

## Karyotypes of Coralsnakes (Reptilia: Elapidae) from the Western Hemisphere, with Comments on Intraspecific Variation and Centric Fission of Chromosomes

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### ABSTRACT

Karyotypes are described for *Micruroides euryxanthus* from Arizona and *Micrurus tener* from Texas. These are compared with karyotypes of other elapids from around the world, which exhibit significant interspecific variation. The largest macrochromosome of *M. euryxanthus*, which is metacentric, is shared by only two other species of coralsnakes from the New World. This may be a shared ancestral chromosome homologous to the largest macrochromosome that occurs in most other snakes, including some of the Australian elapids. The karyotype of *M. tener* from Texas has a ZZ:ZW<sub>1</sub>W<sub>2</sub> sex chromosome system, which differs from individuals of this species reported previously from Louisiana. Over the relatively young 35-million-year global history of the Elapidae, karyotypes appear to have varied more than those of most other snakes throughout a 140-million-year history.

### INTRODUCTION

As mentioned by Cole and Hardy (2019), we karyotyped particularly interesting individuals of snakes as they became available to our laboratories over several decades, and most snakes compared to date have highly conserved karyotypes, extending back about 140 million years. Although there are significant exceptions to this rule of conservatism, the biggest exception is in the family Elapidae, which has quite extensive interspecific karyotypic variation (see references

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in Discussion). Here I report the karyotypes of *Micruroides euryxanthus* (the Sonoran Coral-snake) and *Micrurus tener* (the Texas Coralsnake) and compare them with other elapids.

## MATERIALS AND METHODS

Chromosomes in metaphase spreads were examined in cells from spleen, testes, and bone marrow, following Hardy (1976), but using colchicine instead of velban before preparing sodium citrate cell suspensions. Cells were fixed in methanol and glacial acetic acid and applied to glass slides with flame-drying. I followed Cole (1970, 1979) for chromosome morphology. Details on the specimens examined are in the appendix.

## RESULTS

*Micruroides euryxanthus*: Results were obtained from one male, for which 17 cells provided consistently similar results. The karyotype (fig. 1A) has a diploid chromosome number of 34, with 14 macrochromosomes + 20 microchromosomes. For this description, individual pairs of macrochromosomes are designated specific numbers according to decreasing size in the karyotype. Chromosome number 1 is a large metacentric that is shown (fig. 1A) in the middle position among the macrochromosomes in order to illustrate the possible evolutionary relationship to specific macrochromosomes of *Micrurus tener* and other species (discussed below; fig. 1B). The second largest chromosome (number 2, the leftmost pair in fig. 1A) is submetacentric, with a secondary constriction near the middle of the long arm. Chromosome number 3 is metacentric and number 4 is submetacentric to subtelocentric. Chromosome 5 is metacentric to submetacentric, number 6 is subtelocentric, and number 7 is telocentric. Microchromosomes are too small for distinguishing the centromere position clearly, but in one cell, several were resolved as subtelocentric to telocentric. No pair was heteromorphic in this male, which is to be expected. Snakes usually have a ZW sex chromosome system (females being heterogametic). In comparison with other coralsnakes, the pair of sex chromosomes may be homologous to what is chromosome 5 in this species (see below).

*Micrurus tener*: Results were obtained from one female, for which 14 cells were examined and one male, for which 7 cells were examined. The karyotype of the female (fig. 1B) has a diploid chromosome number of 33, with 16 macrochromosomes + 17 microchromosomes. Chromosome number 1 is submetacentric, with a secondary constriction near the middle of the long arm, apparently identical to chromosome number 2 of *M. euryxanthus* (fig. 1A). Chromosomes number 2 and 3 are metacentric, the size and centromere position of number 2 similar to number 3 in *M. euryxanthus* and number 3 similar to number 4 in *M. euryxanthus*, allowing for an apparent unequal pericentric inversion. Chromosomes 4 and 5 are subtelocentric and of a size that they could be homologous to the two arms of chromosome 1 of *M. euryxanthus*, following a possible centric fission event (fig. 1). Chromosome 6 appears to represent the sex chromosomes in the female (fig. 1B), with three sex chromosomes; one larger submetacentric to subtelocentric may be the Z chromosome, one intermediate-sized telocentric

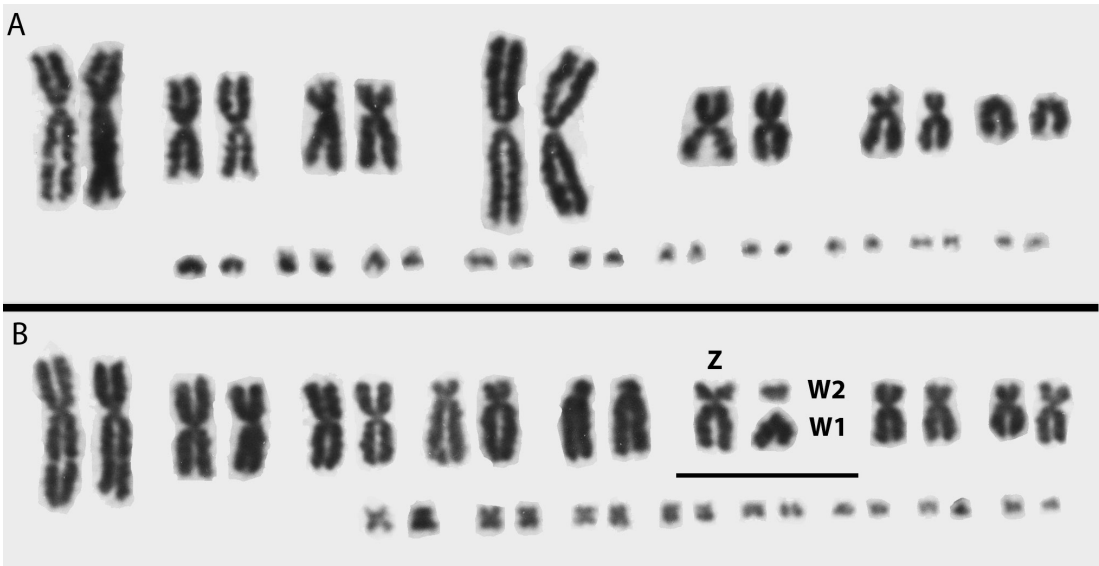


FIGURE 1. Snake chromosomes. **A.** Karyotype of *Micruroides euryxanthus* ( $2n = 34$ , with 14 macrochromosomes and 20 microchromosomes), AMNH R-109413, male. **B.** Karyotype of *Micrurus tener* ( $2n = 32$  in males, 33 in females, with 16 macrochromosomes and 16 microchromosomes in males, 17 microchromosomes in females), AMNH R-110075, female illustrated with ZW1W2 sex chromosome heteromorphism. Scale bar = 10  $\mu\text{m}$ .

may be the W1, and a microchromosome may be the W2, in an apparent multiple sex chromosome system. Chromosomes 7 and 8 are submetacentric to subtelocentric. The microchromosomes are too small to be resolved clearly, but up to four pairs were seen to be banded in the clearest cells. The karyotype of the male has a diploid number of 32 chromosomes (16 macrochromosomes + 16 microchromosomes), apparently with two Z chromosomes (pair number 6) and no recognizable W chromosomes.

## DISCUSSION

### THE ANCESTRAL KARYOTYPE OF ELAPIDAE

The Elapidae are one of the youngest families of snakes. The family arose approximately 35 million years ago and shared a common ancestor with the Colubridae approximately 50 million years ago (Pyron and Burbrink, 2012). As reviewed in detail by Cole and Hardy (2019), many colubrids apparently have one and the same karyotype ( $2n = 36$  with 16 macrochromosomes and 20 microchromosomes) that is basically similar to karyotypes in other families of snakes and appears to represent the ancestral karyotype for the Serpentes. If the same basic, conservative snake karyotype occurs in some elapids, it would seem likely that this is the ancestral karyotype for the family. Consequently, I searched the global literature on elapid karyotypes and compared them, using only literature with clear photographs that I could interpret with my usual comparative methods, following Cole and Hardy (2019). Although these comparisons are based on non-

differentially stained chromosomes, recent research (e.g., Schield et al., 2019) suggests that such studies indicating chromosome conservatism among widely diverged taxa can be valuable.

The conservative ancestral snake karyotype, sometimes with small modifications in some centromere positions, is known to occur in the following Australian elapids reported by Mengden (1985), who also suggested that this is the ancestral karyotype for elapids: four species of *Pseudechis*, three species of *Acanthophis*, *Echiopsis curta*, *Rhinoplocephalus bicolor*, *Elapognathus minor*, *Vermicella annulata*, two species of *Neelaps*, *Drysdalia coronata*, and *Cacophis kreftii*. Mengden et al. (1986) examined karyotypes for all six species of *Pseudechis* that occur in Australia and New Guinea and agreed with Mengden (1985).

The apparent occurrence of the conservative ancestral snake karyotype in the diverse elapids listed above strongly suggests that it is the ancestral karyotype for the family Elapidae (Mengden, 1985; Mengden et al., 1986). Nevertheless, the family has considerable karyotypic variation, as shown in the following papers: Singh (1972, 1974); Graham (1977); Gutiérrez and Bolaños (1979, 1980, 1981); Gorman (1981); Toriba (1987); Gutiérrez et al. (1988); Luykx et al. (1992); and Serafim et al. (2007).

#### COMPARISONS BETWEEN *M. EURYXANTHUS* AND OTHER ELAPIDS

None of the karyotypes reported for elapids from the New World represents the ancestral karyotype in all details ( $2n = 36$ , with 16 macrochromosomes and 20 microchromosomes). However, the karyotype of *M. euryxanthus* ( $2n = 34$ , with 14 macrochromosomes and 20 microchromosomes) is close to this condition, with only one fewer pair of macrochromosomes. In addition, the first five pairs of macrochromosomes appear to be very similar to those in the common, ancient snake karyotype as found in many members of the Colubridae (reviewed by Cole and Hardy, 2019). In particular, pair 1, the largest metacentric chromosome in *M. euryxanthus*, appears to be the same as pair 1 in the ancestral karyotype, and this chromosome has been found in only two other species of coralsnakes. It occurs in the South American *Micrurus surinamensis* (see Gutiérrez et al., 1988) and the Central American *Micrurus mipartitus* (see Gutiérrez and Bolaños, 1979), but none of the other coralsnakes of the Western Hemisphere that have been karyotyped. In fact, the karyotype of *M. mipartitus* is basically identical to that of *Micruroides euryxanthus*. This is consistent with the phylogenetic conclusion (Slowinski, 1995; Castoe et al., 2007) that *Micruroides euryxanthus* represents the earliest clade of New World coralsnakes that is the sister taxon to all the others, and suggests that the presence of the large metacentric macrochromosome is retained from the ancestral condition. The presence of apparently the same macrochromosome in *M. surinamensis* and *M. mipartitus* may also be from retention of the ancestral condition, and its absence from other New World coralsnakes may reflect a historic transformation by means of centric fission to being represented in the other species as in *M. tener* (fig. 1B).

#### INTRASPECIFIC VARIATION IN *M. TENER*

A karyotype for several specimens of *M. tener* from Louisiana was reported by Graham (1977). Details of the karyotype are identical to what I report here, except in the sex chromo-

TABLE 1. Karyotypic data for terrestrial elapids from the Western Hemisphere.

Species	Area <sup>a</sup>	2n	Macros <sup>b</sup>	Micros <sup>b</sup>	References
<i>Micruroides uryxanthus</i>	US	34	14	20	This paper
<i>Micrurus tener</i>	US	32–33	16	16–17	Graham, 1977; this paper
<i>M. mipartitus</i>	CA	34	14	20	Gutiérrez and Bolaños, 1979
<i>M. elegans</i>	CA	30	16	14	Gutiérrez et al., 1988
<i>M. n. mosquitoensis</i>	CA	30	16	14	Gutiérrez and Bolaños, 1979, 1981
<i>M. n. nigrocinctus</i>	CA	26–28	16	10–12	Gutiérrez and Bolaños, 1979, 1981
<i>M. browni</i>	CA	26	16	10	Gutiérrez et al., 1988
<i>M. ruatanus</i>	CA	26	16	10	Luykx et al., 1992
<i>M. diastema</i>	CA	30	14	16	Gutiérrez et al., 1988
<i>M. hippocrepis</i>	CA	30	14	16	Gutiérrez et al., 1988
<i>M. alleni</i>	CA	34	20	14	Gutiérrez and Bolaños, 1979
<i>M. surinamensis</i>	SA	38	18	20	Gutiérrez et al., 1988
<i>M. corallinus</i>	SA	40	20	20	Serafim et al., 2007
<i>M. iboboboca</i>	SA	42	22	20	Serafim et al., 2007
<i>M. lemniscatus</i>	SA	42	22	20	Beçak and Beçak, 1969

<sup>a</sup> Source of specimens: US (United States); CA (Central America); SA (South America).

<sup>b</sup> Macros = number of macrochromosomes; Micros = number of microchromosomes.

somes. Specimens of both sexes from Louisiana have a diploid chromosome number of 32, in which males are like those from Texas but females have a Z and one W chromosome, the W being a subtelocentric chromosome that is smaller than the Z. The specimens from Texas have a multiple-sex-chromosome system (fig. 1B). The differences between specimens from Louisiana and Texas should be investigated further for confirmation and to determine whether these forms are genetically compatible. Also, a rangewide study of karyotypes in *M. tener* may be valuable.

#### COMPARISONS BETWEEN *M. tener* AND OTHER SPECIES OF *MICRURUS*

Diploid chromosome numbers reported for species of *Micrurus* vary from 26–42, with some intraspecific variation in *M. nigrocinctus* and *M. tener* (table 1). Interspecific variation in number of macrochromosomes ranges from 14–22 and variation in number of microchromosomes ranges from 10–20. Variation among the macrochromosomes apparently results largely from centric fissions of ancestral biarmed chromosomes into derived uniarmed or telocentric chromosomes, but there is no simple hypothesis that explains the variation in number of microchromosomes. In addition, there is a general geographic tendency in coralsnakes of the Western Hemisphere to have experienced an increase in the number of fissioned ancestral macrochromosomes trending from species occurring in northern temperate regions to species occurring in southern equatorial regions (table 1). In the South American species *M. iboboboca* and *M. lemniscatus* all macrochromosomes are telocentric, except for one pair (subtelocentric; table 1) and there are changes additional to centric fissions.

## COMMENTS ON CENTRIC FISSION OF CHROMOSOMES

It has long been known that interspecific differences in karyotypes involve evolution by means of many types of chromosomal aberrations, the most obvious of which are Robertsonian rearrangements called centric fusion and centric fission (reviewed by Sites, 1983). These are apparent in karyotypes as whole-arm changes in which two nonhomologous ancestral telocentric chromosomes become one large metacentric chromosome (centric fusion) or, alternatively, an ancestral large metacentric chromosome becomes two smaller telocentric chromosomes (centric fission). In the 20th century, fusion was favored over fission for explaining the direction of karyotype evolution because there was no mechanism visualized for fission and the formation of new centromeres. Nevertheless, there are many examples in which centric fission clearly occurred (see below). Recent work provides new details on more than one mechanism for the occurrence of centric fission (e.g., centric preduplication, direct transverse breakage, events preceded by chromosomal rearrangements) as well as information on its potentially negative consequences in evolution, as it is associated with diseases, including cancer, in humans (e.g., Perry et al., 2004, 2005; Shim et al., 2007; Martínez and van Wely, 2011).

Not only is karyotypic evolution by means of centric fission becoming more apparent in amphibians and reptiles, but also it is most frequently associated with recent changes in ancient karyotypes that had been stable in lineages extending for tens of millions to 200 million years in age. In extreme cases, all or nearly all of the biarmed macrochromosomes in a karyotype appear to become divided into smaller telocentric chromosomes in a short period of time. Examples include the following: treefrogs (Cole, 1974); turtles (Bickham, 1981); lizards (Webster et al., 1972, for *Anolis*; Cole, 1979, for *Cnemidophorus* or *Aspidoscelis*; Porter and Sites, 1986, 1987, Arévalo et al., 1991, 1993, and Sites et al., 1992, 1993, for *Sceloporus*); and snakes (Baker et al., 1971).

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## APPENDIX

### SPECIMENS EXAMINED

The specimens are cataloged in the herpetological collections of the American Museum of Natural History (AMNH) as follows:

*Micruroides euryxanthus*: UNITED STATES: *Arizona*: Cochise County; Chiricahua Mountains, Cave Creek Canyon, 1.5 mi S and 1.8 mi W (linear) Portal; 5,600 ft elevation (AMNH R-109413).

*Micrurus tener*: UNITED STATES: *Texas*: Walker County; no additional locality data available (AMNH R-110075 and R-110076).