A pericentric-inversion polymorphism and a ZZ/ZW sex-chromosome system in *Varanus acanthurus* Boulenger analyzed by G- and C-banding and Ag staining

M. King[1], G. A. Mengden[1] & D. King[2]

[1] *Department of Population Biology, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra, ACT 2601, Australia*

[2] *Agriculture Protection Board of Western Australia, Bougainvillea Avenue, Forrestfield, W. Australia 6058*

Abstract

A chromosomal analysis of the monitor *Varanus acanthurus* Boulenger has been made using G- and C--banding and silver-staining techniques. This species has two cytotypes, one of which has a pericentric-inversion polymorphism, whereas the other is chromosomally monomorphic. A ZZ/ZW sex-chromosome system is also present in both cytotypes of this species. The banding patterns of these mechanisms are described and their evolution is discussed.

Introduction

Many karyotypic analyses have been made on lizards, but very few cases of chromosomal polymorphism are recorded. Webster *et al.* (1972) described a fusion/fission polymorphism in *Anolis monticola*, whereas Beçak *et al.* (1972) found what may be a similar system in *Amphisbaena dubia*. Cole (1977) described a polymorphism involving an additional segment in *Sceloporus undulatus*, and in an earlier study reported polymorphisms involving addition and presumed pericentric inversions in *S. clarkii* and *S. melanorhinus* (Cole, 1970). To our knowledge these are the only described inversion polymorphisms in bisexual lizards. There are, however, two cases of sex-correlated inversion polymorphisms. King & Rofe (1976) showed, with the aid of G banding, that a ZZ/ZW sex-chromosome system had been established in *Phyllodactylus marmoratus* by a pericentric inversion. Similarly, Cole *et al.* (1969) found an XX/XY sex-chromosome system in *Cnemidophorus tigris* which had similarly been established by an inversion. Bull (1978) subsequently confirmed this with G- and C--banding and meiotic analyses. In these cases, chromosome-banding techniques have been instrumental in defining both the nature of the polymorphism and the sex-chromosome system.

In a chromosomal and electrophoretic study of the lizard genus *Varanus* (King & King, 1975; Holmes *et al.*, 1976) it was proposed that this genus had differentiated into a series of monomorphic karyotype groups characterized by presumed pericentric inversions. Additionally, four of these species had established ZZ/ZW sex-chromosome systems in which the heteromorphic W had increased in size, presumably by the addition of heterochromatin (see King, 1977).

In the present paper we use G- and C-banding and Ag-staining techniques to describe the chromosome morphology of the lizard *Varanus acanthurus*. This species has an unusual geographically delimited pericentric-inversion polymorphism and a ZZ/ZW sex-chromosome system.

Material and methods

Specimens were collected from the localities shown in Table 1 and Figure 1. Each of the 26 specimens were karyotyped using short term leucocyte cultures as described by King & King (1975). Standard air-dried preparations were made for each specimen and stained with 10% Giemsa solution. In addition, the following staining procedures were applied:

(A) G banding – as described by Sites *et al.* (1979) but with a reduction in treatment time with trypsin.

Genetica 58, 39–45 (1982). 0016-6707/82/581 0039 $1.40.

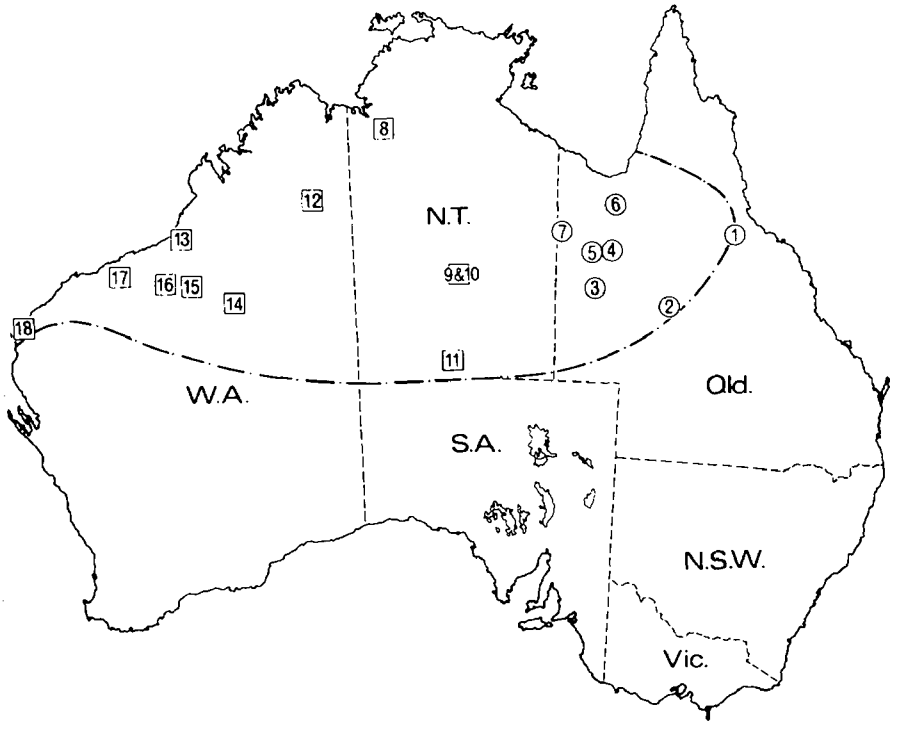

Fig. 1. The distribution of *V. acanthurus* in Australia. The numbers ringed correspond to localities at which the monomorphic karyotype (Fig. 2a) occurs. Those numbers encased in squares correspond to localities at which the polymorphic karyotype (Fig. 2b) is found. The numbers correspond to localities seen in Table 1.

Table 1. The localities, number, and sex of specimens karyotyped in the study. The morphology of chromosome pair 6 for each specimen is also described: MM (homozygous metacentric), MA (heterozygous metacentric/acrocentric), AA (homozygous acrocentric).

Loc. no. on Fig. 1		No. of Specimens		Morphology of Chrom. pair 6		
		♂	♀	MM	MA	AA
1	Charters Towers, Qld	1		1	–	–
2	Opalton, Qld	2	2	4	–	–
3	25 km S of Dajarra, Qld	–	1	1	–	–
4	13 km SE of Cloncurry, Qld	–	1	1	–	–
5	Mt Isa, Qld	–	1	1	–	–
6	96 km N of Dismal Crossing, Qld	–	3	3	–	–
7	20 km W of Camooweal, Qld	1	–	1	–	–
8	Bullo River, VRD, NT	1	–	1	–	–
9	6 km S of Barrow Ck., NT	1	1	–	1	1
10	5 km S of Barrow Ck., NT	1	1	1	1	–
11	120 km N of Kulgera, NT	1	–	–	1	–
12	17 km SW of Halls Ck., WA	–	1	–	1	–
13	Sandfire Flat, Anna Plains, WA	1	1	–	1	1
14	Lake Pellew, WA	1	–	1	–	–
15	9 km N of Abydos Stn, WA	–	1	1	–	–
16	Meentheena Stn, WA	1	–	–	–	1
17	14 km E of Barradale, WA	1	–	1	–	–
18	120 km S of Port Hedland, WA	1	–	–	–	1

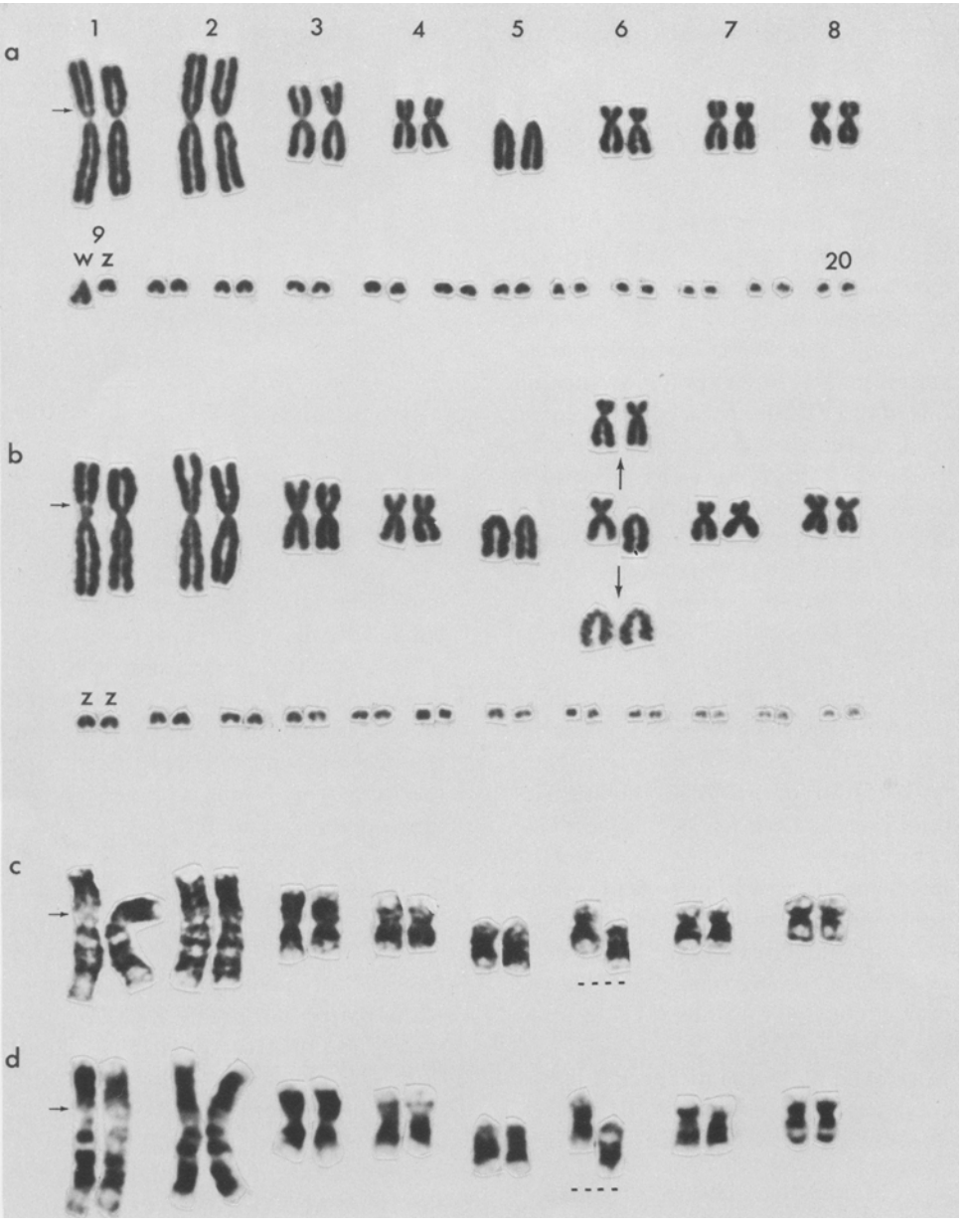

Fig. 2. V. acanthurus (a) female karyotype from the monomorphic race in Queensland. Note the secondary constriction on the short arm of pair 1 (arrowed), the monomorphic submetacentric pair 6 and the heteromorphic WZ pair 9 (sex-chromosome pair); (b) male karyotype from the polymorphic race in the Northern Territory and Western Australia showing the three possible forms of chromosome pair 6. Note the constriction on pair 1 (arrowed) and the homomorphic ZZ pair 9; (c and d) partial G-banded karyotypes of an animal heteromorphic for the pair-6 inversion polymorphism. Note the asymmetrical banding in pair 6 and the interbands corresponding to pair-1 constrictions. The telomeric C blocks on the long arm of pair 1 are also interbands.

(B) C banding – as described by King (1980).
(C) Silver staining – as described by Goodpasture & Bloom (1975)

Results

All specimens of *V. acanthurus* had 2n = 40 and the complement included two pairs of large (1–2), six pairs of medium (3–8) and twelve pairs of small chromosomes. Chromosomes 1 to 4 and 7 to 8 were metacentric whereas pair 5 was invariably acrocentric. Chromosome pair 6 was polymorphic and occurred in one of three states, namely: metacentric homozygous; metacentric/acrocentric heterozygous and acrocentric homozygous. In all cases the two homologues were identical in length. This polymorphism was found in specimens collected in the Northern Territory and Western Australia, whereas those animals from Queensland were all homozygous for the metacentric form (Figs. 1, 2a and b)

A secondary constriction is present on the short arm of pair 1 close to the centromere. This constriction is present in all varanids so far karyotyped (King & King, 1975). In some cells a faint constriction was also seen on the long arm of this pair, also close to the centromere.

Female specimens of *V. acanthurus* all possessed a ZW sex-chromosome pair in which the acrocentric W (pair 9) was approximately twice the length of its homologue (Fig. 2a). This heteromorphism has the same basic morphology as that observed in other varanids (King & King, 1975).

The following banding techniques were applied to the chromosomes of this species to more fully delimit the karyomorph characteristics described above:

(a) G banding

This technique was used to determine whether the polymorphism encountered in chromosome pair 6 was due to a pericentric inversion as appeared likely from conventional staining. The G-banding pattern is shown in Figures 2c and d which is from an animal heterozygous for the polymorphism. In the metacentric 6 chromosome G bands are concentrated in the central area of the chromosome, whereas the telomeric areas are unbanded at both ends. The acrocentric homologue has bands associated with the centromeric end of the chromosome, whereas the telomeric end distal to the centromere is unbanded. Moreover, a small interband area is present near the centromere. This banding pattern is consistent with the hypothesis that the observed rearrangement is due to a pericentric inversion as illustrated in Figure 3.

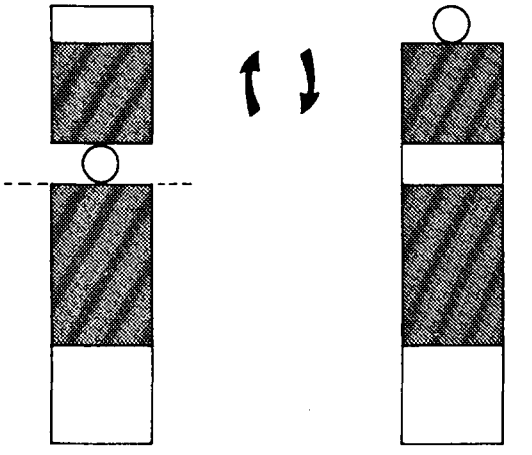

Fig. 3. A diagrammatic representation of the G banding of the polymorphic pair 6 showing the site of chromosome break and the resultant pericentric inversion.

(b) C banding

This technique was used to determine the predominant C-banding pattern, and the relationship of this pattern to both the ZZ/ZW sex-chromosome system and the structure of the secondary constriction. The C-banding distribution of both sexes is shown in Figures 4, b and c. The most outstanding feature is the very intensely G-banded W chromosome present in all female cells. Most of this chromosome is C-banded except for a small region at the telomere. A second feature is the presence of C blocks on the distal end of the long arm of chromosome pair 1. These are present in both sexes and are less intensively stained than the W. These C-banded blocks are also G-band negative (Fig. 2, c and d). The remaining C bands include small centromeric spots or pairs of spots located proximally to the centromere in the long arm of pairs 1 and 2.

Faint C bands appear to be associated with the secondary contriction on the short arm of chromo-

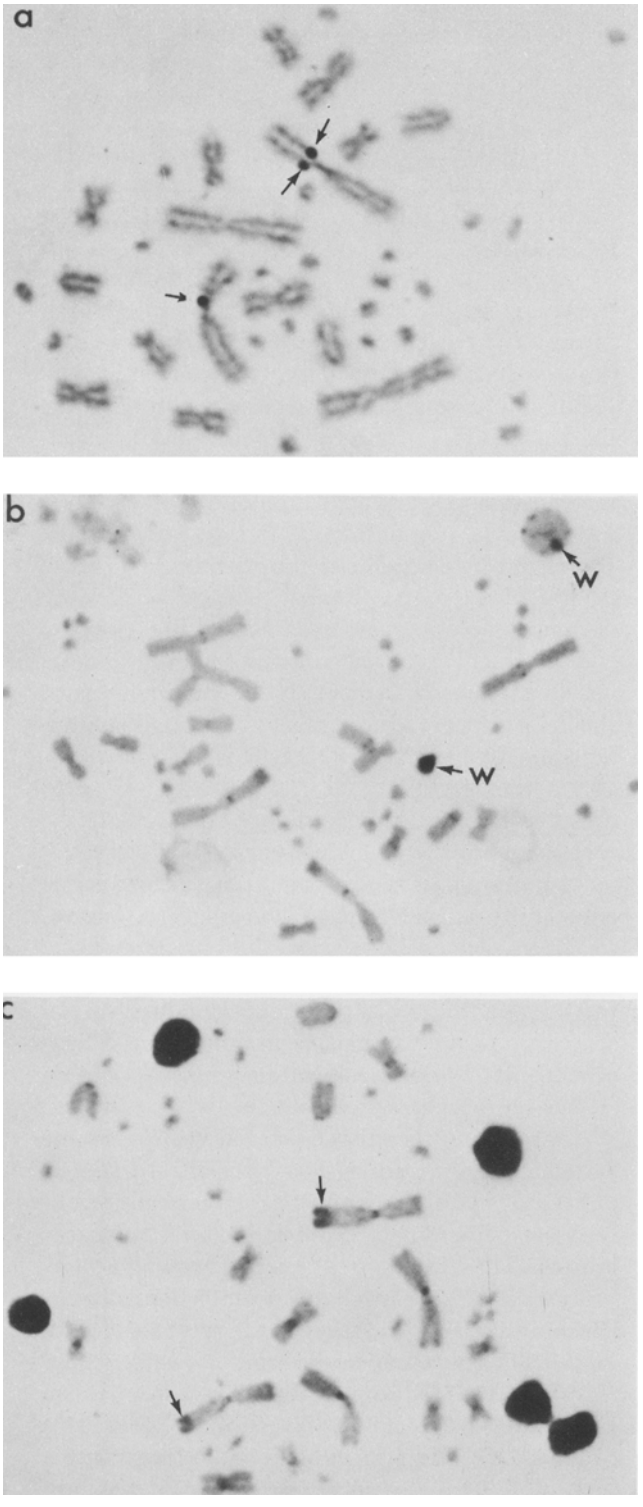

Fig. 4. (a) *V. acanthurus*. Silver staining showing a prominent reaction with the pair-1 constriction site. Note that one of the homologues in this cell has only one silver-positive site; (b) C-banded cell of a female. Note the very prominent W chromosome (arrowed), the small centromeric and procentric bands, and telomeric blocks on pair 1. A heterochromatic block corresponding to the W is also present in an interphase nucleus (arrowed); (c) C-banded male cell showing the absence of a prominent W. Note the C blocks on pair 1 (arrowed).

some pair 1, however difficulties in resolution prevent an accurate positioning of these bands in relation to the achromatic gap.

(c) Silver staining

This technique was used to localize the probable nucleolar organizer site in this species. In all cases, silver bands were restricted to the achromatic gap of the major secondary constriction on chromosome pair 1 (see Fig. 4a). Both homologues invariably stained with silver, though there was often a differential intensity between them. In the cell shown, only one of the chromatids in one homologue showed a silver spot; this was a consistent feature of this specimen.

Discussion

Evolution of a geographically delimited polymorphism

A karyotypic comparison of 18 species of the 35 known in the genus *Varanus* indicates that all of them have $2n = 40$ chromosomes but that certain groups of species can be differentiated on the basis of fixed differences involving presumed pericentric inversions in chromosome pairs 3–8 and the microchromosomes (King & King, 1975). The following groupings can be recognised: *V. gouldii* group (A); *V. varius* group (B); *V. salvator* group (C); Subgenus *Odatria* group (D); *V. griseus* group (E) and *V. niloticus* group (F). Group-A species occur in Australia, whereas group-B, -C and -D species are found in South East Asia and Australia. The group-E species occur in the Middle East and North Africa, whilst group-F species are present throughout Africa.

The chromosomal data combined with an electrophoretic study of these species (Holmes *et al.*, 1976) suggested that group-C species were closest to the ancestral karyomorph, and that the other groups diverged from C in three independent lineages which corresponded to major colonizing radiations within Australia and from South East Asia to Africa. These data imply that the direction of chromosome change has been from metacentricity to acrocentricity in pairs 3 to 8 since the alternative assumption would have predicted that

the most closely related karyotypic groups were, in fact, polyphyletic in origin.

The distribution of the monomorphic and polymorphic forms of *V. acanthurus* across Australia is of considerable interest for they provide us with an independent insight into the direction of chromosome change. The monomorphic karyomorph we see in eastern Australian specimens is essentially the same as that seen in other species of the *Odatria* group (D), all of which have an acrocentric pair 5. Indeed, the presence of this pair in both the *V. salvator* (C) and *V. varius* (B) groups was used as a basis for defining the ancestral karyotype in *Varanus* (King & King, 1975). Thus, the pericentric-inversion polymorphism we see in pair 6 in the central and western populations of *V. acanthurus*, and which is confirmed by G banding (Fig. 2, c and d; Fig. 3), is essentially a new rearrangement in which metacentric chromosomes have been converted to additional acrocentric chromosomes. That is, we have good evidence in this case that the direction of change has been from metacentricity to acrocentricity, thus inferentially supporting the model proposed by King & King (1975).

The geographic distribution of eastern and western karyomorphs is of some interest in terms of the mode of origin of the polymorphism in *V. acanthurus*. Although the central and western populations are separated by sandy deserts (Tanami, Gibson and Great Sandy Desert), it is doubtful whether these are effective barriers to gene flow. In desert areas these essentially rock-dwelling forms have been found living in hollow logs, *Triodia* clumps and even in grasslands. The only conceivable barrier between eastern and central/western karyomorphs is thus the Barkly Tablelands; a vast area of grasslands prone to extensive flooding and dissected by many rivers. However, even the effectiveness of this area as a means of isolation is open to question.

Clearly the mode of origin of the polymorphism cannot yet be determined. It is not clear if the inversion system was developed during an east-to-west colonizing radiation, or whether the polymorphism has spread throughout the central and western populations of a pre-existing distribution. Nevertheless, what is quite clear is the fact that the eastern populations are in some sense effectively genetically isolated from central and western populations.

Sex chromosomes

It is of particular interest that the ZZ/ZW sex-chromosome system described in *V. acanthurus* is of essentially the same morphology as that described in the African *V. niloticus* group and the Australian *V. varius* group (King & King, 1975). In each case the W is an acrocentric pair 9 about twice the length of the Z. The C-banding pattern of the W with its pronounced C block extending from the centromere to all but a small distal segment, confirms the likelihood of its origin by the addition of heterochromatin (see King, 1977) at a site associated with the centromere. A similar type of amplification is present in a population of the gekkonid lizard *Gehyra australis* in which a C-positive short arm has been added to the longest pair of acrocentric chromosomes in female specimens (King, 1977).

The sex-chromosome systems found in varanid lizards are unusual in that they occur in a number of species on separate continents. This suggests that female heterogamety of this type is of some antiquity. Other groups of lizards in which sex-chromosome systems are consistently observed are the North American Iguanidae where male heterogamety predominates and in which the Y chromosome is generally reduced by loss of chromatin (Gorman, 1973). (Cole, 1970; 1971). The predominantly Australian Pygopodidae also have male heterogamety and in this family the production of multiple-sex-chromosome systems by amplification of heterochromatin and translocation is not uncommon (King, 1977; King, unpublished).

Apart from the above, the sex-chromosome systems encountered in most other lizard species appear to have arisen independently on a number of separate occasions by a series of different mechanisms. Thus in the gekko *Phyllodactylus marmoratus* a ZZ/ZW mechanism which has differentiated by a pericentric inversion is present (King & Rofe, 1976), whereas in the teiid *Cnemidophorus tigris*, Bull (1978) described an XX/XY mechanism also established by such an inversion. Wright (1973) found an XX/XY system and related forms with a multiple system in *Scincella laterale*. Bhatnagar & Yonis (1976) described an instance of female heterogamety in a lacertid *Ophisops elegans*, while Chevalier *et al.* (1979) described a similar although multiple case of female heterogamety in the lacertid *Lacerta vivipara* which, however, involved different chromosomes.

The sex chromosomes of reptiles thus fall into two distinct categories. In groups such as *Varanus*, the Pygopodidae and many Iguanidae, recognizable sex chromosomes have been maintained for a considerable evolutionary period. In other lizard families, however, sex chromosomes are of more recent origin, evolving by different mechanisms and in some cases giving rise to chromosome races which may form part of a speciation process (King, 1977).

Acknowledgements

The authors thank Professor B. John for reading the manuscript. Dr Max King thanks the Genetics Department at the University of Adelaide for extending to us the use of their facilities during part of this work. Many thanks to Pamela King, Dr Hal Cogger, Simon Whitehouse and John Wombey, who helped collect or loaned us specimens for this study.

References

Beçak, M. L., Beçak, W. & Denaro, L., 1972. Chromosome polymorphism, geographic variation and karyotypes in Sauria. Caryologia 25, (3): 313-326.

Bhatnagar, A. N. & Yonis, Y. Th., 1976. A proof of female heterogamety in a lacertid lizard Ophisops elegans. Cytologia 41: 507 511.

Bull, J., 1978. Sex chromosome differentiation: an intermediate stage in a lizard. Can. J. Genet. Cytol. 20: 205-209.

Chevalier, M., Dufaure, J. P. & Lecher, P., 1979. Cytogenetic study of several species of Lacerta (Lacertidae, Reptilia) with particular reference to sex chromosomes. Genetica 50, 1: 11 18.

Cole, C. J., 1970. Karyotypes and evolution of the spinosus group of lizards in the genus Sceloporus. Am. Mus. Novit. 2431: 1 47.

Cole, C. J., 1971. Karyotypes of the five monotypic groups of lizards in the genus Sceloporus. Am. Mus. Novit. 2450: 1-17.

Cole, C. J., 1977. Chromosomal aberration and chromatid exchange in the North American fence lizard, Sceloporus undulatus (Reptilia: Iguanidae). Copeia 1: 53-59.

Cole, C. J., Lowe, C. H. & Wright, J. W., 1969. Sex chromosomes in teiid whiptail lizards (genus Cnemidophorus). Am. Mus. Novit. 2395: 1-14.

Goodpasture, C. & Bloom, S. E., 1975. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma 53: 37-50.

Gorman, G. C., 1973. The chromosomes of the Reptilia, a cytotaxonomic interpretation. In: 'Cytotaxonomy and vertebrate evolution', edited by A. B. Chiarelli and E. Capanna. Academic Press, New York. pp. 349-424.

Holmes, R. S., King, M. & King, D., 1976. Phenetic relationships among varanid lizards based upon comparative electrophoretic data and karyotypic analyses. Biochem. Syst. Ecol. 3: 257-262.

King, M., 1977. The evolution of sex chromosomes in lizards. In: 'Evolution and reproduction'. Proc. 4th Int. Conf. Reproduction Evolution. pp. 55-60. Aust. Acad. Sci.

King, M., 1980. C-banding studies on Australian hylid frogs: secondary constriction structure and the concept of euchromatin transformation. Chromosoma 80: 191-217.

King, M. & King, D., 1975. Chromosomal evolution in the lizard genus Varanus (Reptilia). Aust. J. Biol. Sci. 28: 89 108.

King, M. & Rofe, R., 1976. Karyotypic variation in the Australian Gekko Phyllodactylus marmoratus (Gray) (Gekkonidae: Reptilia). Chromosoma 54: 75 87.

Sites, J. W., Bickham, J. W., Haiduk, M. W. & Iverson, J. B., 1979. Banded karyotypes of six taxa of kinosternid turtles. Copeia 4: 692 698.

Webster, T. P., Hall, W. P. & Williams, E. E., 1972. Fission in the evolution of a lizard karyotype. Science 177: 611-613.

Wright, J. W., 1973. Evolution of the X_1X_2Y sex chromosome mechanism in the scincid lizard Scincella laterale (Say). Chromosoma 43: 101-108.

Received 8.5.1981 Accepted 1.10.1981.