Stochastic and factorial components of phenotypic variation in morphological traits in laboratory populations of *Drosophila ananassae.*

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Introduction

Phenotypic variation is a universal requirement for evolution upon which natural selection acts. It has been observed in a wide variety of traits across the populations and species (Woods *et al*., 1999; Belade *et al*., 2005). But how does this variation arise? How do new variants evolve? What constrains variations? These are the central questions of interest to ecologists and evolutionary biologists. Phenotypic variation reflects the genetic differences among individuals and the diversity of the environment (Lajus and Alekseev, 2000). Most of the studies have focused much on the genotypic variation than non-genotypic variation, which provides the basis for population's adaptive response to change in environmental factors by means of selection (Lajus and Alekseev, 2004). But some experiments have shown that even when both genetic and environmental factors have been minimized variation still exists suggesting that there is some "independent variation" which can be neither reduced due to genotypic differences nor due to direct effect of environmental factors (Belade *et al*., 2005; Astaurov, 1930). Later, it has been proposed that this kind of variation results from developmental instability, which reflects the deviations of developmental trajectories from the target determined for a particular genotype and environment (Lajus and Alekseev, 2000). It is measured by fluctuating asymmetry (FA), *i.e*., variance in random deviations from the perfect bilateral symmetry (Van Valen, 1962) and its levels increase due to either genetic or environmental stresses (Leamy and Klingenberg, 2005). There are different ways to partition total phenotypic variation, but most of them require special experimentation and are very difficult to apply to the populations of out-breeding species in natural and laboratory conditions (Lajus and Alekseev, 2000). In non-symmetrical structures, two kinds of the variation, heterogeneity among individuals and within-individual variation, impact total phenotypic variation, but it is impossible to separate them (Lajus, 2001). In symmetrical traits total phenotypic variation can be divided into two components: stochastic and factorial components using a special index of fluctuating asymmetry (Lajus and Alekseev, 2000). The stochastic component reflects developmental instability while the factorial component reflects the heterogeneity of individuals within a population and includes genotypic, macro- and micro-environmental, and ontogenetic variations (Kozhara, 1994; Lajus *et al*., 2003a). Genotypic variations reflect the genetic differences among individuals. Macro-environmental variations are due to environmental variations between distinct environments, whereas micro-environmental variations are due to relatively minor and local environmental variations. The ontogenetic component represents variation among different stages of growth, *e.g*., juveniles and senescent adults differ in their phenotypes (Lajus *et al*., 2003a). However, there is no report on the relative contribution of stochastic and factorial components of phenotypic variance in any bilateral trait in *Drosophila* (D. Lajus, pers. comm.). In view of this, we have made an attempt to analyze the phenotypic variation in *D. ananassae* Doleschall, a cosmopolitan and domestic species, which occupies a unique status among the *Drosophila* species due to certain unusual genetic features (Singh, 2000).

Materials and Methods

Fly stocks and Trait measurements

The details of the eighteen mass culture stocks of *D. ananassae* used in the present study have been described elsewhere (Vishalakshi and Singh, 2006). The stocks were maintained in the laboratory on simple yeast-agar medium at approximately 24^{o}C temperature. Virgin flies collected from these mass culture stocks were aged for 4 days in food vials. Different morphological traits *viz*., thorax length, wing length, wing to thorax ratio and sternopleural bristle number, sex comb tooth number, and ovariole number were measured as described earlier (Vishalakshi and Singh, 2006). Except thorax length, all the traits were measured on both left and right sides in a total of 1800 individuals (50 males and 50 females from each population).

Data analyses

Variation among populations and sexes was tested by analysis of variance (ANOVA) for each morphological trait. The variability of each population was estimated using the coefficient of variation (CV). Comparisons of phenotypic variability among populations in both sexes were performed using test of homogeneity for coefficient of variation (Zar, 2005).

For the asymmetry analysis, the framework laid by Palmer (1994) was followed. Measurement error (ME) will artificially inflate the estimates of FA. Therefore, for this reason, it is important to have the confidence that there are differences in R-L among individuals and not simply an artifact of ME. For measurement error, 32 flies randomly collected from the cultures and two replicate counts were made for different traits per fly, each on different day. Measurement error was assessed using two-way mixed model ANOVA in which, sides were entered as fixed factor and individuals as a random factor (Palmer, 1994). The tests for FA differences are only justified if interaction (Side × Individual) variance is significant. In all ANOVA (not shown), the interaction between side and individual is highly significant ($P < 0.001$), indicating that the measurement error in all the traits is negligible compared with the variation between sides. In all ANOVA (not shown), the interaction between side and individual is highly significant ($P < 0.001$), indicating that the measurement error in all the traits is negligible compared with the variation between sides. Further, individual asymmetry was measured as D = R - L, where R is the value of the trait on the right side and L is the value of the trait on the left side.

Partitioning total phenotypic into stochastic and factorial components

The partitioning of stochastic and factorial components is justifiable only if the asymmetry is fluctuating asymmetry and not directional asymmetry and antisymmetry. Therefore, it is important to check the kind of asymmetry before proceeding with an analysis (Lajus *et al*., 2003a). Thus, one sample t – test on the signed differences (R-L) for each trait was performed to determine whether the mean values differ from zero for directional asymmetry (Palmer, 1994). For antisymmetry, we checked departures from normality of the distribution of the signed differences (R-L) using the Kolmogorov-Smirov test. Fluctuating asymmetry in bilateral traits (wing length, W/T ratio, sternopleural bristle number, sex comb tooth number and ovariole number) in 18 laboratory populations of *D. ananassae* was calculated. Further, phenotypic variance of bilateral traits, *e.g*., wing length, W/T ratio, sternopleural bristle number, sex comb tooth number, and ovariole number was partitioned into stochastic (σ^2_S) and factorial (σ^2_F) components (Kozhara, 1989, 1994) $\sigma^2 = \Sigma (Xi - M)^2/ 2 (n-1)$; $\sigma^2_S = \Sigma (R-L)^2/ 2 n$; $\sigma^2_F = \sigma^2 - \sigma^2_S$ where σ^2 is the total phenotypic variance, σ^2_S is the stochastic component of total variance, σ^2_F is the factorial component, X_i is both right and

left manifestation of the trait, M is the mean value, R is the right manifestation, and L is the left manifestation of the trait, and n is the number of individuals. Variations among the populations for both stochastic (σ^2_S) and factorial (σ^2_F) components in both sexes were tested by test of homogeneity of variance for all the traits separately. To compare the difference in the components (stochastic and factorial) and sexes (males and females), F-test was performed.

Table 1. Comparisons of morphological traits between populations and sexes in laboratory populations of *D.ananassae* by two –way Analysis of Variance.

Traits	Source of variation	df	MS	F
Thorax length	Sex (S)	1	19529.5	211.26***
	Populations (P)	17	152.44	25.74***
	S x P	17	92.44	15.61***
	Error	1764	5.921	
SBN	Sex (S)	1	991.63	58.47***
	Populations (P)	17	46.02	8.32***
	S x P	17	16.96	3.07***
	Error	1764	5.52	
WL	Sex (S)	1	47228	235.18***
	Populations (P)	17	250.88	21.59***
	S x P	17	200.82	17.28***
	Error	1764	11.62	
W/T ratio	Sex (S)	1	0.0346	7.208*
	Populations (P)	17	0.095	256.76***
	S x P	17	0.0048	12.98***
	Error	1764	0.00037	
ST	Sex (S)	1	791533.6	949.39***
	Populations (P)	17	1447.28	4.203***
	S x P	17	833.72	2.421**
	Error	1	344.32	

*P<0.05; **P<0.01; ***P<0.001.
TL-Thorax length; SBN-Sternopleural bristle number; WL-Wing length; W/T– Ratio of wing length and thorax length; ST- sexual traits (in females- Ovariole number and in males - Sex-comb tooth number)

Results and Discussion

The mean value of different morphological traits *viz*., thorax length, wing length, wing to thorax ratio and sternopleural bristle number, sex comb tooth number, and ovariole number vary significantly among populations (Table 1) providing the evidence for genetic heterogeneity among laboratory populations of *D. ananassae*. There is significant difference between the males and females for all traits (Table 1). Test of homogeneity of coefficient of variation (CV) reveals that there are significant differences among individual variation for all the morphological traits in both

Table 2. Population variance (Var) and test for homogeneity for coefficient of variations (χ^2) among populations for different traits in *D.ananassae*.

Traits	MALES		FEMALES	
	Var	χ^2	Var	χ^2
TL	8.59	65.51**	7.63	123.57**
SBN	1.89	30.99*	3.781	108.15**
WL	24.44	69.73**	14.48	1302.66**
W/T ratio	0.004	134.79**	0.0036	363.9**
ST	74.16	33.00*	17.47	69.04**

* Significant at $P<0.05$, ** Significant at $P<0.001$

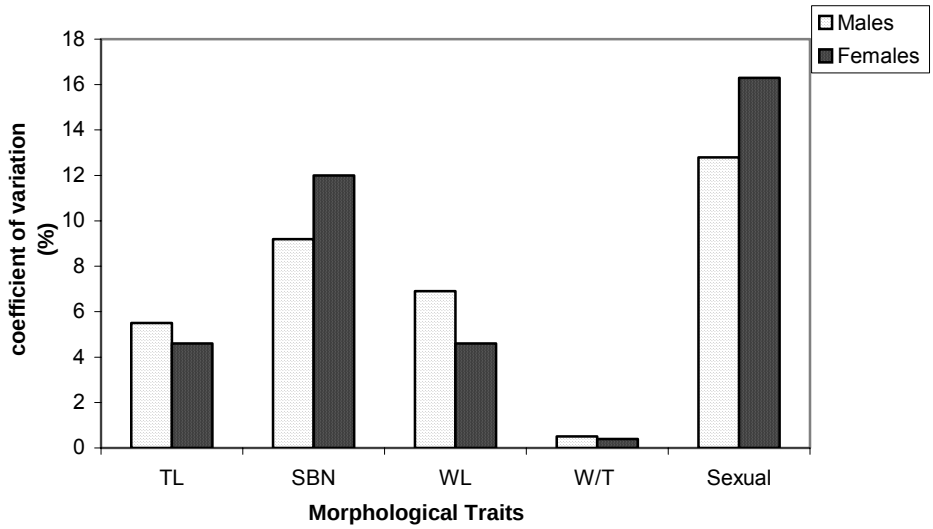

Figure 1. Phenotypic coefficient of variation (CV) for morphological traits in males and females. (The abbreviations used: TL - Thorax length, SBN - Sternopleural bristle number, WL – Wing length, W/T- Ratio of wing length and thorax length, sexual traits (SCTN-Sex comb tooth number and ON- Ovariole number).

sexes (Table 2). The phenotypic variability of sexual traits is more than that of the non-sexual traits (Figure 1). To compare phenotypic variations between traits, we considered trait CVs separately in males and females. Traits differed significantly in their CVs by Kruskal-Wallis tests in both the sexes (males: $F = 50.822$, df = 4, $P < 0.001$; females: $F = 21.33$, df = 4, $P < 0.001$). This difference in CV arises due to enormous diversity of the genetic and environmental factors difficult to disentangle (Houle, 1992) and also depends on the forces acting on them. For example, in the present study the traits that we have selected can be categorized into: meristic traits (SBN, SCTN, and ON) and metric traits (WL, W/T ratio, and TL) and also in non-sexual traits (TL, SBN, WL, and W/T ratio), and sexual traits (SCTN and ON). Across the populations (Figure 1), the CVs of the meristic traits (SBN, SCTN, and ON) are relatively higher than the metric traits (WL, W/T ratio, and TL) suggesting that meristic traits are more prone to phenotypic variability than metric traits (Woods *et al*., 1999). Trait differences in CVs also reflect the forces acting on them, which is evident from the larger CV of sexual traits (SCTN and ON) than non-sexual traits (SBN, WL, W/T ratio, and TL). This suggests that any trait that enhances mating and fertilization opportunities will be favored by sexual selection (Andersson, 1994) and also are sensitive to the effect of environmental perturbations leading to high phenotypic variability (M∅ller and Cuervo, 2003) than the non-sexual traits, which are supposed to be under natural selection. Interestingly, the variability is more in ovariole number (in females) than in sex comb tooth number (in males), suggesting intense sexual selection will reduce variability in males, which supports the theory that strong directional selection reduces underlying genetic and phenotypic variation (Falconer and Mackay, 1996). Moreover, in the non-sexual traits, the lower CVs in the wing traits (WL and W/T ratio) than bristle number in both sexes suggests that the traits that are more closely related to fitness are expected to be better buffered against environmental effects (Lerner, 1954; Woods *et al*., 1999).

Directional asymmetry and antisymmetry

One-sample t-test reveals that mean values of each trait did not differ significantly from zero. For example, in males, for SBN (t = -1.273, df = 1798, P = 0.203), WL (t = 0.238, df = 1798, P = 0.814), W/T ratio (t = 0.697, df = 1798, P = 0.486) and SCTN (t = 0.505, df = 1798, P = 0.614) and in females for SBN (t = -0.093, df = 1798, P = 0.926), WL (t = 1.633, df = 1798, P = 0.103), W/T ratio (t = -0.693, df = 1798, P = 0.488) and ON (t = -1.552, df = 1798, P = 0.121). The distribution of the signed differences (R-L) showed normal distribution in the Kolmogorov-Smirov test for normality. Moreover, none of skewness (t = -1.509, df = 8, P = 0.170) and kurtosis (t = 1.752, df = 8, P = 0.118) values differed from zero for all the traits. This indicates that we are observing true FA rather than directional asymmetry and antisymmetry in our data. Fluctuating asymmetry have been calculated as mean of absolute trait asymmetry (|R-L|) for males and females.

Partitioning of stochastic (σ^2_S) and factorial (σ^2_F) components

Further, we have partitioned out the total phenotypic variance into stochastic (σ^2_S) and factorial (σ^2_F) components for all the bilateral traits (sternopleural bristle number, wing length, wing to thorax ratio, ovariole number, and sex comb tooth number). There are significant differences among the populations for stochastic component of variance for all the traits in males (except SCTN) and females (Table 3). The populations also differ significantly for the factorial components of all traits except WL and SCTN (Table 3). The cause of this variability is due to genotypic variation and internal component of environment (Zhang and Hill, 2005). The percentage contribution of factorial component to the total phenotypic variation is more than that of the stochastic component (Table3). Interestingly, the percentage contribution of the stochastic variance for wing traits decreases in comparison to the SCTN, ON, and SBN supporting the view of Lajus *et al.,* (2003a), that the contribution of stochastic variance to the total phenotypic variance decreases as the mean increases. In males, factorial and stochastic component of variance differ significantly for WL and W/T ratio but not for SBN and SCTN (Table 3 A). Similarly, in females the two components differ significantly for WL, W/T ratio, and ON except SBN (Table 3 B).

Table 3. Magnitude of stochastic (σ^2_S) and factorial (σ^2_F) components of the variance of different morphological traits in *D. ananassae.*

Traits	Variance Among populations (χ^2)		F-test
	σ^2_S	σ^2_F	
a) Males			
SBN	91.22*	918.22*	0.782
WL	914.76*	3.289	511.17*
W/T	1055.45*	1019.20*	128.50*
SCTN	0.505	1.125	2.57
b) Females			
SBN	112.94*	862.27*	1.22
WL	628.51*	3.21	100.65*
W/T	1050.9*	443.33*	72.61*
ON	111.82*	4.082*	7.259*

* Significant at P<0.05,

However, the magnitude of factorial component in all the traits is more than that of the stochastic component in all the traits except SBN in both the sexes (Figure 2). In order to test the difference statistically between the two sexes, F –test was employed. Males and females have a similar level of stochastic component of variation for sternopleural bristle number (F = 1.299, P > 0.05), wing length (F = 1.069, P > 0.05) and wing to thorax ratio (F = 2.08, P > 0.05) but not for the sexual traits (F = 6.357, P < 0.001). These results support the previous findings (Vishalakshi and Singh, 2006) in these laboratory populations where the levels of fluctuating asymmetry (|R-L|) are similar in non-sexual traits (SBN, WL, and W/T ratio).

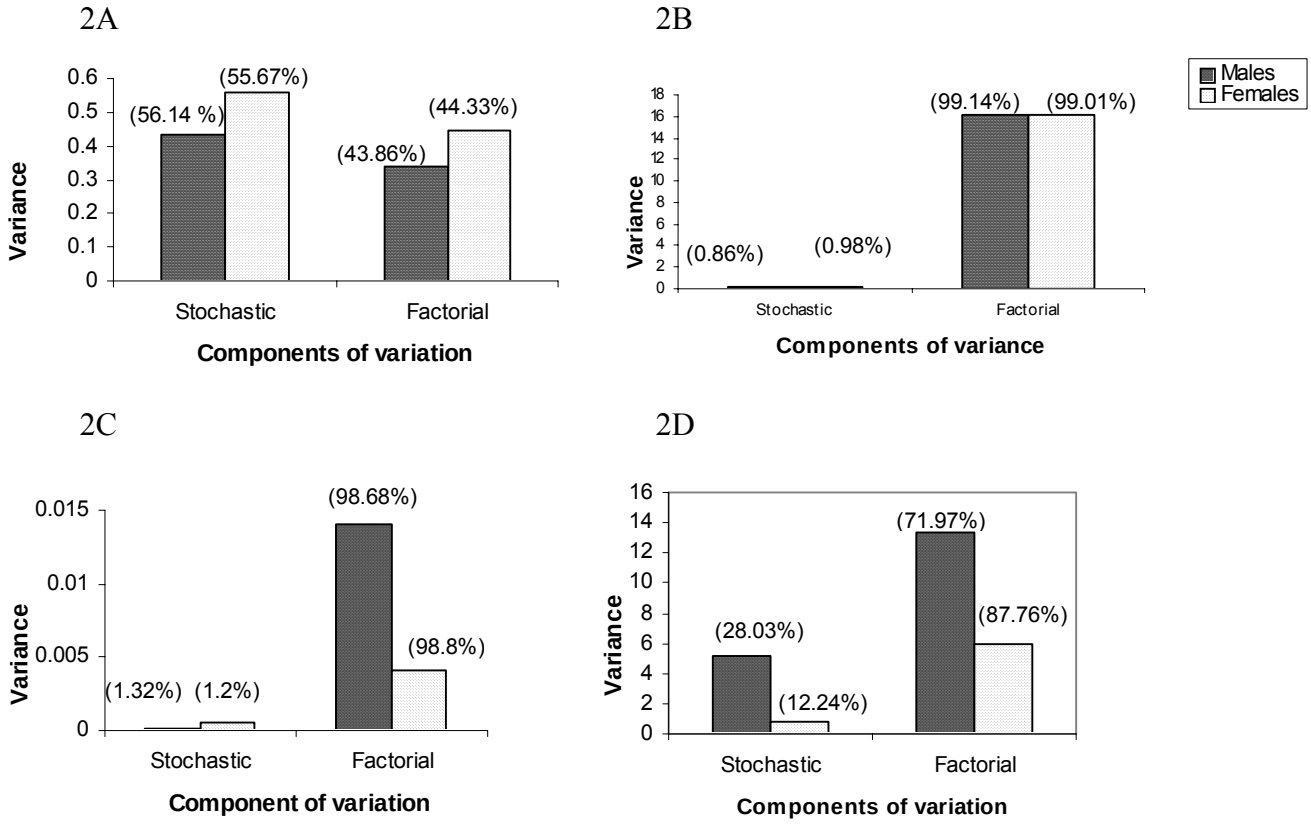

Figure 2. Magnitude of stochastic and factorial components of phenotypic variation in different populations of *D.ananassae* for different morphological traits, A) sternopleural bristle number, B) Wing length, C) Wing to thorax ratio D) Sexual traits. In the parentheses, the percentage values of the contribution of the stochastic and factorial component of the total variance are given).

Similarly, both sexes have the similar level of the factorial component for sternopleural bristle number ($F = 1.368$, $P > 0.05$), wing length ($F = 1.069$, $P > 0.05$), and sexual traits ($F = 2.248$, $P > 0.05$). Moreover, the magnitude of stochastic components is higher in males for SCTN than in female for ON (See Figure 2D) suggesting that sexual traits in males are more prone to developmental instability than in females (Vishalakshi and Singh, 2006).

The relationship between stochastic and factorial components was tested for all the traits in males and females. In males, there is negative correlation between stochastic and factorial components for SBN($r = -0.584$, $P < 0.01$), W/T ratio ($r = -0.305$, $P < 0.219$) and SCTN($r = -0.302$, $P < 0.224$) where as positive correlation for WL ($r = 0.114$, $P = 0.651$). Interestingly, in females there is negative correlation between the two components for WL ($r = -0.394$, $P = 0.106$), W/T ratio (-0.168, $P = 0.504$) and ON ($r = -0.433$, $P = 0.072$), whereas as positive correlation for SBN($r = 0.282$, $P < 0.257$). When the data of males and females were pooled for both stochastic and factorial components there is a negative correlation for WL ($r = -0.354$, $P < 0.05$), W/T ratio ($r = -0.156$, $P = 0.585$), whereas there is a positive correlation for SBN ($r = 0.007$, $P = 0.967$) and sexual traits ($r = 0.366$, $P < 0.05$). The association between the stochastic and factorial variance of the same traits is positively correlated (Lajus, 1991; Lajus and Alekseev, 2000). In constrast to this, there is negative correlation for wing traits but positive for SBN and sexual traits. The positive significant association of stochastic and factorial component for sexual traits suggests that the underlying mechanisms are

responsible for buffering the traits development against both external and internal environmental variations are interrelated (Lajus *et al.,* 2003b).

In conclusion, the present study provides the evidence for 1) phenotypic variability among populations and individuals which is caused due to genotypic and micro environmental diversity and within-individual variation is caused due to the developmental instability, 2) trait variability arises due to the evolutionary forces (sexual and natural selection) acting on them at individual level, and 3) the contribution of stochastic variance in comparison to the factorial variance to the total phenotypic variance is small, but is considerable. This is the first report on the relative contribution of stochastic and factorial components of phenotypic variance in any bilateral trait in the genus *Drosophila*. However, present study provides new avenues for further research in order to get more information regarding the contribution of factorial and stochastic variance in other *Drosophila* species with a much wider range of traits.

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Parallel trend in pigmentation and desiccation tolerance: altitudinal and latitudinal effects in *Drosophila melanogaster*.

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