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Copeia, 1994(2), pp. 302–313

Evolution and Phylogenetic Significance of Ribosomal Gene Location in Chromosomes of Squamate Reptiles

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Using *in situ* hybridization with a biotin-labeled probe, we determined the chromosomal location of ribosomal genes in 56 species of squamate reptiles, including representatives of nine major taxa. Where possible, these data were examined in a phylogenetic context, and in several cases they provided phylogenetically useful shared derived character states. The ribosomal genes in *Sceloporus variabilis* are found on a single pair of microchromosomes, which seems to be primitive for the phrynosomatids. In the remainder of species of *Sceloporus* we examined, the ribosomal genes are found on the long arm of pair 2. We also found that *Holbrookia*, *Cophosaurus*, and *Callisaurus* share a derived condition not found in *Uma*. Two species of the viperid genus *Agkistrodon* share a condition that may be derived relative to other pit vipers. A third species of *Agkistrodon* differs from all other reptiles we examined in that ribosomal genes are located on the sex chromosomes. Location of rDNA also provides systematic information in several other clades of squamates.

DNA sequences coding for ribosomal RNA in eukaryotes consist of tandemly repeated copies that may be found on one or several chromosome pairs. The transcription of these genes in interphase nuclei can be recognized by the presence of one or more nucleoli within the nucleus. The nucleoli are formed from a complex of rRNA and proteins assembled into functional ribosomes. Silver-staining techniques can

be used to visualize ribosomal sequences that are being actively transcribed and can determine the location of nucleolar organizing regions (NORs) on metaphase chromosomes.

The procedure of *in situ* hybridization also has been used to locate ribosomal RNA genes (=rDNA) in interphase or metaphase nuclei. After chromosomal preparations on microscope slides have been denatured to separate

the DNA strands, a single-stranded probe molecule is hybridized to the chromosomal DNA in areas of partial sequence identity. The probe can be labeled in various ways to visualize regions of hybridization. In this study, probes were labeled with biotin and viewed under ultraviolet light. The ribosomal sequences are sufficiently conserved that a probe isolated from one species may be used to locate rDNA sequences in species from different classes, phyla, and even different eukaryotic kingdoms. Because this technique does not depend on the presence of proteins and transcription products, it can be used to locate rDNA sequences that may have been inactivated but are still retained in the genome (Martini et al., 1982; Ward and Cole, 1986; McMullen et al., 1991).

Bickham and Rogers (1985) and King et al. (1986) found that variation in location of NORs in turtles and crocodylians was between distantly related rather than closely related species. Moritz (1986) found variation in the number and location of NORs between populations of the gekkonid genus *Gehyra*. However, this level of variation may not be typical of squamates. Porter et al. (1991) found variation within squamate families, but closely related genera often had identical locations of rDNA. In this study, which is a continuation of that of Porter et al. (1991), we used a probe molecule isolated from the 28S ribosomal sequence of the laboratory mouse (Arnheim, 1979) to identify chromosomes with ribosomal repeats. The in situ hybridization procedures used in this study cannot locate single-copy ribosomal genes. It, therefore, cannot be determined whether observed variation in rDNA location is due to translocation of multiple tandem repeats in the genome or to amplification and reduction in copy number of already existing sites. However, either case represents an alteration in the genomic organization of rDNA which can be analyzed in a systematic or evolutionary context. The primary objectives of this study were (1) to document patterns of evolution of rDNA location based on previously accepted phylogenies; and (2) to use rDNA data to help determine the systematic relationship in other taxa.

MATERIALS AND METHODS

Fifty-six species of squamate reptiles were examined (Appendix), including 16 of those previously studied by Porter et al. (1991). Four snakes were purchased commercially, and two lizards were karyotyped from blood drawn from captive specimens (see Material Examined). All other specimens were collected from natural

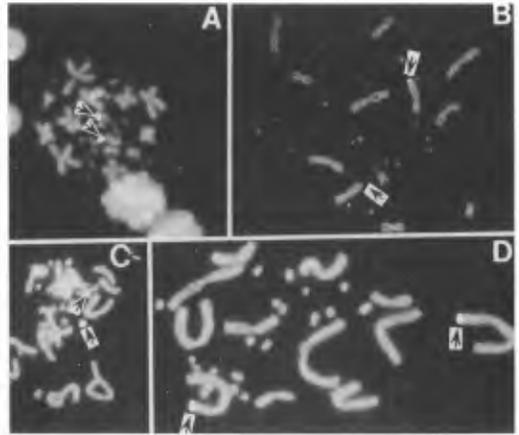


Fig. 1. Hybridized karyotypes of representative phrynosomatid lizards. In Figures 1–3, regions of hybridization appear as a light color and are indicated with arrows. (A) *Petrosaurus mearnsi* (UTEP13698). (B) *Holbrookia propinqua* (LUK439). (C) *Sceloporus variiabilis* (LUK437). (D) *Sceloporus jarrovi* (LUK339).

populations. Chromosome preparations were made from bone marrow, testes (Porter and Sites, 1986), cell culture (Sites et al., 1979), or blood (Baker et al., 1971). Because of the difficulty often encountered in G-banding lizard chromosomes, it was not possible to confirm the homology of chromosome arms in different species. Therefore, determination of homology was based on size and morphology of chromosomes, and a minimum number of rearrangements was assumed. Vouchers of specimens not maintained in captivity are deposited at institutions indicated in the Material Examined.

In situ hybridization was performed with a biotin-labeled probe according to the procedures of Moyzis et al. (1987) as modified by Baker and Wichman (1990) and Hamilton et al. (1990). Detailed description of these procedures is given by Porter et al. (1991). Preparations viewed and photographed at 436 nm show regions of hybridization as yellow fluorescence, and the remainder of the chromosome is orange when counterstained with propidium iodide. Figures 1–3 are monochrome reproductions of the original color photographs. These reproductions were prepared by exposing Kodak Technical Pan Film through a green filter at exposure index 160 and developing in Kodak HC-110 developer (dilution B) for 8.5 min. Color prints of these figures are included in Porter (1992).

Hybridization was performed with the I-19 probe isolated by Arnheim (1979) from the 28S ribosomal gene sequence of the laboratory mouse, *Mus musculus*. This probe is conserved

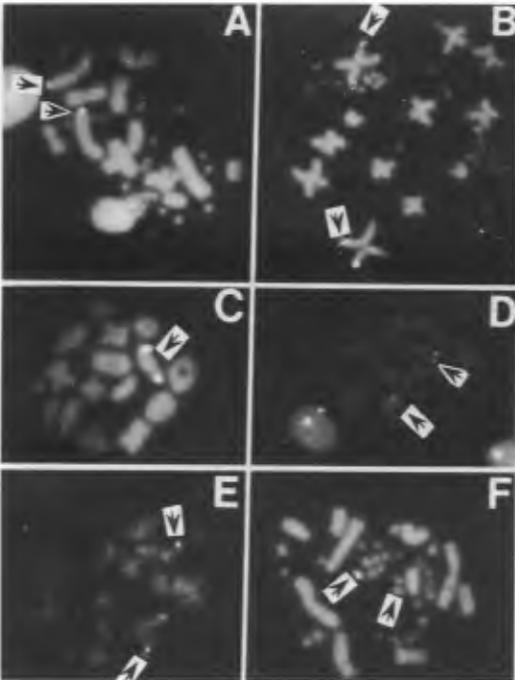


Fig. 2. Hybridized karyotypes of various lizards and snakes. (A) *Gambelia wislizenii* (CAS174477). (B) *Anolis carolinensis* (TTU11329). (C) *Coleonyx variegatus* (UTEP13699); the cell shown is in diakinesis, and the bivalent with the ribosomal genes is indicated with an arrow. (D) *Varanus niloticus* (TK28350). (E) *Thamnophis sirtalis* (TTU11432) haploid secondary spermatocyte. (F) *Naja naja* (TTU11433).

to the extent that it produces hybridization to mollusc rDNA with the methods used in this study. Therefore, it is unlikely that any species of amniote vertebrate has diverged from *Mus* to a point where there is insufficient sequence similarity to produce hybridization. This technique reveals the presence of tandemly repeated sequences but is not sufficiently sensitive to reveal single-copy genes. It is not known exactly how many repeats are required to produce a visible signal. However, Baker et al. (1992; R. J. Baker, pers. comm.) found strong signals with the I-19 probe even when the approximately 300 ribosomal gene copies found in the haploid genome of rodents were distributed among as many as 10 chromosome pairs. This suggests that the procedure is at least sensitive enough to detect ribosomal sequences of as few as 30 copies.

RESULTS AND DISCUSSION

A tabular description of the karyotype and the location of hybridization of the rDNA probe

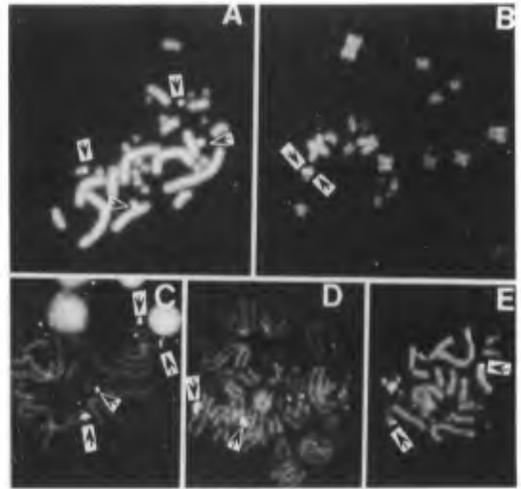


Fig. 3. Hybridized karyotypes of representative pit vipers. (A) *Calloselasma rhodostoma* (TTU11314). (B) *Aghistrodon intermedius* (TTU11434). (C) *Crotalus atrox* (TTU11435). (D) *Aghistrodon piscivorus* (LUK487); chromosomes of two cells are shown, but arrows indicate regions of hybridization in only one of the two diploid chromosome sets. (E) *Aghistrodon contortrix* (LUK506).

for each species is given in the Appendix. Representatives of four major iguanian clades (Iguaninae, Polychrotidae, Phrynosomatidae, and Crotaphytidae) were included in the study. The presumed ancestral karyotype of iguanian lizards consists of 12 meta- or submetacentric macrochromosomes and 24 microchromosomes (designated 12 + 24; Paull et al., 1976; Bickham, 1984). Among the macrochromosomes, pair 1 is metacentric; pair 2 is slightly smaller and submetacentric; pairs 3 and 4 are metacentric and usually identical to each other in size and morphology; pairs 5 and 6 are both metacentric and of progressively smaller size (Fig. 1). This macrochromosomal morphology is found in many species representing various iguanian families and is thought to be primitive for iguanians.

Iguanine and crotaphytid lizards.—All of the iguanines and crotaphytids thus far karyotyped possess an identical 12 + 24 karyotype. We have probed the rRNA genes of representatives of both genera of crotaphytids and three of eight iguanine genera. All of these lizards have rDNA sequences on the end of the long arm of pair 2 (Fig. 2A). The same pattern is found in most species of the phrynosomatid genus *Sceloporus* (Fig. 1D) and in modified form in the tropidurid genus *Leiocephalus* (Porter et al., 1991).

The occurrence of rDNA on pair 2 in cro-

taphytids, iguanines, tropidurids, and some phrynosomatids seems to suggest a close relationship among these taxa; however this hypothesis should be considered cautiously. Although rDNA is found on pair 2 in most *Sceloporus*, for the reasons discussed below, it is unlikely that this condition is retained from a common ancestor shared with crotaphytids, iguanines, and tropidurids. Instead, the presence of rDNA on pair 2 probably is derived within the phrynosomatids. If so, its presence at that location in Tropiduridae, Iguaninae, and Crotaphytidae would represent at least one instance of convergence. It is also possible that the presence of rDNA both on pair 2 and on a pair of microchromosomes, as seen in *Leiocephalus* (Porter et al., 1991), is ancestral for the iguanians with the subsequent loss of rDNA from microchromosomes in iguanines and crotaphytids and from the macrochromosomes in the ancestral phrynosomatid.

Phrynosomatid lizards.—We have examined rDNA sequences in representatives of nine phrynosomatid genera. *Sator* has not been examined, but this small insular genus is often considered congeneric with *Sceloporus* (Wyles and Gorman, 1978; Etheridge and de Queiroz, 1988; but see Wiens, 1993). Much of the rDNA variation observed in this study was found within the phrynosomatids (Fig. 1). With the exception of the genus *Sceloporus*, all phrynosomatid species thus far karyotyped have a 12 + 22 karyotype consisting of 12 meta- or submetacentric macrochromosomes and 22 microchromosomes. This 12 + 22 karyotype is thought to be ancestral for the phrynosomatids (Hall, 1973; Paull et al., 1976; Bickham, 1984). Although number of microchromosomes differs, the morphology of the macrochromosomes in these lizards is identical to that found in other iguanian lizards that have the 12 + 24 karyotype. The karyotypes of *Sceloporus* exhibit considerable variation both among and within species (see review in Sites et al., 1992), but all karyotypes of *Sceloporus* can easily be derived from the 12 + 22 pattern, most commonly by reduction in the number of microchromosomes or by centric fissions of the macrochromosomes (Bickham, 1984; Sites et al., 1992).

Within phrynosomatids, three locations of rDNA have been found (Appendix). In *Callisaurus*, *Cophosaurus*, and *Holbrookia*, rDNA is located on pair 3 or 4 (these pairs are indistinguishable in nondifferentially stained chromosomes). In *Petrosaurus*, *Phrynosoma*, *Uma*, *Uta*, *Urosaurus*, and *Sceloporus variabilis*, rDNA is located on a single pair of microchromosomes. In

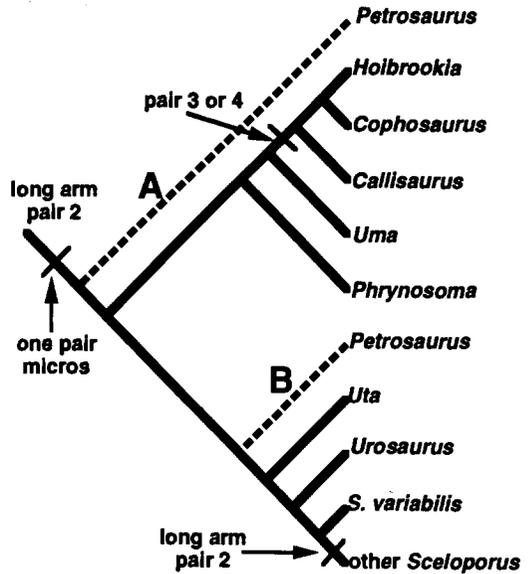


Fig. 4. Phylogeny of phrynosomatids with indication of rDNA location. Alternative placements of *Petrosaurus* (dashed lines) are (A) according to R. E. Etheridge in Paull et al. (1976) and Etheridge and de Queiroz (1988); and (B) according to Frost and Etheridge (1989) and Wiens (1993). Changes in rDNA location are superimposed on the previously proposed phylogenies. Comparison with other major iguanian clades as outgroups shows rDNA on the long arm of pair 2.

all other species of *Sceloporus* examined, rDNA is located on the long arm of pair 2 (or, in the case of *S. merriami*, on a pair of large telocentric chromosomes, which are probably homologous with the long arm of pair 2 in other *Sceloporus*).

Current views on phrynosomatid phylogeny differ in whether they consider *Petrosaurus* to be the sister group of all other phrynosomatids (Fig. 4A; R. E. Etheridge in Paull et al., 1976; Etheridge and de Queiroz, 1988) as opposed to being the sister group of the clade stemming from the most recent common ancestor of *Sceloporus*, *Urosaurus*, and *Uta* (Fig. 4B; Frost and Etheridge, 1989; Wiens, 1993). Examination of the rDNA data in the context of each of these two alternative phylogenies under maximum parsimony (Fig. 4) yields identical conclusions concerning the evolution of rDNA location within phrynosomatids. Under both phylogenies (1) rDNA was located on one pair of microchromosomes in the stem lineage of phrynosomatids; (2) location of the ribosomal genes on pair 3 or 4 evolved in the common ancestor of *Callisaurus*, *Cophosaurus*, and *Holbrookia*; and (3) location of rDNA on the long arm of pair 2 is a derived condition that evolved within *Sc-*

loporus subsequent to the diversification of the extant species.

The location of rDNA on the long arm of pair 2 in many species of *Sceloporus* is similar to the condition seen in outgroup taxa such as iguanines, crotaphytids, and tropidurids (in the third instance, rDNA is also located on a pair of microchromosomes, Porter et al., 1991). Nevertheless, rDNA on the long arm of pair 2 in many species of *Sceloporus* does not appear to be a retained ancestral state. The karyotype and rDNA organization in *S. variabilis* (Fig. 1C) are identical to those found in the genera most closely related to *Sceloporus*. G-banding data (M. W. Haiduk, unpubl.) indicate that *S. variabilis* has retained a karyotype identical to that found in the phrynosomatid genera most closely related to *Sceloporus*. It would appear that *S. variabilis* has retained both the primitive G-banded karyotype and the rDNA distribution primitive for the genus. Furthermore, morphological evidence (Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989; Wiens, 1993) indicates that *Sceloporus* is embedded well within the phrynosomatid clade. The occurrence of microchromosomal rDNA in other genera of phrynosomatids and their phylogenetic relationships suggests that this is the ancestral condition and that the location of rDNA on pair 2 in other species of *Sceloporus* represents a character reversal.

It is generally agreed (Presch, 1969; Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989) that *Phrynosoma* is the sister taxon to the four genera (*Uma*, *Callisaurus*, *Cophosaurus*, and *Holbrookia*) collectively known as sand lizards. However, the relationships among the sand lizard genera have been controversial (reviewed by de Queiroz, 1989, 1992). One of the primary differences among the phylogenetic trees of sand lizards proposed by previous authors concerns whether *Uma* or *Holbrookia* was the first lineage to diverge from the rest of the clade. Based on outgroup comparisons with other phrynosomatids, the presence of ribosomal genes on chromosome pair 3 or 4 (Fig. 1B) in *Callisaurus*, *Cophosaurus*, and *Holbrookia* is derived relative to the condition in *Uma*. Therefore, this character state is an additional synapomorphy uniting *Callisaurus* with the earless lizards and placing *Uma* as the sister group of the remaining sand lizards.

Polychrotid lizards.—We have examined three species of *Anolis*. The karyotype of *A. homolechis* differs considerably from the karyotype of other iguanians, and so it is difficult to determine chromosome homologies. *Anolis porcatum* and *A. carolinensis* have a 12 + 24 karyotype that occurs

widely within the genus as well as in other iguanian families. However, these two species of *Anolis* are unique among iguanians so far examined in that the ribosomal genes are on the largest chromosome pair. Chromosome pair 1 is slightly submetacentric in these species, and Porter et al. (1991) reported ribosomal genes on the end of the long arm of pair 1 in *A. porcatum*. Although the chromosomes of *A. carolinensis* are indistinguishable from *A. porcatum*, the ribosomal genes appear to be on the short arm of pair 1 in *A. carolinensis* (Fig. 2B). In both *A. porcatum* and *A. carolinensis*, the two arms of chromosome 1 are nearly the same size. Upon re-examining material from both species, it seems the apparent difference can be attributed to technical difficulties and is most likely due to variation in the degree of contraction of the arms in different cells. However, if the apparent difference in rDNA location reflects a real difference between the two species, the change could result from either a small pericentric inversion or an addition/deletion in one arm. Either scenario could alter the designation of long and short arms without altering the relationship of rRNA genes to other sequences in the same chromosome. Alternatively, it is possible that the rDNA has been translocated to the opposite arm of chromosome 1.

Gekkonid lizards.—The karyotype of gekkonids differs substantially from that of iguanians. The most common gekkonid karyotype consists primarily of acrocentric chromosomes with no division between macro- and microchromosomes (Gorman, 1973; Bickham, 1984). The ribosomal genes are near the telomere of one of the largest chromosome pairs in *Coleonyx variegatus* (Fig. 2C). In the Australian gekkonid, *Heteronotia binoei*, in situ hybridization revealed that the ribosomal genes are on the sixth largest chromosome (Hillis et al., 1991). Moritz (1986) found NORs on chromosome 10 in *Gehyra*, but the location of this NOR was variable, and additional NORs were sometimes found on other chromosomes.

Varanid lizards.—We examined material from one specimen of *Varanus niloticus*. The ribosomal genes in this species are found near the centromere of pair 1, which is a large metacentric chromosome (Fig. 2D). The region of hybridization corresponds with a distinct secondary constriction, which King and King (1975) correctly interpreted as the nucleolus organizer. The secondary constriction is found near the centromere of pair 1 in all karyotyped species of the genus (King and King, 1975). Assuming that rDNA can be accurately located by the

secondary constriction, it would appear that the ribosomal genes have remained in the same location throughout the evolution of *Varanus*. The proximal location of NORs in varanid chromosomes is unusual. Other squamate reptiles typically have the sites of rDNA located near the chromosome ends.

Colubrid, elapid, and viperid snakes.—The most common snake karyotype is $2n = 36$ with 16 macrochromosomes and 20 microchromosomes (Baker et al., 1972; Gorman, 1973). This karyotype is typical of widely divergent families, including Leptotyphlopidae, Boidae, Colubridae, and Viperidae, and it has been suggested that this $16 + 20$ karyotype is ancestral for snakes (Beçak and Beçak, 1969; Baker et al., 1972; Gorman, 1973). However, G-banding data suggest that substantial rearrangement of genes may have occurred without altering the chromosomal morphology (Mengden and Stock, 1980).

The New World natricines (Colubridae) possess a $2n = 36$ karyotype that differs substantially from the $16 + 20$ karyotype commonly found in other snakes. The typical American natricine karyotype consists of 34 biarmed macrochromosomes and two microchromosomes ($34 + 2$) and is considered derived relative to other colubrids (Baker et al., 1972; Rossman and Eberle, 1977). Porter et al. (1991) examined two natricines (*Thamnophis marcianus* and *Nerodia fasciata*), and in both, the ribosomal genes were found on the long arm of chromosome pair 1 or 2. We have examined two additional natricines (*N. rhombifer* and *T. sirtalis*) and found that both also have rDNA on the long arm of pair 1 or 2. However, *T. sirtalis* has ribosomal genes on the microchromosome pair in addition to a macrochromosome (Fig. 2E). Because the chromosomal location of rDNA in *T. marcianus* also is found in at least two species of *Nerodia*, it is likely that this character is primitive for *Thamnophis* and that the presence of rDNA in the microchromosomes is derived in *T. sirtalis*. A survey of rDNA location in other garter snakes could help elucidate the relationships among the species of this genus.

Nonnatricine colubrids possess the widely distributed $16 + 20$ karyotype. Porter et al. (1991) found the ribosomal genes to be located on a single pair of microchromosomes in *Masticophis flagellum*. We have since examined three additional nonnatricine colubrids (*Elaphe obsoleta*, *E. guttata*, and *Pituophis melanoleucus*) and found that the rDNA distribution in these species is indistinguishable from that reported for *Masticophis*.

Elapid species that have been karyotyped show a moderate amount of chromosomal variation.



Fig. 5. Giemsa-stained karyotypes of two species of Asian pit vipers. The sex chromosomes are indicated as Z and W. (A) Karyotype of female *Agkistrodon intermedius* (TTU11434). (B) Karyotype of female *Calloselasma rhodostoma* (TTU11314).

An examination of rDNA in additional elapids may shed light on the patterns of karyotypic evolution in this family. The cobra *Naja naja*, has a diploid number of 38 (Nakamura, 1935; Singh et al., 1970), but there is disagreement as to which elements should be properly designated as microchromosomes. The ribosomal genes were restricted to a single pair of the 22–24 microchromosomes in this species.

Karyotypes have not previously been reported for two Asian pit vipers *Calloselasma rhodostoma* and *Agkistrodon intermedius* (Cole, 1990), and giemsa-stained karyotypes of these species are shown in Figure 5. As has been reported for other pit vipers, both species have a diploid number of 36 (16 macrochromosomes and 20 microchromosomes) with the sex chromosomes being the fourth largest pair (pair 4). Although we have not examined males of these species, it appears that the female is the heterogametic sex, as in other pit vipers (Cole, 1990). In both species, the Z chromosome is slightly submetacentric. The W chromosome of *A. intermedius* is metacentric or slightly submetacentric. In *C. rhodostoma*, the W chromosome is subtelocentric. Unlike other pit vipers (Cole, 1990), chromosome 3 of *C. rhodostoma* is submetacentric, rather than metacentric. The morphology of the other macroautosomes in *A. intermedius* and *C. rhodostoma* is similar to that of other pit vipers (Cole, 1990).

We have now studied seven species representing four genera of pit vipers. Porter et al. (1991) found rDNA on two microchromosome pairs in *Crotalus viridis*. The same organization (Fig. 3C) is found in two other rattlesnakes (*C. atrox* and *Sistrurus catenatus*) and in the Malayan pit viper *Calloselasma rhodostoma* (Fig. 3A). However, the rDNA probe hybridized to only one pair of microchromosomes in the Asian species *A. intermedius* (Fig. 3B) and its American congener, *A. piscivorus* (Fig. 3D).

Ribosomal genes in *A. contortrix* are located

on the Z chromosomes (Fig. 3E), but we have not yet examined a female of *A. contortrix*, so we do not know whether the W chromosome carries ribosomal genes in that species. Ribosomal DNA is found infrequently on sex chromosomes of other vertebrates. Using both in situ hybridization and silver staining, rDNA has been found on sex chromosomes in the phyllostomid bat genus *Carollia* (Hsu et al., 1968, 1975; Goodpasture and Bloom, 1975). Moritz (1986) described sex-linked NORs in the Australian gecko *Gehyra*.

The interpretation of rDNA evolution in pit vipers is hindered by lack of a generally accepted phylogeny (Gloyd and Conant, 1990; but see the proposed phylogenies of Brattstrom, 1964). Therefore, variation in rDNA location may be helpful in reconstructing crotaline phylogeny. Outgroup comparison with colubrids and elapids suggests that the presence of rDNA on two pairs of microchromosomes in *Calloselasma* and rattlesnakes is derived relative to presence on only one pair of microchromosomes in some *Aghkistrodon*. However, both Gloyd and Conant (1990) and Knight et al. (1992) have proposed that *C. rhodostoma* diverged early from both Old and New World representatives of *Aghkistrodon*, and none of the trees published by Knight et al. (1992) indicate a close relationship of *Calloselasma* to rattlesnakes. If *Calloselasma* is the sister group of either *Aghkistrodon* or of *Aghkistrodon* plus rattlesnakes, then it is possible that the presence of rDNA on two pairs of microchromosomes in *Calloselasma*, *Crotalus*, and *Sistrurus* is ancestral for pit vipers. Study of rDNA in viperines should help determine the primitive location of ribosomal genes in pit vipers.

The three American species of *Aghkistrodon* are thought to represent a monophyletic group (Minton, 1990; Van Devender and Conant, 1990; Knight et al., 1992), but the monophyly of the Asian and American *Aghkistrodon* has been questioned (Hoge and Romano-Hoge, 1981; Knight et al., 1992). If the condition seen in *Calloselasma* and rattlesnakes is primitive for pit vipers, then the presence of rDNA on a single pair of microchromosomes in *A. intermedius* and *A. piscivorus* would be derived and would be a potential synapomorphy uniting the Asian and American species of *Aghkistrodon*. But regardless of which condition is ancestral and which is derived, at least two separate pit viper lineages may have invaded the New World. One of these carried its rDNA on two pairs of microchromosomes and gave rise to the rattlesnakes. The other lineage carried its rDNA on a single pair of microchromosomes and gave rise to the American species of *Aghkistrodon*. And in either

case, the location of rDNA on the sex chromosomes in *A. contortrix* evolved in the New World.

Knight et al. (1992), suggested that the New World *Aghkistrodon* may be more closely related to the American genus *Porthidium* than to Asian *Aghkistrodon* and that Asian *Aghkistrodon* may be more closely related to at least some species of *Trimeresurus* than to New World *Aghkistrodon*. Although rDNA on one pair of microchromosomes is a possible synapomorphy uniting Old and New World *Aghkistrodon* relative to *Calloselasma* and the rattlesnakes, the location of rDNA in *Porthidium* and *Trimeresurus* remains to be determined. Examined of rDNA location in these and additional crotalines should provide valuable data toward the resolution of questions concerning the phylogeny and biogeography of pit vipers.

Conclusions.—Evolutionary changes in the chromosomal location of rDNA in squamate reptiles have often occurred at a rate that allows inferences regarding its evolution as well as providing potential systematic characters at various hierarchical levels. For example, interpretation of rDNA location among phrynosomatid genera in the context of their phylogenetic relationships permitted us to draw several conclusions about evolutionary transformations in the location of rDNA. However, additional study is warranted to document rDNA location in other species of *Sceloporus*. In addition, the location of rDNA apparently provides systematic characters relevant to the elucidation of pit viper phylogeny and biogeography. We have suggested some tentative interpretations of these data. However, additional species of both viperines and crotalines will need to be examined before firm conclusions can be made. Although relatively few species have been examined, variation in rDNA location was noted among major clades of iguanians and in polychrotid lizards and natricine snakes. Future studies should explore the possibilities of using rDNA location as a systematic character in these taxa.

MATERIAL EXAMINED

Specimens deposited in the collection at Lamar University in Beaumont, Texas, are indicated with the acronym LUK. The acronym EDHEM represents specimens deposited in the Ecología de la Herpetofauna del Estado de México collection at the Escuela Nacional de Estudios Profesionales—Iztacala in Tlalnepantla, México, México. Other museum acronyms are as recommended by Leviton et al. (1985). Specimens that are currently maintained in captivity are identified by their Texas Tech University voucher (TK) number until the voucher specimen is deposited. Some specimens were examined previously by Porter et al. (1991).

Colubridae: *Elaphe guttata* - *moryi* TTU11444, Texas: Garza County; *E. obsoleta quadrivittata* TTU11417, Locality unknown (purchased com-

mercially); *Masticophis flagellum testaceus* TTU11419, Texas: Garza County; *Nerodia fasciata pictiventris* TTU11421, Florida: Collier County; *N. rhombifer rhombifer* TTU11424, Texas: Colorado County; *Pituophis melanoleucus sayi* TTU11426, Texas: Lubbock County; *Thamnophis marcianus marcianus* TTU11431, Texas: Lubbock County; *T. sirtalis sirtalis* TTU11432, Maryland: Allegany County.

Crotaphytidae: *Crotaphytus collaris collaris* TTU11344, Texas: Garza County; *Gambelia wislizenii wislizenii* CAS174477, California: San Bernardino County.

Elapidae: *Naja naja naja* TTU11433, Pakistan (purchased commercially).

Gekkonidae: *Coleonyx variegatus* UTEP13699, Arizona: Maricopa County.

Iguaninae: *Dipsosaurus dorsalis dorsalis* CAS174475, California: San Bernardino County; *Iguana iguana* TK28384, Captive bred specimen (purchased commercially); *Sauromalus obesus obesus* LUK432, Arizona: Mohave County.

Phrynosomatidae: *Callisaurus draconoides* CAS174473, California: San Bernardino County; *Cophosaurus texanus texanus* LUK176, Texas: Kimble County; *C. t. scitulus* CAS174483, Arizona: Cochise County; *Holbrookia lacerata subcaudalis* LUK435, Texas: Webb County; *H. maculata maculata* TTU11348, Texas: Lubbock County; *H. propinqua* LUK439, Texas: Hidalgo County; *Petrosaurus mearnsi* UTEP13698, California: Imperial County; *Phrynosoma cornutum* TTU11357, Texas: Lubbock County; *P. modestum* TTU11358, Texas: El Paso County; *P. solare* CAS174487, Arizona: Pima County; *Sceloporus clarki* CAS17486, Arizona: Cochise County; *S. graciosus graciosus* TTU11359, Utah: Washington County; *S. grammicus* 1BHED7163, Mexico: Tlaxcala; *S. grammicus microlepidotus* BYU39797, Mexico: Distrito Federal; *S. jarrovi* LUK339, Arizona: Cochise County; *S. magister uniformis* TTU11360, Utah, Washington County; *S. merriami* LUK443, Texas: Brewster County; *S. occidentalis biseriatus* LUK405, LUK406, Utah: Washington County; *S. olivaceus* LUK231, LUK254, Texas: Kimble County; *S. orcutti* UTEP13696, California: Riverside County; *S. palaciosi* 1BHED7165, Mexico: Michoacan; *S. poinsetti* LUK302, Texas: Zapata County, LUK469, Texas: Real County; *S. scalaris* LUK348, Arizona: Cochise County; *S. serrifer cyanogenis* LUK467, Texas: Zapata County; *S. undulatus elongatus* TTU11365, Utah: Washington County; *S. variabilis* LUK 436-437, Texas: Hidalgo County; *S. virgatus* CAS174490, Arizona: Cochise County; *Uma notata* CAS174488, California: Imperial County; *U. scoparia* CAS174470, California: San Bernardino County; *Urosaurus graciosus* CAS174480, California: San Bernardino County; *U. ornatus* LUK172, LUK226, Texas: Kimble County, CAS174481, Arizona: Cochise County; *Uta stansburiana stejnegeri* TTU11367, New Mexico: Doña Ana County.

Polychrotidae: *Anolis carolinensis* TTU11329, Texas: Brazoria County; *A. homolechis* TTU11332, Cuba: Guantánamo Province; *A. porcatum* TTU11337, Cuba: Guantánamo Province.

Varanidae: *Varanus niloticus* TK28350, Captive specimen of unknown origin.

Viperidae: *Aghistrodon contortrix laticinctus* LUK506, Texas: Bandera County; *A. intermedius saxatilis* TTU11434, Russia (purchased commercially); *A. piscivorus leucostoma* LUK487, Louisiana: Natchitoches Parish; *Calloselasma rhodostoma* TTU11314, Malaysia (purchased commercially); *Crotalus atrox* TTU11435, Texas; *C. viridis viridis* TTU11439, Texas: Castro County; *Sistrurus catenatus edwardsi* TTU11440, Locality unknown.

ACKNOWLEDGMENTS

This paper was part of a dissertation submitted by CAP in partial fulfillment of the requirements of the Doctor of Philosophy degree at Texas Tech University. R. J. Baker, L. D. Densmore III, R. C. Jackson, C. Jones, and the late J. K. Jones, Jr. served as committee members. N. Arnheim provided the I-19 probe. The following people provided specimens or assisted in the fieldwork: P. Adams, R. J. Baker, R. D. Bradley, L. D. Densmore, D. K. Eades, K. Geheb, R. A. Knight, C. S. Lieb, L. C. Lyons, M. Maltbie, J. P. O'Brien, L. G. Porter, P. C. Porter, R. D. Porter, S. D. Porter, J. W. Sites, Jr., S. White, and D. Worner. We thank R. J. Baker, R. D. Bradley, M. J. Hamilton, L. L. Janeczek,

J. L. Longmire, M. Maltbie, M. S. Powell, and R. A. Van Den Bussche for their assistance and advice in the laboratory work. R. J. Baker, L. D. Densmore, R. C. Jackson, C. Jones, J. K. Jones, R. A. Knight, M. J. Hamilton, L. L. Janeczek, J. L. Longmire, M. Maltbie, S. Robert, and R. A. Van Den Bussche reviewed the manuscript.

This study was supported by a National Science Foundation grant to R. J. Baker, by grants to M. W. Haiduk from Lamar University Organized Research, and by grants from ASIH (Gauge Award), the Society for the Study of Amphibians and Reptiles (Grants-in-Herpetology), Sigma Xi (Grant-in-Aid of Research), and from a Helen Hodges Educational Scholarship.

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1992. Accepted 16 July 1993. Section editor: D. G. Buth.

APPENDIX. KARYOTYPES AND LOCATION OF HYBRIDIZATION OF THE rDNA PROBE FOR EACH SPECIES STUDIED. The table includes data from Porter et al. (1991). For some species studied by Porter et al. (1991), we examined additional individuals from different localities (see Material Examined). The number of acrocentric (A) and biarmed (B) macrochromosomes is indicated, along with the number of microchromosomes (m).

| Taxon | A | B | m | Location of hybridization | Karyotype references |
|--------------------------------|----|----|-----------------|---|--|
| Iguaninae | | | | | |
| <i>Dipsosaurus dorsalis</i> | 0 | 12 | 24 | long arm of pair 2 ^a | Paull et al., 1976 ^b |
| <i>Iguana iguana</i> | 0 | 12 | 24 | long arm of pair 2 ^a | Cohen et al., 1967 |
| <i>Sauromatus obesus</i> | 0 | 12 | 24 | long arm of pair 2 ^a | Gorman et al., 1967 |
| Crotaphytidae | | | | | |
| <i>Crotaphytus collaris</i> | 0 | 12 | 24 | long arm of pair 2 ^a | Cohen et al., 1967 |
| <i>Gambelia wislizenii</i> | 0 | 12 | 24 | long arm of pair 2 ^a | Montanucci, 1970 |
| Phrynosomatidae | | | | | |
| <i>Callisaurus draconoides</i> | 0 | 12 | 22 | pair 3 or 4 ^a | Gorman et al., 1969 |
| <i>Cophosaurus texanus</i> | 0 | 12 | 22 | pair 3 or 4 ^a | Painter, 1921 |
| <i>Holbrookia lacerata</i> | 0 | 12 | 22 | pair 3 or 4 ^a | Unpubl. data ^c |
| <i>Holbrookia maculata</i> | 0 | 12 | 22 | pair 3 or 4 ^a | Porter et al., 1991 |
| <i>Holbrookia propinqua</i> | 0 | 12 | 22 | pair 3 or 4 ^a | Unpubl. data ^c |
| <i>Petrosaurus mearnsi</i> | 0 | 12 | 22 | one pair of microchromosomes | Gorman et al., 1969 |
| <i>Phrynosoma cornutum</i> | 0 | 12 | 22 | one pair of microchromosomes | M. Robinson, pers. comm. to Gorman, 1973 |
| <i>Phrynosoma modestum</i> | 0 | 12 | 22 | one pair of microchromosomes | M. Robinson, pers. comm. to Gorman, 1973 |
| <i>Phrynosoma solare</i> | 0 | 12 | 22 | one pair of microchromosomes | M. Robinson, pers. comm. to Gorman, 1973 |
| <i>Sceloporus clarki</i> | 16 | 4 | 20 | long arm of pair 2 ^{a,d} | Lowe et al., 1967 |
| <i>Sceloporus graciosus</i> | 0 | 12 | 18 | long arm of pair 2 ^a | Jackson and Hunsaker, 1970 |
| <i>Sceloporus grammicus</i> | 0 | 12 | 20 ^e | long arm of pair 2 ^a | Hall, 1973; Arévalo et al., 1991 |
| <i>Sceloporus jarrovi</i> | 0 | 12 | 20 ^e | long arm of pair 2 ^a | Cole et al., 1967 |
| <i>Sceloporus magister</i> | 0 | 12 | 14 | long arm of pair 2 ^a | Lowe et al., 1967 |
| <i>Sceloporus merriami</i> | 24 | 0 | 22 | large pair of telocentrics ^{a,f} | Cole, 1971 |
| <i>Sceloporus occidentalis</i> | 0 | 12 | 10 | long arm of pair 2 ^a | Cole et al., 1967 |
| <i>Sceloporus olivaceous</i> | 0 | 12 | 10 | long arm of pair 2 ^a | Cole, 1970 |
| <i>Sceloporus orcutti</i> | 0 | 12 | 22 | long arm of pair 2 ^a | Cole, 1970 |
| <i>Sceloporus palaciosi</i> | 4 | 10 | 20 ^e | long arm of pair 2 ^a | Hall, 1973; Arévalo et al., 1991 |
| <i>Sceloporus poinsetti</i> | 0 | 12 | 20 ^e | long arm of pair 2 ^a | Cole et al., 1967 |
| <i>Sceloporus scalaris</i> | 0 | 12 | 12 | long arm of pair 2 ^a | Lowe et al., 1966 |
| <i>Sceloporus serrifer</i> | 0 | 12 | 20 ^e | long arm of pair 2 ^a | Hall, 1973 |
| <i>Sceloporus undulatus</i> | 0 | 12 | 10 | long arm of pair 2 ^a | Lowe et al., 1966 |
| <i>Sceloporus variabilis</i> | 0 | 12 | 22 | one pair microchromosomes | Hall, 1973 |
| <i>Sceloporus virgatus</i> | 0 | 12 | 10 | long arm of pair 2 ^a | Cole and Lowe, 1968 |
| <i>Uma notata</i> | 0 | 12 | 22 | one pair microchromosomes | Gorman et al., 1967 |
| <i>Uma scoparia</i> | 0 | 12 | 22 | one pair microchromosomes | Gorman et al., 1969 |
| <i>Urosaurus graciosus</i> | 0 | 12 | 22 | one pair microchromosomes | W. P. Hall, unpubl. in Gorman, 1973 |
| <i>Urosaurus ornatus</i> | 0 | 12 | 22 | one pair microchromosomes | Unpubl. data ^c |
| <i>Uta stansburiana</i> | 0 | 12 | 22 | one pair microchromosomes | Pennock et al., 1968 |

APPENDIX. CONTINUED.

| Taxon | A | B | m | Location of hybridization | Karyotype references |
|--------------------------------|-----------------|----|----|--|---------------------------------------|
| Polychrotidae | | | | | |
| <i>Anolis carolinensis</i> | 0 | 12 | 24 | pair 1 ^a | Matthey, 1931 |
| <i>Anolis homolechis</i> | 0 | 14 | 14 | pair 2 or 3 ^a | Gorman and Atkins, 1968 |
| <i>Anolis porcatus</i> | 0 | 12 | 24 | pair 1 ^a | Gorman and Atkins, 1968 |
| Varanidae | | | | | |
| <i>Varanus niloticus</i> | 8 | 8 | 24 | near centromere of pair 1 | King and King, 1975 |
| Gekkonidae | | | | | |
| <i>Coleonyx variegatus</i> | 32 ^a | 0 | — | one pair macrochromosomes ^a | Matthey, 1933 |
| Colubridae | | | | | |
| Colubrinae | | | | | |
| <i>Elaphe guttata</i> | 2 | 14 | 20 | one pair microchromosomes | Baker et al., 1971 |
| <i>Elaphe obsoleta</i> | 2 | 14 | 20 | one pair microchromosomes | Fischman et al., 1968 |
| <i>Masticophis flagellum</i> | 2 | 14 | 20 | one pair acrocentric microchromosomes | Bury et al., 1970; Baker et al., 1972 |
| <i>Pituophis melanoleucus</i> | 2 | 14 | 20 | one pair microchromosomes | Bury et al., 1970 |
| Natricinae | | | | | |
| <i>Nerodia fasciata</i> | 0 | 34 | 2 | long arm pair 1 or 2 ^a | Eberle, 1972 |
| <i>Nerodia rhombifer</i> | 0 | 34 | 2 | long arm pair 1 or 2 ^a | van Brink, 1959 |
| <i>Thamnophis marcianus</i> | 0 ^b | 34 | 2 | long arm pair 1 or 2 ^a | Baker et al., 1972 |
| <i>Thamnophis sirtalis</i> | 0 | 34 | 2 | long arm pair 1 ^a and smallest pair | Baker et al., 1972 |
| Viperidae | | | | | |
| <i>Agkistrodon contortrix</i> | 2 | 14 | 20 | Z chromosomes ^a (pair 4) | Baker et al., 1972 |
| <i>Agkistrodon intermedius</i> | 2 | 14 | 20 | one pair microchromosomes | This study |
| <i>Agkistrodon piscivorus</i> | 2 | 14 | 20 | one pair microchromosomes | Baker et al., 1972 |
| <i>Calloselasma rhodostoma</i> | 2 | 14 | 20 | two pairs microchromosomes | This study |
| <i>Crotalus atrox</i> | 2 | 14 | 20 | two pairs microchromosomes | Baker et al., 1972 |
| <i>Crotalus viridis</i> | 2 | 14 | 20 | two pairs microchromosomes | Monroe, 1962; Baker et al., 1972 |
| <i>Sistrurus catenatus</i> | 2 | 14 | 20 | two pairs microchromosomes | Baker et al., 1972 |
| Elapidae | | | | | |
| <i>Naja naja</i> | 0 | 16 | 22 | one pair microchromosomes | Nakamura, 1935; Singh et al., 1970 |

^a Hybridization restricted to telomeric end.

^b Paull et al. (1976) recognized three species of *Dipsosaurus* and cited unpublished chromosomal data for one species without indicating which of the three was karyotyped. Although W. P. Hall worked in the Baja California area, the data cited by Paull et al. (1976) are probably from the widespread species *D. dorsalis*. The other two nominal species of *Dipsosaurus* are restricted to islands in the Gulf of California and may be conspecific with *D. dorsalis* (Soulé and Sloan, 1966; Etheridge, 1982). Our data for *D. d. dorsalis* are consistent with the karyotype reported by Paull et al. (1976).

^c Unpublished data for these species are from M. W. Haiduk, L. C. Lyons, and K. Geheb. Paull et al. (1976) previously cited unpublished data for two species of *Holbrookia* and five species of *Urosaurus* but did not indicate which species were studied.

^d The chromosome with the ribosomal genes is designated pair 1 in the terminology of Lowe et al. (1967) and Cole (1970). The morphology of the chromosome indicates that it is homologous to pair 2 of other *Sceloporus* (Hall, 1973).

^e 19 microchromosomes in males.

^f The chromosomes that bear the ribosomal genes have terminal satellites, and are designated as pair 1 by Cole (1971). This pair is probably homologous to the long arm of pair 2 in other *Sceloporus*, although it is also similar in size to either arm of pair 1.

^g All chromosomes are acrocentric, but there is no distinct break between macrochromosomes and microchromosomes.

^h In females, the W chromosome is acrocentric.