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Additional evidence for cis-acting regulation of ADH activity.

Variation in levels of alcohol dehydrogenase (ADH) activity have been observed for many strains of Drosophila melanogaster (McDonald & Ayala 1978; Maroni et al. 1982; Sampsell & Steward 1983). Both genetic and epigenetic factors may contribute to this variation. Mutations at the structural locus have been shown to yield proteins with different specific activities, while changes in putative regulatory regions have been proposed to explain differences in quantity of ADH produced by strains with the same Adh genotype. Evidence for both closely-linked, cis-acting elements as well as unlinked, trans-acting regions has been reported. Here we offer additional evidence for the presence of cis-acting genes which appear to affect the quantity of slow ADH subunits produced in flies heterozygous for Adh-Fr and Adh-Sm.

The three strains (CS4, CS20, and CS19) were derived from wild flies collected in Chicago, Illinois. By the standard breeding schemes involving the Cy/Pm, D/Sb marker stock, each strain was made homozygous for a different wild second and third chromosome. All three strains are homozygous for Adh-Sm, and so far as can be determined from cellulose acetate electrophoresis, thermostability tests, and activity ratios (Sampsell 1977; Sampsell & Steward 1983) they code for identical ADH-Sm proteins. The three strains do show consistent and significant differences in ADH activity (for data in Table 1, $F_{strain} = 131$, $p<0.001$).

To test whether these activity differences were the consequence of cis-acting regulatory elements, we crossed each of the Adh-Sm strains to a variety of strains homozygous for an Adh-fast allele. Extracts from the heterozygous progeny were subjected to disc-gel electrophoresis according to the methods of Cooper (1977). The gels were stained for ADH using the standard nitro-blue tetrazolium stain and were scanned densitometrically to determine the proportion of ADH activity contributed by each of the dimers. Areas under the two peaks representing the NAD-bound and unbound forms of each dimer were combined. The relative band intensities (area of the scanned peaks) will be a function of the relative number of slow and fast subunits synthesized, the relative dimerizing ability of fast and slow subunits, and the relative specific activities of the various dimers. By making comparisons among heterozygotes with the same ADH subunits, the latter two factors (which are unknown) can be ignored. From the proportions of the three kinds of dimers the relative quantities of fast and slow subunits were determined (given as F/S ratio in Table 2). A one-way ANOVA of these ratios indicated significant differences among the progeny of the three different slow strains. For the crosses to Ric110, $F=6.56$, $p=0.02$; while for the crosses to Si44, $F=14.88$, $p=0.001$.

The relative number of slow subunits produced in the various heterozygotes reflected the different activity levels of the parental slow strains; CS4 consistently had the highest ADH activity and had the lowest F/S ratio among the heterozygous progeny indicating a higher production of slow subunits. CS19 which had the lowest ADH activity, had the highest F/S ratio

Table 1. ADH activity levels in three Adh-Sm strains. Averages of two groups of male flies aged 5-7 days post-eclosion. Methods are those of Sims and Sampsell (1982).

	ADH activity (ΔOD_{340}/min/mg)	
Strain	on 2% isopropanol	on 10% ethanol
CS4	19.4	12.9
CS20	17.3	9.5
CS19	15.3	7.3

Table 2. Proportion of ADH dimers in Adh-Fr/Sm hybrids. Four separate groups of flies were tested for each hybrid cross. Averages are given below.

		Relative amounts of			F/S
Strain	Activity	FF	FS	SS	subunits
CS /Ric110	22.0	0.29	0.46	0.26	1.06
CS20/Ric110	22.2	0.34	0.46	0.20	1.33
CS19/Ric110	17.6	0.35	0.46	0.19	1.38
CS4/Si44	25.6	0.32	0.45	0.23	1.18
CS20/Si44	24.5	0.34	0.46	0.20	1.31
CS19/Si44	22.5	0.37	0.47	0.17	1.53

suggesting a lower production of slow subunits. CS20 was intermediate in parental ADH activity and in F/S ratio. CS4 and CS20 have been crossed to three Adh-Fm homozygous strains, and among the heterozygous progeny the F/S ratio was inversely proportional to the Adh-Sm activity in the parental strain (data not shown). Although we have not yet tested ADH levels directly in the CS strains by immunodiffusion techniques, these results are consistent with the observations on other strains in which differences in ADH activity among strains with the same Adh genotype were shown to be associated with differences in the quantity of ADH (Maroni et al. 1982; Sampsell & Steward 1983).

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References: Cooper,T.G. 1977, The Tools of Biochemistry, Wiley & Sons, New York; Maroni, G. et al. 1982, Genetics 101:431-446; McDonald,J.F. & F.J.Ayala 1978, Genetics 89: 371-388; Sampsell,B. 1977, Biochem.Genet. 15:971-987; Sampsell,B. & S.Sims 1982, Nature 296:853-855; Sampsell,B. & E.Steward 1983, Biochem.Genet. 21:1071-1088.

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Contaminating microorganisms interfere with Southern Blot analysis of Drosophila melanogaster DNA.

During our studies of restriction enzyme polymorphism of the small heat shock genes at the 67B locus in Drosophila we discovered that one of the restriction enzyme fragments which we were observing was actually hybridizing to pBR322, the vector that our heat shock probe was cloned in. In several isofemale lines of Drosophila melanogaster we found one band of about 4.7 kb which showed up when probed with pBR 322 (Fig. 1). We believe our finding may serve as a useful warning to other investigators who are using whole flies as a source of genomic DNA for study of restriction enzyme polymorphism. We would also like to report on the properties of this contaminating fragment which were observed incidentally to our studies of the small heat shock genes.

Our first impression upon discovering this additional fragment was that this was merely a simple case of accidental contamination of some of our genomic DNA stocks by trace quantities of plasmid. If this was true then independent DNA extracts of these lines of flies should be free of the additional fragment. In independent DNA extracts we found that the additional fragment was present in precisely the same lines in which it was found in the first place, and absent in lines in which it was not previously found. This observation could have two possible causes: either there are pBR322 homologous sequences in the Drosophila genome or this fragment was derived from microorganisms which are associated with flies

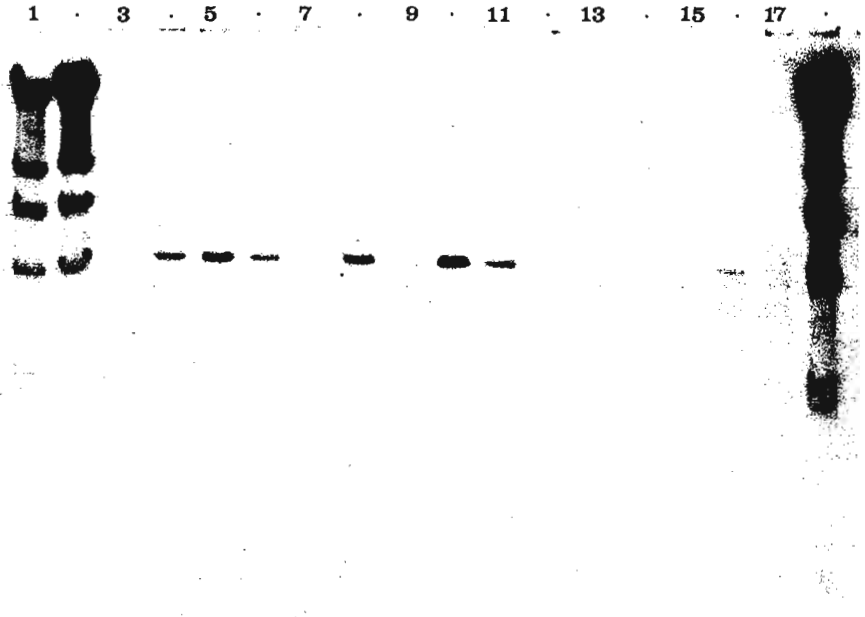

Fig. 1. A Southern Blot of fly DNA from 15 different strains of D.melanogaster. Lane 1, 2 and 18 contain DNA mol. weight markers. Lane 3-9 represent strains from Hamilton, Ontario and lane 10-17 from Brownsville, Texas. The fly DNA is cut with EcoR I and probed with the plasmid (pBR 322) DNA. Migration is from top to bottom. Lane 4, 5, 6, 8, 10, 11 16 show the contaminating 4.7 kb DNA fragment which hybridizes with pBR 322 DNA.