

(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** (Ménsua & 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 & Ménsua 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.

Table 1. Mean survival in inner, outer and total populations, and mean developmental time in inner and outer subpopulations of the *Drosophilid Chymomyza costata*.

Strain	Overfeeding (days)	Survival			Developmental Time	
		Inner	Outer	Total	Inner	Outer
VKL	Uncrowded control	--	17.0±1.3	17.0±1.3	--	32.18±0.44
	13	0.0±0.0	8.2±2.1	8.2±2.1	--	40.84±0.98
	17	0.7±0.5	11.5±2.9	12.5±2.8	34.50±0.50	41.91±2.10
	21	1.5±0.9	5.5±3.0	7.0±2.9	36.83±0.17	47.93±1.65
	25	2.0±1.0	10.7±0.7	12.7±0.7	42.10±1.45	53.42±0.64
TODA	Uncrowded control	--	17.7±0.9	17.7±0.9	--	32.11±0.17
	17	0.0±0.0	11.5±3.5	11.5±3.5	--	46.39±1.87
	21	0.0±0.0	13.5±1.3	13.5±1.3	--	44.35±1.94
	25	0.5±0.5	13.7±2.8	14.2±2.6	38.50± *	47.57±1.07
	29	2.0±2.0	10.7±3.9	12.8±2.3	38.75± *	52.01±1.94
	33	3.0±2.4	14.5±2.5	17.5±4.6	40.50±2.51	61.29±1.20

* values obtained from a single vial.

References: Basden, E.B. 1954, Proc. Royal Ent. Soc. London 29:7-9; Hackman, W., S. Lakovaara, A. Saura, M. Sorsa & K. Vepsäläinen 1970, Ann. Ent. Fenn. 36:1-9; Lakovaara, S. 1969, DIS 44:128; Mensua, J.L. & A. Moya 1983, Heredity 51:347-352; Moya, A. & J.L. Mensua 1983, DIS 59:90-91.

Botella, L.M. and J.L. Ménsua. University of Valencia, Spain. A comparison of the urea and uric acid content between crowded and uncrowded cultures of *D.melanogaster* throughout development.

Uric acid, main biotic residue coming from the nitrogen catabolism in *Drosophila* (Botella et al. 1984a), plays an important role in competition phenomena for food, in such a way that it may account at least partially for the low survival and delayed development, as well as larval stop obtained in these conditions (Botella et al. 1984). On the other hand, urea, also present in small quantities in *Drosophila* cultures (Botella et al. 1984, 1985) is able to mimic the kinds of responses obtained in crowded cultures, for it decreases larva-adult survival and increases mean developmental time (Botella et al. 1983). An analysis of urea and uric acid has been carried out in the present work in order to compare uric acid and urea contents in crowded and uncrowded cultures throughout development. Crowded cultures consisted of 5 x 0.8 cm vials with 0.5 ml of Lewis medium seeded with 70 larvae. Uncrowded cultures consisted of 10 x 2.5 cm vials with 5 ml of Lewis medium seeded with 70 larvae. Both kinds of cultures were incubated at 19°C at 85% relative humidity and constant light. Analysis of urea and uric acid were carried out following the methods described elsewhere (Botella et al. 1984). The expression giving larval, pupal and adult content of both products is:

$$C(\text{mg}/100\text{ml}) = (\text{Sample absorbance}/\text{Standard Absorbance}) \times (C \text{ Standard}) \times (1/\text{dilution factor}),$$
 where dilution factor (d.f.) is:
$$\text{d.f.} = (\text{Sample Volume})/(\text{Sample Volume} + \text{Sodium Acetate Volume}).$$

Sample volumes were estimated from larval, pupal and adult densities, as well as their respective body weights. These data appear in Table 1. The results of uric acid and urea determinations throughout the different stages of development are also shown in Table 1. As can be seen, when comparing the results between crowded and uncrowded cultures the following observations deserve to be pointed out: (i) uric acid concentrations in larvae from uncrowded cultures are higher than those of larvae bred in crowded conditions; (ii) uric acid concentrations in pupae rise as their development progress (as a consequence of a lack of external excretion), while urea concentration remains more or less the same, this being true for both crowded and uncrowded cultures. It is worth mentioning here that the level of both products is higher in crowded than in uncrowded conditions in pupal stage, but for the urea content in mature pupae where both kinds of cultures show approximately the same level; (iii) the main component of the newly born adults excretion is uric acid present in high levels, and close to that obtained in 33-day old pupae from crowded cultures. Recently emerged adults, when incubated at 25°C for 20 hr show a high uric acid content, similar in both kinds of cultures, and close to the uric acid concentration at the end of the pupal stage. Urea concentrations are slightly above this level.

The conclusion is that larvae are in diapause, but the mechanism which makes them enter diapause is different from that previously described in literature so far for this species (Basden 1954; Hackman et al. 1970). In relation to this view, it seems that larval stop might be regarded as a kind of diapause in non-diapausing species, such as *D.melanogaster*, but that is also present in diapausing species such as *Chymomyza costata*, promoted by crowding.