POLYMORPHISMS, LINKAGE AND MAPPING OF FOUR ENZYME LOCI IN THE FISH GENUS XIPHOPHORUS (POECILIIDAE)[1]

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Manuscript received March 19, 1979
Revised copy received August 8, 1979

ABSTRACT

Electrophoretic variants at four additional enzyme loci—two esterases (*Est-2*, *Est-3*), retinal lactate dehydrogenase (*LDH-1*) and mannose phosphate isomerase (*MPI*)—among three species and four subspecies of fish of the genus Xiphophorus were observed. Electrophoretic patterns in F_1 hybrid heterozygotes confirmed the monomeric structures of MPI and the esterases and the tetrametric structure of LDH in these fishes. Variant alleles of all four loci displayed normal Mendelian segregation in backcross and F_2 hybrids. Recombination data from backcross hybrids mapped with Haldane's mapping function indicate the four loci to be linked as *Est-2*—0.43—*Est-3*—0.26—*LDH-1*—0.19—*MPI*. Significant interference was detected and apparently concentrated in the *Est-3* to *MPI* region. No significant sex-specific differences in recombination were observed. This group (designated linkage group II) was shown to assort independently from the three loci of linkage group I (adenosine deaminase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase) and from glyceraldehyde-3-phosphate dehydrogenase and two isocitrate dehydrogenase loci. Evidence for conservation of the linkage group, at least in part, in other vertebrate species is presented.

THE assessment of genetic linkage relationships among protein-coding loci in animal species is still in its infancy. The assignment of an appreciable number of enzyme loci to linkage groups is an even rarer event, having been accomplished in only a few mammal, bird and insect species. Since homology among loci coding for specific enzymes can often be established or presumed, even in widely divergent organisms, the expansion of linkage group assignments, particularly in lower vertebrates, can be expected to provide important insights into such evolutionary problems as the stability and function of gene arrangements. The present work demonstrates a linkage group comprised of four enzyme loci in a teleost fish genus.

Linkage studies in nonpoeciliid fishes are few, but some progress has been made assessing linkage of biochemical loci in sunfish, trout and salmon. Utilizing inter-

[1] Supported in part by Public Health Service Research Grants GM-19513 and ES/CA-02411, and Training Grant GM-02237.

specific sunfish hybrids, WHEAT and WHITT (1973) demonstrated the linkage of liver α-glycerophosphate dehydrogenase and 6-phosphogluconate dehydrogenase loci, and the independent assortment of loci coding for malate dehydrogenase (*A* and *B*), tetrazolium oxidase, and a skeletal muscle esterase. Also in sunfish, the presumably duplicated glucosephosphate isomerase loci (*A* and *B*) were found to be unlinked (WHITT *et al.* 1976). By contrast, in the pink salmon, the reduplicated malate dehydrogenase *A* loci appear to be linked or pseudolinked (ASPINWALL 1974), and in trout hybrids pseudolinkage between *LDH-A* and *LDH-B* has been reported (MORRISON 1970; DAVISSON, WRIGHT and ATHERTON 1973).

The poeciliid fishes, *Xiphophorus maculatus* (platyfish) and *X. helleri* (swordtail), have been the objects of genetic research for over five decades (recently reviewed by KALLMAN 1975), due in part to the genetically controlled melanosis and melanomas exhibited by interspecific hybrids. Recently (SICILIANO, MORIZOT and WRIGHT 1976), our laboratory has begun to utilize electrophoretically variant proteins to identify the number of factors responsible for the melanosis and melanomas and to determine their position in the genome. These experiments have led to the assignment of ten enzyme loci to five linkage groups. Linkage group I (MORIZOT, WRIGHT and SICILIANO 1977) is comprised of loci coding for adenosine deaminase (*ADA*), glucose-6-phosphate dehydrogenase (*G6PDH*), and 6-phosphogluconate dehydrogenase (*6PGD*) and was shown to assort independently from loci coding for a glyceraldehyde-3-phosphate dehydrogenase (*G3PDH-1*; WRIGHT, SICILIANO and BAPTIST 1972) and two isocitrate dehydrogenases (*IDH-1* and *IDH-2*; SICILIANO and WRIGHT 1973).

Linkage group II, to be described here, is comprised of loci coding for two nonspecific carboxylesterases (*Est-2* and *Est-3*; E.C.3.1.1), retinal lactate dehydrogenase (*LDH-1*; E.C.1.1.1.27), and mannosephosphate isomerase (*MPI*; E.C.5.3.1.8).

MATERIALS AND METHODS

Animals used: Crosses were made *via* artificial insemination (CLARK 1950) using the following species, strains and pedigrees of Xiphophorus.

X. maculatus: Strain Jp 163 A, collected in 1939 from the Rio Jamapa, Mexico, and inbred by brother-to-sister matings for 50 generations; pedigree 2856, collected in 1971 from the Rio Coatzacoalcos, Mexico, and maintained in closed colony since capture; strain Cp, collected in 1948 from the Rio Coatzacoalcos, Mexico, and inbred by brother-to-sister matings since capture. *X. h. helleri*: strain Cd, collected in 1943 from the Rio Jamapa, Mexico, and inbred by brother-to-sister matings for 29 generations. *X. h. strigatus*: 501, collected in 1963 from the Rio Sarabia, Mexico, and maintained in closed colony since capture; pedigree 2977, collected in 1968 from the Rio Sarabia, Mexico, and maintained in closed colony since capture. *X. h. guentheri*: pedigree 3062, collected in 1971 from the Belize River, Belize, and maintained in closed colony since capture. *X. clemenciae*: pedigree 2985, collected in 1968 from the Rio Sarabia, Mexico, and maintained in closed colony since capture; and pedigree 3258, collected in 1968 from the Rio Sarabia, Mexico and maintained in closed colony since capture.

All stocks were obtained from KLAUS KALLMAN of the New York Zoological Society. Three types of crosses were used for the segregation and linkage analyses: (1) *helleri* $\times$ *maculatus* (Jp 163 A) F_1 hybrids backcrossed to *helleri* (HHM-BC); (2) *helleri* $\times$ *maculatus* (Jp 163 A) F_1 hybrids backcrossed to *maculatus* (HMM-BC); and (3) *maculatus* (2856) $\times$ *maculatus* (Jp 163 A) F_1 hybrids backcrossed to Jp 163 A *maculatus* (MMM-BC). Additionally, segregation

data from intraspecific F_2 broods resulting from intercrosses between two *X. maculatus* F_1 hybrids (Jp 163 A × Cp), were included in the segregation analysis totals and separate tests for linkage were performed. Crosses involving *X. clemenciae* with *X. maculatus* were also made. The information provided by the backcross to *X. clemenciae* was considered equivalent to that obtained from HHM crosses. Table 1 lists the F_1, F_2, and backcross broods obtained from the matings.

Sample preparation: Crude extracts of blood plasma, liver, muscle and brain-eye were prepared for the electrophoretic detection of the four enzymes. Esterase-2 was best visualized in blood plasma, with brain-eye extracts also producing informative electrophoretic patterns; liver extracts showed the highest esterase-3 activity, MPI was resolved using muscle extracts, and

TABLE 1

F_1, F_2 and backcross broods analyzed

Brood number	Cross type*	Female parent	Male parent
58	F_1	*X. h. strig.* 501	*X. mac.* Jp 163 A
77	F_1	*X. mac.* Jp 163 A	*X. mac.* Cp
82	F_1	*X. mac.* Jp 163 A	*X. h. hel.* Cd
85	F_1	*X. mac.* Jp 163 A	*X. h. strig.* 2977
86	F_1	*X. mac.* Jp 163 A	*X. h. guenth.* 3062
93	F_1	*X. mac.* Jp 163 A	*X. clem.* 2985
3075	F_1	*X. mac.* Jp 163 A	*X. mac.* 2856
89	F_2	F_1 77	F_1 77
91	F_2	F_1 77	F_1 77
70	H(HM)-BC	*X. h. strig.* 501	F_1 58
72	M(HM)-BC	*X. mac.* Jp 163 A	F_1 58
90	M(MM)-BC	*X. mac.* Jp 163 A	F_1 3075
98	M(MM)-BC	*X. mac.* Jp 163 A	F_1 3075
99	M(MM)-BC	*X. mac.* Jp 163 A	F_1 3075
100	H(HM)-BC	*X. h. strig.* 2977	F_1 85
101	(MH)M-BC	F_1 86	*X. mac.* Jp 163 A
102	(MH)M-BC	F_1 82	*X. mac.* Jp 163 A
103	(MH)H-BC	F_1 86	*X. h. guenth.* 3062
104	M(MM)-BC	*X. mac.* Jp 163 A	F_1 3075
105	(MH)M-BC	F_1 82	*X. mac.* Jp 163 A
106	(MH)H-BC	F_1 82	*X. h. hel.* Cd
107	(MH)M-BC	F_1 86	*X. mac.* Jp 163 A
108	(MH)H-BC	F_1 86	*X. h. guenth.* 3062
108b	Mixed†	F_1 86	(MH)H-BC 108
109	M(MH)-BC	*X. mac.* Jp 163 A	F_1 85
116	(MH)M-BC	F_1 86	*X. mac.* Jp 163 A
117	(MH)M-BC	F_1 86	*X. mac.* Jp 163 A
126	(MH)M-BC	F_1 82	*X. mac.* Jp 163 A
133	(MC)C-BC	F_1 93	*X. clem.* 3258

* F_1, F_2 are 1st and 2nd filial generations, respectively. Backcrosses (-BC) follow a female-first convention. The F_1 parent is indicated in parenthesis with its female parent listed first where H symbolizes *X. helleri*, M symbolizes *X. maculatus* and C symbolizes *X. clemenciae.* For example, brood 70 was produced by mating a female *X. helleri* with a male F_1 that was produced from a female *X. helleri* and male *X. maculatus.*

† After producing brood 108, the F_1 86 female parent was inadvertently allowed to mate with a maturing male of brood 108. Her subsequent broods were thus mixed with respect to enzyme locus segregation, some loci segregating as backcross types and some exhibiting F_2 segregation patterns.

LDH-1 activity was restricted to brain and eye extracts. Blood samples were drawn in capillary tube pipettes from orbital sinuses; pipettes and sample tubes were coated wtih sodium heparin (Upjohn, 1000 units per ml) and 0.3% sodium citrate solutions sequentially to inhibit coagulation. Whole blood samples were centrifuged for 3 min at 1300 × g to pack the red cells, and the decanted plasma was diluted 1:1 v/v with distilled water.

The following procedures were carried out at 4°. Tissues were homogenized in a medium containing 0.01 M Tris-HCl pH 7.5, 0.001 M EDTA, and 0.001 M β-mercaptoethanol. Tissue weight to volume of homogenizing medium was 1:2. Brain and eye and liver samples were homogenized by hand in glass homogenizers or by a short burst of a motor-driven Dounce-type homogenizer; muscle samples were homogenized in the latter apparatus by high-speed homogenization of 10–30 sec duration. All samples were centrifuged at least once at 10,000 × g for 50 min to yield a clear supernatant for electrophoresis.

Electrophoresis and histochemical staining: Supernatants were subjected to vertical starch gel electrophoresis as described in SICILIANO and SHAW (1976) with the following particulars: Est-2 and LDH-1 were best resolved on the tris-citrate, pH 7.0, buffer system; Est-3 and MPI were usually best resolved on the tris-versene-borate, pH 8.0, buffer system.

Gels were run for 5 hr at 400V (not exceeding 30 milliamperes) at 0°–4°, after which they were sliced horizontally at a thickness not less than 1 mm. Histochemical stain was applied to the cut surface of the gel slice to visualize the enzyme bands; after sufficient staining the gel slices were photographed with a 35 mm camera. Stain recipes for the enzyme are described in SICILIANO and SHAW (1976).

Data analysis: Since Jp 163 A is the most highly inbred line and was the only parental genome involved in the backcrosses, the electrophoretic mobilities of its enzymes were used as standards. For each locus and for every cross the Jp 163 A allele is designated *a* and the protein produced considered to have a relative electrophoretic mobility (rm) of 1.00. The rm's of the allele products of the other parental stocks were calculated merely by: distance (mm) of migration of the allele product in question divided by the distance of migration of the Jp 163 A allele product for that locus. For the purposes of statistical analysis, all allelic variants from Jp 163 A are referred to as the products of a *b* allele, since all crosses involved the Jp 163 A genome with only one other parental type.

Based upon a co-dominant model of inheritance, genotypic assignment of the electrophoretic patterns was made and the resulting data subjected to segregation and linkage analysis. In the segregation analysis, a chi-square goodness-of-fit was calculated against the expected ratio under independent assortment of 1 homozygote to 1 heterozygote (MATHER 1957). The F_2 crosses were first tested for deviation from a 1 *aa*: 2*ab*: 1 *bb* expectation and then against a 1 homozygote to 1 heterozygote expectation before inclusion with backcross data. 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 χ^2) analysis (MATHER 1957; ROBINSON 1971). Estimation of the proportion of recombinant chromosomes, and thus of distance, was made using the method of maximum likelihood and the product formula (MATHER 1957; BAILEY 1961). Homogeneity of recombination frequency estimates between HHM-BC, HMM-BC, and MMM-BC crosses was tested using the *z* test for the difference between the parameters of two binomial distributions (REMINGTON and SCHORK 1970).

RESULTS

Electrophoretic alleles and subunit structures of linkage group II loci

The electrophoretic alleles observed in the parental stocks of Xiphophorus used in this study are listed in Table 2. This table is not meant to be an exhaustive list of populational variation, and only a few salient points will be emphasized. *Est-2* and *Est-3* are particularly variable loci, each population and often each

TABLE 2

Electrophoretic alleles and relative electrophoretic mobilities at linkage group II loci in Xiphophorus parent stocks

Taxon, strain or pedigree number	*Est-2* Allele	Rm	*Est-3* Allele	Rm	*LDH-1* Allele	Rm	*MPI* Allele	Rm
X. mac. Jp 163 A	*a*	1.00	*a*	1.00	*a*	1.00	*a*	1.00
X. mac. 2856	*b*	1.05	*c*	1.06	*a*	1.00	*c*	0.91
X. mac. Cp	*a*	1.00	*c*	1.06	*a*	1.00	*c*	0.91
X. h. hel. Cd	*b*	1.05	*e*	0.88	*a*	1.00	*b*	0.93
X. h. strig. 501	*d*	0.93	*d*	0.94	*a*	1.00	*b*	0.93
X. h. strig. 2977	*g*	0.86	*d*	0.94	*a*	1.00	*b*	0.93
X. h. guenth. 3062	*b*	1.05	*f*	1.04	*b*	0.95	*a*	1.00
	e	0.94					*b*	0.93
	f	0.90						
	h	0.83						
X. clem. 2985	*a*	1.00	*a*	1.00	*a*	1.00	*b*	0.93
	c	1.07	*c*	1.06				
X. clem. 3258	*a*	1.00	*d*	0.94	*a*	1.00	*b*	0.93

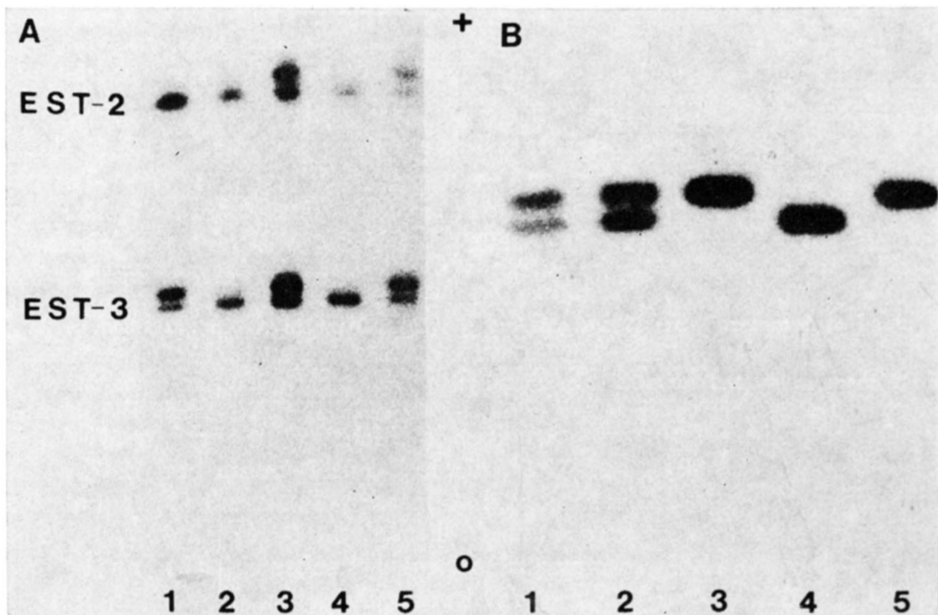

FIGURE 1.—Starch gel slices stained histochemically for enzyme activity following electrophoresis (zymograms). Origins are marked by "O". Direction of electrophoresis is towards anode (+).

(A) Esterase zymogram of brain-eye homogenates from 5 MMM-BC hybrids. Zones of EST-2 and EST-3 activity are marked. Homozygotes for the *a* alleles for each locus are seen as single bands. Double banded patterns represent heterozygotes for the loci in the samples which they appear. Sample #1 is the only recombinant in this group of 5 backcross hybrids.

(B) MPI zymogram of muscle homogenates from five F_2 hybrids. Type *a* homozygotes are seen as a single fast migrating band (channels 3 and 5). A type *b* homozygote is in channel 4. Channels 1 and 2 contain material from *ab* heterozygotes.

inbred strain having its own distinct electrophoretic allele. *MPI* is somewhat less variable, the stocks represented here having apparently only three different forms. The *guentheri* swordtail stock (3062) possesses both the *a* and *b* alleles, resulting in one mating (which produced brood 103, Table 1) that was uninformative with respect to linkage analysis since neither parent was doubly heterozygous. *LDH-1* is the least variable locus of the four loci, the only electrophoretic variant observed occurring in *guentheri* swordtails.

Est-2, *Est-3* and *MPI* exhibit electrophoretic patterns in heterozygotes characteristic of monomeric molecules (Figure 1), *i.e.*, yielding two bands equivalent to the parental bands with no association into intermediate hybrid molecules. These results are in agreement with the observations of WOMACK and SHARP (1976) that most esterases in the mouse and rat are monomeric and with the

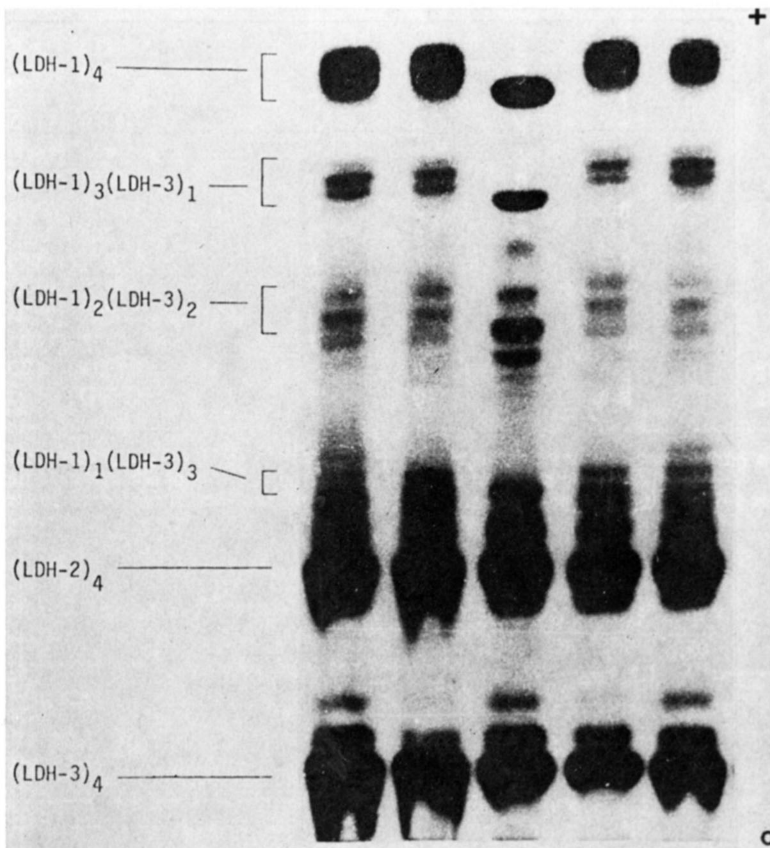

FIGURE 2.—LDH zymogram of brain-eye homogenates from 5 HHM-BC hybrids. Zones representing the homopolymer products of the three LDH loci are indicated to the left as $(LDH\text{-}1)_4$, $(LDH\text{-}2)_4$ and $(LDH\text{-}3)_4$. The middle sample is the only homozygote (for the *b* allele at the *LDH-1* locus) shown. Separation of LDH_1^b and LDH_1^a is not sufficient to resolve the five expected bands in heterozygotes in the $(LDH\text{-}1)_4$ zone, and the phenotype is read there as only a broad smear. However, the four-banded patterns expected in the $(LDH\text{-}1)_3(LDH\text{-}3)_1$ zone can be readily observed in heterozygotes.

previously observed subunit structure of MPI in the mouse (NICHOLS, CHAPMAN and RUDDLE 1973).

Since the electrophoretic separation of the products of the *LDH-1* locus was not sufficient to resolve heteropolymers between them, evidence for the tetrameric structure of LDH-1 is the production of the expected four-banded pattern of interlocus hybrid molecules with LDH-3 (Figure 2), in the molecules containing 3 LDH-1 subunits and 1 LDH-3 subunit (SHAW 1964). This result also is in agreement with previously published subunit structures of LDH, including studies of the fish retinal isozyme (MARKERT and MØLLER 1959; WHITT 1969).

Segregation analyses of linkage group II loci

The segregation analyses of *Est-2*, *Est-3*, *LDH-1* and *MPI* are presented in Table 3. The *Est-2*, *Est-3* and *MPI* data in the MMM–BC include F_2-type segregations that were first analyzed against a 1 *aa*: 2 *ab*: 1 *bb* segregation expectation and were found to be in agreement. None of the segregations in Table 4 deviates significantly from the tested hypothesis, 1 homozygote: 1 heterozygote, and no significant heterogeneity exists between cross types. Segregation results for *ADA*,

TABLE 3

Segregation analysis of linkage group II loci

Enzyme locus	Cross type*	Homozygotes	Heterozygotes	χ^2	P†
Est-2	HHM-BC	95	90	0.14	0.80–0.70
	HMM-BC	43	37	0.45	0.60–0.50
	MMM-BC	44	47	0.10	0.80–0.70
	Total	182	174	0.18	0.70–0.60
	Heterogeneity			0.51	0.80–0.70
Est-3	HHM-BC	78	100	2.72	0.10–0.05
	HMM-BC	35	39	0.22	0.70–0.60
	MMM-BC	45	46	0.01	0.95–0.90
	Total	158	185	2.13	0.20–0.10
	Heterogeneity			0.82	0.70–0.60
LDH-1	HHM-BC	75	81	0.23	0.70–0.60
	HMM-BC	29	18	2.57	0.20–0.10
	Total	104	99	0.12	0.80–0.70
	Heterogeneity			2.68	0.20–0.10
MPI	HHM-BC	105	108	0.04	0.90–0.80
	HMM-BC	13	9	0.72	0.40–0.30
	MMM-BC	93	74	2.16	0.20–0.10
	Total	211	191	1.00	0.40–0.30
	Heterogeneity			1.92	0.40–0.30

* HHM-BC represents all interspecific backcrosses to *X. helleri*.
HMM-BC represents all interspecific backcrosses to *X. maculatus*.
MMM-BC represents all intraspecific backcrosses among *X. maculatus* populations.
† *P* is the probability that the observed deviation from expectation is due to chance.

G6PDH, *6PGD*, *G3PDH-1*, *IDH-1*, and *IDH-2* have been published previously (MORIZOT, WRIGHT and SICILIANO 1977).

Linkage analyses of Est-2, Est-3, LDH-1 *and* MPI

Since segregation in all crosses is normal, orthogonal function analysis (MATHER 1957; ELANDT-JOHNSON 1971) can be used to detect linkage most efficiently (contingency table analysis was also performed upon all the data, and results agreed closely with the method presented here). The results of the linkage analyses are presented in Table 4. Highly significant excesses of parental types occur in the *Est-2–Est-3*, *Est-2–LDH-1*, *Est-3–LDH-1*, *Est-3–MPI* and *LDH-1–MPI* pairs. The lack of heterogeneity between cross types indicates the

TABLE 4

*Linkage analysis of linkage group II locus pairs**

Locus pair	Cross type	Parentals	Recombinants	p	Linkage χ^2	P
Est-2–Est-3	HHM-BC	66	26	0.28	17.39	<0.001
	HMM-BC	55	18	0.25	18.75	<0.001
	MMM-BC	62	29	0.32	11.97	<0.001
	Total	183	73	0.29	47.29	<0.001
	Heterogeneity				0.84	0.70–0.60
Est-2–LDH-1	HHM-BC	59	30	0.34	9.46	<0.01
	HMM-BC	32	15	0.32	6.15	<0.05
	Total	91	45	0.33	15.56	<0.001
	Heterogeneity				0.04	0.60–0.50
Est-2–MPI	HHM-BC	45	32	0.42	2.19	0.20–0.10
	HMM-BC	11	11	0.50	0.00	>0.99
	MMM-BC	34	43	0.56	1.05	0.40–0.30
	Total	90	86	0.49	0.09	0.80–0.70
	Heterogeneity				3.15	0.30–0.20
Est-3–LDH-1	HHM-BC	69	14	0.17	36.45	<0.001
	HMM-BC	29	11	0.28	8.10	<0.01
	Total	98	25	0.20	43.33	<0.001
	Heterogeneity				1.22	0.30–0.20
Est-3–MPI	HHM-BC	39	22	0.36	4.74	<0.05
	HMM-BC	14	8	0.36	1.64	0.30–0.20
	MMM-BC	59	18	0.23	21.83	<0.001
	Total	112	48	0.30	25.60	<0.001
	Heterogeneity				2.61	0.30–0.20
LDH-1–MPI	HHM-BC	76	14	0.16	42.71	<0.001
	Total	76	14	0.16	42.71	<0.001

* Parental and recombinant classes are identified in MORIZOT, WRIGHT and SICILIANO (1977) for double-backcross types; informative genotypes that could not have been produced by crossover events are parentals, and informative genotypes that must have been produced by crossing over are recombinants in single backcross types. The lower case p indicates the recombination fraction. The upper case P is the probability that the deviation from the expected values is due to chance. Other symbols as in Table 3.

conservation of the linkages in all the populations studied. That such nonindependent assortment is not due to a general assortment in the F_1 gametes of the chromosomes of the heterogeneous parents resulting, *e.g.*, in gametes containing mostly swordtail chromosomes and gametes containing mostly platyfish chromosomes similar to a process known as hybridogenesis in Poeciliopsis (SCHULTZ 1969), is shown in Table 5: *Est-2*, *Est-3*, *LDH-1* and *MPI* assort independently from the three loci of linkage group I (*ADA*, *G6PDH*, and *6PGD*) and from *G3PDH-1*, *IDH-1*, and *IDH-2*. The latter three loci assort independently from the linkage group I loci and from each other (MORIZOT, WRIGHT and SICILIANO 1977). None of the locus pairs in Table 5 differs significantly from the 1 parental: 1 recombinant ratio expected under independent assortment.

Thus, it is concluded that *Est-2*, *Est-3*, *LDH-1* and *MPI* are linked and represent markers of linkage group II in at least three species, *X. maculatus*, *X. helleri* and *X. clemenciae*.

Recombination frequencies among linkage group II loci

Recombination fractions between pairs of linkage group II loci are presented in Table 4. No significant differences in recombination frequency estimates exist between cross types, allowing the data to be pooled. The gene order indicated by the data is *Est-2–Est-3–LDH-1–MPI*. Since no cross was an informative backcross for all four loci, double crossovers must be analyzed in each of the informative systems: *Est-2–Est-3–LDH-1*, *Est-2–Est-3–MPI*, and *Est-3–LDH-1–MPI*. In the In the *Est-2–Est-3–LDH-1* group of data, five double crossovers were observed in 99 individuals, very close to the expected 6.23. Interference appears much stronger in the *Est-2–Est-3–MPI* data, where only three double crossovers were observed in 115 individuals considerably less than the 9.76 expected. When tested by an interference χ^2 test (BAILEY 1961), significant ($P < 0.01$) interference was detected, yielding a coincidence coefficient of 0.30. Presumably such interference is concentrated in the *Est-3–MPI* region. The data for *Est-3–LDH-1–MPI* are much more meager; no double crossovers were observed in 32 individuals, but less than one (0.75) double crossover was expected. A tentative chromo-

TABLE 5

*Independent assortment of linkage group II loci from other enzyme loci in Xiphophorus**

	Linkage group II locus							
	Est-2		*Est-3*		*LDH-1*		*MPI*	
	N	*p*	*N*	*p*	*N*	*p*	*N*	*p*
Other loci								
ADA	268	0.50	234	0.51	178	0.46	225	0.44
G6PDH	212	0.48	175	0.50	191	0.46	124	0.42
6PGD	223	0.47	197	0.47	178	0.49	141	0.45
G3PDH-1	293	0.48	271	0.49	149	0.44	194	0.49
IDH-1	186	0.53	150	0.49	134	0.51	112	0.48
IDH-2	221	0.57	186	0.56	171	0.56	152	0.51

* Data presented represent totals from all crosses. *N* is the number of individuals analyzed for the locus pair; *p* is the recombination fraction for the locus pair.

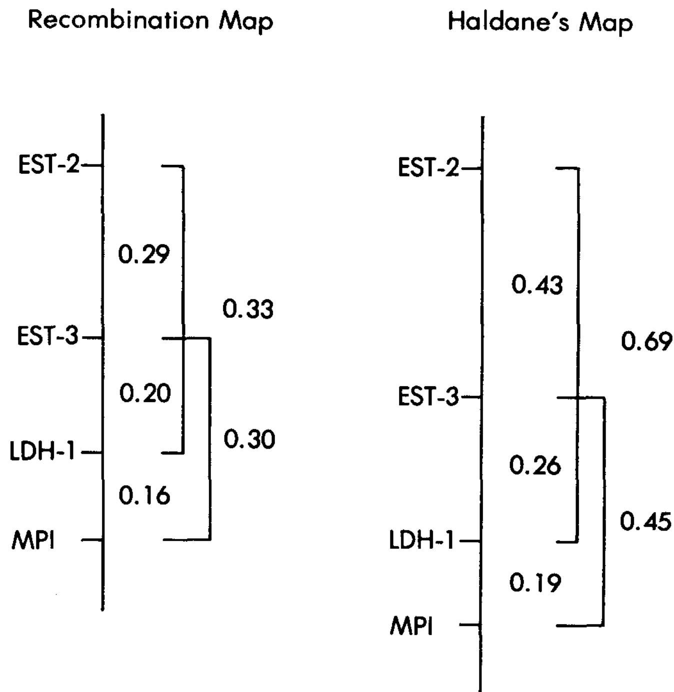

FIGURE 3.—Recombination map and Haldane's map of Xiphophorus linkage group II.

some map of linkage group II, using Haldane's mapping function (BAILEY 1961), is presented in Figure 3.

Sex differences in recombination between linkage group II loci

The effect of sex of the F_1 parent on recombination frequency is assessed in Table 6. While the data are obviously incomplete (due primarily to the skewed sex ratios in interspecific hybrid broods) and in some cases (*e.g.*, *Est-2–MPI* and *Est-3–MPI*) suggestive, no significant differences in recombination fractions exist. The collection of further data to answer more conclusively the question of whether sex-specific recombination occurs in linkage group II is in progress.

DISCUSSION

The existence of linkage groups in other organisms that can be considered comparable to all or part of linkage group II of Xiphophorus is difficult to establish, since proof of homology of esterase loci with loci of higher vertebrates probably will require sequence data. However, as in the case of Xiphophorus linkage group I (MORIZOT, WRIGHT and SICILIANO 1977), some suggestive genetic linkages have been elucidated in other organisms. WOMACK and SHARP (1976) present evidence for homology of part of a rat and a mouse linkage group containing clusters of esterase loci, five loci in linkage group XVIII (chromosome *8*) of the mouse and four loci in linkage group V of the rat. They further suggest that linkage of two

TABLE 6

*Effect of sex of the F_1 hybrid backcross parent upon recombination among linkage group II loci**

Locus pair	Cross type	Male F_1 parent Parentals	Male F_1 parent Recombinants	p	Cross type	Female F_1 parent Parentals	Female F_1 parent Recombinants	p
Est-2–Est-3	HHM-BC	30	8	0.21	HHM-BC	36	18	0.33
	HMM-BC	12	3	0.20	HMM-BC	43	15	0.26
	MMM-BC	62	29	0.32				
	Total	104	40	0.28	Total	79	33	0.29
Est-2–LDH-1		No data			HHM-BC	59	30	0.34
					HMM-BC	32	15	0.32
					Total	91	15	0.32
Est-2–MPI	HHM-BC	18	18	0.50	HHM-BC	27	14	0.34
	HMM-BC	3	3	0.50	HMM-BC	8	8	0.50
	MMM-BC	34	43	0.53				
	Total	55	64	0.54	Total	35	22	0.39
Est-3–LDH-1		No data			HHM-BC	69	14	0.17
					HMM-BC	29	11	0.28
					Total	98	25	0.20
Est-3–MPI	HHM-BC	11	7	0.39	HHM-BC	28	15	0.35
	HMM-BC	4	2	0.33	HMM-BC	10	6	0.38
	MMM-BC	59	18	0.23				
	Total	74	27	0.27	Total	38	21	0.36
LDH-1–MPI		No data			HHM-BC	86	14	0.16

* Headings and symbols as in Table 4.

erythrocyte esterase loci in the rabbit (SCHIFF and STORMONT 1970) and of two plasma esterase loci in voles (SEMENOFF 1972) may indicate further conservation of the autosomal linkage group in mammals. Thus, the linkage of *Est-2* and *Est-3* in at least two species of Xiphophorus may represent an extension to lower vertebrates of the conservation of linkages of esterase loci in particular and of functionally related enzymes in general.

The establishment of linkage relationships of the retinal *LDH* locus with other biochemical markers in Xiphophorus should prove to be especially useful in future studies of the the evolution of linkage groups in fishes. MARKERT, SHAKLEE and WHITT (1975) have demonstrated that most bony fishes possess a third *LDH* locus (*LDH-C*) coding either a retinal-specific or a liver-specific isozyme. They further postulate that this locus arose from the *LDH-B* locus by a single duplication event early in fish evolution. Thus, the ascertainment of the linkage relationships of retinal or liver *LDH* loci in diverse fish species should yield valuable information concerning the evolutionary stability of linkage groups. The only other such data collected to date are from another genus of Poeciliid fishes, Poeciliopsis. Two carboxylesterase loci, almost certainly homologous by tissue specificity cri-

teria to those in linkage group II of Xiphophorus, were found to be linked to the *LDH* eye locus with about the same recombination frequencies as in Xiphophorus (LESLIE and VRIJENHOEK 1978). Thus, at least part of Xiphophorus linkage group II seems to be conserved in a noncongeneric species.

The linkage relationships of *MPI* are known only in the mouse and man in vertebrates other than Xiphophorus. In the mouse, *MPI* has been assigned to linkage group II (NICHOLS, CHAPMAN and RUDDLE 1973), and in man to chromosome *7* (McMORRIS *et al.* 1973). Thus, in neither species is *MPI* syntenic with presently mapped esterase loci; neither is it syntenic with *LDH-A* (chromosome *11*) or *LDH-B* (chromosome *12*) in man (BOONE, CHEN and RUDDLE 1972; CHEN *et al.* 1973). One of the presently mapped esterase loci in man (esterase-A*4*) is syntenic with *LDH-A* on chromosome *11* (SHOWS 1972); homology with any of the rodent esterases has not been established. No *LDH* polymorphism has been reported thus far in mice (NICHOLS and RUDDLE 1973). Since the homology of the retinal *LDH* locus in fishes is presumed to be closest to the *LDH-C* (or *LDH-X*) of mammals (MARKERT, SHAKLEE and WHITT 1975), such a lack of synteny is hardly surprising. Unfortunately, *LDH-C* has not yet been mapped in mouse or man.

Due to the sharp contrast between the data concerning possible sex effects on recombination for linkage group II with those assembled for linkage group I of Xiphophorus (MORIZOT, WRIGHT and SICILIANO 1977) and the frequency with which such sex effects are observed in mammals (ROBINSON 1972), the data presented here are of some interest. It is entirely possible that the situation in fishes of sex effects on recombination will reflect the wide evolutionary experimentation apparent in the sex determination mechanisms seen in Xiphophorus (KALLMAN 1965). Comparisons of intraspecific crosses in several species, as well as in interspecific crosses, of recombination frequencies in reciprocal crosses with respect to sex will be necessary before the final decision of whether sex-specific recombination exists in these fishes can be made. The only conclusion that can be drawn from the present data is that such a sex-specific difference in recombination remains to be demonstrated in fishes.

In conclusion, the assignment of seven enzyme-coding loci to two linkage groups and the demonstration of independent assortment of three additional enzyme loci in Xiphophorus provides a starting point for comparative gene mapping in lower vertebrates. The results of such mapping of homologous loci in other vertebrate species should yield at least provisional estimates of the rate of evolution of linkage groups in vertebrates and the stability over evolutionary time of particular arrangements of genes.

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