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Sexual dimorphism in mouse gametogenesis

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INTRODUCTION

There is very little information in the literature on the frequency and behaviour of chiasmata in the female mouse. Crew and Koller (1932) give counts of chiasmata at diplotene of forty-two randomly recorded tetrads and at metaphase of five oöcytes. Since their paper was published no other information has appeared on the subject.

The present paper deals with chiasma frequency in female mice. There are several limitations inherent in the material which make this study more difficult and less exact than the corresponding study in males.

The diplotene stage is exceedingly rarely observed in females (Guenin, 1948), and in those few cases in which it has been found, the tetrads could not be satisfactorily analysed. Consequently, after several unsuccessful attempts, the study of chiasma frequency in earlier stages of meiotic division had to be abandoned, and it was necessary to limit the study of chiasmata to the metaphase stage.

MATERIAL AND METHODS

The material for this study was obtained through the kindness of Dr D. S. Falconer, Mr J. Isaacson and Dr M. Latyszewski from the mouse colony in this Laboratory. The material is by no means uniform in any respect; it consists of animals of various ages, strains and outcrosses.

Mice were killed mechanically, and the ovaries were removed as quickly as possible. After the removal of the covering membrane, they were subjected to water pretreatment for 5 minutes. Fixation in acetocarmine was followed by squashing on an albuminized slide. After detachment of the cover slip, the preparation was hydrolysed, stained in a water solution of basic Fuchsin, bleached in sulphurous acid, dehydrated in an increasing series of alcohol solutions and finally mounted in Euparal.

About 10% of metaphases were analysable. It has been found that in searching for metaphases the size of the oöcyte is not a valid criterion, since there is not much correlation between the size of the oöcyte and the stage of meiosis its nucleus can show. To illustrate this point a microphotograph has been taken of two oöcytes of similar size but with different stages of meiosis in them ; one shows the metaphase plate, the other what is probably the end of dictyotene (Plate I, fig. 1).

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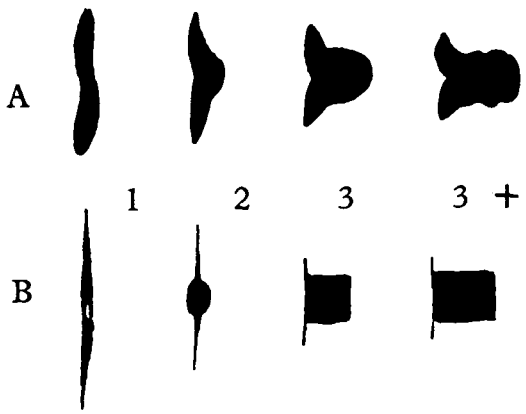
The diagram (Text-fig. 1 and Plate I, figs. 2 and 3) illustrates the definitions accepted in the interpretation of the number of chiasmata in a tetrad. Not all metaphase plates recorded are exactly in the same stage. Undoubtedly there is a relation between the shape and configuration of the tetrad, and consequently between the number of chiasmata it appears to contain and the stage of metaphase. Therefore two substages were distinguished: and earlier (early prometaphase) in which the poleward tendency of bivalents is only at the beginning, and a later (full metaphase) in which it is more pronounced. In the first stage, the centromeric ends of the bivalents (ends which are not involved in chiasmata) are blunt or even rounded, while in the second substage they are very much attenuated, forming long thin threads. A one-chiasma tetrad is almost uniformly thick throughout its length in prometaphase, but in metaphase it clearly shows a very thin region in



Text-fig. 1. Diagram showing the definitions accepted in interpretation of the number of chiasmata: A—earlier; B—later substage of meiotic metaphase. The figures give the assessed number of chiasmata.

which the bivalents are held together. Thus any small thickening in the middle of the tetrad was taken to indicate a single chiasma when seen in prometaphase, but probably indicated the occurrence of two chiasmata in metaphase.

The method applied for the assessment of the number of chiasmata in each tetrad was taken from Huskins & Hearne (1936) who wrote: 'Each bivalent configuration was assessed as having the minimum number of chiasmata necessary for the formation of such a configuration. Certain bivalents have quite certainly had more chiasmata than assessed, but the arbitrary minimum standard is the only one that can be maintained successfully.'

RESULTS

The estimated numbers of chiasmata in 39 oöcytes are shown in Table 1, which also gives the corresponding data on 10 spermatocytes of a CBA male recorded for comparison, and the data of Crew & Koller on 5 oöcytes and 11 spermatocytes. In the column marked '3+', are tetrads having probably more than 3 chiasmata;

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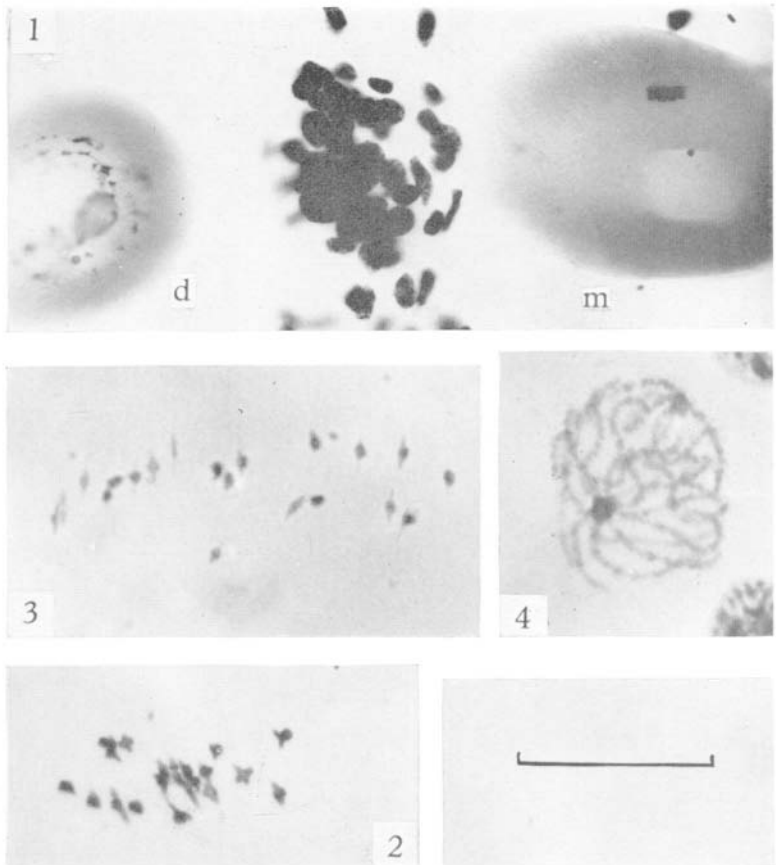

Microphotographs of: (1) Two oöcytes of similar size but differing in the state of their nuclei—d, dictyotene; m, metaphase. (2)—Earlier and (3)—later substage of meiotic metaphase. (4)—Chromocenters in mouse pachytene nucleus.
The scale represents 20 microns for photo Nos. 2 and 3, 35 microns for No. 4 and 50 microns for No. 1.

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the number could not be determined more exactly. In calculating the total number of chiasmata and in all subsequent calculations, these figures were taken as representing 3·5 chiasmata. For Crew & Koller's data this column includes cases of 4, 5 and 6 chiasmata. The assumption that the 3+ class has an average of 3·5 chiasmata may of course lead to some bias, but there is no way of checking this point.

Table 1. *Distribution of metaphase chiasmata in mice*

	Chiasmata				
Females	1	2	3	3+	Total
1	3	16	1	—	38·0
2	5	10	4	1	40·5
3	5	5	8	2	46·0
4	5	6	7	2	45·0
5	3	10	6	1	44·5
6	5	6	9	—	44·0
7	4	7	8	1	45·5
8	2	10	8	—	46·0
9	4	10	6	—	42·0
10	2	11	7	—	45·0
11	6	10	2	2	39·0
12	2	10	8	—	46·0
13	4	15	1	—	37·0
14	5	14	1	—	36·0
15	6	7	6	1	41·5
16	4	16	—	—	36·0
17	4	7	7	2	46·0
18	4	7	7	2	46·0
19	3	7	10	—	44·0
20	2	11	7	—	45·0
21	6	12	2	—	36·0
22	2	13	5	—	43·0
23	7	9	4	—	37·0
24	4	9	7	—	43·0
25	6	12	2	—	36·0
26	7	10	3	—	36·0
27	4	10	5	1	42·5
28	7	7	5	1	39·5
29	6	12	2	—	36·0
30	7	9	4	—	37·0
31	6	12	2	—	36·0
32	5	12	3	—	38·0
33	2	13	4	1	43·5
34	4	12	2	2	41·0
35	3	11	6	—	43·0
36	6	11	2	1	37·5
37	4	16	—	—	36·0
38	7	7	4	2	40·0
39	3	11	4	2	44·0
Mean	4·5	10·4	4·5	0·6	40·9

	Chiasmata				
Males	1	2	3	3+	Total
1	10	6	4	—	34·0
2	4	14	2	—	38·0
3	8	10	2	—	34·0
4	10	7	3	—	33·0
5	8	12	—	—	32·0
6	8	9	3	—	35·0
7	6	12	2	—	36·0
8	11	7	2	—	31·0
9	5	13	2	—	37·0
10	9	10	1	—	32·0
Mean	7·9	10·0	2·1	—	34·2

Crew & Koller's data

	Chiasmata				
Females	1	2	3	3+	Total
1	4	13	1	2	40·0
2	8	8	2	2	37·0
3	7	10	2	1	36·5
4	6	11	1	2	38·0
5	4	12	2	2	41·0
Mean	5·8	10·8	1·6	1·8	38·4

	Chiasmata				
Males	1	2	3	3+	Total
1	7	12	1	—	34·0
2	11	9	—	—	29·0
3	10	9	1	—	31·0
4	13	7	—	—	27·0
5	14	6	—	—	26·0
6	6	13	1	—	35·0
7	9	10	1	—	32·0
8	8	9	2	1	35·5
9	11	8	1	—	30·0
10	7	11	2	—	35·0
11	12	7	1	—	29·0
Mean	9·8	9·2	0·9	0·1	31·3

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As can be seen from Table 1, the oöcytes contain more chiasmata than do the spermatocytes; this applies to the distribution of chiasmata per nucleus and to the mean chiasma frequency per bivalent. Chiasma frequency in females at metaphase is similar to that of males at diplotene. Table 2, containing data calculated from Table 1, and taken partly from Crew & Koller (1932), and from Huskins & Hearne (1936) and for diplotene from Slizynski (1955) illustrates these points.

Table 2. *Mean chiasma frequencies and map lengths*

Stage and cells	Mean chiasma frequency per nucleus	Mean chiasma frequency per bivalent	Map length Mean	Map length Total	Reference
Metaphase:					
39 oöcytes	40·9	2·05	102·5	2050	Present data
5 oöcytes	39·4	1·97	98·5	1970	Crew & Koller
10 spermatocytes	34·2	1·71	85·5	1710	Present data
11 spermatocytes	31·3	1·56	78·0	1520	Crew & Koller
Diakinesis:					
400 spermatocytes	31·3	1·54	79·0	1540	Huskins & Hearne
Diplotene:					
41 spermatocytes	36·4	1·82	91·0	1822	Slizynski
42 loose bivalents from oöcytes	—	2·80	140·0	2800	Crew & Koller

DISCUSSION

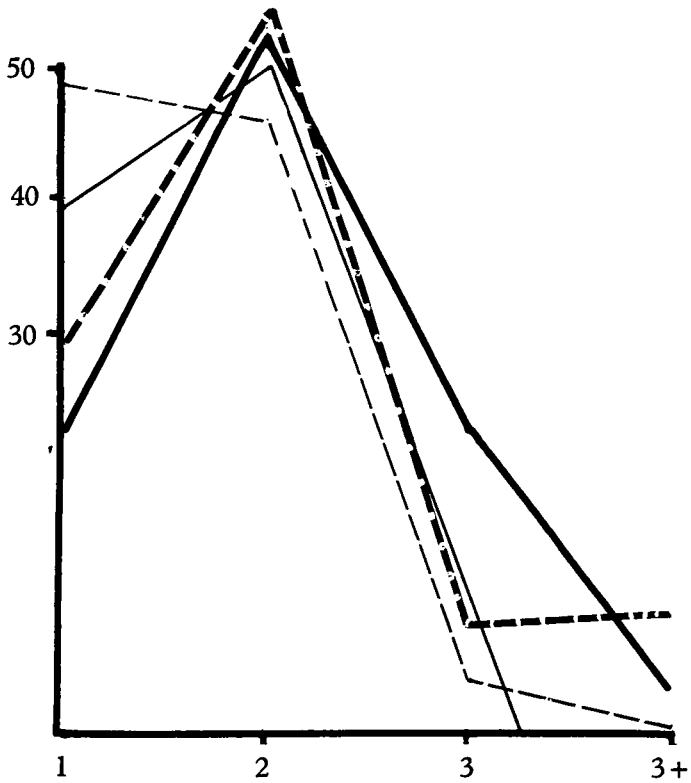
The primary object for which this study was originally undertaken, namely to obtain data on chiasma frequency at diplotene in female mice, could not be achieved by direct methods. However, using the available data on diplotene chiasma frequency in the male, and the mean chiasma frequency per bivalent in males and in females at metaphase, and finally assuming that the changes between diplotene and metaphase with regard to the behaviour of chiasmata are of similar nature and degree in the two sexes, the diplotene chiasma frequencies can be deduced for females. As a result of these deductions we obtain 2·3 as the mean chiasma frequency per bivalent in diplotene in females, which is about 15% higher than the corresponding figure for males (2·0).

If a bivalent shows a mean chiasma frequency of 1·0, it means, as is generally accepted, that two of the four chromatids have exchanged parts and that crossing-over has taken place in 50% of cases between the two ends of the chromosome. Its map length is 50 cM. The formula for calculating map length may then be given as the mean number of chiasmata in a bivalent multiplied by 50.

On the basis of the above data and assumptions the total diplotene map length of 20 bivalents is about 2300 cM. for the female mouse, in comparison with 1954 cM. for the male.

This difference is probably related to the sexual dimorphism in genetical map length found in the mouse by several authors.

A comparison of proportions of bivalents with 1, 2, 3, 3+ chiasmata in the two sexes shows that females have less 1-chiasma bivalents and more (3+)-chiasmata bivalents than do the males. These results are in agreement with Crew & Koller's



Text-fig. 2. Sexual dimorphism in distribution of chiasmata in mice. Thick lines—chiasmata in females; thin lines—in males. Continuous line—present data; broken line—data of Crew and Koller (1932). Ordinate—percentage of bivalents containing 1, 2, 3, 3+ chiasmata which are marked on the abscissa.

data (1932) in which the sexual dimorphism of chiasma distribution is even more pronounced. Table 3 shows the relevant figures and Text-fig. 2 presents them graphically.

Table 3. *Proportions of bivalents containing 1, 2, 3, and 3+ chiasmata*

	Chiasmata %				
Cells	1	2	3	3+	Reference
Meiotic metaphase:					
39 oöcytes	22·3	52·1	22·5	3·1	Slizynski
5 oöcytes	29·0	54·0	8·0	9·0	Crew & Koller
10 spermatocytes	39·5	50·0	10·5	—	Slizynski
11 spermatocytes	49·0	46·0	4·6	0·4	Crew & Koller

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Sexual differences in crossing-over and chiasma frequency have been under discussion for some time. Haldane (1922) wrote: 'If in any animal sex is determined by one factor, there is probably no sex linkage or chromosome difference between the sexes. As soon as another factor becomes necessary, complete sex linkage between the two must appear in the heterozygous sex and the same mechanism which prevents them from crossing-over may be expected to hinder or prevent crossing-over of all factors in that sex. Sex-linked factors must be completely linked in the heterozygous sex but linkage between autosomal factors is also always strong in that sex.'

From the frequencies of bivalents with different numbers of chiasmata we can calculate the distribution of crossover frequencies with the help of Mather's formula in which 'the results of n chiasmata in terms of frequencies of strands with various numbers of crossovers are given by the expansion of $(\frac{1}{2}+\frac{1}{2})^n$, assuming that there is no chromatid interference' (Mather, 1936). Carter (1954) came to the conclusion that there is little or no chromatid interference in the male mouse. In view of this, Mather's formula can probably be applied to the present material to obtain the frequency of crossovers in females. Mather (*loc. cit.*) gives a two-way table by which chiasma frequency can be expressed in crossing-over frequency and vice versa. The following results were obtained (Table 4).

Table 4. *Proportions of single, double, etc., crossovers based on the chiasma distribution given in Table* 1

	Females	Males
Non-crossovers	22·5	28·5
Crossovers:		
single	44·4	47·7
double	27·1	21·5
triple	5·5	2·3
quadruple	0·4	—

The data of this table show that, taken as a whole, crossover frequencies in females are higher than in males. There is also a tendency for females to have more multiple crossovers.

It has been known for some time in *Drosophila* (see Schultz & Redfield, 1951) that the amount of crossing-over can be increased or decreased by changes in genetic constitution. Moreover, a decrease of the frequency of crossing-over in one chromosome may be accompanied by an increase in that of another chromosome.

Recently a very interesting study of Oksala (1958) has thrown new light on this problem. According to Oksala's hypothesis, non-homologous pairing of heterochromatic segments may lead to retardation of chiasma formation in euchromatic segments. The distribution of heterochromatic material in the karyotype is then one of the factors responsible for the frequency of chiasmata. For instance, an inversion changing the normal distribution of heterochromatic material in *Drosophila* karyotype and thus influencing non-homologous pairing may change the pairing and chiasma formation in euchromatic segments.

Theoretically the distribution of heterochromatic material can be of two ex-

treme types. Either it may be equally apportioned to all chromosomes or it may be mainly concentrated on one chromosome. It is obvious that in the case of extremely unequal distribution the chromosome with most of the heterochromatic material will as a rule pair legitimately in the heterochromatic region, since it will only be able to pair with its homologue. Thus there will be no difficulties in the pairing of euchromatic regions and the frequency of chiasmata will not be reduced by delays in pairing. On the other hand, in the case of equal distribution of heterochromatic material, heterochromatic segments of any chromosome may pair with those of any other chromosome. This will delay the legitimate pairing of euchromatic segments and cause a reduction in chiasma frequency.

It follows that, all other conditions being equal, the number of chromosomes has an effect on the frequency of chiasmata. Of two species having the same total length of chromosomes, and a similar amount and distribution of heterochromatic material, the one with the higher number of chromosomes will be expected to have less chiasmata per bivalent.

The facts that a chromocentre occurs regularly in the mouse and that all chromosomes in females take part in it with much the same frequency indicate that the chromosomes all contain roughly the same amount of heterochromatic material. In the male the autosomes behave in the same way but the sex bivalent does not join the chromocentre. This may indicate that its heterochromatic regions do not pair illegitimately. Thus the distribution and amount of heterochromatic material in both sexes is the same, but the number of chromosomes which may be involved in illegitimate pairing is smaller in males.

In this way, on the basis of Oksala's hypothesis, male mice are expected to show more chiasmata than females. The results of this paper show the opposite.

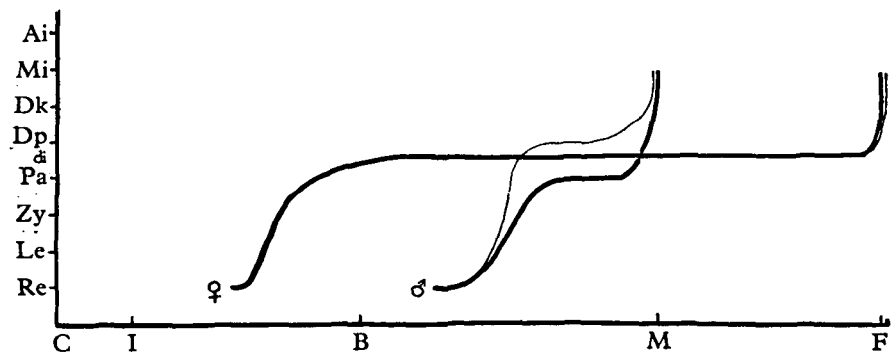
It is possible that the explanation of this discrepancy may be found in the time allowed for the formation of chiasmata.

There is a profound difference in the duration of various stages of gametogenesis between the two sexes. In males, the whole of the pachytene stage lasts about 7 days (Oakberg, 1956), of which possibly half is taken up by the formation of chiasmata. In females the pachytene stage appears at about the fifteenth day of embryonic life and when the pachytene stage is ending the chromosomes uncoil and appear as a fine network inside the nuclear vesicle. This is called the dictyotene stage, which lasts till just a few hours prior to ovulation. Chiasmata are formed during the dictyotene stage and its duration at its minimum takes about 35–40 days. If again half of this time be assumed for the actual formation of chiasmata, then clearly chiasmata have much more time for their formation and terminalization in females than they have in males (Text-fig. 3).

In female gametogenesis, chiasmata are formed in the oöcytes of the ovary of the embryo. These chiasmata are determining the maternal contribution to the genetic constitution of an individual while it is an oöcyte in the ovary of its mother, who is at that time herself an embryo in the uterus of the grandmother of this individual. This complicated system certainly ensures complete independence of chiasma formation from all uncontrolled influences.

There is nothing comparable in the males, where chiasma formation is exposed to various external influences, and any detrimental effect of these is probably balanced by the very high number of gametes produced. In the male mouse the ratio of released gametes to the offspring produced is about 5000 : 1, while in females it is only about 2 : 1.

The time factor is of importance also in another aspect of gametogenesis. Guenin (1948) found that in the oöcytes of young female mice which had hormonally induced super-ovulation, the sex chromosomes can be distinguished from the autosomes by being retarded in the anaphase of the first meiotic division; the sex bivalent shows constantly two chiasmata which have their terminalization delayed. This agrees very well with White (1959) who, starting from Callan's idea of



Text-fig. 3. Diagram representing the time differences in mouse between female and male gametogenesis. For females only the first ovulation is given, for males the period of spermateleosis is not included. Ai—first anaphase separation; B—birth; C—copulation; Dk—diakinesis; di—dictyotene; Dp—diplotene; F—female maturation division; I—implantation; Le—leptotene; M—male maturation division; Mi—first metaphase; Pa—pachytene; Re—end of postmitotic resting stage; Zy—zygotene. Thick lines refer to the autosomes—thin lines to heterochromosomes. (Based on Oakberg, 1956, for time relations in spermatogenesis.)

chromosome ends being undivided, expressed a belief that 'regularity of disjunction is best served by extreme terminalization only where there is a maximum of one chiasma per arm, and that selection has operated to produce these regularities of meiotic mechanism'. Two chiasmata recorded by Guenin (*loc. cit.*) are thus associated in young oöcytes with poorly developed terminalization, which in turn works for delayed separation and possibly for non-disjunction. This possibility is supported by the fact that the Guenin phenomenon, i.e. delayed separation of the sex bivalent observable in induced ovulation, is not detectable in old females ovulating naturally.

No chiasmata are seen in oöcytes at the end of the pachytene stage and no diplotene stage is seen before prometaphasic appearance of condensed chromosomes, and from this it follows as a necessary deduction that chiasmata are formed and their terminalization is nearly completed during the diffuse stage of dictyotene. From this it can be concluded that stainable material of the chromosomes is concomitant to pairing, but not necessary for chiasma formation and movement.

All oöcytes enter the chiasma formation stage almost at the same time—the variation does not exceed 2–4 days. However, the duration of the dictyotene stage is variable, depending on when the cell completes development and is shed as an ovum. Thus dictyotene in the mouse may last any time between a few weeks and 1½ years, while in the human female it may last as long as 30–40 years.

A very long dictyotene stage, such as would occur in oöcytes used late in the reproductive life of the female, might be expected to cause so complete a terminalization of chiasmata, that in the first meiotic anaphase the bivalents of a tetrad would be liable to fall asunder, leading to disturbed separation and to non-disjunction. Such a mechanism may possibly be the cause of the non-disjunction responsible for Mongolism in man, since this is known to occur much more frequently in children born to old mothers.

In male gametogenesis the situation is different. The XY tetrad develops in advance of the autosomes and chiasmata in it can be studied at the time when autosomes are in early pachytene stage. This is probably due to the fact that in males time is a limiting factor for chiasma formation in the autosomes. In females there are 25·1% of bivalents with three or more chiasmata in contrast to males where such bivalents form only 10·5% of the total. The sex bivalent is less dependent on the time factor because of the precocity of its prophase, due probably to a higher content of heterochromatic material in the Y chromosome.

On the other hand, this precocity in development of the sex bivalent may sometimes have too much effect on the time of terminalization of chiasmata: namely, in a fair proportion of cases (30–40%) the sex bivalent is the first to separate at the time when autosomes are still held on the metaphase plate. This speeding up may even go so far that occasionally (with a frequency probably less than 1%) there is a non-disjunction of the sex chromosomes detectable cytologically.

SUMMARY

Diplotene and diakinesis chiasma frequency in oöcytes of the mouse cannot be studied successfully with the present technique. Metaphase chiasmata have been examined in thirty-nine oöcytes. It is deduced that the total diplotene map length in females is about 2300 cM. compared with 1950 cM. in males. There is sexual dimorphism in the frequency of chiasmata, which is paralleled by similar dimorphism in frequencies of crossing-over, measured genetically.

The two sexes differ in the duration of various stages of meiosis. In adult males the pachytene stage, lasting for about 7 days, is directly followed by diplotene and diakinesis, after which the metaphase stage sets in. The sex bivalent in males develops visible chiasmata much earlier than do the autosomes and it precedes them in anaphase separation. Quick terminalization of chiasmata in it leads in a fair proportion of cases to precocious separation and in less than 1% of cases to cytologically detectable non-disjunction of sex chromosomes.

In females the pachytene stage appears in oöcytes of the embryo and is followed by the dictyotene stage, which last still ovulation, i.e. between 35–40 days and

several months. Since in the oöcyte chiasmata are formed and move during the dictyotene stage, it follows that stainable materials of the chromosomes are not necessary for the formation and movement of chiasmata and are concomitant with pairing and anaphase separation. It follows also that the time for chiasma formation and movement is in females at least five to six times longer than in males. In old oöcytes in which time is available for maximum terminalization of chiasmata, non-disjunction may appear with detectable frequency. This mechanism may also operate in cases of Mongolism in man, where non-disjunction of an autosome has been recently cytologically established and higher frequency of incidence of the condition for old mothers has been known for some time.

It is possible that the differences in duration of various stages of gametogenesis are connected with the period at which gametic selection is operating: in spermatogenesis after the second meiotic division, in oögenesis prior to first meiotic metaphase.

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