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Intraspecific hybridization, developmental stability and fitness in *Drosophila mercatorum*

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ABSTRACT

One of the possible effects of intraspecific hybridization is outbreeding depression, due to a breakdown of coadapted gene complexes, which can lead to reduced fitness and decreased developmental stability in hybrids. Alternatively, increased fitness and increased developmental stability in hybrids (hybrid vigour) may be a result of hybridization, probably due to increased heterozygosity. Developmental stability is assumed to be correlated with fitness and is commonly measured as fluctuating asymmetry or phenotypic variance. *Drosophila mercatorum* is capable of reproducing sexually, but also parthenogenetically in the laboratory. When selecting for parthenogenesis, the flies become homozygous in one generation; strong selection, therefore, is acting on the genome of these flies for coadaptation among genes. Intraspecific hybridization is therefore expected to have an impact when coadaptation is disrupted. Intraspecific hybridization between a parthenogenetic and a sexually reproducing strain of *Drosophila mercatorum* resulted in significant changes in fecundity as well as fluctuating asymmetry and phenotypic variance for the number of sternopleural bristles and in the length of two wing traits over three generations after hybridization. We found a 'hybrid vigour effect' in F1 females with an increase in fecundity relative to their parental populations. The F2 and F3 females showed increased fluctuating asymmetry in several traits and reduced fecundity compared with the F1 females, probably due to a breakdown in coadapted gene complexes. The males followed the same pattern of fluctuating asymmetry for bristles but there was no increase in wing fluctuating asymmetry in the F2 and F3 generations. Trait differences in phenotypic variance were found between wings and bristles. We found an increase in phenotypic variance in the F1 generation for both sexes and all traits, which could be due to increased genetic variance after hybridization. The phenotypic variance increased further in generations F2 and F3 for bristle number. For the wings, phenotypic variance generally decreased in generations F2 and F3 when compared with F1, which we attribute to canalization and selection on the wings.

Keywords: developmental stability, *Drosophila mercatorum*, fitness, hybridization, outbreeding.

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INTRODUCTION

Hybridization and developmental stability

In disturbed habitats, previously isolated populations may come in contact (Dowling and Secor, 1997). If individuals from two such populations mate, there will be a hybridization of the two gene pools in the progeny (Ross and Robertson, 1990). Hybridization most commonly refers to matings by individuals that differ taxonomically (interspecific hybridization) but the term has also been applied to matings between individuals of populations that differ genetically but which are not taxonomically distinguishable (intraspecific hybridization) (Barton and Hewitt, 1985; Rhymner and Simberloff, 1996).

Because of habitat fragmentation, migration between populations of the same species can become almost impossible, which can be a threat to many populations. Because of differences in selection regimes and drift, small endangered populations may differ genetically or become susceptible to inbreeding depression (Lynch, 1996). To avoid inbreeding depression, conservationists have used translocation of animals between populations and have introduced captive reared animals to solve this problem (Marshall and Spalton, 2000). However, it is important to examine the populations involved very carefully, both genetically and demographically, before any translocation to avoid outbreeding depression (Miller *et al.*, 1999), which is a reduction in fitness due to mating of genetically divergent individuals (Lynch, 1991).

Hybridization can lead to outbreeding depression within the affected populations due to a breakdown in coadapted gene complexes (Dobzhansky, 1950). Dobzhansky (1950) defined coadaptation as the balance between loci in the genome. Such coordination within the genome protects the individual from developmental accidents, which can be of environmental or genetic origin or both (Parsons, 1990). A breakdown in coadaptation might be displayed by the individual as a decreased ability to develop an optimal phenotype due to reduced developmental stability (Leary *et al.*, 1985). Developmental stability refers to the ability of an organism to buffer its developmental processes against environmental and genetic disturbances to ensure common developmental outcomes under specified conditions (Mitton, 1993). Two principal methods are commonly used to estimate developmental stability. Some studies have used the phenotypic variance of different morphological traits, where the estimate can be blurred by genetic variance and environmental variance. Other studies have used fluctuating asymmetry, which is the difference in value between paired bilateral traits. This dissimilarity in expression of a given character on the left and the right side, observed in the case of fluctuating asymmetry, cannot be explained by either genotype or environmental differences, since the development of bilateral characters in an individual is ensured by the same genotype under identical environmental conditions (Palmer and Strobeck, 1986). Fluctuating asymmetry tends to become elevated due to both environmental stress, such as pollution (Østbye *et al.*, 1997) and extreme temperatures (Imasheva *et al.*, 1997), and genetic factors, including the loss of genetic variation (Vøllestad *et al.*, 1999), the extent of protein heterozygosity (Leary *et al.*, 1983; Mitton, 1993), hybridization (Ross and Robertson, 1990), episodes of directional selection (Markow and Ricker, 1992) and mutations (Clarke and McKenzie, 1987). Much of the current interest in fluctuating asymmetry stems from its potential as an indicator of fitness. Some studies have found fluctuating asymmetry to be negatively correlated with fitness components, whereas others have found a very weak correlation or no correlation (Møller, 1999).

A disruption of coadaptation has been observed by crossing individuals representing differently coadapted genomes. This can be reflected in decreased fitness, increased phenotypic variance and increased fluctuating asymmetry (Graham and Felly, 1985). These effects might be displayed immediately after hybridization in the F1 generation, due to very distinct genomes of the hybridizing individuals (Markow and Ricker, 1991), and is therefore often seen as a result of interspecific hybridization (Ross and Robertson, 1990). In other cases, disruption of coadaptation might not be observed before the F2 generation. The strong interruption of coadaptation in F2 is a result of recombination and segregation events during meiosis in the F1 generation. This produces an F2 generation containing genomes that are recombinations of the parental F1 genomes (Graham, 1992). The F2 genomes, therefore, consist of genes with different evolutionary histories, which have not undergone selection for coadaptation together (Felley, 1980). The disruption of coadapted gene complexes might, therefore, produce individuals that have lower fitness than either parental type (Vetukiv, 1955, 1957).

Another common observation is that progeny in the F1 generation after hybridization exhibit enhanced fitness and decreased fluctuating asymmetry relative to their parents (referred to as hybrid vigour), which generally is believed to originate from increased heterozygosity (Ferguson *et al.*, 1987).

Drosophila mercatorum is capable of reproducing sexually, but also parthenogenetically in the laboratory. When selecting for parthenogenesis, the flies become homozygous in just one generation, as they reproduce by pronuclear duplication (Templeton *et al.*, 1976). Therefore, strong selection is acting on the flies and only flies with a coadapted genome will be able to establish a parthenogenetic strain that can persist in time. The parthenogenetic flies are totally homozygous and, therefore, do not possess any recessive lethal or sublethal alleles; therefore, it is impossible for any F1 hybrid to be homozygous for these alleles. Performing intraspecific hybridization using this strain seemed a good way of maximizing the effects of both hybrid vigour and a breakdown of coadaptation. We assessed the effects by using estimators of individual developmental stability and fitness. We did this over three generations after hybridization to determine if the possible impact of hybridization would change over generations.

MATERIALS AND METHODS

Experimental design

We used two different strains of *Drosophila mercatorum*, a sexually reproducing outbred strain and a parthenogenetic strain Iv-23-olm isolated from the sexual population in 1990 (Kramer and Templeton, 2001). The parthenogenetic flies should be completely homozygous because they reproduce by pronuclear duplication (Templeton *et al.*, 1976).

In this experiment, we made the intraspecific hybridization by mixing a sexual male with a parthenogenetic female. In each of 10 vials, we placed one parthenogenetic female and one male and, when mated, the parthenogenetic females reproduced sexually. In this way, we obtained 10 strains. The flies were kept at 25°C in vials containing instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC, USA) and were fed with live yeast. They were allowed to lay eggs for 2 days in one vial before being moved to a new vial, which was done four times. We moved the flies every second day to minimize any effects of crowding and competition between the larvae. We only used progeny from mothers 4–8 days

old to minimize any maternal effects, as both fluctuating asymmetry and phenotypic variance in the progeny have been shown to increase with maternal age in *Drosophila* (Parsons, 1962). The F2 and F3 generations were reared following the same procedure as the F1 generation. The hybrid flies that were used to produce the next generation were taken at random from separate strains to avoid any possibility of mating between related individuals.

In each generation, when the flies hatched, males and females were separated (as virgins). After 7 days, about 70 males and 70 females from each of the 10 strains were chosen at random and individually mated with flies from the other nine strains, giving around 700 matings in total. One female and one male were placed in a vial with a plastic spoon that contained a small amount of medium covered with a layer of live yeast. Then egg laying was allowed for 24 h before the flies were frozen at −18°C. We counted the number of eggs laid by each female and the number of sternopleural bristles on the right and left side. The wings were removed, mounted on a glass slide in a drop of lactic acid and a cover slip was placed over them. The wings were measured using a camera attached to a dissecting microscope, a Macintosh computer and the software package Object Image 1.62p2 (Vischer, 2000). We measured two wing traits (traits A and B) on each wing using three landmarks (Fig. 1).

The fecundity of the parthenogenetic females when mated with the sexual males was lower than that of the sexual females (results not shown). Probably because of the lower propensity of parthenogenetic females to mate than sexual females (Templeton, 1983), this resulted in fewer than the desired 700 matings of F1 flies (Table 1).

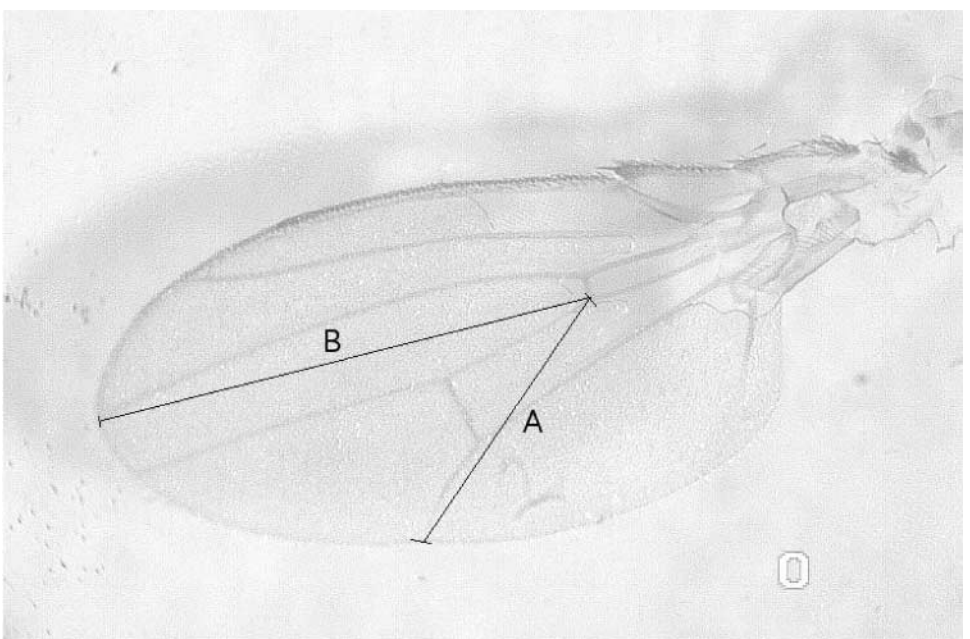

Fig. 1. Wing landmarks used for measuring the two wing traits. A = length between the end of the fifth longitudinal vein and link between the anterior cross vein and the fourth longitudinal vein. B = length between the link between the anterior cross vein and the fourth longitudinal vein and the end of the third longitudinal vein.

Table 1. Results of one-way ANOVA comparing the mean fluctuating asymmetry (FA) of the number of bristles and length of wing traits A and B among the parental sexual and parthenogenetic populations and generations F1, F2 and F3

Traits	Partheno-genetic (*n*), Mean ± s.e.	Sexual (*n*), Mean ± s.e.	F1, (*n*), Mean ± s.e.	F2, (*n*), Mean ± s.e.	F3, (*n*), Mean ± s.e.	Source of variation	*df*	MS	Levene's test (*F* value)	*P*	F_{max} test	*P*	K-W test (H-value)	*P*	Scheffé's *F*-test
FA bristles:															
Females:															
Mean IFAI	(109)	(124)	(681)	(763)	(769)	between	4	5.21	4.54	**	1.44	**	12.57	*	(F1 < F3)**
	1.14 ± 0.09	1.15 ± 0.08	1.13 ± 0.04	1.28 ± 0.04	1.35 ± 0.04	within	2441	1.15							
Males:															
Mean IFAI		(124)	(680)	(762)	(769)	between	3	4.98	3.75	*	1.42	***	9.12	*	(F1 < F3)*
		1.34 ± 0.09	1.19 ± 0.04	1.27 ± 0.40	1.39 ± 0.05	within	2331	1.33							
FA wings:															
Females:															
IFAI A	(100)	(121)	(663)	(750)	(759)	between	4	5.98	99.78	***	4.87	***	632.53	***	(sex < F2)***, (par < F2)***, (par < F3)**, (F1 < F2)***, (F1 < F3)***, (F2 > F3)***
	0.07 ± 0.02	0.14 ± 0.03	0.07 ± 0.01	0.32 ± 0.01	0.17 ± 0.01	within	2388	0.06							
IFAI B	(100)	(121)	(663)	(750)	(758)	between	4	2.98	59.16	***	3.52	***	213.85	***	(sex < F2)***, (par < F2)***, (F1 < F2)***, (F2 > F3)***
	0.06 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.20 ± 0.01	0.05 ± 0.01	within	2387	0.05							
Males:															
IFAI A		(118)	(660)	(749)	(760)	between	3	0.17	2.79	*	2.02	***	12.10	**	
		0.11 ± 0.03	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	within	2283	0.06							
IFAI B		(118)	(660)	(749)	(760)	between	3	0.09	4.74	**	12.77	***	15.50	**	(sex > F1)*
		0.06 ± 0.02	0.02 ± 0.003	0.03 ± 0.004	0.04 ± 0.01	within	2283	0.19							

Note: F_{max} test for homogeneity of variance of absolute fluctuating asymmetry, Kruskal-Wallis (K-W test) one-way non-parametric analysis of variance and Scheffé's *F*-test for multiple comparisons. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Measurement error

Measurement error can cause extreme bias in studies of fluctuating asymmetry; therefore, accurate estimates of measurement error are essential (Palmer, 1994). When sample sizes are large, repeated measurements of the whole sample may not be practical, in which case the effect of measurement error should be calculated from repeat measures of a subsample of at least 30 individuals. To estimate measurement error in this study, a subsample of 50 parthenogenetic flies chosen at random was measured two times. The second set of measurements was made within 24 h, without reference to the first set, and all measurements were recorded by the first author only. Between measurements of the sternopleural bristles, the flies were frozen in Eppendorf tubes. The sternopleural bristles were measured without error. For the wings, a two-way analysis of variance was conducted to test for the significance of fluctuating asymmetry relative to measurement error (the difference between two independent estimates of fluctuating asymmetry) following Palmer and Strobeck (1986). We found that the wing traits were measured with high repeatability. The interaction mean square (MS) containing information about fluctuating asymmetry was tested against error mean square (reflecting measurement error), which showed that fluctuating asymmetry was significantly larger than measurement error in all cases ($0.33 <$ interaction MS < 1.16; $0.0001 <$ error MS < 0.04; d.f. $= 49$, $P < 0.0001$).

Statistical properties of fluctuating asymmetry

In the following statistical analysis, males and females in each generation were analysed separately and, because of the large number of tests conducted, we applied the sequential Bonferroni test (Rice, 1989).

Fluctuating asymmetry is characterized by a normal distribution of right-side minus left-side (r – l) differences with a mean of zero (Palmer and Strobeck, 1986). Antisymmetry occurs when a significant difference exists between sides, but the larger side is randomly distributed within a sample (Graham *et al.*, 1993). Fluctuating asymmetry distributions for the sternopleural bristles and for the two wing traits were inspected graphically for normality and antisymmetry. No deviation from true fluctuating asymmetry was found. However, in 11.1% of the fluctuating asymmetry distributions for the sternopleural bristles and 94.4% of the wing traits investigated, we found leptokurtic distributions.

We tested for directional asymmetry, which occurs when there is a consistent bias in a more pronounced development of a character towards one side (Graham *et al.*, 1993). If directional asymmetry occurs, the mean (r – l) character value has a normal distribution, with a mean value deviating from zero. We tested for directional asymmetry using a one-sample *t*-test, which tests for significant deviation of the mean value of (r – l) from zero. No significant directional asymmetry was detected.

Measurement of fluctuating asymmetry

Asymmetry was estimated as the difference in value between each bilateral pair of traits (r – l). Fluctuating asymmetry was calculated as an absolute value at the individual level and as a mean value at the population level (Palmer and Strobeck, 1986).

Statistical analysis

Dependence of fluctuating asymmetry on trait size

We tested for possible associations between trait size and fluctuating asymmetry because these may affect the interpretation of directional asymmetry studies (Palmer, 1994). We tested for the dependence of fluctuating asymmetry on trait size by linear regression, polynomial regression and the Spearman rank correlation test.

Correlation of fluctuating asymmetry among traits and with the number of eggs

As individual fluctuating asymmetry values of the number of sternopleural bristles and wing traits could be correlated, we tested for a correlation between fluctuating asymmetry for the different traits at an individual level using linear regression analysis. The females from the sexual strain, the F1, F2 and F3 generations were examined for correlations between fluctuating asymmetry in each of the traits and number of eggs laid.

Furthermore, to quantify the effect of body size on fecundity, the females from the sexual strain, the F1, F2 and F3 generations were pooled and examined for correlations between trait B and the number of eggs laid. Trait B is correlated with thorax length in *Drosophila*, which is used as a measure of body size (Robertson, 1959); therefore, we considered trait B to be a reliable estimate of body size in this study.

Comparison of fluctuating asymmetry and phenotypic variance among generations

A one-way analysis of variance (ANOVA) was conducted to test for differences in mean fluctuating asymmetry for the different traits among generations. Because fluctuating asymmetry is half-normally distributed, comparisons between generations were also performed using a non-parametric Kruskal-Wallis test (Zar, 1984). Multiple comparison tests were performed using Scheffé's *F*-test; this compares the differences between generations.

As a measure of phenotypic variance, we used the coefficient of variation. A two-tailed test for differences between two coefficients of variation was conducted to determine whether the coefficient of variation for the number of sternopleural bristles and that for the length of the two wing traits, on the right wing, was significantly different among generations.

Comparison of mean and coefficient of variation for the number of eggs among generations

A one-way ANOVA and a non-parametric Kruskal-Wallis test were conducted to test for differences in the mean number of eggs among generations. We used log-transformed data to homogenize variances (Zar, 1984). Multiple comparisons were performed using Scheffé's *F*-test to test for differences between generations. The conservative Scheffé's *F*-test was used to reduce the possibility of a Type 1 error. Type 1 error is typically associated with investigations dealing with large amounts of data, as in the present study.

A two-tailed test for differences between two coefficients of variation was conducted to test for significant changes in the coefficient of variation for the mean number of eggs laid in each generation.

Comparison of mean values of traits among generations

Tests for significant changes in the mean value of the investigated traits were performed using one-way ANOVA and the non-parametric Kruskal-Wallis test. Multiple comparison

tests were performed using Scheffé's *F*-test to compare the differences between the generations.

RESULTS

Dependence of fluctuating asymmetry on trait size

For the number of sternopleural bristles and its fluctuating asymmetry, only the polynomial regression analysis was found to be highly significant, showing a U-shaped relationship, for both sexes in all generations.

Correlations between wing trait length and its fluctuating asymmetry were not so clear-cut. We found significant differences both for the linear and the polynomial regression analysis, but in all cases the latter was more significant, with higher r^2-values. There was a general pattern of a significant U-shaped relationship in 80% of the traits for the females and in 87.5% of the traits for the males.

Correlation of fluctuating asymmetry among traits and with the number of eggs

We found no significant correlation between fluctuating asymmetry for the sternopleural bristles and fluctuating asymmetry in the two wing traits for the females ($0.001 < r < 0.119$; $100 < n < 759$; $0.1 < P < 0.97$) or the males ($0.003 < r < 0.17$; $118 < n < 760$; $0.16 < P < 0.93$). Highly significant correlations were found between wing traits; therefore, no average fluctuating asymmetry over traits was measured.

We did not find any significant correlation between the number of eggs laid by each female and fluctuating asymmetry for the sternopleural bristles, but we found highly significant correlations between fluctuating asymmetry in trait B for the wings in the F2 generation ($r = 0.243$, $n = 750$, $P = 0.0001$). There was a highly significant positive relationship between trait B and the number of eggs laid ($r = 0.1$, $n = 2293$, $P = 0.0001$), $r^2 = 0.01$ indicating that body size accounted for 1% of the variation in fecundity.

Comparison of fluctuating asymmetry and phenotypic variance among generations

There were significant changes in mean fluctuating asymmetry of the number of sternopleural bristles among generations (see Table 1), with a significant increase from the F1 to the F3 generation, both for males and females (Table 1, Fig. 2a, Fig. 3a).

For the wing traits, the changes in mean fluctuating asymmetry among generations showed a different pattern than that for the bristles. For the females (Table 1, Fig. 2b,c), we found for both trait A and B a highly significant increase in fluctuating asymmetry in the F2 generation when compared with the parental sexual and parthenogenetic populations and the F1 generation, and a highly significant decrease in fluctuating asymmetry in the F3 generation when compared with the F2 generation (Table 1). For the males, the changes in mean fluctuating asymmetry between generations were not of the same magnitude as for the females (significant changes were only found in trait B; Table 1, Fig. 3c), with significant highest fluctuating asymmetry found in the sexual population (Table 1, Fig. 3c). Using the non-parametric test, significant changes were noted among generations for all wing traits (Table 1).

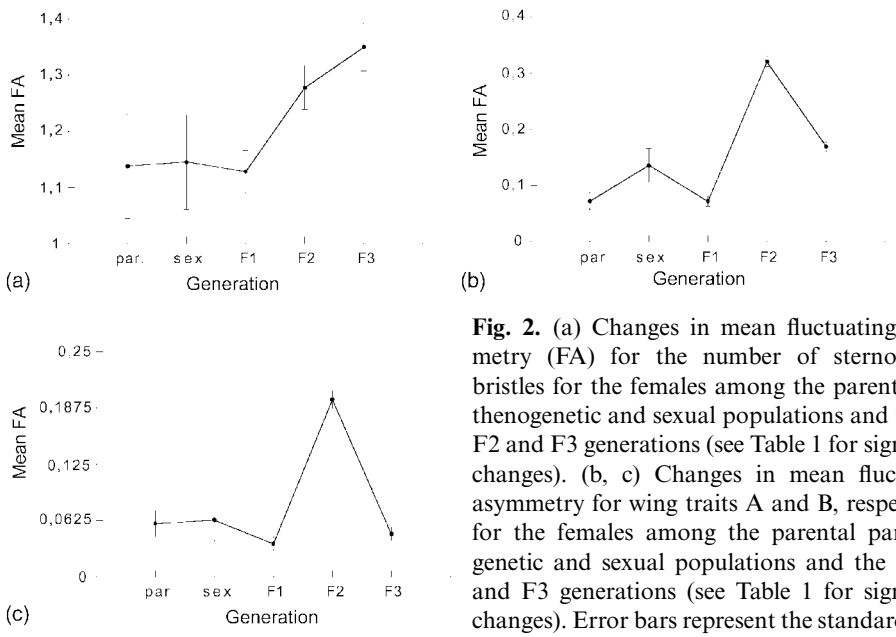

Fig. 2. (a) Changes in mean fluctuating asymmetry (FA) for the number of sternopleural bristles for the females among the parental parthenogenetic and sexual populations and the F1, F2 and F3 generations (see Table 1 for significant changes). (b, c) Changes in mean fluctuating asymmetry for wing traits A and B, respectively, for the females among the parental parthenogenetic and sexual populations and the F1, F2 and F3 generations (see Table 1 for significant changes). Error bars represent the standard error.

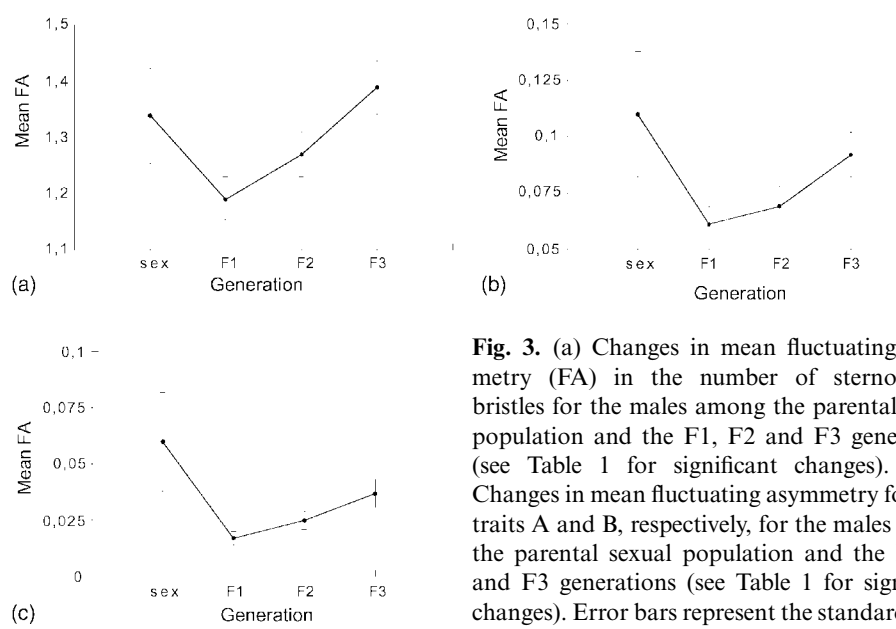

Fig. 3. (a) Changes in mean fluctuating asymmetry (FA) in the number of sternopleural bristles for the males among the parental sexual population and the F1, F2 and F3 generations (see Table 1 for significant changes). (b, c) Changes in mean fluctuating asymmetry for wing traits A and B, respectively, for the males among the parental sexual population and the F1, F2 and F3 generations (see Table 1 for significant changes). Error bars represent the standard error.

For the females, the smallest coefficient of variation was for the number of (r + l) sternopleural bristles in the parthenogenetic population, followed by a significant increase in the F2 and F3 generations when compared with the parthenogenetic population (Fig. 4a). The same trend was found for the males, with the smallest coefficient of variation in the sexual population followed by a significant increase in the F2 and F3 generations relative to

the sexual population (Fig. 5a). For the wings of females (measured on the right wing), the smallest coefficient of variation for both wing traits was seen in the parthenogenetic population (Fig. 4b,c). A significant increase from the parthenogenetic population to the sexual population was found and a further significant increase in F2 (trait A) or in F1 (trait B), and a decrease in F3 (trait A and B). The same trend was found for the males (Fig. 5b,c), with an increase in the coefficient of variation in the F1 generation when compared with the sexual population, followed by a significant decrease in F2 and F3 compared with F1 (see Fig. 5b,c).

Comparison of mean numbers of eggs among generations

We found a highly significant difference in the mean numbers of eggs between generations (Table 2, Fig. 6). The mean number of eggs laid in the F1 generation increased when compared with the parental sexual generation, followed by a highly significant decrease from the F1 to the F2 generation, and a further significant decrease from the F2 to the F3 generation.

Comparison of mean values of traits among generations

The changes in the mean number of bristles between generations were similar for both males and females (Table 2), with the highest mean value in the F1 generation, a significant decrease in the F2 generation followed by a significant increase in the F3 generation compared with the F2 generation.

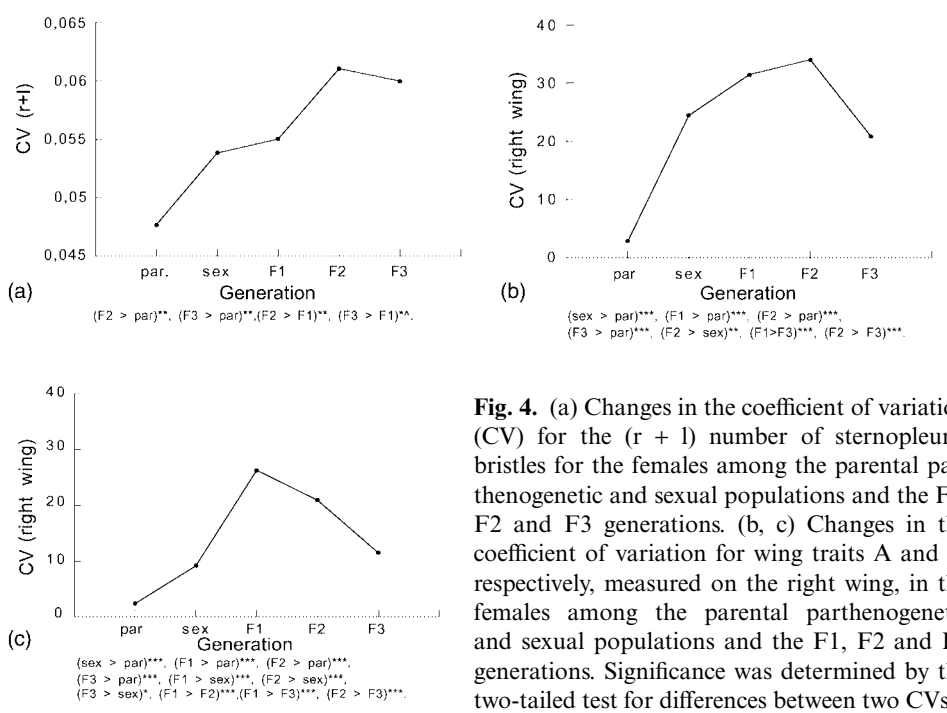

Fig. 4. (a) Changes in the coefficient of variation (CV) for the (r + l) number of sternopleural bristles for the females among the parental parthenogenetic and sexual populations and the F1, F2 and F3 generations. (b, c) Changes in the coefficient of variation for wing traits A and B, respectively, measured on the right wing, in the females among the parental parthenogenetic and sexual populations and the F1, F2 and F3 generations. Significance was determined by the two-tailed test for differences between two CVs.

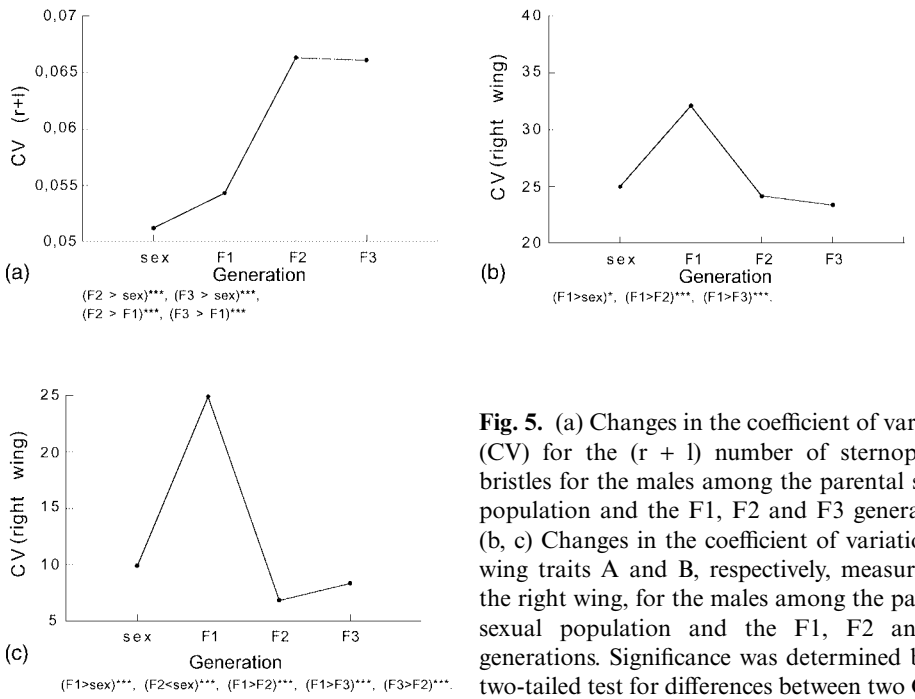

Fig. 5. (a) Changes in the coefficient of variation (CV) for the (r + l) number of sternopleural bristles for the males among the parental sexual population and the F1, F2 and F3 generations. (b, c) Changes in the coefficient of variation for wing traits A and B, respectively, measured on the right wing, for the males among the parental sexual population and the F1, F2 and F3 generations. Significance was determined by the two-tailed test for differences between two CVs.

The mean length of the two wing traits (right wing) in the parthenogenetic females was significantly greater than that for the sexual population and the three hybrid generations (Table 2). The changes in mean length were similar for both traits with a decrease in mean length in the F1 generation when compared with both the parental populations, although it was not significant. We found a significant decrease in the F2 compared with the F1 generation and a significant increase in the F3 compared with the F2 generation (Table 2). For the males, we found significant changes among generations; however, no significant pairwise comparisons using Scheffé's *F*-test were found (Table 2).

DISCUSSION

Metric and meristic traits

In this study, we assessed fluctuating asymmetry of the number of sternopleural bristles (meristic trait) and of the wings (metric trait). The underlying genetic mechanisms controlling these two systems are very different (Woods *et al.*, 1999). We found deviations from normality in the investigated traits, seen as a leptokurtic distribution of fluctuating asymmetry.

For the sternopleural bristles, the leptokurtic distribution could have been produced by chance, as it was only found in the F3 generation and only in males. For the wings, however, the leptokurtic distribution was probably due to selection on the wing traits, which results from a lot of individuals having very low fluctuating asymmetry. The wings are believed to be a fitness-related trait and, therefore, the development of the wings might be subject to

Table 2. Results of one-way ANOVA for the mean number of bristles, length of wing traits A and B and number of eggs among the parental sexual and parthenogenetic populations and generations F1, F2 and F3

Traits	Partheno-genetic (*n*), Mean ± s.e.	Sexual (*n*), Mean ± s.e.	F1, (*n*), Mean ± s.e.	F2, (*n*), Mean ± s.e.	F3, (*n*), Mean ± s.e.	Source of variation	*df*	MS	Levene's test (*F* value)	*P*	F_{max} text	*P*	K-W test (H-value)	*P*	Scheffé's *F*-test
Analysis of mean number of bristles:															
Females:															
Mean number	(109) 38.52 ± 0.19	(124) 38.17 ± 0.17	(681) 38.92 ± 0.08	(763) 37.59 ± 0.08	(769) 38.22 ± 0.08	between	4	161.06	32.61	***	2.45	***	126.04	***	(sex > F2)**, (par < F1)*, (F1 > F2)***, (F1 > F3)***, (F2 < F3)***
						within	2441	4.94							
Males:															
Mean number		(124) 38.44 ± 0.18	(680) 39.78 ± 0.08	(762) 38.02 ± 0.09	(769) 39.05 ± 0.09	between	3	384.08	65.86	***	1.72	***	194.65	***	(sex < F1)***, (F1 > F2)***, (F1 > F3)***, (F2 < F3)***
						within	2331	5.83							

Analysis of mean length of wing traits:															
Females:															
A	(73)	(122)	(663)	(750)	(759)	between	4	6.10	82.42	***	92.86	***	827.61	***	(sex > F2)***,
	1.15 ± 0.004	1.04 ± 0.023	1.01 ± 0.012	0.84 ± 0.01	1.08 ± 0.01	within	2362	0.07							(par > F1)**,
															(par > F2)***,
															(F1 > F2)***,
															(F1 < F3)***,
															(F2 < F3)***
B	(73)	(122)	(663)	(750)	(758)	between	4	6.16	64.10	***	89.84	***	991.73	***	(sex < par)***,
	1.92 ± 0.01	1.71 ± 0.01	1.66 ± 0.02	1.47 ± 0.01	1.65 ± 0.01	within	2361	0.10							(sex > F2)***,
															(par > F1)***,
															(par > F2)***,
															(par > F3)***,
															(F1 > F2)***,
															(F2 < F3)***
Males:															
A		(118)	(660)	(749)	(760)	between	3	0.02	0.29	*	1.89	***	728.99	***	
		0.94 ± 0.02	0.93 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	within	2283	0.06							
B		(118)	(660)	(749)	(760)	between	3	0.11	2.16	*	13.61	***	900.519	***	
		1.54 ± 0.01	1.53 ± 0.02	1.50 ± 0.004	1.52 ± 0.01	within	2283	0.02							
Eggs:															
Females:															
log(1 +x)		(124)	(682)	(763)	(769)	between	3	21.81	60.77	***	1.56	***	295.23	***	(sex < F1)***,
of eggs		1.41 ± 0.06	1.70 ± 0.02	1.39 ± 0.02	1.29 ± 0.02	within	2334	0.36							(F1 > F2)***,
number															(F1 > F3)***,
															(F2 > F3)*

Note: F_{max} test for homogeneity of variance, Kruskal-Wallis (K-W test) one-way non-parametric analysis of variance and Scheffé's *F*-test for multiple comparisons. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

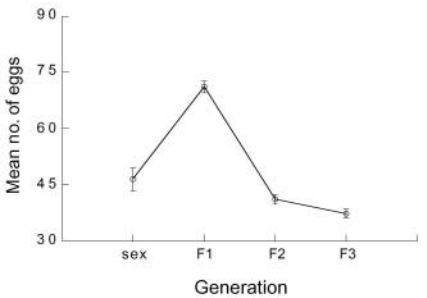

Fig. 6. Changes in the mean number of eggs per female among the parental sexual population and the F1, F2 and F3 generations (see Table 2 for significant changes). Error bars represent the standard error.

stabilizing selection for an optimal function that can only be achieved by ensuring similar developmental patterns in both wings.

Effects of intraspecific hybridization on fecundity, fluctuating asymmetry and phenotypic variance

The changes in mean fluctuating asymmetry and phenotypic variance differed depending on the trait investigated, bristles or wing traits. However, as previously discussed, the discrepancy in the results could be due to differences in stabilizing selection and canalization acting upon the traits. Furthermore, we found differences between the sexes for the effects of hybridization on changes in mean fluctuating asymmetry for the wings.

Developmental instability results when developmental noise or stress affects the buffering capacity of the processes that provide developmental stability (Lens *et al.*, 2000). Phenotypic variance reflects developmental instability, but is influenced by other factors: $\sigma^2 p = \sigma^2 g + \sigma^2 e + (G \times E) + \text{cov}(GE) + DI$ (Pertoldi *et al.*, 2001a), where $\sigma^2 g$ is the genetic variance, $\sigma^2 e$ is the environmental variance, $(G \times E)$ is the genotype × environment interaction, cov(GE) is the covariance between genotypic and environmental sources of variance and DI is developmental instability. The interaction term expresses the extent to which genotypic variants differ in their sensitivity to environmental effects. The covariance between genotypic and environmental sources of variance is the source of experimental error (for instance, when the fastest growing animals are given the best diet). In the parthenogenetic flies, genetic variance is zero ($\sigma^2 g = 0$); if there is no environmental variance ($\sigma^2 e = 0$), phenotypic variance in the parthenogenetic strain should mainly reflect developmental instability. Phenotypic variance in the parthenogenetic flies is expected to be low under optimal environmental conditions, because there is no genetic variance and developmental instability is low (Pertoldi *et al.*, 2001b). This is also in agreement with the result that the parthenogenetic flies always had the smallest coefficient of variation for all traits, when compared with the other generations (Fig 4). When considering phenotypic variance in sexually reproducing populations, the results are influenced by both genetic and environmental variance. In this study, we tried to minimize environmental variance; by doing so, we can ignore this variance ($\sigma^2 e = 0$), which simplifies the previous equation to $\sigma^2 p = \sigma^2 g + DI$.

That there was a (non-significant) decrease in fluctuating asymmetry in the F1 generation for all traits investigated for both sexes, and a highly significant increase in fecundity, when compared with the sexual population, indicates a hybrid vigour 'effect' in the F1 generation. This could be due to the complete homozygosity of the parthenogenetic flies. These flies do not possess any recessive sublethal or recessive lethal alleles. As parthenogenetic flies reproduce by pronuclear duplication (Templeton *et al.*, 1976), any mutation creating such alleles would make the progeny homozygous for the new alleles and they would be expressed immediately. It is therefore impossible for an individual in the F1 generation to be homozygous for any sublethal alleles.

The reduced fecundity in the F2 and F3 generations for females and increased fluctuating asymmetry of sternopleural bristles for both males and females, in these two generations (relative to the F1 generation), and increased wing fluctuating asymmetry in the F2 generation for females (relative to the parental populations and F1), could be due to a breakdown in coadapted gene complexes under the meiosis in the F1 flies. The increase in fluctuating asymmetry in bristles from generations F1 to F3 probably reflects the fact that the breakdown of the coadapted genome is not fully expressed before the F3 generation. That there is no crossing over in *Drosophila* males does not make it possible for two recombinant alleles to be joined in the same individual before the F3 generation. Furthermore, there is a possibility, in the F2 and F3 generations, for two sublethal alleles to become homozygous in an individual and this might have some effect on fluctuating asymmetry and fecundity. However, the main effect, reducing fecundity and increasing fluctuating asymmetry, must be due to a breakdown in coadapted gene complexes, because for the sexual population the fecundity is higher, although not significantly so, and fluctuating asymmetry for the wing traits is significantly lower when compared with the F2 generation for the females. We can consider the effect of body size on fecundity minimal as the relationship between trait B and fecundity accounted for only 1% of the total variability.

The increase in phenotypic variance in both sexes, for the sternopleural bristles, from the F1 to the F3 generation could be due to a disruption of genetic canalization processes that normally depress additive genetic variation by epistatic modifiers (Debat and David, 2001), or an increased contribution of developmental instability to phenotypic variance, or a combination of the two. Characters of greater functional significance to the organism are subject to stronger selection for canalization and, therefore, reduced phenotypic variance (Palmer and Strobeck, 1986; Woods *et al.*, 1999). Sternopleural bristles are generally not regarded as a fitness-related trait and we do not, therefore, expect the sternopleural bristles to be a very canalized trait. Therefore, the main reason for the increase in the coefficient of variation from F1 to F2 and F3 must be attributed to an increased contribution from developmental instability to phenotypic variance, reflected in the increase in fluctuating asymmetry.

For the wings, the pattern seen for phenotypic variance was different. The increase in the coefficient of variation for generation F1 (Fig. 4b,c, Fig. 5b,c) compared with the sexual population appeared, in three of four cases, to arise from an increase in genetic variance after hybridization, as fluctuating asymmetry decreased in F1 and an increase in environmental variance appears unlikely. The wing traits are generally believed to be closely related to fitness and, therefore, the development of the wings may be influenced by genetic canalization processes. Therefore, another explanation may be disruption of genetic canalization processes, operating in the parental populations reflected in an increase in phenotypic variance in the hybrids (Blows and Sokolowski, 1995). It was only in the females that an

increased contribution from developmental instability could have increased phenotypic variance in generation F2. However, phenotypic variance decreased (relative to F1) for trait B and there was no significant increase from generation F1 to generation F2 for trait A, so the contribution of developmental instability must have been lower (or not significantly higher) than the effect of decreased genetic variance. This decrease in genetic variance might be due to selection on the additive genetic variation, which is a component of genetic variation.

We expect the breakdown of coadapted gene complexes to be equal for males and females. However, the effect of the breakdown appears to differ between the sexes. For the sternopleural bristles, a trait expected not to be under strong selection, we found the same result. For the wing traits, on the other hand, a difference was found between the sexes, with lower developmental stability in the wings of the females.

Much theory assumes that the fluctuating asymmetry of a trait is the result of an organism-wide propensity for developmental imprecision (Lerner, 1954), due to underlying stress factors displayed in the extent of fluctuating asymmetry. In this study, we found no correlation between the fluctuating asymmetry of wing traits and that for the number of sternopleural bristles. There may be several explanations for this. Different traits have trait-specific developmental windows, in which the developmental stability of a trait is more vulnerable to stress factors, because the development of distinct traits is probably controlled by different gene complexes (Parsons, 1990). A stress factor may also be specific to particular metabolic pathways and may not affect the fluctuating asymmetry of all traits (Parsons, 1990). Furthermore, different traits are exposed to different degrees of stabilizing selection and canalization that tends to decrease fluctuating asymmetry (Palmer and Strobeck, 1986). This implies that strong fluctuating asymmetry in one trait does not necessarily mean a strong fluctuating asymmetry in other traits in the same individual or population. Therefore, one should be careful when using the fluctuating asymmetry of one trait as a measure of individual fitness and developmental stability for the whole individual. If strong stabilizing selection acts on a trait, the most asymmetrical individuals may be removed during development, and the measured fluctuating asymmetry will not show the real effect on developmental stability in the given trait. Therefore, the choice of trait investigated for fluctuating asymmetry must be considered carefully, as selection could obscure and confound the result.

Mean length of traits

If the genetic factors that control the development of the wings and the bristles only are under additive genetic control, then hybrid progenies are expected to exhibit intermediate values between their parents. This, however, was not observed for the females for any of the traits (Table 2). Therefore, epistatic and dominant interactions also appear to be involved in the development of the traits (Blows and Sokolowski, 1995).

CONCLUSIONS

It is generally believed that developmental stability in hybrid populations is theoretically related to the genetic distance between hybridizing populations, and results from a balance between the stabilizing effect due to increased heterozygosity and the disruptive effect caused by the breakdown in coadaptation (Markow and Ricker, 1991). Several studies did

not find any correlation between developmental stability, reduced fitness and hybridization (Ferguson *et al.*, 1987). The lack of any reducing effect on developmental stability and fitness has led to the conclusion that, in such hybridizations, the divergence between the genomes in the parental populations and the breakdown in coadaptation have less of an effect than increased heterozygosity. But it is very important to note that most of these studies only considered the F1 generation (Ferguson *et al.*, 1987; Lu and Bernatchez, 1999). This may give misleading results, because it is not until meiosis in the F1 generation that the real disruption to the coadapted gene complexes occurs. Future research should follow several generations after hybridization, before making any conclusions about the effect on developmental stability and fitness.

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