Effect of directional selection for body size on fluctuating asymmetry in certain morphological traits in *Drosophila ananassae*

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Variation in the subtle differences between the right and left sides of bilateral characters or fluctuating asymmetry (FA) has been considered as an indicator of an organism's ability to cope with genetic and environmental stresses during development. However, due to inconsistency in the results of empirical studies, the relationship between FA and stress has been the subject of intense debate. In this study, we investigated whether stress caused by artificial bidirectional selection for body size has any effect on the levels of FA of different morphological traits in *Drosophila ananassae.* The realised heritability (h^2) was higher in low-line females and high-line males, which suggests an asymmetrical response to selection for body size. Further, the levels of FA were compared across 10 generations of selection in different selection lines in both sexes for sternopleural bristle number, wing length, wing-to-thorax ratio, sex comb-tooth number and ovariole number. The levels of FA differed significantly among generations and selection lines but did not change markedly with directional selection. However, the levels of FA were higher in the G10 generation (at the end of selection) than G0 (at the start of selection) but lower than the G5 generation in different selection lines, suggesting that the levels of FA are not affected by the inbreeding generated during the course of selection. Also, the levels of FA in the hybrids of high and low lines were significantly lower than the parental selection lines, suggesting that FA is influenced by hybridisation. These results are discussed in the framework of the literature available on FA and its relationship with stress.

[Vishalakshi C and Singh B N 2009 Effect of directional selection for body size on fluctuating asymmetry in certain morphological traits in *Drosophila ananassae*; *J. Biosci.* **34** 275–285]

1. Introduction

Over the past three decades, fluctuating asymmetry (FA, small random deviations from perfect bilateral symmetry, Van Valen [1962]) has received special attention in ecological and evolutionary studies. This is due to the resurgence of interest in FA as an indicator of genetic or environmental stress (Parsons 1990, 1992), an important variable in sexual selection and a measure of the nature of selection of a trait (Møller and Pomiankowski 1993). The underlying assumption of FA is that both sides of a bilateral trait are under the control of the same genes and any deviation from the bilaterally symmetrical phenotype would be due to perturbations of either environmental or genetic origin during ontogeny (Mpho *et al.* 2002). Thus, the left–right asymmetry of morphological traits implies perturbations in developmental homeostasis at the molecular, chromosomal and epigenetic levels (Parsons 1992). However, many studies have examined how FA responds to changes in genetic and environmental parameters, but no clear-cut pattern has been found due to inconsistent results (Waldman 1999; Leamy and Klingenberg 2005; Van Dongen 2006).

It has been suggested that directional selection decreases the level of developmental precision or developmental stability (Soule 1967; Parsons 1992; Møller and Pomiankowski 1993) because it may prevent the evolution of canalisation and possibly favour those mechanisms that increase the phenotypic variation (Pelabon

Keywords. Body size; directional selection; *Drosophila ananassae*; fluctuating asymmetry; hybridisation; morphological traits

Abbreviations used: FA, fluctuating asymmetry; h^2, realised heritability; ME, measurement error

et al. 2006 and references therein). In addition to this, many hypotheses linking directional selection with a decrease in developmental stability have been proposed. For example, a genetic correlation exists between the expression of a trait and its sensitivity to developmental noise (Gavrilets and Hastings 1994), occurrence of developmental homeostasis, i.e. a trade-off between growth rate and regulatory processes during ontogeny (Arendt 1997), or an indirect effect of directional selection on developmental stability due to the negative effect of homozygosity resulting from selection, on developmental stability (Lerner 1954; Leamy 1986). Each hypothesis assumes a particular mechanism with distinct predictions for the relationship between selection and developmental stability (for details, *see* Pelabon *et al.* 2006).

The genetic control of developmental stability remains poorly understood (Pelabon *et al.* 2006) and, despite empirical evidence suggesting that developmental stability can evolve (Clarke and McKenzie 1987), attempts to find genetic variance in developmental stability have proved to be unsuccessful (Pelabon *et al.* 2006). Therefore, understanding the relationship between directional selection and developmental stability may provide insights into the genetic control of developmental stability, and may help us to better understand the variational properties of organisms as suggested by Pelabon *et al.* (2006).

In view of this, we tried to investigate whether the levels of FA in different morphological traits (sternopleural bristle number, wing length, wing-to-thorax ratio, sex comb-tooth number and ovariole number) are affected by the stress caused by artificial bidirectional selection using a drosophilid fly as model organism. *Drosophila ananassae* is a cosmopolitan and domestic species and belongs to the *ananassae* subgroup of the *melanogaster* species group (Singh 1996, 2000). It occupies a unique status among the *Drosophila* species due to certain peculiarities in its genetic behaviour (Singh 2000). Further, we also investigated the effect of two genetic factors, viz. inbreeding which is generated along with selection, i.e. within selection lines across generations from G0 to G10, and hybridisation by crossing both the high- and low-selection lines reciprocally at the end of selection. Previous studies in *D. ananassae* have shown that FA exists in controlled laboratory conditions (Vishalakshi and Singh 2006) and there is a negative relationship between FA and sexual selection (Vishalakshi and Singh 2008a). Further, FA is affected by different environmental stressors (Vishalakshi and Singh 2008b, c) and mutations in *D. ananassae* (Vishalakshi and Singh 2008d) but not by interspecific hybridisation between two sibling species, *D. ananassae* and *D. pallidosa* (Vishalakshi and Singh 2009).

2. Materials and methods

2.1 Drosophila *stocks*

In order to test the effect of artificial bidirectional selection for body size in *D. ananassae*, a base population was constructed by using five mass culture stocks of different geographical origins, namely, Siliguri (SL), Shaktinagar (SK), Jabalpur (JB), Itarsi (IT) and Pondicherry (PC). The details of these populations are given elsewhere (Vishalakshi and Singh 2006).

2.2 *Selection regimen*

The artificial bidirectional selection experiment was started from the base population. The latter was constructed from the F1 flies of twenty reciprocal crosses of five laboratory populations (SL, SK, JB, IT and PC). The base population was maintained in duplicate by taking 100 pairs of flies in the laboratory for six generations of random mating before the start of the selection experiment. By employing the base population (at generation seven), artificial bidirectional selection for small and large body size was initiated at G0 generation. Two replicates were maintained for small body size and two for large body size. Thorax length was measured in both sexes and data were collected separately. One control line was also maintained along with the selection lines by taking 10 males and 10 females randomly for the next generation from the 50 virgin flies collected randomly for the measurement of body size. Virgin flies in each generation were collected every 2–4 h till their number reached 70 for each sex and line. The density of flies per vial was maintained at 10 flies per vial at a time for ageing for 5–7 days. Before measurement of thorax length, flies of each sex were pooled from different vials of that particular line to avoid any chance factor and, from them, the thorax length of 50 flies was measured from the anterior portion of the thorax to the tip of the scutellum (Norry *et al.* 1997) using an ocular micrometer (1 unit = 16.67 μm). Twenty flies of each sex with the highest and lowest body sizes were selected for the high and low line, respectively, of each replicate. For the control line, in each sex, 10 flies out of 50 were selected randomly for body size measurement as well as for initiation of the next generation; the remaining 40 flies were measured afterwards and the data were pooled. All the selection lines and control line were allowed to lay eggs for 24 h for initiation of the next generation. This was repeated in every generation of selection experiment. The selection experiment was continued for 10 generations. Throughout the study, a simple yeast agar culture medium containing agar-agar, crude sugar, dried yeast and active yeast (50:50), maize powder, nipagin, propionic acid and water was used. Flies were maintained in a BOD incubator at 25°C temperature and 65% humidity,

and were raised simultaneously to eliminate the possibility of environmental effects.

2.3 *Measurement of traits*

Different morphological traits, viz., sternopleural bristle number, wing length, wing-to-thorax ratio, sex comb-tooth number and ovariole number were measured in different generations of the control and selection lines in both the sexes (for details of measurement, *see* Vishalakshi and Singh 2006) on both the left and right sides of the 50 individuals per line (high, low and control) and sex across the ten generations.

2.4 *Statistical analyses*

As measurements of morphological traits showed no significant deviation from normality in the Kolmogorov–Smirov test for goodness of fit, no transformation was used for the traits studied (data not shown). At the end of the selection, differences for mean thorax length among different selection lines (high, control and low) were tested by one-way ANOVA for a fixed effects model (i.e. Model I, Zar 2005 [p.184]). The regression of offspring on parents is a useful measure of the degree of resemblance because it is simply related to the casual components of variance (Falconer and Mackay 1996). According to Yadav and Singh (2006), the regression coefficient (b) is calculated using means of parents selected for initiation of the next generation as dependent variables (10 flies) and the mean number of total flies (50 flies) as an independent variable in each selection line. Furthermore, the validity of significant differences observed in high and low lines is tested by the Tukey post hoc test. At the end of the selection, the trait sizes of different morphological traits were compared among different selection lines by one-way ANOVA followed by the Tukey post hoc test in males and females (Sokal and Rohlf 2000). Pearson correlation test was employed to test the relationship between different morphological traits and thorax length in different selection lines in both the sexes.

The realised heritability (h^2) is calculated as $h^2 = R/S$, where R is the response in offspring (gain) and S is the summation of the selection differential in parents (Falconer and Mackay 1996). Genetic variance is calculated by the formula $h^2 = VA/VP$, where VA is the additive genetic variance; VP is the phenotypic variance and h^2 the heritability (Falconer and Mackay 1996).

2.5 *Asymmetry data analyses*

The framework laid by Palmer (1994) and Palmer and Strobeck (1986, 2003) was followed for the analyses of FA. The analyses of measurement error (ME), repeatability, directional asymmetry and antisymmetry have been described in detailed elsewhere (Vishalakshi and Singh 2006). FA (FA1 of Palmer 1994, which is the FA measure reported in most of the studies) has been calculated for a given trait as the mean of the absolute value of the difference in trait size between the right and left sides of the body, |(R–L)| for sternopleural bristle number, wing length, wing-to-thorax ratio, ovariole number and sex comb-tooth number. To know whether trait FA co-varies with the trait size as it may affect the interpretation of studies on developmental stability (Palmer 1994), we obtained non-parametric Spearman correlation coefficient for all the traits between absolute trait asymmetry |(R–L)| and trait size (R+L)/2. The levels of FA among different generations (G0–G10) in males and females were tested by one-way ANOVA. To test the differences among different selection lines and sexes, two-way ANOVA was performed for different morphological traits.

2.6 *Effect of hybridisation*

In order to test the effect of hybridisation on FA in different morphological traits, we reciprocally crossed both the replicates of high and low lines, viz. H1♀ x L1♂, H1♀ x L2 ♂, H2 ♀ x L1♂, H2♀ x L2 ♂, L1♀ x H1 ♂, L1♀ x H2 ♂, L2 ♀ x H1♂ and L2♀ x H2 ♂ at the end of selection. The levels of FA in hybrids and their corresponding parents were compared by one-way ANOVA.

3. Results

3.1 *Selection regimen*

It is apparent from figure 1 that the response to selection was immediate from the fifth generation of selection, with rapid divergence in the mean thorax length in both high and low lines. Although there were some fluctuations between the two replicates of high and low lines, the selection was effective. At the end of the selection, the differences in the mean thorax length of the control and selection lines were significant in both males ($F_{4, 245} = 264.48$, $P<0.001$) and females ($F_{4, 245} = 291.12$, $P<0.001$). The results of the Tukey test also showed that there was significant difference ($P<0.05$) between the high and low lines (data not shown). In males, the cumulative selection differentials at G10 were 13.52 and 15.1 for high line (H1 and H2, respectively), and –20.55 and –18.86 (negative sign is used to represent decrease in size) for low lines (L1 and L2, respectively). Likewise, in females, cumulative selection differentials were 15.42 and 15.62 at G10 for high line (H1 and H2, respectively), and –27.32 and –24.82 for low lines (L1 and L2, respectively)

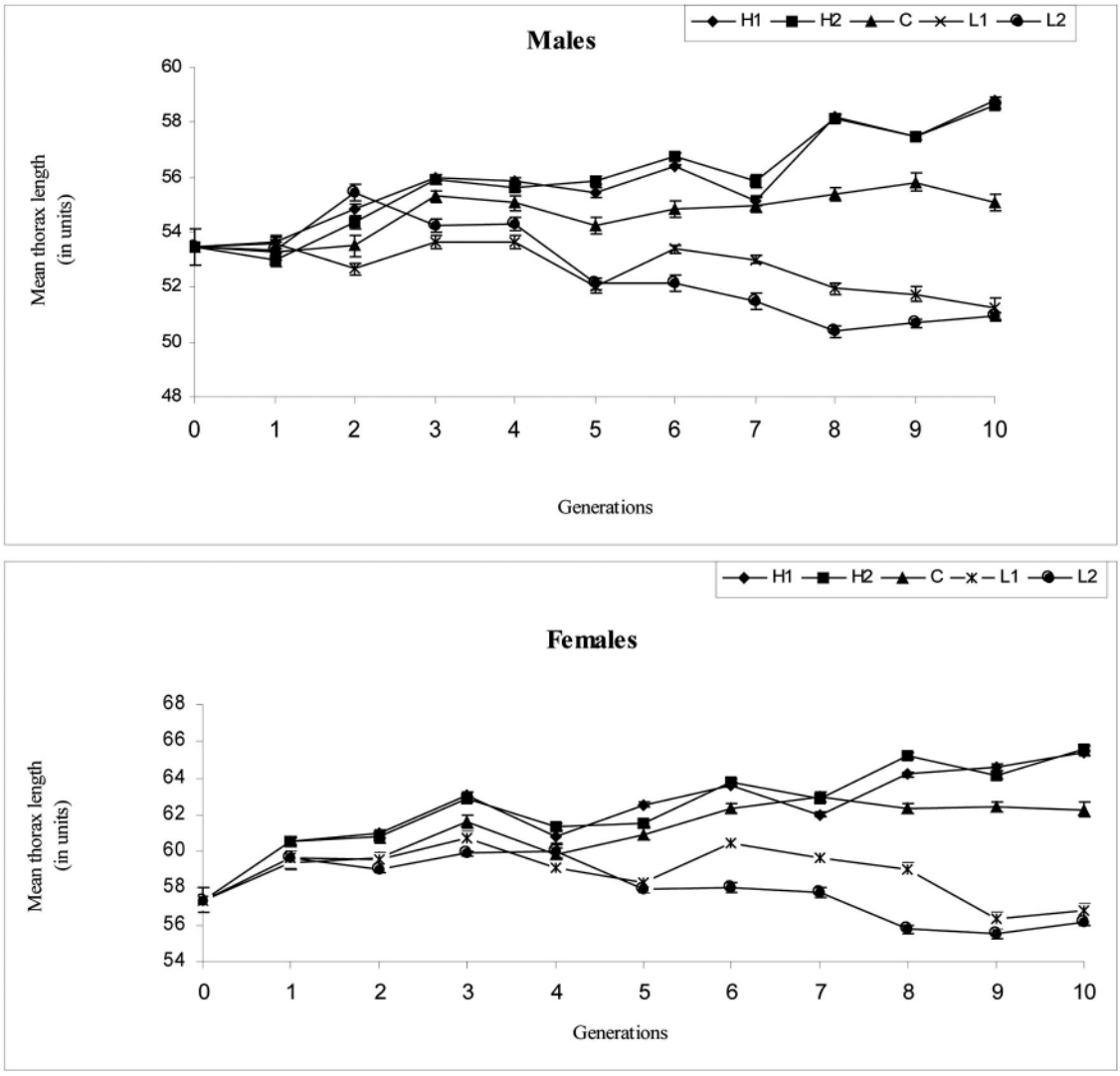


Figure 1. Mean thorax length in different generations of selection experiments in males and females of *D. ananassae*. Thorax length is given in units (1 unit =16.67 μm). H1 and H2, replicates of the high line; L1 and L2, replicates of the low line; C, control line.

(*see* figure 2). Over the 10 generations of selection, the mean thorax length changed by 3.84 to 4.14 units (1 unit =16.67 μm) in low-line and 3.56 to 3.74 units in high-line males with respect to the control flies (*see also* figure 1). The low-line females showed more drastic reduction in body size (–3.48 to –6.16 units) compared with control-line flies, whereas high-line females showed an increase in body size of up to 3.08–3.32 units compared with females of control lines, thus showing a clear asymmetry in selection response.

The realised heritability (hereafter mentioned as h^2) of the offspring on midparent, standard error of regression coefficient and test of significance of regression coefficient in males and females of different selection lines at G10 are presented in table 1. In females, the h^2 was higher in low-line (L1 and L2) than in high-line females (H1 and H2), whereas the values of h^2 were higher in high-line (H1 and H2) than in low-line males (L1 and L2), suggesting that the response of selection is asymmetrical and more pronounced in low-line females and high-line males (table 1). Further, *t*-test showed significant differences in all the selection lines of both sexes, which suggests that the response to selection for thorax length is positive in *D. ananassae*. This is in agreement with earlier findings (Yadav and Singh 2006). It is evident from figure 3 that there was a drastic decrease in the values of genetic variance from the G0 to the G1 generation in both males and females for the selection and control lines. However, the genetic variance values were more or less similar across the generations from G1 to G10 in all the selection lines in both males and females (figure 3).

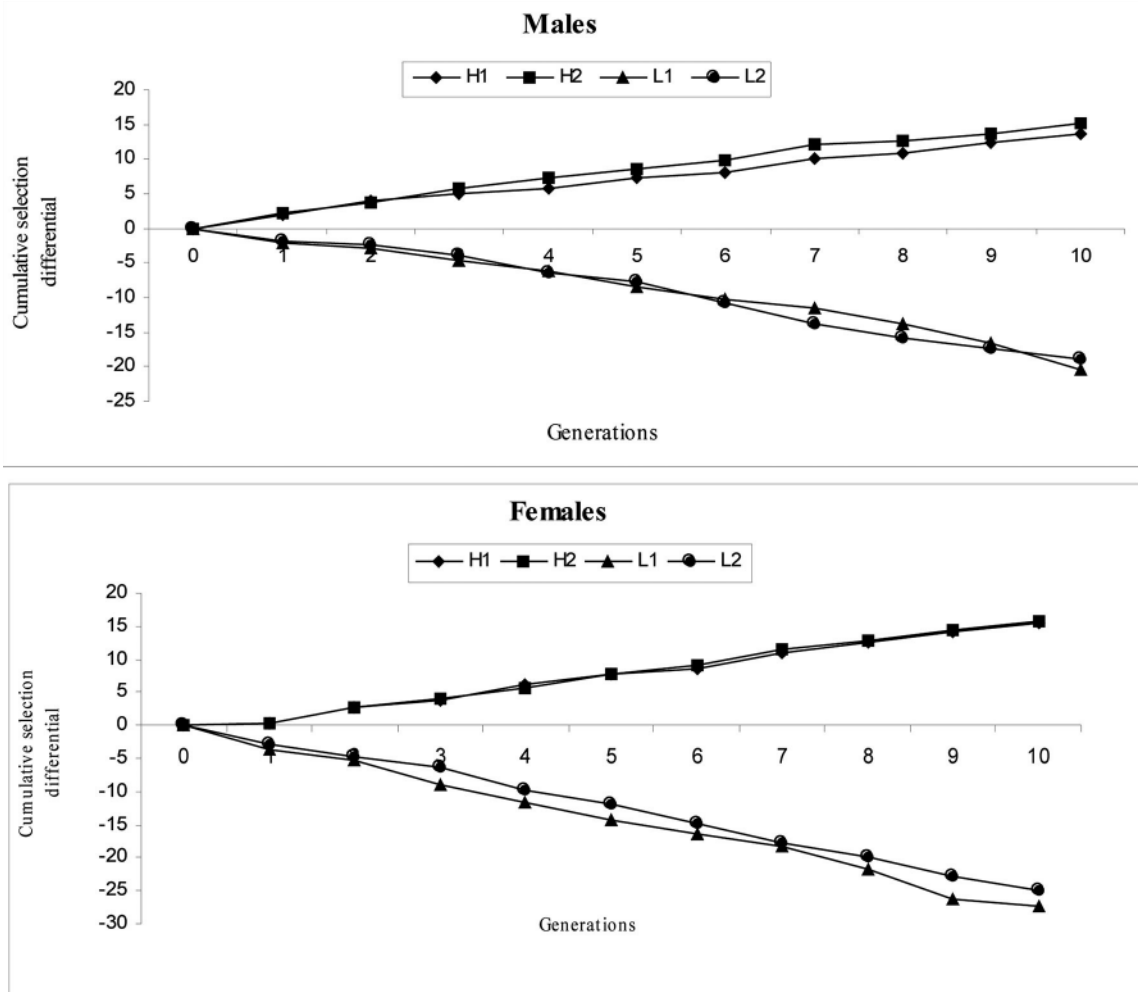


Figure 2. Response to bi-directional selection plotted against cumulative selection differential in males and females of *D. ananassae*. Negative values for low line show decrease in thorax length. H1 and H2, replicates of the high line; L1 and L2, replicates of the low line

3.2 *Asymmetry analyses*

3.2.1 *Measurement error and repeatability:* In all ANOVA, interaction between the sides and individuals was highly significant ($P<0.001$, data not shown) indicating that the ME in all the traits was negligible compared with the variation between sides. Repeatability of FA (R–L) values of all four bilaterally symmetrical traits were higher (for sternopleural bristle number, repeatability was 1.00 [$F_{98, 99}=0.0$, $P=1.00$]; for wing length 0.938 [$F_{98, 99}=0.08$, $P=0.777$], for ovariole number 1.00 [$F_{98, 99}=0.0$, $P=1.00$] and for sex comb-tooth number 0.911 [$F_{98, 99}=0.272$, $P=0.603$]). Thus, the high repeatability and the relatively low levels of ME accounted for a minor part of the total variance in asymmetry and therefore asymmetries were measured with sufficient precision.

3.2.2 *Directional asymmetry and antisymmetry:* For directional asymmetry, one-sample *t*-test revealed that the mean values of each trait did not differ significantly from zero ($P>0.05$) in all the selection lines across the different generations. The distribution of the signed differences (R–L) showed normal distribution in the Kolmogorov–Smirov test for normality. Moreover, none of skewness and kurtosis values differed from zero ($P>0.05$) for all the traits in different selection lines (results not shown). This indicates that our data represented true FA rather than directional asymmetry and antisymmetry. FA has been calculated as the mean of absolute trait asymmetry (|R–L|) for males and females. There were significant differences among different selection lines for trait size of different morphological traits in males and females ($P<0.001$, data not shown). Pearson correlation coefficients revealed that thorax length was positively correlated with sternopleural bristle number, wing length, sex comb-tooth number and ovariole number but negatively with wing-to-thorax ratio in both males and females (data not shown). In order to test the trait size

Table 1. Realized heritability (h^2), regression coefficient (b), standard error of regression (SE_b) and results of the test of significance of regression coefficients in males and females of different selection lines in *D. ananassae*

Selection line	h^2	b	SE_b	*t*-value	*P*-value
Males					
L1	0.109	0.467	0.122	3.82	0.005*
L2	0.135	0.893	0.072	12.34	1.73×10^{-6} *
H1	0.395	0.390	0.162	2.40	0.042*
H2	0.342	1.036	0.104	9.95	8.78×10^{-6}*
Females					
L1	0.223	0.974	0.148	4.534	0.001*
L2	0.271	0.77	0.179	4.323	0.002*
H1	0.161	0.441	0.164	2.68	0.02*
H2	0.174	1.043	0.111	9.33	1.41×10^{-5}*

* significant, df = 8; H1 and H2, replicates of the high line; L1 and L2, replicates of the low line.

dependence of FA and trait size, Spearman correlation was performed between absolute trait asymmetry (|R–L|) and trait size (R+L)/2 of different morphological traits. None of the correlation coefficients were significant (data not shown); therefore, we used absolute FA for further analysis.

3.2.3 *FA analyses:* The details of mean FA values in different morphological traits in G0 and G10 (the first and last generations of selection) are presented in table 2. The mean FA for sternopleural bristle number varied significantly among generations for all the selection lines and control line in males and in females (except for the C line, *see* table 2). For wing length, the degree of FA also differed significantly among generations in the H1, H2 and C lines but not for both the replicates of low lines in males. In contrast to this, in females, the magnitude of FA differed significantly among generations in the low lines and control lines but not in the high lines. Nevertheless, wing-to-thorax ratio also followed a similar pattern as that of wing length in both sexes. In sex comb-tooth number, the levels of FA differed significantly for the H2 and L1 lines but not in the other selection lines. In females, the levels of FA in ovariole number differed significantly among generations in selection lines, except in the H2 line (table 2). For comparison of individual trait FA among different selection lines and between sexes, two-way mixed model ANOVA with factors sexes (fixed) and lines (random) were used (data not shown). The levels of FA differed significantly ($P<0.05$) among the selection lines for sternopleural bristle number, wing length, wing-to-thorax ratio and sexual traits (sex comb-tooth number in males and ovariole number in females). Although the magnitude of FA was similar in males and females for sternopleural bristle number, wing length and wing-to-thorax ratio, it differed significantly for sexual traits (sex comb-tooth number and ovariole number; data not shown).

3.3 *Effect of inbreeding and hybridisation*

In order to test the effect of inbreeding on the levels of FA, we used the data of only three generations – G0 (start of selection experiment), G5 (middle, i.e. when the selection line was stabilised) and G10 (at the end of selection). The mean FA of sternopleural bristle number was higher in H2 males and L1 females of the G10 generation than G0 and G5 generations (table 2). For wing length, except in H1 and L2 males and H2 females, the levels of FA were higher in the G5 generation. As with wing length, the levels of FA in wing-to-thorax ratio were more in the G5 generation than G0 and G10. For sexual traits, the levels of FA were more or less similar in all the three generations among the selection lines (table 2). There were significant differences in the levels of FA of different morphological traits in parental selection lines and their hybrids in both the sexes (data not shown). In general, the degree of FA was lower in hybrids than the parental lines, suggesting a role of heterozygosity. Interestingly, there was also a significant difference between the levels of FA of two reciprocal crosses in both males and females (data not shown).

4. Discussion

4.1 *Response of selection*

It is evident from the results that ten generations of directional selection for thorax length in *D. ananassae* produced pronounced changes in thorax length in both high and low lines in both sexes (figure 1). However, the response to selection was asymmetrical and was more pronounced in low-line females and high-line males (table 1). Asymmetrical responses are caused due to random genetic drift, selection differential, inbreeding depression, maternal effects, genetic

Table 2. Mean fluctuating asymmetry in different morphological traits in different selection lines (G0 and G10) in males and females of *D. ananassae*

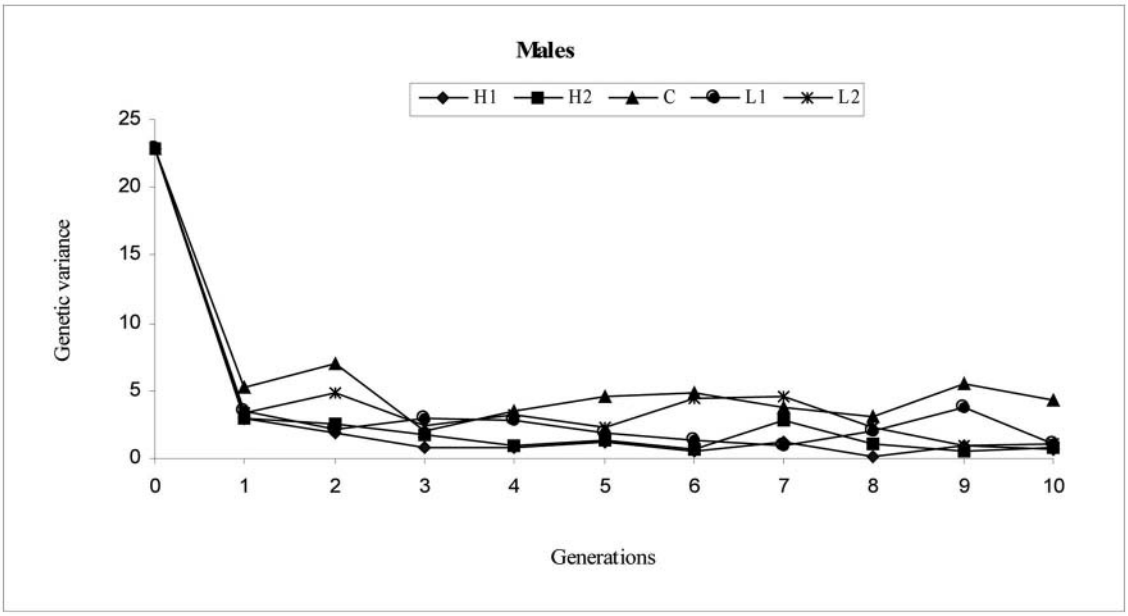
Traits	Generations	Females					Males				
		H1	H2	C	L1	L2	H1	H2	C	L1	L2
SBN	0	0.66 ± 0.133	0.66 ± 0.133	0.66 ± 0.133	0.66 ± 0.133	0.66 ± 0.133	0.74 ± 0.098	0.74 ± 0.098	0.74 ± 0.098	0.74 ± 0.098	0.74 ± 0.098
	10	0.78 ± 0.100	0.70 ± 0.098	0.86 ± 0.111	1.72 ± 0.211	0.66 ± 0.104	0.76± 0.105	3.02±0.29	1.22±0.11	1.04±0.14	0.42±0.08
	$F_{9, 490}$	1.949*	2.099*	1.258	4.47***	2.175*	35.698***	19.874***	2.763*	1.950*	3.854***
WL	0	0.62± 0.151	0.62± 0.151	0.62± 0.151	0.62± 0.151	0.62 ± 0.151	0.3 ± 0.22	0.3 ± 0.22	0.3 ± 0.22	0.3 ± 0.22	0.3 ± 0.22
	10	0.56± 0.10	0.98± 0.27	0.78± 0.15	0.76± 0.17	0.14± 0.04	1.28± 0.14	0.4 ± 0.08	0.52± 0.14	0.54± 0.09	0.82± 0.12
	$F_{9, 490}$	0.444	1.482	14.280***	6.412***	4.837***	10.801***	4.611***	6.380***	1.339	1.758
W/T	0	0.01± 0.002	0.01± 0.002	0.01± 0.002	0.01± 0.002	0.01± 0.002	0.006± 0.004	0.006± 0.004	0.006± 0.004	0.006± 0.004	0.006± 0.004
	10	0.008±0.001	0.015±0.004	0.014±0.003	0.006±0.002	0.003±0.001	0.022±0.002	0.007±0.002	0.0094±0.003	0.0111±0.002	0.016±0.002
	$F_{9, 490}$	0.862	0.9117	2.412**	6.29***	1.605	9.854***	4.537***	6.731***	0.976	0.792
ST	0	1.44 ±0.201	1.44 ±0.201	1.44±0.201	1.44±0.201	1.44±0.201	3.32±0.509	3.32±0.509	3.32±0.509	3.32±0.509	3.32 ± 0.509
	10	1.2±0.189	1.26±0.176	1.68±0.279	0.88±0.186	0.92±0.153	3.62±0.566	3.06±0.352	3.12±0.424	3.66±0.431	2.60 ± 0.433
	$F_{9, 490}$	2.196*	0.725	2.265*	3.212**	4.183***	1.773	1.882*	1.044	5.34***	0.699

*P<0.05; **P<0.01; *** P<0.001.

$F_{9, 490}$, F values after one-way ANOVA to test the difference among generations (0 to 10) in different selection lines and control line in males and females; SBN, sternopleural bristle number; WL, wing length; W/T, wing-to-thorax ratio; ST, sexual traits; H1 and H2, replicates of the high line; L1 and L2, replicates of the low line; C, control line.

asymmetry, scalar asymmetry, genes with large effects and indirect selection (Falconer and Mackay 1996). The measure of the selection applied is the average superiority of the selected parents and is known as the selectional differential. The difference in selectional differential influences the response per generation and may also affect h^2 (Yadav and Singh 2006). In our study, the differences in h^2 in low- and high-line males and females (table 1) suggest that the reason for an asymmetrical response to selection may be attributed to the selection differential, supporting previous findings in *D. ananassae* (Yadav and Singh 2006).

The trait size of sternopleural bristle number, wing length, wing-to-thorax ratio, sex comb-tooth number and ovariole number varied significantly in the different



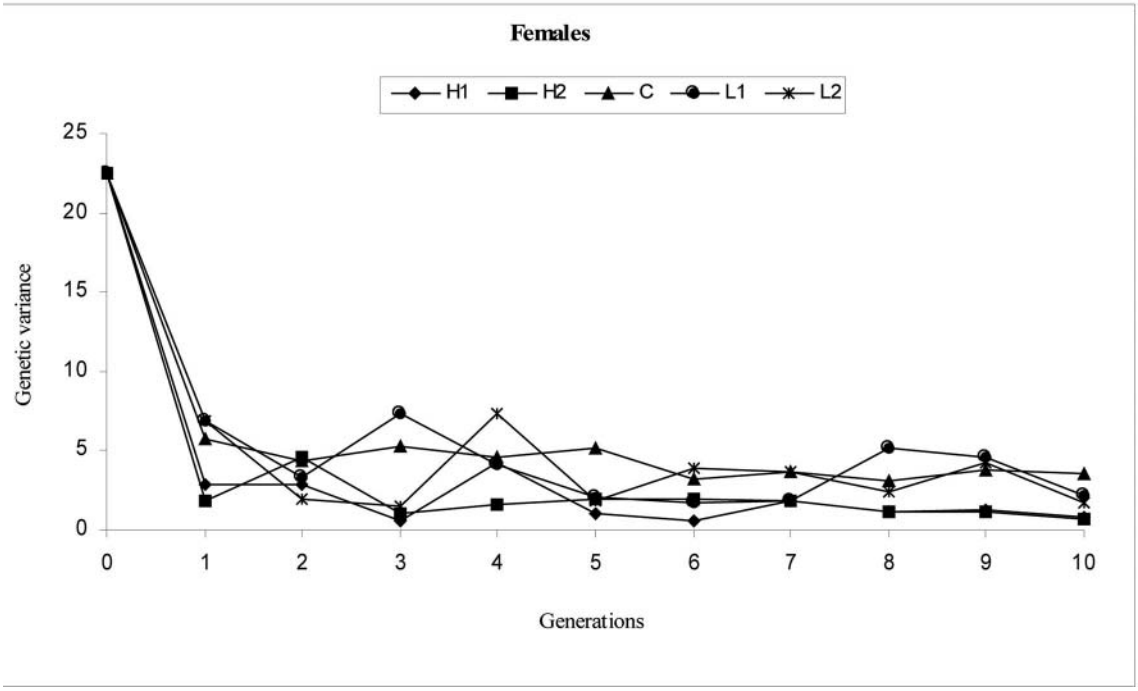


Figure 3. Dynamics of genetic variance values for thorax length throughout the selection experiment in males and females of *D. ananassae*. H1 and H2, replicates of the high line; L1 and L2, replicates of the low line.

selection lines (data not shown). As the selection experiment progresses, favourable alleles are selected and give their response with different combinations of gene action and epistasis to produce phenotypic divergence, i.e. large and small thorax length (Yadav and Singh 2006). Figure 3 shows that there was a drastic decrease in genetic variance values from the G0 to the G1 generation. This can be explained by the fact that the base population was maintained by taking 100 pairs of flies whereas the selection lines were started by taking 10 pairs of flies per generation. Therefore, this might be the reason for the sharp decrease in genetic variance from G0 to G1; after G1, the values were more or less similar in the selection lines and across the ten generations of selection among both males and females in all the selection lines as well as in the control line (figure 3).

4.2 *Directional selection and fluctuating asymmetry*

Despite the marked changes in trait size of different morphological traits – sternopleural bristle number, wing length, wing-to-thorax ratio, sex comb-tooth and ovariole numbers – changes in the levels of FA were small and inconsistent across the generations and selection lines in males and females of *D. ananassae* (data not shown). Similarly, Pelabon *et al.* (2006) found no effect of directional selection for wing shape (which was maintained for 8–9 generations) on the levels of FA in wing characters in *D. melanogaster* and they suggested that the generation time of 8–9 generations of selection was insufficient to significantly affect developmental stability. The vast changes in the morphological traits themselves suggest strong effects of selection on the genetics of the traits (Pelabon *et al.* 2006). However, Shakarad *et al.* (2001) found no effect of selection for faster development (selection lines were maintained for more than 70 generations) on the levels of FA in sternopleural bristle number in *D. melanogaster,* suggesting that directional selection has very little effect on the levels of FA. A review of the few available studies that have experimentally tested the hypothesis that directional selection reduces developmental stability (Pelabon *et al.* 2006) suggests that this hypothesis is weakly supported (Leamy 1986; Shakarad *et al.* 2001; Pelabon *et al.* 2006). The increase or decrease in levels of FA in high and low lines in comparison with control lines indicated an asymmetrical response of FA to selection in the opposite direction (data not shown). Our results on directional selection and FA are inconsistent with the different hypotheses as described in the Introduction section. First, our results do not support the 'homozygosity model' that predicts a decrease in developmental stability regardless of the direction of the selection, since it is the loss of heterozygosity in selected lines that is assumed to affect the levels of developmental stability. Furthermore, our design should not have led to a very big difference in homozygosity between the control and selection lines as all the lines were maintained by 10 pairs of flies. Therefore, the level of homozygosity was similar in selection as well as in control lines. Our results refute the 'classical canalisation model' in which a decrease in developmental stability is expected with directional selection, whatever the direction of selection because both high- and low-selection lines should represent a departure from the wild (most canalised) type. Also, our results go against the 'genetic–developmental model hypothesis', which suggests that selection for an increasing trait size increases the trait's sensitivity to developmental noise and therefore decreases its developmental stability, while selection for a decreasing trait size induces the opposite effects (Gavrilets and Hastings 1994). However, in our study, the asymmetrical response of FA to selection in opposite directions can be explained by the 'developmental–homeostasis model' (Arendt 1997; Pelabon *et al.* 2006) that links developmental stability to trait size.

4.3 *Effect of inbreeding and hybridisation*

In our study, bidirectional selection was performed using the 'extreme' 20% of flies with the largest or the shortest thorax length for high and low lines, respectively, and 20% of control line flies were chosen randomly. Thus, there is evidence for homozygosity by inbreeding in the high and low lines. It is known that developmental stability is affected by inbreeding because homozygotes lack the enzymatic diversity that allows heterozygotes to buffer their development from perturbation during development (Lerner 1954). An effect of inbreeding was expected, which was generated along with directional selection in the selection and control lines. Therefore, we tested the above hypothesis by comparing the levels of FA in different morphological traits in three different generations – G0 (start of the selection experiment), G5 (after the lines had stabilised for body size) and G10 (at the end of selection). As expected, the levels of FA should be higher in the G10 generation in both males and females. Except in a few, we found that the levels of FA were higher in the G10 than in the G0 generation but lower than in the G5 in all the selection lines and for different morphological traits, suggesting that inbreeding has no effect on FA, thereby supporting earlier findings (Fowler and Whitlock 1994; Vishalakshi and Singh 2006, 2008e). This increase in FA in the G5 generation as compared with the G0 and G10 generations may have some relation to the directional selection for thorax length. As the genetic variance was more in G0 due to the random mixing of flies, and the population size was more than 100 pairs (*see* Materials and methods section), the levels of FA were lower in the different traits studied. From G0 to G5, the selection lines had not stabilised (*see* figure 1), which

resulted in an increase in the levels of FA but after the G5 generation, the lines stabilised and, as a result, there was a decrease in the magnitude of FA. This indicates that directional selection can act as a canalising force (Pelabon *et al.* 2006). The levels of FA differ significantly among hybrids of different selection lines in comparison with their parents. Moreover, the magnitude of FA is lower in hybrids as compared with the parents. This decrease in FA levels is caused by an increase in heterozygosity; heterozygotes have the ability to buffer themselves against developmental perturbations due to allelic dominance (which masks the expression of deleterious recessive alleles) and overdominance (heterozygote superiority *per se*), which is thought to increase the efficiency of physiological and biochemical processes (Mitton 1993; Alibert and Auffray 2003). There is also a significant difference between two reciprocal crosses, which indicates the role of the maternal effect. As a whole, in this study, the degree of FA was similar in males and females for non-sexual traits (sternopleural bristle number, wing length, wing-to-thorax ratio) but differed significantly for sexual traits (sex comb-tooth number in males and ovariole number in females), supporting previous findings (Vishalakshi and Singh 2006). When the FA values were compared among both the sexual and non-sexual traits, it was higher in the sexual traits, suggesting that sexual traits are more prone to developmental instability (Vishalakshi and Singh 2006, 2008a, b, c, d, e, 2009).

One may speculate why certain traits are more susceptible to increased FA than others. Trait susceptibility has normally been attributed to different degrees of developmental stability which could be caused by different modes of selection, functionality or the stress experienced during the developmental process, as suggested by Aparicio and Bonal (2002). The degree of FA of a trait could depend on its functional importance, because stabilised development should be more strongly selected in traits that perform certain critical functions in an organism. For example, in our study, wing length and wing-to-thorax ratio, which are inversely proportional to wing loading and, presumably, related to flight capacity (Barker and Krebs 1995), have a lower FA than the other traits. The increase in the levels of FA in sexual traits can be explained by the fact that these traits are directly under sexual selection, and since this selective process acts against genetic modifiers that control the expression of the genotype, it increases the level of developmental instability (Clarke 1997). Also, different traits develop in part at different time points, such that variation in stress over time may have a different effect on the degree of asymmetry as well as on fitness (Van Dongen 2006). Further, FA is more in males than in females, suggesting that males are more prone to developmental instability due to hemizygosity in males of loci on the X chromosome (Palmer and Strobeck 1986; Vishalakshi and Singh 2006, 2008a, b, c, d, e, 2009).

In summary, (i) directional selection for thorax length has a small effect on the developmental stability of the different morphological traits studied, (ii) there is no effect of inbreeding on FA, (iii) hybridisation decreases the levels of FA due to an increase in heterozygosity, and (iv) the effect of directional selection, inbreeding and hybridisation seems to be trait- and sex-specific in *D. ananassae*.

Acknowledgements

The authors thank the two anonymous reviewers for their helpful comments on the original draft of the manuscript. Financial assistance in the form of a Senior Research Fellowship of the Center of Advanced Study, Department of Zoology, Banaras Hindu University to CV is gratefully acknowledged.

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MS received 21 October 2008; accepted 27 February 2009

*e*Publication: 2 April 2009

Corresponding editor: Ellen Larsen