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EVOLUTIONARY GENETICS OF DIPLOID—TETRAPLOID SPECIES OF TREEFROGS OF THE GENUS *HYLA*

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Several theories have been advanced to explain why polyploidy plays a lesser role in animal evolution than in that of plants. White (1973) favors the view that the prevalence of obligatory cross-fertilization is the principal barrier to bisexual polyploidy in animals. Bisexual polyploidy is unknown in amniote vertebrates, being precluded perhaps by the existence of well-developed chromosomal mechanisms of sex determination (Muller, 1925; Ohno, 1970; Jackson, 1976), but it has been a factor in the evolution of fishes and amphibians. Among amphibians, 12 bisexual polyploid species of anurans representing five families have been identified (Bogart and Tandy, 1976). Three of these are New World tetraploid species with morphologically similar diploid counterparts (Beçak et al., 1970; Wasserman, 1970; Bogart and Wasserman, 1972; Batistic et al., 1975).

The treefrog *Hyla versicolor* is tetraploid ($2n = 48$) and bisexual throughout its range in the eastern United States and Canada. Its distribution is largely allopatric with that of its diploid ($2n = 24$) sibling species *H. chrysoscelis* (Ralin, 1968, 1977; Bogart and Wasserman, 1972). As shown in Figure 1, *H. versicolor* occurs in the Northeast, the Appalachian region, the Great Lakes region, and in an area from southwestern Missouri south through eastern Oklahoma to south-central and eastern Texas and southwestern Louisiana. The range of *H. chrysoscelis* in the southern United States apparently is divided into western (central Texas) and eastern parts by the interposition of *H. versicolor*. The species are extensively sympatric in southern and extreme northwestern Wisconsin (Jaslow and Vogt, 1977), and they are known to occur together at localities in central Texas, southwestern Louisiana, eastern Oklahoma, Minnesota, Ohio, and Virginia, although the details of their distributions and the full extent of sympatry in these regions remain to be worked out.

The species differ in mean values of some morphological characters (Johnson, 1966; Ralin, 1968), but chromosome number, cell size, and the pulse rate of the mating call (corrected for temperature) are the only known characteristics by which individuals may reliably be identified (Ralin, 1968, 1976*b*, 1977; Bogart and Wasserman, 1972).

Diploid-tetraploid complexes like *H. chrysoscelis* and *H. versicolor* provide an opportunity to study the population genetic effects of relatively recent duplications of entire genomes. Here we survey electrophoretically demonstrable variation at structural gene loci and analyze phenotypic and inferred genotypic variation, frequency distributions of alleles, heterozygosity, and genetic similarity within and between populations. Hypotheses concerning the origin and evolution of the tetraploid species are considered, and the significance of polymorphism at structural gene loci in relation to the evolutionary potential provided by bisexual polyploidy is discussed.

MATERIALS AND METHODS

Collections made at 12 localities on various days from March through July, 1969, include a total of 370 specimens (Table 1), almost all of which were males. Species identifications of populations initially were made on the basis of mating call and later were confirmed by karyotypic analysis (Bogart and Wasserman, 1972). *H.*

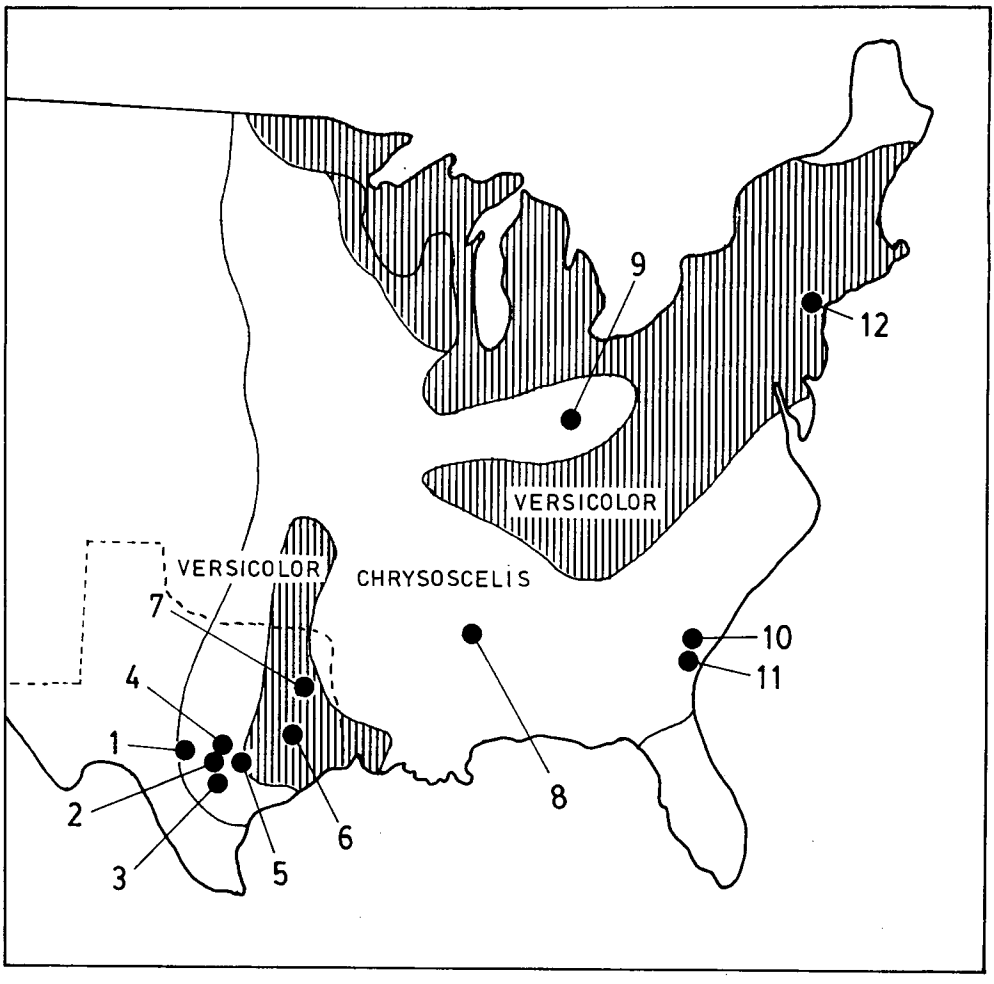

FIG. 1. Distribution of *Hyla chrysoscelis* (diploid) and *H. versicolor* (tetraploid), and locations of populations sampled. Numbered localities correspond to those listed in Table 1.

chrysoscelis was represented by 171 specimens from five populations in Texas, distributed from the western extremity of its range (locality 1) east through central Texas (2, 3, and 4) to an area of sympatry with *H. versicolor* at Bastrop (5); and by 67 specimens from four populations east of the Mississippi River (8, 9, 10, and 11). Populations of *H. versicolor* for which we have data are one sympatric with *H. chrysoscelis* in central Texas (locality 5), two in eastern Texas (6 and 7), and one in southeastern New York (12).

Starch-gel electrophoresis was performed on aqueous extracts of kidney, liver, and heart according to methods described by Selander et al. (1971). Twelve consistently scorable enzymes, each presumably encoded by a separate locus, were assayed: a phosphoglucomutase (*Pgm-2*), two isocitrate dehydrogenases (*Idh-1, Idh-2*), three malate dehydrogenases (*Mdh-1, Mdh-2, Mdh-3*), malic enzyme (*Me*), two lactate dehydrogenases (*Ldh-A, Ldh-B;* abbreviated in allele designations as *Lda* and *Ldb*), (cathodally migrating) phosphoglucose isomerase (*Pgi-2*), glutamic oxaloacetic transaminase (*Got-2*), and superoxide dismutase (*Sod-1*).

TABLE 1. *Collecting localities for* H. chrysoscelis *and* H. versicolor.

Code[1]	Locality	Species	Number of individuals
1	Texas: Fredericksburg, Gillespie County	*H. chrysoscelis*	30
2	Texas: Utley, Bastrop County	*H. chrysoscelis*	24
3	Texas: Palmetto State Park, Gonzales County	*H. chrysoscelis*	30
4	Texas: Elgin, Bastrop County	*H. chrysoscelis*	74
5	Texas: Bastrop State Park, Bastrop County	*H. chrysoscelis* *H. versicolor*	13 77
6	Texas: Sam Houston National Forest, Montgomery County	*H. versicolor*	47
7	Texas: Trawick, Nacogdoches County	*H. versicolor*	5
8	Mississippi: Starkville, Okitebbeha County	*H. chrysoscelis*	16
9	Ohio: Miami-Whitewater County Park, Hamilton County	*H. chrysoscelis*	15
10	South Carolina: Tillman, Jasper County	*H. chrysoscelis*	26
11	Georgia: Savannah area, Bryan and Chatham counties	*H. chrysoscelis*	10
12	New York: Harriman State Park, Rockland County	*H. versicolor*	3
		Total:	370

[1] See Figure 1.

Electromorphs of an enzyme were assigned numbers corresponding to their mobilities relative to the most anodal one (*100*). In designating tetraploid genotypes, we used superscripts to indicate the number of alleles presumed to be represented. Thus, for example, $(Sod\text{-}1^{58})^2(Sod\text{-}1^{95})^2$ indicates a tetraploid, heterozygous genotype of two each of the $Sod\text{-}1^{58}$ and $Sod\text{-}1^{95}$ alleles.

Unless otherwise noted, samples used in Chi-square tests of Hardy-Weinberg proportions of genotypic classes were those of *H. chrysoscelis* from Elgin (locality 4, $N = 74$) and *H. versicolor* from Bastrop (locality 5, $N = 77$). In these analyses, cells with expectations of less than five individuals were combined with adjacent ones.

Nei's (1972) coefficients of genetic iden-

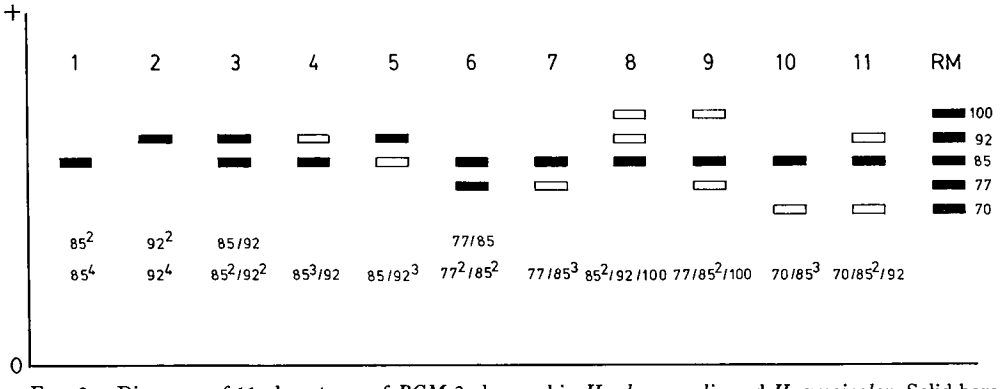

FIG. 2. Diagrams of 11 phenotypes of *PGM-2* observed in *H. chrysoscelis* and *H. versicolor*. Solid bars represent bands of greater intensity, hollow bars bands of lesser intensity. Relative mobility of electromorphs indicated in column RM. Presumed genotypes of *H. chrysoscelis* (upper row) and *H. versicolor* (lower row) indicated below phenotypes. Anode denoted by +, origin by O.

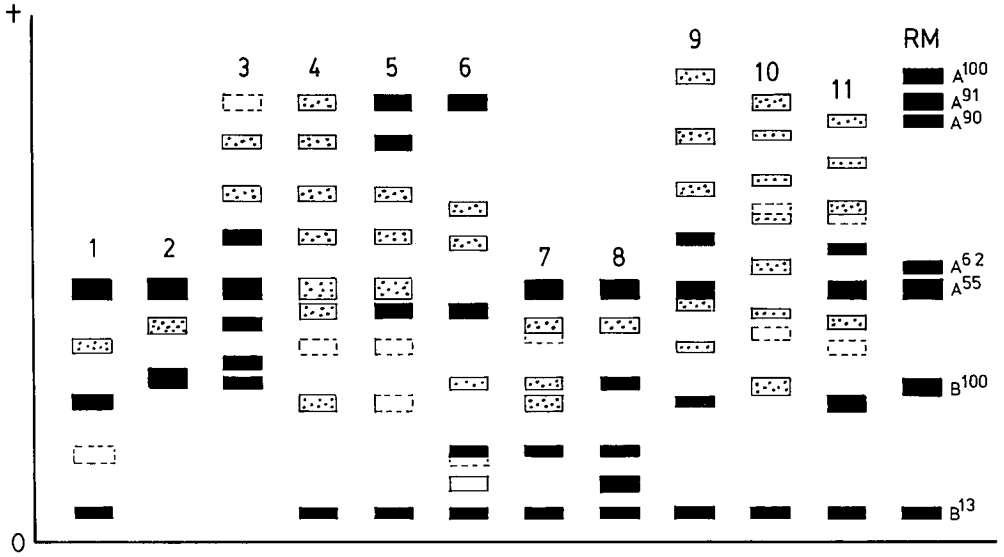

FIG. 3. Diagrams of common allozyme patterns of LDH (in kidney extracts) observed in *H. chrysoscelis* and *H. versicolor*. Solid, stippled, hollow, and dashed bars indicate decreasing intensity of staining. Relative mobility of *Lda* and *Ldb* homotetramers indicated in column RM. See text for details.

tity (or similarity) (I) and genetic distance (D) were calculated for pairs of populations.

RESULTS

Allelic and Genotypic Variation

Allozyme patterns of polymorphic enzymes in *H. chrysoscelis* were in all respects similar to those described for other diploid bisexual vertebrates. But electromorphs of heterozygotes in *H. versicolor* often were of quite different intensities, presumably reflecting tetraploid dosage effects (Figs. 2–4). We have therefore assumed that all individuals of *H. versicolor* carry and express four alleles at each locus. Allele frequencies are presented in Table 2.

Phosphoglucomutase.—Phenotypes and presumed genotypes at the *Pgm-2* locus are shown in Figure 2. Heterozygous individuals of *H. chrysoscelis* exhibited two bands of equal staining intensity (phenotypes 3 and 6); and the observed distribution of genotypes agreed with Hardy-Weinberg expectations, as calculated from the allele frequencies in Table 2 ($\chi^2_{(2)} = 0.34$; $P > .80$). Some heterozygous individuals of *H. versicolor* had a similar pattern, but in many the intensity of the two bands was unequal. We interpreted phenotypes 4, 5, and 7 as "asymmetrical" heterozygotes exhibiting different dosages of two alleles. Other heterozygotes of *H. versicolor* had three-banded patterns (phenotypes 8, 9, and 11), indicating the presence of three different alleles. In all cases, three-banded patterns involved a relatively rare allele, *Pgm-2*^{70} or *Pgm-2*^{100}, in combination with two of the more common alleles, *Pgm-2*^{77}, *Pgm-2*^{85}, and *Pgm-2*^{92}. The asymmetrical heterozygote (*Pgm-2*^{70}) (*Pgm-2*^{85})^3 also was observed (phenotype 10). Genotype frequencies matched Hardy-Weinberg expectations ($\chi^2_{(3)} = 6.08$; $0.20 > P > .05$).

Isocitrate dehydrogenases.—Two loci (*Idh-1* and *Idh-2*) encode isozymes of isocitrate dehydrogenase. Heterozygotes at either locus were three-banded, and the band of intermediate mobility (heterodimer) was more intense than either homodimeric band. At the *Idh-1* locus, all populations of *H. versicolor* were

TABLE 2. *Allele frequencies at twelve loci in populations of* H. chrysoscelis *and* H. versicolor.

Locus and allele		Population group and locality											
		Western *H. chrysoscelis*					*H. versicolor*				Eastern *H. chrysoscelis*			
		1	2	3	4	5	5	6	7	12	8	9	10	11
Pgm-2	*N* =	30	14	30	60	13	76	47	5	3	15	15	26	10
100							0.03	0.02	0.10					
92		0.02	0.04	0.10	0.17	0.11	0.20	0.23	0.15	0.25	0.13	0.20		0.05
85		0.97	0.93	0.90	0.75	0.85	0.75	0.71	0.65	0.75	0.83	0.73	1.00	0.95
77			0.04		0.08	0.04	0.02	0.02	0.10		0.03	0.07		
70								0.02						
Idh-1	*N* =	30	24	30	74	13	75	47	5	3	15	15	26	9
100		0.70	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	0.58	0.50
88											0.03		0.42	0.50
82		0.30												
Idh-2	*N* =	30	24	23	71	12	59	46	5	3	15	15	26	10
100													0.02	
71		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00
36													0.10	
Mdh-1	*N* =	30	24	30	74	13	77	47	5	3	15	15	26	10
100			0.17	0.02	0.14	0.19	0.01				0.03			
95							0.14	0.07	0.10					
83		1.00	0.83	0.96	0.84	0.73	0.85	0.92	0.85	1.00	0.97	1.00	0.98	1.00
66				0.02	0.03	0.08		0.01	0.05				0.02	
Mdh-2	*N* =	30	24	30	65	10	50	46	0	0	13	15	26	10
100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	—	1.00	1.00	1.00	1.00
Mdh-3	*N* =	30	17	0	11	3	1	2	0	0	15	15	26	10
100		1.00	1.00	—	1.00	1.00	1.00	1.00	—	—	1.00	1.00	1.00	1.00
Me	*N* =	30	24	15	60	10	31	4	0	0	13	15	12	10
100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	—	1.00	1.00	1.00	1.00
Lda	*N* =	30	24	30	74	13	77	47	5	3	15	15	26	10
100							0.01		0.15					
91							0.42	0.37	0.25	0.58	0.02			
90							0.04	0.02	0.20					
62							0.01			0.17				
55		1.00	1.00	1.00	1.00	1.00	0.52	0.61	0.40	0.25	0.98	1.00	1.00	1.00
Lbd	*N* =	30	24	30	74	13	77	47	5	3	15	15	26	10
100							0.44	0.54	0.67	1.00	1.00	1.00	1.00	1.00
13		1.00	1.00	1.00	1.00	1.00	0.56	0.46	0.33					
Pgi-2	*N* =	30	24	30	70	13	77	47	5	3	15	15	26	10
−100			0.02	0.02		0.04		0.01		0.17				
−71		1.00	0.92	0.95	0.93	0.85	0.90	0.90	1.00	0.67	1.00	1.00	0.96	0.95
−63			0.06	0.03	0.07	0.11	0.11	0.09		0.16			0.04	0.05
Got-2	*N* =	0	0	0	4	6	61	13	2	2	16	1	16	4
100		—	—	—			0.01			0.12	0.03		0.22	
56		—	—	—	1.00	1.00	0.98	1.00	1.00	0.88	0.97	1.00	0.78	1.00
30		—	—	—			0.01							
Sod-1	*N* =	30	24	30	70	13	77	47	5	3	14	15	26	10
100							0.01	0.03						
95							0.21	0.36	0.50		0.25	0.10		
79				0.07	0.08					0.50			0.12	0.30
58		1.00	1.00	0.93	0.92	1.00	0.77	0.60	0.50	0.50	0.75	0.90	0.88	0.70
52							0.01	0.01						

In calculations of *H* and percentage polymorphic loci, *Got-2* was assumed to be monomorphic in populations 1, 2, and 3; and *Mdh-2*, *Mdh-3*, and *Me* were assumed to be monomorphic in populations 3, 7, and 12.

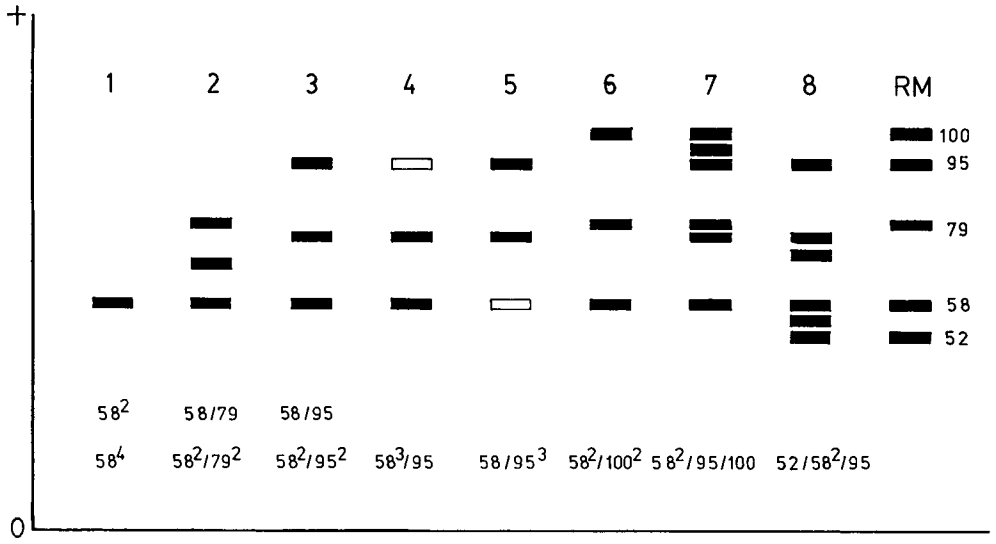

FIG. 4. Diagrams of 8 phenotypes of *SOD-1* observed in *H. chrysoscelis* and *H. versicolor.* Solid bars represent bands of greater intensity, hollow bars bands of lesser intensity. Relative mobility of homodimers indicated in column RM. Presumed genotypes of *H. chrysoscelis* (upper row) and *H. versicolor* (lower row) indicated below phenotypes. Anode denoted by +, origin by O.

monomorphic for $Idh\text{-}1^{100}$; but two additional alleles were detected in populations of *H. chrysoscelis* (Table 2). Frequencies of genotypes in the sample of *H. chrysoscelis* from Fredericksburg (locality 1) were consistent with Hardy-Weinberg expectations ($\chi^2_{(2)} = 1.28$; $P > .50$), as were those in the sample from South Carolina (locality 10) ($\chi^2_{(2)} = 3.50$; $P > .50$). The latter sample was the only one polymorphic at the *Idh-2* locus ($\chi^2_{(1)} = 0.04$; $P > .80$).

Malate dehydrogenases.—Of the three *Mdh* loci examined, only *Mdh-1* was polymorphic (Table 2). Heterozygotes of *H. chrysoscelis* were three-banded, and the intermediate (heterodimeric) band was the most intensely staining. For genotype frequencies, $\chi^2_{(4)} = 2.0$; $P > .50$. In *H. versicolor,* heterozygotes were either two-banded or three-banded, sharing the heterodimeric band and one or both of the homodimeric bands with the three-banded heterozygotes of *H. chrysoscelis*. We interpret the two-banded patterns as asymmetrical heterozygotes, and the three-banded patterns as symmetrical heterozygotes. A Chi-square test of four genotypic classes $(Mdh\text{-}1^{83})^4$, $(Mdh\text{-}1^{83})^3(Mdh\text{-}1^{95})$, $(Mdh\text{-}1^{83})^2(Mdh\text{-}1^{95})^2$, and "other" was not significant ($\chi^2_{(3)} = 4.60$; $P > .20$).

The *Mdh-2* and *Mdh-3* loci were monomorphic, each for the same allele, in both species.

Malic enzyme.—There was no variation at the *Me* locus in either species.

Lactate dehydrogenases.—In order to score the complex phenotypes of lactate dehydrogenase, it was necessary to electrophorese both kidney and liver extracts from each individual. Because kidney extracts of all specimens of *H. chrysoscelis* from the five central Texas localities exhibited a five-banded pattern (Fig. 3, phenotype 1), we infer that lactate dehydrogenase is a tetramer of subunits encoded by two loci, *Lda* and *Ldb,* as in other species of *Hyla* (Maxson and Wilson, 1974; Case et al., 1975). In zymograms of liver extracts, the most anodal band (number 1) was very intense, and bands 4 and 5 were absent; and in heart extracts, bands 1 and 2 were missing, and bands 3, 4, and 5 were intense.

Phenotype 2 of Figure 3 was observed

in 64 of 65 specimens of *H. chrysoscelis* from populations east of the Mississippi River; the corresponding genotype was interpreted as $(Lda^{55})^2/(Ldb^{100})^2$. The single exception, an individual from Mississippi (locality 8), had bands identical in mobility to those of phenotype 3, but all bands were equal in intensity; the presumed genotype was $(Lda^{55})(Lda^{91})/(Ldb^{100})^2$. All individuals of *H. chrysoscelis* from central Texas were of the genotype $(Lda^{55})^2/(Ldb^{13})^2$. Thus, western and eastern populations of *H. chrysoscelis* apparently are monomorphic for different *Ldb* alleles, but they are nearly monomorphic for the Lda^{55} allele (Table 2).

Some individuals of *H. versicolor* from the Texas populations also had phenotype 1 (Fig. 3) and, hence, were presumed to be of the genotype $(Lda^{55})^4/(Ldb^{13})^4$. Phenotype 3, which was observed in samples of *H. versicolor* from Texas and New York, was scored as an asymmetrical heterozygote at the *Lda* locus and a homozygote at the *Ldb* locus, represented genotypically as $(Lda^{55})^3(Lda^{91})/(Ldb^{100})^4$. Phenotypes 4 and 5 also were represented in samples of *H. versicolor* from Texas. Since the bands of these phenotypes were identical in mobility but differed in intensity, they were scored, respectively, as $(Lda^{55})^2(Lda^{91})^2/(Ldb^{13})^4$ and $(Lda^{55})(Lda^{91})^3/(Ldb^{13})^4$. Phenotype 6 was scored as $(Lda^{91})^4/(Ldb^{13})^3(Ldb^{100})$, and phenotypes 7 and 8 were interpreted as genotypes $(Lda^{55})^4/(Ldb^{13})^3(Ldb^{100})$ and $(Lda^{55})^4/(Ldb^{13})^2(Ldb^{100})^2$, respectively. Phenotypes 9, 10, and 11, which apparently represent one of three different minor alleles (Lda^{62}, Lda^{90}, and Lda^{100}) in combination with the two major alleles at the *Lda* locus, were scored genotypically as $(Lda^{55})^3(Lda^{100})/(Ldb^{13})^4$, $(Lda^{62})^2(Lda^{91})^2/(Ldb^{13})^4$, and $(Lda^{55})^3(Lda^{90})/(Ldb^{13})^4$, respectively.

By using liver extracts, we were able to make reasonably accurate estimates of the frequencies of the five *Lda* alleles in populations of *H. versicolor* (Table 2). A large number of genotypic classes is predicted by expansion of the expression $(p + q + r + s + t)^4$, but our Chi-square test was limited to the six genotypic classes $(Lda^{55})^4$, $(Lda^{55})^3(Lda^{91})$, $(Lda^{55})^2(Lda^{91})^2$, $(Lda^{55})(Lda^{91})^3$, $(Lda^{91})^4$, and "other" ($\chi^2_{(5)} = 4.17$; $P > .50$).

Because Ldb^{100}-Ldb^{13} heterotetramers and some *Lda-Ldb* heterotetramers have similar electrophoretic mobilities, we had difficulty scoring the *Ldb* phenotypes in all individuals. However, Ldb^{100} homozygotes were easily diagnosed in kidney extracts; and crude estimates of the frequencies of the Ldb^{100} allele were obtained (Table 2) by taking the fourth root of the frequency of Ldb^{100} homozygotes.

Phosphoglucose isomerase.—Heterozygotes were three-banded in *H. chrysoscelis*. Observed numbers of genotypes agreed with Hardy-Weinberg expectations ($\chi^2_{(1)} = 0.02$; $P > .50$). The Chi-square test for the Bastrop population of *H. versicolor* bordered on significance ($\chi^2_{(2)} = 6.01$; $P > .05$); but the genotypic proportions in the sample of *H. versicolor* from Sam Houston National Forest (locality 6) did not deviate significantly from Hardy-Weinberg expectations ($\chi^2_{(2)} = 0.07$; $P > .95$).

Glutamic oxaloacetic transaminase.—The slower migrating of two anodal *Got* isozymes, *Got-2,* was polymorphic. Heterozygotes of *H. chrysoscelis* were three-banded, and the heterodimeric band of intermediate mobility was the darkest. Observed proportions of genotypes in the South Carolina population reflected equilibrium ($\chi^2_{(1)} = 0.01$; $P > .80$). The few heterozygotes observed in samples of *H. versicolor* were two-banded, and of two types. Because the bands of one of these heterozygotes were identical in mobility to the second and third bands of heterozygotes of *H. chrysoscelis,* we assumed that it was an asymmetrical heterozygote of the genotype $(Got\text{-}2^{56})^3(Got\text{-}2^{100})$.

Superoxide dismutase.—The more anodal of the two *Sod* isozymes appeared as light bands on the blue background of gels stained for *Mdh* or *Ldh*. Phenotypes and inferred genotypes are shown in Figure 4. Heterozygotes of *H. chrysoscelis* were three-banded. For genotype frequencies, $\chi^2_{(2)} = 0.48$; $P > .50$. Heterozygotes of

TABLE 3. *Genetic distance* (D) *and identity* (I) *between pairs of populations of* H. chrysoscelis *and* H. versicolor.[a]

	H. chrysoscelis					*H. versicolor*			*H. chrysoscelis*			
	1	2	3	4	5	5	6	7	8	9	10	11
1	×	0.11	.009	.014	.016	.055	.069	.108	.108	.105	.116	.121
2	.989	×	.003	.003	.001	.045	.060	.098	.102	.099	.123	.131
3	.991	.998	×	.003	.005	.042	.054	.092	.098	.095	.119	.124
4	.986	.997	.997	×	.003	.043	.055	.093	.102	.097	.129	.132
5	.985	.999	.995	.998	×	.047	.062	.101	.107	.103	.131	.138
5	.946	.956	.959	.958	.954	×	.005	.021	.051	.052	.087	.089
6	.934	.942	.948	.946	.940	.995	×	.012	.036	.041	.079	.077
7	.898	.907	.912	.911	.904	.979	.988	×	.040	.049	.091	.087
8	.898	.903	.907	.903	.898	.951	.965	.961	×	.003	.025	.029
9	.900	.906	.910	.908	.902	.949	.960	.952	.997	×	.028	.033
10	.891	.884	.888	.879	.877	.917	.924	.913	.975	.972	×	.010
11	.886	.876	.884	.877	.871	.915	.926	.917	.972	.968	.991	×

[a] *D* values above diagonal; *I* values below diagonal.

H. versicolor differed in several respects from those of *H. chrysoscelis*. Phenotypes 3, 4, and 5 were interpreted as representing, respectively, the symmetrical heterozygote $(Sod\text{-}1^{58})^2(Sod\text{-}1^{95})^2$ and the asymmetrical heterozygotes $(Sod\text{-}1^{58})^3(Sod\text{-}1^{95})$ and $(Sod\text{-}1^{58})(Sod\text{-}1^{95})^3$. Additionally, several individuals from Texas exhibited six-banded patterns (phenotypes 7 and 8), presumably reflecting the presence of three different alleles: a relatively rare allele, $Sod\text{-}1^{52}$ or $Sod\text{-}1^{100}$, in combination with the common alleles $Sod\text{-}1^{58}$ and $Sod\text{-}1^{95}$. Genotypic frequencies were consistent with Hardy-Weinberg expectations ($\chi^2_{(3)} = 2.21$; $P > .50$).

Genetic Relationships

Coefficients of genetic distance (*D*) and similarity (*I*) between pairs of populations are presented in Table 3. To simplify matters by having all coefficients based on 12 loci, missing values in Table 2 were set equal to 1.00. (Populations of *H. chrysoscelis* at localities 1, 2, and 3 were assumed to be monomorphic for $Got\text{-}2^{56}$.) The population of *H. versicolor* from New York (locality 12), which was represented by only three specimens, was not included in this analysis.

The five western populations of *H. chrysoscelis* (localities 1–5) are genetically very similar although not homogeneous; $\bar{I}$ for paired samples over 12 loci is 0.994. The most notable variation is the unique occurrence of the $Idh\text{-}1^{82}$ allele in population 1 (Table 2). Variation in allele frequencies among populations also occurs at the *Pgm-2, Pgi-2, Mdh-1,* and *Sod-1* loci. Samples of the eastern populations of *H. chrysoscelis* are slightly less uniform ($\bar{I} = 0.979$) than those of the western populations, but understandably so, since they represent a much larger geographic area. The level of heterogeneity among populations of *H. versicolor* in Texas ($\bar{I} = 0.987$) is similar to that among western populations of *H. chrysoscelis*.

For comparisons of western and eastern populations of *H. chrysoscelis*, $\bar{I} = 0.892$ ($\bar{D} = 0.114$). The most marked difference involved the *Ldb* locus, at which alternative alleles apparently are fixed in the eastern and western populations (Table 2). Other differences include the presence of $Idh\text{-}1^{88}$, $Got\text{-}2^{100}$, and $Sod\text{-}1^{95}$ in eastern *H. chrysoscelis* and their absence in western populations, and the apparent absence of $Pgi\text{-}2^{100}$ in eastern populations.

In mean genetic character, southern populations of *H. versicolor* are almost precisely intermediate between eastern and western populations of *H. chrysoscelis,* the mean coefficients of similarity being 0.938 and 0.934, respectively. At the *Ldb* locus, *H. versicolor* is polymorphic for the two alleles that are alternatively fixed in eastern and western *H.*

TABLE 4. *Genic variation in* H. chrysoscelis *(C) and* H. versicolor *(V).*

Locus	Proportion of populations polymorphic[a]		Average genic heterozygosity/population		Mean number of alleles/population		Number of unique alleles[b]	
	C	V	C	V	C	V	C	V
Pgm-2	.89	1.00	.206	.732	2.4	3.7	0	2
Idh-1	.44	.00	.164	.000	1.4	1.0	2	0
Idh-2	.11	.00	.023	.000	1.2	1.0	1	0
Mdh-1	.67	.75	.127	.310	2.0	2.5	0	1
Mdh-2	.00	.00	.000	.000	1.0	1.0	0	0
Mdh-3	.00	.00	.000	.000	1.0	1.0	0	0
Me	.00	.00	.000	.000	1.0	1.0	0	0
Lda	.11	1.00	.002	.894	1.1	3.7	0	3
Ldb	.00	.75	.000	.630	1.0	1.8	0	0
Pgi-2	.67	.75	.090	.372	2.0	2.3	0	0
Got-2	.33	.50	.066	.119	1.2	1.8	0	1
Sod-1	.67	1.00	.161	.812	1.7	3.0	0	2
Means								
All loci	.324	.479	.068	.322	1.43	1.98	.25	.75
Polymorphic loci	.432	.639	—	—	1.57	2.31	.33	1.00

[a] A locus is considered polymorphic if the frequency of the common allele ≤0.99.
[b] Alleles found exclusively in populations of one species.

chrysoscelis; and at most other loci (*Idh-1* is exceptional), alleles occurring commonly in either or both groups of populations of *H. chrysoscelis* also are represented in *H. versicolor.* Hence, its general genetic character is that of a hybrid incorporating alleles from two sources. However, nine alleles occurring in low (or, in one case, moderate) frequency at five loci (*Pgm-2, Mdh-1, Lda, Got-2,* and *Sod-1*) in *H. versicolor* were not represented in samples of *H. chrysoscelis.*

Genic Variability

In Table 4, the species are compared with respect to four measures of genic variability. Mean values for all measures are greater for *H. versicolor* than for *H. chrysoscelis.* Only in *Idh-1* and *Idh-2* does *H. chrysoscelis* exceed *H. versicolor* in proportion of populations polymorphic (P); and mean values over all loci are 0.479 and 0.324 for the two species, respectively. Average genic heterozygosity ($\bar{H}$) is nearly five times greater in *H. versicolor;* major contributions to this increased variability are made by the *Lda* and *Ldb* loci, but heterozygosity also is greater in *H. versicolor* at five of the seven other polymorphic loci. *Idh-1* and *Idh-2* again are exceptional, being weakly polymorphic in *H. chrysoscelis* and monomorphic in *H. versicolor.* $\bar{P} = 0.27$ and $\bar{H} = 0.057$ for the five western populations, and $\bar{P} = 0.38$ and $\bar{H} = 0.083$ for the four eastern populations of *H. chrysoscelis.* Hence, the eastern populations may be, on the average, slightly more variable than those at or near the western limit of the range of the species.

DISCUSSION

Origin and Differentiation of the Tetraploid

Because of the generally intermediate genetic character of the tetraploid *H. versicolor,* any attempt to understand its origin inevitably must focus on the problem of the evolutionary history and present relationship of eastern and western populations of the diploid *H. chrysoscelis.* Recently, Maxson et al. (1977) applied the quantitative immunological technique of micro-complement fixation to serum albumins of *H. chrysoscelis* in an effort to determine the time of divergence. When tested against antiserum prepared to albumin from an individual of *H. chrysoscelis* from Mississippi, albumins of 15 *H.*

chrysoscelis from Ohio, Mississippi, Georgia, and South Carolina yielded an average immunological distance of 0.4 ± 0.7 units (*IDU*) and were considered to be identical. But an average distance of 4.5 ± 1.7 *IDU* was obtained when these albumins were reacted with antiserum prepared from the albumin of an individual of *H. chrysoscelis* from Bastrop County, Texas, representing the western population. Reciprocal tests involving 18 *H. chrysoscelis* from Bastrop, Gonzales, and Gillespie counties, Texas, gave corresponding distances of 0.9 ± 0.9 and 9.3 ± 1.5 *IDU*. According to Maxson et al. (1977), "the deviation from perfect reciprocity, 9 and 5 *IDU* respectively, is attributable to a different number of amino acid substitutions in the albumins of the eastern and western lineages since they last shared a common ancestor." By taking an average distance of 7 *IDU* for the eastern and western populations of *H. chrysoscelis* and assuming that the mean evolutionary rate of albumin is 1 *IDU* per 0.6 million years, Maxson et al. (1977) estimated that these populations diverged roughly four million years ago.

Using the approach developed by Nei (1975), we can estimate time of divergence of eastern and western *H. chrysoscelis* from the mean genetic distance between pairs of populations. This value is $\bar{D} = 0.114$, which yields an estimate of some 570,000 years, by the formula $t = 5 \times 10^6 \bar{D}$. However, because all 12 enzymes that we studied belong to the slowly evolving group defined by Sarich (1977), an adjustment based on his calibration of "electrophoretic clocks" is required. The adjusted estimate is three and one-half million years.

Our genetic data clearly suggest that southern populations of *H. versicolor* evolved from *H. chrysoscelis* after the latter had differentiated geographically into eastern and western groups of populations. One of several plausible evolutionary schemes postulates a disjunction in the distribution of *H. chrysoscelis,* followed by secondary contact of differentiated populations (Ralin, 1977). But, of course, the geographic differentiation in *H. chrysoscelis* may have developed in situ, without geographic isolation (see discussion in Endler, 1977). In any case, our data suggest that variation from west to east in *H. chrysoscelis* is clinal, for the two easternmost populations of *H. chrysoscelis* (localities 10 and 11, in South Carolina and Georgia) are less similar to western *H. chrysoscelis* than are those in Mississippi (8) and Ohio (9) (see Table 3). Populations of *H. chrysoscelis* in extreme eastern Texas, Louisiana, Arkansas, and western Mississippi (and, perhaps, northward into Missouri and Illinois) may be truly intermediate, with allele frequencies resembling those of the southern populations of *H. versicolor.* The two individuals of *H. chrysoscelis* from extreme eastern Texas identified as "hybrids" by Maxson et al. (1977) were more probably heterozygotes in a population segregating for albumin alleles that may be fixed in western and eastern populations of that species (see critique by Ralin, 1978).

Because of uncertainties regarding the evolutionary and distributional history of *H. chrysoscelis* and patterns of geographic variation in allele frequencies among its populations, it does not seem possible to decide whether southern *H. versicolor* arose through allopolyploidy or autopolyploidy, or whether, in either case, contemporary populations have descended from one, several, or many individuals that developed tetraploidy. About all we can conclude is that *H. versicolor* carries most of the alleles represented in its progenitor species, but that none of the populations of *H. chrysoscelis* we have sampled has a genetic composition that would be expected in a population directly ancestral to the southern population of *H. versicolor.*

Curiously, genetic similarity between western *H. chrysoscelis* and *H. versicolor* increases from east to west, being .905, .942, and .955, respectively, for localities 7, 6, and 5. Because similarity is greatest where the species occur together (locality 5), we have considered the possibility that introgression is occurring as a result of

hybridization. If triploids were formed in nature by hybridization and produced unreduced eggs, fertilization by haploid sperm could reconstitute the tetraploid condition. Thus, triploid hybrids could serve as a one-way bridge for gene flow from the diploid to the tetraploid. Zohary and Nur (1959) have described such a situation in the orchard grass *Dactylis glomerula;* but for the species of *Hyla,* gene exchange is unlikely for two reasons. First, hybridization would be expected to occur only rarely because of differences in male mating calls and the associated discriminative abilities of the females. Among hundreds of individuals that have been examined karyotypically, triploids have not been found. Second, even if triploids occasionally were formed, there would be little chance of their reproducing, since studies of laboratory crosses have demonstrated severely reduced viability in F_1 hybrids (Johnson, 1959; Ralin 1976*a*) and, more importantly, complete mortality of backcross progeny before the 15th day of development (Johnson, 1963).

Whatever may have been the chronology of differentiation of *H. versicolor* and *H. chrysoscelis,* our analysis indicates that differences between them at the level of the structural gene are relatively minor. There is greater genetic similarity between the southern population of *H. versicolor* and either the western or eastern populations of *H. chrysoscelis* than between the two groups of populations of *H. chrysoscelis*. The reduced viability of *H. chrysoscelis* × *H. versicolor* hybrids (50% mortality before hatching stage) and the 100% mortality in the backcrosses to either parental species observed in laboratory breeding experiments (Johnson, 1959, 1963; Ralin, 1976*a*) probably reflect disruption of developmental regulation caused by gene-dosage imbalances in the triploid F_1 and the aneuploid backcross progeny (Ralin, 1976*a*; Bogart and Wasserman, 1972). In any event, the behavioral differentiation and the reproductive iolation resulting from developmental incompatibility of *H. versicolor* and *H. chrysoscelis* need not be viewed as inconsistent (Ralin, 1977) with their relatively close genetic and morphological similarity (Johnson, 1966). Blair (1965) suggested that speciation occurred prior to the Wisconsin glacial period of the Pleistocene, roughly 150,000 years ago, but, of course, there is no evidence ruling out the possibility of a more recent origin for *H. versicolor.* Because the albumins of southern *H. versicolor* were immunologically indistinguishable from those of western and eastern populations of *H. chrysoscelis,* Maxson et al. (1977) concluded that *H. versicolor* evolved rather recently.

Until the northern populations of *H. versicolor* are adequately characterized genetically, we will have no firm basis for deciding whether they were derived independently from an eastern population of *H. chrysoscelis* or are phylogenetically more closely related to southern *H. versicolor.* The data for our small sample from New York (Table 2) are not incompatible with either hypothesis.

Genic Variability and Polyploidy

Measures of genic variability for *H. chrysoscelis* are within the normal range reported for other small vertebrates (Selander and Johnson, 1973; Powell, 1975; Selander, 1976). Mean heterozygosity ($\bar{H}$ = 0.068) is similar to that reported for the Pacific treefrog, *Hyla regilla* (Case et al., 1975); and mean numbers of alleles per locus per population also are similar in *H. chrysoscelis* and *H. regilla* (1.43 vs. 1.50, as calculated from data presented by Case et al., 1975).

The tetraploid, *H. versicolor,* is much more variable than its diploid ancestor. The mean $\bar{H}$ value of 0.322 estimated for *H. versicolor* is well beyond the normal range for vertebrates, being, in fact, as high as any yet reported for bisexual animal species (Powell, 1975; Selander, 1976). (Average heterozygosity in the sexual diploid beetle *Otiorrhynchus scaber* reportedly is 0.304 [Suomalainen and Saura, 1973].)

The high heterozygosity of *H. versicolor* relative to that of its diploid sibling species is attributable to two factors.

First, *H. versicolor* carries a larger number of alleles per locus as a result of the previously discussed geographical-historical events peculiar to the species complex. The geographic differentiation of *H. chrysoscelis* into eastern and western population groups involves alleles at several loci (*Sod-1*^{95}, *Pgi-2*$^{-100}$, *Got-2*^{100}, *Lda*^{91}, and *Ldb*^{100} and *Ldb*^{13}) that reach polymoprhic frequencies in only one of the two diploid groups and in *H. versicolor*. The increased heterozygosity of allopolyploid species of plants (Gottlieb, 1976) and parthenogenetic lizards of hybrid origin (Parker and Selander, 1976) relative to their diploid ancestors is an analogous situation. In *H. versicolor,* we did not find "fixed heterozygosity" at any locus. *H. versicolor* forms quadrivalents during metaphase of meiosis (Bogart and Wasserman, 1972), in contrast to the situation in many allopolyploid plants, in which fixed heterozygosity is maintained by "preferential" pairing of chromosomes in meiosis (Gottlieb, 1976). If, as suggested earlier, some of the alleles in *H. versicolor* are present in polymorphic frequencies in populations of *H. chrysoscelis* as yet unsampled in extreme eastern Texas, Louisiana, and western Mississippi, genic heterozygosity in populations in these areas may be somewhat higher than in those of *H. chrysoscelis* we have sampled.

A second and relatively minor cause of increased genic variability in *H. versicolor* relative to *H. chrysoscelis* is the presence of nine apparently unique alleles at five loci in the tetraploid (Tables 2 and 4). And it is this factor that holds greater interest in relation to the evolutionary significance of genic variation in natural populations. The pattern of occurrence of unique alleles over loci in *H. versicolor* is as follows: Six of the nine alleles occur at loci that are polymorphic in both eastern and western populations of *H. chrysoscelis* (*Pgm-2, Mdh-1* and *Sod-1*) or in eastern *H. chrysoscelis* alone (*Got-1*). The other three were detected at the *Lda* locus, which is monomorphic in western *H. chrysoscelis* and apparently only weakly polymorphic in eastern *H. chrysoscelis*. Unique alleles were not found at three loci (*Mdh-2, Mdh-3,* and *Me*) that are monomorphic in *H. chrysoscelis* or at two loci that are strongly (*Idh-1*) or weakly (*Idh-2*) polymorphic in either western or eastern *H. chrysoscelis*.

What is the origin of the "unique" alleles in *H. versicolor*? If we assume that they are not represented in populations of *H. chrysoscelis* that we have not sampled, they must represent either new mutations (or possibly, intra-cistronic recombinations) that occurred in *H. versicolor,* or alleles derived from a common ancestor of contemporary populations of *H. versicolor* and *H. chrysoscelis* that have not persisted in the latter species.

It has been suggested that, following gene duplication, one locus of the pair is free to accumulate new mutations and regulatory modifications because the other member maintains its original function and thus reduces the selective constraints normally imposed on a single gene-enzyme locus (Ohno, 1970; Avise and Kitto, 1973; Markert et al., 1975). This hypothesis predicts that, in the early stages of this process, duplicated loci will be more variable than unduplicated ones because new and initially deleterious mutations are shielded from the effects of selection. The mean number of unique alleles per locus is much larger in *H. versicolor* than in *H. chrysoscelis* (Table 4). Similarly, the South American tetraploid species of ceratophrydid frog *Odontophrynus americanus* is polymorphic for two alleles at an albumin locus, while its congeneric diploid relative *O. cultripes* is monomorphic (Beçak et al., 1968). It seems reasonable to assume that similar mutations occur in equivalent frequencies per locus in the diploid, but that selection maintains their frequencies at lower levels. In populations of *H. versicolor,* the probability of observing adult individuals carrying three of four presumed "mutant" alleles at any one polymorphic locus is very low (see allele frequencies in Table 2). But the accumulation of deleterious alleles in the tetraploid would produce a significant genetic load. Perhaps this is why mortality of in vitro

control crosses of *H. versicolor* is two to three times higher than in control crosses of *H. chrysoscelis* and other diploid North American hylids (Ralin, 1976*a*).

The very high level of genic variability in *H. versicolor* may contribute to its success, even though differential specialization of duplicate genes, which ultimately may be one of the major advantages of polyploidy, probably has not yet occurred to a great extent if at all. Gottlieb (1976) discusses a number of different, but not necessarily exclusive, selectionist models linking enzyme variability to enhanced adaptation in allopolyploid plant species. These include heterotic effects, novel biochemical properties, homeostatic advantage, and the production of a variety of enzymes suited to maintaining biochemical efficiency in a variety of environments. The same models can be applied to *H. versicolor.*

Summary

Electrophoretic variation in enzymes encoded by 12 structural gene loci was examined in populations of the diploid treefrog *Hyla chrysoscelis* and its sibling bisexual tetraploid derivative *H. versicolor.* At polymorphic loci, *H. versicolor* exhibits patterns of electromorphs consistent with allele dosage effects expected in a tetraploid in which four alleles at each locus are expressed. *H. versicolor* has a greater mean number of alleles per locus per population, and an average individual heterozygosity ($\bar{H}$ = 0.322) nearly five times that of *H. chrysoscelis* ($\bar{H}$ = 0.068). The tetraploid also has an average of three times as many unique alleles per locus.

Interspecific genetic similarity ($\bar{I}$ = 0.922; range, 0.82–0.97) is well above the normal range recorded for sibling species, semispecies, and even subspecies of other vertebrates, suggesting a relatively recent origin of the tetraploid species. Western (central Texas) and eastern (Mississippi, Ohio, South Carolina, and Georgia) populations of *H. chrysoscelis* are weakly differentiated genetically ($\bar{I}$ = 0.892). Southern (central and eastern Texas) populations of *H. versicolor* are genetically more similar to either diploid population group ($\bar{I}$ = 0.934, 0.938) than the diploid groups are to each other ($\bar{I}$ = 0.892). The general genetic character of *H. versicolor* is that of an intermediate incorporating alleles from eastern and western populations of *H. chrysoscelis.* Geographic and genetic relationships are considered relative to the probable time and place of differentiation and speciation in the complex. The genetic data suggest that the southern population of *H. versicolor* evolved from *H. chrysoscelis* after the latter had differentiated into eastern and western groups of populations. Following differentiation, *H. versicolor* arose either through allopolyploidy or by autoploidy in a population of *H. chrysoscelis* genetically intermediate between the eastern and western groups.

The presence of apparently unique alleles in appreciable frequencies in the tetraploid species supports the hypothesis that duplication releases structural gene loci from normal selective constraints, thus potentially permitting differential specialization of duplicated genes.

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