Rendiconti Lincei 20, 77 – 82 (2009)
DOI: 10.1007/s12210-009-0005-4

Riccardo Castiglia · Andrés García ·
Alexandra M.R. Bezerra ·
Oscar Flores-Villela · Ekaterina Gornung

Karyotypic Diversification Due to Robertsonian Rearrangements in *Phyllodactylus lanei* Smith, 1935 (Squamata, Gekkonidae) from Mexico

Received: 10 July 2008 / Accepted: 11 September 2008 – © Springer-Verlag 2009

Abstract We analyzed chromosomes of male and female individuals of *Phyllodactylus lanei* Smith, 1935 (Squamata, Gekkonidae) from Chamela-Cuixmala Biosphere Reserve, Jalisco state, Mexico. The karyotype constructed for these specimens is composed of 19 pairs of telocentric chromosomes (2n = 38, FN = 38). This karyotype, due to Robertsonian fusions/fissions, differs from the one previously reported in samples from the State of Guerrero, which probably belonged to a different subspecies (2n = 33-34, FN = 40-41). Moreover, a presumed ZW sex chromosome system was not confirmed in the presently studied individuals.

Keywords Chromosomal evolution, Reptilia, Robertsonian fusions/fissions, Sex chromosomes.

Subject codes L25007, L25023, L2504X

R. Castiglia (✉)
Dipartimento di Biologia Animale e dell'Uomo, Università di Roma "La Sapienza", via A. Borelli 50, 00161, Rome, Italy
Tel.: +39 06 49910122, Fax: +39 06 4457516, E-mail: castiglia@uniroma1.it

A. García
Estación de Biología Chamela, Instituto de Biología, UNAM. Apdo. Postal 21. San Patricio, Jalisco, 48980, México

A.M.R. Bezerra
Depto. de Zoologia, Universidade de Brasília, ICC Sul, CEP 70910-900, Brasília, DF, Brasil
E-mail: abezerra@fst.com.br

O. Flores-Villela
Museo de Zoología, Fac. de Ciencias, UNAM A.P. 70-399, México D.F. 04510

E. Gornung
Dipartimento di Biologia Animale e dell'Uomo, Università di Roma "La Sapienza", via A. Borelli 50, 00161, Rome, Italy

The family Gekkonidae (Reptilia, Squamata) represents a highly heterogeneous group of species. Formerly, the genus *Phyllodactylus,* included a diversity of leaf-toed geckos occurring all-over the world. However, today, on the basis of morphological and allozyme phylogenetic analyses, several lineages of Old World gecko lizards are proposed as distinct genera, such as *Afrogecko* (southern Africa), *Christinus* (Australia), *Cryptactites* (southern Africa), *Goggia* (southern Africa), *Dixonius* (Southeast Asia), *Euleptes* (Mediterranean), *Haemodracon* (Sokotra), and *Matoatoa* (Madagascar) (Bauer 1997; Gamble et al. 2008). The species of the genus *Phyllodactylus sensu stricto* are now restricted to the New World.

Karyological data are rather intricate in Gekkonidae. In fact, along with the temperature-dependent sex determination, a genotypic sex determination was also found in the family (Viets et al. 2005). Furthermore, intraspecific chromosome variation has been reported mainly due to Robertsonian (Rb) fusions/fissions, e.g., in many species of *Diplodactylus* (King 1977; Oliver et al. 2007), *Gehyra* (King 1984), and in *Gekko smithii* (Ota, 1989).

Considering the four karyotyped species of *Phyllodactylus sensu lato* (Tab. 1), the Rb variation has been found only in *Christinus* (*Phyllodactylus*) *marmoratus* (King and Rofe 1976). This species shows four distinct chromosome races: 2n = 36, 2n = 36 with a pair of heteromorphic chromosomes, 2n = 34 (King and Rofe 1976), and 2n = 32 (King and King 1977). Specimens from these races are morphologically discernible, show a degree of habitat specialization, and are distributed non-randomly in isolated environmental pockets (Donnellan et al. 2000).

The Mexican endemic *Phyllodactylus lanei* is distributed along the Pacific coast of Mexico from Nayarit down to Guerrero. Currently, the species includes four subspecies: *P. l. lanei* Smith 1935, in the state Guerrero, *P. l. rupinus*

Table 1 Karyological data in *Phyllodactylus*. 2n = diploid number, FN = fundamental number.

Species	2n	FN	Geographic area	Reference
Christinus (*Phyllodactylus*) *marmoratus*	32–36	40–41	Australia	King & Rofe 1976; King & King 1977
Phyllodactylus lanei lanei	33–34	40–41	Guerrero, Mexico*	King 1981
Phyllodactylus lanei rupinus	38	38	Chamela-Cuixmala, Jalisco, Mexico	This work
Phyllodactylus gerrhopygus	40	40	South America	Navarro et al. 1981
Phyllodactylus ventralis	32	32	Anzoategui, Boca de Uchire, Venezuela	McBee *et al.* 1984

* King (1981) did not specify a voucher specimen. Both *P. delcampi* and *P. lanei lanei* occur in the same area.

Dixon 1964, distributed Nayarit, coastal Jalisco, South Michoacan, and two insular subspecies *P. l. lupitae* and *P. l. isabelae* (Castro-Franco & Uribe-Pena 1992). Karyological data in *P. lanei* were restricted to a report that described karyotypes of specimens from the state of Guerrero, 2n = 33-34 and FN = 40-41 (King 1981). Except for this notion, no other helpful information concerning these specimens has been reported. Given that the holotype of *P. lanei lanei* was "collected near Tierra Colorada, Guerrero, Mexico…" (Smith, 1935), the samples studied by King (1981) most likely belong to this nominal subspecies.

In the present study, we analyzed three males and three females of *P. l. rupinus* from Chamela-Cuixmala Biosphere Reserve (19°22′03″–19°35′11″N and 104°56′13″–105°03′25″W), Jalisco, Mexico. Specimens were caught around the buildings within the Chamela Biological Estación. The subspecific identification of the specimens from this population was performed according to Castro-Franco & Uribe-Pena (1992). The analyzed specimens, all adults, are housed at the herpetological collection of the Dipto. Biologia Animale e dell'Uomo, Università di Roma "La Sapienza" (CEAC) as numbers: CEAC1, CEAC2, and CEAC3 (males) and CEAC4, CEAC5, and CEAC6 (females).

The specimens were injected with a 1:1000 solution of Velbe (Lilly) for one hour. The femurs, vertebral column and testes were removed, crushed and left in hypotonic solution (0.075 M KCl) for 40 minutes at room temperature. Cells were collected after centrifugation and were fixed with methyl alcohol – acetic acid (3:1). Metaphase plates were prepared using the air-drying method. Fifteen methaphases for individuals were studied and photographed.

The karyotype of *P. lanei rupinus* (2n = 38, FN = 38) is composed of 19 pairs of telocentric chromosomes (Fig. 1). Tiny short arms, visible in two or three chromosome pairs after a substantial elongation of the chromosomes, were not considered for the fundamental number. No significant karyological differences were observed among the specimens after conventional Giemsa staining. Secondary constrictions are visible at the paracentromeric region of chromosomes 3, 4, 5 and 6. The presently constructed karyotype of *P. l. rupinus* differs from the karyotype reported by King (1981). The presence of two pairs of large metacentric chromosomes in *P. l. lanei* strongly indicates that the chromosomal rearrangements are due to Rb fusions/fissions. Furthermore, the slight difference in the fundamental number found in the two samples is probably due to a different interpretation of the very small short arms.

However, we cannot rule out another possibility concerning this karyotypic variation. Another species of *Phyllodactylus* occurs in Guerrero, *P. delcampoi*, whose holotype was "collected at Tierra Colorada, Guerrero…", Mexico (Mosauer 1936), and it is also possible that the karyotype analyzed by King (1981) belongs to this species, rather than to *P. lanei.*

Karyological analyses of more than 70 species from the most likely polyphyletic subfamily Gekkoninae showed that eight distinct putative ancestral *karyomorphs* are present, 2n = 32, 34, 36, 38, 40, 42, 44 and 46, each of

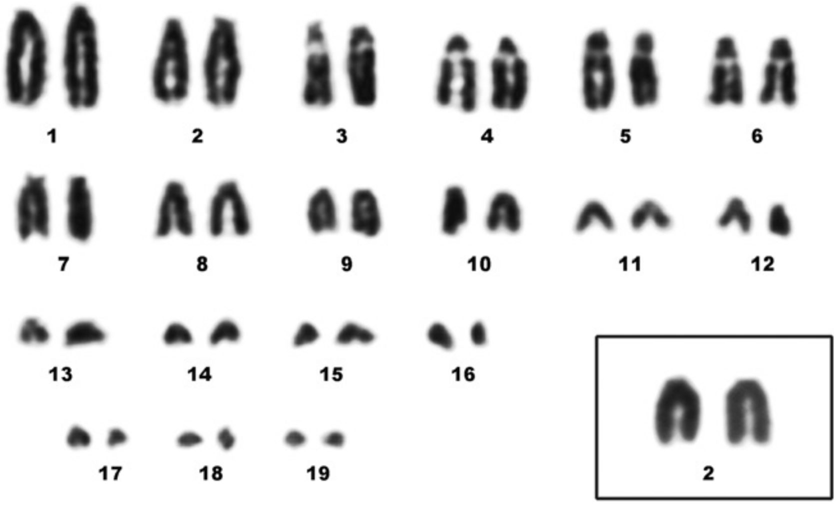

Fig. 1 Karyotype of a male *P. lanei rupinus* from Jalisco, Mexico. The insert contains chromosomes 2 of a female individual.

which is acrocentric or telocentric. Within each of these categories, numerous chromosomal rearrangements – fusions, inversions, additions and tandem fusions – have occurred (King 1987). The presently described all-acrocentric karyotype of *P. lanei rupinus*, 2n = 38, easily belongs to one of the ancestral categories. Plausibly, the 2n = 33-34 karyotype of *P. lanei* described by King (1981) should be a derived form. The large metacentric chromosomes were probably derived after Rb fusion of medium sized telocentrics of the *P. lanei rupinus* karyotype. Moreover, in the karyotype from Guerrero a pair of heteromorphic chromosomes was also observed. In females, one of the homologues of this pair was described as bi-armed (with tiny short arms) and this was considered, by the author, a possible ZW sex chromosome system. In males, this chromosome resulted additionally fused with another telocentric chromosome in a heterozygous condition.

A comparison of the two available karyotypes suggests that the heteromorphic chromosome of the Guerrero specimens (King 1981) most likely corresponds to chromosomes 2 in our karyotype. However, in the presently studied individuals, neither chromosomes 2 nor other chromosome pairs showed visible heteromorphic condition. Moreover, the chromosome in the two studies shows a similar condensation state and the differences are not due to technical artifacts.

The occurrence of cytologically distinguishable sex chromosomes appears to be relatively recent in geckos. Sex chromosomes have been reported in some species: *Gehyra australis*, *Heterontia binoei* and *Christinus marmoratus* show female heterogamety of the ZW type, while male XY heteromorphism has been described in *Gekko gecko* (Olmo 1986).

Moreover, in the three cases of heterogametic females, intraspecific variation for ZW sex chromosome was also observed (King 1977; Bull 1980; Bickham 1984; Moritz 1984). In fact, in *Christinus marmoratus*, the presumed sex chromosome shows interracial variation for a pericentric inversion (King and Rofe 1976).

In this study, we show that in the Mexican *Phyllodactylus lanei,* there is a remarkable intraspecific chromosome variation which involves both autosomes and presumed sex chromosomes. Additional data from different localities, as well as molecular and morphological studies, are needed to investigate this interesting case of chromosomal evolution in Gekkonidae.

Acknowledgements. Funding in support of this project was provided by "Fondi Musei Naturalistici Lincei" (Progetto Messico, Ernesto Capanna-Riccardo Castiglia). A.M.R. Bezerra receives postdoc fellowship from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

References

1. Bauer AM, Good DA, Branch WR (1997) The taxonomy of the Southern African leaf-toed geckos (Squamata: Gekkonidae), with a review of Old World "*Phyllodactylus*" and the description of five new genera [*Haemodracon, Dixonius, Cryptactites, Goggia, Afrogecko*; reinstatement of *Euleptes*]. Proc. Calif. Acad. Sci. 49: 447–497
2. Bickham JW (1984) Patterns and modes of chromosomal evolution in reptiles, pp. 13–40. *In*: Chromosomes in Evolution and Eukaryotic groups, edited by A.K. Sharma & A. Sharma. Vol. 2. CRC, Boca Raton
3. Bull JJ (1980) Sex determination in reptiles. Quart. Rev. Biol. 55: 3–21
4. Castro-Franco R, Uribe-Pena Z (1992) Two new subspecies of *Phyllodactylus lanei* (Sauria: Gekkonidae) [in Spanish]. Anales del Instituto de Biologia, Universidad Nacionl Autonoma de Mexico. Serie Zoologia 63(1): 113–123
5. Dixon JR (1964) The systematics and distribution of lizards of the genus *Phyllodactylus* in North and Central America. Research Center New Mexico State University Scientific Bulletin 64: 1–139
6. Donnellan SC, Aplin KP, Dempsey PJ (2000) Genetic and morphological variation in Australian Christinus (Squamata: Gekkonidae): preliminary overview with recognition of a cryptic species on the Nullarbor Plain. Aust. J. Zool. 48(3): 289–315
7. Gamble T, Bauer AM, Greenbaum E, Jackman TR (2008) Out of the blue: a novel, trans-Atlantic clade of geckos (Gekkota, Squamata). Zool. Scripta, 37(4): 355–366
8. King M (1977) Chromosomal and morphometric variation in the gecko *Diplodactylus vittatus* (Gray). Aust. J. Zool. 21: 21–32
9. King M (1981) Chromosome change and speciation in lizards. Pp. 262–265. *In: Evolution and Speciation.* W.R. Atchley & D.S. Woodruff. Cambridge, University Press
10. King M (1984) Karyotypic evolution in *Gehyra* (Gekkonidae: Reptilia) IV. Chromosome change and speciation. Genetica 64: 101–114
11. King M (1987) Monophyleticism and polyphyleticism in the Gekkonidae – a chromosomal perspective. Aust. J. Zool. 35(6): 641–654
12. King M, King D (1977) An additional chromosome race of *Phyllodactyllus marmoratus* (Gray) (Reptilia: Gekkonidae) and its phylogenetic implications. Aust. J. Zool. 25(4): 667–672

13. King M, Rofe R (1976) Karyotypic variation in the Australian Gekko *Phyllodactylus marmoratus* (Gray) (Gekkonidae: Reptilia). Chromosoma 54: 75–87
14. McBee K, Sites Jr. JW, Engstrom MD, Rivero-Blanco C, Bickham JW (1984) Karyotypes of four species of Neotropical gekkos. J. Herpetol. 18(1): 83–84
15. Moritz C (1984) The origin and evolution of parthenogenesis in *Heteronotia binoei* (Gekkonidae). I. Chromosome banding studies. Chromosoma 89: 151–162
16. Mosauer W (1936) Description of a new *Phyllodactylus* from Mexico, with remarks on the status of *P. tuberculosus*. Copeia 3: 141–146
17. Navarro J, Sallaberry M, Veloso A, Valencia J (1981) Diversidad cromosómica en lagartos (Squamata-Sauria). I: Avances citotaxonómicos. Perspectiva de estudios evolutivos en Iguanidae. Medio Ambiente (Chile) 5: 28–38
18. Oliver P, Hutchinson M, Hutchinson R (2007) Karyotypic variation in the Australian gecko *Diplodactylus tessellatus*, with the description of a new karyotypic complement for diplodactyline geckos. J. Herpetol. 41: 540–543
19. Olmo E (1986) A. Reptilia. In: John B, editor. Animal cytogenetics 4, Chordata 3. Berlin: Gebrüder Borntraeger
20. Ota H (1989) Karyotypes of five species of *Gekko* (Gekkonidae: Lacertilia) from east and southeast Asia. Herpetologica. 45(4): 438–443
21. Smith HM (1935) Miscellaneous notes on Mexican lizards. Univ. Kansas Sci. Bull. 22 (6): 119–155
22. Viets BE, Ewert MA, Talent LG, Nelson CE (2005) Sex-determining mechanisms in squamate reptiles. J. Exp. Biol. 270: 45–56