Meiosis in *Dasyurus viverrinus*

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Introduction

The methods of analysis introduced by Darlington and other into the study of meiotic phenomena in plants have been extended to various species of animals, and recently the spermatogenesis of the rat (Bryden 1932) and of the mouse (Crew & Koller 1932) have been investigated with special reference to the distribution and behaviour of chiasmata in relation to genetic crossing over. In such forms where there is a large number of chromosomes showing little diversity in shape or size, chiasma frequency in individual chromosomes cannot be analysed. In this respect many marsupials offer more favourable material for such investigations. The number of chromosomes is smaller, and often there is such diversity within the complex that particular chromosomes can be recognised at all stages of meiosis. The following account deals with some aspects of the spermatogenesis of *Dasyurus viverrinus*, particularly those relating to the frequency of chiasma formation. The observations have been interpreted according to the chiasmotype theory of Jannsens as modified by Darlington (1931).

Material and methods. The material consisted of the testes fixed in Allen's Bouin at 37°C. After dehydration it was cleared in cedar wood oil and embedded in paraffin (M.P. 42°C). The most useful sections were cut at thickness of 12–15 microns and stained with gentian violet. Iron haematoxylin and Feulgen's method were also used. For staining thick sections gentian violet is superior to haematoxylin but its use has the disadvantage that the preparations fade.

Spermatogonial chromosomes. The diploid number of chromosomes is fourteen, including the sex chromosomes which are of the XY type in the male. With few exceptions, homologous chromosomes can be identified with certainty at metaphase. The haploid set of chromosomes is represented in text figure 1. Chromosome A, the longest, has a median spindle attachment. B and C are approximately equal in length. B has a submedian attachment and C a sub-terminal one. The position of the attachment in the remaining chromosomes

is: sub-medial in D, sub-terminal in E and median in F. Both sex chromosomes are considerably smaller than the smallest autosome and therefore easily identified. The X chromosome, which is about half the length of the autosome F, has a terminal attachment. The Y is very minute and does not attain the width of an autosome. As is commonly found, the small chromosomes, including the sex chromosomes, typically lie towards the centre of the equatorial plate.

Text fig. 1. Diagram of the sex chromosomes and haploid set of autosomes.

Dasyurus viverrinus has the same number of chromosomes as *D. maculatus* (Greenwood 1923, Koller 1936) and the morphology of the complement is similar in the two species.

Meiotic divisions. The leptotene nucleus presents the appearance typical of this stage of meiosis. The chromosomes are in the form of fine beaded threads which, however, can not be counted. Synapsis and contraction take place together, and although it is not possible to observe the process in all the chromosomes in one nucleus, it appears that synapsis commences at the ends of the chromosomes and, therefore, is not initiated at the spindle attachments. At later stages synapsis extends to various intermediate points and finally is complete along the whole length. The sex chromosomes were not observed at zygotene. The longitudinal contraction of the threads during synapsis is accompanied by a slight synizetic contraction.

The cytoplasm of the pachytene cells contains a variable number of small chromatoid bodies. These are distributed at random at the first meiotic division.

Diplotene. It has been established that in many organisms, following synapsis, each paired homologue divides into two chromatids, but in the present material the quadruple nature of the bivalents is not evident. According to the chiasmatype theory, the holding together of chromosomes at a chiasma is due to the occurrence of an exchange of partners in the system of paired chromatids. The chiasmata indicate the number of points at which an exchange has taken place, but at diplotene do not necessarily show the actual loca-

tions of the exchanges, because chiasmata may terminalise, that is, move away from the point of spindle attachment towards the ends of a chromosome (Darlington 1931).

Diplotene is marked by the condensation of the pachytene threads and a gradual contraction to about one quarter of their respective pachytene lengths. The rate of contraction is not uniform throughout a nucleus. With the exception of the smallest one, corresponding bivalents cannot be individually recognised in a series of nuclei during this stage. The three large bivalents can always be distinguished from the remaining bivalents, but not from one another. The two medium sized bivalents are similarly recognisable.

Text fig. 2. Drawing of the autosomal bivalents from two nuclei at diplotene. The numbers in this and the next figure refer to the number of chiasmata.

Counts of chiasmata were made on twenty-four complete nuclei i.e. nuclei in which the full haploid number of bivalents could be analysed. The nuclei varied from diplotene to diakinesis, but they show no significant reduction in number of chiasmata over this period, and can be considered together. In the autosomes chiasmata are distributed at random, and vary in number from five in the long ones to one in the shortest (Text figs. 2 & 3). The distribution of chiasmata

in the six bivalents is shown in Table 1. Bivalents A, B and C (following the notation adopted in Text fig. 1), considered together, have on the average four chiasmata each, the range of variation being between three and five. More than five or less than three chiasmata

Text fig. 3. Drawing of the autosomal bivalents from two nuclei at diakinesis.

were not observed in any one of these three bivalents. The shorter bivalents D, E and F, all have fewer chiasmata, as shown in the table. The relationship between chiasma frequency and length will be discussed later. The total number of chiasmata per nucleus varies between sixteen and twenty two, (Table 3) with the mode at eighteen. The average number per nucleus is 18.7.

Metaphase. At metaphase the average number of chiasmata is slightly, but possibly significantly, reduced below that of diplotene, and there is an increase in the proportion terminal or nearly terminal in position. The number of chiasmata in the various bivalents at metaphase is shown in Table 2. The slight reduction in chiasma frequency occurs in the A, B, C group of large bivalents. At metaphase bivalent C can be distinguished from A and B on account of the position of the spindle fibre attachment; and similarly D can be distinguished from E; but the analysis of the reduction is incomplete in regard to the individual bivalents of the groups A, B, C and D, E. For this reason the bivalents have been grouped in Table 2 in the same way as in Table 1.

Table 1. Chiasma frequency at diplotene 24 nuclei.

Bivalent	Number of chiasmata					Average No. of chiasmata per bivalent
	1	2	3	4	5	
A, B, C			9	52	11	4.03
D, E		24	23	1		2.52
F	9	15				1.62

It has been shown that metaphase configurations are determined by the position of the spindle attachment and by the number and position of the chiasmata. In most of the bivalents the chiasmata are distributed on each side of the spindle attachment in only two or three ways, and there is a corresponding limitation to their possible

Text fig. 4. Drawings of metaphase bivalents from three nuclei. The sex chromosomes are shown on the left. In one case the Y chromosome is not shown.

metaphase configurations (Text fig. 4). Bivalents A and B have three, four, or five chiasmata which are always distributed on both sides of the spindle attachment, with not more than three on one side. In C there is always a single chiasma in the short arm and approximately terminal in position. In D chiasmata occur on both sides of the attachment. E shows distributions similar to those in D and a further one in which chiasmata are restricted to the long arms, the short arms being free. In other metaphase nuclei not analysed in Table 2, bivalent E has only one chiasma, whereas at diplotene it always has at least two. In the nuclei in question the single chiasma is in the distal arm. But probably E is in an early

Table 2. Chiasma frequency at metaphase. 21 nuclei.

Bivalent	Number of chiasmata					Average No. of chiasmata per bivalent
	1	2	3	4	5	
A, B, C			19	40	4	3.76
D, E		23	19			2.45
F	6	15				1.71

anaphase stage in these nuclei, the single chiasma condition following one in which two chiasmata were present, one in each arm; and for this reason these nuclei are not included in Table 2. At anaphase the proximal arms separate in advance of the distal so that only one chiasma would be evident. Bivalent F shows the two expected configurations, according to whether one or two chiasmata are present. When there are two, they are terminal in position, resulting in a diamond shaped tetrad. When one chiasma is present it remains interstitial.

Table 3. Total chiasma frequency at diplotene and metaphase.

Stage	Total chiasmata per nucleus							Total chiasmata	Average and S. E. of average
	16	17	18	19	20	21	22		
Diplotene	1	4	9	3	3	3	1	448	18.7±31
Metaphase	2	5	9	4		1		376	17.9±24

Anaphase. The early separation of small chromosomes seen in spermatogonial divisions is also characteristic of first meiotic divisions (Text fig. 5a). It is more pronounced in the latter, probably due to the fact that long bivalents have more interstitial chiasmata than the small ones. The retarding effect of interstitial chiasmata, as opposed to terminal chiasmata, on the rate of bivalent separation,

Text fig. 5. Anaphase. (a) early anaphase showing the early separation of the small bivalents. (b) two bivalents from another nucleus, showing interlocking.

is seen best in organisms in which an occasional bivalent shows terminalisation to a degree sharply different from that of the rest of the complement. (Darlington 1931). Bivalent F always separates first, but in E there may be an earlier separation of the short arms as mentioned above.

In the longer bivalents, when two chiasmata occur in one arm, it is possible to determine the relationships of chromatids at successive chiasmata. Adjacent chiasmata may be compensating or

non-compensating (Darlington 1931). As far as mammals are concerned, both types have been observed in the rat (Bryden 1932) and the mouse (Crew and Koller 1932); but in the present material, anaphase configurations resulting from non-compensating chiasmata have not been observed. The compensating relationship may be revealed by interlocking at anaphase (Text fig. 5b).

Throughout the preceding stages the four chromatids of each bivalent have not been generally visible. At anaphase, as a result of the occurrence of interstitial chiasmata at metaphase, the chromatids of the longer chromosomes are often widely separated over most of their lengths. In the small chromosomes the component chromatids may not be visible until late anaphase (Text fig. 6). Ultimately, the paired chromatids separate completely, except in the region of the spindle attachment where they remain in close association. The paired condition of the chromosomes is temporarily obscured when they clump together at early telophase, but it is again evident as the secondary spermatocyte nuclei pass into the resting stage.

Sex chromosomes. As described above, the sex chromosomes form a karyosome throughout pachytene. In mid-pachytene this karyosome has a narrow, elongated shape, but as diplotene is reached it becomes rounded. The component parts X and Y cannot be identified until metaphase, when it is seen that they are terminally associated at the distal ends. They segregate to opposite poles, the separation preceding that of the autosomes. The sex chromosomes do not show a division into chromatids.

The first division results, therefore, in the formation of two types of secondary spermatocytes, and these can be distinguished by the fact that one has a karyosome formed from the X chromosome. In the second type no karyosome can be observed, though it is possible that the Y remains in a compact form but is too small to identify with certainty.

Second division. From the telophase of the first division the nucleus passes into a resting condition, the autosomes becoming diffuse. During interphase the spermatocytes increase in size. At the following prophase the haploid number of autosomes reappears as long thin threads, and from the beginning, daughter chromatids

Text fig. 6. Late anaphase. Paired chromatids are now clearly visible.

are widely separated except at the point of spindle attachment. Apparently the chromatids have remained in this loose association since the telophase of the preceding division. The prophase of the second division is therefore entirely different in appearance from the prophase of a spermatogonial mitosis, when the chromosomes appear as single threads.

As the chromosomes contract, daughter chromatids come to lie more or less parallel, or may be slightly twisted together. When the equatorial plate is forming they are in contact along their whole lengths, but, shortly after, the spindle attachments move apart, leaving the chromatids in contact only at the ends. The parallel association is closer than that at a similar stage in the rat, where the chromatids are in contact only at the spindle attachment (Koller and Darlington 1934). These authors did not observe the chromosomes at the moment of separation, but from their figures it appears that, in contrast to *Dasyurus*, the final separation in the rat must take place at the spindle attachments and not at the ends. The equatorial plates formed in the two types of spermatocytes can be distinguished by the sex chromosome present, one type having the X, the other the Y. At anaphase the further separation of the spindle attachments is followed immediately by that of the ends of the chromatids, so that each daughter complement passes to the pole as a more or less flat plate. There is no evidence, at anaphase or telophase, that the chromosomes are double structures. The sex chromosomes do not exhibit any precocity at the second division and may lag behind the autosomes.

The contraction of the chromosomes at second division metaphase is very pronounced. On the average they are only two thirds of the length of spermatogonial chromosomes. This cannot be a direct outcome of the first meiotic contraction since this is lost during interphase.

Discussion

Chiasmata. In *Dasyurus* chiasmata are distributed at random in the autosomes, and the terminal position of some at diplotene shows that a certain amount of movement towards the ends has already taken place. When only one chiasma is formed, as sometimes happens in bivalent F, it is always interstitial and remains so at metaphase. In the other metaphase bivalents there is an increase in the proportion of terminal or nearly terminal chiasmata, due to the repulsion between spindle attachments of paired chromosomes. That this localised repulsion is not marked before metaphase is shown by the fact that at diakinesis chiasmata are evenly spaced. If

spindle attachments were repelling one another, the loops containing them would enlarge at the expense of adjacent loops. (Darlington 1931). Comparison of chiasma frequency in A and B, and C, at diplotene and metaphase, suggests that terminalisation is dependent to some extent on the position of the spindle attachment, and is greater when the attachment is towards the end of a chromosome.

In some organisms it has been found that the mean number of chiasmata per bivalent is approximately proportional to length. In the majority of cases the ratio breaks down for small bivalents, and this is particularly evident when there is a wide range in chromosome size (Darlington and Dark 1932). In *Dasyurus*, with a restricted range (the largest chromosome is less than three times the length of the shortest), chiasma frequency is proportional to length, as shown in Table 4. In

Table 4. Relationship between chiasma frequency and length. Lengths measured at spermatogonial metaphase and expressed in microns.

	Bivalent		
	A, B, C	D, E	F
Average number of chiasmata at diplotene	4.03	2.52	1.62
Average length of mitotic chromosome	7.4	4.4	2.8
Chiasmata per unit length	.54	.57	.58

this table the frequency at diplotene has been used in preference to that at metaphase, because, when the latter stage is reached, the various bivalents have been subjected to different degrees of terminalisation.

Sex chromosomes. The terms "equational" and "reductional" divisions, though of restricted application to the division of autosomes, are still conveniently used to describe the division of an unequal bivalent such as that formed by the sex chromosomes. X and Y are homologous for only part of their lengths, and pairing must be incomplete. The sex bivalent will consist of paired and unpaired portions, chiasma formation being restricted to the former. Depending on the position of the spindle attachment and on the number of chiasmata, the first division may be reductional or equational for the non-homologous parts.

Using the terms in this sense, the first division in *Dasyurus* is always reductional. This is typical of the majority of mammals in which sex chromosomes have been observed, but recently, equational divisions of the sex bivalent have been recorded in the mouse (Crew and Koller 1932) and rat (Koller and Darlington 1934). In another rodent, *Apodemus*, in which there is no Y chromosome, the first division is equational for the X chromosome (Oguma 1934). In the rat

and mouse chiasmata are formed in the sex bivalent, but in *Dasyurus* pairing is probably restricted to the terminal particles without the formation of chiasmata. This would prevent the occurrence of equational divisions. Koller (1936) is of the opinion that pairing without the formation of chiasmata is characteristic of the sex chromosomes of marsupials.

The apparent singleness of the sex chromosomes at the first meiotic anaphase and the second prophase, and their delayed separation at second anaphase, may be related to the earlier failure to form chiasmata.

Summary

1. The diploid number of chromosomes in *Dasyurus viverrinus* is 14, or $12 + XY$, in the male.
2. The sex chromosomes form a karyosome during the prophase of the first meiotic division. At metaphase they are associated terminally and the first division is always reductional.
3. The frequency and distribution of chiasmata in various bivalents have been analysed. In the autosomes chiasmata are distributed at random, with a frequency proportional to length.
4. There is a slight but possibly significant reduction in chiasma frequency at metaphase.

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