Pipkin, S. B. Howard University, Washington, D.C. A genetic study of octanol dehydrogenase isozyme patterns in D. pellewae.

In a genetic analysis of complex ODH isozyme patterns in D. metzii, Pipkin (in press, Genetics) concluded that the enzyme is probably a tetramer, dependent on two structural genes. Using a protein enriched culture medium, a genetic study of ODH isozyme patterns of a sibling species, D. pellewae, supports the tetramer hypothesis. A class A line extracted from the Rio Raposo, Colombia, strain showed an isozyme at position #3 when cultured on standard corn meal medium (Fig. 1) but displayed isozymes at positions #3, 5, 6, 7 when cultured on this medium enriched by the addition of Kellog K breakfast food (Fig. 2). Similarly, an extracted class B line derived from the Rio Raposo strain, showed a single isozyme at position #5 when cultured on ordinary corn meal medium but three isozymes, at positions #5, 6, 7 (Fig. 3) when cultured on the Kellogg K enriched medium. This suggests that the product of one of the genes responsible for the ODH isozyme patterns is inducible with the enriched medium and is further evidence that the ODH molecule may depend on two different structural loci.

Crossing the class A Rio Raposo with the class B Rio Raposo lines in pair matings using Kellogg K enriched medium, gave F_1 hybrids with 5 isozymes at positions #3, 4, 5, 6, 7 (Fig. 4). The appearance of the new darkly staining isozyme at position #4 in the F_1 heterozygotes indicates that it is a hybrid isozyme coded by distinct structural genes. Since 5 isozymes are present in the F_1 heterozygotes, the three isozymes at positions #4, 5, 6 are believed to be hybrid isozymes of the tetramer. The isozymes at positions #3 and #7 are considered the homotetramers coded by the structural genes ODH_2 and ODH_1 respectively.

A class B line extracted from the Darien, Panama, strain showed isozymes with the same mobilities as those of the class B Rio Raposo line; i.e., at positions #5, 6, 7. F_1 hybrids between these class B lines of different geographic origin, and their F_2 progeny likewise showed isozymes at positions #5, 6, 7. The staining intensity of the isozyme at position #7 is variable, depending partly on the degree of induction of ODH-1 subunits, and partly because histochemical staining of isozymes represents an end point reaction. The latter cause of variation in intensity of staining of isozymes has been pointed out by Ursprung and Carlin (1968).

When the Darien class B line was crossed with the Rio Raposo class A line, F_1 hybrids showed isozymes at positions #4, 5, 6, and sometimes 7. In these hybrids, staining was strong at position #5 (Fig. 5), in contrast with the pattern of Rio Raposo class B / Rio Raposo class A hybrids which showed strongest staining at position #4 (Fig. 4). This suggests that the loci responsible for ODH isozyme patterns are controlling genes, ODH_{1c} and ODH_{2c}, which regulate the subunit synthesis by corresponding structural loci, ODH_1 and ODH_2. Whether or not each controlling locus is linked with its corresponding structural locus is not known, but distinct loci control the production of ODH-1 and ODH-2 subunits. Change in intensity of staining of isozymes has been used to indicate differential subunit synthesis in dosage studies in maize endosperm by Schwartz (1960) for esterase variants and by Beckman and Scandalios (1964) for catalase variants.

Inbreeding F_1 hybrids of Rio Raposo class B / Rio Raposo class A hybrids gave an F_2 progeny composed of 24 individuals with either the heterozygote pattern or the class A homozygote pattern and 9 individuals with the class B homozygote pattern, a ratio not differing from 3 : 1 (chi square, 0.0909; 0.98>P>0.95). The F_2 progeny of Darien class B / Rio Raposo class A F_1 hybrids, intercrossed, yielded 31 individuals with the heterozygote plus class A homozygote patterns and 13 individuals with the class B homozygote pattern. This ratio does not differ significantly from 3 : 1 (chi square, 0.4848; 0.8>P>0.7). Thus unifactorial inheritance distinguishes class A and class B variants. This work has been supported by PHS Grant GM 14937.

References: Pipkin, S. B., in press, 1968, Genetics; Schwartz, D. (1960). Proc. N.A.S. (U.S.) 46: 1210-1215; Beckman, L. and J. G. Scandalios (1964) Science 146: 1174-1175; Ursprung, H. and L. Carlin, in press, 1968, Ann. N.Y. Acad. Sci.
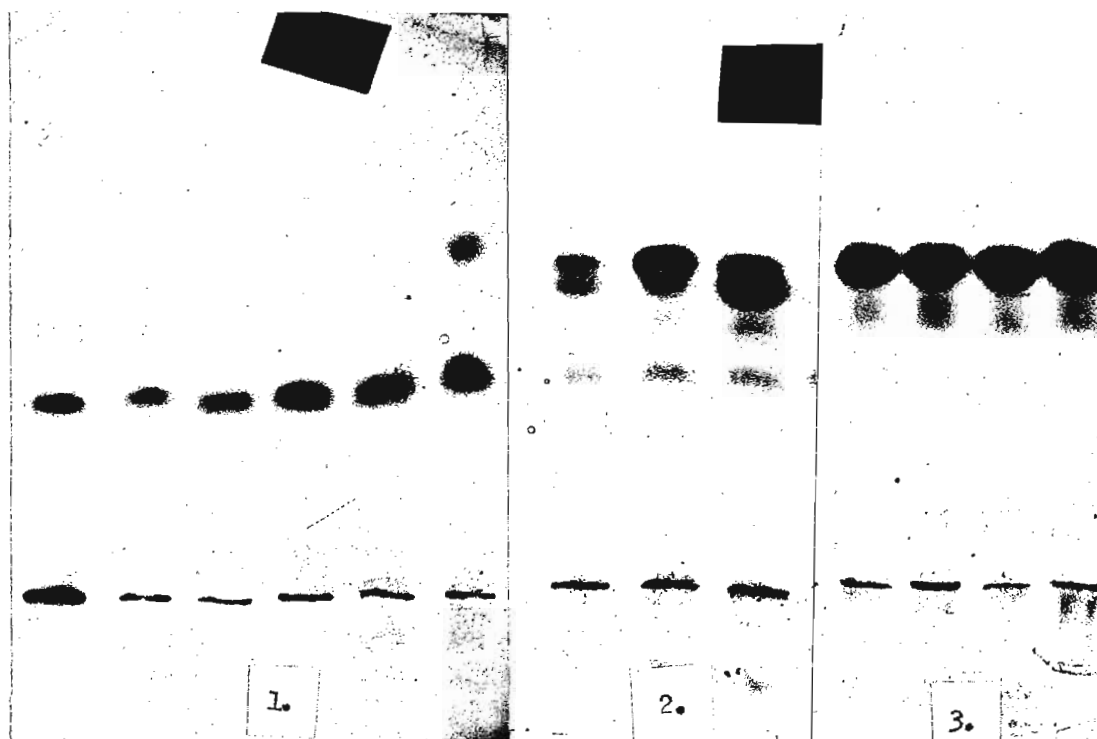
Fig. 1. single females of the Rio Raposo class A line cultured on corn meal medium, showing an isozyme at position #3, and in the sixth female, an additional isozyme at position #7.

Fig. 2. Single females of the Rio Raposo class A line cultured on corn meal medium enriched by the addition of Kellogg K breakfast food, showing isozymes at positions #3, 5, 6, 7.

Fig. 3. Single females of the Rio Raposo class B line, cultured on Kellogg K enriched corn meal medium, showing isozymes at positions #5, 6, 7.
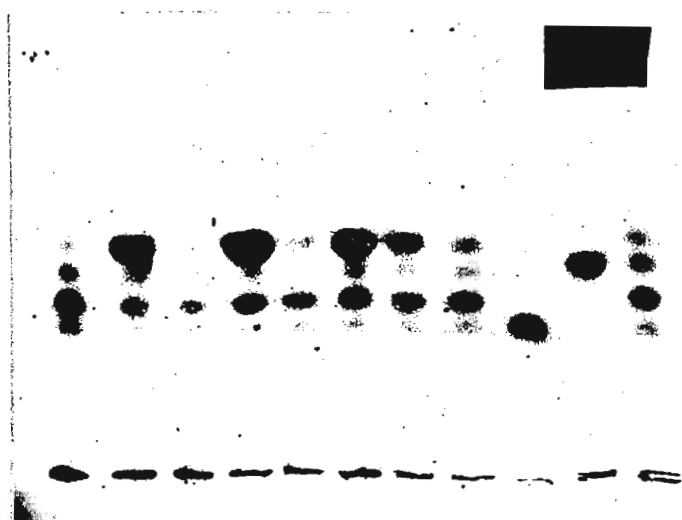

Fig. 4. F_1 hybrid progeny of a cross of a Rio Raposo class B line with the Rio Raposo class A line. The ninth individual to the right is a control class A individual with an isozyme at position #3; the tenth individual is a control class B individual with an isozyme at position #5. Both control individuals were taken from crowded stock bottles and therefore show only single isozymes.
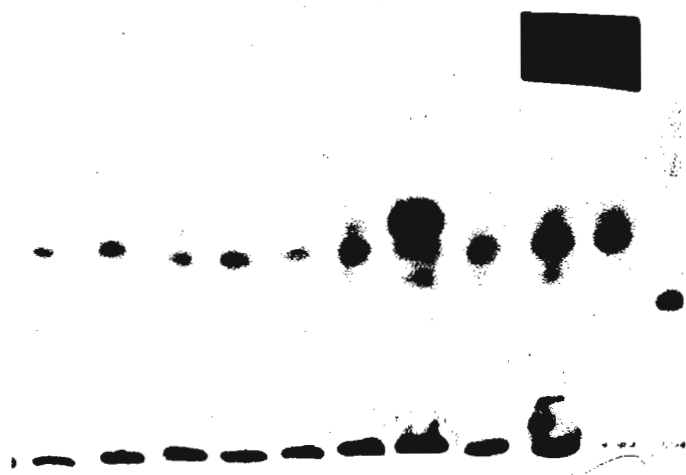
Fig. 5. F_1 hybrid progeny of a cross of Darién class B line with the Rio Raposo class A line. The right most individual is a control female of the Rio Raposo class A line, showing an isozyme at position #3.

Sanger, W. G. and D. D. Miller. University of Nebraska. Further study of hybrids between D. azteca and D. tolteca.

Patterson (1954) reported axteca ♀ x tolteca ♂ and tolteca ♀ x azteca ♂ hybrids, those from the former cross being sterile, those from the latter fertile in both sexes. Recent crosses in our laboratory between azteca females (Arizona, California, Mexico) and tolteca males (Bolivia) yielded sterile males; however, female hybrids mated to tolteca males sometimes produced offspring that died as pupae. Out of 66 attempts at mating azteca ♀♀ by tolteca males (Bolivia, Colombia), each involving from 1 to 60 pairs (x̄=7.67), 7 were successful, producing a total of 126 females and 81 males. D. azteca males normally possess and tolteca males normally lack a second tarsal segment sex comb tooth on each foreleg (e.g. Sulerud and Miller, 1966); however, we found one tolteca male (Bolivia) to possess a second tarsal segment tooth. Our observation of a second tarsal segment tooth in fifteen out of eighteen azteca ♀ (Arizona) x tolteca ♂ (Bolivia) male hybrids differs from Patterson's report of an absence of this tooth in males from this combination. The first and second tarsal segment length ratios (f/s) were intermediate to those of the parent species, as indicated below (eyepiece micrometer units);

	N	f	s	f/s
D. azteca (Arizona)	20			
Mean		32.20	27.40	1.1755±0.0025
Range		30-34	26-29	1.1071-1.2592
D. tolteca (Bolivia)	20			
Mean		20.65	27.40	0.7272±0.0114
Range		19-24	25-31	0.6551-0.8571
D. azteca ♀ (Arizona) x D. tolteca ♂ (Bolivia)				
F_1 males	20			
Mean		31.67	32.73	0.9676±0.0068
Range		30-34	31-35	0.9117-1.0000

Hybrids from the reciprocal cross, tolteca ♀ x azteca ♂, were obtained in 2 out of 69 attempts, each involving from 1 to 60 pairs (x̄=7.52) and yielding a total of only one female (Bolivia x Mexico) and four males (Colombia x California). The female was very weak and died after two days; her ovaries were small. One of the four males possessed a second tarsal segment sex comb tooth, the others none. Male hybrids from both reciprocal crosses possessed small testes with no signs of sperm.