Boerema, A.C. and R. Bijlsma. University of Groningen, Haren, Netherlands. The effect of substituting artificial laboratory food by orange food on allozyme polymorphisms.

Little is known about the natural breeding sites of **Drosophila melanogaster**, but it is generally assumed to breed mainly on rotting and fermenting fruit. Boerema & Bijlsma (1984) measured egg-to-adult survival of **D.melanogaster** on different kinds of fruit. Reasonably good viabilities were observed on most fruits, even when mixed with agar. In the present study the influence--if any--of substituting the standard laboratory food by orange food on a number of allozyme polymorphisms was examined.

For the experiments two cage populations were used: the Death Valley population (DV) and the Riverside population (RV). These populations were both established in March 1981 by mixing the offspring of a number of isofemale lines (10 pairs per line) which had been caught in Death Valley and Riverside (for a description of the sites, see Coyne et al. 1983) in March and June 1980, respectively. The DV population was started with 83 and the RV population with 94 isofemale lines. After establishment the cages were kept undisturbed on standard laboratory food for more than one year at 25°C in order to ensure random mixing of the lines. Thereafter both cages were quadruplicated: of each population two cages were still provided with standard laboratory food, the other two with orange food. The standard food contained 18 g agar, 54 g sucrose, 32 g dried dead yeast and 13 ml of a nipagin solution (10 g nipagin in 100 ml ethanol 96%) in 1 liter water. The orange food consisted of 720 g ground orange (without peel) and 450 ml of agar solution (1% w/v) in which 6.5 ml nipagin solution.

The allele frequencies of the following allozyme loci were monitored at intervals by electrophoresis: glucose-6-phosphate dehydrogenase (G6pd), 6-phosphogluconate dehydrogenase (Pgd), alcohol dehydrogenase (Adh) and α-glycerophosphate dehydrogenase (α-Gpdh). All four loci were found to be polymorphic in both of the original populations and each exhibited a fast (F) and a slow (S) allele (for electrophoretic procedures, see Eisses et al. 1979). Significance of allele frequency changes in the cages in relation to time was tested by Spearman rank coefficient.

The results are shown in Table 1. The data for Pgd are omitted from this table as all cages turned out to be nearly fixed for the F allele with frequencies fluctuating between 0.95 and 1.00 F. The other three loci showed less extreme allele frequencies. The results differed between enzymes and/or populations.

Table 1. F frequency determined at intervals for the G6pd, Adh and α-Gpdh loci for the two populations both on standard food and orange food. Gene frequencies were determined on 84 females (G6pd) or 84 males (Adh and α-Gpdh).

DEATH VALLEY POPULATION:

	G6pd-F				Adh-F				α-Gpdh-F			
Time in	Standard food		Orange food		Standard food		Orange food		Standard food		Orange food	
months	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2
0	0.67	0.67	0.67	0.67	0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.79
5	0.64	0.77	0.61	0.67	0.80	0.79	0.81	0.80	0.87	0.83	0.75	0.73
9	0.68	0.78	0.59	0.51	0.73	0.82	0.65	0.68	0.87	0.84	0.82	0.64
12	0.73	0.72	0.57	0.75	0.74	0.73	0.69	0.67	0.83	0.79	0.83	0.64
15	0.63	0.74	0.64	0.55	0.74	0.73	0.68	0.58	0.86	0.77	0.86	0.67
18	0.55	0.66	0.57	0.60	0.75	0.74	0.64	0.65	0.85	0.86	0.86	0.60
21	0.66	0.69	0.65	0.40	0.76	0.68	0.55	0.55	0.89	0.82	0.87	0.70
24	0.60	0.71	0.63	0.58	0.69	0.76	0.67[a]	0.65[a]	0.92	0.82	0.83[b]	0.51[a]

RIVERSIDE POPULATION:

	G6pd-F				Adh-F				α-Gpdh-F			
Time in	Standard food		Orange food		Standard food		Orange food		Standard food		Orange food	
months	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2
0	0.57	0.57	0.57	0.57	0.84	0.84	0.84	0.84	0.95	0.95	0.95	0.95
2	0.74	0.63	0.61	0.80	0.71	0.83	0.80	0.81	0.87	0.96	0.96	0.94
6	0.75	0.54	0.70	0.67	0.81	0.81	0.85	0.71	0.88	0.95	0.96	0.82
9	0.63	0.54	0.55	0.55	0.70	0.82	0.73	0.74	0.88	0.95	1.00	0.82
12	0.61	0.61	0.56	0.68	0.71	0.78	0.80	0.72	0.88	0.96	1.00	0.89
15	0.64	0.61	0.63	0.61	0.63	0.77	0.70	0.69	0.83	0.88	0.98	0.92
18	0.52	0.61	0.65	0.61	0.63	0.68	0.71	0.69	0.82	0.92	0.99	0.83
21	0.65	0.61	0.71	0.62	0.63[a]	0.73[a]	0.72[a]	0.74	0.84[a]	0.94	0.91	0.99

a = significant decrease, $P < 0.05$. b = a significant increase, $P < 0.05$.

(1) For G6pd the allele frequencies were found to be fairly constant over the two year period for both populations; there were some fluctuations but none of the cages showed a significantly consistent change. At the end of the experiment no divergence between the two food situations was observed.

(2) For Adh the RV populations showed a decrease in F frequency in the course of the experiments; this decrease was significant for both cages with standard food and for cage 1, with orange food. The change was in the same order of magnitude in all cages, so again no divergence between standard food and orange food was observed. The DV populations also showed a decrease in F frequency, but the decrease was found to be only significant for the cages provided with orange food. This indicated some divergence between the two types of food, but the difference in allele frequency at the end of the experimental period was, however, small.

(3) For α-Gpdh the situation was ambiguous. The RV population showed a significant decrease in F frequency in one standard food cage only (cage 1) while the other three cages did not change significantly. The DV population showed significant changes for both orange food cages. The change, however, was in opposite directions: cage 1 showed an increase while cage 2 showed a decrease in F frequency. These results also did not indicate a consistent divergence between the two food situations.

In conclusion, it can be said that, compared to standard food, orange food did not significantly alter the behavior of these allozyme polymorphisms and that it did not change selective differences--if any--with respect to these loci and/or linked fitness genes. Only in one of the cases (DV: Adh) a possible divergence was observed.

References: Boerema, A.C. & R. Bijlsma 1984, DIS 60:62-63; Coyne, J.A., J. Bundgaard & T. Prout 1983, Am. Nat. 122:474-488; Eisses, K.T., H. van Dijk & W. van Delden 1979, Evol. 33:1063-1068.

Botella, L.M. and J.L. Ménsua. University of Valencia, Spain. Can crowding promote larval diapause in Drosophilids?

Chymomyza costata is a Drosophilid closely related to **Drosophila** genus which shows light-dependent larval diapause (Hackman et al. 1970). This species is located in Northern European regions. Larval diapause appears in 3rd instar larvae when the period of light is under a certain minimum according to the strain. This diapause can only be interrupted by cold treatment for two months. The present work has analyzed the relationship between diapause and other phenomenon of larval arrest promoted by crowding, the larval stop in 3rd instar larvae of **Drosophila melanogaster** (Mensua & Moya 1983). For this purpose **Chymomyza costata** was bred under crowded conditions which give rise to a larval stop in Drosophila. Two different strains of **Chymomyza costata** (VKL & TODA), kindly supplied by Dr. Rihimaa, were employed. TODA comes from a mass capture carried out in Tomazaki (Japan) in August 1983, while VKL was captured in Kuopio (Finland) in June 1981. Crowded cultures were set up by seeding 30 larvae in 5 x 0.8 cm vials with 0.5 ml of Lakovaara medium (Lakovaara 1969). Uncrowded controls were also taken by seeding 30 larvae in 10 x 2.5 cm vials with 5 ml of the same medium. Temperature was kept at 19°C and light was constant to avoid diapause. Crowding was interrupted by overfeeding (Moya & Mensua 1983) on days 17th, 21st, 25th and 29th in VKL strain, and on days 17th, 21st, 25th, 29th and 33rd in TODA strain. The total population was divided in this way in inner and outer subpopulations. A total of five replicates were made for each strain and overfeeding.

Table 1 shows survival obtained in inner populations (adults emerged in the small vials), in outer subpopulations (adults emerged in the overfeeding vials) and total survival, as well as developmental times corresponding to inner and outer populations. As can be seen from this table, hardly were flies recovered in inner populations. Moreover, most of total survival is due to outer populations which nevertheless is below 50% in all the overfeedings. Developmental times are progressively delayed as overfeedings are later. In the VKL strain, the highest elongation of development obtained by difference between the longest time in crowded experiments and the uncrowded culture is about 21 days, while in TODA where overfeedings were prolonged until the 33rd day, the maximum lengthening is about 29 days. These elongations, though noticeable, would seem too short to be considered as a true diapause. In order to enhance the developmental arrest, the following experiment was designed. Ten crowded cultures for each strain were seeded as usual on the 3rd November 1984. On the 40th day of culture, the remaining larvae were extracted and seeded again in fresh food (5 x 0.8 cm vials with 0.5 ml food) by groups of 30 larvae keeping in this way the same degree of initial crowding. This operation has been repeated every 40 days. The results obtained have been striking in both strains, but especially in VKL. A large number of larvae does not pupate, remaining as 3rd instar larvae for a period of time in principle not determined. In fact, as of this date (15th March 1985),120 larvae from VKL strain (40% of the number initially seeded), and 30 larvae from TODA strain (10%) remain alive in these cultures.