	0.015	(−)***	0.012	(−)**	0.003	(−)***	4.0	(+)**

Note: For each trait, the σ_p^2 and DI of the different temperature groups are compared (Levene's test) with the σ_p^2 and DI of the control temperature. The significance of ECV was tested by a Pearson product–moment correlation test, correlating right and left wing trait size. The σ_e^2 for each temperature was tested if it was significantly different from the σ_e^2 of the control temperature by z-transforming all the correlation coefficients between right and left of each trait for each temperature. The z-values were tested for differences using an unpaired t-test. A sequential Bonferroni analysis (following Miller, 1981) ($k = 4$) was performed to correct for the large number of tests.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2. Results (mean ± standard error) of one-way ANOVA for comparing the mean length of wing traits, among the control, 36°C, 37°C, 37.5°C and 38°C temperature treatments, and Scheffé's *F*-test for multiple comparisons

Traits	Pearson *R*	*P*	Control (25°C)	36°C	37°C	37.5°C	38°C	Source of variation	d.f.	MS	ANOVA (*F*-value)	*P*	Scheffé's *F*-test
AB	[0.938]	(–)*	5.66 ± 0.008	5.794 ± 0.009	5.935 ± 0.01	6.002 ± 0.008	6.016 ± 0.007	Between	4	6.514	325.269	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.02			
AC	[0.839]	(–), N.S.	6.856 ± 0.009	7.004 ± 0.01	7.167 ± 0.011	7.263 ± 0.009	7.28 ± 0.007	Between	4	9.534	421.652	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.023			
AD	[0.974]	(–)*	4.564 ± 0.007	4.677 ± 0.007	4.789 ± 0.008	4.833 ± 0.006	4.834 ± 0.006	Between	4	4.082	317.024	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.013			
AE	[0.256]	(–), N.S.	1.22 ± 0.06	1.233 ± 0.004	1.251 ± 0.004	1.253 ± 0.004	1.258 ± 0.004	Between	4	0.093	23.792	***	(25 < 37)***, (25 < 38)***, (36 < 37)*, (36 < 38)***, (37.5 < 38)**
								Within	1245	0.004			
BC	[0.676]	(–), N.S.	1.729 ± 0.003	1.758 ± 0.004	1.793 ± 0.004	1.822 ± 0.003	1.83 ± 0.003	Between	4	0.606	219.201	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.003			

BD	[0.372]	(–), N.S.	3.738 ± 0.007	3.861 ± 0.006	3.949 ± 0.007	3.991 ± 0.005	3.974 ± 0.006	Between	4	2.622	306.318	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***
								Within	1245	0.009			
BE	[0.370]	(–), N.S.	4.5 ± 0.007	4.622 ± 0.008	4.743 ± 0.008	4.827 ± 0.007	4.816 ± 0.006	Between	4	5.391	404.266	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.013			
CD	[0.237]	(–), N.S.	3.669 ± 0.007	3.774 ± 0.006	3.859 ± 0.006	3.918 ± 0.006	3.906 ± 0.006	Between	4	2.556	295.655	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.009			
CE	[0.486]	(+), N.S.	5.64 ± 0.007	5.776 ± 0.008	5.92 ± 0.009	6.032 ± 0.008	6.027 ± 0.006	Between	4	8.51	514.513	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.016			
DE	[0.908]	(–)*	3.499 ± 0.006	3.604 ± 0.006	3.702 ± 0.006	3.755 ± 0.005	3.74 ± 0.004	Between	4	3.27	397.222	***	(25 < 36)***, (25 < 37)***, (25 < 37.5)***, (25 < 38)***, (36 < 37)***, (36 < 37.5)***, (36 < 38)***, (37 < 37.5)***, (37 < 38)***
								Within	1245	0.008			

Note: The correlation coefficient (R) of the square-root of DI and the mean trait size was calculated, together with its level of significance (P), with a Pearson product–moment correlation test. The signs of the slope of the regression (b) are shown in parentheses.

*$P < 0.05$, ***$P < 0.001$.

The negative maternal effect observed in this study appears to be mainly due to the change of the mean trait size of the wings, rather than changes in σ_p^2. If the wing traits hadn't changed in size, the maternal effect would have shifted from negative to positive at the two highest temperatures, because σ_p^2 became significantly smaller at 37.5°C and 38°C compared with the control group. It might therefore be important to consider at which point it could be advantageous to differ phenotypically from the parental generation. In populations living in a stable environment, a positive maternal effect will help to ensure that the offspring are well suited for the environment (Schluter and Gustafsson, 1993). However, in a stochastically changing environment, a positive maternal effect can be deleterious, since the low σ_p^2 in the population will minimize the number of offspring capable of surviving the changes. Whereas negative maternal effects in a stochastically changing environment will ensure the production of phenotypically different offspring, that can increase the chance of survival of at least some individuals. If all offspring are alike, the probability of them surviving is decreased, because their preferred habitat is identical to that of their mothers, and a very slight change could be deleterious for the population. If the offspring are phenotypically very different, many offspring will be maladapted to the environment in each generation, but for a few of them the habitat will be suitable, securing the survival of the population.

The results of the current study suggest that DI, σ_e^2 and their interaction (ECV) can cause changes in σ_p^2. Although each of these components separately might be adaptive in extremely different scenarios, the combination is consistent with the selective pressures imposed by a highly heterogeneous and unpredictable environment. Developmental instability is traditionally viewed as the inability of a genotype to direct its development, but it has also been interpreted as a bet-hedging strategy in an unpredictable environment (Simmons and Johnston, 1997). Phenotypic plasticity, which is reported to be adaptive only when environmental change is predictable (Scheiner, 1993; Pigliucci, 2001), could also be adaptive in habitats where σ_e^2 is unpredictable (Robe and Grifiths, 2000). The increased σ_p^2 with increased temperature could also be considered (in evolutionary terms) to be a mechanism of defence for populations in fluctuating environments. The apparent lack of selection on σ_g^2 observed in some sexually reproducing species could be explained by a high degree of phenotypic plasticity, developmental instability and/or maternal effects that would obscure selective differences among genotypes (Sultan, 1996). Phenotypic plasticity, developmental instability and maternal effects can mask genetic differences among individuals. In this way, the population protects itself against fast potential changes of genetic structure under environmental changes. However, when the magnitude of genetic variation is insufficient to create a diversity of phenotypes that can be exposed to selection, developmental instability – by producing stochastic variation – will enrich the evolutionary potential of a population. Stochastic fluctuations will contribute to the development of extreme phenotypes by exposing them to selection, thus affecting frequencies of the corresponding genotypes (and contributing to lower phenotypic variation) (Lajus *et al.*, 2003).

This study is the first to provide an accurate estimate of the maternal effect and its components and their interactions (σ_p^2, DI, ECV, σ_e^2 and mean trait size). We have also seen another example of the importance of being able to estimate σ_e^2 and its effect on σ_p^2 due to the ECV effect, since it is almost impossible to estimate such parameters correctly when conducting experiments with sexually reproducing organisms. The control of σ_e^2 is crucial in many investigations, but only rarely is it possible to estimate it with some precision. The present study found evidence that σ_e^2 affects wing traits in offspring from different maternal

temperature treatments and showed the unpredictability of ECV and its effect on σ_p^2 and DI estimates. Hence, the detection and estimation of σ_e^2 is crucial for future investigations dealing with clonal strains. Furthermore, it should also be borne in mind that σ_e^2 can also affect the estimates of mean trait size. Unfortunately, there is no statistical test for determining how σ_e^2 alters mean trait size. We only know that the smaller σ_e^2 is, the more reliable is the estimate of trait sizes.

ACKNOWLEDGEMENTS

This work was supported by grants from the Danish Natural Sciences Research Council (21-01-0526 and 21-03-0125) and the Marie Curie Fellowship of the European Community Host Development program under contract number HPMD-CT-2000-00009 (C.P.). Thanks to Jürgen Tomiuk, Charles Fox, Céline Teplitsky and an anonymous reviewer for their comments on an earlier draft of the manuscript.

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