PROTEIN POLYMORPHISMS, SEGREGATION IN GENETIC CROSSES AND GENETIC DISTANCES AMONG FISHES OF THE GENUS XIPHOPHORUS (POECILIIDAE)

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ABSTRACT

The products of 49 protein-coding loci were examined by starch gel electrophoresis for populational variation in six species of Xiphophorus fishes and/or segregation in intra- and interspecific backcross and intercross hybrids. Electrophoretic variation was observed for 29 of the 35 locus products in a survey of 42 population samples. The highest frequency of polymorphic loci observed in noninbred populations was 0.143. After ten or more generations of inbreeding, all loci studied were monomorphic. Inbred strains generally exhibited the commonest electrophoretic alleles of the population from which they were derived. An assessment of genetic distances among Xiphophorus populations reflected classical systematic relationships and suggested incipient subspeciation between X. *maculatus* from different drainages as well as several species groups. Thirty-three loci were analyzed with respect to segregation in hybrids. The goodness of fit of segregations to Mendelian expectations at all loci analyzed (except loci in linkage group I) is interpreted as evidence for high genetic compatibility of the genomes of Xiphophorus species. It is anticipated that these data will result in a rapid expansion of the assignment of protein-coding loci to linkage groups in these lower vertebrate species.

POPULATIONAL and genetic analysis of pigment and meristic characters of platyfishes and swordtails (genus Xiphophorus, family Poeciliidae) has resulted in the grouping of these North and Central American viviparous bony fishes into eight species and numerous subspecies (ROSEN 1960). These species have relatively large ranges in Mexico and Central America (Figure 1). The fish have proven to be a model system for the study of a wide range of genetic, evolutionary, physiological and ecological problems (KALLMAN 1975).

Data concerning electrophoretic variation of proteins within the genus are less extensive. SCHOLL (1973) and SCHOLL and ANDERS (1973) studied nine inbred strains of four of the eight species for electrophoretic variation at 10 enzyme loci and concluded that much less variability between species existed in Xiphophorus than in other congeneric vertebrate species. However, SICILIANO *et al.*

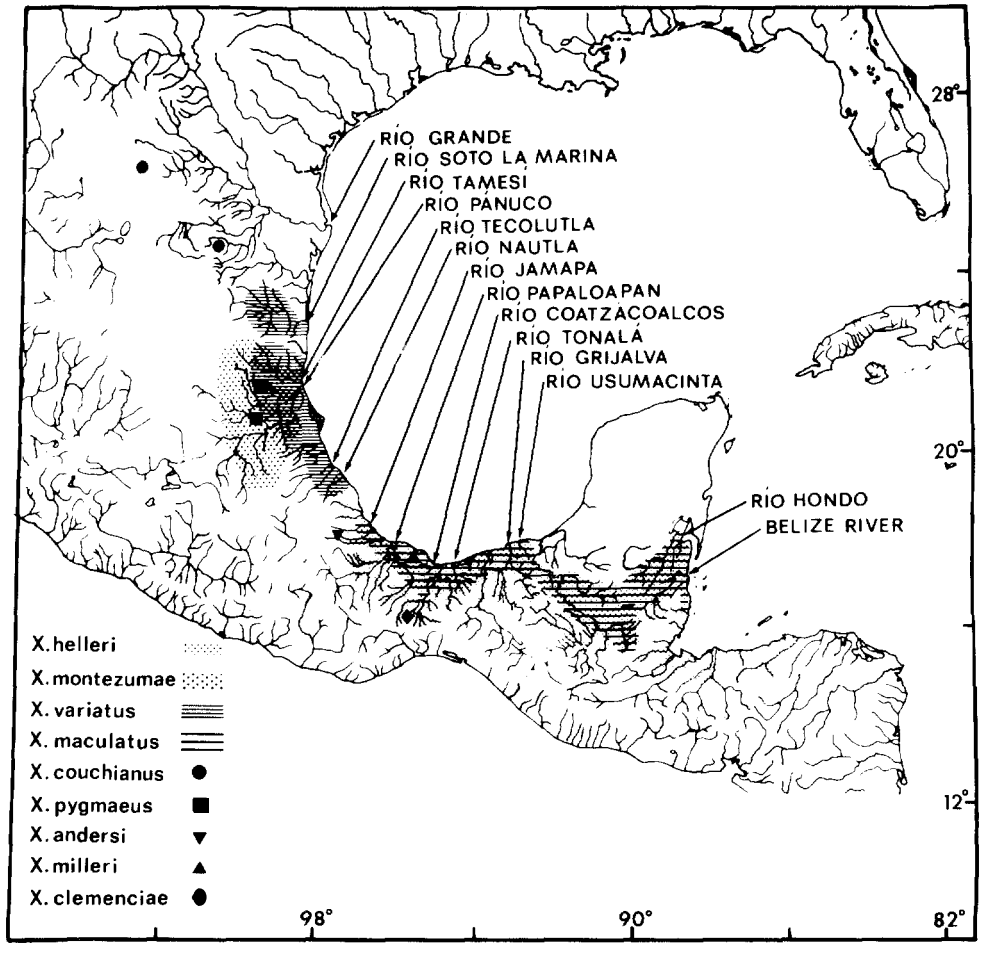

FIGURE 1.—Map of Mexico and adjacent parts of Central America showing distribution of fishes of the genus Xiphophorus. X. *maculatus* occurs considerably farther south of Belize River than indicated. Modified from a map obtained from DR. KLAUS KALLMAN of the New York Zoological Society and used with his permission.

(1973) studied 14 populations of three species of Xiphophorus for 24 protein-coding loci and noted substantial divergence between species as measured by genetic dissimilarity indices.

Perhaps the major impetus in assessing the linkage relationships of biochemical loci in Xiphophorus is the ability to utilize electrophoretic variants as markers to identify genes involved in the regulation of the severity of genetically controlled melanomas in hybrids. SICILIANO, MORIZOT and WRIGHT (1976) studied the relationships of melanotic severity in backcross hybrids with more than 20 segregating protein-coding loci and found a significant association with only the locus coding for esterase-1. AHUJA, SCHWAB and ANDERS (1980) confirmed this result. One of the major directions, then, of biochemical genetic studies of Xiphophorus populations has been to expand the number of markers that can

be used to detect regulatory genes important in genetically conditioned neoplasia.

Here we present the results of an electrophoretic survey of 42 Xiphophorus stocks of varying degrees of inbreeding, representing six of the nine species of the genus. Analysis of genetic distance relationships among the inbred strains and population samples reveals four clearly defined species groups. We also present data documenting normal segregation of electrophoretic alleles in intra- and interspecific hybrid crosses. The products of 49 protein-coding loci were analyzed in this study, of which 35 were utilized in the population survey and 33 were informative in the segregation analyses. These data provide a large series of potential markers for investigators performing genetic research using Xiphophorus strains, and they provide an extensive background for future studies assessing the linkage relationships of the many polymorphic genes thus far identified in these species.

MATERIALS AND METHODS

Animals used: The stocks, strains and pedigrees of Xiphophorus used in these studies are listed in Table 1. All stocks were originally obtained from DR. KLAUS KALLMAN of the New York Zoological Society with the exception of N27, N39, N48, N49 and N50, which were provided by DR. RICHARD BOROWSKY of New York University. Several general types of crosses were made, either by artificial insemination (CLARK 1950) or selected matings, to provide informative data for segregation analyses. Three cross types involved the production of interspecific F_1 hybrids between X. *helleri* or X. *clemenciae* (swordtails) and X. *maculatus* (platyfish): (1) *helleri* × *maculatus* F_1 hybrids backcrossed to *helleri* (HHM-BC); (2) *helleri* × *maculatus* F_1 hybrids backcrossed to *maculatus* (HMM-BC); and (3) intercrossed *helleri* × *maculatus* F_1 hybrids (HM-F_2). Two types of intraspecific crosses were made using F_1 hybrids produced by mating *maculatus* individuals from different river systems: (1) *maculatus* × *maculatus* F_1 hybrids backcrossed to one of the *maculatus* parents (MMM-BC) and (2) *maculatus* × *maculatus* F_1 hybrids intercrossed with each other (MM-F_2). Additionally, a few loci were found to be polymorphic within the parental stocks (designated P_1), providing in some cases nonhybrid segregation data. A few crosses were complex in yielding several different segregation patterns for the set of variant loci. Table 2 lists the F_1, F_2 and backcross broods obtained from the matings.

Sample preparation: Animals were sacrificed to obtain samples of blood, brain and eye, liver and skeletal muscle tissues. Sample preparation procedures are described in MORIZOT, WRIGHT and SICILIANO (1977) and MORIZOT and SICILIANO (1979).

Electrophoresis and histochemical staining: Supernatants were subjected to vertical starch gel electrophoresis as described in SICILIANO and SHAW (1976). Optimal electrophoretic conditions (such as buffer system, duration and electrical current of the run, and cofactor requirements) are summarized for each protein product in Table 3. Gels were run for 4–6.5 hr at 400 V, not exceeding 30 mA or 14–18 hr at 200 V 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 protein bands; after sufficient staining, the slices were photographed with a 35-mm camera. Recipes for stains follow SICILIANO and SHAW (1976) except for *GALT*, *PGK*, *UMPK* and *GUK*, which are in HARRIS and HOPKINSON (1977).

Data analysis: Since X. *maculatus* strain Jp 163 A is the most highly inbred line (maintained by brother-to-sister matings for more than 50 generations) and was a parental genome involved in almost every backcross and intercross, the electrophoretic mobilities of its proteins were used as standards. For each locus and for every cross or stock, the Jp 163 A allele is designed *a* and the protein produced considered to have a relative electrophoretic mobility (rm) of 1.00. The rms of the allele products of other stocks were calculated by: distance (in millimeters) 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 of segregation data, all allelic variants from Jp 163 A

TABLE 1

Inbred strains, stocks or pedigrees of fishes analyzed for electrophoretic variation

Taxon	Strain, stock or pedigree number	Drainage, country of origin	Year collected	Minimum number of generations of inbreeding when analyzed[a]
X. *clemenciae*	2826	Rio Coatzacoalcos, Mexico	1968	4
	2985	Rio Coatzacoalcos, Mexico	1968	5
	3258	Rio Coatzacoalcos, Mexico	1968	7
X. *h. helleri*	Cd	Rio Jamapa, Mexico	1932	25
X. *h. alvarezi*	2427	Rio Usumacinta, Guatemala	1968	1
	2288	Rio Usumacinta, Guatemala	1968	2
X. *h. guentheri*	Bx	Belize River, Belize	1949	25
	Gx	Rio Grijalva, Mexico	1951	27
	3062	Belize River drainage, Guatemala	1968	3
X. *h. signum*	Ch	Rio Usumacinta, Guatemala	1963	9
X. *h. strigatus*	3B	Rio Papaloapan, Mexico	1939	32
	501	Rio Coatzacoalcos, Mexico	1963	9
	2977	Rio Coatzacoalcos, Mexico	1968	5
X. *maculatus*	Jp 163 A	Rio Jamapa, Mexico	1939	35
	Jp 30	Rio Jamapa, Mexico	1939	54
	2852	Rio Jamapa, Mexico	1971	0
	2862	Rio Jamapa, Mexico	1971	0
	2891	Rio Jamapa, Mexico	1971	0
	Hp	Rio Hondo, Mexico	1954	26
	Cp	Rio Coatzacoalcos, Mexico	1948	28
	2848	Rio Coatzacoalcos, Mexico	1971	0
	2856	Rio Coatzacoalcos, Mexico	1971	0
	2940	Rio Coatzacoalcos, Mexico	1971	0
	2811	Belize River, Belize	1966	4
	3163	Belize River, Belize	1966	8
	2844	Rio Papaloapan, Mexico	1971	0
	2885	Rio Tonala, Mexico	1971	0
	3008	Rio Tonala, Mexico	1971	0
	2599	Rio Usumacinta, Mexico	1963	8
	3026	Rio Usumacinta, Mexico	1963	13
X. *m. montezumae*	2983	Rio Panuco, Mexico	1965	7
X. *m. cortezi*	38	Rio Panuco, Mexico	1939	24
X. *p. pygmaeus*	3046	Rio Panuco, Mexico	1972	0
X. *p. nigrensis*	3043	Rio Panuco, Mexico	1972	0
X. *v. variatus*	2984	Rio Tamesi, Mexico	1957, 1965	0
	3035	Rio Tamesi, Mexico	1957, 1965, 1968	1
	N27	Rio Tamesi, Mexico	1970	0
	N39	Rio Tamesi, Mexico	1970	0
	N48	Rio Tamesi, Mexico	1973	0
	N49	Rio Tamesi, Mexico	1973	
	N50	Rio Tamesi, Mexico	1973	
X. *v. xiphidium*	2822, 2857	Rio San Carlos X Rio Purificacion hybrids, Rio Soto la Marina drainage, Mexico	1965	8
	2910	Rio Purificacion, Rio Soto la Marina drainage, Mexico	1965	9

[a] Inbreeding usually was accomplished by either of two strategies, brother-to-sister matings or maintenance in closed colony. In a few cases, more complicated pedigree histories obtain: pedigree 2984, e.g., was derived from animals collected in 1957, inbred for 12 generations, hybridized with individuals collected in 1965 from a nearby river, and subsequently inbred for six generations before analysis. Such complex pedigrees are excluded from the analyses present in Figure 2.

TABLE 2

F_1, F_2 and backcross broods used in segregation analysis[a]

Brood numbers	Cross type	Female parent	Male parent
7, 58	F_1	X. *h. strigatus* 501	X. *maculatus* Jp 163 A
64, 67, 81, 84, 85			
87, 154, 155, 158	F_1	X. *maculatus* Jp 163 A	X. *h. strigatus* 501
77	F_1	X. *maculatus* Jp 163 A	X. *maculatus* Cp
82, 88	F_1	X. *maculatus* Jp 163 A	X. *h. helleri* Cd
86	F_1	X. *maculatus* Jp 163 A	X. *h. guentheri* 3062
93	F_1	X. *maculatus* Jp 163 A	X. *clemenciae* 2985
120	F_1	X. *maculatus* Jp 163 A	X. *maculatus* 3163
121	F_1	X. *h. strigatus* 501	X. *maculatus* 3163
3075	F_1	X. *maculatus* Jp 163 A	X. *maculatus* 2856
73, 74	F_2	F_1 67	F_1 64
89, 91	F_2	F_1 77	F_1 77
111	F_2	F_1 82	F_1 85
133	CCM-BC	F_1 93	X. *clemenciae* 3258
140, 143	Mixed[b]	MMM-BC 137	MMM-BC 137
142, 145	Mixed[b]	MMM-BC 135	MMM-BC 135
157, 172, 174, 161	HHM-BC	X. *h. strigatus* 501	F_1 87, 121, 154
160	MMM-BC	X. *maculatus* Jp 163 A	F_1 120
163, 175	HMM-BC	X. *maculatus* Jp 163 A	F_1 84, 158
164,[c] 165,[c] 181	HHM-BC	F_1 155	X. *h. strigatus* 501
164,[c] 165[c]	HMM-BC	F_1 155	X. *maculatus* Jp163A

[a] F_1 and F_2 are first and second filial generations, respectively. Backcrosses (-BC) are of three general types, HHM-BC (or CCM-BC), HMM-BC and MMM-BC, where H symbolizes X. *helleri*, M symbolizes X. *maculatus*, and C symbolizes X. *clemenciae*. Thus, HHM-BC describes a backcross obtained by mating a *maculatus* × *helleri* F_1 to a *helleri* parent, etc.

[b] 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. Similar mixed segregation patterns were obtained in crosses produced by mating two MMM-BC siblings.

[c] Broods from F_1 females that were inseminated with mixtures of X. *maculatus* and X. *helleri* sperm produced HHM-BC and HMM-BC offspring.

are referred to as the products of a *b* allele, since most crosses involved the Jp 163 A genome with only one other parental type.

Genotypic assignment of electrophoretic phenotypes was made assuming a codominant model of inheritance. In the survey of populational variation among Xiphophorus stocks, gene frequency estimates were calculated by gene counting. In the segregation analyses a chi-square goodness-of-fit was calculated against the expected backcross ratio under independent assortment of one homozygote to one heterozygote (MATHER 1957). The F_2 crosses were first tested for deviation from a 1*aa*:2*ab*:1*bb* expectation before inclusion with backcross data. Testing for the presence of segregation distortion was performed utilizing the criterion of statistical significance of heterogeneity chi-squares (MATHER 1957) calculated among cross types.

For electrophoretically separable products of multiple gene loci, isozymes are numbered from the most anodal to the most cathodal. Tissue specificities allowing homology comparisons of isozymes in other organisms are summarized in Table 3. Genetic distance estimates were calculated as in NEI (1972), and the estimates of *D* were clustered using an unweighted pair group (UPG) computer program obtained from DR. MASATOSHI NEI.

RESULTS

The products of 49 protein-coding loci were analyzed in this study (Table 3). Of these loci, 19 were examined for both population variation and segregation,

TABLE 3

Listing of 49 protein-coding loci of Xiphophorus used in the study, including parameters important to adequate electrophoretic resolution of allelic variation

Locus	Symbol	E.C.[a]	Tissue[b]	Buffer[c]
Acid phosphatase	*ACP*	3.1.3.2.	L	TC
Aconitase	*ACON*	4.2.1.3.	L	TC
Adenosine deaminase	*ADA*	3.5.4.4.	M, B&E, L	TVB or TC
Adenylate kinase	*AK*	2.7.4.3.	M	TC
Esterase-1	*ES1*	3.1.1.	B&E, M	TVB
Esterase-2	*ES2*	3.1.1.	Pl, B&E(M)	TC (TVB)
Esterase-3	*ES3*	3.1.1.	L (B&E, M)	TVB (TC)
Esterase-5	*ES5*	3.1.1.	L	TVB
Fumarase	*FUM*	4.2.1.2	M	TVB or TC
Galactose-1-phosphate uridyl transferase	*GALT*	2.7.7.12	L, B&E	TC (TVB)
α-Galactosidase	*αGAL*	3.2.1.22	L	TC
Glucose-6-phosphate dehydrogenase	*G6PD*	1.1.1.49	B&E (M, L)	TVB
Glucosephosphate isomerase-1	*GPI1*	5.3.1.9	M (B&E)	TVB
Glucosephosphate isomerase-2	*GPI2*	5.3.1.9	B&E, M, L	TVB
Glutamate-oxaloacetate transaminase-1	*GOT1*	2.6.1.1	B&E, M	TC (TVB)
Glutamate-oxaloacetate transaminase-2	*GOT2*	2.6.1.1	B&E, M, L	TC (TVB)
Glyceraldehyde-3-phosphate transaminase-2	*GOT2*	2.6.1.1	B&E, M, L	TC (TVB)
Glyceraldehyde-3-phosphate dehydrogenase-1	*GAPD1*	1.2.1.12	B&E, M	TVB
Glyceraldehyde-3-phosphate hydrogenase-2	*GAPD2*	1.2.1.12	M (L)	TVB or TC
Glycerate-2-dehydrogenase	*G2DH*	1.1.1.29	L	TC
α-Glycerophosphate dehydrogenase	*αGPD*	1.1.1.8	M	TC or TVB
Guanylate kinase-2	*GUK2*	2.7.4.8	B&E	TC
Isocitrate dehydrogenase-1	*IDH1*	1.1.1.42	L	TVB
Isocitrate dehydrogenase-2	*IDH2*	1.1.1.42	L	TVB
Isocitrate dehydrogenase-3	*IDH3*	1.1.1.42	B&E, M	TVB
Lactate dehydrogenase-1	*LDH1*	1.1.1.27	B&E	TC
Lactate dehydrogenase-2	*LDH2*	1.1.1.27	M (B&E)	TC
Lactate dehydrogenase-3	*LDH3*	1.1.1.27	L (B&E, M)	TC
Malate dehydrogenase-1	*MDH1*	1.1.1.37	M	TC
Malate dehydrogenase-2	*MDH2*	1.1.1.37	M (B&E)	TC
Malate dehydrogenase-3	*MDH3*	1.1.1.37	M	TC
Malic enzyme	*ME*	1.1.1.40	B&E, M	TVB
Mannosephosphate isomerase	*MPI*	5.3.1.8	M (B&E, L)	TVB (TC)
Muscle protein-1	*MP1*		M	TVB
Muscle protein-2	*MP2*		M	TVB
Muscle protein-3	*MP3*		M	TVB
Muscle protein-4	*MP4*	2.7.3.2	M	TVB (TC)
Muscle protein-5	*MP5*		M	TVB
Peptidase-2	*PEP2*	3.4.13	M (B&E, L)	TVB
Peptidase-3	*PEP3*	3.4.13	M (B&E, L)	TVB
Phosphoglucomutase	*PGM*	2.7.5.1	M (B&E, L)	TVB
Phosphoglycerate kinase	*PGK*	2.7.2.3	B&E, M, L	TVB (TC)
Phosphoglycerate mutase-1	*PGAM1*	2.7.5.3.	B&E, M, L	TVB or TC

TABLE 3—*Continued*

Locus	Symbol	E.C.[a]	Tissue[b]	Buffer[c]
Phosphoglycerate mutase-2	*PGAM2*	2.7.5.3.	M	TVB or TC
6-Phosphogluconate dehydrogenase	*6PGD*	1.1.1.44	B&E, M, L	TC
Superoxide dismutase-1	*SOD1*	1.15.1.1	B&E, L, M	TVB
Superoxide dismutase-2	*SOD2*	1.15.1.1	B&E, L, M	TVB
Transferrin	*Tf*		Pl (B&E)	TC
Triosephosphate isomerase-1	*TPI1*	5.3.1.1	B&E (M)	TVB
Uridine monophosphate kinase-1	*UMPK1*	2.7.4	B&E	TVB

[a] Numbers recommended by Commission on Biological Nomenclature ("Enzyme Nomenclature," Elsevier, Amsterdam, 1973).

[b] First entry is tissue of choice; if multiple entries separated by commas, all tissues listed provide adequate activity and resolution. Entries in parentheses are usable but less desirable tissues. Abbreviations: B&E = brain and eye; L = liver, M = skeletal muscle, Pl = blood plasma.

[c] Buffer compositions are described in SICILIANO and SHAW 1976. Abbreviations: TVB = Tris-Versene-borate, pH 8.0; TC = Tris-citrate, pH 7.0.

16 were utilized in the population study alone (usually because they were invariant in the crosses designed for segregation analysis), and 14 were utilized only in segregation analyses (primarily because the histochemical methods for visualizing the protein products were developed subsequent to the populational variation analyses).

Segregation of electrophoretic alleles in Xiphophorus crosses

Data concerning segregation of electrophoretic alleles in intra- and/or interspecific crosses were obtained for 33 protein-coding loci. Summaries of these data for each locus are presented in Table 4.

No segregation chi-square on the total data at any locus is significantly different from a one homozygote:one heterozygote backcross expectation at the 0.01 level of significance. Thus, the hypothesis that Mendelian segregation occurred at all 33 biochemical loci is not rejected. Such a result is of paramount importance when considering interspecific crosses, where particular chromosomal incompatibility could confer reduced viability upon some hybrid classes with accompanying segregation distortion. Sample sizes for the 33 loci range from 56 to 987 individuals, with only eight loci having $N < 145$.

Only three segregations yield chi-squares with associated probabilities of <0.10, which is no more than the number expected by chance. These results indicate that it is improbable that a minor segregation error caused by hybrid inviability occurs at more than a few of the 33 loci.

Although the general conclusion is unquestionable that Mendelian segregation is observed at most protein-coding loci in Xiphophorus hybrids, three rather highly deviant segregations deserve comment. First, in the case of *IDH2*, the observed heterogeneity between cross types is negligible, and it is suggestive that in every cross type a substantial excess of heterozygotes is observed. Whether a significant component of heterosis exists at this locus can only be answered through a considerably enlarged data base.

Second, a rather simple explanation exists for the large excess of homozygotes for *MP1* in intraspecific X. *maculatus* crosses. On many gels stained for *MP1*,

TABLE 4

Segregation analysis of loci in intraspecific and interspecific Xiphophorus crosses[a]

Locus	Cross type	Homozygotes	Heterozygotes	N	χ^2
ACP	MM-F_2, MMM-BC, HHM-BC, HMM-BC	104	86	190	1.71
	1 d.f. intraspecific—interspecific heterogeneity				0.37
ACON	HM-F_2, HHM-BC, HMM-BC	166	164	330	0.01
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.29
ADA	HM-F_2, MMM-BC, HHM-BC, HMM-BC	285	280	565	0.44
	1 d.f. intraspecific—interspecific heterogeneity				0.04
	1 d.f. HHM-BC—HMM-BC heterogeneity				10.15**
ES1	HM-F_2, HHM-BC, HMM-BC	175	178	353	0.03
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.06
ES2	HM-F_2, MMM-BC, HHM-BC, HMM-BC	293	329	622	2.08
	1 d.f. intraspecific—interspecific heterogeneity				0.16
	1 d.f. HHM-BC—HMM-BC heterogeneity				1.27
ES3	HM-F_2, MMM-BC, HHM-BC, HMM-BC	301	338	639	2.14
	1 d.f. intraspecific—interspecific heterogeneity				0.26
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.42
ES5	HHM-BC	33	29	62	0.26
GALT	HHM-BC	32	41	73	1.11
αGAL	HHM-BC, HMM-BC	101	106	207	0.12
	1 d.f. HHM-BC—HMM-BC heterogeneity				2.53
G6PD	HM-F_2, HHM-BC, HMM-BC	320	333	653	0.26
	1 d.f. HHM-BC—HMM-BC heterogeneity				9.11**
GPI1	HM-F_2, MM-F_2, MMM-BC, HHM-BC, HMM-BC	405	447	852	2.07
	1 d.f. intraspecific—interspecific heterogeneity				2.00
	1 d.f. HHM-BC—HMM-BC heterogeneity				1.01
GAPD1	HM-F_2, MM-F_2, MMM-BC, HHM-BC, HMM-BC	493	494	987	0.00
	1 d.f. intraspecific—interspecific heterogeneity				1.39
	1 d.f. HHM-BC—HMM-BC heterogeneity				4.32*
GAPD2	MM-F_2, MMM-BC	125	137	262	0.55
GUK2	HHM-BC	27	39	66	2.18
IDH1	HM-F_2, HHM-BC, HMM-BC	251	271	522	0.77
	1 d.f. HHM-BC—HMM-BC heterogeneity				1.60
IDH2	HM-F_2, HMM-BC, HHM-BC	300	354	654	4.46*
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.34
LDH1	HMM-BC, HHM-BC	135	132	267	0.04
	1 d.f. HHM-BC—HMM-BC heterogeneity				1.20
MDH2	HH-P_1, HM-F_2, HMM-BC, HHM-BC	208	210	418	0.01
	1 d.f. HHM-BC—HMM-BC heterogeneity				4.53*
MPI	MM-F_2, HM-F_2, MMM-BC, HMM-BC, HHM-BC	377	373	750	0.02
	1 d.f. interspecific—intraspecific heterogeneity				3.61
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.02
MP1	MM-F_2, MMM-BC	133	97	230	5.63*
MP2	HM-F_2, HMM-BC, HHM-BC	131	124	255	0.19
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.37
MP4	HMM-BC, HHM-BC	71	74	145	0.06
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.14
MP5	HMM-BC, HHM-BC	117	119	236	0.02
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.42
PEP2	HM-F_2, HMM-BC, HHM-BC	182	209	391	1.86
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.02

TABLE 4—*Continued*

Locus	Cross type	Homozygotes	Heterozygotes	N	χ^2
PEP3	MMM-BC, HMM-BC, HHM-BC	45	49	94	0.17
PGM	HM-F_2, HMM-BC, HHM-BC	297	313	610	0.42
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.18
PGK	HHM-BC	34	40	74	0.49
PGAM1	HHM-BC	28	32	60	0.27
PGAM2	HHM-BC	32	38	70	0.51
6PGD	MM-P_1, MM-F_2, MMM-BC, HM-F_2, HMM-BC, HHM-BC	400	458	858	3.92*
	1 d.f. intraspecific—interspecific heterogeneity				0.32
	1 d.f. HHM-BC—HMM-BC heterogeneity				1.65
Tf	HM-F_2, MMM-BC, HMM-BC, HHM-BC	256	274	530	0.48
	1 d.f. intraspecific—interspecific heterogeneity				1.15
	1 d.f. HHM-BC—HMM-BC heterogeneity				0.03
TPI1	HM-F_2, HMM-BC, HHM-BC	87	110	197	2.69
	1 d.f. HHM-BC—HMM-BC heterogeneity				3.70
UMPK1	HHM-BC	29	27	56	0.07

[a] Abbreviations and symbols are as follows: N = total number of individuals, d.f. = degrees of freedom. Cross-type symbols and locus name abbreviations are as explained in text.

* Probability that a given χ^2 is due to chance <0.05

** Probability that a given χ^2 is due to chance 0.01

the identification of heterozygotes has proven to be difficult: the protein is highly anodal in migration, and as a result, failure to separate the two bands in heterozygotes could produce misclassification in the absence of repeated reruns.

The third instance of a large deviation from the expected segregation ratio is at *6PGD*. Here, a more biologically relevant explanation seems to obtain. The linkage of *6PGD* with *ADA* and *G6PD* in Xiphophorus linkage group I in the gene order *ADA-G6PD-6PGD* has been established (MORIZOT, WRIGHT and SICILIANO 1977). When the segregations of these three loci are examined (Table 4), a significant pattern of segregation distortion emerges in interspecific backcrosses. An excess of swordtail alleles is observed that is highly significant at *ADA* and *G6PD* (as judged by heterogeneity chi-square). The swordtail allele excess is reduced or absent at *6PGD*. The simplest explanation for the observed heterogeneity is that, at a locus linked to *ADA* (or at the *ADA* locus itself) on the opposite side from the *G6PD* and *6PGD* loci, an allelic or chromosomal difference between swordtails and platyfish exists which in backcross hybrids confers enhanced viability on hybrids carrying more swordtail than platyfish alleles or, conversely, that a platyfish gene at the locus confers reduced viability on the hybrids. The lack of segregation distortion in intraspecific backcrosses between *maculatus* strains indicates a species-specific difference. When the data are pooled with respect to sex of the F_1 parent (Table 5), it appears that in both sexes a large excess of swordtail alleles are represented in the backcross progeny, although the sample sizes are as yet too small for unequivocal acceptance of this conclusion.

No other segregation exhibits significant heterogeneity in interspecific crosses. Thus, although one case of segregation distortion resulting from species-

TABLE 5

Electrophoretic alleles at 35 biochemical loci in Xiphophorus stocks

Locus and rm of allelic products[a]	Stock, strain or pedigree: 2826	2985	Cd	2288	2427	Bx	Gx	3062	Ch	3B	501	2977	Jp 163 A	Jp 30	2852	2862	2891	Hp	Cp	2848	2856	2940	2811	2844	2885	3008	2599	3026	2983	38	3046	3043	2984	3035	N27	N39	N48	N49	N50	2822	2857	2910
	Number of alleles observed																																									
ACP																																									
−1.00				6	8				2				30		6	16	1						6																			
−1.37	6	6	4				4	14			30	3																	16		16	20							2	6	6
−1.80						6				4		1		6	4		7	4	24	20	8	16	14	16	2	2	24	2		4			4	14	8	10	28	14	30			
AK																																									
1.00	—	4	2	6	8	6	—	2	2	2	10	2	20	—	—	—	—	2	16	4	—	—	—	—	—	—	—	—	16	4	6	—	4	8	8	10	28	14	30	2	6	6
ES1																																									
0.89								7																																	
0.93							4				30	14																													
0.96			4																																						
1.00				6	8	6		5	2	4			30	6	10		18	4	26	20	6	16	22	16		12	26	2	16	4	16	20	4	12	6		28	14	30	2	6	6
1.03	6	4														22					2				12											10						
ES2																																										
0.83	—							5						—							—	—				—				3	—										
0.86	—											2		—							—	—				—					—										
0.90	—					6		4						—							—	—				—	—		16		—									
0.94	—						2	1	1	4	2			—							—	—				—	—			3	—							2		6
0.96	—													—							—	—				—	—										1		
1.00	—	6		6	8				3				20	—	8	8	14		16	1	—	—	4	8	1	—	—	2		4		—			8	10	15	7		5	
1.05	—		4					2						—				2		9	—	—			1	—	—					—	4	14			13	7	30			
ES3																																									
0.88	—		—	—	—										—	—	—				—	—			—	—		—				—	2	3	4		3				
0.91	—		—	—	—										—	—	—				—	—			—	—		—	16	4		—								6
0.94	—		—	—	—	6				2	2	8			—	—	—				—	—			—	—		—				—						2	6	
1.00	—	2	—	—	—			2	4				30	2	—	—	—	2		10	—	—		8	—	—		—			6	—	2	9	4	10	25	14	30			
1.06	—	2	—	—	—		2								—	—	—		4		—	—	8		—	—		—				—									
1.11	—		—	—	—										—	—	—				—	—			—	—	24	—				—									
FUM																																									
0.69																													16												
0.90										4																															
1.00	6	4	4	6	8		4	14	2		30	14	30	6	10	22	18	4	26	14	8	14	22	16	12	12	26	2		4	16	20	4	12	6	10	28	14	30	2	6	6

[a] Locus and rm of allelic products

1.03						6																																				
1.11																				6	8	2																				
G6PD																																										
0.93																																								2	6	6
1.00	6	4	4							4			30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20	4	12	8	10	28	14	30			
1.08				6	8	6	4	14	2		30	14																														
GPI1																																										
0.51																										3																
0.71																																								2	6	6
0.76			4	6	8	6	4	14	2	4	30			6	10	21	18	4	26	20	16	16		1	11	9			16	4	16	20	4	12	6	10	28	14	30			
0.96	6	4																																								
1.00												14	30			1							22	15	1		26	2														
GPI2																																										
0.45															3	3																										
1.00	6	4	4			6	4	14	2	4	30	14	30	6	7	19	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20	4	12			28	14	30	2	6	6
1.55																																			6	10	10					
2.00				6	8																																					
GOT1																																										
1.00	—	2	2	6	8	6	2	6	2	—	4	2	14	2	—	—	—	—	—	4	—	2	—	2	—	2	—	—	18	4	10	6	—							2	6	—
1.44	—									—					—	—	—	—	—		—		—		—		—	—					—	4	6	10	28	12	30			—
GOT2																																										
1.00	—	2	4	6	8	6	2	6	2	2	4	2	14	2	—	—	—	2	—	4	—	2	—	2	—	2	—	—	18	4	10	6	—	4	6	10	26	12	30	2	6	—
GAPD1																																										
1.00													30	6	10	22	18							16					16	4	16	20	4	12	6	10	28	14	30	2	6	6
1.26	6	4	4	6	8	6	4	14	2	4	30	14						4	26	20	16	16	22		12	12	26	2														
GAPD2																																										
0.00																					4																					
1.00	6	4	4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	12	16	22	16	12	12	26	2	16	4	16	20	4	12	6	10	28	14	30	2	6	6
G2DH																																										
0.92		—		—	3	—	—	2	—			—				—	—				—	—			—			—					—		—	—				—	—	—
1.00	4	—	2	—		—	—		—	2	10	—	10	2	2	—	—	2	10	16	—	—	18	16	—	2	26	—	12	4	16	20	—		—	—				—	—	—
1.15		—		—	3	—	—		—			—				—	—				—	—			—			—					—		—	—				—	—	—
1.22		—		—		—	—		—			—				—	—				—	—			—			—	6				—	4	—	—	22	14	30	—	—	—
αGPD																																										
0.27																									—	2		—														
0.93																									—			—						10								
1.00	6	4	4	6	8	6	4	8	4	2	20	2	30	6	8	16	6	4	8	16	8	16	16	14	—		10	—	18	4	10	6	4		6	10	16	14	30	2	6	6
IDH1																																										
1.00	4	6		—	—		6						30	2	10	16	18	4	26	20	16	16	22	16	14	14	24	2	16	4	10	20								2	4	6
1.11			8	—	—			1	6	6	15	8																					4	14	8	10	24	14	28			
1.22					—	—	6		17			15	6																													
IDH2																																										
0.63	6	6	8				6	12				2											7																			
0.79				6	8	6		6	6	6	30	12																					4	14	8	10	25	12	18	2	4	6
0.91																																					3	2	2			

TABLE 5—Continued

Locus and rm of allelic products[a]	Stock, strain or pedigree / Number of alleles observed: 2826	2985	Cd	2288	2427	Bx	Gx	3062	Ch	3B	501	2977	Jp 163 A	Jp 30	2852	2862	2891	Hp	Cp	2848	2856	2940	2811	2844	2885	3008	2599	3026	2983	38	3046	3043	2984	3035	N27	N39	N48	N49	N50	2822	2857	2910
1.00													30	6	10	22	18	4	26	18	16	16	15	16	12	14	26	2	16	4	16	20										
IDH3																																										
1.00	6	6	8	6	8	6	6	18	6	6	30	14	30	6	10	22	18	4	26	18	16	16	22	16	12	14	26	2	16	4	16	20	4	14	8	10	28	12	18	2	4	6
LDH1																																										
0.95								14																						4												
1.00	6	4	4	6	8	6	4		2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16		16	20	4	12	8	10	28	14	30	2	6	6
LDH2																																										
1.00	6	4	4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20	4	12	8	10	28	14	30	2	6	6
LDH3																																										
1.00			4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16		4	12	8	10	28	14	30	2	6	6
1.05	6	4																																								
4.00																																20										
MDH1																																										
0.73																																				6						
0.79																																			8	4						
0.89																															6											
0.90																																8										
0.96																																							1			
0.99																																	4	12						2	6	
1.00	6	4	4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2		4	10	12					28	14	29		10	
1.14																													16													
MDH2																																										
0.69																															16	20										
0.71																													16													
0.72	6	4							2																																	
0.84											15	9																														
1.00			4	6	8	6	4	14		4	15	7	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2		4			4	12	8	10	28	14	30			
1.08																																								2	6	6
MDH3																																										
1.00	6	4	4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20	4	12	8	10	28	14	30	2	6	6
ME																																										
0.91				6	8	6																																				
0.94									2																																	
0.98	6	4																																								
1.00			4				4	14		4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20	4	12	6	10	28	14	30	2	6	6

MPI																																										
0.79	—			3	3		—		—					—	—	—	—		—		—	—	—	—	—		—	—					—									—
0.84	—						—		—					—	—	—	—		—		—	—	—	—	—		—	—	16				—									—
0.92	—	2	4	3	5	6	—	1	—	2	8	2		—	—	—	—	2	—	4	—	—	—	—	—	1	—	—			16	20	—	4	6	10	28	14	30	2	6	—
0.96	—						—		—					—	—	—	—		—		—	—	—	—	—		—	—		4			—									—
1.00	—						—	5	—				20	—	—	—	—		—		—	—	—	—	—	1	—	—					—									—
MP1																																										
0.74																													16	4												
0.76																																20										
0.78																															16											
0.97																			26	20	16	16			12	12																
1.00	6	4	4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4					22	16			26	2					4	12	8	10	28	14	30	2	6	6
MP2																																										
0.85			4																																							
0.88											30																															
1.00	6	4		6	8	6	4	14	2	4		14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20										
1.02																																	4	12	6	10	28	14	30	2	6	6
MP3																																										
0.97	6	4																																								
1.00			4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20	4	12	6	10	28	14	30	2	6	6
MP4																																										
0.88			4							4		14																														
0.92					8	6	4	14	2		30																															
1.00				6									30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2					4	12	6	10	28	14	30	2	6	6
1.07	6	4																											16	4	16	20										
MP5																																										
1.00	6	4	4	6	8	6	4		2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	9	4	16	20										
−1.40								14																																		
−1.92																													7				4	12	6	10	28	14	30	2	6	6
PGM																																										
0.88			4	6	8	6		14	2	4	30	14																	14	4												
1.00	6	4					4						30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2				20	4	12	6	10	28	14	30	2	6	6
1.18																															16											
6PGD																																										
0.89																					6																					
1.00													30	6	10	22	18	4	26	8	10	11	22	16	12	12	24	2			1											
1.06	6	4		6	8	6	2	14	2	4	30	14								10		5							16	4	15	20	4	12	6	10	28	14	30	2	6	6
1.12			6																																							
SOD1																																										
1.00	6	4	4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2	16	4	16	20	4	12	8	10	28	14	30	2	6	6
SOD2																																										
0.19																													16	4	16	17										
1.00	6	4	4	6	8	6	4	14	2	4	30	14	30	6	10	22	18	4	26	20	16	16	22	16	12	12	26	2				3	4	12	8	10	28	14	30	2	6	6

[a] Relative mobilities (rm) of allelic products compared to strain Jp 163 A, whose allelic products are considered to have an rm of 1.00.

specific genetic differences has been demonstrated, the notable lack of segregation distortion at most loci is convincing evidence for the genetic compatibility of the *maculatus* and *helleri* genomes.

Electrophoretic variation in Xiphophorus stocks

The results of the population variation survey of Xiphophorus stocks are presented in Table 5. *AK*, *GOT2*, *IDH3*, *LDH2*, *MDH3* and *SOD1* were monomorphic in all Xiphophorus stocks studied. Thus, 29 of the 35 loci surveyed for population variation exhibited electrophoretic variation within the genus.

The efficiency of inbreeding in producing strains homozygous for all protein-coding loci tested is well documented in these data. Variability within all inbred stocks has been eliminated by the 10th generation of inbreeding by brother-to-sister matings. Since no stock tested was polymorphic at more than five loci and since most noninbred stocks were even less polymorphic (Table 5), the achievement of substantial homozygosity by directed inbreeding with respect to the polymorphic loci could be accomplished quite rapidly.

Comparisons of several sets of stocks allow the assessment of allelic changes in closely related populations over time and/or the frequency of fixation of uncommon alleles by inbreeding. Two sets of populations are particularly informative in this regard (Table 5): (1) the Jp 163 A and Jp 30 strains compared to 2852, 2862 and 2891 stocks; (2) the Cp strain *vs.* 2848, 2856 and 2940 stocks. In the first case, that of X. *maculatus* from the Rio Jamapa drainage, the two inbred strains were derived from collections made in 1939; 2852, 2862 and 2891 are samples of offspring of Rio Jamapa fish collected in 1971. At 26 loci, both Jp 163 A and Jp 30 were fixed for the same allele monomorphic in the Jamapa populations collected more than 30 years later. In the 1971 collections, four loci (*ACP*, *ES1*, *GPI1*, *GPI2*) exhibited variation either within or between population samples. At two of these loci (*ES1*, *GPI2*) both inbred strains (Jp 163 A and Jp 30) had become fixed for the more common allele. At the other two loci, the two inbred strains had become fixed for different alleles: at *ACP*, both alleles are in high frequency in the noninbred populations; at *GPI1*, the rather rare 1.00 allele was fixed in Jp 163 A.

Similar results are obtained when the inbred strain Cp, derived from X. *maculatus* individuals taken from the Rio Coatzacoalcos in 1948, is compared with Rio Coatzacoalcos population samples (2848, 2856, 2940) collected in 1971. The Cp strain had become fixed for the same allele as the 1971 samples at the 24 monomorphic loci analyzed in all three populations. Of the four loci found to be polymorphic and analyzed in all three 1971 collections, the Cp strain had become fixed for the commonest allele.

Thus, in three inbred strains compared with syntopic population samples collected decades later, only one uncommon allele had become fixed among the total of 88 loci studied.

On this evidence, it can be concluded that inbred strains of Xiphophorus generally reflect the commonest electrophoretic alleles of the populations from which they were derived. Thus, when substantial electrophoretic variation is observed between two inbred strains of Xiphophorus species, it must be ex-

pected that similar levels of differentiation could be observed in natural populations, rather than being attributable to inbreeding artifacts.

Genetic distances among Xiphophorus populations

The dendrogram generated by UPG clustering of genetic distance estimates is presented in Figure 2. Several levels of genetic differentiation within and among Xiphophorus species are clearly exhibited. These parallel very closely the taxonomic relationships generally accepted by systematists. The evolutionary implications of the genetic distance analysis will be considered in DISCUSSION.

DISCUSSION

The survey of electrophoretic variation among Xiphophorus stocks has yielded results in disagreement with the conclusion of SCHOLL and ANDERS (1973) that genetic dissimilarity among Xiphophorus species is much lower than in other congeneric vertebrate species. The lowest genetic distance between any two Xiphophorus species in our data is ~0.23, and most estimates are considerably higher. This is in striking contrast to the results of SCHOLL and ANDERS (1973), who obtained many estimates of similarity between pairs of species of 0.90 or even 1.0. We have studied nine of the ten loci included in their analysis and have found substantial variability of *G6PD* (Table 5) that cannot be resolved on the buffer system used in the SCHOLL and ANDERS study. Due to the small number of loci studied by SCHOLL and ANDERS, the effects of undetected variability of even one locus were serious in overestimating genetic similarity.

At least three levels of genetic differentiation are identifiable in Figure 2. The first level reflects possible incipient subspeciation occurring within populations of X. *maculatus* sampled from the three major river drainages—the Jamapa-Papaloapan drainage in the West (stocks Jp 163 A, 2844, Jp 30, 2891, 2852 and 2862), the Usumacinta-Hondo-Belize drainage in the East (stocks 2811, 2599, 3026 and Hp), and the centrally located Coatzacoalcos-Tonala drainage (stocks 2848, 2940, Cp, 2856, 2885 and 3008). In general, stocks from within a drainage show low levels of dissimilarity ($P < 0.10$) and cluster with each other before clustering with members of any other drainage group. (The only exception among the 16 X. *maculatus* stocks sampled was 2862 from the Rio Jamapa. This stock, having been sampled early was not studied for five loci developed later in the program. It was also fixed for an *ES1* allele not seen in other Jamapa-Papaloapan fish. Results from it may, therefore, be attributed to sampling error.) These results imply true genetic differentiation between the fish from the three drainages. The presence of mutually exclusive alleles in the groups suggests little or no gene flow between them. We conclude that incipient subspeciation is occurring between X. *maculatus* drainage groups. This conclusion is consistent with the abnormal proliferation of pigment cells observed in hybrids between platyfish from the different drainages (GORDON 1951), since the degree of abnormal melanosis in hybrids is strongly correlated with genetic differences in parental populations (GORDON 1950).

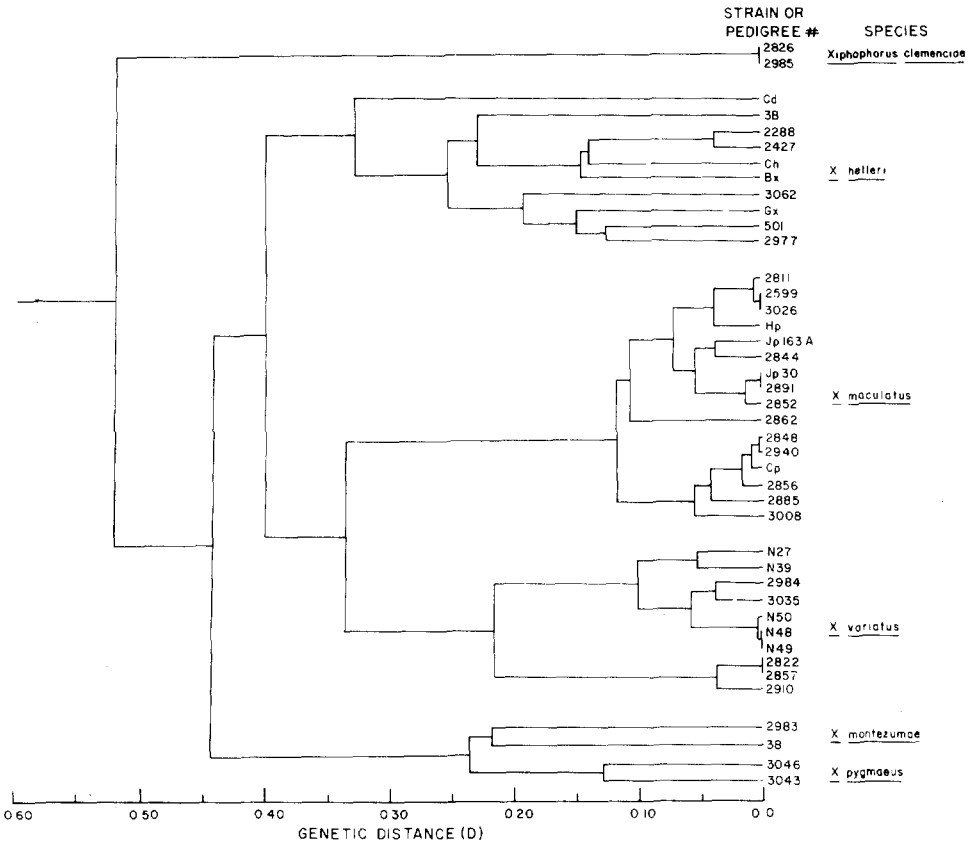

FIGURE 2.—Dendrogram generated by clustering of genetic distance estimates derived from the data in Table 4.

TABLE 6

Effect of sex on segregation distortion at ADA in Xiphophorus linkage group I[a]

Cross type	Sex of F_1 parent	Homozygotes	Heterozygotes	N	χ^2
HHM-BC	Male	82	63	145	2.49
HMM-BC	Male	12	24	36	4.00*
		94	87	181	0.27
		1 d.f. total heterogeneity			6.22*
HHM-BC	Female	48	30	78	4.15*
HMM-BC	Female	34	51	85	3.40
		82	81	163	0.01
		1 d.f. total heterogeneity			7.54*

[a] Symbols and abbreviations as in Table 4.

The second level of genetic differentiation observable in Figure 2 correlates well with the subspecies described using morphological analysis (ROSEN 1960). For example, X. *maculatus* and X. *clemenciae* are monotypic species, whereas two or more subspecies have been described for X. *variatus*, X. *montezumae*,

X. pygmaeus and X. *helleri*. The genetic distances between different subspecies range from ~0.13 in X. *pygmaeus* to ~0.32 in X. *helleri*. X. *helleri*, which has no less than five described subspecies, also exhibits by far the greatest genetic variability of any Xiphophorus species studied.

The third apparent level of genetic differentiation is the clustering of the Xiphophorus species studied into four well-defined species groups. X. *montezumae* and X. *pygmaeus* are swordtails that make up a species group with the lowest level of dissimilarity between species and appear to be as closely related as some X. *helleri* subspecies. The platyfish, X. *maculatus* and X. *variatus* appear to make up a species group that clusters at a dissimilarity level not different from the more distantly related X. *helleri* subspecies. The third and fourth species groups we recognize contain only one swordtail species each, X. *helleri* and X. *clemenciae*. We predict that the inclusion of more extensive samples from natural populations and of X. *milleri*, X. *couchianus* and the recently discovered and described X. *andersi* will strengthen these apparent relationships. In summary, the genetic distance relationships are fully consistent with the inclusion of all of the species studied into an individual genus Xiphophorus (ROSEN 1960), rather than the designation of the species of platyfishes and swordtails as separate subgenera or genera (SCHOLL and ANDERS 1973).

The data regarding segregation of electrophoretic alleles indicate the almost universal normal Mendelian segregation at the biochemical loci in inter- and intraspecific hybrids. This provides a large number of markers for use in establishing a detailed genetic map of the species. Since more than 10 loci known to exhibit variation in Xiphophorus stocks have not as yet been included in crosses informative for segregation and linkage, the rapid expansion of the linkage maps of these fishes is anticipated.

The segregation distortion observed at linkage group I loci differs from most other well-studied segregation distortion systems. Meiotic drive mechanisms of segregation distortion usually are limited to one or the other sex, such as the male-only distortion exhibited by the *SD* locus of Drosophila (CROW 1979). The *AR* locus in corn represents perhaps the single case of a meiotic drive mechanism operating in both sexes (ZIMMERING, SANDLER and NICOLETTI 1970). Thus, the question of whether a postfertilization viability difference or a meiotic drive mechanism is producing the Xiphophorus linkage group I segregation distortion is of fundamental genetic interest as an example of preferential transmission of species-specific alleles in hybrid offspring. Future experiments hopefully will establish the genetic mechanism for this case of segregation distortion.

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