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.

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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 AdhF 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.

Parallelling the decreased enzymatic activity of the hybrid adults was their decreased survival on ethanol supplemented media. Whereas AdhF 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 AdhF 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

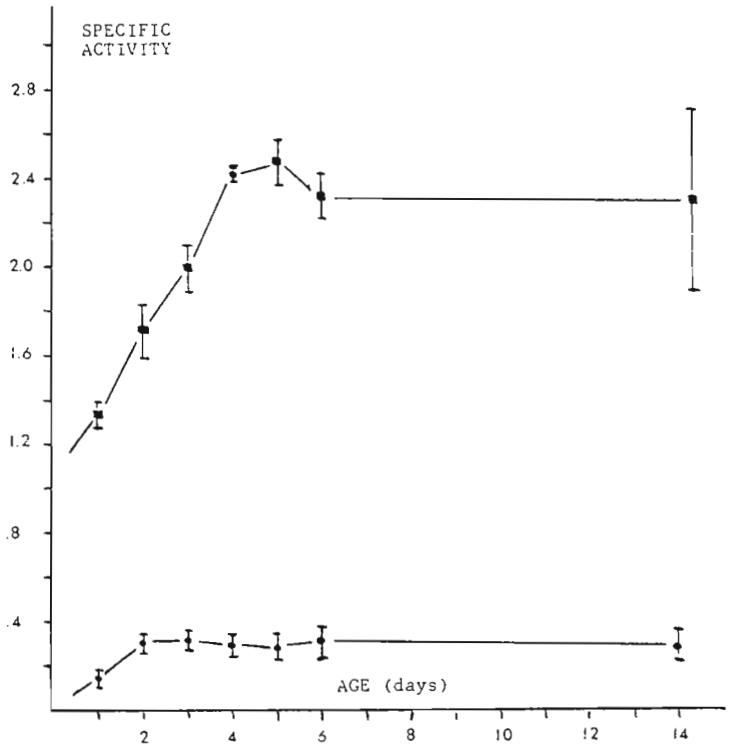

Figure 1. Developmental profile for alcohol dehydrogenase specific activity in AdhF (squares) and hybrid (circles) adults. Each point represents the mean ± standard deviation for at least 3 separate determinations.

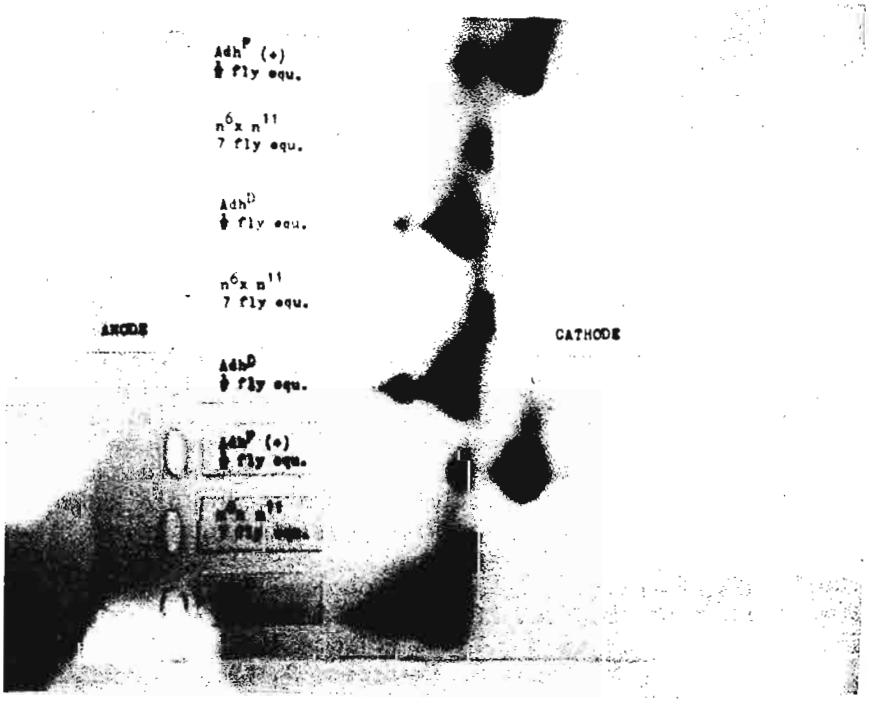

Figure 2. Agar gel electrophoresis of 20,000 x g supernatants from bulk fly homogenates. The bands were visualized with ADH activity stain. 1 'fly equivalent' is that amount of bulk supernatant containing protein equivalent to that from a single fly.

than normal due to an increased rate of protein degradation. The shorter time necessary for the hybrid enzyme to reach its steady state level of activity could be explained if it too showed a similar rate of synthesis and an increased rate of degradation when compared to wild type.

A more direct test of enzyme stability was performed by using the technique of heat denaturation on extracts partially purified from AdhF and hybrid flies by salt fractionation and hydroxylapatite chromatography. This procedure produces a preparation of approximately 35 fold greater purity than a crude homogenate. Equal volumes of the extracts were prepared which contained 100 enzyme units of activity. After 2 minutes at 42°C, the hybrid enzyme had completely lost its catalytic activity while the F enzyme still retained 15% of its initial activity. In fact, after 4 minutes at the elevated temperature, the F enzyme still retained 12% of its activity. At this temperature, the hybrid enzyme lost activity more rapidly than the wild type, but the F extract appeared to contain a small percentage of ADH molecules more heat resistant than average. Schwartz et al. determined that the ADH 1 isozyme is more heat stable and catalytically less active than the ADH 3 or ADH 5 isozymes due to the binding of a small NAD+ carbonyl adduct (Schwartz et al. 1979). Thus, our data suggests that although the hybrid protein denatures more quickly than the F enzyme, the prolonged stability of a small portion of the F extract may be due to an increased proportion of adduct when compared to the hybrid.

As a first step in resolving this issue, we used agar gel electrophoresis to separate the isozymes produced by AdhF, hybrid, and AdhD flies. The AdhD allele was obtained by Grell (1965) from AdhF flies by EMS mutagenesis and produces a protein which migrates more slowly toward the cathode. This is reasonable as the amino acid change in ADH D has been found to be from a glycine in ADH F to a glutamic acid (Schwartz & Jornvall 1976). This change accounts for an overall charge difference of 2 in the dimeric ADH molecule. Adh n11 protein also differs from ADH F by a single amino acid as it has an aspartic acid instead of a glycine at position #14 (Thatcher 1980). Thus, if we assume that the n6 protein does not have a charge difference from F, then the hybrid enzyme would have an intermediate mobility between that of ADH F and ADH D. Figure 2 demonstrates that this is, indeed, true. Since the n6 and n11 homodimers would therefore differ from the heterodimer in mobility, it should prove possible to quantitate the amounts of the three different species produced by a heterozygous fly.

Note the presence of the different isozymic forms for the F and D proteins. In certain overstained gels, these forms were also seen for the hybrid, indicating that this protein also has the capacity to bind the NAD+ carbonyl adduct. However, we were not able to estimate whether the hybrid flies maintained similar levels of the three isozymic forms as the F strain.

We again used the partially purified extracts described above to determine the kinetic parameters for the substrates ethanol and 2-butanol. The data is summarized in Table 1. ADH F protein shows a higher Vmax for the secondary alcohol as compared to the primary. The hybrid enzyme shows the same pattern. What is interesting is that whereas, for the F enzyme, Vmax for ethanol is about 30% that of Vmax for 2 butanol; the difference is markedly greater for the hybrid as here Vmax for ethanol is only 2.1% as large as it is for 2 butanol. In addition, the hybrid protein binds ethanol more tightly than the F enzyme while this is reversed for 2-butanol. Thus, the interaction of the two different subunits which restores a functional catalytic site seems to produce one with altered substrate binding properties. We are testing other substrates to determine if other differences exist.

Table 1. Kinetic parameters for F and hybrid ADH Vmax is expressed in enzyme units. The units for Km are mM.

	Hybrid		F	
Substrate	Km	Vmax	Km	Vmax
Ethanol	65	0.4	73	8.5
2 Butanol	48	19	29	28

Finally we used the technique of antibody competition to measure the number of cross reactive molecules in extracts from AdhF and hybrid flies. 100 enzyme units of the hybrid extract contained approximately 12% more cross reacing molecules than a similar F extract. We suspect that this is due not only to the presence of the inactive homodimers (which should be present at low levels) but also to the reduced catalytic efficiency of the hybrid enzyme which would therefore require more molecules to produce 100 enzyme units. Again, as these molecules are electrophoretically separable, it should be possible to determine the relative contributions of these two ideas. Complete purification of the heterodimer enzyme would also allow a direct comparison of catalytic activity per molecule.

In conclusion, intragenic complementation at the Adh locus of the fly can produce a functional enzyme which has several interesting properties when compared to the wild type protein. Since the amino acid differences in certain null activity ADH proteins are known (Thatcher 1980) information correlating the change in sequence with physical parameters of the enzyme should be possible.

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