Pipkin, S.B. and T.A. Bremner. Howard University, Washington, D.C. Coordinate activity of octanol dehydrogenase isozymes and its breakdown in Drosophila interspecific hybrids.

The octanol dehydrogenase (ODH) isozyme complex of the sibling species D. metzii, D. pellewae, and D. leticiae is observed in zymograms using agar gel electrophoresis as at least 15 bands, including those seen in different developmental stages of variant strains (Fig. 1). Genetic evidence has been interpreted as supporting the hypothesis of a tetramer subunit structure of isozymes in positions 3 to 7 which are supposed to contain subunits coded by two structural genes, ODH_1 and ODH_2 (Pipkin, 1968, 1969a, 1969b). ODH isozymes anodal to position 3 and cathodal to position 7 have been hypothesized to depend on duplicate ODH structural genes (Pipkin, 1969b). Genetic studies indicate that isozyme patterns of true breeding A and B type variants (Fig. 2) depend on regulatory alleles ODH^A_{1c} and ODH^B_{1c} affecting the time and rate of subunit synthesis by the ODH structural genes, ODH_1 and ODH_2 (Pipkin, 1968, 1969a, 1969b). In the progeny of crosses of A and B type variants extracted from the Barro Colorado Island strain of D. pellewae,

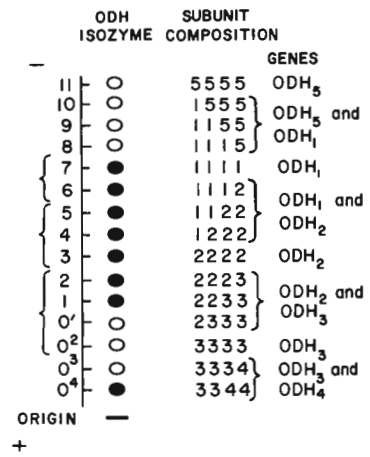

Fig. 1. Duplicate gene hypothesis explaining the subunit structure of ODH isozymes in D. metzii & D. pellewae.

the maternal ODH pattern is seen in A♀/B♂ hybrid embryos 24 hours old (Fig. 3a) and in B♀/A♂ hybrid embryos of the same age (Fig. 3e). In addition, these embryos display new slowly migrating isozymes at positions 1, 2 and 0^1. Both the maternal pattern affecting isozymes in positions 3 to 7, and the new embryonic isozymes disappear in late first instar larvae. At this time synchronous activity of both paternal and maternal regulatory alleles is indicated by the appearance of a 3, 4,5 triplet pattern in both A♀/B♂ (Fig. 3b,c) and in B♀/A♂ (Fig. 3f-j) hybrid first instar larvae.

Coordinate activity of two groups of isozymes is observed in the 24 hour A♀/B♂ and B♀/A♂ embryos of D. pellewae, respectively. In A♀/B♂ embryos (Fig. 3a), the isozymes at positions 3 and 1 show strong staining, and the #5 isozyme is weak or sometimes undetectable. In B♀/A♂ embryos, on the other hand, the #5 isozyme shows strong staining and the #3 and #1 isozymes are faint (Fig. 3e). The correlation of activity as judged by the intensity of formazan staining of the isozymes at positions 3 and 1 can be explained by assuming that in embryos, ODH_2 and duplicate gene ODH_3 share subunits in the #1 isozyme of the A variant, whereas a strongly staining isozyme at position 5 in the B variant indicates subunit sharing by ODH_2 and ODH_1.

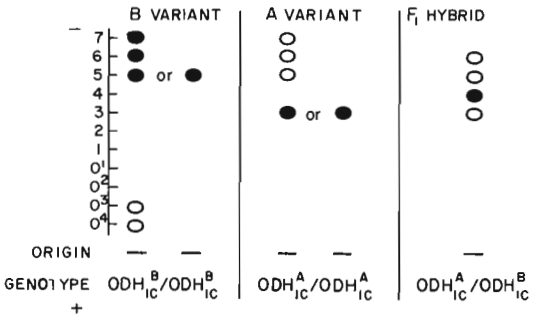

Fig. 2. ODH isozyme patterns of B and A type variants and of their hybrid.

In interspecific hybrids all development is retarded. Moreover, the 3,4,5 triplet pattern expected in post-embryonic stages is not always observed. This is believed to be due to the failure of operation of either the maternal or the paternal regulatory alleles or to their acting with altered timing. As a result, certain third instar larvae of leticiae B♀/metzii A♂ hybrids showed only a single isozyme at position 3 instead of the expected 3,4,5 hybrid pattern, indicating absence of detectable action of the maternal regulatory allele, ODH^B_{1c}. In brown pupae of the same hybrids (Fig. 4,c,d) both maternal and paternal regulatory alleles were apparently acting to cause structural genes to code for subunits in isozymes at positions 4 and 5, but the rate and/or time of activity of structural gene ODH_2 was altered so that the #3 isozyme, the supposed homotetramer composed of "2" subunits, was undetectable. Third instar larvae of the reciprocal cross, metzii A♀ x leticiae B♂, showed isozymes at positions 3 and 4 but not at 5 (Fig. 4e,f), indicating reduced or faulty activity of the paternal regulatory allele, ODH^B_{1c}. In brown pupae of metzii A♀/leticiae B♂ hybrids, an expected 3,4,5 triplet

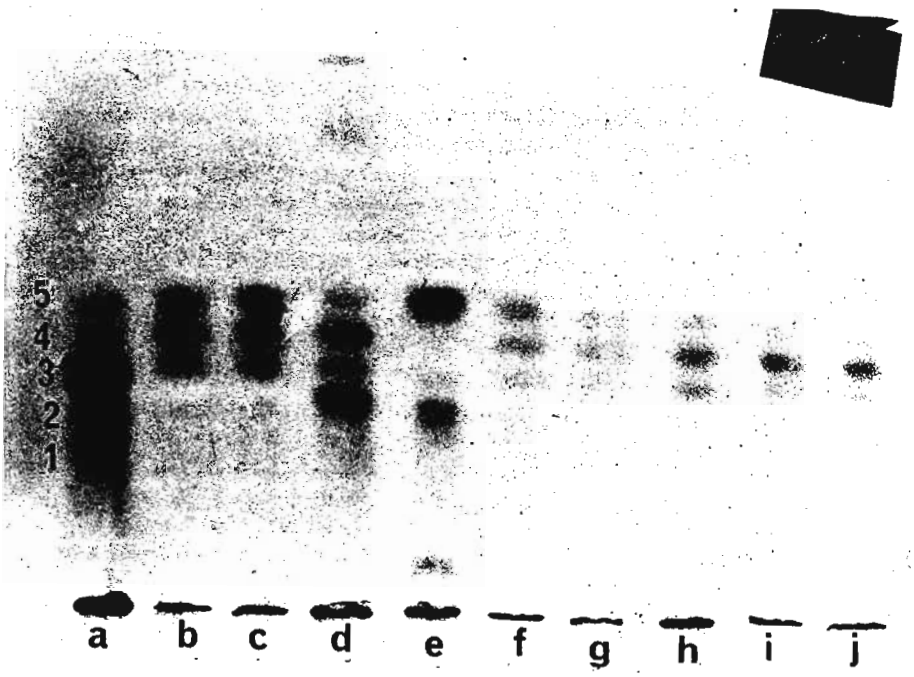

Fig. 3. Reciprocal hybrids of A and B type variants of D. pellewae: a, A♀/B♂; e, B♀/A♂ 24 hour embryos; b,c, A♀/B♂ first instar larvae; f-j, B♀/A♂ first instar larvae.

pattern was observed in the individuals assayed in Fig. 4g,h, indicating synchronous activity of maternal and paternal regulatory alleles. However, a difference in pupal ODH patterns of reciprocal hybrids of D. metzii and D. leticiae was sometimes observed, suggesting a breakdown of normal regulation of the structural gene ODH_2 and its duplicate gene ODH_3. Normally the slowly migrating embryonic isozymes at positions 2,1,0^1, and 0^2 are undetectable in post-embryonic stages of both D. metzii and D. leticiae except in concentrated mass homogenates (i.e., electrophoresed aliquots of 100 females per 0.25 ml of 0.2 M tris buffer) of D. metzii. This suggests that the ODH_3 structural gene is active in the embryonic period when it shares subunits with ODH_2 but shows little or no activity in post-

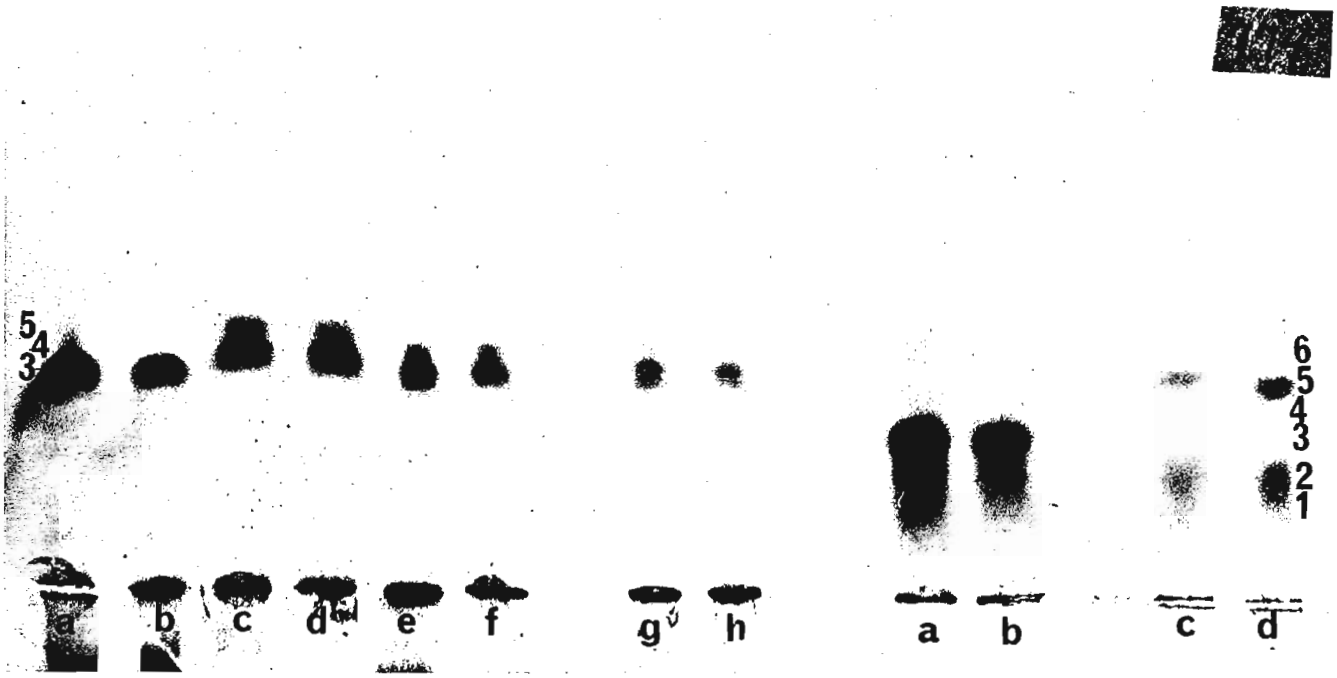

Fig. 4 (left). leticiae B♀/metzii A♂ hybrids: a,b single third instar larvae; c,d single pupae metzii A♀/leticiae B♂ hybrids: e,f single third instar larvae; g,h single pupae.
Fig. 5 (right). a,b metzii A♀/leticiae B♂ hybrids: single pupae; c,d leticiae B♀/metzii A♂ hybrids; single pupae.

embryonic stages. In interspecific hybrid pupae of D. metzii and D. leticiae, strong formazan staining of isozymes at positions 2,1, and 0^1 is sometimes observed, indicating abnormally high activity of structural gene ODH_3 during the pupal stage. For example, the pupae of both metzii A♀/leticiae B♂ hybrids in Fig. 5a,b and of leticiae B♀/metzii A♂ hybrids (Fig. 5c,d) showed strongly staining isozymes at positions 2,1, and 0^1, instead of the expected 3,4,5 pattern. Metzii A /leticiae B hybrids assayed as single adult females sometimes showed the expected 3,4,5 triplet pattern, but often only a single isozyme at position 3 was observed, indicating that activity of the paternal ODH^B_{1c} allele was undetectable. In conclusion our studies indicate that regulation of subunit sharing between ODH structural genes may be disturbed in third instar larvae, pupae, and adult stages of hybrids of D. metzii and D. leticiae. The parental species were shown by Pipkin (1968) to differ in multiple translocations. Similar results regarding ODH isozyme patterns have been obtained for D. metzii/D. pellewae hybrids.

This work was supported by PHS Grant 14937 and National Science Foundation Grant GB 8770.

References: Pipkin, S.B., 1968, Evol. 22: 140-156; Pipkin, S.B., 1968, Genetics 60: 81-92; Pipkin, S.B., 1969a, DIS 44: 59-61; Pipkin, S.B., 1969b, In press, Oct. Genetics.

Mukherjee, A.S. and A. Das. University of Calcutta, India. A recombination associated segregation - distortion in D. ananassae.

A case of segregation distortion has been observed in the inbred laboratory strain, px-pc, of D. ananassae. It is comparable to the SD action of D. melanogaster (Sandler et al, 1959), but unlike SD, this phenomenon is associated only with the recombinant classes and in both sexes. The recombinant classes px + and + pc from the px pc/+ + (male or female) parent are not complementary to each other; px flies appear in the progeny in a much higher proportion than the pc. The proportion of the complementary non-recombinant classes is close to 1:1 (the mean K values, i.e. the proportion of px pc among all non-recombinants, vary from 0.46 to 0.55). There is considerable inequality of the complementary recombinant classes in both sexes (i.e. px pc/+ + male as well as female) but it is unusually high in the male (K values, i.e. the proportion of px among all recombinants, are always close to 1.0)(Table 1). In testcrosses with px +/+ pc,

Table 1
Distribution of testcross progeny and K values in heterozygous males and females. Genotypes of F_1 parents: px pc/+ + x px pc/px pc in Expts. 1-6 and px +/+ pc x px pc/px pc in Expts. 7-8

Expt. No.	Sex of heterozygote parent	$+_a+$	$px_b pc$	$\bar{K}$ b/a+b	px/c	pc/d	$\bar{K}$ c/c+d	No. of crosses
1	Female	1892	1694	0.48	1028	323	0.76	45
2	Female	460	448	0.49	345	75	0.83	13
3	Female	681	750	0.52	631	84	0.88	19
4	Male	1091	979	0.46	399	1	0.99	29
5	Male	709	746	0.51	405	4	0.99	20
6	Male	370	451	0.55	215	2	0.99	12
7	Female	219	355	0.62	859	740	0.53	21
8	Male	38	147	0.80	982	931	0.53	26

the non-recombinant px and pc classes were in equal proportion and the recombinant px pc and + + classes were highly disproportionate, thus conforming with the data of the previous set (Table 1). Tests on viability and penetrance of the mutants px and pc, in relation to the wild type (a6+) or px pc double recessive, do not show any abnormality. It is, therefore, suggested that there may be certain genetic factor or factors closely associated with the px locus, whose function is to prevent the recovery of that recombinant class which is separated from the factor following the exchange. It may be noted that this case of segregation distortion perhaps records the first example of the phenomenon in a species in which spontaneous crossing over in males is quite frequent, unlike other species of Drosophila. The results presented above, however, do not exclude the possibility of a type of nonrandom disjunction (Novitski, 1967, Ann. Rev. Genet., 1: 71-86), somehow operating in both sexes.

Fahrig, R.* Genetisches Institut der Justus-Liebig-Universität, Giessen, Germany. The influence of temperature upon the concentration of the free amino acids of D. melanogaster.

The free amino acids of Drosophilae cultivated for some generations at a distinct temperature are very constant in their concentrations. A change of the temperature is correlated with a change of the concentration of many amino acids. In this work we have determined the concentration of 19 different amino acids by using an automatic amino acid analyzer of Beckman. The concentration changed in nine amino acids in larvae (96 h old), in ten in pupae (24 h old) and only in one in adults (72 h old). The concentration of ammonia which has also been determined is not influenced by temperature.

AMINO ACIDS	LARVAE			PUPAE			ADULTS		
umol wt/100mg wet weight	18°C	24°C	30°C	18°C	24°C	30°C	18°C	24°C	30°C
Histidine	0.28	0.28	0.28	0.38	0.33	0.30	0.49	0.47	0.48
Lysine	0.27	0.18	0.05	0.27	0.20	0.07	0.06	0.05	0.05
Arginine	0.30	0.34	0.38	0.37	0.30	0.22	0.36	0.37	0.35
Ammonia	0.24	0.23	0.24	0.25	0.24	0.26	0.43	0.40	0.42
Aspartic acid	0.12	0.11	0.12	0.15	0.14	0.15	0.17	0.17	0.17
Glutamic acid	0.42	0.40	0.39	0.56	0.56	0.55	0.53	0.54	0.53
Threonine	0.18	0.11	0.05	0.13	0.10	0.08	0.08	0.08	0.08
Serine	0.16	0.21	0.31	0.18	0.13	0.10	0.17	0.17	0.18
Proline	0.36	0.34	0.35	0.17	0.18	0.17	0.32	0.30	0.32
Glycine	0.23	0.23	0.22	0.18	0.16	0.17	0.29	0.29	0.30
Alanine	0.64	0.63	0.64	0.35	0.26	0.16	0.39	0.40	0.41
Valine	0.05	0.04	0.05	0.22	0.17	0.12	0.05	0.06	0.05
Methionine	0.01	Traces	Traces	Traces	Traces	Traces	Traces	Traces	Traces
Isoleucine	0.01	Traces	Traces	0.12	0.07	0.01	Traces	Traces	Traces
Leucine	0.07	0.07	0.07	0.40	0.24	0.11	0.04	0.04	0.05
Tyrosine	0.37	0.34	0.32	0.16	0.16	0.17	0.08	0.08	0.08
Phenylalanine	0.01	0.01	0.01	0.07	0.05	0.03	0.02	0.02	0.02
β-Alanine	0.02	0.02	0.01	0.03	0.03	0.03	0.45	0.38	0.33
γ-Aminobutyric acid	0.02	0.02	0.03	Traces	Traces	Traces	0.07	0.06	0.06
Ornithine	0.03	0.03	0.04	Traces	Traces	Traces	Traces	Traces	Traces
TOTAL	3.79	3.59	3.55	3.99	3.32	2.70	4.00	3.88	3.88

A rise of temperature in the cultures results in a decline of the concentration of all amino acids being influenced in larvae, pupae and adults with exception of arginine and serine in larvae.

Amino acids	Larvae	Pupae	Adults
Histidine		+	
Lysine	+	+	
Arginine	-	+	
Ammonia			
Aspartic acid			
Glutamic acid	+		
Threonine	+	+	
Serine	-	+	
Proline			
Glycine			

Amino acids	Larvae	Pupae	Adults
Alanine		+	
Valine		+	
Methionine	+		
Isoleucine	+	+	
Leucine		+	
Tyrosine	+		
Phenylalanine		+	
β-Alanine			+
γ-Aminobutyric acid			
Ornithine			
TOTAL	8	10	1

The total amount of all amino acids shows little differences in larvae, high differences in pupae (in accordance with Anders, Drawert, Anders and Reuther 1964) and no differences in adults.

References: Anders, F., Drawert, F, Anders, A and Reuther, K.H., 1964, Z. Naturforschung 19b: 495-499.

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