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Developmental stability in hybrids between the sibling species pair, *Drosophila melanogaster* and *Drosophila simulans*

T. A. Markow & J. P. Ricker
Department of Zoology, Arizona State University, Tempe, AZ 85287, USA

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Abstract

Drosophila melanogaster and its sibling species *D. simulans* were hybridized in the laboratory to test the hypothesis that developmental homeostasis in hybrids between two species having no prior gene flow would be significantly reduced. Developmental stability was assessed by measuring fluctuating asymmetry for three bilateral traits: sternopleural chaetae, wing length, and fronto-orbital plus frontal chaetae. Male F_1 hybrids showed no decrease in developmental stability compared to males of parental species. Female hybrids showed significant fluctuating asymmetry compared to other flies. The results are discussed with respect to ideas about coadaptation and gene flow based upon previous studies of hybrid developmental stability.

Introduction

Developmental stability, or homeostasis, is the ability of an organism to execute its ontogenetic program despite adverse environmental conditions (Waddington, 1957). A variety of measures are typically utilized to assess developmental stability. These include the frequency of morphological variants or phenodeviants, fitness or its components, and fluctuating asymmetry (nondirectional right-left differences) in paired bilateral traits (Rasmusson, 1960).

Two phenomena are believed to influence developmental stability: heterozygosity and coadaptation. The relationship between high levels of heterozygosity and greater developmental stability is supported by a number of observations. Lerner (1954) discussed the repeated appearance of phenodeviants upon inbreeding or artificial selection in fowl, *Drosophila*, and mice. Fluctuating asymmetry has been shown to increase with laboratory schemes designed to reduce genetic variation (Leamy, 1984; Reeve, 1960; Thoday, 1955; Van Valen, 1962). A positive correlation between heterozygosity at allozyme loci and developmental stability has been reported in rainbow trout (Leary & Allendorf, 1989), marine bivalves (Mitton & Grant, 1984; Mitton & Koehn, 1985) and *Poeciliopsis* (Vrijenhoek & Lerman, 1982; Quattro & Vrijenhoek, 1989) and man (Livshitz & Koblianski, 1985).

The relationship between coadaptation and developmental stability is supported by crosses between different populations of the same species in which a decline in components of fitness appears following recombination in F_2 generation (Vetukhiv, 1954, 1956, 1957; Anderson, 1968; King, 1955). These observations are interpreted as a breakup of coadaptation when two coadapted gene pools recombine and segregate, leading to the prediction that in zones of hybridization and introgression between two species, a decrease in developmental stability should exist relative to the two parental species.

Tests of this prediction have yielded conflicting results. Jackson (1937a, 1937b) reported no excess of fluctuating asymmetry in zones of hybridization between *Sceloporus woodi* and *S. undulatus*. An intergrade zone between two bluegill subspecies, *Lepomis machrochirus* and *L. m. purpurescens*, also failed to show the expected increase in fluctuating asymmetry (Felley, 1980). On the other hand, in freshwater sticklebacks, Zakharov (1981) found greater asymmetry to accompany gene flow from marine popula-

tions.

In contrast, in hybrid populations between two sunfish, *Enneacanthus gloriosus* and *E. obesus*, fluctuating asymmetry was positively correlated with introgression (Graham & Felley, 1985). Recently hybridized populations also show high levels of developmental instability (Leary *et al.* 1985). Graham and Felley (1985) suggested that the seemingly conflicting findings on hybrids reflect the relative age of the hybrid zones such that when introgression has been of long duration, coadaptation has had time to evolve in the hybrid population. They suggest that the hybrid populations in the work of Jackson (1973a, b) and Felley (1980) were much older than the hybrids in their own study (Graham & Felley, 1985).

In an extension of this interpretation, we ask to what degree developmental stability is compromised in F_1 hybrids made between two species that never hybridize in nature and thus in which no coadaptation could have evolved. *Drosophila melanogaster* and *D. simulans* are sibling species of fruit fly. Despite their frequent sympatry, F_1 sterility prevents gene flow between them. However, hybrids can be made in the laboratory. To address the above question, fluctuating asymmetry in F_1 flies from reciprocal laboratory crosses of *D. melanogaster* and *D. simulans* was compared to fluctuating asymmetry of flies from the parental species.

Methods

Drosophila strains. Initially, flies were mass collected (n = hundreds) in nature off of fallen fruit in Tempe, AZ, and brought into the laboratory where they underwent several generations of domestication before being used. The *melanogaster* population (MEL) used was collected in May 1988. There were two *simulans* populations used: In replications 1 and 2, subjects were from a population (SIM-1) started from flies collected in May 1988 at the same location as the *melanogaster* population; in replications 3 and 4, the subjects were from a population (SIM-2) started from flies collected in October 1988 about 1.5 miles from the original site. Therefore the *D. melanogaster* strain had been in the laboratory several generations longer than the *D. simulans* in the third and fourth replication.

Crosses. Asymmetry measures were obtained from the offspring of four different types of mating: *melanogaster* females by *melanogaster* males (offspring of both sexes), *melanogaster* females by *simulans* males (only female offspring), *simulans* females by *melanogaster* males (only male offspring), and *simulans* females by *simulans* males (offspring of both sexes). Although the reciprocal hybrid crosses sometimes produced offspring of both sexes, only one of the sexes in each cross was produced in numbers large enough to perform meaningful statistical analyses. Four replications of the crosses were performed: Replication 1 was from a cross of the 6th laboratory generation of SIM-1 and MEL: Replication 2 was from a cross of the 10th laboratory generation of SIM-1 and MEL; Replication 3 was from a cross of the 12th generation of MEL and the 2nd generation of SIM-2; Replication 4 was from a cross of the 18th generation of MEL and the 8th generation of SIM-2.

Rearing. In replications 1-3, adult flies and their developing offspring were housed at low densities in 1/2 pint bottles containing agar-molasses-corn starch medium. In replication 4, flies were reared in a softer medium containing banana. The bottles were kept at 25°C under a light/dark schedule of 14/10 h and uncontrolled humidity.

Asymmetry measurements. Not all traits may show the effect of a decrease in developmental stability. As Palmer and Strobeck (1986) argued, characters having greater functional significance to the organism would be subject to stronger selection for canalization. To overcome this particular problem, three different bilateral traits were measured: the number of sternopleural chaetae (replications 1-4), the length of the wings (replications 1-4), and the number of frontoorbital plus frontal chaetae (hereafter referred to as head chaetae; replications 3-4). The number of chaetae were counted under a dissecting microscope. The wing lengths were measured by mounting wings on double-sided tape on a projector slide and projecting the image onto a screen. The length measured was from the anterior crossvein to the end of the second longitudinal vein and was in arbitrary units. Asymmetry scores were calculated by subtracting the value of the left side from that of the right side.

Results

Scaling. A positive correlation is sometimes found between the magnitude of a trait measure and the amount of asymmetry. In that case, transforming the data by dividing the asymmetry score of each individual (R – L) by the average magnitude of the trait measure on each side of the body (R + L/2) will often remove the correlation. Thus, correlations were calculated between the absolute value of asymmetry for each trait and the average number of chaetae or average wing length on each side of the body. Table 1 shows the significant correlations for untransformed and transformed data (there were no significant correlations for untransformed wing data so no transformations were performed). For sternopleural chaetae, 4 of 24 correlations (both sexes considered together) using untransformed data are statistically significant ($p < 0.05$), which is a number greater than chance (16.7%). After transformation, only one of these is still statistically significant, which is less than chance level (4.2%). For head chaetae, 2 of 12 correlations using untransformed data are statistically significant (16.7%). After transformation, one of these – a negative correlation – is still statistically significant (8.3%). The transformation used is for positive correlations, and it seems to have worked for those. We decided to leave this significant negative correlation because we are unable to remove both positive and negative correlations with the same transformation.

Table 1. Significant correlations between the magnitude of the trait value and asymmetry. An X indicates data was not available, 0 indicates no significant correlation.

	Absolute value					
	Rep	Stern	TrStern	Head	TrHead	Wing
Males						
MEL	1	0	0	X	X	0
	2	0.297	0	X	X	0
	3	0	0	0	0	0
	4	0.322	0	0.324	0	0
SIM	1	0	0	X	X	0
	2	0.380	0.295	X	X	0
	3	0	0	0	0	0
	4	0	0	0	0	0
F_1 SIM♀ × MEL♂	1	0	X	X	0	
	2	0	0	X	X	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Females						
MEL	1	0	0	X	X	X
	2	0	0	X	X	X
	3	0	0	0	0	X
	4	0.378	0	0	0	0
SIM	1	0	0	X	X	X
	2	0	0	X	X	X
	3	0	0	0	0	X
	4	0	0	0	0	0
F_1 MEL♀ × SIM♂	1	0	0	X	X	X
	2	0	0	X	X	X
	3	0	0	–0.422	–0.609	X
	4	0	0	0	X	0
Pooled replications						
MEL Males		0.152	0	0.220	0	0
MEL Females		0	0	0	0	0
SIM Males		0	0	0	0	0
SIM Females		0	0	0	0	0
F_1 SIM♀ × MEL♂		0	0	0	0	0
F_1 MEL♀ × SIM♂		0	0	–0.380	–0.602	0

Table 2. Significant directional asymmetriers after analysis with a paired t-test (Significant values are indicated by mean of R-L, transformed for stern and head, untransformed for wing). An X indicates data were not available, 0 indicates no significant correlation.

		Males			Females		
	Rep	Stern	Head	Wing	Stern	Head	Wing
MEL	1	0	X	0	–0.044	X	X
	2	0	X	0	0	0	X
	3	0	–0.054	0	0	0	X
	4	0	0	0	0	0	0
SIM	1	0	X	0	0	X	X
	2	0	X	0	0	X	X
	3	0	0	0.196	0	0	X
	4	0	0	0	0	0	–0.120
F_1 SIM♀ × MEL♂	1	0	X	–0.143			
	2	0	X	0			
	3	0	0	0			
	4	0	0	0			
F_1 MEL♀ × SIM♂	1				0	X	X
	2				0	X	X
	3				0	0	X
	4				0	0	0

Reliability. For the three measures of asymmetry, inter-experimenter reliabilities were estimated by having two experimenters independently measure asymmetry; 100 *melanogaster* flies of each sex were measured and reliabilities (using scores scaled by individual character size; see below) of 0.92 and 0.98 were obtained for males and females, respectively. For head asymmetry, 50 *melanogaster* flies of each sex were measured and reliabilities (using scaled scores) of 0.97 and 0.97 were obtained for males and females, respectively. For wing asymmetry, 100 *melanogaster* flies of each sex were measured and respectively reliabilities were 0.96 and 0.97.

Directional asymmetry and antisymmetry. Fluctuating asymmetry is distinguished from directional asymmetry (DA) and antisymmetry: DA refers to greater trait magnitudes on a particular side of the body; the latter refers to nondirectional deviations from bilateral symmetry that form a platykurtic or bimodal distribution. DA was measured by comparing trait measures on each side of the body with a paired *t*-test. We used the transformed scores for sternopleural and head chaetae, and untransformed scores for wing lengths. In only 1 of 24 instances is there a statistically significant ($p < 0.05$) DA of sternopleural chaetae (less than chance level). In only 1 of 12 instances is there a statistically significant DA of head chaetae. Thus, for these two traits, DA seems to be observed rarely if at all. For wing lengths, however, DA was observed in 3 of 15 instances (20%) and our analysis of other types of asymmetry took into account this finding (see the section on statistical analysis).

Because of the small range of possible scores and the fact that meristic characters are being used (so that there may be many ties), we know of no statistical test that will be a good indicator of antisymmetry. However, antisymmetry is rare (Palmer & Strobeck, 1986). In addition, it is not clear how our interpretations would be changed if we found greater antisymmetry in hybrids instead of FA: both would seem to indicate a problem in developmental stability.

Statistical analysis. Indices of FA using the variance of $R - L$ are most useful for detecting differences in FA (Palmer & Strobeck, 1986). Statistical testing of the variances of $R - L$ among groups using the Levene Test (Snedecor & Cochran, 1980) is little affected by DA (Palmer & Strobeck, 1986). The Levene test is also sensitive primarily to differences among within-group variances but little affected by nonnormality (hence, antisymmetry). Hence we tested for differences in asymmetry using the Levene test.

Asymmetry. In Tables 3 and 4 are presented the variances of asymmetry scores for males and females, respectively. Also presented are the results of statistical testing for differences among the *melanogaster, simulans*, and hybrid populations in each replication. No male progeny were produced from the MEL♀ × SIM♂ cross and only a few females were produced from the SIM♀ × MEL♂ cross (these were not analyzed). Wing data was not analyzed for females in replications 1 through 3 because hybrid females had undeveloped wings. In replication 4, banana medium was used which seemed to allow the development of full-sized wings, although these were still often missing veins and misshapen.

Table 3. Variances of asymmetry for the three traits for males in each replication and the results of Levene tests for differences among the hybrid and parental populations.

		Population			
	Rep.	MEL	SIM	SIM♀× MEL♂	Levene test
Sternopleural	1	.020 (n=50)	.015 (n=50)	.016 (n=50)	n.s.
	2	.016 (n=50)	.061 (n=50)	.010 (n=50)	n.s.
	3	.016 (n=50)	.014 (n=50)	.018 (n=50)	n.s.
	4	.018 (n=50)	.014 (n=50)	.016 (n=50)	n.s.
	Pooled	.018	.015	.015	n.s.
Head	3	.019 (n=50)	.019 (n=50)	.026 (n=50)	n.s.
	4	.022 (n=50)	.030 (n=50)	.019 (n=50)	n.s.
	Pooled	.021	.029	.022	n.s.
Wing	1	.285 (n=49)	.020 (n=50)	.125 (n=49)	n.s.
	2	.129 (n=47)	.083 (n=49)	.100 (n=49)	n.s.
	3	.100 (n=49)	.339 (n=46)	.288 (n=46)	n.s.
	4	.267 (n=49)	.360 (n=49)	.102 (n=50)	n.s.
	Pooled	.195	.200	.152	n.s.

Table 4. Variances of asymmetry for the three traits for females in each replication and the results of Levene tests for differences among the hybrid and parental populations.

	Rep.	Population MEL	SIM	MEL♀ × SIM♂	Levene test
Stern	1	.013 (n=50)	.016 (n=50)	.036 (n=24)	*
	2	.014 (n=50)	.015 (n=50)	.030 (n=38)	*
	3	.014 (n=50)	.011 (n=50)	.018 (n=50)	n.s.
	4	.020 (n=50)	.014 (n=50)	.014 (n=50)	n.s.
	Pooled	.016	.014	.024	*
Head	3	.023 (n=50)	.030 (n=50)	.294 (n=29)	*
	4	.015 (n=50)	.024 (n=50)	.029 (n=50)	*
	Pooled	.020	.027	.125	*
Wing	4	.098	.149	3.653	*

* $p < 0.05$

For males, no statistically significant differences ($p > 0.05$) in asymmetry were found in any replication; nor were any differences found when all replications were pooled. For females, statistically significant differences in asymmetry of head chaetae were found for both replications separately as well as when they were pooled: MEL♀ × SIM♂ females had more asymmetry than flies of either parental species. Statistically significant differences in asymmetry of wing lengths was also found, with MEL♀ × SIM♂ females having much greater asymmetry than either parental species. The results for sternopleural chaetae are less consistent, with two of the replications (1 and 2) showing statistically significant differences and the remaining two (3 and 4) showing none (although the third replication shows differences in the direction expected). However, there exist statistically significant differences in asymmetry when the four replications are pooled, with the hybrid females showing the greatest asymmetry.

Discussion

Hybridization of *melanogaster* and *simulans* seems to have little effect on the asymmetry of any of the three traits in F_1 males but does have an effect on asymmetry of all three traits in females. The overall appearance of the females is also abnormal (i.e., many phenodeviants) whereas that of males is difficult to distinguish from either of the parental species. Because similar results were found across several replications using sample sizes large relative to other studies, and with three different trait measures of high reliability, it is unlikely that the developmental instability of hybrids can be explained by chance.

The sex difference in developmental stability is interesting in light of the Haldane rule (Haldane, 1922). When the absence, sterility, or inviability of a given sex is observed on crossing two species, it is usually the heterogametic sex that is affected. The sibling species pair *D. melanogaster* and *D. simulans* provide an exception to the rule in two ways. First, it is well known that when *D. simulans* are the mothers, it is the female offspring that are absent, although males are sterile. The production of female offspring from the reciprocal cross reveals the other exceptional observation that hybrid females are less viable and developmentally stable compared to hybrid males produced by *D. simulans* mothers. These observations are compatible with a situation in which developmental stability of interspecific hybrids is controlled at least in part by a different mechanism than the sterility or absence of the heterogametic sex hybrid. In hybrids between *D. virilis* and *D. lummei*, developmental anomalies are associated with a loss of a microchromosome (Orr, 1990) but the F_1 of *D. melanogaster* mothers have normal chromosome members.

Our observations also support the hypothesis that developmental instability or fluctuating asymmetry reflects the presence of environmental stressors (Parsons, 1990; Leary & Allendorf, 1989) especially in vulnerable genotypes. Female hybrids from *D. melanogaster* mothers showed severely abnormal wing development on cornmeal medium while banana medium was more favorable to the development of full-size wings. Thus a vulnerable genotype (F_1 female) in a poor environment (cornmeal) resulted in more extreme developmental difficulties for wings.

The present findings, together with other studies, are consistent with the idea that the relative importance of heterosis and coadaptation for developmental homoestasis is related to the degree of re-

productive isolation and genetic divergence between the populations in question. If isolated populations of the same species are hybridized for the first time, increased developmental homeostasis may be seen in the F_1 only to decrease in the F_2, and recover in the F_3 (Anderson, 1968; Vetukhiv, 1954, 1956, 1957; King, 1955). When hybrids of different species are being examined, their levels of developmental stability may depend upon their degree of isolation and genomic incompatibility. If there has been gene flow between them, especially if, accompanying introgression, selection has allowed similar coadaptational complexes to arise in each, there may be no evidence of compromised developmental homeostasis. This is what was observed by Felley (1980) for subspecies of blue gill and by Jackson (1973a, 1973b) in lizards.

On the other hand, if hybridization and introgression are recent as in natural populations of the banded and blue spotted sunfish of the genus *Enneacanthus* (Graham & Felley, 1985), an increase in developmental instability may be observed, even in the presence of heterozygosity (Vrijenhoek & Lerman, 1982; Graham & Felley, 1985). The most extreme example is provided in the present study in which the species were hybridized initially in the laboratory and the genomic incompatibilities were so large as to manifest themselves in the F_1, not requiring recombination or segregation to break up coadaptation.

Developmental homeostasis can be viewed as a long continuum. At one end, extreme disruption of homeostasis may be manifest in increased fluctuating asymmetry, phenodeviance, sterility and inviability when the hybridizing populations represent highly diverged and incompatible genomes as in separate species (Hedrick *et al.*, 1978; Endler, 1977). More moderate or transitory disruptions are seen when less genetic divergence has occurred or under particular environmental conditions. No changes in developmental homeostasis may also be observed especially if introgression and selection have produced similar coadaptation in the hybridizing populations. At the other extreme, as in isolated populations of the same species, developmental stability and fitness of certain hybrids may actually increase. Thus, depending upon its level, developmental homoestasis may act as a reproductive isolating mechanism, or, conversely, given the absence of significant prezygotic isolation, may, among F_1s at least, promote gene flow.

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