Variations in morphological and life-history traits under extreme temperatures in *Drosophila ananassae*

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Using half-sib analysis, we analysed the consequences of extreme rearing temperatures on genetic and phenotypic variations in the morphological and life-history traits of *Drosophila ananassae*. Paternal half-sib covariance contains a relatively small proportion of the epistatic variance and lacks the dominance variance and variance due to maternal effect, which provides more reliable estimates of additive genetic variance. Experiments were performed on a mass culture population of *D. ananassae* collected from Kanniyakumari (India). Two extremely stressful temperatures (18°C and 32°C) and one standard temperature (25°C) were used to examine the effect of stressful and non-stressful environments on the morphological and life-history traits in males and females. Mean values of various morphological traits differed significantly among different temperature regimens in both males and females. Rearing at 18°C and 32°C resulted in decreased thorax length, wing-to-thorax (w/t) ratio, sternopleural bristle number, ovariole number, sex comb-tooth number and testis length. Phenotypic variances increased under stressful temperatures in comparison with non-stressful temperatures. Heritability and evolvability based on among-sires (males), among-dams (females), and the sum of the two components (sire + dam) showed higher values at both the stressful temperatures than at the non-stressful temperature. These differences reflect changes in additive genetic variance. Viability was greater at the high than the low extreme temperature. As viability is an indicator of stress, we can assume that stress was greater at 18°C than at 32°C in *D. ananassae*. The genetic variations for all the quantitative and life-history traits were higher at low temperature. Variation in sexual traits was more pronounced as compared with other morphometric traits, which shows that sexual traits are more prone to thermal stress. Our results agree with the hypothesis that genetic variation is increased in stressful environments.

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1. Introduction

There has been growing awareness of the fact that environmental stress plays a major role in the evolution and adaptation of living organisms. Stressful environmental conditions can be defined as those that lead to a sharp reduction in the fitness in populations. Environmental stress constitutes changed environmental conditions that cause a drastic reduction in reproductive output, and persistence of the conditions lead to permanent damage (Hoffmann and Hercus 2000). It has been suggested that stressful environments induce an increase in both phenotypic and genetic variations in quantitative characters, particularly in those related to fitness. This increase in variation is likely to be manifested under severe stress when mortality in the population 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. In particular, a change in genetic variation of quantitative traits induced by exposure to stressful factors is a focus of attention for evolutionary biologists. Evolutionary responses to selection depend on the amount of genetic variation expressed in the population. Because of this, the effect of environmental changes on the expression of genetic

Keywords. *Drosophila ananassae*; genetic variation; morphometric and life-history traits; stressful temperatures

Abbreviations used: CV, coefficient of variation; FA, fluctuating asymmetry; Hsp, heat-shock protein; w/t, wing/thorax

variation in quantitative traits has important evolutionary implications.

It has been proposed that some environmental conditions are likely to result in the expression of genetic variability, which can then be selected. This follows the early work of Waddington and others (*see* Waddington 1961) who argued that exposure to stressful environmental conditions can result in phenotypic changes due to the expression of new genetic variation, whose expression eventually remains 'switched on' in the absence of any stress. There is also evidence that heritable variation in quantitative traits can be increased by stressful conditions (Parsons 1987; Hoffmann and Parsons 1991). There is ample evidence indicating that the expression of genetic variation changes with changing environments. Numerous experiments have shown that the genetic and environmental components of the variance tend to change in adverse environments, which is often interpreted in terms of changes in heritability with stress level. According to a well-known hypothesis (Hoffmann and Parsons 1991), narrow-sense heritability, i.e. the proportion of the additive genetic variance in the phenotypic variance increases in ecologically extreme environments, thus promoting more rapid evolutionary change. An alternative hypothesis (Johnson and Frey 1967; Blum 1988) assumes that heritability decreases under stress, which may lead to deceleration of the evolutionary process. At present, it seems that neither of these hypotheses can fully account for the results of experiments comparing genetic variation between stressful and non-stressful environments (Sgro and Hoffmann 2004).

A related explanation is that genetic variation under conditions of stress may be increased because these conditions are novel for an organism. Because such conditions are not normally encountered, there is no history of selection on a trait decreasing levels of genetic variability by selecting against extreme phenotypes. This 'selection history' hypothesis has been advocated by a number of workers (Jink *et al.* 1973; Holloway *et al.* 1990; Kawecki 1995; Pigliucci *et al.* 1995) to explain changes in genetic variance in novel and often stressful environments. In *Drosophila,* numerous experiments have provided heritability estimates for a range of traits, and a few experiments have considered estimates under different conditions, particularly those resulting in stress. The evolution of natural populations is known to depend on the presence of additive genetic variation in quantitative traits.

Temperature is a critical determinant of the distribution and abundance of ectotherms (Andrewartha and Birch 1960). It affects most physiological functions and numerous mechanisms that compensate for temperature variation are known (Leather *et al.* 1993). Recently, the emphasis has been on investigating extreme conditions and tolerance to cold or heat stress (Hoffmann and Parsons 1991; Hoffmann *et al.* 2003). The behaviour of quantitative genetic variation in different environments has been the focus of extensive research on *Drosophila*, with growth temperature as the most common stress factor (Tantawy *et al.* 1964; Giesel *et al.* 1982; Murphy *et al.* 1983; Gebhardt and Stearns 1992; Neifakh and Hartl 1993; David *et al.* 1994; Barker and Krebs 1995; Noach *et al.* 1996; Imasheva *et al.* 1998; Sgro and Hoffmann 1998; Loeschcke *et al.* 1999; Karan *et al.* 1999; Bubliy *et al.* 2000; Bubliy and Loeschcke 2001, 2002). Generally, the evidence indicates that stressful temperature may affect genetic variation.

Drosophila ananassae, a cosmopolitan and domestic species belonging to the *ananassae* subgroup of the *melanogaster* species, is stenothermic and circumtropical in distribution. Previous studies in *D. ananassae* have shown the effect of temperature on survival and longevity (Sisodia and Singh 2002), various life-history and fitness traits (Yadav and Singh 2005), and fluctuating asymmetry (FA) (Vishalakshi and Singh 2008a), as well as the effect of short-term heat stress on survival and productivity (Sisodia and Singh 2006). In the present study, we consider the effect of environmental stress on genetic variation in *D. ananassae* 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 (Falconer and Mackay 1996) and thus provides more reliable estimates of the additive genetic variance (Bubliy and Loeschcke 2001, 2002). We aimed to test the effect of thermal stress on genetic and phenotypic variations in the quantitative characters of *D. ananassae.* As a stress factor, we examined high (32°C) and low (18°C) temperatures, which are close to the physiological tolerance limits of *D. ananassae*. We scored three types of characters: (i) life-history characters, i.e. fitness components (viability and developmental time); (ii) body size components (thorax length, wing length, wing/thorax [w/t] ratio, ovariole number, testis length and sex comb-tooth number); in *Drosophila,* these are closely associated with different aspects of fitness; and (iii) characters that are less closely associated with fitness (sternopleural bristle number). We followed the methods of Bubliy and Loeschcke (2001, 2002).

2. Materials and methods

2.1 Drosophila *stock*

In the present study, a mass culture stock was established from flies collected from Kanniyakumari, India in April 2006. Wild inseminated females were used to start 20 isofemale lines, which were maintained in a standard culture medium (containing agar-agar, dried yeast, maize powder, crude sugar, nipagin, propionic acid and plain water) for 25 generations (5 generations at 25°C and 20 generations at

20°C). To obtain flies for the experiment, a mass population was established and maintained as discrete generations at 15 pairs with regular mixing of adults among bottles.

2.2 *Experimental design*

From the third generation of the mass populations, a sample of 90 virgin males and 270 virgin females was taken and aged for 6 days. Each male was then placed in an individual food vial together with three females. The vial contained the standard medium supplemented with live yeast to enhance 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 the spoon to six new vials – size 3 x 1 inch, 1 inch = 25.4 mm (10 larvae per vial), three vials on the first day (block I) and another three on the second day (block II). All vials contained 7 ml of standard medium without live yeast. Immediately after transferring the larvae, the two sets of vials were placed at both the stressful temperatures, i.e. 18±0.5°C and 32±0.5°C and the other set at the control temperature of 25±0.5°C. Each set consisted of 540 vials and represented 30 sire families. Eclosed adults were collected and counted every 12 h until all the flies had emerged. Females and males from each vial were kept separately for the measurement of morphological traits.

2.3 *Measurement of traits*

Morphological traits such as thorax length, left wing length, w/t ratio, and sternopleural bristle number on the left side of each fly were measured (one male and one female per vial). Ovariole number and testis length were measured in females and males, respectively, and sex comb-tooth numbers were counted in males. The left wing of each etherised adult fly was kept horizontally and the length measured from the anterior cross vein to the distal tip of the third longitudinal vein. The thorax was measured from the anterior margin of the thorax to the posterior tip of the scutellum. All measurements were performed under a compound microscope fitted with an ocular micrometer at 50x magnification. The ocular micrometer has 100 divisions and one division of the micrometer is equivalent to 16.67 μ measured with the help of a stage micrometer. On the sternopleuron of males and females, two sets of bristles are present. The anterior bristles are arranged in an oblique row from the forecoxa towards the midline, whereas the transverse bristles run in a thin line toward the centre of the fly just anterior to the middle leg. The anterior and transverse sternopleural bristles were counted under a stereo binocular. The total number of sternopleural bristles was taken as the sum of the anterior and transverse bristles. W/t ratio was calculated from the data on wing and thorax lengths.

In females, the ovaries were dissected in insect saline (0.67% NaCl), stained with 2% acetocarmine stain, and mounted in 45% acetic acid; the ovariole number was counted under a microscope at 50x magnification. The sex combs in males of *D. ananassae* are characterised by several transverse rows of stout, blackish bristles on the ventral surface of the first, second and third tarsal segments of the prothoracic legs. The foreleg of males was dissected and mounted in insect saline and the number of teeth on the first (C1), second (C2) and third tarsal segments (C3) was counted under a microscope. The total sex comb-tooth number per leg includes the teeth on the C1, C2 and C3 segments. The testes along with the seminal vesicles were dissected out in insect saline (0.67% NaCl) without disturbing their integrity and then uncoiled carefully. The length of the testes was measured in the stretched condition by an ocular micrometer at 25x magnification.

Development 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 for use as an individual observation in statistical analyses. 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 culture. Thus, the number of observations for viability was equal to the number of vials.

3. Results

Mean values and phenotypic variances of the morphological and life-history traits examined in *D. ananassae* reared under stressful and non-stressful conditions are presented in table 1. Means of all the traits were lower under both the stressful conditions except wing length in both the sexes. Thermal stress had a strong effect on the phenotypic variation of the traits. Rearing at 18°C and 32°C resulted in decreased thorax length, sternopleural bristle number, ovariole number, testis length and sex comb-tooth number. Temperature had a significant effect on the means of all the characters. Development time was decreased at 32°C but increased at 18°C. Thus, the increase in wing length and development time appears to be trait specific. To estimate phenotypic variation, we used the coefficient of variation (CV). In all the characters examined (figures 1, 2 and 3), the CV showed a significant increase at both the extreme temperatures. The CV of thorax length, wing length, w/t ratio, sternopleural bristle number and testis length showed greater phenotypic variation at 18°C than at 32°C, but the

Table 1. Mean ± SE and phenotypic variance (s^2) of morphometric and life-history traits in *D. ananassae* reared at different temperatures

		18°C		25°C		32°C		
Trait	Sex	Mean ± SE	s^2	Mean ± SE	s^2	Mean ± SE	s^2	*F*
Thorax length	M	51.85±0.24	10.14	52.78±0.23	9.89	50.04 ±0.29	10.81	102.93**
	F	57.61±0.25	21.73	61.91±0.34	8.36	56.52±0.22	10.34	369.03**
Wing length	M	76.21±0.06	11.79	69.29±0.43	8.75	66.43±0.25	10.24	122.20**
	F	85.37±0.26	11.54	82.59±0.44	6.00	74.76±0.25	0.004	250.04**
Wing/thorax ratio	M	1.47±0.006	0.006	1.31±0.008	0.001	1.32±0.005	0.004	105.16**
	F	1.48±0.006	0.007	1.33±0.007	0.002	1.32±0.004	0.005	262.50**
Sternopleural bristle number	M	7.21±0.69	0.790	7.51±0.06	0.670	7.34±0.075	0.690	4.30*
	F	7.58±0.66	0.880	8.06±0.07	0.790	7.89±0.08	0.840	10.41**
Ovariole number	F	9.47±0.01	9.40	15.34±0.22	4.80	10.33±0.22	8.41	50.71**
Sex comb	M	29.5±0.24	17.96	35.16±0.32	9.5	30.01±0.33	13.47	122.39**
Testis length	M	46.56±0.64	82.29	57.98±0.59	62.35	52.78±0.81	69.84	48.61**
Development time (days)		26.54±0.10	5.17	9.14±0.01	0.02	7.26±0.05	3.25	142.72**
Viability		0.42±0.02	0.035	0.92± 0.01	0.006	0.57±0.017	0.012	172.41**

*P<0.01; **P<0.001.
M, male; F, female.

CV of ovariole number showed greater variation at 32°C than at 18°C.

The results of three-way nested ANOVA for females and males are shown in tables 2 and 3, respectively. Statistically significant among-sire and among-dam variation was found for all the traits in females at all the three temperature regimens. There was significant among-dam variation for all the traits among males at all the three temperatures. Among-block variations reflected the effect of a common environment. For all the traits studied under stressful and non-stressful environments, among-block variations, which reflect the effect of the common environment, were non-significant. In males, except for sex comb-tooth number and testis length, there was significant variation for among-sires at higher temperatures.

Tables 4 and 5 give estimates of heritability and evolvability based on the among-sire and among-dam variances as well as on the sum of the two components (sire + dam) for females and males, respectively. Heritability and evolvability tended to have higher values at both the stressful temperatures. For each trait, h^2 values were higher under stressful conditions. In females, h^2 sire values were higher for thorax length, w/t ratio and ovariole number at 18°C, for thorax length at 25°C and for thorax length and w/t ratio at 32°C. For males, h^2 sire values were higher for all the traits at 18°C, for all the traits except wing length at 25°C and similarly for all the traits at 32°C. Evolvability for morphological traits tended to have higher values at 18°C and 32°C regardless of which variance component (sire, dam, sire + dam) they were based on. Table 6 presents estimates of heritability and evolvability based on the among-sire and among dam variances as well as on the sum of the two components (sire + dam) for life-history traits. Values of heritability and evolvability are higher at both the extreme temperatures than at control temperature.

4. Discussion

A significant increase in phenotypic variation at both the extreme temperatures was recorded for all morphological traits. Rearing flies under stressful temperatures had a strong effect on phenotypic variation. Phenotypic variation was greater with regard to thorax length, wing length, ovariole number, sex comb-tooth number, testis length, development time and viability at both the extreme temperature regimens but phenotypic variation was lesser with regard to w/t ratio and sternopleural bristle number. There was also a significant increase in the CV under stressful conditions for all the morphological traits. These results are in complete agreement with those obtained earlier for *D. melanogaster* at extreme temperatures, which also show increased phenotypic variation in the analysed traits (Imasheva *et al.* 1997, 1998; Bubliy and Loeschcke 2001, 2002). A similar pattern was also found for nutritional stress (Imasheva *et al.* 1999; Bubliy *et al.* 2000, 2001 [low yeast concentration]) and stress combined with a nutritional component (De Moed *et al.* 1997 [low yeast concentration and temperature]; Hoffmann and Schiffer 1998; Woods *et al.* 1999 [low yeast

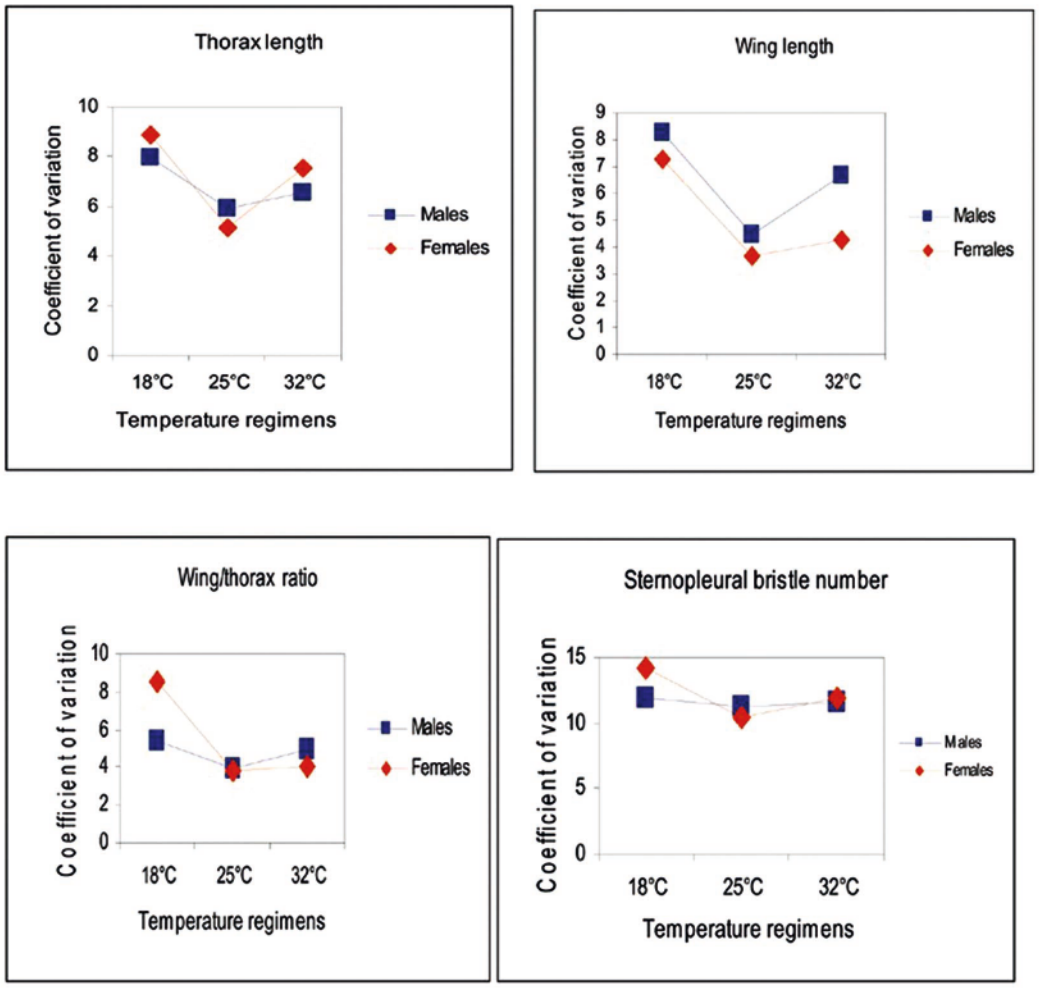


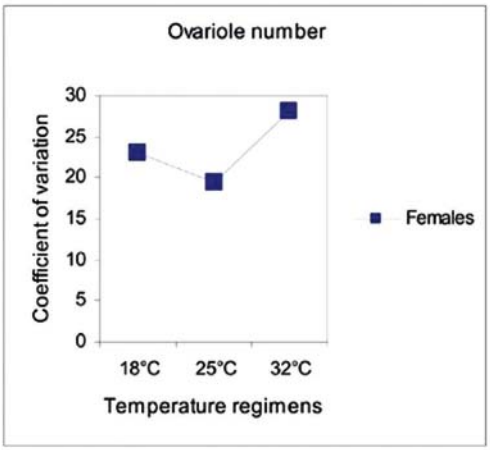
Figure 1. Phenotypic variation of morphometric traits at different rearing temperatures in *Drosophila ananassae.* SBN, sternopleural bristle number.

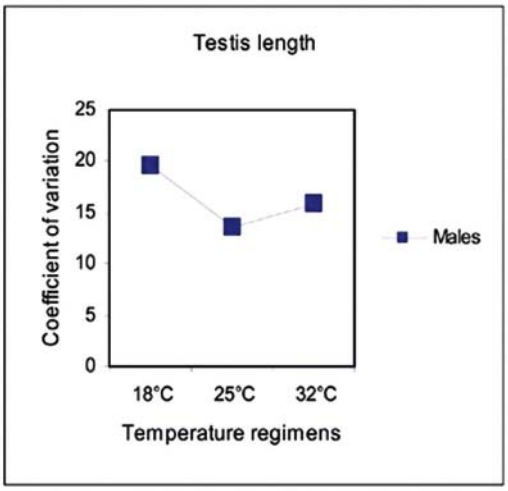
concentration, ethanol added to the medium and repeated cold shock]; Imasheva and Bubliy 2003 [larval densities], and Vishalakshi and Singh 2008b [nutritional and larval crowding]).

Estimates of additive genetic variation for all the morphometric characters examined were higher at both the extreme temperatures. It has been suggested that a stressful environment induces an increase in both phenotypic and genetic variations in quantitative characters, particularly those related to fitness (Parsons 1989; Hoffmann and Parsons 1991). In view of this, we further categorised morphometric characters into sexual and non-sexual traits. In females, ovariole number and in males, sex comb and testis length were taken as sexual traits. A review of the literature on the effects of stress on variation in quantitative characters showed that testis length has been taken as a quantitative trait for the first time. Phenotypic variation was highest in testis length at both the temperature regimens but the low temperature had a greater effect on testis length than the high temperature.

Both the size as well as structure of the testis in *Drosophila* is affected by temperature. High temperature makes the testis smaller and results in abnormal spermatogenesis but low temperature has the opposite effects. Testis size is highly sensitive to temperature especially in hybrids, but also in pure races in *D. subobscura* (Baker 1935). In *D. ananassae,* testis length was shorter at both the stressful temperatures, which indicated that spermatogenesis is highly abnormal in males having smaller testis. Whether Bergman's rule applied to ectotherms is an adaptive response or a physiological constraint is under debate (Partridge and Coyne 1997). Blanckenhorn and Hellriegel (2002) provide the first experimental evidence that the size of an important type of cell, i.e. sperm, increases (rather than decreases) with temperature in a cold-blooded animal, the yellow dung fly.

Imasheva *et al.* (1997) considered the effect of temperature on several meristic and metric traits in two *Drosophila* species. Although the data suggested a general overall increase in phenotypic variability at extreme temperatures,





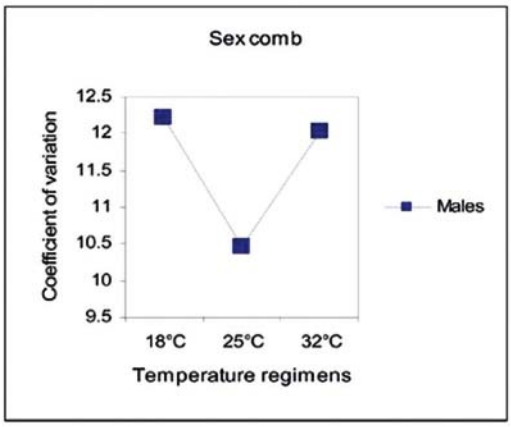
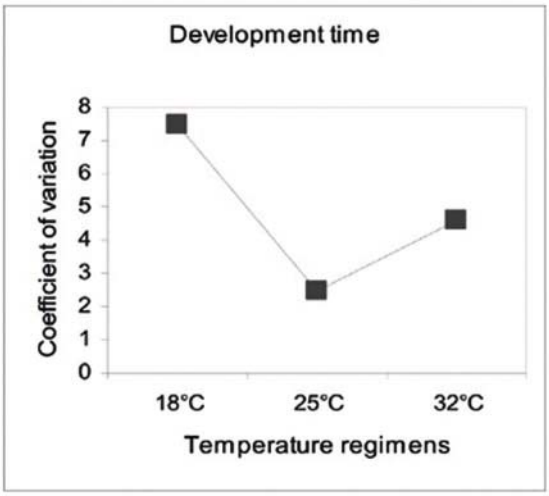


Figure 2. Phenotypic variation of sexual traits at different rearing temperatures in *Drosophila ananassae.*



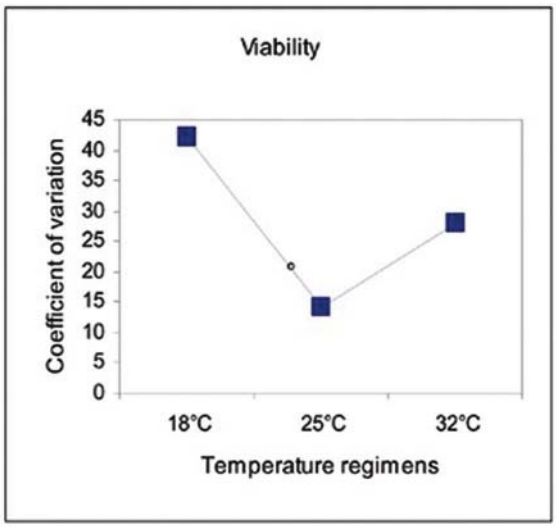


Figure 3. Phenotypic variation of life-history traits at different rearing temperatures in *Drosophila ananassae.*

there were often sharp changes in CVs even at favourable temperatures and weak changes under stress for some traits. Phenotypic variation is generally increased by exposure to extreme conditions, as in the case of characters such as thorax length, wing length, number of sternopleural chaeta and number of arista branches (David *et al.* 1994). There are several reasons for an increase in phenotypic variability (Parsons 1987; Hoffmann and Parsons 1991). It may reflect the expression of genetic variation at the phenotypic level due to exposure to novel or extreme environmental conditions; conversely, stressful conditions may increase phenotypic variability because of environmental effects; under stressful conditions, minor changes in environmental conditions may have a large effect on traits, whereas they may have little impact under more favourable conditions (Blum 1988).

We found that genetic and phenotypic variabilities were higher at both the extreme temperatures for sternopleural bristle number. Swindell and Bouzat (2006) studied the association between environmental stress, selection history and quantitative genetic variation using *D. melanogaster* and

Table 2. Three-way nested ANOVA for females of *D. ananassae* at different temperatures regimens

		18°C			25°C			32°C		
Traits	Source	df	ms	F	df	ms	F	df	ms	*F*
Thorax length	Among sires	24	41.28	2.72**	29	76.60	2.78***	26	25.83	1.71*
	Among dams	48	15.16	1.79*	60	27.51	2.44***	54	15.07	1.74*
	Among blocks	72	8.43	1.21	90	11.25	1.21	81	8.62	1.43
	Within blocks	288	6.92		360	9.25		324	6.00	
Wing length	Among sires	24	53.39	2.21**	24	53.39	2.21**	26	34.49	3.69***
	Among dams	48	24.07	2.12**	60	32.74	3.58***	54	20.34	2.02**
	Among blocks	72	11.33	1.24	180	9.12	1.20	81	0.02	1.17
	Within blocks	288	9.12		360	7.59		324	0.002	
Wing/thorax ratio	Among sires	24	65.23	1.97*	29	34.7	2.59***	26	3.08	59.23***
	Among dams	48	43.09	1.94*	60	13.41	2.19**	54	0.52	13.00***
	Among blocks	72	22.15	1.31	180	6.12	0.88	81	0.004	0.006
	Within blocks	288	6.85		360	7.1		324	0.66	
Sternopleural bristle number	Among sires	24	3.08	2.56***	29	95.67	2.24**	26	6.68	5.30 ***
	Among dams	48	1.20	2.79***	60	42.52	1.75*	54	2.43	1.88*
	Among blocks	72	0.43	0.81	180	24.25	0.66	81	1.29	1.95
	Within blocks	288	0.5		360	36.58		324	0.66	
Ovariole number	Among sires	24	18.13	2.09*	29	29.32	1.95*	26	85.09	6.35***
	Among dams	48	8.66	1.58*	60	15.03	1.52*	54	13.38	2.20**
	Among blocks	72	5.47	1.14	180	9.84	1.15	81	6.07	1.16
	Within blocks	288	4.78		360	8.55		324	5.20	

*$P<0.05$; **$P<0.01$; ***$P<0.001$. df, degrees of freedom; ms, mean square.

sternopleural bristle number as a model. High temperature stress was found to increase both additive genetic variance and heritability, but did not support the selection history hypothesis as an explanation for this effect. A large and significant increase in phenotypic variance was also found to occur under high temperature stress. Levels of environmental variation associated with sternopleural bristle number have often been found to increase under stress (Woods *et al.* 1999; Bubliy and Loeschcke 2002) and possibly contributed to the increase in bristle number that has been observed at high temperatures in several earlier studies (Tantawy and Mallah 1961; David *et al.* 1994; Imasheva *et al.* 1997; Loeschcke *et al.* 1999; Bubliy *et al.* 2000).

The w/t ratio is inversely proportional to wing loading and wing beat frequency. In our study, w/t ratio was higher at both the extreme temperatures. Barker and Krebs (1995) examined the effect of temperature on the development of genetic variation in wing length, thorax length and w/t ratio in *D. aldrichi* and *D. buzzatii.* In both the species, heritability for body size characters was higher in stressful environments. David *et al*. (1994) reported a slight increase in the coefficient of intraclass correlation of wing and thorax length in *D. melanogaster* reared at 12°C and 31°C compared with that at intermediate temperature. They also reported that the consistency of genetic differences over environments indicates that the response of w/t ratio to temperature is highly regulated, which indicates a strong relationship between w/t ratio and fitness.

In our study, we considered three variances that reflect genetic variation. Among-sire (parental half-sib) variance is similar to genetic variance from the parent–offspring regression analysis and includes, in addition to an additive component, a small proportion of non-additive (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 also that due to maternal effects) but with a relatively smaller proportion of 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 stressful temperatures for all the traits. Our findings support the results of De Moed *et al*. (1997), Imasheva *et al*. (1998, 2000), and Bubliy and Loeschcke (2001, 2002). The minimum requirements for an evolutionary change are the occurrence of natural selection and the presence of heritable

Table 3. Three-way nested ANOVA for males of *D. ananassae* at different temperatures

		18°C			25°C			32°C		
Traits	Source	df	ms	*F*	df	ms	*F*	df	ms	*F*
Thorax length	Among sires	24	34.84	1.66*	29	69.6	2.41**	18	88.74	4.65***
	Among dams	48	20.93	2.41***	60	32.49	2.08**	42	19.74	5.41***
	Among blocks	72	8.66	1.18	180	15.58	0.55	126	3.52	0.78
	Within blocks	288	7.29		360	28.11		252	4.50	
Wing length	Among sires	24	54.96	3.48***	29	104.43	1.79*	18	154.62	6.89***
	Among dams	48	15.76	1.66*	60	58.02	2.98***	42	22.46	2.68***
	Among blocks	72	9.48	1.32	180	19.44	0.56	126	8.37	0.84
	Within blocks	288	7.14		360	34.53		252	9.94	
Wing/thorax ratio	Among sires	24	0.301	2.25**	29	63.62	2.53**	18	0.004	2.01**
	Among dams	48	0.134	2.03**	60	25.05	2.85***	42	0.002	2.22***
	Among blocks	72	0.066	1.24	180	8.78	0.36	126	0.34	0.57
	Within blocks	288	0.65		360	24.15		252	0.003	
Sternopleural bristle number	Among sires	24	3.35	5.87***	29	24.78	1.83*	18	3.11	2.82**
	Among dams	48	0.57	1.67*	60	13.54	1.64*	42	1.10	3.23***
	Among blocks	72	0.34	0.52	180	8.25	0.69	126	0.34	0.57
	Within blocks	288	0.65		360			252		
Sex comb	Among sires	24	3.35	5.87***	29	135.34	1.82*	18	138.93	1.87
	Among dams	48	0.57	1.62*	60	74.25	2.01***	42	73.95	10.18***
	Among blocks	72	0.35	0.692	180	34.94	0.77	126	7.26	0.71
	Within blocks	288	0.65		360	45.37		252	10..21	
Testis length	Among sires	24	302.69	2.13*	29	95.07	1.67*	18	395.1	1.75
	Among dams	48	141.85	2.44**	60	56.78	1.63*	42	225.28	12.57***
	Among blocks	72	58.01	1.03	180	34.94	0.77	126	17.91	0.88
	Within blocks	288	55.85		360	45.37		252	20.41	

*P<0.05; **P<0.01; ***P<0.001. df, degrees of freedom; ms, mean square.

variation in the selected traits. It is well known that trait heritability is not constant but can vary with environmental conditions, as well as with changes in gene frequency. Other measures of genetic variability, particularly measures of evolvability that define the additive genetic variance relative to the mean, also vary with environmental conditions. Understanding this is important in determining the evolutionary potential and dynamics of populations inhabiting heterogeneous environments and in predicting the fate of populations under environmental change. Finally, changes in heritability due to genetic variance may occur because of the way genes control the response to environmental conditions.

Negative genetic correlations among traits are often used as evidence for trade-offs that can influence evolutionary trajectories in populations. While there may be evidence for negative correlations within a particular environment, genetic correlations can shift when populations encounter different environmental conditions. A fundamental assumption underlying the life history theory is that evolution is constrained by universal trade-offs between traits affecting fitness (for references, *see* Sgro and Hoffmann 2004). The thermotolerance effect of heat hardening, knock-down resistance to high temperature with and without heat hardening, and chill–coma recovery are important phenotypes of thermal adaptation in insects and other organisms. Noory *et al*. (2008) studied Quantitative Trait Loci for the thermotolerance effect of heat hardening, knock-down resistance to heat and chill–coma recovery in an intercontinental set of recombinant inbred lines of *D. melanogaster*. In contrast to morphological traits, heat stress appears to reduce rather than increase genetic variation in thermal stress-resistant lines. The heat-shock protein Hsp90 supports diverse but specific signal transducers and lies at the interface of several developmental pathways. Rutherford and Linquist (1998) found Hsp90 to be a

Table 4. Heritability (h^2) and evolvability (I_A) of morphometric traits among females of *D. ananassae* at different temperature regimens

		18°C		25°C		32°C	
Traits	Variance	h^2	$I_A \times 10^2$	h^2	$I_A \times 10^2$	h^2	$I_A \times 10^2$
Thorax length	Sire	0.552	2.45	0.379	1.99	0.539	2.97
	Dam	0.503	2.68	0.278	0.84	0.437	1.07
	Sire + Dam	0.512	1.72	0.328	1.15	0.482	2.23
Wing length	Sire	0.701	5.86	0.722	1.76	0.872	4.79
	Dam	0.931	7.00	0.763	2.34	0.833	4.56
	Sire + Dam	0.712	4.15	0.313	2.10	0.462	3.42
Wing/thorax ratio	Sire	0.781	8.56	0.41	5.33	0.582	3.02
	Dam	0.613	3.30	0.48	0.38	0.934	4.16
	Sire + Dam	0.757	8.18	0.18	0.45	0.116	2.46
Sternopleural bristle number	Sire	0.46	4.47	0.13	1.41	0.64	3.60
	Dam	0.81	5.03	0.78	2.28	0.54	4.46
	Sire + Dam	0.26	1.74	0.16	1.46	0.09	1.47
Ovariole number	Sire	0.94	17.31	0.59	3.57	0.88	6.96
	Dam	0.67	4.59	0.64	6.19	0.93	9.19
	Sire + Dam	0.83	8.36	0.33	2.14	0.77	4.59

Table 5. Heritability (h^2) and evolvability (I_A) of morphometric traits among males of *D. ananassae* at different temperature regimens

		18°C		25°C		32°C	
Trait	Variance	h^2	$I_A \times 10^2$	h^2	$I_A \times 10^2$	h^2	$I_A \times 10^2$
Thorax length	Sire	0.63	8.43	0.33	4.27	0.45	3.26
	Dam	0.53	3.35	0.25	2.95	0.41	3.05
	Sire + Dam	0.58	6.72	0.19	2.58	0.49	5.53
Wing length	Sire	0.74	11.60	0.32	3.16	0.54	9.84
	Dam	0.56	6.79	0.43	7.80	0.49	4.56
	Sire + Dam	0.35	4.16	0.22	2.93	0.31	3.28
Wing/ thorax ratio	Sire	0.54	13.16	0.45	7.64	0.51	11.36
	Dam	0.41	9.47	0.22	6.02	0.35	8.45
	Sire + Dam	0.46	9.72	0.33	7.21	0.40	8.63
Sternopleural bristle number	Sire	0.781	7.45	0.325	3.01	0.582	6.89
	Dam	0.613	5.16	0.224	2.91	0.445	5.28
	Sire + Dam	0.797	9.16	0.125	2.26	0.316	4.76
Testis length	Sire	0.931	34.67	0.675	13.21	0.872	22.32
	Dam	0.701	30.62	0.564	12.10	0.833	26.42
	Sire + Dam	0.712	31.79	0.322	6.51	0.462	20.24
Sex comb	Sire	0.552	11.50	0.482	7.25	0.539	9.56
	Dam	0.503	8.025	0.346	2.19	0.437	4.31
	Sire + Dam	0.512	7.92	0.304	1.12	0.482	4.50

Table 6. Heritability (h^2) and evolvability (I_A) of life-history traits in *D. ananassae* at different temperature regimens

		18°C		25°C		32°C	
Traits	Variance	h^2	$I_A \times 10^2$	h^2	$I_A \times 10^2$	h^2	$I_A \times 10^2$
Development time	Sire	0.734	3.01	0.302	0.45	0.453	2.56
	Dam	0.198	1.09	0.168	0.08	0.125	1.35
	Sire + Dam	0.436	2.70	0.235	0.23	0.321	2.01
Viability	Sire	0.342	17.89	0.25	3.54	0.431	27.65
	Dam	0.543	51.39	0.35	13.27	0.423	25.76
	Sire + Dam	0.675	64.89	0.22	16.81	0.487	28.78

capacitor for morphological evolution in *Drosophila.* Temperature also has an effect on adult size trait in many ectotherms including *Drosophila* species. The adult size is increased by prolonging development at lower temperatures, although size may be reduced as a lower lethal temperature is approached. Sgro and Hoffmann (2004) have suggested that genes influencing a trait in one environment may not be important in a different one. In our study, variation was higher at 18°C than at 32°C. An interesting point in our study is that in *D. ananassae,* cold stress seems to have a greater effect on variation than heat stress as *D. ananassae* is very cold sensitive and stenothermic in distribution. During winter, the availability of *D. ananassae* becomes zero. Both the stressful temperatures used in our study were at the extreme physiological limits possible for survival of the species. The effect of extreme low temperature on *D. melanogaster* was investigated by Imasheva *et al*. (1998), who analysed quantitative genetic variation using the isofemale line technique. They reported that among-line variance for all 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 towards an increase in the among-line variances at low temperature was also demonstrated. However, in the experiment with parent–offspring comparisons, Sgro and Hoffmann (1998) did not find a difference in the genetic variance of wing length between 14°C and 28°C, while for development time this variance was significantly lower at 14°C. David *et al.* (1994) and Imasheva *et al.* (1997, 1998) reported that, if only one temperature had an effect on variation under stress, it was always the lower temperature.

Hoffmann and Parsons (1991) discuss several mechanisms that can underlie the phenomenon. A likely explanation is the past history of selection. Natural selection (directional or stabilising) acting on a trait under the non-stressful 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 occasionally experienced in nature, then the expression of phenotypic and genetic variance under these conditions may increase. According to Zhitovsky *et al*. (1996, 1997) low values of genotypic variance of adaptive character (and high values of fitness) are observed in environments that 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.

Our results suggest that high- and low-temperature stresses affect the genetic variation of a trait and 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. In summary, the results of our study are in agreement with the view that exposure to stressful environments can have a substantial effect on the expression of phenotypic and genetic variations. The effects of stress on different morphological and life-history traits can be viewed as an indication of the trait-specific effects of extreme temperatures.

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