Genetic Linkage Map of Fishes of the Genus *Xiphophorus* (Teleostei: Poeciliidae)

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ABSTRACT

Analysis of genotypes of 76 polymorphic loci in more than 2600 backcross hybrid individuals derived from intra- and interspecific genetic crosses of fishes of the genus *Xiphophorus* (Poeciliidae) resulted in the identification of 17 multipoint linkage groups containing 55 protein-coding loci and one sex chromosome-linked pigment pattern gene. Multipoint linkage analyses identified highly probable gene orders for 10 linkage groups. The total genome length was estimated to be ~18 Morgans. Comparisons of the *Xiphophorus* linkage map with those of other fishes, amphibians and mammals suggested that fish gene maps are remarkably similar and probably retain many syntenic groups present in the ancestor of all vertebrates.

THE construction of genetic linkage maps has proved to be a powerful tool in genetic studies of animal and plant genomes. The availability of detailed linkage maps can allow identification and localization of genes controlling simple and complex traits. The localization of specific genes on the linkage map can lead to molecular cloning of these genes by "reverse genetics" approaches (ORKIN 1986). Of more immediate benefit is the utilization of linked flanking markers to trace the inheritance of introgressed genes through subsequent generations. While the most rapid method for development of detailed linkage maps has involved the utilization of restriction fragment length polymorphisms (RFLPs) at anonymous DNA sequences, it is equally important to include sequences coding for proteins of known function. A primary benefit of linkage maps of protein-coding loci is the study of the origin and evolution of gene arrangements in a variety of taxa.

Gene maps of mammals, especially mouse and man, have expanded exponentially during the past decade, featuring identification of at least one multipoint syntenic or linkage group in more than 30 species (LALLEY *et al.* 1988). This rapid development has sparked renewed interest in comparative gene mapping with the consequent identification of a number of autosomal and sex chromosome-linked segments which have been highly conserved during the approximately 100 million years of mammalian evolution (STALLINGS and SICILIANO 1983; WOMACK 1987). By contrast, gene mapping in nonmammalian vertebrates has proceeded much more slowly: substantial numbers of loci, almost invariably coding for histochemically visualized proteins, have been assigned to linkage groups only in salmonid fishes (JOHNSON, WRIGHT and MAY 1987), poeciliid fishes (MORIZOT 1989; MORIZOT, SCHULTZ and WELLS 1989), and in frogs of the genera *Rana* (WRIGHT and RICHARDS 1987) and *Xenopus* (GRAF 1989).

Comparisons of fish and amphibian gene maps with those of mammals have identified several syntenic associations apparently conserved through more than 400 million years of vertebrate divergence. The synteny of glucosephosphate isomerase and peptidase D genes residing on human chromosome *19* and conserved in at least 10 other mammals finds counterparts in *Rana, Xenopus,* salmonid fish and poeciliid fish. Apparent human chromosome *15* homologs also have been demonstrated in frogs and fishes; mitochondrial isocitrate dehydrogenase (*IDH*), sorbitol dehydrogenase (*SORD*), and mannosephosphate isomerase (*MPI*) genes are syntenic in *Xenopus laevis* (GRAF 1989), and mitochondrial *IDH,* muscle pyruvate kinase (*PKM2*), and *MPI* genes are linked in poeciliid fishes (LESLIE 1982; MORIZOT 1983; MORIZOT and SICILIANO 1979). The numerous examples of conserved linkages among fishes have been reviewed previously (MORIZOT 1984, 1987, 1989, 1990; MORIZOT and SICILIANO 1983a; MORIZOT and SICILIANO 1984).

It seems likely that gene arrangements conserved between fishes and mammals reflect the pattern of the ancestral vertebrate. Thus, expansion of fish gene maps will provide a valuable tool for reconstruction of chromosome rearrangement events in mammalian lineages. Partly for this reason, we have performed numerous intra- and interspecific genetic crosses using

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TABLE 1

Strains of *Xiphophorus* species used in genetic crosses

Taxon	Strain symbol	Drainage, country of origin	Year(s) collected or obtained
I. Platyfish species group			
X. andersi	and	Rio Atoyac, Veracruz, Mexico	1981
X. couchianus	Xc	Rio Santa Catarina, Rio Grande drainage, Nuevo Leon, Mexico	1961
X. maculatus	Jp 163 A	Rio Jamapa, Veracruz, Mexico	1939
	Jp 163 B	Rio Jamapa, Veracruz, Mexico	1939
	w-Jp	Rio Jamapa, Veracruz, Mexico	1971
	Cp	Tributary to Rio Coatzacoalcos, Veracruz, Mexico	1971
	Bp	Belize River, Belize	1966, 1979
	Ghost	Domesticated stock	1979
X. milleri	mil	Tributary to Laguna Catemaco, Veracruz, Mexico	1982
II. Northern swordtail species group			
X. montezumae	mont	Rio Ojo Frio, Rio Panuco system, San Luis Potosi, Mexico	1981
X. n.sp. cf. *montezumae*	cf. mont	Rio Salto de Agua, Rio Panuco system, San Luis Potosi, Mexico	1965
X. nigrensis	nig	Rio Choy, Rio Panuco system, San Luis Potosi, Mexico	1972, 1979
X. n.sp. cf. *nigrensis*	cf. nig	Rio Coy, Rio Panuco system, San Luis Potosi, Mexico	1974
X. pygmaeus	pyg	Rio Huichihuayan, Rio Panuco system, San Luis Potosi, Mexico	1972
III. Southern swordtail species group			
X. alvarezi	Dol	Rio Dolores, Rio Usumacinta system, Alta Verapaz, Guatemala	1968
X. clemenciae	clem	Rio Sarabia, Rio Coatzacoalcos system, Oaxaca, Mexico	1968
X. helleri	Cd	Rio Jamapa, Veracruz, Mexico	1932
	Mah[a]	Mahogany Creek, Belize	1974
	Pop	Tributary to Rio de la Pasion, Rio Usumacinta system, Alta Verapaz, Guatemala	1968
	Sa	Rio Sarabia, Rio Coatzacoalcos system, Oaxaca, Mexico	1963, 1968

[a] Collected by DAVID M. WILDRICK and generously donated to our laboratory.

platyfishes and swordtails (*Xiphophorus,* Poeciliidae). In addition to their value as model systems for a variety of human neoplasms (VIELKIND, KALLMAN and MORIZOT 1989), the almost complete interfertility and high level of genetic variability (KALLMAN and ATZ 1966; MORIZOT and SICILIANO 1982c) and the availability of a number of highly inbred strains (KALLMAN 1975) make *Xiphophorus* an ideal organism for gene mapping using classical genetic methods. Extensive genetic analysis of pigment pattern and other polymorphisms (BOROWSKY 1984; KALLMAN 1975, 1984) further enhances the number of genes available for linkage analyses.

Previous gene mapping studies in *Xiphophorus* have utilized hybrids between 15 species of the genus (LECHNER and RADDA 1987; MEYER and SCHARTL 1979; RAUCHENBERGER, KALLMAN and MORIZOT 1990; ROSEN 1979; SCHARTL and SCHRODER 1987), but have centered upon melanoma-producing *X. helleri* × *X. maculatus* hybrids backcrossed to *X. helleri* (AHUJA, SCHWAB and ANDERS 1980; SICILIANO, MORIZOT and WRIGHT 1976; VIELKIND, KALLMAN and MORIZOT 1989). For this reason only a subset of the more than 70 identified protein and enzyme polymorphisms (MORIZOT and SICILIANO 1984) has been studied for joint segregation in genetic crosses. To date, descriptions of six autosomal linkage groups comprising some 20 protein-coding loci have been published (MORIZOT 1983; MORIZOT, GREENSPAN and SICILIANO 1983; MORIZOT and SICILIANO 1979, 1982a, 1982b, 1983b; MORIZOT, WRIGHT and SICILIANO 1977); other assignments have been summarized in the review literature (MORIZOT 1984, 1987, 1989) to maximize utilization by other investigators of the linkage map during its construction.

We present here the results of linkage analyses of 76 loci in a total of more than 2600 backcross hybrids produced from 87 different intra- and interspecific matings. Fourteen multipoint, independently assorting linkage groups are described and three additional multipoint linkage groups are designated as unassigned due to incompleteness of independent assortment tests. Multipoint linkage analyses suggest definitive gene orders for 10 linkage groups. Eight additional independently assorting loci could provide markers for the remainder of the 24 pairs of acrocentric chromosomes (HINEGARDNER and ROSEN 1972). Data supporting 32 new assignments to the *Xiphophorus* gene map are presented and recombination estimates among loci previously assigned to linkage groups I–VI are updated.

MATERIALS AND METHODS

Animals used: Parental stocks and species used to produce hybrids are listed in Table 1. Cross types, mostly first backcrosses, and numbers of offspring used for linkage analyses are presented in Table 2. Most informative are hybrids between taxa belonging to different species groups in which genetic differences are maximized and often fixed.

TABLE 2

Hybrid pedigrees contributing to offspring used for linkage analyses

Type of cross[a]	Pedigrees[b]	No. of hybrids
I. Intraspecific crosses		
A. *Xiphophorus maculatus*		
(Jp 163 A × Bp) × Jp 163 A	160, 4076, 4080, 4126	125
(Jp 163 A × Bp) × Bp	4104, 4112	25
(Jp 163 B × Bp) × Bp	4093, 4097	44
(Cp × Bp) × Bp	4083, 4091	41
(Ghost × Bp) × Ghost	4101	75
(Jp 163 A × Cp) × Jp 163 A	90, 98, 99, 104, 137	99
B. *Xiphophorus helleri*		
(Mah × Cd) × Cd	192	111
II. Interspecific crosses		
A. Within platyfish species group		
((Xc × w-Jp) × Xc) × Xc	4035, 4043	53
(Jp 163 A × and) × and	4779	138
(Jp 163 A × and) × Bp	4736	97
(Jp 163 A × mil) × mil	4953, 5050, 5057	80
(Jp 163 A × mil) × Jp 163 A	176, 187, 5011	76
(Jp 163 A × mil) × w-Jp	4972	75
B. Within northern swordtails		
(mont × cf. mont) × mont	4794, 4809	109
(mont × cf. mont) × cf. mont	4750	25
(pyg × nig) × nig	5010, 5012	63
((pyg × nig) × nig) × nig	5162	15
(pyg × nig) × pyg	4245	24
(pyg × cf. nig) × nig	4234, 4327	45
(nig × cf. nig) × cf. nig	4237, 4264, 4280, 4286, 4291, 4295, 4315	117
(nig × cf. nig) × cf. nig	4191, 4247, 4253, 4306, 4313	133
((pyg × nig) × nig) × pyg	5049	25
C. Between platyfishes and northern swordtails		
(mil × nig) × nig	5055	17
((mil × nig) × nig) × mil	5094	30
D. Between platyfishes and southern swordtails		
(Dol × mil) × Dol	4989, 4991, 4997	150
(clem × mil) × clem	5353, 5357, 5384, 5489	157
((clem × mil) × clem) × clem	5662	16
(clem × Jp 163 A) × clem	133	18
(Sa × Bp) × Sa	161, 180	19
(Sa × Jp 163 A) × Sa	44, 70, 100, 157, 164,[c] 165,[c] 172, 174, 181	241
((Sa × Jp 163 A) × Sa) × Sa	76	12
(Sa × Jp 163 A) × Jp 163 A	72, 109, 163, 164,[c] 165,[c] 175	95
(Pop × Jp 163 A) × Pop	103, 108	70
((Pop × Jp 163 A) × Pop) × (Pop × Jp 163 A)	108b[d]	96
(Pop × Jp 163 A) × Jp 163 A	101, 107, 116, 117	74
(Cd × Jp 163 A) × Cd	106	5
(Cd × Jp 163 A) × Jp 163 A	102, 105, 126	19

[a] Cross types indicate only type of parents and do not imply sex; many sex-reciprocal crosses were constructed.

[b] Pedigree numbers <2000 are numbers assigned at the University of Texas Science Park Fish Genetics Laboratory; numbers >2000 are numbers assigned at the Fish Genetics Laboratory of the New York Aquarium.

[c] Pedigrees 164 and 165 are found in two cross types because they were produced by artificial insemination of a hybrid female with a mixture of *X. helleri* and *X. maculatus* sperm.

[d] Pedigree 108b was derived by an inadvertant mating of an early-maturing backcross hybrid male with his hybrid mother; loci segregating as intercrosses were excluded from linkage analyses.

For this reason it is usually not particularly important how many generations a stock has been inbred. Most matings were made utilizing artificial insemination (CLARK 1950). In many cases sex- and/or parental strain-reciprocal backcross hybrids were produced. Fish were maintained at densities no greater than 1.4 individuals/liter in aquaria at the New York Aquarium and University of Texas Science Park and fed two to three times daily.

Tissue preparation: Fish were sacrificed following ice anesthesia and some or all of the following tissues were dissected immediately upon death: blood plasma, erythrocytes, brain, eye, gill, skeletal muscle, liver, pancreas, head kidney, spleen, foregut, hindgut, testis, ovary, fin, and heart. Tissue preparation procedures follow those described by MORIZOT, WRIGHT and SICILIANO (1977). Stored tissues and/or extracts were held at −80°.

TABLE 3

Protein-coding loci polymorphic in *Xiphophorus* genetic crosses

Gene, protein symbol[a]	Fish gene, protein symbol[b]	Gene, protein name	EC No.[c]	Subunits	Tissue[d]	Buffer system[e]
ACO1	sAH	Aconitase-1 (cytosolic)	4.2.1.3	1	L	TC
ACO2	mAH	Aconitase-2 (mitochondrial)	4.2.1.3	1	M	TEB
ADA	ADA	Adenosine deaminase	3.5.4.4	1	M	TEB, TC
AMY	AMY	α-Amylase	3.2.1.1	1	P	TEB
ATP	None	Adenosine triphosphatase	3.6.1.3	>2	B&E	TEB
CA1	CAH-1	Carbonic anhydrase-1	4.2.1.1	1	B&E	TEB
CKMM	CK-A	Creatine kinase, muscle form	2.7.3.2	2	M	TEB
ENO2	ENO-2	Enolase-2 (muscle)	4.2.1.11	2	M	TEB
ES1	EST-1	Esterase-1	3.1.1.	1	M	TEB
ES2	EST-2	Esterase-2	3.1.1.	1	M, B&E	TEB, TC
ES3	EST-3	Esterase-3	3.1.1.	1	L	TEB, TC
ES4	EST-4	Esterase-4	3.1.1.	1	L	TEB
ES5	EST-5	Esterase-5	3.1.1.	1	L	TEB
ES6	EST-6	Esterase-6	3.1.1.	1	M	TEB
ES7	EST-7	Esterase-7	3.1.1.	1	M, B&E	TEB
FH	FH	Fumarate hydratase	4.2.1.2	4	M	TEB
GAA	aGLU	α-glucosidase, acid	3.2.1.20	4	L	TC
GALT1	UGHUT-1	Galactose-1-phosphate uridylyltransferase-1	2.7.7.12	2	L	TC
GALT2	UGHUT-2	Galactose-1-phosphate uridylyltransferase-2	2.7.7.12	2	L	TC
GAPD1	GAPDH-1	Glyceraldehyde-3-phosphate dehydrogenase-1	1.2.1.12	4	B&E, M	TEB, TC
GAPD2	GAPDH-2	Glyceraldehyde-3-phosphate dehydrogenase-2	1.2.1.12	4	M	TEB, TC
GAPD3	GAPDH-3	Glyceraldehyde-3-phosphate dehydrogenase-3	1.2.1.12	4	T	TEB, TC
GDA	GDA	Guanine deaminase	3.5.4.3	2	L	TEB
GDH	GDH	Glucose dehydrogenase	1.1.1.47	2	L	TC
GLA	aGAL	α-Galactosidase	3.2.1.22	2?	L	TC
GLNS	GLAL	Glutamine synthetase	6.3.1.2	8?	B&E	TEB
GLO	LGL	Glyoxalase I	4.4.1.5	2	M	TM
GLYDH	GLYDH	Glycerate dehydrogenase	1.1.1.29	2	L	TC
GOT1	AAT-1	Glutamate-oxaloacetate transaminase-1 (cytosolic)	2.6.1.1	2	M	TEB
GOT2	AAT-2	Glutamate-oxaloacetate transaminase-2 (mitochondrial)	2.6.1.1	2	M	TEB, TC
GOT3	AAT-3	Glutamate-oxaloacetate transaminase-3 (cytosolic)	2.6.1.1	2	L	TEB, TC
G6PD	G6PDH	Glucose-6-phosphate dehydrogenase	1.1.1.49	2?	B&E	TEB
GPI1	GPI-1	Glucose phosphate isomerase-1	5.3.1.9	2	M	TEB, TC
GPI2	GPI-2	Glucose phosphate isomerase-2	5.3.1.9	2	M	TEB, TC
GUK1	GUK-1	Guanylate kinase-1	2.7.4.8	1	B&E	TEB, TC
GUK2	GUK-2	Guanylate kinase-2	2.7.4.8	1	B&E	TC
GUK3	GUK-3	Guanylate kinase-3	2.7.4.8	1	M	TEB
HB	HB	Hemoglobin		4?	RBC	TEB
HEX	bGLUA	Hexosaminidase	3.2.1.30	>1	L	TEB

Electrophoresis and histochemical staining: Vertical starch gel electrophoresis was performed using previously described methods (MORIZOT and SCHMIDT 1990; MORIZOT, WRIGHT and SICILIANO, 1977; SICILIANO and SHAW 1976), using lots of hydrolyzed potato starch supplied by Connaught Laboratories (Toronto, Ontario) and StarchArt (Smithville, Texas). Tissues and buffer systems used for resolution of each protein product are listed in Table 3. Polyacrylamide gels (7%) were used for separation of α-amylase allozymes (HARRIS and HOPKINSON 1977). Histochemical visualization procedures followed the methods of MORIZOT and SCHMIDT (1990). We have chosen to follow standard human nomenclature where possible for locus names (MCALPINE *et al.* 1988), but have included in Table 3 the symbols recommended for fish loci in a proposed fish gene and protein nomenclature system (SHAKLEE *et al.* 1989).

Electrophoretic phenotypes and genotype assignments: Most heterozygous electrophoretic patterns in F_1 hybrids agreed with expectations predicted from subunit structures in other vertebrates (MANCHENKO 1988). Because phenotypes of parental stocks were known in almost every case, genotypic assignments as homozygotes or heterozygotes were unambiguous, even in the few cases where three or four alleles were segregating. Illustrations of more than 50 polymorphic locus products have been presented in previous publications (*e.g.*, MORIZOT and SCHMIDT 1990; MORIZOT and SICILIANO 1984). Probable subunit structures of polymorphic proteins are listed in Table 3.

Segregation and linkage analyses: Goodness-of-fit to 1 homozygote: 1 heterozygote backcross segregation expectations was tested at each locus by chi-square analysis in each cross type and in the total data. All loci segregating as intercrosses were excluded from segregation and linkage analyses. Because 76 loci were studied, the effect of multiple comparisons was taken into account. Each test was considered to be significant if the goodness-of-fit χ^2 exceeded 11.57 ($P = 0.00067$) to yield an overall 5% level of significance.

Two tests for genetic linkage were used. In preliminary

Gene, protein symbol[a]	Fish gene, protein symbol[b]	Gene, protein name	EC No.[c]	Subunits	Tissue[d]	Buffer system[e]
IDH1	sIDH-1	Isocitrate dehydrogenase-1 (cytosolic)	1.1.1.42	2	L	TEB
IDH2	sIDH-2	Isocitrate dehydrogenase-2 (cytosolic)	1.1.1.42	2	L	TEB
LDHB	LDH-B	Lactate dehydrogenase B	1.1.1.27	4	L	TC
LDHC	LDH-C	Lactate dehydrogenase C	1.1.1.27	4	B&E	TC
MAN	aMAN	α-Mannosidase	3.2.1.24	>2?	B&E	TEB
MDH1	sMDH-1	Malate dehydrogenase-1 (cytosolic)	1.1.1.37	2	M	TC
MDH2	sMDH-2	Malate dehydrogenase-2 (cytosolic)	1.1.1.37	2	M	TC
ME	ME	Malic enzyme	1.1.1.40	4	M	TEB
MP5	MP-5	Muscle protein-5 (troponin?)		4?	M	TEB
MPI	MPI	Mannose phosphate isomerase	5.3.1.8	1	M	TEB, TC
NP2	PNP-2	Nucleoside phosphorylase-2	2.4.2.1	3	B&E	TEB
PEPA	PEPA	Peptidase A	3.4.11.	2	M	TB
PEPB	PEPB	Peptidase B	3.4.11.	2	M	TEB
PEPC	PEPC	Peptidase C	3.4.11	2	B&E	TB
PEPD	PEPD	Peptidase D	3.4.13.9	2	M	TB
PEPS	PEPS	Peptidase S	3.4.11.	6?	M	TEB
PEPX	PEPX	Peptidase "X"	3.4.11	2?	M	TB
PGAM1	PGAM-1	Phosphoglycerate mutase-1	5.4.2.1	2?	L	TEB
PGAM2	PGAM-2	Phosphoglycerate mutase-2	5.4.2.1	2	M	TEB
PGD	PGDH	Phosphogluconate dehydrogenase	1.1.1.43	2	M	TEB, TC
PGK	PGK	Phosphoglycerate kinase	2.7.2.3	1	M	TEB
PGM	PGM	Phosphoglucomutase	5.4.2.2	1	M	TEB
PK1	PK-1	Pyruvate kinase-1	2.7.1.40	4	B&E	TEB
PK2	PK-2	Pyruvate kinase-2	2.7.1.40	4	M	TEB
PP1	PP-1	Inorganic pyrophosphatase-1	3.6.1.1	2?	M	TC
PP2	PP-2	Inorganic pyrophosphatase-2	3.6.1.1	2?	B&E	TC
PVALB1	PARV-1	Parvalbumin-1		1	M	TEB
PVALB2	PARV-2	Parvalbumin-2		1	M	TEB
PVALB3	PARV-3	Parvalbumin-3		1	M	TEB
SOD1	sSOD-1	Superoxide dismutase-1 (cytosolic)	1.15.1.1	2	M	TEB
TF	TF	Transferrin		1	Pl, B&E	TC
TPI1	TPI-1	Triose phosphate isomerase-1	5.3.1.1	2	B&E	TEB
UMPH1[f]	None	Uridine 5′-monophosphate phosphohydrolase	3.1.3.	2?	L	TC
UMPK	NPK	Uridine monophosphate kinase	2.7.4	1	B&E	TEB
UP1[g]	None	Uncharacterized phosphatase-1	3.1.3.2?	1?	B&E	TC

[a] Gene and protein symbols usually are designated in agreement with standardized human genetic nomenclature (see McAlpine *et al.* 1988).

[b] Standard nomenclature for fish genes and proteins proposed by Shaklee *et al.* (1989).

[c] Standard enzyme nomenclature of the International Union of Biochemistry, Nomenclature Committee (IUBNC 1984).

[d] Tissues are those routinely used for isozyme resolution: B&E, combined brain and eye samples; L, liver; M, skeletal muscle; P, pancreas; Pl, blood plasma; RBC, red blood cells (hemolysates); T, testis.

[e] Buffer systems and histochemical staining procedures are presented in detail in Morizot and Schmidt (1989): TEB, tris-EDTA-borate, pH 8.0; TC, tris-citrate, pH 7.0; TM, tris maleate, pH 7.4; TB, tris-borate, pH 8.0.

[f] Previously termed "acid phosphatase" by Morizot and Siciliano (1984).

[g] Probably an "acid phosphatase" locus.

analysis pairwise comparisons in individual cross types were assessed by contingency χ^2 analysis (Mather 1957) using the LINKAGE-1 computer program of Suiter, Wendel and Case (1983). The entire data set was then analyzed for two-point and multipoint linkage by log-likelihood methods using the computer program MAPMAKER (Lander *et al.* 1987). For any number of segregating loci MAPMAKER evaluates all gene orders and computes the log likelihood of the observations and the maximum likelihood estimate of recombination value for each adjacent interval. For two-point data, the evidence for linkage can be presented as the lod score (Morton 1955),

$$z(\theta) = \log_{10}\{L(\theta)/L_{(1/2)}\}$$

where θ ($0 \leq \theta \leq \frac{1}{2}$) is the recombination value and $L(\theta)$ the likelihood of the observations at an assumed recombination value θ. The lod score is the $\log_{10}$ of the odds of the evidence for linkage ($\theta < 0.5$) *vs.* no linkage ($\theta = 0.5$). By maximizing the above function the maximum likelihood estimate of θ ($\hat{\theta}$) and the lod score at $\hat{\theta}$, $z(\hat{\theta})$, can be calculated. A lod score of 3.0 is considered to be significant evidence for linkage (Morton 1955).

Initial evidence for linkage groups was obtained from MAPMAKER by grouping sets of markers that had pairwise lod scores ≥ 3.0 and θ values of 0.4 or smaller. Multipoint mapping of these suggested groupings was performed by choosing a set of loci and considering all possible gene orders and their associated log likelihoods. An order was considered definite if its likelihood was 10^4 times larger than the likelihood of any other gene order; such a set of loci was called an anchor map. Subsequently, other markers were

TABLE 4

Segregation of polymorphic loci in *Xiphophorus* backcross hybrids

Locus	No. of informative matings	Homozygotes	Heterozygotes	$\chi^2_{1\ d.f.}$[a]	Locus	No. of informative matings	Homozygotes	Heterozygotes	$\chi^2_{1\ d.f.}$[a]
ACO1	31	496	437	3.73	*HB*	1	6	4	0.40
ACO2	19	217	241	1.26	*HEX*	1	68	70	0.03
ADA	45	732	748	0.17	*IDH1*	23	195	193	0.01
AMY	4	69	72	0.06	*IDH2*	41	580	616	1.08
ATP	3	71	102	5.56	*LDHB*	15	169	187	0.91
CA1	4	129	157	2.74	*LDHC*	8	165	184	1.03
CKMM	28	542	593	2.29	*MACR*[b]	41	645	665	0.31
DIFF	11	101	86	1.20	*MAN*	2	24	22	0.09
ENO2	1	66	71	0.18	*MDH1*	19	364	366	0.01
ES1	22	281	282	0.00	*MDH2*	20	335	375	2.25
ES2	36	365	380	0.30	*ME*	5	103	133	3.81
ES3	44	542	539	0.01	*MP5*	8	117	116	0.00
ES4	5	67	60	0.39	*MPI*	40	618	603	0.18
ES5	5	72	64	0.47	*NP2*	9	254	268	0.38
ES6	1	5	4	0.11	*PEPA*	5	143	134	0.29
ES7	11	151	150	0.00	*PEPB*	22	297	302	0.04
FH	2	58	51	0.45	*PEPC*	3	73	60	1.27
GAA	1	7	8	0.07	*PEPD*	4	100	129	3.67
GALT1	22	316	326	0.16	*PEPS*	50	679	718	1.09
GALT2	1	16	18	0.12	*PEPX*	3	56	49	0.47
GAPD1	41	558	547	0.11	*PGAM1*	1	29	31	0.07
GAPD2	4	85	55	6.43	*PGAM2*	6	112	112	0.00
GAPD3	7	44	44	0.00	*PGD*	37	577	605	0.66
GDA	22	420	452	1.17	*PGK*	7	67	71	0.12
GDH	14	208	160	6.26	*PGM*	39	511	515	0.02
GLA	13	183	182	0.00	*PK1*	12	126	174	7.68
GLNS	15	272	301	1.47	*PK2*	24	506	515	0.08
GLO	3	64	86	3.23	*PP1*	4	131	156	2.18
GLYDH	13	231	223	0.14	*PP2*	1	9	9	0.00
GOT1	4	94	76	1.91	*PVALB1*	16	209	231	1.10
GOT2	14	311	338	1.12	*PVALB2*	33	468	470	0.00
GOT3	3	126	141	0.84	*PVALB3*	5	75	78	0.06
G6PD	29	490	500	0.10	*SOD1*	3	78	52	5.20
GPI1	44	681	713	0.74	*TF*	47	644	669	0.48
GPI2	14	378	372	0.05	*TPI1*	25	561	570	0.07
GUK1	13	307	321	0.31	*UMPH1*	27	441	407	1.36
GUK2	6	73	107	6.42	*UMPK*	15	281	292	0.21
GUK3	3	135	119	1.01	*UP1*	3	54	79	4.70

[a] For 76 comparisons, $\chi^2_{0.05} = 11.57$.
[b] Macromelanophore pigment pattern genes (sex chromosome-linked) of platyfish species group.

added to this map one at a time and allowed to take any location relative to the existing map of anchor loci. Likelihood calculations were made for each possible location and all maps with likelihoods 100 times less than the most likely map were considered to be excluded. For each multipoint cross, recombinants were also counted and an order was determined parsimoniously by minimizing the frequency of multiple recombination events.

An estimate of the total map length of the genome was calculated from the numbers of recombinants and nonrecombinants for each locus pair (A. CHAKRAVARTI, L. LASHER and J. REEFER, unpublished results). This method used the maximum likelihood approach and is a variation of the method proposed by HULBERT *et al.* (1988).

RESULTS

Backcross hybrids: Three broad cross type categories resulting from 87 matings produced a total of 2614 backcross hybrids for the current analysis (Table 2). The number of polymorphic loci per cross varied from two to 41 but averaged more than 20 loci per cross (augmented by the high proportion of hybrids between species groups). Detailed presentation of informative loci and alleles in each cross is prohibitively space consuming, but is available for crosses of interest from the first author.

Electrophoretic phenotypes of protein-coding loci: Table 3 lists resolution conditions and brief characterizations of the 74 polymorphic protein products assayed. Most heterozygous electrophoretic patterns agreed with predictions from previously determined subunit structures (MANCHENKO 1988), with several notable exceptions. PGAM1, GLA, MDH2 and CKA are presumed to be dimeric in most verte-

TABLE 5

Genetic linkages supported by significant LOD scores ($Z > 3.0$), maximum likelihood recombination frequency estimates, and provisional linkage group assignments

Locus pair		$\hat{\theta} \pm$ SE	Z	Linkage group
ACO2	*PP1*	0.28 ± 0.04	6.6	X
ACO2	*PVALB2*	0.02 ± 0.01	34.5	X
ADA	*G6PD*	0.17 ± 0.01	85.7	I
ADA	*PGD*	0.29 ± 0.01	39.2	I
AMY	*GLNS*	0.10 ± 0.03	15.2	VI
AMY	*GUK3*	0.04 ± 0.02	22.0	VI
AMY	*NP2*	0.20 ± 0.06	3.4	VI
AMY	*TF*	0.15 ± 0.03	16.7	VI
AMY	*UMPK*	0.23 ± 0.04	6.5	VI
ATP	*GAPD1*	0.00 ± 0.00	13.8	III
ATP	*GUK2*	0.02 ± 0.02	11.2	III
ATP	*ME*	0.07 ± 0.04	8.5	III
CA1	*MACR*	0.13 ± 0.02	39.1	XXIV
ENO2	*GPI2*	0.04 ± 0.02	30.5	II
ENO2	*PK2*	0.04 ± 0.02	31.4	II
ES1	*ES4*	0.03 ± 0.02	23.4	V
ES1	*GLYDH*	0.18 ± 0.03	13.2	V
ES1	*MDH2*	0.31 ± 0.03	11.4	V
ES2	*ES3*	0.24 ± 0.02	40.7	II
ES2	*ES5*	0.30 ± 0.05	3.1	II
ES2	*LDHC*	0.34 ± 0.04	3.0	II
ES3	*ES5*	0.01 ± 0.01	38.4	II
ES3	*LDHC*	0.23 ± 0.04	7.8	II
ES3	*MPI*	0.28 ± 0.02	34.3	II
ES3	*PK2*	0.16 ± 0.02	39.7	II
ES5	*MPI*	0.20 ± 0.04	8.0	II
ES5	*PK2*	0.10 ± 0.04	8.3	II
ES7	*GAPD1*	0.26 ± 0.04	7.8	III
ES7	*GUK2*	0.26 ± 0.04	5.3	III
ES7	*ME*	0.31 ± 0.04	5.0	III
GALT1	*HEX*	0.29 ± 0.04	4.8	VIII
GALT1	*PGAM2*	0.04 ± 0.02	41.9	VIII
GALT2	*IDH2*	0.09 ± 0.05	5.6	VII
GAPD1	*GUK2*	0.24 ± 0.03	10.2	III
GAPD1	*ME*	0.05 ± 0.02	30.8	III
GAPD2	*PEPC*	0.34 ± 0.04	3.1	U3
GADP3	*TPI1*	0.01 ± 0.01	23.8	XIII
GDA	*PEPS*	0.22 ± 0.02	49.5	XII
GDH	*PEPX*	0.09 ± 0.03	16.2	U2
GLNS	*GUK3*	0.09 ± 0.02	25.4	VI
GLNS	*NP2*	0.29 ± 0.04	6.3	VI
GLNS	*TF*	0.12 ± 0.02	60.6	VI
GLNS	*UMPK*	0.21 ± 0.03	20.1	VI
GLYDH	*MDH2*	0.09 ± 0.02	63.1	V
GLO	*GPI1*	0.33 ± 0.04	3.7	IV
GLO	*PEPD*	0.30 ± 0.05	3.4	IV
GOT2	*GPI1*	0.13 ± 0.01	78.1	IV
GOT2	*PEPD*	0.18 ± 0.03	14.4	IV
GOT2	*PK1*	0.17 ± 0.03	16.2	IV
GOT3	*MDH1*	0.35 ± 0.03	5.2	U1
G6PD	*PGD*	0.16 ± 0.01	72.7	I
GPI1	*PEPD*	0.05 ± 0.01	49.8	IV
GPI1	*PK1*	0.06 ± 0.02	54.7	IV
GPI2	*PK2*	0.15 ± 0.02	70.1	II
GUK1	*PGM*	0.23 ± 0.04	10.1	IX
GUK2	*ME*	0.09 ± 0.03	17.2	III
GUK3	*NP2*	0.20 ± 0.04	9.0	VI
GUK3	*TF*	0.10 ± 0.02	24.2	VI
GUK3	*UMPK*	0.17 ± 0.03	19.8	VI
NP2	*TF*	0.19 ± 0.03	22.8	VI
PEPA	*TPI1*	0.07 ± 0.02	25.2	XIII
PGAM1	*PGK*	0.13 ± 0.04	7.8	XI
PP1	*PVALB2*	0.27 ± 0.04	6.2	X
TF	*UMPK*	0.24 ± 0.02	25.5	VI

brates, but in *Xiphophorus* hybrids interallelic heterodimers are only sometimes (MDH2) or never observed (MORIZOT and SICILIANO 1984). Lack of heterodimer formation has been noted in CKA (=CKMM) isozymes of other fishes, and in vitro heterodimer production has been achieved (FERRIS and WHITT 1978). Assignment of variants in some presumably multilocus systems (especially hexosaminidase and hemoglobin) will require further characterization in fishes. Subcellular localization has rarely been studied directly in fishes through organelle purification; inferences usually are based upon relative activity variation among tissues.

Pigment pattern phenotypes: Expression in hybrids of macromelanophore pigment patterns of the platyfish species group ranged from complete absence to malignant melanoma (KALLMAN 1975; KALLMAN and ATZ 1966; VIELKIND, KALLMAN and MORIZOT 1989). Data from crosses with reduced expression were excluded from segregation and linkage analyses of macromelanophore genes. Benign and malignant melanoma phenotypes controlled by the *DIFF* gene (AHUJA, SCHWAB and ANDERS 1980), previously called *Mel Sev* by SICILIANO, MORIZOT and WRIGHT (1976) and MORIZOT and SICILIANO (1983b), were scored only in some *X. helleri* × *X. maculatus*- and *X. clemenciae* × *X. maculatus*-derived backcrosses; other regulatory elements appear to be involved in *X. couchianus* × *X. maculatus* and *X. andersi* × *X. maculatus* melanomas (VIELKIND, KALLMAN and MORIZOT 1989).

Inheritance of phenotypes in backcross hybrids: Segregation analyses of the 76 loci studied are presented in Table 4. Overall, assayed loci segregate in all informative cross types in excellent agreement with Mendelian expectations; the observed level of segregation distortion probably is insufficient to interfere significantly with accurate assessment of genetic linkage.

Linkage analyses: Joint segregation data were obtained for more than 60% of the 2850 possible pairwise comparisons, ranging from one to more than 1000 informative individuals. The complete data set can be obtained from the first author upon request.

Statistically significant lod scores, recombination estimates and standard errors are presented in Table 5. From these data 56 loci can be assigned to 17 multipoint linkage groups, for which the most likely gene orders are illustrated in Figure 1. Pairwise comparisons are sufficient to document independent assortment of 13 of these linkage groups, which we designate as *Xiphophorus* multipoint linkage groups I–XIII. Three other groups, designated U1–U3 (unassigned linkage groups) are as yet incompletely tested for independent assortment from LGs I–XIII. All 16 of the above groups assort independently from LG XXIV, the sex chromosome linkage group to which the macromelanophore locus and carbonic anhydrase-

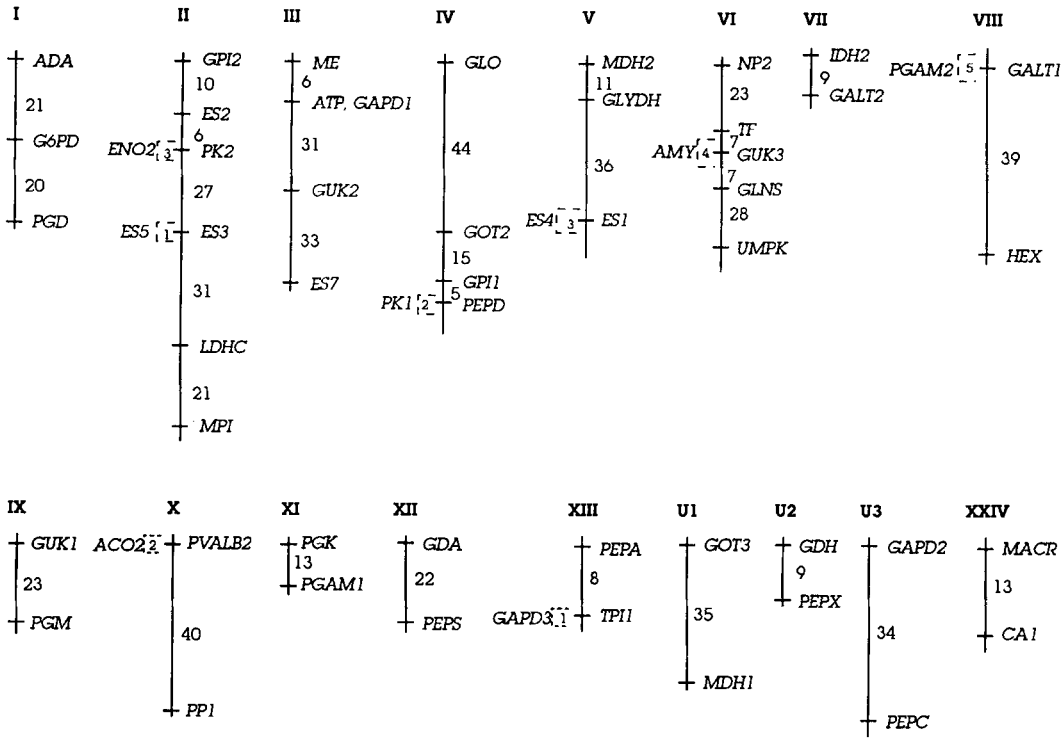

FIGURE 1.—Summary of the *Xiphophorus* linkage map. Key to symbols is given in Table 3. Arabic numbers are recombination percentages. Linkage group designations are given as Roman numerals or boldface symbols above chromosome segment representations.

1 are assigned. The linkage groups designated here are intended to supplant those defined in earlier *Xiphophorus* gene map summaries.

Alternative gene orders with relatively high likelihood are noted in Figure 1. Many crosses lack one or more informative markers in particular multipoint linkage groups, resulting in few data to distinguish between likely orders. Additional cross types currently being produced should provide further definition of correct gene orders.

Genome map length: The estimate of the total map length of the *Xiphophorus* genome was 18.25 Morgans (M). By considering all map lengths that have likelihoods which are at most 100 times less than the maximum likelihood, the total map length can be estimated to be between 14 and 26 M with approximately 95% confidence.

DISCUSSION

The development of fish gene maps, while painfully slow in the past, promises to undergo rapid expansion in the future as DNA-level technologies become widely utilized. The large number of chromosomes marked by isozyme loci in *Xiphophorus* interspecific hybrids should allow very rapid linkage group assignment of DNA restriction fragment length polymorphisms cross-hybridizing either with heterologous probes or probes cloned from *Xiphophorus* cDNA or genomic libraries (SCHARTL 1988; VIELKIND and DIPPEL 1984; ZECHEL *et al.* 1988). A saturated linkage map will facilitate identification and cloning of genes controlling tumorigenesis and tumor progression (MAUELER, RAULF and SCHARTL 1988; SCHWAB *et al.* 1978, 1979; VIELKIND, KALLMAN and MORIZOT 1989; WITTBRODT *et al.* 1989), genes controlling age and size of sexual maturation (KALLMAN and BORKOSKI 1978), and perhaps genes involved in quantitative and meristic traits used for species discrimination.

Two loci, *IDH1* and *DIFF*, previously assigned to *Xiphophorus* linkage groups do not appear in the gene map presented here. *IDH1* was assigned to LG IV (MORIZOT and SICILIANO 1982a) on the basis of loose linkage (41% recombinants, $N = 305$) to GPI1. Log-likelihood analysis results in a lod score of <3.0, failing to meet our criterion for significance. Additional crosses are required to confirm the earlier assignment. Collection of linkage data is made difficult by the extreme lability of IDH1, a cytosolic liver-specific isozyme which loses activity rapidly in frozen tissues and extracts.

Three studies have suggested linkage of *DIFF* of *X. maculatus* to LG V markers (AHUJA, SCHWAB and ANDERS 1980; MORIZOT and SICILIANO 1983b; SICILIANO, MORIZOT and WRIGHT 1976). *DIFF* has been proposed to promote macromelanophore pigment cell precursor differentiation (VIELKIND 1976) and to be nonfunctional or absent in *X. helleri*. The *DIFF* phenotype in particular backcross hybrids is benign mel-

anomas (loss of one functional copy) or malignant melanomas (loss of both *DIFF* functional alleles). The *DIFF* gene has always been narrowly discussed in the context of pigment cell abnormalities in *X. helleri* × *X. maculatus*-derived backcross hybrids. But melanosis and melanoma are of regular occurrence also in other interspecific hybrids not involving the above two species (ATZ 1962) and it is immaterial whether or not the recipient species has its own set of macromelanophore genes. For this reason a more correct view of *DIFF* may imply a locus with species-specific alleles that is present in all members of the genus. This view also circumvents the difficulty of having to postulate a segment on a *maculatus* chromosome that has no homolog in *X. helleri*. Whether in other hybrids a similar relationship exists between melanoma severity and the absence of one or both species-specific *DIFF* factors remains to be explored.

As in the case of *IDH1*, the loose linkage of *DIFF* with *ES1* (36.9% recombinants, $N = 130$) fails to meet our criterion for significance. Highly variable recombination between *DIFF* and *ES1* has been discussed previously (VIELKIND, KALLMAN and MORIZOT 1989). In view of the consistent findings by several laboratories of association with LG V, we view the linkage group assignment as confirmed but the map position as uncertain.

Our estimates of total map length of the *Xiphophorus* genome suggest that tests of linkage of any new, randomly located markers with the endpoint loci of currently identified linkage groups should allow linkage detection in ~76% of cases. Completeness of pairwise recombination estimation will be enhanced significantly by backcrosses currently being produced using hybrids between northern and southern swordtails and between northern swordtails and platyfishes. New linkages from these crosses should increase the number of genes assigned to the 17 multipoint linkage groups and further reduce the number of crosses required for linkage group assignment of new pigment, protein, or DNA markers.

With regard to future studies, our estimate of the total map length of the *Xiphophorus* genome allows some interesting predictions. Given a genome length of ~18 M and a difference in size of the largest and smallest chromosome of no more than 8-fold, the largest and smallest chromosomes can be estimated to have genetic lengths of 1.33 M and 0.17 M, respectively, assuming proportionality between physical and genetic distances. Furthermore, since *Xiphophorus* genomes contain approximately 20% of the DNA of the average mammalian cell (HINEGARDNER and ROSEN 1972), the physical lengths of the largest and smallest chromosomes can be roughly estimated as 44 Mb and 6 Mb, respectively; thus, the largest *Xiphophorus* chromosome may be smaller than the smallest human chromosome. More importantly, the apparently small physical length per map unit in *Xiphophorus* (~0.3 Mb/cM) suggests that molecular analysis by physical techniques is well within current capabilities. Identification of particular genes by reverse genetic strategies thus could be considerably simpler in *Xiphophorus* than in mammalian species.

Many of the linkages listed in Table 5 have been demonstrated in multiple cross types, suggesting virtually no chromosome rearrangement among *Xiphophorus* species (but see the comments of KALLMAN and ATZ (1966) and KALLMAN (1971) concerning the existence of several unlinked macromelanophore loci in *X. cortezi*). Comparison of the *Xiphophorus* map with the five linkage groups identified in the confamilial genus *Poeciliopsis* (LESLIE 1982; MORIZOT, SCHULTZ and WELLS 1989) identifies homologues of *Xiphophorus* LGs I, II, and IV, the only groups with loci mapped in common. Extension of such comparisons to gene maps of salmonid fishes (JOHNSON, WRIGHT and MAY 1987; WRIGHT, JOHNSON and MAY 1987) identifies four probable homologues to *Xiphophorus* LGs I, II, IV and XII and no certain cases of linkage group divergence. Homology of salmonid LG 6 with *Xiphophorus* LG I can be predicted by comparison of each to LG I of *Lepomis* sunfishes (PASDAR, PHILIPP and WHITT 1984; WHEAT, WHITT and CHILDERS 1973).

The striking similarities among fish gene maps suggest substantial retention of primitive vertebrate gene arrangements during teleost evolution. Therefore, comparison of fish, amphibian, and mammalian gene maps promises to allow identification of plesiomorphic syntenic groups present in the ancestor of vertebrates. This hypothesis has been discussed in detail by MORIZOT (1990) and we will limit discussion here to new data confirming long term evolutionary conservation of specific autosomal segments. The linkage of a glucose phosphate isomerase locus to peptidase D in *Xiphophorus* LG IV, in *Poeciliopsis* (MORIZOT, SCHULTZ and WELLS 1989), salmonids (WRIGHT, JOHNSON and MAY 1987), and frogs of the genera *Rana* (WRIGHT and RICHARDS 1987) and *Xenopus* (GRAF 1989) identify homologues of a segment of human chromosome *19* conserved throughout vertebrate evolution. Human chromosome *15*, to which mitochondrial isocitrate dehydrogenase, sorbitol dehydrogenase, muscle pyruvate kinase, alpha-mannosidase A, and mannose phosphate isomerase have been assigned (MCALPINE *et al.* 1988), likewise has apparent homologues both in fishes and amphibians. In both poeciliids (composited from homologous *Xiphophorus* and *Poeciliopsis* linkage groups) and salmonids, mitochondrial isocitrate dehydrogenase and mannose phosphate isomerase genes are syntenic; in *Xiphophorus*, muscle pyruvate kinase also resides in LG II. Linkage group 3 of

the frog *Xenopus laevis* comprises mitochondrial isocitrate dehydrogenase, a mannose phosphate isomerase locus, and sorbitol dehydrogenase (GRAF 1989). Finally, in *Rana pipiens,* mannose phosphate isomerase is linked to an alpha-mannosidase locus (WRIGHT and RICHARDS 1987). Thus in amphibians or fishes linkages of five genes syntenic on human chromosome *15* are observed, indicating persistence of an ancestral vertebrate syntenic association.

Map locations of expressed gene duplicates in fishes suggest a further retention of ancestral chordate patterns produced by chromosome or chromosome set duplications. For example, duplicate isocitrate dehydrogenase genes (coding for mitochondrial and cytosolic isozymes), pyruvate kinase genes, and glucose phosphate isomerase genes are located in *Xiphophorus* LGs II and IV. *Xiphophorus* LG IV also contains a glutamate-oxaloacetate transaminase locus, as does LG 13 of salmonids, the homolog of *Xiphophorus* LG II. Distribution in "lower" vertebrates of syntenic associations of human chromosome *15* and *19* markers further suggest their origin by chromosome duplications: linkage of sorbitol dehydrogenase to glucose phosphate isomerase and peptidase D loci in salmonids (WRIGHT, JOHNSON and MAY 1987) and of sorbitol dehydrogenase to isocitrate dehydrogenase and mannose phosphate isomerase loci in *Xenopus* (GRAF 1989) exemplifies this type of nonrandom association. Lactate dehydrogenase and peptidase loci further evidence such associations: peptidase B in *Xenopus* is linked to mannose phosphate isomerase and mitochondrial isocitrate dehydrogenase; the apparently homologous linkage group in poeciliid and salmonid fishes contains lactate dehydrogenase A, while in ranid frogs lactate dehydrogenase B is syntenic with mannose phosphate isomerase. Alternately, lactate dehydrogenase B and peptidase B are syntenic in many mammals (*e.g.,* human chromosome *12*) and in salmonid fishes. An argument could be made that these apparently nonrandom associations arise from mistaken presumptions of homology across taxa, but to us this seems unlikely for well studied isozyme systems such as lactate dehydrogenase (MARKERT, SHAKLEE and WHITT 1975) and peptidases (FRICK 1983), or for apparently single gene coded isozymes such as sorbitol dehydrogenase and mannose phosphate isomerase. A second hypothesis (MORIZOT 1990) suggests that human chromosomes *11, 12, 15* and *19* arose by two duplications of an ancestral vertebrate chromosome (consistent with OHNO's (1970) hypothesis of multiple tetraploidization events in chordate lineages) and that translocations occur preferentially among ancestral homeologues. Such preferential translocations could account for the linkage in *Xiphophorus* LG XIII of triosephosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase loci to peptidase A and peptidase C and a glyceraldehyde-3-phosphate dehydrogenase locus in LG U3, rather than the frequently observed synteny of peptidase B and glyceralydehyde-3-phosphate dehydrogenase in mammals (*e.g.,* human chromosome *12,* cat syntenic group B4 and bovine group U3 (LALLEY *et al.* 1988)). Far too few data are as yet available to test such hypotheses convincingly, but mapping of more duplicated, expressed genes in fishes (such as glyceraldehyde-3-phosphate dehydrogenase-4 and triosephosphate isomerase-2) should provide new insights into primitive vertebrate genome organization. Regardless of whether evolutionarily conserved chromosome segments are maintained because of functional constraints (WAGNER, COX and SCHOEN 1985) or reflect historic variability in translocation frequencies, the fact that some segments have persisted throughout vertebrate evolution has been amply documented. The new problem to be addressed is estimation of the rates and types of chromosomal rearrangements which have occurred during the divergence of vertebrate lineages.

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