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The use of fluctuating asymmetry and phenotypic variability as indicators of developmental instability – testing maternal effect employing clonal organisms.

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Abstract

The impact of maternal effects on developmental stability (DS) was investigated in *Drosophila mercatorum* by measuring fluctuating asymmetry (FA) and phenotypic variability (Vp). Maternal effects are normally difficult to estimate, as both genetic and environmental components affect the phenotype; however, by using a homozygous parthenogenetic strain of *D. mercatorum*, we could exclude any genetic variance among the individuals. In order to estimate the amount of environmental variance (Venv) in a monoclonal population a new methodology was used. The impact of the maternal environment on FA and Vp for three wing traits was measured in the progeny. This new method has been applied for quantifying the produced "bias" of Venv on FA and Vp and may give insight into why non-reproducible results have been obtained in DS studies so far. The maternal flies were treated in a water bath at different temperatures (36°C, 37°C, 38°C and 25°C as control temperature). The results showed a tendency towards an increase of FA and Vp for all three traits in the progeny from mothers being stressed at high temperatures. This shows maternal effects for temperature stress resulting in an increased developmental instability (DI) in the offspring of heatstressed mothers.

Introduction

Much effort has been made to separate the impact of environmental components from genetic components in the expression of a phenotype. Investigations have shown that a mother (or father) can influence the phenotype of their offspring by inherited environmental effects, that is those components of the phenotype that are derived from the parent, apart from nuclear genes (Rossiter, 1996). This environmental effect is also called the maternal effect because it is mostly transmitted from the mother by cytoplasmic genes (mitochondria or plastids) which are present in the egg but not in the sperm from the father, although exceptions occur (Rossiter, 1996).

Stress may constitute an important part of the environment of many living organisms, and has been shown, in several investigations, to affect DS (Zakharov, 1992). DS refers to the ability of an organism to buffer its developmental processes against environmental and genetic disturbances and ensures common developmental outcomes under specified conditions (Zakharov, 1992).

The aim of this investigation is to measure the impact of maternal effect on FA and Vp in the progeny from mothers stressed at various temperatures. To measure the maternal effect by the use of FA and Vp, the genetic and environmental variance among the progeny must be eliminated. This was possible by using a parthenogenetic strain of *D. mercatorum*, which is totally homozygous (pronuclear duplication) (Templeton *et al.,* 1976). Therefore the genetic variance (Vg) among the individuals is eliminated (Vg = 0). To minimise Venv the progeny were exposed to identical conditions. If differences in FA and Vp are observed between offspring from mothers tested at different temperatures this can only be due to maternal effects or due to Venv as Vg = 0. As both FA and Vp are affected by the Venv, another aim of this investigation is to check if the experimental conditions allow us to consider Venv as nonsignificant. This was possible by measuring the covariation (cov) between the right (r) and left (l) sides of bilateral traits (for a description of this method see Pertoldi *et al*., 2001).

Materials and methods

Experimental design: The investigation was performed by the use of a parthenogenetic strain of *D. mercatorum* which is totally homozygous, due to their way of reproducing (Templeton *et al.,* 1976). The strain originates from a single individual belonging to stock (Iv-23-01m).

Parental flies used for the experiment were transferred to new vials each containing 15 flies when they were 48 hours old. These flies were stressed in a water bath for 30 min twice a day, at a 12 hour interval, for four days. Before each stress period the stoppers were moistened to eliminate any desiccation during stress exposure. The temperatures of the water baths were 36°C, 37°C or 38°C, and a control temperature was at 25°C. These temperatures are known to have an impact on fitness in *D. mercatorum* (Kristensen, pers.comm.).

Four days after the last water bath treatment the surviving flies were transferred to new vials, to make sure that all eggs were laid after the mothers had been exposed to all eight stress periods.

The density was kept constant with 15 flies in each vial. There can, however, be a difference in the conditions under which the progeny developed, due to differences in the number of larvae between the four temperatures. This may produce stress among the larvae, due to lack of the group feeding "facilitation effect" (Bundgaard *et al*., unpublished). This effect arises from the fact, that there need to be a certain amount of larvae to penetrate the media sufficiently. Between vial differences in the number of larvae also result in different concentrations of waste products in the vials as well as differences in the level of crowding.

Only the flies hatched within the first three days were used for phenotypic measurements, as these flies have been exposed to the lowest amount of stress caused by waste products and crowding. For each temperature approximately 120 flies were collected. This, however, does not relate to the progeny from mothers stressed at 38°C. These were collected in a period of six days and only 35 flies were obtained.

The wings were removed and placed in a droplet of lactic acid, on a microscope slide and covered with a cover slip. The wings were measured by using a camera attached to a dissecting microscope and a Machintosch computer, and by the use of the software package object image 1.62p (Vischer, 2000). By using three landmarks, we measured three wing traits on each wing (see Figure 1).

Statistical properties of FA: Three types of asymmetry are known: directional asymmetry (DA), antisymmetry (AS), and true FA, which is characterized by a normal distribution of (r-l) characters with a mean of zero (Palmer and Strobeck, 1986). When measuring FA in populations, it is important to test for DA and AS, to be sure that the deviations found in a given trait is true FA. FA distributions for the

three wing traits were inspected graphically for normality and AS. No deviation from true FA were found. Skewness and kurtosis were tested for deviations following D'Agostino (Zar, 1999). In 80% of the FA distributions investigated we found significant deviations from normality (results not shown), due to a significant positive kurtosis. The graphical inspections confirmed that the deviations from normality were due to leptokurtic distributions of the FA values. Testing for DA was done by a one-sample t-test (Sokal and Rohlf, 1981) for significant deviation of the mean value of (r-l) from zero. No significant DA was detected. (results not shown). Because of the large number of tests conducted in the following analyses a sequential Bonferroni test was applied (Rice, 1989).

FA and maternal stress temperature: FA was calculated for the three wing traits as the absolute differences in value between each bilateral pair of traits (r-l) (FA1 following Palmer, 1994). No significant correlations among wing traits FA at the individual level were found (results not shown). Therefore, also the mean FA value of the three wing traits for each group of progeny from mothers exposed to the four different temperatures was calculated. A Levene's test (Zar, 1999) was conducted to test if the absolute values of traits FA and the mean FA value were significantly different among the different temperatures. A multiple comparison test among the progeny from each maternal temperature treatment was made with a Scheffé *F*-test (Zar, 1999).

Vp and maternal stress temperature: Vp was measured as the variance of the length of the three wing traits on the right wing. An *F*-test was conducted to compare Vp of the three wing traits from progeny from each group of maternal stress temperature with Vp of progeny from mothers of the control group.

Wing size and maternal stress temperature: The length of the three wing traits was measured on the right wing. Tests for significant changes in mean value were performed by a Levene´s test and multiple comparison tests were made with a Scheffé *F*-test (Zar, 1999).

Environmental variability: To calculate the produced "bias" on FA and Vp produced by the presence of Venv the 2cov(r,l) were calculated for each trait and for each temperature treatment.

Results

FA and maternal stress temperature: The Scheffe's *F*-test showed significant effects of test treatment temperature on mean FA measured in all three traits. Mean FA in progeny from mothers exposed to 38°C had higher FA when compared to progeny from the mothers exposed to control conditions (25°C) and mothers exposed to 37°C (Table 1).

The Scheffe´s F-test showed significant changes among the mean FA for each of the three traits, with a significant increase in FA for trait ac in progeny from mothers exposed to 38°C compared to progeny from mothers exposed to 25°C. For trait bc an increase in FA for progeny from mothers exposed to 38°C where found when compared to progeny from mothers exposed to 36°C and 37°C (Table 1).

Vp and maternal stress temperature: The pairwise comparisons (*F*-test) of the variance of the length of the three wing traits on the right wing, showed generally a significant difference in Vp between offspring from temperature stressed mothers and offspring from the control group (Table 2). For trait ab

Table 1. Levene's test for comparing the length ab, ac and bc and the fluctuating asymmetry of the mean length (ab, ac and bc) within the different temperatures. The table also shows the Scheffe's *F* - test for multiple comparisons.

Traits	25 °C Mean±SD(n)	36°C Mean±SD(n)	37 °C Mean±SD(n)	38 °C Mean±SD(n)	Source of variation	df	Mean square	Levene's test (*F* value)	*P*	Scheffe's *F*-test
					Between	3	0.002			
length ab	1.133±0.036(105)	1.133±0.154 (105)	1.134±0.03 (106)	1.118±0.053(30)	Within	342	0.026	0.283	ns	
					Between	3	0.212			(38°C>25°C)*, (38°C>36°C)*,
length ac	1.148±0.026(105)	1.137±0.196(105)	1.168±0.161(106)	1.287±0.442(30)	Within	342	0.036	5.808	***	(38°C>36°C)*, (36°C>37°C)*
					Between	3	0.052			(25°C>36°C)*, (37°C>36°C)*,
length bc	1.785±0.053(105)	1.748±0.033(105)	1.796±0.037(106)	1.796±0.036(30)	Within	342	0.002	29.491	***	(36°C>25°C)*, (36°C>36°C)*
					Between	3	0.051			
Mean F/A	0.02±0.027(105)	0.044±0.131(105)	0.031±0.099(106)	0.095±0.225(30)	Within	342	0.012	4.095	***	(38°C>25°C)*, (38°C>37°C)*
					Between	3	0.035			
F/A of length ab	0.015±0.026(105)	0.049±0.161(105)	0.025±0.053(106)	0.063±0.199(30)	Within	342	0.014	2.11	ns	
					Between	3	0.197			
F/A of length ac	0.015±0.014(105)	0.066±0.276(105)	0.056±0.232(106)	0.175±0.444(30)	Within	342	0.037	3.476	*	(38°C>25°C)*
					Between	3	0.015			
F/A of length bc	0.023±0.04(105)	0.015±0.012(105)	0.015±0.011(106)	0.057±0.219(30)	Within	342	0.005	3.396	*	(38°C>36°C)*, (36°C>37°C)*

P<0.05 = *, P<0.01 = **, P<0.001 = ***

and ac we found some significant increases of Vp with increasing stress temperature, whereas for trait bc we found a significant decrease of Vp at 36°C and 37°C compared to the control temperature.

Wing size and maternal stress temperature: Significant difference in mean length of the three wing traits between the four temperatures was found. However, there was no tendency of a reduction in the traits with increasing temperature treatments of the mothers (Table 1).

Discussion

FA and maternal stress temperature: The finding of an increase of FA in the progeny from females stressed at the highest temperatures compared to females being stressed at lower temperatures or exposed to control conditions indicates that the stress put upon the mothers has a negative effect on the progeny. This can be seen by a decrease in DS. The level of FA measured in this investigation is, however, an under-estimation of the real FA level, due to the presence of Venv measured as the cov between the r and l side (Table 2).

Vp and maternal stress temperature: The fact that no linear relationship between Vp and temperature was found could be due to the uncontrolled effect of Venv affecting the phenotype (in trait ab and ac). Another reason could be that the range of variability of Vp in wing trait length is too narrow. The narrow range could be due to the absence of Vg which, if present, would introduce an additional source of variability and thereby increase Vp. From Table 2 it is also clear that every time cov is positive, the relative "bias" is high as compared to the Vp value. From positive values of cov it is obvious that environmental variance is present among individuals and that in all but one case it acts to increase Vp (Table 2). However, the bias produced by Venv on Vp should only affect the pairwise comparions in two cases (Table 2).

The results show that by stressing the maternal flies, there is a tendency to produce offspring which are phenotypically more diverse (Table 2). This can only be caused by maternal effect. Wing measures are positively correlated to life history traits, because the symmetry of the wing traits probably is under direct selection for aerodynamic stability (Woods *et al.,* 1999). Generally characters more closely related to fitness are expected to be better buffered against developmental disturbances (Palmer and Strobeck, 1986). However, in our investigation the stress put upon the mothers is sufficient to affect the development of canalised traits

(the wing traits) in their offspring, by increasing the Vp among the offspring. An explanation for the increase in Vp in progeny from temperature stressed females can be due to the presence of Venv. Another factor contributing to the increased Vp could be a disruption of the environmental canalisation process. Environmental canalisation can be interpreted as: Vp = Va + Vna + Venv, with Va being the additive genetic variance and Vna non-additive genetic variance (Debat and David, 2001)..

Wing size and maternal stress temperature: An explanation for the significantly longer wing size in progeny from mothers stressed at 38°C in the length ac could be that there has been some kind of maternal selection among the progeny (Kirkpatrick and Lande, 1989). In spite of the fact that the flies are parthenogenetic and totally homozygous, maybe the few surviving progeny at 38°C were better able to cope with the stress due to non-mendelian inheritance.

Overall in the experiment there seems to be no connection between wing size in the progeny and the stress level at which the mothers were exposed.

Environmental variability: Evidence was found for the presence of Venv at several temperature treatments and in the different traits (Table 2). This is interesting because it was attempted to reduce Venv as much as possible. The absence of a clear pattern between increasing maternal stress and Venv can only be explained by the unpredictability and complexity of Venv.

Table 2. Stress temperatures and total phenotypic variance (Vp) with their respective p-values which indicate the significance of the F-test of the pairwise comparisons of the variances at the different stressing temperatures, versus the variances at the control temperature (25 °C). The Venv values are calculated as 2cov(r,l). The sign (+) indicates a higher variance of the numerator as compared to control temperature, the sign (-) indicates a higher variance of the control temperature.

Treatment	Vp length ab	Vp length ac	Vp length bc	Venv length ab	Venv length ac	Venv length bc
25°C control	0.001444	0.000784	0.002809	0.001	0.001	0.003
36°C	(0.023716)***	(0.038416)***	(-0.001089)***	0.003	(-0.003)	0.001
37°C	(-0.0009)"	(0.025921)***	(-0.001369)***	0.001	2.37E-04	0.001
38°C	(0.002809)"	(0.195364)***	(-0.001444)"	0.001	0.003	0.001

$p < 0.001$ = ***, " = results no longer significant after Bonferroni's correction (k = 9).

Conclusion

The temperature stress experienced by the mothers clearly affects FA and Vp in the progeny. This environmental effect is transferred to the progeny through maternal effects, which shows that Vp and FA can be used in the study of maternal effects.

Two types of maternal effect are known, positive maternal effect (offspring resembling their mothers) and negative maternal effect (offspring not resembling their mothers) (Palmer, 1996), which are both found in this investigation. Mothers surviving the high temperature stress on average get offspring with higher DI. The type of maternal effect can influence population dynamics. Positive maternal effect will act similarly to additive genetic effects. This may be important for small populations with a very low effective population size (Ne). Small populations are not able to respond to environmental changes in an evolutionary way as they are governed by drift and mutation and not selective forces. A positive maternal effect can therefore be very important for future adaptations to environmental changes in populations where selection is not effective anymore.

Negative effects may produce offspring maladapted for the environmental conditions for which their mothers were selected (Boonstra and Hochacke, 1997). The negative maternal effect found in this investigation is an expression of the environmental stress the maternal population has been exposed to and can be used as an instrument to predict future survival of the population. If a population is living in a slowly changing environment, a positive maternal effect is advantageous, due to an evolutionary adaptation. However, if a population is living in a stochastically changing environment a negative maternal effect can ensure the survival of some progeny in future generations. This might be the case for populations living in fragmented and disturbed habitats since individuals with phenotypically variable offspring are more likely to leave at least some survivors every generation in environments that vary in time and space (Cullum, 2000).

Future directions: It is well known from quantitative genetics how biased the estimates of Venv and Vg may be. As a consequence, also the heritability (broad and narrow sense and realised heritability) estimates are biased. Furthermore, Vg can probably never be considered zero in sexually reproducing populations, even when working with inbred isofemale lines. It has also been hypothesised that the level of Vg may change with environmental conditions (Hoffmann and Parsons, 1991). Together with the amount of epistatic interactions this contributes to further complexity.

The method proposed here and in Pertoldi *et al*. (2001) for partitioning out the different components of Vp is expected to increase the value of FA and Vp as indicators of environmental stress. From this study, however, it has become clear that the exact amount of a DS of a group of individuals can only be precisely estimated for traits considered singularly. Furthermore it is necessary to be aware that the method used here for correcting the "bias" produced by Venv on the two estimators of DS operates on the mean value of DS of the individuals. This is important as the differences in DS among individuals are due to the fact that they have experienced different microenvironments during development.

In the future it would also be interesting to follow the maternal effect for several generations to see if the effect is carried on for more than two generations.

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References: Boonstra, R., and W.M. Hochachka 1997, Evol. Ecol. 11: 169-182; Cullum, A.J., 2000, Evol. Ecol. Res. 2: 841-855; Debat, V., and P. David 2001, TREE 16: 555-561; Graham, J.H., D.C. Freeman, and J.M. Emlen 1993, Genetica 89: 121-137; Kirkpatrick, M., and R. Lande 1989, Evolution 43(3): 485-503; Palmer, A.R., and C. Strobeck 1986, Annu. Rev. Ecol. Syst. 17: 391-421; Palmer, A.R., 1994, in: *Developmental Instability: Its Origins and Evolutionary Implications*. (Markow, T.A., ed.) Pp. 335-364. Kluwer Academic Publishers, Dordrecht; Palmer, A.R., 1996, BioScience 46(7): 518-532; Pertoldi, C., T.N. Kristensen, and V. Loeschcke 2001, Jour. Theor. Biol. 210: 407-410; Rice, W.R., 1989, Evolution 43: 223-225; Rossiter, M.C., 1996, Annu. Rev. Ecol. Syst. 27: 451-476; Sokal, R.R., and F.J. Rohlf 1981, *Biometry*. W.H. Freeman and Co., San Francisco; Templeton, A.R., H.L. Carson, and C.F. Sing 1976, Genetics 82: 527-542; Vischer, N.O.E., 2000, University of Amsterdam. Program available on the site: ftp://codon.nih.gov/pub/nih-image/nih-image161_docs.hqx; Woods, R.E., C.M. Sgró, M.J. Hercus, and A.A. Hoffmann 1999, Evolution 53: 493-505; Zakharov, V.M., 1992, Acta Zool. Fenn. 191: 7–30; Zar, J.H., 1999, *Biostatistical Analysis*. Prentice Hall, New Jersey.