Studies of Selective Mating Using the Melanistic Mutants of *Drosophila melanogaster*

The sexual isolation between *ebony* (e^{11}), *black*, and *wild* (Canton special) flies was measured according to a 'multiple choice method'. The flies of both strains were introduced through a funnel into a small wooden box, circular on the inside (diameter 9 cm), with bevelled edges, and covered by a thick glass. A chequered canvas was extended beneath. 100 flies (25 pairs of both strains), 4 or 5 days old, were used in each test. The observations were recorded at intervals of 5 min on a paper reproducing the same grid as the chequered canvas. Observation was easy because copulating pairs generally do not move (for more details see [1]). In an attempt to measure locomotor activity, we used a series of 6 glass tubes similar to those described by EWING[2]. 25 flies of the same sex and strain were introduced together into the first tube and the number of flies in each of the 6 tubes was recorded at 3 min intervals, the figures obtained being expressed as 'percentage possible moves' occurring within the 3 min periods according to the procedure of EWING.

The results of the observations concerning selective mating are given in Table I as cumulated percentage of females fertilized in each mating type. The graphic expression of such data would be exponential curves approaching a plateau, and the result of such comparisons could be biased by the time at which the observation is ended; a few copulations can certainly take place later. Consequently, it seemed interesting to use a method recently proposed by WATTIAUX[3], where such exponential curves are turned in straight lines by logarithmic transformation, the values of measures becoming independent of time. WATTIAUX's formula is

$$dx_A/(n_A - x_A)\,dt = b\,K\,e^{-Kt},$$

where n_A is the total number of individuals of one sex and genotype, dx_A the number of flies mating in the intervals of time dt, and K and b constants which can be calculated from the regression line fitting to the transformed data. By analogy with the well-known MERREL 'isolation estimate', 'male mating ratio', and 'female mating ratio'[4], one can effect such comparison between regression lines concerning: (1) homogamic and heterogamic matings, (2) matings of males of the first and of the second genotype, (3) matings of females of the first and of the second genotype. Figure 1 (time in abscissa and values $dx_A/n_A - x_A$ in ordinate) gives the regression lines permitting such comparison (through lack of space, their formulae are not given).

The application of a χ-square test to the classical 'isolation estimate' seemed to indicate a certain isolation between *wild* and *black* types, and between *black* and *ebony* too. But the differences between homogamic and heterogamic regression lines are never significant. Between the female *black* and *ebony* types, the comparison of regression lines indicates a significant difference of activities ($P < 0.005$) as well as the χ-square test concerning 'female mating ratio'. The major differences concern the male activities of the three genotypes. The differences between the regression lines are highly significant in all cases ($P < 0.005$), which is in concordance with the χ-square test (Table II).

Table I. Cumulate percentage of fertilized females

Time in min:	20	40	60	80	100	120
wild × *ebony* (20 × 25 pairs)						
♂ *wild* × ♀ *wild*	24.6	33.4	42.4	45.2	45.8	45.8
♂ *wild* × ♀ *ebony*	16.8	24.6	33.4	37.9	39.0	39.2
♂ *ebony* × ♀ *ebony*	2.2	4.4	6.6	7.4	7.4	7.4
♂ *ebony* × ♀ *wild*	1.6	3.2	5.2	6.0	6.0	6.0
wild × *black* (20 × 25 pairs)						
♂ *wild* × ♀ *wild*	31.4	42.0	45.4	48.8	50.8	50.8
♂ *wild* × ♀ *black*	23.8	30.0	35.6	37.8	39.8	39.8
♂ *black* × ♀ *black*	20.6	25.4	29.0	30.2	31.4	32.0
♂ *black* × ♀ *wild*	10.6	14.0	16.4	18.4	19.0	19.0
black × *ebony* (5 × 25 pairs)						
♂ *black* × ♀ *black*	22.4	30.4	36.0	38.4	40.0	40.0
♂ *black* × ♀ *ebony*	4.0	10.4	12.8	13.6	13.6	13.6
♂ *ebony* × ♀ *ebony*	0.0	0.8	2.4	3.2	3.2	3.2
♂ *ebony* × ♀ *black*	0.0	4.0	7.2	8.0	8.8	8.8

Table II. Calculated χ^2 of the isolation estimate, female mating ratio male mating ratio derived from the data of Table I

	I	χ^2	M♂	χ^2	M♀	χ^2
wild × *ebony*	1.17	0.64	1.11	0.26	6.34	52.08[c]
wild × *black*	0.89	4.06[a]	0.97	0.02	1.77	11.00[c]
black × *ebony*	1.92	6.40[a]	2.90	15.60[c]	4.46	26.38[c]

[a] $P < 0.05$. [b] $P < 0.005$. [c] $P < 0.0025$.

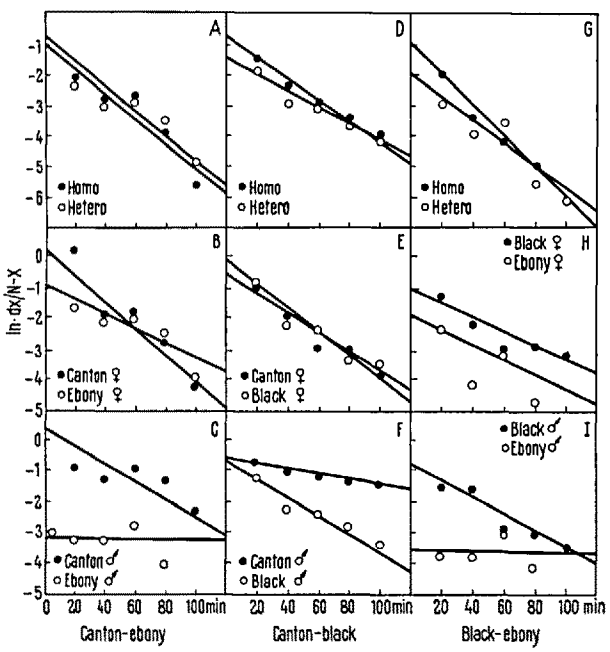

Fig. 1

[1] A. A. ELENS and J. M. WATTIAUX, Drosoph. Inf. Serv. *39*, 118 (1964).
[2] A. W. EWING, Anim. Behav. *11*, 369 (1963).
[3] J. M. WATTIAUX, Z. Vererb. *95*, 10 (1964).
[4] D. J. MERREL, Evolution *4*, 326 (1950).

Figure 2 gives the regression lines fitting the data concerning the motor activity levels (for each genotype and sex the means of the 15 repetitions are plotted as percentage of possible moves in function of time). For males as for females, the differences between the three genotypes are always significant or highly significant, the order of superiority being the same as for the sexual activity of the males. Perhaps the common level of agitation could be a stimulating factor for the courting initiatives of the males.

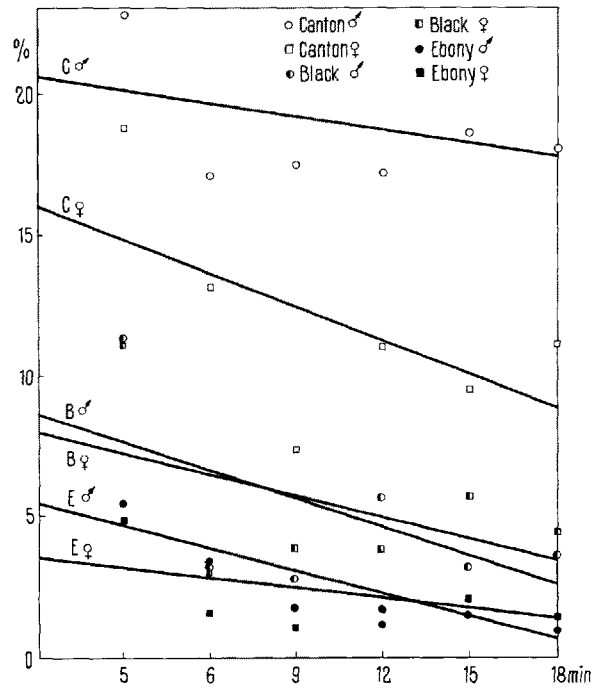

Fig. 2

The two melanistic mutants *ebony* and *black*, phenotypically very similar, are certainly different in selective value. The second one is rapidly eliminated in competition with its wild type allele, but, under the same conditions, *ebony* may be present in the population for many generations. The explanation of its maintenance can certainly not be found in the difference of activity: the *ebony* flies are the least active. But, in a previous paper, we have shown that the sexual activity of heterozygote flies from father *wild* and mother *ebony* is significantly higher than that of the *wild* ones[5].

Résumé. L'observation directe a montré qu'il n'y a pas d'isolement sexuel entre le type *sauvage* et les mutants mélaniques *ebony* et *black* de *Drosophila melanogaster*, mais il existe de notables différences entre l'activité sexuelle et motrice des mâles des trois génotypes. L'importance sélective de ces différences est soulignée.

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[5] A. A. ELENS, Exper. *14*, 274 (1958).

The Regeneration of Accessory Limb Parts Following Epidermal Cap Transplantation in Urodeles

Introduction. Recently, interest in the functions of the wound epidermis in amphibian limb regeneration has focused on the phagocytic and histolytic activities of the epithelial cells[1], and on their apparent ability to influence the aggregation of mesenchymatous cells to form the regeneration blastema[2,3]. The purpose of this communication is to supply new information on the influence of the apical epidermal cap on the formation and outgrowth of the limb blastema, and in particular, to describe the production of accessory limb outgrowths following transplantation of epidermal caps to the basal areas of limb blastemata.

Material and methods. Two species of urodele larvae were used in these experiments: *Ambystoma talpoideum* 70–80 mm long, and *A. mexicanum* 80–90 mm long. The right forelimbs were amputated through the distal condyles of the humerus. After 11 days (*A. talpoideum*) or 14 days (*A. mexicanum*) a mound-stage blastema had formed at the end of the limb stump. At this stage the larvae were anesthetized with MS 222 and placed on a pad of sterile gauze moistened in Ringer's solution. A section of epidermis, equivalent in area to the apical epidermal cap, was excised from the base of each blastema on its pre-axial surface. Immediately after the epidermis was removed the apical cap was cut away from the blastema tip, cleared of adhering blastema cells and placed autoplastically over the proximal wound in the blastema. Each larva was placed for 6 h in a refrigerator, held at 6°C, in order to insure healing of the edges of the apical cap with the cut edges of the blastemal epidermis. Within a week a new apical cap regenerated at the blastema tip to replace the one which had been removed and transplanted. In 27 of 51 blastemata both epidermal caps (the grafted and the regenerated) continued their individual growth. In these cases, therefore, the limb blastemata continued to develop in association with the regenerated epidermal caps while a secondary blastema formed beneath each of the grafted epidermal caps. These accessory blastemata differentiated supernumerary limb parts. In the remaining 24 cases the graft cap was suppressed or its outgrowth fused indistinguishably with that of the regenerated apically situated cap so that single, typical limbs were produced.

Control experiments consisted of the transplantation of whole head skin (dermis and epidermis – 10 cases) and

[1] M. SINGER and M. M. SALPETER, Basic Books Inc. (New York 1961).
[2] C. S. THORNTON, Devel. Biol. *2*, 551 (1960).
[3] T. P. STEEN and C. S. THORNTON, J. exp. Zool. *154*, 207 (1963).