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Mating propensities and variations in enzyme activities in long-term cage populations of *Drosophila melanogaster*

ABSTRACT: Environment-dependent reproductive isolation was established between cage populations (B_s) of *Drosophila melanogaster* originated from a Greek natural population (summer 1973) and maintained for about five years under different diets (poor-rich). The detected deviation from random mating involved no homogametic or heterogametic preference but rather, a significantly increased activity of males from populations maintained on the rich food medium. This observation indicates that the male parental investment is not negligible and under certain conditions sexual isolation can be a function not only of female behavior but also of male behavior. Differences also were found in various enzyme activities on the inter- and intra-population levels. Given those observations as well as the observed different behavioral patterns of B_s and C_s-D_s populations[19], a preliminary attempt was made to associate adaptive evolution with differences in enzyme activities. The differences in enzyme activities between populations reared on different media are not due to allozymic differences. It also was shown that in some populations environmental effects do not always ellicit differences in enzyme activity. It was concluded, therefore, that the observed variations were the result of environmental effects interacting with modifier genes.

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ONE OF THE important questions in evolutionary theory, relates to how species are formed and what kind and magnitude of genetic differentiation characterizes species formation. Heretofore, several mechanisms have been emphasized, e.g. that speciation involves a genetic reorganization dependent on a founder event, followed by one or more cycles of exponential population growth and a sudden contraction or crash[9-12]. Moreover, according to Prakash[33] founder events, inbreeding, and geographic isolation are considered as major factors in the development of reproductive isolation. In contrast, speciation also is considered a by-product of the process of adaptation, characterized by changes in behavioral and structural gene (allozymic) variation[6,17,29]. Experimental evidence exists for all the above views[6,30,33], a fact indicating that the development of reproductive isolation is not unimodal.

We recently demonstrated[19] that stable environment-dependent sexual isolation had been established between cage populations of *Drosophila melanogaster* maintained under different environmental conditions (temperature and humidity) for about five years, whereas the isolation of populations alone did not lead to ethological isolation. Since the isolation of populations is considered to be an important factor in the development of reproductive isolation[33], we investigated a set of four additional cage populations (possessing a common gene pool at their origin) maintained (in duplicate) under different food media (poor-rich)[1], for about six years, in order to assess: 1) whether or not reproductive isolation was established and, if so, whether it was correlated to environmental manipulation or to the isolation of populations. 2) Since the environmental factor used in the present study (food medium) differs from

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those utilized before[19] (temperature-relative humidity), a comparison also was made regarding the patterns of ethological isolation.

The ecological factor food medium (poor-rich) was found to induce dramatic genetic differentiation in *Adh* and α-*Gpdh* allozymic and inversion frequencies[3] as well as in the frequencies of lethal bearing chromosomes[1] during the initial 30 generations. Taking into account the situation already revealed in the populations, we thought it of interest also to measure the quantitative genetic variation of enzyme activities in our preliminary effort to see whether or not correlation exists between changes in enzyme activities and in reproductive isolation. Since it was suggested[21] that in natural populations there may be several polymorphic loci affecting the expression of a given structural gene and therefore contribute to variation in the enzyme activity, this study may ultimately prove useful to test the adaptive significance of enzyme quantitative variability. At the same time, it also will have a bearing on the suggestion that regulatory variation of enzyme activity is a more important source of adaptive variation than structural variation[8,14,21,34].

Materials and Methods

Four cage populations designated $1B_1$, $1B_2$, $1B_3$, $1B_4$ were studied. Enzyme activities were measured in flies from two additional cage populations (1C, 1D). The last two populations have been studied and described previously[19]. The B_s populations originated from a common parental population (1B) by replication, 10 generations after the latter originated. Consequently, we may regard the four derived populations as possessing practically the same gene pool at their origin. Population 1B was established in the autumn of 1973 from flies captured during the summer of 1973 from the Greek island of Cephalonia (for more details see Alahiotis[1]). All B_s populations were maintained at 25 ± 0.5° and a mean relative humidity of 43 ± 4 percent, in 12-hour daylight cycles. Populations $1B_1$ and $1B_2$ were kept on the dead-yeast-sugar-agar medium, which is considered a rich food medium (RFM)[1], while populations $1B_3$ and $1B_4$ were maintained on the cornmeal-sugar-agar medium, which is characterized as a poor food medium (PFM)[1]. The size of the PFM populations was estimated at 1.100 (average)[1] flies, and that of the RFM populations at 2.800 (average) flies[1]. Sexual isolation index was measured according to the method followed in our previous study[19], i.e., random mating was tested by chi-square and the joint-isolation index of Mologolowkin-Cohen et al.[23]:

$$I = (X_{AA} + X_{BB} - X_{AB} - X_{BA})/N$$

where X_{AA}, X_{BB}, X_{AB}, X_{BA} stand for the four types of matings, ♀A × ♂A, ♀B × ♂B, ♀A × ♂B and ♀B × ♂A, respectively, and $N = X_{AA} + X_{BB} + X_{AB} + X_{BA}$

$$SE \text{ of } I = \sqrt{(1 - I^2)/N}.$$

A value of zero for this index indicates random mating; <0, negative assortative; and >0, positive assortative mating. The flies of each sex that were mated were tested by chi-square if females and males of one population mated more frequently than those from other populations, and if assortative mating occurred. Experiments were carried out 210 weeks after the origin of populations.

The enzyme activities were measured in about eight replicates in at least two separate experiments. The flies used were 3 to 4 days old in all assays. The enzyme assays have been described elsewhere (ACHE and IDH[2], ADH[15], αGPDH[3], NAD-SoDH[s][7], 6PGD and G6PD[22]). The enzymes assayed in this study are: α-Glycerophosphate dehydrogenase (α-glycerol 3-phosphate:NAD$^+$ oxidoreductase, EC 1.1.1.8.), glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate: NADP$^+$ oxidoreductase, EC 1.1.1.49.), 6-phosphogluconate dehydrogenase (6PGD; 6-phospho-D-gluconate: NADP$^+$ oxidoreductase, EC 1.1.1.44.), alcohol dehydrogenase (ADH; alcohol: NAD$^+$ oxidoreductase, EC 1.1.1.1.), isocitrate dehydrogenase, NADP (IDH-NADP$^+$; L_s-isocitrate:NADP$^+$ oxidoreductase (decarboxylating, EC 1.1.1.42.), acetylcholinesterase (ACHE

Table I. Mating preferences in crosses between isolated cage populations of *Drosophila melanogaster*

Cross A × B	A♀ × A♂	A♀ × B♂	B♀ × A♂	B♀ × B♂	χ^2(1df)♀	χ^2(1df)♂	χ^2(1df) assort.	χ^2(1df) total	Isolation index ± SE
1. $1B_1 \times 1B_2$	29	31	35	34	0.703	0.616	0.073	0.703	−0.023 ± 0.080
2. $1B_1 \times 1B_3$	32	14	18	15	2.138	5.582	1.870	10.569	+0.189 ± 0.110
3. $1B_1 \times 1B_4$	59	27	35	39	0.900	4.900	7.450	13.900	+0.225 ± 0.077
4. $1B_2 \times 1B_3$	41	16	26	18	1.672	10.782	1.830	15.300	+0.168 ± 0.098
5. $1B_2 \times 1B_4$	45	19	26	11	7.218	16.642	0.000	25.040	+0.108 ± 0.098
6. $1B_3 \times 1B_4$	12	10	12	12	0.333	0.333	0.000	0.664	+0.000 ± 0.144
7. $1B_1 \times 1B_4$ (comm. cond.)	26	13	12	10	0.013	0.013	0.012	0.038	+0.013 ± 0.113

Table II. Mating preferences in crosses between Or-k flies raised on RFM or PFM

Cross (A × B) Or-k^{RFM} × Or-k^{PFM}	A♀ × A♂	A♀ × B♂	B♀ × A♂	B♀ × Bo♂	χ^2(1df)♀	χ^2(1df)♂	χ^2(1df) assort.	χ^2(1df) total	Isolation index ± SE
Generations									
1	25	26	24	26	0.010	0.089	0.010	0.305	0.010 ± 0.099
3	23	22	21	22	0.045	0.000	0.045	0.023	0.022 ± 0.106
5	23	22	23	22	0.000	0.014	0.000	0.044	0.000 ± 0.105
Or-k × Or-k (in common conditions)	19	22	20	22	0.012	0.301	0.014	0.326	−0.012 ± 0.109