Studies on the Chromosomes of the Rainbow Lizard *Agama agama agama* (L.) with Notes on Polypoidy in the Spermatocytes

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The Rainbow lizard, *Agama agama agama* has been described as the West African subspecies of *A. agama* (L.) (Harris 1963). Other *Agama* species from Africa which have been karyotyped are *A. atra* (S. Africa) and *A. stellio* (N. Africa) (De Smet 1981a).

Cytogenetic studies of the reptiles, particularly the suborder Lacertilia (Sauria) have been done by several workers (Matthey 1949, Gorman 1965, Becak and Becak 1969, Dutt 1969, King and Rofe 1976, Bull 1978, De Smet 1978, 1981a, 1981b, Chevalier *et al.* 1979, Cole 1979, Mengden and Stock 1980, Adegoke 1984, 1985), and these studies reveal a number of interesting features. There is a wide variation in the karyotypes, although fairly close relationships exist between members of a particular family. Thus it was possible for Matthey (1949) to categorise the reptilian karyotypes into three complexes, the scincolacertid, gekkonid and iguanid. Of the three complexes only members of the scinco-lacertid have an appreciable number of large metacentric chromosomes while the karyotypes of the gekkonid and iguanid complexes contain a high proportion of telocentric and acrocentric chromosomes. The family Agamidae belongs to the iguanid complex.

Another common feature of the reptilian karyotypes is the presence of small to very small chromosomes generally referred to as “microchromosomes.” These are relatively more prevalent among the iguanid complex than the other two complexes. Reptiles share this feature with birds.

Chromosomal sex differentiation is not always obvious, even in species where the male animal is normally morphologically distinguishable from the female. However, cases of both male and female heterogamety have been reported (Chevalier *et al.* 1979, Becak and Becak 1969, Bull 1978).

The meiotic cells from the testes of reptiles offer good materials for karyotypic studies, particularly in species with substantial numbers of very small chromosomes whose numbers are often difficult to obtain from somatic preparations. In the present studies, the diploid chromosome number in *A. agama agama* has been confirmed from testis preparations. Furthermore the phenomenon of tissue polyplloidisation in the spermatocytes has been studied and the possible origin of these polyplloid cells is discussed.

Materials and methods

Source of animals

Several male and female animals of various ages were caught at different times and brought to the laboratory where they were injected intraperitoneally with 0.5-0.8 ml of 0.20% colchicine or 10 μg/ml colcemid (GIBCO) for 2-3 hours before sacrificing with chloroform.

Cell fixation

The femur, with or without the humerus was dissected out, cut at both ends and the marrow aspirated into a centrifuge tube with 4-8 ml of warm (37°C) hypotonic buffer of 0.55%
KCl. Cell fixation and spreading were as described earlier (Adegoke 1985).

Testes from adult males were homogenized at low speed in the hypotonic buffer using a Polytron PT-20-OD homogenizer and thereafter treated in the same manner as the marrow tissue.

Cells were stained for 10–20 minutes with acetoorcein or 6% of Giemsa solution (0.5 g in 66 ml of a 1:1 mixture of glycerine and methanol). The Giemsa solution was diluted with phosphate buffer to give a pH of 6.8.

Results and discussion

The karyotype of *A. agama agama*

Well over three hundred well spread metaphase cells were scored from bone marrow preparations of twelve male and ten female animals collected from Ile-Ife and Benin City over a period of two years. A chromosome number of 2n=44 was obtained for both sexes as shown in Figs. 3–5, and the fundamental number is 46. From the testis, cells in diakinesis give 22 bivalents (Fig. 6), to confirm a diploid number of 44. This number is consistent with other findings for members of the family Agamidae in which also the diploid number tends to decrease with increase in the number of biarmed chromosomes (Sokolovsky 1975).

An idiogram was constructed from chromosome measurements (Fig. 2), and from this, it was possible to divide the chromosomes into three size classes. Class one includes chromosome 1 which is conspicuously the largest, and described as submetacentric with a centromeric index of 32.8. Furthermore this is the only chromosome with a secondary constriction which is located on the short arm (see arrow in Fig. 3). A second class consists of chromosomes 2 to 11 which are large to medium sized and which are always clearly distinguishable in all the good metaphase spreads. A third class consists of the small chromosomes numbered from 12 to 22. Chromosome Nos. 14 to 22 are however too small to be accurately matched and the pairing shown in Fig. 1 for these elements is largely arbitrary.
The chromosomes in classes 2 and 3 show rather uniform intergading of size and there is no evidence of small arm in any of the elements and these chromosomes would be described as truly telocentric. Close observations of the karyotypes of both sexes did not reveal any differences that might be attributable to sex. If there is sex differentiation in the karyotypes this might be revealed by banding techniques provided such sex chromosomes are large.

A study of the karyotypes of five lizard species from the family Agamidae by De Smet (1981a) showed that *A. agama* (L.) and *A. atra* Daud. have diploid chromosome numbers of 50 and 44 respectively. Apart from number, other features of the karyotype of *A. atra* Daud. resembles very closely those of *A. agama agama* (L.) (this study). It is therefore suggested that both *A. atra* and *A. agama agama* are most probably subspecies of the same species, while the Rainbow lizard is most probably not a subspecies of *A. agama* (L.) as the current classification indicates (Harris 1963).

![Fig. 2. Idiogram of the karyotype to show size variation among the chromosomes.](image)

**Polyploidy in the spermatocytes of A. agama agama**

While wholly polyploid animals are rare, many tissues in various animal groups show varying degrees of polyploidy. Polyploid nuclei are observed in somatic tissues of various Dipteran, Hymenopteran and Heteropteran larvae (White 1977) and in the peripheral blood cells of mammals (Sinha, *et al.* 1973). Apparent polyploid primary spermatocytes at diakinesis or meiotic metaphase from the testis of some mammalian species have previously been reported (Sasaki and Makino 1965, Hulten *et al.* 1970, Ford and Evans 1971).

In this study, presumably normal polyploid spermatocytes have been observed in prophase I and metaphase I stages. Most of them are derived from tetraploid cells while a few are from octoploid and 16-ploid cells (Figs. 7–9). Table 1 shows the incidence of normal polyploid cells in diakinesis from eight animals in relation to total number of cells at the same stage of meiosis. A total of 1,377 cells were scored, and the results show that 4.8% of these were tetraploid while only 0.4% had ploidy levels higher than 4n. This is consistent with earlier
observations (Ford and Evans 1971). Furthermore there is no evidence to show that the incidence of polyploidy in the spermatocytes is greater in animals treated with colchicine or colcemid than untreated animals. A number of apparent polyploid spermatocytes were

Figs. 3-10. Chromosome spreads from bone marrow and testis. 3, metaphase spread from bone marrow of a male animal. Arrow shows the secondary constriction on the short arm of the largest chromosome. 4, from bone marrow of a female animal. Encircled is an artefact. 5, mitotic metaphase from testis. 6, diakinesis of a diploid (2n) cell. 7-9, diakinesis of normal polyploid cells; 7 is 4n, 8 is 8n and 9 is 16n. 10, tetraploid cell in mitotic metaphase from the testis. Figs. 3-8 are Giemsa stained; Figs. 9 and 10 are orcein stained. Figures on bars (except Fig. 9) are in microns.
also observed in all the preparations as shown in Figs. 12-14.

Normal polyploid cells were defined as those in which the chromosomes are "mixed" as shown in Figs. 7-11, and the ploidy levels are what would be expected from endoreduplication of the chromosomes, i.e. 4n, 8n, 16n. Apparent polyploidies are those that arise by fusion of cells, and these always show areas of organisation of the chromosomes of the contributing cells at any stage of prophase I or metaphase I. Moreover they may give nongeometric levels of polyploidy. In Fig. 12 is shown 'a cell' presumably derived from fusion of four cells whose areas of chromosome organisations are marked a-d. The 'cells' shown in Figs. 13, 14 and 16 are derived from such fusions. Although there appears to be interconnection of the nuclear materials of fusing cells particularly in the leptonema and zygonema stages, nevertheless no quadrivalents were observed in diakinesis. Abnormal pairings were also absent in the diakinesis stage of presumed normal polyploids. One could surmise from these observations that:

Table 1. Incidence of normal polyploid cells at the diakinesis stage in eight animals

<table>
<thead>
<tr>
<th>Animal and date</th>
<th>2N</th>
<th>4N (as %)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 December 1985</td>
<td>165</td>
<td>15 (8.3%)</td>
<td>2</td>
</tr>
<tr>
<td>2 December 1985</td>
<td>99</td>
<td>5 (4.7%)</td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td>3 January 1986</td>
<td>405</td>
<td>13 (3.1%)</td>
<td>0</td>
</tr>
<tr>
<td>4 February 1986</td>
<td>51</td>
<td>4 (7.24%)</td>
<td>0</td>
</tr>
<tr>
<td>5 April 1986</td>
<td>94</td>
<td>6 (5.9%)</td>
<td>1 (1.0%)</td>
</tr>
<tr>
<td>6 January 1986</td>
<td>207</td>
<td>1 (5.0%)</td>
<td>0</td>
</tr>
<tr>
<td>7 April 1986</td>
<td>78</td>
<td>7 (8.2%)</td>
<td>0</td>
</tr>
<tr>
<td>8 June 1986</td>
<td>207</td>
<td>5 (2.3%)</td>
<td>2 (0.9%)</td>
</tr>
<tr>
<td>Totals</td>
<td>1,306</td>
<td>66 (4.8%)</td>
<td>5 (0.4%)</td>
</tr>
</tbody>
</table>

Animals 1-5 were injected with colcemid while 6-8 were untreated prior to sacrifice. For each animal the proportion of the polyploid cell is given as percentage in brackets.

1) The polyploid cells (whether normal or apparent) probably have an effective mechanism that prevents abnormal pairings in the presence of two or more pairs of homologous chromosomes at the onset of meiosis. And it is presumed that these cells complete meiosis and produce normal spermatids.

2) Normal polyploid cells could arise by two rounds of endoreduplication of the chromosomes without a separation of the chromatids before the onset of meiosis. For example, an otherwise normal diploid spermatogonium will, after two rounds of endoreduplication followed by initiation of meiosis, have two bivalents of each chromosome at diakinesis. The cell would appear as shown in Fig. 7 and be classified as 4n. A similar event occurring to a tetraploid cell such as shown in Fig. 10 would yield an octoploid at diakinesis (Fig. 8).

Figure 15 shows a tetraploid cell in which the homologous chromosomes are paired, and illustrates an abnormal pairing which was frequently observed in most preparations. The meiotic stage at which such cells are is not yet certain. In many cases apparently fused cells are at the same stage of meiosis (e.g. Figs. 13, 14). However in some cases they are not synchronous as shown in Fig. 16 where cell m is in metaphase II and cell n in anaphase II.
Summary

The chromosomes of the Rainbow lizard, *A. agama agama* have been studied from bone marrow and testis preparations. The animal has a diploid chromosome complement of 44 with a fundamental number of 46. The karyotype bears very close relationship to that of *A. atra* from South Africa. Several spermatocytes in diakinesis and metaphase I show polyploidy which may be regular or apparent. The incidence of the regular polyploids varies.
from one animal to the other, although averagely about 4.8% of all cells in diakinesis are of the tetraploid origin while other ploidy levels account for 0.4%. Quadrivalents were not found in these polyploid cells and a process of endoreduplication of chromosomes without separation of sister chromatids is suggested for their origin.

References