Lamborot, M. and S. Koref-Santibañez. Universidad de Chile, Santiago, Chile. Temperature and sexual isolation between D. gaucha and D. pavani.

A study of mating activity of the sibling species D. pavani and D. gaucha (DIS 1966 42: 106; Biológica 1967 61: 3-6) revealed that the optimum temperature for sexual activity was lower (18°C) in the Chilean than in the Brazilian species (25°C). Interspecific crosses showed a differential receptivity of the females. As the isolation indices could not be obtained, a new set of experiments was set up, using the "male choice method" at nine different temperatures: 6°C, 8°C, 12°C, 16°C, 20°C, 24°C, 28°C, 32°C and 34°C). For each temperature the activity of approximately 100 males was studied. 5 males were placed for 6 hours with 5 females of their own and 5 of the sibling species in 5 x 20 cm. vials. The ventral receptacle and the spermathecae of the females were examined for the presence of sperm.

Tables 1 and 2 summarize the results obtained, together with the isolation coefficients (K) (Malogolowkin-Cohen et al. Evolution 19: 95-103). While D. pavani males show little isolation throughout the temperature range, having even greater preferences for the foreign female, D. gaucha males reveal marked preferences for their own females, specially notorious at extreme temperatures; at the temperature at which the activity of D. gaucha was optimal, isolation was lowest. The results point to the conclusion that female receptivity seems to be more responsible for sexual isolation than the activity of the male.

Table 1. ♂ pavani

Temp. °C	Homog. %	Heterog. %	K
6	32.0	16.0	o.43±0.19
8	48.9	34.8	0.22±0.11
12	42.3	39.6	0.05±0.08
16	71.2	72.5	0.01±0.07
20	77.3	79.2	0.01±0.07
24	72.5	83.2	0.16±0.08
28	59.6	61.5	0.02±0.09
32	23.6	34.6	0.22±0.04
34	6.0	24.0	0.63±0.01

Table 2. ♂ gaucha

Temp. °C	Homog. %	Heterog. %	K
6	24.0	17.0	0.16±0.22
8	48.9	17.2	0.51±0.10
12	51.2	21.2	0.49±0.07
16	66.2	22.8	0.61±0.06
20	75.7	27.9	0.59±0.06
24	89.1	72.2	0.27±0.08
28	79.4	40.4	0.45±0.08
32	71.8	20.0	0.69±0.07
34	40.4	4.7	0.85±0.14

Homogamic, heterogamic preferences and isolation index (K) of D. gaucha and D. pavani males at different temperatures

Kuroda, Y. National Institute of Genetics, Misima, Japan. Effects of X-irradiation on the differentiation of eye-antennal discs of D. melanogaster in organ culture.

Eye-antennal discs were irradiated with 0 R, 500 R, 1,000 R, 1,500 R and 2,000 R of X-rays (180 kV, 25 mA, 1.0 mm Al filter, distance 40 cm, dose rate 300 R/min) immediately after their preparation in hanging drop cultures from mature third-instar larvae of the Oregon-R strain of D. melanogaster. After irradiation the discs were cultured in a chemically defined medium containing 10^{-4} mg/ml rubrosterone and examined for the effects of X-rays on the differentiation of ommatidia. When eye-antennal discs were irradiated with 500 R or 1,000 R no marked inhibition was observed in the differentiation of ommatidia after 24 hours of cultivation. The organization of ommatidium-forming cells into cell clusters was observed in the eye disc portion as seen in eye-antennal discs in non-irradiated control cultures. With 1,500 R the differentiation of ommatidia was partially inhibited 24 hours after explantation. 2,000 R inhibited almost completely the differentiation of ommatidia when examined after 24 hours of cultivation.

When eye-antennal discs were irradiated first with a dose of 1,000 R immediately after explantation, then they were exposed to a second dose of 1,000 R at 2 or 4 hours after explantation, the effects of X-ray were found to be different depending on the extent of the intervals between the first and second doses. With the second dose given at 2 hours after explantation the differentiation of ommatidia was partially inhibited after 24 hours of cultivation; whereas with the second dose given at 4 hours after explantation no inhibitory effect of X-ray was observed on the differentiation of ommatidia.

These results suggest two possibilities; the presence of repair 4 hours after the first irradiation, and alternatively, the differential sensitivity of the eye-antennal discs at different stages of cultivation. To examine these alternatives, eye-antennal discs were irradiated with single dose of 2,000 R 2 hours or 4 hours after explantation. When eye-antennal discs were irradiated with 2,000 R 2 hours after explantation, the differentiation of ommatidia was partially inhibited. However 2,000 R of X-ray had no inhibitory effect on the differentiation of ommatidia when given 4 hours after explantation. These results suggest that the organization of ommatidium-forming cells was inhibited by 2,000 R of X-ray when eye-antennal discs were irradiated at 0-2 hours after explantation. After 4 hours of cultivation eye-antennal discs showed no pronounced changes in morphology but they had a lesser sensitivity to X-ray and resulted in the full organization of ommatidium-forming cells following 2,000 R of X-irradiation.

Kuroda, Y. National Institute of Genetics, Misima, Japan. Effects of BUdR, actinomycin D and puromycin on the differentiation of eye-antennal discs of D. melanogaster in organ culture.

Eye-antennal discs dissected from mature third-instar larvae of the Oregon-R strain of D. melanogaster were cultured in chemically defined medium as described in the previous paper (1). In the medium supplemented with 10^{-4} mg/ml rubrosterone (an ecdysone analogue isolated from plants) a pronounced differentiation of ommatidia was observed in 92% of eye-antennal discs after 24 hours of cultivation at 28°C (2). When 10^{-5} M BUdR (5-bromodeoxyuridine, Sigma Chem. Co., crystaline) was added to the medium containing 10^{-4} mg/ml rubrosterone, eye-antennal discs showed differentiation of ommatidia similar to that in control cultures without BUdR. Similarly, the addition of 1 μg/ml actinomycin D (Daiichi Pure Chem. Co., Ltd.) to the medium containing 10^{-4} mg/ml rubrosterone also had no effect on the hormone-induced differentiation of ommatidia. The presence of 10 μg/ml puromycin (Nutritional Biochem Corp.) also did not inhibit the hormone-induced differentiation of ommatidia. These results are summarized in Table 1.

Table 1. Effects of BUdR, actinomycin D and puromycin on the differentiation of ommatidia in eye-antennal discs cultured in chemically defined medium containing 10^{-4} mg/ml rubrosterone

	No. of explants tested	No. of explants in which ommatidia differentiated	Percent of differentiation
Control	12	11	92
BUdR (10^{-5} M)	7	6	86
Actinomycin D (1 μg/ml)	14	11	79
Puromycin (10 μg/ml)	10	7	70

This suggests that the organization of ommatidium-forming cells in eye-antennal discs in organ culture promoted by an ecdysone analogue was not inhibited by inhibitors of RNA and protein synthesis and that the process of the formation of ommatidial cell clusters may be conducted by pre-existent macromolecules which were activated into their functioning by ecdysone analogue.

1. Kuroda, Y. and Tamura, S. 1956, Med. J. Osaka Univ., 7: 137. 2. Kuroda, Y., 1969, Japan. J. Genetics, 44, Suppl. 1: 42.