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A SEXUAL DIFFERENCE IN THE CHROMOSOMES OF TWO SPECIES OF AGAMID LIZARDS.[1]

By

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With 14 figures in the text.

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Heterogametic condition in the female sex has been well established in birds on both cytological and genetic evidence. According to the general conceptions of phylogeny the reptiles form a group standing in very close relationship to the birds, not only in anatomical structure but also in a number of embryological features. On account of this fact, both the birds and reptiles are included in a group called *Sauropsida*. Thus, the close relationship in phylogeny naturally suggests the occurence of female heterogamety in the reptiles as in the birds. Less progress has so far been made concerning the sexual difference of chromosomes in the reptiles than in the birds; there have been published three papers which deal with this subject. OGUMA (1934) was the first to prove the occurrence of heterogamety in the reptilian female with indisputable clearness, based on a chromosome survey in the lizard, *Lacerta vivipara*. Somewhat later he (OGUMA, 1937) showed a similar condition to occur in the soft-shelled turtle, *Amyda japonica*, by demonstrating an unpaired element in the female cell. Quite recently, NAKAMURA (1946) reached a similar conclusion in the chelonian, *Caretta olivacea*, based on the comparison of chromosomes between the two sexes. The present authors wish to present in this paper the results proving heterogamety in the female sex, which were obtained from the study of the chromosomes in two species of Indian lizards belonging to the *Agamidae*. This paper will thus furnish additional evidence on sex-determination in the reptiles.

Here it is the authors' pleasant duty to express sincere gratitude to Dr. KAN OGUMA for his valuable suggestions. The authors are also under obligation to Prof. F. SCHRADER for his kind service in revising the manuscript for publication.

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Material and methods.

Calotes versicolor and *Sitana ponticeriana* with which the present investigation was carried on, are members of the *Agamidae*, and very common in the suburb of Ahmedabad, Western India, where they were collected. The specimens of *Calotes* were secured during August and September, 1934, and those of *Sitana* in the latter part of August, 1934. in the above-mentioned locality. In both species the chromosomes of the male sex were studied in both adult and embryonic testes. but those of the female were investigated exclusively in the embryonic ovaries. The embryonic gonads were obtained by dissecting the embryos out of developing eggs (age unknown) which were collected in the field. As fixatives, NAKAMURA's modification of CHAMPY's mixture was used for adult testes, and HERMANN's fluid with reduced acetic acid for the gonads of embryos, with satisfactory results in both cases.

The material was fixed and embedded in paraffin by the senior author in India (J. J. A.), and then was sent to the junior author (S. M.) for further work. The sections were prepared by means of the iron-haematoxylin method after Heidenhain. The cytological work on the chromosomes and the arrangement of the data in the manuscript was done by the junior author (S. M.).

The chromosomes of Calotes versicolor.

The chromosomes of the male: The male diploid chromosomes were investigated in germ cells observed in both adult and embryonic testes. After study of the spermatogonial cells in division it became evident that the diploid complement of the male sex consists of 34 chromosomes as given in figs. 1—2. The diploid complement is characterized by showing a marked demarkation into two distinct groups of chromosomes, namely macro- and micro-chromosomes, according to their size and shape. The morphological analysis of the elements shows that the macro-chromosomes, which are 12 in number, assume a distinct V-shape; they always occupy in the metaphase arrangement a peripheral position in the equatorial plate, directing their points of fibre attachment towards the centre. By the mating up of the homologous chromosomes on the basis of their shape and size. it was found that the macro-chromosomes consist of six distinct pairs of homologous elements, showing a nearly graded series in reduction of size. Among them the three larger pairs of chromosomes are provided with submedian spindle fiber attachment, the lengths of the arms forming the V's being dissimilar; while the other three pairs of macro-chromosomes, smaller in size than the former, seem to be of median attachment, since the arms of the V's are seemingly

equal in length. The micro-chromosomes exhibit a striking contrast to the former in being small in size and in being of a simple rod-nature. They are 22 in number and vary in shape from short rods to minute spheroidal bodies. They arrange themselves in the central space of the equatorial plate with their inner ends pointing towards the middle, and are surrounded by the macro-chromosomes. Considering their external form, it appears that the point of the spindle

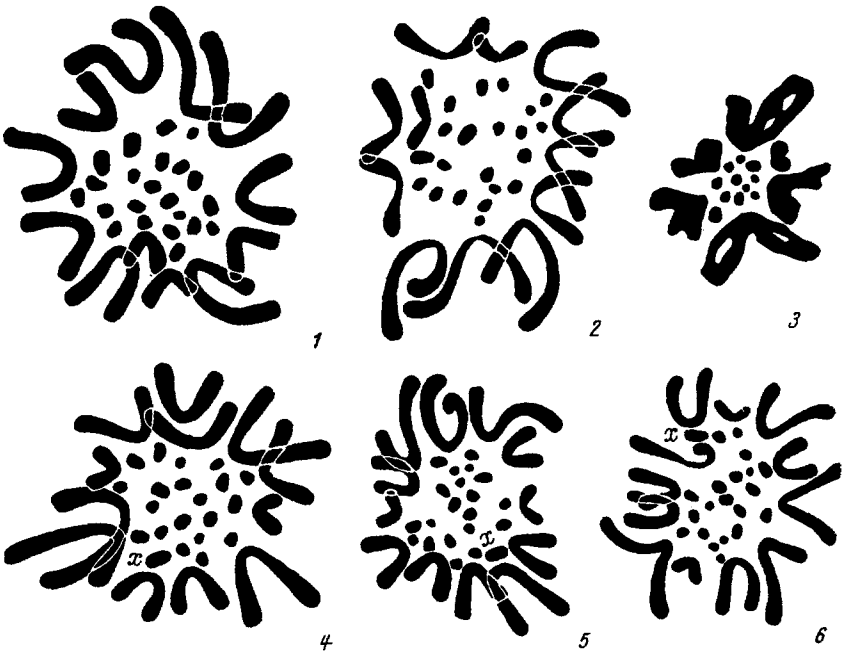

Fig. 1–6. Chromosomes of *Calotes versicolor*. × 4200. 1–2, Spermatogonial metaphases, 34 chromosomes; 3, primary spermatocyte metaphase, 17 bivalents; 4, oogonial metaphase, 33 chromosomes 5–6, metaphases of the epithelial cells of the intestine, 33 chromosomes.

fibre attachment is terminal in every micro-chromosome. Morphological analysis by means of the comparison of their shape and size reveals that they comprise eleven pairs of homologous elements and that there are present no elements which form an unequal pair. From the above observations the conclusion was drawn that the diploid complement of the male contains 34 chromosomes which consist of six homologous pairs of macro-chromosomes having a distinct V-shape and eleven homologous pairs of micro-chromosomes of telomitic nature, and that there are present no elements which form an unequal pair.

The haploid number of chromosomes was clearly established as 17 in the primary spermatocyte, as observed in the adult testis (fig. 3).

They are all ordinary bivalents in structure. Six of them are very conspicuous on account of their distinct V-shape, their large size, and their arrangement at the periphery of the equatorial plate; these are obviously the bivalents formed by the macro-chromosomes. Surrounded by the latter, there are eleven elements with a compact dot-like form; they are evidently the bivalents originating from the micro-chromosomes. Throughout the first maturation division of the male there are observed no chromosomes which may be regarded as forming a heteromorphic bivalent, since all bivalents are made up of two equal halves. It follows, therefore, that there must be present in the male a sex-chromosome bivalent derived from the conjugation of two homologous components, thus showing that this sex is probably of the XX type.

The chromosomes of the female: The observations on the chromosomes of the female were made principally in the ovaries derived from embryonic individuals, with special attention to the diploid complement as seen in the oogonial divisions. The oogonial cells show 33 distinct chromosomes in every case under study. An example is given in fig. 4. The oogonium thus contains one chromosome less in total number than does the spermatogonium in which 34 elements were found. The odd number of chromosomes in the oogonium obviously indicates the occurrence of an unpaired element. To identify the unpaired chromosome, a morphological analysis of the chromosomes was made. The diploid complement is here also classified into two sorts, macro- and micro-chromosomes, as was done in the male cell. The macro-chromosomes are 12 in number and assume a distinct V-shape. Compared with the corresponding ones of the male cell, those of the female are quite identical in form and number: there are present six homologous pairs, three of which are made up of chromosomes with submedian attachment, while the elements forming the other three pairs have median attachment. This clearly shows, therefore, that there occur no elements forming a heteromorphic pair in the group of the macro-chromosomes. Thus the macro-chromosomes of the female correspond exactly with those of the male, not only in number but also in morphological characteristics. The evidence will be clear by reference to fig. 13 in which the elements 1 to 6 represent the macro-chromosomes placed in order of size; these elements are easily identifiable in both male and female diploid complements (figs. 1, 2 and 4, 5, 6).

In contrast with the macro-chromosomes, the number of the micro-chromosomes is odd, being 21: the odd number suggests the existence of an unpaired element having no homologous mate among these micro-chromosomes. The mating up of the homologous elements was

made by a careful comparison of their shape and size, after which corresponding chromosomes were placed in pairs as shown in Nos. 7—17, of fig. 13. It becomes evident from this study that chromosome No. 7 is destitute of its appropriate partner. It is therefore this chromosome which is responsible for the numerical difference between the chromosome numbers of the two sexes, and is regarded as the sex-chromosome of this species. Noticeable is the fact that the unpaired element thus identified seems to rank first in the micro-chromosome group, being the largest among them.

Additional evidence, supporting that derived from the oogonial chromosomes described above, is afforded by the somatic cells of the embryos, which were abundantly found in process of division. The somatic cells show in metaphase the same number of chromosomes as do the oogonia. Examples are given in figs 5—6 which are taken from epithelial cells of the intestine. Each contains 33 chromosomes, identical in form with those of the oogonia. The unpaired element is identified as the 13th in order of size; it is a short rod in shape and is the largest of the micro-chromosomes, as was the case in the oogonial cell (x in figs. 5—6).

From the foregoing evidence on the female chromosomes, one thing stands out clearly: heterogamety occurs in the female of this lizard.

The chromosomes of Sitana ponticeriana.

The chromosomes of the male: Observations were carried out on the germ cells contained in the adult testes. The diploid chromosome number of the male was determined to be invariably 46 in the spermatogonial divisions (figs. 7—8). In striking contrast to the former species, the present species is characterized by the fact that all members of the diploid complement are provided with terminal attachments of the spindle fibres: there is demonstrated in the complement no element which takes the shape of a V or J. They vary considerably in length, ranging from long rods to minute spheroidal bodies. By the mating up of the homologous chromosomes having equal length, the diploid chromosomes are assorted into 23 homologous pairs; neither a solitary unmated element nor any pair of unequal size is present.

Though it is less evident than in the former species, the chromosomes may here also be divided into two groups, namely the macro- and micro-chromosomes. The macro-chromosomes are 24 in number and are found to form 12 homologous pairs, the members of each of which are identical in size. They form a graded series in respect to length, ranging from long rods to short ones. The number of the

micro-chromosomes is 22, and they may be arranged into 11 homologous pairs. They also are gradated in respect to length, but this is not so conspicuous as in the former group. Demarkation between the macro-chromosomes and micro-chromosomes is not sharp, since the members of the smallest pair in the macro-chromosomes do not show an outstanding difference in length from those of the largest pair of micro-chromosomes. In general the macro-chromosomes are distributed

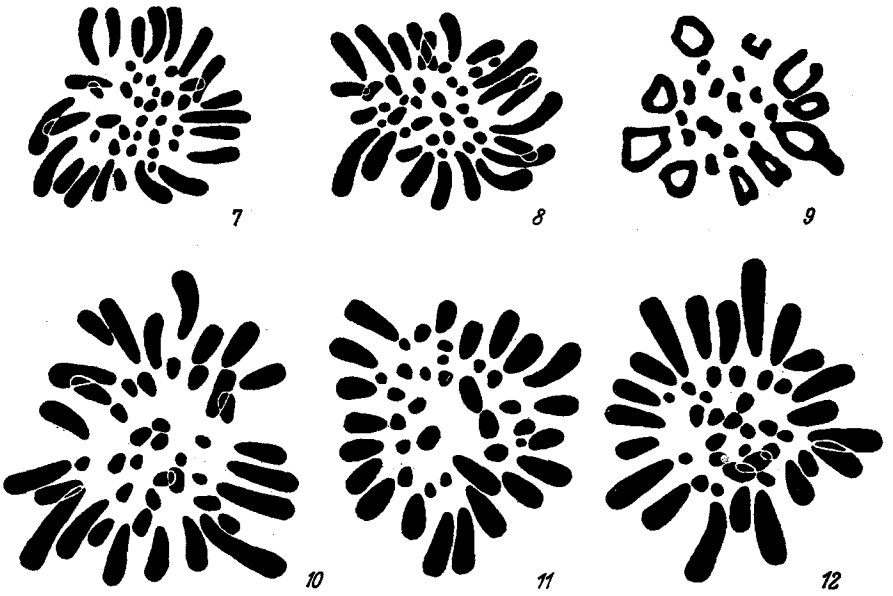

Fig. 7–12. Chromosomes of *Sitana ponticeriana*. × 4200. 7–8, spermatogonial metaphase, 46 chromosomes; 9, primary spermatocyte metaphase, 23 bivalents; 10–12, oogonial metaphases, 45 chromosomes.

at the periphery of the equatorial plate, surrounding the micro-chromosomes which lie in the central space.

The haploid number of chromosomes was observed to be 23 in the primary spermatocyte division (fig. 9). Ten to twelve of them are larger than the others; they are undoubtedly the bivalents formed by the macro-chromosomes. These larger ones take the form of the horizontal ring and usually occupy the peripheral circle of the equatorial plate. The remaining smaller ones, the bivalents of the micro-chromosomes, which always scatter in the central space of the equatorial plate, are 11 in number and assume a bipartite outline. The chromosomes of the primary spermatocyte are all ordinary bivalents in structure, each comprising two equal components; there is no bivalent consisting of unequal components.

The evidence is thus sufficient to imply that the male is homogametic as regards the sex-chromosomes, although the con-

clusive identification of the sex chromosomes is impossible from a study of the male chromosomes alone.

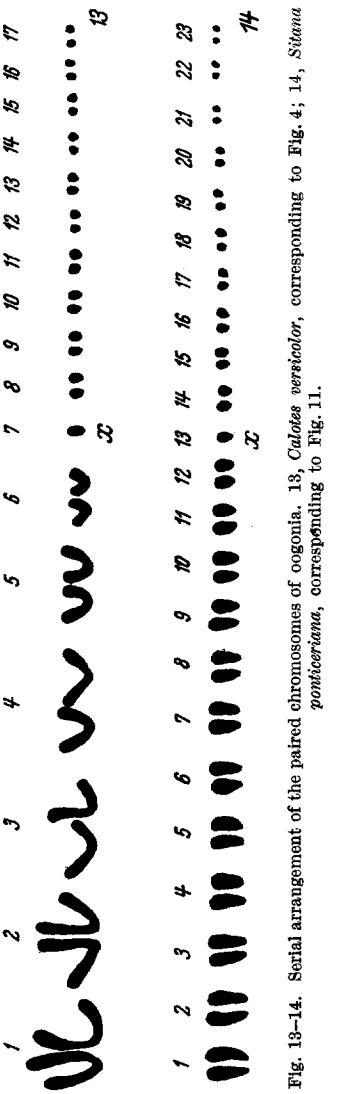

Fig. 13–14. Serial arrangement of the paired chromosomes of oogonia. 13, *Calotes versicolor*, corresponding to Fig. 4; 14, *Sitana ponticeriana*, corresponding to Fig. 11.

The chromosomes of the female: The female chromosomes were studied in the oogonial divisions encountered in the embryonic ovaries. The diploid number of chromosomes determined in many cases was definitely 45, one fewer than that of the male. Three examples of the metaphase plates are given in figs. 10—12. The composing elements of the diploid complex are all telomitic in nature. The general morphological characteristics of the chromosomes are identical with those of the male. The complement is made up of two different sorts of components as in the male; one is the group of the macro-chromosomes and the other is that of the micro-chromosomes which are shorter and form a striking contrast to the former. From the odd number of chromosomes the presence of an unpaired sex chromosome may again be inferred. For identifying the particular chromosome the morphological analysis of chromosomes was usually utilized. The pairing of the homologous chromosomes by comparison of their size and shape was first attempted in the macro-chromosomes. The macro-chromosomes proved to be 24 in number and to constitute 12 homologous pairs (Nos. 1—12 in fig. 14). They form a closely graded series from long rods to short ones. Thus, the members of the macro-chromosomes are seen to show no difference from those of the male, but to agree with them, not only in their number but also in their morphological features.

In contrast to the above, the micro-chromosomes show an odd number 21, in the female, while it is 22 in the male. The odd number is suggestive of the occurrence of an unpaired element and therefore

the sexual difference may be detectable in the micro-chromosome group. The serial arrangement of chromosomes after the mating up of the homologous pairs reveals the fact that the chromosome ranking first in size in the micro-chromosome group remains without a mate of corresponding size. When all chromosomes are taken into account, the chromosome numbered 13 in the serial arrangement is that which lacks its partner, as shown in fig. 14. Thus the sexual difference of chromosomes is attributable to the fact that the chromosome No. 13 is unpaired in the female, while it is in a paired state in the male. The chromosome No. 13 is, therefore, nothing other than the sex chromosome.

The sex chromosome.

As shown in the foregoing descriptions, the total number of chromosomes is one less in the female than in the male. In *Calotes* the diploid number of the male is 34 and that of the female is 33. *Sitana* possesses 46 chromosomes in the male diploid complex and only 45 in the female. The cause of the numerical difference in these species lies in the fact that one particular chromosome is always unpaired having no mate in the female cell, whereas it is in a paired condition having a homologous partner in the male. It is this particular chromosome which is responsible for the sexual difference between the chromosomes of the two sexes, and therefore this fact can be understood only by assuming the particular element to be the sex chromosome. The sex chromosome thus identified was proved to be the chromosome No, 7 in *Calotes* and the chromosome No. 13 in *Sitana*, as shown in figs. 13 and 14. From this account the following is self-evident: the male is homogametic and the female is heterogametic as regards the sex chromosome.

The sex chromosome (No. 7) of *Calotes* is represented by the largest member of the micro-chromosomes. The condition is similar in the case of *Sitana* where the chromosome No. 13 —the sex chromosome— is also the largest in the micro-chromosomes. Corroborative evidence has previously been furnished by OGUMA (1934, 1937) in the cases of *Lacerta vivipara* (a lizard) and *Amyda japonica* (the soft-shelled turtle). He demonstrated that the sex chromosomes of these reptiles were represented by one of the medium sized chromosomes which correspond in size to the larger members of the micro-chromosome group. These findings seem to be sufficient to indicate that the sex chromosomes of reptiles are generally not of large size but rather minute, being represented by one of the micro-chromosomes.

In this connection, one cannot overlook the evidence furnished by NAKAMURA (1928, 1931, 1932, 1935), in his studies of snakes, lizards

and geckos. Though his studies were exclusively confined to the chromosomes of the males, and consequently any conclusive statement was impossible regarding female heterogamety, he was still successful in finding that the sex chromosomes of the males of these reptiles are represented by an equal (homomorphic) pair of short rod-like chromosomes, based on the fact that they form a heteropycnotic karyosome with a bipartite heart-shape in the nucleus of the growth period. Moreover, according to NAKAMURA, the karyosome found in the meiotic nucleus is composed of a certain pair of the micro-chromosomes. Thus in the case of *Eumeces* (NAKAMURA 1931), one of the *Scincidae*, the karyosome is made up of two of the 14 micro-chromosomes, probably of two of the four rod-shaped elements which rank among the larger members of the micro-chromosome group. Conditions in the snakes and geckos were shown by him to be closely comparable to the above (NAKAMURA 1928, 1932, 1935). It is noteworthy that the sex-chromosomes identified by NAKAMURA in these reptiles from observations of the male only, show in this feature a close approach to the sex chromosomes as described by OGUMA (1934, 1937) and by the present author in this study, which gives a final proof for their conclusive identification by actual observations of the chromosomes of both sexes.

Chromosome relations in related species.

Previous to the present study, the investigations of the chromosomes in the Family *Agamidae* have been carried out on three species, two by MATTHEY (1931) and one by NAKAMURA (1931, 1935). MATTHEY (1931) has studied the male chromosome complexes of *Agama stellio* and *Uromastix hardwicki*. He found in these two forms a similar chromosome complex; the number of chromosomes reported by him is 36 in the male, which comprises 12 V-shaped macro-chromosomes and 24 dot-like micro-chromosomes. NAKAMURA (1931, 1935) has given 46 chromosomes for the male complex of *Japarula swinhonis;* they are made up of 24 rodlike macro-chromosomes and 22 dot-like micro-chromosomes. The results from the ather two species, *Calotes versicolor* and *Sitana ponticeriana*, dealt with in the present study can now be added to the above.

In these five species of agamid lizards, the karyotypes of *Japarula swinhonis* and *Sitana ponticeriana* are identical with each other in that the diploid number of chromosomes is 46 (♂), and consists of 24 rod-shaped macro-chromosomes and 22 dot-like micro-chromosomes.

On superficial observation the karyotype of *Calotes versicolor*

seems to differ from those found in *Japarula* and *Sitana* above mentioned. But close examination and morphological analysis of the chromosomes disclose a close relationship in the karyotypes of these forms. The chromosome complement of *Calotes*, as already noted, consists of 12 V-shaped macro-chromosomes and 22 dot-like micro-chromosomes, being 34 (♂) in total. Comparison of chromosomes shows that the 12 V-shaped chromosomes of *Calotes* fairly correspond in their size relations to 12 rod-shaped pairs of chromosomes occuring in *Japarula* and *Sitana*, and that the micro-chromosomes exhibit no visible difference among these three species. If it be assumed here that each of the 12 V-shaped elements may be conceived as double, consisting of two rods associated at their inner points of fibre attachment, the total number of *Calotes* becomes 46, that is the number shown by *Japarula* and *Sitana*. The apparent reduction in the chromosome number, therefore, is possibly the result of the fusion of 24 rod-shaped chromosomes, two by two into 12 V-shaped mutiples. A close karyological relationship is thus possible between *Calotes* and the two forms, *Japarula* and *Sitana*.

According to MATTHEY (1931), the chromosomes of *Uromastix hardwicki* and *Agama stellio* are 36 in number and comprise 12 V-shaped macro- and 24 dot-like micro-chromosomes. In the comparison with those of *Calotes*, the 12 V-shaped macro-chromosomes of *Uromastix* and *Agama* express no visible difference from the corresponding elements of *Calotes*, either in number or in relative magnitude and shape. But the number of the micro-chromosomes of the former two species is 24 while in *Calotes* it is 22, two less than the former. Referring to the original drawings of *Uromastix* and *Agama* given in MATTHEY's paper (1931, figs. 21—22, figs. 36—41), it becomes evident that the number of the micro-chromosomes is not exactly as indicated in each. There thus arises some question concerning the number of the micro-chromosomes and a reinvestigation is desirable. Fortunately, the authors have had an opportunity to reinvestigate the chromosomes of *Uromastix hardwicki* in material coming from Ahmedabad, India. A close examination of this material demonstrated that *Uromastix* possesses a karyotype quite identical with that of *Calotes:* namely, the diploid number of this species is definitely 34 (♂), and comprises 12 V-shaped macro and 22 dot-like micro-chromosomes.

A detailed account of this point will be given in another paper now in press (ASANA and MAKINO 1947). From the above account it is evident that the chromosome number of *Uromastix* formerly reported by MATTHEY (1931) is not correct. Considering the close taxonomical relationship, it is not surprising that the chromosome

complex of *Agama stellio* should be like those of *Uromastix* and *Calotes*, all having 34 chromosomes.

In conclusion, there are two types of chromosome complexes, closely related with each other, in the agamid lizards thus far investigated. The one is the *Japarula*-type in which 46 telomitic chromosomes are found; to this type *Japarula swinhonis* and *Sitana ponticeriana* belong. The other is the *Calotes*-type which shows 34 chromosomes consisting of 12 atelomitic V-shaped chromosomes and 22 dot-like ones of telomitic nature. The chromosomes of *Calotes versicolor*, *Uromastix hardwicki* and probably *Agama stellio*, are included in the latter type.

Summary.

The diploid number of chromosomes in *Calotes versicolor* is 34 in the male and 33 in the female. The numerical difference in the two sexes is based on the condition of the sex chromosome; in the male it is present as a homologous pair, while in the female it remains unpaired. The sex chromosome was identified as one of the micro-chromosomes (the chromosome No. 7) in this species.

Sitana ponticeriana possesses the diploid number of 46 in the male and 45 in the female. The sex chromosome identified in this form is also one of the micro-chromosomes (chromosome No. 13), which has no partner in the female, whereas in the male it is in the paired state having a homologous mate.

It was established therefore that, in both species studied, the female is heterogametic as regards the sex chromosome.

The chromosome complement of *Calotes versicolor* consists of 12 V-shaped macro-chromosomes and 22 dot-like micro-chromosomes. while in *Sitana ponticeriana* there are 24 rod-shaped macro-chromosomes and 22 dot-like micro-chromosomes. The difference in the karyotypes of these two species can be accounted for on the basis of the formation of V-shaped multiple chromosomes by means of the fusion of two rods at the point of fibre attachment.

The karyological relationships in the Family *Agamidae* were discussed. The chromosome number previously reported for *Uromastix hardwicki* was found to be erroneous; the diploid number in this species is 34.

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