Karyotypic Homology and Evolution of the Agamid Lizards

G. P. Sharma and Usha Nakhasi

Department of Zoology, Panjab University,
Chandigarh-160014, India

Received August 24, 1978

The family Agamidae has received very little attention of the cytologists (Arronet 1965, Matthey 1949, Makino and Asana 1950, Dutt 1968, 1969, Hall 1970, Gorman and Shochat 1972). The chromosome data are available only for about sixteen species of which many reports are incomplete and need further elucidation. The present communication establishes the karyotype morphology and inter-specific relationships of four species of agamids and also provides some clues to the problem of speciation in this group of lizards.

Materials and technique

Three species viz., *Calotes versicolor* (Daudin), 1802; *Calotes jerdoni* Günther, 1870 and *Ptyctolaemus gularis* Peters, 1864 were collected from the Meghalaya (Shillong) and Assam (Khanapara) states and one species, *Uromastix hardwickii* Gray, 1827, was collected from U. P. (Agra) in the months of April and May of the years 1975 and 1976.

The animals were injected with 0.90% colchicine intra-peritoneally 24 hours prior to dissection. The tissues like spleen, intestine, bone-marrow and testes were pre-treated with 0.90% sodium-citrate, for 45 minutes at 30°C. The fixation was done in Carnoy's fixative (methanol: glacial acetic acid, 3:1). The slides were prepared by the usual air-drying technique and stained in carbol-fuchsin.

Observations

Calotes versicolor: The diploid number of chromosomes is $2n=34$. The diploid karyotype consists of twelve macro-chromosomes and twenty-two micro-chromosomes. The macro-elements occasionally show differential heteropycnosis, terminal or sub-terminal achromatic zones and a wavy contour. Leaving aside the second largest pair, all the macro-chromosomes are metacentric. The second pair is sub-mediocentric and in some cells its long arms bear satellites. All the twenty-two micro-elements are bi-armed and range in size from 0.84 to 1.47 microns. The karyotypes of the male and female diploid complements are identical (Figs. 1 and 2). The morphometric analysis is presented in Table. 1.

An early metaphase I cell (Fig. 3) reveals six large and eleven small bivalents. The largest two bivalents have, on an average, four chiasmata each, the third and fourth bivalents have three chiasmata each and the fifth and sixth macro-

bivalents have two terminal chiasmata each. All the micro-bivalents retain a single terminal chiasma each. Metaphase II (Fig. 4) is constituted by six macro- and eleven micro-elements. The morphology of the macro-elements is identical to their counter-parts in the diploid complement. The second largest element, here too, carries satellites on its long arms.

Figs. 1-5. 1, karyotype of spermatogonial metaphase of *C. versicolor*. 2, karyotype of female macro-complement of *C. versicolor*. 3, early metaphase I of *C. versicolor*. 4, metaphase II of *C. versicolor*. 5, karyotype of female *C. jerdoni*.

Table 1. Relative length (RL), centromeric index (CI), arm ratio (AR) and nomenclature of the macro-complement in *Calotes versicolor*

Chromosome pair number	RL	CI	AR	Nomenclature
1	146.58	0.48	1.07	m
2	134.78	0.43	1.30	sm
3	113.70	0.49	1.03	m
4	106.67	0.48	1.08	m
5	95.13	0.48	1.04	m
6	79.06	0.49	1.01	m

Calotes jerdoni The diploid number of chromosomes is $2n=34$. The diploid complement is constituted by twelve macro- and twenty-two micro-chromosomes. The second and fifth largest macros are submetacentric, whereas the remaining four pairs of macro-elements are metacentric. The differential heteropycnosis and achromatic zones have been observed in many of these macros. The second largest pair of this species also bears satellites on its long arms similar to those described in the former species. The twenty-two micro-elements are meta-/submetacentric and range in size from 0.63 to 1.37 microns (Fig. 5). The quantitative data on the macro-complement are given in Table 2.

Table 2. Relative length (RL), arm ratio (AR), centromeric index (CI) and nomenclature of the macro-complement in *Calotes jerdoni*

Chromosome pair number	RL	AR	CI	Nomenclature
1	194.93	1.06	0.48	m
2	164.83	1.58	0.38	sm
3	129.22	1.08	0.47	m
4	124.81	1.19	0.46	m
5	105.35	1.65	0.37	sm
6	67.73	1.10	0.47	m

Ptyctolaemus gularis: The diploid number of chromosomes is retained here too as $2n=34$. Except for the second largest pair, all the remaining five pairs of macro-chromosomes are metacentric. The second pair is submetacentric. The size range of the twenty-two micro-elements is from 0.46 to 1.26 microns (Fig. 6). The morphometric analysis of the macro-complement is given in Table 3.

Table 3. Relative length (RL), arm ratio (AR), centromeric index (CI) and nomenclature of macro-complement in *Ptyctolaemus gularis*

Chromosome pair number	RL	AR	CI	Nomenclature
1	177.89	1.01	0.49	m
2	159.56	1.43	0.40	sm
3	124.38	1.00	0.50	m
4	123.90	1.00	0.49	m
5	110.50	1.18	0.46	m
6	77.05	1.07	0.46	m

The metaphase I cell (Fig. 7) reveals six macro- and eleven micro-bivalents. The largest four bivalents carry four to two chiasmata each, the fifth and sixth bivalents have two terminal chiasmata each, and each micro-bivalent characteristically retains a single terminal chiasma. The seventeen elements of metaphase II (Fig. 8) comprise six macro- and eleven micro-elements. The morphology of the macro-chromosomes is identical to that of their counter-parts in the diploid complement.

Figs. 6-11. 6, karyotype of spermatogonial metaphase of *P. gularis*. 7, early metaphase I of *P. gularis*. 8, metaphase II of *P. gularis*. 9, karyotype of spermatogonial metaphase of *U. hardwickii*. 10, early metaphase I of *U. hardwickii*. 11, karyotype of haploid complement of *U. hardwickii*.

Uromastix hardwickii: The diploid number of chromosomes is $2n=36$. The macro-complement is constituted by six large homomorphic pairs, whereas the twenty-four small elements compose the micro-complement. The first, third and fourth largest pairs are metacentric and the second, fifth and sixth largest macros are submetacentric. The size range of the twenty-four small elements is from 0.54 to 1.11 microns (Fig. 9). The quantitative analysis of the macro-complement is given in Table 4.

Table 4. Relative length (RL), arm ratio (AR), centromeric index (CI) and nomenclature of macro-complement in *U. hardwickii*

Chromosome pair number	RL	AR	CI	Nomenclature
1	202.51	1.07	0.48	m
2	157.14	1.52	0.39	sm
3	139.90	1.05	0.48	m
4	110.36	1.12	0.47	m
5	106.70	1.35	0.42	sm
6	63.37	1.33	0.42	sm

The metaphase I cell (Fig. 10) reveals six macro- and twelve micro-bivalents. The largest bivalent carries four chiasmata. Each of the second, third and fourth bivalents bears three chiasmata. The fifth and sixth have two terminal chiasmata each. On the other hand, each micro-bivalent has a single terminal chiasma. The haploid complement, as depicted by the karyotype of metaphase II cell (Fig. 11), possesses eighteen chromosomes. Out of the six macro-elements, the first, third and fourth largest are metacentric, the second, fifth and sixth largest chromosomes are submetacentric.

Discussion

Two kinds of chromosomal constitutions are reflected by the species of the family Agamidae (Table 5). In group-I, the diploid number is either $2n=34$ or $2n=36$ and in group-II, it is 44, 46 or 48. In both the kinds of complements there are present twenty-two or twenty-four micro-chromosomes and in general there is a conservativeness for the total number of arms which is $N.F.=46$ or 48. The two groups differ mainly in the number and morphology of the macrochromosomes.

Group-I: The macro-chromosomes are twelve in number except in *Agama atricollis*, where only ten macros have been observed. All the macro-chromosomes are bi-armed, either meta- or submetacentric.

The four species of Agamids analysed in the present studies belong to this group. *Calotes versicolor* and *Ptyctolaemus gularis* are identical to each other in general morphology of their macros. However, on comparing the relative lengths of the corresponding macro-chromosomes of these two species (Tables 1 and 3; Fig. 12), except the pair number six, all the macros of *P. gularis* have a much higher value of relative lengths than their counter-parts in the karyotype of *C.*

versicolor. Hence it is evident that the percentage relative length of the whole macro-complement of *P. gularis* is higher than that of *C. versicolor* (Fig. 13). It is interesting to note that this increase in percentage relative length is accom-

Figs. 12–13. 12, composite idiogram of the macro-complement of four species of family Agamidae (A—*C. versicolor*, B—*C. jerdoni*, C—*P. gularis*, D—*U. hardwickii*). 13, histograms showing RL of macro-complements (a) and micro-complements (b) of A—*C. versicolor*, B—*C. jerdoni*, C—*P. gularis*, D—*U. hardwickii* (Family Agamidae).

panied by a relative decrease in the perecentage relative length of the micro-complement of *P. gularis* and with a decrease in the % RL of macro-complement there is an almost equal increase in the % RL of micro-complement of *C. versicolor* (Fig. 13). The result is that the total genome of the two species remains the same. It is suggested that *Ptyctolaemus gularis* has evolved from a *C. ver-*

Table 5. Cytological details of the species so far reported from the family Agamidae

Group number	Species	Number and morphology of the macros	N. F.	References
I:				
a, 2n=34	<i>Agama caucasica</i>	12, m	46	Arronet (1965)
(12 macros, 22 micros)	<i>Agama stellio</i>	12, m	46	Gorman and Shochat (1972)
	<i>Agama tuberculata</i>	12, m	46	Dutt (1969)
	<i>Calotes jerdoni</i>	12, m/sm	46	Present study
	<i>Calotes versicolor</i>	12, m/sm	46	Singh <i>et al.</i> (1970) and Present study.
	<i>Ptyctolaemus gularis</i>	12, m/sm	46	Present study.
	<i>Uromastix hardwickii</i>	12, m/sm	46	Makino and Asana (1950)
I:				
b, 2n=36	<i>Agama stellio</i>	12, m	48	Gorman and Shochat (1972)
(12 macros, 24 micros)	<i>Leiolepis belliana</i>	12, m	48	Hall (1970)
	<i>Uromastix hardwickii</i>	12, m/sm	48	Present study.
II:				
a, 2n=44	<i>Agama sinaita</i>	22, acro	44	Gorman and Shochat (1972)
	<i>Sitana ponticeriana</i>	22, acro	44	Dutt (1968)
	<i>Phrynocephalus helioscopus</i>	20: 2m. 18 acro	46	Arronet (1965)
II:				
b, 2n=46	<i>Agama pallida</i>	24, acro	46	Gorman and Shochat (1972)
(24 macros, 22 micros)	<i>Agama savignii</i>	24, acro	46	Gorman and Shochat (1972)
	<i>Japalura polygonata</i>	24, acro	46	Makino and Momma (1949)
	<i>Japalura swinhonis</i>	24, acro	46	Nakamura (1935)
	<i>Sitana ponticeriana</i>	24, acro	46	Makino and Asana (1950)
II:				
c, 2n=48	<i>Agama planiceps</i>	26: 6 st, 20 acro	48	Gorman and Shochat (1972).
(26, macros, 22 micros)				

sicolor type ancestor through translocation of some micro-complement genome on to the macro-complement in such a manner that the centromeric index of the individual macros remains undisturbed.

Calotes jerdoni differs from its congeneric species, *C. versicolor* in having a submetacentric pair number five of the macro-chromosomes. Furthermore, a

study of the histogram (Fig. 13) reveals a considerably higher value of % RL of the macro-complement of *Calotes jerdoni*. Also it is noteworthy that the % RL of the micro-complement is the lowest in *C. jerdoni* and this relative decrease is directly proportional to the relative increase in its macro-complement genome. Thus, it is postulated that *Calotes jerdoni* has probably a *Calotes versicolor*-like ancestor and its evolution has occurred through a pericentric inversion in the fifth largest pair, followed by the translocation of a definite percentage of micro-complement genome onto the macro-complement, the distribution being fairly regular in a manner which has not disturbed the apparent morphology of the macros. The sixth pair has remained unaffected.

The quantitative study of the chromosomes of *Uromastix hardwickii* reveals that this species is closely related to *Calotes jerdoni*. The only difference lies in the morphology of sixth largest pair. The submetacentric state of this pair in the karyotype of *U. hardwickii* has certainly evolved through a pericentric inversion in the original more conserved metacentric form as revealed by *Calotes jerdoni*. The increase in the number of micros in *U. hardwickii* by two elements can be explained through either a centric fission in the largest pair of micro-chromosomes of *Calotes jerdoni*-like micro-complement or translocation of a small genome from macro-complement onto the micro-complement. The latter hypothesis finds its support from the histogram data (Fig. 13) which shows a higher value of micro-complement and a lower value of macro-complement in *Uromastix hardwickii* than their corresponding values in *Calotes jerdoni*. Both of these, however, i.e., centric fission and translocation accompanied by an increase in the numerical value of the micro-chromosomes involve duplication of the centromere which is relatively a rare feature of the evolutionary pathway.

The fundamental number of arms is N. F.=46 in all the three species viz., *Calotes versicolor*, *C. jerdoni* and *Ptyctolaemus gularis* and it is N. F.=48 in *Uromastix hardwickii*. The Robertsonian forces do not seem to be operating in these species, because both the diploid number and N. F. are more or less conserved in group-I. Therefore, it seems that speciation has occurred in these forms through relative shifts in the genome and through sporadic pericentric inversion in one or two pairs of macro-chromosomes.

The characteristic feature of the species in group-II (Table 5) is that their macros are all acrocentric. The large bi-armed elements are either completely absent or are one or two in number as in *Phrynocephalus helioscopus* and *Agama planiceps*. Gorman and Shochat (1972) have reported the absence of bimodality in the karyotypes of five species of *Agama*. It is, therefore, obvious that the twenty-four acrocentric macros in species of group-II have evolved through centric fissions in twelve meta-/submetacentric macros of the species in group-I. The reverse can also be true i.e., the group-I karyotype has evolved from that of group-II in general, through the centric fusions of twelve medium-sized acrocentric and twelve large acrocentric chromosomes of the group-II karyotype, thereby resulting also in a bimodal form characteristic of the group-I karyotype.

So long both the sexes have been reported to be homogametic in all the species of the family Agamidae. However, Makino and Asana (1950) claimed

X-O female heterogamety in *Calotes versicolor* and *Sitana ponticeriana*. But now Singh *et al.* (1970), Dutt (1968) and the present report have specifically denied these results. During the present investigations, the males of *Calotes versicolor*, *Ptyctolaemus gularis*, *Uromastix hardwickii* and the females of *Calotes versicolor* and *Calotes jerdoni* have all been found to be homogametic.

Summary

In the family Agamidae, there has been observed a conservativeness for the total number of arms in the karyotype. All the diploid species have twenty-two (*Calotes versicolor*, *Calotes jerdoni*, *Ptyctolaemus gularis*; present report) or twenty-four (*Uromastix hardwickii*, present report) micro-chromosomes and all but three species have twenty-four macro-chromosomal arms, twelve meta-/submetacentric (group-I) or twenty-four acrocentric chromosomes (group-II). The group-I karyotype has evolved from group-II karyotype through the centric fusions of twelve medium sized acrocentric and twelve large acrocentric elements of the group-II karyotype.

Acknowledgements

The authors wish to extend their sincere gratitude to the Council of Scientific and Industrial Research for financial assistance and to the Director, Zoological Survey of India for providing identification of the material.

References

- Arronet, V. N. 1965. Tsitologia 7: 237-239 (Quoted from Gorman, 1973).
- Dutt, K. 1968. Pre-fixation treatment and squashing for reptilian chromosomes. The Microscope 16: 355-358.
- 1969. Studies on the chromosomes in two species of Indian lizards. The Microscope 17: 213-218.
- Gorman, G. C. 1973. The chromosomes of the Reptilia: A cytotaxonomic interpretation. Chapter 12 In: Cytotaxonomy and Vertebrate Evolution, Chiarelli and Capanna. 1973: 349-422.
- and Shochat, D. 1972. A taxonomic interpretation of chromosomal and electrophoretic data on the agamid lizards of Israel with notes on some east African species. Herpetologica 28: 106-112.
- Hall, W. P. 1970. Three probable cases of parthenogenesis in lizards (Agamidae, Chamaeleonidae, Gekkonidae). Experientia 26: 1271-1273.
- Makino, S. and Asana, J. J. 1950. A sexual difference in the chromosomes of two species of agamid lizards. Chromosoma (Berl.) 3: 208-129.
- and Momma, E. 1949. An idiogram study of the chromosomes in some species of reptiles. Cytologia 15: 96-108.
- Matthey, R. 1949. "Les Chromosomes des Vertebres". F. Rouge, Lausanne.
- Nakamura, K. 1935. Mem. Coll. Sci. Kyoto Univ, Ser. B 10: 355-360. (Quoted from Gorman, 1973).
- Singh, L., Sharma, T. and Ray-Chaudhuri, S. P. 1970. Chromosome numbers and sex-chromosomes in few Indian species of amphibia and reptiles. Mamm. Chrom. Newslett. 11: 91-94.