STUDIES ON THE SAUROPSID CHROMOSOMES

I. THE SEXUAL DIFFERENCE OF CHROMOSOMES IN THE PIGEON

By

KAN OGUMA

(With two plates)

INTRODUCTION

Cytologists who once intended to study the avian chromosomes would have always come across a difficulty in getting any good preparations of their dividing figures, enough available to distinguish each chromosome separately in the metaphase plate. The avian chromosomes show in general strong tendency of agglutination to become closely contact or to fuse with each other, as one very often met with in the case of the mammalian chromosomes. Still more, in the birds in general, there are found a lot of extremely small chromosomes which occupy mostly the central part of the spindle, but scatter often among the larger ones surrounding them, and it is these that make confusion of vision upon the determination of the chromosome-number.

To find out any favourable method of fixation of avian chromosomes, on one hand, and to discover, if possible, the mode of sex-determination as regard to the chromosomes, on the other hand, the author has undertaken the investigation of chromosomes of the pigeon, since five years ago. A great interval, however, has been inserted in the course of the study, as I have been about three years in Europe, where I had to spend time chiefly in the study of the human chromosomes, although some parts were still carried on at Liége in the laboratory of Prof. H. DE WINIWARTER, to whom I express my sincerest thanks for his courtesy. The study has been continued after my returning to Japan and was able to accomplish it recently.

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During this interrupted interval some valuable results of revised studies of the avian chromosomes have been recorded by several cytologists; as rewards of their enthusiastic and ceaseless efforts they have thrown a light and suggestion, in a great deal, upon the course of my present investigation. But many points, important and interesting, concerning the avian chromosomes seem to remain still in puzzle, not to be satisfactorily solved. Moreover, the studies are almost confined to the chromosomes of the domestic fowl, and not extended to the bird in question. Our knowledge on the chromosomes of the pigeon is little added since the studies of GUYER (1900), HARPER (1904) and SMITH (1912). Only some suggestions may be found in the recent study of HANCE (1925) which will be brought under consideration later.

As the material of the present study I choosed the Belgian race of the homing pigeon which were reared in the Japanese Military Colombier at Nakano, near Tokyo. They were at first introduced from France after the Great World War, and are considered as the hybrids between the races "Anversois" and "Liégois." The domestic dove reared in Japan since long time seems to have similar condition in chromosome to those of the Belgian races, but it can not be settled, at present, as the study is still carrying on the way.

From the present study I gained the results, (1) that in the pigeon, the unmber of chromosomes is not so small as repeatedly recorded hitherto, (2) that the sex-chromosome is represented by a member of the largest chromosomes, and (3) that the female is heterozygous concerning to the constitution of chromosomes.

MATERIAL AND TECHNIQUE

Under conception that any of revised studies of the avian chromosomes must be undertaken with the material prepared by technique which will preserve the natural condition of dividing chromosomes, as the agglutinated condition of chromosomes, as we very often met with, being considered not to be natural, new methods of fixation have been applied by several authors during these years. And thus SHIWAGO (1924) and HANCE (1923, 1925 and 1926) succeeded to get the excellent figures of avian chromosomes in course of division. The technique employed by the present author seems to be one of the best methods too, as the plasmolysis took place in least degree and consequently the chromosomes show no sign of agglutination to make obscure their individuality. Especially when I applied it to the embryos I have never failed to obtain good figures of dividing chromosomes.

While in testes, on the contrary, it has shown no such constant results as seen in the embryos, sometimes it showed excellent results but sometimes was in vain, the cause is quite unknown at present. At any rate, the results being described in this paper were gained from the material which had been fixated in best of condition. In such a material the chromosomes are seen to form a beautiful rosette in metaphase plate and each of them can be distinguished very clearly as are shown in the annexed plates.

The material was consisted of young embryos and testes of several different ages. The fluid used for fixation is HERMANN's mixture with small amount of urea (1% against the total volume of the mixture), and previously warmed about 40° C. before employed. HEIDENHAIN's iron-haematoxyline was applied for staining of chromosomes and light green as the counter staining in usual way.

For Embryos: As is well known the pigeon lays in general only two eggs as any one clutch. The eggs used in this study were selected from four clutches of different parents. They may be labelled as A, B, C and D in the following description, and the clutch D was destitute of its sister egg. All the eggs were removed into an incubator from the nest as soon as they were laid. After about twenty hours they were taken out and operated. The embryos were grown in best of condition, but they were so young as the sex could not be determined by observing the sexual organs. The egg was opened in warm RINGER's solution (ca 40°C) and the embryonal part was cut free from the yolk mass by aid of scisors and a spatula, then were imbedded into a vial containing the HERMAN's mixture with urea as mentioned above. The mixture previously was warmed in the same incubator with eggs for a long time, until to attain about the same temperature as the material. After immersing the embryos in the fixing fluid, the vial containing it was kept still more about three hours in the incubator, and then let it gradually cool until to get the same temperature with that of the laboratory. In this state the embryos remained more than ten hours, then were washed by running water with very slow velocity to avoid to be broken into fragments. Nothing should be especially mentioned here as to the methods for sectioning and staining, as the usual way was properly applied.

For Testes: The same method was employed, but in cutting material into small pieces I tried two different ways with purpose of comparison of the results obtained. In the first place the testis was cut into possiblest small pieces by means of scisors as usual, and in the second place, I tried to use a knife with a very thin blade, with which very thin slices in total diameter of the testis were made. The latter was applied only in young testis with small diameter. Observing the results obtained from these two different methods, I found that the method of making slices is preferable to making pieces by use of scisors. Dividing figures of chromosomes were abundantly found in the tubules closely applied to the outer envelope of the testis, and all of them were fixated in excellent good condition. It may be considered, therefore, that making thin slices by sharp knife causes less disturbance of the living state of protoplasm than using scisors which may press the cells to some extent. At any rate, the best figures of chromosomes, which were properly used in the present study, were chiefly selected from this kind of material.

THE CHROMOSOMES FOUND IN THE EMBRYONAL CELLS

CLUTCH A

Embryo No. I (figs. 3 and 4).

Excellent figures of chromosomes in division are found in plenty number everywhere in sections. Figures 3 and 4 are two representatives of them. At a glance one can easily recognize that the chromosomes are represented in a great number and they vary in magnitude with gradatory relation. The larger ones seem like loops, while the smaller ones are so small as dots. The latter are situated in the central part of the spindle, and the former are arranged radially along the marginal area of the spindle. The total number of them is constantly sixty one, if we take the dot-like minute chromosomes as distinct ones. Such expectation would be soon arisen in one's mind that this odd number of chromosomes might be due to absence of a single mate of a particular homologous pair.

As the chromosames being arranged in metaphase plate mostly separated from one another, and being, at the same time, not so difficult to pick up any homologous pairs, with exception of some of intermediate size, it should be facile to find out what pair is destitute of the partner, so long as it would not belong to such chromosome of ambiguous magnitude. Under such consideration, I labelled every supposed homologous pairs of chromosomes with the letter *a*, *b*, *c* etc. according to size, and thus every supposed pairs are to be distinguished by the same letter. Then it will be soon noticed that there are present three considerably large chromosomes which should be considered as to form one homologous pair and a half. These three chromosomes under consideration all assume the similar shape; they are bent, without exception, near the middle point of their entire length, representing V-shape with asymmetrical arms, as being considered as possessing submedian attachment of spindle fibre. It seems to be not so easy, however, to determine which one, out of the three, is in solitary state and wanting its homologous partner which are composing a pair. They are so closely allied to each other in their shape as well as in their size, but repeated comparison of them in many different cells compelled us to descide that the longest and largest one is the very chromosome that wanting its partner, as being labelled *a* in the figure. Of course the difference between *a* and *b*'s, next to the largest, is by no means great, sometimes I can hardly distinguish the *a* from *b*'s merely by the length. In this case the shape of the chromosome would be brought under consideration. The chromosome *a* shows its fibre-attachment in most cases as more median than in the case of *b*'s, so its entire form appears like V with equal arms, while the *b*'s assuming in most cases V's with markedly asymmetrical length of arms, as the fibre-attachment shows tendency to become more and more sub-median.

The chromosomes labelled *c*'s are tolerably long; they possess in some circumstances about similar length with *b*'s, but in such cases they appear always much slender. So long they represent similar thickness with *b*'s, they are much shorter and this may be the normal condition of them. In strong contrast to *a* or *b*'s, the chromosome *c*'s have in any cases terminal attachment of spindle fibre, assuming the form of straight rods as shown in fig. 3. In rare cases, however, they are curved strongly, as if they may have submedian fibre-attachment (fig. 4), eventhough in such cases they take still constantly terminal or telomitic attachment of fibres. This mode of the fibre-attachment is a remarkable characteristic of the chromosome *c*; in this respect the chromosome *c* can never be mistaken as *a* or *b*.

The next coming ones in gradation of size are *d*'s, which show again V-shape like *a* or *b*. Their length, however, is much shorter than the latters, being not more than two thirds of *c*'s. The chromosomes *e*'s, *f*'s, *g*'s etc. diminish their length successively according to the alphabetical order as shown clearly in the figure. In brief, besides *b*'s there are no chromosomes which represent allied configuration to *a* by any means, yet they are distinguishable from *c*'s by their length and shape as already mentioned above. Thus we may conclude from present observation of chromosomes of the embryo No. I, (1) the chromosome number is odd (61), (2) there are three markedly large V-shaped ones of allied length, (3) in all probability the largest one of the three is destitute of its mate, so resulting the odd number in the diploid garniture.

Embryo No. II (figs. 5. and 6).

This embryo is of a little advanced stage in development as compared with the sister embryo of the clutch A. The cell body, and the nucleus as well, seems accordingly much smaller than those of the preceding embryo as the cell growing smaller, at least in this stage of development, through successive divisions. As the matter of fact, this will be quite clear if we compare the figs 5 or 6 with the figures 3 or 4. Beside this fact, there are found a remarkable difference between these two embryos as regards the number of chromosomes. They are represented, in this embryo, by sixty two of distinct ones, one more than in the preceding embryo. And at the same time, it can be soon found that the V-shaped chromosomes of huge size are four, instead of three, which may compose two distinct homologous *aa* and *bb* pairs. They show quite similar appearance in shape and size, to those found in cells of the embryo No. I, having submedian attachment of the spindle fibres and taking their position in arrangement unexceptionally in peripheral part of the metaphase plate, as being readily recognizable in the figures.

The chromosome *c*'s are also represented by a pair of straight (fig. 6) or curved (fig. 5) rods with invariably terminal attachment of fibres, just like those we have already found in the embryo No. I. The chromosomes *d*'s again appear as small V's, but the chromosomes *e*'s take the from of rods in fig. 5, while V-shape in fig. 6. The another smaller chromosomes may vary in their shape in different cells, and every two compose a homologous pair like the preceding embryo.

The embryo No. II is thus characterized in feature of the chromosome; (1) the chromosome number is even (62), (2) there are four considerably large V-shaped ones with allied length, and (3) these four compose two homologous pairs.

From the data described above such consideration must be born naturally in one's mind, that the difference of chromosomes garniture in these two embryos from one and the same clutch may indicate, at the same time, the difference of sex between them. If the embryos were of much advanced stage of development, or grown enough available to distinguish the sex, the problem should be solved without great difficulty. The matter is, however, not actually so, but they are in fact too young to determine their sex from the sexual organs. Before discussing this point in detail I wish to examine the embryos from another clutches with purpose to see, whether the similar chromosomal difference stills occurs between embryos of the same clutch or not.

CLUTCH B

Two embryos (No. III and IV) of this clutch represent the similar composition of chromosomes in contrast to the preceding clutch. In both embryos every cell possesses sixty one chromosomes as the embryo No. I of the preceding clutch, as the chromosome *a* being found always without mate. In fig. 7 are shown the three chromosomes of the largest size which may be considered as one *a* and two *b*'s, from a retina cell of the embryo No. III, and in fig. 8 the corresponding chromosomes from a nerve cell of the sister embryo (No. IV). The chromocome *a* represents a close resemblance to that of the preceding clutch both in shape and size, the two arms of its V-shaped body being much alike each other than in the *b*'s, and in magnitude it seems to be the first in the three. In this respect, therefore, the two embryos of this clutch must be considered as of the same category as the embyro No. I of the clutch A.

CLUTCH C

Again, in this clutch, I met with the chromosomal difference existing between two sister embryos as in the case of the clutch A. In the embryo No. V (fig. 9) the chromosome garniture of a cell is composed of sixty one distinct chromosomes as seen already in the embryos No. I, No. III and No. IV. The chromosome *a* is found exclusively in solitary condition without the partner, while two *b*'s form a complete pair. Fig. 9 represents three largest chromosomes, single *a* and two *b*'s, picked from a nerve cell.

In the cell of the sister embryo, No. VI, on the contrary, I can count without doubt sixty two chromosomes, among which four of huge sized and V-shaped chromosomes can be easily distinguished as shown in fig. 10. The cell is one of the mesenchyme cells and the chromosomes seem much smaller than those dealt with above. Still we can recognize the two of them are larger than the others, as being identified as a pair of *a*'s. The embryo, therefore, must belong to the same category as the embryo No. II, instead of its sister embryo No. V.

CLUTCH D

In the present clutch I could found no sister egg at all, as stated previously; the mother pigeon laid eggs no more after laying the first egg, as we have occasionally experienced. The embryo developed from this egg, No. VII, possesses sixty two chromosomes as their diploid group. As has been tried above, four of the largest chromosomes are drawn in fig. 11 in series. They are taken from a nerve cell of larger size. All these four represent similarly V-shape having asymmetrical arms, and we can readily identify them as two *a*'s and two *b*'s. Thus the embryo No. VII is of the same sort as the embryo No. II and VI as regards constitution of the chromosomes.

Looking through the fasts discovered by observation on the seven embryos from four different clutches, it becomes clear that there are two distinct kinds of individuals possessing different composition of chromosomes; *viz.* one having sixty two with the chromosome *a* in pair, and the other only sixty one in lacking one of *a*'s. The numerical ratio of these two kinds of embryos seems to be nearly equal so far as the present study shows, although it has not been extended to a great number, enough to descide this important point. Four embryos, I, III, IV and V, out of the total seven, are the individuals of sixty one chromosome type, and the remaining three, II, VI and VII, belong to the another type, in which sixty two chromosomes are to be found.

The difference of the two types is merely due to the condition of the chromosome *a*, and nothing is concerned to the nature of the remaining chromosomes. If the difference, consequently, denotes the sexual diffence at the same time, as expected previously and as widely observed in other kinds of animals, the chromosome *a* should be taken as the X-chromosome. It is still obscure, however, to take which type as the male or the female, until we can make clear the chromosome group in the adult pigeons, in which the sex has been already settled. To determine this point I studied the condition of the chromosomes included in the male germ cells as described in detail in the following chapter.

THE CHROMOSOMES FOUND IN THE MALE GERM CELLS

Everywhere in my preparations, especially in those prepared by slicing method of fixation of testes, I could find plenty splendid figures of chromosomes in metaphase of division. Not only the spermatogonia but also the spermatocytes, both primary and secondary, are found in a great number in favorable condition to study. All the dividing cells were found in several parts of the seminal tubules, but those found in the tubules in close contact with the outer envelope of the testis are the best in condition to allow the close investigation of chromosomes, for, in such tubules all the cells and the chromosomes as well, were preserved without a least shrinkage of protoplasm. The following data have been gathered only from the observation of such cells and not of the cells lying in deeper parts of the testis, otherwise any correct observations would not be expected.

THE SPERMATOGONIUM

The diploid group of the chromosome is first brought under consideration. In a spermatogonium the chromosomes are generally much thicker and shorter, as compared with those found in embryonal cells, as one can readily recognize in figs. 1 and 2.

The chromosomes of larger kind take their position, in arrangement upon the equator of dividing cells, always in the peripheral part of spindle and those of the smaller size usually occupy the central part, surrounded by the former, as seen in any kind of embryonal cells already dealt with. Individual chromosome can be distinguished from one another without slightest doubt, excepting a few of minute ones. Even the latter, as I am sure, shall not be able to count up otherwise than I did.

My careful counting shows the diploid number of chromosomes is sixty two, and the chromosomes *a*'s, *b*'s and *c*'s are obviously identified as in the embryonal cells. Similar to the case of the latter cells four chromosomes of huge size, *a*'s and *b*'s, also assume V-shape, with the arms of asymmetrical length, or having submedian attachment of the spindle fibres, while the chromosomes *c*'s unexceptionally take appearance of straight rods. In striking contrast to the embryonal cells, there are found none of the small sized V's as represented often by the chromosomes *d*'s, *e*'s etc. All the chromosomes, smaller than *c*'s in size, take invariably rod-shape, straight in most cases, but rarely bent a little, while the minute ones appear like dots. Consequently the chromosomes *a*'s and *b*'s are to be observed as marked configurations with extraordinal clearness among the remaining chromosomes, not only by their huge size but also by their shape.

THE PRIMAY SPERMATOCYTE

A lot of the metaphase plates of the first maturating division were observed. The chromosomes of the bivalent condition are so arranged as shown in the four figures (figs. 12, 13, 14 and 15) of the plate. Here we find thirty one chromosomes in their reduced number, among which three of remarkably large size call our attention. These three are arranged widely apart from each other. The largest one takes the form of thick V-shape in every cell, while the second and the third ones represent often the form of closed ring (fig. 14, *b*). The remaining smaller chromosomes assume various form of tetrad nature; some one ring (fig. 15, *e* and *f*), some one cross (figs. 12, *f* and 15, *g*). But those of very small size, on the other hands, show nothing of tetrad constitution, in as much as they appeare as extremely short rods or dots.

Our special attention will be turned first upon the largest forms of chromosomes. Judging from their relative magnitude the three must be *a*, *b*, and *c*, respectively in their bivalent condition. When the anaphase of division sets in, the bivalent *a*, *b* and *c* separate themselves into similar halves as shown in fig. 17. In this stage they are characterized by the largest ones among daughter garniture of the divided chromosomes. Fig. 18 is a polar view of such garniture, in which they can be identified with clearness. They are found often retard in the course of removing to the poles, while nearly all the remaining smaller ones have reached the poles destined to go. This phenomenon seems to be due only to the weight and magnitude of the chromatic substance, from which they are camposed; the smaller and lighter ones remove usually much sooner than the larger and heavier ones like *a* or *b*. By this time, of course, all the chromosomes of smaller size separate themselves into similar halves as the larger forms, as being recognizable in fig. 18, where we can count thirty one, just the same as in the case of metaphase.

There are no particular chromosomes to be considered as the heterotrophic chromosome as observed in the fowl by GUYER (1916). And thus all the daughter cells possess equal number of chromosomes.

THE SECONDARY SPERMATOCYTE

As expected, from the mode of division of chromosomes during the course of the first maturating division, the resulted secondary spermatocytes should possess all kinds of chromosomes, which once have been found in their mother cells. In fact, we can find thirty one univalent chromosomes in every secondary spermatocytes. Figs. 19 and 20 are the example of the equatorial plates of the second maturating division. The chromosomes *a*, *b* and *c* can be soon pointed out of the smaller ones by their considerably large size. But in this case they appear all like rods, though they are sometimes curved more or less.

In the anaphase of the division they are divided into similar halves which are migrating to the opposite poles, although it is quite difficult to count them accurately in the daughter garnitures, as the minute ones are becoming, through successive divisions, smaller and smaller. At any rate, there can be found no other fashion of division to be considered otherwise than an equational division. Thus I believe that all the spermatids produced as a result of the present division, contain the similar kinds of chromosomes in equal number.

GUYER (1900) and SMITH (1912) record a phenomenon of so-called double reduction, in which the chromosomes are to be reduced again in number in the second spermatocyte division. Such phenomenon in question, however, must be due to agglutinated condition chromosomes resulted by their incomplete mode of fixation. For, in my preparations, the chromosomes are observable quite distinctly in the same number as those of the primary spermatocytes. In the same way, the occurrence of double reduction in man reported by GUYER (1910) has been denied by PAINTER (1923), and WINIWARTER and myself (1926) recently.

From the facts observed in the male germ cells we can induce the following conclusions:

(1) in the male, the diploid number of chromosomes is sixty two,

(2) the chromosome *a* is represented by two homologous ones,

(3) all the spermatids, the spermatozoa eventually, possess the similar number of chromosomes, thirty one, including one *a* in each garniture.

THE X-CHROMOSOME AND THE MALE HOMOZYGOSITY FOR X

By the present study of chromosome, embryional and testicular cells, we know that there are two different garnitures of chromosomes in different individuals of the pigeon. The one kind consists of sixty one chromosomes and the other of sixty two. The former type is found in some embryos studied and the latter type in the remaining embryos as well as in testes. A precise conclusion will be valid thereby, that the embryos whose chromosomes make a garniture of even number are the male individual, since in the testicular cells we find always the similar garniture of chromosomes as mentioned in the preceding chapter. Eventually the remaining embryos having odd number of chromosomes may be taken as the females, eventhough I could not determine their sex by actual observation of the reproductive organs. Thus, a rational interpretation on such discrepancy to chromosome garnitures between individuals can be accepted by us only by supposing the chromosome *a* is nothing but *X*. In the other words, a garniture composed of 60 autosomes plus 2 *X*'s or *a*'s, belongs to the male, while that of 60 autosomes plus one *X* or *a* belongs to the female. Or the female heterozygosity for *X* may be brought under cytological evidence, as already discovered in the domestic fowl. From the genetical point of view, the female heterozygosity for sex in the pigeon has been known through repeated experiments of making hybrids between individuals with plumage of intense colour and those of dilute colour that is considered as a sex-linked factor. We are thus justified to maintain this view with RIDDLE (1917) and COLE and KELLY (1912, 1919), notwithstanding some authors, as STRONG (1912) or BRIDGE (1913) holding an opposed view.

The maturation division of the female cell has not been made clear in the present study, but from possibility of the female heterozygosity for sex, as mentioned above, it will be expected that during maturation processes the single *X*-chromosome would behave hetrotropically, since it has no partner to conjugate with and then segregate from. Two different kinds of mature eggs, therefore, may be produced, one having 30 autosomes plus one *X* and the other merely 30 autosomes without *X* at all.

HARPER (1904) is the only investigator who reports what happens in the maturating divisions in female germinal cells of the pigeon. In his paper the author describes, however, no occurrence of a particular chromosome which exhibits heterotropical nature. Not only such kind of chromosome but also the total number of chromosomes shows a marked difference from the fact discovered in my present study. He informs only eight bivalents as a reduced garniture of chromosomes. This minority of the number of chromosomes, as I believe, is evidently due to the fusion of some chromosomes as occured usually in the preparations of poor fixation. A revised study must be needed, therefore, to ascertain this most important question whether the single *X*-chromosome would display its heterotropical nature during the polar body formation, especially in material prepared by any improved methods of fixation, by which slightest agglutination of chromosomes does not take place.

At any rate, in the present state of our knowledge, it seems not to be precautious to expect the existence of two kinds of eggs, whose chromosome garniture are different in respect to the *X*-chromosome, so far as its unpaired condition in the female cells is to be taken in mind. And at the same time, we have a strong reason to expect the occurrence of a chromosome that behaves heterotropically at a division of maturating process of the egg, just like the case of Lepidopteran eggs discovered by SEILER (1917, 1920, 1921, 1922).

Then, it is quite unnecessary to doubt, the fertilization of an egg with one *X*-chromosome produce a male, having two *X*'s in addition to autosomes, because every spermatozoon possesses similarly one *X*-chromosome, and the fertilization of an egg without *X*-chromosome gives rise to a female, as one *X*-chromosome should be brought in merely by the spermatozoon.

The male homozygosity for *X* in pigeon, on the contrary, can be clearly suggested even from the previous studies. When GUYER (1900) published his study on spermatogenesis of normal and hybird pigeons, he described already equal distribution of chromosomes at any maturating division. But he informed, in the case of normal pigeon, that a chromatic body having an appearence of a chromosome is eliminated from a nucleus so early at the time when the nucleus is ready to commence its maturating process. This extraordinal phenomenon has not been cleared up in the present study. I have found no such occurrence in my material. Whatever the fact may be, there is no evidence in adverse to the homozygosity of the male in pigeon.

The paper of SMITH (1912), appeared next to that of GUYER, on the spermatogenesis of the pigeon, also proves the facts found by Guyer. He found just equal number of chromosomes, as the latter author did, and no occurrence of heterotropic chromosomes, all the chromosomes being divided symmetrically.

Turning our attention upon the other kind of birds, we have a similar example of the male homozygosity and the female hetrozygosity in the domestic fowl. It has been long believed by majority of authors, that in fowl the female must be heterozygous as in pigeons, as regards the genetic data. Recently it has been proved by actual existence of a single *X*-chromosome in the female cells. In fact HANCE (1923) reports the sexual difference of chromosomes in the chick embryos in which the *X*-chromosome without partner is found only in the female individual, while two in the male. The *X*-chromosome is represented by one of the largest chromosomes. In this respect too, the *X*-chromosome of the fowl reveals a similar feature to that of the pigeon.

In the next year, SHIWAGO (1924) informed a somewhat different result from HANCE's findings in the fowl. In his view the female embryo possesses a small *Y*-chromosome as the partner of the *X*. But still the heterozygosity of the female is not to be altered.

Quite recently WERNER (1925) describes the sexual difference of chromosomes in the Indian runner duck; in the female of this bird he counts 76 autosomes and one *X*-chromosome. By this way, the heterozygosity of the birds in general has been gradually brought under microscopical evidence in favour of theoretical interpretation of genetical data.

SEX-RATIO OF THE SISTER PIGEONS OF A SINGLE CLUTCH

For a long time it has been generally said that one of the two sister pigeons from a single clutch is male, and the other is female. Some breeders believe, on the other hand, the first egg of a clutch produces generally a male pigeon, while the second a female. From extensive experiments of the crossing of pigeons, WHITMAN and RIDDLE conclude that in the case of crossing between two distinct species or genera the first egg shows high tendency to give rise to male and the second to female. How this can be interpreted? I examined with great interest, whether similar occurrence of sex-relation is also to be found in my material dealt with. At least, however, present study has revealed nothing of data favourable to support such conception of sex-relation.

If we acknowledge the heterozygosity in the female, any given one cluth of pigeon eggs produce both sexes in the ratio as shown in the following table, since the single *X*-chromosome may or may not be cast off by chance as the polar bodies which degenerate, just like the case of Lepidoptera, during the maturating process.

Clutch	First egg	Second egg	%
1	♂	♂	25%
2	♀	♀	25%
3	♀	♂	50%
4	♂	♀	

In brief, the case where one male and one female are produced should be 50% in total, and the case of two males and that of two females are 25% respectively. To examine the matter of fact I shall refer here a result observed in the Japanese Military Pigeon Experiment Station. In this station K. TAKESHITA, a veterinary surgeon, has studied under my suggestion the actual sex-ratio of the pigeon reared up in the last year (1925). He selected for this study one hundred examples of dead sister pigeons constituting fifty different clutches, and then dissected them with purpose of determination of their sex only by actual observation of the reproductive organs.

As the table I shows, twenty six out of total fifty clutches produced one male and one female, and in just half the remaining clutches both produced males (clutch 27 to 38), while in the remaining half the both females (clutch 39 to 50). The percentage obtained is just coincide with the expected ratio, 50% ♂♀. 25% ♂♂, and 25% ♀♀.

TABLE I

Clutches in which one male and one female were produced.

No. of Clutch	No. of Pigeon	Date of Laying	Date of Hatching	Sex
1	1505 1506	IV 12 IV 13	IV 30 IV 30	♂ ♀
2	1517 1518	IV 12 IV 13	IV 30 V 1	♀ ♂
3	89 90	IV 5 IV 6	IV 23 IV 23	♀ ♂
4	111 118	II 17 II 18	III 7 III 7	♂ ♀
5	2102 2103	IV 4 IV 6	IV 20 IV 20	♀ ♂
	2134 2135	IV 9 IV 11	IV 28 IV 28	♀ ♂
7	2563 2564	VI 7 VI 8	VI 21 VI 21	♂ ♀
8	2746 2747	IV 30 V 1	V 18 ?	♀ ♂
9	3795 3796	VII 18 VII 19	VIII 5 ?	♂ ♀
10	1140 1141	V 1 V 3	V 20 V 20	♀ ♂
11	3892 3893	VIII 30 IX 1	IX 16 ?	♀ ♂
12	3896 3897	VIII 30 IX 1	? ?	♂ ♀
13	3899 3900	IX 4 IX 5	IX 21 ?	♂ ♀
14	3911 3912	IX 8 IX 9	IX 29 ?	♀ ♂
15	982 983	III 29 III 30	IV 16 IV 16	♂ ♀
16	984 985	IV 4 IV 5	IV 21 ?	♂ ♀

TABLE I (continued).

No. of Clutch	No. of Pigeon	Date of Laying	Date of Hatching	Sex
17	2447 2448	V 12 V 13	V 29 V 29	♀ ♂
1	3727 3728	VI 29 VI 30	VII 16 VII 18	♀ ♂
19	3645 3646	VII 9 VII 10	VII 27 VII 27	♂ ♀
20	3506 3513	VI 11 VI 12	VI 29 VI 29	♀ ♂
21	3575 3576	VI 29 VII 1	VII 18 VII 19	♂ ♀
22	3686 3687	VI 29 VII 1	VII 18 VII 18	♂ ♀
23	2377 2378	V 26 V 25	VI 17 ?	♀ ♂
24	2480 2481	V 21 V 22	VI 7 VI 8	♂ ♀
25	3187 3188	VI 12 VI 13	VI 25 VI 25	♀ ♂
26	3882 3883	VIII 29 VIII 30	IX 15 IX 15	♀ ♂

TABLE II

Clutches in which two males were produced.

No. of Clutch	No. of Pigeon	Date of Laying	Date of Hatching	Sex
27	2092 2093	V 27 V 29	VI 15 VI 15	♂ ♂
28	1624 1625	V 9 V 10	V 27 V 27	,, ,,
29	2141 2190	V 24 V 22	VI 9 VI 9	,, ,,
30	2167 2166	V 7 V 8	V 25 V 26	,, ,,

TABLE II (continued).

No. of Clutch	No. of Pigeon	Date of Laying	Date of Hatching	Sex
31	3928 3929	IX 5 IX 6	IX 22 IX 23	♂ ♂
32	2375 2376	V 29 V 30	VI 16 VI 17	,, ,,
33	2455 2456	V 12 V 13	V 30 V 30	,, ,,
34	3275 3276	VII 11 VII 13	VII 29 VII 31	,, ,,
35	3638 3637	VII 9 VII 8	VII 26 VII 26	,, ,,
36	2512 2513	IV 9 IV 10	IV 26 IV 27	,, ,,
37	3297 3298	VII 28 VII 29	VIII 14 VIII 15	,, ,,
38	2982 2983	VI 15 VI 17	VII 2 VII 2	,, ,,

TABLE III

Clutches in which two females were produced.

No of Clutch	No. of Pigeon	Date of Laying	Date of Hatching	Sex
39	1740 1741	V 28 V 29	VI 14 VI 15	♀ ♀
40	1878 1888	VI 4 VI 5	VI 22 VI 22	,, ,,
41	2957 2958	VI 1 VI 3	VI 18 VI 19	,, ,,
42	3515 3514	VI 16 VI 15	VII 4 VII 4	,, ,,
43	3438 3439	VII 22 VII 23	VIII 8 VIII 8	,, ,,
44	3688 3662	VI 28 VI 27	VII 15 VII 14	,, ,,

TABLE III (continued).

No. of Clutch	No. of Pigeon	Date of Laying	Date of Hatching	Sex
45	2460	V 10	V 29	♀
	2461	V 11	V 29	♀
46	3024	VI 6	VI 25	,,
	3025	VI 7	VI 25	,,
47	3541	VII 4	VII 21	,,
	3542	VII 5	VII 21	,,
48	2675	VIII 13	VIII 30	,,
	2699	VIII 14	VIII 30	,,
49	1094	V 9	V 27	,,
	1093	V 10	V 27	,,
50	3305	VII 11	VII 28	,,
	3306	AII 12	VII 28	,,

Further, another important result can be found from the table I, where one male and one female were produced. The case in which the first egg was male are to be found in thirteen clutches (1, 4, 7, 9, 12, 13, 15, 16, 19, 21, 22, 23 and 24) out of total twenty six. And in the remaining thirteen clutches (2, 3, 5, 6, 8, 10, 11, 14, 17, 18, 20, 25 and 26) the females were produced from the first eggs. In short, the first male case is just 50% of the total cases. So far as the present investgation shows, therefore, the common conception regarding the sex-relation between the sister eggs of a clutch, and the supposed male of the first egg as well, can be hold truth no more. A similar conclusion also can be found in the case of the ring dove studied by STRONG (1912).

The total number of the male is just equal to the females in our material studied. We find namely fifty males against fifty females. The previous records, however, seem to be somewhat different. For example CUÉNOT (1899)* reports the sex-ratio in the pigeon as 105 ♂ : 100 ♀ and the similar ratio seems to be accepted by COLE and KIRKPATRIK (1915)* as the average ratio of sex. The male is a little excess against the female, like majority of other higher vertebrates. Furthermore, a great preponderance of the male was recorded by RIDDLE (1917) in the

* Their original papers were not accessible to me.

case of crossing between two distinct genera. If our observation, therefore, would be extended to a greater number of cases, then the sex-ratio now obtained may suffer in more or less alteration.

It can be expected, on the contrary, that the sex-ratio of the pigeon is really 100 ♂ : 100 ♀ like the present result, so far as we examine it in the birth rate, not in examination of grown birds taken at random.

I have a ground to expect this, when we turn our attention to the results investigated on a coccid insect by SCHRADER (1923). A coccid, *Pseudococcus*, has been believed to have much preponderance of female, if we examine the insects taken at random from nature. But the study of SCHRADER, who examined the sex-ratio in descendants of definite mothers reared in laboratory, instead of random capture from nature, disproved the fact. His result shows that the sex-ratio of this insect in question holds nearly equal ratio as 100 ♂ : 100 ♀ ; no preponderance of the female insect was actually observed.

I shall be able to have an opportunity to report again other results concerning the sex-ratio of the pigeon, as the further investiigations are now carrying on.

SOME REMARKS ON THE CHROMOSOME OF THE PIGEON

As has been cited already, GUYER (1900) informed 16 chromosomes in a spermatogonium and 8 in a primary spermatocyte. Such number of chromosomes was also made out in the female germinal cell by HARPER (1904), and again in the male cell by SMITH (1912). Inspite of such coincidence in counting chromosomes by these three authors, I have doubted their results concerning the actual number of chromosomes in pigeon. For I found in other species of birds usually a great number of chromosomes including considerably minute ones which should be expected to occur also in the cells of pigeon. When he investigated the methods of fixation of avian chromosomes in general, HANCE (1925) found too such minute chromosomes in the testicular cells of the pigeon, but he says " the smallest ones are almost beyond the limits of vision ", and consequently the accurate counting of the total number of chromosomes has not been succeeded.

In fact, there are found those chromosomes of considerably small size not less in number. And one can hardly count them unless he observe such excellent figures as I showed in the present paper. At first I thought that the minute ones with dot-like appearence might not be true distinct chromosomes, but fragmental masses of chromatin substance. Repeated and cautious observations, however, revealed at last that they retain always their individuality as chromosomes as those of larger sized ones do. The most minute ones are twelve in diploid number (figs. 1 to 6) and six in haploid condition (figs. 12 to 15 and 18 to 20). At the time of division they divide themseves into two similar halves (figs. 16 to 18), so they are found in every cells in constant number. They are in fact, therefore, nothing other than the distinct chromosomes, being only composed of a very small amount of chromatin substance. Whenever we examine such preparations in which the process of differentiation of colour by iron-alume advances a little excess, the minute ones usually become disappeared from our vision. It is due, as I am sure, not to less affinity with haematoxyline, but to less amount of chromatin substance in volume.

The central granules of the spindle drawn by HARPER (1912) in the oocytes of the pigeon seem to be no more than the minute chromosomes under consideration. Similar figures also can be found in the drawings of the fowl-chromosomes by GUYER (1916), but nothing is shown by the same author (1900) in the pigeon chromosomes. Existence of the minute chromosomes has been thus noticed by previous authors, eventhough they did not take them as chromosomes. If they were counted as distinct chromosomes, therefore, then the number of the chromosomes should have been naturally altered.

Between the group of the rank of largest chromosomes, namely *a*'s, *b*'s and *c*'s, and the minute 12 chromosomes, there are 44 chromosomes constituting 22 homologous pairs of the intergrading magnitude. The larger ones of the latter series, *d*'s, *e*'s, *f*s, *g*'s for instance, show still conspicuous configuration, but the smaller ones represent no striking difference to be distinguished from the minute chromosomes. It is evident, however, there may be allowed by no means to draw a demarkation line, by which whole member of the chromosomes is divided into two categories, large or macro-chromosomes and small or microchromosomes, as seen in the case of the American lizards studied by PAINTER (1923). A glance at the chromosomes of the lizards may call one's notice to remind of an apparent resemblance with those of the birds. Thus the fact in question has been already emphasized by BORING (1923) who published the notes and figures left by late STEVENS. But in truth this idea can be hold no more, for in the pigeon, probably birds in general, there are present the third kind of chromosomes of intermediate magnitute ranked between the macro- and micro-chromosomes as mentioned above. In this respect, therefore, the avian chromosomes should be sharply distinguished, from morphological point of view, from the reptilian chromosomes, so far as the American lizard is concerned.

The number of chromosomes counted by GUYER (1900), HARPER (1908) and SMITH (1912) must be a result of counting only some larger sized intergrading ones in addition to three largest chromosomes. Or, in their preparations, a strong reduction of number of chromosomes might have taken place by agglutination resulted by reagent they employed. At least in the figures shown by GUYER (1900) and SMITH (1912) I can find no such chromosomes as stretched radially to form a rosette as in the case of my present study. It proves that the preparations of above named authors were not appropriated for a close study of small chromosomes. We find similar examples in mammalian chromosomes. The known number of chromosomes in the pig was thus revised by HANCE (1917) and in the rat by ALLEN (1918) through their highly improved technique by which agglutination of chromosomes took place in least degree.

The number 61 (♀) and 62 (♂) of chromosomes are, as I believe, the definite number characteristic to the pigeon dealt with. It shows no variation in number either in germinal cells or somatic. Of course, I experienced that a less number of chromosomes was to be the case, when short, rod-like chromosomes overlapping one another were overlooked, but in none of cases I could count the number more than 61 or 62. We know from this fact, therefore, that any kind of fragmentation of chromosomes does not occur in pigeon, unlike the case of fowl as well as the pig discovered by HANCE (1926, 1917).

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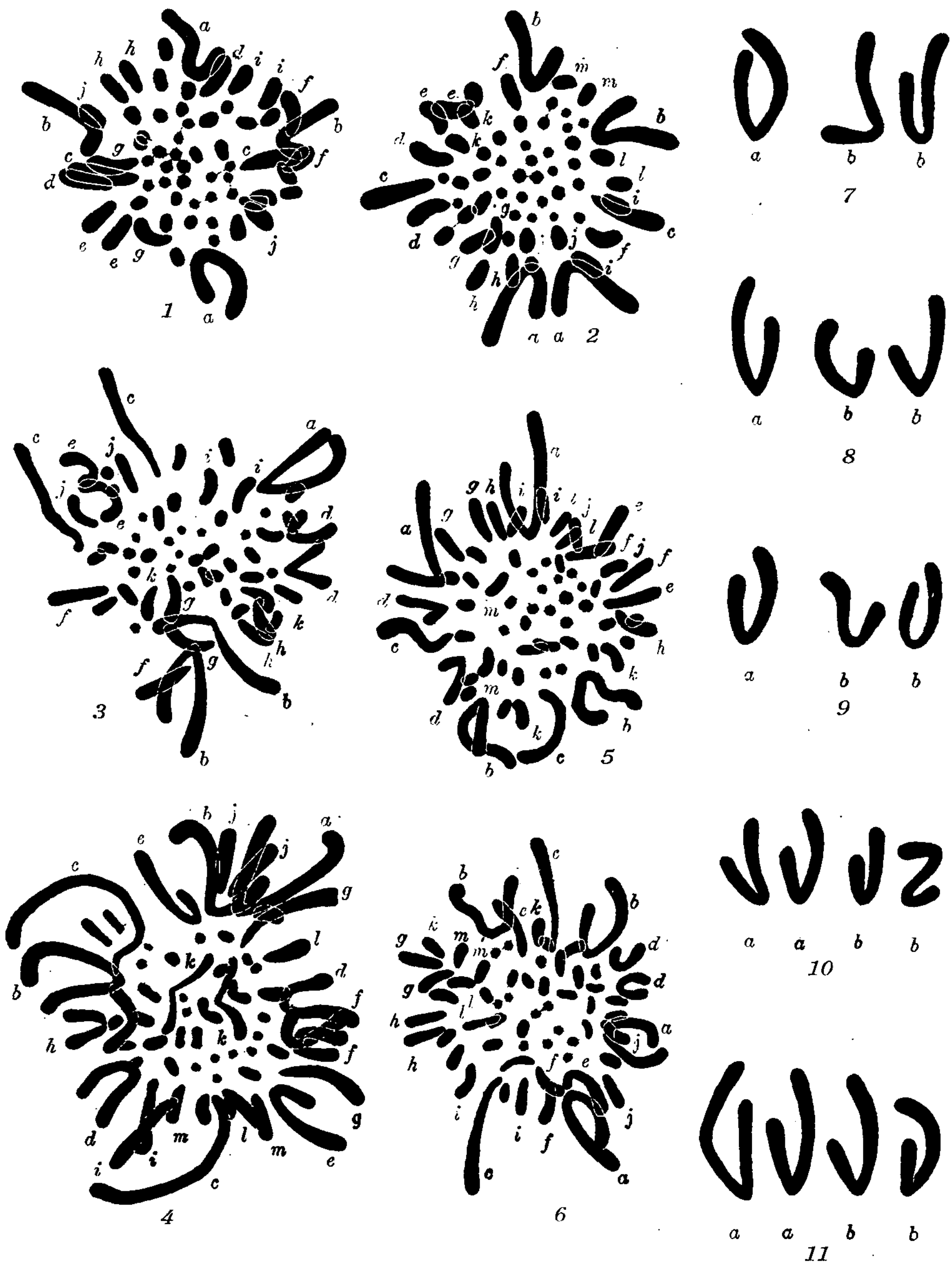
EXPLANATION OF PLATES.

All the figures were drawn at the level of desk, in aid of Abbe's drawing apparatus under magnification in using Zeiss apochromatic objective ·1,5 mm and compensating ocular 18. The magnification is 5000 times.

Fig. 1. Spermatogonial chromosomes.
Fig. 2. The same.
Fig. 3. Chromosomes of a mesenchyme cell, Embryo No. I. Clutch A.
Fig. 4. Chromosomes of an ectoderm cell, Embryo No. I. Clutch A.
Fig. 5. The same, Embryo No. II. Clutch A.
Fig. 6. The same, the same embryo.
Figs. 7. to 11. The chromosomes *a*'s and *b*'s selected.
Fig. 7. From a retina cell, Embryo No. III. Clutch B.
Fig. 8. From a nerve cell, Embryo No. IV. Clutch B.
Fig. 9. From a nerve cell, Embryo No. V. Clutch C.
Fig. 10. From a mesenchyme cell, Embryo No. VI. Clutch. C.
Fig. 11. From a nerve cell, Embryo No. VII. Clutch D.
Fig. 12. Chromosomes of primary spermatocyte, polar view of metaphase plate.
Fig. 13. The same.
Fig. 14. The same.
Fig. 15. The same, oblique view of metaphase plate.
Fig. 16. The same, lateral view of the first division.
Fig. 17. The lateral view of anaphase of the same division.
Fig. 18. A daughter garniture of chromosomes of the first division.
Fig. 19. Chromosomes of secondary spermatocyte.
Fig. 20. The same.



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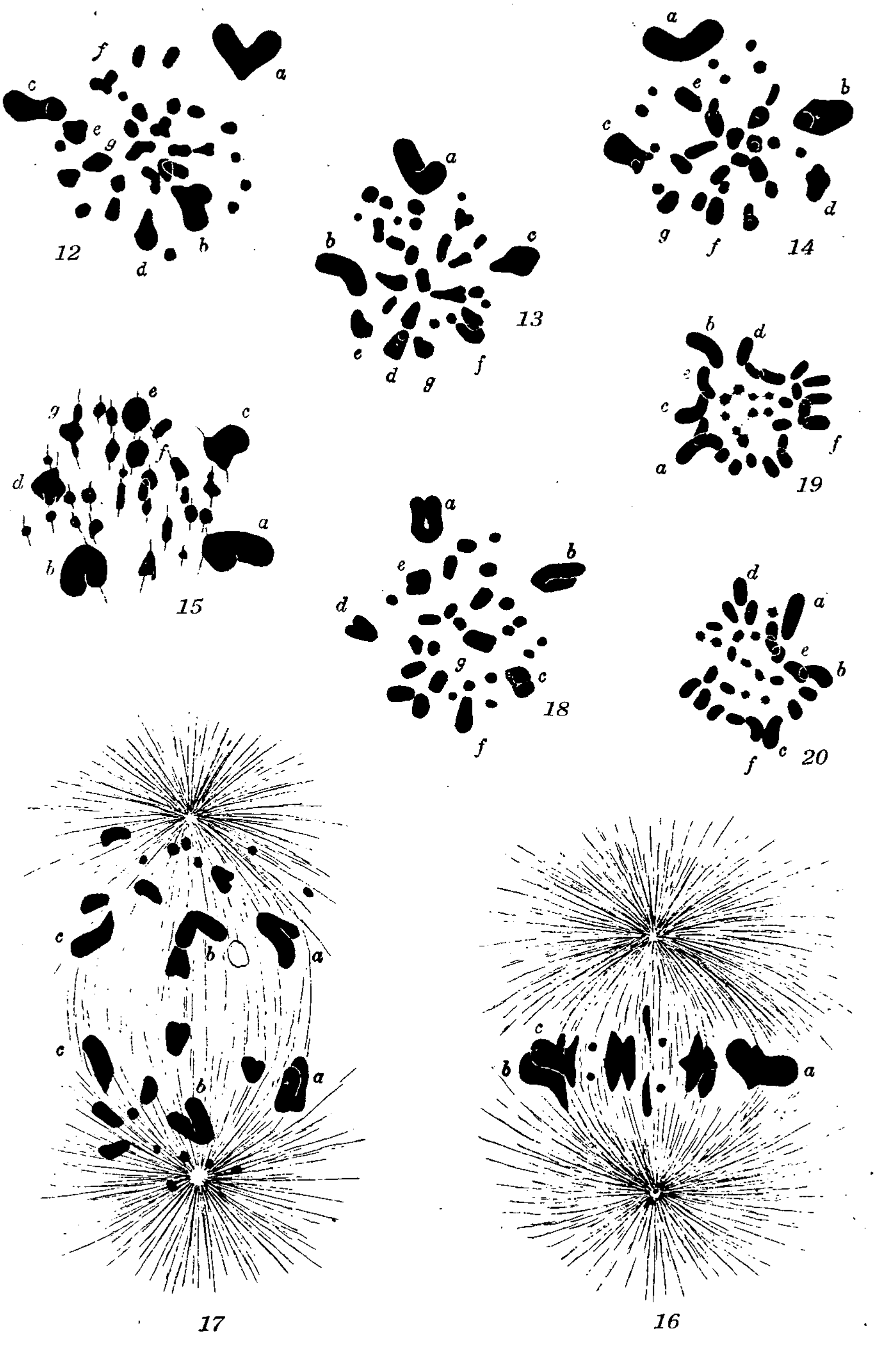

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