Evolution of faster development does not lead to greater fluctuating asymmetry of sternopleural bristle number in *Drosophila*

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Abstract

Both strong directional selection and faster development are thought to destabilize development, giving rise to greater fluctuating asymmetry (FA), although there is no strong empirical evidence supporting this assertion. We compared FA in sternopleural bristle number in four populations of *Drosophila melanogaster* successfully selected for faster development from egg to adult, and in four control populations. The fraction of perfectly symmetric individuals was higher in the selected populations, whereas the FA levels did not differ significantly between selected and control populations, clearly indicating that directional selection for faster development has not led to increased FA in sternopleural bristle number in these populations. This may be because: (i) development time and FA are uncorrelated, (ii) faster development does result in FA, but selection has favoured developmentally stable individuals that can develop fast and still be symmetrical, or (iii) the increased fraction of symmetric individuals in the selected populations is an artifact of reduced body size. Although we cannot discriminate among these explanations, our results suggest that the relationship between development time, FA and fitness may be far more subtle than often thought.

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Introduction

The development of a stable phenotype, buffered against environmental and developmental noise, is thought to be of major importance for optimal performance of individual organisms (Møller 1999a). The ability of an individual to develop a stable phenotype despite adverse environmental or genetic conditions reflects developmental stability, which counteracts deviations from an optimal ontogenetic trajectory due to developmental upsets (Møller and Swaddle 1997; Møller 1999b). In recent years, there has been increasing interest in the use of various measures of developmental stability to understand a wide range of ecological and evolutionary problems, as measures of developmental stability/instability are thought to provide reliable information about the quality (fitness) of individuals (Leung 1999), and of the degree of environmental stress to which they were exposed (Waddington 1960; Parsons 1961; Hurtado *et al*. 1997). The most popular measure of developmental stability has been the departure from perfect symmetry of a bilateral character (usually measured as its fluctuating asymmetry, FA), and it has been suggested that measures of developmental instability like FA are reliable predictors of fitness (Møller and Swaddle 1997; Møller and Thornhill 1998; Waynforth 1998; Møller 1999b), although this relationship is controversial (e.g. Clarke 1998; Cadée 2000), as is the reliability of FA as an indicator of stress (Blanckenhorn *et al*. 1998; Lu and Bernatchez 1999; Woods *et al*. 1999; Bjorksten *et al*. 2000; Bourguet 2000).

Many factors are thought to affect developmental stability, although empirical evidence for their effects is

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often tentative (extensive review in Møller and Swaddle 1997). One of the major factors thought to affect developmental stability and FA is the growth rate or development time (Møller 1997), although there is considerable disagreement about the nature of the effect of development time on developmental stability, leading to mutually contradictory predictions as exemplified by the following quotation (Møller 1997, p. 921): 'In conclusion, studies of growth rates demonstrate that fast growth generally is associated with a symmetrical phenotype. Alternatively, there may be a trade-off between developmental stability and growth rate . . . .' Another suggestion is that development rate is optimized in populations, and any deviations from this optimum will tend to lower developmental stability (Clarke 1998). Strong directional selection is another factor thought to lead to increased FA (Parsons 1992; Møller and Pomiankowski 1993).

If both directional selection and faster development tend to result in increased FA, one would expect populations that have been under intense directional selection for faster development for many generations to exhibit reduced developmental stability and greater FA. Previous studies addressing the relationship between development time and FA have relied either on phenotypic manipulations (Parsons 1961) or comparisons of different breeds of animals (Møller *et al*. 1995). It is, however, known that phenotypic correlations do not necessarily reflect underlying genetic correlations (Falconer 1981; Rose and Charlesworth 1981; Chippindale *et al*. 1993, 1994). Moreover, comparisons of different breeds of animals with varying developmental time (Møller *et al*. 1995) are difficult to interpret because the breeds are likely to differ genetically for any number of traits, other than development time, that could have a direct effect on FA. Laboratory selection experiments in which either FA or development time were directly subjected to selection have not yet been used to investigate the genetic relationship between development time and FA.

In our laboratory, we have successfully selected four populations of *Drosophila melanogaster* for reduced preadult development time, with a reduction of ~ 36 hours (20%), relative to controls, being seen over 70 generations of selection (Prasad *et al*. 2000). Hence we might expect higher levels of FA in these populations that are under intense directional selection for reduced development time relative to controls. We use sternopleural bristle number (henceforth, bristle number) as the indicator trait to compute FA since this trait has been used previously as a measure of developmental stability in *Drosophila*, and also appears to be correlated with fitness (Kearsy and Barnes 1970).

Materials and methods

***Experimental populations*:** This study was done on eight laboratory populations of *D. melanogaster* of which four served as controls and four were subjected to selection for faster development and early reproduction relative to the controls. The control populations employed here were the four populations (JB_1, JB_2, JB_3, JB_4) described in detail by Sheeba *et al*. (1998). The JB populations were maintained on a 21-day discrete generation cycle at 25°C, about 90% relative humidity and under constant light, at moderate densities of 60–80 eggs per 8-dram vial containing about 6 ml banana–jaggery food. The number of breeding adults was about 1800 per population and the adults were maintained in plexiglass cages (25 cm × 20 cm × 15 cm) with abundant food. The four populations selected for faster development and early reproduction were derived from the four JB populations and designated as FEJ_1, FEJ_2, FEJ_3, FEJ_4 (F, faster development; E, early reproduction; J, JB-derived). Each FEJ population was derived from one JB population; thus, selected and control populations with names bearing identical numerical subscripts are more closely related to each other, than to other populations with which they share a selection regime (JB_i and FEJ_i are more closely related than JB_i and JB_j or FEJ_i and FEJ_j; $i, j = 1–4$). The selected populations were maintained on a regime similar to the JB populations except that 80 vials of 60–80 larvae were collected per population and monitored closely for eclosions once the pupae began to darken. The first 15 or so flies that eclosed in each vial were dumped into plexiglass cages with abundant food and a generous smear of live yeast – acetic acid paste. Typically the breeding adult number is about 1000–1200 per population. The protocol adopted for selection has been described in detail by Prasad *et al*. (2000), and we have therefore restricted ourselves here to details pertinent to the present study. We emphasize that with such high numbers of breeding adults per population, inbreeding, which is known to affect FA, is not a problem in our experimental setup.

***Measurement of sternopleural bristle number*:** Imposition of different maintenance regimes on populations can induce nongenetic parental effects that get confounded with evolved genetic differences between selected and control populations. Consequently, all selected and control populations were maintained under common rearing conditions for one complete generation prior to assaying to eliminate all such nongenetic effects. Eggs were collected from the running cultures at generation 69 of selection and dispensed into vials containing about 6 ml of food at a density of 60–80 eggs per vial. On the 12th day after egg collection, by which time all normally developing individuals would have eclosed, the flies were dumped into plexiglass cages with abundant food and supplied with live yeast – acetic acid paste for two days prior to egg collection for assay. The adult numbers were usually around 1200–1800 per population at this point. The progeny of these flies, hereafter referred to as

standardized flies, were used for the assay. Eggs were collected from the standardized flies at a density of 50 eggs per vial containing 6 ml banana–jaggery food, and five such vials were set up per population. As there was a large difference in the developmental time of the JB and the FEJ population (Prasad *et al.* 2000), the emergence of flies from the selected and control populations was synchronized by staggering the egg collection for the two types of populations by the developmental time difference. Hence, the flies used in the assay were all of the same age. Three-day-old flies were killed by immersing them in soapwater and the number of sternopleural bristles on the right and left sides of 30 flies of each sex from each population was counted under a stereo-zoom microscope. Thus, data from 480 flies were used for the analyses.

***Checking for directional asymmetry, antisymmetry and measurement error*:** Bristle number being a discrete variable, the methods suggested by Palmer and Strobeck (1986) for assessing directional asymmetry were not applicable to our data. Since there is no possibility of measurement error in bristle number, replicate measurements on the same individual were not made. We assessed directional asymmetry by constructing 95% confidence intervals around the mean of the signed $(R_i - L_i)$ values (where L is the number of sternopleural bristles on the left side and R the number of sternopleural bristles on the right side) for each sex in each population separately and testing for significant deviations of the mean from zero. We graphically checked for antisymmetry by constructing frequency histograms of the $(R_i - L_i)$ values for each sex in each selection regime pooled across all four populations.

***Size dependence of FA*:** As suggested by Palmer and Strobeck (1986), we regressed the absolute value of $(R_i - L_i)$ on the trait size $((R_i + L_i)/2)$ for each sex in each population separately. A three-way mixed-model analysis of variance (ANOVA) (with selection regime and sex as the fixed factors crossed among themselves and with random blocks) on the slopes of these individual regressions indicated no significant main effects or interactions of any of the factors. Hence data from all the populations and both sexes were pooled and regressed as described earlier. The overall regression showed a significant positive slope ($b = 0.16$; $P = 0.0002$), thus making it necessary to correct for trait size while computing FA.

***Computation of FA*:** Of the nine FA indices listed by Palmer and Strobeck (1986), we have chosen two: **index 1** (index 2 in Palmer and Strobeck 1986),

$$\mathrm{FA} = \frac{\sum_{i=1}^{30} \frac{|R_i - L_i|}{(R_i + L_i)/2}}{N};$$

index 2 (index 6 in Palmer and Strobeck 1986),

$$\mathrm{FA} = \mathrm{Var}\left[\frac{(R_i - L_i)}{(R_i + L_i)/2}\right],$$

where L is the number of sternopleural bristles on the left side, R the number of sternopleural bristles on the right side, $i = 1$ to 30 for each sex in each population, and $N = 30$.

The FA indices were calculated separately for each sex in each population. Index 1 is commonly used and is based on the means of the absolute right–left differences. We also calculate index 2 since it is claimed to have higher discriminatory power, being based on the variance of the right–left differences (Palmer and Strobeck 1986).

***Statistical analysis*:** Selected and control populations with names bearing identical subscripts were treated as blocks in the statistical analyses as they were closely related. The data on total bristle number and the two FA indices for each population were subjected to separate mixed-model ANOVA, treating selection regime and sex as fixed factors crossed among themselves and with block as a random factor. All statistical analyses were done using STATISTICA™ for Windows Release 5.0 B (Statsoft Inc., Tulsa, USA).

Results

There was a clear difference in the total bristle number in the flies from selected (FEJ) and control (JB) populations (table 1). The ANOVA indicated significant main effects of selection regime ($F = 370.970$, d.f. = 1, $P = 0.0003$) and sex ($F = 113.105$, d.f. = 1, $P = 0.002$), as well as a significant selection regime × sex interaction ($F = 35.937$, d.f. = 1, $P = 0.009$). The FEJ males and females had significantly fewer bristles (~ 13 in males, ~ 15 in females) than the JB males (~ 18) and females (~ 19), respectively. In the FEJ populations, females had significantly higher number of bristles than males, whereas in the JB populations the difference between sexes was not significant (table 1).

The means of the signed $(R_i - L_i)$ values did not differ significantly from zero in either of the sexes in any of the populations, indicating absence of directional asymmetry. The frequency distributions of the $(R_i - L_i)$ values for each selection regime × sex combination show a unimodal distribution with a distinct mode at zero, thus ruling out antisymmetry for the trait studied (figure 1). The FA indices for FEJ and JB populations did not differ significantly, irrespective of whether index 1 or index 2 was used for the computation of FA (tables 2 and 3).

Table 1. Mean total bristle number of each sex in each population. CI is the 95% confidence interval around the mean for each selection regime × sex combination, calculated using the least-squares estimate of the appropriate error mean squared term in the ANOVA.

		Pop 1	Pop 2	Pop 3	Pop 4	Mean	CI
FEJ	Male	13.10	12.97	12.90	13.13	13.03	0.49
	Female	14.97	14.87	14.73	14.97	14.88	0.49
JB	Male	17.77	18.50	18.77	18.17	18.30	0.49
	Female	18.17	19.23	18.77	19.20	18.84	0.49

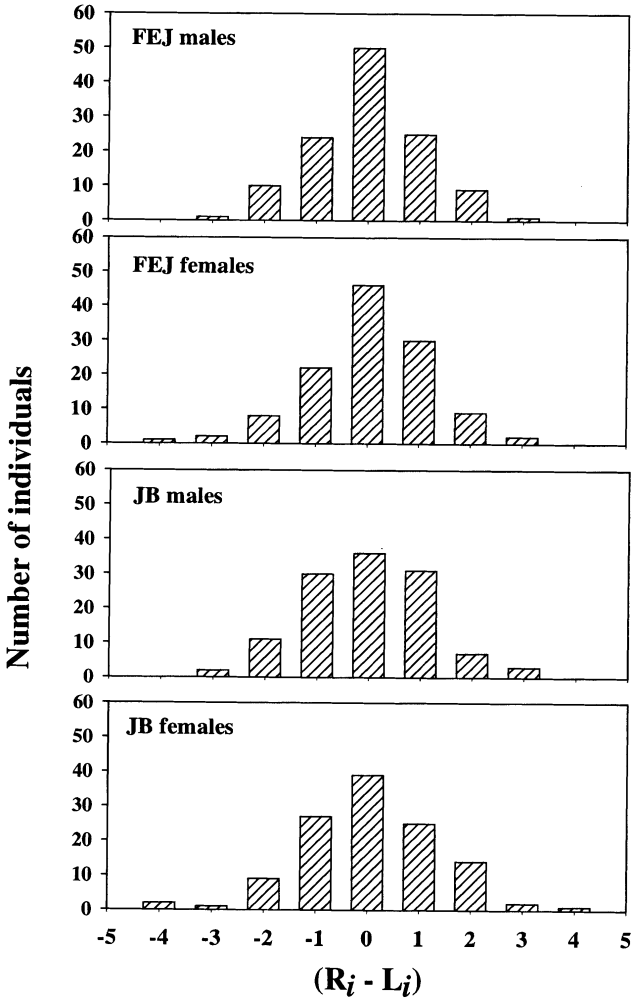

Figure 1. Frequency distributions of the ($R_i - L_i$) values pooled over all four blocks within each selection regime × sex combination.

The fraction of perfectly symmetric individuals ($R_i - L_i = 0$) was higher in the FEJ populations (0.58 for males and 0.38 for females) compared to the JB populations (0.30 for males and 0.32 for females). This difference was, however, not statistically significant owing to the anomalous behaviour of one block (block 4). If the data from this block are excluded from the analysis, then there is a significant main effect of selection ($F = 22.192$, d.f. = 1,2, $P < 0.05$) but no significant effect of sex ($F = 0.309$, d.f. = 1,3, $P = 0.6$) or selection × sex interaction ($F = 2.106$, d.f. = 1,3, $p = 0.3$), despite reduced degrees of freedom. Overall, it is clear that FA in bristle number is not higher in the FEJ populations that have been subjected to intense directional selection for faster development.

Discussion

Studies using FA as a measure of developmental stability have typically used phenotypic manipulations, especially with respect to the effect of stresses like poor nutrition and parasites on FA (Møller and de Lope 1998; Martel *et al*. 1999; Møller 1999a; Roy and Stanton 1999). In *D. melanogaster*, the effects of larval stress on FA are particularly well studied: in general, larval stress decreases the trait value but increases the FA, and the effect of stress on FA is often trait specific (Parsons 1961; Kearsy and Barnes 1970; Woods *et al*. 1999; Cadée 2000). The heritability of FA for many traits is also known to be very low (Woods *et al*. 1999). However, the evolution of developmental stability in response to directional selection is not well studied. This is a serious lacuna given that selection studies on life history traits clearly indicate that results from phenotypic manipulations are not necessarily good indicators of correlated responses to selection (Rose *et al*. 1996; Chippindale *et al*. 1993).

Our study concentrates on a single trait, sternopleural bristle number. We realize that it is desirable to assess several traits for FA if one wishes to draw broad conclusions about faster development affecting the FA of an organism. We regard the present study as a beginning in that direction. Indeed, this study is the first attempt to assess correlated changes in FA of any trait in response to strong directional selection on development time. The point we want to stress is that our results clearly suggest that the relationship of development time, developmental stability and FA is likely to be more complex and subtle than previously thought.

Table 2. Values of the two FA indices for each sex in each population. CI is the 95% confidence interval around the mean for each selection regime × sex combination, calculated using the least-squares estimate of the appropriate error mean squared term in the ANOVA.

	Selection	Sex	Pop 1	Pop 2	Pop 3	Pop 4	Mean	CI
Index 1	FEJ	Male	0.112	0.119	0.133	0.120	0.121	0.030
		Female	0.088	0.117	0.145	0.113	0.116	0.030
	JB	Male	0.091	0.110	0.102	0.100	0.101	0.030
		Female	0.097	0.098	0.093	0.124	0.103	0.030
Index 2	FEJ	Male	0.030	0.030	0.039	0.023	0.031	0.009
		Female	0.018	0.029	0.041	0.022	0.028	0.009
	JB	Male	0.014	0.019	0.016	0.017	0.017	0.009
		Female	0.016	0.017	0.017	0.028	0.020	0.009

Table 3. Summary of the two separate three-way mixed-model ANOVAs on the two FA indices. Here selection regime and sex were treated as the fixed factors crossed among themselves and with the random blocks. Since the effect of blocks or their interactions cannot be tested for in this design, the table shows only the main effects and interactions of the fixed factors.

	Effect	d.f.	MS effect	*F*	*P*
Index 1	Selection	1	0.001	3.689	0.151
	Sex	1	0.000	0.138	0.735
	Selection × Sex	1	0.000	0.310	0.617
Index 2	Selection	1	0.001	5.158	0.108
	Sex	1	0.000	0.000	1.000
	Selection × Sex	1	0.000	2.182	0.236

Table 4. Fraction of perfectly symmetric individuals in each sex in each population. CI is the 95% confidence interval around the mean for each selection regime × sex combination, calculated using the least-squares estimate of the appropriate error mean squared term in the ANOVA.

Selection	Sex	Pop 1	Pop 2	Pop 3	Pop 4	Mean	CI
FEJ	Male	0.500	0.433	0.433	0.300	0.417	0.142
	Female	0.500	0.366	0.333	0.333	0.383	0.142
JB	Male	0.366	0.233	0.266	0.333	0.300	0.142
	Female	0.333	0.300	0.366	0.300	0.325	0.142

In the FEJ populations, over 70 generations of selection, the total bristle number has been decreased by about 6 in males and 4 in females (table 1), along with a large reduction in the adult size measured as adult dry weight at eclosion (Prasad *et al*. 2000). Hence the positive correlation between fly size and bristle number reported by studies using phenotypic manipulations (Parsons 1961; Kearsy and Barnes 1970) seems to be quite robust. A reduction in surface area of smaller adults has been suggested as a mechanistic reason for the reduction in bristle number (Parsons 1961). In the FEJ populations, the larval resource provisioning is very low and the larvae have evolved several mechanisms to minimize energy expenditure (Prasad *et al*. 2001). Hence it is also quite possible that the reduction in bristle number in the FEJ populations is due to low resources or due to the available resources being utilized for faster development, a trait that has the highest fitness premium under the FEJ selection regime. The selection regime × sex interaction with respect to bristle number agrees with an earlier report that males have fewer bristles than females (Reeve and Robertson 1954), though this difference is not significant in the JB populations. The males of the FEJ populations having lost significantly more weight than the females over 70 generations of directional selection (Prasad *et al*. 2000)

may correspondingly have also lost more bristles than the females.

The nearly 20% reduction in the total bristle number and development time in the FEJ populations over 70 generations of directional selection is, however, not accompanied by a significant increase in the levels of FA. In fact, the fraction of perfectly symmetric individuals in the FEJ populations appears to be greater than in the controls. This is contrary to what would be expected on the basis of the phenotypic correlation between development time and FA in bristle number obtained by increasing or decreasing the development time by adding a tyrosine inhibitor (phenylthiocarbamide), or increasing the rearing temperature (Parsons 1961). As opposed to the results of these studies in which we have to contend with the potentially confounding effects of temperature and chemicals on FA directly, our results are easier to interpret since development time is under direct selection and the reduction of development time in the FEJ populations is genetic. Moreover, our results do not support the notion that directional selection and faster development should lead to greater developmental instability (Parsons 1992; Møller and Pomiankowski 1993; Clarke 1998). We offer three possible explanations for the observed increase in the fraction of symmetric individuals and no significant change in the levels of asymmetry in the FEJ populations.

(i) The evidence linking development time and developmental stability is very tentative, since it is based on phenotypic manipulations and among population comparisons. Selection experiments and within-population comparisons on development time and FA have never been done. Moreover, whether there is a relationship between FA and fitness of an individual is itself not clear (Møller 1997; Clarke 1998). Hence it is possible that development time and FA are not causally related and the expectation of greater FA under directional selection for faster development is therefore without foundation.

(ii) If we assume that faster development does in fact lead to increased asymmetry in individuals, and that asymmetric individuals in the FEJ populations do have lower fitness, it is likely that selection would have favoured those individuals that can develop fast and yet have a high developmental stability. The prediction from such a scenario is, thus, opposite of what has been proposed by Parsons (1992), Møller (1997) and Clarke (1998), namely that selection for faster development should lead to greater symmetry and developmental stability.

(iii) In *D. melanogaster* adult size critically depends on the duration of the post-critical feeding period in the middle and late third instar (Robertson 1963). It is therefore likely that the total bristle number also depends on the duration of the post-critical feeding period. If one assumes that a particular minimum bristle number that cannot be lower is associated with the minimum critical size that a larva has to attain to complete development, then it is possible that greater symmetry in the FEJ populations is simply an artifact of reduced adult size. In the FEJ populations the minimum critical size of larvae has been reduced, along with a large reduction in the third larval instar duration (Prasad *et al*. 2001). If the minimum bristle number is the same for both left and right sides, then the bristle number in the FEJ populations is likely to be close to the minimum bristle number, thereby leading to a greater proportion of symmetric individuals in these populations.

The work reported here is the first to look at the effect of faster development on developmental stability through a long-term laboratory selection experiment. In earlier studies, using phenotypic manipulations, there is confounding of factors that can possibly affect developmental stability directly rather than through their effect on development time. Our experimental setup does not allow us to discriminate between the effects of faster development and directional selection on FA separately. One way to overcome this problem is to use development-time mutants instead of selecting for faster development time. But even under such a situation, there is still a confounding effect of mutation since it is known that many mutations tend to decrease asymmetry. Thus we see no other cleaner way of assessing the effect of development time on FA, although FA measurements on flies selected for traits that do not affect development time could yield more insight on the effects of directional selection on FA in general.

Our study indicates that genetically decreased development time per se does not increase FA and, if anything, tends to increase the fraction of perfectly symmetric individuals at a populational level with respect to bristle number. The results therefore suggest that the relationship between development time and FA in bristle number is not causal, and that faster development in the selected populations does not destabilize the developmental processes that concern bristle development. We conclude that it is prudent to be circumspect about drawing broad generalizations regarding development time, developmental stability and evolutionary change from a mere handful of studies. Reciprocal studies in which bristle number is selected for can further clarify this issue though it is known that correlated responses to selection can be asymmetric. Whether faster development, in general, is a destabilizing factor or not can be better appreciated by incorporating more traits that are bilaterally symmetrical in studies addressing this issue.

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