The Origin and Behaviour of Chiasmata

XI. *Dasyurus* and *Sarcophilus*

by

P. C. Koller

Institute of Animal Genetics, University of Edinburgh

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Introduction

Two genera of Marsupials, *Dasyurus maculatus* and *Sarcophilus ursinus*, which, according to Wood Jones (1932), belong to the same family of Dasyuridae, were studied in respect of chromosome behaviour during mitosis and meiosis. The advantage of these genera lies mainly in the great size of the cell and nucleus, and in the small number of the chromosomes. It is expected that by these studies a better insight will be gained into the mechanism of chromosome behaviour during mitotic and meiotic division.

Material and Technique

The fragments of testes from *Dasyurus* and *Sarcophilus* were obtained through the kindness of Professor F. A. E. Crew from W. E. Agar, F. R. S., Professor of Zoology in the University of Melbourne, Australia. They were fixed in Flemming solution and embedded in wax for sending.

The sections were cut 20–30 micra in thickness and stained with gentian violet by the method elaborated by La Cour (1931). The new brazilin, which was so advantageously used by Belling (1931) was also tried and gave fairly good result, though inferior to the first, mainly because it obscures the structure of the bivalents while de-staining is difficult.

Dr. A. W. Greenwood was kind enough to hand over some slides which he used in his studies on Marsupial spermatogenesis (1923). The sections of the slides were 10–20 micra in thickness and stained with iron haematoxylin. The present writer is greatly indebted to Professor Agar and Dr. Greenwood for their courtesy which alone made the following study possible.

The drawings were made with the aid of camera lucida, using 90, 1.3 oil immersion objective, $\times 18$ and $\times 30$ eyepiece. The initial magnification was $\times 3300$ and $\times 4500$ respectively, and reduced in reproduction to $2/3$, except the figures 20–23, which were reduced to $1/3$. The scale of magnification is given with the figures.

Mitosis

Dasyurus maculatus and *Sarcophilus ursinus* have six pairs of autosomes and the usual XY chromosomes in the heterozygous sex

male) as reported by Greenwood (1932). The length and shape of the chromosomes show a definite range of variation in both genera (Fig. 1. A, B). In *Dasyurus* there is a gradual decrease in length as can be seen in Fig. 2, where the chromosomes are arranged in serial order to illustrate the difference in their length. In *Dasyurus* the first four chromosomes (A, B, C, and D) represent the major group, and the two others (E and F) the minor group. The minimum difference between these two groups of chromosomes is at least two

Fig. 1. Mitotic metaphase in (A) *Dasyurus* and (B) *Sarcophilus*. $\times 2800$.

micra. In *Sarcophilus* the range of variation in the length of chromosomes is smaller. The first three chromosomes (A, B and C) are about the same size and the minimum difference between this and the minor group of chromosomes (D, E and F) is not more than one micron. The locus of the spindle attachment constriction (centromere), and hence the shape of the chromosomes, vary greatly. In *Dasyurus* chromosome B and perhaps A (the two arms are 3.7 and 3.3 micra long respectively), and in *Sarcophilus* chromosomes A, D and F have median attachment constrictions. The chromosomes are v-shaped with equal arms. Chromosomes C, D and F of *Dasyurus* and B of *Sarcophilus* have submedian, and the others (E in both and C in *Sarcophilus*) subterminal attachment constrictions.

Fig. 2. The size and shape of different chromosomes: top row *Dasyurus*; second row *Sarcophilus*. The length of chromosomes is given in micra. $\times 2800$.

Secondary constrictions were found in the A and B chromosomes of *Dasyurus* and probably also in *Sarcophilus*.

The sex chromosomes are very small; the length of the X was estimated as being $1.3\text{--}1.0\mu$. The position of the attachment constriction is most probably terminal in both sex chromosomes.

During mitotic metaphase the chromosomes are arranged in the same plane, the attachment constriction is turned towards the centre of the equatorial plate, the arms lying outside the

spindle. Fig. 1, which illustrates the arrangement of chromosomes at metaphase during mitosis in *Dasyurus* and *Sarcophilus*, shows very well that the homologous chromosomes lie close to each other. Such an arrangement is called somatic pairing and was described as characteristic in the Diptera (Metz, 1916), which have few and short chromosomes in their complement. In the genera of Marsupials under investigation where the chromosomes are long, the secondary association may be obscured more or less by the movement of the arms following the cytoplasmic currents, although the relative position of the attachment constrictions of chromosomes always remain fixed. The permanent and characteristic metaphase pattern of chromosomes during mitosis is an obvious outcome of attraction, operating as an inherent property between two homologous constituents. The conditions in the Marsupials are very favorable, the number of chromosomes is small, the size of the cell is large and the somatic pairing of homologous chromosomes can be easily expressed.

In *Dasyurus* and *Sarcophilus* very remarkable differences are found during mitotic prophase which are perhaps, to a certain extent, responsible for the differences in chiasma behaviour at meiotic prophase. The prophase in *Dasyurus* is of short duration and very few spermatogonia were observed at this stage. At the beginning of mitosis the uncoiling chromosomes are fine spiral threads, the condensation of which gradually proceeds. Fig. 3A illustrates this process; the unravelling is almost completed and the condensation is already far advanced as the strong staining indicates. The disappearance of the nuclear membrane

Fig. 3. (A) Mid-prophase of mitosis in *Dasyurus*; (B) pro-metaphase in *Sarcophilus*. $\times 2800$.

almost coincides with the maximum condensation and contraction of the mitotic chromosomes. Without further delay they move towards the equator and form the metaphase plate.

This process of mitotic prophase is different in *Sarcophilus*. The rate of contraction of chromosomes is slower than in *Dasyurus*. At the time of the disappearance of the nuclear membrane the prophase chromosomes are still thin threads, and there follows a long pro-metaphase. The chromosomes lie in the cytoplasm, showing no tendency to move towards the equator. At

the beginning of pro-metaphase the chromosomes are about twice as long as in metaphase. In *Sarcophilus* several spermatogonia were found in this stage which is of considerable duration. Fig. 3B illustrates the pro-metaphase of mitosis in *Sarcophilus*.

Comparison of the mitotic prophase in *Dasyurus* and *Sarcophilus* shows that the contraction or condensation of chromosomes is much slower in the latter.

It is known that the length of the metaphase chromosome is permanent and is an indication of the degree of their longitudinal contraction. The rate of contraction may differ in different species, consequently the time taken by division varies. In *Dasyurus* and *Sarcophilus* there exists a very definite difference in the prophase contraction, and the fact that it is constant for the genera suggests that its ultimate cause lies in the genotype. It was demonstrated by Hollingshead (1930) that the degree of chromosome contraction is controlled genotypically. It is therefore reasonable to assume that similarly the rate of contraction is regulated by the genotype. In the two closely related genera of Marsupials under observation there is a genetic differentiation of the factors controlling the rate of chromosome contraction.

The sex chromosomes are usually arranged at the centre of the equatorial plate during mitotic metaphase. At prophase no precocious condensation of sex chromosomes are found. The number and the size of the nucleoli varies. Table 1 shows the size of cells, nucleus and chromosomes in *Dasyurus* and *Sarcophilus*.

Table 1. The approximate size of cells, nuclei and nucleoli in *Dasyurus* and *Sarcophilus*.

Genera	Size of cell		Size of nucleus		Total length of chromosomes at metaphase	Size of nucleoli
	longest diameter	shortest diameter	longest diameter	shortest diameter		
<i>Dasyurus</i>	21.7 μ	17.7 μ	12.2 μ	11 μ	31.7 μ	1.5-3 μ
<i>Sarcophilus</i>	21.1 μ	16.6 μ	11.1 μ	10 μ	28.2 μ	1-2.5 μ

(20 spermatogonia were measured and the average is given.)

Chromosome Pairing at Meiosis

At the onset of meiotic prophase the chromosomes are represented by fine, single threads, with clearly defined chromomeres. The number of these threads is 12. It was found that the size of the chromomeres and the distances between adjacent ones vary along the chromosome thread. There is no chromosome with evenly distributed chromomeres of the same size. At late prophase the single threads stain more deeply; most probably owing to contraction, the distances between chromomeres are reduced and the connective fibre of

adjacent chromomeres becomes much thicker than previously. The length of the leptotene chromosome could not be measured, therefore the degree of contraction could be estimated only from the reduction of the internodes between chromomeres.

Chromomeres of the same chromosome show differential staining capacity. This is obviously not due to size, since small chromomeres may sometimes stain more deeply than larger ones. The differential staining is specially well seen in the zygotene and pachytene stages. If the staining capacity is an outcome of chemical reactions, then the differential staining of chromomeres is a proof of a qualitative linear differentiation of the chromosome. The consistency of chromomere behaviour shows that it must be regarded as a characteristic property of chromosomes. There is no evidence that chromomeres arise accidentally as overlappings of or twists in the chromatids (Kaufmann, 1931). The number, position and size of chromomeres are the same in the pairing chromosome threads, a fact difficult to explain on Kaufmann's interpretation.

The time of chromosome association varies in the same nuclei, partially and completely paired homologous chromosomes being present (Fig. 4A). This variation is due, most probably, to chance of association

and to the length of chromosomes. If short chromosome threads come into contact at the early zygotene, they will pair completely before the longer chromosomes.

Fig. 4. (A) Zygotene stage in *Dasyurus*; (B) early pachytene stage in *Sarcophilus*. $\times 2800$.

The leptotene threads lie in the nucleus, more or less twisting around each other, and there is no sign of polarisation. The manner of chromosome pairing was followed in

several instances and it was found that pairing is initiated most frequently at the terminal segment. Only very few cases were seen where the chromosomes paired in the middle region. The association of chromosomes proceeds from the region of primary pairing along the threads. The number of primary associations, which may be called "pairing blocks" (Darlington and Mather, 1932), is one in the short, and two in the longest chromosomes. In plants higher numbers of pairing blocks have been found, but the length of leptotene threads in those plants that have been studied is greater.

The arrangement of chromomeres in the paired chromosomes shows a very remarkable similarity, a definite proof that they are homologous if it is assumed that external similarity represents similar internal organisation. Fig. 5 illustrates partially paired chromosomes, the unpaired segments showing similar arrangement of chromosomes.

The duration of zygotene and pachytene stages may be estimated indirectly from the number of spermatocytes found in these stages in comparison with diplotene, diakinesis and metaphase. The number of spermatocytes in zygotene-pachytene and in the following "diffuse stage" is so great that those showing diplotene and post-diplotene stages are really insignificant. This is definite proof that the process of chromosome association is

Fig. 5. Pairing of homologous chromosomes in *Dasyurus* showing association at both ends. $\times 2800$.

of long duration. In leptotene and zygotene, as already mentioned, the chromosomes show complicated intertwisting, overlapping, interlocking (either true or false) and considerable time is required to allow the completion of pairing of homologues. The long duration of zygotene-pachytene stages in *Dasyurus* and *Sarcophilus* is apparently an adaptation to this condition.

The relationship between pairing and contraction of chromosomes was studied. The diameter of the early pachytene threads is about twice as great as that of the leptotene chromosomes. But soon after partial or complete pairing the contraction proceeds in the paired threads, they become thicker and their staining capacity is greatly increased. The contraction seems to be enhanced by the pairing. This is one of the causes of the variation of length of the same chromosome found in different nuclei. In *Dasyurus* the limit of variation is 4μ for the long and short chromosome pair during the same stage. If the chromosome pair A is associated at the onset of zygotene, then, at the end of pachytene this chromosome will be shorter than in other nuclei where the association of this chromosome A was delayed. The great unevenness and beaded appearance of paired chromosomes disappear partly or entirely at the late pachytene owing to contraction. Sometimes the chromosomes at this stage appear as deeply stained rods with blurred outlines.

The pachytene chromosomes of *Dasyurus* and *Sarcophilus* are illustrated in Fig. 6 at the completion of pairing. The association of chromosomes is not very intimate; there are segments showing doubleness (the

third chromosome pair in Fig. 6 a). The centre of some chromomeres is stained more deeply than the periphery. Great care was taken to locate the attachment chromomeres (centromeres) of the chromosomes, but with the technique employed their position could not be ascertained. In *Dasyurus* there is a large chromomere about the centre of chromosome B, and a very deeply stained chromomere was found in chromosome F of *Sarcophilus*; these may perhaps be interpreted as attachment chromomeres.

Fig. 6. Pachytene bivalents in (a) *Dasyurus*, (b) *Sarcophilus*. The complete nuclei are represented. $\times 2800$.

The nucleolus or nucleoli lie usually on the periphery of the nucleus as strongly stained, round bodies of nearly equal size. The sex chromosomes can be seen at pachytene as short oblong dark aggregates.

At the end of pachytene stage two important changes occur in the spermatocytes: (1) the gradual loss of staining capacity of chromosomes, and (2) the progressive increase in the volume of the nucleus and cell. This stage is called the growth, diffused or "resting" period; it is common in animals, but is not found in plants. It is reasonable to assume that the inability of paired chromosomes to respond to stains and dyes during the diffuse period is a proof that changes must have taken place in the internal organisation of the chromosomes. The maximum increase of the nucleus coincides with the disappearance of chromosomes. The pale outlines of paired chromosomes can be seen for a while at the beginning of this period, but soon they become invisible.

Striking differences were found in the behaviour of spermatocytes of *Dasyurus* and *Sarcophilus* during the diffuse period. Table 2 illust-

rates the approximate volume of nuclei during prophase of meiosis. In both genera the volume during the resting stage shows a great range of variation owing to rapid growth. The variation is greater in *Sarcophilus* than in *Dasyurus*. In the preceding stages (before the growth period) there is a gradual decrease in *Dasyurus* but a slight increase in *Sarcophilus*. In the following stages there

is a decrease in the volume of nuclei in both genera but the rate is different and the data obtained suggest that the change in nuclear volume of spermatogonia in *Sarcophilus* during meiotic prophase is slower in rate and less in degree than in *Dasyurus*. This difference very closely corresponds to those found in the two genera at mitotic prophase in respect of the rate and degree of contraction and condensation of chromosomes.

Inside the increased nuclei only fine granules can be seen during the diffuse period beside the darkly-stained aggregates of the sex chromosomes. Apart from the gradual increase in volume the spermatocytes closely resemble cells at the onset of either mitotic or meiotic division. They may be regarded as cells which have entered upon a temporary resting stage. The splitting of the chromosomes most probably occurs during this period. Previous to that the paired chromosomes are double, but in the following diplotene stage they are quadruple, each chromosome being constituted of two sister chromatids. The essential difference between mitosis and meiosis is that the prophase condensation and contraction begins before the chromosomes have divided (Darlington, 1932). It is most reasonable to assume that the diffuse period coincides with the delayed time of splitting which occurs in the resting stage before contraction during mitosis. In meiosis, contraction takes place first and proceeds in the chromosomes to a certain degree until the delayed time of splitting has arrived. The division of paired chromosomes necessarily involves a qualitative rearrangement in their internal organisation, and is associated with changes, such as the loss of staining capacity, the dynamical alteration in linear direction, and so on.

The approximate length of chromosomes at mitosis and meiosis is given in Table 3. There is a disproportionate difference in length at pachytene in the two genera, owing to the differences in the rate and

Table 2. The volume of nuclei during prophase of meiosis.

Stages	<i>Dasyurus</i>	<i>Sarcophilus</i>
Resting stage	5200-6400	4800-5400
Zygotene	6400	4800
Early pachytene	4800	5600
Late pachytene	4200	6200
Growth period	14200	8800
Early diplotene	13200	9200
Late diplotene	6320	7200
Diakinesis	6000	6500

(25 nuclei were measured, the cube of the mean radius in micra being multiplied by 3.4 π .)

degree of contraction. The chromosomes of *Dasyurus* contract more than do those of *Sarcophilus* and are therefore shorter at pachytene in spite of their greater length at mitotic metaphase. During the period

Table 3. The lengths of chromosomes in *Dasyurus* and *Sarcophilus* in micra.

	<i>Dasyurus</i> Types of chromosomes						<i>Sarcophilus</i> Types of chromosomes					
Stages	A	B	C	D	E	F	A	B	C	D	E	F
Mitotic metaphase	7	6	5.5	5.3	3.0	2.6	5.6	5.5	5.1	3.8	3.6	3.2
Pachytene	13-17	12-15	12-14	8-12	6-8	6	20	18	16	12	7	6
					Diffuse stage							
Diplotene	23	20	18	10	4.5	4	17	15	12	10	8	7
Metaphase II.	5.3	4	3.5	2.8	2.4	1.7	4.2	4	3.3	2.6	2.6	2.2

(The camera lucida drawings were measured and the approximate length of chromosomes calculated.)

of growth, or diffuse stage, a dynamic change ensues in the linear direction of the chromosomes, and the stress brought about by contraction is somewhat released. In *Dasyurus* the length of the major chromosomes is greater in diplotene bivalents than it was at pachytene. They appear at the beginning of diplotene as very thin, slightly condensed double threads, their diameter being less than was found at pachytene. The minor bivalents of *Dasyurus*, however, show further contraction at the beginning of diplotene. The differential behaviour of the major and minor bivalents with regard to contraction suggests that the completion of splitting coincides with the beginning of the second period of condensation. The short chromosomes complete their splitting sooner than the major chromosomes, hence the difference in their relative length. Furthermore, the data obtained support the theory that the splitting takes place simultaneously in every chromosome of the same complement. The length of bivalents of *Sarcophilus* during diplotene is less than it is in pachytene, most probably owing to contraction which continuously operates during the diffuse stage, although the rate or the degree of such contraction is very small.

Behaviour of Chiasmata

The splitting of paired chromosomes into two pairs of sister chromatids, accompanied by exchange of partner chromatids, was inferred to occur during the diffuse stage. In the following diplotene the chromosomes appear as bivalents held together only by chiasmata. At the

beginning of this stage the contraction and condensation are very slight in both genera, and the bivalents either do not stain at all, or only very slightly. Figs. 7 and 8 illustrate diplotene at mid-stage, where the bivalents and their structure could be seen owing to the advanced contraction.

There also occurs at diplotene a definite decrease in the number and alteration in the position of chiasmata. At diakinesis and metaphase there are less chiasmata in both genera than at diplotene. The diplotene and post-diplotene genera in *Dasyurus* and *Sarcophilus* are illustrated in figs. 7 and 8 and show very clearly the reduction in number.

The chiasmata at mid-diplotene are interstitial. In the long bivalents of *Sarcophilus* occasionally a terminal chiasma was found, which is proof that there is a movement of chiasmata from the earliest diplotene. When the bivalents become visible by contraction, the originally sub-terminal chiasma has already shifted into the terminal position. The movement of chiasmata is due to repulsion operating between the paired chromosomes, but this repulsion is not yet a strong force; the loops show twisting, overlapping, and the attachment loop cannot be distinguished from the others. The slight repulsion at early and mid-diplotene is an adaptation to the great length of chromosomes. The volume of

Fig. 7. Mid-diplotene, diakinesis and metaphase in *Dasyurus*. The total number of chiasmata and the number of terminal chiasmata are given for each bivalent. $\times 2800$.

Fig. 8. Mid-diplotene, diakinesis, metaphase and anaphase in *Sarcophilus*. The number of total and terminal chiasmata is given for each bivalent. $\times 2800$.

nuclei is sufficient to contain the diplotene bivalents only if they bend and turn. Sometimes it was found that the adjacent loops are turned at 90 degrees. Strong repulsion at early diplotene would lead to such mechanical difficulties that the normal chromosome behaviour would be interfered with.

The diakinesis bivalents exhibit a much greater contraction, but their staining is difficult, the fixation is not satisfactory and they tend to collapse. In some spermatocytes of *Sarcophilus*, however, this post-diplotene stage was represented quite well, showing the larger attach-

ment loops. The equilibrium between those loops was brought about by the movement of chiasmata, resulting from the repulsion between the paired chromosomes and between the attachment constrictions. The contraction is accompanied by very strong condensation and results in a compact bulk and relatively smaller size of metaphase bivalent than was expected. The long bivalents at diplotene are very delicate and thin; the fine threads of chromatids and their chromomeres can sometimes be seen clearly.

Table 4. Chiasma frequency of *Dasyurus* and *Sarcophilus* in diplotene and metaphase.

Stages	Genera	Number of chiasmata							Total no. of Xta	Total no. terminal Xta	Mean no. Xta per bivalent	Terminalisation coefficient
		1	2	3	4	5	6	7				
Diplotene	<i>Dasyurus</i>	—	17	12	7	—	—	—	103	16	2.8	0.14
	<i>Sarcophilus</i>	—	13	7	15	6	1	—	145	15	3.4	0.10
Metaphase	<i>Dasyurus</i>	22	32	6	—	—	—	—	104	30	1.6	0.29
	<i>Sarcophilus</i>	10	13	8	11	—	—	—	104	18	2.4	0.17

The diplotene-metaphase chiasma frequency of both genera is given in Table 4, and illustrated by graph in Fig. 9. The bivalents of *Dasyurus* have less chiasmata at diplotene than these of *Sarcophilus*, but the movement of chiasmata is more intensive in the first genus, giving a higher terminalisation coefficient.

The graph of chiasma frequency at diplotene and metaphase in *Sarcophilus* shows a small percentage of bivalents with three chiasmata. This is due to the median, or nearly median, position of attachment constriction, the long chromosomes having arms of equal length which leads to a more or less

Fig. 9. Graph illustrating the chiasma frequency in diplotene and metaphase of *Dasyurus* and *Sarcophilus*. (D^d = diplotene of *Dasyurus*; D^m = metaphase of *Dasyurus*; S^d = diplotene of *Sarcophilus*; S^m = metaphase of *Sarcophilus*.)

symmetrical arrangement in the position of chiasmata. It is a further proof that chiasmata move away from the attachment constriction. In Fig. 8 the metaphase bivalents of *Sarcophilus* with median attachment have about equal numbers of chiasmata in both arms, and the terminalisation being slight, the bivalents at different metaphase plates show great similarity. In *Dasyurus*, however, it was found that the metaphase bivalents exhibit a wide range of types in different spermatocytes. Fig. 10 illustrates three complete nuclei with nearly the same number of total and terminal chiasmata, but the types of bivalents vary greatly in

Fig. 10. Different types of metaphase bivalents in three nuclei of *Dasyurus*. The number of total and terminal chiasmata is given. $\times 2800$.

the different nuclei. It strongly suggests that there is a definite difference in *Dasyurus* and *Sarcophilus* regarding the loci and number of chiasmata formed at diplotene and the degree of terminalisation.

Analysing the above-mentioned differences in the types of metaphase bivalents, and comparing the diplotene and metaphase chiasma frequencies of *Dasyurus* and *Sarcophilus*, one must infer that there is a different cytological interference in these genera. The curve of chiasma frequency in *Dasyurus* is a strong modification of the Poisson curve, the mean being favoured at the expense of the extremes, indicating that the formation of one chiasma operates against the occurrence of another in the same bivalent as was demonstrated by Haldane (1931). In *Sarcophilus* the curve is of the reverse type, the extremes being favoured. This observation indicates that in *Dasyurus* there is a strong cytological interference which is absent in *Sarcophilus*.

This difference in chiasma formation may be seen directly by comparing the types of diplotene bivalents in the two genera. Fig. 11 illustrates the long bivalents of *Dasyurus* and *Sarcophilus* respectively. In the former the distance between two chiasmata is relatively a long one, chiasma or chiasmata are not formed in the region adjacent to the first chiasma. In the latter, the internodes between chiasmata are short, either equal or unequal in size. The number of chiasmata is high, showing that chiasmata are formed more or less freely in the neighbourhood of the first chiasma. The lower number and the position of chiasmata in *Dasyurus* at diplotene is an outcome of the strong cytological interference.

Fig. 11. Diplotene bivalents, showing interference in *Dasyurus* (A and B), which is absent in *Sarcophilus* (C and D). $\times 2800$.

The effect of interference upon chiasma frequency may be seen best in the different relationship of length of chromosomes and their chiasma frequency in the two genera. Table 5 shows this relationship between length and chiasma formation. In *Dasyurus* there is very definite disproportionate relationship, the greater length is thus accompanied by

Table 5. The relationship between length and chiasma frequency

Genera	Length and X frequency	Types of bivalents					
		A	B	C	D	E	F
<i>Dasyurus</i>	Length at diplotene	23 μ	20 μ	18 μ	10 μ	4.5 μ	4 μ
	X frequency at diplotene (observed)	3.8	3.6	3	2.5	2	2
	X frequency at diplotene (calculated)	5.3	5	4	2.5	2	2
	Length at mitosis	7 μ	6 μ	5.5 μ	5.3 μ	3.0 μ	2.6 μ
<i>Sarcophilus</i>	Length at diplotene	17 μ	15 μ	12 μ	10 μ	8 μ	7 μ
	X frequency at diplotene (observed)	4.8	4.2	4	2.8	2	2
	X frequency at diplotene (calculated)	5	4.2	3.6	2.8	2	2
	Length at mitosis	5.6 μ	5.5 μ	5.1 μ	3.8 μ	3.6 μ	3.2 μ

stronger interference. Leaving out of consideration the last two bivalents E-F, because they show very often precocious condensation, and hence their length may not be representative, the bivalent D is taken as an appropriate 'yard-stick' for this purpose. The diplotene length of this bivalent is 10μ , with an approximate chiasma frequency of 2.5. Bivalents A and C, according to this ratio, ought to have at least 4.2-4 chiasmata. In *Sarcophilus* there is a direct relation between length and chiasma frequency, if the diplotene length is taken into consideration. But at mitotic length the relationship is altered; the number of chiasmata is much higher than expected. This very important difference between the two genera must be due to genotypic differences, and it is probable that there is a close causal connection with other variations between *Dasyurus* and *Sarcophilus* which have already been mentioned.

The rate of terminalisation in the major and minor bivalents was compared in the two genera. Fig. 12 illustrates the terminalisation

Fig. 12. The terminalisation coefficient in diplotene and metaphase in *Dasyurus* and *Sarcophilus* for the major and minor bivalents.

coefficient at diplotene and metaphase. In *Dasyurus* the major bivalents show a higher degree of terminalisation compared with that found in the minor group. It was expected that the long bivalents, having more chiasmata at diplotene, would have an increased number of terminal associations in comparison with the minor bivalents. Furthermore, it is not improbable that the sub-median

position of the attachment constriction in bivalents C and D is primarily responsible for the disproportionate increase of terminal association in the major group of bivalents. In *Sarcophilus* the terminalisation coefficient increases towards metaphase in the same degree for both groups of bivalents. In each group there are two chromosomes with median and one with submedian attachment constrictions, hence the terminalisation coefficients are similar.

The structure and type of chiasmata at metaphase are difficult to analyse in both genera owing to the marked contraction in the bivalents. At anaphase, however, it was possible to discriminate chiasmata of compensating and non-compensating types the latter being less numerous. Fig. 8 shows a non-compensating chiasma in bivalent D at

anaphase. It is obvious that the number and type of chiasmata interfere with the separation of metaphase bivalents. Fig. 13 illustrates anaphase with lagging bivalents, caused by chiasmata. The short bivalents with one or two chiasmata will separate prior to others which have more chiasmata.

Fig. 13. Anaphase, showing lagging of bivalents in (A) *Dasyurus* and (B, C) *Sarcophilus*. $\times 2800$.

In both genera the segregated homologues form the second metaphase plate without any interphase. In *Dasyurus* the sister-chromatids of the chromosome lie closely parallel, in *Sarcophilus* they are held together at the attachment constriction only, and the chromosomes appear in polar view as a cross with equal or unequal arms. Fig. 14 illustrates the second metaphase plates of *Dasyurus* and *Sarcophilus*. The small chromosomes usually lie at the centre of the equator. The length of chromosomes is less than it is in mitotic metaphase. The increased diameter shows that the shortening is due to greater contraction during meiosis as compared with that found in mitosis. The time taken by second metaphase in both genera is quite long, several secondary spermatocytes being found in this stage.

Fig. 14. Second meiotic metaphase in (A, B) *Dasyurus*; (C, D) *Sarcophilus*. The X is present in A and C, the Y in B and D. $\times 2800$.

Sex Chromosomes during Meiosis

At the early prophase stages the sex chromosomes appear as dark round bodies of unequal size. During the diffuse stage they remain

visible, usually at the periphery of the nuclei. In most cases they are closely associated or even connected by fine threads. In diplotene the sex chromosomes constitute one more or less deeply stained aggregate, placed amongst the autosome-bivalents. The structure of the associated

Fig. 15. Types of associated sex chromosomes at meiotic metaphase in *Dasyurus* and *Sarcophilus*. $\times 3100$.

sex chromosomes can be seen best during the first metaphase. Fig. 15 illustrates the most common type of metaphase association.

Dasyurus and *Sarcophilus* show very great similarity regarding the behaviour of the sex complex during meiosis. In metaphase the X and Y chromosomes can be stained very well with iron-hematoxylin and new brazilin, but remain pale with gentian violet. They always differ from the autosomes in the degree of staining. The analysis of the metaphase complexes of the sex chromosomes suggests that the X is composed of two ball-shaped chromatids with a terminal attachment constriction. At mitotic metaphase the doubleness of the X chromosomes can be seen clearly (Fig. 1 A, B). It is most probable that the Y chromosome is homologous with the terminal segment of X. The metaphase configuration suggests that they are associated at this region, but it is rather difficult to determine whether or not this association is due to chiasmata. The segregation of the sex chromosomes is reductional at the first anaphase, which favours the view that they are associated only terminally.

Several spermatocytes were found in which the sex chromosomes were strongly repelled from each other during metaphase. Sometimes they move precociously towards opposite poles. Commonly the sex chromosomes lie off the equatorial plate at the boundary of the spindle. This behaviour of the sex chromosomes must be considered as an inherent mechanical property, which differentiates them from the autosomes (Koller and Darlington, 1934).

The secondary spermatocytes are of two kinds one containing the X, the other the Y chromosome. The Y is very small, but it stains much better than in mitotic metaphase, and consequently can be distinguished clearly in the secondary spermatocyte. During the

Fig. 16. Second meiotic anaphase, showing lagging of (A) X in *Sarcophilus*, (B) Y in *Dasyurus*. $\times 2800$.

second meiotic anaphase the X and Y chromosomes commonly show lagging as illustrated in Fig. 16.

Chromosome Interlocking

Several hundreds of zygotene and pachytene nuclei were studied in both genera to gain a better insight into the problem of chromosome pairing and accidental interlocking. Only a few cases were found with obvious chromosome interlocking, the rarity probably being due to the long duration of the zygotene and pachytene stages and to the method of chromosome conjugation. As already mentioned the number of 'pairing blocks' is very small—an adaptation to avoid mechanical interference of interlocking during the zygotene pairing.

The few cases of chromosome interlocking, however, offer evidence regarding the method of such interlocking. They can be classified as false and true interlocking, the first being much more common than the second. The different types of pachytene interlocking are illustrated in Fig. 17. The true interlocking can be either internal or external. Fig. 17 A illustrates the internal interlocking, where only the same bivalents are involved. This type was seen by Gelei (1922) in *Dendrocoelum*, and the possibility of such interlocking was discussed by Gairdner and Darlington (1932) in *Campanula*. It is possible that chiasmata are formed at the intercalary segment which will lead to inversion or other structural changes. Fig. 17 B represents true but external interlocking which involves four chromosomes in such a way that they remain unassociated at the point of interlocking. Fig. 17 E shows double false interlocking, where two pairs of already paired chromosomes prevent the association of a third one. The true and false interlocking may be combined in one configuration as it is illustrated in Fig. 17 C. The most important type of interlocking is the double and true chromosome interlocking. This can be seen in Fig. 17 D. The same chromosome is interlocked twice by a second chromosome. Chiasma formation in

Fig. 17. Interlocking in *Dasyurus* during pachytene. (A) internal interlocking, two homologues are involved; (B) true and single; (C) true combined with false; (D) true double; (E) false double. $\times 2300$.

the interlocked segment provides a definite proof of Janssen's partial chiasma type theory, as pointed out by Mather (1933).

The pachytene interlocking leads to diplotene interlocking which, in accordance with the position of chiasmata and interlocking, can be

Fig. 18. Interlocking at (A, B) diplotene; (C, D) metaphase. (A) distal-proximal; (B) proximal; (C) false; (D) proximal-proximal; (E) distal-distal. $\times 2800$.

distal-distal, proximal-proximal or distal-proximal (Gairdner and Darlington, 1932). Fig. 18 A is a distal-proximal, and B is a proximal-proximal type. It is possible that the first type may disappear later, owing to terminalisation of the distal interlocking. True and false metaphase interlocking is illustrated in the same figure, C, D, and E.

In *Dasyurus* a very interesting case was

found which is illustrated and diagrammatically explained in Fig. 19. Most probably this was a true distal-proximal interlocking. At late diplotene one loop broke entirely, but in the other bivalent only one chromatid of the same segment had broken. The following fusion of the two sister chromatids of the first broken chromosome with the two ends of the non-homologous chromatid will give rise to the type of chromosome configuration which is illustrated in the same figure D. According to the position of the attachment constriction and breakage, new types of chromosomes may arise carrying translocations, duplications or deficiencies. This case may be

Fig. 19. Segmental interchange in *Dasyurus* through interlocking. (A) pachytene interlocking of two non-homologous chromosomes; (B) chiasma formation in the bivalents; (C) breakage of one loop, the two sister chromatids are fused with the two ends of the broken chromatids of non-homologous chromosomes; (D) the observed configuration at late diplotene.

taken as proof that the formation of new chromosomes through single or reciprocal interchange occurs after interlocking as suggested by Belling (1927).

Multinucleate Cells during Meiosis

In *Sarcophilus* multinucleate spermatogonia are formed very frequently. Giant spermatocytes were found, their diameter being approximately 60μ – 80μ . Similar cells are common in other species of Marsupials and reported by Drummond (1933). Eisentraut (1926), studying the abnormal giant cells in *Gomphocerus maculatus* arrived at the conclusion that they originate by fusion of two independent cells and not by syndiploidy. His proofs are (i) the presence of two separate spindles, and (ii) the formation of equatorial plates in the same plane. In *Sarcophilus* sometimes three meiotic metaphase plates were found within the giant spermatocyte the fourth nucleus degenerated, but the planes of the plates were different, as illustrated in Fig. 20. In cells with two chromosome complements at meiotic metaphase the equatorial plate of the two complements are turned at 90 degrees or shifted, as can be seen in Figs. 21 and 22.

Figs. 20–23. 20, Multinucleate spermatocyte with three active nuclei in *Sarcophilus*. 21, Binucleate spermatocyte in *Sarcophilus*; the spindle is turned at 90 degrees. 22, Binucleate spermatocyte in *Sarcophilus*. The equatorial plate of the nuclei is in the same plane but shifted. 23, Diplotene stage in binucleate spermatocyte in *Sarcophilus*. Note the variation in number of chiasmata in the two nuclei. $\times 2000$.

The chiasma formation and frequency were studied in these giant spermatocytes. Fig. 23 illustrates diplotene stage with slightly different chiasma frequencies in the two nuclei. Similar differences were found at metaphase (Table 6). The difference in chiasma frequency of the

Table 6. Chiasma frequency in multinucleate spermatocytes of *Sarcophilus*.

Stage	Cell	Nucleus	Number of Xta in bivalents						Total no. of Xta	Total term. Xta
			A	B	C	D	E	F		
Diplo- tene	I.	a	5/1	5/0	4/1	3/0	2/1	2/0	21	3
		b	6/0	4/0	4/0	3/0	2/0	2/1	21	1
Meta- phase	II.	a	4/0	3/1	3/2	2/1	1/0	1/0	14	4
		b	4/0	3/0	3/1	2/1	2/1	1/0	15	3
	III.	a	4/0	4/0	3/0	2/0	2/1	1/0	16	1
		b	4/1	3/0	3/1	2/1	2/0	1/0	15	3
	IV.	a	4/0	4/0	4/0	2/1	1/0	1/0	16	1
		b	4/2	3/1	3/0	2/0	1/0	1/0	14	3
		c	4/0	3/0	2/0	2/1	1/0	1/1	13	2

separate nuclei within the same cell strongly suggests that in spite of the identical cytoplasmic environment there is a variation in chiasma which formation is an inherent property of a chromosome.

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Summary

1. *Dasyurus* and *Sarcophilus* have six pairs of autosome chromosomes. In *Sarcophilus* they show slight variation in length; in *Dasyurus* this is greater. The loci of attachment constrictions are median, submedian or subterminal.

2. The chromosomes exhibit somatic pairing at mitotic metaphase.

3. The contraction of prophase chromosomes at mitosis is less in *Sarcophilus* than in *Dasyurus*, and the time required to arrive at the metaphase stage is longer.

4. At meiotic prophase the chromosomes contain well defined chromomeres. The number of varying sizes of chromomeres is the same in homologous chromosomes.

5. The zygotene and pachytene stages are very long. The pairing of homologous chromosomes begins in one of the terminal regions, and never more than two separate segments of the same chromosome are involved.

6. At the end of pachytene a sudden change takes place in the first spermatocyte; the chromosomes lose their staining capacity and the volume of nuclei and cell increases. It is suggested that this stage is equivalent to the resting stage in that the delayed splitting of chromosomes occurs.

7. In *Dasyurus* there is a high chiasma interference with lower chiasma frequency than in *Sarcophilus*.

8. The relationship between the length of chromosomes and the chiasma frequency is disproportionate in *Dasyurus*, but is independent in *Sarcophilus*.

9. The different types of pachytene interlocking are described. One case is illustrated where interlocking leads to segmental interchange.

10. Chiasma frequency in multinucleate spermatocytes shows slight variation within the same cell, which variation must be genetic.

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