

GROUP III: Puffs active during normal development at this stage which tended to maintain or to increase their activity. A total of 38 loci belong to this group. The loci 5D, 16B, 40D-41A, 63BC, 65B, 74A, 85AB, 86A and 98C could be distinguished as significantly increasing their activity after the shock.

GROUP IV: Puffs active during normal development at this stage which regressed markedly in their activity after the heat shock. A total of 20 loci belong to this group.

In addition to the puffs in these four groups several puffs were found which showed little or no activity either in the control individuals or in the shocked ones.

Finally, it must be mentioned that the length of time under heat shock does not seem to have a decisive effect on puff formation at the times investigated. The greatest development of puffs tends to appear between 20 and 45 min.

References: Ashburner, M. 1970, *Chromosoma* 31:356-376; Berendes, H.D. & Th.K.H.Holt 1964, *Genen en Phaenen* 9:1-7; Ritossa, F.M. 1962, *Experientia* 18:571-573.

Pechan, P.A. and M.L.Tracey. Florida International University, Miami, USNA. Passive anti H-Y immunization of *Drosophila melanogaster* females reduces progeny sex ratio.

Tissue grafts among members of highly inbred populations are accepted, in general, as readily as autografts. Eichwald & Silmsker (1955) detected a weak rejection reaction of male skin grafts by female C57BL/6 mice; all other skin grafts were readily accepted. They hypothesized that the observed male to female

rejection was governed by a male specific transplantation antigen, H-Y antigen. Serological identification of H-Y antigen was first demonstrated by Goldberg et al. (1971) who used serum from male grafted female mice to kill sperm in the presence of complement. Subsequent in vitro studies provide further support for the hypotheses of H-Y antigen male specificity and an early developmental role in sex determination: (1) in anti H-Y antibody cytotoxicity assays, male eight cell stage embryos are lysed; female embryos are not lysed (Krco & Goldberg 1976; Epstein et al. 1980; Ohno 1979). (2) When cultured testicular cells are lysostripped of H-Y antigen, they organize ovarian follicle-like aggregates (Ohno et al. 1978). Similarly, the addition of H-Y antigen to cultured ovarian cells induces the formation of testicular-like tubules (Zenzes et al. 1978). (3) XX bovine gonad primordia undergo testicular conversion in whole organ cultures which contain H-Y antigen (Ohno et al. 1979). Moreover, surveys of both vertebrates and invertebrates report the detection of H-Y antigen in heterogametic individuals (Wachtel 1983).

These reports suggest that similar effects should be detectable in vivo. For example, female mice producing H-Y antibodies should produce fewer male progeny than mothers who are not producing H-Y antibodies. Comparison of sex ratios between litters from H-Y antibody producing C57BL/6 mothers (40% male progeny) and non H-Y producing mothers (52% male progeny) supports this hypothesis ($t=2.35$; $P<0.05$; Pechan unpubl.). Given the ubiquity of H-Y antigen, we decided to attempt a similar in vivo test using passively immunized *D.melanogaster* females.

Three to four day old virgin females were injected with 0.5 μ l of mouse monoclonal H-Y antibody, mouse monoclonal dinitrophenol antibody, 1/64 dilution of mouse monoclonal H-Y antibody (the antibody is not detectable at this dilution), polyclonal H-X serum from females previously injected with female spleen cells, and female mouse serum. Uninjected females were also used. Two males were added immediately after injection and the first 24 h egg

Table 1. Progeny Sex Ratios.

Immunization	Number	Sex Ratio	Number	Sex Ratio
1. H-Y antibody	797	0.44 <.001	1524	0.48 ns
2. H-X	491	0.52 ns	1309	0.46 <.01
3. DNP antibody	226	0.52 ns	309	0.51 ns
4. 1/64 H-Y dilution	381	0.50 ns	334	0.49 ns
5. female serum	301	0.51 ns	252	0.47 ns
6. uninjected	2344	0.48 ns	2707	0.50 ns
pooled 2-6	3743	0.49 ns	4911	0.49 ns

collection or brood was collected 24 h later, that is 24-48 h post-injection. A second brood was collected 48-72 h post-injection. The progeny sex ratios (#males/total) were computed by counting all males and females in broods one and two between days eight and sixteen after egg laying. Differences in the sex ratio between broods as well as among treatments were tested for significance, because we did not expect the passive immunization effect to last more than 24 h due to protein degradation.

Among treatments within the first brood, only the H-Y immunized mothers produced fewer sons than expected. All other sex ratios were in agreement with a 0.50 expectation (Table 1). In the second brood the progeny sex ratio of mothers immunized with serum from mice injected with female spleen cells (H-X) differed significantly from 0.50. All others were in agreement with the 0.50 expectation. Sex ratios from females of increasing age have been shown to increase with maternal age (Lauge 1980); however, the differences are not significant over a 48 h period, and we did not observe an effect in our controls. The decreased production of sons by H-Y immunized females is consistent with the mouse data and supports the hypothesis of an early male differentiation role for H-Y antigen. The low second brood sex ratio among progeny of H-X immunized mothers is problematical.

References: Eichwald, E.J. & C.R. Silmser 1955, *Transp. Bull.* 2:148-149; Epstein, C.J., S. Smith & B. Travis 1980, *Tissue Antigens* 15:63-67; Goldberg, E.H., E.A. Boyse, D. Bennett, M. Scheid & E.A. Carswell 1971, *Nat.* 232:478-480; Krco, C.J. & E.H. Goldberg 1976, *Sci.* 193:1134-1135; Lauge, G. 1980, in M. Ashburner & T.R.F. Wright, *Genetics and Biology of Drosophila* 2d: 33-106; Ohno, S. 1979, *Major Sex-Determining Genes*; Ohno, S., Y. Nagai & S. Ciccarese 1978, *Cytogenet. Cell Genet.* 20:351; Ohno, S., Y. Nagai, S. Ciccarese 1978, *Cytogenet. Cell Genet.* 20:351; Ohno, S., Y. Nagai, S. Ciccarese & H. Iwata 1979, *Rec. Progr. Horm. Res.* 35:449-476; Wachtel, S. 1983, *H-Y Antigen and the Biology of Sex Determination*; Zenzes, M.T., U. Wolf & M. Engel 1978, *Hum. Genet.* 44:333-338.

Pelliccia, J.G. and D.G. Couper. Bates College, Lewiston, Maine USNA. Intragenic complementation at the Adh locus.

Intragenic complementation is a process where, in a multiple subunit protein, two or more non-functional subunits produced by null activity alleles, interact to produce an active enzyme.

We are interested in determining some of the properties of enzymes produced by this process as compared to their wild type counterparts.

A large number of null activity mutations of the alcohol dehydrogenase (Adh) gene have been isolated and the properties of their respective protein products have been studied (Sofer & Hatkoff 1972; O'Donnell et al. 1975). Heterozygotes for certain pairs of CRM positive Adh null activity mutations show levels of enzyme activity ranging from 1% of normal up to almost 23% (W. Sofer, unpubl. data). All such animals have either the Adhⁿ¹¹ or n18 mutation as one member of their complementing pair of alleles. ADH enzyme is active only as a dimer so we assume that the heterodimer is the active form in these hybrid animals.

One such combination of complementing alleles results from crossing an Adhⁿ⁶ cn vg male with a b Adhⁿ¹¹ cn vg female with the resulting F1 having approximately 13% of the enzyme activity found in the b Adh^F cn vg strain from which these mutant strains were derived (F indicates the 'FAST' electrophoretic variant). The results were similar when the reciprocal cross was done.

Paralleling the decreased enzymatic activity of the hybrid adults was their decreased survival on ethanol supplemented media. Whereas Adh^F flies have an LD₅₀ at 6½% ethanol under our conditions of testing, (25 four day old males placed in a plastic shell vial with Carolina instant media reconstituted with an ethanol solution of known concentration and covered with parafilm for 24 hr), the hybrid flies had an LD₅₀ of 1%. Homozygous n6 or n11 males showed 100% mortality when fed media supplemented with 1% ethanol. Thus, the enzyme activity levels predict the in vivo susceptibility to environmental alcohol.

Adults of the b Adh^F cn vg strain show a pattern of accumulating enzyme activity as they age. Enzyme specific activity (units of enzyme per mg soluble protein) rises to a maximum between days 4 and 5 and remains constant thereafter. As shown in Figure 1, the specific activity of the hybrid flies peaks at day 2 and then remains constant. Thus, not only is a lower level of activity maintained, but that level is reached earlier in the developmental profile of the adult. Pelliccia & Sofer (1982) showed that both the n6 and n11 strains produced inactive ADH at rates similar to wild type but maintained steady state levels lower