Vol. 130, No. 6 The American Naturalist December 1987

PARALLEL ELECTROMORPH VARIATION IN THE DIPLOID-TETRAPLOID GRAY TREEFROG COMPLEX

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Submitted November 29, 1984; Revised October 27, 1986; Accepted April 24, 1987

For nearly two decades intense scientific inquiry has focused on biochemical polymorphisms in natural populations (Lewontin 1974; Kimura 1979). Two distinct schools of thought have developed to explain their widespread occurrence. The neutralist hypothesis states that the bulk of protein polymorphisms are selectively neutral and maintained by stochastic forces; selectionists favor the viewpoint that biochemical variation is maintained primarily by some sort of balancing or frequency-dependent selection. Although it seems likely that the actual situation is represented by a third alternative encompassing elements of both theories, attempts to discredit one or the other hypothesis have dominated the literature (Yamazaki et al. 1983). Proof regarding the definitive hypothesis for the majority of protein polymorphisms remains equivocal and controversial (Ayala and Kiger 1984). In this study we use a natural system involving a diploid-tetraploid sibling-species pair of treefrogs to determine the extent to which biochemical polymorphisms are maintained by natural selection.

Hyla versicolor and its diploid progenitor, *H. chrysoscelis*, are a sibling-species pair found in the eastern United States and Canada. *Hyla chrysoscelis*, which has a diploid number of 24 chromosomes (Wasserman 1970), occurs throughout large portions of the southern, southeastern, and central United States. *Hyla versicolor*, however, is a bisexual tetraploid ($4n = 48$) found throughout the northeastern United States and Canada. Its range extends into eastern and central Texas and apparently divides *H. chrysoscelis* into western (central Texas) and eastern populations (fig. 1; Blair 1958; Johnson 1961, 1966; Ralin 1968, 1977). Although the two species are largely allopatric, numerous narrow contact zones in a variety of habitats are broadly distributed throughout North America (Jaslow and Vogt 1977; Ralin and Selander 1979; Ralin et al. 1983).

In addition to broad ecological and geographical distribution of sympatric zones, the two species are morphologically, behaviorally, and ecologically indis-

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Am. Nat. 1987. Vol. 130, pp. 864–878.
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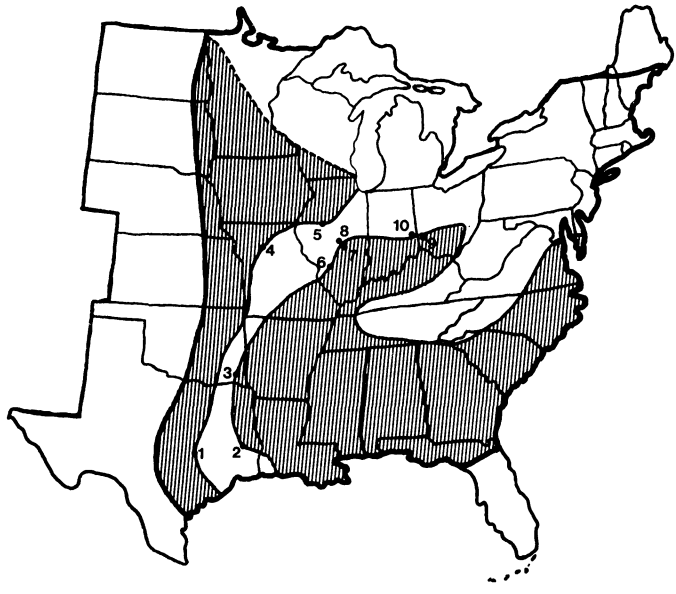

FIG. 1.—Tentative distribution of *H. versicolor* and *H. chrysoscelis* in the United States. *Hyla versicolor*, represented by the clear area, splits the *H. chrysoscelis* range, represented by the hatching. The broken line indicates less certainty about the relative distribution of both species in the upper Midwest.

tinguishable (except for mating-call pulse rate, karyotype, and relative cell size) and share nearly all of the major electrophoretically detectable alleles or electromorphs (Johnson 1966; Ralin 1968, 1981; Jaslow and Vogt 1977; Ralin and Rogers 1979; Ralin and Selander 1979; Danzmann and Bogart 1982*a*,*b*; Ralin et al. 1983). In an investigation of ecological and reproductive behavior in sympatry, Ralin (1968) reported significant, but not absolute, differences in mating-call perch positions and slight but inconclusive differences in food and humidity preferences that might enable the two species to avoid complete niche overlap. Ralin (1981) provided evidence that the two species are capable of ecophysiological convergence: sympatric populations of *H. chrysoscelis* and *H. versicolor* did not differ in body mass, water content, or mean dehydration tolerance, but they were significantly more tolerant to dehydration than an *H. versicolor* population from a higher-rainfall locality 150 km to the east.

Ralin et al. (1983) presented electromorph distribution data that strongly implied that the tetraploid had but one geographic origin from the diploid. Vermont and New York populations of *H. versicolor* contained electromorphs at several loci (*Lda*, *Ldb*, *Sod*) that are found in Texas and Illinois populations of *H. versicolor* and *H. chrysoscelis*, but not in eastern *H. chrysoscelis*. Although a multiple-origin hypothesis could not be categorically rejected, it requires a complex series of parallel independent mutations between geographically distant tetraploid populations. It also requires that the northeastern and southern tetraploids must have arisen simultaneously from geographically distant diploid popu-

TABLE 1

COLLECTING LOCALITIES FOR *H. chrysoscelis* (*chr.*) AND *H. versicolor* (*ver.*)

Code	Locality	Species	No. of Individuals
1	Bastrop, Bastrop Co., Tex.	*chr.*	18
		ver.	23
2	Town Bluff, Tyler Co., Tex.	*chr.*	20
		ver.	24
3	Broken Bow, McCurtain Co., Okla.	*chr.*	19
		ver.	14
4	Miami, Carroll Co., Mo.	*chr.*	20
		ver.	12
5	Manito, Mason Co., Ill.	*chr.*	20
		ver.	25
6	Ramsey, Fayette Co., Ill.	*chr.*	17
		ver.	14
7	Bethany, Moultrie Co., Ill.	*chr.*	13
8	Decatur, Macon Co., Ill.	*ver.*	23
9	Oxford, Butler Co., Ohio	*chr.*	20
10	Connersville area, Fayette Co. and Union Co., Ind.	*ver.*	17
TOTAL			299

lations, followed by a third, much later, origin from *H. chrysoscelis* in the central part of the range. Ralin et al. (1983) observed similar interspecific electromorph frequencies in the eastern, central, and southeastern portions of the range of the complex. Their simplest explanation for these data was that parallel patterns of electrophoretic variation are the result of convergent selection pressures subsequent to a single geographic origin of the tetraploid from the diploid in the central portion of the diploid's range.

In order to test this hypothesis and to elucidate the role of natural selection in maintaining biochemical polymorphisms in natural populations, we analyzed electrophoretic variation in pairs of populations from eight geographically distinct sympatric or parapatric localities. Gene flow has been considered unlikely because of pre-mating isolation (Ralin 1977; Gerhardt 1978) and the high mortality and sterility of hybrids (Johnson 1958, 1963); therefore, given a single origin of the tetraploid (Ralin et al. 1983), any parallel geographic patterns of biochemical variation could only result from similar selection pressures.

METHODS

Collections were made at 10 localities from April to July, 1981, 1982, and 1983 (table 1). The species were found breeding in the same ponds at 6 of the localities. Two pairs of parapatric populations, comprising the remaining 4 localities, were treated as sympatric in the absence of better information about an actual contact zone. The first pair of localities, 7 and 8, are 28 km apart; the second pair, 9 and 10, are 32 km apart. Species identification was based on temperature-corrected mating call (Ralin 1977; Gerhardt 1978). Representative calls and site or cloacal

temperatures were recorded for most of the specimens collected at these localities.

Starch-gel electrophoresis was performed on aqueous extracts of kidney and liver. The tissues were homogenized separately in equal volumes of 2% 2-phenoxyethanol and centrifuged at 25,000 × g at 0°C for 45 min. The supernatant solution was stored at −70°C until used. Eleven polymorphic loci were analyzed in this study: two lactate dehydrogenases (*Lda*, *Ldb*), mannose phosphate isomerase (*Mpi*), phosphoglucomutase (*Pgm*), glucosephosphate isomerase (*Gpi-2*), superoxide dismutase (*Sod*), 6-phosphogluconate dehydrogenase (*6-Pgd*), malate dehydrogenase (*Mdh-1*), glutamate oxalate transaminase (*Got-1*, *Got-2*), and isocitrate dehydrogenase (*Idh-1*).

Techniques of electrophoresis were similar to those employed by Ralin and Selander (1979), with the following modifications: *Mpi* and *Mdh-1* were resolved on an n-(3-aminopropyl)-morpholine-citrate buffer, pH 6.0 (Clayton and Tretiak 1972); *6-Pgd* and *Mdh-1* were resolved on the Tris-citrate, sodium borate buffer (pH 8.7 gel, pH 8.2 electrode) of Selander et al. (1971); *Got-2* was examined on the Tris-EDTA-borate buffer, pH 9.1 (Ayala et al. 1973); *Mdh-1* was also examined on the continuous Tris-citrate-buffer (pH 8.0) and the Tris-citrate buffer (pH 6.7 gel, pH 6.3 electrode) of Selander et al. (1971). *Mdh-1* was examined on four different buffer systems in order to elucidate cryptic variants that did not resolve using any single buffer system. All gels were 15% Sigma starch except those made with the Tris-citrate (pH 8.7) buffer, which were 13% Sigma starch.

In a previous study of natural populations of *Hyla chrysoscelis* and *H. versicolor*, the distribution of phenotypes for allozymes at a particular locus were shown to fit Hardy-Weinberg expectations (Ralin and Selander 1979). In addition, the allelic nature of electromorph variation in both species has been confirmed from investigations of Mendelian inheritance in laboratory-reared offspring (Danzmann and Bogart 1982*a*,*b*, pers. comm.). These studies demonstrate that the electrophoretic variation in this study represents a sample of allelic variation at structural gene loci.

Electromorphs of an enzyme were assigned numbers corresponding to their mobilities relative to the most common electromorph found in central Texas populations of the readily identifiable green treefrog, *H. cinerea*. Texas populations of *H. cinerea* are easily obtainable and relatively invariant compared to other populations. Therefore, *H. cinerea* populations serve as reliable markers when dealing with many populations of other *Hyla* whose common electromorphs vary from population to population. Tetraploid genotypes are designated by superscripts to indicate the number of alleles presumed to be present on the basis of visual estimation of dosages on the gel. For example, an *Mdh-1* phenotype with the 78 electromorph approximately three times as intense as the 100 electromorph would be genotyped $(Mdh\text{-}1^{78})^3$ $(Mdh\text{-}1^{100})^1$. For multiple-isozyme systems, the isozyme with the greatest anodal migration is designated as 1, with progressively slower-migrating isozymes receiving progressively higher designations (e.g., *Mdh-1*, *Mdh-2*). The one exception involves lactate dehydrogenase (*Lda*, *Ldb*); its nomenclature follows that of Ralin and Selander (1979).

Allozyme data were analyzed by means of a distance analysis proposed by

Borowsky (1977). This approach is based on the premise that if parallel selection exists, then sympatric populations of closely related species should be more similar than allopatric populations of these same species. Borowsky devised an index (S) to determine the importance of parallel selection. If species A from area I is denoted as AI and species B from area I as BI, then the genetic distance between the two species at a particular locus can be represented as AIBI. S derived from four interspecific distances would be $S = \frac{1}{2}[(\text{AIBII} + \text{AIIBI}) - (\text{AIBI} + \text{AIIBII})]$. In the procedure outlined by Borowsky, S varies between -1.0 and $+1.0$; S would have an expected value of zero for cases in which parallel selection does not determine allelic frequencies because allopatric populations will, on the average, be no more or less similar than sympatric populations.

More than one S value can be calculated for a given species-locus combination for cases in which there are more than two localities (eight in this study). A mean value of S, $\overline{S}$, which measures the importance of differential selection, is calculated from the individual S values. The significance of an individual $\overline{S}$ value is determined by generating a family of S values, $\hat{S}$, from the data set by rearranging the locality orders (mispairing sympatric populations) repeatedly and then comparing the $\overline{S}$ value with the entire distribution of $\hat{S}$. If there is no selection, then proper ordering is unimportant and $\overline{S}$ should lie near the center of distribution $\hat{S}$. The null hypothesis (H_0), that ordering has no effect upon $\overline{S}$, can be tested by determining the proximity of $\overline{S}$ to the center of the distribution $\hat{S}$. If $\overline{S}$ falls in the range greater than 95% ($P < 0.05$) then H_0 can be rejected. In this case, parallel patterns of electromorph variation at a given locus would not be predicted by the neutrality hypothesis, and selective forces would be strongly implicated as the explanation.

RESULTS

Allele-frequency data are given in table 2. Three distinct patterns emerge from the geographic distributions. Throughout the range of the two species, *Got-1*, *Got-2*, and *Lda* exhibit a major allele around which one or more minor alleles appear randomly distributed. In this study, *Got-2*$^{-190}$ appears to be unique to sympatric locality 3. However, the allele is known to occur at a 0.02 frequency in a population of *Hyla versicolor* from Montgomery County, Texas (unpubl. data). The major allele at each of these loci varies somewhat throughout the range, but, with a few exceptions (e.g., *Lda* in population 9), the frequency is essentially the same in all populations. In the second pattern, exhibited by *Gpi-2*, *Mdh-1*, and *Pgm*, the major allele at a locus varies from region to region but is similar where the two species are found together in sympatry or parapatry.

The third pattern of variation is more complex than the previous two in that two or more alleles, present at high frequency at a locus, vary similarly in sympatric or parapatric populations. This pattern, demonstrated by *Idh-1*, *Ldb*, *Mpi*, *6-Pgd*, and *Sod*, is particularly striking since the major allele may vary from locality to locality as well. For example, *Ldb-1*^{18} is fixed in *H. chrysoscelis* from locality 1 and is the major allele in *H. versicolor*. In a short span of 245 km, frequencies shift to near fixation of the alternative allele in *H. versicolor* and fixation in *H.*

TABLE 2

ALLELE FREQUENCIES AT ELEVEN LOCI IN POPULATIONS OF *H. chrysoscelis* (*chr.*) AND *H. versicolor* (*ver.*)

	LOCALITY AND SPECIES															
	1		2		3		4		5		6		7	8	9	10
LOCUS	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*
N	18	23	20	24	19	14	20	12	20	25	17	14	13	23	20	13
Got-1																
66	.06	.02		.02												.02
37	.94	.98	1.0	.98	1.0	1.0	1.0	1.0	1.0	.99	1.0	1.0	1.0	1.0	1.0	.98
20										.01						
Got-2																
−190					.10	.05										
−100	1.0	1.0	1.0	1.0	.90	.95	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Gpi-2																
−184			.02	.05			.08									
−100	.92	.95	.98	.95	1.0	.98	.92	.89	1.0	1.0	1.0	1.0	1.0	1.0	.98	.97
−18	.08	.05				.02		.11							.02	.03
Idh-1																
109										.01						
100	1.0	1.0	1.0	1.0	.90	.75	1.0	.96	.85	.88	1.0	1.0	1.0	.98	.95	1.0
93					.10	.25		.04	.15	.11				.02	.05	
Lda																
165							.02			.02						.02
151		.36		.28		.46	.05	.27		.27		.35		.24		.50
149		.01						.02						.01		
103										.01				.02		
91	1.0	.63	1.0	.72	1.0	.54	.92	.71	1.0	.70	1.0	.65	1.0	.73	1.0	.48
Ldb																
115		.41	1.0	.98	.96	.64	.05	.42	.38	.52	1.0	.50	.97	.64	1.0	.66
18	1.0	.59		.02	.04	.36	.95	.58	.62	.48		.50	.03	.36		.34

(*continued*)

TABLE 2 (*Continued*)

	Locality and Species															
	1		2		3		4		5		6		7	8	9	10
Locus	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*	*chr.*	*ver.*
Mdh-1																
120	.17	.03														
109		.06	.02	.02	.08	.12					.03					.02
100	.78	.90	.98	.98	.92	.84	.78	.75	.52	.89	.97	.89	1.0	.98	.78	.79
90												.11			.22	
78	.05	.01				.04	.22	.25	.48	.11				.02		.19
Mpi																
111					.05		.08	.02		.03		.02				
106								.02		.05				.01		.02
100	.97	.47	.22	.32	.29	.30	.65	.81	.62	.64	.47	.64	.42	.46	.60	.67
97													.08			
84			.15		.61	.36					.06		.08			
82	.03	.48	.42	.55	.05	.12	.28	.15	.38	.15	.12	.25	.23	.29	.20	.15
71		.05	.08	.05		.05							.04			
66			.12	.07		.16				.13	.35	.09	.15	.25	.20	.15
Pgm																
198		.08		.01												
182	.14	.18	.18	.27	.21	.23		.07	.08	.08	.08		.02	.09	.55	.18
168	.83	.71	.82	.67	.76	.73	1.0	.89	.92	.85	.92	1.0	.84	.85	.38	.75
164				.01												
152	.03	.02		.04	.03	.04		.04		.07			.14	.06	.07	.07
138		.01														
6-Pgd																
109						.02										
100	.53	.64	.70	.66	.87	.75	.53	.39	.68	.77	.79	.84	.75	.67	.70	.66
85	.47	.36	.30	.34	.13	.23	.47	.61	.32	.23	.21	.16	.25	.33	.30	.34
Sod																
111		.01														
109		.23	.50	.48	.29	.34		.04		.08	.44	.04	.31	.36	.08	.09
98	.03	.01				.05						.05	.04	.03		.16
86	.97	.75	.50	.52	.71	.61	1.0	.96	1.0	.92	.56	.91	.65	.61	.92	.75

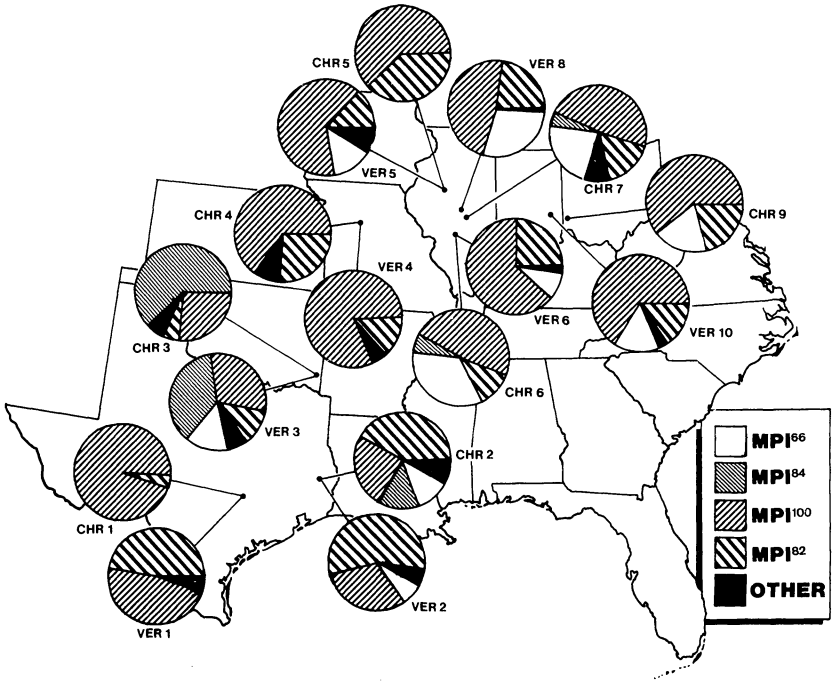

FIG. 2.—Geographic distribution of *Mpi* allozymes in *H. chrysoscelis* (CHR) and *H. versicolor* (VER). The pie graphs indicate the similarity of predominant allele frequencies at sympatric or parapatric localities.

chrysoscelis (table 2). More-northern populations exhibit intermediate frequencies with both alleles varying together. In fact, in all but one instance (locality 5), the allele found in highest frequency in one species is also found in highest frequency in the other species.

Mpi presents an even more striking example of this situation since more than two alleles vary. Figure 2 illustrates the geographic distribution of the four predominant alleles. Additional, less frequent, alleles were lumped into an "other" category in order to simplify the figure. These less-frequent alleles may be found in table 2 and were included in subsequent statistical analyses.

Values of $\overline{S}$ and their respective probability values are reported in table 3. At 5 of the 11 loci (*Gpi*, *Ldb*, *Mdh-1*, *Mpi*, *Pgm*), ordering has a significant effect on $\overline{S}$. *Mpi* shows the most dramatic as well as the most consistent parallel shifts in allele frequency ($P = 0.017$), although, as previously noted, *Ldb* shows the sharpest difference in frequency. However, *Ldb* showed a weak parallel shift in allele frequency at locality 5 (table 2).

Therefore, H_0 is rejected, and we conclude that ordering in fact affects $\overline{S}$. The pattern of electromorph variation at 5 of 11 loci over eight sympatric or parapatric localities in *H. chrysoscelis–H. versicolor* suggests that allele frequencies at these loci converge when the two species come into contact or near contact. Three additional loci, *Got-2*, *6-Pgd*, and *Sod*, border on significance (table 3).

TABLE 3

STATISTICS OF THE PARALLEL-SELECTION ANALYSIS INVOLVING EIGHT SYMPATRIC OR PARAPATRIC LOCALITY PAIRS

Locus	$\bar{S}$	P	Locus	$\bar{S}$	P
Got-1	0.003	0.319	*Mdh-1*	0.051	0.030
Got-2	0.014	0.061	*Mpi*	0.111	0.017
Gpi-2	0.022	0.021	*Pgm*	0.050	0.016
Idh-1	0.024	0.123	*6-Pgd*	0.050	0.076
Lda	0.002	0.188	*Sod*	0.099	0.052
Ldb	0.127	0.030			

DISCUSSION

Available evidence suggests that *Hyla versicolor* probably arose once from a geographically central ancestral population of *H. chrysoscelis* approximately 375,000 years ago, during the close of the Illinoian glaciation and the onset of the Sangamon interglacial (Ralin et al. 1983). Since that time both species have expanded into their present ranges. Explanations that could account for the parallelism of allele frequencies reported here (table 2; fig. 2) are (1) hybridization and gene flow between the two species at each locality; (2) multiple geographic origins of the tetraploid from differentiated populations of the diploid; (3) selection operating directly on electromorphs in a similar manner at the same locality; and (4) electromorphs acting as neutral markers linked to genes or blocks of genes under similar selection pressures at the same locality.

A precedent for explanation (1) comes from evidence supporting two possible mechanisms for gene flow. The first mechanism involves introgressive hybridization and subsequent gene flow previously documented between diploid and tetraploid plants (Zohary and Nur 1959; Vardi 1974). Triploid hybrids are capable of backcrossing to diploids to form new tetraploids, since the triploids produce some nonreduced gametes. Bogart (1979) proposed that a similar mechanism might be possible in *H. chrysoscelis* and *H. versicolor*. Nishioka and Ueda (1983) obtained bisexual tetraploids from *H. arborea japonica*; they suppressed extrusion of the second polar body by refrigeration to produce diploid eggs and fertilized the eggs with haploid sperm to produce triploids. When triploid females were backcrossed to diploid males, a few of the surviving offspring proved to be bisexual tetraploids. Available field and laboratory data suggest that this mechanism is not currently operating between *H. chrysoscelis* and *H. versicolor*. Although mortality was high, triploid hybrids between *H. versicolor* and *H. chrysoscelis* have been produced in the laboratory (Johnson 1963; Ralin 1976; Bogart 1979); however, multiple backcrosses between male triploids and *H. chrysoscelis* females resulted in 100% mortality by the fourteenth day (Johnson 1963). Both Johnson (1963) and Bogart (pers. comm.) were unable to cross female triploids because they never produced mature eggs.

The second mechanism involves matings between *H. versicolor* males and *H. chrysoscelis* females that produce a number of nonreduced eggs. The resulting

offspring would be tetraploid containing two haploid genomes from the diploid. Bogart (pers. comm.) concluded that *H. chrysoscelis* females do produce some nonreduced eggs since laboratory crosses involving *H. chrysoscelis* females and other hylid species, such as *Pseudacris triseriata* and *P. crucifer*, sometimes result in triploid hybrids. Nevertheless, Bogart has failed to produce anything but triploids in crosses involving *H. versicolor* males and *H. chrysoscelis* females. Even if this mechanism could be demonstrated in the laboratory, it has not been demonstrated in the field; and pre-mating isolation by means of mating call is extremely effective, making it unlikely that females respond to anything but conspecific calls (Gerhardt 1978; Bogart 1979).

Given the possibility that mistakes occur despite pre-mating isolation, one would expect to find triploids in sympatric populations of both species as well as allopatric populations of the diploid. No triploids have been karyotypically detected from natural populations after extensive surveys (Bogart 1979; Wiley 1982, 1983; Wiley et al. 1986); nor have they been detected in laboratory crosses utilizing *H. chrysoscelis* females and conspecific diploid males (Ralin 1976; Bogart, pers. comm.). It seems likely that both pre-mating and post-mating isolation mechanisms are effective enough to prevent gene flow by means of the previously discussed triploid intermediate or suppressed maturation of eggs from diploid (*H. chrysoscelis*) females in current populations of the gray treefrog.

However, the parallel patterns may be a result of gene flow that took place before isolation mechanisms were firmly established. This may be a plausible explanation for the great number of alleles in common between the diploid and tetraploid. The emergence of definite patterns of allele frequencies, identified according to the direction and relative level of gene flow, supports a hypothesis of historical gene flow. If particular alleles are unique to diploids from certain localities, one-way gene flow would place the alleles at lower frequencies in the tetraploids from sympatric localities. Several examples of this kind of pattern are present in the allele-frequency data (table 2). From locality 1, $Got\text{-}1^{66}$, $Mdh\text{-}1^{120}$, $Mdh\text{-}1^{78}$, and Sod^{98} are representative. Two alleles, Mpi^{84} and $Mdh\text{-}1^{78}$, are rare in most *H. chrysoscelis* populations but common in one locality. In population 3, Mpi^{84} is present at a frequency of 0.61 but it is present in sympatric *H. versicolor* at a considerably lower frequency (0.36). The $Mdh\text{-}1^{78}$ allele is present at a 0.48 frequency in population 5 and drops to 0.11 in sympatric *H. versicolor*.

There are two independent lines of evidence supporting explanation (2). The first comes from relatively recent karyological data involving a population of *H. versicolor* from Virginia polymorphic for two chromosomal positions for the nucleolar organizing region (NOR; Wiley et al. 1988). Frogs were found with NOR's detectable through silver staining or Biotin-labeled DNA on chromosomes 6, 8, or both. Until recently, populations of *H. versicolor* had demonstrated an NOR position only on the short arm of chromosome 6 (Wiley 1982), although *H. chrysoscelis* exhibited two NOR positions (Wiley 1982, 1983). Populations of *H. chrysoscelis* from eastern North Carolina exhibited the NOR on the long arm of chromosome 8, whereas *H. chrysoscelis* west of the North Carolina populations had the chromosome-6 morph. Even more chromosomal polymorphisms have been recently documented in *H. chrysoscelis* (Wiley et al. 1988). From these data

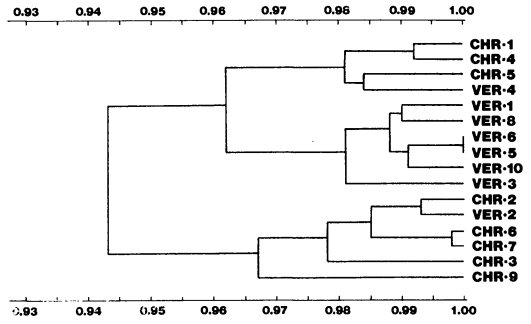

FIG. 3.--Phenogram for all populations using Nei's (1978) unbiased genetic identity.

one could conclude that the Virginia *H. versicolor* had an additional origin along with gene flow from surrounding populations of 6-morph tetraploids or a previous history of gene flow from the diploid as discussed above. Electrophoretic data from Virginia *H. versicolor* and North Carolina *H. chrysoscelis* are not available.

The other line of evidence, which is speculative, is based on the previously documented laboratory study on *H. arborea* (Nishioka and Ueda 1983) and the geographic similarities in gene frequencies. Information above regarding the absence of natural triploids argues against multiple origins involving triploid intermediates. However, given the possibility that the gene frequencies reported here result from multiple geographic origins, regardless of mechanism, one can make predictions about the geographic patterns of *H. versicolor* populations. One would expect to find isolated pockets of *H. versicolor* within the range of *H. chrysoscelis*. Large areas of the southeast have dense populations of *H. chrysoscelis* devoid of any *H. versicolor* (Johnson 1966; Ralin 1968; Ralin and Selander 1979; Ralin et al. 1983).

Furthermore, every population of *H. versicolor* sympatric with *H. chrysoscelis* that has been sampled thus far has contained alleles present in other *H. versicolor* populations but not in the *H. chrysoscelis* population sympatric with it (Ralin and Selander 1979; Ralin et al. 1983). As previously mentioned, this suggests that all *H. versicolor* resulted from a single ancestral population (with the possible exception of the Virginia population), because the multiple-origin hypothesis would require a complex series of parallel, independent mutations between geographically distant populations of the tetraploid and simultaneous origins of the tetraploid from eastern and southwestern diploid populations, followed by a third, later origin in the central portion of the diploid's range (Ralin and Selander 1979; Ralin et al. 1983). This is certainly not the most parsimonious explanation of the available data.

Under assumptions of multiple origins, one would expect an examination of genetic relationships to show individual *H. versicolor* populations consistently fragmenting and pairing with their sympatric diploid populations. However, the tetraploid populations do not fragment in this manner in the phenogram (fig. 3), where all but two *H. versicolor* populations cluster together (see also Ralin and Rogers 1979; Ralin et al. 1983). In addition, *H. versicolor* is continuously distrib-

uted through localities 5, 6, and 8 (fig. 1; Ralin and Romano, unpubl. data). If one is to propose multiple origins for the similarity of allele frequencies at *Mdh-1*, *Ldb*, and *Mpi* (fig. 2; table 2) between the two species, then one must assume de novo that tetraploid isolation would have to be more rapid than intraspecific gene flow.

Support for explanation (3) comes from the finding that natural selection has recently been invoked to explain parallel geographic patterns of electromorph variation at *Est-6* and *Pgm* in *Drosophila simulans* and *D. melanogaster* (Anderson and Oakeshott 1984). In that study, *D. simulans* and *D. melanogaster* were sampled from Australasia, North America, and Eurasia. For every 10° increase in latitude from the equator, $Est\text{-}6^{100}$ increased in frequency by 14% in both species. The Pgm^{100} allele did not exhibit a clinal pattern of variation but did show a correlation of allele frequency between *D. simulans* and *D. melanogaster* on all three continents. Gene flow is not possible between these two species; hence, the authors concluded that selection was responsible for the observed geographic variation.

Although selection may be acting directly on electromorphs, Powell and Wistrand (1978) expressed favor for explanation (4) by suggesting that loci mark "coadapted tightly linked blocks of genes." Their conclusions are based on laboratory studies indicating that a variable environment of temperature and medium shows a significant effect on increasing genetic variation as determined by mean heterozygosity. However, no correlations of environmental variables and specific electromorphs were evident. Powell and Wistrand suggested that, instead of neutral markers, however, electromorphs may represent integral parts of coadapted "supergenes."

Inheritance patterns of several polymorphic loci in *H. chrysoscelis–H. versicolor* (*Got-2*, *Mdh-1*, *Sod*, *Lda*, and *Ldb*) demonstrate that these loci are not in the same linkage groups (Danzmann and Bogart, pers. comm.). An inspection of the allele frequencies in this study (table 2) reveals no apparent correlations that suggest linkage disequilibria among the loci exhibiting parallel patterns of variation (table 3). Mukai and Voelker (1977) investigated a population of *D. melanogaster* from Raleigh, North Carolina, for linkage disequilibrium. They determined that there were no linkage disequilibria between the genes for different electromorphs, but linkage disequilibria were detected between two enzyme loci and two different chromosomes with inversion polymorphisms. Such chromosome inversions have not been reported for *H. chrysoscelis* or *H. versicolor* (Bogart 1979; Wiley 1982, 1983).

Regardless of whether selection is maintaining parallel patterns of electromorph variation by acting on genes linked to neutral electromorph markers, on coadapted gene complexes, or on the electromorphs themselves, convergence for ecophysiological characteristics has been documented in this sibling-species pair of treefrogs. Ralin (1981) collected adult frogs from two localities in eastern and central Texas, *H. versicolor* and *H. chrysoscelis* from Bastrop County (average annual rainfall, 91.4 cm) and *H. versicolor* from Montgomery County (average annual rainfall, 106.7 cm). There was no water-content-mediated relationship between body mass and dehydration tolerance in either species, indicating that body size, percentage of body water, and dehydration tolerance can vary indepen-

dently of one another in different populations, as in other hylid frogs. The sympatric populations *H. chrysoscelis* and *H. versicolor* did not differ significantly in body mass, water content, or mean dehydration tolerance. Individuals of *H. versicolor* from the higher-rainfall locality were significantly less tolerant to desiccation. Ralin concluded that the diploid and tetraploid are capable of converging ecophysiologically when they occupy the same habitat.

In addition to the above example of ecophysiological convergence, neither gene flow nor multiple origins of the tetraploid appear sufficient to account for all of the electromorph patterns shown here. Allele frequencies remain remarkably close between *H. versicolor* and *H. chrysoscelis* populations that are not in physical contact (populations 7 and 8, 9 and 10; table 2). Although it is possible that these populations were in physical contact and have not had time to differentiate, inconsistencies with a hypothesis of gene flow remain. *Hyla chrysoscelis* in population 9 possesses an allele $Mdh\text{-}1^{90}$ at a 0.22 frequency. The common allele $Mdh\text{-}1^{100}$ is in almost perfect congruence between populations 9 and 10 (table 2). If gene flow had been extensive enough to account for the congruence, then it is improbable that the $Mdh\text{-}1^{90}$ would not be present in *H. versicolor*. Likewise, if the *H. versicolor* population resulted from an ancestral population in the area of populations 9 and 10, then both *H. versicolor* and *H. chrysoscelis* would share the major alleles at *Mdh-1*, including $Mdh\text{-}1^{90}$. Furthermore, since *H. versicolor* is continuously distributed within the area bounded by localities 5, 6, and 8 (fig. 1; Ralin and Romano, unpubl. data), interspecific gene flow, historical or otherwise, would had to have been much greater than intraspecific gene flow in order to explain the frequency changes in *H. versicolor* from locality 5 to localities 6 and 8 (table 2).

Therefore, given the likelihood of selection in *Drosophila*, the currently available evidence against gene flow between *H. chrysoscelis* and *H. versicolor*, and the ecophysiological convergence of *H. versicolor* with *H. chrysoscelis* (Ralin 1981), the most probable explanation is that natural selection is the predominant force causing similar gene frequencies in sympatric populations from geographically distant sites. Nevertheless, the alternative explanations cannot be ruled out categorically and may, in fact, have played a significant role. One means of assessing the possible role of gene flow is to analyze restriction endonuclease maps of mitochondrial DNA (mtDNA) from the sympatric or parapatric populations of the diploid and tetraploid in this study. If *H. versicolor* share unique restriction sites with nearby *H. chrysoscelis*, then it is virtually certain that gene flow from the diploid has taken place. At the present time, mtDNA from two sympatric sites (table 1, localities 1 and 5) have been isolated by one of the authors (M.A.R.), but the restriction maps are not yet available. If gene flow has taken place, a comprehensive study of mtDNA from treefrogs at the remaining sites would indicate the pervasiveness of this phenomenon. Continued research should firmly establish whether gene flow and/or selection is primarily responsible for the parallel variation observed in this study. Should selection continue to be indicated as the primary explanation for the parallel patterns in these two species, correlations will be made for such environmental variables as temperature, precipitation, and humidity, with electromorphs demonstrating parallel geographic patterns of variation.

SUMMARY

The tetraploid treefrog *Hyla versicolor* and its diploid progenitor, *H. chrysoscelis*, are extremely similar morphologically and ecologically, and they share virtually all of the same major protein polymorphisms. Sympatric populations of *H. versicolor* and *H. chrysoscelis* were sampled from widely separated localities in Texas, Oklahoma, Missouri, Illinois, and the Indiana-Ohio area. The data were analyzed by means of a statistic that measured the degree to which the two species parallel one another in terms of electromorph frequency. Of 11 polymorphic loci, 5 were significantly correlated ($P < 0.05$), and 3 more bordered on significance. Several explanations for these results are possible: parallel selection for shared electromorphs in similar environments, extensive gene flow between ploidy levels in areas of contact, multiple origins of the tetraploid in different areas of the diploid range, and some combination of these possibilities. Available data suggest that natural selection is the major factor responsible for parallel patterns of electromorph frequencies.

ACKNOWLEDGMENTS

We wish to thank E. DeVillez, T. Gregg, C. W. Kilpatrick, U. Nur, R. K. Selander, and D. H. Taylor for their comments and careful review. D. Day, C. Maher, S. P. Romano, and D. Shepard provided technical assistance. We also thank C. Gerhardt, M. McLeod, M. Rapp, and S. Taylor for their assistance in the collection of specimens. National Science Foundation grant DEB-8209327 (to M.A.R. and S.I.G.), a Society of the Sigma Xi Grant-in-Aid of Research (to M.A.R.), and Miami University (M.A.R. and S.I.G.) supported this work.

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