The Karyotype of *Plestiodon anthracinus* (Baird, 1850) (Sauria: Scincidae): A Step Toward Solving an Enigma

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Abstract - The cosmopolitan lizard genus *Eumeces* was first revised in 1936 and consisted of 15 species-groups comprising a total of 50 species. Nine species in North America were later recognized as belonging to the genus *Plestiodon* and all contained the diploid chromosome number of 26. Modern cladistic techniques indicated that *Plestiodon anthracinus* (Coal Skink) was near the ancestral form for the *fasciatus* group. We employed the hypotonic citrate method to study chromosomes of 5 Coal Skink specimens from Louisiana and Arkansas and found them to have a diploid number of 24 (12 macrochromosomes, 12 distinctly smaller chromosomes, all biarmed) and a fundamental number of 48. The diploid number of 24 is probably derived by some chromosome rearrangements in the evolution of *Plestiodon* and of the *Plestiodon anthracinus* group.

Introduction

Taylor (1936) recognized 15 species-groups within the cosmopolitan genus *Eumeces* (= *Plestiodon*), which he regarded as monophyletic. Within *Eumeces*, Taylor (1936) identified the *anthracinus* group, which contained 3 species: *Plestiodon anthracinus* Baird (Coal Skink), *P. copei* (Taylor), and *P. septentrionalis* Baird (Prairie Skink), all endemic to North America. Dixon (1969) removed *copei* from the *anthracinus* group and placed it in the *brevirostris* group, based on several morphological characters. Lieb (1985) upheld removing *P. copei* from the *anthracinus* group, but added *P. tetragrammus* Baird (Four-lined Skink) to *P. anthracinus* and *P. septentrionalis* in this group. Schmitz et al. (2004) reported that the *laticeps* species-group includes the *obsoletus* and *anthracinus* species-groups in a clade that comprises *laticeps*, *inexpectatus*, *fasciatus*, *obsoletus*, and *septentrionalis*. However, they determined that "*E*." *anthracinus* is not part of this group, because it is consistently placed outside of the latter clade and is mostly recovered as a sister species to "*E*." *egregius* (Schmitz et al. 2004). This view was supported by Brandley et al. (2012) who placed *P. anthracinus* as the sole member of the *anthracinus* species-group within the *fasciatus* species series. *Plestiodon tetragrammus* was included in the *fasciatus* species-group (Brandley et al. 2012). Multiple studies have concluded that *Eumeces* (sensu lato) is, in fact, polyphyletic (Brandley et al. 2005, Caputo, et al. 1993, Griffith et al. 2000, Schmitz et al. 2004), with the east Asian/North American species of "*Eumeces*" being designated as *Plestiodon* by Brandley et al. (2005). Brandley et al. (2012) included *P. anthracinus* as the sole member of the *anthracinus* group in their revised phylogeny and placed the *anthracinus* group

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as a sister taxon to the *fasciatus* group (their Clade C5). The mtDNA data (Brandley et al. 2012) provides strong support for the sister relationship of *P. anthracinus* with *P. egregius* and *P. reynoldsi* (C4 clade) and the exclusion of *P. septentrionalis* from close relationship with *P. anthracinus*. The cosmopolitan lizard genus *Eumeces* (sensu lato) contains at least 25 valid species (according to ITIS 2016), including 8 species referred to *Plestiodon* as invalid. We follow Brandley et al. (2005:388) and Brandley et al. (2012:182) for the use of *Plestiodon*.

Despite the large number of species in the family Scincidae, the karyotypes of this family are comparatively poorly known (Giovannotti et al. 2009). All of the species that have been karyotyped share the characteristics of having a relatively low diploid number, with the first 4 pairs of chromosomes being metacentric and larger than the remainder of the chromosome complement (e.g., Gionvannotti et al. 2009). We had access to several live specimens of the uncommon Coal Skink in northwestern Louisiana and Arkansas, which allowed us to compare the karyotype of *Plestiodon anthracinus* to other species in the genus. We tested the hypothesis that the diploid number of chromosomes in *P. anthracinus* is 26, the known diploid number reported for other species of *Plestiodon* (Dowling 1975).

Materials and Methods

We euthanized specimens by an intraperitoneal injection of 10% Nembutal and processed for analysis of mitotic and meiotic cells sampled from bone marrow from crushed vertebrae and/or testes. We prepared chromosomes by the hypotonic citrate method of Patton (1967) and used the modification by Cole and Leavens (1971). We made an intraperitoneal injection of 10% Velban as a mitotic inhibitor, instead of colchicine. We examined chromosomes under a Leitz Dialux microscope and photographed appropriate Giemsa-stained images with a 10.16 cm x 12.70 cm (4” x 5”) black and white, high-contrast film. We prepared the karyotype from a scanned image (positive) of the 10.16 cm x 12.70 cm (4” x 5”) negative. Chromosome terminology follows Cole (1970).

Specimens examined: LSUS 4545, female, Caddo Parish: 4.02 km (2.5 mi) W, 1.61 km (1.0 mi) S Blanchard, 14 September 1980 (LMH 8933); LSUS 5742, male, Caddo Parish: Walter Jacobs Nature Park (T18N, R15W, Sec 7), 26 March 1981 (LRR 929); LSUS 5743, female, Caddo Parish: Walter Jacobs Nature Park (T18N, R15W, Sec 7), 12 March 1980 (LRR 712); LSUS 5744, male, Caddo Parish: Walter Jacobs Nature Park (T18N, R15W, Sec 7), 27 February 1980 (LRR 706); LSUS 8911, male, Arkansas, Polk County, 2.90 km (1.8 mi) S, 2.57 km (1.6 mi) W Big Fork (Ouachita Mountains Biological Station), 21 July 2003 (LMH 12954).

Results

Our chromosome analysis of 4 specimens (2 males, 2 females) of *P. anthracinus* from northwestern Louisiana and 1 specimen (male) from the Ouachita Mountains of Arkansas indicated a diploid number of 24 chromosomes, including 12 macrochromosomes and 12 microchromosomes. The macrochromosomes include,

from largest to smallest, 1 metacentric to submetacentric, 1 submetacentric, and 4 metacentrics. The microchromosomes are metacentrics and submetacentrics. No chromosomes are telocentric and no secondary constrictions or satellites were observed. Twelve pairs of chromosomes are shown in metaphase (Fig. 1). Both macrochromosomes and microchromosomes are biarmed, and the fundamental number is 48. We observed no obvious heteromorphic sex chromosomes.

We counted chromosomes from more than 380 cells, using both mitotic and meiotic preparations; in all cases, the total diploid number was 20–26. We assume that the cells containing 20 or 22 chromosomes were incomplete cells because those numbers were recorded in specimens that also showed the modal number of 24. The 2 cells with 26 chromosomes were part of 384 cells analyzed that included 336 cells containing 24 chromosomes from 1 specimen (LSUS 5744). This result indicates that the correct diploid number for our sample is 24. In addition, the reduction in chromosome number from 26 seen in all other species of *Plestiodon* to 24 in *Plestiodon anthracinus* is due to a reduction of the microchromosome number (from 14 to 12) and not a reduction of the macrochromosome number.

Discussion

Nakamura (1931a, 1931b) reported a diploid number of 26 chromosomes for *E. latiscutatus,* a member of the *fasciatus* group and, according to Taylor (1936), the only species from Japan. Talluri (1968) reported a diploid number of 32 chromosomes for *Eumeces algeriensis* (see Caputo et al. 1993) a member of the North African/Southeast Asia clade of Brandley et al. (2005). Deweese and Wright (1970) reported chromosome data for 6 New World species of *Plestiodon* (all with a diploid number of 26), including *P. copei* from Morelos, Mexico, which is 1 of 3 species of *Plestiodon* placed by Taylor (1936) in the *P. anthracinus* species group, but later removed from this group (Brandley et al. 2012, Dixon 1969, Lieb 1985). Dowling (1975) listed diploid chromosome numbers for 9 species of *Plestiodon,* all

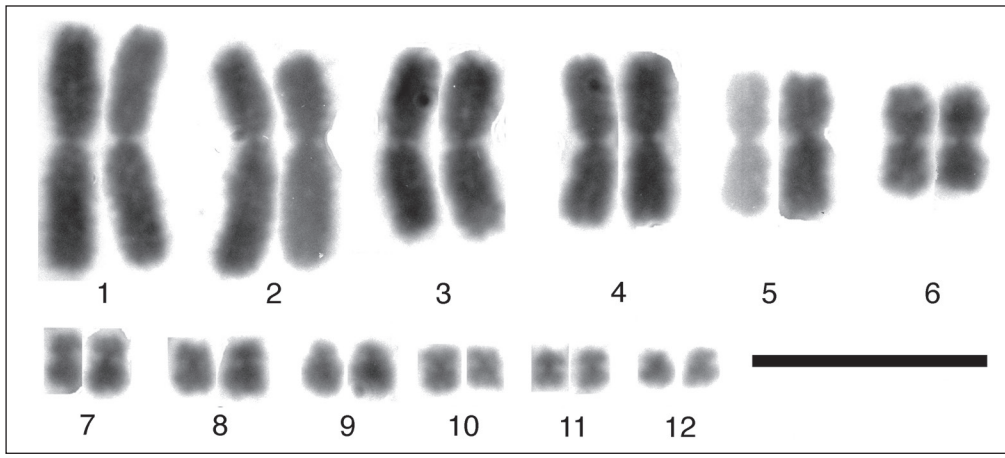

Figure 1. Karyotype of an adult male *Plestiodon anthracinus* (LSUS 5744); 2*n* = 24; bar = 0.01 mm.

of which had a diploid number of 26. A review of chromosomal data for *Eumeces* included 7 North American species (not *P. anthracinus*); all of which have a diploid number of 26 (Caputo et al. 1994). The karyotype of *Plestiodon anthracinus* differs from that of *P. copei* studied by DeWeese and Wright (1970) by having a diploid number of 24 (only 6 pairs of microchromosomes, not 7 as in *P. copei* and all other species of *Plestiodon* for which karyotypes have been published). Chromosome change during evolution can be a useful indicator of phylogenetic relationships, especially when used together with molecular and morphological data (Giovannotti et al. 2009). However, we do not know the significance of the loss of a microchromosome, which appears to indicate the loss of genetic material. We do know that microchromosomes contain genetic material and are probably important to the genome. For example, a microchromosome is, in some species, one of the sex chromosomes and, therefore, very important for that species (Cole et al. 1967). Microchromosomes may be translocated onto a macrochromosome and, therefore, easily misinterpreted as a chromosome loss; however, it is not lost and the genetic material is still present and probably functional.

Scincidae have highly conserved karyotypes (Giovannotti et al. 2009) and all *Plestiodon* in the North American clade have a diploid number of 26, except for *P. anthracinus*. By having a unique karyotype for the genus *Plestiodon,* based on present knowledge*, P. anthracinus* is somewhat of an enigma. Its biogeographic location would not suggest any particular evolutionary event to explain this, and it has not previously been associated with any more-derived species group. However, in the results of Brandley et al. (2011, 2012), *P. anthracinus* appeared in one of the more divergent positions of the cladogram, and was nested between the *fasciatus* and *egregius* groups. The most conservative conclusion suggests that the chromosome rearrangement to a diploid number of 24 in *P. anthracinus* occurred after the divergence of *P. anthracinus* from the *fasciatus*-species group, and the *P. anthracinus* karyotype is independent of all of the North American *Plestiodon* containing a diploid number of 26.

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Literature Cited

Brandley, M.C., A. Schmitz, and T.W. Reeder. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of Scincid lizards. Systematic Biology 54:373–390.

Brandley M.C., Y. Wang, X. Guo, A.N. Montes De Oca, M. Feria-Ortiz, T. Hikida, and H. Ota. 2011. Accommodating heterogenous rates of evolution in molecular-divergence dating methods: An example using intercontinental dispersal of *Plestiodon* (*Eumeces*) lizards. Systematic Biology 60: 3–15.

Brandley, M.C., H.O. Fls, T. Hikida, A.N. Montes De Oca, M. Feria-Ortiz, X. Guo, and Y. Wang. 2012. The phylogenetic systematics of blue-tailed skinks (*Plestiodon*) and the family Scincidae. Zoological Journal of the Linnean Society 165:163–189.

Caputo, V., G. Odierna, G. Aprea, and T. Capriglione. 1993. *Eumeces algeriensis*, a full species of the *E. schneiderii* group (Scincidae): Karyological and morphological evidence. Amphibia-Reptilia 14:187–193.

Caputo, V., G. Odierna, and G. Aprea. 1994. A chromosomal study of Eumeces and Scincus, primitive members of the Scincidae (Reptilia, Squamata). Bolletino di zoologia, 61(2):155–162.

Cole, C.J. 1970. Karyotypes and evolution of the *spinosus* group of lizards in the genus *Sceloporus*. American Museum Novitates 2431:1–47.

Cole, C.J., and C.R. Leavens. 1971. Chromosome preparations of amphibians and reptiles: Improved technique. Herpetological Review 3:102.

Cole, C.J., C.H. Lowe, and J.W. Wright. 1967. Sex chromosomes in lizards. Science 155:1028–1029.

Deweese, J.E., and J.W. Wright. 1970. A preliminary karyological analysis of scincid lizards. Mammal Chromosome Newsletter 11:95–97.

Dixon, J.R. 1969. Taxonomic review of the Mexican skinks of the *Eumeces brevirostris* group. Natural History Museum of Los Angeles County Contributions to Science 168:1–30.

Dowling, H.G. (Ed.). 1975. Yearbook of Herpetology. Herpetological Information Search Systems Publications., American Museum of Natural History, New York, NY. Pp. i–vi, 1–256.

Giovannotti, M., V. Caputo, P.C.M. O'Brien, F.L. Lovell, V. Trifonov, P.N. Cerioni, E. Olmo, M.A. Ferguson-Smith, and W. Rens. 2009. Skinks (Reptilia: Scincidae) have highly conserved karyotypes as revealed by chromosome painting. Cytogenetic Genome Research 127:224–231.

Griffith, H., A. Ngo, and R.W. Murphy. 2000. A cladistic evaluation of the cosmopolitan genus *Eumeces* Wiegmann (Reptilia, Squamata, Scincidae). Russian Journal of Herpetology 7:1–16.

Integrated Taxonomic Information System (ITIS). 2016. ITIS database. Available online at http://www.itis.gov. Accessed 13 April 2016.

Lieb C.S. 1985. Systematics and distribution of the skinks allied to *Eumeces tetragrammus* (Sauria, Scincidae). Natural History Museum of Los Angeles County Contributions to Science 357:1–19.

Nakamura, K. 1931a. Preliminary note on reptilian chromosomes. III. The chromosomes of some lizards. Proceedings of the Imperial Academy 7:26–38.

Nakamura, K. 1931b. Studies on reptilian chromosomes. II. On the chromosomes of Eumeces latiscutatus (Hallowell), a lizard. Cytologia 2:385–401.

Patton, J.L. 1967. Chromosome studies of certain pocket mice, genus *Perognathus* (Rodentia: Heteromyidae). Journal of Mammalogy 48:27–37.

Schmitz, A., P. Mausfeld, and D. Embert. 2004. Molecular studies on the genus *Eumeces* Wiegmann, 1834: Phylogenetic relationships and taxonomic implications. Hymadryad 28:73–89.

Talluri, M.V. 1968. I Chromosomi di Eumeces schneiderii algeriensis (Scincidae-Reptilia). Instituto di Zoologia dell'Universita di Siena, Siena, Italy. Pp. 1959–1964.

Taylor, E.H. 1936. A taxonomic study of the cosmopolitan scincoid lizards of the genus *Eumeces* with an account of the distribution and relationships of its species. The Kansas University Science Bulletin 23:1–643.