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STUDIES IN CYTOGENETICS.

The somatic chromosomes, meiosis, chiasma frequency and the effect of sex on the chiasma frequencies in *Rattus norvegicus albinus*

by

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CONTENTS.

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TEXT FIGURES.

TABLES.

1. Introduction.

The rat has been used frequently for genetical and cytological studies. Castle (1919), Dunn (1920) and Castle and Wachter (1924) have examined differences in the sex incidence of genetical crossing over. Cytological studies have been made by Painter (1926, 1927), Pincus (1927), and Swezy (1928). Hitherto however, no attempt has been made to correlate the data derived from genetical studies of crossing over and those which relate to chromosome behaviour. The present study endeavours to interpret the cytological data relating to the rat, in terms of Janssens' partial chiasmatype hypothesis (1909 1924) as elaborated by Darlington (1931 1932), and to compare the chiasma frequency with the frequency of crossing over as determined by the authors mentioned above.

2. Materials and Methods.

The Wistar strain of white rats - Rattus norvegicus albinus - kept by this Institute, provided the material for study. Males and females from 3 weeks to 9 months old were used. Sections were made to study oogenesis while both smears and sections were prepared in the case of the males. The sections were cut from 8u to 16u, those at 10u to 12u being best. The following fixatives were used: 2BD (La Cour 1931), 2BE (La Cour 1931), 2BD (La Cour 1931) and Carnoy: medium and strong Flemming.

La Cour's 2BD gave excellent sections in late prophase studies but for smears and the study of metaphase plates, the results with medium Flemming were much better than any of the other fixatives. The material, after fixing and washing, was taken through the alcohol series, through alcohol and chloroform to pure chloroform and finally embedded in wax. After removal of the paraffin with xylol, the slides were taken from absolute to 70% alcohol and then bleached with a solution of 70% alcohol and hydrogen peroxide, after which the slides were taken down the alcohol range before being stained with the standard gentian violet stain. This stain was found very suitable for observation and though objections have been raised against it on the score of fading, it is a simple matter/

matter to remove the coverslip and re-stain the sections.

Drawings were made with a Zeiss camera lucida and an H.I. 90, 1.30 apochromate oil-immersion objective. The x18 compensating eye piece gave a magnification of 3500 diameters, while the x30 gave 5500 diameters. All drawings have been reduced and in some metaphase stages of meiosis, bivalents have been separated for clearness.

3. The somatic chromosome complex.

(a) Number and morphology of the chromosomes.

The chromosome number of the rat has been studied in many instances and the results have varied greatly. It is only comparatively recently that reliable counts have been made.

Von Lenhossek (1898), one of the earliest investigators, determined the haploid number as 12. His result was contradicted by von Ebner (1899), who established counts giving a diploid of 16 and a haploid of 8. Regaud (1901, 1910) located 20 and 30 chromosomes respectively, but on each occasion found the haploid with only 12. Moore and Arnold (1905) gave the numbers as 32 diploid and 16 haploid.

These results were followed by those of Duesberg (1908) who found about 24 and 12. Later Sobotta and Burchard (1910) found 32 in the diploid and in the second spermatocyte stage counted 8. In 1917 Pratt and Long suggested 40 from their studies of the oocyte and Allen (1919) established the diploid as consisting of 37 chromosomes and in the haploid could locate 18 plus the sex chromosomes.

Great improvement in technique enabled Painter (1926) to establish some definite figures in which he gave 42 as the diploid number and 21 as the haploid. These results were confirmed later by Pincus (1927) and Swezy (1928).

In common with the results of Painter, Pincus and Swezy, the number of chromosomes found in this study, in the somatic cells, was 42. Fig. 1. shows the somatic chromosome complement of the male, the members of which are shown in serial alignment in Fig. 2. Two striking features are the size differences, and the morphology of the sex-chromosomes. There is a gradual decrease in size from the first to the twentieth pairs, and three pairs, the first, eleventh and nineteenth, show a curved outline. The first pair is decidedly longer than any other pair. In the male the sex-chromosomes are of the XY type and in many cases are comparatively easy to identify. The identification of the XX-chromosomes in the female was impossible, since they could easily be arranged in any aligned series as an autosomal pair. The XX-chromosomes and the longer autosomes are, to all outward appearances, identical. The majority of the chromosomes exhibit terminal or nearly terminal attachments, a fact which is further borne out in the form of some bivalents (see Fig. 9a) at meiotic metaphase and anaphase. The shape exhibited by the large chromosome pair in Painter's (1926) figure of a serial alignment, suggests that it is this pair that may show subterminal attachments.

(b) Behaviour of the chromosomes at mitosis.

(i) Prophase.

The prophase in mitotic division is comparatively/

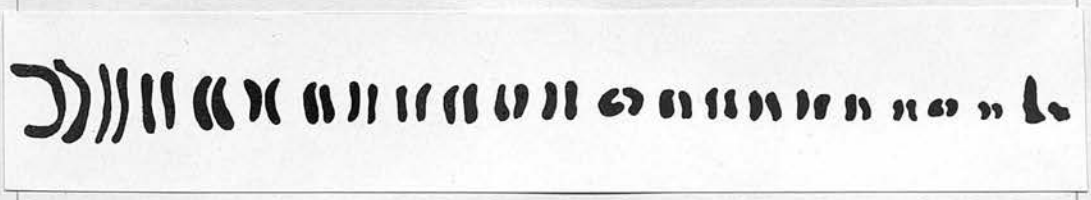


Fig. 1. The somatic chromosomes from a cell of the male rat.

Fig. 2. Serial alignment of the somatic chromosomes shown in Fig. 1.

comparatively short, and consequently is not easily followed. The cells in which such stages were observed showed that the chromosomes were long threads, which stained deeply and appeared denser than the elements in early prophase stages of meiosis. The number of chromosomes is large, and it was not possible to follow their arrangement within the nucleus. Of the chromosomes at mitosis Darlington (1932) says, "An increasing number of observations of the best material show the chromosomes as double threads at the earliest prophase. It has often been contended that the chromosomes have already split at the anaphase or the telophase for the next division (e.g. McClung 1927, Robertson 1931a). The evidence is valueless.....The permanent thread is double at prophase (except in meiosis) and single at telophase". In this study however the chromosomes appeared as single units, the doubleness due to splitting could not be observed.

(ii) Metaphase.

In metaphase plates the chromosomes were seen with their ends, probably the location of the attachment constriction, towards the centre of the metaphase plate. The spindle was very well marked. In early metaphase stages some chromosomes were seen dividing precociously, as was also the case in meiosis.

(iii)/

(iii) Anaphase.

The beginning of the separation of the half chromosomes was not observed. In the earliest anaphase stages seen the chromosomes were already separated and were on the way to the poles. Occasionally at this stage some chromosomes showed a lagging tendency. A similar condition was found in meiosis where both the precocious and lagging elements were commonly found, the most extreme forms of which were associated with sex. (Fig. 10).

4. Meiosis.

The most exact and detailed account of meiosis ever given was prepared by Janssens (1924) from observations on Mecostethus and Stenobothrus in support of his chiasmatype theory. In the present work rats of different ages were used for the study of the meiotic phases. The sections of the testes produced excellent figures of all stages. Sections through the ovaries gave different meiotic phases, but only the diplotene and metaphase stages were studied. The nomenclature of Darlington (1931, 1932) has been followed throughout and the following stages were observed;

(1) Prophase (a) leptotene, (b) zygotene, (c) pachytene (d) diplotene, (e) diakinesis.
(2) Metaphase
(3) Anaphase and Telophase
(4) Second Metaphase
(5) Second Anaphase
(6) Second Telophase

(1) Prophase
(a) Leptotene.

At this stage the chromosomes of the first spermatocyte division resembled a series of fine bead-like threads lying in the nucleus. It was not possible to follow the complete outline and length of the threads owing to their large number, their length and to the smallness of the nucleus. The number of separate threads observed is sufficient to realise that each is a single chromosome.

(b)/

(b) Zygotene.

Pairing of the chromosomes takes place at this stage and the threads get thicker due to pairing and to contraction, the two processes going on simultaneously. The observations here show that pairing generally commences at the ends of the chromosomes, but occasionally it appears that some chromosomes exhibit the commencement of pairing in a subterminal position. This is in accord with the findings of Wenrich (1917). "The comparison of the behaviour of chromosomes during the prophase of meiosis in diploids and polyploids shows certain of the conditions of their pairing as follows: (i) Zygotene pairing occurs only between similar chromosomes. (ii) The unit of pairing is not the chromosome but the chromomere. Each chromosome is potentially independent of its neighbours at zygotene as shown by changes of partner in the polyploids". (Darlington 1932). From observations on triploids and tetraploids (Darlington 1932), it is concluded that the pairing properties of the chromosomes are specific to their constituent particles (chromomeres) and further, the affinities of the chromosomes are satisfied by association in twos. "The qualitative differentiation of the chromosomes is shown by their specific pairing properties at all stages of meiosis. It was first found by Sutton and Montgomery that particular chromosomes/

chromosomes pair at meiosis and that these chromosomes were similar in size and shape. This meant that the different chromosomes showed specific and different affinities at meiosis". (Darlington 1932).

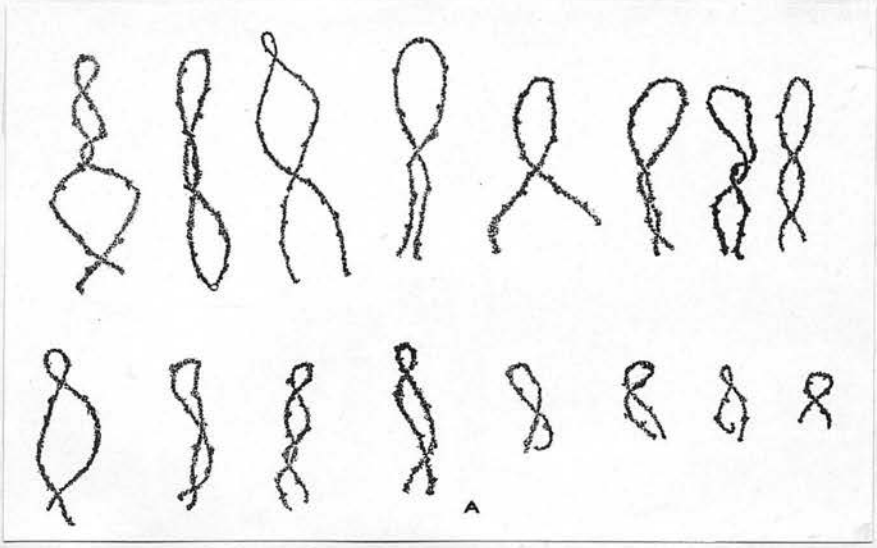
(c) Pachytene.

The paired threads or chromosomes showed marked differences in size very clearly. Some are long, some intermediate, while others are very short. "The length of the chromosome must be a function of contraction and thread length. Since contraction is constant for the race, length is constant for the individual chromosome". (Darlington 1932). In stages earlier than pachytene no such size differences, if there are any, could be seen, the twisted nature of the threads and their length making it almost impossible to follow any individual chromosome threads throughout. During the pachytene stage contraction has advanced to a greater degree, resulting in shorter and more compact chromosome pairs which are built up from two homologous threads.

(d) Diplotene.

The next stage of the prophase revealed the quadruple nature of the chromosome pairs. The homologous chromosomes become separated by loops, while at relatively few points they hold together. Each chromosome thus becomes constituted of two half chromosomes - the so-called chromatids. The initial points/

points at which the chromatids appear to hold together are, by Darlington's theory, where they exchange partners, and these points are known as chiasmata. It is considered, on the partial chiasmatype hypothesis, that these points bear some relation to the physical basis of crossing over. "Evidence showing a parallelism between frequencies and distributions of chiasmata and variations in these, on the one hand, and similar properties of occurrences of crossing over on the other, supports a hypothesis relating chiasmata to crossing over". (Darlington 1932). The splitting of each homologous chromosome into two chromatids and the exchange of partner chromatids, is the characteristic feature of the diplotene, (Figs. 3a and 3b), and it would appear that chiasmata arise initially, in an interstitial position, not at the ends. Discussing 'Structural Units', Darlington (1932) comments: "Gametes produced by triploid Drosophila with three homologous chromosomes have one or two of these; and when they have two they are sometimes identical in one part although the three homologues all differed in this part; they are therefore derived from the chromatids of one chromosome. But the chromosomes are sometimes dissimilar in other parts; they must then be the result of crossing over between chromatids, not between chromosomes. In genetical language, these 'equational exceptions' are said to prove crossing over in the 'four-strand'



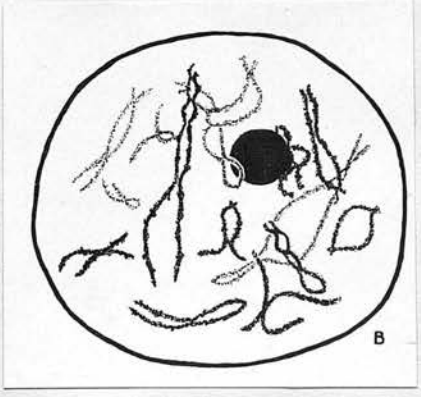


Fig. 3(a). Some configurations from diplotene of the male and female rat.

Fig. 3(b). Cell showing diplotene bivalents.

'four-strand' stage i.e. after the division of the chromosomes into chromatids, and never between all four strands at the same point (Bridges and Anderson 1925; Redfield 1930). Such is the method of crossing over demanded by the chiasmatype interpretation of the available cytological evidence". The loops are produced by repulsion between the partner chromosomes, while the bivalents are held together in the chiasmata by the exchange between partner chromatids. On Darlington's 'precocity theory' one may assume that the urge of pairing between chromosomes was satisfied by the association of homologous members, but this balance between them has been upset by the longitudinal splitting of each constituting member. The chromosomes after the splitting would be repelled each from the other if the exchange between their chromatids did not hold them together at the chiasmata. These points where the exchange takes place appeared at random. The length of the chromosomes to some extent governs the number of chiasmata formed in them. Examples have been quoted from Vicia faba Hyacinthus and Frittilaria imperialus. Darlington and Dark (1932) have quoted a case in Stenobothrus parallelus in which the chiasmata are distributed the longer chromosomes being more than five times the length of the shortest, but in spite of that the chiasma frequencies recorded were only about three times as great.

(e) Diakinesis.

The linear contraction of the chromosomes nears completion and the chromosomes appear relatively short and massive. In diakinesis the nucleolus, which has been a very definite body in the earlier prophase stages, is still represented by a very small spherical body. Owing to further contraction, the configurations of the paired chromosomes or bivalents are greatly changed, and the looping is not as prominent as in diplotene; and further, the number of chiasmata has decreased. This fact is discussed later. Figs. 4a and 4b show bivalents at diakinesis; it will be seen that the form of one pair shows great size differences. It is suggested that they represent the sex-chromosomes - the X and the Y. The chromosomes at diakinesis appear to be distributed at random in the nucleus due to the fact that the paired chromosomes repel one another sharply at this stage.

The repulsion between all the chromosomes weakens after the diakinesis and the whole complement draws closer together. This stage has been designated the 'pro-metaphase'. Kuwada and Sugimoto (1928), from their staining experiments, have suggested that the chromosomes change from being electro-negative within the nuclear membrane, to electro-positive in the spindle. If a weakening occurred at such a stage the metaphase arrangement could possible be accounted for.

(2)/

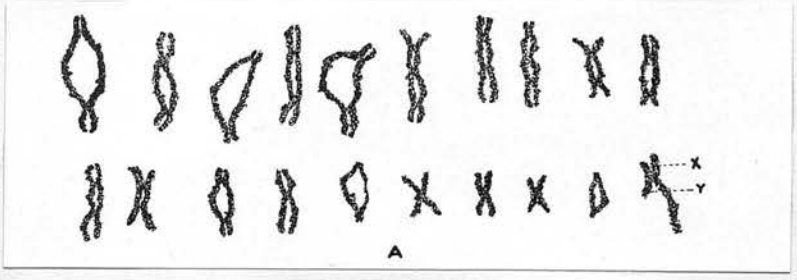


Fig. 4(a). Elements from several cells in diakinesis stage.

Fig. 4(b). A cell with nineteen diakinesis bivalents.

(2) Metaphase

At metaphase, condensation and contraction of the chromosomes reaches the maximum, the spindle appears and the nuclear membrane and nucleolus are lost. The bivalents arrange themselves on the equatorial plate and the configurations adopted by the bivalents differ in the early and late metaphase stages. The most remarkable differences are the altered positions and altered number of chiasmata. Fig. 5. Most members of the bivalents are held together by terminal and subterminal chiasmata, while in earlier stages the chiasmata are formed in the main, interstitially. The decrease in the number of chiasmata from prophase to metaphase is explained on Darlington's hypothesis by movement of chiasmata away from the attachment constriction towards the ends of the chromosomes. This process, the so-called 'terminalisation' is discussed later.

Typical metaphase bivalents are shown in Fig. 6. Some workers have referred to bivalents as 'hat-shaped' 'Derby hat-shaped', 'propellor' etc. Examples of these with line drawings of the chromatid arrangement are given in Fig. 7. These have in all probability originated through the assumed terminalisation from types in such a series as shown in Fig. 8. Here 'a' represents the possible arrangement at diplotene, 'b' the early metaphase, 'c', 'd' and 'e' later metaphase stages/

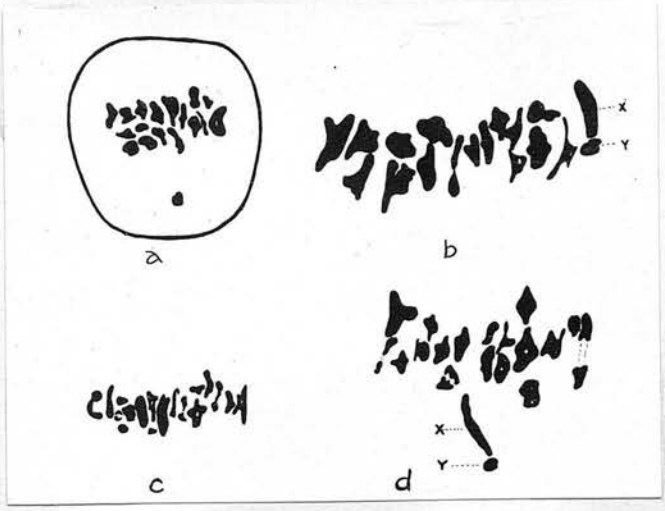


Fig. 5. Four figures showing metaphase bivalents. 'a' shows the precocious separation of a sex-chromosome; in 'b' the bivalents have been separated to show the terminal association of the sex-elements; 'c' is a plate from the metaphase of a female, and 'd' represents a male cell exhibiting early and irregular separation of the sex-chromosomes.
All types of bivalents described in the text are shown and attention is drawn to the smaller and precociously dividing forms.

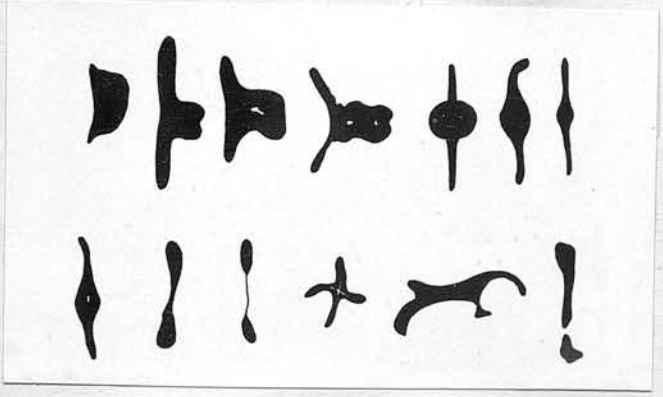


Fig. 6. The most frequent types of metaphase bivalents. The sex-chromosomes are shown on the extreme right of the lower row.

stages. The bivalents shown in Fig. 9 are in the process of separation; the types are typical. In Fig. 5 some bivalents, especially the short ones, are seen to have already divided, while the remainder, mostly the long bivalents, are still in the metaphase plate. This precocious separation is characteristic of some short bivalents of this species and has also been observed in Mus (Painter 1927 Crew and Koller 1932), and already noticed in the rat by Pincus (1927). The terminalisation of the short bivalents soon becomes complete and the members of these short bivalents are held together by a chiasma at each end. In most cases the short chromosomes terminalise in advance of the longer ones. There is evidence that the chromosomes associated only terminally separate more readily than those associated by interstitial chiasmata; in some species in which the chiasmata usually show complete terminalisation, lagging chromosomes in anaphase are always associated with the presence of exceptional interstitial chiasmata at metaphase.

"In organisms having complete terminalisation, as a rule, an occasional bivalent has an interstitial chiasma; it is sharply distinguished at anaphase by its lagging (Darlington 1931d).....The other side of the picture is shown by the precocious separation of bivalents in certain organisms. This is particularly noted where most of the bivalents have interstitial chiasmata/

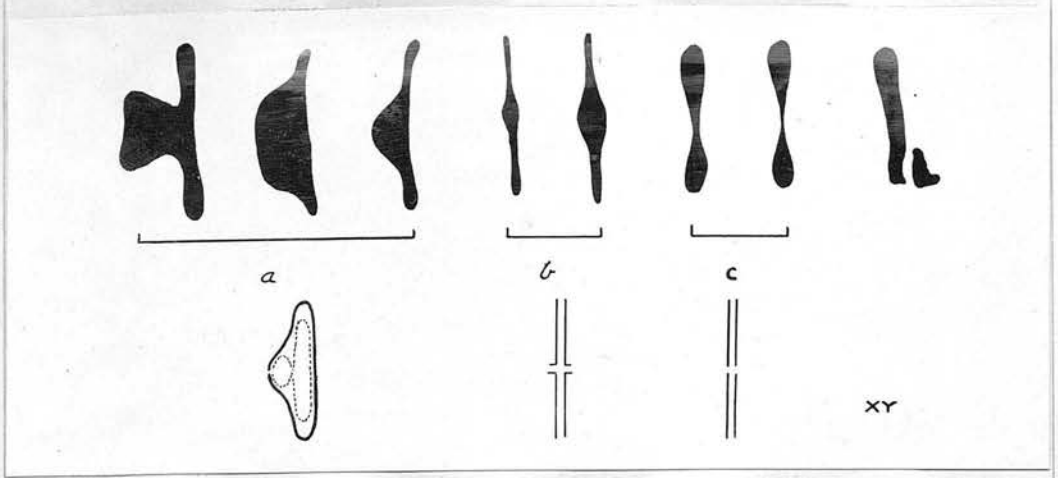


Fig. 7. Metaphase bivalents and their chromatid constitution. The types at 'a' are the 'hat-shaped' forms while those at 'b' and 'c' are the 'propellor' forms referred to by Painter. On the extreme right is the sex pair.

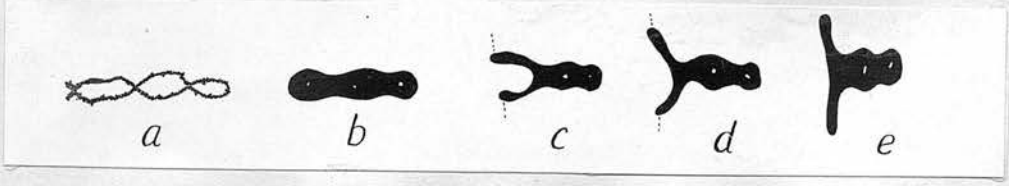


Fig. 8. Possible origin of metaphase bivalents similar to the type shown in Fig. 7 'a'. 'a' is the possible diplotene; 'b' the early metaphase; 'c', 'd' and 'e' represent the later metaphase stages of which 'e' is typical in most plates.

chiasmata. If then an exceptional one has a terminal chiasma, and especially if this is a short chromosome, it divides precociously (e.g. Mus musculus Painter 1927; Ranunculus acris Larter 1932)" (Darlington 1932).

The sex-chromosomes can be seen in some metaphase plates. Fig. 5. If the X and the Y are associated at metaphase, this association appears terminal, and owing to their pairing properties the sex-chromosomes can separate precociously. Fig. 5. In some cases the opposite behaviour of the sex-chromosomes was observed viz. lagging in the metaphase plate. Fig. 10. This could be produced if the pairing of the two unequal chromosomes took place by interstitial chiasmata as was observed in the mouse by Crew and Koller (1932). This unusual behaviour of these chromosomes cannot be due entirely to inequality in size, but is possibly also connected with qualitative differences between them and the autosomes. Discussing the sex-chromosomes, Schrader (1928) writes: "It may safely be stated that it is impossible to isolate any one feature of behaviour and regard it as diagnostic of sex-chromosomes. The confusion that has often arisen in the study of the sex-chromosomes is attributable in large part to the more or less arbitrary assumption that one or a few reactions are specific for them and this despite the fact that several of the earlier

workers/

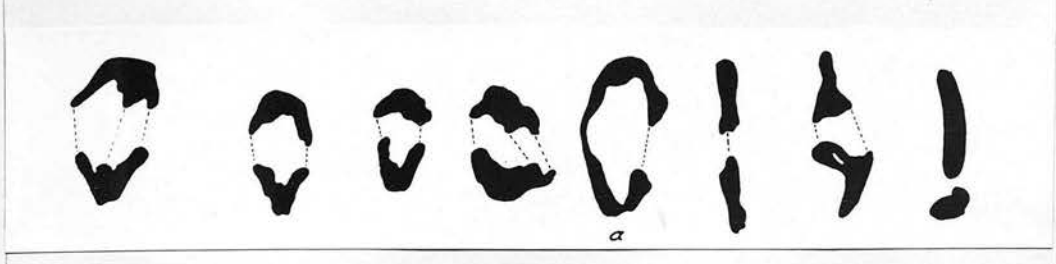


Fig. 9. Early anaphase stage. At 'a' is the bivalent with possible subterminal attachment constriction (referred to in section 3).

Fig. 10. Separation of the metaphase bivalents has taken place, but the sex-chromosomes are found lagging.

workers like Wilson were aware of this danger and warned against it....."

(3) Anaphase and Telophase.

In mitosis the change from metaphase to anaphase is sudden. In meiosis the separation is a gradual one and it is considered that the repulsions recover their previously lost force again. Thus the chromosomes appear to continue the effect of repulsion of the paired attachments. From the types of separating bivalent observed at anaphase it is sometimes possible to infer the types of chiasmata that have originally taken place between the chromatids, but owing to the small size of some of the chromosomes in the rat, the arrangement could not be inferred in a satisfactory manner. However in a few cases the compensating and non-compensating (Fig. 11) types of formation could be made out from the anaphase configurations. The doubleness of the separating chromosomes has been observed in a few instances, especially where the bivalents were associated by chiasmata of the non-compensating type. In anaphase it is seen (usually) that bivalents having the greatest length of chromatid to pull apart, lag behind the others. These are in contradistinction to the short and precociously dividing forms.

The members of each bivalent pass to the poles where the chromosomes fuse together into a compact chromatin/

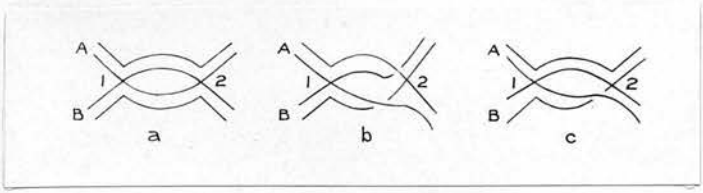


Fig. 11. The illustrations 'a' and 'b' represent compensating chiasma formation while 'c' represents the non-compensating type. (after Crew and Koller 1932).

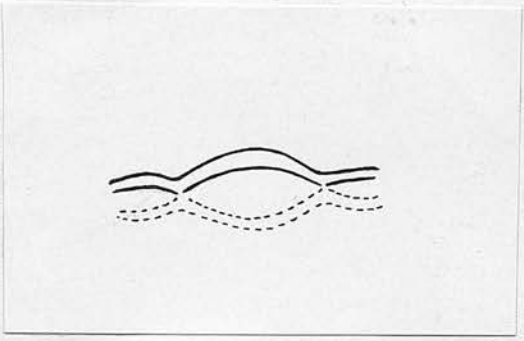


Fig. 12. The arrangement of chromatids in diplotene by Darlington's hypothesis.

chromatin mass, and it is then no longer possible to distinguish any of the separated chromosomes. The daughter nuclei, and the cells originated in this way are the second spermatocytes. They remain in close association for a very short time (interphase) when the second metaphase begins. This division is a mitotic one - the so-called homotypic division.

(4) Second metaphase.

The chromosomes in the bulked chromatin masses at the poles are irregularly arranged. These finally form out on a metaphase plate in the process of the second division, which is an ordinary division.

Each chromosome is constituted of two half-chromosomes, the splitting taking place as described during the prophase of the first spermatocyte. The double structure of these chromosomes could not be followed, the chromosomes appearing as one.

(5) and (6) Second Anaphase and Telophase

From the metaphase plate the chromosomes segregate. The half-chromosomes are pulled apart by the attachment constrictions. The process is quicker and shorter than in ordinary mitosis, and the separating chromosomes build up the nucleus of the spermatids in a very short time.

5. Chiasma frequency and Genetical crossing-over.

The observations on triploid Drosophila (Anderson 1925; Redfield 1930, 1932) and on maize (Beadle 1932), show that crossing-over, that is the separation of the linked factors, takes place in the four strand stage. By Darlington's hypothesis each chiasma recorded in the early prophase stages results from crossing over, and the two chromatids derived by division of a single chromosome always remain associated. A chiasma results when two that are not identical undergo a mutual exchange at a given locus. This hypothesis has not gone without criticism. Belling (1928, 1931a, 1931b) and Maeda (1930) have put forward the suggestion that every point of contact at diplotene is not necessarily a chiasma but may be an overlap or temporary fusion of the paired chromatids.

McClung (1927) in his outline of chromosome behaviour has pointed out that when crossing over occurs, by Janssens' theory, an asymmetrical arrangement of the chromatids should exist, but from his studies McClung has stated that the clearest figures show that symmetrical figures are the usual and most prevalent types.

Perhaps the greatest rival to Darlington's theory is found in Sax with his 'Breakage Hypothesis' (1930)/

(1930). "Another assumption is that chiasmata arise between two chromatids through breakage and reunion after diplotene, in such a way as to resolve the chiasma (Darlington 1929b). This is analogous to Janssens' third assumption, except that chiasmata were supposed to have arisen without crossing-over either through the meeting of reductional and equational loops or through the meeting of two equational loops.. The discovery of the reduction in the number of chiasmata during terminalisation has led to the revival of the hypothesis of breakage after diplotene (Sax 1930, 1931a)" (Darlington 1932).

Sax (1930) put forward a hypothesis accounting for the crossing over mechanism. The hypothesis is based on the usual assumption that chiasmata are formed by the alternate opening out of sister and non-sister chromatids at diplotene. "A crossover is caused by a break in the two crossed chromatids at a chiasma between diplotene and late diakinesis.....A crossover occurs only when two chromatids break at a chiasma".

In the 'Breakage Hypothesis', Sax has assumed that crossing over is to some extent conditioned by chiasmata, i.e. the chiasmata are formed first and where the chromatids cross over each other, a break will occur there with re-attachment between the partner chromatids. Figs. 12 and 13.

Sturtevant (1933) has drawn attention to the fact/

fact that the regular pairing in the male Drosophila, where it is generally reckoned there is no crossing-over, appears to make it clear that chiasmata are not necessarily associated with crossing over, "a conclusion avoided by Darlington only by the expedient of a very special and improbable accessory hypothesis. It follows that chiasmata are not due to crossing over....."

On Darlington's hypothesis a high frequency of crossing over would be paralleled by a high number of chiasmata at metaphase. Belling (1928) found difficulty in accounting for the disappearance of chiasmata between diplotene and late diakinesis, a phenomenon which happens to be very widespread and of decided importance. It is assumed, on Darlington's hypothesis, that the chiasmata move from the attachment constriction between diplotene and metaphase, and that the decreased number so resulting by movement is due to the so-called terminalisation. Belling has pointed out that coiling of chromonemata would prevent any appreciable movement of the chiasmata after diakinesis.

Sax (1932) has stated that Darlington has not described chromonemata in his studies, "probably due to inadequate fixation or staining for showing this structure, rather than the absence of coiled chromonemata in the species studied". It is generally assumed/

assumed that the position of the chiasmata, formed in early diplotene, cannot be satisfactorily recorded at the moment of origin, but when the frequencies of formation are compared with the frequencies in diakinesis and metaphase, it appears that the chiasmata have changed in position. It is considered that chiasmata move down the bivalent and of this Darlington says: "All the chiasmata, both near to the attachment and further away, move at the same time towards the ends. The movement is a simultaneous one of all chiasmata towards the end and leading to fusion only at the ends.....The chiasmata do not break in the course of terminalisation. This possibility i.e. that two of the four chromatids break and the four free ends rejoin in such a way that there is no longer an exchange of partners among the chromatids, was suggested before exact records were made". And further; "In a word, it has become impossible to reconcile observation with the assumption of breakage".

The length of the chromosomes and the original distribution of chiasmata as well as the frequency, position of the spindle attachment, and the rate of movement are all conditions which affect terminalisation. Further it is considered that all the chiasmata move to the ends at the same time so that distal chiasmata become terminal without reduction in the total/

total number through fusion. "The movement is not therefore, merely an opening out of the attachment constriction loop at the expense of the distal loops, but a simultaneous movement of all chiasmata towards the ends, and leading to fusion only at the ends". (Darlington 1932).

That the movement is away from the position of the attachment of the spindle is shown by some of the metaphase bivalents which become rod-shaped, with one terminal chiasma, and also by the other smaller (and precociously dividing) bivalents having two terminal chiasmata. Fig. 5. Darlington, discussing this movement of chiasmata says: "In some organisms a change takes place. This is seen in its extreme form in Primula and Campanula. It has three obvious characteristics:

(1) The total number of chiasmata is reduced in each bivalent

(2) The chiasmata come to be concentrated nearer the ends

(3) These changes taking place pari passu eventually leave all the bivalents associated terminally and the number of chiasmata reduced to the number of ends associated.....if this end-to-end association be indeed a chiasma...."

On Sax's hypothesis the decrease in number from diplotene to metaphase would be the result of breakage in/

in the chiasmata, i.e. the frequency of crossing over.

The bivalents shown in Fig. 6 are typical of the types found in the metaphase stages of both male and female sections. Practically all these exhibit terminal chiasmata but some present interstitial chiasmata as well. The chromatid arrangement of three types is shown in Fig. 7 along with the sex- chromosomes. In Fig. 8 the possible origin of types similar to those shown in Fig. 7a is given. Bivalents similar to those shown in Fig. 7a(having one interstitial and a terminal chiasma) could be produced from that shown in Fig. 8e (two interstitial and one terminal chiasma), by complete terminalisation i.e. the arms locating the attachment constriction have parted still further with consequent movement of chiasmata down the bivalent. The process progressing still further would produce types having only a terminal chiasma similar to those shown in Fig. 7c. The short (and precociously dividing) bivalents already discussed exhibit a terminal chiasma at each end. In these the attachment constriction appears at the highest point of the convex surface as can be seen from those precociously dividing forms shown in Fig.5. This suggests, that, though in the early prophase where pairing appeared to be either terminal or subterminal, some forms may commence pairing in a different position. This question is further discussed in Section/

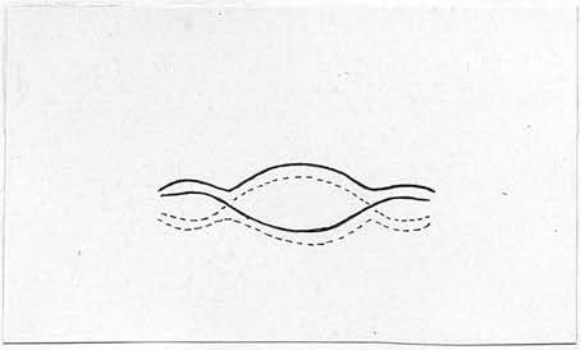


Fig. 13. The arrangement of chromatids in diplotene by Sax's hypothesis.

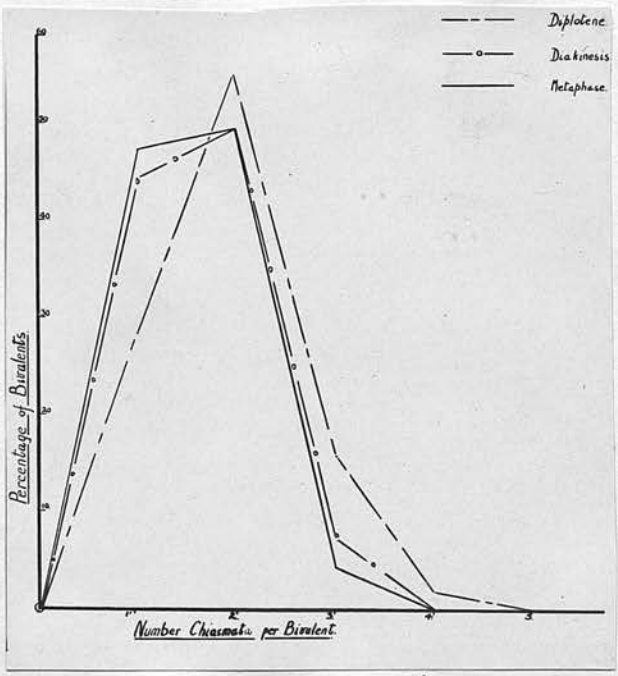


Fig. 14. Graphical representation of the chiasmata in the male rat at diplotene, diakinesis and metaphase.

Section 8. The complex nature of the mass, due to the large number and length of the chromosome threads, makes such observation difficult and at the same time somewhat speculative.

The rat presents very good material for genetic observation. There are several well-known factors linked in the autosome chromosomes and detailed accounts are known regarding the sex-incidence in the difference in crossing-over. The present study offers good opportunity to describe and discuss the detailed mechanism of chiasma formation and crossing-over. In calculating the chiasma frequencies animals of similar age have been used (or closely so - see Bryden 1933). The large chromosome number in the rat makes it difficult to find a quantity of cells containing the entire complement at any stage of meiosis. To overcome this difficulty the frequencies have been made out from counts of bivalents from those cells exhibiting not less than fifteen configurations, the cells being taken at random throughout the sections. Counts have been made in diplotene, diakinesis and metaphase in the male rat, the results being shown in Table I.

Table I/

Table I.

The frequency of chiasma formation in prophase and metaphase together with the terminal coefficients.

Stage	Bivalent Number	Chiasmata per Biv. 1	2	3	4	Chiasma Freq.	Term. Coeff
Early Diplotene	320	90	175	50	5	1.906	0.288
Diakinesis	320	141	156	23	-	1.631	0.388
Metaphase	320	150	156	14	-	1.575	0.527

The graphical results of the table are shown in Fig. 14.

This table shows the differences recorded in chiasma frequencies at diplotene and so on through the stages. It is evident that changes have taken place which are revealed in the terminalisation coefficient calculated by dividing the number of terminal chiasmata recorded, by the total chiasma number. The table indicates that as the chiasma frequency decreases so the coefficient of terminalisation increases. i.e. the movement of chiasmata increases the terminal associations.

6. The effect of sex on the frequency of chiasma formation.

Variability in crossing over among male and female gametes has been shown by Stadler (1926) with maize. He studied the C-Sh-Wx regions of the chromosome, the cross-over value known from other workers to be 3.5 for the region C-Sh and 21.88 for the Sh-Wx region. In each region, in the studies recorded, the female gametes registered a higher coefficient of variation than the male gametes, the variability being statistically significant beyond that which might be due to fluctuations in sampling. Previous to this work, Bregger (1918), reported a cross-over value in male gametes between waxy endosperm and aleurone colour to be 2.46 compared with the 2.9 of Stadler.

Studies with Pisum have been carried out but no significant differences have been obtained in crossing over values for male and female gametes. De Winton (1928) working on the factors y--gr (yellow cotyledon, green pod) finds differences which are not statistically significant.

In Drosophila, it is generally considered that there is no crossing over in the male. This is a phenomenon made to appear all the more remarkable by the fact that pairing occurs regularly in the male. Discussing this, Eloff (1932) after mentioning cross-over modifiers etc. suggests that the phenomenon is due/

due to a secondary enzyme action preventing crossing over. The experiments of Stern (1927) with the factor bb, however, have shed a little light in a new direction on the Y-chromosome and with extension it is probable that new knowledge of the constitution of the X- and the Y-chromosomes will be gained. Even though Stern has demonstrated the presence of a factor in the Y-chromosome, this chromosome must still be regarded as differing essentially from the autosomes since

(i) excess or defect of the Y-chromosome produces completely normal phenotypes whereas similar irregularities in the autosomes result in far-reaching changes in the organism.

(ii) Metz (1926), has demonstrated the cytological differences in the behaviour of the Y-chromosome during the growth period of the spermatocytes.

Working with the grouse locust, Nabours (1919) reported that crossing-over occurs to a greater extent in the female than in the male. With Paratettix texanus Nabours found a linkage between certain factors to have a crossing over value of 24% in the male and 46% in the female.

The crustacean Gammarus chevreuxi has been studied by Huxley (1928) and he found stronger linkage in the male than in the female, the cross-over value in the former/

former being 25.4% compared with 50.6% in the latter.

Tanaka (1915) found that in the silk-worm, Bombyx mori, crossing-over was of frequent occurrence in the male but was not observed in the female.

The values of crossing over for definite characteristics in birds has been investigated by Cole (1912) who reported no crossing-over in the female but Christie and Wriedt (1924) found results closely related for the male and the female.

Snell (1931) studied the linkage relations in mice between hairless and piebald and observed a difference in the sexes, finding a cross-over value of 2.6% in the male compared with 9.8 in the female.

Observations on linkage results with rats have been made by Dunn (1920) and Castle and Wachter (1924). These results are discussed at a later stage.

Detlefsen and Clemente (1924) in experiments with mice obtained a higher crossing-over value for females than males. Detlefsen (1925) considers, however, that the difference may not be due to sex, but to pre-natal elimination.

Castle (1926) finds a difference between the sexes of the rabbit but the difference is not of true statistical significance.

Eloff (1932) has presented a table summarising the cases and includes with it the chromosome number. His table is produced in Table II below.

TABLE II.

(From Eloff 1932) of a summary of cases of comparisons of crossing-over in the male and female.

Object studied	Number of chromosomes	Observer	Remarks pertaining to sex diff. in c.o.
Zea mays	10 hapl. No sex diff.	Kuwada	c.o.v. in the one case lower in the other higher for same sex. Diff. usually observ.
Pisum sativum	7 hapl. No sex diff.	Cannon Sakamura	Equal frequency for both ♂ and ♀
Primula sinensis	9 hapl. No sex diff.	Gregory	c.o.v. higher in ♀ for one region but higher in ♂ for other region of the same chromosome.
Pharbitus Nil	14 hapl.	Imai	c.o. prob. equal freq.
Drosophila melanogaster and D. simulans	4 haploid ♂ XY type	Belar and others	Except for few doubtful cases no c.o. in ♂
Tettigidea parvipennis	All spp. 6 hapl. in ♂ 7 hapl. in ♀	Robertson	c.o.v. in ♀ higher than in male.
Paratettix	do		do
Bombyx mori	28 hapl. ♀ heterogam.	Yatsu	No c. o. in ♂
Gammarus chevreuxi	? ♂ heterogam	?	c.o.v. higher in ♀
Colombia livia domestica	8 hapl. ♀ heterogam.	Harper	Contrary results. Cole and Kelly c.o. only ♂. Christie and Wriedt no diff. in male and female.
Gallus domesticus	9 hapl. ♀ heterogam.	Guyer	Uncertain. Dunn says equal in ♂ and ♀
Mus norvegicus	18 and 19 hap. ♂ is heterogametic	Allen	Both rats and mice have higher c.o.v. in ♀ than in the ♂
Lepus cuniculus	11 hapl. for ♀ and ♂ ♂ heterogametic	Bachhuber	Uncertain. Sometimes c.o. higher for ♂ and sometimes higher for ♀

The rat offers good material for a study of the chiasma frequencies in the sexes, since definite and conclusive results have been presented for genetical crossing-over.

In the male all stages of meiotic division were found and results have been recorded. In the female, however, the dividing stages were more rare and con-:figurations from early diplotene were not observed. It has been stated however that the metaphase config-:urations may give some indication of the changes that have taken place in early stages, q.v., see Figure 8. By endeavouring to work back from some of the metaphase figures observed, a figure has been obtained which may give some indication of the likelihood of frequency at diplotene stage. The number of bivalents that could be used for such a calculation has necessarily been small. This calculation was merely for gaining some indication of the early stages and may be taken for what it may be worth.

Working on similar lines (for comparison here only) results have been given for the male and are shown in Table III.

TABLE III. A possible theoretical value of the chiasma frequencies (from metaphase configura-:tions) in the male and female.

Sex	Chiasma Frequency
Male	2.0
Female	2.5

This calculation favours the female, similar results for which have been found at metaphase - See Table IV.

TABLE IV.

The number and frequency of chiasmata in the male and female rat at metaphase together with the mean terminal chiasma number.

Sex	Bivalent Number	Number chiasmata per bivalent				Chiasma Frequency	Mean No. Term. Chiasma per Bivalent
		1	2	3	4		
Male	320	150	156	14	-	1.562	0.81
Female	80	24	40	10	6	1.976	0.79

Fig. 15 shows the graphical representation of Table IV.

From Tables III and IV it is seen that the frequency of chiasma formation is greater for the female in both the diplotene and metaphase stages. Further, the mean numbers of terminal chiasmata per bivalent is practically identical in the sexes; the coefficient of terminalisation is approximately equal in the sexes. If real environmental differences exist in the cells of the two sexes, this phenomenon can be looked upon with surprise. If terminalisation can be regarded as a definite feature throughout the late prophase and metaphase stages of meiosis then it is possible that the differences existing in chiasma frequencies in the male and female is dependent on amount and/or rate, and/or time of terminalisation.

Sax, in his hypothesis considers that the decrease in number of chiasmata between diplotene and metaphase is due to breaks at the nodes. If this is so it should therefore be a smaller value in these females at metaphase when the diplotene values were also higher - provided the nodes seen at diplotene all represent true chiasmata.

Owing to the extensive research work of Dunn, and Castle and Wachter, the genetical crossing-over value between certain factors is known in both sexes. Dunn (1920), in his results, has shown that the crossing over/

over in the male is appreciably reduced as compared with the female for the three linked genes, albinism, red-eyed yellow and pink-eyed yellow. His results are given in Table V.

TABLE V/

TABLE V.

Data from Dunn (1920) on crossing-over in the male and female rat for the genes albinism, red-eyed yellow and pink-eyed yellow.

Sex	Total Number	Cross-overs	Non-Crossovers	%	P.E.
Male	2063	321	1742	15.56	± 0.538
Female	2683	549	2134	20.46	± 0.525

Similar results have been given by Castle and Wachter (1924). They verified that the crossing-over value for the same factors is higher in the female than in the male. Their results are given in Table VI.

TABLE VI.

Summary of the young produced by F_1 males and females heterozygous for albinism and pink-eyed yellow. (Castle and Wachter 1924).

Sex	Total	Percentage cross-overs	P.E.
Male	21324	18.39	0.32
Female	11186	21.69	0.45

These differences in genetical crossing-over of the two sexes were pointed out by Dunn, and he mentioned as an assumption that the difference exists not only in rats and mice but in other animals (see Haldane 1922), and must be due to sexual differences "not yet discovered in either the structure or functioning of the chromatin".

In both the genetical and cytological studies there appears to be a suppression of crossing-over in the sex-heterozygote. The reason has been debated by many workers, the general assumption being that in the XY-complex the 'genic balance' is disturbed. Crew and Koller (1932) in their study on the mouse, discussed/

discussed the question of the evolution of the sex-chromosomes and the lower crossing-over value for the male. Darlington (1932) discussing the question of the sex-chromosomes and the sex-heterozygote says: "Suppression of crossing-over might equally be supposed to be genetically determined. This suppression would permit the development of structural differences which would themselves secondarily prevent crossing-over. The evidence that this is the origin of differentiation is both genetical and cytological as follows:

(i) Precocity of the sex-chromosomes combined with pairing by terminal affinity, or precocity of parts of them, leading to localisation of chiasmata, and crossing-over, is a reaction of the chromosomes to a genetic property of the organism.

(ii) Abnormal forms of meiosis are characteristic of one sex in the Coccidae and in Sciara and affect equally like and unlike chromosomes. They must therefore be determined by a genetic property and not by hybridity as such.

(iii) Crossing-over is reduced in sex-heterozygotes relative to the homozygotes in the autosomes as well as in the sex-chromosomes. This points to a unitary and therefore genetic control of the difference (Haldane 1922; Huxley 1928).....The incidence/

incidence of localisation is less easily detectable than precocity and abnormal meiosis. Most commonly sex-chromosomes that appear to have paired normally during prophase, are associated by terminal chiasmata at metaphase, as in all plants. In these the localisation must be near the distal ends of the chromosomes and therefore terminalisation will occur rapidly and the original position of the chiasmata will not be ascertainable....."

The combined results recorded appear to be in line with Huxley's (1928) statement that where crossing-over is reduced it is in the heterozygous sex.

7. The Sex-Chromosomes.

In the study of the somatic chromosomes, it was found that the twenty-first pair in the male are very unlike, whereas the corresponding pair in the female are very similar. "As far as present knowledge goes it seems evident that there is little basic difference between the sex-chromosomes and the autosomes." Schrader (1928) considers that such a conclusion seems warranted in view of the fact that in the homogametic sex, the sex-chromosomes behave in every observable way like the autosomes and also that no exceptional behaviour of any chromosome has been discovered in either sex of a great many, if not the majority of forms and this, despite the fact that in some of them the genetic evidence indicates the presence of sex-chromosomes.

In further support of the view that there is little basic difference between the sex-chromosomes and the autosomes, fusion of the X-chromosome with an autosome takes place in Orthoptera (de Sinèty 1901, McClung 1905, 1917) and Macropus (Agar 1923).

Boveri considers the chromosomes of the complement qualitatively different from experiments he carried out on doubly fertilised sea urchin eggs.

The sex-chromosomes are found to be either like or unlike and in some cases the sex is determined by the absence of one chromosome. The following are

some examples (from Darlington 1932) in which the sex-chromosomes have been determined:

(a) Where the X- and the Y-chromosomes are similar.

Oncopeltus (Wilson 1905, 1911)

Drosophila obscura B. (Metz 1914, 1916)

Drosophila Willistoni (Metz 1926)

(b) Where the X is smaller than the Y

Drosophila melanogaster (Metz 1914)

Asilus notatus (Metz and Nonidez 1923)

Macropus (Agar 1923)

(c) In most cases the X-chromosome is larger than the Y.

Mus musculus, Rattus etc.

(d) Complete disappearance of the Y-chromosome - the XO type.

Examples of this group are mainly confined to the insects. e.g. Coleoptera, Hemiptera and Orthoptera (also in Nematoda).

In many genetical studies recorded, a difference in crossing-over has been found in the sexes and the question arises - what causes the difference? Investigations on Drosophila have shown that the sex-chromosomes do not entirely hold the characters concerning sex determination but that a character may be dependent on several genes situated in several chromosomes/

chromosomes. Bridges (1921, 1922) has pointed out that the genes may be classified as female and male determining and the two types are, in a sense, opposed to one another. Genes for femaleness outweigh the genes for maleness in the X-chromosome, so this chromosome becomes female determining.

In Drosophila it is generally considered that, as a carrier of hereditary units, the Y is almost valueless. But the individuals produced experimentally with an XO constitution are sterile, which fact would point to the conclusion that the Y-chromosome must play some part as confirmed by Stern (1927).

If we are to conclude that originally the sex-chromosomes were of the same type, many changes must have taken place to produce forms in which one of the pair is missing or almost so. In the XO cases it is possible that the Y has either fragmented and disappeared or has been translocated permanently to another chromosome. In cases of partial loss of the Y-chromosome, such a transference is incomplete over the whole chromosome. This is the case with the rat - the Y-chromosome is about one fourth the size of the X-chromosome. Haldane (1932), however, has suggested that diminution of the chromatin would require Localisation of certain genes.

Painter (1925) discussing the comparison of the chromosomes of mammals writes:- "From a theoretical point/

point of view we should expect the elements which determine sex to be the most stable of the chromosomes during the course of evolution, for the reason that the great change in the making of the X-chromosome would doubtless result in normal individuals".

Goldschmidt (1923) suggested that sex, like any other hereditary character, is normally determined by specific genes which are definitely located in the chromosome material. Where the sexes are separate, the difference between them is determined by a single gene, or by a gene complex behaving as one. Working from this hypothesis it would seem possible that in cases where the Y-chromosome differs from the X, its size may be determined by the location of the gene or the gene complex. If the genes in the complex are close together, that portion of the chromosome could remain intact; if widely separated, the immediate portion, when rid of its factors, may disintegrate, or the sex carrying portions may remain apart as in Phragmatobia.

Aida (1921, 1930) has given examples from his work with the fresh water fish Aplocheilus latipes in which the sex-chromosomes are of the Drosophila XY type. He found that the factor (R), red, is sex-limited and is borne on the Y-chromosome. This factor occasionally goes over to the X and from the X occasionally back to the Y. It transfers from Y to X with/

with a frequency ratio of 1:300 and from X to Y with a frequency ratio of 1:1200. Aida (1930) adds:-(from Eloff 1932.)
"If the crossing-over between the X-and the Y-chromosomes takes place simply by chance and no other cause interferes with it, the frequencies of transfer of the (R) gene in both directions should be equal and no preponderance on either side should take place. At present I am unable to elucidate the cause of this difference in the direction of crossing-over. Whether it is effected by a dissimilarity in structure of the X- and the Y-chromosomes or by the action of some gene or genes situated in one of them is not known. However, the tendency of the Y-chromosome more frequently to lose the dominant gene and less often to regain it, is well established in our own fish as well as in Lebistes, and if this difference is supposed to occur also in other animals we have perhaps a plausible explanation of the origin of the so-called empty Y-chromosome of Drosophila where crossing-over must have taken place so repeatedly between the X- and the Y-chromosomes, that the Y-chromosome has gradually lost its dominant genes, eventually to become quite empty".

After such results taking place, that portion which is devoid of factors may, under certain conditions, either fragment or become lost in the cytoplasm or become translocated. Discussing the question/

question of autosomal characters in the sex-chromosomes Morgan (1926) has stated that if no crossing-over takes place between the autosomal parts of this pair, the inheritance of characters whose genes are carried in the autosomal parts will be expected to show partial linkage to sex and to the characters whose genes are in the X-component.

Working with Drosophila, Painter and Muller (1929) have shown that when chromosomes are exposed to X-rays, in appropriate doses they exhibit a tendency to break in one or more places and as a consequence two or more fragments are formed. These fragments may remain detached but they very often unite at their broken surfaces with other chromosomes or chromosome parts. Any detached fragment with no fibre attachment will not be transported properly at division and will eventually be lost, but when an attachment is present the fragment can persist.

Hamlett (1926) has pointed out that crossing-over is reduced at points of translocations and consequently the general frequency reduced. If the Y-chromosome owes its smaller size to fragmentation and translocation in some portions, then there is every reason to expect that the frequency of crossing-over would be reduced in the sex in which heterozygosity appears. On the other hand, in the cases where all the factors have possibly been removed from the Y-chromosome/

chromosome, fragmentation may have taken place and the fragments disorganised.

Eloff (1932) prefers to reason that the difference in crossing-over between the sexes, though possibly due to a factor present in the Y-chromosome preventing crossing-over is more likely to be due to a secondary enzyme action preventing crossing-over in the chromosome.

The differences in crossing-over values in the sexes of the rat have been observed genetically, and in the results reported here, there are differences in the frequencies of chiasma formation. It has been suggested by Bridges that the genic balance of the group has been changed. The amount of change may to some extent depend on the amount of the Y-chromosome lost. This being so, the cell components, in times of division, may perhaps be expected to act in the hope of restoring such a balance. It is suggested that such a restoration would result in decreased crossing over. This, accompanied by any reduction in crossing-over by translocation, should be sufficient to account for the very significant difference between the two sexes.

In the female on the other hand, no such balance needs restoring and the amount of crossing-over is greater. Further, it would appear that the restoration of balance has not been completed, from observation/

observation on the sex-chromosomes; they sometimes separate precociously and at other times exhibit a tendency to lag in separating.

Pairing of the sex-chromosomes has been observed at diakinesis and also in metaphase. In the case under consideration the sex elements are of unequal size and it would be expected that metaphase bivalents would sometimes be formed consisting of unequal chromatids. (See Fig. 5). In the metaphase plates examined some cells exhibited twenty-one bivalents while in others the sex-chromosomes were seen to have separated earlier than the autosomes. Again, in other cases, the sex elements sometimes exhibited a lagging tendency and they could be observed separating later than the autosomes. From these facts it seems that, at times, there is definite exchange between the sex-chromosomes in prophase. Discussing the question, Crew and Koller (1932), from studies on the mouse, point out "According to the partial chiasma-type hypothesis, it is necessary to assume that the paired sex-chromosomes are qualitatively different from the autosomes, though not amongst themselves. The exchanges between the unequal sex-chromosomes would result in the elimination of any qualitative differences in the pairing segments, though they do not destroy quantitative dissimilarity. Thus it can be concluded that it is the quantitative difference between/

between the sex-chromosomes of the mouse, rather than the qualitative, which is more concerned with sex-determination; a view which is in close agreement with the genic balance theory of Bridges (1925). If the X and the Y differ in their internal structure, then maintenance of this qualitative dissimilarity would be a pre-requisite to any precision in sex determination".

8. Reconciliation of results with those of other workers.

Pincus (1927) has given figures of the somatic chromosomes in the rat and has arranged them in serial alignment, while Painter (1928) in his work has done likewise.

Painter's alignment agrees very closely with the one given in the present study. There are two pairs of large chromosomes and a gradually decreasing size series to the twentieth, after which are the unequal sex-chromosomes. Pincus's figure does not show great size differences in the first and second pairs, nor does he show such a large Y-chromosome. The series agree in other respects.

In his figures of spermatogonial stages, Pincus has given some excellent metaphase plates for the male rat. Most of his configurations agree with those found in this study. His observations will be compared with those cited here and his figures will be interpreted on the partial chiasmatype hypothesis.

Adopting terminology from Painter (1926), Pincus mentions the occurrence of 'hat-shaped' elements. These are shown with typical ones from this study in Fig. 16 D--I (Using letters similar to Pincus). The possible origin of such 'hat-shaped' elements is shown in Fig. 8 and the line drawing representing the chromatid arrangement is shown in Fig. 17 D to I.

All/

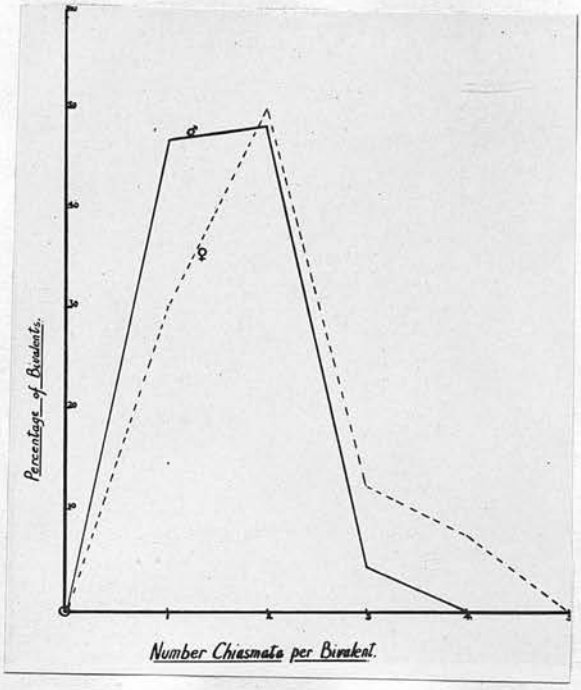


Fig. 15. Graphical representation of chiasmata in the male and female rat at metaphase.

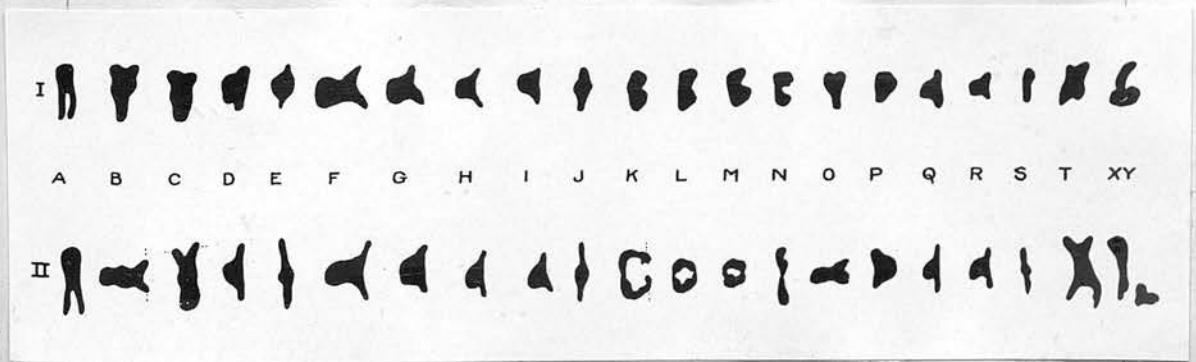
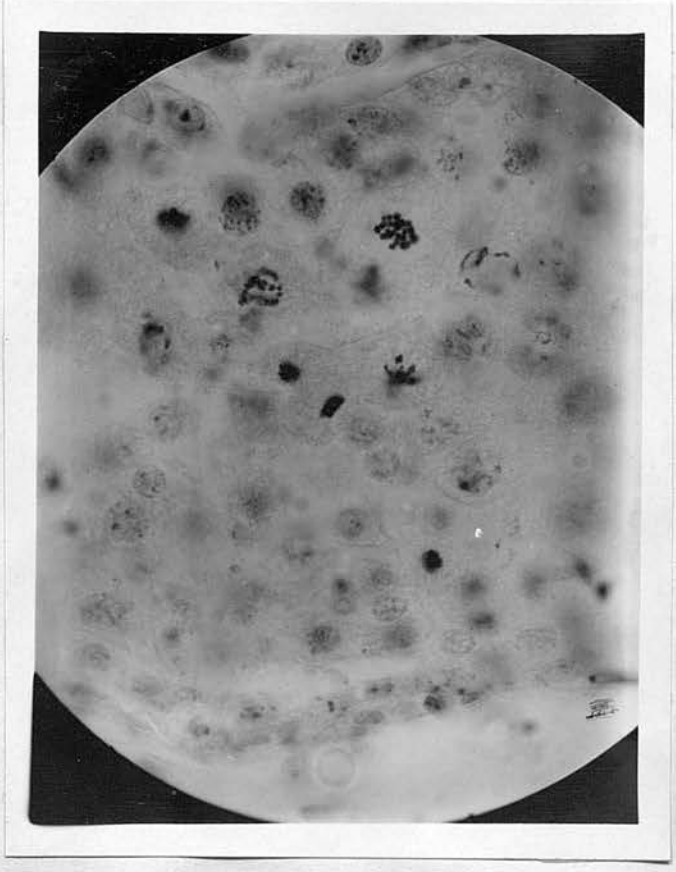


Fig. 16. I. Configurations from Pincus (1927).
II. Similar configurations found in this study. The lettering is similar to that given by Pincus in his figure.

All these types can be accounted for by different degrees of terminalisation i.e. by different degrees of movement of the interstitial chiasmata to the distal end of the bivalent. As chiasmata in the course of terminalisation are not considered to catch up the one on the other, and so cancel some out, the types of configuration shown can be imagined. Type E in the Pincus series is not a typical 'hat-shaped' element but is similar in chromatid arrangement to that shown in Fig. 17 E - a subterminal chiasma. The difference in bulk of bivalent E shown in Fig. 16, I and 16 II, is due to the different angle from which the bivalent is seen. Though different in bulk the configurations are similar. Q and R in the Pincus series are again similar to the 'hat-shaped' types D--I. In his text discussion Pincus says that bivalents "C and J are more or less diamond-shaped elements". Their constitution is shown in Fig. 17 C and J. The type C exhibits a terminal chiasma and at least two interstitial chiasmata, while J shows only a subterminal chiasma. Bivalent B which Pincus calls "a large twisted thread having a characteristic telomitic attachment and dividing much later than the others" is similar in chromatid arrangement to that shown in Fig. 17 C. Such a later dividing form may be due to length of chromosome, to interstitial chiasmata not terminalising as quickly as in other chromosomes/

PLATE I.



Plates I to III showing meiotic divisions and somatic chromosomes.

PLATE II.

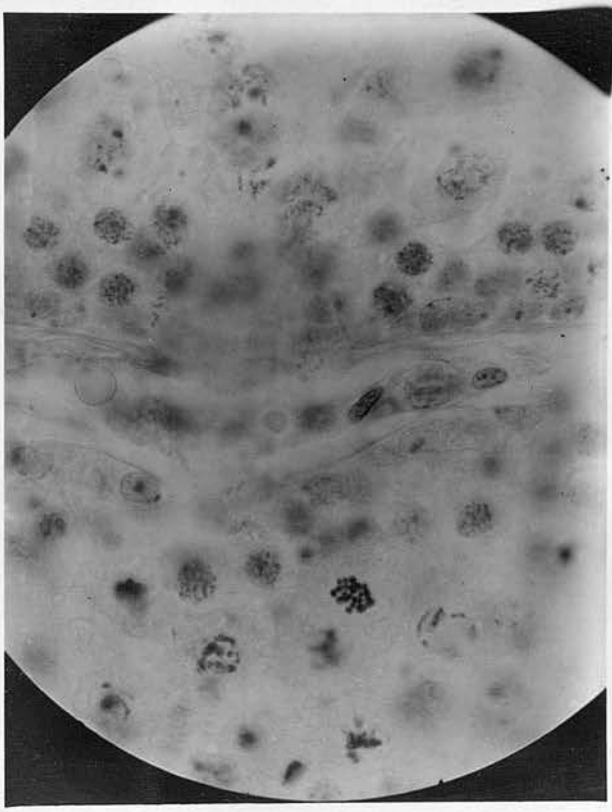
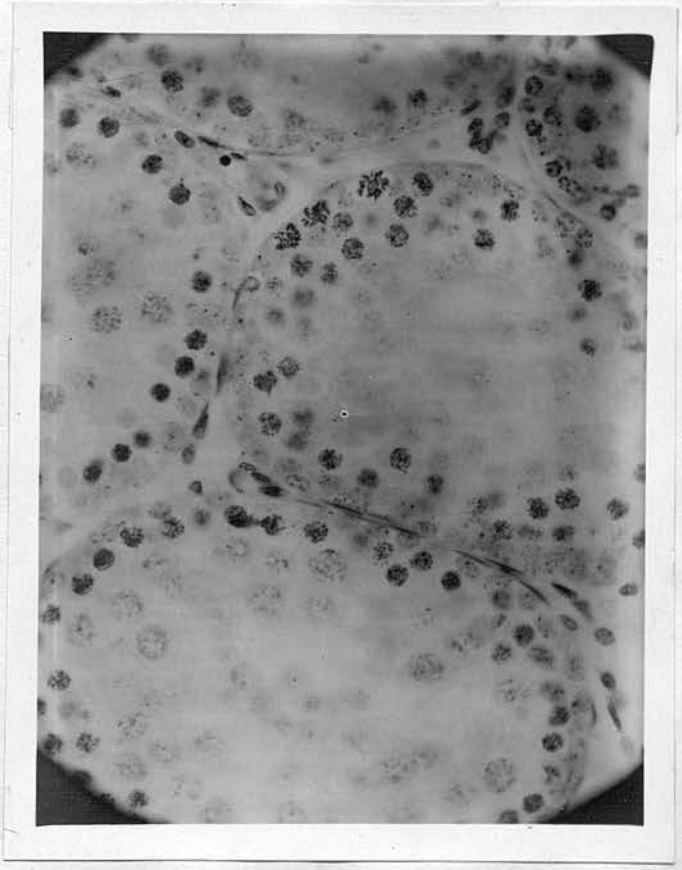


PLATE III.



chromosomes(possibly to length) or to the association of the chiasmata.

L and M appear to be of the type exhibiting subterminal attachments. The terminal chiasma near the attachment constriction has broken and the chromosomes are separating. The type of configuration shown could be easily derived from this by the terminalisation of any distal interstitial chiasmata. Bivalent N may be of a type similar to LM or it may be due to a terminal chiasma with a subterminal attachment constriction as shown in Fig. 17 N.

Type O of Pincus is possibly the first stage of the type LM or N. Its possible arrangement is shown at Fig. 17 O (compare this with Fig. 17 LM, which has possibly arisen from such a type).

The small rod-shaped element S is somewhat similar to J (see Fig. 17 S). This is a more advanced stage, regarding the movement of chiasmata, which in this case is nearly terminal, while in J the chiasma is definitely subterminal.

The element T, which Pincus regards as a 'K-shaped' element, is one in which the chromatid structure is difficult to make out. The bivalent shown at T in Fig. 16 II is represented in chromatid arrangement in Fig. 17 T. It exhibits interstitial chiasmata. The configuration shown at T in Fig. 16 I, may be due to an interstitial chiasma with the complete/

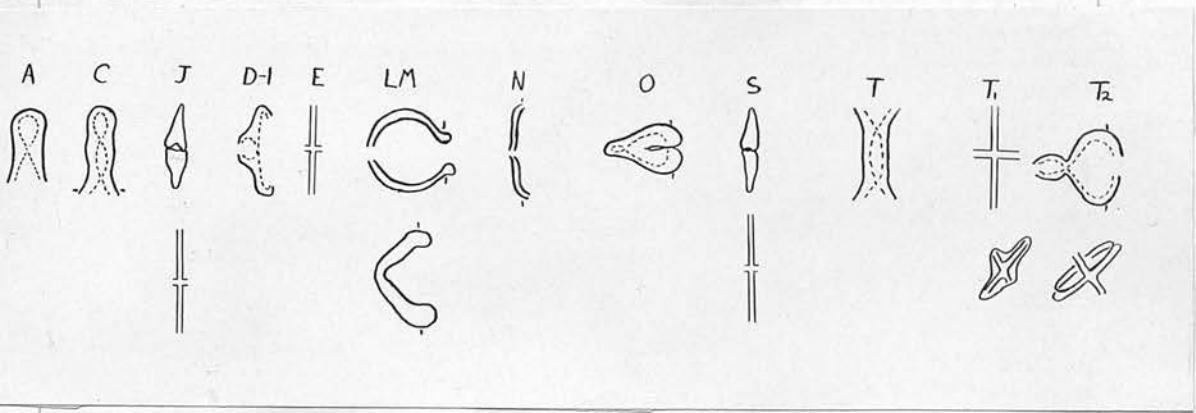


Fig. 17. The possible chromatid arrangement of some configurations shown in Pincus's figures.

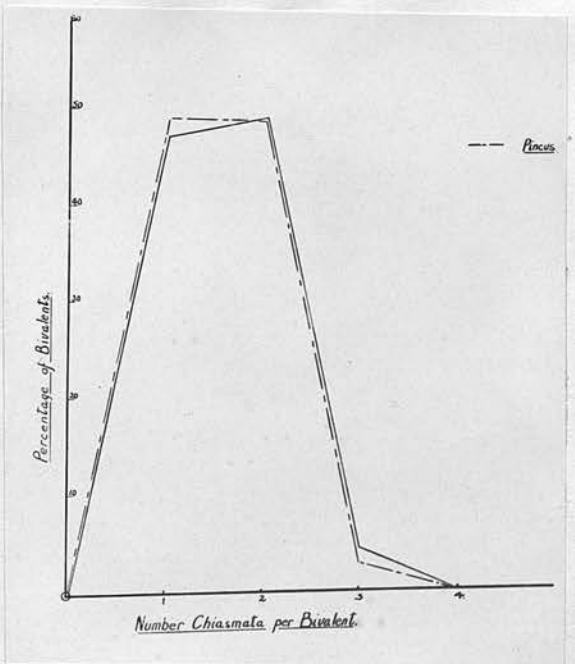


Fig. 18. Graphical comparison of chiasmata of Pincus with the results from this study of the male rat.

complete bivalent twisted as shown in Fig. 17 T_1, or, on the other hand, it may be similar to that shown in Fig. 17 T_2 where both terminal and interstitial chiasmata are exhibited - a type which bears favourable relation to Fig. 17 LM.

The chiasma frequency and terminalisation coefficient for the metaphase stage in the bivalents from the male rat shown in Pincus's figures, has been calculated on the partial chiasmatype hypothesis interpretations and compared with the results obtained in this study. The results are as follows:-

TABLE VII.

Comparison of chiasma frequencies of male rats from this study and from the figures of Pincus (1927).

Origin	% chiasmata per bivalent				Chiasma Frequency	Term. Coeff.
	1	2	3	4		
Pincus	48.8	48.4	2.8	-	1.584	0.532
Present Study	47.0	48.7	4.3	-	1.571	0.527

From Pincus's figures it has not always been possible to determine the exact configuration for interpretation on the partial chiasmatype hypothesis. The figures, however, are close enough to give every indication of the amount of terminalisation and general chiasma formation in the male.

The graphical representation of these chiasma frequency/

frequency results is shown in Fig. 18. Comparatively few bivalents were taken to calculate the chiasma frequency of Pincus's figures. As pointed out previously, such a method of calculation seems within the limits of error.

From the comparison of these figures it will be seen that the general results agree closely. The configurations found in the two studies bear a close resemblance and when both are interpreted in numbers of chiasmata, terminal and otherwise, results which bear a favourable relation to one another are found.

9. Summary.

1. A study has been made of the somatic chromosome complex, the chiasma frequency and the relationship of sex to crossing-over and chiasma frequency in the male and female rat - Rattus norvegicus albinus.

2. The stages of mitosis in relation to the somatic chromosomes have been discussed.

3. The number of chromosomes in the cells of both males and females have been determined as 42. which is in accord with the findings of Painter (1926, 1927), Pincus (1927) and Swezy (1928).

4. Stages of meiosis have been followed, the nature of pairing, formation of chiasmata etc. being studied throughout.

5. The chiasma frequencies have been calculated for both the male and the female in diplotene and metaphase, by interpreting the configurations in the light of the partial chiasmatype hypothesis. (Janssens 1909, 1924 and Darlington 1931, 1932).

6. In addition, in the male where mitotic stages were more abundant, the chiasma frequency has been calculated in the diakinesis stage as well.

7. It has been noticed that as meiosis progresses, the/

the frequency of chiasma formation decreases and the terminal associations of the chromosomes increases. The apparent movement of chiasmata and the accompanying 'terminalisation' have been discussed.

8. The theories of Darlington and Sax have been considered from the presentation of configurations in diplotene and metaphase stages.

9. The various types of bivalent found have been interpreted, and the origin of various types in metaphase, from configurations in diplotene, is suggested.

10. The precocious separation of some bivalents accompanied by the tendency of others to lag has been discussed.

11. Suggestions have been made of the relation between genetical and cytological crossing-over and a parallel has been drawn between them.

12. The genetical results of various workers on the effect of sex on crossing-over has been summarised.

13. It has been found that in many forms there is a suppression of crossing-over in one sex - usually the sex-heterozygote.

14/

14. The results recorded in this study of the frequencies of chiasma formation, have been compared with the genetical crossing-over results.

15. In common with the genetical results in rodents and in other examples, the frequency of chiasma formation is lower in the heterozygous sex - which in the rat is the male.

16. The cause of the suppression of crossing-over in the sex-heterozygote has been discussed and the results of some other workers have been summarised.

17. The forms of sex-chromosomes commonly met with have been listed.

18. The evolution of sex-chromosomes, the characters carried by them, and the genetical aspect of sex in individuals have been discussed.

19. The results given by Pincus (1927) have been compared with those found in this study.

20. The types of bivalent found have been compared and interpretations offered of some of Pincus's forms.

21. The chiasma frequencies have been calculated from each form and the results shown in graphical form.

14. The results recorded in this study of the frequencies of chiasma formation, have been compared with the genetical crossing-over results.

15. In common with the genetical results in rodents and in other examples, the frequency of chiasma formation is lower in the heterozygous sex - which in the rat is the male.

16. The cause of the suppression of crossing-over in the sex-heterozygote has been discussed and the results of some other workers have been summarised.

17. The forms of sex-chromosomes commonly met with have been listed.

18. The evolution of sex-chromosomes, the characters carried by them, and the genetical aspect of sex in individuals have been discussed.

19. The results given by Pincus (1927) have been compared with those found in this study.

20. The types of bivalent found have been compared and interpretations offered of some of Pincus's forms.

21. The chiasma frequencies have been calculated from each form and the results shown in graphical form.

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