

Studies on Reptilian Chromosomes VI. Chromosomes of some Snakes

By

Kenji NAKAMURA

With 144 Text-figures

(Received October 10, 1934)

Introduction

In spite of the remarkable advancement of our knowledge of the chromosome-complexes of lizards in the last few years (MATTHEY 1929-33, NAKAMURA 1927-34, OGUMA 1934a), the cytology of snakes has been worked out very little. The following is a list of the works which have been published concerning the chromosomes of snakes ;

Species	Authority	Chrom. No. in Male	Sex-chrom.
<i>Thamnophis butleri</i>	THATCHER '22	37	XXY
<i>Natrix tigrina</i>	NAKAMURA '27, '28a	40	XX
<i>Elaphe quadrivirgata</i>	" '27	36	"
<i>Tropidonotus natrix</i>	MATTHEY '31	36	"
<i>T. viperinus</i>	" "	"	"
<i>Zamenis gemonensis</i>	" "	"	"
<i>Coronella austriaca</i>	" "	"	"
<i>Tarbophis fallax</i>	" "	"	"
<i>Coelopeltis lacertina</i>	" "	42	"
<i>Aghistrodon blomhoffii</i>	NAKAMURA '27	36	"
<i>Vipera aspis</i>	MATTHEY '29a	41	XO
"	" '31, '33	42	XX
<i>V. berus</i>	MAKINO, unpubl.	36	XX

Further study is required in order to make any generalization concerning the chromosome-complex of this group. The present study deals with male chromosome-complexes of the following 13 species of snakes :—

Macropistodon rudis carinatus
Dinodon rufozonatum
Holarchus formosanus
Zoacys nigrorginatus oshimai
*Elaphe quadrivirgata**
E. climacophora
Bungarus multicinctus
Naja naja atra
Laticauda semifasciata
Agkistrodon acutus
*A. halys blomhoffii**
Trimeresurus mucrosquamatus
T. gramineus stejnegeri

Before going any further I wish to express my cordial thanks to the Imperial Academy of Japan, which accorded me a grant for the present study. My thanks are also due to Professor Taku KOMAI and Dr. Osamu MINOUCHI for their kind suggestions and criticism throughout the work. I am indebted to Mr. Sajiro MAKINO of Hokkaido Imp. Univ. for the privilege of consulting his unpublished figures and manuscript on the spermatogenesis of *Vipera berus*.

Material and Method

For materials many individuals of snakes obtained in the season extending from April to July were used. Of these *Elaphe quadrivirgata*, *E. climacophora* and *Agkistrodon halys blomhoffii*, were caught in the vicinity of Kyoto; while all the others were collected in Formosa. Besides these, a sea-snake, *Laticauda semifasciata*, was brought from Okinawa.

Most of the Formosan snakes were obtained from dealers, so that some of them may have been kept in captivity for a month or more. Keeping snakes in confinement is apt to affect spermatogenesis by restraining divisions of sperm-cells. But I have noticed no irregular divisions of chromosomes in the present material. It is true that there were some cases in which division figures of certain cell generations were not observed. But similar facts were met with in materials taken from animals directly after capture.

* A brief account of the chromosomes of these two snakes was published in my previous paper "Preliminary notes on reptilian chromosomes I" (1927).

Moreover, the testes of the sea-snake furnished an abundance of well-preserved division figures in all cell-generations, notwithstanding the fact that the snake had apparently been kept for weeks under very unnatural conditions. In my previous studies on lizard chromosomes I found that the spermatogenetic cycles of different species do not coincide with one another. So that it is likely that the diversity of the stages found in the testes of these snakes is due to the diversity of spermatogenetic cycles.

The materials were treated in the usual way. From the animals killed by decapitation, the testes were removed, cut into small pieces, dropped into a bottle filled with the fixative.

Previous investigators of reptilian chromosomes have employed customary fixatives for mammalian chromosomes such as FLEMMING's strong, modifications of BOUIN's solution, etc. This mainly accounts for the fact that the results obtained are far from satisfactory and the conclusions drawn from these materials are diverse.

In the study of the chromosomes of *Natrix tigrina*, I recommended a modification of CHAMPY's mitochondrial fixative, concentrated 1.5 times as strong as the original formula (1928a). Since then this formula has been adopted by some other investigators in their studies on chromosomes of many reptiles and very satisfactory results have been obtained. CHAMPY's fluid is an excellent fixative for mitochondria, and the application of the fluid for the preservation of chromosomes was first made by MINOUCHI (1928). He employed it chiefly for the preservation of mammalian chromosomes. It was also used by IRIKI (1932d, etc.) and MAKINO (1934) for fixation of amphibian and fish chromosomes and they found it very satisfactory. This fixative, however, often gives unsatisfactory results if the original formula is used. It is therefore preferable to modify the formula according to the kind and condition of the material to be fixed.

In the present study the following modifications have been used.

A. One, concentrated 1.5 times as strong as the original.

3 %	Osmic acid	1 part
1.5 %	Chromic acid	2 „
4.5 %	Potassium dichromate	2 „

As 2-percent osmic acid is usually provided, it is more convenient to alter the formula as follows.

2 %	Osmic acid	3 parts
1.5 %	Chromic acid	6 „
6 %	Postassium dichromate	4 „

B. In the second modification the percentage of osmic acid is increased :

2 %	Osmic acid	7 parts
1.5 %	Chromic acid	6 „
6 %	Potassium dichromate	3 „

Some materials contain a great quantity of substance which is blackened with osmic acid so that the percentage of osmic acid to the fixative diminishes very rapidly. Therefore I prefer to use this modification containing a high percentage of osmic acid.

C. The third, concentrated 2 times as strong as the original.

2 %	Osmic acid	2 parts
4 %	Chromic acid	2 „
6 %	Potassium dichromate	1 „

In using these fixatives it is necessary to immerse materials in a sufficient quantity of the fixative.

After the materials had been fixed with the fluid for 24 hours, they were washed thoroughly in running water, then they were dehydrated with a series of alcohol baths and imbedded in paraffin. The sections were cut in 7-8 micra thick. Before staining, the sections were bleached and soaked into CHURA's micro-acetic bath at room temperature or at about 30°C. to get rid of cellular inclusions and at the same time to intensify the affinity of chromosomes for the dyes. In some materials soaking in dilute chromic acid before staining seems to be helpful for the latter purpose. For staining chromosomes HEIDENHAIN's iron-haematoxylin was employed.

In most of the materials, chromosomes, both in prophase and metaphase, were preserved very nicely in the fluid A ; others were fixed beautifully in the fluid B or C. There were also a few cases in which I never obtained satisfactory results by any of the fluid. In well preserved materials, metaphase chromosomes of all cell generations are individually distinct with no tendency to stick together. It is most difficult, however, to preserve fine structures of the tetrads, as they are often condense to such an extent that they become mere masses of chromatin. Individual chromosomes have less tendency to fuse with one another.

Staining of chromosomes is somewhat difficult. Even in well-fixed preparations, individual chromosomes often stain in different degrees. In some cases, they stain well showing very smooth contours ; in other cases, however, they are almost unstainable ; rarely they are stained only faintly and with ragged contour, sug-

gesting that only the spiral chromonemata in the chromosomes have been stained.

All the figures in this paper have been drawn with the aid of camera lucida, using Zeiss 1.5 mm. apochromatic oil-immersion objective and a K. 18 ocular, tube-length 16 mm., at a level about 25 mm. below the stage.

Observations

Family Colubridae

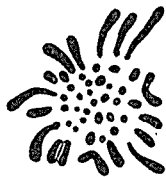
Series Aglypha

Subfamily Colubrinae

1. *Macropistodon rudis carinatus* (DENBURGH).

Text-figs. 1-3.

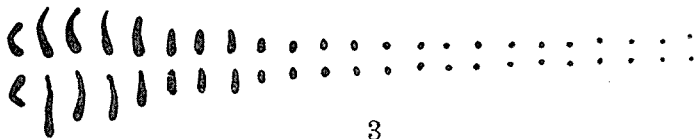
The materials were fixed in June 1929, in Formosa in solution A. There are found a few dividing figures of the spermatogonia and the first spermatocytes but none of the second spermatocyte.



1



2



3

Text-figs. 1-3. Chromosomes of *Macropistodon rudis carinatus*. 1, polar view of the equatorial plate of the spermatogonial division; 2, that of the first maturation division; 3, the spermatogonial chromosomes arranged into pairs.

Spermatogonial chromosomes show somewhat ragged contours and are not stained very deeply. The spermatogonial chromosomes are 46 in number; they are divided into 16 macro- and 30 micro-chromosomes. The macro-chromosomes consist of a pair of V-shaped and seven pairs of rod-like chromosomes. The V-shaped

chromosomes are centromitic and are shorter than the largest rod-like ones; the arms of V's are not longer than the shortest rod-like ones. Of the rod-like chromosomes three pairs are the longest, a single pair come next and the remaining three pairs are the shortest. Most of the 15 pairs of the micro-chromosomes are spherical in shape but two or three pairs are larger than the rest and somewhat elongated.

The arrangement of the spermatogonial chromosomes in the equatorial plate is a very usual one: the larger ones come in the periphery forming an outer circle and the small dot-like ones are scattered in the central space. The rod-like chromosomes are arranged radially and the V's lie with their apexes toward the centre.

In the first maturation division 23 tetrads appear in the equatorial plate. Eight of these are larger than the others. The largest dumbbell-shaped tetrad probably represents a pair of V's; while the remaining ring, V-shaped or heart-shaped tetrads are formed each by two rod-like chromosomes. The 15 small tetrads are heart-shaped, bipartite or dot-like.

2. *Dinodon rufozonatum* (CANTOR)

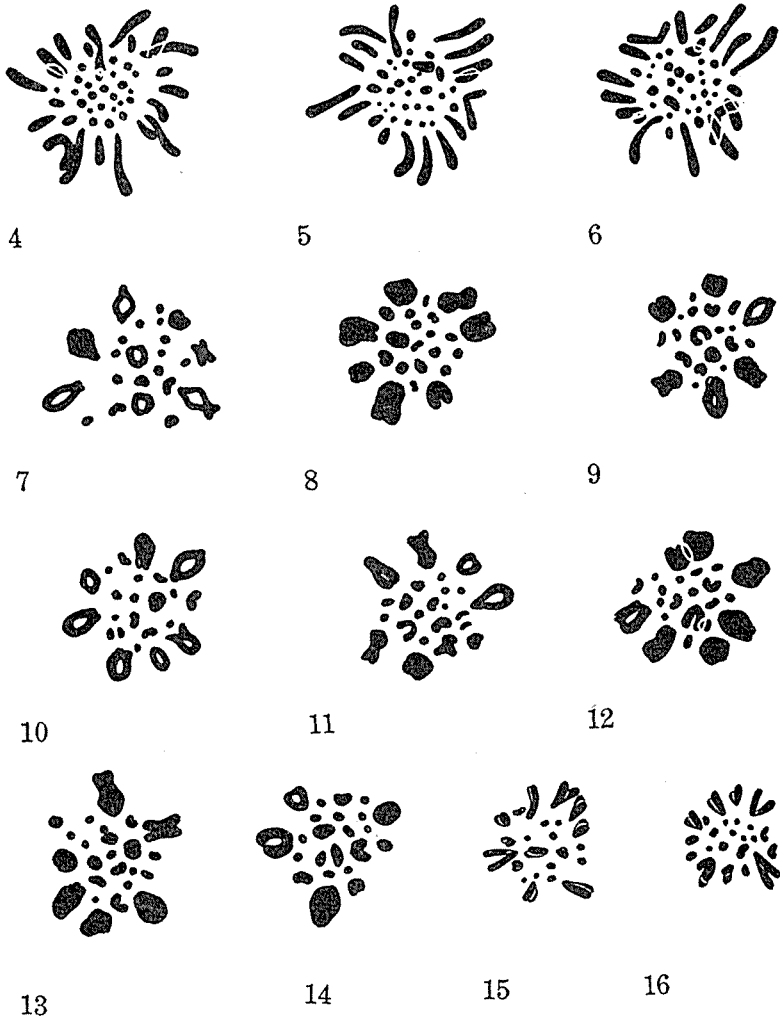
Text-figs. 4-21.

The materials were fixed in May 1929, in Formosa in solution A. The results are very favourable.

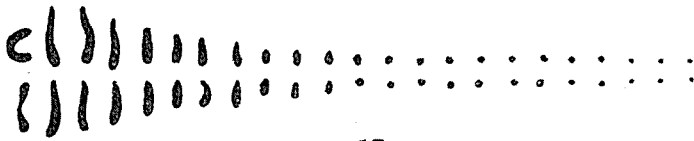
The spermatogonial complex is composed of 46 chromosomes. There are one pair of V-shaped, seven pairs of rod-like, and 15 pairs of small chromosomes. On the whole, the complex is strikingly similar to that found in *Macropistodon* but differs slightly in details. The V-shaped chromosomes are as long as the largest rod-like one, and the rod-like chromosomes are of intergrading sizes. Of the small chromosomes some pairs are elongate-oval or very short rods, so that the demarcation between the macro- and the micro-chromosomes is not so clear as in some other reptiles.

In the equatorial plate the larger chromosomes are arranged radially in the periphery, enclosing the smaller ones in the centre. The former vary from 18 to 20 in number. In usual cases, the V-shaped chromosomes lie in the periphery of the equatorial plate with their apexes toward the centre of the spindle. Sometimes, and not seldom, the arms of the V are so widely open that the chromosome assumes an almost rod-like form with one arm pointing to the centre and with the other arm pointing to the periphery of the plate.

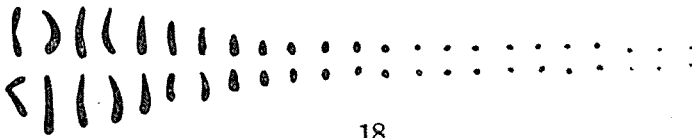
In the first maturation division 23 tetrads appear in the equatorial plate. Of these the larger ones tend to come in the periphery. The precise structures of these tetrads have not been observed; but as shown in Text-fig. 20, most of larger tetrads are rings. In several cases one can discriminate a large dumbbell or X-shaped tetrad, which apparently corresponds to a pair of V-shaped spermatogonial chromosomes. The remaining smaller tetrads are bipartite or dot-like.



Text-figs. 4-16. Chromosomes of *Dinodon rufozonatum*. 4-6, polar views of the equatorial plates of the spermatogonial divisions; 7-14, those of the first maturation divisions; 15 & 16, those of the second maturation divisions.



17



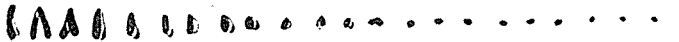
18



19



20



21

Text-figs. 17-21. Chromosomes of *Dinodon rufozonatum*. 17 & 18, two sets of the spermatogonial chromosomes arranged into pairs; 19 & 20, the linear arrangements of two sets of the tetrads; 21, that of the dyads.

The second maturation division shows 19 dyads in every equatorial plate. The larger dyads are vertical V's or horizontal double V's; the others are bipartite and dot-like dyads. The dyads which are in the periphery are 13-15 in number, and most of them are larger than those scattered in the centre.

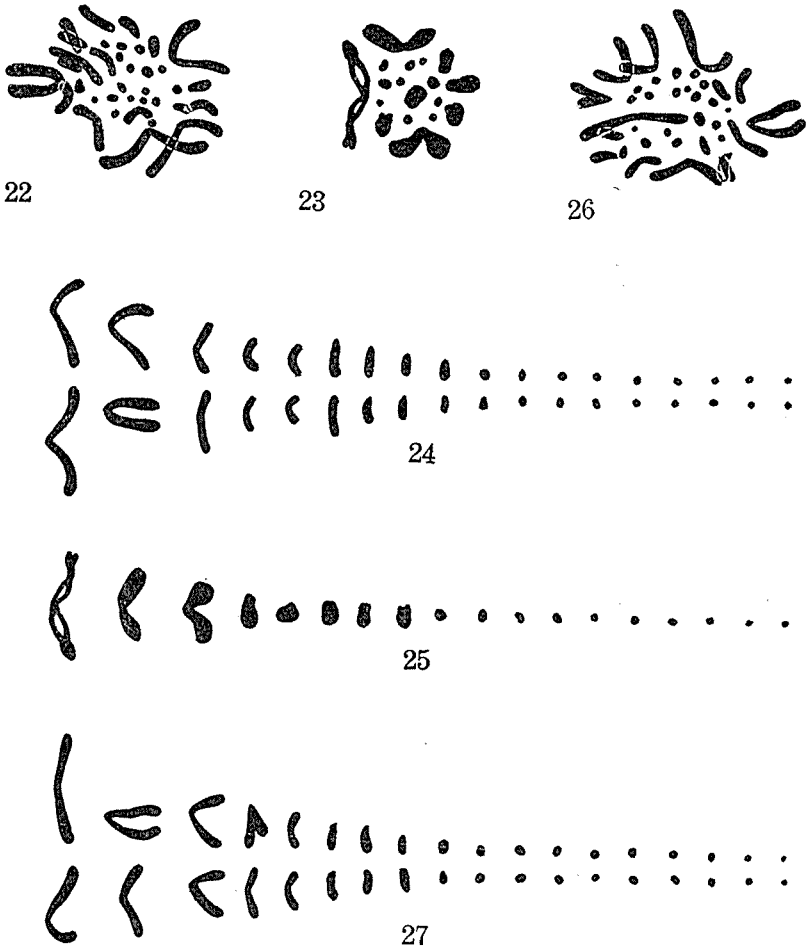
3. *Holarchus formosanus* (GÜNTHER)

Text-figs. 22-25.

The materials were taken in May 1929, in Formosa. As the fixative solution A was used; but they furnish less satisfactory results than we have obtained from other snakes.

The spermatogonial chromosomes seem to be 36 in number. The number is just twice as many as that of the tetrads in the

first maturation division, so that this is probably the right number. The chromosomes consist of five pairs of V-shaped, three pairs of rod-like, and ten pairs of oval and dot-like ones. The V-shaped chromosomes may be sorted into two largest pairs, a medium-sized pair and two smallest pairs. Of the ten pairs of small chromosomes, one pair is more rod-like than dot-like. This pair, however, is shorter than the other rod-like chromosomes; and in the first



Text-figs. 22-25. Chromosomes of *Holarchus formosanus*. 22, polar view of the equatorial plate of the spermatogonial division; 23, that of the first maturation division; 24, the spermatogonial chromosomes arranged into pairs; 25, the linear arrangement of the tetrads.

Text-figs. 26 & 27. The spermatogonial chromosomes of *Zoacys nigroarginatus oshimai*. 26, polar view of the equatorial plate; 27, the chromosomes arranged into pairs.

maturation division the tetrad which represents this pair is decidedly smaller than those formed by other pairs, and for this reason I do not group this pair together with the other rod-like ones.

In the first maturation division there appear 18 tetrads—eight larger and ten smaller. The three largest tetrads are bent in the middle and they are probably multiple-ring tetrads. In Text-fig. 23 the larger tetrads are arranged in the periphery of the equatorial plate, except the one which lies in the centre.

For the second maturation division no well-preserved equatorial plate was found in my material.

4. *Zoacys nigroroginatus oshimai* (STEJNEGER)

Text-figs. 26 and 27.

The materials were taken in May and June 1929, in Formosa. Various modifications of CHAMPY's fixative were tried, and solution B has proved to be best.

Only one equatorial plate of the spermatogonial division was examined. There are 36 chromosomes, which consist of five V-shaped pairs, three short rod-like pairs and 10 oval and dot-like pairs. The V's may be classified as two largest pairs, one next largest pair and two smallest pairs.

5. *Elaphe quadrivirgata* (BOIE)

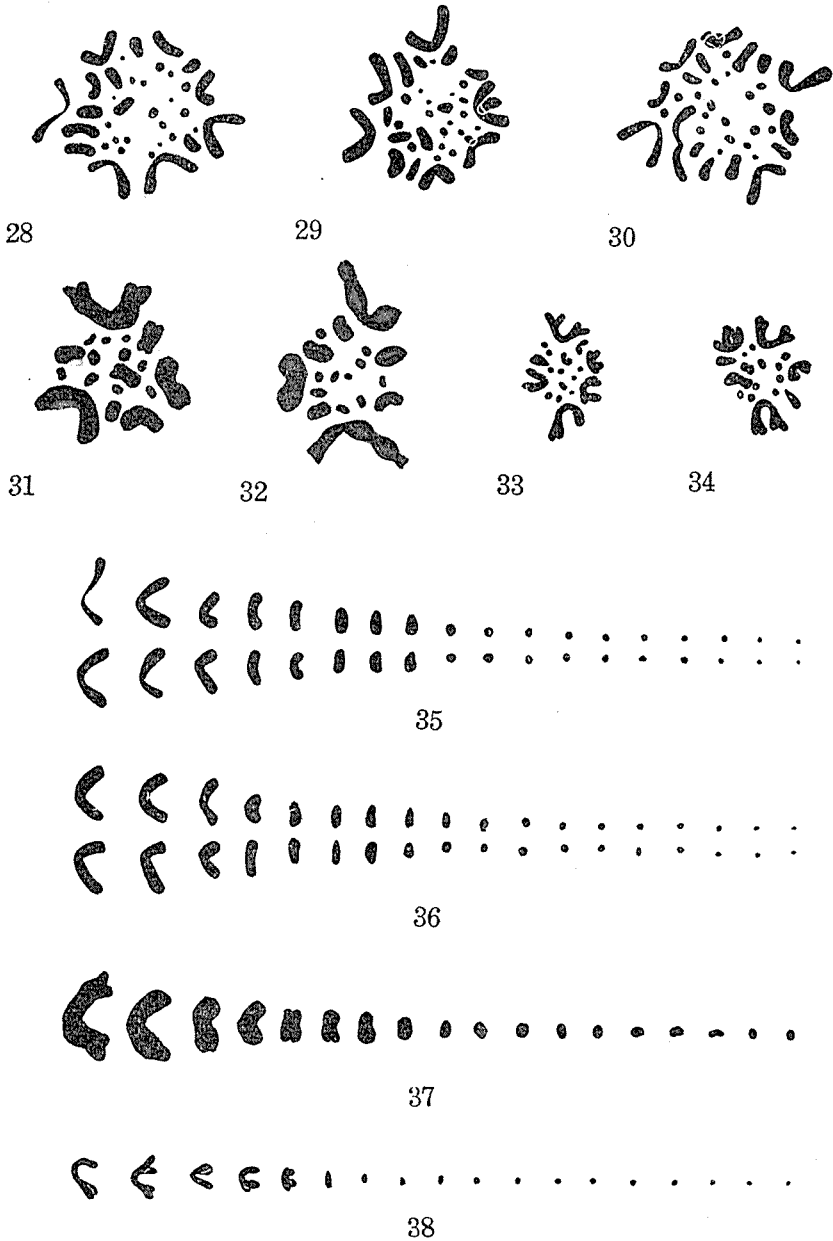
Text-figs. 28-38.

Several individuals caught in the vicinity of Kyoto in May, June and July were employed as materials. For preserving chromosomes solution A was found to be the best.

The spermatogonial divisions show 36 chromosomes in the equatorial plate. The spermatogonial complex consists of five V-shaped pairs, three short rod-like pairs and ten dot-like pairs. Of the V-shaped ones two pairs are the largest, though they are only slightly larger than the third pair, and the remaining two pairs are the smallest.

The tetrads which lie on the equatorial plane of the first maturation division are 18 in number. Of these eight are noticeably larger than the remaining ten. As shown in Text-fig. 37, the largest four are somewhat bent in the middle and are probably multiple-ring tetrads. The fine structures of the remaining tetrads are obscure.

On the equatorial plane of the second maturation spindle there appear 18 dyads—five double V's, three single V's or bipartite bodies and ten dots.

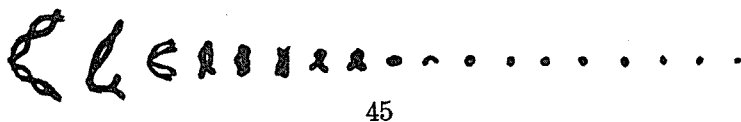
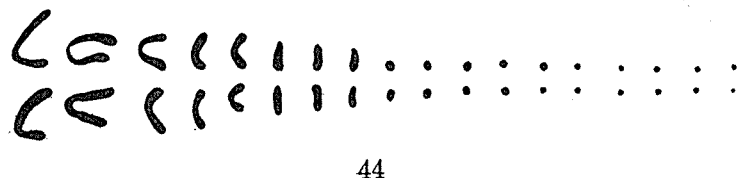
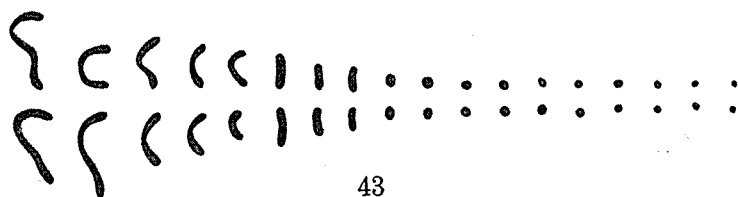
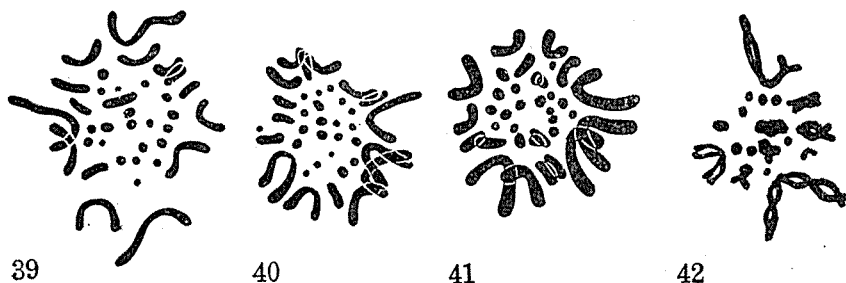


Text-figs. 28-38. Chromosomes of *Elaphe quadrivirgata*. 28-30, polar views of the equatorial plates of the spermatogonial divisions; 31 & 32, those of the first maturation divisions; 33 & 34, those of the second maturation divisions; 35 & 36, two sets of the spermatogonial chromosomes arranged into pairs; 37, the linear arrangement of the tetrads; 38, that of the dyads.

6. *E. climacophora*

Text-figs. 39-45.

The materials were taken in June and July in the vicinity of Kyoto. For preservation of chromosomes solution A was used.



Text-figs. 39-45. Chromosomes of *Elaphe climacophora*. 39-41, polar views of the equatorial plates of the spermatogonial divisions; 42, that of the first maturation division; 43 & 44, the spermatogonial chromosomes arranged into pairs; 45, the linear arrangement of the tetrads.

The chromosome-complex of this species coincides strictly with that found in *E. quadrivirgata*. However, the chromosomes are more slender than those of the latter species which show a very rigid appearance.

Series Proteroglypha

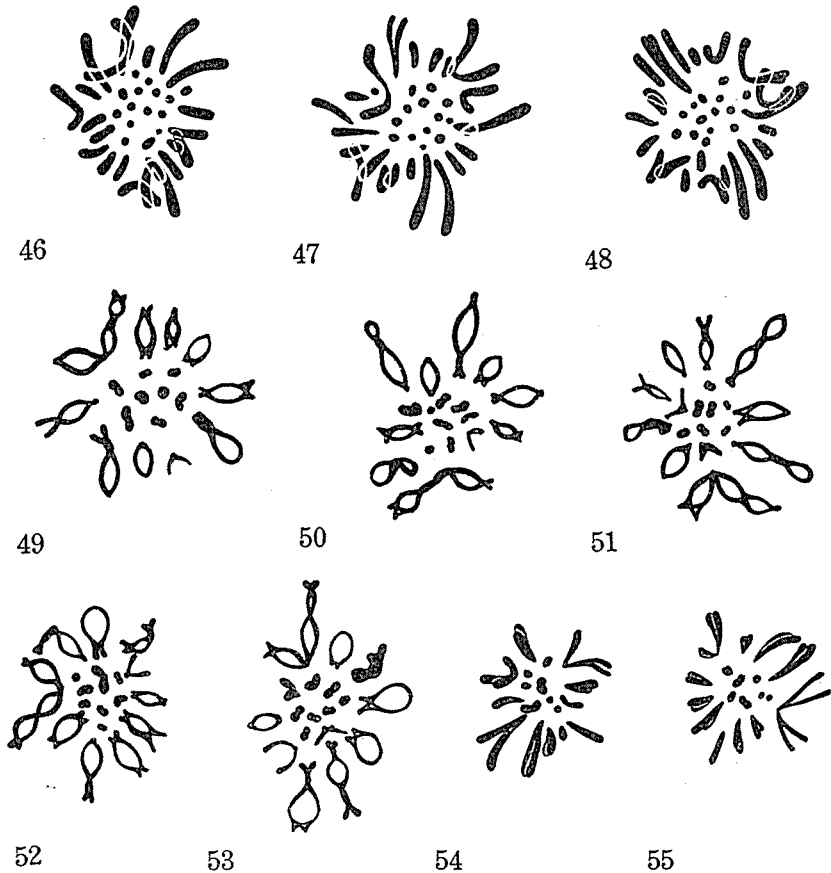
Subfamily Elapinae

7. *Bungarus multicinctus* BLYTH

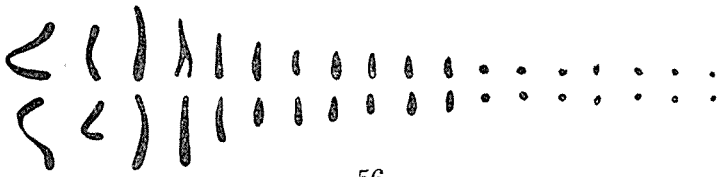
Text-figs. 46-62.

The materials were taken in June 1929, in Formosa; they were fixed in solution A. The results are extremely good.

The spermatogonial chromosomes which appear in the equatorial plate are 36 in number. They are clearly divided into macro- and micro-chormosomes; the former comprise two V-shaped and 9 rod-like pairs while the latter comprise seven dot-like pairs. Of the V-shaped chromosomes, one pair is very large and centromitic, while the other is smaller than the former and seems to have sub-



Text-figs. 46-55. Chromosomes of *Bungarus multicinctus*. 46-48, polar views of the equatorial plates of the spermatogonial divisions; 49-53, those of the first maturation divisions; 54 & 55, those of the second maturation divisions.



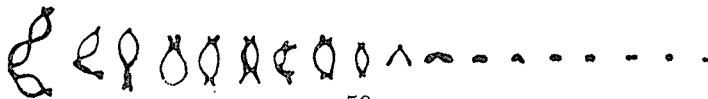
56



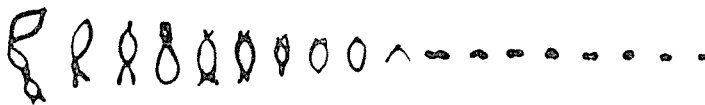
57



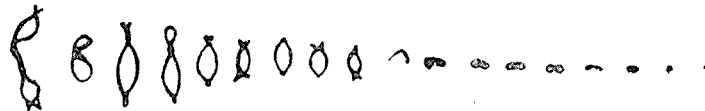
58



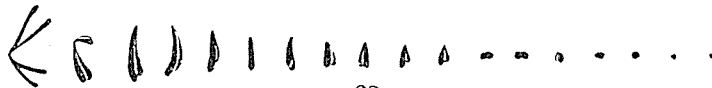
59



60



61



62

Text-figs. 56-62. Chromosomes of *Bungarus multicinctus*. 56-58, the spermatogonial chromosomes arranged into pairs; 59-61, the linear arrangements of the three sets of the tetrads; 62, that of the dyads.

median spindle-fibre attachment. Of the rod-like chromosomes we can clearly distinguish two longest pairs and one next longest pair, while the others are rather short and of intergrading sizes. The micro-chromosomes are almost spherical in shape.

The arrangement of the chromosomes in the equatorial plate is in the usual manner; from 20 to 22 larger chromosomes lie radially in the peripheral part and the rest are scattered in the central space.

In the equatorial plate of the first maturation division, 18 tetrads appear; nine rings, one V-shaped, one large bipartite and seven small bipartite or dot-like tetrads. Nine or ten larger tetrads lie in the periphery of the equatorial plate and the small tetrads are enclosed in the centre. Of the ring tetrads some are multiple-rings, of which two are somewhat flexed in the middle. One of these is the largest of the tetrads and the other is the next largest. Consequently, it is clear that the flexed multiple-ring tetrads correspond to the two pairs of the largest V-shaped chromosomes, and the other ring and V-shaped tetrads correspond to the eight pairs of rod-like chromosomes, while the large bipartite tetrad corresponds to the smallest rod-like chromosomes and the remaining seven tetrads correspond to the seven pairs of the micro-chromosomes of the spermatogonial complex.

The second maturation division shows 18 dyads forming the equatorial plate. Of these dyads, two are double V's, nine are vertical single V's and seven are bipartite or dot-like. 11 or 12 dyads, which are mostly larger dyads, lie in the periphery of the plate while the rest are in the central space.

8. *Naja naja atra* (CANTOR)

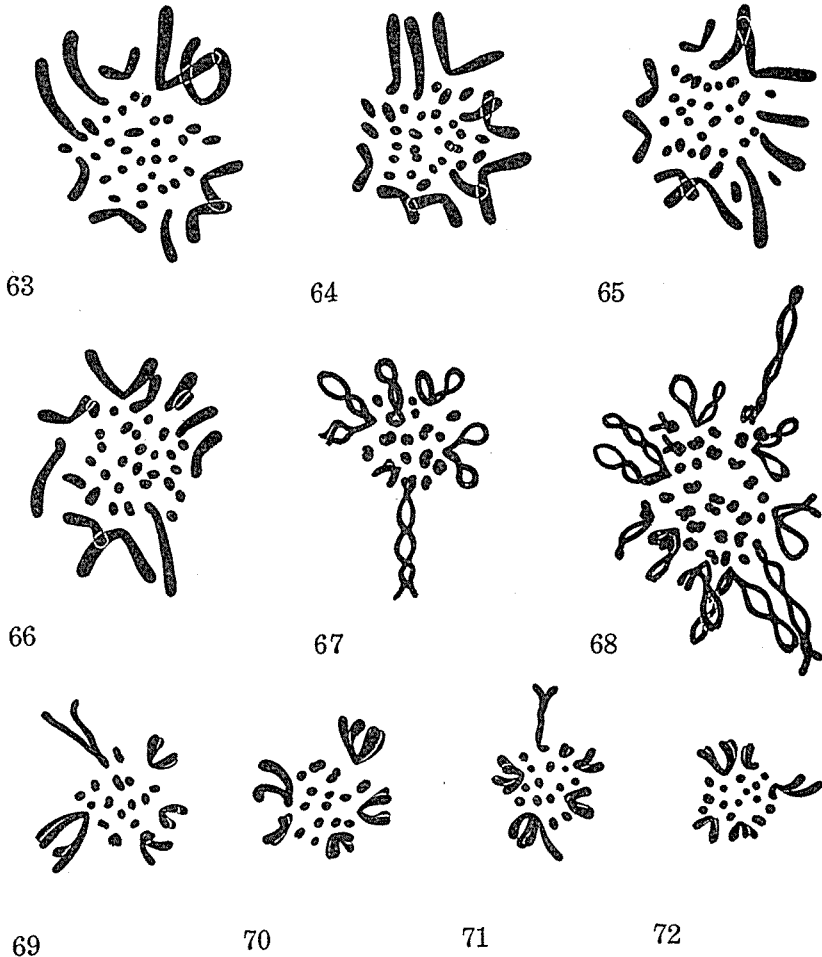
Text-figs. 63-78.

The materials were fixed in May 1929, in Formosa in solution A. The chromosomes in all cell generations are preserved as well as in *Bungarus*.

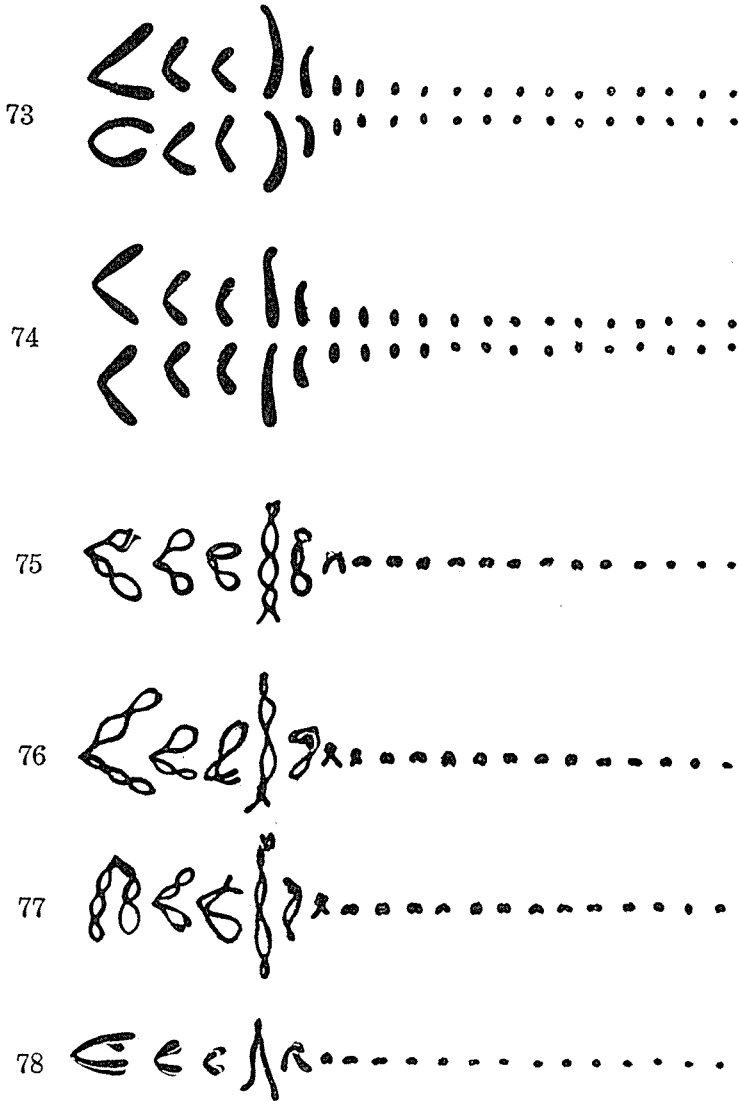
The spermatogonial complex consists of 38 chromosomes, which may be divided into ten macro- and 28 micro-chromosomes. The macro-chromosomes are made up of three pairs of V's, and two pairs of J's. The micro-chromosomes include 14 pairs of short rods and dots. The V-shaped chromosomes are centromitic, and one pair is very large, two pairs are similar in size and smaller than the former. The J's have subterminal attachment of spindle-fibre; one pair is very long—twice as long as the other pair. The J's

are rods with constrictions at the points of spindle-fibre-attachment. Of the micro-chromosomes two or three pairs are very short rods and the rest are nearly oval or spherical in shape.

In the first maturation division 19 tetrads form the equatorial plate. Of these tetrads five are much larger than the others and are multiple-ring tetrads. Three of them are bent in the middle, and the remaining two are nearly straight. In some cases, these straight tetrads are flexed near the proximal end at points where



Text-figs. 63-72. Chromosomes of *Naja naja atra*. 63-66, polar views of the equatorial plates of the spermatogonial divisions; 67, that of the first maturation division; 68, that of the first spermatocyte which includes tetraploid chromosomes; 69-72, those of the second maturation divisions.



Text-figs. 73-78. Chromosomes of *Naja naja atra*. 73 & 74, the two sets of the spermatogonial chromosomes arranged into pairs; 75, the linear arrangement of the tetrads; 76 & 77, those of the two sets of tetrads included in a tetraploid first spermatocyte; 78, the same of the dyads.

spindle-fibres are attached. The majority of the smaller tetrads are bipartite but one or two take shapes of V or γ .

Rarely, cells having tetraploid chromosomes are found. Text-figs. 68, 76, 77 show the views of such cells in the first matura-

tion division ; there is one more set of tetrads than in the normal cells.

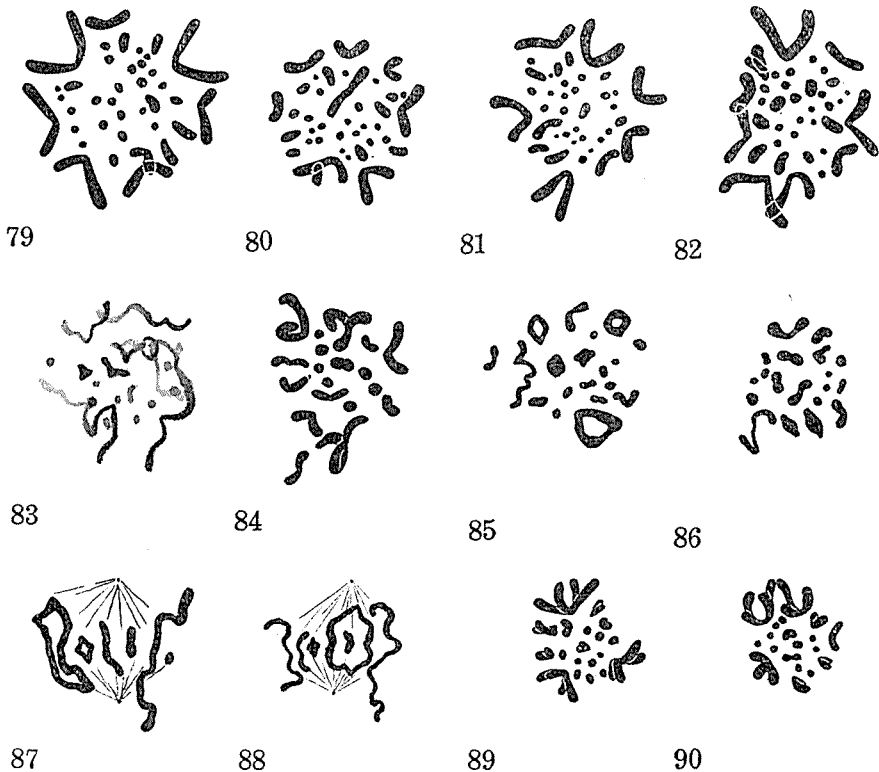
The dyads which appear in the second maturation division are 19 in number, including three double V's, two double J's and 14 bipartites or dots. The double V's and J's are very conspicuous by their large size, and they consist of two V's and J's respectively. In some cases the proximal end of the J is not hooked, and the chromosomes look more like a vertical simple V ; but the component monads are associated at subterminal points.

Subfamily Hydrophiinae

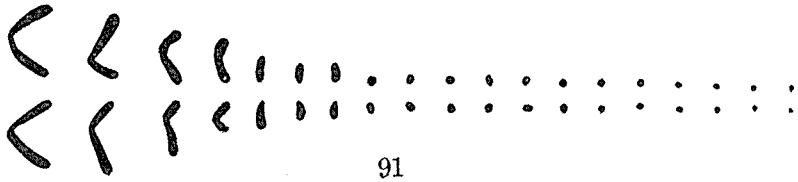
9. *Laticauda semifasciata* (REINWARDT)

Text-figs. 79-95.

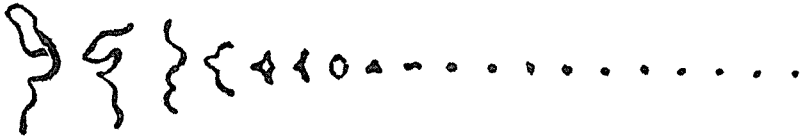
The snakes used as material were sent alive from Okinawa in June 1930. For the fixation of chromosomes solution A furnished



Text-figs. 79-90. Chromosomes of *Laticauda semifasciata*. 79-82, polar views of the equatorial plates of the spermatogonial divisions ; 83-86, those of the first maturation divisions ; 87 & 88, side views of some tetrads in the metaphase ; 89 & 90, polar views of the equatorial plates of the second maturation divisions.



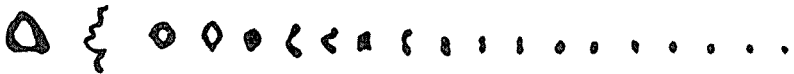
91



92



93



94



95

Text-figs. 91-95. Chromosomes of *Lalicauda semifasciata*. 91, the spermatogonial chromosomes arranged into pairs; 92-94, the linear arrangements of three sets of tetrads; 95, that of the dyads.

favourable results. Notwithstanding the fact that the snakes had been kept for many days under very unnatural and unfavourable conditions, there is no irregular division figure visible in any cell generation.

The spermatogonial chromosomes are 38 in number; and there are four pairs of V-shaped, three pairs of short rod-like and 12 pairs of small chromosomes. Of the V-shaped chromosomes two pairs are the largest, one pair is of medium size and one pair is the smallest. The three pairs of short rods are clearly distinguish-

able from the 12 small pairs, but sometimes, a pair of small ones simulate short rods as shown in Text-figs. 82.

The tetrads which appear in the metaphase of the first maturation division are 19 in number. These tetrads are very peculiar in form as compared with those of other reptiles. In the polar views, the tetrads appear as irregularly-crooked rods, vertical rings, V's, dumb-bells or dots of various sizes. Of these tetrads the four which have the shape of a crooked rod or of a vertical ring are always distinguished by their large size. The side views of these tetrads reveal that they are composed each of two centromitic chromosomes which are joined at one end into an irregularly-crooked rod or at both ends into a vertical ring. The other tetrads also consist of two rods or dots. The rods are fused with each other at one end and form vertical rods or V's. The dot-like chromosomes tend to become very short rods. The two components of each tetrad are wide apart and are almost vertical to the equatorial plane of the spindle. Thus vertical rings, rods, V's, dumb-bells and dots are formed. Sometimes, some medium-sized tetrads simulate vertical rings or diamond-shape which are comparable to diaschistic rods and double crosses in the early anaphase. One might imagine that these peculiar tetrads are nothing but the tetrads with ordinary forms in the early anaphase. But the fact that the small ones remain still undivided indicates that they are not in the anaphase. As clearly shown in *Natrix tigrina* (1928a), all the dyads usually move toward the poles simultaneously. Accordingly, the points of spindle-fibre-attachment in all dyads lie nearly in one plane in each set.

In contrast with the previous division, the dyads which appear in the second maturation division are of a quite ordinary form. They are 19 in number—four double V's, 15 vertical V's and bipartite bodies or dots.

Family Viperidae
Subfamily Crotalinae

10. *Agkistrodon acutus* (GÜNTHER)

Text-figs. 96-98, 113-115.

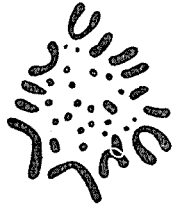
The materials were taken in June 1929, in Formosa. For fixation solution C was found to be the most satisfactory.

The spermatogonial chromosomes are 36 in number; comprising five V-shaped, three short rod-like and ten dot-like pairs. Of the

V's, two pairs are the largest, a single pair the next largest and two pairs are the smallest. The smallest pairs often appear as curved-rods but they are slightly longer than the six short rod-like pairs.



96



97



98



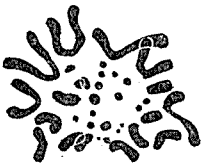
99



100



101



102



103



104



105



106



107



108



109



110



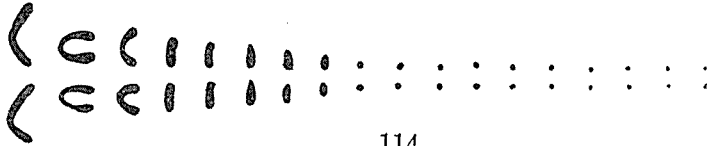
111



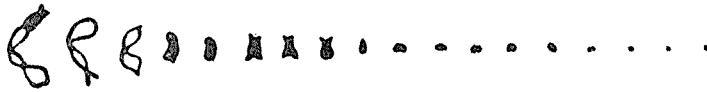
112



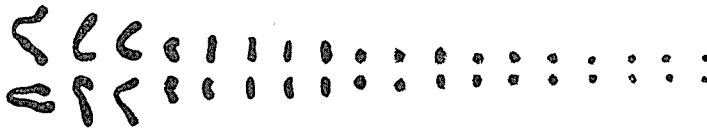
113



114



115



116



117



118

Text-figs. 96-98 & 113-115. Chromosomes of *Agkistrodon acutus*. 96 & 97, polar views of the equatorial plates of the spermatogonial divisions; 98, that of the first maturation division; 113 & 114, two sets of the spermatogonial chromosomes arranged into pairs; 115, the linear arrangement of the tetrads.

Text-figs. 99-112, & 116-118. Chromosomes of *A. halys blomhoffii*. 99-104, polar views of the equatorial plates of the spermatogonial divisions; 105-108, those of the first maturation divisions; 109-112, those of the second maturation divisions; 116, the spermatogonial chromosomes arranged into pairs; 117, the linear arrangement of the tetrads; 118, that of the dyads.

In the first maturation division the 18 tetrads lie on the equatorial plane. They may be sorted into eight larger ones and ten smaller ones; the largest three of the former are multiple ring tetrads of the shape of V. The structure of the other large tetrads is obscure; but two are somewhat bent in the middle and may correspond to the two pairs of the small V-shaped chromosomes.

The second maturation division is not found in my materials.

11. *A. halys blomhoffii* (BOIE)

Text-figs. 99-112, 116-118.

The materials were taken in July in the vicinity of Kyoto and fixed in solution A.

The conditions of chromosomes found in this species coincide strictly with those in the preceding species. So there is no need of description of any stage except the second maturation division which was not found in that species.

In this division 18 dyads appear on the equatorial plane of the spindle. They are two large, one medium-sized and two small double V's, three vertical V's and ten dots of various sizes. In the figures vertical V's are drawn like single rods, curved or straight, because one arm of each V overlaps the other.

12. *Trimeresurus mucrosquamatus* (CANTOR)

Text-figs. 119-133.

The materials were taken in May 1929, in Formosa. They were fixed in solution A. The results are satisfactory.

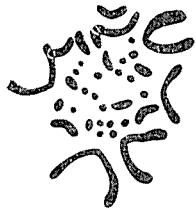
The spermatogonial chromosomes are 36 in number; five pairs of V's, three pairs of short rods and ten pairs of dots. Among the V's we can distinguish two pairs of the largest, one pair of the next largest and two pairs of the smallest chromosomes. One pair of the smallest V's seems to be slightly larger than the other pair. The rod-like chromosomes are of nearly the same length; while the dot-like ones are spherical or oval in shape.

The arrangement of the chromosomes in the equatorial plate is as usual; about ten larger ones occupy the peripheral part of the plate.

The tetrads which appear in the first maturation division are 18, of which eight are larger than the other bipartite and dot-like ones. In my materials, tetrads show a tendency to be condensed into masses so that fine structures are concealed; still it is clear that the largest three tetrads which are bent in the middle are multiple-ring tetrads. In the equatorial plate the larger tetrads tend

to lie in the periphery; but some of them which are smaller than the rest often come in the central space together with the small tetrads.

In every second spermatocyte 18 dyads appear in the metaphase. As in the preceding division eight dyads are clearly distinguishable from the remaining ten dot-like ones. Of the former, five larger ones are double V's and three smaller ones are single V's. Except the two largest ones, the double V's often appear as consisting of two curved rods, of which one overlies the other.



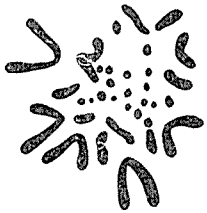
119



120



121



122



123



124



125

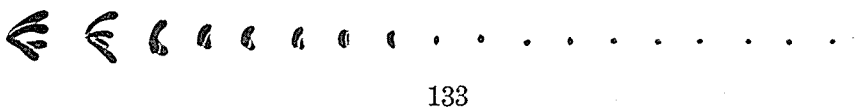
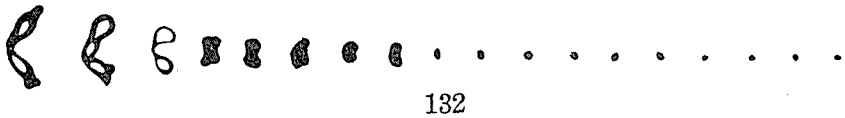
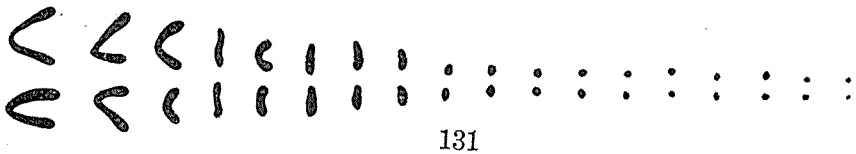
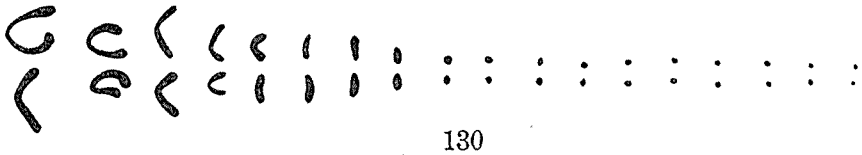
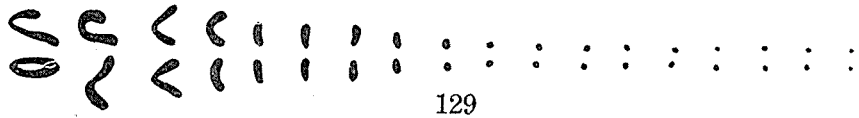
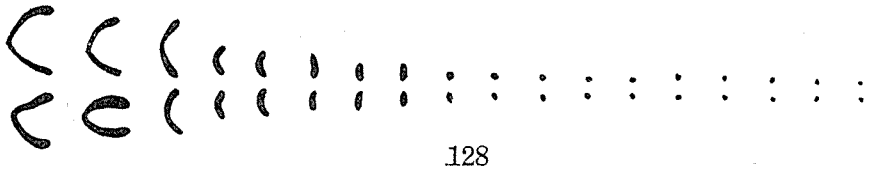


126



127

Text-figs. 119-133. Chromosomes of *Trimeresurus microsquamatus*. 119-122, polar views of the equatorial plates of the spermatogonial divisions; 123-125, those of the first maturation divisions; 126 & 127, those of the second maturation divisions; 128-131, three sets of spermatogonial chromosomes arranged into pairs; 132, the linear arrangement of the tetrads; 133, that of the dyads.

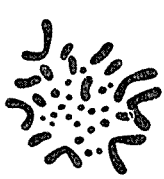


13. *T. gramineus stejnegeri* (SCHMIDT)

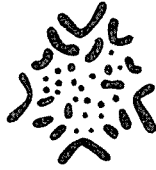
Text-figs. 134-143.

The materials were fixed in May 1929, in Formosa in solution A.

The spermatogonial chromosome-complex of this species is very similar to that of *T. mucrosquamatus*. The spermatogonial chromosomes are 36 in number: five pairs of V's, three pairs of rods and ten pairs of dots. The V's are centromitic chromosomes, and we can sort them into three groups according to their relative size; two largest pairs, one medium-sized and two smallest. The smallest



134



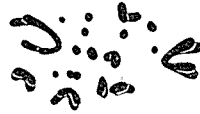
135



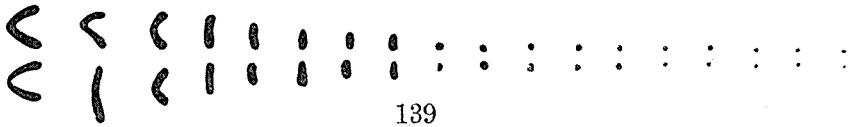
136



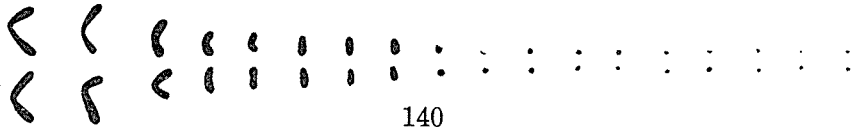
137



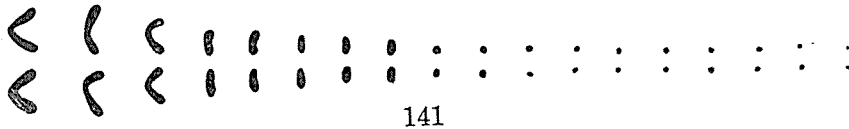
138



139



140



141



142



143

Text-figs. 134-143. Chromosomes of *T. gramineus stejnegeri*. 134-136, polar views of the equatorial plates of the spermatogonial divisions; 137, that of the first maturation division; 138, that of the second maturation division; 139-141, three sets of the spermatogonial chromosomes arranged into pairs; 142, the linear arrangement of the tetrads; 143, that of the dyads.

two pairs often take the form of curved rods and are arranged parallel to the radius of the equatorial plate; but there is no doubt that they are centromitic.

In the first maturation division 18 tetrads appear; of these eight can be distinguished from the other bipartite or dot-like ones by their large size. As shown in Text-fig. 142 the largest three and one of the next largest are V-shaped, and are probably multiple-ring tetrads. The other four appear as masses of chromatin so that fine structures are invisible, but it is clear that one of them represents a pair of small V-shaped chromosomes and the rest the three pairs of short rods.

In the second maturation division 18 dyads form an equatorial plate; two large, one medium-sized and two small double V's, three small single V's which are perpendicular to the equatorial plane and ten small bipartite or dot-like ones.

Discussion

1. Chromosome-complexes of snakes

In the foregoing chapters male chromosome-complexes of 13 species of snakes were described. Besides these, several kinds of snakes have been investigated by previous authors. THATCHER (1922) was the first to describe the chromosome-complex of a snake. His results on *Thamnophis butleri* may be summarized as follows:— In the male of this snake the chromosomes seem to be 37 in number and the condition of the sex-chromosomes is XXY. In contrast to THATCHER's results I found in the male of the snake, *Natrix tigrina* (1928a) that the diploid number of chromosomes is 40 and also that the male seems to be homogametic as regards the sex-chromosomes. Next in *Elaphe* and *Agkistrodon* I found 36 spermatogonial chromosomes and also reported the condition of sex-chromosomes. MATTHEY (1929a) observed that the male of *Vipera aspis* has 41 diploid chromosomes and the sex-formula is XO-type. In later studies (1931–33), however, he revised the above results, and confirmed the presence of 42 chromosomes and the XX-type of the sex-chromosome formula. He also observed 36 chromosomes in the spermatogonial divisions of some snakes. Recently, MAKINO found that spermatogonial chromosomes of *Vipera berus* are 36 in number. The chromosome-complexes of these snakes as well as the 13 species dealt with in the present paper may be formulated and arranged in systematic order as in the following table. The V-shaped, J-

shaped, rod- and dot-like chromosomes are designated as V, J, R and D respectively.

Family Colubridae

Series Aglypha

Subfamily Colubrinae

<i>Natrix tigrina</i>	10V's + 6R's + 24D's = 40	(NAKAMURA '28a)
<i>Macropistodon rudis carinatus</i>	2V's + 14R's + 30D's = 46	
<i>Dinodon rufozonatum</i>	"	
<i>Holarchus formosanus</i>	10V's + 6R's + 20D's = 36	
<i>Zocys nigroroginatus oshimai</i>	"	
<i>Elaphe quadrivirgata</i>	"	
<i>E. climacophora</i>	"	
<i>Tropidonotus natrix</i>	"	(MATTHEY '31)
<i>T. viperinus</i>	"	"
<i>Zamenis gemonensis</i>	"	"
<i>Coronella austriaca</i>	"	"

Series Opisthoglypha

Subfamily Dipsadomorphinae

<i>Tarbophis fallax</i>	10V's + 6R's + 20D's = 36	(MATTHEY '31)
<i>Coelopeltis lacertina</i>	6V's + 36R's and D's = 42	"

Series Proteroglypha

Subfamily Elapinae

<i>Bungarus multicinctus</i>	4V's + 18R's + 14D's = 36
<i>Naja naja atra</i>	6V's + 4J's + 4R's + 24D's = 38

Subfamily Hydrophiinae

<i>Laticauda semifasciata</i>	8V's + 6R's + 24D's = 38
-------------------------------	--------------------------

Family Viperidae

Subfamily Viperinae

<i>Vipera aspis</i>	4V's + 18R's + 20D's = 42	(MATTHEY '33)
<i>V. berus</i>	10V's + 6R's + 20D's = 36	(MAKINO unpubl.)

Subfamily Crotalinae

<i>Agkistrodon acutus</i>	10V's + 6R's + 20D's = 36
<i>A. halys blomhoffii</i>	"

Trimeresurus mucrosquamatus 10V's + 6R's + 20D's = 36
T. gramineus stejnegeri „

In *Tropidonotus*, *Zamenis*, *Coronella* and *Tarbophis*, MATTHEY states that eight chromosomes seem to be V-shaped. But judging from his figures, I rather take the view that there are ten V-shaped or centromitic chromosomes in each complex. In MATTHEY's paper, *Tarbophis* is included in Series Aglypha; but according to BOULENGER (1896) it is a member of Series Opisthoglypha.

As shown in the above list, there are five genera, *Elaphe*, *Aghkistrodon*, *Trimeresurus*, *Vipera* and *Tropidonotus*, in each of which chromosome-complexes of two species are known. When the chromosome-complexes found in the different species of the same genus are compared, we find that they are very much alike except that of *Vipera*. Such a coincidence in chromosome-complex is not restricted to members of the genus. The formula, 10V's + 6R's + 20D's = 36, occurs not only in the members of the family Colubridae but also in the family Viperidae. Precise study of the chromosome-complexes of these snakes shows that there is striking coincidence among them; the complex consists of two pairs of large V's, a pair of V's of medium size, two pairs of small V's, three pairs of short rods and 10 pairs of dots. Such coincidence in chromosome-complexes exists in *Macropistodon rudis carinatus* and *Dinodon rufozonatum* also, though they belong to different groups of the family. The chromosome-complex consists of one pair of V's, seven pairs of rods and 15 pairs of dots; the rods are of intergrading sizes and demarcation between rods and dots is not so sharp. Thus there is striking correspondence among the chromosome-complexes of many snakes. In my opinion this fact reveals an intimate relationship existing among these snakes, because the correspondence is too far-reaching to be mere coincidence.

At the same time, discrepancies may be found among chromosome-complexes of various snakes. How can these discrepancies be accounted for? Among the chromosome-complexes of the members of certain groups of animals—both invertebrate and vertebrate—the following relation is often found: a V-shaped chromosome of one member corresponds to two rod-like chromosomes of the other member, as ROBERTSON (1916) has emphasized in his study on some orthoptera. If a V-shaped chromosome is counted as two, the number of chromosomes in these snakes becomes as follows.

	Family Colubridae	
	Series Aglypha	
	Subfamily Colubrinae	
<i>Natrix tigrina</i>		50
<i>Macropistodon rudis carinatus</i>		48
<i>Dinodon rufozonatum</i>		”
<i>Holarchus formosanus</i>		46
<i>Zoacys nigroroginatus oshimai</i>		”
<i>Elaphe</i> spp.		”
<i>Tropidonotus</i> spp.		”
<i>Zamenis gemonensis</i>		”
<i>Coronella austriaca</i>		”
	Series Opisthoglypha	
	Subfamily Dipsadomorphinae	
<i>Tarbophis fallax</i>		46
<i>Coelopeltis lacertina</i>		48
	Series Proteroglypha	
	Subfamily Elapinae	
<i>Bungarus multicinctus</i>		40
<i>Naja naja atra</i>		48
	Subfamily Hydrophiinae	
<i>Laticauda semifasciata</i>		46
	Family Viperidae	
	Subfamily Viperinae	
<i>Vipera aspis</i>		46
<i>V. berus</i>		”
	Subfamily Crotalinae	
<i>Agkistrodon</i> spp.		46
<i>Trimeresurus</i> spp.		”

For the sake of convenience, let us call the above numbers “hypothetical numbers.” The chromosome-complex, which may be formulated as $10V's + 6R's + 20D's = 36$, corresponds with the 46 in the hypothetical number. Besides the complexes of this type, there are two additional types which have the same hypothetical number.

These occur in two species of different groups, *Vipera aspis* and *Laticauda semifasciata*. In the sea-snake, *Laticauda*, the chromosome-complex may be formulated as $8V's + 6R's + 24D's = 38$. More precisely, the complex consists of two pairs of large V's, one pair of V's of medium size, one pair of small V's, three pairs of short rods and 12 pairs of dots. Comparing this complex with the former complex, we find that there is a remarkable correspondence between them. The four pairs of V's, three pairs of short rods and 10 of 12 pairs of dots in the sea-snake may be identified with the larger four of the five pairs of V's, three pairs of short rods and the ten pairs of dots in the former. In the sea-snake, accordingly, a pair of small V's are gone, and two pairs of dots occur in their place. This discrepancy, however, may easily be explained by supposing that one of the smallest V's in the former group corresponds to two dots of the sea-snake; in other words, the arms of the two small V's of the former have separated and become the two pairs of dots of the latter. The difference between the former complex, $10V's + 6R's + 20D's = 36$ and that of *Vipera aspis* can be explained in the same way. In *V. aspis*, the chromosome-complex consists of two pairs of V's, nine pairs of rods of intergrading sizes and ten pairs of dots, while there are five pairs of V's, three pairs of short rods and ten pairs of dots in the former complex. The V's of *V. aspis* are apparently rather small, so that these two pairs of V's seem to correspond to the smaller two pairs among the five pairs of V's in the former complex, while the six larger pairs among the nine pairs of rods in *V. aspis* seem to correspond to the larger three pairs of V's in the former complex. Accordingly, the remaining three pairs of short rods and ten pairs of dots of *V. aspis* seem to correspond to the rods and dots of the former complex respectively. As *V. berus* has the chromosome-complex, $10V's + 6R's + 20D's = 36$, such an explanation seems to be probable.

In his study on the chromosomes of the Rhynchocephalia, KEENAN (1932) has concluded that the basal number of the chromosomes of reptiles is 48, which number coincides with that of mammalian chromosomes according to PAINTER (1925). KEENAN's view is supported by MATTHEY (1933) who has studied the chromosomes of many lizards. I also have agreed to this conclusion as far as saurian chromosomes are concerned. Of the chromosome-complexes of snakes mentioned above, we find some in which the hypothetical number is 48. But the actual number is so varied

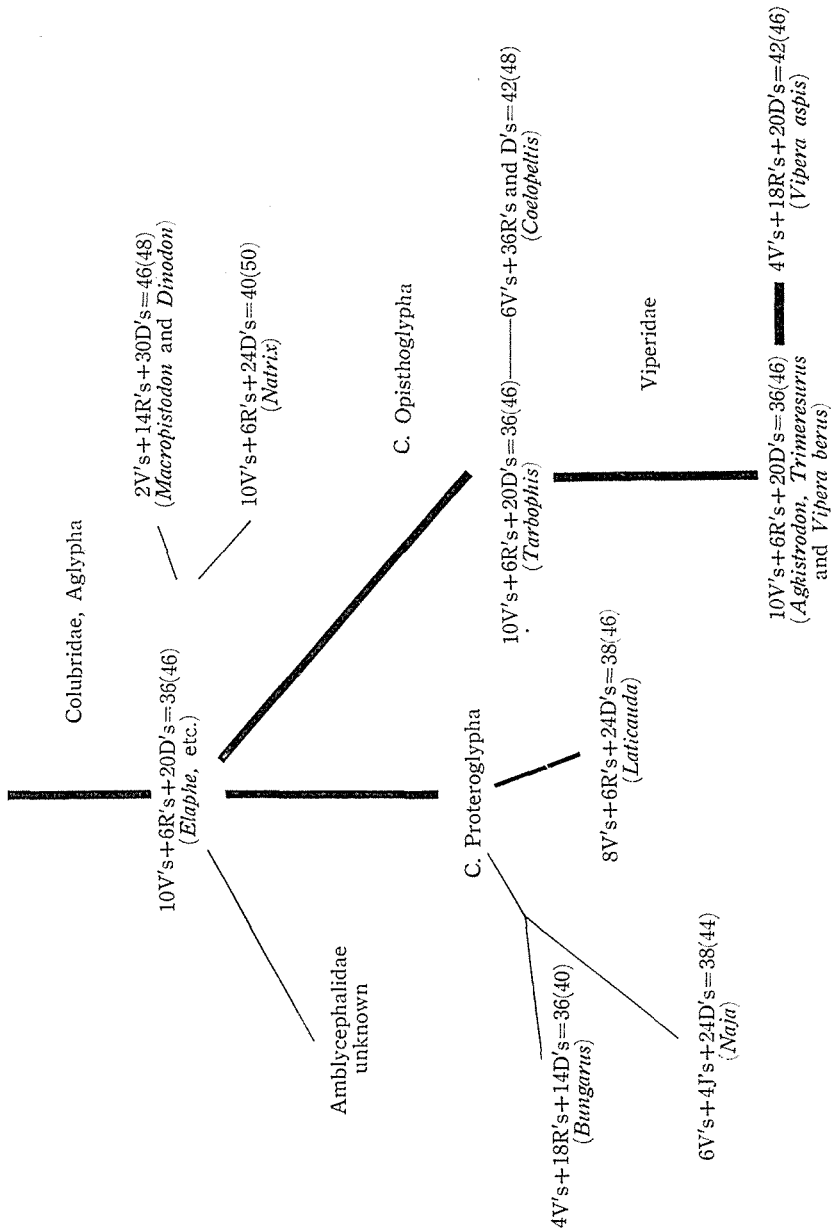
that it is difficult to find out the relationship among them. There is another type of chromosome-complexes which may be formulated as $10V's + 6R's + 20D's = 36$ and where the hypothetical number is 46. This type occurs in the snakes of several subfamilies, Colubrinae, Dipsadomorphinae, Viperinae and Crotalinae. The complexes found in Hydrophiinae and Viperinae are intimately related to this type, although slightly different. Thus, this type or its modification is common and is distributed widely among various groups of snakes; and it seems to represent the basic type from which the other types have been derived.

Relations between this fundamental complex and the complex found in each species are somewhat more complicated. Besides the interrelation between V and rods stated above, variation in chromosome-complexes may be brought about by fragmentation, association, diminution of chromosomes as illustrated in *Lacerta vivipara* (OGUMA 1934a), duplication, translocation, etc. However, it is almost impossible to know exactly which of these has been responsible for the development of these complexes.

Concerning the development of chromosome-complexes of snakes, my view presented above is consistent with BOULENGER's view (1869) regarding the phylogeny of snakes. He is of opinion that Proteroglypha, Opisthoglypha and Amblycephalidae have been derived from Aglypha, while Viperidae originated from Opisthoglypha. My view is illustrated in Text-fig. 144, a diagram which I have constructed after BOULENGER's scheme.

As to the hypothetical basic chromosome number, 48 seems to be most common in the Sauria and Rhychocephalia. In snakes, however, 46 seems to be the fundamental number so far as the families, Colubridae and Viperidae, are concerned. Our knowledge of the chromosomes of snakes of other groups is meagre, and it is premature to state that this number is fundamental for all the snakes. Yet, this number is very similar to the basic number found in other groups of reptiles, being only two less than the latter. Moreover, as stated before, the hypothetical number 48 occurs in some snakes which belong to different groups. Consequently it seems reasonable to assume that 48 is the fundamental number for the whole reptile group.

Thus, I agree with KEENAN and MATTHEY on the opinion that the fundamental number of the reptilian chromosomes is 48. But I do not mean that the prototype of the reptilian chromosome-com-



Text-fig. 144. Diagram illustrating the phygenetical development of chromosome-complexes in the families Colubridae and Viperidae. Numbers enclosed in brackets are the hypothetical number of chromosomes.

plex consists of 48 rod-like and dot-like chromosomes. MATTHEY is of opinion that variations in number of chromosomes in reptiles are due chiefly to the association of two rods into a V. OGUMA (1934b) also has maintained that V-shaped chromosomes found in certain mammals have been formed by the union of rod-like and other chromosomes two by two. ROBERTSON is of the opinion that the V-shaped chromosome which is composed of two telomitic rods united at one end, retains a bridge of unstained material between the two arms. But I have never found such a bridge in any V-shaped chromosome occurring in reptiles. MATTHEY (1933) attributes this to the difference of materials. I have never met with any case in chromosomes of this group which might provide positive evidence for MATTHEY's opinion.

On the other hand, many amphibians have chromosome-complexes consisting of 24 V's (IRIKI 1930-32, MAKINO 1932, MINOUCHI and IRIKI 1931, etc.). Most of them are frogs; but urodeles like *Diemyctylus pyrrhogaster* (IRIKI 1932b, SATO 1932), *Triton* (JANSSENS 1900, MOORE and ARNOLD 1905) have also 24 V-shaped chromosomes. In other urodeles also V-shaped chromosomes occur, though they vary in number from 12 to 22. Whether in reptiles or in other vertebrates, there is no positive evidence that the V-shaped chromosome has been formed by association of two rod-like chromosomes. On the contrary, some V- or J-shaped chromosomes in the lower vertebrates have constriction at the point where the spindle-fibre is attached. Also, amputation of chromosomes at such constriction is often observed in various animals and plants. So, it does not seem very unreasonable to surmise on the basis of these facts that the prototype of reptilian chromosome-complex consists of 24 V-shaped or atelomitic chromosomes, which may be divided into 12 larger and 12 smaller ones. The chromosome-complexes of higher vertebrates, such as recent reptiles, birds and mammals, have originated probably from this complex. In the course of evolutionary change, fragmentation must have taken place. Since the number of V's does not exceed 12 in any reptilian complex, dissociation of arms of V's might have occurred first in the 12 smaller chromosomes and then in larger ones.

The fact that the primitive reptiles of to-day, such as Spheonodon and Geckos, have many telomitic chromosomes and that some other lizards have many V-shaped chromosomes, appears to be incompatible with my opinion. However, this is probably due to the

fact that there is no accurate correspondence between the shape of chromosomes and the hereditary potency of the animal. In support of this view, we can point out some cases of closely-related lizards which have widely different chromosome-numbers. In the family Agamidae, for example, *Japarula* has 46 rod-like and dot-like chromosomes (NAKAMURA 1934b), while *Agama* and *Uromastix* have 36 chromosomes consisting of 12 V-shaped and 24 dot-like ones (MATTHEY 1931).

From the phylogenetic point of view the ancestral reptiles are closely related to urodeles. So that one may imagine that the prototype of reptilian chromosome-complex resembles the complexes found in urodeles more than those found in anurans. Many urodeles have chromosomes exceeding 60 in number (IRIKI 1932b, c, MAKINO 1932c, SMITH 1929, etc.). IRIKI (1932c), however, states that general phylogenetic relations in urodela can hardly be considered from the standpoint of the number of chromosomes only.

Sex-chromosomes in snakes

In my previous works on reptilian chromosomes I have found that XX-chromosomes occur in the males of some snakes and lizards (1927-34). Other authors also have reported the same type of sex-chromosomes in certain reptiles (KEENAN 1932, MATTHEY 1929b-33). In all of these studies, only the male chromosome-complexes were dealt with. Recently, OGUMA (1934a) studied the chromosome-complexes in both sexes of the lizard, *Lacerta vivipara*, and confirmed the female heterogamety, namely, XO-type in the female and XX-type in the male. Thus, his observation has given conclusive evidence of the correctness of my view.

In all the snakes thus far studied, the chromosomes are in even number, and may be assorted into homologous pairs, so that there occurs no pair which consists of two heteromorphic chromosomes. From the systematic point of view, the snakes and lizards are very intimately related; it is therefore likely that the condition of the sex-chromosomes of the snake does not differ much from that of the lizard. In fishes, both female heterogamety and male heterogamety occur in closely related forms. In reptiles, however, the sex-chromosomes are specialized to such a degree that co-existence of both the types within the group is hardly to be expected. Thus, it seems probable that the males of the snakes are homogametic as regard the sex-chromosomes.

In my previous papers* heteropycnosis of XX-chromosomes has been reported. In the present materials also, nucleoli which are apparently karyosomes have been recognized. But the descriptions of the phenomenon of heteropycnosis, and of nucleoli will be deferred to another paper.

Morphology of chromosomes in reptiles

Reptilian chromosomes are often classified into two categories, the macro- and the micro-chromosomes (DALCQ '21, PAINTER '21, MATTHEY, NAKAMURA). Such classification is only for convenience' sake, and there is no fundamental difference between the two categories. So that it is a matter of course that the macro- and micro-chromosomes of one animal do not correspond accurately to those in another animal. All through my studies the macro-chromosomes include all V-shaped and most of the rod-like ones, while the micro-chromosomes include dot-like ones. But, in such special cases as where certain rods are much shorter than the others, they are also included in the latter group together with dots.

OGUMA (1934b) has stated that the saurospid chromosome-complex belongs to the dimorphic type, that is: chromosomes may be sorted into the macro- and micro-groups. In some reptiles, however, the distinction between these two categories is obscure, and their complexes belong rather to the polymorphic than to the dimorphic type.

Morphologically, the reptilian chromosomes may be sorted into three groups.

A. Chromosomes which have non-terminal attachment of spindle-fibre.

Both centromitic chromosomes and the chromosomes having subterminal spindle-fibre-attachment are included in this group. The former are V-shaped and the latter are J-shaped chromosomes. The J's are of rather rare occurrence as compared with V's, being found only in *Sphenodon* (KEENAN 1932, HOGBEN 1921) and in *Naja*. These two kinds of chromosomes are rather large; and when the whole complex is divided into the macro- and micro-chromosomes, they always belong to the former category. The number of these chromosomes does not exceed 12; on the equatorial plate they lie in the peripheral zone with their apexes or the points of spindle-

* In my earlier papers (1927, 1928a) the male sex-chromosomes were designated ZZ; but in my later studies I have substituted the symbol XX.

fibre-attachment toward the centre of the plate. Sometimes, certain of these chromosomes have the arms widely open and lying parallel to the radii of the plate. When the V's are very small, they often appear like curved rods.

Concerning these V- and J-shaped chromosomes, MATTHEY is of opinion that each consists of two telomitic chromosomes which are connected at the point of the spindle-fibre-attachment. His conclusion is based upon ROBERTSON'S hypothesis. But as I have explained in the preceding chapter, I cannot agree with him in this.

In the metaphase of the first spermatocyte, V's and J's form multiple-ring tetrads. The tetrads are also V- or J-shaped, somewhat flexed at the point of spindle-fibre-attachment. The number of rings appearing in each tetrad is variable. Each ring lies at right angles with the adjacent ring, and they seem to be formed by alternate widening of clefts between synaptic mates and by longitudinal splitting of each mate. But, as I have illustrated in *Natrix*, an apparent ring may result from the twisting of a tetrad. In some cases, certain small tetrads assume an X-shape in the polar view.

In contrast with these cases, V-shaped chromosomes in the sea-snake, *Laticauda semifasciata*, form tetrads of very peculiar shape. In this snake the tetrads which represent the V-shaped spermatogonial chromosomes are in the form of vertical rings and irregularly-crooked vertical rods. The rings are formed by two centromitic chromosomes fused at both ends, while the vertical rods consist of such chromosomes united at one end. Tetrads of this type are often found in insects and other invertebrates as well.

In higher vertebrates V-shaped chromosomes generally become V-shaped multiple-ring tetrads. In amphibia tetrads of this type occur in urodeles only; in anurans vertical rings and V's are formed in the manner found in the sea-snake. According to SWINGLE (1921) there are two larval sexual cycles in the bullfrog, *Rana catesbiana*, and in the first cycle the tetrads are of urodelan type, while in the second cycle they are of a different type. IRIKI (1932c) has pointed out that the tetrads in the second cycle are of the anuran type. These two authors agree in the opinion that the first ripening of the germ cells is possibly a recapitulation, just as the development of its body shows a recapitulation of an earlier phylogenetic stage. Moreover, IRIKI has concluded that the multiple-ring tetrads must be regarded as an ancestral type of tetrads of am-

phibians and also that the similarity in the forms of tetrads between the urodela and the higher vertebrates implies a phylogenetic significance. In reptiles, however, the tetrads of vertical rods and rings which are of the anuran type occur together with the multiple-ring tetrads in members of one group, so that it is doubtful whether such dissimilarity in tetrads has any profound significance.

The anaphase dyads of all these tetrads are double V's or J's and each dyad is composed of two monads united at the point of spindle-fibre-attachment.

In the metaphase of the second maturation division, the dyads take the form of horizontal double V's or J's in which the composing monads overlie one another. In the anaphase these monads separate and go to the poles.

B. Telomitic chromosomes.

I have called these rod-like chromosomes. There are intergradations from long rods to very short ones. Most of the chromosomes of this type belong to the macro-chromosomes, but some are classified among the micro-chromosomes. The number of rods in each complex is variable. In some case, there is no V and the chromosomes are of intergrading sizes from long rods to small dots, while in other cases there are many V's and only a few rods. As stated before, there is a definite relation between the V's and rods found in reptiles. I have assumed that the arms of V's found in one form have dissociated and formed two rod-like chromosomes in the other form, while MATTHEY considers that the V's have been formed by the fusion of two rods.

In *Natrix* and *Eumeces*, I have suggested that a certain pair of short rod-like chromosomes are the sex-chromosomes. This view has been confirmed by OGUMA in his observation on *Lacerta vivipara*.

In the equatorial plate rod-like chromosomes are arranged radially along the axes in usual cases.

In the first spermatocyte, the rod-like chromosomes form ring, V- and heart-shaped tetrads. When the chromosomes are very long, the resulting tetrads are double or triple rings. When they are shorter, the tetrads are simple rings with or without lateral prominences, horse-shoes, V's and heart-shaped or bipartite bodies. All these, except the ring tetrads, belong to the diaschistic tetrads of WILSON (1925).

In the sea-snake, vertical rods, ring and diamond-shaped tetrads are formed. The vertical rods are diaschistic rod-tetrads having

terminal attachment and consist of two rod-like dyads united at one end. The diamond-shaped tetrads are identical with the diachistic rod-tetrads with median attachment or double crosses in the early anaphase.

In the metaphase of the second maturation division the rods turn into vertical V's which are composed of two single rods. Sometimes they simulate single rods in the polar view, one arm of the V overlying the other. The composing monads of each V dissociate and go to the poles in the anaphase.

C. Dot-like chromosomes.

In this group not only spherical chromosomes but also oval chromosomes are included. OGUMA (1934b) has stated that it is more reasonable to call these chromosomes "spherule, spherochromosome or spherosome"; but I have adopted the usual name in the present study.

Throughout the stages of spermatogenesis, the behavior of these dot-like chromosomes is quite ordinary. There is no irregularity which suggests a degenerating tendency in these chromosomes. In *Lacerta vivipara*, however, there are apparently two kinds of individuals. In the individuals caught in Saghalien, OGUMA (1934a) found that the two very minute chromosomes which are very characteristic of the chromosome-complexes of the European individuals of the same species and also of all other members of the same family, are lacking. He has concluded that they might have disappeared through gradual reduction of chromatin. In no other reptile has such a phenomenon been observed. The number of dot-like chromosomes is not constant for all reptiles, but in most of them they are 20-24 in number. As stated before, I am of opinion that these dot-like chromosomes have originated by dissociation of the arms of 12 small V's found in the prototype of the reptilian chromosome-complex.

On the equatorial plane the dot-like chromosomes are scattered in the central space of the spindle enclosed by the larger chromosomes. In well-fixed materials they are quite distinct from one another and there is neither fusion nor any material connecting them together.

In the first maturation division every pair fuses into a small dumb-bell or bipartite body. The components of these tetrads seem to lie side-by-side along the equatorial plane of the spindle. In the sea-snake, components of some tetrads become elongated and lie in

a vertical plane forming vertical rods or V's. In the second maturation division they appear almost as mere dots. Through these divisions four dot-like monads result from each pair and they are distributed into the four spermatids.

Summary

1. The male chromosome-complexes of 13 species of snakes which belong to the two families, Colubridae and Viperidae, are dealt with.
2. The chromosome-complex, $10V's + 6R's + 20D's = 36$, seems to be the fundamental complex as far as the members of these two families are concerned.
3. In most snakes, chromosome-complexes are dimorphic, viz. the chromosomes may be classified into macro- and micro-chromosomes. In some snakes, however, the complexes are polymorphic, viz. the classification into the two groups is less clear-cut.
4. The chromosomes of reptiles may be sorted into three types, namely, V- and J-shaped, rod-like and dot-like chromosomes. In the first maturation division the V- and J-shaped chromosomes form multiple-ring tetrads of the shape of V, J or X; the rod-like chromosomes form multiple-ring, single-ring or bipartite tetrads and the dot-like chromosomes form bipartite or dot-like tetrads. In the second maturation division, the dyads appear in the shape of double V's, bipartites or dots.
5. In the sea-snake, *Laticauda semifasciata*, V-shaped chromosomes form tetrads of vertical rings and irregularly-crooked rods. The rod-like chromosomes form diaschistic rod-tetrads having spindle-fibre-attachment at both ends and vertical rings or diamond-shaped tetrads which can be identified with diaschistic rod-tetrads and double crosses in the early anaphase. The structures of dyads, however, do not differ from those of the other reptiles.
6. The sex-chromosomes of snakes are of the XX-type, so that the males are homogametic in respect to sex.
7. Concerning the prototype of the reptilian chromosome-complex, it may be surmised that the original complex consists of 24 V-shaped chromosomes and the complexes of higher groups, such as recent reptiles, birds and mammals, have developed from this type by dissociation of the arms of V's.

Literature Cited

- BOULENGER, G. A. (1896). Catalogue of the snakes in the Brit. Mus. Nat. Hist. London.
- DALCQ, A. (1921). Étude de la spermatogénèse chez l'orvet (*Anguis fragilis* Linn.). Arch. Biol., 31.
- HOGGEN, L. T. (1921). A preliminary account of the spermatogenesis of *Sphenodon*. J. Roy. micr. Soc., 1921.
- IRIKI, S. (1930). Studies on amphibian chromosomes. 1. On the chromosomes of *Hyla arborea japonica* GUENTHER. Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, 5.
- (1932a). —4. On the chromosomes of *Rana rugosa* and *Rana nigromaculata*. Sci. Rept. Tokyo Bunrika Daigaku, 1.
- (1932b). —6. On the chromosomes of *Diemyctylus pyrrhogaster*. Ibid.
- (1932c). —7. On the chromosomes of *Megalobatrachus japonicus*. Ibid.
- (1932d). —8. On the fixation of the chromosomes of *Hyla arborea japonica* and some other animals. Ibid.
- KEENAN, R. D. (1932). The chromosomes of *Sphenodon punctatum*. J. Anat., 67.
- MAKINO, S. (1932a). Notes on the chromosomes of *Rhacophorus schlegelii schlegelii* (GUENTHER). Proc. Imp. Acad. Japan, 8.
- (1932b). Notes on the chromosomes of *Rana temporalis* L. and *Bufo sachaliensis* (NIKOLSKI). Ibid.
- (1932c). The chromosome number in some salamanders from northern Japan. J. Fac. Sci., Hokkaido Imp. Univ., Ser. VI, 6.
- (1934). The chromosomes of the sticklebacks. Cytologia, 5.
- MATTHEY, R., (1929a). Les chromosomes de la Vipère mâle (*Vipera aspis* Linn.). Biol. Zentralblatt, 49.
- (1929b). La spermatogénèse du lézard (*Lacerta muralis* Linn.). Zeitschr. Zellf. mikr. Anat., 8.
- (1931). Chromosomes de reptiles. Rev. Suisse de Zool., 38.
- (1932). Le chromosomes de l'Amphisbaénien acrodonte: *Trogonophis wiegmanni* KAPUP. Arch. Zool. Exp., 74.
- (1933). Nouvelle contribution à l'étude des chromosomes chez les Sauriens. Rev. Suisse Zool., 40.
- MINOUCHI, O. (1928). On the fixation of chromosomes in mammalian and some other animals. Japan. J. Zool., 1.
- MINOUCHI, O. and IRIKI, S. (1931). Studies on amphibian chromosomes. 2. On the chromosomes of *Bufo bufo japonicus* SCHLEGEL. Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B., 6.
- NAKAMURA, K. (1927). Preliminary notes on reptilian chromosomes. I. The chromosomes of some snakes. Proc. Imp. Acad. (Tokyo), 3.
- (1928a). On the chromosomes of a snake, *Natrix tigrina* (Studies on reptilian chromosomes I). Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, 4.
- (1928b). Preliminary notes on reptilian chromosomes II. The chromosomes of a lizard, *Takydromus tachydromoides*. Proc. Imp. Acad. (Tokyo), 4.
- (1931a).—III. The chromosomes of some lizards. Ibid. 7.
- (1931b). Studies on reptilian chromosomes II. Chromosomes of a lizard, *Eumeces latiscutatus* (HALLOWELL). Cytologia, 2.
- (1932). —III. Chromosome of some geckos. Ibid. 3.
- (1934a). —IV. Chromosomes of *Takydromus* spp. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. in press.

- (1934b). —V. Chromosomes of *Japarula swinhonis*, a lizard. Ibid. in press.
- OGUMA, K., (1934a). Studies on the sauropsid chromosomes II. The cytological evidence proving female heterogamety in the lizard (*Lacerta vivipara*). Arch. Biol., 45.
- (1934b). A revision of the mammalian chromosomes (in Japanese). Japanese J. Genetics, 9.
- PAINTER, T. S. (1921). Studies in reptilian spermatogenesis I. Spermatogenesis of lizards. J. exp. Zool., 34.
- (1925). A comparative study of the chromosomes of mammals. Amer. Nat., 59.
- ROBERTSON, Wm. R. B. (1916). Chromosome studies. I. J. Morph., 27.
- SATO, I. (1932). Chromosome behaviour in the spermatogenesis of Urodele Amphibia. *Diemyctylus pyrrhogaster* (BOLE). J. Sci. Hiroshima Univ. Ser. B, 2.
- SMITH, B. G. (1929). The history of the chromosome vesicles in the segmenting egg of *Cryptobranchus allegheniensis*. J. Morph., 47.
- SWINGLE, W. W., (1921). The male sexual cycle of *Rana catesbiana* larvae. J. exp. Zool., 32.
- THATCHER, L. E. (1922). Spermatogenesis of the garter snake (*Thamnophis*). Science, N. S., 56.
- WILSON, E. B. (1925). The cell in development and heredity. New York.
- WU, SU-HSUEN, (1933). Spermatogenesis in Gecko Japonicus (DUMERIAL and BIERON). J. Morph., 54.