

Stress temperatures and quantitative variation in *Drosophila melanogaster*

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Using an isofemale line analysis, we analysed the consequences of extreme rearing temperatures for genetic variation in quantitative characters in *Drosophila melanogaster*. Three types of characters were used: life history (viability and developmental time), body size (thorax length and wing length) and meristic (number of sternopleural chaetae and number of arista branches). Phenotypic variation significantly increased under stress conditions in all morphological characters studied; for viability, it increased at the low stress temperature. Genetic variation, measured by the coefficient of intraclass correlation, was generally higher at both low and high stress temperatures for thorax length and sternopleural chaeta number. For wing length and viability, genetic variation was higher at the low extreme temperature. No consistent trend was found for genetic variation in arista branch number and developmental time. Our results agree with the hypothesis that genetic variation is increased in stressful environments. A possible mechanism underlying this phenomenon is briefly discussed.

Keywords: *Drosophila melanogaster*, life history, morphological characters, stress, variation.

Introduction

The genetic response of populations to changing environments is one of the key issues of population biology. Of particular interest are situations where the environmental conditions are extreme, i.e. where they are experienced as stressful. Understanding the genetic changes underlying responses to such challenges may shed light on mechanisms enabling organisms to survive drastic environmental changes, including those induced by human activity.

It has been suggested that stressful environments induce an increase in both phenotypic and genetic variation in quantitative characters, particularly in those related to fitness (Parsons, 1982, 1987, 1989; Hoffmann & Parsons, 1991). This increase in variation is likely to be manifested under severe stress when mortality in populations is high. The evolutionary implications of this phenomenon are obvious. By increasing the amount of genetic variation expressed in the population, stress would increase the adaptive potential of this population and thereby the probability of its survival in adverse environments.

At the phenotypic level, an increase of variation resulting from stress has been demonstrated for

several quantitative characters in *Drosophila*. Rearing under stressful conditions has been shown to increase phenotypic variation in developmental time (Parsons, 1961; Gebhardt & Stearns, 1988, 1992), dry weight at eclosion (Gebhardt & Stearns, 1988), thorax length (Robertson, 1960; Imasheva *et al.*, 1997), wing length (Tantawy & Mallah, 1961; David *et al.*, 1994; Lazebny *et al.*, 1996), ovariole number (Delpuech *et al.*, 1995), and sternopleural chaeta number (Parsons, 1961; Imasheva *et al.*, 1997). Types of stress used were extreme temperatures (Parsons, 1961; Tantawy & Mallah, 1961; David *et al.*, 1994; Imasheva *et al.*, 1997), deficient larval diet (Robertson, 1960; Gebhardt & Stearns, 1988, 1992), and larval crowding (Parsons, 1961; Lazebny *et al.*, 1996).

The increase of phenotypic variance in *Drosophila* populations reared under adverse environmental conditions can result from disrupted developmental homeostasis, from genetic causes, or both. The former cause seems plausible in view of accumulating evidence that fluctuating asymmetry of morphological characters, which is a conventional measure of disturbances of developmental stability, tends to increase with deteriorating environment (Markow, 1995). However, the effect of stress on genetic variation is under dispute (Barton & Turelli, 1989) as the

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available evidence is less clear-cut and the results are often inconsistent (see, e.g. Murphy *et al.*, 1983; Gebhardt & Stearns, 1988, 1992).

The aim of the present study was to test the effects of rearing temperature on genetic variation of quantitative characters of *Drosophila melanogaster*. We scored three types of characters: (i) life history characters, i.e. fitness components (viability and developmental time); (ii) body size characters (thorax and wing length); in *Drosophila*, these are closely associated with different aspects of fitness: fecundity and mating success (see Partridge *et al.*, 1987; Partridge, 1988 and references therein); (iii) characters that are less closely associated with fitness (number of sternopleural chaetae and arista branches). To estimate the genetic component of variation, the isofemale line technique was employed (Hoffmann & Parsons, 1988).

Materials and methods

Isofemale lines were set up from inseminated females collected in March 1995 near Armidale, New South Wales, Australia. In this experiment, we used 10 isofemale lines. The lines were maintained in the laboratory on standard *Drosophila* medium at 25°C. Each line was maintained as a separate culture (≈ 100 flies per culture) and transferred to fresh medium every two weeks; several dozens of flies were used to start each generation. About 15 generations after establishing the lines, mass cultures of the lines were started. Two bottles were set up for each line, with about 10 pairs of parents for each bottle. The newly emerged flies from each isofemale line were placed in separate population cages (≈ 700 – 800 flies per cage). The flies were aged for seven days; fresh medium supplemented with live yeast was provided every other day. Then the flies were allowed to oviposit on Petri dishes supplemented with live yeast. After 4 h of oviposition, the Petri dishes were removed from the cages and incubated for 24 h at 25°C. First instar larvae were collected from the Petri dishes and transferred to vials with fresh medium with 50 larvae per vial. For each isofemale line, 40 vials were established. The vials were randomly distributed between temperature regimes with five vials per line per temperature.

Four temperature regimes were used: 12°, 21°, 25° and 31°C. The two extreme temperatures are known to be stressful for *D. melanogaster* (David *et al.*, 1983).

Emerging flies were collected and counted every 12 h until the end of emergence (the emergence was considered finished when no flies had emerged for

72 h). All flies were stored in fixing liquid (1/2 glycerine: 1/2 ethanol) separately for each vial culture. From all flies emerging from each vial, five females were randomly chosen for analysis.

As in our previous work (Imasheva *et al.*, 1997), four morphological characters were scored: thorax length, wing length (metrical characters) and sternopleural chaeta number and arista branch number (meristic characters). Thorax length and wing length were measured as described in Imasheva *et al.* (1997). Bilateral characters were measured on the left side of the fly. All measurements were made with an ocular micrometer and given in arbitrary micrometer units (1 mm = 70 units).

As life history characters, we estimated viability and developmental time. Viability was calculated as the percentage of flies that emerged from the vial relative to the number of first instar larvae used to set up the cultures. Developmental time was estimated as the time interval in hours from the midpoint of the oviposition period to the recorded time of emergence.

All statistical analyses were carried out with SYSTAT (Wilkinson, 1988). In the two-way nested ANOVA for morphological characters, we used a balanced design for all temperatures except 12°C. At this temperature, high mortality led to an unbalanced number of individual data points as sometimes fewer than five females emerged from a vial. In this case, variance components were calculated using the procedure for unequal sample sizes; significance was tested using the Satterthwaite approximation (Sokal & Rohlf, 1981).

The proportion that genetic variation comprised of the total variation was measured by the coefficients of intraclass correlation (Falconer, 1981; Hoffmann & Parsons, 1988), which were estimated in the one-way ANOVA after combining data for cultures within each isofemale line. Data on viability (proportions) were arcsine-transformed for ANOVA to improve normality.

Confidence limits for coefficients of variation were calculated according to Sokal & Rohlf (1981; p. 147).

Results

Morphological characters

The reaction norms of the four morphological characters examined are presented in Fig. 1. As expected, temperature had a significant and large effect on the means of all the characters. The shapes of the reaction norm curves were similar to those reported earlier (David *et al.*, 1983; Imasheva *et al.*,

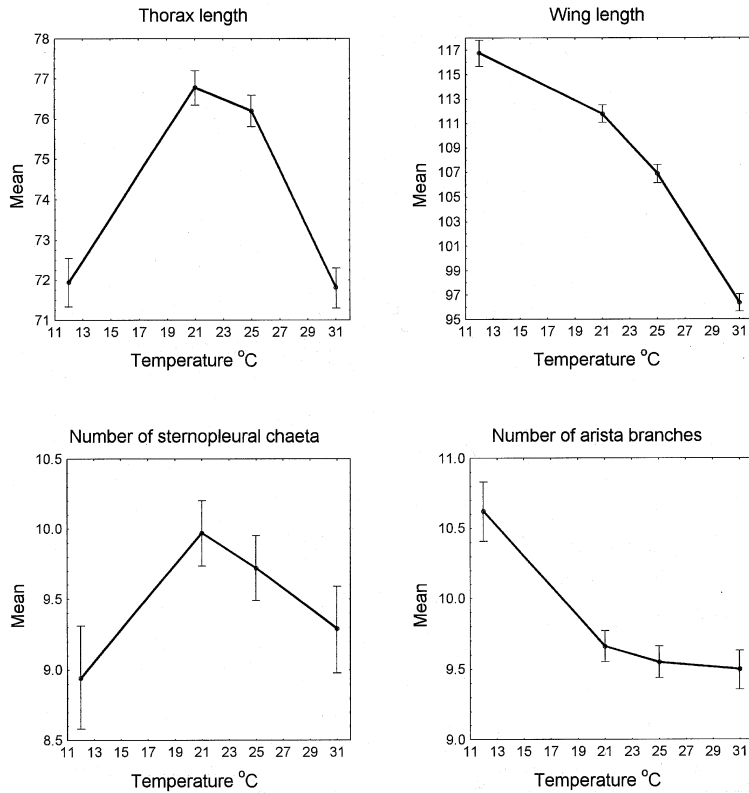


Fig. 1 Reaction norms of four morphological characters in relation to rearing temperature in *Drosophila melanogaster*. Here and in further figures, error bars are 95% confidence intervals.

1997). In all the characters, these shapes were different and nonlinear.

Phenotypic variation of the four morphological characters examined (thorax length, wing length, sternopleural chaetae number and arista branch number) in relation to rearing temperature is presented in Fig. 2 (the data for isofemale lines were combined). To estimate phenotypic variation, we used the coefficient of variation (CV) rather than the variance, because the variances of some of the characters were correlated with the means. In all the characters examined except wing length, CV showed a significant increase at both extreme temperatures; in wing length, the effect of high temperature was not significant. Note that at 12°C, CVs were somewhat higher than at 31°C.

The results of the two-way nested ANOVA of the morphological characters are presented in Table 1. The isofemale line effect on variation of the characters was highly significant ($P < 0.001$, except $P < 0.01$ for arista branch number at 12°C, and $P < 0.05$ for thorax length at 21°C). No significant culture effects were observed except in two cases: thorax length at 12°C ($P < 0.01$) and wing length at 25°C ($P < 0.05$).

Table 2 gives estimates of the intraclass correlation coefficients (r_i) and between-line components of variance for each character at each temperature esti-

mated from the one-way ANOVA (the data for cultures within each line were pooled). No statistically significant differences between temperatures were observed for either parameter. However, the general trend for thorax length and sternopleural chaeta number was for higher values of the intraclass correlation coefficients at both extreme temperatures. For wing length, the coefficient of intraclass correlation was higher at the low extreme temperature. For arista branch number, intraclass correlation coefficients did not exhibit any consistent pattern of change with regard to temperature. The between-line component of variance showed the same trend as r_i in all the characters examined.

Life history characters

Mean values of viability and developmental time at the four rearing temperatures are presented in Table 3. Viability was higher at the high than at the low extreme temperature ($t = 7.9$; $P < 0.001$). As viability is an indicator of stress, we can assume that the stress was stronger at 12°C than at 31°C. Viability estimates at the intermediate (optimal) temperatures were also rather low (0.66 at 21°C and 0.74 at 25°C). A possible explanation of this is inbreeding depression of the isofemale lines, which

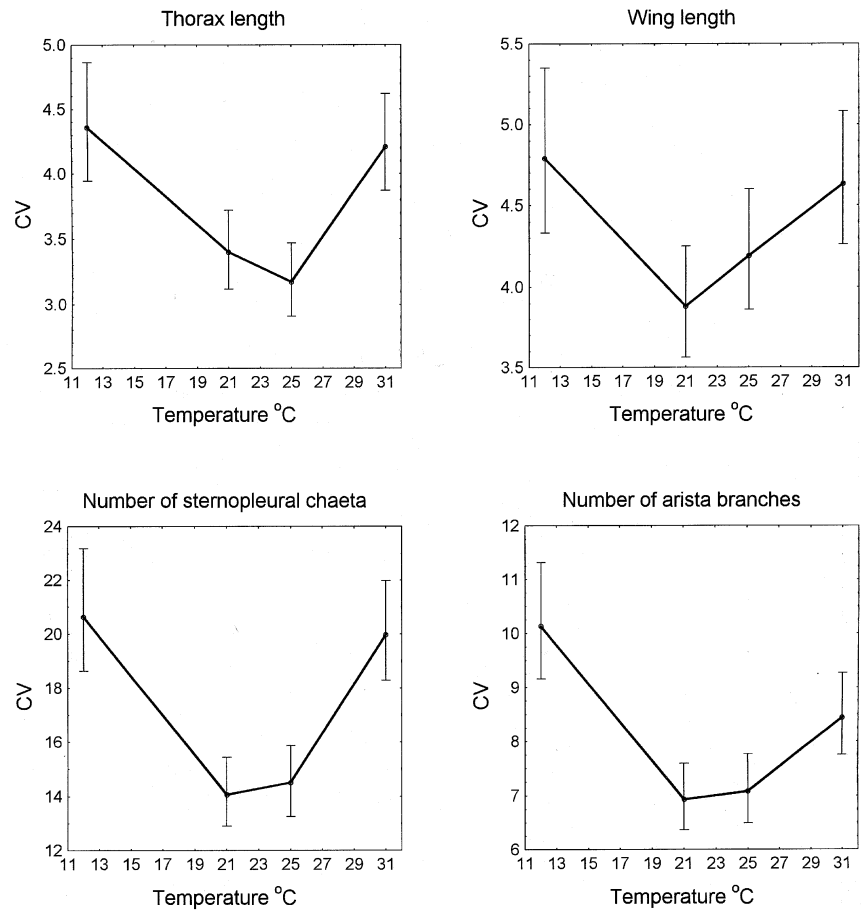


Fig. 2 Phenotypic variation of four morphological characters at different rearing temperatures in *Drosophila melanogaster*.

Table 1 Two-way nested ANOVA for four morphological characters in *Drosophila melanogaster* at different rearing temperatures

Character	Source of variation	12°C		21°C		25°C		31°C	
		d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS
TL	Lines	9	59.210***	9	18.304*	9	30.031***	9	56.337***
	Cultures	36	11.502**	40	7.614	40	4.118	40	9.535
	Error	131	6.067	200	6.118	200	5.098	196	6.914
WL	Lines	9	337.847***	9	217.432***	9	186.820***	9	195.039***
	Cultures	36	18.225	40	11.668	40	19.518*	40	15.232
	Error	131	13.796	200	11.232	200	12.700	196	12.833
SCN	Lines	9	38.524***	9	21.723***	9	23.698***	9	43.537***
	Cultures	36	1.157	40	1.316	40	1.658	40	2.079
	Error	131	1.598	200	1.208	200	1.490	196	1.873
ABN	Lines	9	4.255**	9	3.300***	9	3.376***	9	4.692***
	Cultures	36	0.838	40	0.232	40	0.386	40	0.602
	Error	131	1.030	200	0.364	200	0.340	196	0.466

TL, thorax length; WL, wing length; SCN, sternopleural chaeta number; ABN, arista branch number.

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

Table 2 Coefficient of intraclass correlation ($r_i \pm SE$) and the between-line component of variance (V_B) for four morphological characters in *Drosophila melanogaster* at different rearing temperatures

Character	12°C		21°C		25°C		31°C	
	r_i	V_B	r_i	V_B	r_i	V_B	r_i	V_B
TL	0.294 ± 0.115	2.999	0.070 ± 0.048	0.477	0.169 ± 0.081	1.004	0.213 ± 0.093	2.050
WL	0.559 ± 0.125	18.629	0.422 ± 0.124	8.245	0.333 ± 0.115	6.919	0.358 ± 0.119	6.704
SCN	0.586 ± 0.122	2.134	0.401 ± 0.122	0.820	0.369 ± 0.120	0.887	0.470 ± 0.125	1.826
ABN	0.160 ± 0.085	0.188	0.257 ± 0.102	0.118	0.258 ± 0.103	0.121	0.259 ± 0.103	0.176

TL, thorax length; WL, wing length; SCN, sternopleural chaeta number; ABN, arista branch number.

were maintained in the laboratory for a number of generations.

In Fig. 3, mean estimates of phenotypic variation in viability and developmental time at the four rearing temperatures are given. Phenotypic variation in viability was significantly higher at 12°C, but did not exhibit any differences among the remaining temperatures including 31°C. Variation in developmental time sharply decreased at 25°C, but was not significantly different among the other temperatures.

The one-way ANOVA of the life history characters (Table 4) demonstrated a highly significant effect of isofemale lines in both characters at all temperatures ($P < 0.001$) except for viability at 25°C and developmental time at 31°C ($P < 0.05$). Table 5 gives intraclass correlation coefficients and the between-line component of variance for viability and develop-

mental time at the four temperatures. The differences between temperatures and between characters were not statistically significant. In general, the intraclass correlation coefficient of viability was the highest at 12°C and dropped at 25°C. For developmental time, it was also the highest at 12°C and steadily decreased with an increase in temperature.

Discussion

A significant increase of phenotypic variation at both extreme temperatures was recorded for all morphological characters examined except for wing length at high temperature. This confirmed and extended the results of our earlier work (Imasheva *et al.*, 1997), in which we reported an increase in phenotypic variation of the same characters with temperature, but

Table 3 Means \pm SE of viability and developmental time in *Drosophila melanogaster* at different rearing temperatures

Character	12°C	21°C	25°C	31°C
Viability	0.21 ± 0.020	0.66 ± 0.017	0.74 ± 0.016	0.42 ± 0.017
Time of development	938 ± 2.12	271 ± 0.33	220 ± 0.20	187 ± 0.30

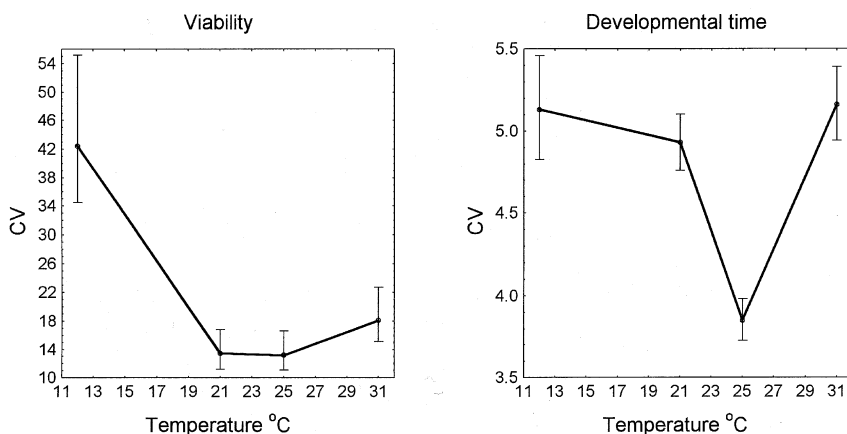
**Fig. 3** Phenotypic variation of viability and developmental time at different rearing temperatures in *Drosophila melanogaster*.

Table 4 One-way ANOVA for viability and time of development in *Drosophila melanogaster* at different rearing temperatures

Character	Source of variation	12°C		21°C		25°C		31°C	
		d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS
Viability	Lines	9	432.81***	9	161.60***	9	121.00*	9	150.90***
	Error	40	45.82	40	30.02	40	48.57	40	30.11
Time of development	Lines	9	0.00528***	9	0.00200***	9	0.00053***	9	0.00062*
	Error	39	0.00058	40	0.00018	40	0.00008	40	0.00028

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

this increase was not statistically significant for sternopleural chaeta number. With life history characters, however, the situation was less clear-cut. For viability, phenotypic variation increased only at the low extreme temperature; for developmental time, there was a sharp decrease at 25°C, but variation was high at all other experimental temperatures including 21°C.

Estimates of additive genetic variation (represented by coefficients of intraclass correlation) for two of four morphological characters examined (thorax length and sternopleural chaeta number) were generally higher at both extreme temperatures although no significant differences between temperatures were shown, because of the large standard error inherent to this estimate. For wing length, the intraclass correlation coefficient was higher at the low extreme temperature. As this trend was followed by the between-line component of variance, it was caused by changes in the additive genetic variance rather than in the environmental one. For arista branch number, no effect of stress on genetic variation was detected. As to life history characters, no clear trend of changes with temperature appeared for either of them. For viability, the coefficient of intraclass correlation was higher at the low extreme temperature and low at all other temperatures. For developmental time, it progressively decreased with temperature being lowest at the high stress temperature.

Thus, the most clear-cut effect of stress on genetic

variation was obtained for thorax length and sternopleural chaetae, whereas for life history characters the pattern was not clear even in the case of phenotypic variation.

Literature evidence dealing with stress effects on variation of quantitative characters in *Drosophila* is rather scarce. Murphy *et al.* (1983) analysed heritabilities of life history characters of *D. simulans* at different rearing temperatures. For three out of five analysed characters, consistent effects of temperature on the narrow-sense heritabilities were lacking; based on this, the authors concluded that their results were in conflict with the suggestion of an increase in genetic variation under stressful conditions. Heritabilities of two characters in their study (instantaneous birth rate and average per day fecundity) were higher at the extreme than at the intermediate temperatures. The range of temperatures used by these authors (20°, 24°, 28° and 30°C) included only high stress temperatures (30°C and possibly 28°C) and the increase in heritabilities of some characters was recorded at both temperature extremes. Because of this and of large standard errors of heritability estimates, the results of Murphy *et al.* seem inconclusive.

Using a diallel experimental design, Gebhardt & Stearns (1992) estimated genetic and environmental components of variance for developmental time and dry weight at eclosion in *D. melanogaster* at different temperatures and yeast concentrations in nutritional medium. No trend in genetic variance or heritability

Table 5 Coefficient of intraclass correlation (r_i (SE)) and the between-line component of variance (V_B) for viability and time of development in *Drosophila melanogaster* at different rearing temperatures

Character	12°C		21°C		25°C		31°C	
	r_i	V_B	r_i	V_B	r_i	V_B	r_i	V_B
Viability	0.628 ± 0.138	77.398	0.467 ± 0.161	26.315	0.230 ± 0.156	14.488	0.445 ± 0.163	24.159
Time of development	0.626 ± 0.139	0.00096	0.673 ± 0.127	0.00037	0.518 ± 0.156	0.00009	0.196 ± 0.151	0.00007

of either character was observed for temperature; additive genetic variance of developmental time was generally higher on poor medium, but this was not demonstrated for weight. In experiments with *D. mercatorum*, low concentrations of yeast in the medium also resulted in an increase in genetic variance of developmental time but not of weight (Gebhardt & Stearns, 1988). The lack of temperature effect on genetic variation might be explained by the fact that these authors did not use temperatures that could cause extreme stress. Out of three temperature regimes they used (22°, 25° and 28°C), only one (28°C) can be considered very mildly stressful.

David *et al.* (1994) reported a slight increase of the coefficient of intraclass correlation of wing length and thorax length in *D. melanogaster* reared at 12°C and 31°C compared to that at intermediate temperatures.

Barker & Krebs (1995) examined the effect of temperature of development on genetic variation in wing length, thorax length, and the wing/thorax ratio in *D. aldrichi* and *D. buzzatii*. In both species, heritabilities for the body size characters were higher in stressful environments; in *D. aldrichi*, the additive genetic variance also increased at the extreme temperatures.

The results of the present work together with literature data suggest that the effect of stress on variation is different for different characters. The highest and most clear-cut effect is observed for body size, i.e. thorax length and wing length. As it has been repeatedly reported by several authors (David *et al.*, 1994; Barker & Krebs, 1995; Lazebny *et al.*, 1996; the present work), this effect can be considered as established. We also recorded the same effect for another morphological character, sternopleural chaeta number, although in this case it was less marked. By contrast, searches for the effect of stress on fitness characters often seem to give equivocal results (Murphy *et al.*, 1983; Gebhardt & Stearns, 1992 — temperature effects; the present work). This is somewhat surprising as hypotheses predicting an increase of variation at stress (Parsons, 1982; Hoffmann & Parsons, 1991; Zhivotovsky *et al.*, 1996, 1997) put special emphasis on fitness components.

However, our results do not contradict the suggestion on the prevailing role of fitness-related traits in the evolutionary stress response. Body size in *Drosophila* is a character that is strongly correlated with individual fitness. In addition, it is easily and accurately measured and has high heritability. This facilitates the detection of a stress-induced increase in

the expression of variation of this character. In life history traits, which have low heritability and a high environmental component to the total variation, the effect of stress may be obscured by environmental 'noise.'

Another point to note is that, in *D. melanogaster*, cold stress seems to have a stronger effect on variation than heat stress. Both stressful temperatures used in our work were at the extreme physiological limit possible for the survival of the species. If only one temperature had an effect on variation under stress it was always the low temperature. In the present study, this was shown for wing length and viability; the same has been previously recorded for phenotypic variation of ovariole number (David *et al.*, 1994) and wing length (Imasheva *et al.*, 1997).

A number of stressful agents (including high and low temperatures) induce an increase in genetic variation via increasing mutation and/or recombination rates (see Hoffmann & Parsons, 1997 for references). However, in the present case, where variation was measured in the same generation that was subjected to stress, we deal with a manifestation of the variation that already existed in the population but did not express itself under normal conditions. Hoffman & Parsons (1991) discuss several mechanisms that can underlie this phenomenon. A likely explanation is the past history of selection. Natural selection (directional or stabilizing) acting on a trait under nonstressful conditions commonly experienced by a species will lead to a decrease in variation. However, genes that are not selected under normal conditions may contribute to variance in extreme conditions. If we assume (as is probably the case) that highly stressful situations are only occasionally experienced in nature, then the expression of phenotypic and genetic variance under these conditions may increase. The same predictions are made by Zhivotovsky *et al.* (1996, 1997) in their theory of evolution in a spatially heterogeneous environment. According to this theory, low values of genotypic variance of adaptive characters (and high values of fitness) are observed in environments which predominantly occur in the evolutionary history of the species and are the most productive in terms of survival and reproduction. By contrast, in rare and poor environments, fitness decreases and genotypic variance is higher.

Summarizing, the results of the present work are in agreement with the view that exposure to stressful environments can have a substantial effect on the expression of phenotypic and genetic variation. The effect of stress on genetic variation seems to be character-specific. However, our genetic data are

only tentative, and the problem of genetic effects of stress is far from solved. Further experimental evidence on the issue is clearly needed.

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