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Differences in fluctuating asymmetry in *Drosophila melanogaster* caused by microclimatic contrasts.

Temperature and humidity are among the most important factors affecting insect adaptation and evolution. We have studied the effect of contrasting differences for these factors at a microsite, "Evolution Canyon", Lower Nahal Oren Canyon, Mount Carmel, Israel, on the variation for fluctuating asymmetry of wing scores in *Drosophila melanogaster*. The opposite slopes of the Canyon contrast sharply due to difference in solar radiation (Nevo, 1995). Our previous studies with *D. melanogaster* isofemale lines derived from the Canyon revealed significant interslope differences in a number of adaptively important traits including oviposition temperature preferences, viability and longevity changes caused by short-term and lifetime temperature treatments, and resistance to drought stress at different temperatures (Nevo *et al.*, 1997). A part of these tests was also conducted for the sibling species *D. simulans*, displaying parallel results to those of *melanogaster*. Likewise, we found considerable differences in the characteristics of the "transmission system" in *D. melanogaster*: higher rates of male recombination (Derzhavets *et al.*, 1996a) and sex-linked lethal mutations (unpublished results) in lines of ecologically stressful south-facing slope as compared to those of the less stressful north-facing slope, and different patterns of potential for P-M hybrid disgenesis (Derzhavets *et al.*, 1996b).

Deviation from bilateral symmetry was considered by many authors as an indicator of stress, either external or genetic (e.g. Palmer and Strobeck, 1986; Jones, 1987; Parsons, 1992; Markow, 1995). It was demonstrated that fluctuating asymmetry correlates positively with the level of inbreeding, genetic disbalance, general or specific ecological stresses, although in some cases no clear evidence was obtained (Alibert *et al.*, 1994; Fowler and Whitlock, 1994; McKenzie and Yen, 1995; Freebairn *et al.*, 1996). In a long-term study on the Australian sheep blowfly, *Lucilia cuprina*, it was demonstrated that asymmetry scores may serve as relevant and sensitive indicators of population gene pool adaptation to new environmental challenges (McKenzie and Yen, 1995). Thus, it is of interest to employ the asymmetry test in analysis of microsite population adaptation caused by microclimatic geographic differentiation.

Material and Methods: Wild type inseminated females were collected in June-July 1994 from the two opposite slopes of "Evolution Canyon": ecologically stressful south-facing slope (SFS) and the less stressful north-facing slope (NFS). The resulting isofemale lines were kept under standard laboratory conditions. Flies to be measured were reared at 25°C under controlled low-density conditions. This was achieved by placing 10 pairs of flies in a vial for 24 hours. From the progeny emerging from each of the vials during the first four days, five males and five females were taken at random in order to measure wing parameters. Thus, a total of 10 flies from each of the isofemale lines (five lines from SFS and six from NFS) were examined. Wings were prepared for measurement by laying them on two-sided sticky tape and covering them with a coverslip. The left and right wings from each fly were dissected and mounted on the slide. The measured complex of wing parameters involved the wing length along longitudinal vein and the wing width from the extreme of the fifth vein to the coastal border (see Figure), and several derivative traits. These measurements were conducted using an interactive image analyzing system, WScanArray 3 Image Analyzer (Galai Production Ltd, Israel). In order to reduce the uncontrolled variation caused by manual clicking of the cursor on boundary points on the wing, all wings were scored five times and then the initial scores averaged to obtain mean trait scores per wing. All measurements were taken by the same person. For each fly, trait scores of the two wings were used to derive the 'directional asymmetry' $DA = right\text{-}left$, 'fluctuating asymmetry' $FA = \text{abs}(DA)$, 'relative directional asymmetry' $RDA = 100 \cdot DA/M$, where $M = (right + left)/2$ and 'relative fluctuating asymmetry' $RFA = \text{abs}(RDA)$ indices. Clearly, the indices within the pairs $\{DA$ and $RDA\}$ and $\{FA$ and $RFA\}$ have the same sense, but the preference of the 'relative' indices is in the possibility to make comparisons between different traits. However, the results for these two types of indices may differ if asymmetry is correlated with the initial scores. Thus, all our calculations were conducted for both types. Very close results were obtained. Therefore, we present the results only for the 'relative' indices.

Results and Discussion: One would consider the dissection of the total between-individual phenotypic variation of a trait into genetic (between-line) and non-genetic (within-line) components as a natural way of data analysis and presentation for the considered situation. However, analysis of allozymic variation shows that in spite of about two-

Table 1. Variation of wing mean trait scores and asymmetry indices

Trait	sex	SFS			NFS		
		mean	σ^2_P	σ^2_L	mean	σ^2_P	σ^2_L
trait mean scores M=(*right*+*left*)/2							
L1	f	31.84±0.31	2.40	1.10	30.98±0.35	3.69	2.82
	m	28.01±0.29	2.16	1.56	27.40±0.28	2.28	0.64
L2	f	31.65±0.31***	2.43	1.06	30.04±0.29	2.47	2.19
	m	28.39±0.25*	1.59	0.79	27.61±0.29	2.53	0.46
L3	f	14.44±0.15	0.59	0.25	14.29±0.13	0.47	0.34
	m	13.28±0.17	0.68	0.52	13.22±0.15	0.66	0.26
L4	f	16.56±0.17	0.69	0.34	16.39±0.15	0.65	0.58
	m	15.06±0.17	0.71	0.56	14.94±0.13	0.48	0.17
R1	f	49.86±0.27	1.82	1.28	49.25±0.19	1.10	0.38
	m	50.35±0.24	1.42	1.23	50.19±0.21	1.35	0.67
R2	f	46.59±0.25	1.61**	1.37	46.59±0.14	0.59	0.34
	m	46.85±0.24	1.49	1.35*	46.95±0.16	0.76	0.17
R3	f	48.84±0.29***	2.07	1.02	50.34±0.32	2.96	2.07
	m	50.26±0.38	3.65	3.65	51.22±0.30	2.66	1.54
relative directional asymmetry RDA=(*right*-*left*)/M·100%							
L1	f	-0.79±0.94	22.09	5.11	-0.67±0.94	26.42	5.38
	m	0.11±0.78	15.05	0.87	-0.03±0.72	15.68	1.32
L2	f	-0.59±0.44	4.97	0.64	-0.04±0.49	7.29	2.19
	m	-1.32±0.47^{++}	5.57	0.71	-0.07±0.42	5.20	0.90
L3	f	0.61±0.93	21.72**	1.82	-0.81±0.50	7.62	2.92
	m	-1.16±0.92	21.07*	1.28	-0.33±0.54	8.64	1.00
L4	f	-1.18±0.85	17.98	3.76	-1.42±0.54^{++}	8.88	3.31
	m	-0.40±0.76	14.44	0.79	-0.87±0.64	12.46	1.80
R1	f	0.10±0.52	6.81	1.74	0.29±0.44	5.66	0.56
	m	-0.72±0.41	4.29	0.28	-0.01±0.35	3.65	0.55
R2	f	0.95±0.51	6.40	1.25	0.32±0.36	3.92	1.51
	m	-0.42±0.58	8.41	0.58	0.28±0.38	4.37	0.45
R3	f	0.35±0.83	17.56	1.10	-0.80±0.77	17.98	7.56
	m	-0.14±0.85	17.89	1.80	-0.55±0.57	9.73	0.46
fluctuating asymmetry RFA=abs(RDA)							
L1	f	3.44±0.64	10.37	3.24	3.69±0.65	12.82	3.53
	m	2.99±0.48	5.76	1.10	3.14±0.43	5.48	0.46
L2	f	1.47±0.35	3.10	0.29	1.99±0.33	3.20	0.76
	m	2.12±0.33	2.69	0.72	1.78±0.26	1.96	0.50
L3	f	3.46±0.62	9.67**	3.28*	2.17±0.34	3.46	0.38
	m	3.81±0.54*	7.34**	1.49	2.45±0.29	2.53	1.30
L4	f	3.47±0.53	6.92	2.05	2.47±0.39	4.67	2.37
	m	2.74±0.52	6.81	2.50	2.72±0.43	5.57	1.00
R1	f	1.96±0.34	2.82	0.77	1.47±0.34	3.53	1.02
	m	1.68±0.28	1.90	0.64	1.55±0.20	1.17	0.28
R2	f	1.97±0.36	3.31	0.88	1.48±0.24	1.77	0.53
	m	2.19±0.38	3.57*	1.00	1.66±0.23	1.61	0.64
R3	f	3.34 0.49	6.05	2.86	3.23 0.51	7.78	4.12
	m	3.43 0.48	5.66	0.85	2.56 0.33	3.28	1.44

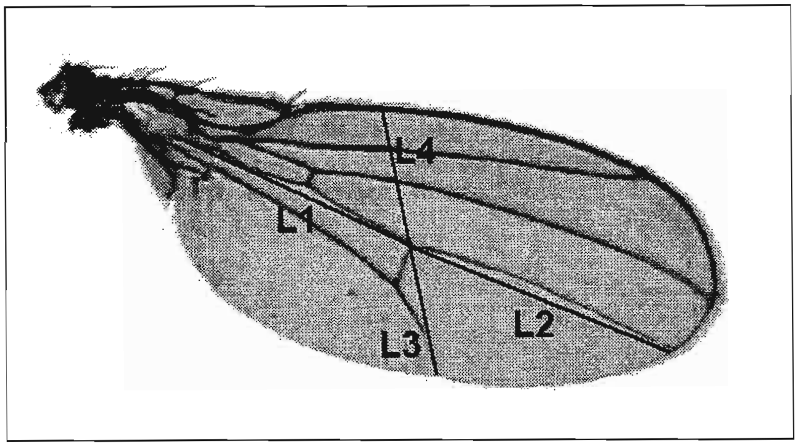

Figure 1. Initial wing traits (L1-L4) measured on the interactive image analyzing system.

year rearing of the isofemale lines as small closed populations (40-100 flies per line per generation), they still manifest rather high genetic variation (unpublished data). In such a case, the 'phenotypic level' is of primary importance when conducting interslope comparisons.

The presented data (Table 1) show significant difference for some wing 'size' and 'shape' mean trait values (L2 and R3) and trait variance (R2). In the latter case, higher variation is characteristic of SFS. The same pattern is manifested by the asymmetry indices: in any case of significant interslope difference in the level of variation, both the directional (*RDA*) and fluctuating (*RFA*) asymmetries are more variable in the material derived from SFS. It is noteworthy, that no significant deviation of *RDA* from zero is observed for the trait L3, where the highest interslope difference for σ^2_{FA} is established. Thus, significantly higher variance of *FA* and for L3 scores in SFS flies reflects higher variation in fluctuating asymmetry, parallel in males and females. Earlier (Nevo *et al.*, 1997), we found a higher intraslope variation of adaptively important traits in isofemale lines derived from the SFS as compared to NFS. This effect appeared to be a result of higher between-station variation on SFS. Our present data (see Table 1) demonstrate that higher variation on SFS is also manifested at the within-station level.

Our results corroborate the conclusion resulted from an experimental study conducted on populations of *D. melanogaster*, derived from laboratory strain kept for 4 years (Cavicchi *et al.*, 1985). The authors found that population adaptation to new temperatures affects wing traits related to our scores L2 and, especially, L3. The observed changes were interpreted in terms of independent units of selection represented by two wing compartments, with determinants of cell size and cell number being the target of selection.

An interesting point is that for all seven traits examined significant fluctuating asymmetry is observed for both slopes for both sexes. Nevertheless, in only one case significant interslope difference was found (for L3 in males, though non-significant difference of the same sign is noted). Our previous studies showed that the lines derived from the opposite slopes are different with respect to their reactions to stressful environments (Nevo *et al.*, 1997; Derzhavets *et al.*, 1996). Nevertheless, if fluctuating asymmetry of the foregoing wing scores can indeed serve as an indicator of stress, it follows from the foregoing results that the conditions of rearing of the scored material were, on the average, nearly equally stressful for the two groups of lines. This allows for further tests to compare the reaction norms of *FA* in the two groups on stress factors characteristic to the natural habitat of "Evolution Canyon".

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Table Footnote: The 'size' traits L1-L4 are as shown in the Figure (the measurements are given in some arbitrary units formed by the Image analyzing system), the 'shape' traits are denoted as follows: $R1 = 100 \cdot L2/(L1 + L2)$, $R2 = 100 \cdot L3/(L3 + L4)$, and $R3 = 100 \cdot (L3 + L4)/(L1 + L2)$. The interslope differences are significant at $^{*}(P < 0.05)$, $^{**}(P < 0.01)$, and *** $(P < 0.001)$, respectively; $^{++}$ significant $(P < 0.01)$ deviation of *RDA* from zero; note that for all traits deviations of *FA* are highly significant for both slopes and both sexes.