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Marking of Drosophila eggs by feeding dyes to larvae and adult females.

In our current studies on interspecific competition between D. melanogaster and D. simulans, we intend to determine the effect of various factors on the fecundity of these species, both in single-species and mixed populations. As their eggs are indistinguishable, the latter would be greatly simplified if eggs of one species could be marked by a dye sufficiently to distinguish them on macroscopic examination. Using D. melanogaster Oregon-R-C and the vermilion mutant of D. simulans, 15 dyes have been tested.

Larval feeding: The dye was blended with hot liquid dead yeast fortified medium. Twenty pairs of adults were left in each bottle for 24 hours. Eggs produced by about 100 progeny from each bottle were collected daily and examined microscopically for at least 4 days post-eclosion.

Adult feeding: For each bottle, dye was added to a mixture of 30 ml cornmeal-agar-treacle medium, 2 ml treacle and 0.5 g dead yeast. Flies (<12 hrs old) were allowed to feed for 12 hrs. Eggs produced over at least the next 4 days were collected and examined daily.

Eggs were examined both whole and squashed. For all ether-soluble dyes, they were soaked in ether. For rhodamine B and fluoroscein, eggs were examined under ultra-violet light. Samples of females were examined for ovary pigmentation.

None of the dyes were apparently grossly toxic with adult feeding. With larval feeding, seven were harmful under some conditions. Where many dead larvae were observed, either toxicity or unpalatability may be involved.

No ovary or egg pigmentation, nor apparent toxicity with larval feeding was observed for the following dyes:

Ether soluble: Calco Oil Red Z-1700 - 0.1 and 1.0% w/v concentrations.

Water soluble: At 0.05 and 0.1% w/v concentrations - Rhodamine B, Fluoroscein, Congo Red, Orcein, Methyl Orange.
At 1.5 and 3.0 ml saturated solution in 30 ml medium - Chrysoidin.

No ovary or egg pigmentation was observed for the ether-soluble dyes Irgacet Scarlet GL and Irgacet Scarlet RL, at 0.1 and 1.0% w/v concentrations. The former was completely toxic to larvae at the higher concentration, while the latter (at 1%) delayed the onset of egg production by D. simulans by 2 days.

For the water soluble dyes, Methylene Blue, Nile Blue and Neutral Red at 0.05 and 0.1%, no egg pigmentation was observed, although all (D. simulans only for Neutral Red) showed ovary pigmentation with adult feeding and with larval feeding at 0.05% for the last two. For these two dyes at 1% and Methylene Blue at both concentrations, no adult females were obtained.

Three ether soluble dyes showed some egg pigmentation. Irgacet Orange GR gave both ovary and egg pigmentation when fed to larvae at 1.0 and 2.0%. Egg pigmentation ceased after 4 days. At 2.0%, emergence of Oregon-R-C was delayed by about 2 days, while no D. simulans were obtained. No pigmentation was observed with adult feeding (1.0%). Sudan III gave both ovary and egg pigmentation with both adult (0.1 and 1.0%) and larval (1.0 and 2.0%) feeding. The proportion of pigmented eggs was highest in the first eggs laid and decreased to zero after 3 days. At 2.0% larval feeding, the numbers of eclosing adults were reduced, and the onset of egg production by D. simulans was delayed by 2 days. Sudan Black fed to larvae (1.0 and 2.0%) also delayed egg production by D. simulans, and although ovaries of both species were pigmented, no pigmented eggs were laid. With adult feeding (1.0%), ovaries of both species again were pigmented, but Oregon-R-C produced pigmented eggs. The proportion was highest in the first eggs laid, and decreased to zero after 4 days.

However, for these three dyes, egg pigmentation was not consistent (only a proportion of early eggs were marked), and the intensity of pigmentation was never great enough to be detectable macroscopically. (Work supported by Australian Research Grants Committee.)