On the Chromosomes of a Snake, Natrix tigrina

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CITATION:

Nakamura, Kenji. On the Chromosomes of a Snake, Natrix tigrina. Memoirs of the College of Science, Kyoto Imperial University. Ser. B 1928, 4(1): 1-18

ISSUE DATE:

1928-03-30

URL:

http://hdl.handle.net/2433/257705

MEMOIRS OF THE COLLEGE OF SCIENCE, KYOTO IMPERIAL UNIVERSITY, SERIES B,
VOL. IV, NO. 1, ARTICLE 1, 1928.

On the Chromosomes of a Snake, *Natrix tigrina*.

By

KENJI NAKAMURA.

With Plates I & II and two Text-figures.

(Received January 12, 1928)

Contents

INTRODUCTION

The chromosomes of snakes have been studied very little, thus far. Although during the last twenty years, several important studies have been published on chromosomes of various reptiles, concerning the chro-

mosomes of snakes, so far as I know, there is but one brief note by THATCHER (1922), which gives certain details of his investigation into the spermatogenesis of the garter snake, *Thamnophis butleri*. His results may be summarized thus: (1) the spermatogonial chromosomes are 37 in number, and (2) the condition of the sex-chromosomes is XXY, of which the double X is disjoined from the single Y at the first maturation division. The results which I have obtained on *Natrix tigrina* and which I propose to describe here, differ greatly from his, especially as to the last-mentioned point. In fact, the chromosomes of *Natrix tigrina*, so far as I have been able to discover, behave in quite an ordinary manner, differing in no way from those of insects.

In regard to the condition of the sex-chromosome of reptiles, three different types have been reported: 1. XXY-type by THATCHER (1922) on the snake just mentioned; 2. XO-type by DALCQ (1921) on a lizard and by JORDAN (1914) on tortoises; 3. compound XX-type by PAINTER (1921) on lizards. Varying in details, these three types agree in the main point, that the male is heterozygous in respect to sex. *Natrix tigrina* belongs to none of these three types, and apparently represents a fourth ZZ-type, which is fundamentally different from all the foregoing three types in the homozygosity of the male.

The present study has been carried on as a graduation thesis, under the direction of Prof. T. KOMAI, to whom I am indebted for valuable criticism. I wish also to acknowledge my obligation to Mr. O. MINOUCHI for criticism and kind assistance especially on the side of technique.

A preliminary account of the present study was published in my "Preliminary Notes on Reptilian Chromosomes. 1. The Chromosome of Some Snakes" (1927).

MATERIAL AND METHODS

The snake which I used in the present study was *Natrix tigrina* (BOIE), one of the commonest snakes on the main land of Japan.

Fully matured individuals in early breeding season (June) were used.

From an animal killed by decapitation, the testes were removed, cut into small pieces and as quickly as possible dropped into the fixative. For fixing chromosomes, a modified solution of CHAMPY'S mixture, concentrated 1·5 time as strong as the original, was found to be best:

3% Osmic acid	.....	4 parts
1·5% Chromic acid	.....	8 〃
4·5% Potassium bichromate	.....	8 〃

After 24 hours of fixation, the material was put into running water for 24 hours, and then gradually dehydrated with alcohol. The alcohol was replaced by cedar-wood oil, this by chloroform, this in turn by toluol, and then the material was imbedded in paraffin. The sections were cut into the thickness of 7·5 micra, and after bleaching, were kept for 12 hours in the following bath:

Glacial acetic acid	.....	50 parts
Saturated picric acid	.....	50 〃

This bath is a modified form of CHURA'S bath (CHURA, 1925), strongly concentrated as compared with the original formula. I used this bath for dissolving cellular inclusions, and at the same time for increasing the affinity of chromosomes to dyes. For this purpose, the above modification was found necessary, because CHAMPY'S mixture preserves too much cellular inclusions, which make detailed observation rather difficult, unless they be dissolved to some extent. For staining, HEIDENHAIN'S iron-haematoxylin was found to be the most adequate, not only for chromosomes but also for nucleoli. For differentiating the karyosome from the plasmosome, FLEMMING'S triple-stain was employed, although it gave no very satisfactory result.

The only deficiency of the above technique lay in the difficulty of staining the chromosomes in all phases, especially those in the nucleus of the growth period. But, the deficiency was compensated for by the advantage of producing a sharp contrast between chromosomes and nucleoli, the latter having much stronger staining capacity than the former, and thus no difficulty was experienced in tracing the complete history of the change of the nucleoli.

OBSERVATIONS

1. *Chromosomes of Spermatogonium*

The polar view of the equatorial plates of the spermatogonial divisions presents 40 chromosomes, consisting of 10 V-shaped, 2 long rod-like, 4 short rod-like and 24 dot-like chromosomes (Figs. 1–5). Of these, the 16 larger chromosomes can easily be classified into 8 homologous pairs and capable of serial alignment according to their relative magnitudes (Fig. 28). The five pairs marked A–E in the figure are V–shaped chromosomes; the pair marked F are long rod-like ones and those marked G and H are short rod-like ones, while the remaining 24 are small dot-like chromosomes none of which has any characteristic feature by which to differentiate it from other similar members. The differences in shape among those eight pairs of chromosomes, A–H, are so obvious that one can distinguish them from one another without any danger of mistaking a long chromosome for a shorter one because of its oblique situation on the equatorial plate. In the equatorial plate the larger twelve chromosomes, A–F, are in most cases arranged in a circle enclosing a central space in which 24 dot-like chromosomes are scattered about, and four short rod-like ones, G and H, lie either near the periphery of the plate among the larger chromosomes (Fig. 4), or in the central space among the dot-like chromosomes (Fig. 2). The spindle fibre attaches to the V-shaped chromosome at the point of its flexure (centromitic), while to the long rod-like chromosome, it attaches terminally (telomitic); and, since all these larger chromosomes lie with their points of fibre-attachment toward the spindle axis, a rosette is formed. However, sometimes, and not seldom, certain of the V-shaped chromosomes turn laterally along the radius of the equatorial plate, one arm pointing to the center, and the other arm, outward. In these cases, the angle between the two arms of such a V is naturally larger than the angle between the arms of ordinary V's.

All these metaphase chromosomes are well separated, and are never connected with one another by a strand of lightly stained material, such

as that described by PAINTER (1921) in his study on lizards.

I have been unable to distinguish the sex-chromosomes in the spermatogonial complexes without referring to the spermatocyte chromosomes; but the presence of the sex-chromosomes in an even number is indicated by the number of the spermatogonial chromosomes which is even.

2. *Chromosomes of First Spermatocyte*

A. Growth period

In the present study no special attention was paid to the striking changes of autochromosomes which take place during the growth period, and the detailed observations on the synapsis and the tetrad-formation remain for later study.

In the daughter cells produced by the final spermatogonial division, the telophase chromosomes dissolve into the nuclei of the first spermatocytes, and consequently form fine leptotene threads or leptonema which are at first entangled and not arranged in any definite way (Fig. 6). This change, from the telophase to the leptotene stage, goes on gradually without any apparent resting stage. In the later leptotene stage the threads show a slight sign of polarization; but in good preparations there appears no marked convergence of the threads toward one pole of the nucleus. As to the synapsis of the leptotene threads, I will not touch upon that subject for the present. The zygotene stage can not be distinguished clearly. At about this stage, however, the thickening of the threads sets in. Then comes a clear pachytene stage, which is characterized by an increase in thickness, and decrease in number, of threads. The pachytene threads do not show any sign of duality, and there is no more polarization (Figs. 7, 8). In the strepsitene stage (Fig. 9) each pachytene thread breaks up into two fine twisted threads; then the threads contract, increase their thickness, undo the twisting, and acquire the specific feature of each tetrad. No confused or cryptosome stage (McClung, 1927) exists, nor any second contraction stage. Through the whole growth period, the secondary split is not observed clearly,

and the strepsinema looks like two long rods twisted loosely about each other. But I am not certain as to whether the crossing of the two rods is formed by simple twisting, or by the intersection of two rings which are due to the opening apart of the pachynema, one in the reduction plane and the other in the equational plane, as usually the case with orthoptera. In the diakinesis (Fig. 10) all the tetrads are scattered about in the peripheral portions of the nuclear space, and only a slight shortening and condensation occur: this stage ends with the formation of the mitotic spindle of the first reduction division.

B. Mataphase and anaphase

In the equatorial plate of the first maturation division, there are always 20 tetrads (Figs. 12–16). The serial alignment of these tetrads is possible according to their size and shape. This is true at least in respect to the eight larger ones (A–H in Fig. 29), as I have done with the spermatogonial homologues. Tetrads A–E are V–shaped and tetrad F is straight and long; while tetrads G and H can hardly be distinguished one from the other by their size; however the latter is heart-shaped and lies always in the peripheral portion of the equatorial plate.

The arrangement of the tetrads in the spindle is similar to that of the spermatogonial chromosomes, that is, the larger six tetrads lie usually in the peripheral portion, small dot-like tetrads are scattered about in the central space, and the oval tetrad G takes its position in the centre of the equatorial plate or among larger tetrads, while tetrad H, as stated above, remains always in the periphery. Occasionally, however, the smallest V–shaped tetrad, E, takes the central position in lieu of tetrad G.

Thus, I have sorted the tetrads into five classes, namely, 5 V–shaped, one rod-like, one heart-shaped, one oval and 12 dot-like, according to their shape, but the case is not so simple as with the chromosomes of spermatogonium, since they do not always exhibit their typical features.

a) *V–shaped tetrads.* There are five tetrads belonging to this type (A–E in Fig. 29). Though they vary in shape and size, they agree in the following features: 1) they are bent more or less in the middle

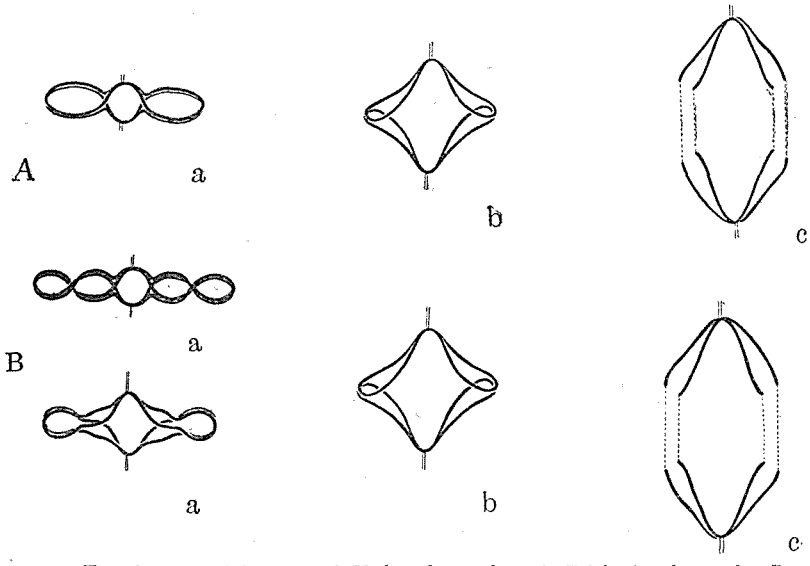
where the spindle fibre is attached, and 2) their tetravalency is not apparent; they appear longitudinally double and the two halves seem to be twisted more or less about each other. These tetrads consist of a series of three, four or five rings. Tetrad A consists of four or five rings, which are all situated in the equatorial plane, except the one in the middle, which is vertical to the plane (Text-fig. 1, B, a). Tetrads B, C and D are formed of three rings, of which the middle ring is vertical to the lateral rings (Text-fig. 1, A, a). To these tetrads, A–D, the spindle fibre attaches at the middle of each half of the middle vertical ring. Contrary to these multiple-ringed tetrads, tetrad E consists of a simple γ-shaped body. The tetravalency of the tetrads is not apparent through the whole metaphase.

The longitudinal rod-tetrad with median fibre-attachment is found in the equatorial plate of the first miosis of many animals and also in that of plants. The V–shaped tetrads of the snake remind one of this kind of tetrad found in higher plants, such as *Trillium*, *Lilium*, etc., and also in urodeles (JANSSENS, 1901), but it is not clear whether this resemblance is real or only seeming.

In the anaphase, the two threads, instead of undoing twist as in the cases of plants and urodeles, split each into two identical parts along the equatorial plane through its whole length. To take a medium-sized tetrad like B for example (Fig. 18, a), the middle vertical ring is divided into an upper and a lower half-ring, while the lateral rings are separated each into an upper and a lower ring by a horizonal cleft. This process begins at the middle of the tetrad and proceeds toward the lateral parts of the same; and it appears as though the two halves of the tetrad were drawn apart by the spindle-fibres (Text-fig. 1, A, b, c). Tetrads C and D also divide in this manner. Anaphase features of all these tetrads suggest that they are of the same structure as the ring tetrad found in many orthoptera, that is, two splits appear in the tetrad, one on the equational plane and the other on the reductional plane, and separate the four chromatids, so that there appear three rings of which the middle ring stands vertical to the plane of the lateral rings (Text-fig.

1, A, a). On the structure of tetrad A no special observation has been made, but from the anaphase figure (Fig. 18 b), it is surmised that its structure is identical in principle with that of the above triple-ringed tetrads. As to the formation of one or two additional rings in that tetrad, it is possibly due to the fact that one or both of the lateral rings of the original three rings remains twisted somewhat in the middle as a remnant of the strepsitene twisting, giving the ring the appearance of two rings instead of one (Text-fig. 1, B).

At the end of the anaphase, all these tetrads divide each into two daughter halves. These are dyads, which are double V's composed each of two V-shaped monads connected at their apices, where the spindle fibre attaches. The dyad travels, in the spindle, with this apex



Text-figure 1. Diagrams of V-shaped tetrads. *A*. Triple-ringed tetrad; *B*. Multiple ringed tetrad.
a. Metaphase; *a'*. Early anaphase; *b*. Anaphase; *c*. Late anaphase.

towards the pole (Fig. 20). From the above fact, it is clear that this tetrad represents the anaschistic V-shaped tetrad of WILSON (1925).

b) *Rod-like tetrad.* There is only one tetrad that belongs to this type (F in Fig. 29). The tetrad of this type seems to be longitudinally double and forms two or three rings in connected series. The

tetrad lies along the radius of the equatorial plate, and the spindle fibres are attached to its inner end. The construction of this tetrad has not been observed minutely. At any rate it divides into two dyads each of which is simple–V–shaped and composed of a pair of monads connected at the point of attachment of the fibre. Thus, the tetrad of this type appears to represent what WILSON calls (1925) the anaschistic rod-tetrad with the terminal attachment of spindle-fibre. This judgment is supported further by the mode of the separation of these two rod-like monads in the second division, as will be mentioned later.

c) *Heart-shaped and oval tetrads.* The distinction between these two tetrads (G and H) has previously been stated, but this distinction is not constant, the condition varying more or less according to the degree of condensation (Figs. 12–16). As to the formation of tetrad H, I will give an account later in the section dealing with the fate of the nucleoli during the growth period. On the equatorial plate, tetrad H takes the form of the ring-tetrad frequently found in orthoptera. In the anaphase stage, it divides along the equatorial plane (Fig. 19 b) into two simple-V-shaped dyads (Fig. 30). The dividing plane of tetrad G is likewise equatorial (Fig. 19 a); but whether the mode of division of this tetrad belongs to the same type as that of tetrad H or not, is not clear.

d) *Dot-like tetrads.* The dot-like tetrads are 12 in number (Fig. 29). Though I have arranged them in series in Fig. 29, it does not mean that I have been able to distinguish one from the others by their size and shape. They are merely small dot-like bodies and show no sign of duality.

Every tetrad of the first spermatocyte divides into two equal parts in the anaphase, as shown in Fig. 20, so that each daughter nucleus receives an equal set of chromosomes. Figs. 21a and 21b are the polar views of two sets of anaphase chromosomes which are daughter halves of one metaphase-complex of the first spermatocyte, and there are 20 dyads, 8 larger and 12 smaller, in each figure.

The attachment of the spindle-fibre is centromitic in the cases of tetrads A–E, G and H, while, as previously stated, to the

tetrad F, the fibre attaches telomitically. The point of the fibre-attachment is easily recognized as a dark spot on each tetrad, when this is faintly stained with haematoxylin (Fig. 17). This spot is probably identical with the "granule proximale" described by JANSSENS (1924) in *Stetophyma grossum*, though I was unable to recognize it in the growth period.

When one makes a comparison with reference to the relative magnitude and the specific feature between each spermatogonial chromosome on the one hand and each tetrad on the other, the correspondence of the homologous pairs of chromosomes of the former with the tetrads of the latter becomes apparent, as shown in Figs. 28 and 29.

3. *Chromosomes of Second Spermatocyte*

The number of the chromosomes which appear in the metaphase of the second spermatocyte is 20 without exception (Figs. 22–26). These chromosomes appear in the identical shape which we noted in the anaphase of the foregoing division (Fig. 21), so that, besides 12 dot-like chromosomes one can easily distinguish five V-shaped A–E, a long rod-like F and two oval G and H (Fig. 31). Sometimes, however, V-shaped dyads undergo a slight change during interkinesis, a slight shortening and thickening occurring in both arms of the V's, and the angle between them becoming larger (Fig. 24). Thus, the V-shaped dyads have a firmer appearance than the anaphase ones of the foregoing division, and take a crescent shape. The larger dyads consist each of two superimposed monads in the polar view connected at the point of attachment of the fibre. They are accordingly double V's composed each of two V-shaped monads connected at the point of flexure. Tetrad F is made up of two long straight monads united at one end into a single V. The plane which includes the two arms of this single V is placed nearly vertical to the equatorial plate, and one arm of the V being placed nearer the pole, V may appear as a simple rod in the polar view. The arrangement of the dyads in the equatorial plate is similar to that in

the first spermatocyte, but in this case G and H are almost always situated among the larger dyads in the outer circle.

Figs. 27a and 27b are polar views of two sets of anaphase chromosomes on the second maturation spindle. We recognize five V-shaped, one rod-like and 14 dot-like monads in each set. Thus, every dyad divides into two monads and each spermatid contains the same set of 20 monads.

4. Fate of Nucleoli during Growth Period

The leptotene nucleus contains three deeply-stained compact bodies; they are nucleoli, two of which are the karyosomes (k) and the third is the plasmosome (p) (Fig. 6). Usually, the karyosomes are oval in shape and lie close together; frequently they are united at the sides. In some cases, one of the karyosomes appears to be spheroidal in shape rather than oval; but this difference in shape is not constant and is often imperceptible in advanced stages. The plasmosome is a uniformly stained spheroidal body rarely appearing hollow, and lies always apart from the karyosomes. All of these nucleoli are situated at the periphery of the nuclear cavity close to the nuclear wall, throughout the growth period.

From the later leptotene stage on, the nuclei increase in size and pass into the pachytene stage (Figs. 7, 8). Meanwhile, both the karyosomes and plasmosome swell into size two or three times the original. In this stage two karyosomes become united, at a point near one end, into a bipartite body; while the plasmosome often appears as an oval body.

The increase of the nucleoli in size reaches its limit in the pachytene stage. Then, the plasmosome gradually decreases in size. The two karyosomes, on the other hand, become more closely united, so that the duality is revealed only at one end; a heart-shaped tetrad is formed in this way by the conjugation of the two karyosomes. In the diakinesis (Fig. 10) the plasmosome still remains; and, though it was impossible for me to

trace its fate further, it probably vanishes into cytoplasm as the nuclear wall disappears.

In the early metaphase (Fig. 11), after the nuclear wall has disappeared, but before autosomal tetrads form the equatorial plate, the karyosomal tetrad (H) is clearly recognizable as a heart-shaped body.

GENERAL DISCUSSION

1. *Chromosome Number*

The difficulty in studying reptilian and avian chromosomes lies mostly in the extreme smallness of the dot-like chromosomes and in their tendency to fuse together. PAINTER (1921) writes that in lizards there are strands of lightly-stained material which connect the small chromosomes. According to HANCE (1926), the number of chromosomes of the fowl is subject to a certain range of variation, this apparently being due to the fusion of some of the smaller chromosomes, and not due to their fragmentation as in the case of the pig (same author 1917).

In the present material all the small chromosomes are well separated and appear in a constant number, so that accurate counting is possible with the same certainty as in the works by previous authors on insect spermatogeneses. As stated above, the spermatogonial metaphase shows 40 chromosomes—five pairs of V-shaped, a pair of long rod-like, two pairs of short rod-like and 12 pairs of dot-like chromosomes (Fig. 28). In the first maturation division these chromosomes are all paired into 20 tetrads (Fig. 29), which divide into 40 dyads (Fig. 30). In the metaphase of the second maturation division all the 20 dyads appear (Fig. 31); and each spermatid receives 20 monads—five V-shaped, one rod-like, two oval and 12 dot-like ones (Fig. 32).

The chromosome-number of an animal can be ascertained with accuracy only when the whole history of each spermatogonial chromosome is traced through all miotic stages, including the stage when the homologous pair of the spermatogonial chromosomes are separated and the resultant two sets of chromosomes (haploid ones) are distributed into

gametes, one to each gamete. Here, in *Natrix tigrina*, each of the spermatogonial chromosomes, A–H and 24 dot-like ones, have been identified all through the reduction divisions. Thus, there is no room for doubt that the diploid number of chromosomes of the male of this snake is 40.

2. *Sex-chromosomes*

The central interest of the present work lies in the question as to how sex is determined in this snake, in other words, whether the male is homozygous or heterozygous in regard to sex. In order to be certain of the condition of the sex-chromosome, the following five points must be made clear: (1) the number of spermatogonial chromosomes for the male, (2) the number of tetrads in the first spermatocyte and their behavior, (3) the number of dyads in the second spermatocyte, (4) the number of chromosomes passing into the spermatid, (5) the diploid number of chromosomes for the female. These points are mentioned by PAINTER (1921) in a study of the chromosomes of some lizards; but, in addition to them, I should like to emphasize a sixth point, namely, the behavior of the karyosome during the growth period.

As characteristic features of the sex-chromosome in the growth period, the following three facts are commonly accepted: that (1) it has a remarkable affinity to the basic dyes, (2) it remains always in the peripheral portion in the nucleus and (3) it grows in size more or less and persists in the chromosome complex of the equatorial plate. Of these three features, the nucleoli answer well to the first and second. With reference to the third feature, however, we are able to identify only the karyosome with the sex-chromosome, because, while the karyosome has this feature also, the plasmosome vanishes entirely previous to the metaphase. In the leptotene nucleus of this snake there are three nucleoli (Fig. 6); and, judging from their later history, which has been stated in the former section, we are able to distinguish one of them as the plasmosome and the other two as the karyosomes. These karyosomes

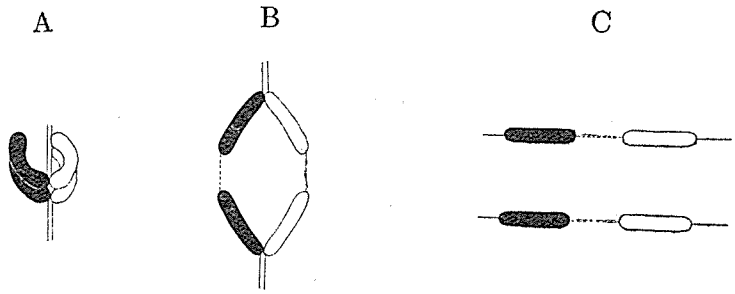
develop into a heart-shaped tetrad (H in Figs. 12–16) found among the metaphase complex of the first maturation division. Therefore, having the characteristic features of the sex-chromosome, this tetrad H has been referred to the sex-chromosome-complex.

Tetrad H is a heart-shaped body in the polar view, and retains the line of the fusion of the two component karyosomes, which might easily be mistaken for the beginning of a longitudinal cleft, (the plane of contact of the synaptic mates of the tetrad are perpendicular to the equatorial plate). In the anaphase this tetrad divides itself along the plane which is parallel to the equatorial plane, and the resultant dyads are again V-shaped or bipartite bodies. Thus, the tetrad is reduced quantitatively by the first maturation division. On the equatorial plate of the second spermatocyte metaphase, dyad H does not appear in the shape of a flat V in the polar view, but as an oval body, showing that it consists of two oval monads one superposed upon the other. Among each complex of the anaphase chromosomes, two oval monads are recognizable one of which evidently being the resultant of the separation of the members of dyad H. This fact shows that, the second maturation division is apparently a qualitative reduction for tetrad H. The mode of the divisions described above, shows that this tetrad H belongs to the tetrad called by WILSON (1925) "diaschistic V-shaped tetrad": Text-fig. 2 illustrates diagrammatically the mode of division of this tetrad.

Since spermatogonial chromosomes are exactly twice as many in number as the tetrads, tetrad H must be composed of two spermatogonial chromosomes. When one compares the tetrad-groups with the spermatogonial groups, it becomes clear that one of the two chromosome pairs, G and H, is represented by tetrad H. Besides this evidence, we know that spermatogonial chromosomes are 40, the tetrads of the first spermatocyte 20, the dyads of the second spermatocyte 20, and the monads passing to the spermatid are 20 in number.

These facts show unequivocally that the sex-chromosomes in the diploid complex are two, and after the two maturation divisions, the resultant four elements are distributed among the spermatids separately,

giving a material proof of what PAINTER has stated (1921) that "the author is of opinion that when the common vertebrates are restudied with improved technique, it will be found that the bipartite X-element



Text-figure 2. Diagram of the formation and division of heart-shaped tetrad H. *A.* Tetrad stage; *B.* First division; *C.* Second division.

arises from two spermatogonial chromosomes, and that its bipartite form during the first maturation division is an expression of this bivalency, and not the precocious appearance of the plane where the second maturation spindle will separate these elements."

When two sex-chromosomes exist in the diploid complex, the formula of the sex-chmomosome must be XY or XX. The Y-chromosome, if present, is usually distinguished without difficulty from X by its smaller size, and often also by a difference in behavior during the growth period, such as, the rate and degree of condensation. Between the two sex-chromosomes of this snake, however, there is no difference whatever, not only in shape and size, but in staining capacity also, by which one is able to differentiate one from the other. Thus, the formula of sex-chromosomes of this snake is very probably XX, and not XY, showing that the snake belongs, in respect to sex, to the type of birds and lepidoptera. If we substitute Z and W for X and Y, the chromosome-complex may be formulated as follows:—

38 + Z + Z = 40 ..... spermatogonium
19 + ZZ = 20 ..... first spermatocyte
19 + Z = 20 ..... second spermatocyte
19 + Z = 20 ..... spermatid

If the male is homozygous in respect to sex, the female should be heterozygous and the chromosome-complex formulated as (AA+ZO) or (AA+ZW). Therefore, the chromosomal formulae of *Natrix tigrina* are (38+ZZ) for male and (38+ZO) or (38+ZW) for female. But the ultimate decision of the validity of this formula must be deferred, until the condition of the chromosomes in the female has been worked out.

SUMMARY

1. In the metaphase of the spermatogonial division there are 40 chromosomes making 20 homologous pairs, consisting of 5 pairs of V-shaped chromosomes, 1 pair of long rod-like, 2 pairs of short rod-like, and 12 pairs of dot-like chromosomes.

2. The number of the tetrads present in the metaphase of the first spermatocyte is 20, including 5 V-shaped, 1 long rod-like, 1 heart-shaped, 1 oval and 12 small dot-like tetrads.

3. The construction of the tetrads is similar to that of the orthopteran tetrads, V-shaped and long rod-like ones being multiple-ringed tetrad, and heart-shaped and oval ones being ring tetrads, while dot-like tetrads have no expression of duality.

4. The number of the dyads which appear in the second spermatocyte is 20; they are of the same size and shape as those in the anaphase of the last division. Each chromosome complex, accordingly, consists of 5 V-shaped or curved rod-like, 1 long rod-like, two oval and 12 small dot-like chromosomes.

5. Each dyad divides into two equal monads passing into the spermatids, so that each spermatid receives an identical set of chromosomes.

6. One of the two short rod-like homologous pairs of spermatogonial chromosomes are the sex-chromosomes.

7. In the leptotene nucleus the sex-chromosomes take the form of karyosomes, which unite and develop into a heart-shaped tetrad.

8. The first maturation division of this tetrad is a quantitative reduction

and the second is qualitative.

9. Between the two sex-chromosomes there exists no difference by which one can distinguish the one as X and the other as Y, so that the condition of the sex-chromosomes in the male seems to be of the ZZ-type, and homozygous in respect to sex.

10. The chromosome-complex of the male may be formulated as 38 + ZZ.

LITERATURE CITED

CHURA, A., 1925. Beitrag zur Fixation der Mitochondrien. *Zeits. wiss. Mikro.*, Bd. 42.

DALCQ, A., 1921. Etude de la spermatogenèse chez l'orvet (*Anguis fragilis* LINN.) *Arch. Biol.*, T. 31.

HANCE, R. T., 1917. The diploid chromosome complexes of the pig *(Sus crofa)* and their variations. *Jour. Morph.*, Vol. 30.

1926. The chromosomes of the chick soma. *Biol. Bull.*, Vol. 51.

JANSSENS, F. A., 1901. La spermatogénèse chez les Tritons. *La Cellule*, 19.

JORDAN, H. E., 1914. Spermatogenesis in *Chrysemys marginata* and *Cistudo carolina*. *Science*, N. S., Vol. 43.

MCCLUNG, C. E., 1927. Synapsis and related phenomena in *Mecostethus* and *Leptysma* (Orthoptera). *Jour. Morph. Physiol.*, Vol. 43.

NAKAMURA, K., 1927. Preliminary notes on reptilian chromosomes I. The chromosomes of some snakes. *Proc. Imp. Acad.* (Tokyo)., Vol. 3.

PAINTER, T. S., 1921. Studies in reptilian spermatogenesis I. The spermatogenesis of lizards. *Jour. Exp. Zoal.*, Vol. 34.

THATCHER, L. E., 1922. Spermatogenesis of the garter snake. *Science*, N. S., Vol. 56.

WILSON, E. B., 1925. The cell in development and heredity. 3rd Edition (New york).

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida using a Zeiss 1·5mm. apochromatic oil-immersion objective and a K. 18 ocular, tube-length 16cm.

Plate I

Figs. 1–5. Polar views of the equatorial plates of the spermatogonia. 40 chromosomes are seen in each figure.

Fig. 6. The first spermatocyte nucleus in the leptotene stage. p=plasmosome k=karyosome.

Fig. 7. The same in the early pachytene stage. p=plasmosome k=karyosome.

Fig. 8. The same in the pachytene stage. p=plasmosome k=karyosome.

Fig. 9. The same in the strepsitene stage. p=plasmosome k=karyosome.

Fig. 10. The same in the diakinesis. p=plasmosome k=karyosome

Fig. 11. Tetrads in the early metaphase, not yet arranged in the equatorial plate. H=sex-chromosome complex

Figs. 12–16. Polar views of the equatorial plates of the first spermatocytes, 20 tetrads are seen in each figure. H=sex-chromosome complex.

Fig. 17. The same, faintly stained. The "granules proximaux" are seen as black spots.

Fig. 18a. Polar view of tetrad B in early anaphase.

Fig. 18b. Polar view of tetrad A in the early anaphase.

Fig. 19a. Polar view of tetrad G in the early anaphase.

Fig. 19b. Polar view of tetrad H in the early anaphase.

Fig. 20. Side view of the first spermatocyte anaphase.

Fig. 21. Polar views of the daughter complexes of the first spermatocyte tetrads in the anaphase. a represents lower and b represents upper sets, in successive sections; 20 dyads are seen in each figure.

Figs. 22–26. Polar views of the equatorial plates of the second spermatocytes; 20 dyads are seen in each figure.

Fig. 27. Polar views of the daughter complexes of the second spermatocyte dyads in the anaphase; 20 monads are seen in each figures.

Plate II

Chromosomes in serial arrangement

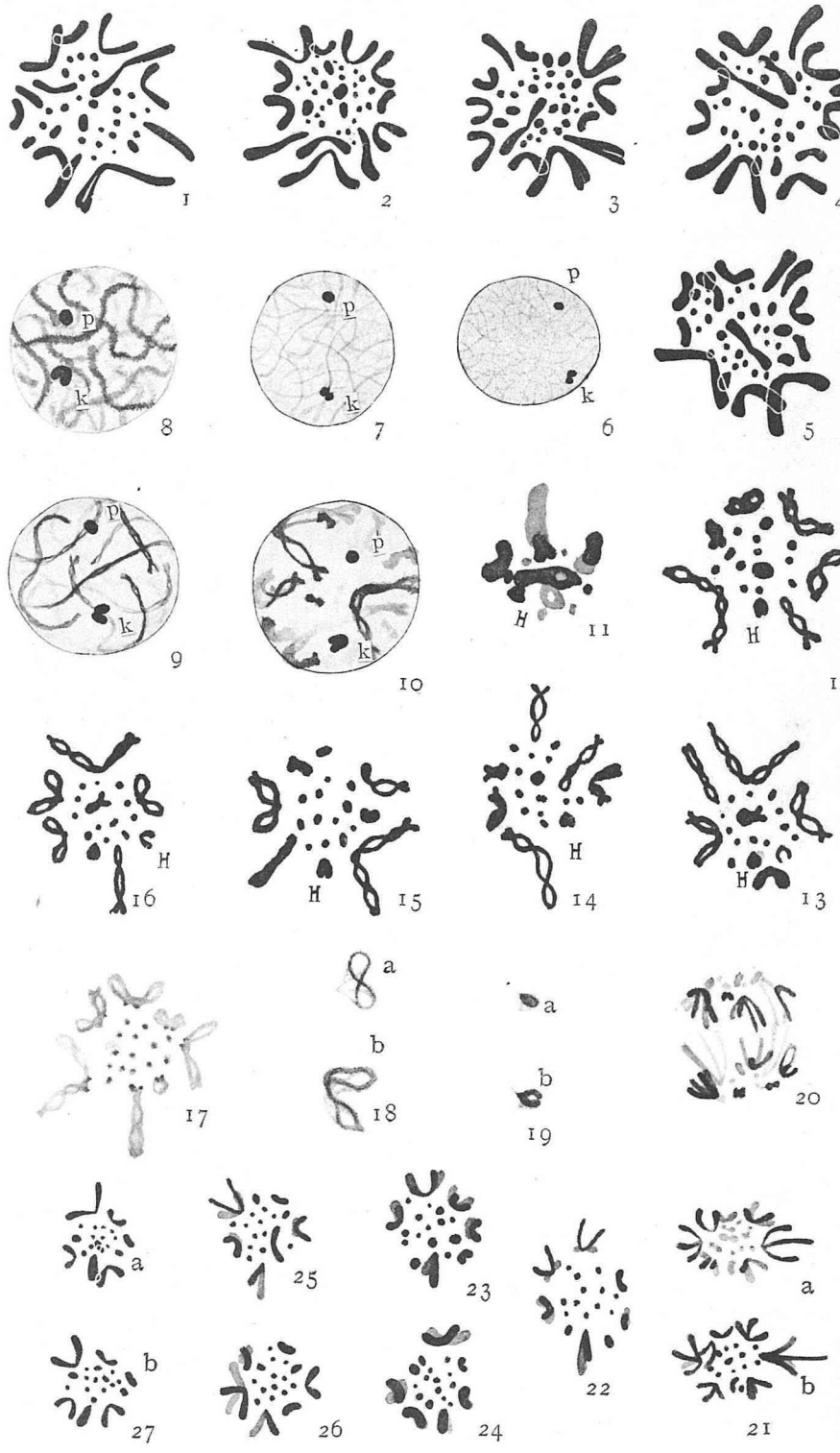
Fig. 28. Spermatogonial chromosomes, which are paired into the synaptic mates (from Fig. 1). The pairing of dot-like chromosomes expresses no more than that they exist in even number.

Fig. 29. The tetrads of the first spermatocyte metaphase (from Fig. 12).

Fig. 30. The two daughter sets of the first spermatocyte tetrads in the anaphase (from Fig. 21a, b).

Fig. 31. The dyads of the second spermatocyte metaphase (from Fig. 23).

Fig. 32. The two daughter sets of the second spermatocyte dyads in the anaphase.



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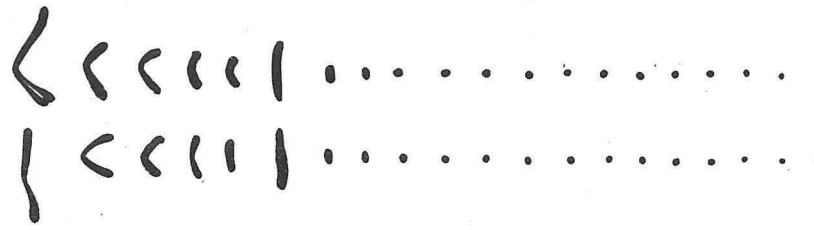


A B C D E F G H

28

A B C D E F G H

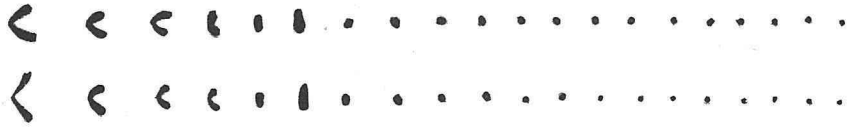
29

A B C D E F G H

30

A B C D E F G H

31



A B C D E F G H

32