Elens, A. Facultés Universitaires N.D. de la Paix, Namur, Belgium. Initiation of an ethological isolation between ebony and wild Drosophila melanogaster?

Three strains of D. melanogaster have been used in the present experiments: a wild one (Canton special), the mutant strain ebony e^{11}, and a new ebony strain "B" selected after 40 generations of crossing between Canton special and ebony e^{11}.

The first object of this work was to investigate the frequency of heterogamic matings between the wild strain and the ebony ones. It is known that in some cases the sexual isolation can be greater between sympatric than between allopatric races of the same species, as e.g. in the Drosophila paulistorum species complex (Ehrman, 1965). Recently, Nikheev and his collaborators have succeeded in selecting for sexual isolation between the black and vestigial mutants of D. melanogaster. The population was started with 25 pairs of each of both mutant strains. Four days after the beginning of the eclosion period, ten males and ten females of each mutant phenotype were taken as founders of the following generation. It is quite probable that some of the females had been inseminated by heterozygote males; however, the selection was very effective: after eleven generations "a reduction of the proportion of heterozygotes down to below 10% has been attained" (Mikheev et al., 1973). Consequently, it was interesting to consider the hypothesis that the ethological isolation could be greater between wild and the new ebony "B" strain than between wild and the original ebony e^{11} strain.

A second object of the present investigation was to draw a comparison between the sexual activity of the males and the females of a strain in competition with flies of another strain and their sexual activity in presence of flies of their own strain only. It is known that in the presence of wild males, the sexual activity of the ebony males is low but remains constant for a longer time than the sexual activity of wild males (1973). It has been established that on some occasions the experimental inhabition of mating of males can induce later matings of such males and more constancy of mating success, as e.g. for the dragonflies studied by Jacobs (1955). Consequently, it was interesting to examine whether the activity of the ebony males was significantly lowered in the presence of wild males.

In each experiment, the mating successes of three groups of 30 virgin pairs of flies, 4 to 5 days old, were recorded in three observation chambers, according to the multiple choice direct observation method which has been previously described elsewhere (1958). The first chamber contained 30 pairs of wild flies, the second one 15 pairs ebony and 15 pairs wild, the third one 30 pairs ebony. The temperature was 25°C, the relative humidity 40-60%, and the light intensity 500 Lux. For each of the competing pairs of strains (wild with ebony e^{11} and wild with ebony "B") the experiments were repeated 10 times. The results are shown in Table 1.

Although the frequency of heterogamic matings does not differ between the experiments with ebony e^{11} and with ebony "B", the mating between wild males and ebony females are much more frequent for the strain e^{11}. Such results are in good agreement with the conclusions of Mikheev et al. (1973). An ethological isolation could be established between ebony and its wild allele.

Compared to previous observations, the sexual activity of the wild flies was rather low when they were not in the presence of ebony flies. On the contrary, the sexual activity of both ebony strains was particularly high when they were not in the presence of wild flies. It is all more surprising that, in the presence of ebony flies, the wild males become very active. On the contrary, the ebony males were inhibited by the presence of wild flies. As previously

Table 1. Matings recorded in four hours observation.

	Wild alone	Both strains in competition				Ebony alone
	♂+ ♀+	♂+ ♀+	♂+ ♀e	♂e ♀+	♂e ♀e	♂e ♀e
1.	131	51	80	10	32	163
Total flies	300 pairs wild	150 pairs wild			150 pairs ebony e^{11}	300 pairs ebony e^{11}
2.	127	66	55	17	32	159
Total flies	300 pairs wild	150 pairs wild			150 pairs ebony "B"	300 pairs ebony "B"

mentioned, the ebony females of the strain e^{11} seemed to be preferred by the wild males; consequently, their level of sexual activity is significantly higher when they are in the presence of wild flies. However, the level of sexual activity of the females of the strain "B" is unchanged.

Further analysis will have to show if this initial frustrating inhibition of the ebony males could be compensated by a more constant success in the ulterior phases of the competition, as it could be conjectured from our previous observations (1973) and by analogy with the observations of Jacobs on the dragonflies (1955).

References: Ehrman, L. 1965, Evolution 19:459; Elens, A. and J.M. Wattiaux 1964, DIS 39:118; Elens, A., J. Van den Haute, J. Delcour 1973, Evolution (in press); Jacobs, M.E. 1955, Ecology 36:566; Mikheev, A.V., A.G. Kreslavsky and V.M. Solomatin 1973, Genetika (Russ.) 9:169.

Rapport, E. Simon Fraser University, Burnaby, Canada. On the action of the vital stain 2,2'-Dipyridyl.

A vital stain which colours imaginal discs, brain and ring gland of Drosophila, has been described (Rapport and Menon, 1973). The stain, 2,2-dipyridal (Dip) is known to chelate ferrous ions (Fe^{++}) and form a red product. Indeed this reaction is used quantitatively in Fe^{++} determinations. We wished to determine if the in vivo staining reaction could be used as a measure of high Fe^{++} concentration.

Larvae were grown on either cream of wheat media seeded with yeast or the same media made with water containing 1 mg $FeCl_2$ per cc. Larvae were removed from the media by flotation with NaCl solution at either 65 or 72 hours and placed in petri dishes with paper pulp and either water or a Dip solution. In addition to usual sites of staining the $FeCl_2$ fed larvae treated with Dip at 72 hours had deep red granules in the base of the gastric caecae. By visual inspection we could not detect enhanced colouration of organs which usually stain. These results are at variance with those of Poulson and Bowen (1952) whose histological studies using fixed material revealed a direct relationship between stainability and iron concentration in the media and who never detected ions in the caecae. The pulp in dishes with $FeCl_2$ treated larvae was stained pink apparently due to excretion of Fe^{++} ions by the larvae.

These results suggest that at least some of the stain in vivo could be the result of Fe^{++} localisation.

To further investigate the action of Dip in the living organism the effect of somewhat toxic levels of Dip on pupation ability and ability to evert imaginal structures was studied in the different feeding regimes. Table 1 gives the results of this study. Larval development was impeded by Dip treatment and this effect could be reversed to some extent by prior

Age removed from feeding	Treatment	Number of organisms	% failing to pupate	95% confidence interval	% pupae failing to evert heads	95% confidence interval
65	H_2O	153	2.0	0 - 4.2	1.3	0 - 3.2
65	Dip	225	89.3	85.5 - 93.1	18.5	3.8 - 33.2
65	Dip + $FeCl_2$	184	67.9	61.2 - 74.7	23.7	12.9 - 34.6
72	H_2O	214	1.9	0.1 - 3.7	0.5	0 - 1.4
72	Dip	218	55.0	48.4 - 61.7	33.7	24.3 - 43.0
72	Dip + $FeCl_2$	208	26.0	20.0 - 32.0	31.2	23.8 - 38.5

feeding with $FeCl_2$. Among the pupae, head eversion was not appreciably influenced by $FeCl_2$ feeding. These results weakly suggest that preventing Dip from chelating with normal cell constituents (by addition of exogenous ions) reduces its toxicity.

Clearly more work needs to be done to determine the substance(s) to which Dip binds in vivo. While detection of stain in gastric caecae only after $FeCl_2$ feeding suggests that Dip will bind to intracellular iron, the failure of our staining results to confirm those of Poulson and Bowen indicates that more work must be done to determine if this stain faithfully reflects ferrous ion concentration in Drosophila organs.

References: Poulson, D.F. and V.T. Bowen 1952, Exp. Cell Res. (Suppl.) 2:161-179; Rapport, E. and M. Menon 1973, Experientia 29:734-735.