Effect of low stressful temperature on genetic variation of five quantitative traits in *Drosophila melanogaster*

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A half-sib analysis was used to investigate genetic variation for three morphological traits (thorax length, wing length and sternopleural bristle number) and two life-history traits (developmental time and larva-to-adult viability) in *Drosophila melanogaster* reared at a standard (25°C) and a low stressful (13°C) temperature. Both phenotypic and environmental variation showed a significant increase under stressful conditions in all traits. For estimates of genetic variation, no statistically significant differences were found between the two environments. Narrow heritabilities tended to be higher at 13°C for sternopleural bristle number and viability and at 25°C for wing length and developmental time,

whereas thorax length did not show any trend. However, the pattern of genetic variances and evolvability indices (coefficient of genetic variation and evolvability), considered in the context of literature evidence, indicated the possibility of an increase in additive genetic variation for the morphological traits and viability and in nonadditive genetic variation for developmental time. The data suggest that the effect of stressful temperature may be trait-specific and this warns against generalizations about the behaviour of genetic variation under extreme conditions.

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Introduction

The evolution of natural populations is known to depend on the presence of additive genetic variation in quantitative traits. Higher levels of this variation accelerate responses to natural selection and increase the probability for successful adaptation to environmental changes. Numerous quantitative genetic experiments indicate that the environment, by itself, can affect the amount of expressed additive variation. Under extreme conditions, estimates of narrow heritability and/or additive genetic variance tend to be higher than under optimal conditions and a hypothesis has been advanced that an enhanced level of additive genetic variation is favoured by environmental stress (Hoffmann and Parsons, 1991).

Quantitative variation in different environments has been extensively studied in the last decade using *Drosophila* (eg, Gebhardt and Stearns, 1992; David *et al*, 1994; Barker and Krebs, 1995; Hoffmann and Schiffer, 1998; Imasheva *et al*, 1998; Loeschcke *et al*, 1999; Bubliy *et al*, 2001). The results of these studies indicated the possibility of both higher and of lower additive genetic variances/heritabilities under stress as well as the absence of changes across environments. Most evidence for increased additive variation has been obtained in experiments (Barker and Krebs, 1995; de Moed *et al*, 1997;

Imasheva *et al*, 1998, 1999, 2000; Karan *et al*, 1999; Loeschcke *et al*, 1999) in which genetic parameters have been estimated using the isofemale line technique, ie, a modified full-sib design with isofemale lines treated as families (Hoffmann and Parsons, 1988). However, the full-sib design is the least precise among all quantitative genetics methods developed to estimate additive genetic variance. Since the covariance among full sibs includes a relatively large proportion of the non-additive component of the genetic variance (Falconer and Mackay, 1996), estimates of the additive genetic variance from full-sib/isofemale line data may be biased.

In the present study, we consider the effects of environmental stress on genetic variation in *D. melanogaster* using a different experimental approach, the paternal half-sib design. The covariance among paternal half-sibs contains a smaller proportion of non-additive genetic variance than does the full-sib covariance (Falconer and Mackay, 1996) and thus provides more reliable estimates of the additive genetic variance. To stress flies, we reared them at 13°C, which is close to the lower tolerance limit for *D. melanogaster* (David *et al*, 1983). Three morphological traits, thorax length, wing length and sternopleural bristle number, and two life-history traits, developmental time and larva-to-adult viability, were analysed. The effect of low rearing temperature on these traits has been examined recently in isofemale lines of *D. melanogaster* by de Moed *et al* (1997, thorax and wing length), Imasheva *et al* (1998, thorax and wing length, sternopleural bristle number, developmental time and viability; 2000, wing length), and Karan *et al* (1999, thorax and wing length). Also, Sgrò and Hoffmann (1998) compared variation for

wing length and developmental time between different temperatures using parent–offspring regression, a method which gives estimates of the additive genetic variance similar to those for the half-sib design. In the isofemale line studies, a trend for an increase in additive genetic variation at low temperatures was reported for all the traits, whereas, in the parent–offspring comparisons, estimates of the additive genetic variance in the stressful environment decreased for developmental time and did not change for wing length.

Materials and methods

The laboratory *D. melanogaster* stock was founded from flies collected in Leiden (the Netherlands) in October 1999. Wild inseminated females were used to start 30 isofemale lines, which were maintained on a standard oatmeal-sugar-yeast-agar *Drosophila* medium for 30 generations (five generations at 25°C and then 25 generations at 20°C). To obtain flies for the experiment, a mass population was established and maintained as discrete generations at 25°C with regular mixing of adults among bottles.

From the third generation of the mass population, a sample of 60 virgin males and 180 virgin females was taken and aged for 5–7 days. Further, each male (sire) was placed in an individual vial together with three females (dams). The vials contained the standard medium supplemented with live yeast to stimulate mating. Thirty-six hours later, each female was transferred for egg laying to a separate empty vial containing a plastic spoon, which was filled with 2 ml of the yeast-seeded standard medium. After another 24 h, the females were transferred to new vials with spoons to continue egg laying. First-instar larvae obtained from each female were transferred from spoons to six new vials (10 larvae per vial), three vials on the first day (block 1) and another three on the second day (block 2). All vials contained 7 ml of the standard medium without live yeast. Immediately after transferring larvae, one set of the vials was placed at the stressful temperature and the other set at the control temperature. Each set consisted of 540 vials and represented 30 sire families. The two temperatures were $13 \pm 0.5^\circ\text{C}$ and $25 \pm 0.5^\circ\text{C}$, respectively.

Eclosed adults were collected and counted every 12 h until all flies had emerged. Females and males from each vial were pooled and stored in an individual plastic microtube filled with a fixative liquid (1/2 glycerine + 1/2 70% ethanol). Three morphological traits, thorax length, left wing length and sternopleural bristle number on the left side of the fly, were measured on fixed females (one per vial). Thorax length (from the anterior margin of the thorax to the posterior tip of the scutellum from the dorsal view) and wing length (from the intersection of the wing margin and vein L3 to the intersection between this vein and the anterior crossvein) were measured in arbitrary micrometer units (70 units = 1 mm) using an ocular micrometer and converted into millimeters. Developmental time was estimated as the time interval in days from the midpoint of the oviposition period to the recorded time of eclosion. For each vial, the mean developmental time (males and females pooled) was computed to be used as an individual observation in statistical analysis. Viability was calculated as the proportion of flies (sexes pooled) that emerged from each vial relative to the number of first-instar larvae used to set up the cultures. Thus,

the number of observations for viability was also equal to the number of vials.

Data treatment was based on standard statistical procedures for the paternal half-sib analysis (Becker, 1992; Falconer and Mackay, 1996). The ANOVA design for three morphological traits at 13°C was slightly unbalanced due to the absence of females among emerged flies in some vials. Because of this, the significance of the mean squares (type III) was tested with Satterthwaite's approximation (Sokal and Rohlf, 1996). Viability data were analyzed as arcsine-square-root-transformed proportions; for other traits, no transformation was needed. Statistical comparisons for variance components and heritabilities were done with *t*-tests, using their approximate standard errors. In addition to these classical measures of genetic variability, coefficients of genetic variation and evolvabilities were calculated as $CV_A = 100\sqrt{V_A}/x$ and $I_A = V_A/x^2$, respectively, where V_A is the additive genetic variance and x is the mean of a trait (Houle, 1992).

Results

Table 1 shows means, phenotypic variances and coefficients of variation for the five traits at 25 and 13°C. There were highly significant differences between the two temperatures for all estimates ($P < 0.001$; means, *t*-test; variances, *F*-ratio test; CVs, *Z*-test). Rearing at 13°C resulted in decreased thorax length, bristle number and viability, whereas wing length and developmental time were higher at this temperature. Phenotypic variances and CVs were higher in the stressful environment and their increase was most pronounced for the life-history traits.

In Table 2, mean squares and variance components from three-way nested ANOVAs with sire, dam and block as random factors are presented. All morphological traits at both temperatures showed significant among-sire and among-dam variation and non-significant variation among experimental blocks. For developmental time, sire and dam effects were significant at 25 and 13°C, respectively, while for viability variation among sires at 13°C was significant and variation among dams at 25°C marginally significant ($P = 0.065$). The effect of block was significant for the life-history traits in both environments, which might be due to unexpected technical problems with maintaining low temperature in the first day of the experiment.

Comparing among-sire and among-dam variances to test for nonadditive genetic effects ($s^2_{\text{dam}} > s^2_{\text{sire}}$) in particular traits did not reveal statistically significant differences (results not presented). Between-temperature comparisons of variance components on the among-sire, among-dam and among-block levels of variation showed differences (13°C > 25°C, two-tailed $P < 0.05$) only for the among-dam variances in wing length and developmental time, but they could not be considered significant taking into account multiple comparisons (the Bonferroni correction). For the morphological traits, the values of s^2_{sire} and s^2_{dam} as well as the sum $s^2_{\text{sire}} + s^2_{\text{dam}}$ (full-sib variance) were generally higher at 13°C than at 25°C. Similar trends were also evident for s^2_{sire} in viability, s^2_{dam} in developmental time and the sum $s^2_{\text{sire}} + s^2_{\text{dam}}$ in both life-history traits. The among-progeny variance was significantly higher (two-tailed $P < 0.001$) at 13°C for each trait, indicating an increase in environmental variation in the stressful environment. This increase was the most pronounced for developmental time and the least pronounced for sterno-

Table 1 Means, phenotypic variances and coefficients of variation for five traits in *D. melanogaster* reared at two temperatures. The morphological traits are measured in females; the life-history traits are estimated using both females and males (pooled samples). For all traits at 25°C and for the life-history traits at 13°C, the number of observations is 540; for the morphological traits at 13°C it is 531

Trait	25°C			13°C		
	mean $\pm$ SE	s ²	CV $\pm$ SE	mean $\pm$ SE	s ²	CV $\pm$ SE
Thorax length (mm $\times$ 100)	107.85 $\pm$ 0.12	8.04	2.63 $\pm$ 0.08	104.00 $\pm$ 0.16	13.09	3.48 $\pm$ 0.11
Wing length (mm $\times$ 100)	157.12 $\pm$ 0.16	13.93	2.38 $\pm$ 0.07	176.64 $\pm$ 0.40	39.93	3.58 $\pm$ 0.11
Bristle number	9.51 $\pm$ 0.05	1.40	12.45 $\pm$ 0.38	8.90 $\pm$ 0.07	2.61	18.15 $\pm$ 0.57
Dev. time (days)	8.69 $\pm$ 0.01	0.02	1.41 $\pm$ 0.04	41.43 $\pm$ 0.10	5.17	5.49 $\pm$ 0.17
Viability	0.961 $\pm$ 0.003	0.006	8.34 $\pm$ 0.26	0.743 $\pm$ 0.008	0.037	25.93 $\pm$ 0.84

Table 2 Mean squares and variance components from three-way nested ANOVAs for five traits at two temperatures. For the morphological traits d.f. is 360 at 25°C and 351 at 13°C

Trait	Source of variation	d.f.	25°C		13°C	
			MS	s ² $\pm$ SE	MS	s ² $\pm$ SE
Thorax length ^a	Among sires	29	22.49*	0.62 $\pm$ 0.34	38.01**	1.24 $\pm$ 0.56
	Among dams	60	11.27*	0.72 $\pm$ 0.38	16.22*	0.91 $\pm$ 0.55
	Among blocks	90	6.95	0.11 $\pm$ 0.38	10.87	-0.04 $\pm$ 0.60
	Among progeny	360/351	6.61	6.61 $\pm$ 0.49	11.00	11.00 $\pm$ 0.83
Wing length ^a	Among sires	29	67.77***	2.55 $\pm$ 0.98	167.74**	5.48 $\pm$ 2.47
	Among dams	60	21.82***	2.46 $\pm$ 0.68	71.48***	7.31 $\pm$ 2.25
	Among blocks	90	7.09	-0.97 $\pm$ 0.43	28.54	0.66 $\pm$ 1.55
	Among progeny	360/351	9.99	9.99 $\pm$ 0.74	26.59	26.59 $\pm$ 2.00
Bristle number	Among sires	29	5.64***	0.21 $\pm$ 0.08	13.65***	0.59 $\pm$ 0.20
	Among dams	60	1.80**	0.13 $\pm$ 0.06	3.19*	0.21 $\pm$ 0.11
	Among blocks	90	1.04	-0.01 $\pm$ 0.06	1.97	0.08 $\pm$ 0.11
	Among progeny	360/351	1.09	1.09 $\pm$ 0.08	1.74	1.74 $\pm$ 0.13
Dev. time ^b	Among sires	29	4.53*	0.11 $\pm$ 0.07	941.20	1.49 $\pm$ 16.11
	Among dams	60	2.48	0.06 $\pm$ 0.09	914.25*	61.94 $\pm$ 30.44
	Among blocks	90	2.10***	0.38 $\pm$ 0.11	542.60*	44.08 $\pm$ 28.54
	Among progeny	360	0.95	0.95 $\pm$ 0.07	410.35	410.35 $\pm$ 30.50
Viability ^c	Among sires	29	7.20	0.08 $\pm$ 0.12	20.29**	0.60 $\pm$ 0.30
	Among dams	60	5.83 ⁺	0.29 $\pm$ 0.20	9.42	0.16 $\pm$ 0.35
	Among blocks	90	4.10***	0.62 $\pm$ 0.21	8.47**	1.08 $\pm$ 0.44
	Among progeny	360	2.24	2.24 $\pm$ 0.17	5.23	5.23 $\pm$ 0.39

^adata in mm $\times$ 100; ^bdata in days $\times$ 10; ^cdata are arcsine-transformed proportions $\times$ 10.* $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$.

pleural bristles. It should be noted, however, that for wing length and especially for developmental time, the between-temperature differences were partly explained by scale effects (see Table 1).

Table 3 gives estimates of heritability, coefficient of additive genetic variation and evolvability based on the among-sire and among-dam variances as well as on the sum of the two components (sire + dam). For each trait, h^2_{dam} was not significantly higher than h^2_{sire} within temperature regimes (a test for the presence of non-additive effects) with the exception of developmental time at 13°C (one-tailed $P < 0.01$). No statistically significant differences were found between stressful and non-stressful environments for the estimates based on the same component of the variance (two-tailed tests, results not shown). The h^2_{sire} values tended to be higher for sternopleurals and viability at 13°C and for wing length and developmental time at 25°C. Trends for changes in h^2_{dam} were most evident for

developmental time and viability (higher at 13°C and at 25°C, respectively). The estimates of $h^2_{\text{sire+dam}}$ were very similar under stressful and non-stressful conditions for thorax length, developmental time and viability. For wing length and bristle number, the direction of changes in $h^2_{\text{sire+dam}}$ with temperature coincided with that for h^2_{sire} but differences in the absolute values between the two environments were relatively small.

Coefficients of additive genetic variation and evolvabilities for the morphological traits tended to have higher values at 13°C regardless of which variance component (sire, dam or sire + dam) they were based on. For the life-history traits, CV_A and I_A obtained from the sum of the among-sire and among-dam variances also tended to be higher under stress. However, the sire- and dam-based estimates for these traits did not reveal a consistent pattern of change with temperature. CV_A and I_A were generally higher at 13°C when estimated using the among-sire

Table 3 Heritabilities, coefficients of additive variation and evolvabilities for five traits at two temperatures based on different variance components

Trait	Variance component	25°C			13°C		
		$h^2 \pm SE$	CV_A	$I_A \times 10^4$	$h^2 \pm SE$	CV_A	$I_A \times 10^4$
Thorax length	Sire	0.309 $\pm$ 0.176	0.73	0.54	0.379 $\pm$ 0.143	1.07	1.15
	Dam	0.357 $\pm$ 0.167	0.79	0.62	0.278 $\pm$ 0.160	0.92	0.84
	Sire + dam	0.333 $\pm$ 0.083	1.07	1.15	0.328 $\pm$ 0.078	1.41	1.99
Wing length	Sire	0.728 $\pm$ 0.189	1.02	1.03	0.547 $\pm$ 0.179	1.33	1.76
	Dam	0.700 $\pm$ 0.197	1.00	1.00	0.731 $\pm$ 0.202	1.53	2.34
	Sire + dam	0.714 $\pm$ 0.098	1.42	2.03	0.639 $\pm$ 0.103	2.02	4.10
Bristle number	Sire	0.603 $\pm$ 0.205	4.85	23.53	0.908 $\pm$ 0.249	8.67	75.16
	Dam	0.358 $\pm$ 0.165	3.73	13.94	0.318 $\pm$ 0.164	5.13	26.30
	Sire + dam	0.480 $\pm$ 0.079	6.12	35.68	0.613 $\pm$ 0.073	10.07	90.56
Dev. time	Sire	0.302 $\pm$ 0.158	0.39	0.15	0.012 $\pm$ 0.075	0.29	0.09
	Dam	0.168 $\pm$ 0.147	0.29	0.08	0.478 $\pm$ 0.176	1.90	3.61
	Sire + dam	0.235 $\pm$ 0.071	0.48	0.23	0.245 $\pm$ 0.095	1.92	3.70
Viability	Sire	0.095 $\pm$ 0.124	1.88	3.54	0.342 $\pm$ 0.146	7.17	51.39
	Dam	0.356 $\pm$ 0.165	3.64	13.27	0.090 $\pm$ 0.139	3.67	17.89
	Sire + dam	0.225 $\pm$ 0.086	4.10	16.81	0.216 $\pm$ 0.065	8.06	64.89

variances for viability and among-dam variances for developmental time, whereas dam-based estimates for the first trait and sire-based estimates for the second trait seemed not to be affected by temperature.

Discussion

Rearing flies under low-temperature stress had a strong effect on phenotypic variation. All phenotypic variances and coefficients of variation were significantly higher at 13°C than at 25°C. The same pattern was found for environmental variation estimated via among-progeny variance. The estimates of genetic variation did not reveal statistically significant differences between the two environments. However, the pattern of their values, considered in the context of results from other experiments on *D. melanogaster*, indicates the possibility of changes in genetic variation at the stressful temperature.

The effect of extreme low temperature on the same set of traits has been recently investigated by Imasheva *et al* (1998), who analysed quantitative genetic variation using the isofemale line technique. In their work, among-line variances for all five traits tended to be higher at 12°C than at 25°C. In other isofemale line studies, in which thorax and wing length were examined (de Moed *et al*, 1997; Karan *et al*, 1999; Imasheva *et al*, 2000), a clear-cut trend for an increase in the among-line variances at low temperatures was also demonstrated. However, in the experiment with parent-offspring comparisons Sgrò and Hoffmann (1998) did not find a difference between 14°C and 28°C (the latter seems to be a suboptimal rather than a stressful temperature; see for example David *et al*, 1983) in the genetic variance of wing length, while for developmental time this variance was significantly lower at 14°C.

In our study, we considered three variances that reflect genetic variation. The among-sire (parental half-sib) variance is similar to the genetic variance from the parent-offspring regression analysis and includes, in addition to the additive component, a small proportion of non-addi-

tive (epistatic) variance (Falconer and Mackay, 1996). The among-dam variance contains the same genetic components as the variance among isofemale lines (additive, dominance, epistatic and the variance due to maternal effects) but with a relatively smaller proportion of the additive variance. The sum of the among-sire and among-dam variances gives the full-sib variance, which is equivalent to the variance among isofemale lines. We found that this sum tended to be higher at the low stressful temperature for all traits, which is in good agreement with the behaviour of among-line variances in the isofemale line studies by de Moed *et al* (1997), Imasheva *et al* (1998, 2000) and Karan *et al* (1999). Moreover, the trend for changes in the among-sire variances for thorax length, wing length, bristle number and viability also coincides with that for the among-line variances reported by the above-mentioned authors. These facts indicate that for these traits the estimates of the additive variance across temperatures based on isofemale line data are quite reasonable and the level of additive genetic variation is likely to increase under low-temperature stress. However, for developmental time, the trend for change in the among-sire variance is consistent with a decrease in genetic variance in the parent-offspring comparisons by Sgrò and Hoffmann (1998) and this warns against using the isofemale line technique to investigate changes in the additive genetic variance in this trait. Our analysis revealed a trend for an increase in the among-dam variance of developmental time in the stressful environment that may be related to the enhanced level of non-additive genetic variation.

In the studies of de Moed *et al* (1997), Imasheva *et al* (1998, 2000) and Sgrò and Hoffmann (1998), heritabilities showed the same trends for changes across temperatures as the genetic variances on which they were based. However, in the present experiment a more complicated pattern was found. The estimates based on the among-sire and among-dam variances were more variable than those obtained from the sum of the two components (ie, full-sib estimates) that reflected opposite trends of changes for

among-sire and among-dam variances within some traits. For thorax length, developmental time and viability, the full-sib estimates seemed to be equal and only for wing length and bristle number did they show a weak trend for changes in the same direction as the sire-based heritabilities (decreased for wing length and increased for bristle number). The sire-based heritabilities, which are theoretically the least biased estimates of the narrow heritability in the half-sib design (Falconer and Mackay, 1996), tended to be higher at the stressful temperature for bristle number and viability and lower for wing length and developmental time, while for thorax length they appeared not to be affected by temperature. Such different trends can be explained by the fact that an increase in environmental variance (which enters the denominator in the expression for heritability) under stress was relatively greater for some traits than for others. Thus, our data illustrates a common situation where evolutionary implications cannot easily be drawn from changes in heritability estimates alone.

To overcome this difficulty, Houle (1992) proposed other measures of the ability of a population to respond to selection, the coefficient of genetic variation (CV_A) and evolvability (I_A). These indices may also be preferable to the additive genetic variance, as they are not affected by trait size. We considered CV_A and I_A based on sire, dam and sire + dam components of genetic variance. Regrettably, standard errors for these estimates are not known and it was not possible to carry out statistical comparisons between environments. Generally, changes in different CV_A and I_A estimates showed the same trends as the variance components used for their calculation with the exception of two cases: the among-sire variability for developmental time and the among-dam variability for viability seemed to be not influenced by temperature when estimated with CV_A and I_A . Thus, we can say that, under low temperature stress, the level of additive genetic variation tends to be higher for thorax length, wing length, bristle number and viability, while for developmental time an increase in nonadditive genetic variation may occur.

The same trends in behaviour of genetic variation in a stressful environment have been recently found in the same *D. melanogaster* stock for the above-listed traits at 32°C and at 25°C (Bubliy and Loeschcke, 2001). A similar situation was also reported in the study of Imasheva *et al* (1998), in which among-line variances for the same traits, except developmental time, tended to increase both at 12°C and 31°C compared with 25°C. In addition, the among-line variances and coefficients of genetic variation of morphological traits analysed in *D. melanogaster* by Karan *et al* (1999) and Imasheva *et al* (2000) tended to be higher at temperature extremes. All these data suggest that high- and low-temperature stress may affect genetic variation of a trait in the same way and for most traits considered here the effect of stress is in agreement with the hypothesis (Hoffmann and Parsons, 1991) of an increase in the level of additive genetic variation in stressful environments.

The difference between developmental time and other traits in response of their genetic variation to the temperature stress can be viewed as an indication of trait-specific effects of extreme temperatures. On the other hand, there is evidence that effects of some stressors on the examined morphological traits may not be consistent with the aforementioned hypothesis. It has been shown (Hoffmann and

Schiffer, 1998; Bubliy *et al*, 2000, 2001) that nutritional stress in *D. melanogaster* did not change or even decreased additive genetic variation for wing length and sternopleural bristle number, while for thorax length this variation displayed a clear trend to be higher under poor nutrition. These facts warn against generalizations about behaviour of genetic variation based on results of testing using single traits and stressors.

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