

the end of the observation period; none of the behaviours of either species actually occurred at this time; therefore, all such values indicate a failure to observe any change. The results were not consistent with the work of Stalker (1942) who observed that substantial mating occurred for this species after only 4 days. Other workers have been more conservative and not used individuals younger than 10 days (Spieth, 1951) or even 14 days (Hoikkala and Lumme, 1984). Generally there appears to be a need to quantify the rate at which species mature under different conditions rather than assume that an arbitrary choice will be acceptable in courtship studies. Clearly the rates of development can vary greatly even within one species as this comparison has shown.

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**Choo, J.K., and C.H. Ahn.** Department of Biology, Chung-Ang University, Seoul 156-756, Korea. Identification of genotype and its relationship with map gene pattern in a population of Korean *Drosophila melanogaster*.

In *Drosophila melanogaster*, the structural gene of alpha-amylase (E.C. 3.2.1.1;  $\alpha$ -1,4-glucan glucanohydrolase) encoding a monomeric enzyme (54,500D) is controlled by allelic, codominant and duplicated genes located near site 78 of the second chromosome. In natural populations, eight variants of the amylase genotype have been reported (Lindsley and

Zimm, 1992), and two regulatory factors in *Amy* gene expression have been well identified. Of them, *mapP*, a regulatory gene, affects the tissue- and age-specific expression of the *Amy* gene in the posterior region of the adult midgut (Klarenberg *et al.*, 1986). The other factor known as the dietary glucose repression depresses the level of *Amy* activity and its product in each developmental stage (Benkel and Hickey, 1987). In our study, the genotype and frequency of the *Amy* variants of *D. melanogaster* collected from a natural population were analyzed and the expression and genetic regulation of alpha-amylase were investigated at the tissue level.

**Materials and Methods:** The flies used in our study were collected at Cheon-An city near Seoul, Korea by sweeping net. To determine genotype and frequency of each variant, polyacrylamide (7.5%) gel electrophoresis was performed. After electrophoresis, activity staining of alpha-amylase needed incubation with separating gel in 2% starch and  $I_2$ -KI solution. The protein content was measured by the method of Bradford (Bollag and Edelstein, 1991). The

specific activity of alpha-amylase of each *Amy* genotype was determined by the method of starch-iodine and DNSA (Doane, 1969). Pattern analysis of amylase activity in midgut (map) was carried out by the method of Abraham and Doane (1978) and each pattern was determined by the method of Doane (1980).

**Results and Discussion:** Frequency, protein content and specific activity of each *Amy* genotype are shown in Table 1. It was revealed that the population analyzed in this study consisted of six *Amy* genotypes designated *Amy*<sup>1</sup>, *Amy*<sup>1\*2</sup>, *Amy*<sup>1\*3</sup>,

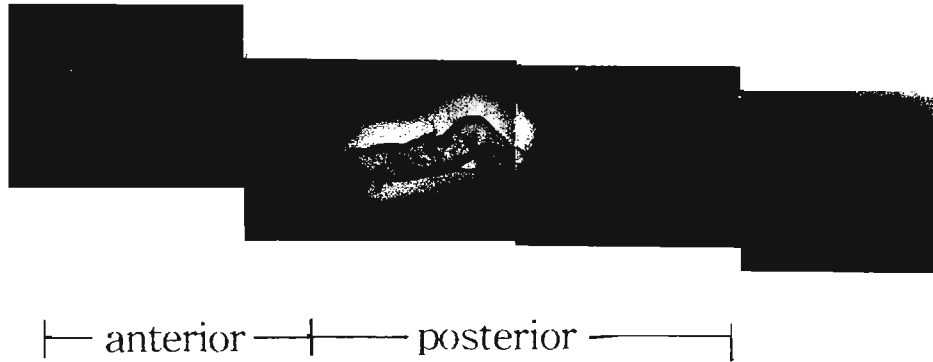
Table 1. Protein content and specific activity of amylase from each *Amy* genotypes in a natural population of *D. melanogaster*.

Genotype	No. of Line	Frequency (%)	Protein content <sup>1</sup> (μg)	Specific activity (unit/min)
<i>Amy</i> <sup>1</sup>	147	75.00	12.9453	2.0179
<i>Amy</i> <sup>1*3</sup>	33	16.84	13.7170	1.8629
<i>Amy</i> <sup>1*2</sup>	7	3.57	13.8156	1.8286
<i>Amy</i> <sup>1*2*3</sup>	4	2.04	13.1786	2.0662
<i>Amy</i> <sup>1*3*6</sup>	4	2.04	13.5442	1.3989
<i>Amy</i> <sup>1*6</sup>	1	0.51	12.8061	1.2432
Total	196	100.00	13.1226 <sup>2</sup>	1.9695 <sup>3</sup>

<sup>1</sup>Protein content of crude extract; <sup>2</sup>Average of protein contents of six genotypes;

<sup>3</sup>Average of specific activities of six genotypes

A)



B)

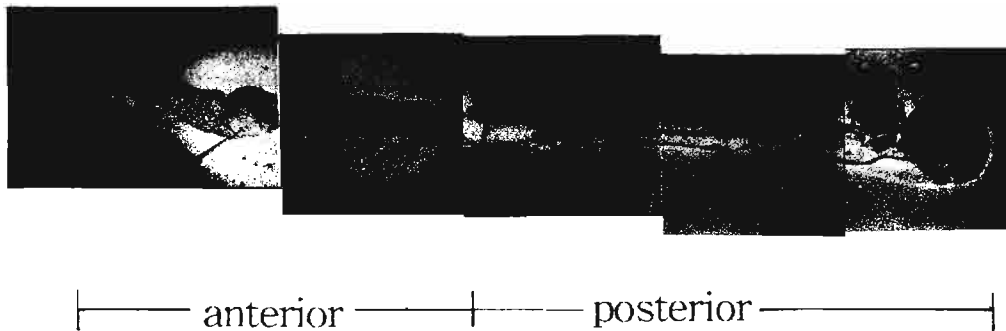


Figure 1: Alpha-amylase expression in the midgut of 120 hr third instar larvae reared on the standard medium. The colorless area indicates the portion containing amylase activity. A)  $\text{mapA}^{000}\text{P}^{12}$ , B)  $\text{mapA}^{123}\text{P}^{00}$ .

$\text{Amy}^{1-3-6}$ ,  $\text{Amy}^{1-2-3}$ , and  $\text{Amy}^{1-6}$ . The frequencies of  $\text{Amy}^1$  and  $\text{Amy}^{1-3}$  appeared to be 75.00% and 16.84%, respectively, indicating that these two genotypes would be the common and ancestral form in this natural population. The protein concentration of each genotype appeared not to be significantly different and the average of six genotypes was 13.1226  $\mu\text{g}$ . The average of the specific activity of amylase was 1.9695 unit/min and  $\text{Amy}^{1-3-6}$  and  $\text{Amy}^{1-6}$  showed relatively low activity.

Figure 1 represents the histological distribution of amylase activity, indicating differential distribution within a genotype (Figure 1, A and B). Also, map pattern of the adult indicated higher activity at the posterior region than at the anterior region (83.5%; 175/209 midgut). This result was shown similarly in Klarenberg *et al.* (1986) which observed *trans*-regulation of amylase activity in larval and adult midgut.

To elucidate the genetic independence of amylase genotype in a natural population, the method of amylase-substrate PAGE was employed. The electrophoretic pattern of amylase appeared to be consistent with genotype and map pattern, suggesting that amylase and map genes are genetically independent.

In this paper, it would be emphasized that the amylase gene showed polymorphism in a natural population and the amylase activity and its gene expression were independent from map gene associated with tissue-specific expression of amylase.

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**Derzhavets, Elena, A. Korol, and E. Nevo.** Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905, Israel, e-mail: korol@esti.haifa.ac.il. 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'  $DA = \text{right-left}$ , 'fluctuating asymmetry'  $FA = \text{abs}(DA)$ , 'relative directional asymmetry'  $RDA = 100 \cdot DA/M$ , where  $M = (\text{right} + \text{left})/2$  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-