

Table 1. Values of hybridized *melanogaster* females in each of the 2x2 factorial combinations, previous larval development and temperature, and results of a weighted analysis of variance of the percentages of hybridization in the logit scale.

| previous development | room temperature | 21°C |
|--|------------------|-------|
| mono-specific cultures | 9/225 | 4/225 |
| bi-specific cultures | 13/225 | 5/225 |
| factorial effect of temperature: 0.862 ± 0.385 ($t=2.23$, $p < 0.05$) | | |
| factorial effect of development: 0.318 ± 0.359 ($t=0.88$ n.s.) | | |

Table 2. Number and percentage of hybridized *melanogaster* females for each species frequency. Data of the 2x2 factorial combinations were pooled.

| number of <i>D.melanogaster</i> pairs | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|-------|-------|-------|-------|-------|-------|------|------|------|
| number of <i>D.simulans</i> pairs | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| hybridized/ total <i>melanogaster</i> females | 0/180 | 1/160 | 2/140 | 2/120 | 7/100 | 3/80 | 6/60 | 4/40 | 6/20 |
| percentage of hybridization | 0% | 0.62% | 1.43% | 1.67% | 7% | 3.75% | 10% | 10% | 30% |

mixture decreased. If we suppose that any female may be simultaneously courted by all present males, then the probability of mating between *melanogaster* females and *simulans* males increases with the decrease of the relative frequency of *D.melanogaster*. This was confirmed by the notable fact that when the relative *melanogaster* frequency was 0.1 the value of hybridization (30%) was not different from that one found in a "no-choice" test carried out with these same strains of *D.melanogaster* and *D.simulans* (Carracedo & Casares 1984).

Summarizing, our results showed that the possibility of hybridization between *D.melanogaster* and *D.simulans* in competition cultures can not be rejected, especially when the relative frequency of *D.melanogaster* is low. Thus, if hybridization occurs, the *melanogaster* progeny may be lower than expected. Furthermore, hybrids could not be detected because they have reduced viability, particularly at the commonly used temperature of 25°C (Sturtevant 1920). In this way, results of competition could be erroneously imputed to factors other than hybridization.

References. Carracedo, M.C. 1984, Doctoral Thesis, Univ. of Oviedo, Spain (unpubl.); Carracedo, M.C. & P. Casares 1985, *Experientia* 41:106-108; Carracedo, M.C. & P. Casares 1984, *Bol. Cien. Nat. I.D.E.A.* 33:15-29; Eoff, M., *Am. Nat.* 107:247-255; Finney, D.J. 1971, *Probit Analysis*, 3rd ed., Cambridge Univ. Press; Manning, A. 1959, *Anim. Behav.* 7:60-65; Sturtevant, A.H. 1920, *Genetics* 5:488-500; Watanabe, T.K. et al. 1977, *Jap. J. Genet.* 52:1-8.

Carracedo, M.C. and P. Casares. Universidad de Oviedo, Oviedo, Spain. A study on the dynamics of crossing between *Drosophila melanogaster* females and *Drosophila simulans* males.

Pontecorvo (1942), Manning (1959) and Barker (1967), among others, have shown that hybridization between *D.melanogaster* females and *D.simulans* males is more frequent when flies are aged a few hours than 3 or more days. As a possible explanation it has been suggested that young females have not well developed

their sexual discriminative sense and may mate with almost any courting male. Nevertheless, it is also probable that when very young male and female flies of both species are kept together and they mature in proximity, they may become accustomed to each other and facilitate interspecific mating, once sexual

of hybridization are related with the relative frequencies of both species. Owing to the low values of hybridization found in some frequencies, we have grouped the data of the four factorial combinations under the supposition that in each combination, the possible effect of species frequency was the same.

Table 2 shows the percentages of hybridized females in each relative frequency and the total number of examined females. Apparently, when the relative frequency of *D.melanogaster* diminished, the number of *melanogaster* females hybridizing with *simulans* males increased. To confirm this, we have obtained a weighted linear regression of the percentages of hybridization in the logit scale on the frequencies of *D.melanogaster*, using an iterative routine that yields maximum likelihood estimates (Finney 1971). The 0.1-0.2 and 0.3 frequencies were grouped to increase the expectatives. Regression was highly significant ($b=5.6 \pm 0.9$ in logits) and the data fit well with the model (chi-square = 7.02 with 5 degrees of freedom, no-heterogeneity). Therefore, the hybridization of *melanogaster* females increased when its frequency in the

Table 1. Average percentage of hybridization between *D.melanogaster* females and *D.simulans* males, in different periods of time. ML and MH = lines of *D.melanogaster*. SL and SH = lines of *D.simulans*.

| Cross | Days | | | | | | | | | |
|---------|------|----|----|----|----|----|----|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ML x SL | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 4 |
| ML x SH | 0 | 0 | 0 | 8 | 4 | 0 | 4 | 0 | 0 | 0 |
| MH x SL | 0 | 64 | 60 | 48 | 40 | 48 | 52 | 44 | 48 | 48 |
| MH x SH | 0 | 16 | 56 | 84 | 68 | 48 | 64 | 76 | 80 | 64 |

examination because we have found that isofemale lines extracted from single populations of *D.melanogaster* and *D.simulans* show large differences in frequency of hybridization (Carracedo & Casares 1985b).

The present study has been carried out with the aim of determining the dynamics of heterospecific matings throughout a 10-day period. We used two isofemale lines of each *D.melanogaster* and *D.simulans* species, chosen on the basis of their showing a low (L) or high (H) value of hybridization (Carracedo & Casares 1985b) and named ML and MH, respectively, for *D.melanogaster* and SL and SH for *D.simulans*. The schedule utilized was as follows: five *D.melanogaster* females and five *D.simulans* males, aged 2 hr, were placed in vials with food in which they remained together for ten different periods of time, ranging from 1 to 10 days. At the end of each period, females were individually placed in vials. The presence of hybrid progeny in the vials was taken as evidence of heterospecific mating. This scheme was initiated for each of the four possible directions of crossing between the 2 x 2 lines of males and females. All the experiments were replicated 5 times in a single block, and carried out at room temperature.

To avoid confusion with the use of the word "age", we must state that there is a clear difference between, for example, saying that maximum hybridization occurs when we use females "aged 3 days", or saying "on the third day of life", since, obviously, in the first case the observation is on day-4 of life; whereas in the second, it is on day-3. In future, we will utilize this last meaning. The table shows the average percentages of hybridization for crossings and periods of time. When the ML-line was utilized as female parent, the frequency of hybridization was, independently of the two male lines, extremely low: from 500 females tested at different periods, only 7 left hybrid progeny. The other *D.melanogaster* line, MH, gave a very different result. In the cross MH x SL, a multiple comparison of percentages revealed that values from 2 to 10 days were not significantly different ($X^2 = 4.52$; $df=9$). That is, the maximum hybridization was attained on day-2 of coexistence, and more time did not increase this value. In the other cross, MH x SH, the percentages from 3 to 10 days were not significantly different ($X^2 = 12.08$; $df=9$) and so, the maximum of hybridization was attained on day-3.

In summary the results show: (1) No interspecific mating occurs on day-1; unpublished data have shown that males of *D.simulans* are sexually active within this period of 24 hr, but females of *D.melanogaster* are practically unreceptive; the above result suggests that young *D.melanogaster* females are not raped by *D.simulans* males. (2) Females play a more important role in hybridization than males, as previously demonstrated (Parsons 1975; Carracedo & Casares 1985b; Casares & Carracedo 1985). (3) Hybridization does not increase with time. This suggests that, in these species, interspecific mating does not depend on male persistence. (4) As previously suggested (Carracedo & Casares 1984), there seems to be a temporal disagreement between the age at which females attain sexual receptivity, and their sexual specific discriminative ability.

Finally, we have obtained for the MH-line the maximum frequency of hybridization with each *D.simulans* line. This has been performed by calculating the average of the non-different percentages. In the cross MH x SL, the maximum hybridization, attained on day-2, is 50.22% (113/225), whereas in the cross MH x SH it is 67.50% (135/200) and it is attained on day-3. These two percentages are significantly different ($X^2 = 4.48$; $df=1$; $p < 0.05$) which point out that "speed" in hybridization is not in agreement with the maximum value attained. This result might be imputed to different behaviour of the male lines, which reveals the complexity of the behavioural mechanisms involved in the precopulatory isolation between these two sibling species.

References: Barker, J.S.F. 1967, Amer. Nat. 101:277; Carracedo, M.C. & P. Casares 1984, Bol. Cien. Nat. I.D.E.A. 33:15; 1985a, DIS 61 (this issue); 1985b, Experientia 41:106; Casares, P. & M.C. Carracedo 1985, DIS 61 (this issue); Manning, A. 1959, Anim. Behav. 7:60; Parsons, P.A. 1972, Can. J. Genet. & Cytol. 14:77; Pontecorvo, G. 1942, DIS 16:66.

maturity has been reached. In previous works, we used adult flies aged 6 hr, which remained together for a period of 5 (Carracedo & Casares 1985b) or 10 days (Carracedo & Casares 1984; 1985a); at the end of these periods of time, we obtained the frequency of hybridized females. But by this procedure, we do not know if the observed hybridization mainly occurred in the first 24-28 hr, or if, on the contrary, the number of hybridized females of *D.melanogaster* increased day after day due to persistent courtship by *D.simulans* males. These two different phenomena deserve