Jallon, J.-M. and Y. Hotta. University of Tokyo, Japan. A proposal for a new genetic scheme to hunt for sex-appealless mutants of D. melanogaster.

We have recently reported genetic and behavioral studies of female sex appeal in D. melanogaster (Jallon and Hotta 1979). The sex appeal was defined as the stimulus (or set of stimuli) which induces wing vibration in courting males. It is most likely to be a measure of sex pheromone released from a female.* Sexual behavior of gynandromorphs was analyzed by means of the blastoderm fate map method (Hotta and Benzer 1972), and we were able to localize the sex appeal focus in the ventroposterior region of the blastoderm fate map. Moreover, studying ontogeny of male courtship, we found that on the first day after eclosion males possess as much sex appeal as females. However, males lose it within a day, while females retain it indefinitely.

Hunting for specific mutations affecting the presence of sex appeal in females is another way to extend the study of sex appeal and its control. A difficulty expected in isolating such mutants is that they will not reproduce. The existence of young males' sex appeal leads us to propose a novel way to overcome this difficulty. The procedure is to look for X-chromosomal sex-appealless mutants among F_1 male progeny of chemically mutagenized males which are mated with attached X-chromosome females. By this genetic scheme, mutagenized paternal X-chromosome is transmitted to sons, so that sex-appealless mutations may be found among F_1 males at their immature stage. If such mutants without ephemeral sex appeal could still function as normal males in later sexual activities, it would be possible to find such genes rather easily. We have so far examined about 4500 such males a few hours after their emergence and tested their ability to induce wing vibrations of male testers. None of them turned out to be a mutant. There are several possible reasons for the present difficulties. It may be that such genes happen to be rare on the X-chromosome. It may also be because such genes become lethals when they are mutated. Finally there might be at least two alternative biochemical pathways to produce sex appeal.

This method is particularly simple and allows a mass screening of sex-appealless mutant candidates. Using the same progeny, one may also investigate male mutants which would maintain female-like sex appeal beyond their usual immature period or ones which would court the wild-type male testers.

References: Hotta, Y. and S. Benzer 1972, Nature 240:527; Jallon, J.-M. and Y. Hotta 1979, Behav. Genetics (in press); *Venard, R. and J.-M. Jallon 1979, submitted to Experientia.

Jha, A.P., B.N. Pandey and D.N. Mishra. Mithila University, Darbhanga, Bihar, India. Substrate specificities of alcohol dehydrogenase in Drosophila.

Alcohol dehydrogenase isoenzymes have been studied qualitatively (Ursprung and Leone, 1965; Jacobson et al., 1970) and quantitatively (Sofer and Ursprung, 1968; Ward, 1974) irrespectively of different substrates in D. melanogaster. Singh (1976) studied substrate specificities in D. pseudoobscura. Here we report on the substrate specificity in relation to developmental changes in D. ananassae, D. malerkotliana and D. bipectinata.

ADH activity was recognized by reduced tetrazolium deposition on 5% polyacrylamide gel. We used ten substrates in our experiment. In D. ananassae, larvae show activity with all ten substrates used in the experiment, pupae with only seven substrates and adults with nine substrates. Third instar larvae of D. malerkotliana and D. bipectinata exhibit activity with all ten substrates. Late pupae of D. malerkotliana reveal activity with all substrates, and those of D. bipectinata with only six. Adults of both species exhibit activity with all ten substrates. Differences in reactivity with substrates indicate that ADH isoenzymes must have some different physiological functions which are stage-specific.

Substrates	Drosophila ananassae			Drosophila malerkotliana			Drosophila bipectinata		
	Larva	Pupa	Adult	Larva	Pupa	Adult	Larva	Pupa	Adult
Ethanol	+	+	+	+	+	+	+	+	+
Methanol	+	-	+	+	+	+	+	-	+
Butanol	+	+	+	+	+	+	+	+	+
n-Propanol	+	+	+	+	+	+	+	+	+
2-Propanol	+	-	+	+	+	+	+	+	+
Benzyl alcohol	+	+	+	+	+	+	+	-	+
Allyl alcohol	+	+	+	+	+	+	+	-	+
Amyl alcohol	+	+	+	+	+	+	+	-	+
Cyclohexanone	+	-	+	+	+	+	+	-	+
Octanol	+	+	+	+	+	+	+	-	+

Presence (+) or absence (-) of activity for alcohol dehydrogenase enzyme in three species of Drosophila acting on a variety of alcohol substrates.

References: Ursprung, H. and J. Leone 1965, J. Exp. Zool. 160: 147; Jacobson, K.B., J.B. Murphy and F.C. Hartman 1970, J. Biol. Chem. 245: 1075; Sofer, W. and H. Ursprung 1968, J. Biol. Chem. 243: 3110; Ward, R.D. 1974, Biochem. Genet. 12: 449; Singh, R.S. 1976, Genetics 82: 507.

Joiner, M. and J.S. Johnston. Baylor University, Waco, Texas. Determination of exact age in Drosophila (1).

In 1971, Van Valen and Van Valen (2) reported failure to find daily growth layers in D. melanogaster. However, in 1973 Van Valen (3) encouraged Drosophila workers to try the aging methods of Schlein and Gratz (4). By following these methods, we found limited areas of banding in thoracic muscle attachments (apodemes). As was shown in other Diptera (4), apodemes of Drosophila exhibit daily growth layers on regions of postmetamorphic growth. The apodemal growth layers are highly variable both in contrast and in maximum number. The best specimens show banding under transmitted light. Phase contrast microscopy improves the contrast, as does staining with Heidenhain's Hematoxylin. None of these methods, however, provide a reliable or repeatably good aging tool. After experimentation with a wide variety of techniques, stains, and counterstains, we developed the following simple and effective method: A fly with legs and head removed is placed into hot 4% KMnO4 for 5 minutes. After the fly is rinsed in distilled water, the apodemes are pulled from the thorax with forceps and placed into Paragon (5) or other water soluble mounting media under a cover slip. Nemarski differential interference contrast (DIC) microscopy shows areas of growth along the apodemes. The first, second, and third furca (ful, fu2, fu3) and the second thoracic phragma (phl, shown in Fig. 1) developed growth layers. However, the third furca shows the most distinct banding (Fig. 2).

The correspondence between fly age and growth band was tested using three homozygous strains of parthenogenetic D. mercatorum supplied by A. Templeton (6). The results are shown in Table 1. We analyzed this by a 3-way ANOVA with unequal subclasses using BMDP. Genetic differences between strains do not affect the banding ($F=0.68$, $P>.50$). Temperature, however, has an effect ($F=4.15$, $P<.05$). The banding was most distinct, and age correspondence closest, with a 22 to 14.5°C day to night temperature fluctuation. It may be important that the 22 to 14.5°C range is close to that experienced by the strains in nature. The actual fly age corresponded closely with the number of bands (F to fit the age effect = 48.89, $p<<.01$). Fifty percent of the flies from the 22 to 14.5° regime were correctly aged. The other 50% were only one day too high or too low. An experiment testing correlation between age and bands on 7-12 day old flies was not as successful. While a 12 day old fly was correctly identified once, most flies had a maximum of 8 growth bands.

The aging method is thus limited to young flies. Yet, the method is important because it permits age determination up to and into sexual maturity. The method is now being applied to ask a variety of questions about age structure of natural populations. In particular, we are interested in age-related dispersal. To date, 10 species have been successfully aged, including 8 repleta species, D. melanogaster, and D. mimica (a Hawaiian species).