Bremner, T.A., W.L. Douglas and G.O. Ogonji. Howard University, Washington, D.C. Substrate-specific differences of alcohol and octanol dehydrogenases in eight species of Drosophilidae.

Of the ten cathodally migrating isozymes of alcohol dehydrogenase (ADH) detected by Ursprung and Leone (1965) in D. melanogaster, the slowest three showed stronger formazan staining with n-octanol than with ethanol. These three bands were shown, on the basis of linkage relationships, substrate specificity, and differential elution from DEAE cellulose columns. to belong to a separate enzyme system, octanol dehydrogenase (ODH) which shows strong formazan staining with n-hexanol, n-heptanol, and n-octanol (Courtright, et al., 1966). Isopropanol and sec-butanol are equally good substrates or better than ethanol for ADH in D. melanogaster (Johnson and Denniston, 1964; Grell et al., 1965).

In an attempt to differentiate these two enzyme systems further and to ascertain whether there might be species-related differences in substrate specificity within each system, a comparison of the substrate requirements of both ADH and ODH in eight members of the family Drosophilidae was undertaken. The eight species belong to two genera, Drosophila and Zaprionus. Of the seven Drosophila species four, robusta, camargoi, metzii, and unipunctata belong to the subgenus Drosophila, while D. lebanonensis casteeli belongs to the subgenus Pholadoris (primitive), D. busckii to the subgenus Dorsiphola, and D. melanogaster to the subgenus Sophophora. The single Zaprionus species is Z. multistriata.

The substrates fall into four categories, primary unbranched alcohols, secondary alcohols, branched primary alcohols, and a cyclic alcohol, cyclohexanol. The method of agar gel electrophoresis and formazan staining of Ursprung and Leone (1965) as modified by Pipkin (1968) was used to assay crude homogenates of single female flies cultured on an enriched medium, and aged according to the following schema: D. busckii, 4-6 days; D. melanogaster, 5-6 days; D. metzii, 5-8 days; D. unipunctata, 7-9 days; D. camargoi, D. robusta, D. l. casteeli, and Z. multistriata, 9-11 days. These were the ages at which the respective species attained their optimum levels of enzyme activity as measured by the intensity of formazan staining. To compensate for the very small size of D. busckii 2-4 females were homogenized in a drop of distilled water.

From Tables I-IV it can be seen that both the ADH and ODH of D. unipunctata show more intense staining when secondary alcohols are used as substrates. The ADH of D. busckii is aberrant in that it shows a preference for the short chain unbranched primary alcohols while its ODH activity is quite low. Both ADH and ODH show a moderate preference for the long chain, unbranched primary alcohols in five of the species assayed. Although the two enzymes have overlapping substrate specificities, that of ODH is distinctly narrower and comprises

Table I Unbranched Primary Alcohols

Substrate	Enzyme Activity	mel.	metz.	busc.	rob.	uni.	car.	Dlc.	Z.mult.
Methanol	ODH	-	*	-	-	-	**	-	*
	ADH	***	-	***	***	**	***	***	***
Ethanol	ODH	-	-	-	-	-	**	-	*
	ADH	**	-	*	***	*	***	***	***
N-propanol	ODH	**	-	-	*	***	**	**	**
	ADH	***	-	***	***	*	***	***	***
N-butanol	ODH	*	*	-	**	**	**	**	*
	ADH	***	-	*	***	*	***	***	***
N-amyl	ODH	**	**	*	*	-	***	***	***
	ADH	***	-	**	***	*	***	***	**
N-hexanol	ODH	**	***	**	**	-		***	***
	ADH	***	-	**	**	*		***	**
N-heptanol	ODH	**	***	*	***	***	***	***	***
	ADH	***	-	*	***	*	***	***	***
N-octanol	ODH	***	***	**	**	-	***	***	***
	ADH	***	-	-	***	*	**	***	***
Nonyl alc.	ODH	*	*	*	**	*	**	***	***
	ADH	***	-	*	***	*	***	***	***
Decyl alc.	ODH	*	*	*	-	-	**	**	**
	ADH	***	-	*	***	*	***	***	**

Table II Secondary Alcohols

Substrate	Enzyme Activity	mel.	metz.	busc.	rob.	uni.	car.	Dlc.	Z.mult.
2-butanol	ODH	-	*	*	*	**	**	-	***
	ADH	***	-	*	**	**	***	***	***
2-hexanol	ODH	-	-	-	-	***	*	-	***
	ADH	***	*	**	***	*	***	***	***
4-heptanol	ODH	-	-	-	-	**	***	-	-
	ADH	***	-	*	***	**	***	*	*
2-octanol	ODH	-	-	*	***	***	***	***	***
	ADH	**	-	*	*	**	**	***	**

Table III Branched Primary Alcohols

Substrate	Enzyme Activity	mel.	metz.	busc.	rob.	uni.	car.	Dlc.	Z.mult.
Iso-propanol	ODH	-	*	-	*	-	-	-	-
	ADH	***	*	*	***	**	***	***	***
Iso-butanol	ODH	*	*	-	***	*	***	**	**
	ADH	***	*	*	**	***	*	***	***
Iso-amyl	ODH	*	**	*	*	**	*	**	**
alcohol	ADH	***	-	*	*	**	**	**	**
Tert-butanol	ODH	*	-	*	-	-	**	-	-
	ADH	**	*	-	***	*	**	***	**
Tert-amyl	ODH	*	-	-	*	-	-	*	*
alcohol	ADH	***	-	***	*	***	*	***	**

Table IV Cyclic Alcohol

Substrate	Enzyme Activity	mel.	metz.	busc.	rob.	uni.	car.	Dlc.	Z.mult.
Cyclohexanol	ODH	*	-	-	*	**	*	-	*
	ADH	***	-	**	***	*	***	*	*

Legend, Tables I - IV.

Species: mel. = D. melanogaster; metz. = D. metzii; busc. = D. busckii; rob. = D. robusta; uni. = D. unipunctata; car. = D. camargoi; Dlc. = D. l. casteeli; Z. mult. = Z. multistriata.

Intensity of formazan staining:

*** = strong; ** = moderate; * = trace; - = negative

primarily the 5 to 8-carbon alcohols, with n-heptanol giving the highest intensity of staining. The ADH of D. unipunctata shows no staining with n-hexanol, but moderate staining with cyclohexanol; its ADH uses all the substrates tested. The ADH of D. metzii does not use any of the unbranched primary alcohols, while that of D. melanogaster uses all of them.

The above findings suggest that the same enzyme in different species shows small but significant differences in substrate specificity which may be related to minor evolutionary differences in the structure of the molecule resulting in different stereochemical requirements for enzyme activity.

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References: Courtright et al, 1966, Genetics 54:1251-1260; Grell et al, 1965, Science 149:80-82; Johnson and Denniston, 1964, Nature 204:906-907; Pipkin, 1968, Genetics 60: 81-82; Ursprung and Leone, 1965, J. Exptl. Zool. 160:147-154.