

Adaptation to larval malnutrition does not affect fluctuating asymmetry in *Drosophila melanogaster*

Biological Journal of the Linnean Society 104(1): 19-28 (2011)

Except for small changes made at proofs, the content of this preprint is identical to the published version.

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Abstract:

Both stress during development and response to directional selection were proposed to lead to reduced developmental stability of an organism, commonly measured as fluctuating asymmetry. Here, we investigated the direct physiological (plastic) effect of larval malnutrition and the effect of evolutionary adaptation to this form of stress on developmental stability, measured as fluctuating asymmetry of several wing measurements. The measurements were made on female *Drosophila melanogaster* from populations which, in the course of 84 generations of experimental evolution, adapted to malnutrition and from non-adapted controls, raised either under standard conditions or under nutritional stress. We detected no changes in the levels of fluctuating asymmetry as neither a plastic nor an evolutionary response. Thus, neither nutritional stress within lifetime nor directional selection it imposes seems to affect developmental stability in flies.

Keywords: Adaptation, developmental stability, *Drosophila*, fluctuating asymmetry, nutritional stress.

1. INTRODUCTION

Many functions of an animal organism (such as locomotion or feeding) rely on precise morphology. Therefore evolution is thought to have favoured specific mechanisms that buffer the developing organism against environmental disturbances, mutations, and random events during development (developmental noise); a phenomenon referred to as canalization or developmental stability (Waddington, 1942; Maynard Smith *et al.*, 1985; Scharloo, 1991; Wagner, Booth & Bagheri-Chaichian, 1997; Flatt, 2005; Van Dongen, 2006). Developmental stability is often quantified in terms of fluctuating asymmetry, *i.e.*, deviation of paired morphological structures from perfect bilateral symmetry. This is based on the notion that the development of left and right sides of such paired structures is controlled by the same genetic and developmental program (Whitlock, 1996), and that asymmetry results from the inability of the organism to buffer development against local disturbances and developmental noise (Van Valen, 1962). Thus, a higher developmental stability would be manifested as lower fluctuating asymmetry (Badyaev, Foresman & Fernandes, 2000; Van Dongen, 2006).

In this study we ask how nutritional stress experienced during larval development affects fluctuating asymmetry in *Drosophila melanogaster*, and how fluctuating asymmetry changes as a result of over 80 generations of experimental evolutionary adaptation to the nutritional stress conditions.

It has been proposed that different forms of stress reduce developmental stability and thus increase fluctuating asymmetry. However, while some empirical studies support this claim, evidence for generality of fluctuating asymmetry as an indicator of stress experienced during development is equivocal (Palmer & Strobeck, 1986; Parsons, 1992; Van Dongen, 2006). Juvenile malnutrition is a common form of stress which directly affects growth and development, and thus may be particularly likely to affect developmental stability (Nosil & Reimchen, 2001; Gronkjaer & Sand, 2003; Vishalakshi & Singh, 2008). The effect of nutritional stress during development on fluctuating asymmetry has been addressed in a range of organisms, including plants (Black-Samuelsson & Andersson, 2003), insects (Breuker & Brakefield, 2003; Stige, Hessen & Vollestad, 2004), fishes (Somarakis *et al.*, 1997; Gronkjaer & Sand, 2003), birds (Searcy, Peters & Nowicki, 2004; Pravosudov & Kitaysky, 2006; Sillanpaa, Salminen & Eeva, 2010) and mammals (Sciulli *et al.*, 1979; Badyaev *et al.*, 2000). While some studies suggest that development under nutritional stress gives rise to individuals with higher fluctuating asymmetry (Swaddle & Witter, 1994; Pravosudov & Kitaysky, 2006; Sillanpaa *et al.*, 2010), other studies demonstrate that fluctuating asymmetry remains unaffected by nutritional stress (Bjorksten *et al.*, 2000; Gronkjaer & Sand, 2003; Searcy *et al.*, 2004; Stige *et al.*, 2004). However, we are not aware of any study that has reported a decrease in fluctuating asymmetry in response to nutritional stress. In *Drosophila*, one study (Imasheva, Bosenko & Bubli, 1999) reported that *D. melanogaster* reared on food which was extremely poor in nutrition (extremely low-yeast-sugar diet) showed increased fluctuating asymmetry for three bilateral traits (wing length, sternopleural chaeta number and arista branch number). In contrast (Vishalakshi & Singh, 2008) found no effect of larval nutrition (low-yeast diet) on fluctuating asymmetry of five bilateral traits (wing length, sternopleural bristle number, wing-thorax ratio, ovariole number in females and sex comb teeth number in males) in *D. ananassae*. However, that study reported greater fluctuating asymmetry for the same traits under larval crowding, which leads not only to nutritional stress but also exposure to increased levels of toxic waste products. Another study (Woods *et al.*, 2002) similarly found no difference in levels of fluctuating asymmetry of five bilateral traits (wing length, wing width, orbital bristle number, sternopleural bristle number and cross-vein length) when *D. melanogaster* reared under low-yeast and high-ethanol conditions where compared to unstressed controls. Thus, the plastic effect of both the intensity and type of nutritional stress on developmental stability seems to be unclear. Furthermore, the effect of adaption to this kind of environmental stress on developmental stability remains unknown.

While developmental stability is thought to have evolved under long-term stabilizing selection, the degree of developmental stability may also undergo short-term evolutionary changes. In particular, strong directional selection has been predicted to reduce developmental stability, because it disrupts gene regulatory networks and possibly favours mechanisms which increase phenotypic variation (Clarke & Mckensie, 1987; Rice, 1998; Kawecki, 2000; Hermisson & Wagner, 2004). Other proposed

mechanisms include a genetic correlation between the expression of a trait and its sensitivity to developmental noise (Gavrilets & Hastings, 1994), and a trade-off between growth rate and regulatory processes during ontogeny (Calow, 1982; Arendt, 1997). Finally, long-term directional selection is likely to lead to homozygosity at loci affecting the trait and a general increase in homozygosity (inbreeding) due to the effect of selection on the effective population size; both were proposed to have adverse effects on developmental stability (Soule, 1967; Leamy, 1986; Pertoldi *et al.*, 2006). The studies supporting and contradicting these four hypothesis linking developmental stability and directional selection have been reviewed in (Pelabon *et al.*, 2006). Bidirectional selection of *D. ananassae* for body size has been shown to increase the levels of fluctuating asymmetry (Vishalakshi & Singh, 2009). Another bidirectional selection study on wing shape failed to support the hypothesis linking directional selection and developmental stability (Pelabon *et al.*, 2006). Similarly, artificial selection of *D. melanogaster* for faster development did not affect fluctuating asymmetry (Shakarad *et al.*, 2001).

In this study we aimed to investigate simultaneously the direct physiological (plastic) effect of larval malnutrition and the effect of evolutionary adaptation to this form of stress on developmental stability (measured as the fluctuating asymmetry of several wing measurements) in *D. melanogaster*. We used flies from six replicate *D. melanogaster* populations subject to experimental evolution under larval nutritional stress, along with six unselected control populations (Kolss *et al.*, 2009), reared under both optimal (standard food) and stressful (poor food) conditions. The nutritional stress involved rearing larvae on a poor food, on which control flies take 70 % longer to develop from egg to adult, show 20 % lower viability, and emerge at half the body weight of flies maintained on standard food. Adaptation to malnutrition in these selected populations included increased egg to adult viability, the evolution of faster growth on poor food, smaller body size and faster development, the latter at least in part resulting from a cut-off on developmental period imposed during selection (Kolss *et al.*, 2009). We concentrate on the wing because wing asymmetry may affect aerodynamic performance and hence in nature more likely selected against than e.g. asymmetry of bristle numbers. Furthermore, we have found plastic and evolutionary reduction of wing size in response to poor larval food (Vijendravarma, Narasimha & Kawecki, 2011).

If nutritional stress indeed led to reduced developmental stability then we would predict the control populations to exhibit higher fluctuating asymmetry when reared on poor food in comparison to those reared on standard food. Furthermore, if directional selection for tolerance to nutritional stress reduced developmental stability, one would expect the selected populations to exhibit higher fluctuating asymmetry than control populations, both when reared on standard and on poor food. Alternatively, as a consequence of the experimental adaptation, the poor food conditions may have become less stressful for the selected than for the control populations. If so, and if nutritional stress increases fluctuating asymmetry, the selected flies would exhibit lower levels of fluctuating asymmetry than control flies when reared on poor food, but not when reared on standard food.

2. MATERIAL AND METHODS

Six populations had been selected for tolerance to chronic larval malnutrition by being maintained on poor larval food for 84 generation; six control populations had been maintained on standard food (15 g agar, 30 g sucrose, 60 g glucose, 12.5 g dry yeast, 50 g cornmeal, 0.5 g MgSO₄, 0.5 g CaCl₂, 30 ml ethanol, 6 ml propionic acid, and 1 g nipagin per litre of water). All 12 populations were derived from a single outbred base population (Kolss *et al.*, 2009). Prior to the assays, all populations were reared for two generations on standard food.

The flies thus raised were allowed to mate for two days and then allowed to oviposit overnight. Two vials with standard food and two with poor food (containing ¼ of the amounts of sugars, yeast and cornmeal of the standard food) were set up per population, each seeded with 200 eggs, and incubated at 25°C and 70% humidity. Five females per vial (10 flies per population) were collected randomly fifteen days later, although only ~40-60 % flies had emerged on poor food. Flies were only collected until day 15 to mimic the conditions imposed during selection. The right and left wing of each fly was dissected and mounted on a glass slide in lactic acid/ethanol (6:5) (Trotta *et al.*, 2007), photographed

at 40× magnification. We thus measured 60 wing pairs per regime × food treatment, i.e. 240 pairs of wings in total. The design thus consisted of two main factors: past selection regime (selected vs. control) and current food treatment (standard vs. poor), with replicate populations nested in selected regime and two replicate vials per population × food combination.

Four wing parameters were measured for each wing to the nearest micrometer (figure 1) using ImageJ (Abramoff, Magelhaes & Ram, 2004): (1) Length A (wing length); (2) Length B (wing width); (3) Length C (distance between the vein tips); (4) Wing Area, (estimated by tracing the outline of each wing and measuring the area of the resulting polygon). These measurements were taken independently twice by two different experimenters. The different linear measurement would potentially reflect the fluctuating asymmetry of different aspects of wing cell proliferation and growth. Furthermore, we used the ratio of Length A and Length B as the fifth trait; a composite index of wing shape roughly corresponding to the length/width ratio.

Statistical analysis

JMP v.7.0 was used for all analysis, and we used the specification for expected mean squares on mixed model ANOVA implemented in this software (it follows the SAS model of Ayres & Thomas, 1990). A separate analysis was performed for each of the five traits.

Effects on trait means. We first analyzed the effects of poor nutrition during larval development and of the selection regime on the mean values of the five wing traits. To simplify this analysis, we first calculated for each trait and individual the average of the four measurements (left and right wing, each measured twice). Values thus obtained were analyzed with a mixed-model ANOVA. The model included selection regime, food and regime × food interaction as fixed factors; the random factors were population (nested within regime), food × population (nested within regime), and vial (nested within regime, population and food).

Measurement error versus directional and fluctuating asymmetry. Subsequently, following the recommendations of Palmer & Strobeck (1986), we carried preliminary analyses aiming test for directional asymmetry, to estimate measurement error and test if it is small enough for non-directional asymmetry (i.e. fluctuating asymmetry + antisymmetry) to be reliably detected, and if antisymmetry significantly contributes to non-directional symmetry. All individuals were pooled for this analysis (i.e. differences between selection regimes and food treatments and variation among populations are absorbed in the variation among individuals). The original four measurements for each trait (left and right wing each measured independently by two experimenters) were examined using a mixed-model ANOVA with sides (left vs right) and the experimenter as fixed factors, and individual and individual × side interaction as random factors (Palmer & Strobeck, 1986). The main effect of experimenter accounts for a systematic bias due to experimenter (e.g., if one person consistently tends to take greater measurements than the other), the main effect of side corresponds to directional asymmetry, which is defined as a general tendency of asymmetry towards one direction (Van Dongen, 2006). The individual × side interaction represents fluctuating asymmetry (plus potential contribution from antisymmetry); if significant, it indicates that non-directional asymmetry can be detected over the measurement error (Palmer & Strobeck, 1986). The residual variance in this analysis gives an estimate of the measurement error. The model initially also included the experimenter × side interaction, but this factor never approached significance and so was excluded from the model.

Antisymmetry (whereby the development "aims" at producing asymmetry in a random direction) may inflate measurements of fluctuating asymmetry (Palmer & Strobeck, 1986). While under fluctuating asymmetry the signed differences between corresponding measurements on the right and left side (R – L) should be normally distributed, antisymmetry would tend to make this distribution platycurtic, or in an extreme case bimodal. Therefore, following the recommendation of Palmer & Strobeck (1986) we used the Shapiro-Wilk test to test for deviations of signed R – L values from normality; this was done for each trait × treatment combination using values of individual asymmetry (signed difference).

Effects on fluctuating asymmetry. To quantify fluctuating asymmetry, we chose two of the several indices described by Palmer & Strobeck (1986), FA4 and FA 6, both based on variance of signed difference between right (R) and left (L) side. $FA4 = \text{var}(R - L)$ estimates the variance of the absolute

signed difference between right (R) and left (L) side measurements, while $FA6 = \text{var}[2(R-L)/(R+L)]$ is the variance of the difference relative to the trait size of each individual. The latter measure takes into account that the magnitude of developmental errors is expected to increase with the size of the morphological structure; being dimensionless, it can be compared among traits. These indices have been widely used in fluctuating asymmetry studies, and in contrast to indices based on unsigned difference between right and left side, are not affected by existence directional asymmetry (reviewed by Palmer & Strobeck, 1986).

We estimated FA4 and FA6 for each population \times treatment \times trait combination. To test for differences in these variance-based fluctuating asymmetry indexes we followed Palmer and Strobeck's (1986) recommendation to use Scheffe's Box approach. This approach involves calculating the fluctuating asymmetry index values for replicate subsamples, log-transforming them, and subjecting to analysis of variance (Sokal & Rohlf, 1995 p. 403). In our cases replication was provided by the selection populations. Thus, the FA4 and FA6 index values for each of 24 population \times food combinations were log-transformed and used as response variables in an ANOVA, with regime and food as fixed factors and populations nested within the regime as a random factor. This analysis thus tests for systematic differences in fluctuating asymmetry between selection regimes and food treatments, which are greater than expected based on variation in fluctuating asymmetry among replicate populations.

3. RESULTS

Effects on trait means. Both the selection regime and the food treatment affected the means of all five wing traits, although for Length A the effect of food was only marginally significant (Table 1). The selected populations had evolved smaller wings (in terms of area and the three linear measurements) than the control populations, irrespective of the food treatment (Figure 2). These wing measurements were also smaller for flies of both selection regimes when raised under the poor food treatment than when raised on standard food (Table 1, Figure 2). Both control and selected populations had a larger Ratio A/B (a simple index of wing shape) when reared on poor food than when reared on standard food (Figure 2): the nutritional stress induced proportionally narrower wings as a plastic response. The effect of selection on the Ratio A/B was parallel to this plastic response: the selected lines showed a greater Ratio A/B (Figure 2). The replicate populations within each regime did not vary significantly for any trait, nor was there any vial effect detected (Table 1).

Measurement error versus directional and fluctuating asymmetry. The magnitude of measurement error was assessed by a mixed-model ANOVA for each trait with sides (left or right) and experimenter (the person measuring) as fixed factors and individuals (combined across all treatments) as a random factor. The interaction between the sides and individuals was highly significant for all five traits examined (Length A: $F_{239, 480}=7.83$, $p<0.001$; Length B: $F_{239, 480}=5.0$, $p<0.001$; Length C: $F_{239, 480}=3.84$, $p<0.001$; Wing Area: $F_{239, 480}=3.33$, $p<0.001$; Ratio A/B: $F_{239, 480}=4.9$, $p<0.001$). This indicates that non-directional asymmetry (fluctuating asymmetry + potential antisymmetry) was greater than the measurement error. The measurement error estimated as the residual variance in this analysis only accounted for a small fraction of total variance for the four directly taken measurements (Length A: error variance = 2×10^{-5} , or 0.4 % of total variance; Length B: 2×10^{-5} or 0.9 %; Length C: 4×10^{-5} or 1.9 %; Wing Area: 4×10^{-5} or 0.6 %). The measurement error for Ratio A/B constituted a greater fraction of total variance than in the case of the other traits (1.1×10^{-4} or 7.7 % of total variance), largely because the total variance for this trait was smaller (Length A and Length B were positively correlated). In the absence of true fluctuating asymmetry the index FA4 should be equal to twice the error variance; the actual estimates of FA4 were one order of magnitude greater for the Ratio A/B, and at least two orders of magnitude greater for the other traits (Fig. 3a). Measurements taken by the two experimenters differed systematically by 0.07-0.17 % for Length A ($F_{1, 479}=8.0$, $p=0.005$), Length B ($F_{1, 479}=9.81$, $p=0.002$) and Wing Area ($F_{1, 479}=7.6$, $p=0.006$). However, the effect of the experimenter does not bias the fluctuating asymmetry indices used here (both experimenters measured each wing; furthermore, experimenter \times side interaction was not significant).

The above ANOVA was further used to test for directional asymmetry. Slight directional asymmetry was detected for Length A (0.16% of the mean trait size; $F_{1, 239}=4.73$, $p=0.031$) and for Wing Area (0.64%; $F_{1, 239}=33.57$, $p<0.0001$), but not for Length B ($F_{1, 239}=0.41$, $p=0.52$), Length C ($F_{1, 239}=0.37$, $p=0.54$) and Ratio A/B ($F_{1, 239}=0.97$, $p=0.33$). Directional asymmetry does not affect the indices of fluctuating asymmetry used in our study (Palmer & Strobeck, 1986).

Effects on fluctuating asymmetry. We found no effect of selection regime or food treatment on either index of fluctuating asymmetry (FA4 and FA6) for any of the five wing traits (Figure 3 a, b, Table 2). Nonetheless, the selected populations raised on poor food tended to show greater fluctuating asymmetry than the other three combinations of selection history and food treatment. Furthermore, the selected populations raised on poor food showed significantly negative kurtosis (i.e., platykurtic distribution) of the signed differences between the left and right wing from zero ($P < 0.05$, Shapiro-Wilk test) for traits Length A, Length B, Length C and Wing Area. No kurtosis was detected for any trait among other combinations of selection regime and food treatment. Inspection of the data revealed that the kurtosis observed in selected populations raised on poor food could be attributed to five highly asymmetric individuals within two replicate populations in this treatment. The measurements on the images of these wings were reconfirmed and the asymmetry in these individuals was found to be three standard deviations greater than the mean asymmetry of the flies within this treatment. We therefore repeated the analysis after removing these five outliers. However, to be conservative, we also excluded data of five individuals with highest asymmetry from the other three treatments. The trend for higher fluctuating asymmetry in selected flies on poor food disappeared in this reduced data set (see figure 3 c); the analysis using this data set (Table 2) again indicated no significant effects on fluctuating asymmetry.

4. DISCUSSION

Larval malnutrition had a significant effect on all wing traits measured in this study at both plastic and evolutionary level. When raised on poor larval food, both control and selected populations had smaller wings than when raised on standard food. Furthermore, the control populations had larger wing measurements than the selected populations on both foods. Similar results were previously found for wing area (Vijendravarma *et al.*, 2011), although in that study the difference in wing area between the selection regimes was only significant on standard food. The effect of food on wing area in the present study tends to be smaller than in Vijendravarma *et al.* (2011). This is probably because only flies emerging until day 15 were assayed here; late emerging flies on poor food were thus not included in the sample, and within populations the late emerging adults tend to be somewhat smaller (Gebhardt & Stearns, 1993). These caveats notwithstanding, our results demonstrate that the wing traits studied here show plasticity in response to the nutritional stress and responded to selection resulting from it.

Nonetheless, we found no evidence for a physiological (plastic) effect of larval malnutrition on fluctuating asymmetry in wing traits. Control populations reared on poor food exhibited similar levels of fluctuating asymmetry to those reared on standard food, thus rejecting the hypothesis that larval malnutrition leads to reduced developmental stability (assessed as increased fluctuating asymmetry), at least for the wing size traits we measured. This result parallels other experimental studies in *Drosophila* and other insects which have similarly failed to observe any effect of nutritional stress on fluctuating asymmetry of different traits (Arnqvist & Thornhill, 1998; David *et al.*, 1998; Bjorksten *et al.*, 2000; Woods *et al.*, 2002; Stige *et al.*, 2004; Vishalakshi & Singh, 2008). However, the variation of fluctuating asymmetry is likely to be specific to the trait measured, organism and population studied, thus limiting our ability to generalise patterns amongst different studies (Van Dongen, 2006). The degree of nutritional stress in our study has been particularly strong; on the poor food the flies take 70 % more time to develop and emerge at less than half the size of flies reared on the standard food (Kolss *et al.*, 2009). Thus, the severity of the nutritional stress is much greater than the modest effect on the means of the wing traits would indicate. It cannot be excluded that particularly asymmetric individuals had been lost to mortality before the measurements were taken. Such developmental selection has been reported in studies where effects of chemicals like arsenic and lead

on fluctuating asymmetry in *Drosophila* were investigated (Polak, Opoka & Cartwright, 2002; Stamenkovic-Radak *et al.*, 2008). Similarly, these particularly asymmetric individuals could have taken too long to develop to be included in the measured sample, although this is unlikely because developmental time does not seem to correlate with fluctuating asymmetry in *Drosophila* (Woods *et al.*, 1999; Shakarad *et al.*, 2001). It is also possible that wing size and shape are particularly strongly buffered against environmental disturbances and genetic changes. Differential effect of environmental stress on fluctuating asymmetry of different traits has been reported in some studies (Blanckenhorn, Reusch & Muhlhauser, 1998; Woods *et al.*, 1999). With this caveat, and occasional reports on the contrary notwithstanding (e.g. Imasheva *et al.*, 1999), the fact that even such a strong stress had no measurable effect on fluctuating asymmetry strongly supports the conclusion that in general larval nutritional stress in *Drosophila* has negligible effects on developmental stability, at least to the extent that the latter is manifested as fluctuating asymmetry (Van Dongen, 2006).

More interestingly, we also found no evidence of fluctuating asymmetry changing as a result of over 80 generations of selection for tolerance to the nutritional stress. (A trend for the selected populations to show greater fluctuating asymmetry on poor food than the control populations was entirely caused by five abnormally asymmetric individuals.) This is despite the strong and effective selection for both survival and fast development on the poor food, which led to a reduction in both body and wing size as a correlated response (Kolss *et al.*, 2009; Vijendravarma *et al.* 2011). It may be argued that in the current experiment the effect of adaptation to nutritional stress on fluctuating asymmetry cannot be tested, because the fluctuating asymmetry of control populations was not affected by nutritional stress. However, the mechanisms proposed to increase fluctuating asymmetry as a result of directional selection (reviewed in the Introduction) do not require that the factor of selection has a direct plastic effect on fluctuating asymmetry. Our result here is in accordance with a study on *D. melanogaster*, reporting no effect of directional selection for faster development on fluctuating asymmetry (Shakarad *et al.*, 2001). However, our result here is in contrast to the increased fluctuating asymmetry observed in response to bidirectional selection for body size in *D. ananassae* (Vishalakshi & Singh, 2009).

Changes in fluctuating asymmetry have been observed in flies exposed to certain stressors and as result of directional selection (Imasheva *et al.*, 1999; Polak *et al.*, 2002; Polak *et al.*, 2004; Vishalakshi & Singh, 2008). However, our results, together with those of (Fowler & Whitlock, 1994; Vishalakshi & Singh, 2008), do not support the notion that fluctuating asymmetry in general increases as a result of plastic response to stress, nor as a result of response to directional selection. Rather, the plastic and evolutionary effects on fluctuating asymmetry seem to depend on the type of stress factor, the selection regime, trait measured, species and possibly even the population. It is also a possibility that, in contrast to what is often assumed, not all differences in developmental stability are reflected in fluctuating asymmetry (Van Dongen 2006).

Acknowledgements

This work has been supported by the Swiss National Science Foundation. We thank the Cellular Imaging Facility of the University of Lausanne and A. Paradis for technical help and the three reviewers for their comments.

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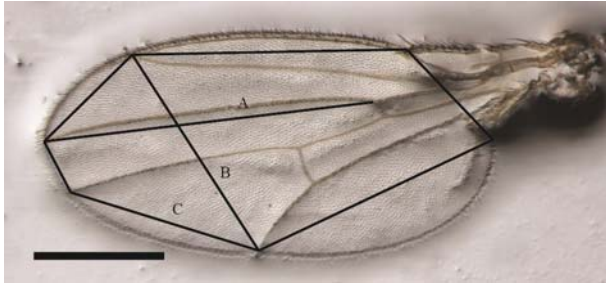


Figure 1: The four trait measurements. Length A, Length B, Length C and Wing area –measured as the area of the polygon traced on the wing (Scale bar =0.5 mm).

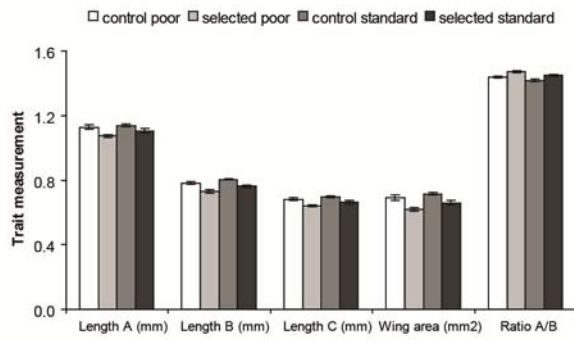


Figure 2: The average trait values for the five traits (Length A, Length B, Length C, Wing area and Ratio A/B) across both selection regimes reared under optimal and stressful conditions.

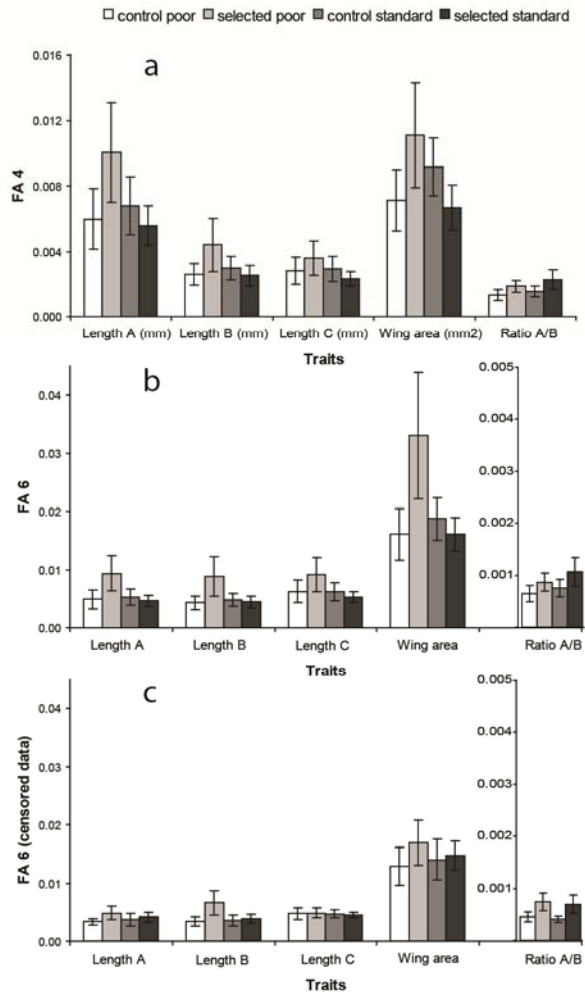


Figure 3: Fluctuating asymmetry measured as (a) FA4 (b) FA6 and (c) FA6 with reduced data (see text), for the five traits measured (Length A, Length B, Length C, Wing area and Ratio A/B) across both selection regimes reared under optimal and stressful conditions.

Tables:**Table 1:** Results of the mixed-model ANOVA on the mean trait values for the five traits (Length A, Length B, Length C, Wing area and Ratio A/B) across both selection regimes reared under optimal and stressful conditions.

Factors	d.f.	Length A		Length B		Length C		Wing area		Ratio A/B	
		F	p	F	p	F	p	F	p	F	p
Regime	1,10	9.5	0.01	25.5	0.001	17.7	0.002	15.1	0.003	20.6	0.001
Population[Regime] ®	10,10	1.6	0.2	1.9	0.2	2.1	0.1	2.2	0.1	2.0	0.1
Food	1,10	4.1	0.07	16.3	0.002	9.7	0.01	9.2	0.01	19.5	0.001
Regime x Food	1,10	0.8	0.4	0.8	0.4	0.2	0.6	0.5	0.5	0.0	0.9
Population x Food[Regime] ®	10,24	1.6	0.2	1.6	0.2	1.2	0.3	1.5	0.2	1.9	0.09
Vial[Regime,Population,Food] ®	24,132	1.3	0.2	1.1	0.3	1.4	0.1	1.1	0.3	1.0	0.5

®: Random factor

Table 2: Results of the mixed-model ANOVA carried out for Log (FA 4) and Log (FA 6) (full and censored data) for the five traits (Length A, Length B, Length C, Wing area and Ratio A/B) across both selection regimes reared under optimal and stressful conditions. "Reduced data" refer to the data with the five most asymmetric individuals for each regime × food combination removed (see text for explanation).

FA Indices	Factors	d.f.	Length A		Length B		Length C		Wing area		Ratio A/B	
			F	p	F	p	F	p	F	p	F	p
Log-FA 4	Regime	1,10	0.34	0.57	0.26	0.62	0.05	0.83	0.11	0.75	1.34	0.27
	Population [regime] ®	10,11	1.40	0.30	0.62	0.77	1.01	0.49	0.67	0.73	1.48	0.26
	Food	1,11	0.11	0.75	0.01	0.94	0.09	0.77	0.03	0.87	0.26	0.62
Log-FA 6	Regime	1,10	0.77	0.40	0.92	0.36	0.36	0.56	1.21	0.30	1.07	0.33
	Population [regime] ®	10,11	1.27	0.35	0.61	0.78	0.98	0.51	0.62	0.77	1.39	0.30
	Food	1,11	0.27	0.62	0.06	0.81	0.20	0.67	0.03	0.87	0.32	0.59
Log-FA 6 Reduced data	Regime	1,10	0.91	0.36	3.18	0.11	0.16	0.70	1.84	0.21	2.94	0.12
	Population [regime] ®	10,11	1.51	0.26	0.51	0.85	0.45	0.89	0.46	0.88	2.50	0.08
	Food	1,11	0.01	0.92	0.46	0.51	0.04	0.84	0.01	0.95	0.18	0.68

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