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CHROMOSOME BANDING PATTERNS OF TREEFROGS (HYLIDAE) OF THE EASTERN UNITED STATES

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ABSTRACT: Chromosomes from 12 species of treefrogs were treated with trypsin and stained with Giemsa stain (G-banded). Banded karyotypes were compared within and between species. Based on this analysis, three species groups were discerned: the *Pseudacris triseriata* group, consisting of *P. t. feriarum, P. brimleyi, P. ornata* and *H. crucifer*; the *Hyla gratiosa* group, consisting of *H. gratiosa, H. squirella, H. cinerea*; and the *Hyla chrysoscelis* group, consisting of *H. andersoni, H. chrysoscelis* (two chromosome morphs), *H. versicolor, H. avivoca* and *H. femoralis*. These groupings generally agree with groupings from morphological, osteological, electrophoretic, immunological and hybridization studies. Chromosome inversions and translocations have played a role in chromosome evolution of the species examined. Populations of *H. chrysoscelis* have probably been involved in the origin of the tetraploid species *H. versicolor*. Two chromosome polymorphisms were discovered in the populations of *H. chrysoscelis* studied.

Key words: Amphibia; Salientia; Hylidae; *Hyla*; *Pseudacris*; Chromosome banding; Taxonomy

ALL holarctic hylids, except *Hyla versicolor* and *Acris* species, have 24 chromosomes which are very similar in morphology (Bogart, 1973). The present study was undertaken to determine if chromosome changes such as inversions and translocations have played a part in the evolution of the holarctic hylids. Treating chromosomes with trypsin and then staining with Giemsa stain induces reproducible cross-bands (G-bands) on chromosome arms. These G-bands can be used as "landmarks" to facilitate the analysis of chromosome changes.

G-banding has been particularly difficult to obtain on anuran chromosomes, and this has been attributed to their tight coiling (Schmid, 1978*a*). This view is supported by the fact that G-bands may be obtained on long prophase chromosomes where coiling is not as tight (Schmid, 1978*b*). Some plant chromosomes rarely show G-bands, and it has been suggested that this is due to their extreme contraction (Greilhuber, 1977).

According to White (1978), 90% of speciation events are accompanied by chromosomal change. Such changes need not be changes in chromosome number but can be pericentric inversions, paracentric inversions, and translocations. Inversions and translocations are not usually detected in mitotic chromosomes by conventional staining but can be detected by banded chromosome analysis. In a study of the G-banded chromosomes of humans and chimpanzee, it was found that in addition to different chromosome numbers and varying amounts of heterochromatin, the chromosomes of the two species differed by nine pericentric inversions (Yunis et al., 1980).

MATERIALS AND METHODS

The specimens used in this study are listed in Table 1. *Hyla versicolor* from Massachusetts, *H. avivoca*, and *H. cinerea* from Rayne, Louisiana were bought from commercial collectors. Chromosome preparations were obtained by the *in vivo* method of Baker et al. (1971). Frogs were injected with 0.1 ml phytohemagglutinin (PHA) per gram of body weight and kept at room temperature for three days. The frogs were then injected with 0.05 ml Colcemid (Gibco) per gram of body weight. After 3–6 h, the frogs were killed with chloroform and the blood was removed with a capillary tube and placed in a 0.05 M potassium chloride solution

TABLE 1.—List of specimens used (abbreviations: NCSM = North Carolina Museum of Natural History; JEW = author's personal collection).

Species	Catalogue number	Sex	Collection locality
Hyla crucifer	NCSM 19680	♂	Sampson Co., NC
	NCSM 22696	♂	Sampson Co., NC
	NCSM 22698	♂	Sampson Co., NC
	NCSM 22728	♂	Wake Co., NC
	NCSM 22679	♂	Wake Co., NC
Pseudacris brimleyi	NCSM 20084	♂	Sampson Co., NC
	NCSM 22687	♂	Sampson Co., NC
	NCSM 22688	♂	Sampson Co., NC
Pseudacris triseriata feriarum	NCSM 22690	♂	Guilford Co., NC
	NCSM 23045	♂	Wake Co., NC
	NCSM 23046	♂	Wake Co., NC
	NCSM 23048	♂	Wake Co., NC
	NCSM 23049	♂	Wake Co., NC
Pseudacris ornata	NCSM 22719	♂	Scotland Co., NC
	NCSM 22720	♂	Scotland Co., NC
	NCSM 20080	♂	Scotland Co., NC
Hyla squirella	NCSM 19663	♂	Wake Co., NC
	NCSM 19665	♂	Sampson Co., NC
	NCSM 22709	♂	Columbus Co., NC
	NCSM 22711	♂	Columbus Co., NC
Hyla gratiosa	NCSM 19658	♂	Wake Co., NC
	NCSM 22664	♂	Wake Co., NC
	NCSM 22692	♂	New Hanover Co., NC
	NCSM 22693	♂	New Hanover Co., NC
	NCSM 22713	♂	Tallahassee, FL
Hyla cinerea	NCSM 22673	♂	Rayne, LA
	NCSM 22674	♂	Rayne, LA
	NCSM 22675	♂	Rayne, LA
	JEW SP-1	♂	Berkeley Co., SC
	JEW SP-2	♂	Berkeley Co., SC
Hyla andersoni	NCSM 20975	♂	Sampson Co., NC
	NCSM 20980	♂	Sampson Co., NC
	NCSM 22712	♂	Sampson Co., NC
Hyla femoralis	NCSM 20975	♂	Bladen Co., NC
	NCSM 20980	♂	Bladen Co., NC
	NCSM 22712	♂	Bladen Co., NC
	NCSM 21043	♂	Sampson Co., NC
	NCSM 22913	♂	Dare Co., NC
Hyla avivoca	JEW Hav1	♂	Reelfoot Lake, TN
	JEW Hav2	♂	Reelfoot Lake, TN
	JEW Hav3	♂	Reelfoot Lake, TN
Hyla chrysoscelis			
Chromosome 8 morph	NCSM 20086	♂	Wake Co., NC
	NCSM 22640	♂	Wake Co., NC
	NCSM 22716	♂	Wake Co., NC
	NCSM 19666	♂	Wake Co., NC
	NCSM 19659	♂	Burke Co., NC
	NCSM 22662	♂	Bladen Co., NC
	NCSM 23054	♂	Rockingham Co., NC
	NCSM 22913	♂	Dare Co., NC

TABLE 1.—Continued.

Species	Catalogue number	Sex	Collection locality
Chromosome 6 morph	NCSM 19648	♂	Jackson Co., NC
	NCSM 21042	♂	Wilson Co., TN
	NCSM 20984	♂	Cumberland Co., TN
	NCSM 20985	♂	Wilson Co., TN
	NCSM 21042	♂	Wilson Co., TN
	NCSM 22668	♂	St. James Parish, LA
	JEW StC1	♂	St. Charles Parish, LA
Hyla versicolor	NCSM 21041	♂	Hampshire Co., MA
	NCSM 22665	♂	Hampshire Co., MA
	NCSM 22666	♂	Hampshire Co., MA
	NCSM 22667	♂	Hampshire Co., MA
	NCSM 22681	♂	Hampshire Co., MA
	NCSM 22682	♂	Hampshire Co., MA
	NCSM 23052	♂	Hampshire Co., MA

for 15 min at room temperature. The cells were then centrifuged, the supernatant removed, and a 3:1 methanol-acetic acid solution (fixative) added. The cells were left overnight in the fixative at 4 C. The next day the fixative procedure was performed three times and the cells were resuspended for a final time in a 6:1 methanol-acetic acid solution. Four drops of the suspension were then placed onto dry slides which had been cleaned with 1:1 methanol-chloroform solution. Slides were allowed to air-dry and were placed on a slide warmer overnight at 60 C. The following day the slides were Giemsa-banded using a procedure developed by Freed (1977). The slides were placed in trypsin for 45 s, in a 1:1 phosphate buffer-formaldehyde solution for 2–4 h, rinsed in buffer, stained in 2% Giemsa, rinsed in acetone, acetone and toluene, and finally toluene before being made permanent.

Metaphase plates were photographed at approximately 1600× on Kodak High Contrast Copy Film or Technical Pan 2415. Negatives were printed on Agfa Gevaert Rapidoprint FPI-2 paper or Kodak Kodabrome RC II paper. Well-banded chromosomes with few or no overlaps were photographed. Due to the large size of anuran prophase chromosomes, some overlaps had to be tolerated. Techniques that produce excellent chromosome spreading in mid-metaphase cells leave prophase chromosomes with some degree of overlap.

At least 50 chromosome spreads from each individual studied were examined for the position of secondary constrictions. Five or more karyotypes from each species were prepared. Idiograms were constructed for each species from the prepared karyotypes, average arm lengths, and the average normalized haploid length. Arm lengths were obtained with a rotary wheel measuring device from the prepared karyotypes and were used to calculate arm ratios and normalized haploid length.

Banding patterns were determined for each species by recording bands that appeared on more than half of the homologues of each chromosome. The composite idiogram that resulted was then compared to the composite idiograms from the other species. Chromosomes were classifed according to Levan et al. (1964), using arm ratio (length of long arm divided by short arm) as the criterion.

RESULTS

Representative karyotypes are shown in Figs. 1–4; idiograms are found in Figs. 5–8. All species examined had 24 chromosomes except *Hyla versicolor,* which

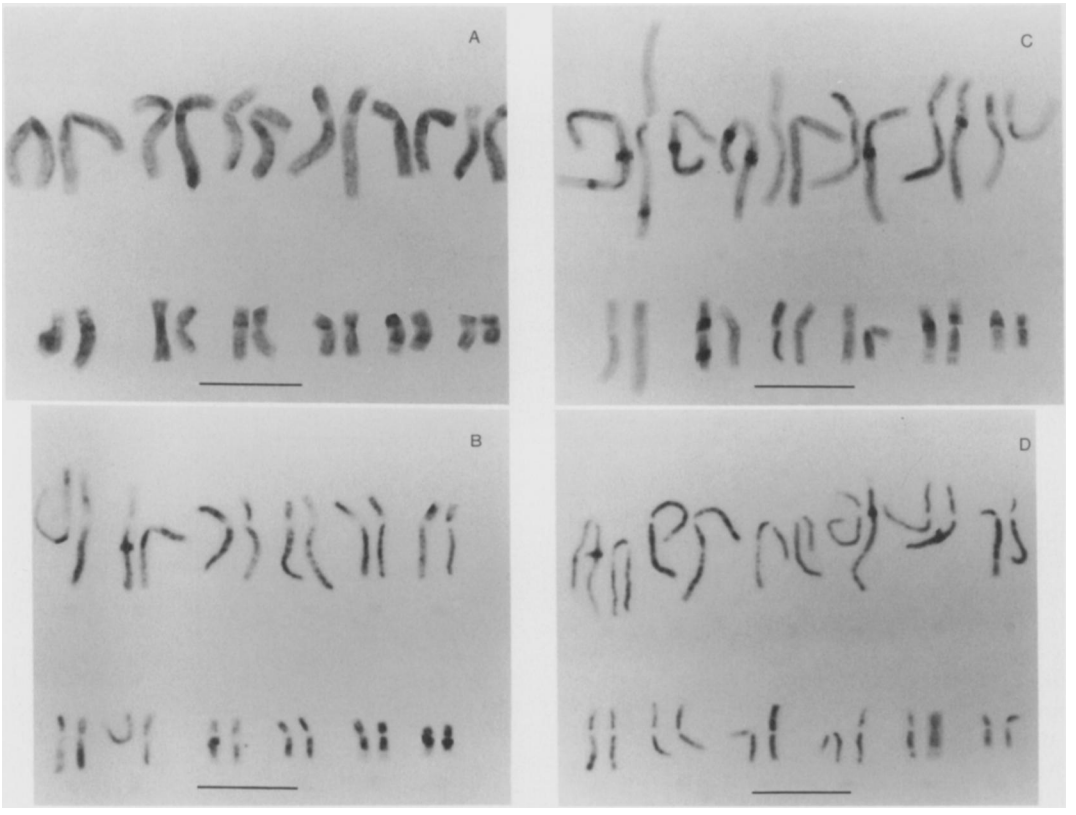

FIG. 1.—(A) G-banded karyotype of *Hyla crucifer*. (B) G-banded karyotype of *Pseudacris brimleyi*. (C) G-banded karyotype of *Pseudacris triseriata feriarum*. (D) G-banded karyotype of *Pseudacris ornata*. Bar represents 10 μ.

had 48 chromosomes. All specimens examined were males; consequently, no attempt was made to analyze differences in banding pattern associated with sex. G-band patterns of all species examined were similar but not identical. Positive identification of chromosomes could be made using the banding pattern. Some of the differences in banding pattern between species were due to differences in average length and resulted from two or three bands on longer chromosomes coalescing into one band on shorter chromosomes (in comparisons of homologous chromosomes). Side by side comparison of chromosomes revealed whether or not a real difference existed or was due to the coalescence of bands.

Based on banding pattern, measurement analysis, and secondary constriction position, the twelve species can be divided into three groups: (1) *Pseudacris triseriata* group: *P. t. feriarum, P. brimleyi, P. ornata, H. crucifer*; (2) *Hyla gratiosa* group: *H. gratiosa, H. squirella, H. cinerea*; (3) *Hyla chrysoscelis* group: *H. andersoni*, chromosome 6 and 8 morphs of *H. chrysoscelis* (Wiley, in press), *H. avivoca, H. versicolor, H. femoralis*. The evidence for placing the species into species groups is shown in Table 2. The following is a comparison of the chromosomes of the species.

Chromosome Pair 1

Chromosome pair 1 was metacentric in all species studied (Figs. 5–8). No major

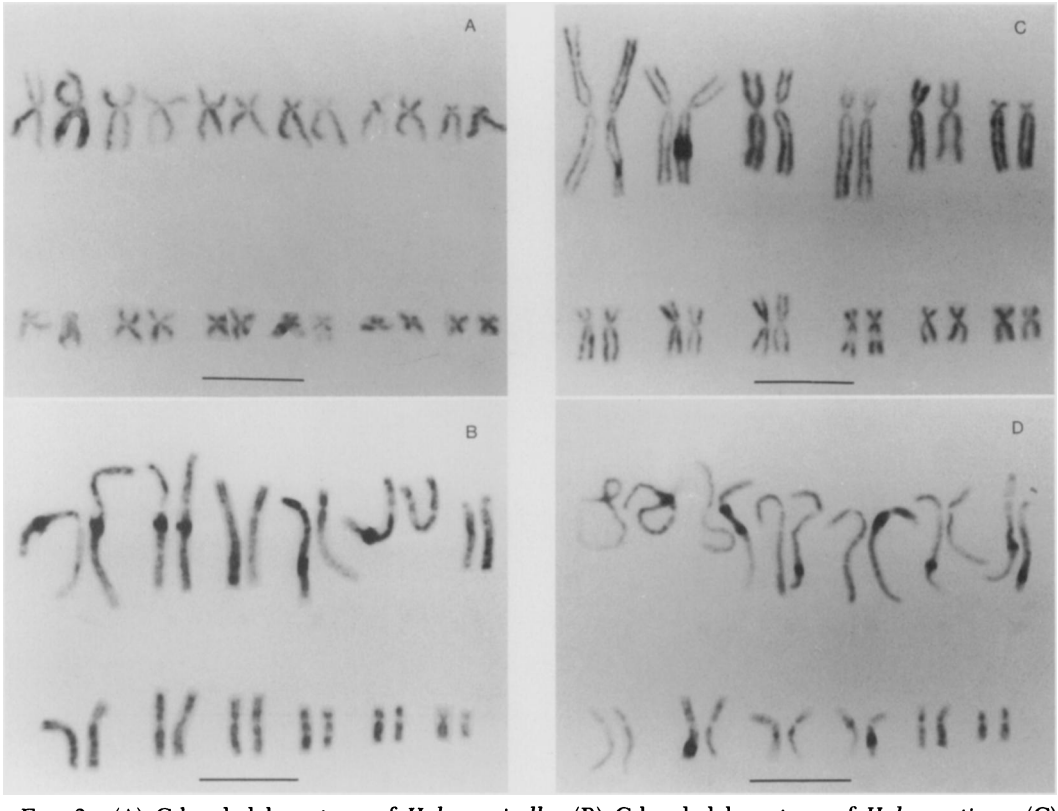

FIG. 2.—(A) G-banded karyotype of *Hyla squirella*. (B) G-banded karyotype of *Hyla gratiosa*. (C) G-banded karyotype of *Hyla cinerea*. (D) G-banded karyotype of *Hyla andersoni*. Bar represents 10 μ.

TABLE 2.—Evidence for species groups. Secondary constrictions (2°) are those common to the group or derived from it. Arm ratios are those that are unique to the majority of the group. Banding patterns are those that are unique to a group. Numbers = chromosome numbers; p = short arm, q = long arm; mt = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric; b = bands.

Species	2°	Arm ratio	Banding patterns
Group 1:			
Hyla crucifer	11q	7st, 11mt	5b, 5p; 3b, 10p
Pseudacris brimleyi	11q	7st, 10mt, 11mt	5b, 5p; 3b, 6p; 3b, 10p
Pseudacris triseriata feriarum	11q	7st, 10mt, 11mt	5b, 5p; 3b, 6p; 3b, 10p
Pseudacris ornata	11q	7st, 10mt, 11mt	5b, 5p; 3b, 6p; 3b, 10p
Group 2:			
Hyla squirella	10q	6t	
Hyla gratiosa	10q	6t	
Hyla cinerea	10q	6t	
Group 3:			
Hyla andersoni	6p	9sm	
Hyla femoralis	2p	9sm	
Hyla avivoca	6p	9sm	
Hyla chrysoscelis Chromosome 8 morph	8q	9sm	
Hyla chrysoscelis Chromosome 6 morph	6p	9sm	
Hyla versicolor	6p	9sm	

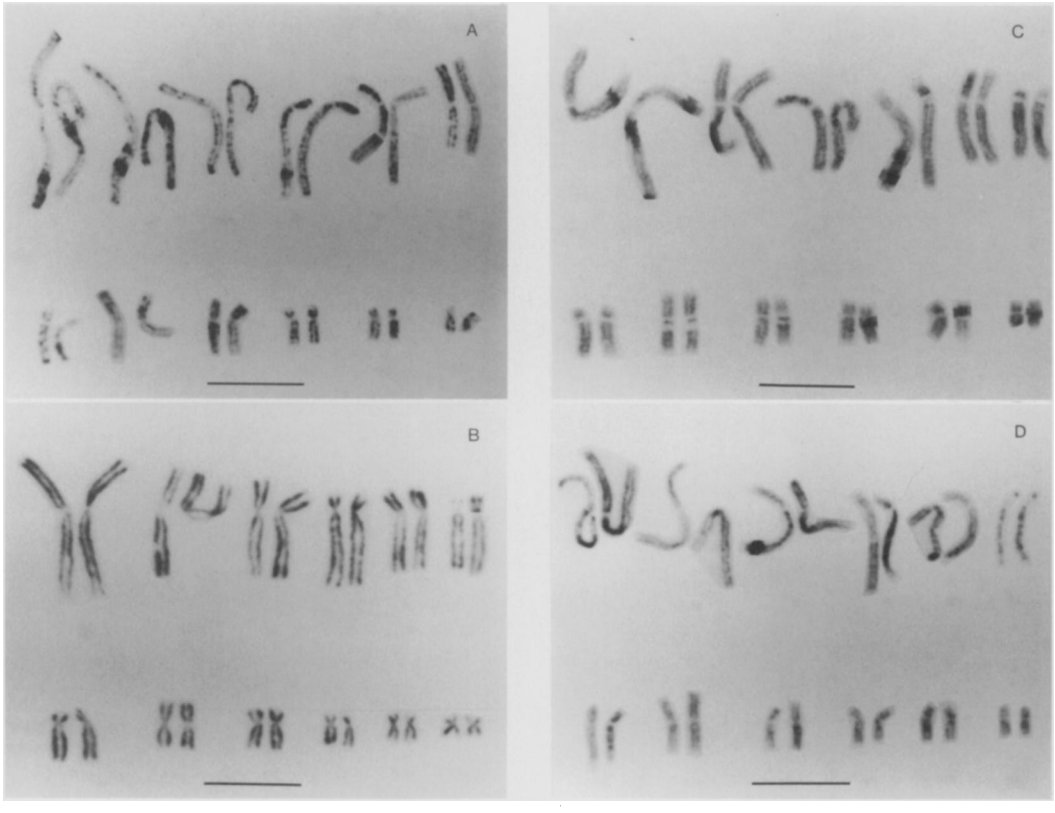

FIG. 3.—(A) G-banded karyotype of *Hyla femoralis*. (B) G-banded karyotype of *Hyla avivoca*. (C) G-banded karyotype of the chromosome 8 morph of *Hyla chrysoscelis*. (D) G-banded karyotype of the chromosome 6 morph of *Hyla chrysoscelis*. Bar represents 10 μ.

differences in banding pattern were evident.

Chromosome Pair 2

Chromosome pair 2 was metacentric in most of the species examined. *H. gratiosa*, *H. crucifer* and *P. t. feriarum* had submetacentric pair 2 chromosomes, but the arm ratios for these species were barely within the submetacentric range and were not significantly different from the arm ratios of the other species (Figs. 6C, 7B, 8). No major differences in banding pattern were evident except for *H. femoralis* which had a secondary constriction on the short arm (Figs. 3A, 7A). This secondary constriction was small and was not always evident, but it could be found on at least half of the homologues examined.

Chromosome Pair 3

Chromosome pair 3 was submetacentric in most species examined. In *H. andersoni* and *H. crucifer*, chromosome pair 3 was metacentric but the arm ratios for these species were barely within the metacentric range and were not significantly different from the submetacentrics of the other species (Figs. 5A, 6D). No major differences in banding pattern were evident.

Chromosome Pair 4

Chromosome pair 4 was subtelocentric in all species examined (Figs. 5–8). No

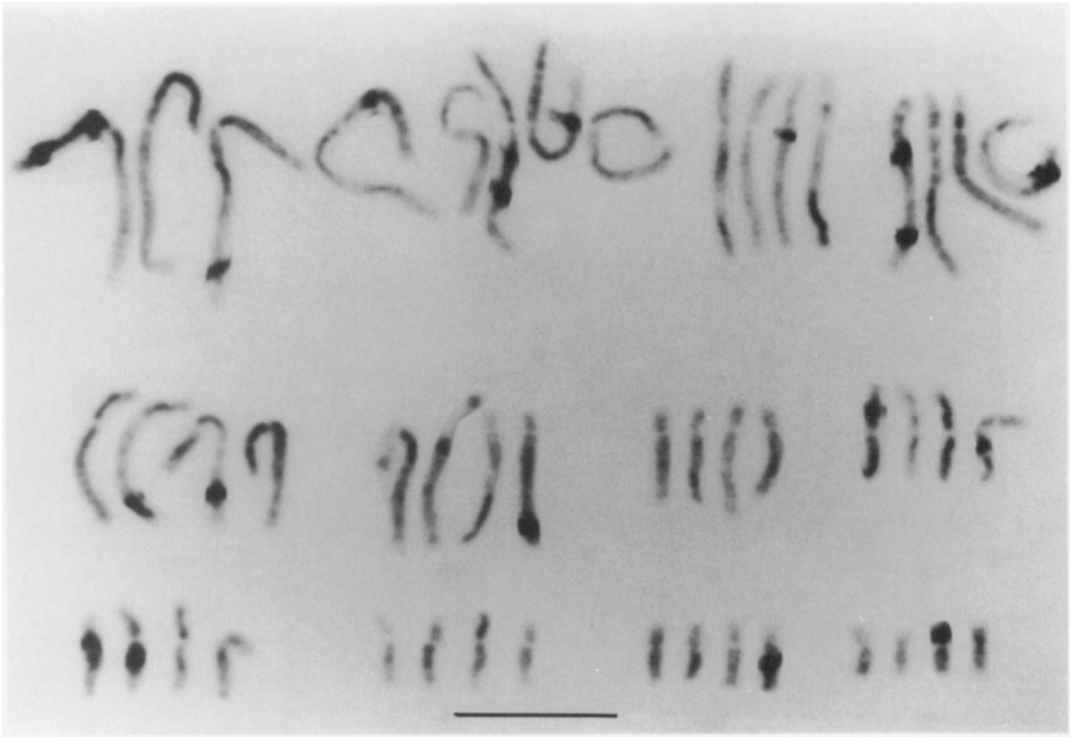

FIG. 4.—G-banded karyotype of *Hyla versicolor*. Bar represents 10 μ.

major differences in banding pattern were evident.

Chromosome Pair 5

Chromosome pair 5 was submetacentric in all species examined (Figs. 5–8). In *P. t. feriarum, P. ornata, P. brimleyi* and *H. crucifer,* five bands were on the short arm (if coalescence of bands was considered) while all the other species had 6 bands (Figs. 5–8).

Chromosome Pair 6

Chromosome pair 6 was subtelocentric in most species examined. *H. gratiosa, H. squirella* and *H. cinerea* had a telocentric chromosome pair 6 (Figs. 6A, 6B, 6C). *H. andersoni, H. avivoca,* the chromosome 6 morph of *H. chrysoscelis* and *H. versicolor* had a secondary constriction on the short arm (K. Anderson, pers. comm.; Figs. 2D, 3B, 3D, 4, 6D, 7B, 7D, 8). In the *H. versicolor* population examined, secondary constrictions were found on only two of the four homologues (Fig. 4).

P. t. feriarum, P. brimleyi and *P. ornata* had three bands on the short arm of chromosome 6 (Figs. 5B–5D); all other species had 2 bands (Figs. 5A, 6–8). *H. femoralis* had a metacentric pair 6 and had a different banding pattern from the other species (Fig. 7A). The uniqueness of the *H. femoralis* karyotype was presumably due to changes in chromosomes 2 and 6 and involved a secondary constriction. *H. femoralis* shared a submetacentric pair 9 with *H. andersoni, H. chrysoscelis* (chromosome 6 morph), *H. versicolor* and *H. avivoca.* Since these species had a secondary constriction on the short arm of chromosome pair 6, it is probable that chromosome changes in an ancestor with the chromosomal characteristics of these species account for the *H. femoralis* karyotype. The probable way this occurred is depicted in Fig. 9A. Al-

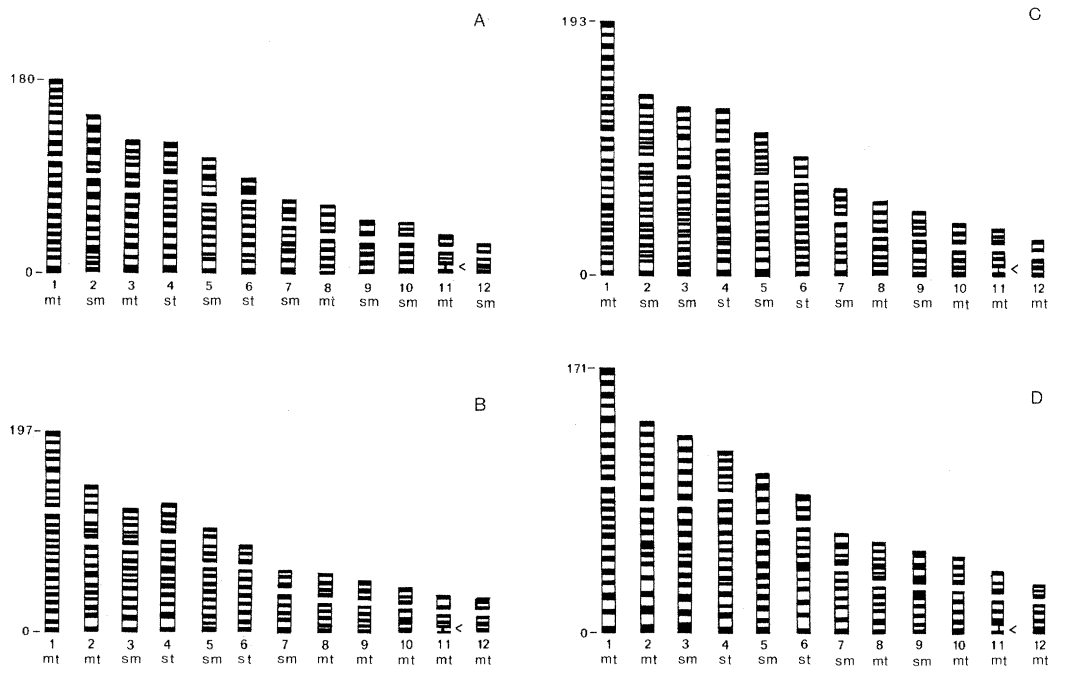

FIG. 5.—(A) Idiogram of *Hyla crucifer* constructed from karyotypes. Dark cross bars represent G-bands, arrows secondary constrictions, spaces denote centromeres, m = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric. Symbols are the same throughout Figs. 5–8. Scale in Figs. 5–8 refer to normalized length of the chromosomes relative to the total complement length. (B) Idiogram of *Pseudacris brimleyi* constructed from karyotypes. (C) Idiogram of *Pseudacris triseriata feriarum* constructed from karyotypes. (D) Idiogram of *Pseudacris ornata* constructed from karyotypes.

though *H. andersoni* was found to have a secondary constriction on the short arm of chromosome 6, the constriction was in a slightly different position than the constrictions of the other species named. An inversion on the short arm of chromosome 6 in a *H. chrysoscelis* group ancestor may have given rise to the current configuration in *H. andersoni*. The probable way this occurred is shown in Fig. 9C.

Chromosome Pair 7

Chromosome pair 7 is subtelocentric in *H. chrysoscelis* (chromosome 6 and 8 morphs), *H. avivoca*, *H. versicolor*, *H. femoralis*, *H. gratiosa* and *H. andersoni* (Figs. 6B, 6C, 7, 8). Chromosome pair 7 was submetacentric in *H. squirella*, *H. cinerea*, *H. crucifer*, *P. brimleyi*, *P. ornata* and *P. t. feriarum* (Figs. 5, 6A, 6C). No major differences in banding pattern were noted.

Chromosome Pair 8

Chromosome pair 8 was metacentric in all species examined (Figs. 5–8). In the *H. chrysoscelis* chromosome 8 morph, a secondary constriction was found on the long arm (Figs. 3C, 7C). Thus *H. chrysoscelis* populations examined were polymorphic for secondary constrictions. This secondary constriction position probably arose from a translocation between chromosomes 6 and 8. The probable way this took place is depicted in Fig. 9B. Since the majority of *H. chrysoscelis* allies (*H. avivoca*, *H. andersoni* and *H. versicolor*) possess the chromosome 6 secondary constriction position, it

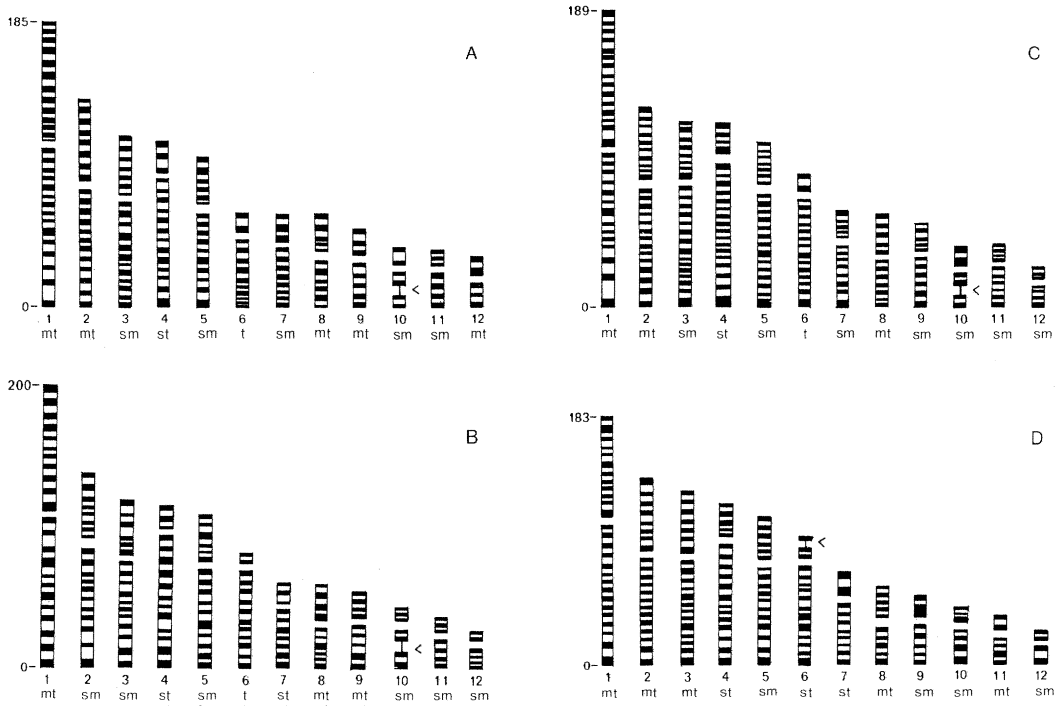

FIG. 6.—(A) Idiogram of *Hyla squirella* constructed from karyotypes. (B) Idiogram of *Hyla gratiosa* constructed from karyotypes. (C) Idiogram of *Hyla cinerea* constructed from karyotypes. (D) Idiogram of *Hyla andersoni* constructed from karyotypes.

is reasonable to assume that this position is ancestral. No major differences in banding pattern were noted in the other species.

Chromosome Pair 9

Chromosome pair 9 was submetacentric in *H. chrysoscelis* (chromosome 6 and 8 morphs), *H. avivoca, H. versicolor, H. femoralis, H. andersoni* and *H. crucifer* (Figs. 5A, 6D, 7, 8). Chromosome pair 9 was metacentric in *H. gratiosa, H. squirella, P. brimleyi* and *P. ornata* (Figs. 5B, 5D, 6A, 6B). *H. cinerea* and *P. t. feriarum* were barely within the submetacentric category, and the arm ratios were not significantly different from the metacentric chromosomes of the other species (Figs. 5C, 6C). No major differences in banding pattern were noted.

Chromosome Pair 10

Chromosome pair 10 was submetacentric in most species examined (Figs. 5A, 6, 7, 8). In *P. brimleyi, P. ornata* and *P. t. feriarum,* chromosome pair 10 was metacentric (Figs. 5B, 5C, 5D). *H. cinerea, H. gratiosa* and *H. squirella* had a secondary constriction on the long arm of chromosome pair 10 (Figs. 2A, 2B, 2C; 6A, 6B, 6C). If coalescence of bands was considered, *H. crucifer, P. t. feriarum, P. brimleyi* and *P. ornata* had three bands on the short arm of chromosome pair 10; the other species had two bands on the short arm of chromosome pair 10 (Figs. 5–8).

Chromosome Pair 11

Chromosome pair 11 was metacentric in most of the species examined. Chro-

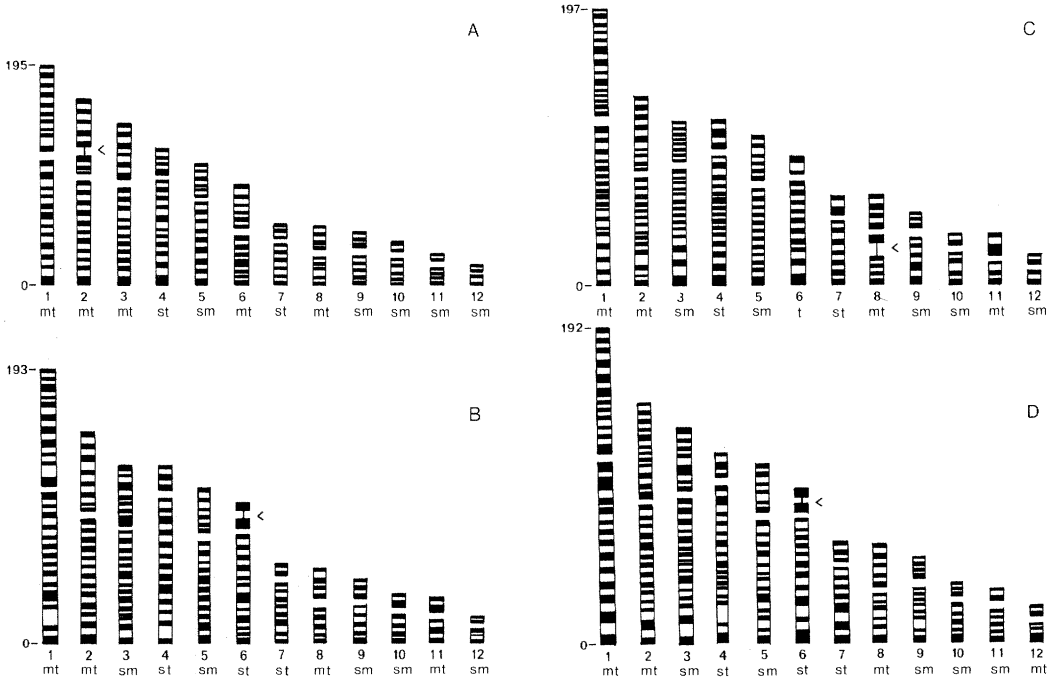

FIG. 7.—(A) Idiogram of *Hyla femoralis* constructed from karyotypes. (B) Idiogram of *Hyla avivoca* constructed from karyotypes. (C) Idiogram of the chromosome 8 morph of *Hyla chrysoscelis*. (D) Idiogram of the chromosome 6 morph of *Hyla chrysoscelis*.

mosome pair 11 was submetacentric in *H. chrysoscelis* (chromosome 6 morph), *H. versicolor*, *H. gratiosa*, *H. squirella* and *H. cinerea* (Figs. 6A, 6B, 6C, 7D, 8). *H. crucifer*, *P. brimleyi*, *P. ornata* and *P. t. feriarum* had a secondary constriction on the long arm of chromosome pair 11 (Figs. 1, 5). No major differences in banding pattern were noted.

Chromosome Pair 12

Chromosome pair 12 was submetacentric in most species examined (Figs. 5A, 5D, 6B, 6C, 6D, 7A, 7B, 7C, 8). Chromosome pair 12 was metacentric in *H. chrysoscelis* (chromosome 6 morph), *H. squirella*, *P. brimleyi* and *P. t. feriarum* (Figs. 5B, 5C, 6A, 7D).

DISCUSSION

The three groups derived from chromosome banding analysis are in close agreement with groupings described by others. Based on experimental hybridization studies, Mecham (1965) found that the potential for gene exchange exists between most species of *Pseudacris* (including all species examined in my

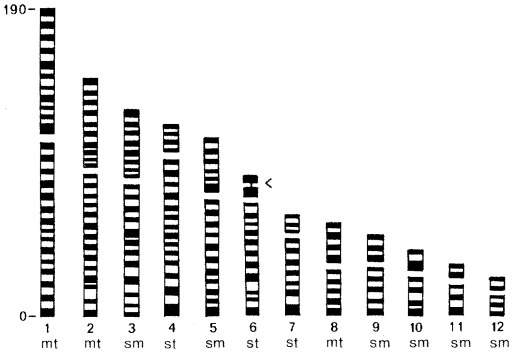

FIG. 8.—Idiogram of *Hyla versicolor* constructed from karyotypes. Only one haploid set is shown since the banding pattern of all four homologues was the same. The secondary constriction shown was found on only two of the four homologues.

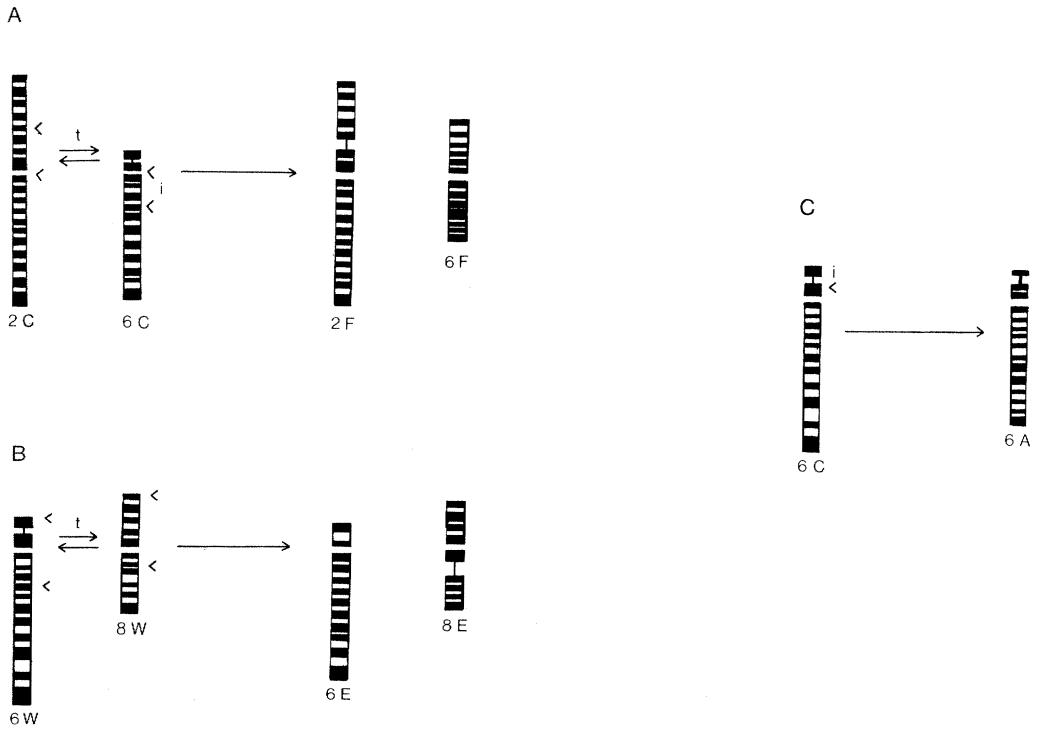

FIG. 9.—(A) Translocation between chromosomes 2 and 6, and inversion in chromosome 6 to form *Hyla femoralis* chromosomes 2 and 6. Dark bands represent G-bands, spaces represent centromeres, and a space with a connecting line represents a secondary constriction. Open arrows represent break points. C = *Hyla chrysoscelis*, F = *Hyla femoralis*; t = translocation, i = inversion. (B) Translocation between chromosomes 6 and 8 in the chromosome 6 morph of *Hyla chrysoscelis* to form chromosomes 6 and 8 of the chromosome 8 morph. W = Chromosome 6 morph, E = Chromosome 8 morph. (C) Inversion in *Hyla chrysoscelis* group chromosome 6 to form chromosome 6 of *Hyla andersoni*. C = *Hyla chrysoscelis* group, A = *Hyla andersoni*, i = inversion.

study), between members of the *H. chrysoscelis* group (*H. chrysoscelis, H. versicolor, H. avivoca* and *H. femoralis*) and between members of the *H. gratiosa* group (*H. gratiosa* and *H. cinerea*). No group had the potential for gene exchange with the other. No distinction was made between *H. chrysoscelis* and *H. versicolor* in Mecham's (1965) study. *H. versicolor* females will produce viable triploid hybrids with most species of North American hylids (J. P. Bogart, personal communication). *H. crucifer* was found marginally compatible with some *Pseudacris* species (Mecham, 1965). Hybridization between *H. crucifer* and other *Hyla* (*H. cinerea, H. chrysoscelis-versicolor*) resulted in high mortality and cessation of development near the larval stage (Mecham, 1965). Other experimental hybridization studies have confirmed Mecham's (1965) results (Fortman and Altig, 1974; Gerhardt et al., 1980; Pyburn, 1960). On the basis of hybridization studies, *H. andersoni* was found to be only marginally compatible with *H. cinerea*. These data appear to be in contradiction with Blair's (1959) inclusion of *H. andersoni* with the *H. cinerea* group. Natural hybrids have been recorded for these combinations: *H. cinerea* × *H. gratiosa*, *H. chrysoscelis* × *H. avivoca* and *H. chrysoscelis* × *H. femoralis* (Mecham, 1965). All of the natural hybrids are

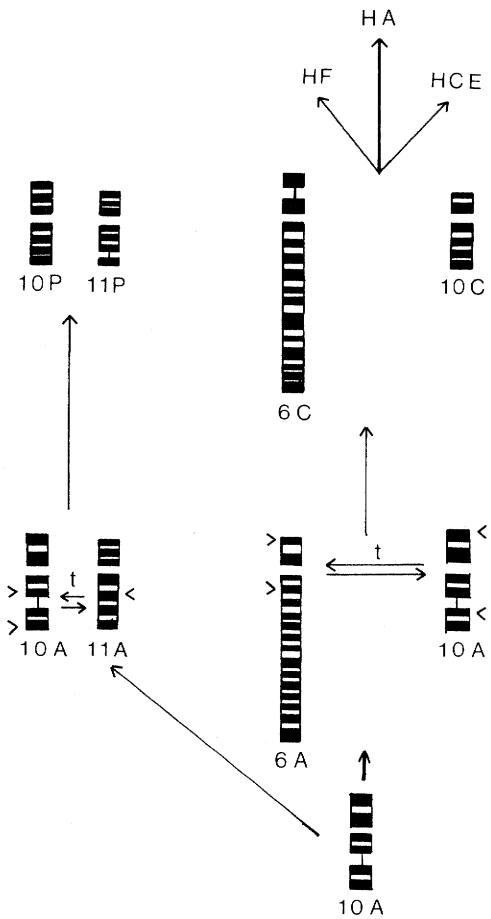

FIG. 10.—Derivation of secondary constriction positions of Hylidae studied from an ancestral chromosome 10. Dark bands represent G-bands, open spaces represent centromeres, and open spaces with connecting lines represent secondary constrictions. Open arrows represent break points. A = ancestral; P = *Pseudacris* group; C = *Hyla chrysoscelis* group; HA = *Hyla andersoni*; HF = *Hyla femoralis*; HCE = chromosome 8 morph of *Hyla chrysoscelis*; t = translocation.

formed by crosses between members of the same species group.

In an osteological analysis of treefrogs, Gaudin (1974) recognized all species of *Pseudacris* as belonging to one group, all *Hyla* species (except *H. crucifer*) used in my study as belonging to another group, and he found *H. crucifer* distinct from both groups. According to albumin similarities, all of the *Hyla* species except *H. versicolor* (which was not studied) and *H. crucifer* (which was distinct) belong to the same group. All *Pseudacris* species used belong to the same group (Maxson and Wilson, 1975). A more detailed analysis, taking into account studies of albumin similarities, external morphology, skin color and pattern, osteology, karyotypes (not banded), mating calls, potential for hybridization, and larval characteristics, was performed by Maxson and Wilson (1975). They grouped *Hyla* and *Pseudacris* into one large group but divided them into subgroups as follows: (1) *H. andersoni, H. cinerea, H. gratiosa*; (2) *H. avivoca, H. femoralis*; (3) *H. chrysoscelis, H. versicolor*; (4) *P. ornata*; (5) *P. brimleyi, P. t. feriarum*; (6) *Incertae sedis*: *H. crucifer, H. squirella*. This grouping is in general agreement with the scheme derived from my study, except for the placement of *H. andersoni* and the fact that *H. crucifer* and *H. squirella* can be reliably placed using chromosome banding analysis. The close homology exhibited by the banded karyotypes is in agreement with Bogart (1973) who stated that holarctic hylid karyotypes have been conservative in their evolution. However, the hylid karyotypes examined are not conservative with respect to secondary constriction position.

It is difficult to determine which of the secondary constriction positions is ancestral. According to immunological distance studies, North American hylids diverged from South American hylids 65 million years ago, and Eurasian hylids diverged from North American hylids 40 million years ago (Maxson and Wilson, 1975). During the Paleocene (62 million years ago) exchange with South America was occurring (Savage, 1973). South America was isolated throughout most of the rest of the Cenozoic until the appearance of the Panamanian land bridge 3 million years ago (Patterson and Pascual, 1972). *Hyla arborea*, a Eurasian species, *Osteopilus septentrionalis*, a West Indian species, and *Phrynohyas venulosa*, a

wide-ranging species found from Mexico to South America, have secondary constriction positions identical to those of the *H. gratiosa* group (Schmid, 1978*a*; Wiley, unpublished). The probable derivations of the secondary constriction positions of *H. femoralis* and the chromosome 8 morph of *H. chrysoscelis* and the derivation of the position of the secondary constriction of *H. andersoni* from the *H. chrysoscelis* group by a paracentric inversion have been demonstrated (Fig. 10). Two populations of *H. chrysoscelis* have been investigated by Maxson et al. (1977) who stated that the divergence of the two gene pools occurred four million years ago. Ralin (1978) has raised objections to the data of Maxson et al. (1977) but agreed that two *H. chrysoscelis* populations can be differentiated by the presence of distinct immunoalleles and that both alleles are found in *H. versicolor*. The origin of *H. versicolor* is even more recent (Ralin, 1976). The genus *Pseudacris* probably arose in North America since it is endemic. Biogeographical and continental drift evidence shows that speciation in North American hylids probably occurred following the Pleistocene glaciations (Blair, 1965) and that the genus *Pseudacris* arose from a *Hyla* progenitor (Savage, 1973). Given the geographic, biochemical, and karyotypic evidence, it is probable that the ancestral group had a secondary constriction position similar to that of the *H. gratiosa* group. It is thus possible to derive all of the secondary constriction positions present in the species studied from the *H. gratiosa* group secondary constriction position by chromosomal changes (Fig. 10).

The divergence of two gene pools of *H. chrysoscelis* and the origin of *H. versicolor* are relatively recent events; Maxson et al.'s (1977) data indicate an origin for *H. versicolor* from hybridization between the eastern and western gene pools of *H. chrysoscelis*. Unfortunately, it cannot be ascertained whether or not the two chromosome morphs of *H. chrysoscelis* described in my study are equivalent to the eastern and western gene pools described by Maxson et al. (1977) because the specimens used in the two studies were not sympatric. It is difficult to state whether or not both chromosome morphs were involved in the origin of *H. versicolor*. In all specimens of *H. versicolor* used in my study, secondary constrictions were found on only two of the four homologues of chromosome pair 6 (Fig. 4). No other secondary constrictions were evident. Possibly the secondary constriction in *H. versicolor* is the nucleolar organizing region (NOR). The secondary constriction in *H. versicolor* resembles the type 4 secondary constriction found in *Litoria* species (King, 1980), which is a NOR. The secondary constrictions of *H. cinerea* and *P. ornata*, which resemble those of *H. versicolor*, are NOR's (Schmid, 1978*a*). The presence of only two secondary constrictions in *H. versicolor* may be due to diploidization, or to amphiplasty (Navashin, 1934) where the NOR of one species suppresses those of the other species in hybrids. There are differences between the two morphs of *H. chrysoscelis* in arm ratios and banding patterns of chromosome pair 11, while this chromosome pair in the chromosome 6 morph of *H. chrysoscelis* and in *H. versicolor* are very similar (Figs. 3C, 3D, 4, 7C, 7D, 8). However, in order to completely exclude the chromosome 8 morph of *H. chrysoscelis* as a possible ancestor to *H. versicolor*, many more populations must be studied.

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