THREE LINKED ENZYME LOCI IN FISHES: IMPLICATIONS IN THE EVOLUTION OF VERTEBRATE CHROMOSOMES[1]

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ABSTRACT

A three-point linkage group comprised of loci coding for adenosine deaminase (ADA), glucose-6-phosphate dehydrogenase (G6PDH), and 6-phosphogluconate dehydrogenase (6PGD) is described in fish of the genus Xiphophorus (Poeciliidae). The alleles at loci in this group were shown to assort independently from the alleles at three other loci—isocitrate dehydrogenase *1* and *2*, and glyceraldehyde-3-phosphate dehydrogenase *1*. Alleles at the latter three loci also assort independently from each other. Data were obtained by observing the segregation of electrophoretically variant alleles in reciprocal backcross hybrids derived from crosses between either *X. helleri guentheri* or *X. h. strigatus* and *X. maculatus*. The linkage component of χ^2 was significant (< 0.01) in all crosses, indicating that the linkage group is conserved in all populations of both species of Xiphophorus examined. While data from *X. h. guentheri* backcrosses indicate the linkage relationship *ADA*—6%—*G6PDH*—24%—*6PGD*, and *ADA*—29%—*6PGD* (30% when corrected for double crossovers), data from backcrosses involving *strigatus*, while supporting the same gene order, yielded significantly different recombination frequencies. The likelihood of the difference being due to an inversion could not be separated from the possibility of a sex effect on recombination in the present data. The linkage of 6PGD and G6PDH has been shown to exist in species of at least three classes of vertebrates, indicating the possibility of evolutionary conservation of this linkage.

THE presence of genetically controlled melanosis and melanoma has led to frequent genetic studies on hybrids involving the Central American freshwater Poeciliid fishes, *Xiphophorus maculatus* (platyfish) and *X. helleri* (swordtails), over the last five decades (see review by KALLMAN 1975). Genetic mapping of loci encoding enzymes and other proteins, utilizing allele products electrophoretically variant within and between Xiphophorus species, has been undertaken only recently (SICILIANO and WRIGHT 1973; WRIGHT, SICILIANO and BAPTIST 1972). Such markers are useful in studying the number and location of factors responsible for melanoma in backcross hybrids (SICILIANO and WRIGHT 1976; SICILIANO, MORIZOT and WRIGHT 1976).

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Since these enzymes are present in the cells of all forms of life, the linkage relationships of the loci that code for them throughout a series of vertebrates is of evolutionary interest. Here we report the first demonstration of linkage in fishes of three of these ubiquitous enzyme loci. This linkage group is comprised of loci coding for the enzymes adenosine deaminase (ADA, E.C.3.5.4.4), glucose-6-phosphate dehydrogenase (G6PDH, E.C.1.1.1.49), and 6-phosphogluconate dehydrogenase (6PGD, E.C.1.1.1.43). Possible intraspecific variation in gene arrangement and the relationship of these findings to the concept of the conservation of linkage groups in vertebrates is discussed.

MATERIALS AND METHODS

Animals used: Crosses were made via artificial insemination (CLARK 1950) using the species, strains and pedigrees of Xiphophorus listed in Table 1. All stocks were obtained from DR. KLAUS KALLMAN of the New York Zoological Society. Three types of crosses were used for the segregation and linkage analysis: *helleri* × *maculatus* (Jp163A) F_1 hybrids backcrossed to *helleri* (HHM-BC); *helleri* × *maculatus* (Jp163A) F_1 hybrids backcrossed to Jp163A *maculatus* (HMM-BC); and *maculatus* (2856) × *maculatus* (Jp163A) F_1 hybrids backcrossed to Jp163A *maculatus* (MMM-BC). Table 2 lists the F_1 and backcross broods obtained from the matings.

Sample preparation: Crude muscle extracts were prepared for the electrophoretic detection of ADA and 6PGD, while brain and eye extracts were used for the detection of G6PDH activity. Muscle was homogenized in a medium composed of 0.01 M Tris-Hcl pH 7.5, 0.001 M EDTA, and 0.001 M β-mercaptoethanol. Brain and eye samples were homogenized in the same medium without mercaptoethanol. Ratio of volume of medium to weight of tissue was 2:1. Brain and eye samples were homogenized by hand in glass homogenizers or by a short burst of a motor-driven Dounce type homogenizer; muscle samples were homogenized by 10–30 second bursts at high speed. Samples were centrifuged twice for 50 minutes at 10,000 × *g* to yield a clear supernatant for electrophoresis. All procedures were carried out between 0–4°.

Electrophoresis and histochemical staining: Supernatants were subjected to vertical starch gel electrophoresis as generally described in SICILIANO and SHAW (1976) with the following particulars: G6PDH and ADA were best resolved on the tris-versene-borate, pH 8.0 buffer

TABLE 1

Strains of platyfish and swordtails used in genetic crosses

Taxon	Strain or pedigree	River, country of origin	Year collected	Degree to which inbred
Xiphophorus maculatus	Jp163A	Rio Jamapa, Mexico	1939	Brother to sister matings for 50 generations
X. maculatus	2856	R. Coatzacoalcos, Mexico	1971	Closed colony since capture
X. helleri helleri	Cd	Rio Jamapa, Mexico	1943	Brother to sister matings for 25 generations
X. h. strigatus	501	Rio Sarabia, Mexico	1963	Closed colony since capture
X. h. strigatus	2977	Rio Sarabia, Mexico	1963	Closed colony since capture
X. h. guentheri	3062	Belize River, Belize	1971	Closed colony since capture

TABLE 2

F_1 and backcross broods used

Brood number	Cross type	Pedigree, strain or brood no. of: Female parent	Male parent
7	F_1	*X.h. strig.* 501	*X. mac.* Jp 163A
58	F_1	*X.h. strig.* 501	*X. mac.* Jp 163A
82	F_1	*X. mac.* Jp 163A	*X.h. hel.* Cd
85	F_1	*X. mac.* Jp 163A	*X.h. strig.* 2977
86	F_1	*X. mac.* Jp 163A	*X.h. guenth.* 3062
3075	F_1	*X. mac.* Jp 163A	*X. mac.* 2856
44	HHM-BC	*X.h. strig.* 501	F_1 7
70	HHM-BC	*X.h. strig.* 501	F_1 58
72	HMM-BC	*X. mac.* Jp 163A	F_1 58
90	MMM-BC	*X. mac.* Jp 163A	F_1 3075
98	MMM-BC	*X. mac.* Jp 163A	F_1 3075
99	MMM-BC	*X. mac.* Jp 163A	F_1 3075
100	HHM-BC	*X.h. strig.* 2977	F_1 85
101	HMM-BC	F_1 86	*X. mac.* Jp 163A
102	HMM-BC	F_1 82	*X. mac.* Jp 163A
103	HHM-BC	F_1 86	*X.h. guenth.* 3062
104	MMM-BC	*X. mac.* Jp 163A	F_1 3075
105	HMM-BC	F_1 82	*X. mac.* Jp 163A
106	HHM-BC	F_1 82	*X.h. hel.* Cd
107	HMM-BC	F_1 86	*X. mac.* Jp 163A
108	HHM-BC	F_1 86	*X.h. guenth.* 3062
109	HMM-BC	*X. mac.* Jp 163A	F_1 85
116	HMM-BC	F_1 86	*X. mac.* Jp 163A

system. Twenty mg of nicotinamide adenine dinucleotide phosphate was added before degassing when G6PDH was to be studied. 6PGD was best resolved on the tris-citrate, pH 7.0 buffer system.

Gels were run for four or five hours between 0–4° at 400 volts (not exceeding 30 milliamperes) after which they were sliced horizontally at a thickness not less than 1 mm. Histochemical stain to visualize the enzyme bands was applied to a cut surface of the gel slice. Stain recipes for the enzymes are contained in SICILIANO and SHAW (1976). Stained gels (zymograms) were fixed in acid-alcohol gel wash (SICILIANO and SHAW 1976) and photographed with a 35 mm camera.

Methods for electrophoretic resolution of the products of the following loci in these fish have been previously described and have also been carried out on tissue extracts from these broods: glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*)-*1* (WRIGHT, SICILIANO and BAPTIST 1972), and isocitrate dehydrogenase (*IDH*)-*1* and *2* (SICILIANO and WRIGHT 1973).

Data analysis: Since Jp163A is the most highly inbred line and was the only parental genome involved in all backcrosses, the electrophoretic mobilities of its enzymes were used as standards. For each locus and for every cross the Jp163A allele is designated *a* and the protein produced considered to have a relative electrophoretic mobility (rem) of 1.00. The rems of the allele products of the other parental stocks were calculated merely by: distance (mm) of migration of allele product in question/distance of migration of Jp163A allele product for that locus. For the purposes of statistical analysis, all allelic variants from Jp163A are referred to as the products of a *b* allele since all crosses involved the Jp163A genome with only one other parental type.

The expected segregation of parental-type and F_1 hybrid-type electrophoretic patterns was observed in reciprocal backcrosses and allowed genotypic assignments to be made based upon a codominant model of inheritance. In the segregation analysis, a chi-square (χ^2) goodness of fit was calculated against the expected backcross ratio under independent assortment, 1 homozygote: 1 heterozygote (MATHER 1957). Since all gene arrangements for the variant loci were known to be in the coupling phase in the F_1 hybrids, two tests of linkage were utilized, orthogonal function analysis (MATHER 1957; ELANDT-JOHNSON 1971) and contingency table (or criss-cross chi-square) analysis (MATHER 1957; ROBINSON 1971). Estimation of the proportion of recombinant chromosomes, and thus of map distance, was made using the method of maximum likelihood and the product formula method; standard errors were calculated from the maximum likelihood estimates (MATHER 1957). Homogeneity of recombination frequency estimates between HHM-BC and HMM-BC crosses and between Belize River swordtail and Rio Sarabia swordtail crosses was tested using the z test for the difference between the parameters of two binomial distributions (REMINGTON and SCHORK 1970).

RESULTS

Enzyme patterns: Four different electrophoretic forms of ADA were identified in the six parental groups used in this study. Besides the product of the *a* allele from Jp163A, 2856 platyfish had an ADA with a rem of 1.07, *guentheri* swordtails an ADA with a rem of 1.30 and the remaining swordtails had the fastest migrating ADA, with a rem of 1.45.

The pattern obtained upon electrophoresis and subsequent histochemical staining for ADA in hybrid heterozygotes is that expected of a monomeric molecule produced by a single genetic locus, *i.e.*, yielding two forms equivalent to the parental types with no intermediate hybrid molecules (Figure 1). Such an interpretation of subunit structure agrees with previously published studies of ADA in other organisms (SPENCER, HOPKINSON and HARRIS 1968).

For 6PGD, only two different allelic products were observed that followed species lines in their distribution. The *a* allele was homozygous in both parental stocks of platyfish, while the *b* allele (product rem = 1.10) was found exclusively in swordtails. In platyfish-swordtail F_1 heterozygotes, 6PGD exhibited electrophoretic behavior characteristic of a dimeric molecule, *i.e.*, a symmetrical three-banded pattern consisting of the three possible dimeric molecules (PARR 1966)—swordtail type homodimer, heterodimer, and platyfish type homodimer (Figure 1).

Two different types of phenotypes were observed for G6PDH among the parental organisms. These are considered products of the *a* allele seen in both platyfish, as well as in the Cd swordtail, and of the *b* allele found exclusively in the remaining swordtails. For both phenotypes there are often two zones of activity. A slow zone consisting of a narrow band is sometimes present and is not used for phenotype determination. A fast zone is always present in brain tissue and is read for phenotype determination. It is composed of three bands in the homozygote. In *bb* fish the three bands are slightly faster (rem = 1.08). In heterozygotes there appears to be a summation of the two three-banded patterns seen in parental fish, resulting in a diffuse area sometimes resolvable into at least five bands. The nature of the banding patterns, while clear enough to identify parental from hybrid types even in gels run <5 hours (Figure 1), is not

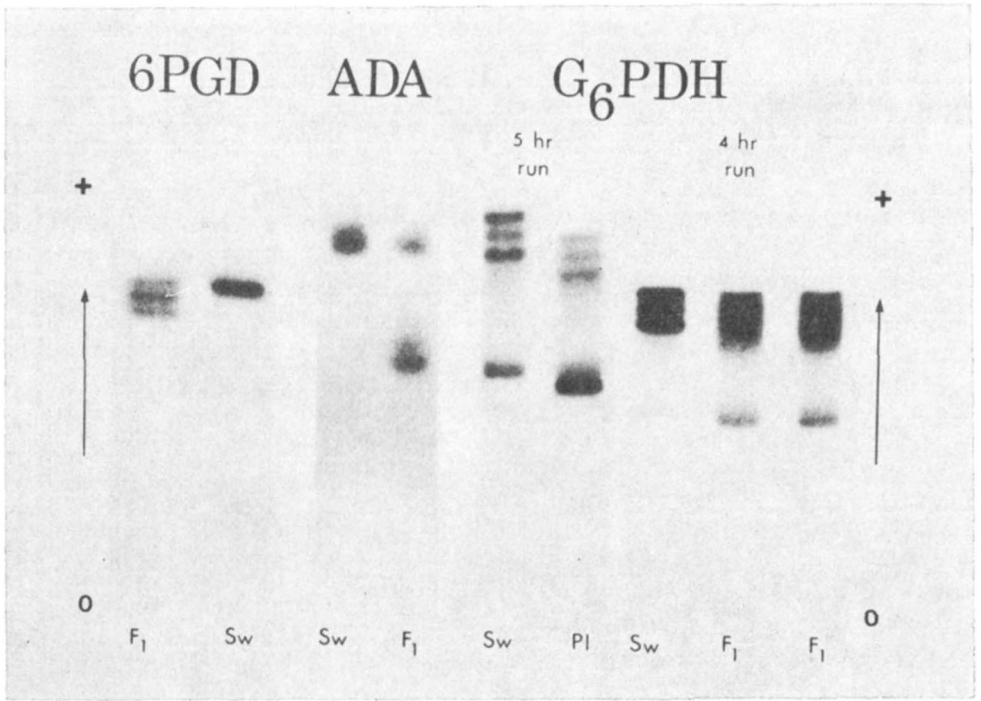

FIGURE 1.—Zymograms showing the electrophoretic patterns of 6PGD and ADA in *strigatus* swordtails (Sw) and F_1 hybrids (between *strigatus* swordtails and Jp163A platyfish). Also shown are the G_6PDH patterns of *strigatus* swordtails (Sw) and platyfish (Pl) after a five hour electrophoresis run as well as patterns from swordtails and F_1 hybrids after only a four hour run.

sufficiently resolved to make a statement with respect to subunit structure of G6PDH in these fish.

As indicated in SICILIANO and WRIGHT (1973), three loci for IDH exist in Xiphophorus. Two produce electrophoretically separable supernatant products (*IDH-1* and *IDH-2*) both of which are polymorphic amongst these fish and therefore informative. For *IDH-1*, three different allele products were observed. Both parental stocks of *maculatus* were homozygous for the *a* allele; faster migrating products of two other alleles were seen in *helleri*. A second allele (product rem = 1.22), as well as a third (product rem = 1.10), were seen in *helleri strigatus* and *h. guentheri*. Cd is homozygous for the 1.10 product. This study also revealed three different alleles for *IDH-2*. The *a* allele was once again homozygous and present exclusively in *maculatus*. The 501 *helleri strigatus* stock was homozygous for a second allele (product rem = 0.80), while 2977 *helleri strigatus* and *h. guentheri* stocks contained both it and a third (product rem = 0.62). Cd was homozygous for the 0.62 product.

WRIGHT, SICILIANO and BAPTIST (1972) demonstrated two tissue-specific loci for G3PDH in Xiphophorus. *G3PDH-1* is informative in these experiments, since Jp163A was homozygous for the *a* allele and all other stocks were homozygous for the *b* allele (product rem = 1.26).

TABLE 3

Segregation analyses for enzymes in backcross hybrids

Enzyme locus	Cross type	Homozygotes	Heterozygotes	χ^2	P
ADA	HHM-BC	80	62	2.28	0.20–0.10
	HMM-BC	26	44	4.63	0.05–0.02
	MMM-BC	33	34	0.02	0.95–0.90
	Combined	139	140	0.00	0.95–0.90
	Heterogeneity			6.93	0.05–0.02
G_6PDH	HHM-BC	103	101	0.02	0.95–0.90
	HMM-BC	37	53	2.84	0.10–0.05
	Combined	140	154	0.67	0.50–0.40
	Heterogeneity			2.19	0.20–0.10
6PGD	HHM-BC	89	100	0.64	0.50–0.40
	HMM-BC	48	58	0.94	0.40–0.30
	Combined	137	158	1.49	0.30–0.20
	Heterogeneity			0.09	0.80–0.70
G_3PDH-1	HHM-BC	102	105	0.04	0.90–0.80
	HMM-BC	62	42	3.85	0.05–0.02
	Combined	164	147	0.93	0.40–0.30
	Heterogeneity			2.96	0.10–0.05
IDH-1	HHM-BC	87	81	0.21	0.70–0.60
	HMM-BC	42	44	0.05	0.90–0.80
	Combined	129	125	0.06	0.90–0.80
	Heterogeneity			0.20	0.70–0.60
IDH-2	HHM-BC	96	104	0.32	0.60–0.50
	HMM-BC	43	55	1.47	0.30–0.20
	Combined	139	159	1.34	0.30–0.20
	Heterogeneity			0.45	0.60–0.50

Segregation and linkage analysis: The analyses of deviation from the expected 1 homozygote: 1 heterozygote segregation ratio at each of the loci in backcross hybrids are presented in Table 3. No significant deviation from the above expectation was observed at our chosen level of significance ($p<0.01$), allowing a linkage analysis to go forward. However, it is recognized that the insignificant heterogeneity observed between cross types in the segregation of *ADA* and *G6PDH* may have impact on the detection and estimation of linkage among the loci. This will be treated in DISCUSSION.

The linkage analyses for pairs of loci are presented in Table 4. For each pair of loci, the linkage component of the χ^2 obtained both through orthogonal function analysis, as well as by a contingency table association test, are given and shown to be in close agreement. For pairs of loci involving *ADA*, *G6PDH* or *6PGD* with each other, linkage chi-square values are highly significant. No significant heterogeneity was detected between cross types, HHM-BC and HMM-BC. Consistent with previous results (SICILIANO and WRIGHT 1973), linkage chi-square values are insignificant when *IDH-1* and *IDH-2* are paired. Both IDH loci and *G3PDH-1* also yield insignificant linkage chi-square values when paired

TABLE 4

Analysis in all crosses of linkage relationships among loci coding for ADA, G_6PDH, 6PGD, G_3PDH-1, IDH-1 *and* IDH-2*

Locus pair	Parentals	Recombinants	Recombination fraction	Linkage tests: Contingency table χ^2	Contingency table P	Orthogonal function χ^2	Orthogonal function P
ADA–G_6PDH	169	20	0.11	117.50	<0.001	117.47	<0.001
G_6PDH–6PGD	230	46	0.17	124.10	<0.001	122.67	<0.001
ADA–6PGD	137	58	0.30	31.96	<0.001	32.01	<0.001
ADA–G_3PDH-1	90	86	0.49	0.05	0.90–0.80	0.09	0.80–0.70
G_6PDH–G_3PDH-1	144	135	0.48	0.31	0.60–0.50	0.29	0.60–0.50
G_3PDH-1–6PGD	144	123	0.46	1.67	0.20–0.10	1.65	0.20–0.10
ADA–IDH-1	86	65	0.43	2.31	0.20–0.10	2.92	0.10–0.05
G_6PDH–IDH-1	131	105	0.44	2.82	0.10–0.05	2.86	0.10–0.05
IDH-1–6PGD	121	111	0.48	0.44	0.60–0.50	0.43	0.60–0.50
ADA–IDH-2	90	101	0.53	0.69	0.50–0.40	0.63	0.50–0.40
G_6PDH–IDH-2	130	137	0.51	0.19	0.70–0.60	0.18	0.70–0.60
IDH-2–6PGD	125	128	0.51	0.04	0.90–0.80	0.04	0.90–0.80
G_3PDH-1–IDH-1	114	133	0.54	1.36	0.30–0.20	1.46	0.30–0.20
G_3PDH-1–IDH-2	145	139	0.49	0.14	0.80–0.70	0.13	0.80–0.70
IDH-1–IDH-2	132	143	0.52	0.56	0.50–0.40	0.44	0.60–0.50

* Parentals include all individuals homozygous or heterozygous at both loci of the tested pair; recombinants include all individuals homozygous at one locus and heterozygous at the other locus of the tested pair. P is the probability that the difference from the tested segregation hypothesis (in orthogonal function analysis) or that row-column associations (in contingency table χ^2 analysis) is due to chance.

with any of the other loci tested in this series of experiments or with each other. Thus *ADA*, *G6PDH* and *6PGD* do not assort independently from each other, but do assort independently from *IDH-1*, *IDH-2* and *G3PDH-1*. The latter three loci also assort independently from each other.

Map distances: Recombination frequency estimates from the backcross data are presented in Table 5. Due to the skewed sex ratios in the F_1 hybrids (all *guentheri* × *maculatus* F_1 offspring obtained were female and most of the *strigatus* × *maculatus* F_1 offspring were male), all of the backcrosses involving *guentheri* swordtails were obtained from female F_1 parents, while all of the backcrosses involving *strigatus* swordtails were obtained from male F_1 parents (see Table 2). Because of the larger numbers of HHM-BC and HMM-BC offspring examined and the lack of significant heterogeneity between cross types in recombination frequency estimates, the *guentheri* backcross data alone will be used in proposing a tentative map. Estimates obtained for the three pairs of loci by the maximum likelihood methods are identical to those calculated by the product method: *ADA-G6PDH*, 0.06; *G6PDH-6PGD*, 0.24; *ADA-6PGD*, 0.29. The data (Table 5) thus support a chromosome arrangement of *ADA-G6PDH-6PGD*. When corrected for double crossovers, the *ADA-6PGD* distance becomes 0.30. The frequency of double crossover (1/120 or 0.008) is not significantly less than that expected (0.014) for the number of animals studied in this group.

TABLE 5

Recombination frequencies for the three pairs of loci in backcrosses involving guentheri *and* strigatus *swordtails*

Locus pair	Cross type	Swordtail parental type: *guentheri* (all F_1 parents ♀) No. of recombinants	Total No.	Recombination fraction	S.E.*	*strigatus* (all F_1 parents ♂) No. of recombinants	Total No.	Recombination fraction	S.E.*	P†
ADA–G_6PDH	HHM-BC	6	109	0.06	0.02	7	39	0.18	0.06	0.06
	HMM-BC	3	47	0.06	0.04	2	6	0.33	0.19	0.17
	Total	9	156	0.06	0.02	9	45	0.20	0.06	0.02
G_6PDH–6PGD	HHM-BC	24	109	0.22	0.04	10	90	0.11	0.03	0.03
	HMM-BC	14	47	0.30	0.07	1	42	0.02	0.02	<0.001
	Total	38	156	0.24	0.03	11	132	0.08	0.02	<0.001
ADA–6PGD	HHM-BC	25	97	0.26	0.04	11	40	0.28	0.07	0.83
	HMM-BC	17	47	0.36	0.07	2	6	0.33	0.19	0.89
	Total	42	144	0.29	0.04	13	46	0.28	0.07	0.90

* S.E. — the standard error of the maximum likelihood recombination frequency estimate.
† P — probability of the difference between recombination fraction in backcrosses involving *guentheri* as opposed to *strigatus* swordtails being due to chance.

Also included on Table 5 are the recombination frequency data from backcrosses involving *strigatus* swordtails. While these are also informative at all three linked loci, there are fewer backcross hybrids and also serious deficiencies of HMM-BC fish for locus pairs involving *ADA*. This is due to the fact that those fish were run before the ADA procedure was routine in our laboratory. Therefore, these are not part of the formal analysis. The data have been included, however incomplete, to point out some interesting comparisons between *guentheri* and *strigatus* backcross hybrids in the recombination frequencies for certain locus pairs. While backcrosses involving *strigatus* reveal the same gene order for the three linked loci and recombination frequency between terminal loci (*ADA-6PGD*) as crosses with *guentheri*, the distance between *G6PDH* and *6PGD* is significantly less. Also, the distance between *ADA* and *G6PDH* is correspondingly, although only marginally, significantly greater. This suggests an apparent difference in the position of the *G6PDH* locus in *guentheri* as opposed to *strigatus* backcrosses.

DISCUSSION

Linkage of ADA-G6PDH-6PGD *in Xiphophorus*: A tendency of an excess of heterozygotes in HMM-BC and homozygotes in HHM-BC is observed in the data, being most pronounced in *ADA*, less so in *G6PDH*, and absent in *6PGD*. Such a segregation disturbance, if real, could indicate superior fitness of the swordtail allele at a locus more closely linked to *ADA* than to *G6PDH* or *6PGD* (or

superior fitness of the swordtail allele of *ADA* itself). While such a possible explanation is of intrinsic interest and may be explored further as additional data are compiled, of present concern is the effect of a segregation disturbance upon the detection of linkage among the three loci.

It appears that the effect is inconsequential. We make this judgment for the following reasons. First, the segregation disturbance is small and insignificant, while the linkage component is large and very highly significant. Second, two methods of linkage analysis were used which gave practically identical linkage chi-square values, even though the tests are based upon quite different hypotheses with respect to the impact of segregation disturbance (MATHER 1957; BAILEY 1961). Furthermore, one of the methods—the contingency table χ^2—has been suggested (BAILEY 1961) as sufficient for linkage detection even when both loci are known to be subject to viability effects.

It might be argued that since the linkage information came from interspecific matings and since all loci were studied in the coupling phase, the loci in question are not linked on the same chromosome at all, but the data merely indicate the tendency of chromosomes from the same species to assort together into the F_1 gametes. However, this does not appear to be the case as indicated by the independent assortment of *IDH-1*, *IDH-2* and *G$_3$PDH-1* from each other as well as from *ADA-G6PDH-6PGD*. If chromosomes from the same species assorted dependently, alleles from the former set of loci should have been affected similarly to those of the *ADA-G6PDH-6PGD* group.

Therefore, it is concluded that *ADA-G6PDH-6PGD* represents the first three-point linkage group of enzyme loci identified in fishes.

Evolutionary stability of linkage relationships: The detection of linkage in reciprocal interspecific backcrosses certainly indicates that these three loci are linked in both of the parental species. While this is not surprising, considering the fertility of interspecific F_1 hybrids in this genus, it does point to the utility of studying the syntenic relationships of enzyme loci in generating data relevant to the question of chromosomal evolution in vertebrates. Since the enzyme loci are ubiquitous throughout the phylum, an analysis of their arrangement through a series of vertebrates enables one to study the evolution of the arrangement of homologous genetic material. This is not generally possible for morphological markers (coat color, tail length, etc.) due to the variable presence and questionable homology of such traits through a series of organisms. It is then of interest to review the linkage relationships of these loci in other animals, where such data exist.

Our finding of the *G6PDH-6PGD* linkage is of interest in this regard. These two loci, in addition to being linked in Drosophila (YOUNG, PORTER and CHILDS 1964) have also been found by one of us (WRIGHT 1975) to be syntenic in frogs (*Rana pipiens*). In mammals two forms of G6PDH have been shown to exist—a sex-chromosome-linked (CHILDS *et al.* 1958), highly specific form and an autosomally coded form with a broad enough substrate specificity to be able to oxidize galactose-6-phosphate (SHAW and KOEN 1968). This latter form has been called hexose-6-phosphate dehydrogenase (H6PDH). CHAPMAN (1975) has recently

reported the tight linkage between the locus coding for H6PDH (*Gpd-1*) and *6PGD* in mice. The long-term stability of the linkage might possibly be indicated. H6PDH has been identified in man (SHAW 1966; OHNO *et al.* 1966) but has not yet been mapped. The third member of our linkage group in fishes, *ADA*, has been mapped only in humans where it does not appear to be associated with *6PGD* (CREAGAN *et al.* 1973; WEITKAMP 1971).

To evaluate the extent of evolutionary stability of linkage groups, further data from fishes would be useful. However, to date, most such fish biochemical genetic studies have revealed linkages only between loci which were the result of apparent chromosomal duplications followed by the centric fusions of duplicates (OHNO 1967, 1970).

Recombination frequency variation: The apparent shift in the position of the *G6PDH* locus seen in backcross hybrids derived from *guentheri* swordtails when contrasted with those derived from *strigatus* swordtails (Table 5) can be explained generally in one of two ways: (1) the difference reflects an actual change in the position of the *G6PDH* locus on the chromosome; or (2) the position of the locus is the same in the two swordtails, but some modification of recombination has been effected.

A mechanism for the first possibility is easily visualized: an ancestral inversion in one of the swordtails between the *ADA* locus and the *6PGD* locus but including the *G6PDH* locus would produce the observed recombination frequency results. NEI (1968) has shown that inversions can produce modifications in linkage intensity between epistatic genes in a population by producing a selective advantage in the inversion carriers. It is interesting in this regard to recall that the minor segregation disturbance noted at the *ADA* locus possibly indicates a swordtail allele with superior fitness at a locus in the region where recombination frequency variation is observed.

The possibility of a sex-specific difference in recombination, often observed in studies of genetic linkage (ROBINSON 1972) also exists in the data. Since all F_1 hybrids between *guentheri* and Jp163A were female and almost all F_1 hybrids between *strigatus* and Jp163A were male, the sex of the F_1 parents in *guentheri* and *strigatus* backcrosses was different (see Tables 2 and 5). Since female *strigatus* × Jp163A F_1 hybrids can be produced, sex-reciprocal backcrosses are in progress to detect sex differences in recombination. The model of CARTER (1954), derived from chiasma frequency differences between sexes, predicts that at a point 30 crossover units from the centromere the sex difference in recombination will be reversed. While the observed recombination frequency variation in our data can be explained by such a hypothesis, and while other hypotheses involving genes modifying recombination can be constructed, at present we prefer the inversion explanation as the most conservative hypothesis. Further data will elucidate the nature of the observed variation in recombination frequency, and experiments to that end are in progress.

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