



CYTOLOGICAL STUDIES IN AUSTRALIAN MARSUPIALS

by

P.J. Sharp, B.Ag.Sc.(Hons.)

Department of Genetics
University of Adelaide

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- APPENDIX 2. Hayman, D. and Sharp, P. (1981) Verification of the structure of the complex sex chromosome system in *Lagorchestes conspicillatus* Gould (Marsupialia: Mammalia). *Chromosoma* 83, 263-274.
- APPENDIX 3. Sharp, P. and Hayman, D. (1981) The distribution of SCEs within and between the genomes of an interspecific hybrid. *Chromosoma* 84, 231-242.
- APPENDIX 4. Hayman, D.L. and Sharp, P.J. (1982) The chromosomes of *Tarsipes spencerae* Gray (Marsupialia). *Aust. J. Zool.* 30, 799-803.

References

SUMMARY

This thesis has two sections considering different aspects of meiosis in Australian marsupials.

The first section is an analysis of meiotic recombination in a wide range of marsupial species. Variation in chiasma frequency in male *Trichosurus vulpecula* was studied and the significant variation found between individuals could not be related either to seasonal factors, or to the age of the animal. Variation between species in male chiasma frequency was studied in thirty-three species, many of which had the $2n=14$ "basic" karyotype of Hayman and Martin (1974). There was a wide range of chiasma frequencies in the different species, which also varied widely with respect to aspects of size, reproduction and development. When these two aspects of the genetic systems of the species were compared, species which were "K-selected" tended to have higher levels of meiotic recombination. Some evidence was also found that chromosome number in marsupials has not evolved due to its contribution to the level of recombination in each species. The distribution of chiasmata was studied in a limited number of species, and the main agency affecting chiasma distribution appeared to be interference within bivalent arms. Within the $2n=14$ species, those with higher chiasma frequencies also had a higher proportion of interstitial chiasmata. The chiasma distribution in the sex trivalents of three species with complex sex chromosomes had little in common. Studies of meiosis in a small number of female marsupials revealed that this process was similar to that in eutherians, but comparisons of chiasma frequencies between the sexes were not possible.

The second section considers the synaptonemal complexes during

meiosis in males. The pairing between the X and Y chromosomes was shown to be different from the normal eutherian pattern since there was no synaptonemal complex formation between these chromosomes. This seems to be a characteristic of the marsupials. Observations were also made of unaligned centromeres on autosomal synaptonemal complexes, and they appear to be due to asymmetric centromeric C-band heterozygosity coupled with the process of "synaptic adjustment".

DECLARATION

Except where due reference is given this thesis contains no material which has been accepted for the award of any degree or diploma in any University and, to the best of my knowledge and belief, contains no material published or written by another person.

I consent to this thesis being made available for photocopying and loan.

P.J. Sharp

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"... De Vries, like Weissmann, considered the "essential purpose" of sexual reproduction to be the increase of variability and the power of individual adaption; and this may be effected, in his view, by the exchange of individual units "and thus cause the creation of those countless combinations of characters of which nature is in need in order to make species as plastic as possible and to enable them to adopt themselves in the highest degree to their ever changing environment'..."

E.B. Wilson (1925), "The Cell in Development and Heredity"
3rd Edition, quoting Hugo De Vries (1903).

SECTION ONE

RECOMBINATION IN AUSTRALIAN MARSUPIALS.



CHAPTER 1: GENETIC SYSTEMS AND THE EVOLUTION OF RECOMBINATION

The question of the evolutionary significance of the variability in chiasma and recombination frequency and localization both between and within species has been approached from two directions. The first is through consideration of the concept of "Genetic Systems", while the second is by theoretical and mathematical treatments concerning the evolution of recombination.

1.1 The concept of genetic systems

Darlington (1939) introduced the concept of the genetic system of a species as the interrelated properties of heredity and variation, method of reproduction and breeding system, all of which can vary genetically. He pointed out that species can have different types or levels of these properties, and that these properties govern the release of genetic variability within the population. Darlington suggested that this released genetic variation is open to the action of natural selection and consequently the genetic system itself is indirectly under the influence of natural selection.

The recombination system (Carlson, 1957) is central to the genetic system, having the most direct influence on the release of genetic variability. In terms of the recombination system, short-term adaption and long-term adaptability are in conflict (Mather, 1943), with high levels of recombination having evolutionary adaptability at the potential cost of producing poorly adapted genotypes by recombination, whereas low levels of recombination have stability of adaption in a stable environment, but are less likely to allow

evolutionary change to new and varying environments. The theory of genetic systems indicates that species will have a level of recombination to allow both immediate fitness and long-term flexibility, but that fitness and flexibility are envisaged to be selected for at different levels depending on the natural history of the species.

Lewis and John (1963) proposed two ways by which species have achieved this result; synthesis and compromise. Synthesis, as a combination of asexual and sexual reproduction can allow both fitness and flexibility. Compromise, which has also been called balance by Grant (1958) is achieved by the interaction of the various factors that affect the amount of variability released. Grant (1958) listed nine factors that regulate the release of variability in plants; chromosome number, frequency and location of crossing-over, sterility barriers, breeding system, pollination system, dispersal range, population size, isolating mechanisms, and length of generation. These factors fall into two basic groups, those concerned with the genetic constitution of the gametes produced by the population (chromosome number and the frequency and location of chiasmata), and those controlling the origin and type of gametes that unite to form the next generation (population ecology and genetics). Grant (1958, 1975) has presented many examples where an increase in recombination due to one factor appears to be balanced by a decrease due to another factor. For example, species which cross-fertilize tend to have lower chiasma frequencies than self-fertilizing species (Grant, 1958; Arranyo, 1973).

Structural hybridity is another possible regulator of recombination. Carson (1955a, b) has shown that central populations of *Drosophila robusta* have higher levels of inversion heterozygosity compared to

peripheral populations, and he has argued that this difference is adaptive, with the central, and presumably well adapted, populations conserving fit gene combinations, while the peripheral populations are releasing genetic variation as a result of continually adapting to new environments. This interpretation is supported by the observation that responses to artificial selection in strains from the marginal populations are greater than those in strains from the centre (Carson, 1958). Other *Drosophila* species follow a similar pattern (Carson and Heed, 1964). However, this distribution of recombination within a species range does not always occur, since in two grasshopper species, B-chromosomes that increase the frequency and variance of chiasma are more common in central regions of the species distribution (Hewitt and John, 1967; Hewitt, 1973; Fontana and Vickery, 1973). In the snail *Cepaea*, chiasma frequencies are not related to the geographic marginality of the population, but there is a negative correlation between chiasma frequency and population density (Price and Bantock, 1975). Clearly, different species can achieve an appropriate compromise by different means.

One of the interactions which has been examined widely is that between chiasma frequency and longevity, and it serves to show that other processes can interact with a balance between two factors. Darlington (1939) and Mather (1943) postulated that recombination should be restricted in short-lived species. Mather (1953) suggested *Drosophila* as an example, having a low level of recombination since it has no recombination in the male, low chromosome number, few chiasmata in the female, but potentially many generations in a year. The resulting genetic stability was argued to be adaptive in preventing

Drosophila populations continually evolving seasonally advantageous strains which would be disadvantageous in the next season. This argument has been supported by the observation that many multivoltine species of arthropod have restrictions on recombination (Halkka, 1964; White, 1973).

In plants, Grant (1958, 1975) and Stebbins (1958) have found that restrictions on recombination are more common in annual plants than in closely related perennial species. Annuals tend to have lower chromosome numbers and fewer chiasmata, and to have higher levels of inbreeding than the perennials. Grant (1958) argues that this difference is adaptive in that for short-lived species the demand is for uniformity so that the offspring, which have to re-establish the population in each generation, are similar to the parental forms adapted to that environment. Despite this general trend some groups have been identified in which the opposite occurs. In species of *Lolium* (Rees and Ahmad, 1963; Crowley, 1969; Rees and Dale, 1974), *Secale* (Sun and Rees, 1964) and *Festuca* (Rees and Dale, 1974), populations of annual habit have higher chiasma frequencies than more perennial populations. In these pasture grasses the annual varieties have been derived from the perennial types by strong selection. Two possible explanations of this situation have been advanced. First, the annual types have reduced genetic variability (in parameters like shoot growth, and flowering time) and Rees and Ahmed (1964) and Crowley (1969) argued that the higher chiasma frequencies might compensate for this reduced variability. In a later publication, Rees and Dale (1974) suggested that the explanation lies in the derivation of the highly specialized annuals from their more perennial

ancestors. Selection of the extreme phenotypes would, they suggest, lead to an inadvertent selection for individuals with high chiasma frequency, as this would tend to generate the extreme phenotypes more frequently. They suggest that any compensatory role is possibly fortuitous, and the high chiasma frequency in these annuals is a "...relic of the cytological prerequisites upon which their evolution was founded". Support for this idea comes from experiments in which selection for extremes (i.e. disruptive selection) was accompanied by an increase in chiasma frequency. This has been found in *Brassica* (Hayinarayana and Munty, 1973) and also in *Drosophila*, where Flexon and Rodell (1982) have found that strong directional selection for DDT resistance brought about an increased recombination level in the resistant lines.

The notion that the biological diversity of the environment in which a species lives might exert an influence over the level of recombination in the species has been explored in recent years (Levin, 1975; Jaenike, 1978; Glesener and Tilman, 1978). Species with many competitors, predators, diseases, pests and parasites are expected to have high recombination in order to escape these pressures. The argument supporting this proposal has similarities to the argument put forward by Clarke (1979) that predators and parasites are partly responsible for the maintenance of genetic variation by frequency-dependent selection. According to Jaenike (1978) recombination is favoured as it produces both novel and less frequent genotypes which will be favoured by the frequency-dependent selection pressure from parasites, etc., since these hit hardest the most common genotypes. Data from plants (Levin, 1975) indicates that recombination restricting

devices are commonest in simple ecosystems, in accord with the theory. Glesener and Tilman (1978) considered only parthenogenesis as a restriction on recombination in terrestrial animals, but showed that it is more common where there are relatively few competing, predatory and pathogenic species, although the pattern they and Levin (1975) observed could also be due to an advantage in parthenogenesis in colonising species (Maynard Smith, 1978).

Grant (1975) has expressed the view that much of the genetic system might be explained within the framework of r- and K-selection (Pianka, 1970). The r-selection occurs when there is high density-independent mortality, so that selection is mainly on high fecundity and rapid development, whereas it is the reverse in K-selection, so that this favours lower fecundity and slower development. The different species form a continuum between these two extremes. However, this suggestion does not appear to have been taken up in any study.

Very little consideration has been afforded to the question of genetic systems in mammals. The review by Hayman (1981) points to how little is known. Hayman (1981) suggests a number of processes which may be important in the genetic system of certain species; differences in chiasma frequency between the sexes, the effects of sex chromosome-autosome fusions on chiasma frequency and distribution, variations from a 1:1 sex ratio, and the effects of heterochromatin and B chromosomes.

1.2 The evolution of recombination

The evolution of recombination has for long been a subject of discussion in evolutionary theory (Fisher, 1930), and it has recently been the subject of two monographs (Williams, 1975; Maynard Smith, 1978).

The genetic reshuffling that takes place between generations consists of two processes; fusion of gametes from different individuals (i.e. sexuality), and genetic recombination at meiosis. Although it is assumed that forces selecting for sexuality will also select for genetic recombination, most mathematical and theoretical arguments advanced to explain the genetic reshuffling have, for simplicity, addressed only one of these two processes at a time. Consequently, it is possible to review ideas concerning the evolution of genetic recombination itself.

Fisher (1930) was the first to point to a constant natural selection against recombination that would occur if alleles at polymorphic loci interact epistatically with respect to fitness. Mathematical treatments showing this can be found in Lewontin (1974) and Maynard Smith (1978). Given that there is genetic variation in recombination frequency, then there would appear to be constant selection against recombination. This led Turner (1967) to ask "Why does the genome not congeal?", with recombination being reduced to zero. There must be factors that select for recombination, and these have been searched for by mathematical analysis. It is not my task to comment on the mathematics involved, but rather to make an examination of the assumptions used and the conclusions reached in these studies in order to point to factors possibly controlling recombination levels.

Long-term group selection is one way in which sex and recombination could be preserved (Fisher, 1930; Muller, 1932), and while it may be the case for sex, it has been argued (Maynard Smith, 1977) that group selection is not an evolutionary force favouring genetic recombination, since there is in nature extensive genetic variation in recombination and chiasma frequency between individuals within a group (see Chapter 2.2).

Explanations based upon short-term selection for recombination that operates at the level of the individual have therefore been sought. One possibility is an unpredictability in the environment. In this situation selection may favour the offspring of parents that produce recombinant gametes as the offspring will possibly face a different environment than their parents. Simple mathematical models (Maynard Smith, 1971) and computer simulations (Charlesworth, 1976) of temporal variation in the environment indicate that it is possible to get weak selection for high recombination, but only in the unlikely situation where there are changes in the sign of correlations between selectively relevant features each generation (e.g., generation 1 = hot-dry, cold-wet; generation 2 = hot-wet, cold-dry) would recombination be selected. Modelling of spatial variation in the environment has also found weak selection for recombination, but only when there is a very specific relationship between the spatial variation and dispersal (Slatkin, 1975; Maynard Smith, 1978) in which the dispersal distance and the distance between discontinuities are approximately equal. Both of these approaches do not provide a constant selection favouring increased recombination as they operate only under very specific conditions, and even then only on some individuals in the population.

Williams (1975) considered that sib competition in varying environments might give an evolutionary advantage to recombination. Computer simulation has shown that competition between sibs can favour recombination (Maynard Smith, 1976), but once again the conditions seem unrealistically^{at} restrictive; sibs competing against each other must experience a different environment from their parents, and 30-40 individuals must compete for a single patch. All of the models based

on varying environments also give selection for increased recombination only if one locus determines adaption to a specific environmental condition (Maynard Smith, 1977), whereas in the real world a large number of loci would be expected to be involved in such adaption.

The models discussed above also assume an infinitely large population. Models of finite populations have considered the effects of the linkage disequilibrium that will occur in these populations by chance, simply because they are finite. Two processes have been considered; hitchhiking and Muller's ratchet, referring to the effects of recombination on favourable and deleterious mutations respectively (Felsenstein, 1974; Maynard Smith, 1978).

When favourable mutations occur they can produce new favourable combinations of genes, and hence these will be selected for. A gene increasing recombination will be associated with the novel favourable combinations, as high recombination helps to produce these combinations. Selection for the new combinations will thus select for recombination - the gene for recombination will "hitch" a ride on the selective advance of the favourable mutation. Computer simulation (Strobeck *et al.*, 1975) indicates that such hitchhiking is only important if the locus controlling recombination is tightly linked to the other loci at which the favourable combinations are produced. If this effect is the reason for recombination, favourable mutations must occur at fairly high rates, as Fisher (1930) suggested.

Felsenstein (1974) also considered the effect that recombination would have on unfavourable mutations. Without recombination, Muller's ratchet (Muller, 1964) would operate, making it difficult to select against these mutants since they will be linked to favourable genes.

Recombination would make it possible to select both for favourable, and against unfavourable genes even though the effect of small population size would tend to place them in linkage disequilibrium. Computer simulations of this effect by Felsenstein (1974) and Felsenstein and Yokoyama (1976) show that it could select for recombination. Maynard Smith (1978) considers the effects of hitchhiking and Muller's ratchet to be the only ones universal enough to counterbalance the natural selection for reduced recombination, but he doubted that these processes explained the high levels of recombination.

A problem with all the models described above is that they consider only very few genes controlling the selected phenotype. Maynard Smith (1980) has studied a model that includes polygenic control of the selected phenotype by computer simulation. In this case normalizing selection with either a moving or a fluctuating optimum, and also directional selection result in strong selection for increased recombination, both in finite and infinite populations. If the selected optimum changes with time, previously deficient gamete types are favoured, and since these are produced by recombination, there is selection for recombination. Maynard Smith (1980) considers this to be a large effect, and to be biologically feasible, in that natural selection can be visualized as normalizing selection with a moving or fluctuating optimum.

One of the most recent models (Hutson and Law, 1981) is based on the premise that antagonistic interactions between species (predation, parasitism, and pathogenicity) might be important in selecting for recombination (e.g. Levin, 1975; Chapter 1.1). Here the "heterogeneous

environment" is biotic, rather than physical, so that the environment is continually changing along with the species in question. Hutson and Law (1981) have used a simple genetic model incorporating frequency-dependent selection (as the predators etc. take proportionally more of the common types) and time delay (as the predators etc. will respond to changes in the species under consideration). This model, and also that of Jaenike (1978) gives strong selection for recombination, but it is unknown whether the parameters used by the authors are biologically feasible.

It is difficult to assess whether any of these models provide an explanation for recombination. However, recombination occurs and its level is subject to variation and regulation (see Chapter 2.2). It is also clearly important. The factors considered in these models might also be responsible for determining the levels of recombination in different species.

CHAPTER 2: RECOMBINATION AND CHIASMA FREQUENCY

2.1 The measurement of recombination

The most informative method of obtaining a measure of the level of recombination in a species is by using genetic means to estimate the length of its genome in terms of centiMorgans. However, this is extremely difficult to do, as it requires the species to be genetically well known. Such estimates are available only in some microorganisms (e.g. yeast, Mortimer and Schild, 1981), *Drosophila* (Lindsley and Grell, 1967), maize (Neuffer and Coe, 1974) and perhaps in the mouse (Davisson and Roderick, 1981). It is clearly not a feasible method of measurement to enable comparisons of recombination levels in a wide variety of organisms.

Estimates of recombination levels obtained by cytological examination are much easier to accumulate and hence to use in a comparative way. This relies on the one to one correspondence between visible chiasmata and the physical exchange of material between non-sister chromatids which is expressed in the partial chiasmatype theory (Darlington, 1965; John and Lewis, 1965). There are many indirect cytological demonstrations of this theory (reviewed in Catcheside, 1977; and in Rees and Jones, 1977). More direct ^3H -thymidine labelling also supported this (Peacock, 1970). The best direct evidence comes from recent studies using BUdR substitution methods to identify individual chromatids in meiotic bivalents. These methods show that chiasmata are the sites of exchange in male *Locusta* (Tease and Jones, 1978), female (Polani et al., 1979) and male (Kanda and Kato, 1980) mice, and in male Armenian hamsters

(Allen, 1979). Chiasma counts expressed as cell chiasma frequency (average number of chiasmata per cell) can therefore be used as a basis for comparisons between groups.

The practice of counting chiasmata at meiosis in order to make comparisons requires that the counts themselves are as accurate as possible. This immediately brings one against two interrelated long-standing problems in cytogenetics; the problem of "terminal" chiasmata, and the possible movement of chiasmata towards the telomeres during meiosis ("terminalization"). The view one takes on these problems will affect both the chiasma count taken from a particular cell, and the stage at which counts are taken.

The controversy over "terminal" chiasmata has a long history (Belling, 1933), and many of the arguments used are relatively pedantic. Since chiasmata are exchanges of material between non-sister chromatids they cannot, by definition, be strictly terminal (John, 1976). The important point is whether the associations between homologues which appear terminal are chiasmate or not. They may either be chiasmata where the terminal region of exchange is too small to be resolved by the light microscope, or be a form of non-chiasmate association based on special properties of the telomeres (reviewed by White, 1973). An important practical point is that chiasma counts are usually not performed on the very earliest diplotene cells, as in these cells the extreme thinness and length of the bivalents makes distinguishing between chiasmata and twists of the homologues around each other difficult. Chiasma counts are either taken from diplotene cells that have contracted sufficiently to show chiasmata clearly, or on later stages which have contracted even further. This contraction may

make near-terminal chiasmata appear terminal. Jones (1978) has clearly shown this in rye, where the telomeric C-bands allow him to demonstrate that the "terminal" chiasmata at metaphase I are, in fact, near-terminal chiasmata which appear terminal due to bivalent condensation and stretching. The study by Douglas *et al.*, (1978) also suggests that terminal chiasmata in male *Schistocerca* are true chiasmata since they were found to behave as chiasmata with respect to chiasma interference. However, not all terminal associations are chiasmate, as terminal heterochromatin blocks can form non-chiasmate associations (King and John, 1980).

Studies using BUdR labelling also impinge on this point. Many of the associations between homologues in the mouse appear terminal (e.g. illustrations in Evans, 1979) and Kanda and Kato (1980) have suggested that they are non-chiasmate based on the similar frequencies of the types of relationships between labelled chromatids of the homologues in both the terminal autosomal associations, and in the X-Y bivalent, where they assumed the terminal associations to be achiasmate. Recent evidence, however, indicates that a chiasma regularly forms between the X and Y during meiosis in the mouse (Evans *et al.*, 1982) (see Chapter 12.2), so that this comparison was not valid. The frequencies of the different spatial orientations observed between labelled chromatids in the terminally associated autosomal bivalents are in fact exactly as expected if the associations were chiasmate, but with the exchanged region being too small to be resolved. Subsequently, Imai and Moriwaki (1982) found that these "terminal" chiasmata dissociate before intersitial chiasmata. The X-Y bivalent in the mouse also dissociates early (Matsuda *et al.*, 1982), so that

this may be a feature of such near-terminally located chiasmata. A possible mechanism for this is the suggestion by Maguire (1974) that sister chromatid "cohesiveness" is necessary for chiasma maintainance until anaphase I. A chiasma close to the telomere might not have sufficient sister chromatid cohesiveness distal to the chiasma to enable it to be maintained.

The second long-standing problem is that of "terminalization", the movement of the visible chiasmata (but not the physical exchange points) towards the telomere which in the extreme case may lead to a consequent reduction in chiasma frequency as meiosis proceeds (Darlington, 1932).

Most evidence advanced in support of this concept is based on a comparison of chiasma frequencies scored at different meiotic stages within the same individual (Zarchi et al., 1972). The differences found may be due to environmentally determined variation in different batches of meiocytes, or to the difficulty of accurately counting chiasmata in condensed metaphase I bivalents (John, 1976). Better indirect evidence based on the distribution of chiasmata at the different stages of meiosis in *Schistocerca* suggests that there is no terminalization (Fox, 1973), and Hultén (1974) has presented similar evidence that there is no terminalization at least until diakinesis in human spermatocytes.

The most direct studies of chiasma terminalization use BUdR labelling methods. Tease (1978) and Tease and Jones (1978) found no detectable movement of chiasmata from the sites of exchange in *Locusta* bivalents. Similarly, there is no terminalization in mouse spermatocytes (Kanda and Kato, 1980), but Polani et al., (1979)

could not decide whether or not chiasma terminalize in mouse oocytes. Muddlin and Evans (1980) have concluded, however, that there is no terminalization in mouse oocytes based on the similar chiasma distributions at different stages. In another BUdR labelling study Allen (1979) found evidence of terminalization in the chiasmate X-Y bivalent of the Armenian hamster. This interpretation has subsequently been questioned by Jones and Tease (1981) who point out that the similarly labelled chromatids were not in close association proximal to the "terminalized" chiasma, and they suggested that the exceptional bivalents were stretching distortions due to the air-drying technique used. The evidence from Orthopterans and mammals is therefore that terminalization either does not occur, or is only rare. BUdR labelling of meiosis has not yet been reported in plants, but Maguire (1979) has used maize plants heterozygous for heterochromatic knobs to demonstrate the occurrence of terminalization in that species, and she proposed that it is related to the strong meiotic prophase condensation in that species. A similar situation possibly occurs in *Secale* (Price, 1959), although the observations of Jones (1978) might provide an alternative explanation in this case.

The localization of exchanges is also important in determining the level of recombination in a species. Chiasma frequency and localization are inter-related (John, 1976). The factors which determine the distribution of chiasmata are not clear, but the concept of chiasma interference is well established (Haldane, 1931; Mather, 1935). Mather proposed sequential chiasma formation, with the first chiasma being fixed some distance from the centromere, and interference distributing the other chiasmata sequentially by decreasing the

probability of formation of the next chiasma near one already formed. Such interference has been detected in studies of Orthopterans (Henderson, 1963; Southern, 1967; Fox, 1973; Shaw and Knowles, 1976), in mouse oocytes (Maddlin and Evans, 1980), and in human spermatocytes (Hultén, 1974), although most of these authors have considered that chiasma formation takes place sequentially from the telomere, and not the centromere (Mather, 1935). Jones (1974), however, has suggested that the peaks of chiasma frequency at the telomeres reflect only a high probability of chiasma formation, and not their early formation at these sites. In many species interference does not operate across the centromere (Hultén, 1974; Maddlin and Evans, 1980; Fox, 1973).

Mather also proposed competition between bivalents for chiasmata, based on significant negative correlations between chiasma frequencies of bivalents within the same cell in some individuals of certain species. This proposal has not been widely accepted as both positive and an absence of any correlation have been found (Rowlands, 1958; Hewitt and John, 1965; Southern, 1963; Fox, 1967; Hultén, 1974; Maddlin and Evans, 1980).

The number of bivalents in an organism also affects the level of recombination through their independent segregation. Darlington (1939) proposed a "Recombination Index" (= sum of the haploid chromosome number and the cell chiasma frequency) as a convenient measure of recombination for comparative studies where there is variation in chromosome number. However, this index is not completely accurate, since an increase in chromosome number is not equivalent to a similar increase in chiasma frequency, as chiasma position may vary from cell to cell. In addition, it does not take into account differences in

chiasma localization, or differences in karyotype geometry between species.

2.2 Variation in recombination and chiasma frequency

Extensive variation in chiasma frequency and location has been observed within and between species. Developmental variation within a species has been well documented, with one of the most common examples being differences between the sexes, referred to by Darlington (1978) as "two track heredity". In many invertebrate species (e.g. *Drosophila*) one sex is without chiasmata and recombination (White, 1973). Less extremely, the sexes may differ in chiasma frequency and/or chiasma location (Callan and Perry, 1977; Perry and Jones, 1974; Fletcher and Hewitt, 1980). In other cases there is no difference in either frequency or localization between the sexes, both in grasshoppers (Jones et al., 1975) and in plants (Bennett et al., 1973; Davies and Jones, 1974). In mammals, the weight of genetic and cytological evidence suggests that recombination is slightly higher in females than in males, but that in Man, the reverse is the case (Dunn and Bennett, 1969; Jagiello et al., 1975). Hultén et al (1978) have questioned this conclusion in the case of Man because of the technical difficulties involved. There might also be a difference between the sexes in chiasma localization in the mouse (Polani, 1972; Lyon, 1976; Speed, 1977), and in Man (Hultén et al., 1978).

Age is another developmental variation which has been demonstrated to affect chiasma frequency. Anther position (and hence age) affects chiasma frequency in rye (Rees and Naylor, 1960) and in *Tulipa* (Conzin

and Fox, 1974). The effect of age can differ between the sexes, as Speed (1977) finds a reduction in chiasma frequency in female but not in male mice with increasing age. In man, chiasma frequencies are not significantly affected by age in males (Lange et al., 1975), but there is little evidence available for females.

Chiasma frequency is often found to vary significantly between individuals within a natural population (Price, 1975; Jones et al., 1975; Shaw and Knowles, 1976), but very few studies have examined inter-individual variation in chiasma location. In *Chorthippus* males, Laurie and Jones (1981) found significant differences in chiasma distribution between the twelve individuals sampled, whereas in man (seven males sampled) there was little difference in chiasma distribution (Laurie et al., 1981). Laurie et al (1981) suggest that the lack of variation in man is a reflection of the high chiasma frequency coupled with a rigidly controlled chiasma distribution.

It is clear that there is a large genetic component controlling variation in recombination frequency and location (Catcheside, 1977). Studies on rye (Rees and Thompson, 1956) and *Hordeum* (Gale and Rees, 1970) lines, and selection experiments in *Schistocerca* (Shaw, 1972; 1974) indicate polygenic systems controlling chiasma frequency. Shaw's studies have revealed a large additive component and a small dominance component directed towards high chiasma frequency. In addition, selection experiments on recombination frequency in both *Drosophila* and silkworms (*Bombyx*) indicate a similar genetic control (Chinnici, 1971a, b; Turner, 1979). Evidence of genetic control exerted over chiasma localization is much less extensive; Jones (1967; 1974b) has found differences in chiasma distribution between different inbred lines of rye.

In mammals, little is known of the genetics of chiasma frequency and localization, but there are significant differences in chiasma frequency between different inbred strains of mice (Kyslikova and Forejt, 1972) and the heritability of chiasma frequency within a heterozygous interchange in the mouse has been estimated by deBoer and Van der Hoeven (1977).

The amount of recombination can also be affected by cytogenetic features. B chromosomes can affect the amount and distribution of recombination in the normal chromosome complement. They increase chiasma frequency in maize (Ayonoadu and Rees, 1968), decrease it in *Lolium* (Cameron and Rees, 1968), while in rye, Jones and Rees (1967) found no effect on chiasma frequency, but the B chromosomes increased the variance in chiasma frequency. In Orthoptera, B chromosomes increase the mean and variance of chiasma frequencies (John and Hewitt, 1980b), and in *Euthystira* apparently reduce the degree of chiasma localization (Fletcher and Hewitt, 1980b). Only one clear study of the effect of B chromosomes in mammals is available. Patton (1977) has found that the B chromosomes in a North American pocket mouse slightly increases the chiasma frequency in the regular chromosome complement.

Another cytogenetic feature which can affect chiasmata is the presence and location of heterochromatin. John and Miklos (1979) have listed a large number of examples from both insects and plants in which changes in heterochromatin result in shifts in the frequency and/or distribution of chiasmata in the euchromatin of the species, and they have argued that one of the controlling factors of the evolution of heterochromatin is its effects on recombination level

and distribution. In mammals, there is very little evidence that heterochromatin affects the recombination system. In hedgehogs, Natarajan and Gropp (1971) have shown that chiasmata do not occur in the few large blocks of telomeric heterochromatin present, but their illustrations give no indication that chiasma frequency or distribution in the euchromatin near these heterochromatic blocks is unusual. Similarly in the rat *Uromys* the presence of telomeric heterochromatin does not appear to be the cause of any change in chiasma frequency or location within the euchromatin, although here the situation is complicated by the presence of B chromosomes in the race without the telomeric heterochromatin (Baverstock et al., 1982). In the mouse, Navotna and Forejt (1975) found no difference in chiasma frequency near varying amounts of centromeric heterochromatin, but they did not investigate chiasma distribution.

Environmental agents such as temperature can modify the frequency and distribution of chiasmata (Elliot, 1955; Shaw, 1971), and this suggests the possibility of seasonal variation in chiasma frequency, especially in poikilothermic species. Such a variation has been found by King and Hayman (1978) in a lizard, and they have suggested that this species uses this variation in order to produce sperm that had a high interstitial chiasma frequency to be utilized for fertilization.

CHAPTER 3: AUSTRALIAN MARSUPIALS

There are three extant lines of mammalian evolution; the monotremes, marsupials, and eutherian mammals. Estimates based on amino acid sequence analysis of myoglobin and haemoglobin suggest that the marsupials and eutherians diverged from each other some 130 million years ago (Air et al., 1971), and the fossil evidence also suggests a similar date (Keast, 1977). Marsupials are now found both in North and South America (although only one species extends as far north as the United States), and in the Australasian region (Australia, the island of New Guinea, and its adjacent islands including Celebes and Timor). The fossil evidence suggests they were never abundant elsewhere (Clements, 1977).

Kirsch and Calaby (1977) have listed approximately 80 extant American and approximately 180 extant Australasian species. This disjunct present distribution of marsupials has been explained in terms of their origin, with the marsupials originating in the Americas, and migrating into Australia via Antarctica before Australia drifted north towards its present position (Keast, 1977). This must have occurred less than 80 million years ago since no evidence of any marsupial has been found in New Zealand (Clements, 1977; Keast, 1977). Significantly, a fossil marsupial estimated to be 40 million years old has recently been discovered in the Antarctic (Woodborne and Zinsmeirer, 1982), and a recent estimate of the physical separation of Australia and Antarctica at 45 million years ago (Falvey and Mutter, 1981) would also be in accord with this hypothesis.

Since their isolation in Australia the marsupials have diversified into a wide range of herbivores, insectivores and carnivores in aboreal, fossorial, subterranean and terrestrial environments, lacking only small granivorous rodent-like and bat-like forms where more recent immigrants fill these niches (Kirsch and Calaby, 1977). These authors also classify the Australian marsupials into six superfamilies reflecting this diversification. Two of these (Notoryctoidea and Tarsipedoidea) are monospecific and highly specialized. The rest of the species fall into the four major superfamilies of Australian marsupials; Dasyuroidea (50 species), Perameloidea (18 species), Vombatoidea (3 species), and Phalangeroidea (116 species).

The marsupials, and especially those in Australia, are well known cytologically; just over half of the species have been examined, and the nuclear DNA contents are known for many species (Hayman and Martin, 1974; Hayman, 1977). In addition, the silver, N, G, and C banding techniques have been applied to the chromosomes of a considerable number and diversity of Australian species (Rofe, 1979; Young et al., 1982; Rofe and Hayman, in press). Compared to eutherian mammals, marsupial chromosome numbers are low, with most of the species having diploid chromosome numbers between 14 and 22. There are two peaks in the distribution of marsupial chromosome numbers at $2n = 14$ and $2n = 22$, so that different authors have considered either $2n = 14$ or $2n = 22$ to be an ancestral chromosome number for the marsupials (Sharman, 1973; Hayman and Martin, 1974). The G-banding analyses of Rofe (1979) and of Rofe and Hayman (in press) have shown that the wide range of species studied (Australian and one South American) with the $2n = 14$ "basic" complement of Hayman and Martin (1974) have

very similar autosomal G-banding patterns, providing strong evidence that $2n = 14$ is indeed ancestral. In this scheme, the higher numbers would initially arise by chromosome fission within the $2n = 14$ complement, but some groups, especially the Macropodidae (many of which have $2n = 16$), have been characterized by subsequent chromosome fusions from a higher number ($2n = 22$) (Rofe, 1978; Rofe, 1979; Rofe and Hayman, in press).

The presence of the $2n = 14$ basic karyotype in all of the Australian superfamilies, and the restricted amount of karyotypic change that has occurred from this format has thus coupled a relatively stable karyotype with a group which has undergone considerable evolutionary radiation in terms of morphological, physiological and ecological characteristics. The Australian marsupials represent a group in which a study of the relationship between chiasma frequency and other aspects of the genetic system should be fruitful. This is because one other chromosomal mechanism varying recombination level - changes in chromosome number, does not appear to have been as important in this group as in other groups of mammals with a comparable diversity of the other factors (such as reproduction, and longevity) that are important in the genetic system of the species (Hayman, 1981).

Meiosis has been studied in the males of a few marsupial species, mainly as a convenient method for determining chromosome number before lymphocyte cultures had been developed (e.g. Altmann and Ellery, 1925; McIntosh and Sharman, 1953), although Koller (1936b), Drummond (1938), and Gunson *et al.*, (1968) have estimated cell chiasma frequency in spermatocytes of a total of five species.

CHAPTER 4: MATERIALS AND METHODS

4.1 The species studied

Chiasma frequency was estimated for the species listed in Table 4.1. These cover the biological and taxonomic diversity of Australian marsupials.

The animals came from varying sources (Table 4.2). The individuals examined from three of the species (*Sminthopsis crassicaudata*, *Dasyuroides brynei*, and *Perameles nasuta*) came from captive laboratory colonies which have been established for a number of years so that the animals (and their ancestors for a number of preceding generations) were laboratory reared and bred. The ancestry of many of the macropods sampled was unknown since they came from private or public fauna parks containing animals from many different geographic areas.

The large number of *Trichosurus vulpecula* examined all came from the Adelaide metropolitan area. They were collected by commercial pest control firms during the course of their normal activities and delivered either to the Genetics Department or to the Central Animal House, Adelaide University, and held until required.

4.2 Animal handling

All surgery was performed under general anaesthesia. In the small species this was induced either with 4% ethrane in a $10_2 : 1N_2O$ mixture or with 4% halothane in O_2 , supplied through a face mask at 1 litre/min with an anaesthetic machine. Anaesthesia was complete after a few minutes. The wombats and macropods were anaesthetised by an intramuscular injection of a 2 : 1 mixture of "Rompun" (20 mg/ml xylazine,

TABLE 4.1: The species studied arranged into Families and Superfamilies according to Kirsch and Calaby (1977)

<u>Superfamily</u>	<u>Family</u>	<u>Species</u>	<u>Common Name</u>
Dasyuroidea	Dasyuridea	<i>Sminthopsis crassicaudata</i>	Fat-tailed Dunnart
		<i>Sminthopsis murina</i>	Common Dunnart
		<i>Dasyuroides byrnei</i>	Kowari
		<i>Dasyurus hallucatus</i>	Northern Quoll
		<i>Dasyurus viverrinus</i>	Eastern Quoll
		<i>Sarcophilus harrisii</i>	Tasmanian Devil
Perameloidea	Peramelidae	<i>Perameles gunnii</i>	Eastern Barred Bandicoot
		<i>Perameles nasuta</i>	Long-nosed Bandicoot
		<i>Isodon obesulus</i>	Southern Brown Bandicoot
		<i>Isodon macrourus</i>	Northern Brown Bandicoot
Vombatoidea	Phascolarctidae	<i>Phascolarctos cinereus</i>	Koala
	Vombatidae	<i>Lasiorhinus latifrons</i>	Southern Hairy-Nosed Wombat
		<i>Vombatus ursinus</i>	Common Wombat

Phalangeroidea

Petauridae

Petaurus breviceps

Sugar Glider

Pseudocheirus peregrinus

Common Ringtail Possum

Phalangeridae

Trichosurus vulpecula

Common Brushtail Possum

Trichosurus arnhemensis

Northern Brushtail Possum

Macropodidae

Potorous tridactylus

Long-nosed Potoroo

Setonix brachyurus

Quokka

Lagorchestes conspicillatus

Spectacled Hare-wallaby

Bettongia penicillata

Brush-tailed Bettong

Bettongia lesueur

Burrowing Bettong

Thylogale billardierii

Red-bellied Pademelon

Wallabia bicolor

Swamp Wallaby

Macropus parma

Parma Wallaby

Macropus eugenii

Tammar Wallaby

Macropus agilis

Agile Wallaby

Macropus parryi

Whiptail Wallaby

Macropus rufogriseus

Red-necked Wallaby

Macropus robustus

Euro

Macropus giganteus

Eastern Grey Kangaroo

Macropus rufus

Red Kangaroo

Tarsipedoidea

Tarsipedidae

Tarsipes rostratus

Honey Possum

TABLE 4.2: Locality and source of the animals examined

<u>Species</u>	<u>Abbreviation</u>	<u>Locality, Source</u>
<i>Sminthopsis crassicaudata</i>	Sc	Genetics Dept. colony, U.A.
<i>Sminthopsis murina</i>	Sm	Shark Bay, A.P.B.
<i>Dasyuroides byrnei</i>	Db	I.M.V.S. colony
<i>Dasyurus hallucatus</i>	Dh	Northern Territory, M.Z.
<i>Dasyurus viverrinus</i>	Dv	Tasmania, Tas. N.P.W.S.
<i>Sarcophilus harrisii</i>	Sh	Tasmania, Tas. N.P.W.S.
<i>Perameles gunnii</i>	Pg	Tasmania, Tas. N.P.W.S.
<i>Perameles nasuta</i>	Pn	Unknown, C.S.I.R.O. Div. of Wildlife Research
<i>Isodon obesulus</i>	Io	South Australia; Western Australia, U.W.A.Z.
<i>Isodon macrourus</i>	Im	Queensland, I.M.V.S.
<i>Phascogale cinereus</i>	Pc	Kangaroo Island, S.A.N.P.W.S.
<i>Lasiornis latifrons</i>	Ll	South Australia, Genetics Dept., U.A.
<i>Vombatus ursinus</i>	Vu	Tasmania, Tas. N.P.W.S.
<i>Petaurus breviceps</i>	Pb	Unknown ^b , S.A.N.P.W.S.
<i>Pseudocheirus peregrinus</i>	Pp	Queensland [†] , South Australia; Victoria, M.Z.
<i>Trichosurus vulpecula</i>	Tv	Adelaide, South Australia
<i>Trichosurus arhemensis</i>	Ta	Northern Territory
<i>Potorous tridactylus</i>	Pt	Tasmania, Tas. N.P.W.S.
<i>Setonix brachyurus</i>	Sb	Rottneest Island, U.W.A.Z.
<i>Lagorchestes conspicillatus</i>	Lc	Barrow Island, U.W.A.Z.
<i>Bettongia penicillata</i>	Bp	S-W Western Australia, A.P.B.
<i>Bettongia lesueur</i>	Bl	Bernier Island, A.P.B.
<i>Thylogale billardierii</i>	Tb	Unknown [†]
<i>Wallabia bicolor</i>	Wb	Unknown, Cleland Cons. Park
<i>Macropus parma</i>	Mp	Unknown, M.Z.
<i>Macropus eugenii</i>	Me	(2) Kangaroo Island; (1) Western Australia ^a

<u>Species</u>	<u>Abbreviation</u>	<u>Locality, Source</u>
<i>Macropus agilis</i>	Ma	Unknown, Adelaide Zoological Gardens
<i>Macropus parryi</i>	Mpy	Unknown, Adelaide Zoological Gardens
<i>Macropus rufogriseus</i>	Mrf	Tasmania, Tas. N.P.W.S.
<i>Macropus robustus</i>	Mrb	Unknown, A.P.B.
<i>Macropus giganteus</i>	Mg	Unknown, M.Z.
<i>Macropus rufus</i>	Mr	Unknown, Cleland Cons. Park
<i>Tarsipes rostratus</i>	Tr	Two Peoples Bay, Murdoch University

U.A.	University of Adelaide
A.P.B.	Agriculture Protection Board, Western Australia
I.M.V.S.	Institute of Medical and Veterinary Science, Adelaide
M.Z.	Department of Zoology, Monash University
N.P.W.S.	National Parks and Wildlife Service of the state concerned
U.W.A.Z.	University of Western Australia, Department of Zoology

† Obtained from the collection of Pastor I. Wittwer, Cleve, South Australia

a This animal was the result of a hybridization between an animal from the mainland of Western Australia, and one from Garden Island, W.A.; obtained from M. Renfree, Murdoch University.

b These animals had been confiscated from an illegal private collector by the S.A.N.P.W.S.

Bayer; a muscle relaxant) and "Ketalar" (100 mg/ml ketamine hydrochloride, Parke-Davis; an anaesthetic) at a dose of 8 mls/10 kg body weight. Using this method anaesthesia was complete after approximately 15 minutes, during which time the scrotal area was shaved and swabbed with 70% alcohol.

In most cases an entire testis was removed. A small vertical incision was made through the scrotum and the testis squeezed out. The testicular vein and artery were tied off a number of times with sterile cotton thread, and the blood vessels were then severed. The wound and the surrounding area were dusted with antibiotic powder, and the animal was given an intramuscular injection of penicillin and streptomycin ("Streptopen", Glaxovet) at the recommended dose. Only two deaths were attributed to the surgery in over one hundred orchiectomies performed.

Testis biopsies were performed on some animals. This involved making a small incision through the scrotum, rotating the testis until no blood vessels were visible through the tunica albuginea, and then puncturing the tunica albuginea with a sterile scalpel blade. The seminiferous tubules which were everted through this puncture were removed with forceps, and the area was dusted with antibiotic powder.

In the cases where animals were killed this was done by an injection of "Lethobarb" (sodium pentobarbitone, 325 mg/ml; Arnold) into the heart after ethrane anaesthesia.

4.3 Meiotic preparations

Male

The testis or testis biopsy was collected into pre-warmed phosphate buffered saline, and the preparations were made as soon as possible after sampling (less than 10 minutes from sampling to starting the preparation). The following method was used.

- (1) Place approximately 1 cm³ of tubules in 10 mls of warm (37°C) 1.5% tri-sodium citrate in a petri dish.
- (2) Macerate the tissue with curved forceps and by repeated pipetting for 10-15 minutes.
- (3) Let the larger tissue fragments settle for 2-3 minutes, and transfer the supernatant cell suspension to four 10 ml centrifuge tubes.
- (4) Centrifuge at 1000 r.p.m. in a bench centrifuge for 5 minutes.
- (5) Resuspend the cells in 0.075 M KCl and incubate at 37°C for 10 minutes.
- (6) Centrifuge at 500 r.p.m. for 7 minutes and remove the supernatant.
- (7) Flick the cell pellet to resuspend the cells in the small volume of remaining supernatant, and fix by gently layering fresh, cool 3:1 methanol-acetic acid (containing 1 drop of chloroform per 10 mls) over the cells.
- (8) Resuspend the cells in the fixative after 5 minutes at room temperature.
- (9) Change the fixative (without chloroform) twice by centrifugation, and store the tubes at 4°C for 12-24 hours before making air-dried slides.

Females

Preparations of female meiosis were either squashes of pouch young ovaries, or air-dried preparations of cultured oocytes taken from ovaries of mature females (in *Sminthopsis crassicaudata*). Squash preparations of pouch young ovaries were made by a modification of the method described by Hsu (1964).

- (1) Immediately after removal the ovaries were fixed in 50% acetic acid for 10 minutes.
- (2) Approximately 0.5 mm^3 of ovarian tissue was macerated in 2 drops of 50% acetic acid on a slide, a $24 \times 60 \text{ mm}$ coverslip applied, and the preparation was squashed between 2 layers of filter paper.
- (3) The slide was quickly plunged into liquid nitrogen, and after it had frozen, the coverslip was flicked off with a scalpel blade.
- (4) The slide was immediately immersed in methanol for 5 minutes and then allowed to air-dry.

Attempts were also made to culture oocytes taken from mature female *Sminthopsis crassicaudata* to induce reactivation of the dictyate oocytes by using modifications of the methods described by Röhrborn *et al.*, (1977).

Female *Sminthopsis* were given intraperitoneal injections of 10 IU PMS (pregnant mare serum gonadotrophin; "Folligon", Intervet) followed 48 hours later by 5 IU HCG (human chorionic gonadotrophin; "Chorulon", Intervet) in an attempt to induce ovulation (Smith and Godfrey, 1970). The females were killed by cervical dislocation 24 hours after the HCG injection, and the ovaries dissected out into a small petri dish

containing culture medium (RPMI 1640 supplemented with 5% foetal calf serum or with 0.5% bovine serum albumin). Oocytes were liberated into the medium by puncturing the follicles with a 26 gauge syringe needle. The oocytes were then taken through two changes of medium using a micropipette (a pasteur pipette drawn out into a fine point) to free them from any adhering follicular cells. A mouth tube was used to control the micropipette. The oocytes were then placed in the medium under equilibrated paraffin oil in a small petri dish, and incubated in a humidified 5% CO₂ incubator at 37°C. A range of incubation times were tried. The few cells which were reactivated (see Chapter 8) were found after 20-24 hours incubation.

The chromosome preparations of these cultured oocytes were made as described by Tarkowski (1966). This involved transferring the oocytes singly or in small groups with a micropipette onto clean slides, removing excess culture medium (no hypotonic treatment was given), and fixing *in situ* by allowing 4-6 small drops of fresh, cool 3:1 methanol-acetic acid to drop from a micropipette onto the oocytes. The slides were then air-dried.

Staining

The slides from males and from pouch young ovary squashes were stained with 10% Gurr's R66 Giemsa in Sörenson's phosphate buffer pH 6.8 for 1-1½ minutes, while those of cultured oocytes were stained with 5% Gurr's R66 Giemsa in the same buffer for 30 seconds, rinsed with distilled water and air-dried. They were mounted with a coverslip with Gurr's Depex. A number of differential staining techniques including G, C, and fluorescent techniques (see Appendix 4) were tried on the meiotic preparations of the males in an endeavor to better

identify the diplotene-diakinesis bivalents, but none of the techniques were reliable enough, or preserved bivalent morphology well enough to be used.

Where considered necessary, either standard PHA stimulated lymphocytes, or bone marrow chromosome preparations were used in order to examine the somatic karyotype of the animals studied.

4.4 Data collection

The slides were scanned systematically to find diplotene-diakinesis meiocytes in which all autosomal bivalents were present, and interpretable. For each animal the aim was to score the number of chiasmata in twenty different cells. The data were then expressed as mean $\pm$ standard deviation. In some species the numbers of chiasmata in the various individual bivalents in the karyotype were also recorded. In some individuals it was not possible to find twenty diplotene-diakinesis cells - usually the small species in which the amount of material available was limited. In a few cases more than twenty cells were scored, and in the species in which chiasma distribution along the bivalents was examined forty or more cells were used to provide the data in each case.

Scoring was done under a 100X, 1.25 N.A. Planapo oil immersion lens and with 12.5X binocular eyepieces. Any photography was performed with the same lens using Copex Pan Rapid Tri 13 high contrast film (Agfa-Gevaert) exposed at a machine setting of ASA 15 and developed in 50% D11 for 5 minutes (or equivalent).

The distribution of chiasmata along bivalents was examined in a number of species. This was done by photographing each diplotene-

diakinesis cell, and printing the negatives at a standard magnification (approximately 6,300X). The course of the chromosomes within the bivalents, the position of chiasmata, and of the centromeres in each bivalent were marked on the print. The length of the bivalent arms and the positions of the chiasmata were then measured in a manner analogous to that of Shaw and Knowles (1976) in arbitrary units using a Numonics Corporation electronic graphics calculator, and all the measurements recorded. Each measurement was performed three times and the sums of these measurements were used in the analysis. For the analysis of chiasmata distribution the positions of each chiasmata were converted to relative positions along the bivalent arms to take account of variation between different cells in arm and bivalent length due to differences in contraction.

4.5 Ageing male *Trichosurus vulpecula*

Some of the male *Trichosurus vulpecula* examined in Chapter 5 were aged by counting the cementum annuli in the lower second or third molar teeth using the method described by Pekelharing (1970). This involved the following steps.

- (1) The teeth were removed from the lower jaw and dried.
- (2) They were glued with "Araldite" onto short lengths of dowelling, using the posterior or anterior surface of the tooth.
- (3) The teeth were then ground using wet and dry carborundum paper to reach the mid-cementum pad between the two roots, taking care to make a clean horizontal cut. The surface was then polished by rubbing on hard cardboard.

(4) They were then soaked in an 18:2 (V/V) solution of 10% formalin and 90% formic acid for 2 hours, washed in running water for 5 minutes, and neutralized with dilute ammonium hydroxide for 5 minutes.

(5) Staining was for 3-5 minutes in 0.1% Thionin, and the teeth were then washed in running water for 5 minutes, and treated with dilute ammonium hydroxide again for 5 minutes.

(6) The cementum annuli could then be counted with a dissecting microscope.

4.6 Statistical analysis

Standard statistical techniques described in Sokal and Rohlf (1969, and 1973) including contingency tables, various analyses of variance and correlations were used. Details are given in appropriate places in the text. One particular statistic was used extensively; Spearman's correlation coefficient (r_s). This correlation coefficient behaves similarly to the more commonly used correlation coefficient (Pearson's product-moment correlation coefficient) in that it ranges from -1 (perfect negative correlation) through zero (no correlation) to +1 (perfect positive correlation). However it is a non-parametric statistic based on the ranks of the sets of data being compared. It therefore does not (unlike Pearson's correlation coefficient) rely on a number of assumptions. These include that both variables are normally distributed, equality of variances, and that there is an underlying linear relationship between the variables being compared. It was therefore an extremely useful statistic for the analysis of much of the data in this thesis.

The 5% level of significance was applied to all statistical tests performed.

CHAPTER 5: CHIASMA FREQUENCY IN MALE *TRICHOSURUS VULPECULA*5.1 *Trichosurus vulpecula*: the species and its chromosomes

Chiasma frequency was studied in detail in the Brushtailed Possum *Trichosurus vulpecula* for a number of reasons. The species is extremely common and widespread and has adapted itself successfully to suburban life. Considerable numbers were therefore available for study. Tyndale-Biscoe (1974) and Strahan (1983) have reviewed many of the ecological and biological features of this species. On the Australian mainland there are two breeding seasons per year; the major peak of births occurs in autumn, and a minor peak occurs in spring. The animals live for a number of years, with an average life expectancy of around six years. Females therefore breed a number of times during their lifetime.

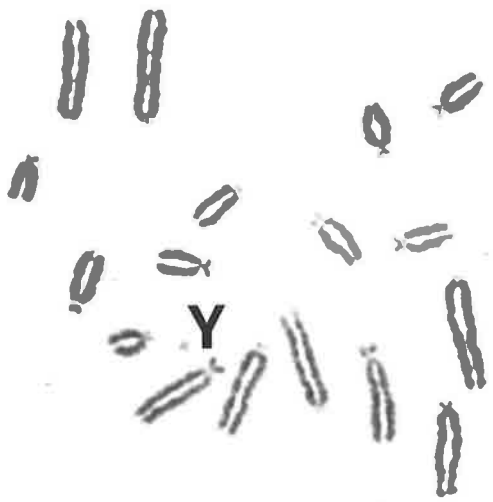
The karyotype of *T. vulpecula* has been described by Hayman and Martin (1974) and Stock and Mengden (1982). There are $2n = 20$ chromosomes, all of which are acrocentric or telocentric (Figure 5.1a). The nine pairs of autosomes cannot be readily distinguished since there is a gradual size diminution from the largest to the smallest pair. C-banding does not help in chromosome identification since all of the chromosomes have small centromeric C-bands.

At diplotene-diakinesis of meiosis the chiasmata are displayed clearly on the autosomes (Figure 5.1b) and can therefore be easily counted. In most diplotene-diakinesis cells the centromere positions are not discernible, so that chiasmata could not be classified as proximal or distal.

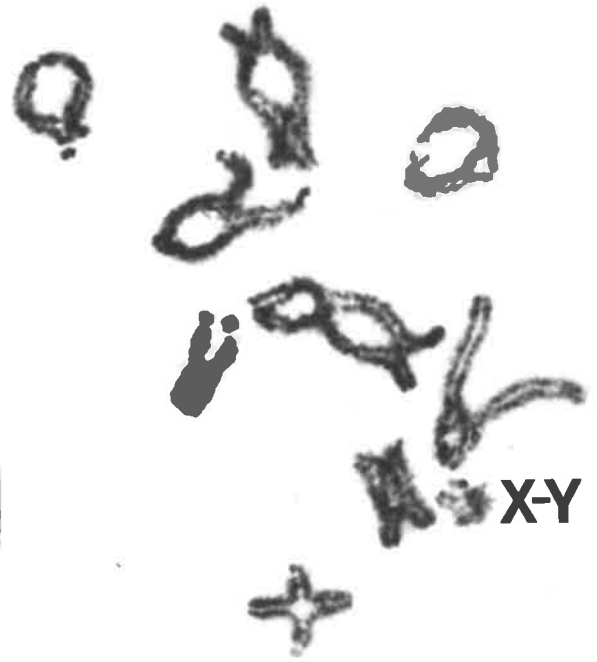
Figure 5.1: *Trichosurus vulpecula*

- a. Chromosomes from a lymphocyte culture of a male. The autosomes are all acrocentric or telocentric and cannot be individually identified. The X and Y chromosomes are indicated.
- b. A diplotene-diakinesis cell showing nine autosomal bivalents and the sex vesicle (X-Y). There are 19 chiasmata.
- c. The diplotene-diakinesis cell with the lowest number of chiasmata found (10 chiasmata). There is a pair of univalents (arrowed).
- d. The diplotene-diakinesis cell with the highest chiasma score found (29 chiasmata).

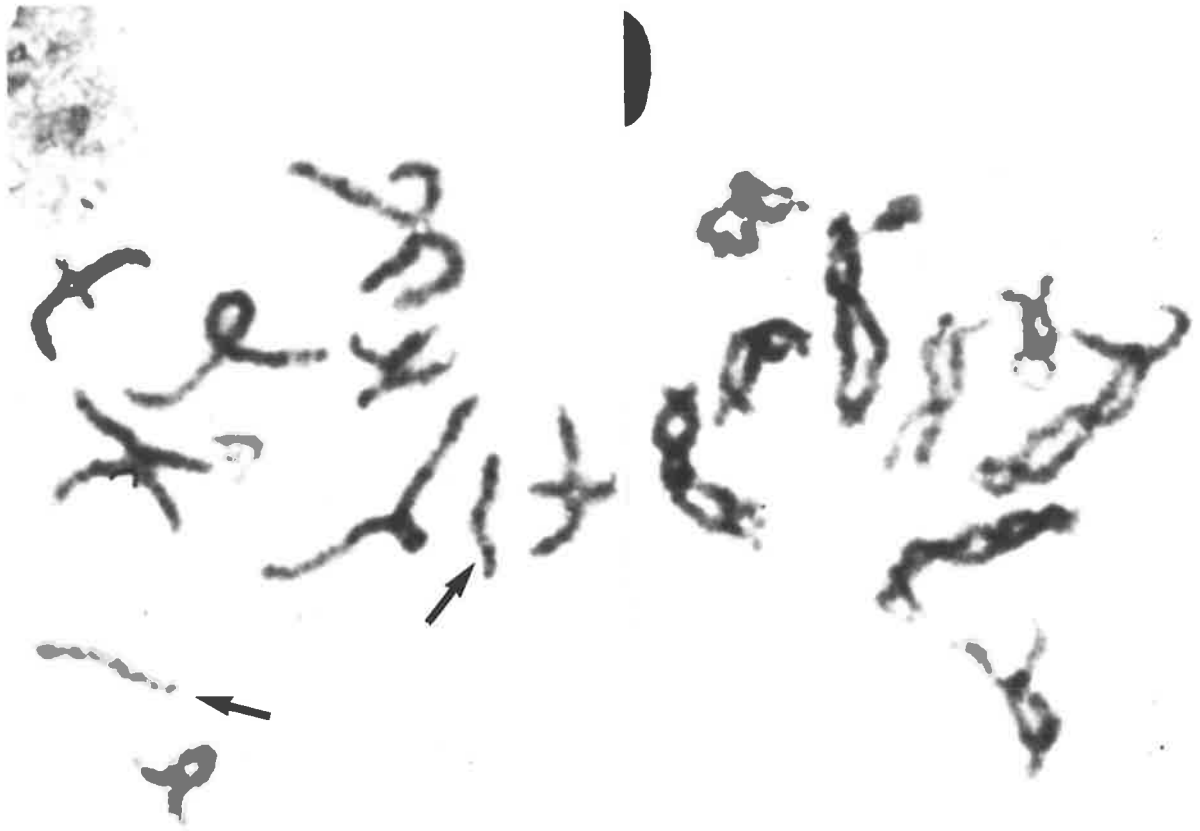
X



a



b



c

d

5.2 Variation in chiasma frequency

Chiasma frequencies were determined from 69 individual *T. vulpecula*. The animals were wild-caught (see Chapter 4.1) and were only held in captivity under natural lighting for short periods (less than 2 weeks) before sampling. These were sampled over an extended time period, beginning in May, 1979 and ending in March 1981. The original scheme was to make meiotic preparations from five individuals per sampling time, with the sampling times being at monthly intervals. The animals within each sampling time were all done within 2 days (the Monday and Tuesday of the week involved). However, the availability of animals and other practical considerations forced the sampling schedule described in Table 5.1 and in Figure 5.2 to be adopted, with the 69 animals coming from 19 sampling times, each containing from one to five animals.

The number of chiasmata present in individual diplotene-diakinesis meiocytes varied from 10 to 29 (Figure 5.1c and d). The cell in Figure 5.1c has a pair of univalents; only 4 cells out of the 1,380 diplotene-diakinesis meiocytes scored had univalents. The chiasma frequencies for the different animals ranged from 14.15 to 21.85, with an overall mean of 18.14 chiasmata per cell. The number of chiasmata in different cells of the one individual varied and this is reflected by the ranges and standard errors given in Table 5.1.

In order to analyse the data for differences in chiasma frequencies between animals within sampling times and between different sampling times by analysis of variance, all chiasma counts were converted to logarithms. The reason for this was the highly significant positive correlation between increased

TABLE 5.1: Chiasma frequency in the 69 *Trichosurus vulpecula* sampled

Sampling Time (week beginning)	Animal	Cell Chiasma Frequency $\pm$ Standard Error	Range
1 (14/5/79)	a	16.70 $\pm$ 1.34	14 - 20
	b	21.55 $\pm$ 2.65	16 - 25
	c	17.80 $\pm$ 1.71	15 - 21
	d	20.65 $\pm$ 2.25	18 - 25
	e	18.50 $\pm$ 1.73	15 - 22
2 (11/6/79)	a	20.80 $\pm$ 3.19*	14 - 28
	b	20.40 $\pm$ 1.67	18 - 24
	c	18.45 $\pm$ 1.50	15 - 21
	d	18.45 $\pm$ 1.91	15 - 24
	e	17.25 $\pm$ 1.71	14 - 20
3 (9/7/79)	a	19.30 $\pm$ 2.58	16 - 26
	b	18.45 $\pm$ 1.64	15 - 21
	c	18.05 $\pm$ 3.02	13 - 26
	d	17.75 $\pm$ 1.71	14 - 21
4 (6/8/79)	a	14.75 $\pm$ 1.86	11 - 19
	b	21.85* $\pm$ 1.27	19* - 24
	c	19.15 $\pm$ 1.76	17 - 22
	d	20.85 $\pm$ 2.23	17 - 26
	e	16.40 $\pm$ 2.23	13 - 22
5 (3/9/79)	a	18.50 $\pm$ 2.37	14 - 22
	b	18.35 $\pm$ 1.79	15 - 22
	c	17.50 $\pm$ 1.70	15 - 21
	d	20.50 $\pm$ 1.32	18 - 22
6 (1/10/79)	a	20.35 $\pm$ 1.76	18 - 24
	b	18.05 $\pm$ 1.47	16 - 20
7 (24/10/79)	a	16.25 $\pm$ 1.62	13 - 20
	b	18.55 $\pm$ 2.46	15 - 25
	c	17.95 $\pm$ 1.96	15 - 22
	d	15.95 $\pm$ 2.06	13 - 21
8 (26/11/79)	a	19.15 $\pm$ 1.87	16 - 23
	b	16.25 $\pm$ 2.00	13 - 20
	c	17.70 $\pm$ 2.41	14 - 23
9 (2/1/80)	a	17.65 $\pm$ 1.46	14 - 20
	b	18.65 $\pm$ 1.50	17 - 21
	c	19.20 $\pm$ 2.19	16 - 24
	d	18.55 $\pm$ 1.47	15 - 21
	e	17.10 $\pm$ 1.33	15 - 20

TABLE 5.1: (cont'd)

Sampling Time (week beginning)	Animal	Cell Chiasma Frequency $\pm$ Standard Error	Range
10 (28/1/80)	a	15.00 $\pm$ 1.81	12 - 18 ^{=†}
	b	18.95 $\pm$ 1.85	17 - 23
	c	17.50 $\pm$ 1.43	15 - 20
	d	16.20 $\pm$ 1.44	14 - 18
	e	18.80 $\pm$ 2.35	15 - 24
11 (25/2/80)	a	17.05 $\pm$ 1.99	14 - 22
	b	18.60 $\pm$ 1.54	16 - 22
	c	16.05 $\pm$ 1.57	13 - 19
	d	17.45 $\pm$ 1.99	14 - 21
12 (24/3/80)	a	20.35 $\pm$ 1.81	17 - 23
	b	21.45 $\pm$ 2.95	17 - 27
	c	16.25 $\pm$ 2.40	13 - 21
	d	18.80 $\pm$ 2.31	15 - 22
13 (21/4/80)	a	19.10 $\pm$ 1.97	15 - 23
	b	21.50 $\pm$ 2.76	17 - 29*
14 (17/6/80)	a	18.20 $\pm$ 1.51	15 - 21
	b	19.60 $\pm$ 2.39	16 - 25
15 (14/7/80)	a	18.05 $\pm$ 1.88	15 - 23
	b	17.90 $\pm$ 1.41	15 - 20
	c	17.20 $\pm$ 1.36	15 - 20 ^{=†}
	d	14.15 [†] $\pm$ 1.81	11 - 18 ^{=†}
16 (8/9/80)	a	18.00 $\pm$ 1.62	15 - 21
	b	17.15 $\pm$ 1.93	15 - 22
	c	18.20 $\pm$ 1.85	15 - 21
	d	16.40 $\pm$ 1.70	13 - 19
17 (6/10/80)	a	18.50 $\pm$ 2.42	15 - 25
	b	17.30 $\pm$ 1.60	13 - 20
	c	17.60 $\pm$ 1.39	15 - 20
	d	16.55 $\pm$ 1.32	13 - 19
18 (10/11/80)	a	15.95 $\pm$ 1.79	13 - 20
19 (9/3/81)	a	18.15 $\pm$ 3.01	10 [†] - 22
	b	16.55 $\pm$ 1.67	14 - 20
Average		18.14 $\pm$ 1.96	14.83 - 21.97

* and † indicate the maximum and minimum values respectively for each variable

chiasma frequency and increased variance in the different animals ($r = +0.329$; Probability = 0.0042) in the raw data (Table 5.1). The logarithmic transformation removed this statistical effect ($r = -0.075$; Probability = 0.54) giving the more homogeneous variances required by the analysis of variance.

Table 5.2 is the result of the analysis of variance performed on the transformed chiasma frequencies. This is a two-way nested analysis of variance with unequal sample sizes since there were varying numbers of animals in the different sampling times. This analysis revealed highly significant differences in chiasma frequency between different animals. Nevertheless, the different sampling times did not differ significantly with respect to chiasma frequency. There is thus no evidence of variation in chiasma frequency related to either the annual seasonal cycle, or the two breeding seasons each year.

This is also apparent when the individual chiasma frequencies are plotted over the sampling period (Figure 5.2). The mean chiasma frequency for each sampling time deviated little from the overall mean (and the largest deviations occurred when few animals were sampled). In addition, the deviations that occurred were irregular.

The positive correlation between chiasma frequency and variance in the different animals had to be taken into account when the data were analysed for differences in variance of chiasma frequency at the different sampling times. This was achieved by calculating the coefficient of variation of chiasma frequency (standard error divided by mean) for each animal. This removes the effect of the correlation and by one-way

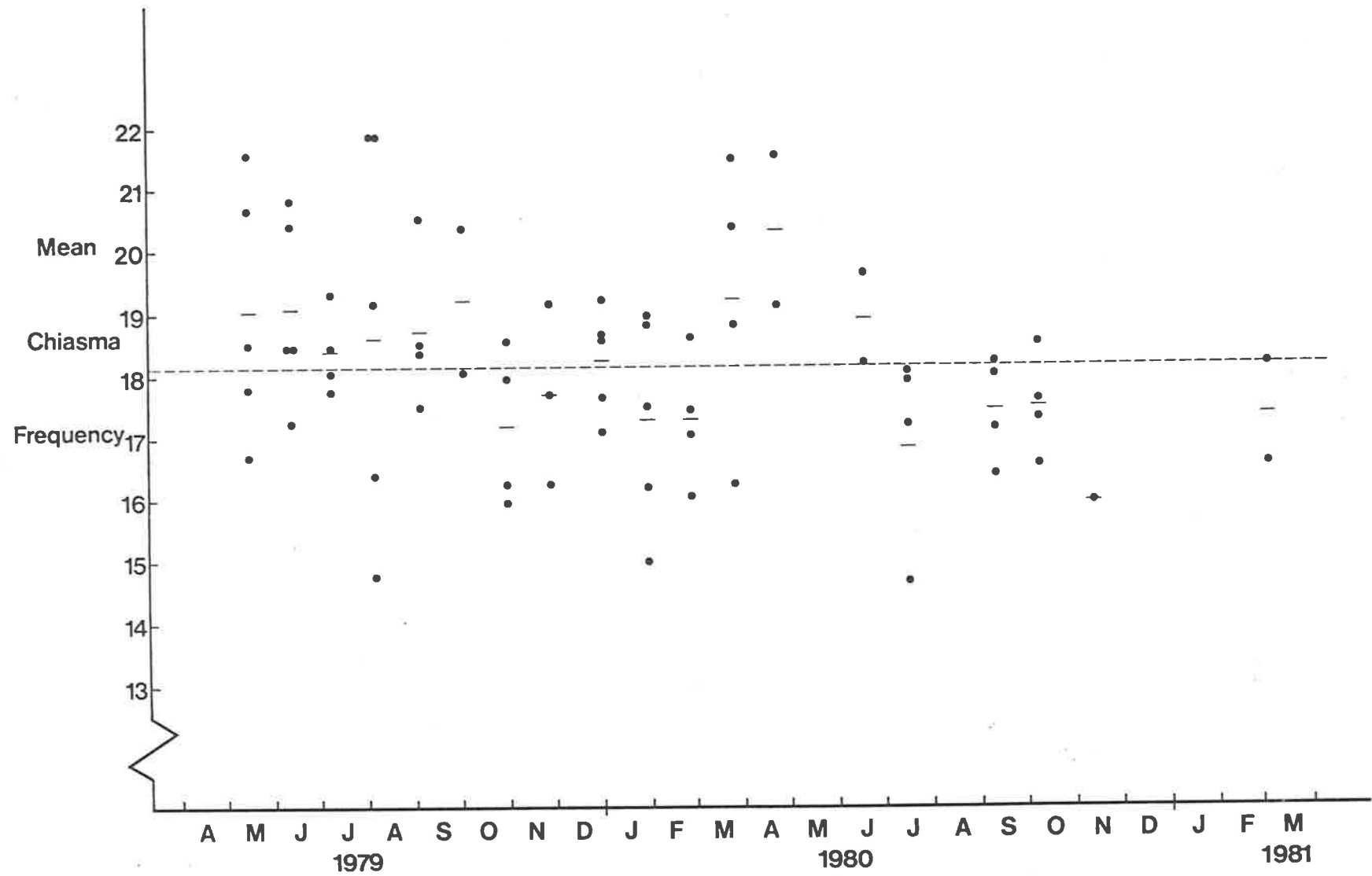
TABLE 5.2: Analysis of Variance of Transformed Chiasma Frequency
in *Trichosurus vulpecula*

Source of Variation	d.f.	SSq	MSq	F	Probability
Between times	18	3.26	0.1811	1.14	>0.20
Between animals (within times)	50	7.92	0.1584	11.31	<0.001
Between cells (within animals)	1,311	18.31	0.0140		
Total	1,379	24.49			

TABLE 5.3: Analysis of Covariance of Chiasma Frequency in
Trichosurus vulpecula

Source of Variation	d.f.	SSq	MSq	F	Probability
Between times	18	0.0093	0.0005	0.83	>0.20
Between animals (within times)	50	0.0278	0.0006		
Total	68	0.0371			

Figure 5.2: The chiasma frequencies of the *Trichosurus vulpecula* sampled plotted against sampling time. The bars indicate the mean chiasma frequency at each sampling time, and the broken line indicates the overall mean chiasma frequency.



analysis of variance (with unequal subclasses) on the coefficients of variation differences in variance between sampling times in addition to the correlation effect could be searched for. The resulting analysis of (co)variance is given in Table 5.3, and it showed that there was no significant difference in variance of chiasma frequency between the different sampling times. Hence there is no evidence of seasonal variation in either chiasma frequency or variance in chiasma frequency.

The animals from sampling times 11 to 19 were also aged by counting cementum layers on teeth prepared according to the method of Pekelharing (1970). The 27 animals from these sampling times had between zero and five cementum layers, so that they were aged from 0-1 years to 5-6 years. An analysis of variance was performed using the transformed data from these animals with the animals being divided into age classes on the basis of their cementum layers. This analysis (Table 5.4) found that there were no significant differences between animals of different ages with respect to chiasma frequency, even though, as in Table 5.2, the individual animals had significantly different chiasma frequencies. An analysis of variance of the covariances of chiasma frequency in the aged animals was also performed (Table 5.5). No significant differences in variance of chiasma frequency was found between the age classes. There was therefore no evidence of age related differences in either chiasma frequency, or variation in chiasma frequency in *T. vulpecula*.

TABLE 5.4: Analysis of variance for aged animals of *Trichosurus vulpecula* (Transformed data).

Source of Variation	d.f.	SSq	MSq	F	Probability
Between ages	5	0.7638	0.1528	1.19	>0.20
Between animals (within ages)	21	2.6983	0.1285	8.86	<0.001
Between cells (within animals)	513	7.4158	0.0145		
Total	539	10.8789			

TABLE 5.5: Analysis of Covariance of chiasma frequency in the aged *Trichosurus vulpecula*

Source of variation	d.f.	SSq	MSq	F	Probability
Between ages	5	0.0016	0.00032	1.13	>0.20
Between animals (within ages)	21	0.0059	0.00028		
Total	26	0.0075			

5.3 Discussion

This study of chiasma frequency variation in the males of *T. vulpecula* has revealed that this metric displays extensive variation between individuals. Such individual variation has been demonstrated in many populations of both animals and plants (Price, 1975; Jones et al., 1975; Gale and Rees, 1970; Shaw and Knowles, 1976; Whitehouse et al., 1981).

The analysis undertaken on the data eliminates two possible factors which might have contributed to the observed variation. Firstly, no evidence was found of a time-of-year effect on chiasma frequency. There was therefore no evidence that either the season of the year (and hence environmental factors relating to this such as temperature, and availability of food), or the time relative to the two breeding seasons in this species have any affect on chiasma frequency. An absence of a seasonal effect is probably as expected, as marsupials are homoeothermic so that their meiocytes would develop under a more uniform environment than those of the poikilothermic gecko *Phyllodactylus marmoratus* which has a cyclical variation in chiasma frequency through the year probably controlled by environmental temperature (King and Hayman, 1978). Male *T. vulpecula* do, however, show evidence of hormonal variation during the year related to the breeding season, with the weight of the prostate glands, the amount of interstitial tissue in the testes (but not testis weight), and the size and development of the sternal glands showing increases at the time of the peaks of conceptions (Tyndale-Biscoe, 1973). This cyclic variation evidently does not result in chiasma frequency variation.

The second possible factor investigated in *T. vulpecula* males, age, showed no association with the chiasma frequency. This is consistent with observations made in the mouse (Henderson and Edwards, 1968; Speed, 1977) and in man (Lange et al., 1975) where no statistically significant trend in chiasma frequency with age could be demonstrated.

Two possible environmental causes of chiasma frequency variation therefore appear not to be important with respect to the amount of chiasma frequency variation observed. By analogy with numerous studies of other species (Shaw, 1972, 1974; Chinmici, 1971a, b; Turner 1979; Kyslikova and Forejt, 1972), it would seem likely that at least a proportion of the observed variation in chiasma frequency between individuals has a genetic (probably polygenic) basis. Only genetic data, such as father-son correlations might demonstrate this. Such genetic data would be extremely difficult to obtain since *T. vulpecula* does not breed well in captivity.

The finding that both the age of the male and the sampling time during the year (with respect to both annual and breeding cycles) have no demonstrable effect on chiasma frequency in male *T. vulpecula* provides a basis for assuming that the male chiasma frequencies reported in the next chapter for a range of marsupial species have not been biased due to the uncontrolled nature of the sampling times (which were determined by the availability of the species) and the usually unknown ages of the individuals sampled. The extent of the between individual variation observed in *T. vulpecula* males does mean, however, that sampling a number

of individuals is important in order to obtain a more accurate estimate of male chiasma frequency in each species.

CHAPTER 6: RECOMBINATION IN MALE AUSTRALIAN MARSUPIALS

6.1 Chiasma frequencies and karyotype features - the observations

In addition to the chiasma frequencies collected from *Trichosurus vulpecula* (considered in Chapter 5), cell chiasma frequencies of 70 male animals from a further 32 species of marsupial were determined (Table 6.1). Examples of diplotene-diakinesis spermatocytes from a number of these species are in Figures 6.1, 6.2 and 6.3. In approximately half of the species a number of individuals were sampled, but in the other species it was only possible to examine one animal.

The species considered had a range of nuclear DNA contents, and their chromosome numbers varied from $2n = 11$ to $2n = 24$ (Table 6.2). A large group of species had the $2n = 14$ "basic" karyotype of Hayman and Martin (1974) (Figures 6.1 and 6.2 a, b, and c), while a smaller group of macropods had $2n = 16$ (Figures 6.3 c and d). Of this group *Macropus eugenii*, *M. agilis*, *M. parryi* and *M. rufogriseus* all have identical G-banding patterns (Rofe, 1977; Rofe and Hayman, personal communication). Three of the species examined had complex sex chromosome systems giving rise to a sex trivalent during meiosis in spermatocytes. *Potorous tridactylus* and *Wallabia bicolor* have a $t(XA), Y, A\delta / t(XA), t(XA)^\circ$ sex chromosome system so that the sex trivalent has a single region of autosomal origin (Figures 6.3a and b), while *Lagorchestes conspicillatus* has a $t(XA_1), t(A_1YA_2), A_2\delta / t(XA_1), t(XA_1), A_2, A_2^\circ$ sex chromosome system giving rise to two regions of autosomal origin within the sex trivalent (see Appendix 2, Figure 5c). Chiasmata within the regions of autosomal origin in these sex trivalents were included in the chiasma frequencies for these species.

TABLE 6.1: Chiasma frequencies from all the individuals sampled.

Where more than one individual was sampled in a species the individual chiasma frequencies are given in the same order as in Table 4.2. Twenty cells were scored in each animal, except where indicated by placing the number of cells scored in brackets preceding the respective chiasma frequency. Standard deviations are given when five or more cells were scored.

† These chiasma frequencies are taken from my Honours B.Ag.Sc. Thesis (unpublished; Genetics Department, University of Adelaide), and have been included in a table by Hayman (1981).

Species Cell Chiasma Frequencies

Sc $\pm 16.95 \pm 1.34$, (11) 13.00 ± 1.55 , (18) 13.06 ± 1.11 , 13.20 ± 1.61 ,
 13.90 ± 1.41 , (2) 12.5 , (5) 13.4 ± 0.89 , (5) 13.6 ± 0.55 , (4) 13.5 ,
(7) 13.24 ± 1.38 , (5) 13.6 ± 1.67 , (16) 13.38 ± 1.09 , (7) 12.71 ± 0.95 ,
(14) 13.9 ± 1.49 , (7) 13.29 ± 1.70 , (15) 13.67 ± 1.63 , (13) 11.31 ± 1.03

Sm 13.50 ± 1.00

Db $\pm 17.05 \pm 1.83$, $\pm 18.05 \pm 2.06$, 16.95 ± 1.76 , 17.55 ± 1.98

Dh 18.75 ± 1.68

Dv $\pm 18.90 \pm 1.52$, 19.00 ± 1.65 , 18.90 ± 2.00

Sh $\pm 21.30 \pm 1.29$, 20.85 ± 2.81 , (50) 20.64 ± 2.20

Pg $\pm 12.20 \pm 1.52$, $\pm 12.95 \pm 2.19$, 12.25 ± 1.25 , (79) 12.82 ± 1.37

Pn 13.60 ± 1.93 , 14.35 ± 1.98

Io $\pm (21) 16.62 \pm 1.47$, 16.00 ± 1.45 , (62) 16.24 ± 1.87

Im $\pm 16.30 \pm 1.97$

Pc 23.40 ± 3.60

Ll $\pm 22.85 \pm 1.61$, 22.00 ± 1.84

Vu 22.20 ± 2.58

Pb (10) 14.60 ± 1.32 , (10) 15.20 ± 1.67

Pp 17.00 ± 1.62 , 16.55 ± 1.23 , 16.85 ± 1.47

Tv 18.14 ± 1.96 (20 cells from each of 69 animals)

Ta 16.55 ± 1.99

Pt (40) 20.95 ± 1.73

Sb 29.40 ± 2.30 , 30.10 ± 2.76 , 28.35 ± 1.87

Lc (48) 16.39 ± 1.54 , 15.85 ± 1.38

Bp 28.40 ± 2.82

Bl 22.25 ± 2.36 , 24.45 ± 2.91

Tb 24.65 ± 2.81

Wb (40) 14.18 ± 1.47

Mp 23.45 ± 2.47

Me 23.10 ± 2.36 , 23.30 ± 2.05 , 22.60 ± 1.98

Ma 17.75 ± 1.20

Mpy (10) 16.90 ± 1.60

Mrf 20.90 ± 2.00

Mrb 31.70 ± 2.79

Mg 32.35 ± 2.91

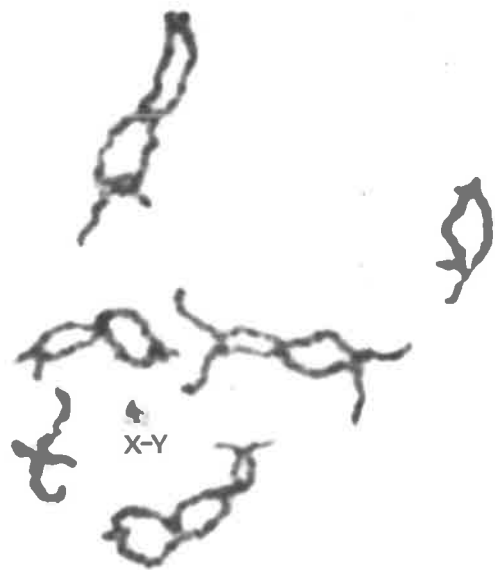
Mr (30) 37.90 ± 3.25

Tr 22.95 ± 1.73

Figure 6.1: Diplotene-diakinesis cells.

- a. *Sminthopsis crassicaudata* $2n = 14$.
- b. *Dasyuroides byrnei* $2n = 14$. The X and Y are separate.
- c. *Sarcophilus harrisii* $2n = 14$.
- d. *Perameles gunnii* $2n = 14$.
- e. *Perameles nasuta* $2n = 14$.
- f. *Isoodon obesulus* $2n = 14$.

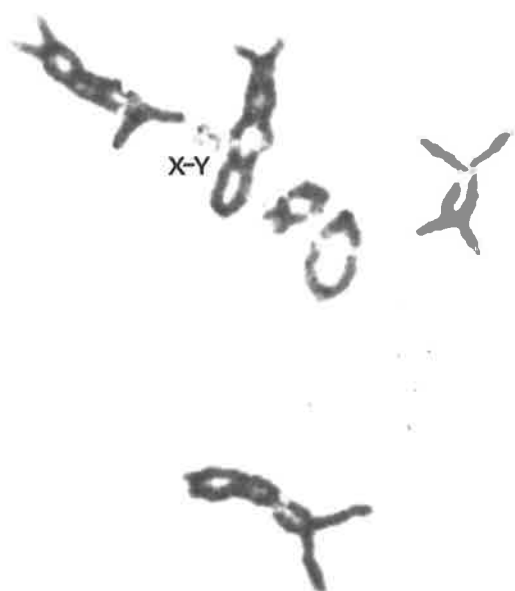
The X-Y pairs are indicated.



a



b



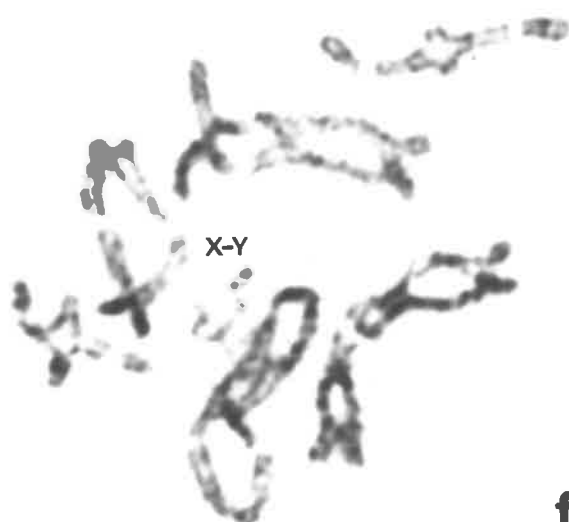
c



d



e



f

Figure 6.2: Diplotene-diakinesis cells

- a. *Isoodon macrourus* $2n = 14$.
- b. *Lasiorhinus latifrons* $2n = 14$.
- c. *Vombatus ursinus* $2n = 14$.
- d. *Phascolarctos cinereus* $2n = 16$.
- e. *Pseudocheirus peregrinus* $2n = 22$.
- f. *Bettongia leseur* $2n = 22$.

The 'X-Y pairs are indicated.

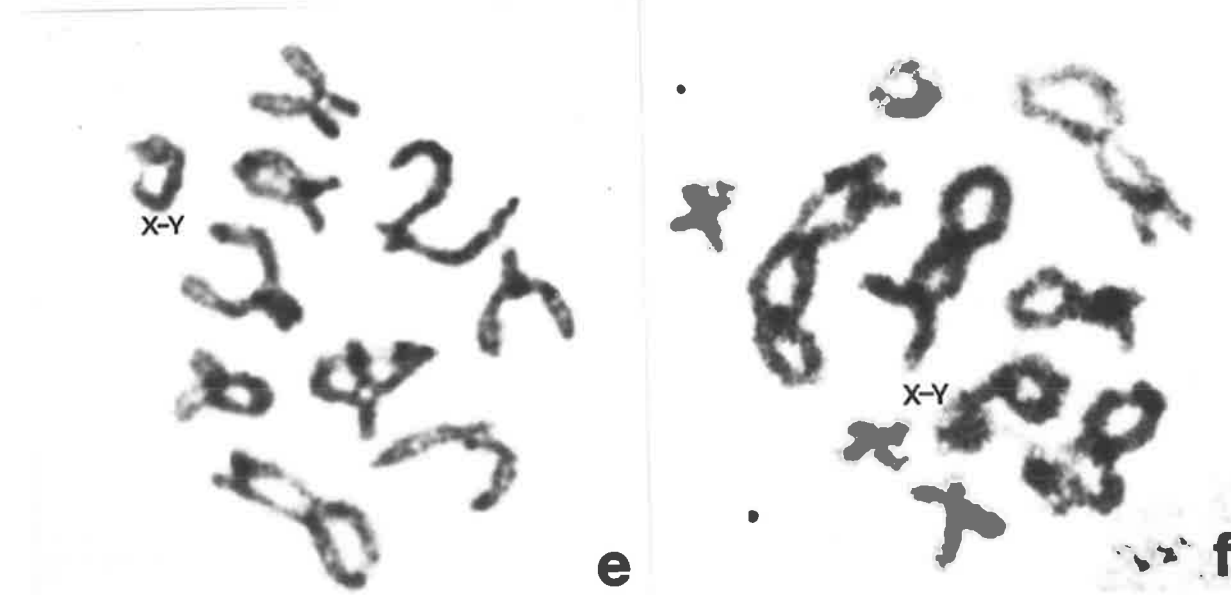
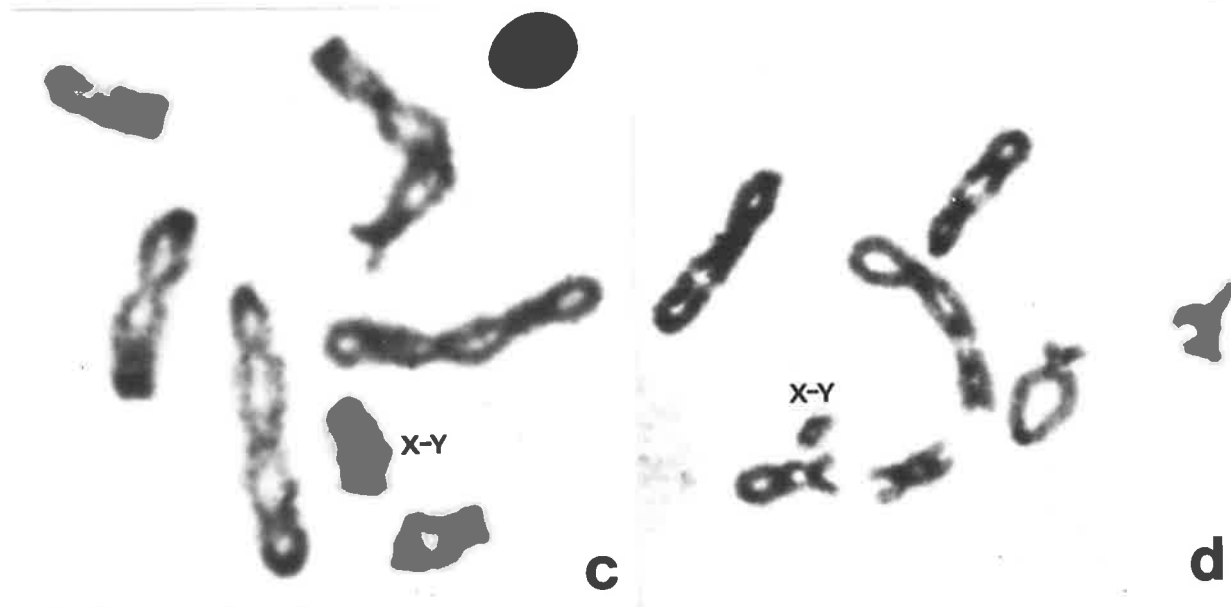
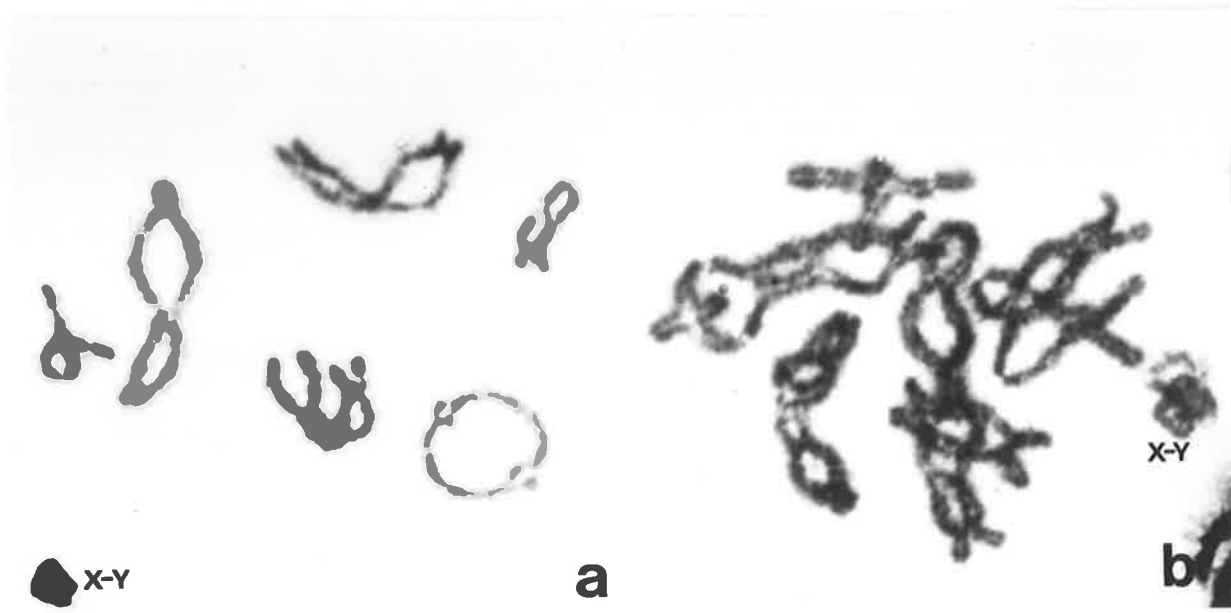
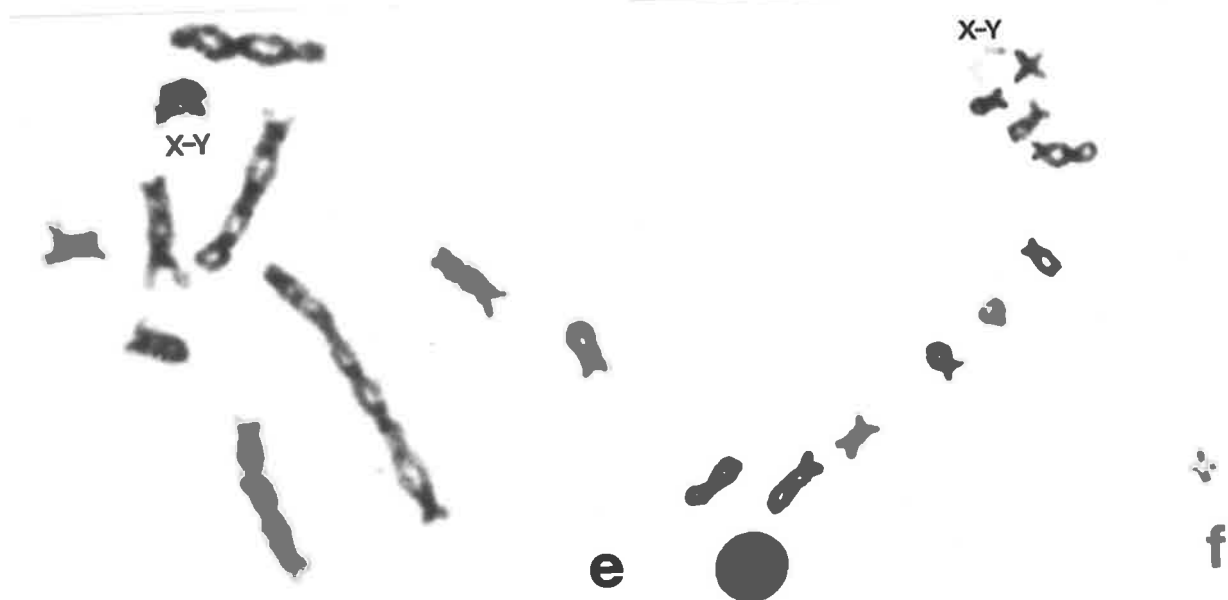
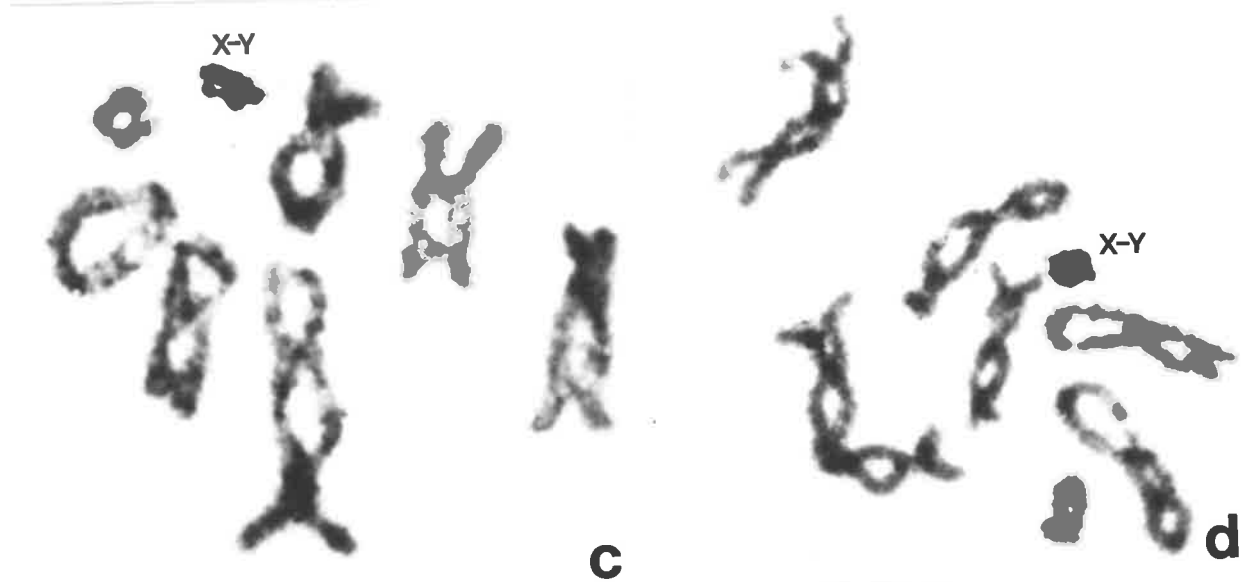
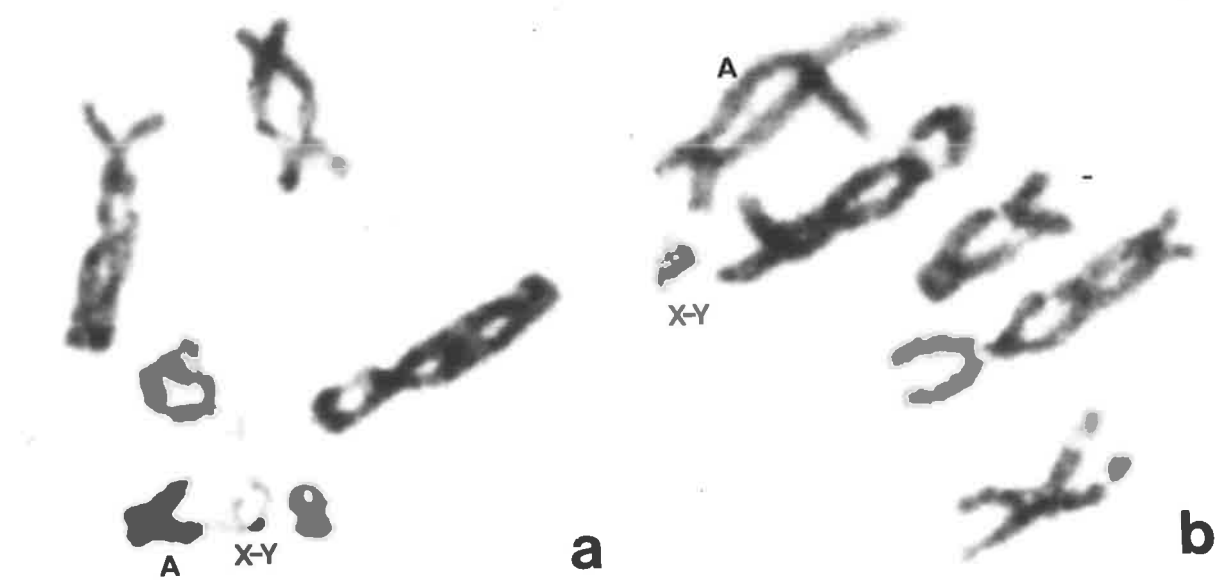


Figure 6.3: Diplotene-diakinesis cells.

- a. *Potorous tridactylus* $2n = 13$. The sex trivalent is indicated with its regions of autosomal and sex chromosome origin being identified (A and X-Y).
- b. *Wallabia bicolor* $2n = 11$. The sex trivalent is indicated with its regions of autosomal and sex chromosome origin being identified (A and X-Y).
- c. *Macropus agilis* $2n = 16$.
- d. *Macropus eugenii* $2n = 16$.
- e. *Macropus rufus* $2n = 20$.
- f. *Tarsipes rostratus* $2n = 24$.

In c-f the X-Y pairs are indicated.



In those species where a number of individuals were sampled there was variation between the different animals. In *Sminthopsis crassicaudata* where 17 males were sampled, the range of chiasma frequencies was comparable to that observed in the large series of *Trichosurus vulpecula* (Chapter 5). Since the *S. crassicaudata* came from the Genetics Department colony, the ages of the animals were known, varying from 425 days to 1,194 days old. A Spearman's rank correlation coefficient between age and chiasma frequency was calculated. There was no evidence of an age-related trend in chiasma frequencies ($r_s = +0.14$, $t = 0.53$, Probability = 0.6 - 0.7), although one must bear in mind that there were very few cells observed in some of these animals (Table 6.1). This result adds weight to the conclusions drawn from the *T. vulpecula* study (Chapter 5).

Where the same species has been studied the chiasma frequencies in Table 6.1 are similar to those that have been published. Drummond (1938) has reported 18.7 ± 1.52 chiasmata/cell (24 cells observed) for *Dasyurus viverrinus* while Koller (1935) gives a value of 20.4 chiasmata/cell (number of cells not given, but probably seven) in *Sarcophilus harrisii*. The other published counts are from species not examined here: *Acrobates pygmaeus*, 14.8 chiasmata/cell (10 cells) and *Burramys parvus*, 22.6 chiasmata/cell (10 cells); *Dasyurus maculatus*, 16.8 chiasmata/cell (probably 6 cells) (Koller, 1935). However, because of possible differences between these previous authors and the present study in the methods of chiasma scoring (the earlier material was sectioned), the published chiasma frequencies were not used in the analysis that follows.

TABLE 6.2: Karyotypic features and meiotic recombination in the species studied.

- a. The nuclear DNA content measurements are taken from Hayman and Martin (1974).

Species	Relative DNA content ^a	2n	Average Cell Chiasma Frequency	Darlington's Recombination index	additional recombination component of R.I.
<i>Sc</i>	86.4	14	13.64	19.64	7.64
<i>Sm</i>	-	14	13.50	19.50	7.50
<i>Db</i>	86.4	14	17.40	23.40	11.40
<i>Dh</i>	86.4	14	18.75	24.75	12.75
<i>Dv</i>	-	14	18.93	24.93	12.93
<i>Sh</i>	86.4	14	20.83	26.83	14.84
<i>Pg</i>	-	14	12.66	18.66	6.66
<i>Pn</i>	109	14	13.98	19.98	7.98
<i>Io</i>	116	14	16.27	22.27	10.27
<i>Im</i>	114	14	16.30	22.30	10.30
<i>Pc</i>	93.3	16	23.40	30.40	16.40
<i>Ll</i>	103.6	14	22.43	28.43	16.43
<i>Vu</i>	102	14	22.20	28.20	16.20
<i>Pb</i>	-	22	14.90	24.90	4.90
<i>Pp</i>	154	20	16.80	25.80	7.80
<i>Tv</i>	102	20	18.14	27.14	9.14
<i>Ta</i>	-	20	16.55	25.55	7.55
<i>Pt</i>	-	13♂, 12♀	20.95	26.95	14.95
<i>Sb</i>	93.8	22	29.28	39.28	19.28
<i>Lc</i>	90.3	15♂, 16♀	16.24	22.24	8.24
<i>Bp</i>	-	22	28.40	38.40	18.40
<i>Bl</i>	-	22	23.35	33.35	13.35
<i>Tb</i>	84.5	22	24.65	34.65	14.65
<i>Wb</i>	97.7	11♂, 10♀	14.18	19.18	9.18
<i>Mp</i>	98.6	16	23.45	30.45	16.45
<i>Me</i>	98.2	16	23.00	30.00	16.00
<i>Ma</i>	97.7	16	17.75	24.75	10.75
<i>Mpy</i>	124.6	16	16.90	23.90	9.90
<i>Mrf</i>	116.1	16	20.90	27.90	13.90
<i>Mrb</i>	104.1	16	31.70	38.70	24.70
<i>Mg</i>	100	16	32.35	39.35	25.35
<i>Mr</i>	103.9	20	37.90	46.90	28.90
<i>Tr</i>	-	24	22.95	44.95	11.95

6.2 Biological features of the species studied

Data on some features of the biology of the studied species were collected (Table 6.3). The information encompasses a number of metrics, including indices of development and of reproduction, since these have been considered to be important in the evolution of recombination levels (see Chapters 1 and 2).

The following metrics are included in Table 6.3.

(1) Size. The physical size of the species is represented by two measurements; head-body length and weight. Head-body length, and not total length (including tail) was used as the metric since not all marsupials have tails, and since it seems likely that tail length has evolved more for reasons of locomotion and habit (i.e. arboreality) than as a possible component of the genetic system of the species. Some of the species are sexually dimorphic with respect to size (especially in the Macropods), and in these cases the values given in Table 6.3 are the means of the values for the two sexes.

(2) Development. Three indices of the speed of development were used; length of gestation, time from birth to weaning, and the age of the females of the species at their first breeding. In some species (especially *Macropus* species) there is delayed implantation or embryonic diapause (Tyndale-Biscoe, 1973). In these cases the gestation time given in Table 6.3 is the length of gestation in which there is no diapause. The time from birth to weaning includes the length of pouch life plus the time the young continue to suckle after leaving the pouch permanently. The information in this classification has some gaps due to the insufficient knowledge of the reproductive cycle of some of the species.

TABLE 6.3: Biological features of the species studied

The information has been collected from the following sources:

Wood Jones (1968); Collins (1973); Tyndale-Biscoe
(1973); Ride (1970); Stonehouse and Gilmore (1977);
Rose (1978); Wells (1978); Hyett (1980); Morton
(1982); Lee et al., (1982); Gemmel (1982);
Rose and McCarteney (1982); and Strahan (1983).
(Additional Reference: Kaufmann (1974)).

Species	Head-Body length (mm)	Weight (gms)	Gestation (days)	Birth to Weaning (days)	No. of young per mating	♀ age at first breeding (months)	Multiple reproduction in a year?	Sociality (rank score)
<i>Sc</i>	75	15	13	65	6	5	✓	-
<i>Sm</i>	85	20	12.5	65	6	5	✓	-
<i>Db</i>	157	110	33	110	6	9	✓	-
<i>Dh</i>	210	525	21	150	6	12	x	-
<i>Dv</i>	355	1010	21	150	6	12	x	-
<i>Sh</i>	610	7000	31	210	4	24	x	-
<i>Pg</i>	305	650	12	60	2.5	3	✓	-
<i>Pn</i>	380	1000	12.5	60	2.5	4	✓	-
<i>Io</i>	315	775	12.5	65	3	4	✓	-
<i>Im</i>	375	1600	12.5	60	3	4	✓	-
<i>Pc</i>	750	8950	35	360	1	24	x	-
<i>Ll</i>	850	26000	-	300	1	36	x	-
<i>Vn</i>	860	20000	-	300	1	24	x	-
<i>Pb</i>	170	130	21	110	2	12	x	-
<i>Pp</i>	325	900	-	180	2	12	✓/x	-
<i>Tv</i>	450	2800	18	180	1	12	✓/x	-
<i>Ta</i>	410	1450	-	180	1	12	✓	-
<i>Pt</i>	360	1100	38	120	1	12	✓	13
<i>Sb</i>	470	3450	27	-	1	12	x	7.5
<i>Lc</i>	425	3500	-	150	1	12	✓/x	13
<i>Bp</i>	330	1300	21	180	1	6	✓	7.5
<i>Bl</i>	280	1000	21	116	1	5	✓	7.5
<i>Tb</i>	590	5500	30	290	1	14	✓/x	10.5
<i>Wb</i>	725	15000	35	450	1	16	x	13
<i>Mp</i>	490	4500	35	315	1	12	x	-
<i>Me</i>	615	6500	28	255	1	9	x	5
<i>Ma</i>	725	15000	30	330	1	12	x	4
<i>Mpy</i>	827	13500	36	450	1	18	x	1
<i>Mrf</i>	745	16900	30	450	1	14	x	10.5
<i>Mrb</i>	960	23300	34	-	1	24	x	7.5
<i>Mg</i>	1100	49000	36	540	1	16	x	2
<i>Mr</i>	1070	46300	33	355	1	18	x	3
<i>Tr</i>	70	10	-	70	2.5	8	✓	-

(3) Reproductive potential. Two indicators of reproductive potential were used; the number of young weaned per mating, and whether there is multiple reproduction by females of the species in a year. The number of young weaned per mating was used instead of average litter size since the ^tlatter depends on the timing of the counts during pouch life due to the excess of births to teat number that occurs in some species, and the wastage of pouch young from large litters in other species (Russell, 1982). In the *Macropus* species this measure is complicated by the possibility of the females having at any one time two young; one "at foot" and still suckling, one pouch young, and also a delayed blastocyst. The value given for these species is therefore the maximum number of young weaned per year. In drought years no young would be successfully weaned in these species, even though breeding (mating, gestation and suckling) may continue for a time (Tyndale-Biscoe, 1973).

The annual reproductive potential of a species can be regarded as the number of young weaned per litter multiplied by the number of litters weaned per year. No numerical values are given for the latter component as the information available is sketchy, but an indication of whether this is common, occasional, or does not occur is given in Table 6.3.

(4) Sociality. Kaufmann (1974) has reviewed what is known of the social organization of macropods. Regrettably, no similar information is available for the other groups of marsupials. He used bar graphs to indicate the degree of sociality displayed by macropod species, and these were used to obtain the rank scores of sociality given in Table 6.3. The degree of sociality might influence the amount of inbreeding in the macropod species, and

hence their genetic systems. Social organization has previously been considered to be important in determining the rate of chromosomal evolution in different groups (Wilson et al, 1975; Bush et al, 1977).

6.3 The metrics of recombination

In many of the species listed in Table 6.1 more than one individual was sampled. In these cases the average cell chiasma frequency for the species was calculated. This was simply the total number of chiasma scored divided by the total number of cells examined for all the different individuals sampled in the species under question. These average cell chiasma frequencies were not themselves used as a metric of recombination since this measure does not take into account the varying chromosome numbers of the different species.

The average cell chiasma frequencies were used to calculate Darlington's recombination index (RI) for each species. This is the sum of the average cell chiasma frequency plus the haploid number of autosomes. This metric takes into account the effect of varying chromosome number on the level of recombination due to the independent segregation of the bivalents, and represents the average number of segments which re-assort at each meiosis.

The general formulation of the RI as the sum of the haploid chromosome number and the average cell chiasma frequency reflects the fact that variation in either of these factors will cause variation in the level of recombination. Although haploid chromosome number can conceivably vary quite widely there is, however, an obvious minimum to the average cell chiasma frequency.

In male marsupials (and indeed in all mammals) where there is no evidence of achiasmate meiosis (except in the X-Y bivalents) this minimum would be one chiasma per autosomal bivalent. The average cell chiasma frequency therefore consists of two components, the minimum number of chiasmata needed for disjunctional reasons (taken to be the haploid number of autosomes) and any additional recombination. This does not necessarily imply that there are two qualitatively different types of chiasmata (Darlington, 1932; Church and Wimber, 1969), but it indicates a possible constraint on the evolutionary adjustment of chiasma frequencies; namely that it is never less than the number of potential bivalents.

A slightly different formulation of Darlington's recombination index would therefore be that it equals twice the haploid chromosome number (n bivalents plus n chiasma essential for disjunction) plus any additional recombination. Darlington (1932) has proposed that genetic variation between populations with respect to recombination was due to variation in this additional recombination component between populations. In considering species with the same karyotype it is also apparent that any differences in cell chiasma frequency between species reflects changes in this component. As John and Lewis (1975) have pointed out, chromosome number can show only unitary change, whereas the additional recombination component can vary fractionally. Both the recombination index and its additional recombination component take no account of possible differences between species with respect to chiasma localization. Species can possibly vary both in the degree of localization, and in the positions of localization. Both of these will have consequences for the generation of variability within the species.

There are reasons for arguing that evolutionary changes in the RI might generally be by changes to this additional recombination component, and not by changes in the chromosome number. Firstly, although visible chromosome rearrangements occur in diverse groups in approximately one in 500 individuals (White, 1973), increases or decreases in chromosome number in the direction which natural selection on the RI favours will be considerably rarer than this. Hence the amount of variation available for selection might be extremely small, and would thus be open to the effects of chance. Changes in the level of recombination by this means must also overcome the effects of the chromosome rearrangements on the fertility of heterozygotes. Secondly, evolutionary changes in chromosome number may also be brought about, in theory at least, by segregation distortion, direct selective advantage (possibly due to position effects), and random genetic drift in small populations (Bengtsson, 1980). Such factors would tend to cause "noise" in any relationship between chromosome number and the biological factors important in the evolutionary determination of recombination levels. It has also been argued that the main reasons for changes in chromosome number and morphology have been inbreeding and random genetic drift in small populations leading to speciation, particularly in groups with low vagility (White, 1978). Finally, the evidence considered in Chapter 2.2 from a wide range of organisms points to extensive variation in chiasma frequency between individuals, and also that this variation has a polygenic basis. Given that selection will favour a change in the recombination index then the relative rate at which such change may be brought about by evolutionary change in the chromosome number would appear to be

less than the rate brought about by change in the chiasma frequency. The additional recombination component might therefore be a better measure than the recombination index of the amount of recombination easily accessible to, and thus the more direct result of the evolutionary forces responsible for the evolution of recombination levels.

Because of this, both Darlington's recombination index and the additional recombination component of the recombination index were used as indices of recombination level. In most of the species the recombination index was calculated as the sum of the average cell chiasma frequency and the number of autosomal bivalents. In the species with complex sex chromosome systems, the $t(XA), Y, A$ sex trivalents were counted as an autosomal bivalent (which with respect to recombination between originally autosomal loci they are), while the $t(XA_1), t(A_1YA_2), A_2$ sex trivalent in *Lagorchestes conspicillatus* was counted as two autosomal bivalents. In this case the adjustment was not completely accurate as the two autosomal portions of the sex trivalent do not segregate independently since they are both attached to the original Y chromosome. The additional recombination component of the recombination index was calculated for each species as the difference between the average cell chiasma frequency and the minimum number of chiasmata needed to ensure regular disjunction. In the species with simple sex chromosomes this was taken to be the number of autosomal bivalents, while in the species with complex sex chromosome systems the autosomal portions of the trivalents were counted as separate autosomal bivalents. The values of the recombination index and the additional recombination component of the recombination index for each species are given in Table 6.2.

6.4 The basis for the analysis of male recombination

The analysis of the levels of recombination in male marsupials has as its primary purpose the testing of the "null hypothesis" that the recombination levels displayed by the various species are not the result of natural selection due to the importance of recombination within their genetic systems. Some of the other components of possible genetic systems in marsupials would be the DNA contents, diploid chromosome number, and the various factors given in Chapter 6.2 making up the life-history of each species.

If the levels of recombination displayed by the different species are the result of natural selection based on the importance of this metric within the genetic system of each species, then one expects there to be associations between the level of recombination and the other possible components of the genetic system given in Chapter 6.2. On the other hand, if the evolution of recombination in the various species was in response to the mechanical requirements of regular bivalent disjunction at meiosis alone, the converse expectation would be that the levels of recombination and the life-history components of the species would not be associated. It is apparent that chromosome number and the components of the life-history of the various species are not correlated, for a comparison of Tables 6.2 and 6.3 reveals that the species with $2n = 14$ show wide variation in components of their life-history. Additionally, in the macropods, species with high chromosome numbers ($2n = 20$ and 22) occur at both extremes, and near the middle of the size range in this group (*Setonix brachyurus*, *Bettongia* species, *Thylogale billardierii* and *Macropus rufus*), and those species with low chromosome

numbers (*Potorous tridactylus* and *Wallabia bicolor*) do likewise.

There is thus no evidence for a correlation between chromosome number and aspects of life-history.

These expectations consequently dictate the general method of analysis undertaken here; the analysis of associations between the levels of recombination in the various species and the properties that the species display in their karyotypes and in their life-history components. In doing this one ignores the fact that it might be that some of the species have evolved different levels of recombination by evolving varying degrees of chiasma localization. The associations were quantified by calculating correlation coefficients between the level of recombination and the other component being considered. This allowed statistical tests of the significance of any association. The non-parametric Spearman's rank correlation coefficient (r_s) was used since the data do not satisfy the requirements (such as normality and equality of variances) for calculating Pearson's correlation coefficients.

These calculations were performed separately for each component of the life-history, as if these various components were independent. However, it is recognized that this is not the case, the various components of life-history of different species are themselves all inter-correlated. This means that caution must be exercised in analysing the correlations calculated. No single overall "Index of Life-History" exists, or seems possible, so that no single correlation coefficient can be used as a measure of the association between recombination and life-history. In mammals increased size is associated with increased lifespan, age at first reproduction, gestation time and decreased number

of young per litter (Stearns, 1977; Western, 1979; Lindstedt and Calder, 1981). Evidence of these associations can be seen in Table 6.3; for example, the smaller species tend to have larger litters.

The general association between size and longevity in mammals (Lindstedt and Calder, 1981) means that one can use size as a rough indicator of longevity. This is useful here since the data available on longevity in marsupials is very incomplete, and was also collected by varying methods. Some data were estimated by measurements of the level of molar progression in shot animals (some from heavily hunted areas which would tend to underestimate longevity), some came from mark-recapture studies, and some information came from zoo specimens (where there would almost certainly be a bias to reports of extreme longevity). Nevertheless, the longevity data reported indicate that the larger species live longer (e.g. *Vombatus ursinus* and *Lasiorhinus latifrons*, 20-26 years; *Macropus rufus*, *M. giganteus*, *M. robustus* and *M. rufogriseus* around 20 years; the smaller *Macropus* species around 10-15 years, and the *Sminthopsis* species around 3-4 years) (Eisenberg 1981; Inns, 1982).

6.5 Correlations between recombination and biological features: Results and Discussion

The recombination indices and the additional recombination components of the recombination indices of each species are given in Table 6.2. Both metrics of recombination varied over a considerable range, the recombination index covered a 2½-fold range from the lowest (*Perameles gunnii*) to the highest (*Macropus rufus*) while the additional recombination

component of the recombination index was more variable, displaying almost a six-fold range from *Petaurus breviceps* to *M. rufus*. This difference in range between these two indices was mainly brought about by a number of species that had high chromosome number (for marsupials), but with a low additional recombination component of their recombination index (*Petaurus breviceps*, *Pseudocheirus peregrinus*, the two *Trichosurus* species, and *Tarsipes rostratus*).

Initially, a set of correlations was calculated using the data from the species with the $2n = 14$ "basic" karyotype (Table 6.4). In this group the chiasma frequencies, recombination indices and the additional recombination components of the recombination index all have the same ranks (since chromosome number does not vary), so that only one set of correlation coefficients was calculated. There were highly significant positive correlations between recombination level and the metrics of size (head-body length and weight) and of developmental speed (gestation time, birth to weaning time, and female age at first breeding). The correlation with the number of pouch young per mating was negative, but not significantly so. It is likely that if the number of young per year per female was used as the index of reproductive potential, that there would be a significant negative correlation since the smaller dasyurids (*Sminthopsis* species and *Dasyuroides byrnei*) and the peramelids have multiple litters per year (Table 6.3) and have low levels of recombination (Table 6.2) in this group. There was no correlation between recombination level and nuclear DNA content.

Table 6.5 shows the results of a similar analysis of correlations in the $2n = 16$ macropod species studied. In this group the number of pouch young is constant so that this was

TABLES 6.4 to 6.11: These tables give the correlations between various biological features of the species studied and different indices of recombination level. The following symbols are used:

r_s = Spearman's rank correlation coefficient.

d.f. = degrees of freedom = no. of species - 2.

This varies within the tables because not all information was available for each species (see Table 6.3).

t = t-test value used to test the significance of the correlation coefficients.

The asterisks in the final columns indicate significant correlation coefficients. Because of the small sample size in Table 6.5 the t-test could not be used so that the significance of the correlations there was tested by reference to Table 87 in Bishop (1980).

TABLE 6.4: Correlations between various features and the level of recombination in the $2n = 14$ species studied.

Feature	r_s	d.f.	t	Probability	Significance
Head-body length	+0.69	10	3.03	0.02 - 0.01	*
Weight	+0.75	10	3.57	0.01 - 0.001	*
Gestation time	+0.80	8	3.81	0.01 - 0.001	*
Birth-weaning time	+0.88	10	5.79	<0.001	*
No. of pouch young	-0.20	10	0.65	0.6 - 0.5	-
♀ age at 1st breeding	+0.88	10	5.95	<0.001	*
DNA content	-0.13	7	0.36	0.8 - 0.7	-

TABLE 6.5: Correlations between various features and the level of recombination in the $2n = 16$ macropods studied.

Feature	r_s	no. of pairs	Significance
Head-body length	+0.32	7	-
Weight	+0.43	7	-
Gestation time	+0.21	7	-
Birth-weaning time	+0.04	6	-
Sociality	-0.26	6	-
♀ age at 1st breeding	+0.17	7	-
DNA content	-0.14	7	-

TABLE 6.6: Correlations between various features and the recombination ^{index} ~~ex~~ in the macropods studied.

Feature	r_s	d.f.	t	Probability	Significance
Head-body length	+0.17	13	0.62	0.6 - 0.5	-
Weight	+0.16	13	0.60	0.6 - 0.5	-
Gestation time	-0.29	12	1.05	0.4 - 0.3	-
Birth → weaning time	-0.01	11	0.02	>0.9	-
Sociality	+0.44	12	1.70	0.2 - 0.1	-
♀ age at 1st mating	+0.09	13	0.31	0.8 - 0.7	-
DNA content	+0.14	10	0.44	0.7 - 0.6	-
2n	+0.73	13	3.82	0.01 - 0.001	*

TABLE 6.7: Correlations between various features and the additional recombination component of the recombination index in the macropods studied.

Feature	r_s	d.f.	t	Probability	Significance
Head-body length	+0.30	13	1.14	0.3 - 0.2	-
Weight	+0.28	13	1.06	0.4 - 0.3	-
Gestation time	-0.02	12	0.08	>0.9	-
Birth → weaning time	+0.08	11	0.27	0.8 - 0.7	-
Sociality	+0.42	12	1.59	0.2 - 0.1	-
♀ age at 1st mating	+0.20	13	0.73	0.5 - 0.4	-
DNA content	+0.28	10	0.93	0.4 - 0.3	-
2n	+0.46	13	1.85	0.1 - 0.05	-

TABLE 6.8: Correlations between various features and the recombination index in the non-macropods studied.

Feature	r_s	d.f.	t	Probability	Significance
Head-body length	+0.53	16	2.52	0.05 - 0.02	*
Weight	+0.51	16	2.38	0.05 - 0.02	*
Gestation time	+0.80	11	4.46	<0.001	*
Birth → weaning time	+0.82	16	5.66	<0.001	*
No. of pouch young	-0.52	16	2.44	0.05 - 0.02	*
♀ age at 1st breeding	+0.80	16	5.27	<0.001	*
DNA content	-0.05	10	0.17	0.9 - 0.8	-
2n	+0.59	16	2.93	0.01 - 0.001	*

TABLE 6.9: Correlations between various features and the additional recombination component of the recombination index in the non-macropods studied.

Feature	r_s	d.f.	t	Probability	Significance
Head-body length	+0.56	16	2.72	0.02 - 0.01	*
Weight	+0.58	16	2.84	0.02 - 0.01	*
Gestation time	+0.67	11	3.00	0.02 - 0.01	*
Birth → weaning time	+0.61	16	3.12	0.01 - 0.001	*
No. of pouch young	-0.08	16	0.33	0.8 - 0.7	-
♀ age at 1st breeding	+0.63	16	3.21	0.01 - 0.001	*
DNA content	-0.24	10	0.77	0.5 - 0.4	-
2n	-0.06	16	0.24	0.9 - 0.8	-

TABLE 6.10: Correlations between various features and the recombination index in the species studied.

Feature	r_s	d.f.	t	Probability	Significance
Head-body length	+0.41	31	2.54	0.02 - 0.01	*
Weight	+0.43	31	2.64	0.02 - 0.01	*
Gestation time	+0.46	25	2.62	0.02 - 0.01	*
Birth → weaning time	+0.49	29	3.02	0.01 - 0.001	*
No. of pouch young	-0.35	31	2.09	0.05 - 0.02	*
♀ age at 1st mating	+0.33	31	1.98	0.1 - 0.05	-
DNA content	-0.01	22	0.04	>0.9	-
2n	+0.68	31	5.12	<0.001	*

TABLE 6.11: Correlations between various features and the additional recombination component of the recombination index in the species studied.

Feature	r_s	d.f.	t	Probability	Significance
Head-body length	+0.60	31	4.20	<0.001	*
Weight	+0.62	31	4.43	<0.001	*
Gestation time	+0.60	25	3.71	0.01 - 0.001	*
Birth → weaning time	+0.57	29	3.74	<0.001	*
No. of pouch young	-0.30	31	1.76	0.1 - 0.05	-
♀ age of 1st mating	+0.52	31	3.41	0.01 - 0.001	*
DNA content	-0.07	22	0.35	0.8 - 0.7	-
2n	+0.21	31	1.20	0.3 - 0.2	-

not considered. Its place in the table is taken by "sociality". None of the correlation coefficients are statistically significant. However, the correlations in common with the $2n = 14$ species analysis (6 comparisons) have the same sign. This (if used as a sign test, although it must be remembered that these features are not independent) possibly indicates that there are similar background associations in the $2n = 16$ macropods to those found in the $2n = 14$ species.

This analysis was then extended to the macropod group as a whole. Here the varying chromosome numbers mean that comparisons can be made both with the recombination index (Table 6.6) and with the additional component of the recombination index (Table 6.7). Only the correlation between diploid chromosome number and the recombination index was significant. This correlation is expected to be positive since chromosome number is a component of the recombination index. As in the $2n = 16$ species, the number of young per mating is constant, so that this was not included, but was again replaced by "sociality". The correlations between the measures of size and female age at first mating and recombination level were the same sign as in the analysis of the $2n = 14$ species, again suggesting a common underlying association. However, the correlations involving the two measures relating to the development of pouch young (length of gestation and time from birth to weaning) were either very close to zero or were negative. This is different to the situation in the $2n = 14$ chromosome species, and it is possibly a reflection of the differences between these two groups with respect to aspects of reproductive biology. In the macropods almost all the species have a maximum

of one weaned young per year (Table 6.3). Under such a system, both the length of gestation and the length of time the young are dependent on the mother may be of different selective significance within the reproductive system. The reproductive systems in the macropods are also characterized by the presence of embryonic diapause in a number of species (Shawman, 1970; Tyndale-Biscoe, 1973), and this would also add to a possible uncoupling of some of the selective constraints on the length of the "true" gestation in this group.

A similar correlation analysis was then performed on the data from the non-macropods (which includes the $2n = 14$ species). The comparisons in this case were also done both with the recombination index and the additional recombination component of the recombination index since chromosome number varies (Tables 6.8 and 6.9). Of the karyotypic features, only the correlation between diploid chromosome number and the recombination index was significant, as expected. The correlations in this group follow the same pattern as that seen in the $2n = 14$ species, with significant positive correlations between recombination (as measured by both indices here) and the metrics of size and developmental time, and negative correlations with the number of pouch young. This last factor has a significant negative value with the recombination index but is very close to zero with the additional recombination component of the recombination index. In both of these comparisons there would almost certainly be a higher absolute value for the correlation coefficients if the number of pouch young per year could be used as the metric since the species with low levels of recombination tend to have multiple litters in a year, although the species with the lowest additional recombination component (*P. breviceps*) has only one litter per year (Table 6.3).

When the same analysis was performed on all the species studied, the results (Tables 6.10 and 6.11) were also very similar to the results with the $2n = 14$ group. Of the karyotypic features only the correlation of recombination index with diploid chromosome number was significant, and there were significant positive correlations between the indices of size, and developmental times, and negative correlations between the number of pouch young and the indices of recombination level.

The direct conclusion from the analysis of the correlations between the level of recombination and biological features is that these variables are associated. This answers the primary question posed. The levels of recombination observed in the different species are thus not the result only of the mechanical requirements of regular bivalent orientation or some proportionality with DNA content, but appear to be due to the importance of recombination in possible genetic systems in the different marsupial species.

Two indices of the level of recombination were used in the correlation analyses when chromosome number varied within the group being considered; Darlington's recombination index, and the additional recombination component of the recombination index. Comparison of the results of the analyses using these two indices (Tables 6.6 and 6.7; Tables 6.8 and 6.9; Tables 6.10 and 6.11) reveals that both indices have similar correlations with the biological features considered. However the additional component of the recombination index seems to be the better metric for two reasons. Firstly, even though the additional recombination component has a larger range, the correlations using this metric are generally of higher absolute value than

those involving the recombination index. Secondly, there were no significant associations between the additional recombination component and chromosome number. This indicates that these two components have not changed in concert with each other during evolution. If the level attained by both components of the recombination index was due to the importance of recombination within possible genetic systems then one would expect an association between the two. Some of the factors mentioned previously (page 47) have possibly been more important in the determination of chromosome numbers in the different species than the effect of chromosome number on the recombination indices. This is underscored by the highest correlations between recombination level and biological features being found in the $2n = 14$ "basic" karyotype group (Table 6.4) where changes in chromosome number have not occurred and changes in morphology have been minor (Hayman and Martin, 1974; Rofo and Hayman, in press).

The correlations calculated between the indices of recombination and biological features in the macropods were all statistically not significant (even when only the $2n = 16$ species were considered alone), whereas most were statistically significant when the $2n = 14$ species or the non-macropods were considered. There are a number of factors which might contribute to this difference. Firstly, the macropods were the most karyotypically variable group considered (Table 6.2). Secondly, in many of the reproductive characters, and especially in the number of young per mating (Table 6.3), the macropods were not as diverse a group as the non-macropods, so that there was a smaller variability between species with respect to biological features which might be components of

possible genetic systems. Thirdly, in most of the macropod species considered only one individual was sampled (Table 6.1). Given the variability between individuals discussed in Chapter 5 and evident in the *Sminthopsis crassicaudata* in Table 6.1 it might be that the low level of sampling in this group has obscured any general trends present. The correlations of highest absolute value found in this group were in fact between Kaufmann's "sociality" and the two metrics of recombination (Tables 6.6 and 6.7). This possibly indicates that other processes concerned with group behaviour might be more important in the evolutionary determination of recombination levels in this group. However, this result might well be fortuitous, since within the $2n = 16$ macropods this correlation is reversed (Table 6.5).

The correlations calculated here therefore show that species with high levels of recombination tend to be large, develop slowly, and produce few pouch young, whereas the species with low levels of recombination tend to be small, develop rapidly and have a greater production of pouch young. Because of the general association between size and longevity in mammals (Lindstedt and Calder, 1981) the longer lived species also tended to have higher levels of recombination.

There is a general agreement between the associations obtained here in the marsupials and the findings from other groups. In plants, annuals tend to have lower levels of recombination than related perennial species (Grant, 1958, 1975; Stebbins, 1958) although in some highly selected grasses this trend is reversed (Rees and Dale, 1974). Additionally, many arthropods with short generation times have restrictions on recombination (Halkka, 1964; White, 1973). The observed association, however, the reverse

of that suggested by Hayman (1977) for the marsupials. The marsupials would thus fit into the scheme of Mather (1953), in that species with short generations would have restrictions on recombination, and there would be an apparent compromise between releasing variation through rapid population turnover, and conserving variation by having low meiotic recombination.

Grant (1975) has suggested that many aspects of the genetic system of different species might be related to the ecological concepts of r- and K-selection. These terms were introduced by MacArthur and Wilson (1967) to describe two kinds of selection possibly operating in nature. The K and r come from the logistic equation for population growth (Roughgarden, 1979). The r-selection was envisaged by MacArthur and Wilson as the result of density-independent mortality, so that selection is mainly on reproductive rate, while K-selection was envisaged as the result of density-dependent mortality, so that selection will favour traits leading to the production of young with high competitive ability. As Pianka (1970) has pointed out, there is a continuum of species between those which are "purely" r-selected and those which are "purely" K-selected. Although the r-K continuum is a one-dimensional plot of life-history strategies, it has been extremely fruitful in its application to the analysis of certain groups (i.e. plant pathogens, Andrews and Rouse, 1982; phytoplankton, Sournia, 1982). Other axes can be used to produce two-dimensional plots of life-history strategies, and other types of selection and strategies have been proposed (e.g. "Adversity" selection, Greenslade, 1982; "bet-hedging", Stearns, 1976). However, they have not yet been as widely applied as the concepts of r- and K-selection.

Pianka (1970) has tabulated the consequences of r- and K-selection. The r-selection favours rapid development, high intrinsic rate of natural increase ($r_{\max}$), early reproduction, small body size, a small number of reproductive episodes per individual, and a short life. K-selection on the other hand leads to slower development, delayed reproduction, larger body size, repeated reproduction and increased longevity.

The correlations found between recombination level and aspects of the life-history of the species studied indicate an association between recombination level and the expected results of r- and K-selection. Species which were large, developed slowly and had few pouch young (characteristics expected under K-selection) tended to have higher recombination levels than those species which were small, had rapid development and which had a greater production of pouch young (characteristics expected under r-selection). There seems to be some basis behind Grant's (1975) suggestion as there is an association between recombination level and the position of the species along the r-K continuum as assayed by the variables considered.

The highest correlation coefficients were generally (except in the macropods) between measures of recombination level and gestation length, time from birth to weaning, and the age of the females at their first breeding. These factors are the main components of generation length, so that it is evident that generation length is an extremely important component of the genetic system. Generation length is the main component of $r_{\max}$, the intrinsic rate of natural increase (Lewontin, 1965), which has been considered to be of central importance in determining the position of a species along the r-K continuum (Pianka, 1970).

The concepts of r- and K-selection have been applied to a small number of marsupial species. Richardson (1975) contrasted *Macropus rufus* and *M. giganteus*, and found that in this pair-wise comparison that *M. rufus* showed characteristics expected under r-selection, while *M. giganteus* appeared to be relatively K-selected. *M. rufus* are adapted for a rapid population recovery following a bad drought, whereas *M. giganteus* are adapted to producing young best able to enter the adult population in the face of competition for limited resources (Poole, 1983). On these assessments and based on the general trend observed here, one would expect *M. rufus* to have a lower level of recombination than *M. giganteus*. This is not the case (Table 6.2), although one must bear in mind that only one individual was sampled from each species, and that one is making a comparison here between species with different chromosome numbers. The results of the analyses in this thesis suggest that this last factor would tend to obscure any relationship between recombination level and life-history attributes. Other pair-wise comparisons of relatively r- and K-selected marsupials have been made (*Pseudocheirus peregrinus* with *Schreinobates volans*, How (1978); *Trichosurus vulpecula* with *T. caninus*, Tyndale-Biscoe (1978); *Sminthopsis crassicaudata* and *Antechinus stuartii*, Morton (1978)), but as each of these comparisons involves a species not studied here no comment can be made as to whether the more r-selected species of each pair has a higher level of recombination. In addition, Lee et al (1982) have reviewed aspects of the life-histories of dasyurids and they identified six types of strategies within this group. However, only one or two species were sampled from each type of strategy so that it is not yet possible to ascertain whether the different strategies, as identified by these authors, are characterized by differences in recombination level.

The theoretical studies on the evolution of recombination considered in Chapter 1.2 proposed a number of factors which might result in selection for increased recombination; sib competition, environmental unpredictability and antagonism and biological diversity (predators, parasites, pathogens, and competitors). The evidence from the marsupials appears not to support sib-competition as a factor since those species with larger litters tended to have lower recombination levels. However one must be careful in making this sort of simple comparison as one does not know the levels of inter-litter sib competition (as opposed to intra-litter competition considered above). Inferences concerning environmental unpredictability and biological diversity are even more difficult to make as there is a paucity of data of this sort, and it is also difficult to decide what scales to use in measuring such quantities. It may also be that these factors are opposing each other, in that environmentally predictable areas tend to have more species, and hence higher biological diversity (Roughgarden, 1979). However, species which inhabit the arid zone of Australia (which might be considered unpredictable, and has a lower species diversity than the wetter areas), such as *Sminthopsis crassicaudata*, *Lasiorhinus latifrons*, *Macropus rufus* and *M. robustus* exhibit the full range of recombination level observed here. Once again, however, one must be careful, in that it may not be that this zone is as "unpredictable" for each of these species. Relating the observed recombination levels to environmental unpredictability, antagonism and biological diversity will have to await much more detailed ecological knowledge of the species, and also the determination of the appropriate scales to use in each species.

CHAPTER 7: CHIASMA DISTRIBUTION IN MALE AUSTRALIAN MARSUPIALS

7.1 The distribution of chiasmata between bivalent classes in the $2n = 14$ chromosome species

In diplotene-diakinesis cells from the $2n = 14$ species, the individual bivalents could not be distinguished, as the positions of the centromeres were usually not apparent in Giemsa stained preparations (see Figures 6.1, 6.2a, b, c) and attempts to band these cells were not successful. In three species the centromeres were identifiable in a proportion of the diplotene-diakinesis cells, and these individuals are considered in detail in Chapter 7.2.

However, it is possible to assign the bivalents of the $2n = 14$ species into three classes on the basis of length; bivalents 1, 2 and 3 formed a group of long bivalents, bivalent 4 was of middle length, and the two smallest bivalents (5 and 6) formed the third group. The numbers of chiasmata in each group in most of the individual animals were recorded. Where more than a single individual was sampled from a particular species, the totals for the different individuals were pooled. This pooling was valid since contingency chi-square tests of homogeneity between animals within species revealed no significant differences between animals.

The data from the different species are shown in Table 7.1, and were tested for homogeneity by a contingency chi-square test. This gave $\chi^2_{20} = 12.6$, with a probability of 0.8 - 0.9. The different species therefore have chiasmata distributed between the bivalent classes in the same way, even though there was a considerable range of average chiasma frequencies (Table 6.3) in the species. At this crude level of resolution, then, the variation in chiasma frequency between the different $2n = 14$ chromosome species has

TABLE 7.1: The distribution of chiasmata between bivalent classes in the $2n = 14$ species. Observed numbers of chiasmata are given, and the expected numbers on the assumption of homogeneity are shown in brackets.

Number of chiasmata in Bivalent Group							
Species ¹	[1,2,3]		4	[5,6]		Total	
<i>Sc</i>	1424	(1427.3)	364	(354.0)	451	(457.7)	2239
<i>Sm</i>	183	(172.1)	39	(42.7)	48	(55.2)	270
<i>Db</i>	440	(439.9)	114	(109.1)	136	(141.0)	690
<i>Dh</i>	249	(239.0)	58	(59.3)	68	(76.7)	375
<i>Dv</i>	487	(483.2)	121	(119.9)	150	(154.9)	758
<i>Sh</i>	922	(923.7)	238	(229.1)	289	(296.2)	1449
<i>Pg</i>	794	(801.9)	198	(198.9)	266	(257.2)	1258
<i>Pn</i>	349	(356.3)	85	(88.4)	125	(114.3)	559
<i>Io</i>	847	(845.9)	189	(209.8)	291	(271.3)	1327
<i>Ll</i>	285	(280.5)	74	(69.6)	81	(89.9)	440
<i>Vn</i>	273	(283.0)	71	(70.2)	100	(90.8)	444
Total	6253		1551		2005		9809

$$\chi^2_{20} = 12.6; \quad \text{Probability} = 0.8 - 0.9$$

¹ Data collected during my Honours year (i.e. marked with a † in Table 6.1) are not included in this table.

not resulted in differences between the species with respect to the distribution of chiasmata between the bivalents.

One indication of chiasma distribution along the bivalents in these species could be obtained without identification of the centromeres. The numbers of chiasmata which appeared "terminal" were scored and recorded for each of the species considered here. The selection criterion for this classification was that no chromatin be visible distal to the chiasmata under the 100X oil immersion lens. The frequency of chiasmata which appeared terminal in these species are tabulated below.

Species	Sc	Sm	Sb	Sh	Dv	Sh	Pg	Pn	Io	Ll	Vu
% Terminal Chiasmata	19.4	18.6	11.9	13.1	12.7	11.3	24.9	21.6	16.4	11.4	10.4

A Spearman's correlation coefficient was calculated between these frequencies and the average cell chiasma frequency in each species (Table 6.3), and this revealed a highly significant negative association between these variables ($r_s = -0.91$, $t_9 = 6.55$, Probability < 0.001). Thus as chiasma frequency increases in these species, the proportion of terminal chiasmata decreases.

7.2 The distribution of chiasmata in three $2n = 14$ species

The distributions of chiasmata along the bivalents were studied in a *Perameles gunnii*, an *Isoodon obesulus* (both peramelids) and a *Sarcophilus harrisii* (a dasyurid) in which a proportion of the diplotene-diakinesis cells had the centromeres visible (Figures 6.1c, d and f). Only complete cells in which all the bivalents were clearly identifiable and interpretable were used

TABLE 7.2: (cont)

Species	Bivalent	Arm	Bivalent	Arm	Bivalent	Arm	Bivalent	Arm	χ^2	d.f.	Probability
Io	1	p q	22.83	29.34 70.66	229.90	68.95 166.05	235	63 172	0.73	1	0.38 - 0.57
	2	p q	21.29	37.63 62.37	214.39	76.77 127.23	204	80 124	0.22	1	0.5 - 0.7
	3	p q	20.11	30.18 69.82	202.51	64.89 150.11	215	60 155	0.53	1	0.3 - 0.5
	4*	p q	15.27	- -	153.77	- -	144	- -	-	-	-
	5	p q	10.85	39.22 60.78	109.26	45.10 69.90	115	38 77	1.84	1	0.1 - 0.2
	6	p q	9.65	28.56 71.44	97.18	26.85 67.15	94	29 65	0.24	1	0.5 - 0.7
			<u>100.00</u>		<u>1007.01</u>		<u>1007</u>		2.41	5	0.7 - 0.8

TABLE 7.2: (cont)

Species	Bivalent	Arm	Bivalent	Arm	Bivalent	Arm	Bivalent	Arm	χ^2	d.f.	Probability
Sh	1	p q	22.32	41.88 58.12	230.34	92.14 127.86	220	93 127	0.01	1	0.90 - 0.95
	2*	p q	21.64	- -	223.32	- -	237	- -	-	-	-
	3	p q	19.94	30.10 69.90	205.78	59.00 137.00	196	56 140	0.22	1	0.5 - 0.7
	4	p q	15.86	43.26 56.74	163.68	75.71 99.29	175	80 95	0.43	1	0.5 - 0.7
	5	p q	11.93	44.85 55.15	123.12	53.82 66.18	120	54 66	0.001	1	0.98 - 0.99
	6*	p q	8.31	- -	85.76	- -	84	- -	-	-	-
			100.00		1032.00		1032		2.67	5	0.7 - 0.8

* These chromosomes are metacentric so that the individual arms cannot be identified.

(79 cells with 1,013 chiasmata from *P. gunnii*, 62 cells with 1,007 chiasmata from *I. obesulus* and 50 cells with 1,032 chiasmata from *S. harrisii*). The average cell chiasma frequencies in these animals were 12.82 ± 1.37 , 16.24 ± 1.87 , and 20.64 ± 2.20 respectively, so that they cover almost the entire range shown in the $2n = 14$ species (Table 6.2).

For each cell the lengths of the bivalents and bivalent arms, and the positions of the chiasmata were measured. The relative positions of each chiasmata along its bivalent arm were then calculated in order to adjust for differences between cells with respect to bivalent contraction. Since the length measurements were taken from the telomere of each arm, more distal chiasmata had lower relative positions on the arm (i.e. a "telomeric" chiasmata has a relative position of zero).

Table 7.2 gives the relative lengths of the bivalents and bivalent arms in each species. In *I. obesulus* and *S. harrisii* there is very close agreement between these meiotic measurements and the mitotic measurements given by Hayman and Martin (1974), since the correlations between the two sets of measurements are greater than 0.99 in each case. Hayman and Martin (1974) did not measure the mitotic chromosomes of *P. gunnii* but a comparison of the meiotic measurements of this species with the mitotic measurements of the closely related *P. nasuta* (Hayman and Martin, 1974) reveals a similar close agreement.

Examination of Table 7.2 also shows the close agreement in karyotypic features in the $2n = 14$ species. Never the less there are some differences between *S. harrisii* and the two peramelid species. Bivalent 1 in *S. harrisii* (unlike the rest of the

dasyurids, Hayman and Martin (1974)) is more mediocentric than the homoeologous bivalent in the peramelids (Table 7.2), but since *S. harrisii* has not been G-banded the exact nature of this difference is not known. *S. harrisii* also has a metacentric bivalent 2 (as do the rest of the dasyurids, Hayman and Martin (1974)), and G-banding has shown that the homoeologous bivalent in *P. gunnii* and *I. obesulus* can be derived from the dasyurid chromosome 2 by a pericentric inversion (Rofe, 1979; Rofe and Hayman, in press). The third bivalent is identical in these species. Both *P. gunnii* and *I. obesulus* have a metacentric bivalent 4, whereas *S. harrisii* has arms of distinguishable length like the rest of the dasyurids (Hayman and Martin, 1974), and this is the result of a pericentric inversion difference (Rofe, 1979; Rofe and Hayman, in press). The three fifth bivalents are similar (Table 7.2 and Hayman and Martin, 1974; Rofe, 1979). The sixth bivalents of *P. gunnii* and *I. obesulus* are identical, but that in *S. harrisii* is metacentric as in most dasyurids (Hayman and Martin, 1974), and G-banding indicates that the dasyurid chromosome 6 is related to the peramelid chromosome 6 by another pericentric inversion (Rofe, 1979; Rofe and Hayman, in press).

The distribution of chiasmata between bivalents and bivalent arms

The numbers of chiasmata observed in the bivalents and bivalent arms are also shown in Table 7.2. The numbers of chiasmata expected on each bivalent and arm on the basis of the relative lengths were calculated (Table 7.2) and compared to the observed numbers by chi-square analyses. These reveal in each of the species that the chiasmata are distributed between the bivalents on the basis of length, and also that within each bivalent chiasmata are distributed between the arms on the basis of length.

This occurs despite the differences in mean all chiasma frequency between the three species. This direct relationship between bivalent length and bivalent chiasma frequency despite differences in average cell chiasma frequency between species, coupled with the relative constancy of the $2n = 14$ karyotype would appear to be the basis for the homogeneous distribution of chiasmata between bivalent classes considered in Chapter 7.1.

Do chiasmata terminalize in these species?

Two methods were used to search for evidence of chiasma terminalization. Firstly, for each species, a Spearman's correlation coefficient between the total autosomal length and the number of chiasmata observed in each cell was calculated (Table 7.3). Each of these were positive, but was not significantly different from zero. There is thus no evidence that as the bivalents contract (within the range of cells considered here) during diplotene-diakinesis that there is any loss of chiasmata from the ends of the bivalents, since this would lead to a significant positive correlation between cell chiasma number and bivalent length.

The second method involved a more direct test for movement of chiasmata during diplotene-diakinesis. This involved calculating Spearman's correlation coefficients between bivalent arm length and the relative position of the first chiasmata from the centromere in each cell. If the chiasmata do move towards the telomeres as meiosis proceeds, then one would expect the cells with more contracted bivalents to have more distally located chiasmata. Since more distal chiasmata have lower relative positions on the bivalent arms, then one expects a negative correlation between the relative position of the chiasma nearest the centromere with

TABLE 7.3: Analysis of the correlation between total autosomal bivalent length and the number of chiasmata in each cell in the three species studied.

Species	r_s	t	d.f.	Probability
<i>Perameles gunnii</i>	+0.06	0.53	77	0.5 - 0.6
<i>Isoodon obesulus</i>	+0.11	0.86	60	0.3 - 0.4
<i>Sarcophilus harrisii</i>	+0.16	1.09	48	0.2 - 0.3

the length of the bivalent arm. Correlation coefficients were calculated separately for each of the different bivalent arm chiasma frequency classes since the number of chiasmata on a bivalent arm affects the chiasma distribution (see later).

The results of this analysis are given in Table 7.4.

Almost all the correlation coefficients are not significantly different from zero, and their calculated values are approximately one half positive and one half negative. Three of the correlation coefficients calculated are significant, but one of these is negative, and the other two are positive. There is therefore no constancy in these significant correlations. It seems likely that these are spurious significant correlations. As the 5% level of significance was being used and 50 tests of significance were performed in this analysis one expects a small number of false significant results, even if there is no underlying relationship between the two variables being considered. The fact that the calculated correlation coefficients are approximately equally distributed between positive and negative values supports this conclusion.

There is therefore no evidence of chiasma terminalization during the diplotene-diakinesis period of meiosis in these three species. The evidence presented does not however, rule out chiasma movement either before diplotene-diakinesis, or between this stage and metaphase 1. Only experiments using BUdR labelling (Tease, 1978) could possibly detect such chiasma movements.

TABLE 7.4: Spearman's rank correlation coefficients between the relative positions of the first chiasma from the centromere and the length of the bivalent arms for the three species studied. The degrees of freedom are two less than the sample size and vary because different numbers of arms have particular chiasma frequencies.

* Significant results

Perameles gunnii

Chromosome	Arm	No. of Chiasmata	r_s	t	d.f.	Probability
1	p	1	-0.99	0.78	75	0.5 - 0.4
	q	1	+0.43	1.58	11	0.2 - 0.1
		2	-0.13	1.36	108	0.2 - 0.1
		3	+0.77	3.62	9	0.01 - 0.001*
2	p	1	+0.20	1.70	69	0.1 - 0.005
	q	1	+0.07	0.44	38	0.7 - 0.6
		2	-0.14	0.81	33	0.5 - 0.4
3	p	1	+0.03	0.24	65	0.9 - 0.8
	q	1	+0.12	0.64	28	0.6 - 0.5
		2	-0.23	1.60	46	0.2 - 0.1
4	both	1	+0.06	0.73	147	0.5 - 0.4
5	p	1	-0.24	1.26	26	0.3 - 0.2
	q	1	-0.18	1.55	74	0.2 - 0.1
6	p	1	-0.35	1.98	28	0.1 - 0.05
	q	1	-0.15	1.29	72	0.3 - 0.2

Isodon obesulus

Chromosome	Arm	No. of Chiasmata	r_s	t	d.f.	Probability
1	p	1	-0.04	0.30	55	0.8 - 0.7
		2	+0.13	0.49	14	0.7 - 0.6
		3	-0.08	0.46	33	0.7 - 0.6
2	p	1	-0.16	1.00	38	0.4 - 0.3
		2	+0.22	0.96	18	0.4 - 0.3
	q	1	+0.42	1.31	8	0.3 - 0.2
		2	+0.07	0.45	41	0.7 - 0.6
3	p	1	+0.15	1.03	46	0.4 - 0.3
		2	-0.10	0.51	26	0.7 - 0.6
		3	-0.03	0.15	26	0.9 - 0.8
4	both	1	-0.11	1.12	102	0.3 - 0.2
		2	+0.38	1.74	18	0.1 - 0.05
5	p	1	-0.21	1.29	36	0.3 - 0.2
		1	-0.07	0.47	45	0.7 - 0.6
		2	-0.36	1.39	13	0.2 - 0.1
6	p	1	+0.25	1.34	27	0.2 - 0.1
	q	1	+0.05	0.36	51	0.8 - 0.7

Sarcophilus harrisii

Chromosome	Arm	No. of Chiasmata	r_s	t	d.f.	Probability
1	p	1	-0.12	0.45	14	0.7 - 0.6
		2	+0.09	0.43	23	0.7 - 0.6
	q	2	+0.10	0.45	20	0.7 - 0.6
		3	-0.28	1.27	19	0.3 - 0.2
2	both	2	-0.04	0.28	49	0.8 - 0.7
		3	+0.13	0.82	38	0.5 - 0.4
3	p	1	+0.33	1.95	32	0.1 - 0.05
		2	-0.73	3.20	9	0.02 - 0.01*
	q	2	+0.28	1.05	13	0.4 - 0.3
		3	+0.16	0.81	25	0.5 - 0.4
4	p	1	-0.08	0.35	19	0.8 - 0.7
		2	+0.32	1.72	26	0.1 - 0.05
	q	1	+0.64	2.63	10	0.05 - 0.02*
		2	+0.18	0.99	29	0.4 - 0.3
5	p	1	-0.14	0.87	38	0.4 - 0.3
		1	-0.18	1.00	30	0.4 - 0.3
	q	2	+0.39	1.64	15	0.2 - 0.1
6	both	1	+0.06	0.49	68	0.7 - 0.6

Interference

Evidence of the effect of interference on chiasma number was found by a consideration of the means and variances of the bivalent and arm chiasma frequencies in the three $2n = 14$ species. If interference was not operating then chiasmata would occur at random in bivalents and bivalent arms, so that the distribution of bivalents and bivalent arms with different numbers of chiasmata would be based on the Poisson distribution. The means and variances of the numbers of chiasmata in the different bivalents and bivalent arms would therefore be expected to be equal.

These statistics were calculated for each bivalent and bivalent arm in all three species and were used to calculate coefficients of dispersion (Sokal and Rohlf, 1969) in each case. This statistic is the variance divided by the mean. If the distribution is Poisson (that is, there is no interference), then one expects this coefficient of dispersion to be close to unity. All the calculated coefficients of dispersion for both bivalents and bivalent arms (Table 7.5) are well below unity, so that the variances were less than the means and the distributions were under-dispersed compared to the Poisson expectations. This indicates that the occurrence of a chiasma in a bivalent or bivalent arm reduces the chances of another forming in the same bivalent or bivalent arm. These three species therefore have negative interference with respect to chiasma number.

Two sets of statistics were calculated in order to test whether such interference operates over the centromere. The first was to look for a negative association between the numbers of chiasmata on the two arms of each bivalent in the different cells.

TABLE 7.5: The coefficients of dispersion (variance divided by mean) of chiasma frequency for the bivalents and bivalent arms of the three species.

Species	Bivalent	C.D.	Arm	C.D.
<i>Perameles gunnii</i>	1	0.103	p	0.026
			q	0.156
	2	0.138	p	0.099
			q	0.215
	3	0.110	p	0.160
			q	0.160
	4 [†]	0.056		
	5	0.173	p	0.654
			q	0.063
	6	0.170	p	0.628
			q	0.065
<i>Isodon obesulus</i>	1	0.163	p	0.080
			q	0.194
	2	0.206	p	0.213
			q	0.180
	3	0.187	p	0.236
			q	0.180
	4 [†]	0.120		
	5	0.237	p	0.393
			q	0.150
	6	0.228	p	0.541
			q	0.139
<i>Sarcophilus harrisii</i>	1	0.241	p	0.295
			q	0.244
	2 [†]	0.110		
	3	0.248	p	0.269
			q	0.175
	4	0.166	p	0.179
			q	0.020
	5	0.153	p	0.183
			q	0.120
	6 [†]	0.205		

[†] These chromosomes are metacentric so that a C.D. cannot be calculated for each arm since the arms cannot be distinguished.

If the interference operated over the centromere, then the presence of chiasmata on one arm would be expected to decrease the frequency of chiasma formation on the other arm. Spearman's correlation coefficients between the numbers of chiasmata on the long and short arms of the non-metacentric bivalents of *S. harrisii* were calculated (Table 7.6). All of these were positive, and one significantly so. There is therefore no evidence on this test of interference extending over the centromere in this species. The same test could not be applied to the results from *P. gunnii* and *I. obesulus* since in these species there was not enough variation in chiasma frequency on many bivalent arms to enable such correlation coefficients to be calculated.

This first analysis does not, however, reveal whether chiasma interference is operating over the centromere and manifests itself by altering the position of the chiasmata. If this occurred one would expect that bivalents with a chiasma close to the centromere on one bivalent arm would tend to have the first chiasmata from the centromere on the other arm further than average from the centromere. This was tested by calculating Spearman's correlation coefficients between the relative positions of the first chiasmata on both arms in the different bivalents in each of the three species (Table 7.7). One of the values (*P. gunnii* bivalent 1) was significantly negative (as expected with interference extending across the centromere). However, of the other correlation coefficients seven were negative and four were positive, though each was not significantly different from zero. This suggests that if interference is operating across the centromere to affect the position of chiasmata, then the effect is very slight and is only present in some bivalents. A more likely

TABLE 7.6: Analysis of chiasma number interference across the centromere in *Sarcophilus harrisii*

Bivalent ¹	r_s^2	t_{48}	Probability
1	+0.07	0.51	0.6 - 0.7
3	+0.41	3.12	0.01 - 0.001*
4	+0.16	1.13	0.1 - 0.2
5	+0.24	1.70	0.05 - 0.1

¹ bivalents 2 and 6 cannot be analysed as they are metacentric

² Spearman's correlation coefficient between the number of chiasmata on the long and short arms of each bivalent.

* Significant result.

TABLE 7.7: Analysis of chiasma position interference across the centromere in the three species studied.

Species	Bivalent ¹	r_s^2	t	d.f. ³	Probability
<i>Perameles gunnii</i>	1	-0.28	2.55	76	0.01 - 0.02*
	2	-0.09	0.78	74	0.4 - 0.5
	3	-0.04	0.37	67	0.6 - 0.7
	5	-0.22	1.11	24	0.2 - 0.3
<i>Isodon obesulus</i>	1	+0.07	0.53	58	0.6 - 0.7
	2	-0.16	1.23	58	0.2 - 0.3
	3	+0.21	1.55	52	0.1 - 0.2
	5	+0.19	1.16	36	0.2 - 0.3
<i>Sarcophilus harrisii</i>	1	-0.28	2.00	48	0.05 - 0.1
	3	+0.25	1.71	43	0.05 - 0.1
	4	-0.08	0.52	48	0.6 - 0.7
	5	-0.21	1.40	44	0.1 - 0.2

¹ The metacentric bivalents are not included. Bivalents 6 from *P. gunnii* and *I. obesulus* are not included as almost all chiasmata on their short arms were telomeric.

² Spearman's correlation coefficient between the relative positions of the 1st chiasma from the centromere on each arm.

³ The degrees of freedom vary since some bivalents had no chiasmata on an arm in particular cells.

* Significant results.

explanation would appear to be that interference is not operating over the centromere and the single significant negative correlation obtained would be a spurious significant result obtained due to the number of tests of significance performed.

Competition between bivalents for chiasmata.

Mather (1936) proposed that there was competition for chiasmata between bivalents since he obtained significant negative correlations between the chiasma frequencies of certain bivalents in some individuals in some species. In the three species considered here two methods of analysis were used to test for competition between bivalents. The first was to calculate a Spearman's correlation coefficient between the number of chiasmata on a particular bivalent and the number in the rest of the complement. If competition between bivalents is operating then these correlations will be negative since the occurrence of chiasmata in one bivalent would tend to decrease the chances of chiasmata forming in the other bivalents in the complement. In many of the shorter bivalents in these three species, however, this method of analysis could not be used as these bivalents almost always had one of two chiasma frequencies. In these cases the mean chiasma frequencies for the rest of the complement were calculated separately for each bivalent chiasma frequency class, and any differences between these means were tested by t-tests (Sokal and Rohlf, 1969). In these cases competition between bivalents within a cell would cause the mean chiasma frequency of the rest of the complement in the lower bivalent chiasma frequency class to be higher than that from the higher bivalent chiasma frequency class.

TABLE 7.8: Analysis for competition between bivalents for chiasmata.

- ¹ Two methods of analysis were used; Spearman's correlation coefficient, or a t-test of difference between two means (see text).

Chromosome 4 from both *P. gunnii* and *I. obesulus* were not analysed as there was too little variation in the numbers of chiasmata on these chromosomes to enable either method of analysis.

* Significant results.

Chromosome	Method of Analysis ¹	Probability

Perameles gunnii

1	$r_s = +0.17$	$t_{77} = 1.49$	0.1 - 0.2
2	$r_s = +0.24$	$t_{77} = 2.21$	0.02 - 0.05*
3	2 Chiasmata Mean (Rest) = 10.18	$t_{75} = 0.59$	0.5 - 0.6
	3 Chiasmata Mean (Rest) = 10.39		
5	1 Chiasmata Mean (Rest) = 11.34	$t_{77} = 1.17$	0.2 - 0.3
	2 Chiasmata Mean (Rest) = 11.72		
6	1 Chiasmata Mean (Rest) = 11.37	$t_{77} = 0.98$	0.3 - 0.4
	2 Chiasmata Mean (Rest) = 11.70		

Isodon obesulus

1	$r_s = +0.21$	$t_{60} = 1.66$	0.1 - 0.2
2	$r_s = +0.15$	$t_{60} = 1.18$	0.2 - 0.3
3	$r_s = +0.30$	$t_{60} = 2.44$	0.01 - 0.02*
5	1 Chiasmata Mean (Rest) = 14.29	$t_{51} = 0.38$	0.7 - 0.8
	2 Chiasmata Mean (Rest) = 14.41		
6	1 Chiasmata Mean (Rest) = 14.63	$t_{57} = 1.11$	0.2 - 0.3
	2 Chiasmata Mean (Rest) = 14.97		

Sarcophilus harrisii

1	$r_s = +0.37$	$t_{48} = 2.79$	0.001 - 0.01*
2	$r_s = +0.28$	$t_{48} = 2.04$	0.02 - 0.05*
3	$r_s = +0.13$	$t_{48} = 0.94$	0.3 - 0.4
4	$r_s = +0.21$	$t_{48} = 1.50$	0.1 - 0.2
5	$r_s = +0.19$	$t_{48} = 1.36$	0.1 - 0.2
6	$r_s = +0.17$	$t_{48} = 1.18$	0.2 - 0.3

These analyses are given in Table 7.8. All the correlation coefficients calculated were positive, and four were significantly so. No significant differences were revealed with the t-tests on the means on the other bivalents, but in each case considered the mean chiasma frequency in the rest of the complement was higher in the higher bivalent chiasma frequency group. These results are therefore the opposite to those expected on the basis of competition between bivalents, so that there is no evidence of competition between bivalents in each of these species. Indeed, the data in Table 7.8 show that high chiasma frequency in a particular bivalent in a cell is associated with a high chiasma frequency in the rest of the complement - cells with high chiasma frequency tend to have high chiasma frequencies in all bivalents.

The distribution of chiasmata along the bivalents

The distributions of chiasmata along the bivalent of *P. gunnii*, *I. obesulus*, and *S. harrisii* are shown in Figures 7.1 to 7.6. Figures 7.1a, 7.3a and 7.5a are the distributions of all the chiasmata observed along the length of the individual bivalents, while in Figures 7.1b, 7.3b and 7.5b the distribution of chiasmata along each bivalent arm in the different bivalent arm chiasma frequency classes are shown separately. In each of these figures each bivalent arm has been divided into twenty segments of equal length.

In general, the telomeric segments of each bivalent arm have a relatively high frequency of chiasmata, and chiasmata are also clumped at certain positions along the length of the bivalents. This gives many of the bivalent arms a bimodal distribution (one telomeric and one interstitial peak) of chiasmata (e.g. *P. gunnii*

Figure 7.1: The distribution of chiasmata along the bivalents of *Perameles gunnii*

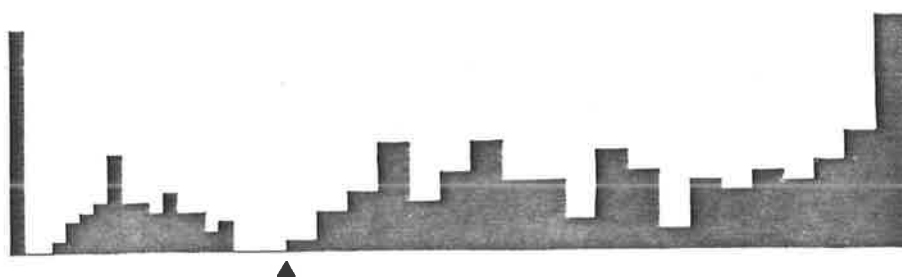
- a. The distribution of all chiasmata along the bivalents. Chromosome 4 is metacentric so that the distribution is shown as symmetrical about the centromere. Centromeres are indicated by arrowheads.
- b. The distribution of chiasmata along the bivalent arms in different arm chiasma frequency classes. The 1st, 2nd, etc., chiasmata from the telomeres are differently shaded. The telomeric and centromeric end of each arm are indicated by a T and C.

The vertical scales are the number of chiasmata observed in each segment.

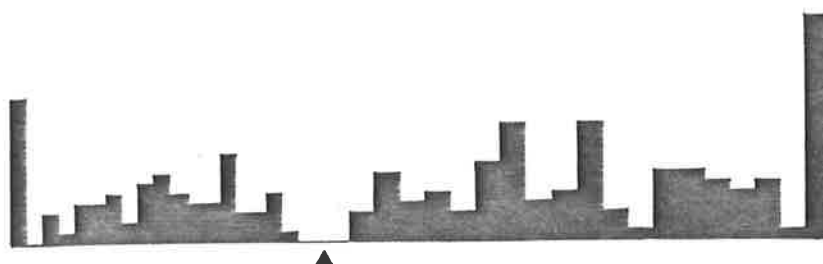
Each chromosome arm has been divided into twenty segments of equal length.

A

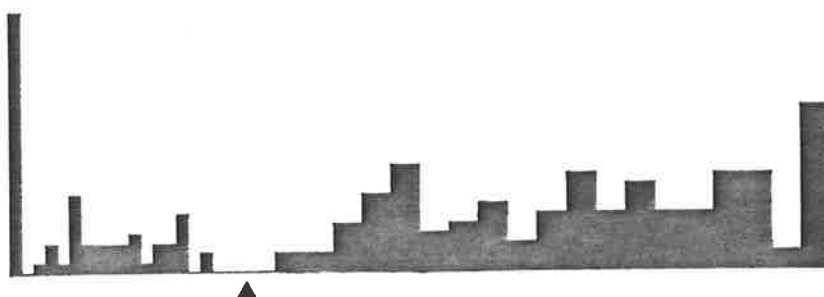
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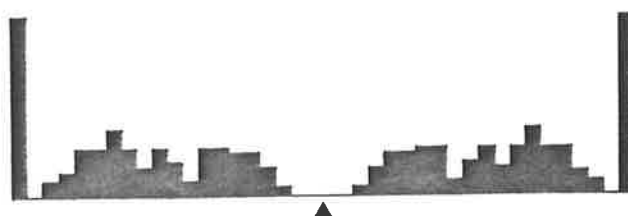
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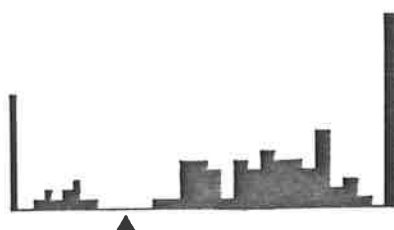
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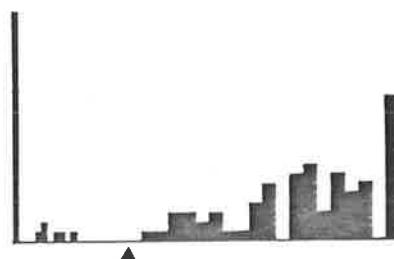
4



5

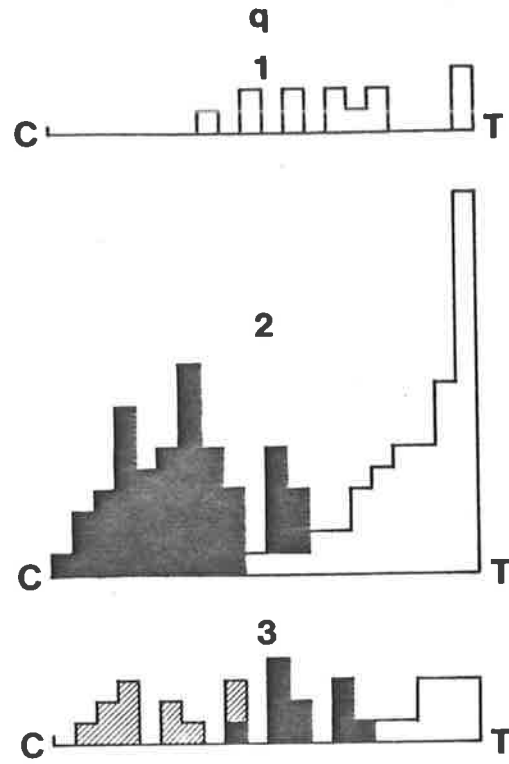
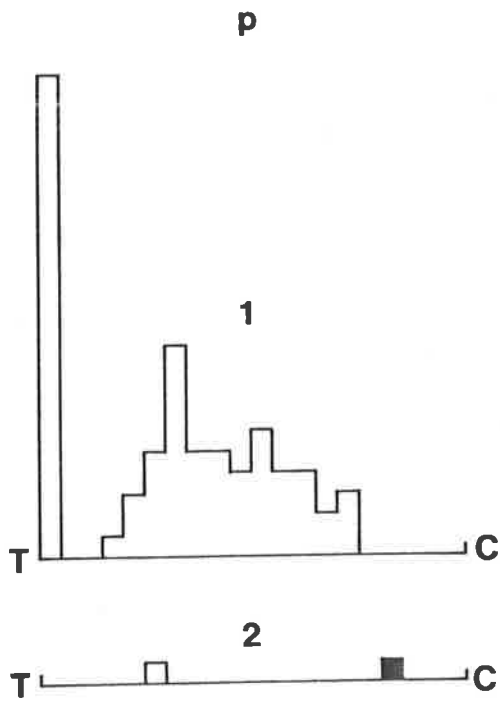


6

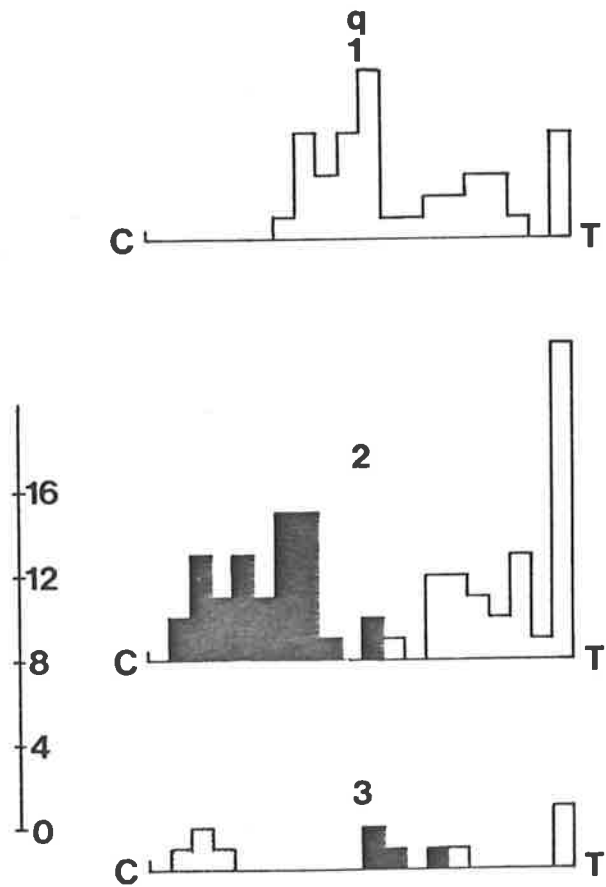
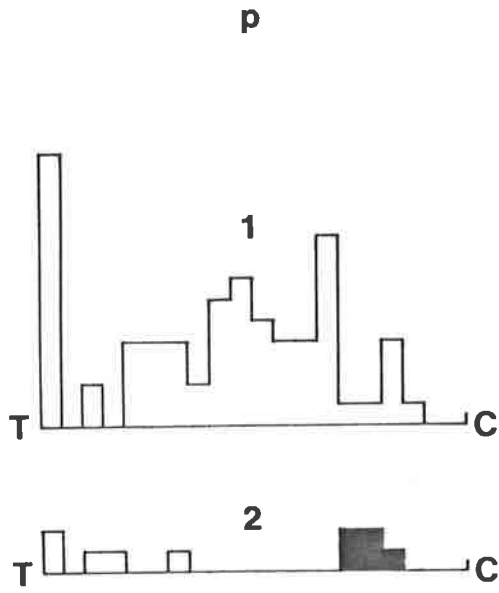


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20
16
12
8
4
0

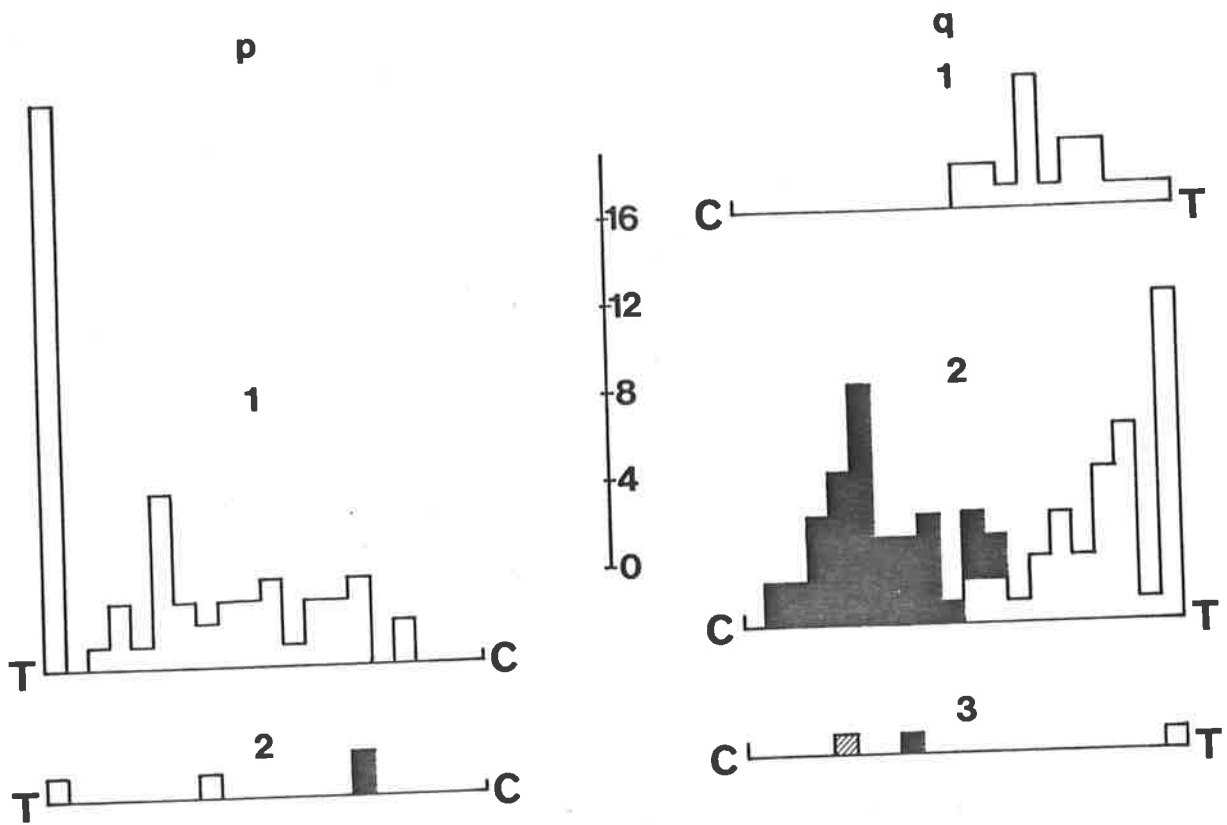
Chromosome 1



Chromosome 2

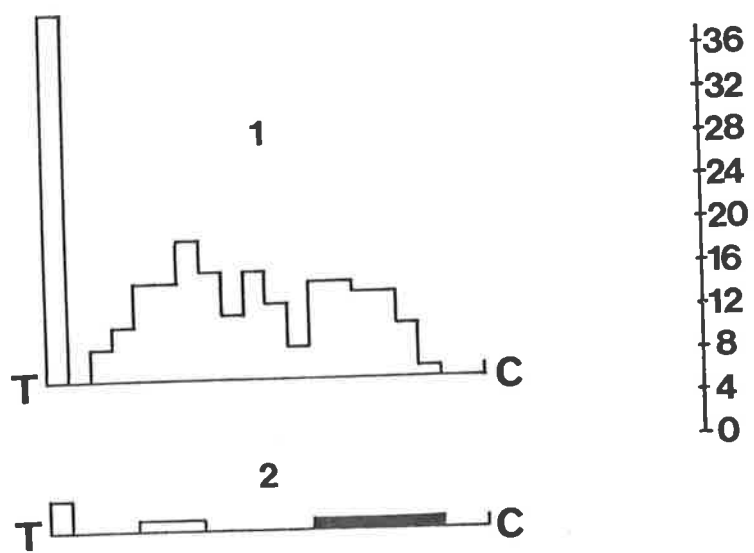


Chromosome 3



Chromosome 4

both



Chromosome 5



Chromosome 6

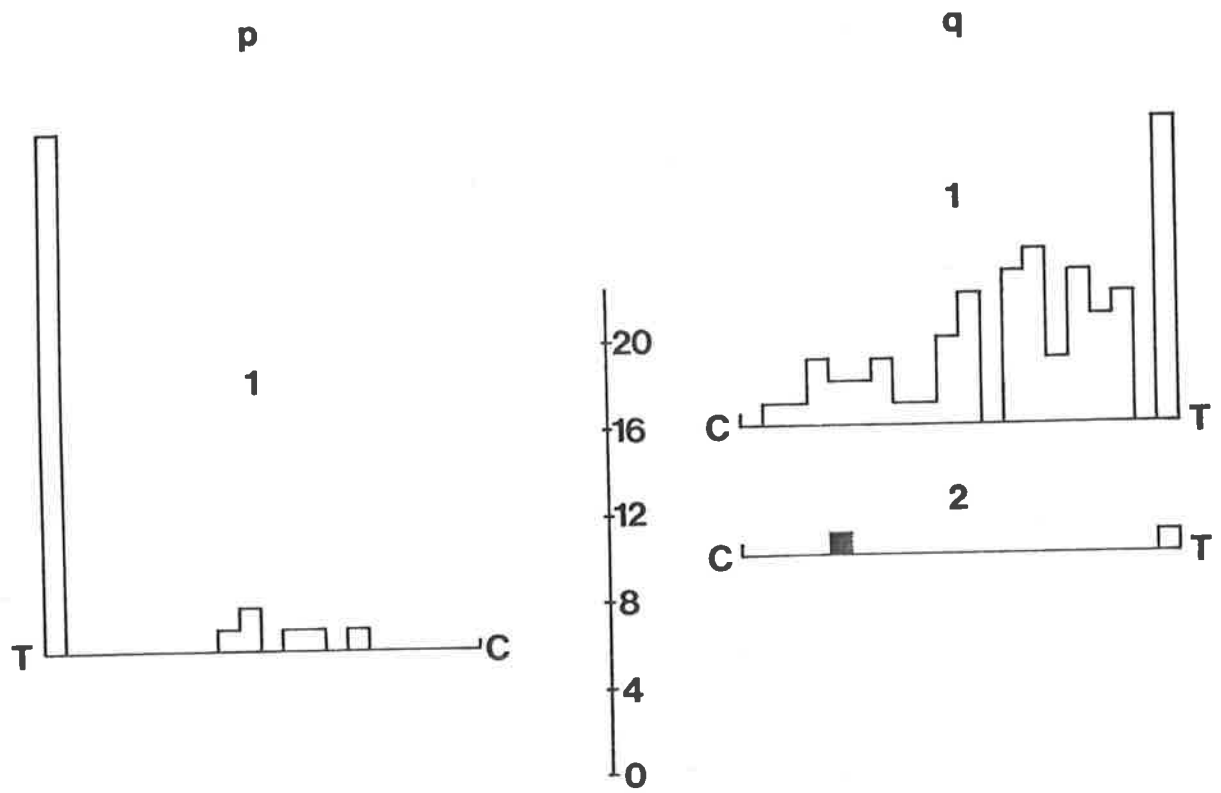
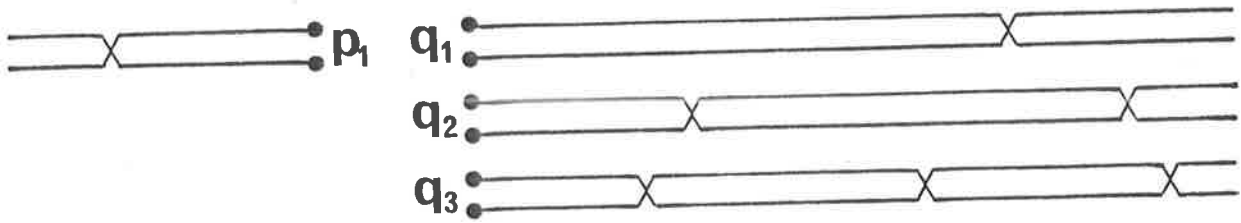


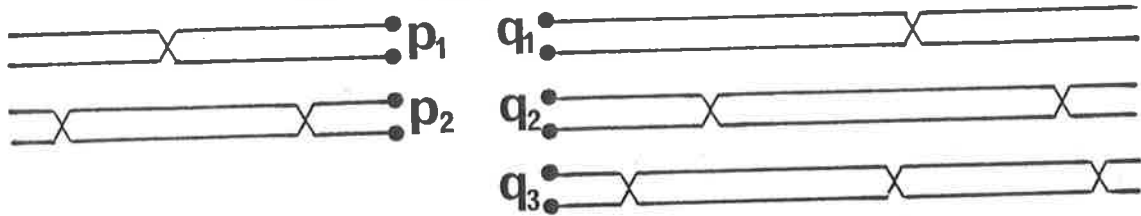
Figure 7.2: The mean positions of chiasmata along the arms of *Perameles gunnii* in the different arm chiasma frequency classes.

In chromosome 4 both arms are combined as this chromosome is metacentric.

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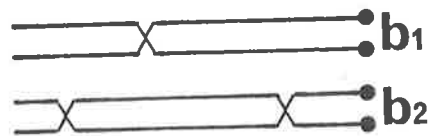
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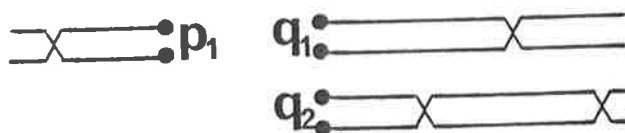
Chromosome 3



Chromosome 4



Chromosome 5



Chromosome 6

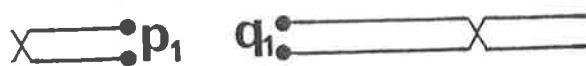
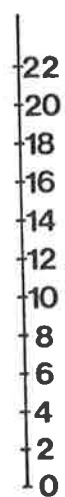
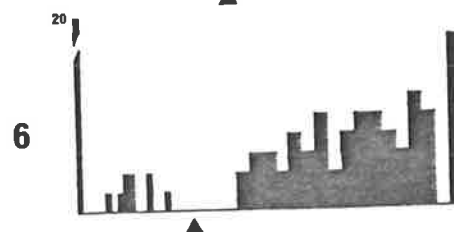
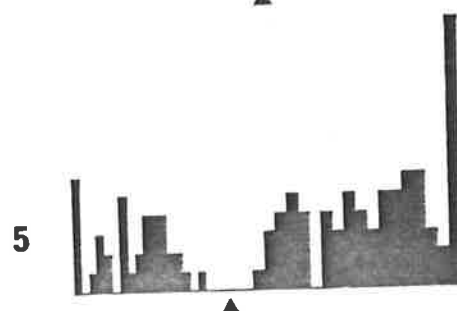
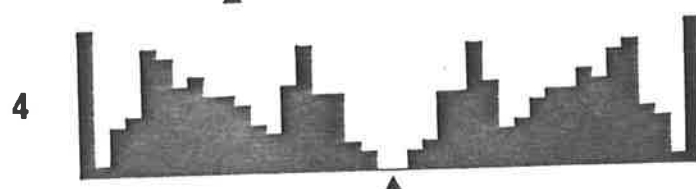
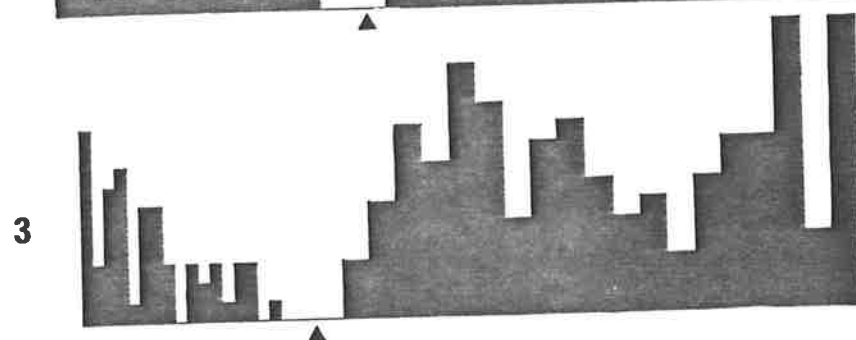
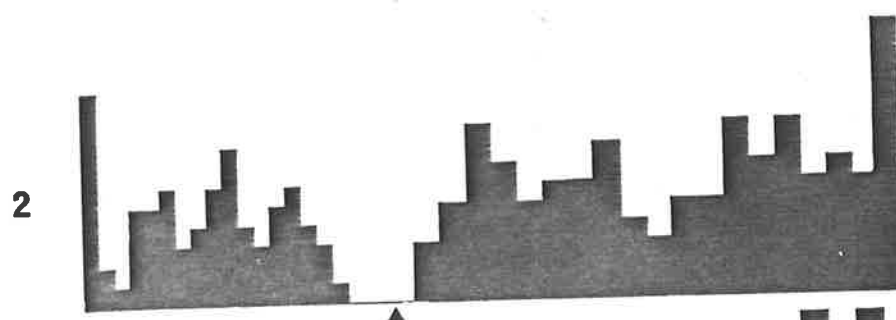
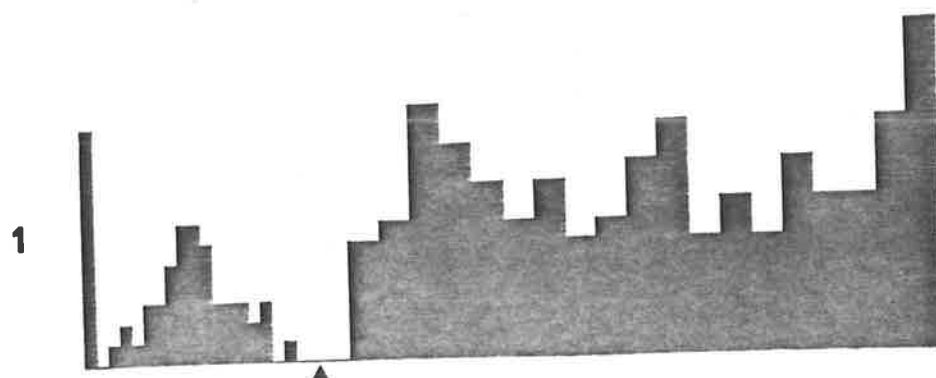


Figure 7.3: The distribution of chiasmata along the bivalents of *Isodon obesulus*

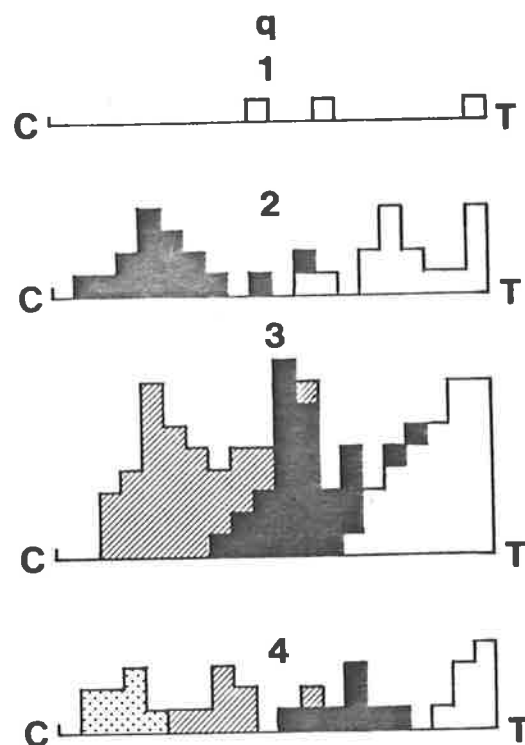
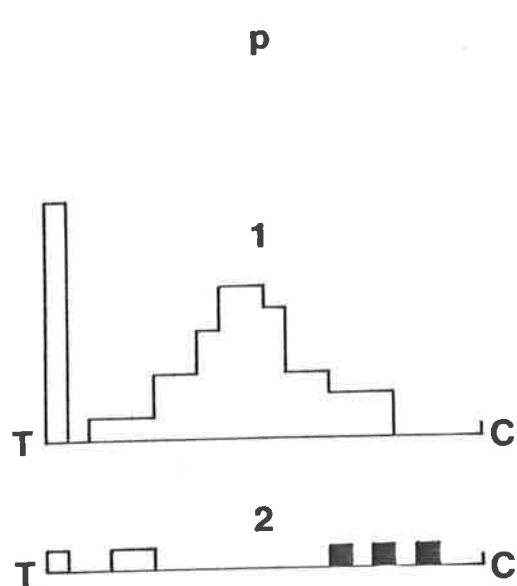
- a. The distribution of all chiasmata along the bivalents. Chromosome 4 is metacentric so that the distribution is shown as symmetrical about the centromere. Centromeres are indicated by arrow heads.
- b. The distribution of chiasmata along the bivalent arms in different arm chiasma frequency classes. The 1st, 2nd, etc., chiasmata from the telomeres are differently shaded. The telomeric and centromeric end of each arm are indicated by a T and C.

The vertical scales are the number of chiasmata observed in each segment..

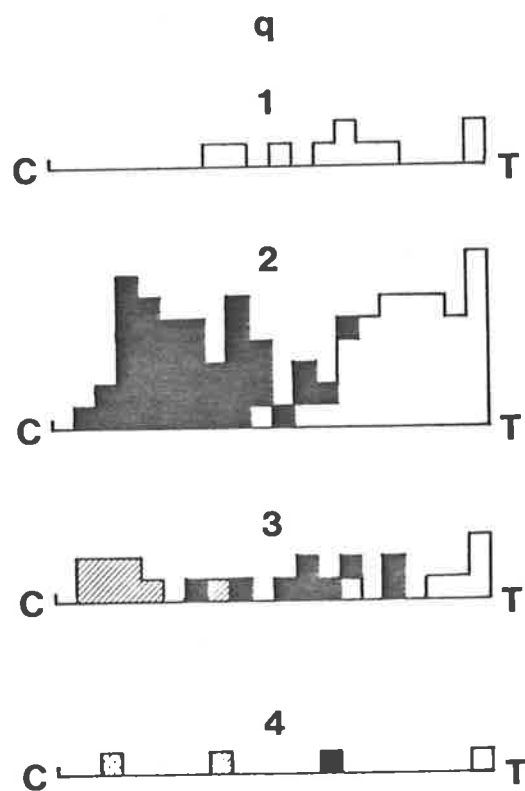
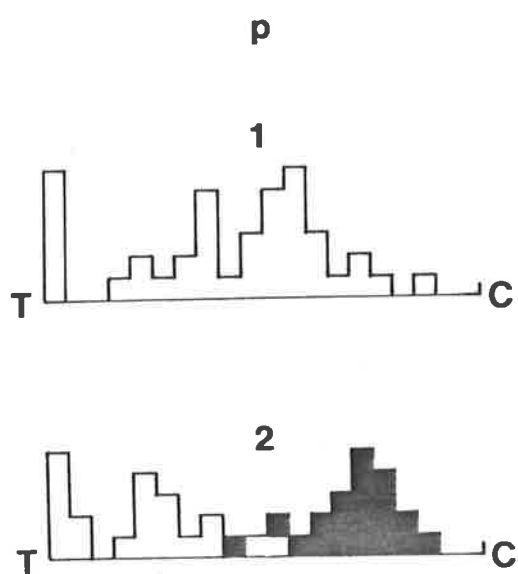
Each chromosome arm has been divided into twenty segments of equal length.



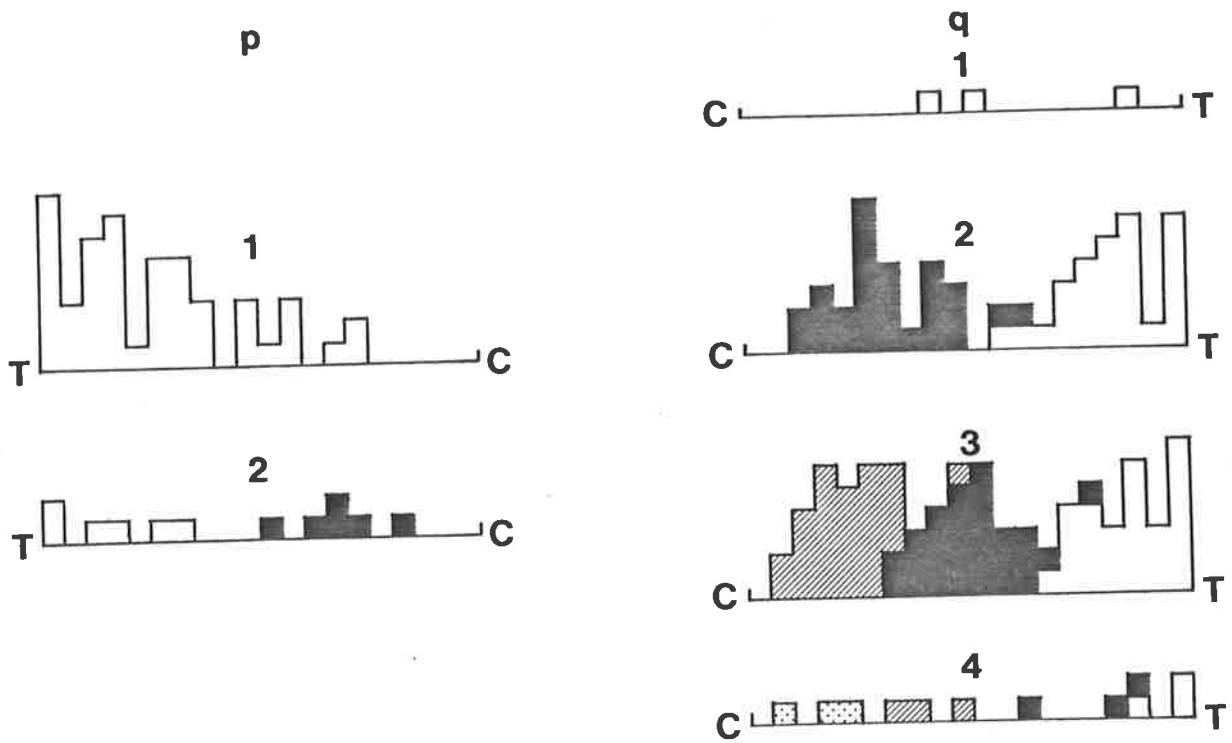
Chromosome 1



Chromosome 2

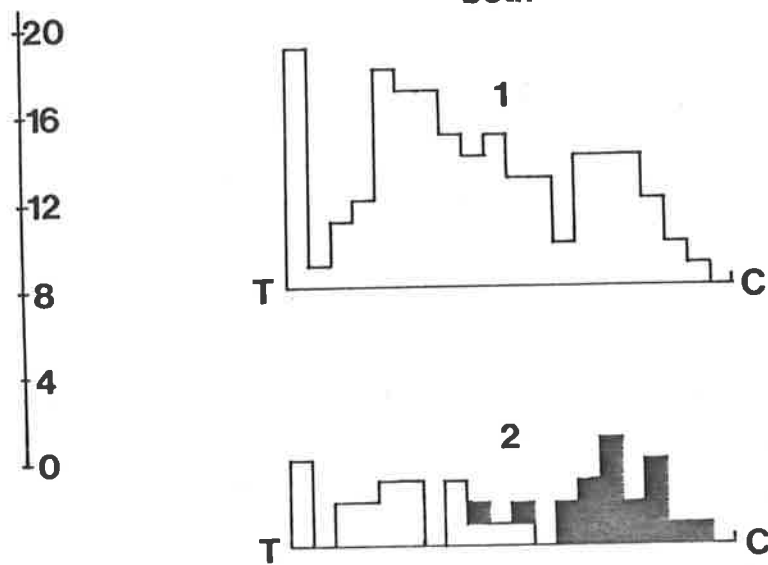


Chromosome 3

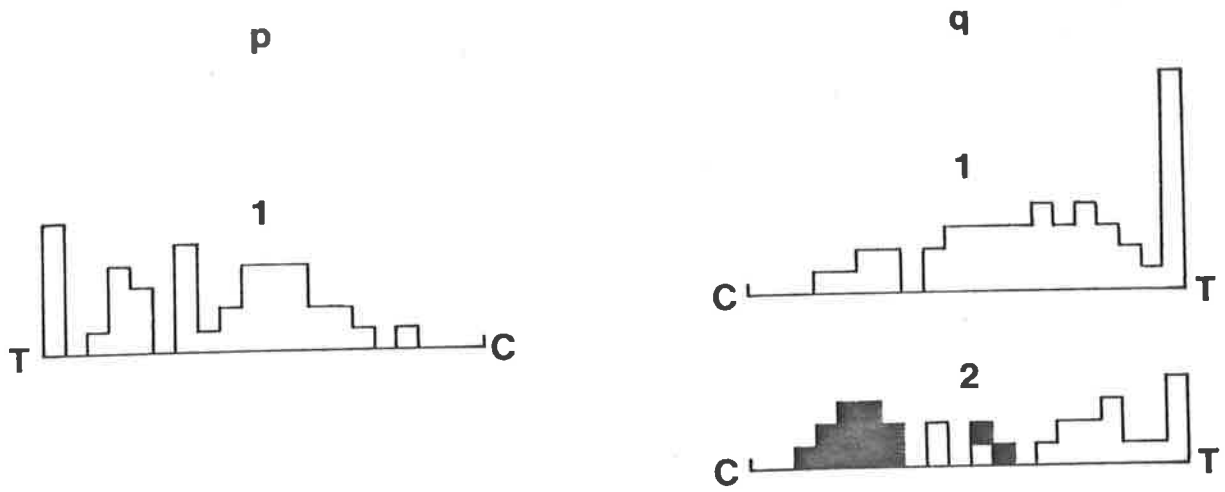


Chromosome 4

both



Chromosome 5



Chromosome 6

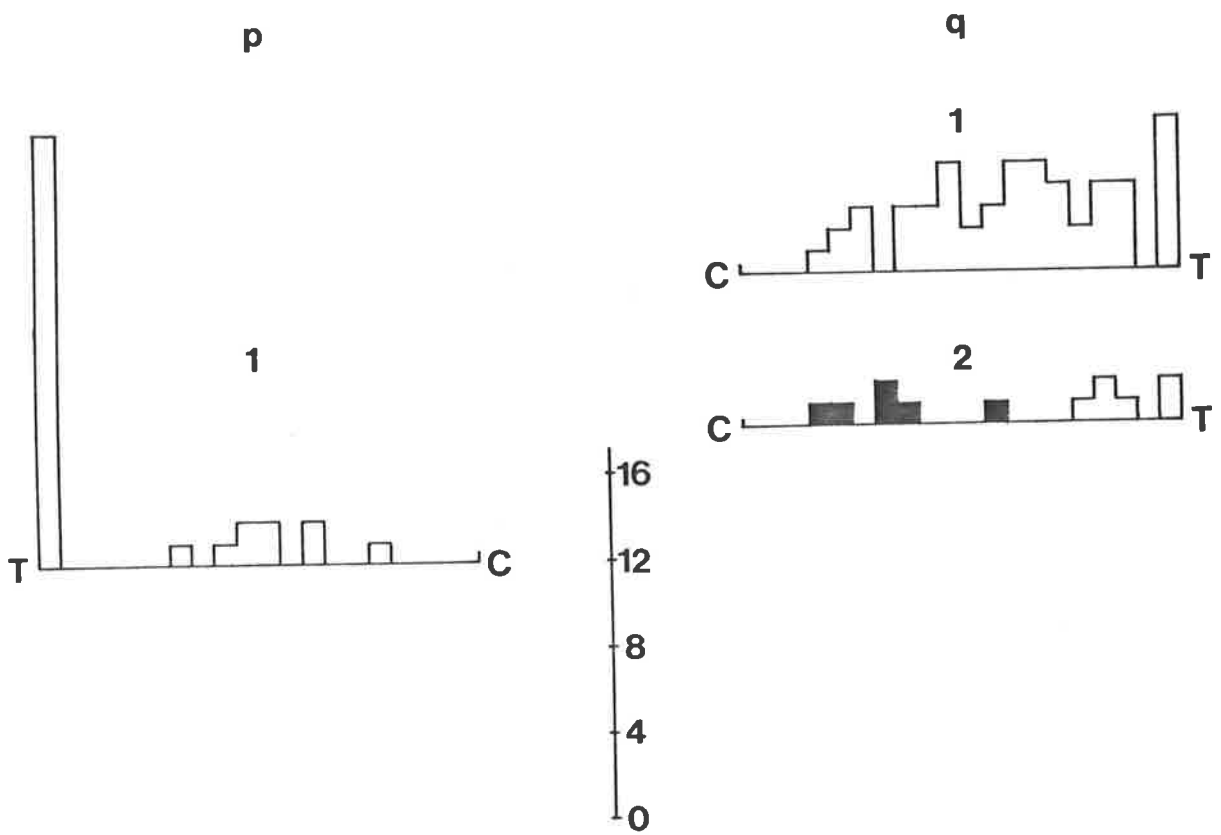
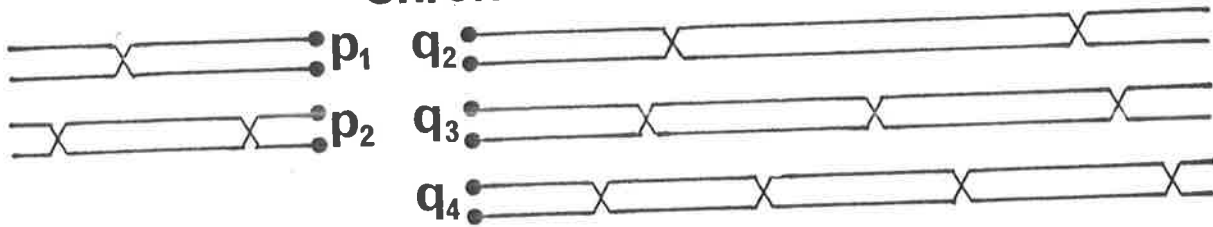


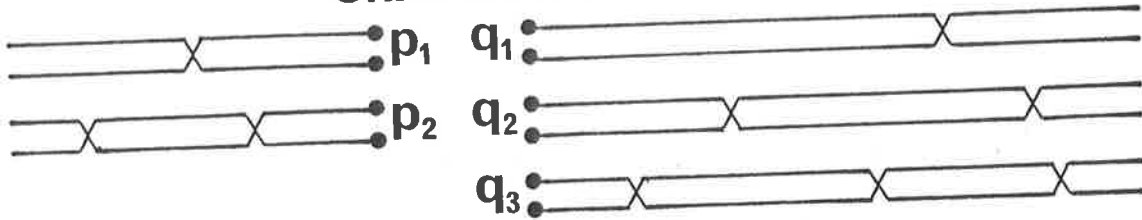
Figure 7.4: The mean positions of chiasmata along the arms of *Isoodon obesulus* in the different arm chiasma frequency classes.

In chromosome 4 both arms are combined as this chromosome is metacentric.

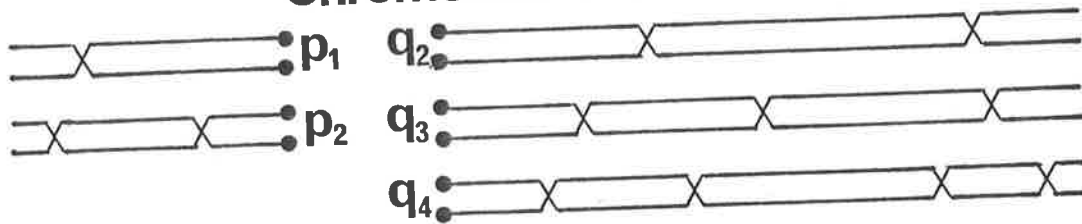
Chromosome 1



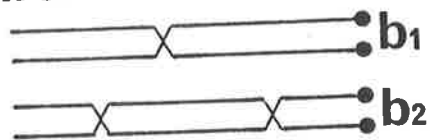
Chromosome 2



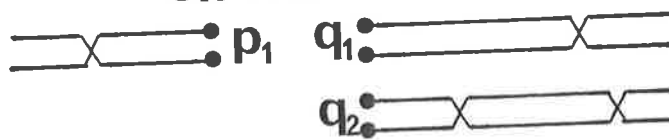
Chromosome 3



Chromosome 4



Chromosome 5



Chromosome 6

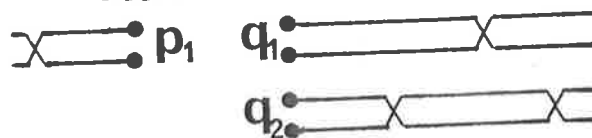
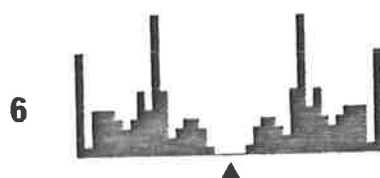
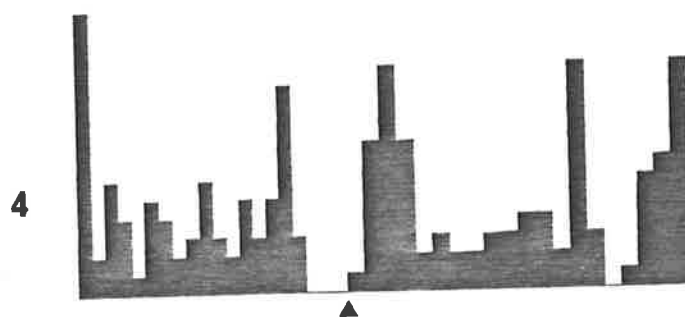
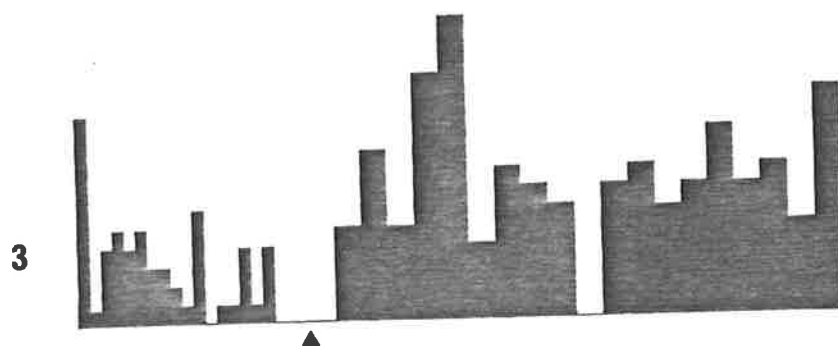
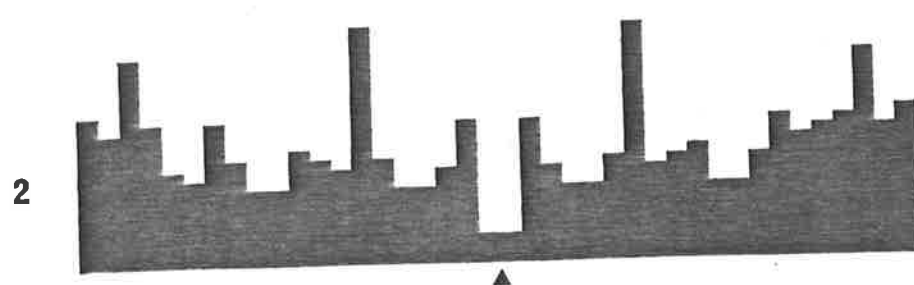
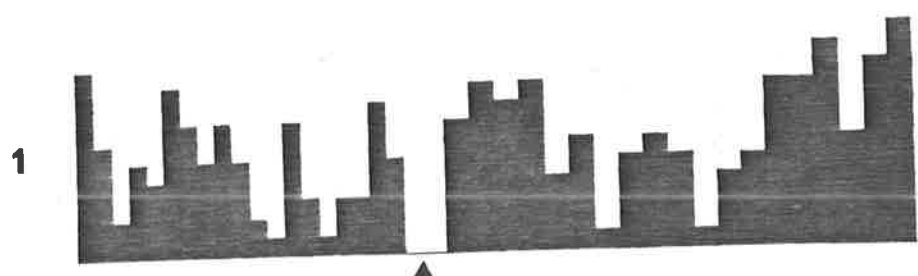


Figure 7.5: The distribution of chiasmata along the bivalents of *Sarcophilus harrisii*

- a. The distribution of all chiasmata along the bivalents. Chromosomes 2 and 6 are metacentric so that their distributions are shown as symmetrical about the centromere. Centromeres are indicated by arrow heads.
- b. The distribution of chiasmata along the bivalent arms in different arm chiasma frequency classes. The 1st, 2nd, etc., chiasmata from the telomeres are differently shaded. The telomeric and centromeric end of each arm are indicated by a T and C.

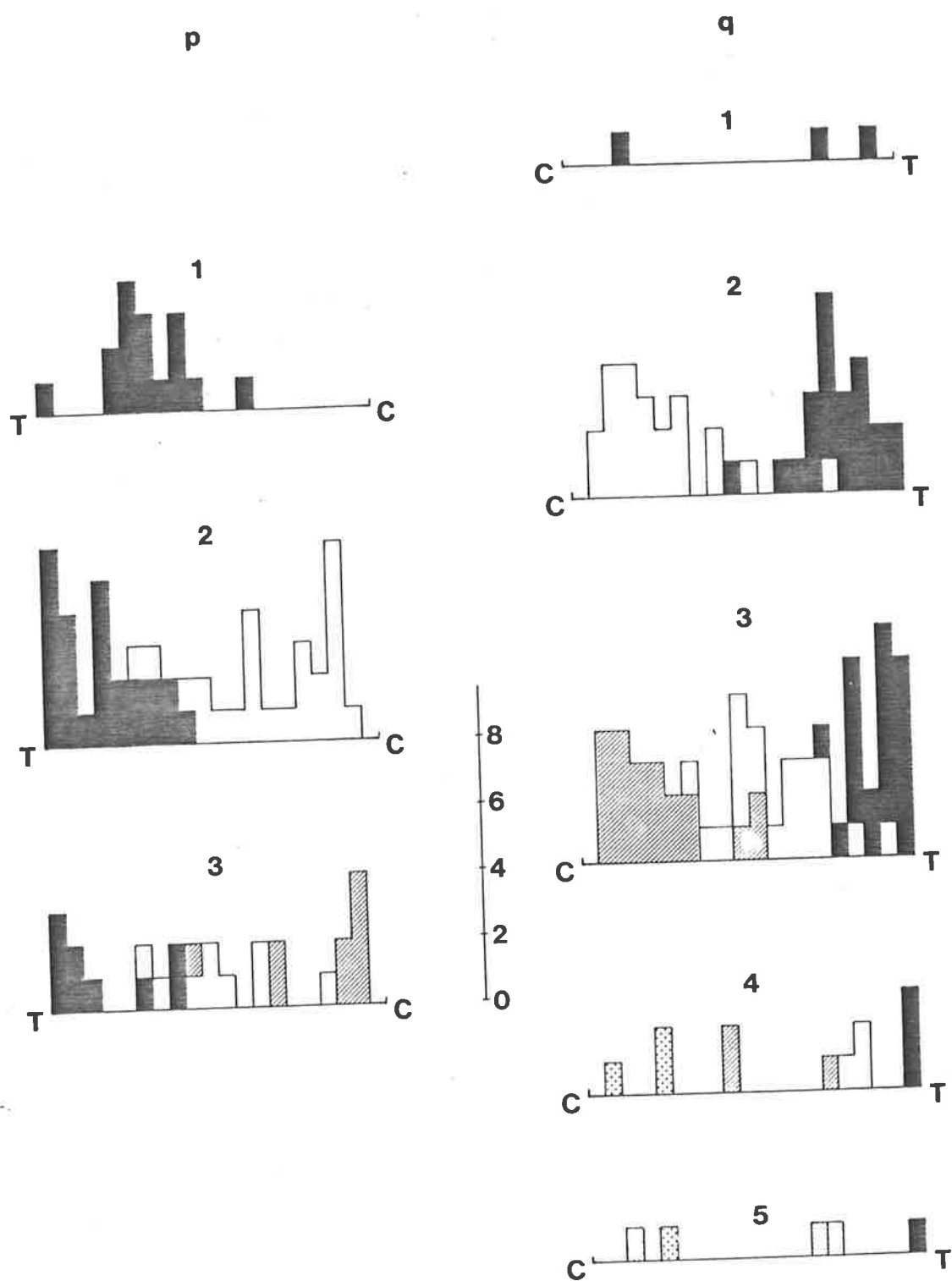
The vertical scales are the number of chiasmata observed in each segment.

Each chromosome arm has been divided into twenty segments of equal length.

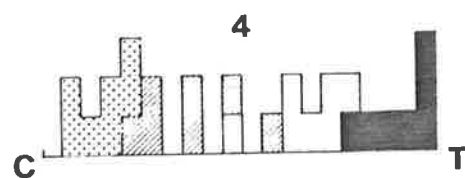
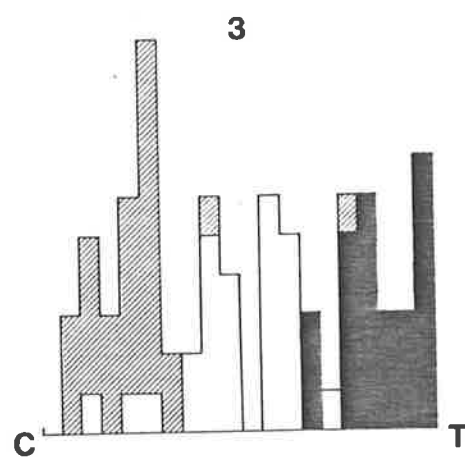
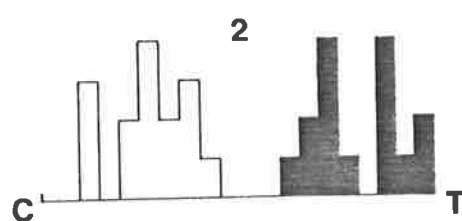
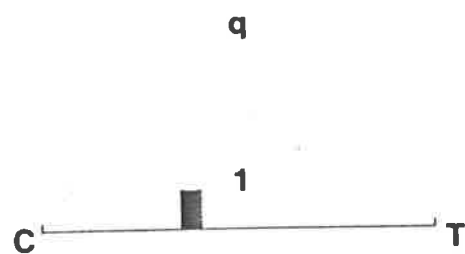
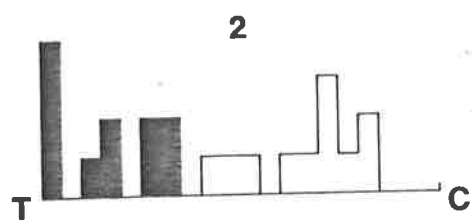
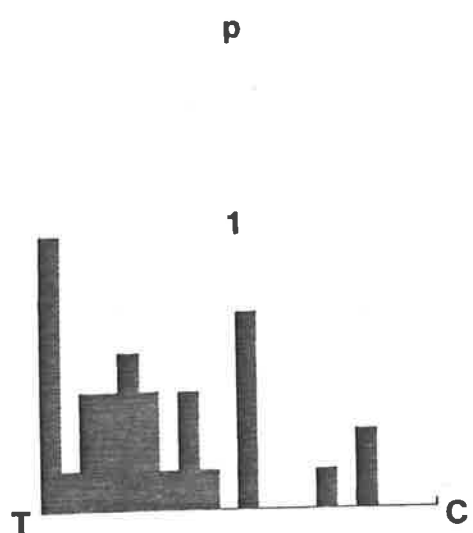


14
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0

Chromosome 1

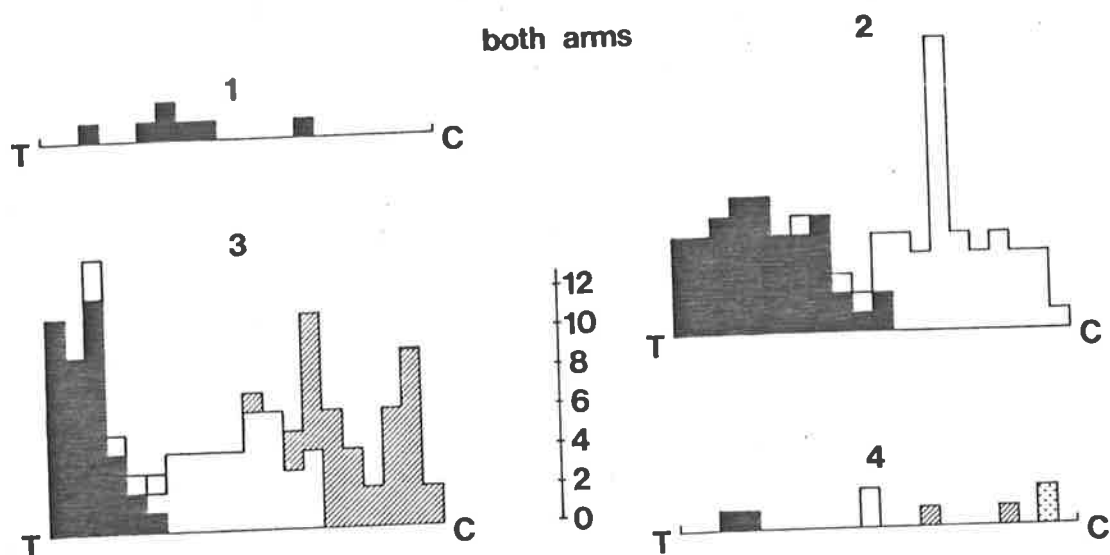


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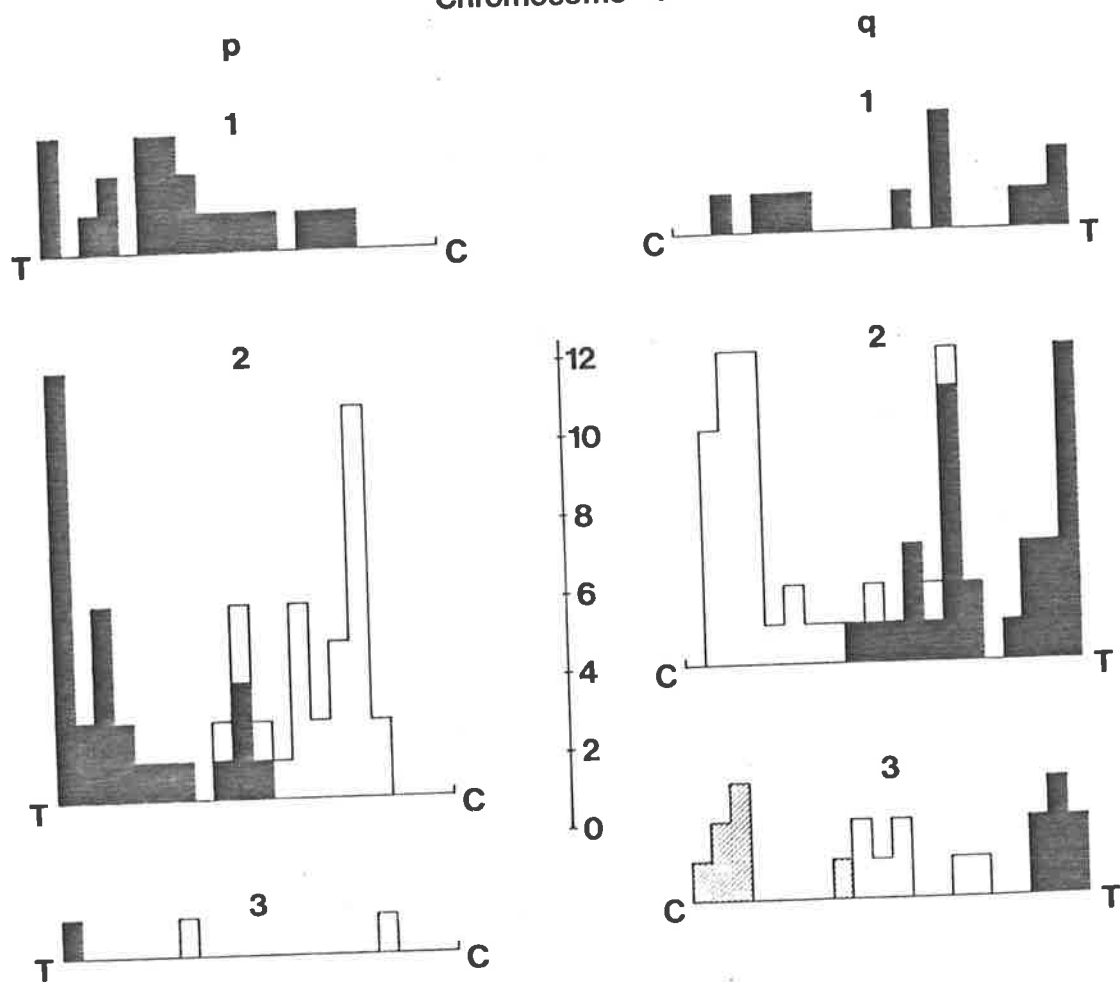


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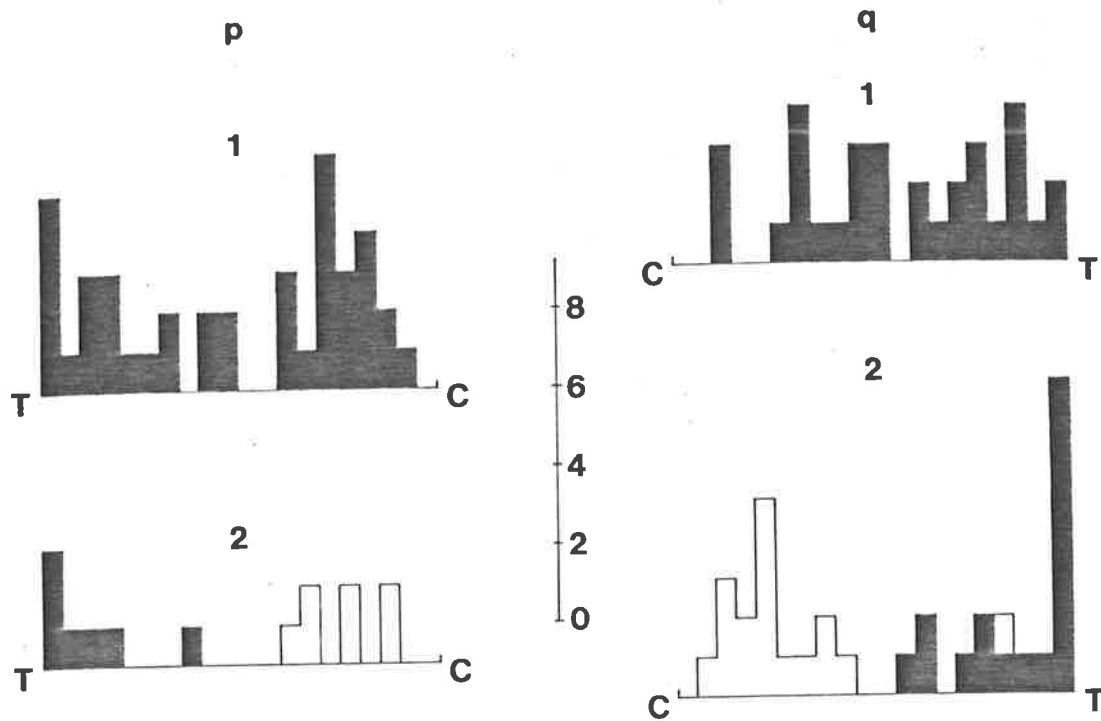
both arms



Chromosome 4



Chromosome 5



Chromosome 6

both arms

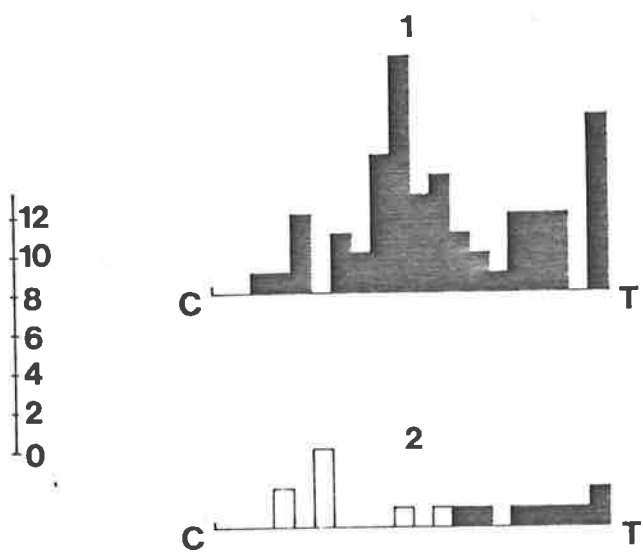
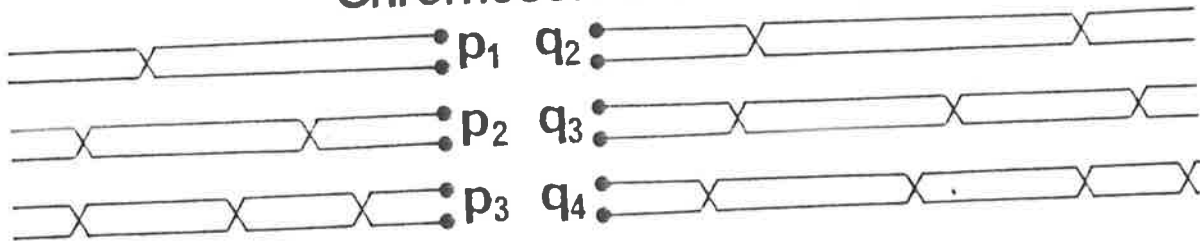


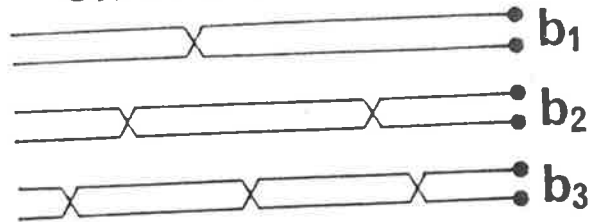
Figure 7.6: The mean positions of chiasmata along the arms of *Sarcophilus harrisii* in the different arm chiasma frequency classes.

In chromosomes 2 and 6 both arms are combined as these chromosomes are metacentric.

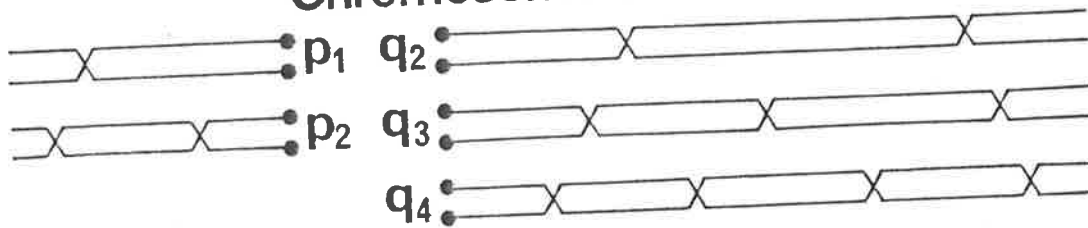
Chromosome 1



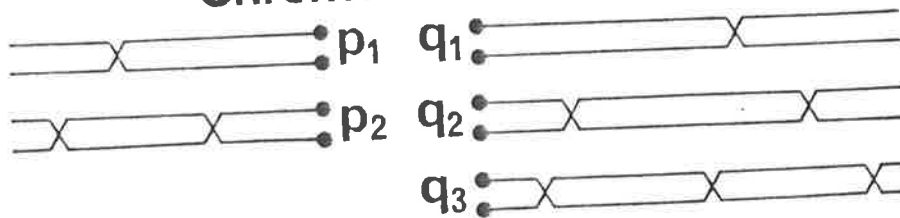
Chromosome 2



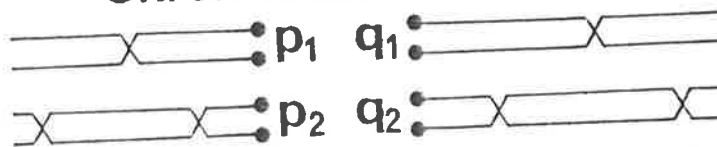
Chromosome 3



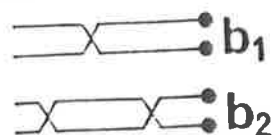
Chromosome 4



Chromosome 5



Chromosome 6



lp, 3p; *I. obesulus* lp, *S. harrisii* 6), and on bivalent arms with higher chiasma frequencies there are more than two areas with higher chiasma frequency (e.g. *S. harrisii* 1q). The presence of more than one peak of chiasma frequency is apparent even when arms with only one chiasma are examined (e.g. *P. gunnii* lp, *I. obesulus* lp, and *S. harrisii* 5p). In contrast to the telomeric regions, the centromeric regions of each bivalent in the three species have a low frequency of chiasmata compared to the rest of the arms (Figures 7.1, 7.3, 7.5).

Examination of the distributions of the chiasmata also reveals evidence of chiasma position interference, since the number of chiasmata on an arm affects the chiasma distribution. There are large differences in the distribution patterns in particular bivalent arms with different numbers of chiasmata. For example, *P. gunnii* 2q arms with one chiasma have a bimodal distribution with peaks at the telomere and at a position approximately half way between the telomere and the centromere, whereas in arms with two chiasmata the second peak of chiasmata is much closer to the centromere, and very few chiasmata at the position at the second peak in one chiasma arms. This effect is apparent on all arms, and is shown in Figures 7.2, 7.4 and 7.6 which illustrate the average positions of the chiasmata in bivalent arms with different arm chiasma frequencies for each species. The average positions of the 1st, 2nd etc. chiasmata from each telomere are shown. In the bivalent arms with a higher number of chiasmata, the 1st chiasmata are closer to the telomere, the chiasmata are closer together, and the last chiasmata are closer to the centromere. This pattern is as expected with negative chiasma position interference whereby the occurrence of a chiasma would decrease the probability of another in the same area.

Comparison of the three chiasma distributions

The distributions of chiasmata along the bivalents of *P. gunnii*, *I. obesulus* and *S. harrisii* were compared. The differences between the karyotypes of *S. harrisii* and the two peramelids outlined before meant that most comparisons had to be limited to the peramelid species. The distributions were compared by constructing contingency tables of the numbers of chiasmata occurring in segments along the length of the bivalents, and the calculation of chi-square value for the homogeneity between the species under comparison. The segments along each bivalent were usually not the twenty equal sized segments used to plot the distributions in the figures, as these had to be pooled in order to maintain each expected number above 5 in each segment (Sokal and Rohlf, 1969). The method of pooling used in each comparison is given with each contingency table (Tables 7.9 to 7.14).

When considering bivalent 1 and bivalent 2 only *P. gunnii* and *I. obesulus* could be compared (Tables 7.9 and 7.10). In both of these bivalents the chi-square analysis indicated that there are no significant differences in chiasma distribution between the two species. The distribution of chiasma along bivalent 3 can be compared for all three species (Table 7.11). Here, the analysis indicates that the three species are homogeneous, with the associated probability between 10 and 5 percent. The segment which contributes most to the rather high (but not significant) chi-square value in this comparison is segment 1-2 on the short arm, contributing a quarter of the value of the calculated chi-square value, but being only less than 5% of the length of the bivalent.

TABLES 7.9 - 7.14: Contingency analysis of the distribution of chiasma along the bivalents of *P. gunnii*, *I. obesulus*, and *S. harrisii*.

The observed numbers had to be pooled in order to make the expected numbers greater than 5 (Sokal and Rohlf, 1969). The method of pooling is indicated in each Table by the segment numbers from 1 to 20, running from each telomere to the centromere. The centromeres are therefore between the two segments number 20.

TABLE 7.9:

Bivalent 1

Bivalent 1		Segment																
Species		1-3	4-6	7-9	10-12	13-15	16-20, 20-19	18-17	16-15	14-13	12-11	10-9	8-7	6-5	4-3	2-1	Total	
<i>Perameles gunnii</i>	Obs.	23	8	20	15	10	8	17	13	18	10	18	9	14	16	36	235	
	Exp.	18.0	7.5	17.5	15.5	9.0	9.0	18.5	16.5	17.0	11.5	20.0	11.5	15.0	16.0	32.5		
<i>Isoodon obesulus</i>	Obs.	13	7	15	16	8	10	20	20	16	13	22	14	16	16	29	235	
	Exp.	18.0	7.5	17.5	15.5	9.0	9.0	18.5	16.5	17.0	11.5	20.0	11.5	15.0	16.0	32.5		
		36	15	35	31	18	18	37	33	34	23	40	23	30	32	65	470	

$$\chi^2_{14} = 8.64; \text{ Prob.} = 0.9 - 0.8$$

TABLE 7.10:

Bivalent 2

<u>Bivalent 2</u>		Segment																Total
Species		1	2-4	5-7	8-10	11-13	14-16	17-20 20-19	18-17	16-15	14-13	12-11	10-9	8-7	6-5	4-3	2-1	
<i>Perameles gunnii</i>	Obs.	15	4	13	15	13	15	9	11	8	20	9	15	8	13	11	24	203
	Exp.	12.97	4.99	14.46	13.97	13.97	14.96	7.98	12.47	9.98	15.96	10.47	11.47	10.97	14.46	11.97	21.95	
<i>Isoodon obesulus</i>	Obs.	11	6	16	13	15	15	7	14	12	12	12	8	14	16	13	20	204
	Exp.	13.03	5.01	14.54	14.03	14.03	15.04	8.02	12.53	10.02	16.04	11.53	11.53	11.03	14.54	12.03	22.05	
		26	10	29	28	28	30	16	25	20	32	21	23	22	29	24	44	

$$\chi^2_{15} = 9.86; \text{ Prob.} = 0.9 - 0.8$$

TABLE 7.11:

Bivalent 3

Species		Segment													Total
		1-2	3-5	6-8	9-11	12-14	15-17	18-20, 20-18	17-15	14-12	11-9	8-6	5-3	2-1	
<i>Perameles gunnii</i>	Obs.	27	5	14	10	7	8	4	24	16	19	21	26	19	200
	Exp.	17.02	11.13	13.09	7.86	5.24	6.87	8.84	28.48	19.64	17.68	18.99	27.17	17.99	215
<i>Isoodon obesulus</i>	Obs.	13	16	15	5	7	4	9	31	25	22	16	33	19	
	Exp.	18.30	11.96	14.08	8.35	5.63	7.39	9.50	30.61	21.11	19.00	20.41	29.21	19.45	196
<i>Sarcophilus harrisii</i>	Obs.	12	13	11	9	2	9	14	32	19	13	21	24	17	
	Exp.	16.68	10.91	12.83	7.79	5.13	6.74	8.66	27.91	19.25	17.32	18.60	26.62	17.56	
		52	34	40	24	16	21	27	87	60	54	58	83	55	611

$$\chi^2_{24} = 34.36$$

$$\text{Prob.} = 0.1 - 0.05$$

TABLE 7.12:

Bivalent 4 (both arms combined)

Species		Segment									Total
		1	2-4	5-6	7-8	9-10	11-12	13-14	15-16	17-20	
<i>Perameles gunnii</i>	Obs.	37	8	20	24	16	10	20	18	8	161
	Exp.	27.45	10.56	23.75	22.70	16.89	11.61	17.42	19.53	11.09	144
<i>Isoodon obesulus</i>	Obs.	15	12	25	19	16	12	13	19	13	
	Exp.	24.55	9.44	21.25	20.30	15.11	10.39	15.58	17.47	9.91	
		52	20	45	43	32	22	33	37	21	305

$$\chi^2_8 = 13.15$$

$$\text{Prob.} = 0.2 - 0.1$$

TABLE 7.13:

Bivalent 5

Species		Segment												Total
		1-2	3-6	7-10	11-14	15-18	19-20, 20-16	15-13	12-10	9-7	6-5	4-3	2-1	
<i>Perameles gunnii</i>	Obs.	12	2	6	7	1	7	10	15	14	10	4	20	108
	Exp.	8.82	5.67	7.87	8.19	6.93	9.45	9.13	11.02	10.08	9.13	6.61	15.10	
<i>Isoodon obesulus</i>	Obs.	6	6	12	12	2	8	9	12	12	11	9	16	115
	Exp.	9.39	6.04	8.38	8.72	7.78	10.06	9.72	11.73	10.73	9.72	7.04	16.09	
<i>Sarcophilus harrisii</i>	Obs.	10	10	7	7	19	15	10	8	6	8	8	12	120
	Exp.	9.79	6.29	8.75	9.09	7.69	10.49	10.15	12.25	11.19	10.15	7.35	16.81	
		28	18	25	26	22	30	29	35	32	29	21	48	343

$$\chi^2_{22} = 52.26; \text{ Prob. } < 0.001$$

TABLE 7.14:

Bivalent 6

Species		Segment							Total
		1	2-20	20-16	15-11	10-6	5-2	1	
<i>Perameles gunnii</i>	Obs.	24	6	8	11	24	18	15	106
	Exp.	23.32	7.95	6.89	14.84	23.32	16.96	12.72	
<i>Isoodon obesulus</i>	Obs.	20	9	5	17	20	14	9	94
	Exp.	20.68	7.05	6.11	13.16	20.68	15.04	11.28	
		44	15	13	28	44	32	24	200

$$\chi^2_6 = 4.60$$

Prob. 0.7 - 0.5

In Table 7.12 the distribution along bivalent 4 in the two peramelids are compared. Both arms had to be combined as the different arms could not be distinguished as the bivalent is meta-centric. The chi-square analysis indicates that the two species have similar chiasma distributions in this bivalent.

Bivalent 5 has the same morphology in all three species, so that the comparison of chiasma distribution involves all three species (Table 7.13). Here there are highly significant differences between the species (Probability <0.001). Examination of Table 7.13 indicates that the largest contribution to the chi-square value comes from the short arm of these bivalents. When similar chi-square analyses were performed on the different arms separately, the short arms alone gives a highly significant difference between species (Probability <0.001) whereas there is not a significant difference between the species in chiasma distribution on the long arm (Probability = $0.7 - 0.5$). The difference between the species on the short arms is mainly caused by *S. harrisii* having many more chiasmata than expected in segments 15-18 (Table 7.3).

Only *P. gunnii* and *I. obesulus* can be compared with respect to chiasma distribution on bivalent 6 (Table 7.14). No significant difference was found between these species (Probability = $0.7 - 0.5$).

In general, then, the limited comparisons that could be made point to little difference between the species with respect to chiasma distribution. Most of these comparisons, however, involve only the two peramelids *P. gunnii* and *I. obesulus* with average cell chiasma frequencies of 12.82 and 16.24 respectively. This only covers part of the range in the $2n = 14$ species. In the two

comparisons which could be performed including *S. harrisii* (with an average cell chiasma frequency of 20.64, so that the range in $2n = 14$ species is almost covered) one comparison had an associated probability of between 0.1 and 0.05, while the other revealed highly significant differences (Probability <0.001). In both of these cases the *S. harrisii* had more chiasmata in interstitial regions than expected, whereas *P. gunnii* had more chiasmata near the telomeres than expected (Tables 7.11 and 7.13). This appears to be a reflection of the negative association between the relative frequency of "terminal" chiasmata and average cell chiasma frequency considered in Chapter 7.1. This effect is also apparent in the comparisons of chiasma distribution involving only *P. gunnii* and *I. obesulus* (Tables 7.9, 7.10, 7.12 and 7.14). In each of the seven telomeric segments, *P. gunnii* has more chiasmata than expected, and *I. obesulus* has fewer. However, this effect is not large enough to give significant chi-square values for these analyses.

7.3 The distribution of chiasmata in complex sex chromosome systems

The distribution of chiasmata along the autosomal portions of the sex trivalents in *Potorous tridactylus*, *Wallabia bicolor*, and *Lagorchestes conspicillatus* were examined. Data ^{were} ~~was~~ collected from forty cells from both a *P. tridactylus* and a *W. bicolor*, while 48 cells were used from the *L. conspicillatus*. The attachment of the autosomal regions to the sex chromosomes in these species makes these regions easily identifiable, and also serves to orientate the regions in the absence of visible centromeres (see Figures 6.3a and b, and Figure 5c in Appendix 2). Descriptions

of these complex sex chromosome systems is given in Chapter 12.1. Since *L. conspicillatus* has two regions of autosomal origin within the sex trivalent, a total of four chiasma distributions are given in Figures 7.7, 7.8 and 7.9. For each species the distributions of chiasmata in each of the different chiasma frequency classes are given, together with the overall distribution of chiasmata.

These distributions are similar to those considered for the autosomes of *P. gunnii*, *I. obesulus*, and *S. harrisii*. The telomeres as well as some interstitial regions have a high frequency of chiasmata. The effect of chiasma position interference is also apparent. The different chiasma frequency classes have different chiasma distributions, and this is especially clear when the average positions of the chiasmata are considered (marked by arrows on Figures 7.7, 7.8 and 7.9).

There are considerable differences between the species with respect to the frequency of chiasmata near the translocation points between the original autosomal and sex chromosome regions. In *P. tridactylus* there is a very low incidence of chiasmata near this junction (Figure 7.7), whereas in *W. bicolor* (Figure 7.8) this region has slightly more chiasmata than expected solely on the basis of length (7 c.f. 5.65). However, the situation in *L. conspicillatus* shows that this is not only a species effect. In this case the two autosomal regions differ quite markedly (Figure 7.9). The longer A_1 segment has an extremely high chiasma frequency near the junction, but in contrast the shorter A_2 segment has no chiasmata near the junction. It is not possible to directly compare the four different regions (e.g. by a contingency table) since they are not homoeologous according to G-banding

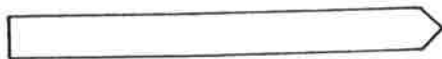
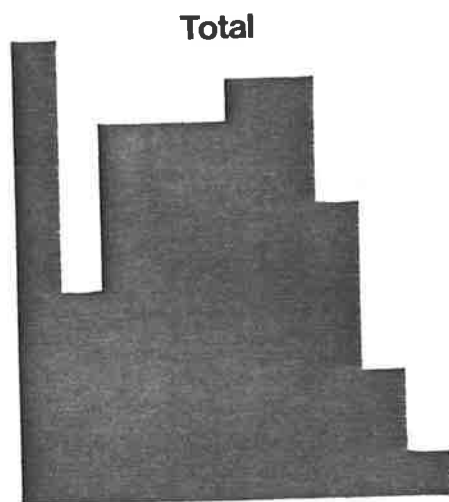
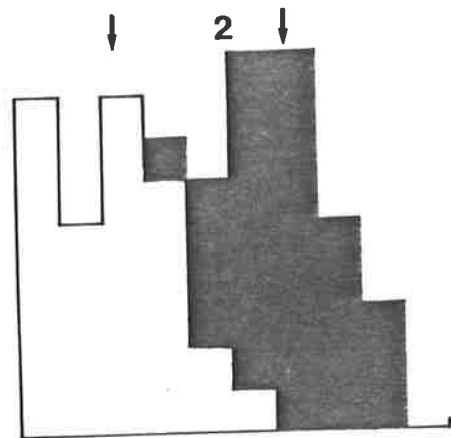
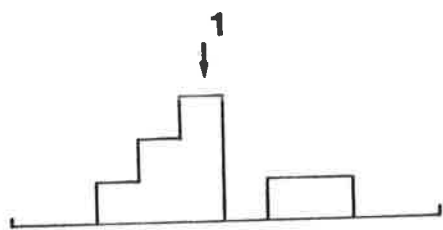
Figure 7.7: The distribution of chiasmata along the autosomal portion of the sex trivalent in *Potorous tridactylus*.

Top. The distributions in the different chiasma frequency classes. The 1st, 2nd, etc., chiasmata from the telomere are differently shaded. The mean positions of these chiasmata are indicated by arrows.

Bottom. The total distribution of all chiasmata.

A schematic diagram of the sex trivalent is underneath the histograms, and has the original sex chromosome regions shaded.

The vertical scale is the number of chiasmata observed in each segment.



X_o

Figure 7.8: The distribution of chiasmata along the autosomal portion of the sex trivalent in *Wallabia bicolor*.

Top. The distributions in the different chiasma frequency classes. The 1st, 2nd, etc., chiasmata from the telomere are differently shaded. The mean positions of these chiasma are indicated by arrows.

Bottom. The total distribution of all chiasmata.

A schematic diagram of the sex trivalent is underneath the histograms, and has the original sex chromosome regions shaded.

The vertical scale is the number of chiasmata observed in each segment.

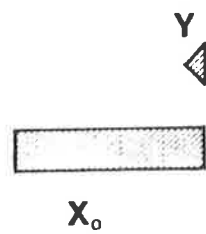
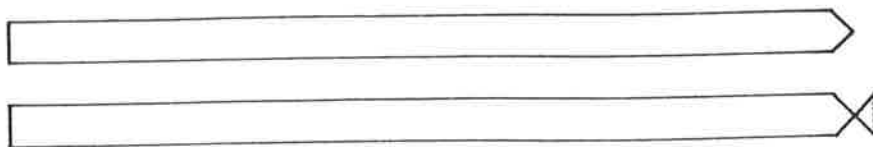
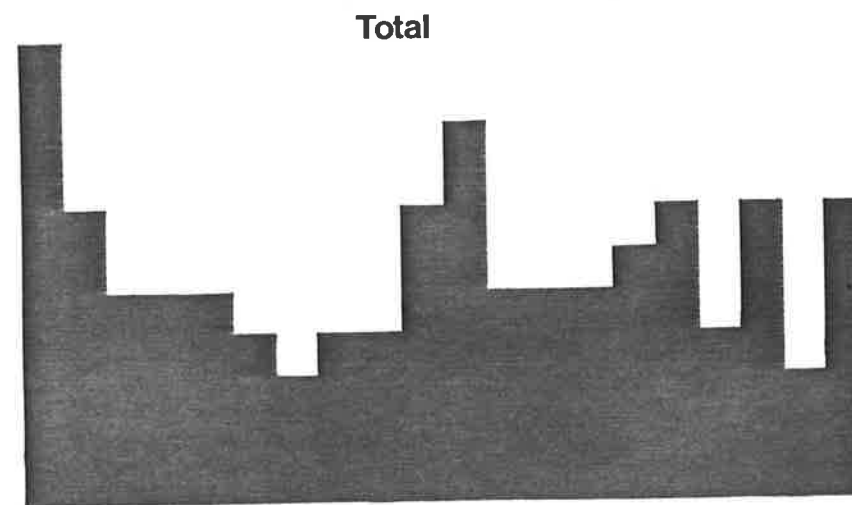
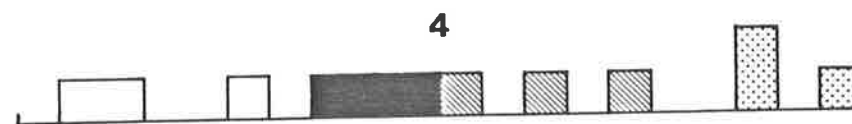
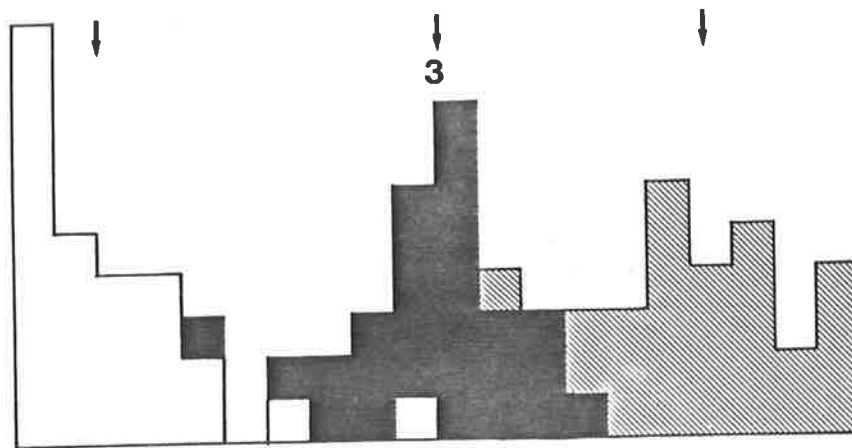
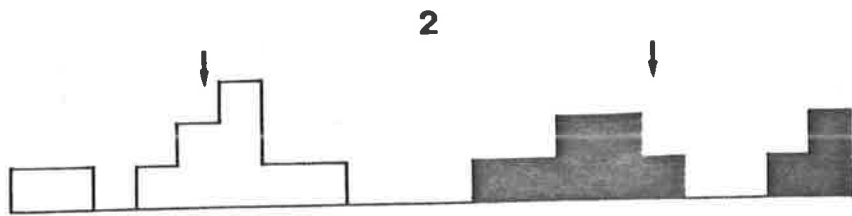


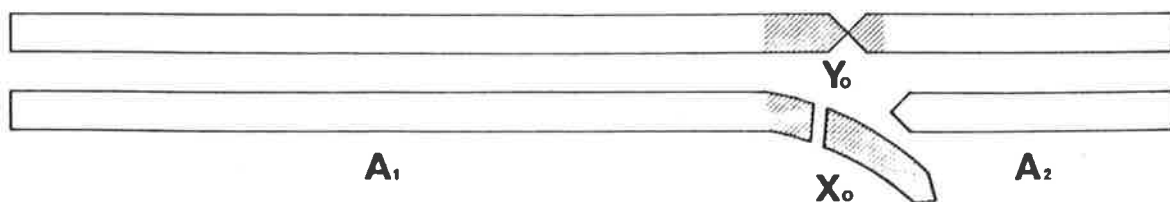
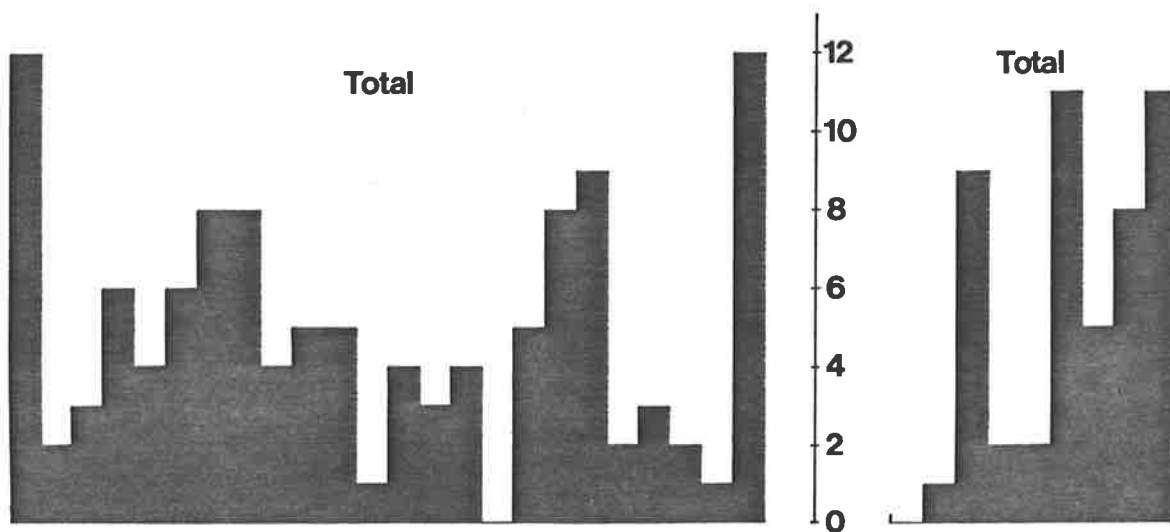
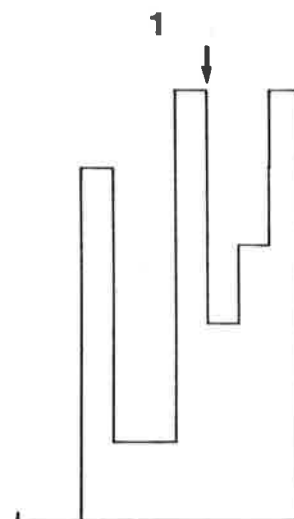
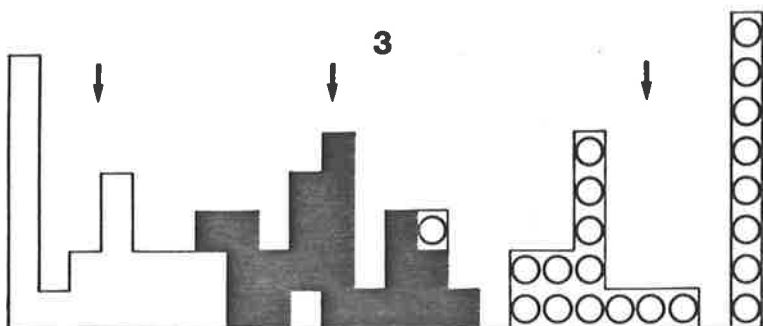
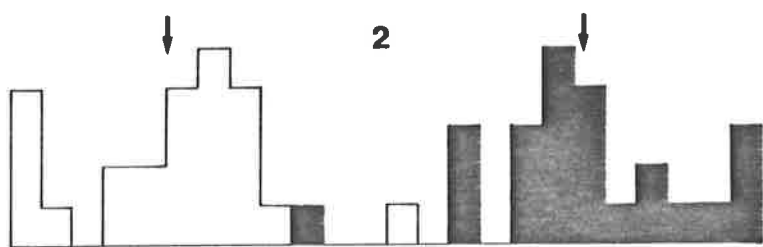
Figure 7.9: The distributions of chiasmata along the two autosomal portions of the sex trivalent in *Lagorchestes conspicillatus*.

Top. The distributions in the different chiasma frequency classes. The 1st, 2nd, etc., chiasmata from the telomere are differently shaded. The mean positions of these chiasmata are indicated by arrows.

Bottom. The total distribution of all chiasmata.

A schematic diagram of the sex trivalent is underneath the histograms, and has the original sex chromosome regions shaded.

The vertical scale is the number of chiasmata observed in each segment.



evidence (Rofe and Hayman, in press), but it is clear that these four regions differ considerably with respect to the frequency of chiasmata in the autosomal regions near the translocation point with the original sex chromosomes.

The positions of the centromeres within these sex trivalents seems to have little relationship to the frequency of chiasmata near the junction. In *W. bicolor*, with a slightly increased chiasma frequency in this region, the centromeres of both the A and the t(XA) chromosomes are in homologous positions and on the t(XA) chromosome it appears to be at the translocation point (Figure 7.8). In *P. tridactylus* only the A chromosome centromere is near the junction as the centromere of the t(XA) chromosome is far from the junction (Figure 7.7), and this region has a low chiasma frequency. In *L. conspicillatus* (Figure 7.9) the A_2 region has both centromeres far from the junction and has an extremely high chiasma frequency, whereas the A_2 region, where the centromere on the telomere of the A_2 chromosome near the junction point, and the centromere of the $t(A_1YA_2)$ chromosome is nearer this junction than the A_1 junction, has a low chiasma frequency. The differences between these junction regions therefore do not appear to be a result of the general low chiasma frequency near centromeres coupled with the varying positions of the centromeres with respect to the translocation points in the four translocations considered here.

7.4 Discussion

The analyses considered in this chapter show that in the $2n = 14$ chromosome species, which have widely varying chiasma frequencies, the chiasmata are distributed between the bivalents according to size. The chiasma frequency variation between species has therefore not been accompanied by large shifts of chiasmata from bivalent to bivalent.

On a finer scale, the distributions of chiasmata in the three $2n = 14$ species studied more intensely show overall similarity to the studies undertaken in other mammalian species (man, Hultén (1974), mouse, Maudlin and Evans (1980)), and in a number of species of grasshopper (Fox, 1973; Shaw and Knowles, 1976; Laurie and Jones, 1981). Chiasma are frequent close to telomeres, and rare near the centromeres. Since there are small C-banding areas around the centromeres in each case considered here (Rofe and Hayman, in press), the rarity of centromeric chiasmata may be due either to an effect of this C-banding material, or to a general centromere effect on chiasma localization. The bimodality of chiasma distributions on bivalent arms with a single chiasmata found in these $2n = 14$ species has also been noted by Maudlin and Evans (1980) who commented that this pattern of distribution seemed to be relatively rare. It is, however, quite common in these three $2n = 14$ species.

The data also indicate that there is no detectable terminalization of chiasmata, and that the different bivalents do not compete for chiasmata. The major agency affecting the chiasma distributions would therefore appear to be interference within bivalent arms, since the interference does not appear to operate

over the centromere. The presence of bimodal distributions in single chiasma arms indicates, however, that a degree of chiasma localization is also affecting the chiasma distributions, as Maudlin and Evans (1980) have noted.

Unfortunately, the level of analysis of the chiasma distributions was limited by the differences in karyotypic features between *S. harrisii* and the two peramelids. The contingency analyses performed only revealed significant differences between the species in one of the two analyses possible including all three species. In both of these comparisons the species with the higher chiasma frequency (*S. harrisii*) had a lower frequency of terminal chiasmata, and this effect, although not significant, was also apparent in the other comparisons. Over the entire range of $2n = 14$ species this negative association between the chiasma frequency and the proportion of terminal chiasmata was highly significant. This would appear to be the result of interference, since extra chiasmata will be localized away from those already present (which are likely to be terminal, due to their high incidence there), so that the overall frequency of terminal chiasmata decreases.

Hayman (1981) has suggested that one reason that complex sex chromosomes have been selected in certain species during evolution is the effect that these translocations have on recombination in the autosomal region, and particularly that this may allow partial sex-linkage of loci near the translocation point due to chiasma localization away from the translocation point. The differences observed here in chiasma frequencies at the translocation points indicate that this does not always occur. However it seems

likely that each of these translocations has involved a shift in chiasma distribution, even if this is not in the same direction in each instance.

CHAPTER 8: MEIOSIS IN FEMALE MARSUPIALS

Meiosis in female eutherian mammals is characterized by (1) the entry by oogonia into meiosis during foetal development, (2) the arrest of meiosis during the diplotene stage to produce "dictyate" or "dictyotene" oocytes which become enclosed in a layer of somatic cells to form follicles, the stage present at birth in most species, and (3) the oocytes being held at this stage until the female matures and until they are hormonally stimulated (a few each oestrous cycle) to proceed through meiosis just prior to ovulation (Baker, 1972; Gondos, 1978; Tsafriri, 1978). The nuclei of dictyate oocytes are extremely large and diffuse so that cytogenetic analysis of these cells is not possible. Consequently, either oocytes from female foetuses, or reactivated oocytes from sexually mature females must be used for cytogenetic observations (Stahl et al., 1975; Röhrborn et al., 1977).

The information available suggests that these same processes occur during oogenesis in female marsupials, except that entry into meiosis as far as the dictyate stage takes place in pouch young females instead of *in utero* as a result of the pseudo-embryonic nature of marsupials at birth (Robinson et al., 1977; Selwood, 1982). Observations have therefore been made on oocytes of pouch young from four species. Additional observations have been made on reactivated oocytes from one of these species, *Sminthopsis crassicaudata*. The early stages of meiosis (leptotene and zygotene) have not been identified in any of the species examined as the quality of the preparations obtained made nuclei of these stages difficult to differentiate from somatic nuclei.

8.1 *Macropus giganteus* ($2n = 16$)

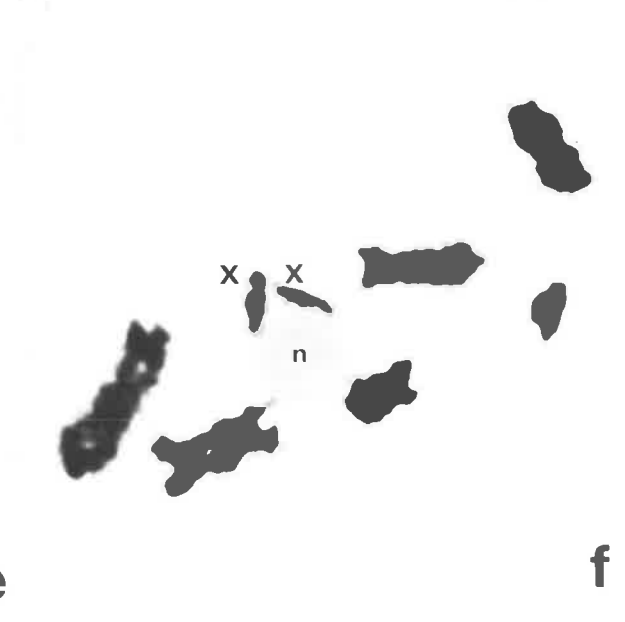
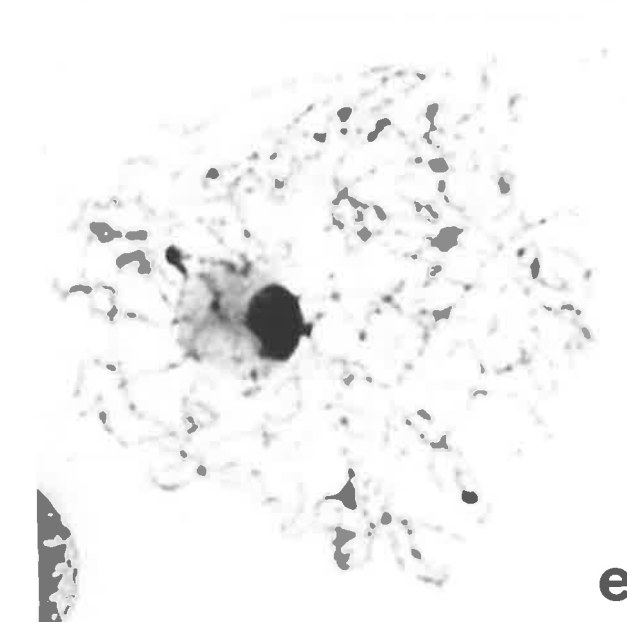
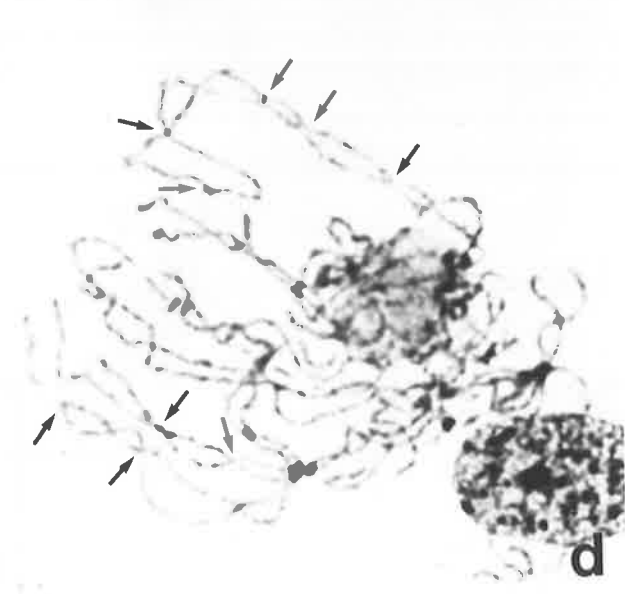
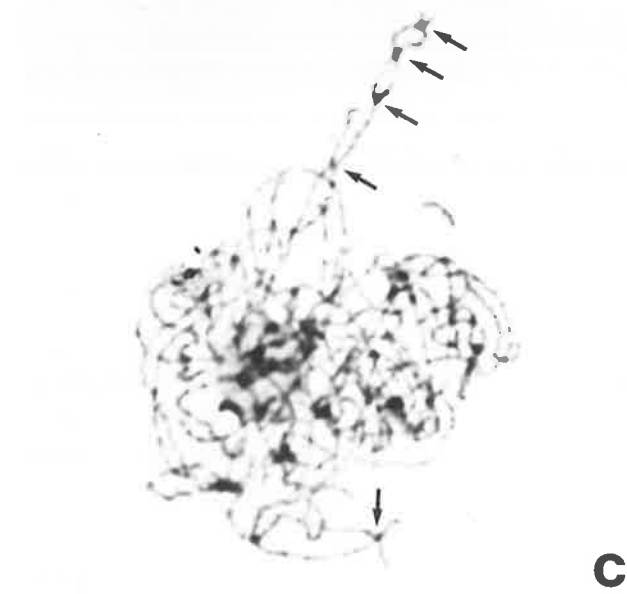
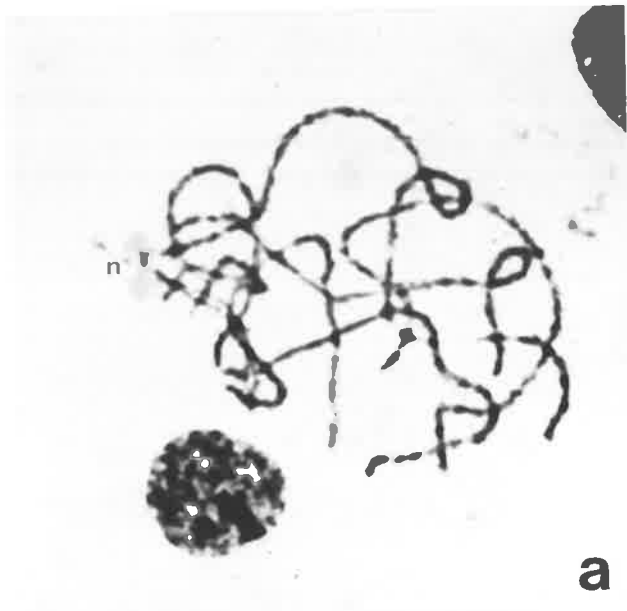
A single female pouch young was examined from this species. It had no fur, the eyes were just starting to open, and the following measurements were taken; weight = 43 grms, head length = 75 mm, tail length = 16 cm, manus length = 25 mm, pes length = 102 mm. Based on these measurements the pouch young was approximately 150 days old (Poole *et al*, 1982; Poole, personal communication).

Oocytes from this animal are shown in Figure 8.1. Most were at the dictyate stage (Figure 8.1e), and were present as follicles, although there were some pachytene and diplotene cells present. The pachytene cells have a prominent nucleolus (Figure 8.1a). The diplotene cells (Figures 8.1c, d) had very long, thin bivalents in which clear examples of chiasmata could be seen, and one nucleolus. Unfortunately, the extreme length of the bivalents resulted in tangling and twisting of the bivalents so that no chiasma count could be made on these cells. The dictyate cells have an extremely large and diffuse nucleus, and a single prominent nucleolus (Figures 8.1e). Only the X chromosomes have nucleolus organizer regions in this species (Rofe, 1979), correlating with the single nucleolus at all stages of meiosis observed here.

Also present in this material was a single cell which appeared to be at metaphase I (Figure 8.1f). It had only six autosomal bivalents, and the two X chromosomes were ~~un~~paired, but both were associated with the single nucleolus. Chiasmata (at least seventeen) were clearly present in the autosomal bivalents. This cell was probably going through the process of "pseudomaturational" (Baker, 1972) whereby oocytes complete the meiotic divisions at an abnormal stage during development.

Figure 8.1: Meiosis in a *Macropus giganteus* female pouch young

- a. A pachytene cell. There is a bilobed nucleolus (n)
- b. A cell during pachytene-diplotene. Separation of the homologues has begun at a number of sites. There is one nucleolus.
- c,d. Diplotene cells. Arrows indicate a number of chiasmata in each. Both have a single nucleolus.
- e. A dictyate oocyte. The nucleus is very large and diffuse, with little detail visible except the single large nucleolus composed of a light and dark region.
- f. A metaphase I cell. An autosomal bivalent is missing, and the two X chromosomes (X) are unpaired, but are both associated with the single large nucleolus (n). Chiasmata are visible in the autosomal bivalents.



8.2 *Petaurus breviceps* ($2n = 22$)

Preparations were obtained from the ovaries of a single female *Petaurus breviceps* pouch young. Observations made during the husbandry of the mother of this animal suggested that the pouch young was approximately 20 days old (D. Golding, personal communication).

Dictyate oocytes were the commonest stage present (Figure 8.3d), but many oocytes at earlier stages of meiosis were also observed, as were cells which resembled mitotic prophase. These latter had very long chromosomes, some with the nucleoli attached (Figure 8.2a). These cells could possibly represent a preleptotene chromosome contraction stage similar to that described in many organisms including foetal mouse oocytes (Hartung and Stahl, 1977), human foetal oocytes (Therman and Sarto, 1977), and *Lilium* microsporocytes (Walters, 1976). The dictyate stage nuclei (Figure 8.3d) were extremely large and diffuse, and had a maximum of three large nucleoli, and many smaller micronucleoli similar to those described from human diplotene oocytes (Stahl *et al.*, 1975; Hartung *et al.*, 1979).

The diplotene oocytes from this species were clearer than those from the *Macropus giganteus* (Figures 8.2c, d; 8.3a, b, c). Chiasmata were clearly present, and numerous. Individual bivalents were occasionally separated sufficiently from the other bivalents to make chiasma counts possible on these bivalents. Bivalents with 2, 3 or 4 clearly chiasmate associations were observed (Figures 8.2 and 8.3). However no individual cell was sufficiently well spread to enable a complete cell count to be made. Nevertheless, a total of 17 separate bivalents from different cells had 49 chiasmata, giving an estimate of 2.88 chiasmata/bivalent or 31.7 chiasmata/cell, since there are

Figure 8.2: Meiosis in a female *Petaurus breviceps* pouch young

- a. A cell during preleptotene chromosome contraction.
A number of nucleoli are indicated (n).
- b. A pachytene cell. There is a distinct "bouquet" arrangement of the bivalents in this cell.
- c,d. Diplotene cells. Chiasmata present in clearly separated bivalents are indicated by arrows.

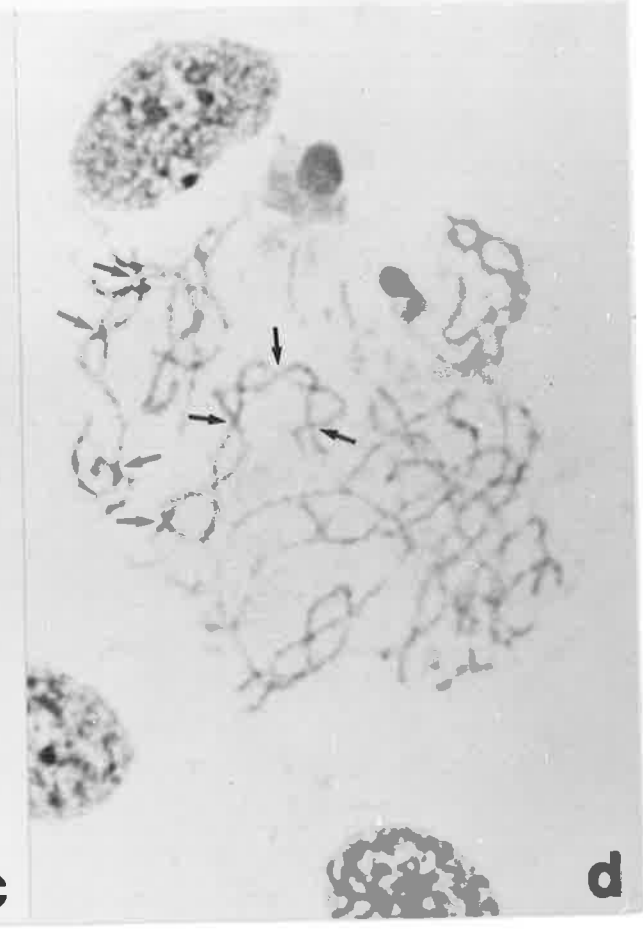
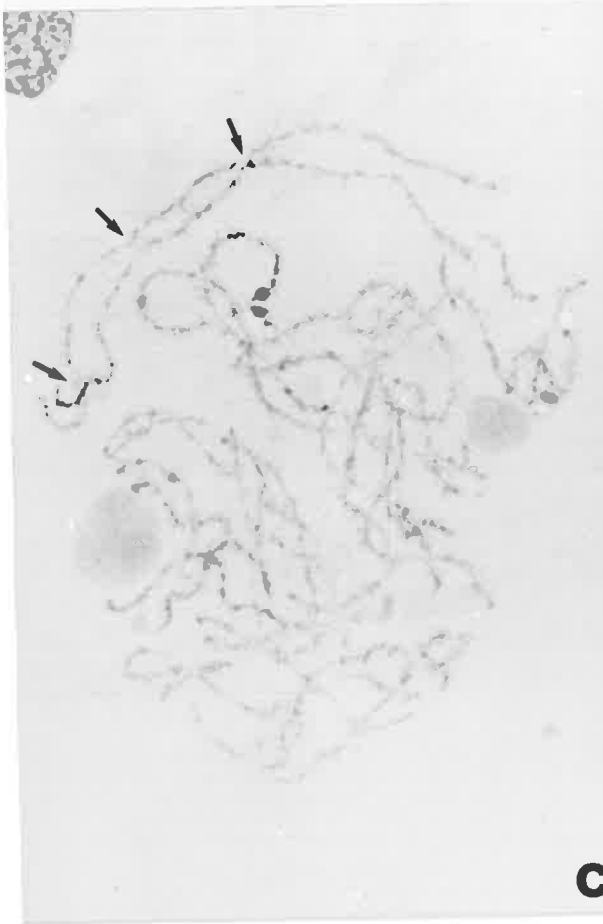
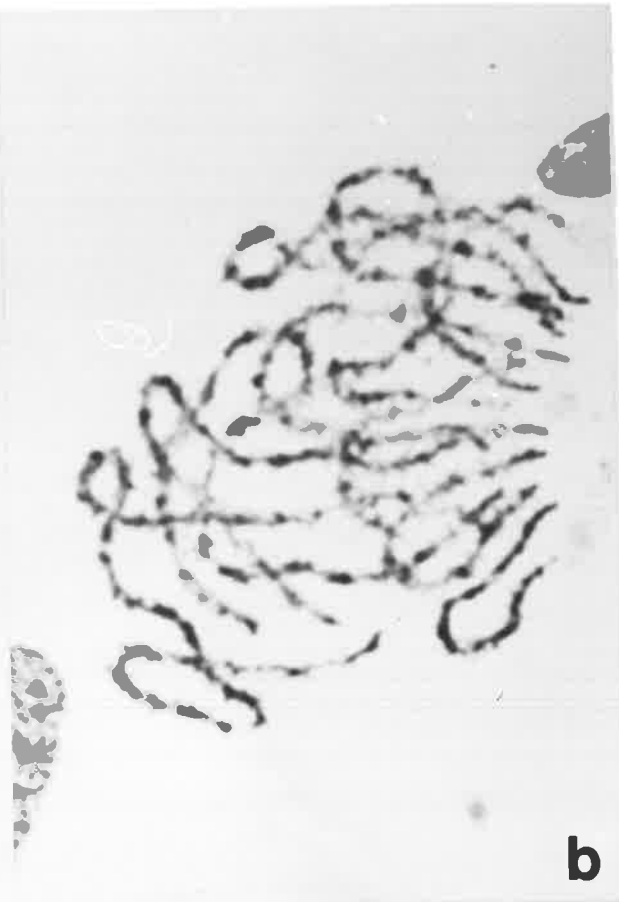
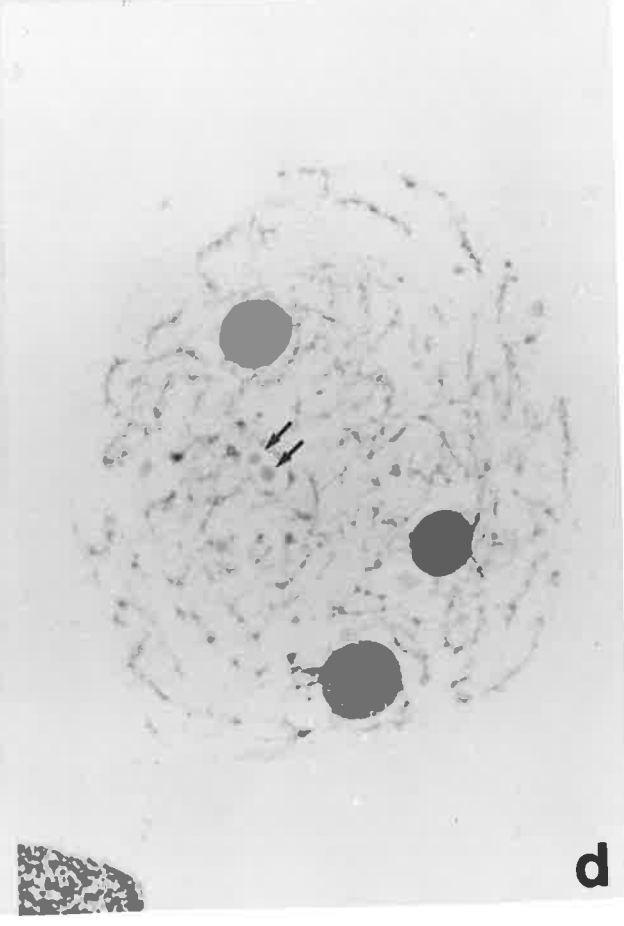
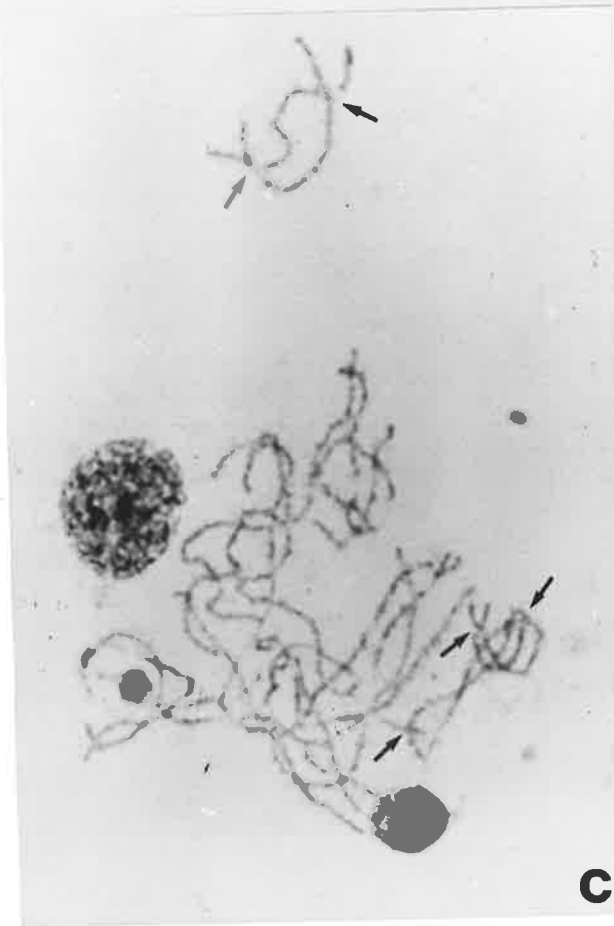
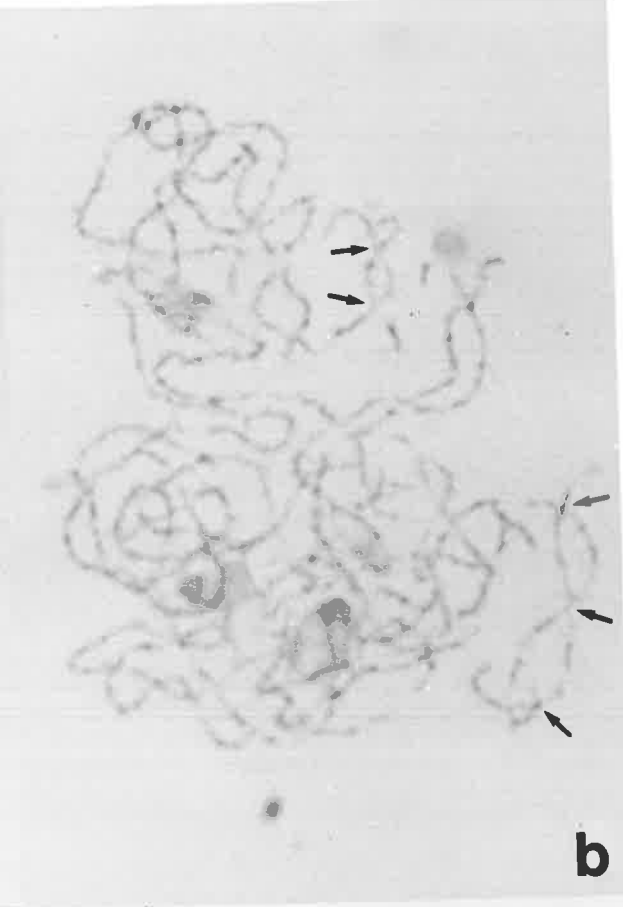
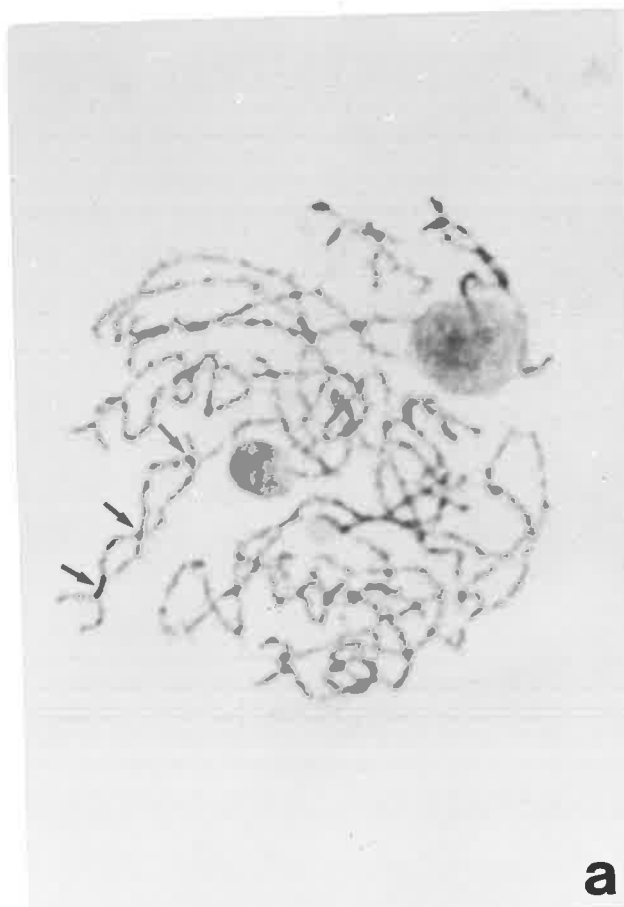


Figure 8.3: Meiosis in a female *Petaurus breviceps* pouch young

- a,b,c. Diplotene cells. Chiasmata present in clearly separated bivalents are indicated by arrows.
- d. A dictyate oocyte. Little detail can be seen apart from the three large nucleoli, and a number of micronucleoli (arrowed).



22 chromosomes in this species. This is a very provisional estimate, since it is based on very few bivalents, the bivalents might not become "free" at random, and there is a difficulty in this material in distinguishing between true chiasmata and twists of the homologues around each other.

8.3 *Trichosurus vulpecula* ($2n = 20$)

A total of twelve female pouch young were examined from this species. Measurements of the weight, head length, tail length, manus length and pes length of each animal were taken and used to obtain an estimate of their respective ages by referring to the age versus "size" nomogram given by Lyne and Verhagen (1957). Details of the animals examined are given in Table 8.1, and examples of the stages found are shown in Figure 8.4.

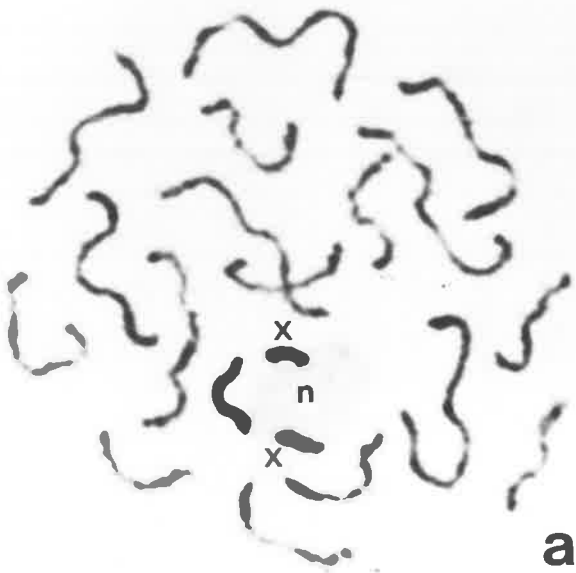
If the presence of the pachytene stage is used as an index of meiotic activity in *Trichosurus*, then it appears to continue over at least sixty days of pouch life, and is thus not highly synchronized, as it is in the mouse and rat (Baker, 1972; Speed, 1982). The cells which appear to be mitotic prophase cells (Figure 8.4a), and are called preleptotene chromosome contraction cells (PLCC) in Table 8.1 are similar to those described previously in *Petaurus breviceps*. The evidence that these might represent a preleptotene stage is in Table 8.1; they appear in the ovary before pachytene cells, but they disappear from the ovary before the later meiotic stages, even though normal mitoses are seen in preparations from the ovaries of these older animals.

TABLE 8.1: Summary of the meiotic activity in the *T. vulpecula* female pouch young examined.

Estimate age (days)	Meiotic stages present
35	no detectable meiotic activity
38	preleptotene chromosome contraction (PLCC)
39	PLCC
58	PLCC, pachytene
60	PLCC, pachytene
69	PLCC, pachytene, diplotene, dictyate, follicles
94	PLCC, pachytene, diplotene, dictyate, follicles
107	- pachytene, diplotene, dictyate, follicles
110	- pachytene, diplotene, dictyate, follicles
112	- pachytene, diplotene, dictyate, follicles
116	- pachytene, diplotene, dictyate, follicles
122	- - - dictyate, follicles

Figure 8.4: Meiosis in female pouch young of *Trichosurus vulpecula*

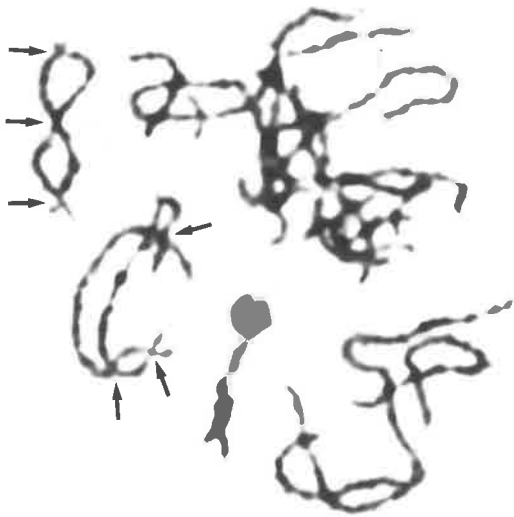
- a. A cell during preleptotene chromosome contraction. The single nucleolus (n) has the X chromosomes (X) and some autosomes associated with it. Taken from a 69 day old pouch young.
- b. A pachytene cell from a 69 day old pouch young.
- c,d,e. Diplotene cells from pouch young aged 69, 69 and 107 days respectively. Chiasmata in separate bivalents are indicated by arrows. The insert in e shows a bivalent which had been widely separated from the rest of the cell.
- f. A dictyate oocyte from a 94 day old pouch young. Two nucleoli of different sizes are visible in the large, diffuse nucleus.



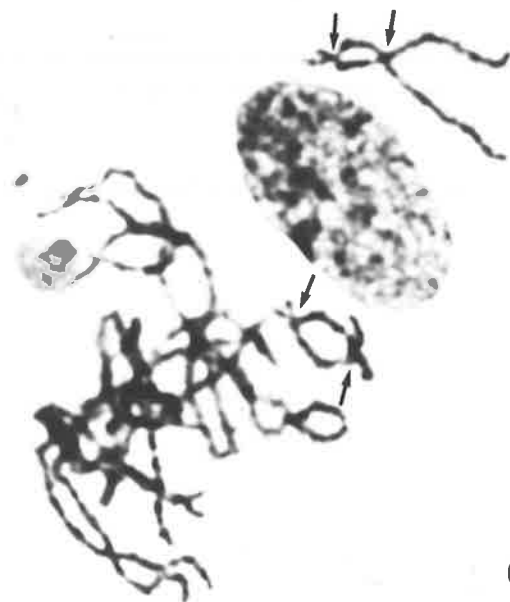
a



b



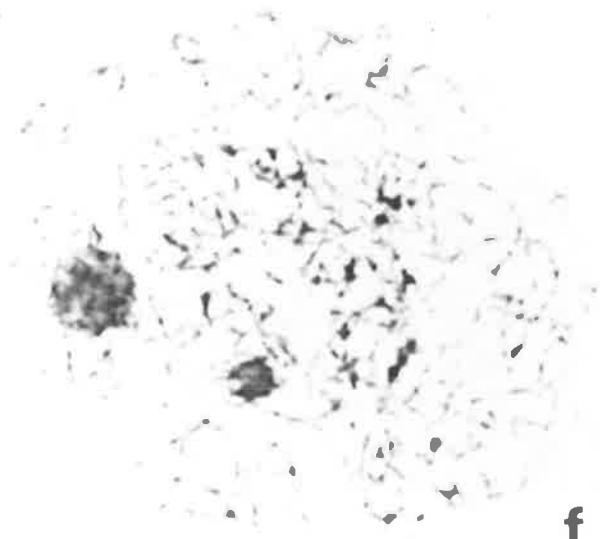
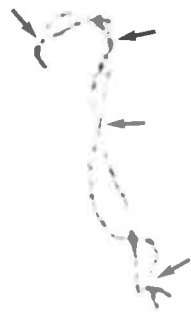
c



d



e



f

The diplotene cells in the *Trichosurus* pouch young also clearly have chiasmata (Figures 8.4c, d, e). Individual bivalents which had separated from the others had 2, 3, or 4 chiasmata, but as in the *Petaurus breviceps* no complete diplotene cell chiasma count could be made. Five separate bivalents had 14 chiasmata giving an average of 2.8 chiasmata/bivalent and an estimate of the cell chiasma frequency of 28/cell ($2n = 20$). The same reservations apply to this estimate as apply to the estimate of cell chiasma frequency in *Petaurus breviceps*.

The dictyate oocyte nuclei usually have 2 nucleoli, one considerably larger than the other (Figure 8.4f), but occasionally has only a single nucleolus. Since *Trichosurus* has nucleolus organizers on the X and a pair of autosomes (Sharp, 1982; Stock and Mengden, 1982), both are probably active in the dictyate oocyte, with occasional nucleolus fusion producing the cells with only one visible nucleolus.

8.4 *Sminthopsis crassicaudata* ($2n = 14$)

Meiosis was examined in both pouch young oocytes and in cultured oocytes from adults of this species. Twenty pouch young females of various ages were examined and details of the animals are given in Table 8.2. The husbandry practices in the Genetics Department colony of *Sminthopsis crassicaudata* enable the age of the animals to be known within 1-2 days. Pouch young are weaned from the mother at 65-70 days (Bennett et al., 1982).

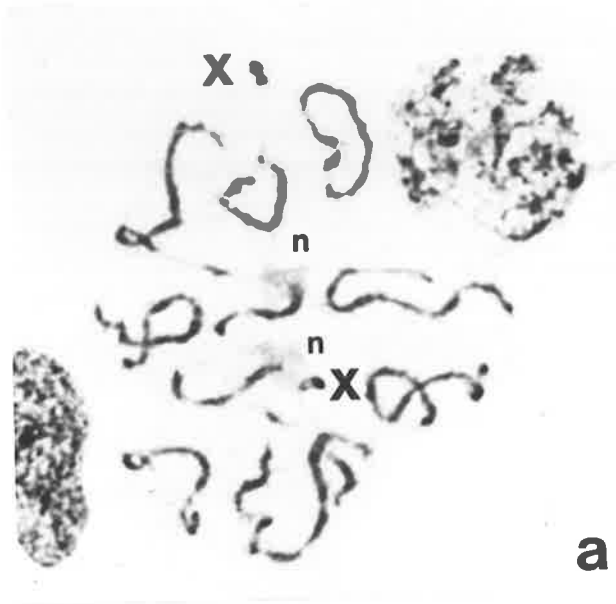
TABLE 8.2: Summary of the meiotic activity in the *Sminthopsis crassicaudata* female pouch young examined. Numbers in brackets indicate the number of animals in which a particular stage was found when it is less than the number of animals examined.

Age (days)	Number examined	Meiotic stages present
32	8	PLCC, pachytene, diplotene (3), dictyate (2)
34	3	PLCC, pachytene, diplotene (1), dictyate
35	2	PLCC, - - dictyate
36	1	pachytene, - dictyate, follicles
38	2	pachytene (1), - dictyate, follicles
41	1	dictyate, follicles
44*	1	dictyate, follicles
48	1	dictyate, follicles
54	1	dictyate, follicles

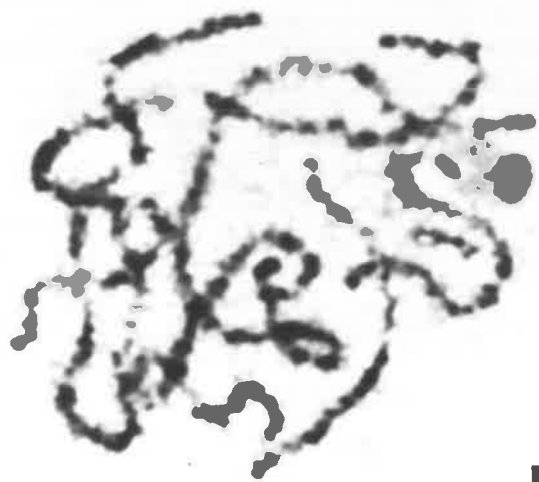
* see text

Figure 8.5: Meiosis in female pouch young of *Sminthopsis crassicaudata*

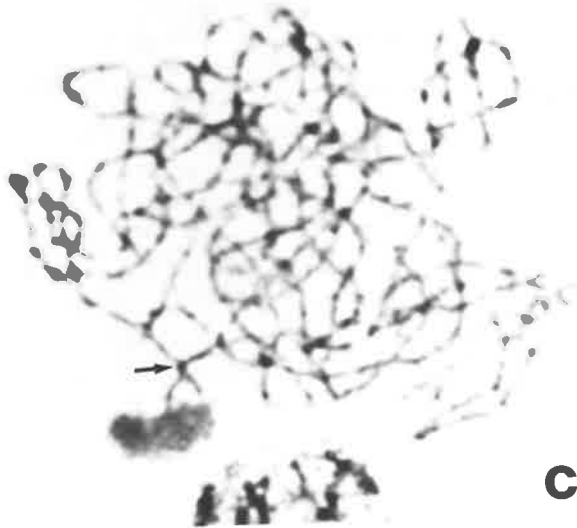
- a. A cell from a 32 day old pouch young during preleptotene chromosome contraction. Two nucleoli are present (n). The X chromosomes are indicated.
- b. A pachytene cell from a 36 day old pouch young. There is a single nucleolus.
- c,d. Diplotene cells from a 34 day old pouch young. Chiasmata are indicated by arrows. The X bivalent in d appears to have one (arrowed) or possibly two chiasmata.
- e. A dictyate oocyte from a 32 day old pouch young. There is a single large nucleolus in the diffuse nucleus.
- f. A metaphase I cell from a 44 day old pouch young. There are 3 unpaired X chromosomes (X).



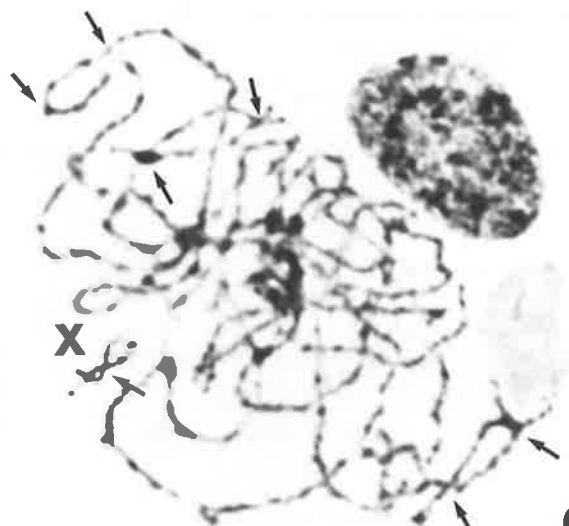
a



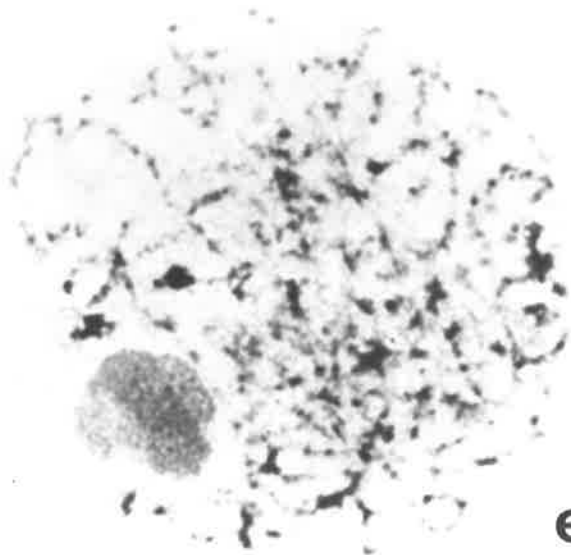
b



c



d



e

.X
a

X.

D.X

f

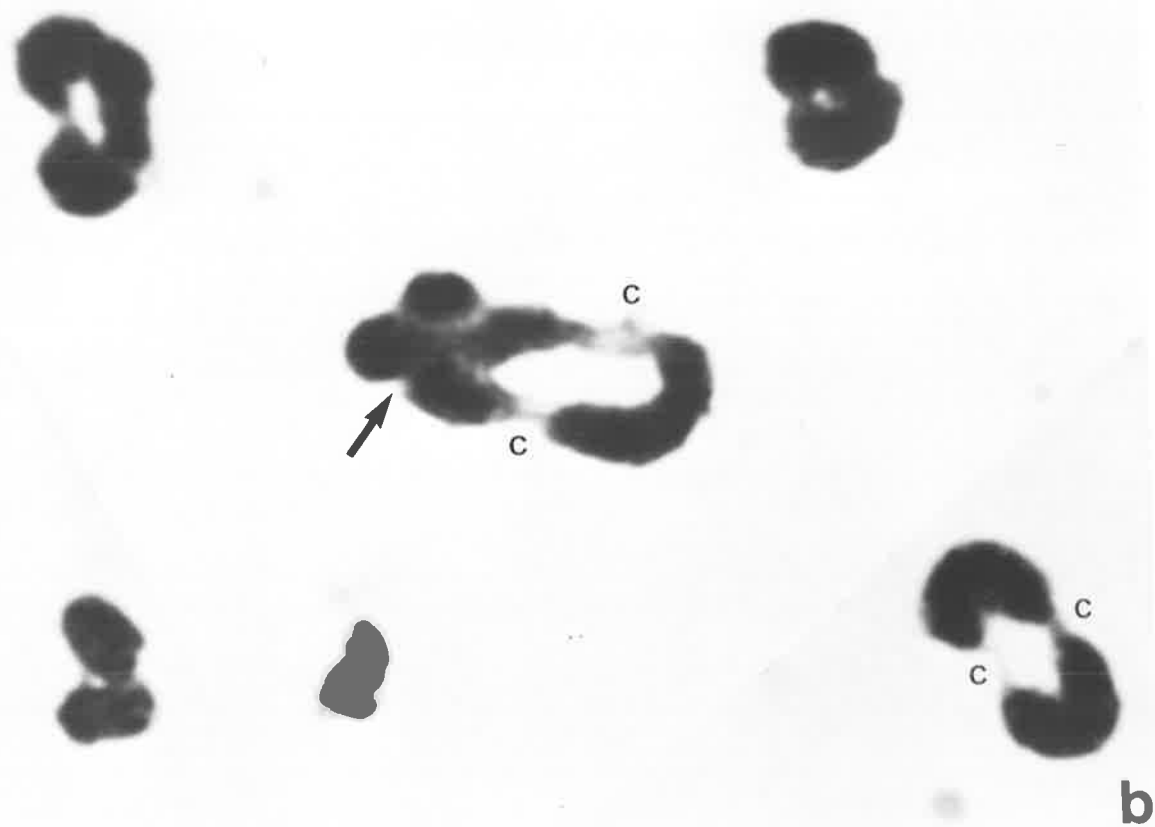
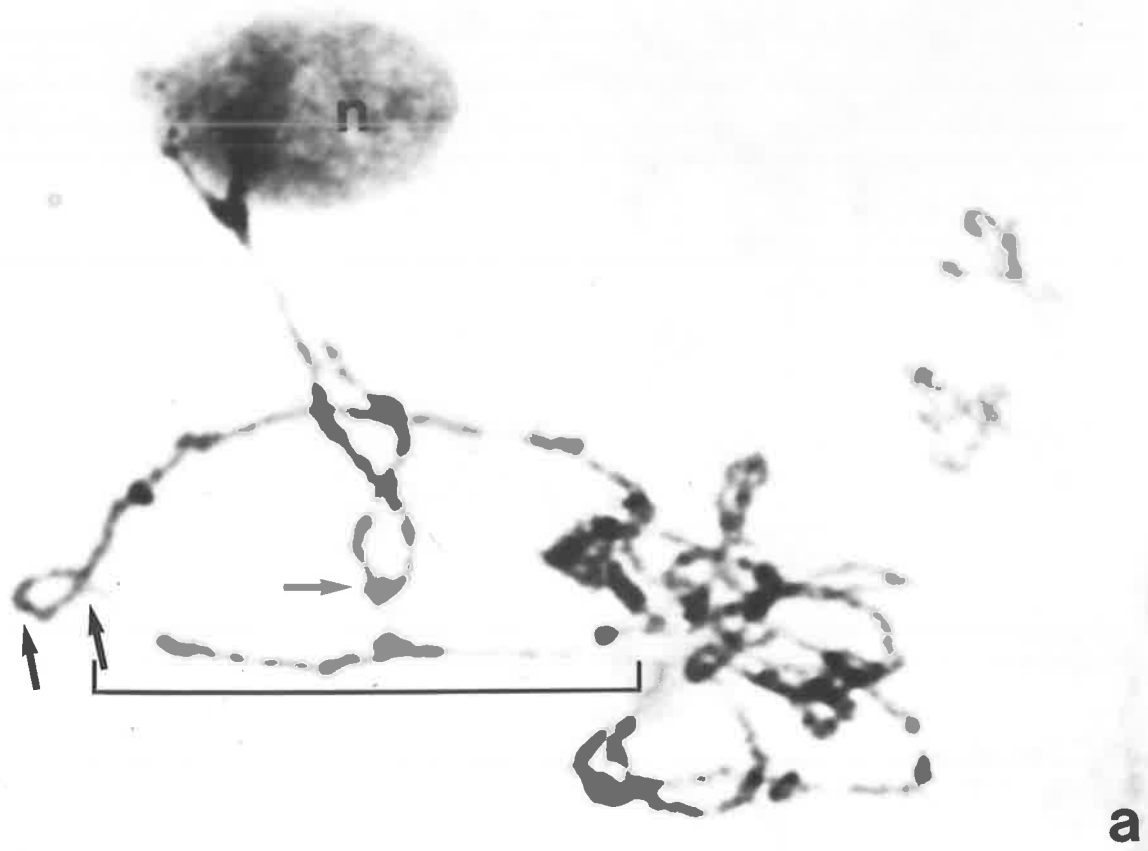
As in *Petaurus breviceps* and *Trichosurus vulpecula*, cells which could represent a preleptotene chromosome contraction stage (Figure 8.5a) were present, but only in the animals less than 35 days old (Table 8.2). Pachytene cells (Figure 8.5b) were present at a low frequency in animals up to 38 days old. These cells had a single nucleolus. Dictyate oocytes were rare in animals 32 days old, but occurred in all older animals and were the only stage present in the animals aged 41 days and over.

Diplotene cells were extremely infrequent and were only found in some animals aged 32 and 34 days. Figures 8.5c and d are examples. The preparations were not as easily interpretable as those in the species discussed above, but chiasmata could be seen. Figure 8.5d was the clearest cell found and here the nucleolus organizing bivalent had at least two chiasmata, and the very small X bivalent had one, possibly two chiasmata. Both the diplotene and dictyate oocytes had only a single nucleolus.

The 44 day old pouch young also had a single cell which appeared to be at metaphase I (Figure 8.5f). This also probably represents a cell which is undergoing "pseudomaturation" (Baker, 1972), similar to the cell observed in the *Macropus giganteus* pouch young. The metaphase I cell was also aneuploid, with three X chromosomes which were all present as univalents. The autosomal bivalents were unusual in that the homologous chromosomes were closely aligned along their entire lengths except where the centromeres appear to have been pulled apart in some bivalents. The cell thus resembles metaphase I of meiosis in achiasmate species (e.g. Figures 13.2, 13.5, and 13.6 in White, 1973). However it is difficult to know what

Figure 8.6: Meiosis in cultured oocytes taken from adult
Sminthopsis crassicaudata

- a. A cell during post-dictyate diakinesis. The bivalent attached to the nucleolus (a) has at least one chiasma (arrowed). The long bivalent segment free from the rest has two clear chiasma (arrowed) and a long segment (bracket) with no chiasmata before the bivalent becomes untraceable within the rest of the nucleus.
- b. A mountage of a widely spread cell at metaphase I. One bivalent is missing from the cell. The attenuated centromeres are marked (c). There is an interstitial chiasma (arrowed) in the largest bivalent present; all other associations appear terminal.



significance to attach to this cell as it was abnormal in two respects, having three X chromosomes, and undergoing "pseudo-maturation".

In the study of adult oocytes in culture, only four oocytes were stimulated to proceed from the dictyate stage (from a total of more than 150 oocytes cultured). Two of these cells were at metaphase II and were therefore of no use for considering chiasmata. One cell (Figure 8.6a) was at very early diakinesis. The nucleolus organizing bivalent was separated from the rest and had at least one terminal chiasma, and possibly another chiasma. A large segment of another bivalent free from the tangle of the rest of the nucleus had a terminal, and a very near terminal chiasma, and a very long interstitial segment free of chiasmata (Figure 8.6a). The other cell was at metaphase I, but was not complete (Figure 8.6b); an autosomal bivalent was missing. The centromeres of the bivalents were greatly attenuated, especially in the largest bivalent present. Only one clear interstitial chiasma could be seen in the cell, the other associations appeared to be terminal chiasmata, although the contraction of the bivalents might mask some details. The type of association in the smallest bivalent in the cell, the X bivalent, was difficult to discern.

8.5 Discussion

The observations reported here of meiosis in oocytes from a number of marsupial species show the essential similarity of this process in both marsupials and eutherians.

The very limited data available from *Trichosurus vulpecula* and *Petaurus breviceps* would possibly indicate that females have a higher chiasma frequency than the males (*T. vulpecula* males 18.14 chiasmata/cell; females 28; *P. breviceps* males 14.9, females ^{31.7}~~31.7~~). Even if the chiasma frequency in the autosomes of the two sexes are the same, one expects a higher chiasma frequency in the female as this sex includes the X-X bivalent which is indistinguishable in its chiasma formation from the autosomal bivalents. However the observed differences are too large to be ascribed to this effect.

The difference observed is in the same direction as that observed by Speed (1977) in the mouse (where its presence depended on mouse strain) and the marmoset monkey, (although Speed did not indicate whether the presence of the X-X bivalent might be the cause of the difference in these cases), but is of a much larger magnitude. This brings into question the accuracy of the chiasma frequency estimates obtained here in females of these two species because of the very small numbers of bivalents observed (and no observations of complete cells), and the high probability that some twists of the bivalents were scored as chiasmata. However, if there is a difference in chiasma frequency between the sexes in these two species, then the females probably have the higher frequency.

Genetic evidence of recombination in both males and females from the Genetics Department *Sminthopsis crassicaudata* colony is available. Studies using the five variable isozyme loci available (3 in one linkage group, and the other 2 in another linkage group) reveal that the females of this species have considerably lower genetic recombination in the measured intervals than the males

(Bennett, personal communication). The observations made here on meiosis in female *S. crassicaudata* are too few to enable a comparison with the males with respect to chiasma frequency, but the two cultured oocytes observed are perhaps suggestive of a high incidence of terminal chiasmata in oocytes.

CHAPTER 9: CHIASMATA IN MARSUPIALS: DISCUSSION

In the marsupials studied the average cell chiasma frequency varies widely from species to species. This variation was greater than the variation observed between animals within a species. The variation within a species could not be related either to seasonal variables, or to the age of the animal.

The marsupials represent the first group of mammals in which there is clear evidence of the evolution of the meiotic recombination component of the genetic system. The constancy of the $2n = 14$ complement appeared to aid this as some evidence was obtained that the chromosome number component of the recombination index was not as highly correlated to other components of the genetic system as was the chiasma frequency component. There were significant associations between the level of meiotic recombination and aspects of the life-history of the different species such as size, reproductive potential and developmental rate. Overall, the species which appeared more "K-selected" were those with higher levels of recombination, and this is in broad agreement with the situation in plants. Never the less, this demonstration does not prove which factors have been important during evolution in determining the level of recombination in the different species.

The evidence on genetic systems depends entirely on partial information; it ignores the possible effects of different chiasma localization between the species, and the possibility of differences between the sexes. Some partial evidence of the distribution of chiasmata in males was obtained, and this pointed to a negative association between the chiasma frequency and the proportion of terminal chiasmata in the different species. This effect would

tend to reinforce the relationship between the level of recombination and life-history characters, since the species with high levels of recombination would tend to have a higher proportion of interstitial chiasmata, which are more effective in terms of genetic recombination within the chromosome than terminal chiasmata.

Much depends on the situation in the females. The evidence obtained here is not sufficient to give any indication of the relative levels of recombination in the two sexes. The important point for the analysis of genetic systems is whether there is a constant relationship in all species between recombination levels in the two sexes (either uniformly higher, lower, or equal), which would not change the associations found by consideration of the data from the males alone. In contrast, a variable, or a reverse trend (i.e. high in male, low in female) in the relationship between male and female chiasma frequencies in the different species would mean that the associations found by consideration of the males alone would not reflect the true situation.

This suggests one avenue, though a difficult one, for further work. Information is needed on recombination in the female sex in order to determine whether the pattern found in the males is maintained. Two groups of marsupials might also be given further attention. Firstly, the macropod species were not covered here as extensively as the non-macropod group and data from further individuals in each species, as well as data from additional species might make trends in recombination level in this group clearer. A group of species not considered here that is potentially informative with respect to the evolution of recombination levels in the genus *Antechinus* (reviewed by Lee et al, 1982). This is a large genus of over a dozen species of small to medium sized

dasyurids, all with $2n = 14$ chromosomes (Hayman and Martin, 1974). A number of these species have the unusual reproductive characteristic of complete male mortality at the conclusion of their first breeding season, whereas the females may breed over a number of years. In other species this does not occur. There are also considerable differences between the species in this genus with respect to litter size (from less than three to more than ten), and even within some species there is geographical variation in litter size (Cockburn *et al*, 1983). In addition some species are seasonal breeders, while others breed all year round. This genus therefore represents a chromosomally conservative, closely related group within which there are quite considerable differences between species in reproductive characteristics.

SECTION TWO

Ultrastructural aspects of meiosis in Australian Marsupials

CHAPTER 10: SYNAPTONEMAL COMPLEXES - LITERATURE REVIEW

The synaptonemal complex was first recognized as a specific structure in electron microscope sections almost simultaneously by Fawcett (1956) in both bird and mammalian spermatocytes, and by Moses (1956) in crayfish spermatocytes. Within a few years of these initial discoveries they were found in the meiocytes of a wide diversity of eucaryotes, and it was demonstrated in species with the meiotic stages spatially ordered within the testis that these structures were only present during the synaptic phase of meiotic prophase (reviewed by Moses, 1968). The final proof that the synaptonemal complex represents an ultrastructural expression of meiotic chromosome pairing came with the reconstructions undertaken by Wettstein and Sotelo (1967) which showed that the number of synaptonemal complexes equalled the haploid number of autosomes, and where a correspondence was found between synaptonemal complex and mitotic chromosome relative lengths (reviewed by Westergaard and von Wettstein, 1972).

The structure and dimensions (apart from length) of the synaptonemal complexes from diverse species with large differences in chromosome size and DNA content are extremely constant, so that it is a structure showing a high degree of evolutionary stability (Westergaard and von Wettstein, 1972). At a higher level of resolution, the main component parts of the synaptonemal complexes (the lateral and central elements) show structural variation between large taxonomic groups. For example, the ascomyceteous fungi are characterized by banded lateral elements, while the insects tend to have a lattice-like central element. More recent work has revealed that some species

have localized variations in lateral element structure, but the significance of these variations is unknown (Gillies, 1981; Zickler and Sage, 1981).

It is also well established that the synaptonemal complex is necessary for meiotic recombination and chiasma formation (reviewed by Westergaard and von Wettstein, 1972; Gillies, 1975). This has been shown by analysis of both natural variants and induced mutants deficient in meiotic recombination. In *Drosophila melanogaster* synaptonemal complexes are present in females (with recombination), but absent from males (which do not have meiotic recombination), and the same situation exists in other dipterans with achiasmate males (Rasmussen, 1973; Moens, 1978a). Homozygous c(3)G mutant female *Drosophila* have no meiotic recombination, and no synaptonemal complexes (Rasmussen, 1975). Additionally, in some species with localized chiasmata the synaptonemal complex only forms in the regions of the bivalents where chiasma are present at later stages of meiosis (Jones and Wallace, 1980; Oakley and Jones, 1982). In other groups, and in other mutants in *Drosophila*, the achiasmatic condition is associated with the presence of synaptonemal complexes (Moens, 1978a; Carpenter, 1979). They are present in both sexes of the silkworm, where the male has chiasmata, but the female is achiasmate (Rasmussen and Holm, 1980). It is thus clear that the presence of a synaptonemal complex does not ensure that recombination will occur, but it is a prerequisite. The studies on the silkworm (*Bombyx mori*) also indicate that a structurally normal synaptonemal complex is associated with chiasma formation, since the lateral elements in the achiasmate female silkworm, although normal early in pachytene, greatly increase in width during pachytene (Rasmussen and Holm, 1980).

There are two basic methods available for the examination of the synaptonemal complexes of whole cells. The first involves serial sectioning for electron microscope observation followed by three-dimensional reconstruction of the nucleus based on these sections (described by Gillies, 1972). Although time consuming, so that it is only practical to analyse a small number of nuclei, this method does preserve the three dimensional arrangement of the chromosomes, and in many cases, the splitting of pachytene into early, mid, and late-pachytene sub-stages can be achieved by taking into account the cellular associations of the analysed nucleus (Moens, 1978b).

The second basic method is that of surface-spreading, based on various modifications of the method originally devised by Counce and Meyer (1973) for grasshopper spermatocytes. Following silver or phosphotungstic acid staining the synaptonemal complexes can be examined with the electron microscope. Silver staining allows the light microscope to be used (Fletcher, 1979), and it is also possible to examine the same cell with both the light and electron microscopes (Dresser and Moses, 1980; Navarro *et al.*, 1981). Surface-spreading has been applied not only to mammalian and insect spermatocytes, but also to mammalian oocytes (Speed, 1982), and to plant pollen mother cells (Gillies, 1981). This method has an advantage over serial-sectioning in that its simplicity enables large numbers of complete nuclei to be analysed, although it does not preserve the three dimensional arrangements of either the chromosomes, or the cells, so that ordering the stages observed cannot be performed directly. Despite this, the large number of nuclei which can be analysed quickly has meant that this method is ideal for obtaining quantitative measurements to enable synaptonemal complex karyotypes to be constructed (e.g. Moses *et al.*,

1977). The method is also extremely useful for analysing individuals heterozygous for chromosome rearrangements, since the heterozygous rearrangements are represented with diagrammatic clarity by the pairing of the lateral elements (Moses, 1977c). Recent observations of the synaptonemal complexes in situations in which the chromosome complement is either not homosequential, or is aneuploid for particular segments, has led to new insights into the process of chromosome synapsis.

When Moses *et al.*, (1977) examined the synaptonemal complexes of mice heterozygous for known translocations (two involving the X and chromosome 7, but with different breakpoints, and one between chromosomes 10 and 18), they found the quadrivalents represented by the pairing of four lateral elements to produce configurations as expected on the basis of cytogenetic theory. Quantitative analysis revealed very close correspondence between the breakpoints of all three translocations as determined from the synaptonemal complexes and those determined from light micrographs of banded mitotic chromosomes.

The behaviour of Robertsonian (centric) fusion/fission heterozygotes was examined by Moses *et al.*, (1979) in hybrids between subspecies of *Lemur fulvus* differing in chromosome number due to Robertsonian rearrangements (as determined by mitotic chromosome banding). In the hybrids the expected trivalent synaptonemal complexes were found, but the trivalent figures had a number of unexpected features. Firstly, the acrocentrics together were slightly longer than the homologous metacentric, and this extra length was due to the short arms of the acrocentrics. Moses *et al.*, (1979) considered these short arms to be heterochromatic, and to be lost from the metacentric produced by fusion of the acrocentrics. Secondly, the short arms of the acrocentrics paired with each other (presumably non-homologously) to form a short segment of synaptonemal

complex after homologous pairing had been completed. Lastly, the centromeres of the acrocentrics were always in a *cis* configuration in the trivalent, on the opposite side of the synaptonemal complex to the centromere of the metacentric. This occurred irrespective of the synaptic state of the short arms of the acrocentrics. This observation suggests that there is a single pairing face on the metacentric lateral element determining the plane of synaptonemal complex formation with the acrocentric lateral elements. As Moses *et al.*, (1977) point out, the synaptic behaviour of homologous bivalents cannot reveal this property. This *cis* arrangement of centromeres, if it were maintained until metaphase, would be expected to predispose the trivalents to balanced segregation. Moses *et al.*, (1977) propose that this contributes to the high fertility observed in the *Lemur* hybrids.

Moses and his colleagues have also examined in detail the synaptonemal complexes of mice heterozygous for other chromosome rearrangements (tandem duplication, paracentric and pericentric inversion), and these studies have been important in revealing additional details of the pairing process.

In a study of a tandem duplication heterozygote (Poorman *et al.*, 1981a; Moses and Poorman, 1981) late zygotene-early pachytene cells displayed the expected synaptonemal complex with lateral elements of unequal length, and the longer lateral element had an unpaired loop of the size and at the range of positions expected on the basis of the mitotic chromosomes. However, as pachytene proceeded (pachytene was divided into sequential substages based on sex vesicle morphology in this and subsequent studies from this group) the difference in length of the lateral elements decreased until in the last half of pachytene, no buckles were observed. This was apparently due to localized

desynapsis near the buckle, equalization of the lengths of the lateral elements, and resynapsis leading to a homomorphic synaptonemal complex through a stage with unequal twists of the synaptonemal complex.

The synaptic behaviour of two different paracentric inversions has also been examined (Poorman *et al.*, 1981b; Moses *et al.*, 1982). In both cases there were synaptonemal complexes at zygotene-early pachytene with a characteristic (in terms of size and position) loop as expected in individuals heterozygous for the particular inversions. As in the tandem duplication, these loops became smaller as pachytene proceeded, and by late pachytene the loops had completely disappeared due to desynapsis within the inversion loop followed immediately by non-homologous resynapsis with the alternate lateral element. The evidence suggested that this desynapsis followed by resynapsis starts at the inversion breakpoints and progresses to the centre of the loop, and that the presence of chiasmata within the inversion loop does not affect this process. A pericentric inversion in the mouse also shows this behaviour (Davisson *et al.*, 1981).

This process was called by Moses and his colleagues "synaptic adjustment" and they viewed chromosome synapsis as having two pairing phases, progressing by the following scheme:

- (1) During zygotene synapsis is strictly by homology ("homosynapsis"), and
- (2) any heterozygosity will lead to local instability in the homosynapsed synaptonemal complex which is resolved by synaptic adjustment involving,
- (3) desynapsis, followed by resynapsis which is not restricted by homology ("heterosynapsis") leading to a simple, straight synaptonemal complex.

However, not all chromosomal rearrangements in different species behave exactly as those described above. Ashley et al., (1981) have described a pericentric inversion in the rat *Psammomys obesus* which does not form a loop in the heterozygous synaptonemal complex, but has this region unsynapsed in early pachytene, and then forms a completely synapsed straight synaptonemal complex (with the centromeres out of alignment) by late pachytene. Synaptic adjustment involving heterosynapsis evidently occurs, but there is no homosynapsis of the inverted segment, and Ashley et al., (1981) suggested that this was because the inversion was so small that topological considerations prevented loop formation.

Different inversions in the mouse also appear to behave differently. Chandley (1982) has examined two overlapping paracentric inversions. One (also studied by Moses et al., (1982)) appeared to behave as described by Moses et al., (1982), while the other did not show any loop formation, and the complex loop formed by the double heterozygote seemed not to be eliminated as approximately 90% of the pachytene cells had the loop. Chandley suggested that the size, position and complexity of the loops and the position of chiasmata might determine the extent of synaptic adjustment. Light microscope observations of an inversion in maize have also revealed that in many cells at both early and late pachytene the relatively inverted segments fail to synapse, and the loops can be retained until diplotene, suggesting that synaptic adjustment is not the rule in maize (Maguire, 1981). Similarly, the study by Gillies (1981) of maize synaptonemal complexes found evidence of asynapsis, and nonhomologous synapsis in inversion heterozygotes, but little evidence of synaptic adjustment. Thus although synaptic adjustment (conversion of one synaptic configuration into another) does

not always occur, heterosynapsis seems to be common to all the cases discussed above. The similarity of the two pairing phases leading to homosynapsis and heterosynapsis with the two types of pairing postulated by Grell (1962) (exchange, and distributive, which can be nonhomologous in the absence of crossing over) is suggestive, but at present the relationship between these systems is unclear. This second pairing phase in meiosis would also appear to be responsible for the high level of non-homologous synaptonemal complex formation in many haploids (maize, Ting, 1973; barley, Gillies, 1974; *Physarum*, and many other species reviewed in Lie and Laane, 1982). In addition, heterosynapsis is common in inversion heterozygotes in some insects, such as grasshoppers and chironomids (Martin, 1967; Nur, 1968; White, 1973; Fletcher and Hewitt, 1978).

Evidence of synaptic adjustment and of two synaptic phases has also come from three dimensional reconstruction of the synaptonemal complexes from a variety of species undertaken at the Carlsberg Laboratory, as reviewed by Rasmussen and Holm (1980), and by Holm, Rasmussen and von Wettstein (1982). In triploid silkworm (*Bombyx*) oocytes at early pachytene there were many trivalents, but by late pachytene these had been converted into bivalents, and the resulting univalents formed many non-homologous synaptonemal complexes (Rasmussen, 1977). Here again, the initial pairing is homologous, synaptonemal complexes with exchanges of pairing partner are unstable, and non-homologous synaptonemal complex formation can occur during pachytene. The instability of synaptonemal complexes with exchanges of pairing partner is further exemplified in autotetraploid *Bombyx* oocytes (Rasmussen and Holm, 1979); at early pachytene there are many quadrivalents which are almost all converted to bivalents by

late pachytene, and only bivalents are seen at metaphase I. In these achiasmate oocytes, then, there is a general drive towards exclusive bivalent formation. Although ultrastructural studies of meiosis in the chiasmatic silkworm autotetraploid spermatocytes have not yet been reported, the quadrivalents remain at high frequency at metaphase I, so that chiasmata appear to stop the synaptic adjustment of quadrivalents into bivalents.

Similarly, in translocation heterozygotes in man (Holm and Rasmussen, 1978), and in the fungus *Coprinus* (Holm et al., 1981), quadrivalents are present in all early pachytene cells, but some late pachytene cells have only bivalents, including partially non-homologous ones. Synaptic adjustment therefore appears not to occur in some cells, and this is possibly prevented by appropriately positioned chiasmata. A high frequency of such chiasmata might also explain the apparent absence of synaptic adjustment in mouse translocation heterozygotes (Moses et al., 1982). In both man and *Coprinus*, there were a few late pachytene cells which had only homomorphic bivalents, and in these cells the lengths of the lateral elements involved appear to have been equalized, as in the tandem duplication in the mouse (Moses and Poorman, 1981), although Holm et al., (1982) have also suggested that the conversion of the quadrivalents to homomorphic bivalents is the result of chromosome breakage and reunion to reverse the original translocations. This extreme view could be tested by constructing synaptonemal complex karyotypes of such cells, since knowledge of the translocation breakpoints should enable the relative lengths and arm ratios of the synaptonemal complexes of the "retranslocated" bivalents to be predicted. With retranslocation one would expect two types of cells, with the retranslocation giving either two normal bivalents, or

two reciprocal translocation bivalents. The exact expectations with synaptic adjustment are unknown, since many of the parameters of this process are unclear, although one might expect that there would be two bivalents of novel size or arm ratio in the synaptonemal complex karyotype.

The importance of the relationship between synaptic adjustment to produce bivalents and the timing of chiasma formation is emphasised by the work of Hobolth (1981) on allohexaploid bread wheat. At zygotene multivalents are common, presumably due to a mixture of homologous and homoeologous synapsis, but by early pachytene only bivalents are present. Hobolth (1981) postulated that the action of the 5B "Ph" gene in wheat is to delay chiasma formation until synaptic adjustment is complete. In addition some mechanism must also ensure that synaptic adjustment of the multivalents leads to homologous bivalents only, and not to homoeologous bivalents.

Examination of synaptonemal complexes has also been important in analysing the nature of pairing between the X and Y chromosomes in mammals. This is reviewed in Chapters 12.1 and 12.2. Related to this are recent studies of chromosomal male-sterility in mammals in which some chromosome aberrations halt spermatogenesis, even though they have no other phenotypic effect. Lifschytz and Lindsley (1972) postulated that the basic chromosomal control mechanism in spermatogenesis consists of X chromosome inactivation during meiosis, and as they suggested, it seems likely that the sterility of sex chromosome autosome translocations is related to an interference in this X chromosome inactivation. However, this hypothesis, *per se*, can not account for the male-sterile effect of purely autosomal rearrangements (de Boer and Searle, 1980). The observations of Forejt and Gregorová

(1977) that translocation multivalents were often associated with the XY pair at metaphase I indicated, however, that the sex chromosomes might be involved even in these cases. They suggested that the multivalents associate with the sex vesicle during pachytene and interfere with X chromosome inactivation at that time. Studies on the synaptonemal complexes of male-sterile tertiary trisomics (de Boer and Branje, 1979), male-sterile translocations (Forejt *et al.*, 1981), and a male-sterile double paracentric inversion heterozygote mouse (Chandley, 1982) indeed reveal a high frequency of association between the heterozygous, or unpaired chromosomal elements and the sex vesicle. This process also appears to occur in man. Johannisson *et al.*, (1983) have found that the extra chromosome 21 in a Down's syndrome male (sterile) is associated with the sex vesicle in almost all cells, and in most cells undergoes similar axial modifications so that it resembles a sex chromosome axis. In contrast, male-fertile rearrangements have few such associations (Forejt *et al.*, 1981; Chandley, 1982). The biochemical results of Hotta and Chandley (1982) also suggest that these associations do indeed lead to a disruption of X chromosome inactivation in the spermatocytes of male-sterile mice since in these mice X-linked enzymes show abnormal activities in these cells whereas autosomally determined enzymes do not.

The association of the male-sterile rearrangements with the sex vesicle would appear to be another example of the non-homologous pairing that can occur during pachytene, since these rearrangements often produce regions of unsynapsed lateral elements (Forejt *et al.*, 1981).

Synaptonemal complex studies have also revealed a structure, the "recombination nodule", which indirect evidence has suggested is an ultrastructural expression of genetic exchange. These electron-dense nodes, which vary slightly between species in size, shape, and exact position relative to the other components of the synaptonemal complex (Moens, 1978a), were first suggested to be the sites of genetic exchange by Carpenter (1975). This hypothesis has received extensive support (Moens, 1978a; Holm et al., 1982), since in all species examined the number and distribution of the nodules is correlated with the number and distribution of crossovers (e.g. Solari, 1980), in recombination-deficient mutants of *Drosophila* the residual crossovers and nodules are similarly correlated (Carpenter, 1979), and the nodules also appear to be sites of pachytene DNA synthesis (Carpenter, 1981), as expected (Stern and Hotta, 1978).

How the recombination nodules and the synaptonemal complexes are related to chiasmata visible at diplotene is unclear, although Moens (1978a, b) has suggested that the recombination nodules are converted into cross-connections between the lateral elements, and in some cases, remnants of the synaptonemal complex appear to be associated with chiasma sites (Holm et al., 1982).

CHAPTER 11: MATERIALS AND METHODS

Synaptonemal complex spreads were made for examination with either the light microscope or the electron microscope. Preparations were usually made as soon as possible after testis biopsy. However, the preparations of *Macrotis lagotis* described in Chapter 12.1 were obtained after the testis tubules suspended in culture medium (see below) had been transported by air from Perth, Western Australia, to Adelaide, so that the preparations were made approximately 12 hours after biopsy. This had little or no effect on the quality of the preparations obtained.

11.1 Light microscope preparations

For light microscopy, preparations were made as described by Fletcher (1979). The following solutions were used.

- (a) Spreading solution, 0.2M sucrose in ddH_2O , freshly filtered.
- (b) Fixative, 4% paraformaldehyde and 3.4% sucrose in ddH_2O . This was made up by dissolving the sucrose in 70mls ddH_2O , adding the paraformaldehyde and 30mls ddH_2O and slowly warming in a water-bath to 60-70°C with constant stirring. Six or seven drops of 1N NaOH were then added and stirring was continued until the solution cleared. After cooling to room temperature, the solution was adjusted to pH8.5-9 with sodium tetraborate buffer (Ajax Chemicals).
- (c) Photoflo. A stock solution of 4% Kodak "Photoflo 600" in ddH_2O was made up, and just before the spreads were made a 0.4% solution was prepared, and adjusted to pH8.5-9 using the borate buffer, and then filtered.

Suspensions of testis cells were made by macerating approximately 0.5cm^3 of the tubules with curved forceps in 5mls RPMI 1640 culture medium supplemented with 20% foetal calf serum. After centrifugation at 500r.p.m. for 5 minutes the pellet was resuspended in a small amount of the remaining medium. The spreads were obtained by touching a drop of the cell suspension from a pasteur pipette onto the surface of spreading solution which had been poured into watchglasses. These had been sprayed with matte black enamel paint (British Paints) to make them more water repellent and to give a dark background.

After spreading for 30 seconds the cells are picked up with clean microscope slides which had been rubbed with silk to give them a static charge. The slides were then left lying on the bench for 5-10 minutes to allow the cells to settle, and then fixed in a Coplin jar for 10 minutes. After the slides were drained of excess fixative, they were dipped in the Photoflo in a Coplin jar for 30 seconds, and then allowed to air-dry in an upright position.

The slides were stained by placing a few drops of freshly prepared and Millipore ($0.22\ \mu\text{m}$) filtered 70% silver nitrate in ddH_2O on the slide, covering the solution with a coverslip and sealing the edges with rubber gum. After overnight incubation of the slides at 60°C in the light, the coverslips were rinsed off with ddH_2O , and the slides were then stained for 1 minute with 5% Gurr's R66 Giemsa in Sørensen's pH6.8 buffer, rinsed and air-dried. They were then mounted with Gurr's Depex.

11.2 Electron microscope preparations .

Spreads for electron microscopy were made by a modification of the method of Solari (1980), derived from that described by Moses (1977a), which is in turn a modification of the original spreading method devised by Counce and Meyer (1973) for grasshopper spermatocytes.

The method was very similar to that outlined above for light microscopy, with the following exceptions.

- (1) The spreading solution was 0.5% NaCl in ddH₂O.
- (2) The fixative did not contain sucrose, but had 0.05% SDS (sodium dodecyl sulphate) added.
- (3) The microscope slides were coated with a plastic film. This was achieved by dipping the slides into a solution of Gurr's Depex mounting medium dissolved in xylene. (Depex is a mixture of polystyrene and a plasticizer dissolved in xylene). The ratio of Depex to xylene was adjusted to produce a film of silver interference colour (approximately seven drops of Depex in 50mls xylene). The slides were dipped in this solution and hung up to air-dry on small clips.
- (4) Because the slides were coated with plastic, no static charge was needed in order to pick up the cells, and the slides were transferred immediately after picking up the cells into the fixative.
- (5) Staining was with ethanolic phosphotungstic acid. This was prepared by mixing 3 (95% ethanol) : 1 (4% phosphotungstic acid in ddH₂O). This mixture was then Millipore (0.22 μ m) filtered, and the slides were stained with this mixture for 7 minutes, and then rinsed with copious amounts of 95% ethanol, and air-dried.

The plastic film with the adherent cells was then removed from the slide by floating the film off onto ddH₂O in a petri dish after scraping the plastic from the edges of the slide with a scalpel blade. Copper-rubidium EM grids (100 mesh) were then placed over the film, and the film and grids were lifted from the water surface with a lens tissue. After air-drying, the grids could be examined.

Electronmicrographs were obtained with a JOEL 100S transmission electron microscope at 60 kV, set up with the smallest objective aperture available (20 μ m) to give high contrast images. The micrographs were taken on Kodak Electron Microscope film 4489 (3 $\frac{1}{4}$ x 4 in.) or on Eastman Fine Grain Release Positive film 5302 (35mm).

Chapter 12.1 has been published as:

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It is reproduced here without alteration, except those necessary to fit it into the general thesis style.

CHAPTER 12: SEX CHROMOSOME PAIRING

12.1 Sex chromosome pairing during male meiosis in marsupials

Introduction

The sex chromosomes in male eutherian mammals associate as a "sex vesicle" or "XY body" during early prophase of meiosis (Sachs, 1954). Usually the axes of the X and Y pair at one end only to form a segment of synaptonemal complex which is indistinguishable from autosomal synaptonemal complex (Solari, 1974a). This "pairing" or "homologous" region has been observed in at least thirteen eutherian mammals (Table 12.1). Among eutherian species only two exceptions to this pattern of X-Y pairing have been described. Solari and Ashley (1977) found that the sand rat, *Psammomys obesus*, forms a sex vesicle in which the axes of the X and Y chromosomes do not pair or form any synaptonemal complex. Instead, the ends of the axes form a terminal (i.e. end to end) association. The other exception is the southern pygmy mouse, *Baiomys musculus* (Pathak et al., 1980). In this species, the X and Y axes within the sex vesicle do not associate with each other.

The behaviour of the sex chromosome axes during meiosis has been described in only three species of marsupial mammals. In *Monodelphis dimidiata*, Solari and Bianchi (1975) found no synaptonemal complex between the X and Y axes. Similarly, no complex forms in the Virginia opossum, *Didelphis virginiana* (Pathak et al., 1980), where the axes of the sex chromosomes within the sex vesicle form various loose associations between their ends. The situation in

TABLE 12.1: Species in which a "pairing region" has been found

Order	Species	Reference
Rodentia	<i>Mus musculus</i>	Pathak et al. (1980)
	<i>Rattus norvegicus</i>	Pathak et al. (1980)
	<i>Sigmodon fulviventer</i>	Pathak et al. (1980)
	<i>Cricetulus migratorius</i>	Solari (1974b)
	<i>Cricetulus griseus</i>	Moses (1977b)
	<i>Mesocricetus auratus</i>	Moses and Counce (1974)
	<i>Millardia melitade</i>	Nanda and Raman (1981)
	<i>Akodon azarae</i>	Oliveira et al. (1979)
Artiodactyla	<i>Bos taurus</i>	Pathak and Hsu (1979)
	<i>Muntiacus muntjak</i>	Pathak and Lin (1981)
Primates	<i>Lemur spp.</i>	Moses et al. (1979)
	<i>Papio sp.</i>	Pathak and Hsu (1979)
	<i>Homo sapiens</i>	Pathak et al. (1980)

the complex sex chromosome system ($t(XA_1), t(A_2YA_1), A_2$) of *Lagorchestes conspicillatus* has been described by Hayman and Sharp (1981b). No sex vesicle is observed, and no synaptonemal complex forms between the axes of the original X and Y regions which are part of the sex chromosome trivalent. Thus marsupials apparently deviate from the pattern which the majority of eutherian mammals follow. Observations have therefore been made on the relationship between the X and Y chromosome axes within the sex vesicle of a number of marsupial species to see if this apparent difference between the marsupials and the eutherians is consistent.

Materials and Methods

A modification of the method of Evans *et al.* (1964) was used for conventional cytological preparations. Testis tubules were initially macerated in 1.5% tri-sodium citrate for 20 minutes, and the resulting cell suspension was then exposed to 0.075M KCl for 10 minutes. After centrifugation, the cells were fixed with 3 methanol:1 acetic acid containing a trace of chloroform. Air-dried preparations were made after 1-2 days, and were stained with 10% Giemsa for 1½ minutes.

Surface spread synaptonemal complexes were examined both with the light microscope (Fletcher, 1979) and the transmission electron microscope. Preparations for the electron microscope were made as described by Solari (1980) with the following modifications;

- (1) the glass microscope slides were coated with Gurr's Depex diluted in xylene to produce a film of silver interference colour,
- (2) the paraformaldehyde fixative contained 0.05% SDS.

TABLE 12.2: The species examined in this study

Superfamily	Species	Method used	
		Light microscope	Electron microscope
Dasyuroidea	<i>Sminthopsis crassicaudata</i> [†]	+	-
	<i>Sarcophilus harrisii</i> [†]	+	+
	<i>Dasyurus viverrinus</i>	-	+
Perameloidea	<i>Isodon obesulus</i> [†]	+	+
	<i>Perameles gunnii</i>	+	-
	<i>Perameles nasuta</i>	+	-
	<i>Macrotis lagotis</i> *	-	+
Wombatoidea	<i>Lasiornhinus latifrons</i>	+	+
	<i>Phascolarctos cinereus</i>	-	+
Phalangeroidea	<i>Trichosurus vulpecula</i> [†]	+	+
	<i>Pseudocheirus peregrinus</i>	+	-
	<i>Petaurus breviceps</i> [†]	+	+
	<i>Thylogale billardieri</i>	+	-
	<i>Macropus rufus</i>	-	+
	<i>Macropus giganteus</i>	+	-
	<i>Macropus rufogriseus</i>	-	+
	<i>Macropus parryi</i>	+	-
	<i>Macropus eugenii</i> [†]	-	+
	<i>Macropus parma</i>	+	-
	<i>Potorous tridactylus</i> *	+	-
	<i>Wallabia bicolor</i> *	-	+
	<i>Lagorchestes conspicillatus</i> * [†]	+	+

* species with complex sex chromosome systems

† more than one individual animal examined

The species examined and the methods used on each are given in Table 12.2. They have been placed into superfamilies according to Kirsch and Calaby (1977).

Results

Conventionally stained cytological preparations

The pairing behaviour of the sex chromosomes of the majority of species studied is identical when observed in conventional cytological preparations stained with Giemsa. In *Trichosurus vulpecula* (used here as an example), the X and Y chromosomes are present as a distinct sex vesicle during pachytene (Figure 12.1a). Very little detail can be seen within the sex vesicle at this stage. It is still present in all diplotene cells, but by diakinesis and metaphase I the X and Y can be recognized. At metaphase I (Figure 12.1b) the X and Y have separated in approximately one half of the cells. The X and Y disjoin normally as all the second metaphases observed had only one sex chromosome. The species with complex sex chromosomes have a sex vesicle involving the original X and Y regions, with the exception of *Lagorchestes conspicillatus* where no sex vesicle is observed. The X-X bivalent is isopycnotic in female marsupials (Robinson et al., 1977; Chapter 8).

Synaptonemal complexes in spread preparations

The appearance of the synaptonemal complexes in spread preparations examined with the electron microscope follows closely the description given by Solari (1980). Figure 12.2a shows a complete spermatocyte of *Trichosurus vulpecula*. The lateral elements end in thickened attachment plaques, and the centromeres show heavy staining, as do the nucleoli which are attached to specific autosomal bivalents, or to the

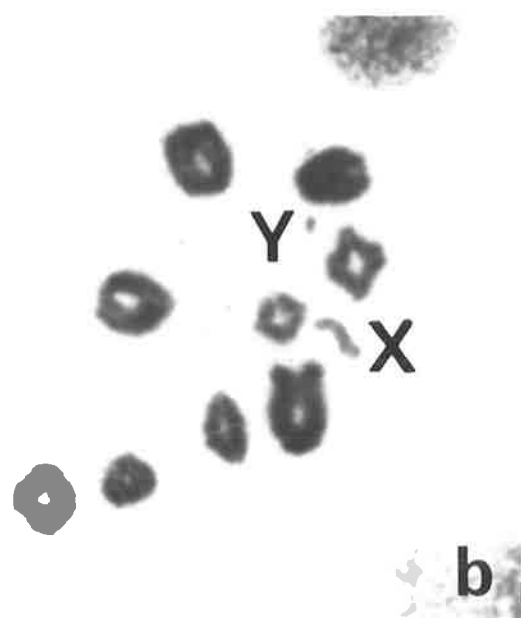
Figure 12.1: Conventional cytological preparations of meiosis
in *Trichosurus vulpecula*.

- a. Pachytene, the sex vesicle is arrowed.
- b. Metaphase I, with the sex chromosomes separate.

The bar = 10 μm for both a. and b.



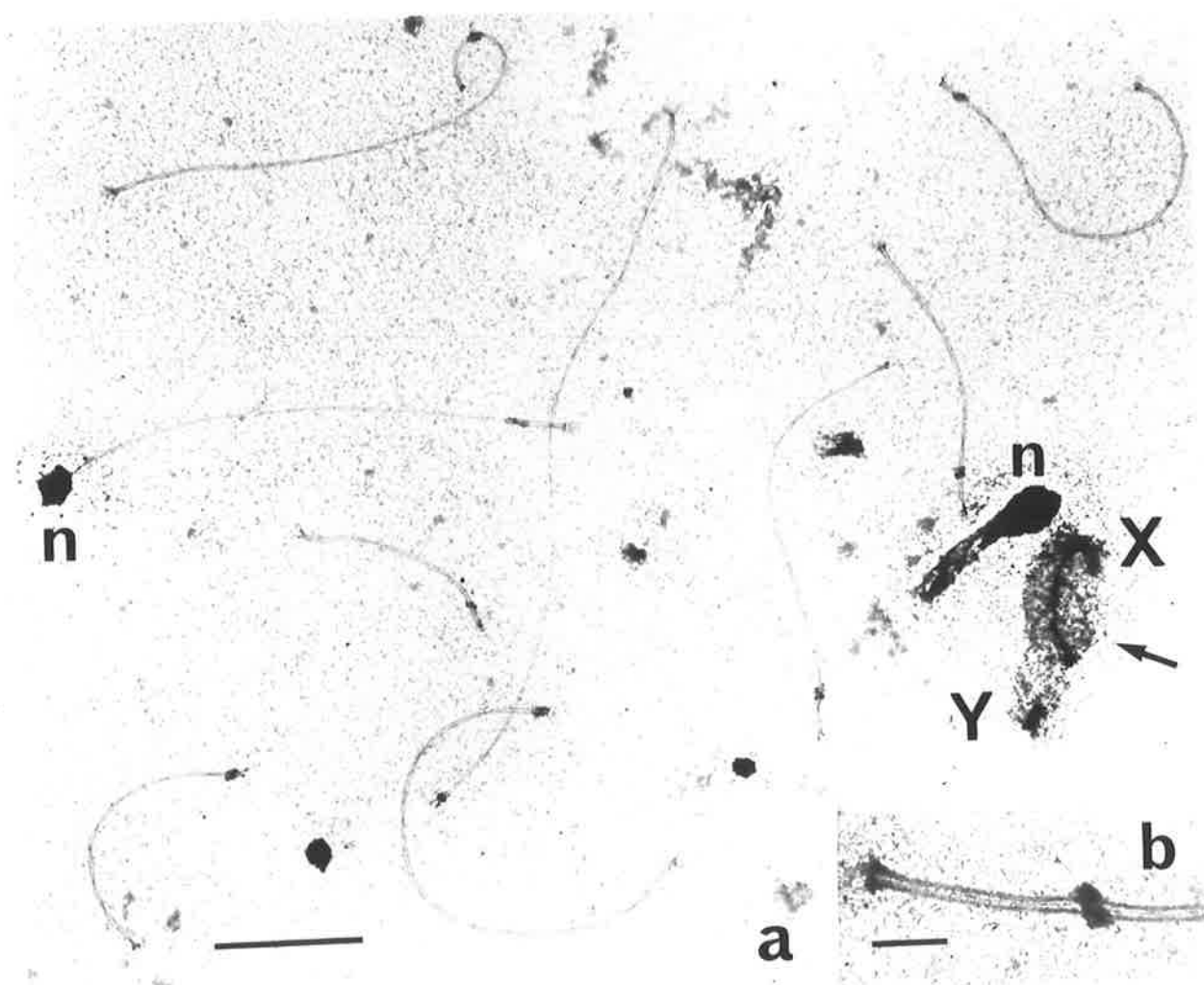
a



b

Figure 12.2:

- a. Complete pachytene EM spread of *T. vulpecula* showing 9 autosomal synaptonemal complexes, the sex vesicle (arrowed) containing the axes of the X and Y, and the nucleoli (n).
Bar = 5 μm .
- b. Detail of an autosomal synaptonemal complex showing attachment plaques, central element and the centromere. EM. Bar = 1 μm .



sex vesicle. The central element is visible at higher magnifications (Figure 12.2b). The sex vesicle shows as a region of condensed chromatin within which the axes of the X and Y chromosomes are embedded. These axes are much thicker than those of the autosomal synaptonemal complexes. Light microscope spreads show virtually the same features, except that the double nature of the autosomal synaptonemal complexes cannot be seen.

Pairing of the sex chromosomes

General

Since observations have been made on spreads of cells obtained from macerated testis tubules, no direct evidence of the sequence of pairing between the sex chromosomes based on the order of cell types in the intact seminiferous tubules has been obtained (Solari and Bianchi, 1975). In spermatocytes which have autosomal synaptonemal complexes incompletely paired and are therefore judged to be at the late zygotene-early pachytene stage, the axes of the sex chromosomes are separate from each other. This observation suggests that the sequence in the species examined is similar to that described by Solari and Bianchi (1975) in *Monodelphis dimidiata*. The X and Y chromosome axes associate after the autosomes have paired, so that during early pachytene, the X and Y axes are separate from each other (e.g. Figures 12.3a, 12.6a). Their axes have thickened ends, indicating that they are attached to the nuclear membrane (Solari and Bianchi, 1975). Later the chromatin of both the X and Y chromosomes associates as a sex vesicle; the axes of the two chromosomes form their characteristic associations, and they develop their characteristic structural modifications. In the species examined there is variation

in the detail of the modifications, and also in the nature of the association between the sex chromosomes which parallel the taxonomic groupings.

Sminthopsis crassicaudata (Fat-tailed Dunnart), *Sarcophilus harrisii* (Tasmanian Devil), *Dasyurus viverrinus* (Eastern Quoll)

These three dasyurids are indistinguishable with respect to the pairing behaviour of their sex chromosomes. Early in pachytene, the X and Y are separate (Figure 12.3a), they then approach each other and their chromatin appears to fuse together. A balloon-like structure then develops around the Y chromosome axis and the two ends of the X axis, and small thickenings appear on the X axis (Figure 12.3b). Later, the ends of the X and Y axes are associated together so that the two axes form a ring. No segment of synaptonemal complex forms between the X and Y chromosomes (Figure 12.3c).

Isoodon obesulus, *Perameles gunnii*, *P. nasuta* (Bandicoots)

The pairing of the sex chromosomes in these three species is very similar. The sex chromosomes approach each other (Figure 12.4c) and their ends associate. In *Isoodon obesulus* the ends are generally near each other (Figure 12.4a), and at a maximum the two ends of the X axis and only one Y axis end associate (Figure 12.4b), whereas in the two *Perameles* species all four ends are most commonly associated (Figures 12.4d, e, f).

No segment of synaptonemal complex occurs between the X and Y chromosomes in these species. The association of the ends appears tighter in *Perameles* as there is often a cross-shaped structure formed involving the four ends (Figures 12.4d, f). In all three species, the X and Y axes develop cross-connections, regions of doubleness,

Figure 12.3: Behaviour of the sex chromosome axes in dasyurids.

- a. *Dasyurus viverrinus*, with the X and Y axes separate.
EM. Bar = 5 μm .
- b. *Sarcophilus harrisii*, with the "balloon" around the
Y axis and both ends of the X axis. EM. Bar = 2 μm .
- c. *Sminthopsis crassicaudata*, the X and Y are associated.
LM. Bar = 2 μm .

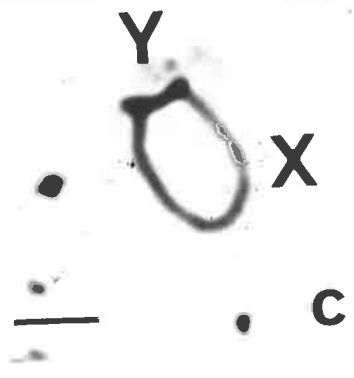
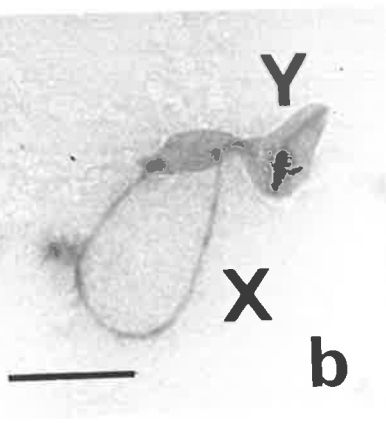
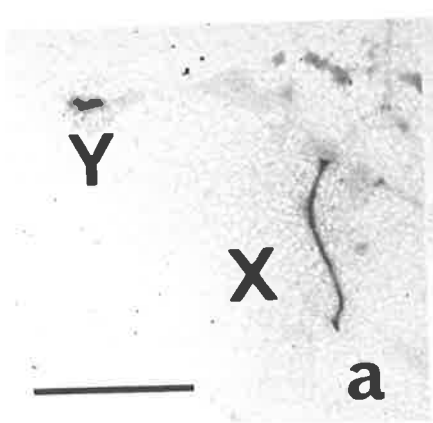
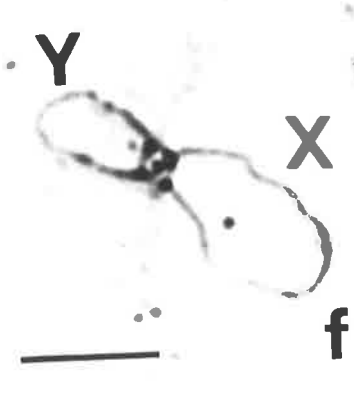
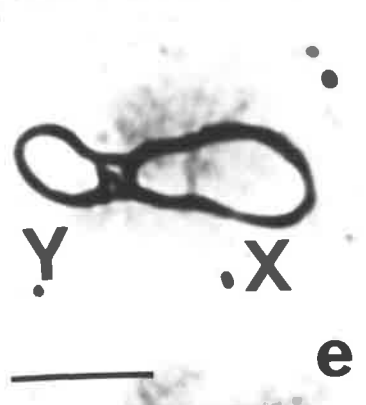
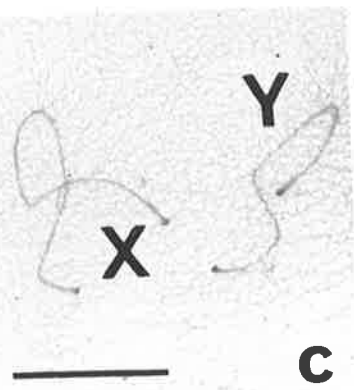
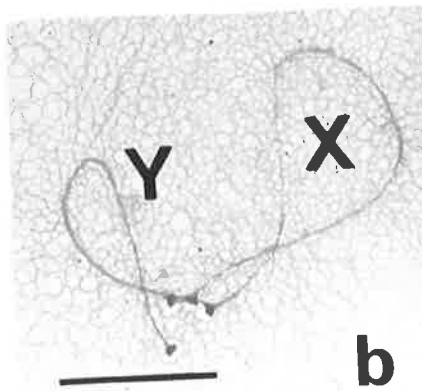
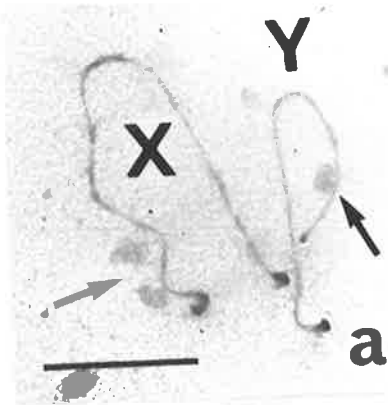


Figure 12.4: Sex chromosome behaviour in peramelids.

- a. *Isoodon obesulus*. The sex chromosome axes are not associated, but show some modifications (arrowed).
EM. Bar = 5 μm .
- b. *I. obesulus*, with one Y axis and both X axis ends associated. EM. Bar = 5 μm .
- c. *I. obesulus*, the sex chromosomes are not associated.
EM. Bar = 5 μm .
- d. *Perameles nasuta*, showing doubleness, cross-connections and modifications of the sex chromosome axes. LM. Bar = 10 μm .
- e. *P. nasuta*. Here the sex chromosome axes are much thickened, and there is one cross-connection.
LM. Bar = 5 μm .
- f. *P. gunnii*. All four axis ends are associated, and there is little modification of the axes.
LM. Bar = 5 μm .



and sites where the axes become much thickened and lateral projections occur (Figures 12.4a, d, e). These structures are more pronounced in the *Perameles* species, especially in *P. nasuta*.

Macrotis lagotis (Rabbit-eared Bandicoot)

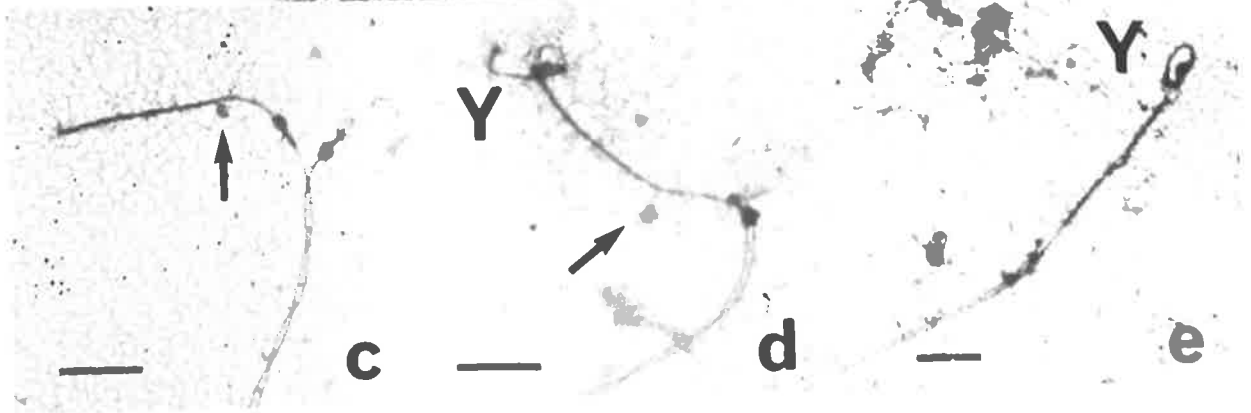
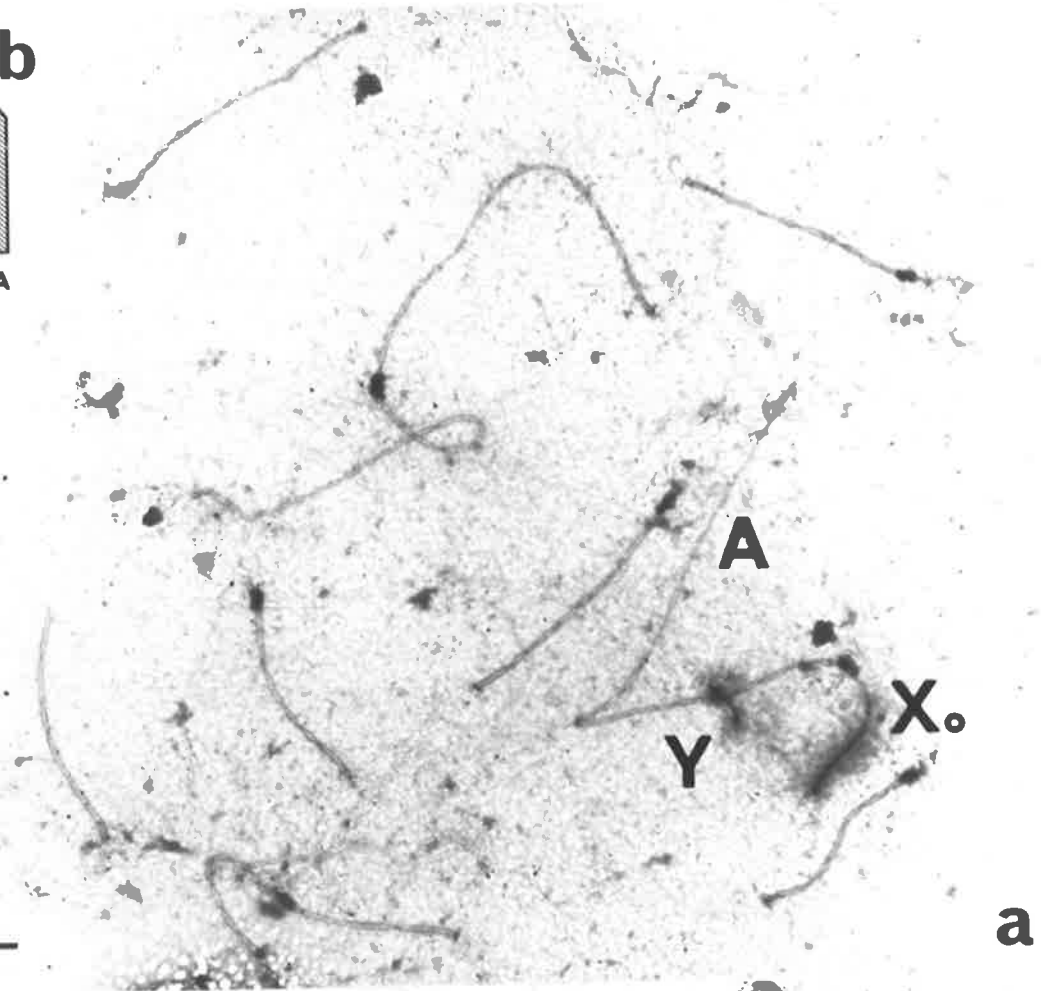
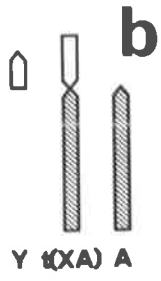
This species has a $2n=19$ t(XA),Y,A $\delta/2n=18$ t(XA),t(XA) δ sex chromosome system (Martin and Hayman, 1967). The structure of the sex chromosomes is shown in Figure 12.5b. At pachytene there are eight autosomal synaptonemal complexes and the sex trivalent (Figure 12.5a). The sex trivalent contains the normal autosomal complex formed by the pairing of the A chromosome with the autosomal portion of the X, and the unpaired axes of the original sex chromosomes which are now the short arm of the t(XA) and the Y chromosome. The axes of the original sex chromosomes have the thickened appearance of the sex chromosome axes (Figures 12.5c, d, e).

After the original X and Y chromosomes approach each other (Figure 12.5a), the free end of the X, and one or both ends of the Y chromosome associate, but no segment of synaptonemal complex is present (Figures 12.5d, e). This is similar to the pairing behaviour of the three other Peramelids described above. The original X and Y show some characteristic structural modifications, the most pronounced being a knob on the original X chromosome (Figures 12.5c, d).

The A chromosome attaches by both telomeres to the nuclear membrane as evidenced by the presence of attachment plaques (Figures 12.5c, e). A small region of asynapsis near the sex vesicle often occurs between the A and the t(XA). The thickening of the Y chromosome axis obscures the location of its centromere, so that its relationship to the orientation of the pairing between the t(XA) and A axes cannot be seen.

Figure 12.5: *Macrotis lagotis*

- a. Complete pachytene spermatocyte showing 8 autosomal complexes (one has broken near its centromere), and the t(XA),Y,A, sex trivalent. The Y and original X(Xo) axes are indicated, with the A synaptonemal complex. EM. Bar = 5 μm .
- b. Schematic diagram of the sex chromosomes. Material of autosomal origin is shaded.
- c. Detail of the sex trivalent, showing asynapsis near the Xo axis, and the knob on the Xo axis (arrowed). EM. Bar = 2 μm .
- d. Detail of the sex trivalent, with the one end of the Y associating with the Xo. The knob is arrowed. EM. Bar = 2 μm .
- e. Detail of the sex trivalent showing the two ends of the Y associating with the free end of the t(XA). EM. Bar = 2 μm .



Lasiiorhinus latifrons (Hairy-nosed Wombat), *Phascolarctos cinereus*
(Koala)

Sex chromosome pairing in these two species is very similar. Both X chromosomes have a nucleolus organizer as the axis of each X has a nucleolus attached to one telomere (Figures 12.6a, d). The X and Y approach each other (Figures 12.6a, d) and the ends associate to form either a ring (Figures 12.6b, e) or all four ends associate together (Figures 12.6e, f). The X and Y axes have no segment of synaptonemal complex between them. In *Lasiiorhinus* the centromeres are visible on both the X and Y axes, which are multistranded although they do not undergo any other structural modifications. The centromeres of the *Phascolarctos* sex chromosomes are not evident, but the X and Y axes show more structural modifications, with certain regions on both becoming much thicker than the rest (Figures 12.6e, f).

Trichosurus vulpecula (Brushtail Possum)

The X chromosome of *Trichosurus* has a nucleolus organizer (Hayman and Rofe, 1977) so that the X axis has an attached nucleolus. As the X and Y chromosomes approach each other, the two chromatin sheaths fuse (Figure 12.6g). The axis of the X chromosome then bends (Figure 12.6h, i), and the Y axis often takes up a position facing the inside of the bend in the X axis (Figure 12.6i). There is no association between the axes of the X and Y chromosomes. Three or four small knobs on the X axis are the only structural modifications, apart from a general thickening of the sex chromosome axes (Figures 12.6h, i).

Figure 12.6: a - c *Lasiorhinus latifrons*

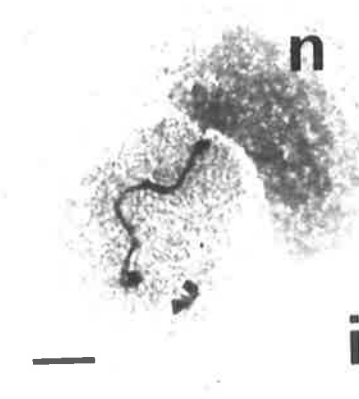
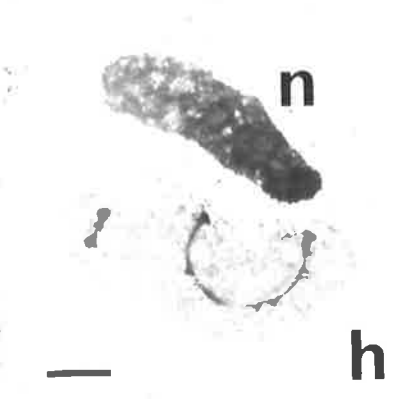
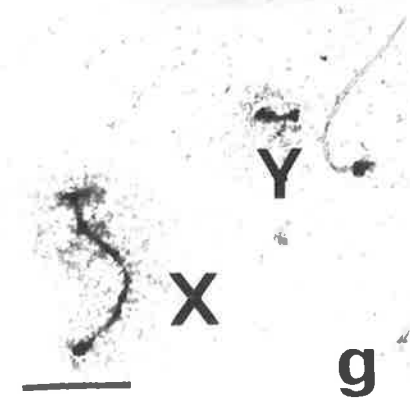
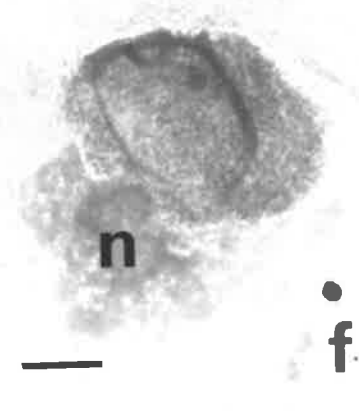
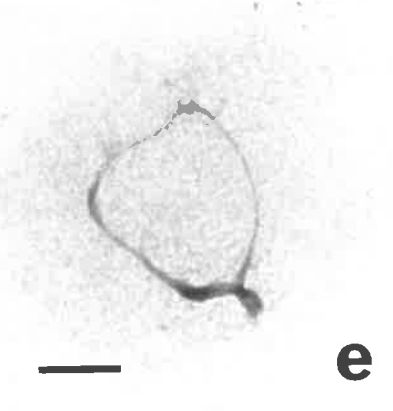
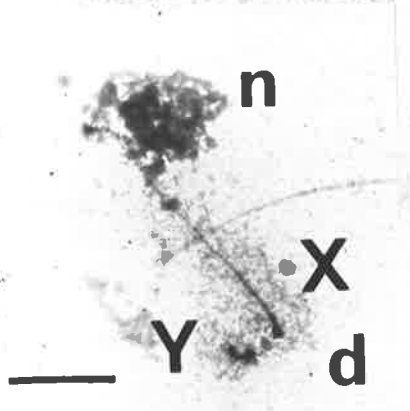
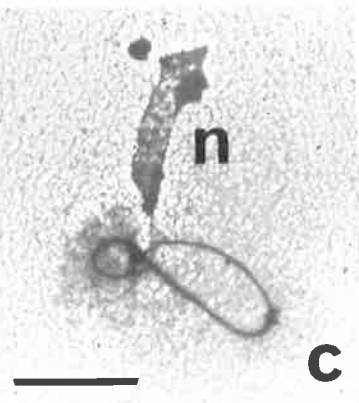
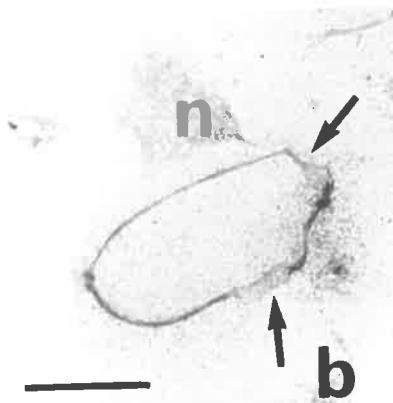
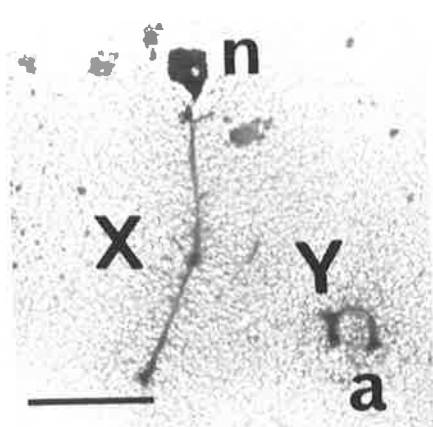
- a. The X and Y axes are separate, the X axis has an attached nucleolus (n). EM. Bar = 5 μ m.
- b. The X and Y axis ends are associated. The nucleolus (n) is indicated. EM. Bar = 5 μ m.
- c. All four axis ends are associated. The nucleolus is indicated (n). EM. Bar = 5 μ m.

d - f *Phascolarctos cinereus*

- d. The X and Y axes are separate, with the X axis having a nucleolus (n). EM. Bar = 5 μ m.
- e. The sex chromosome axes have formed a ring.
EM. Bar = 2 μ m.
- f. All four axis ends are associated, and the nucleolus can be seen. EM. Bar = 2 μ m.

g - i *Trichosurus vulpecula*

- g. The X and Y chromosomes are separate. EM. Bar = 4 μ m.
- h,i. The sex vesicle has formed but there is no association between the axes of the X and Y. The nucleolus is indicated (n). There are a number of knobs along the X axis. EM. Bar = 2 μ m.



Pseudocheirus peregrinus (Ringtail Possum)

In this species the X and Y axes draw near each other (Figure 12.7a) and begin to associate by their ends (Figure 12.7b). They form an association involving all four ends (Figure 12.7c) of the sex chromosome axes. A nucleolus is joined to the axis of the X chromosome (Figure 12.7a, c). The axes do not undergo many structural modifications, although the X axis often becomes twisted. No segment of synaptonemal complex forms between the sex chromosomes.

Petaurus breviceps (Sugar Glider)

After the sex chromosome axes have come close together (Figure 12.7d) they then associate by forming a balloon-like structure which envelopes the Y chromosome axis and both ends of the X axis (Figure 12.7e). This is similar to the structure which develops in the dasyurids. The axes then associate by their ends, with association of the two Y axis ends with one X axis end (Figure 12.7f) being more common than associations of all four ends. There is no synaptonemal complex between the X and Y chromosomes. There is a general thickening of the two axes, and a thickening on the X axis which probably represents its centromere.

Thylogale billardierii (Red-bellied Pademelon), *Macropus rufus*,
M. giganteus, *M. rufogriseus*, *M. parryi*, *M. eugenii*, *M. parma*
 (Kangaroos and Wallabies)

These species have essentially the same mode of association between their sex chromosomes. The sex chromosomes associate with the two ends of the Y often approaching one end of the X (Figures 12.8a, d). No segment of synaptonemal complex forms, although all four ends of the sex chromosome axes become associated together

Figure 12.7: a - c *Pseudocheirus peregrinus*

- a. The sex chromosome axes are separate, with the X having an attached nucleolus (n). LM. Bar = 10 μm .
- b. The X and Y axes begin to associate by their ends. LM. Bar = 5 μm .
- c. All four ends have associated, and the axes are much thicker. The nucleolus (n) is present. LM. Bar = 5 μm .

d - f *Petaurus breviceps*

- d. The axes of the X and Y are separate. EM. Bar = 2 μm .
- e. A "balloon" encloses the Y axis and both ends of the X axis, and a knob develops on the X axis (arrowed). EM. Bar = 2 μm .
- f. Both ends of the Y axis are close to one of the X axis ends. EM. Bar = 2 μm .

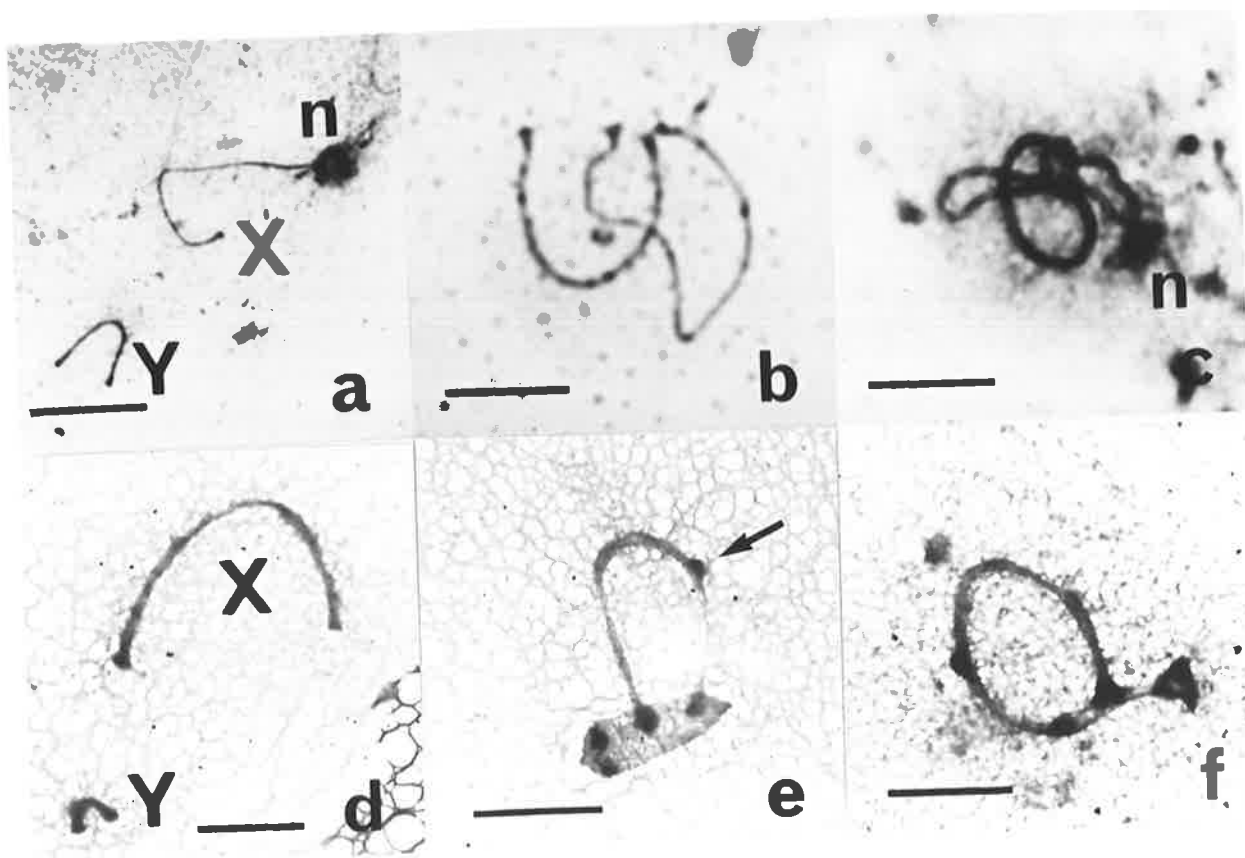
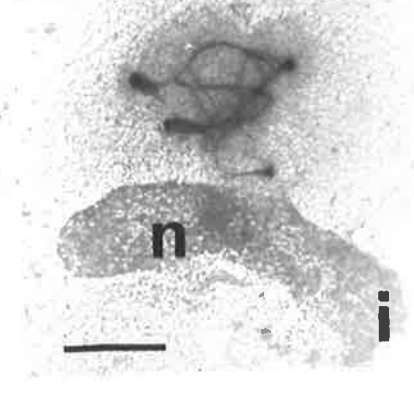
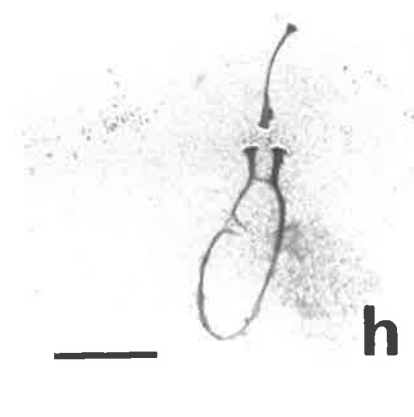
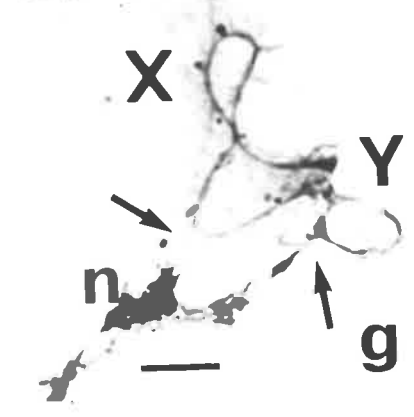
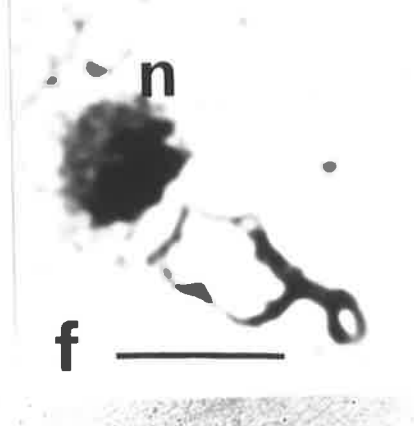
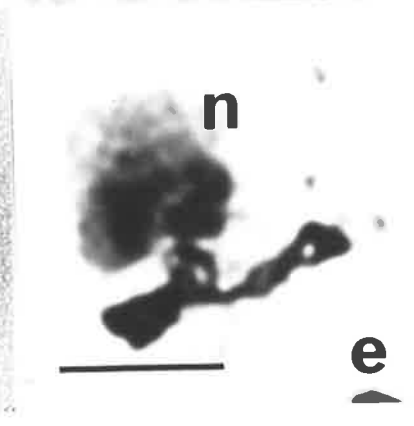
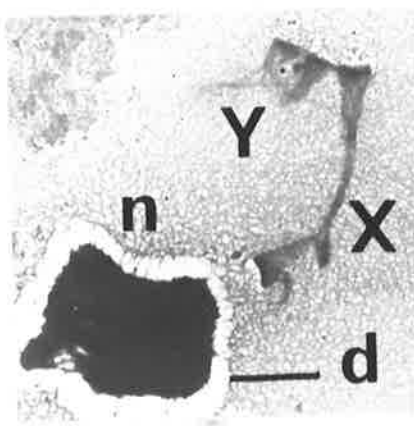
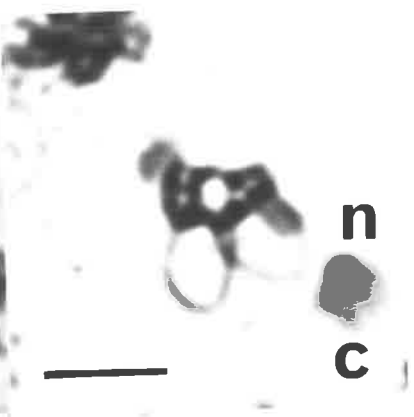
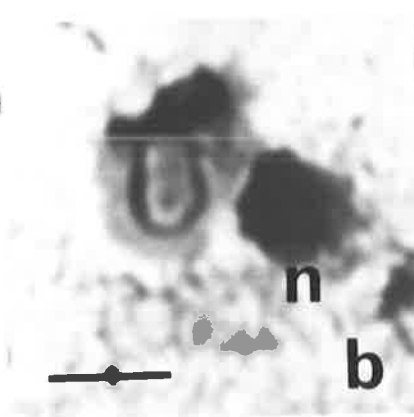
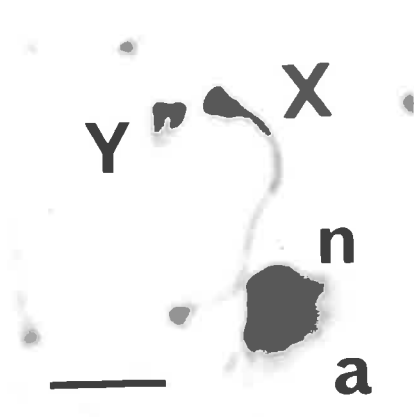


Figure 12.8: Behaviour of sex chromosome axes in macropods.

a - c *Thylogale billardierii*

- a. The Y axis approaches one end of the X axis which has an attached nucleolus (n). LM. Bar = 10 μ m.
- b,c. All four axis ends now associate in a large, deeply staining mass. The nucleolus (n) is still present. LM. Bars = 5 μ m.
- d. *Macropus eugenii*. The Y axis is approaching one end of the X axis. The nucleolus (n) is indicated. EM. Bar = 2 μ m.
- e. *M. giganteus*. The sex chromosome axes are very thick and are associated by all four ends. The nucleolus (n) is present. LM. Bar = 5 μ m.
- f. *M. parma*. All four ends of the X and Y axes are associated, and the nucleolus (n) is present. LM. Bar = 5 μ m.
- g. *M. parryi*. All four axis ends are associated, and the X and Y axes show splitting and excrescences. The nucleolus (n) is attached to both the X and Y axes (arrows). LM. Bar = 5 μ m.
- h. *M. rufogriseus*. The axes have not yet associated. A cross-connection can be seen on the X axis. EM. Bar = 3 μ m.
- i. *M. rufus*. The sex chromosome axes show extensive splitting, and the axis ends are associated. A single nucleolus (n) is present. EM. Bar = 3 μ m.



(Figures 12.8b, c, e, f, g). Later there is massive thickening and splitting of their axes (Figure 12.8g) which becomes so extreme that the X and Y axes cannot be separately distinguished (Figure 12.8i). In *M. rufogriseus* there is often a cross-connection between regions of the X axis (Figure 12.8h). In *M. parryi* 2 nucleoli, or 2 points of attachment of a single nucleolus to the sex vesicle are present, one attachment to the X axis, and the other to the Y axis (Figure 12.8g). The other species have only a single nucleolus, attached to the X axis (Figures 12.8a, d, e, f).

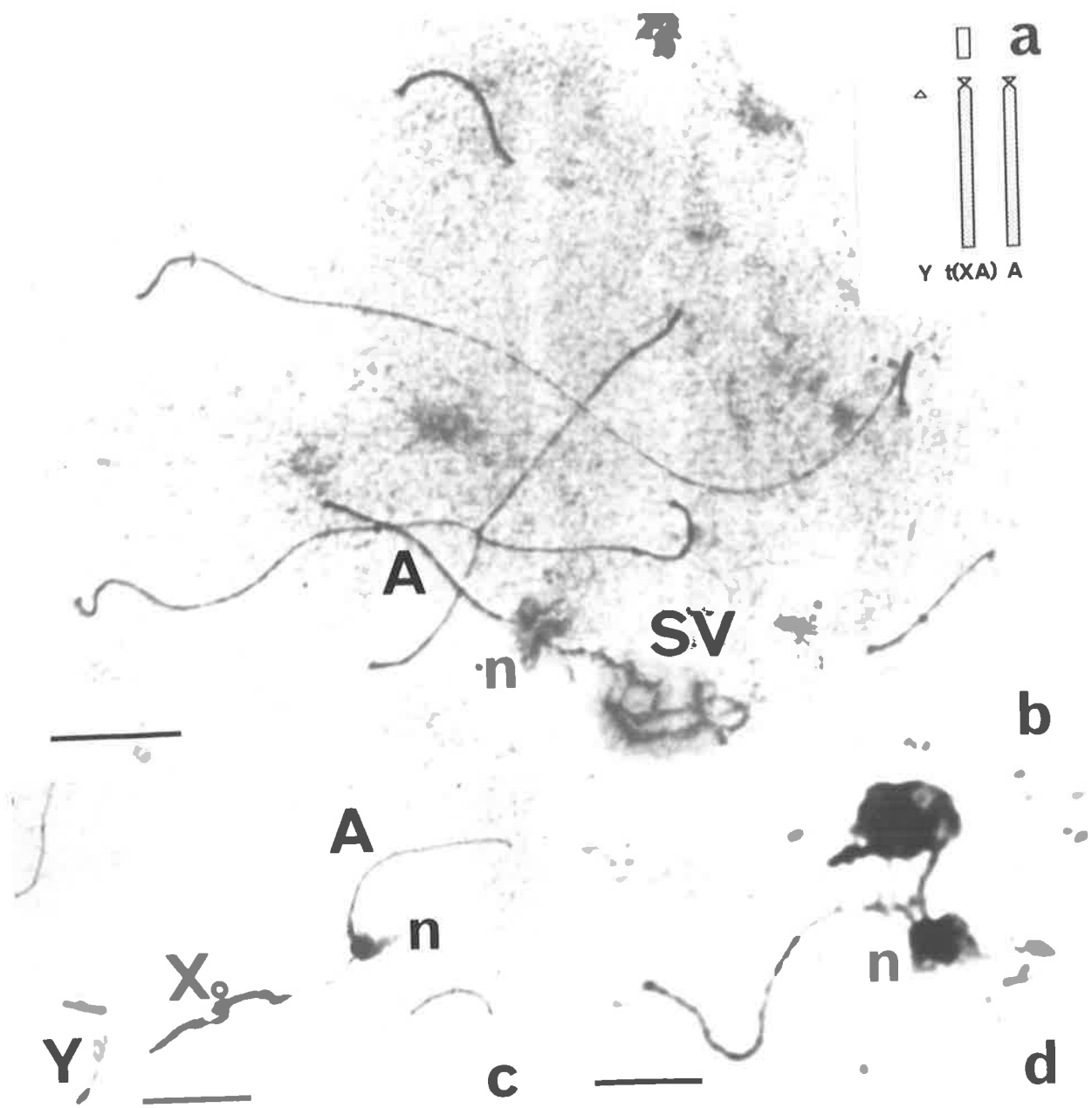
Potorous tridactylus (Potoroo)

Potorous tridactylus has a $2n=13 \text{ t(XA), Y, A} \delta / 2n=12 \text{ t(XA), t(XA)} \delta$ sex chromosome system (Sharman and Barber, 1952), with the t(XA) chromosome having the nucleolus organizer (Hsu et al., 1975). The morphology of the sex chromosomes is shown in Figure 12.9a. At pachytene the sex chromosome trivalent consists of the synaptonemal complex formed between the A chromosome and the autosomal region of the t(XA) chromosome, and the thickened axes of the Y chromosome and the original X in the sex vesicle (Figure 12.9b). The nucleolus is attached to the unpaired axis of the original X (Figures 12.9b, c, d).

The original sex chromosomes move close together (Figure 12.9c), and the axes then undergo extensive splitting and tangle around each other (Figure 12.9b), but form no synaptonemal complex. Later, they become so heavily stained that very little detail can be seen (Figure 12.9d). Since only silver-stained preparations have been examined with the light microscope, the orientation of the centromeres of the sex trivalent with respect to each other has not been determined.

Figure 12.9: *Potorous tridactylus*

- a. Diagrammatic representation of the structure of the sex chromosomes. Material of autosomal origin is shaded.
- b. Spread synaptonemal complexes of a pachytene spermatocyte. There are five autosomal complexes and the sex trivalent, which has a nucleolus (n), a sex vesicle (SV), and an "autosomal" region (A). LM. Bar = 10 μ m.
- c. Sex trivalent where the Y and Xo have not yet associated. LM. Bar = 10 μ m.
- d. Sex trivalent where the Y and Xo have associated and are so darkly staining that no detail can be seen in the sex vesicle. LM. Bar = 10 μ m.



Wallabia bicolor (Swamp Wallaby)

In *Wallabia bicolor* the sex chromosome constitution is $2n=11 \text{ t(XA),Y,A}^\delta/2n=10 \text{ t(XA),t(XA)}^\varnothing$ (Sharman et al., 1950), and the t(XA) chromosome has a nucleolus organizer (Hayman and Rofe, 1977). The sex chromosomes are shown schematically in Figure 12.10a. During meiosis in the male, four autosomal synaptonemal complexes are formed, together with the complex of the sex trivalent (Figure 12.10b). This has the t(XA) chromosome differentiated into a region of autosomal origin which pairs with the A chromosome to form synaptonemal complex, and a region corresponding to the original X which associates with the Y chromosome (Figures 12.10b, d). The thickened axes of the original sex chromosomes draw near each other (Figure 12.10d), the axis of the original X often bends back on itself (Figures 12.10b, c) and while the axes undergo extensive thickening and splitting (Figure 12.10c), the two ends of the Y axis associate with the free end of the original X chromosome (Figures 12.10c, e). As in the other t(XA),Y,A sex chromosome systems described here, the A axis is attached at both ends to the nuclear membrane, and sometimes has a small region of asynapsis with the t(XA) chromosome near the sex vesicle (Figures 12.10e). As the centromere of the Y and t(XA) chromosomes cannot be seen, the relative orientations of the centromeres in the sex trivalent cannot be determined.

Lagorchestes conspicillatus (Spectacled Hare-Wallaby)

Lagorchestes conspicillatus has a $2n=15 \text{ t(XA}_1\text{),t(A}_2\text{YA}_1\text{),A}_2^\delta/2n=16 \text{ t(XA}_1\text{),t(XA}_1\text{)}^\varnothing$ sex chromosome system. The appearance of the silver-stained synaptonemal complexes of the male has been described (Hayman and Sharp, 1981b). Electron microscopy confirms the interpretation of the sex trivalent given there. The increased resolution

Figure 12.10: *Wallabia bicolor*

- a. Schematic representation of the sex chromosomes, with the material of autosomal origin shaded.
- b. Complete spread of a pachytene spermatocyte showing 4 autosomal synaptonemal complexes, and the sex trivalent differentiated into the region of autosomal origin (A), the original X (Xo) and the Y. EM. Bar = 5 μ m.
- c. Detail of a sex trivalent showing a nucleolus (n) and the axis of the Xo bending on itself, and beginning to associate with both Y axis ends. Splitting of the Xo axis is evident. The centromere of the A chromosome can be seen (arrow). EM. Bar = 2 μ m.
- d. The sex trivalent, with the Y axis beginning to associate with the free end of the t(XA) axis. The centromere of the A chromosome is visible. EM. Bar = 2 μ m.
- e. Detail of a sex trivalent, showing a small amount of asynapsis of the A and t(XA) axes near the sex vesicle, which has the nucleolus (n) attached to the Xo axis. EM. Bar = 2 μ m.

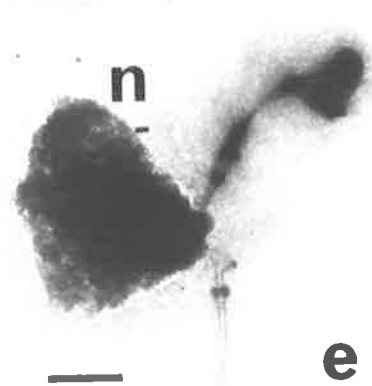
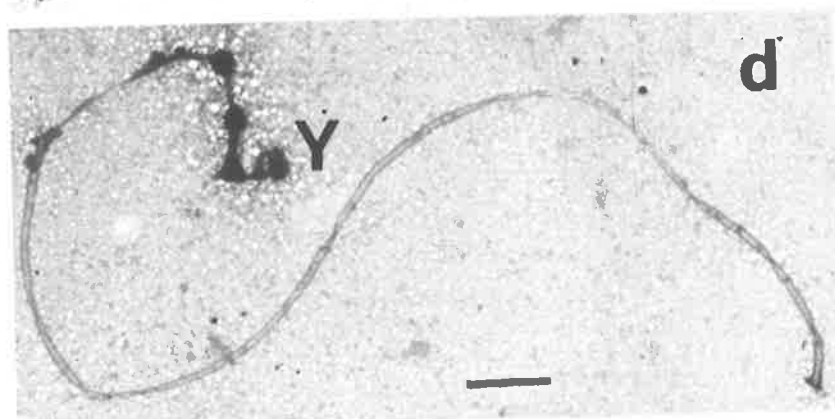
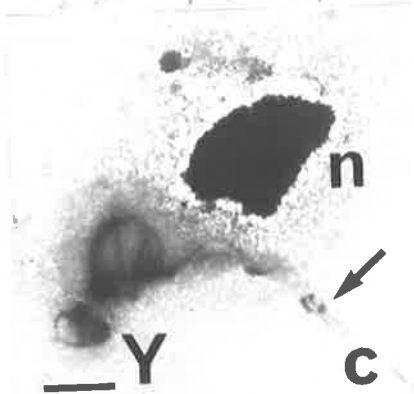
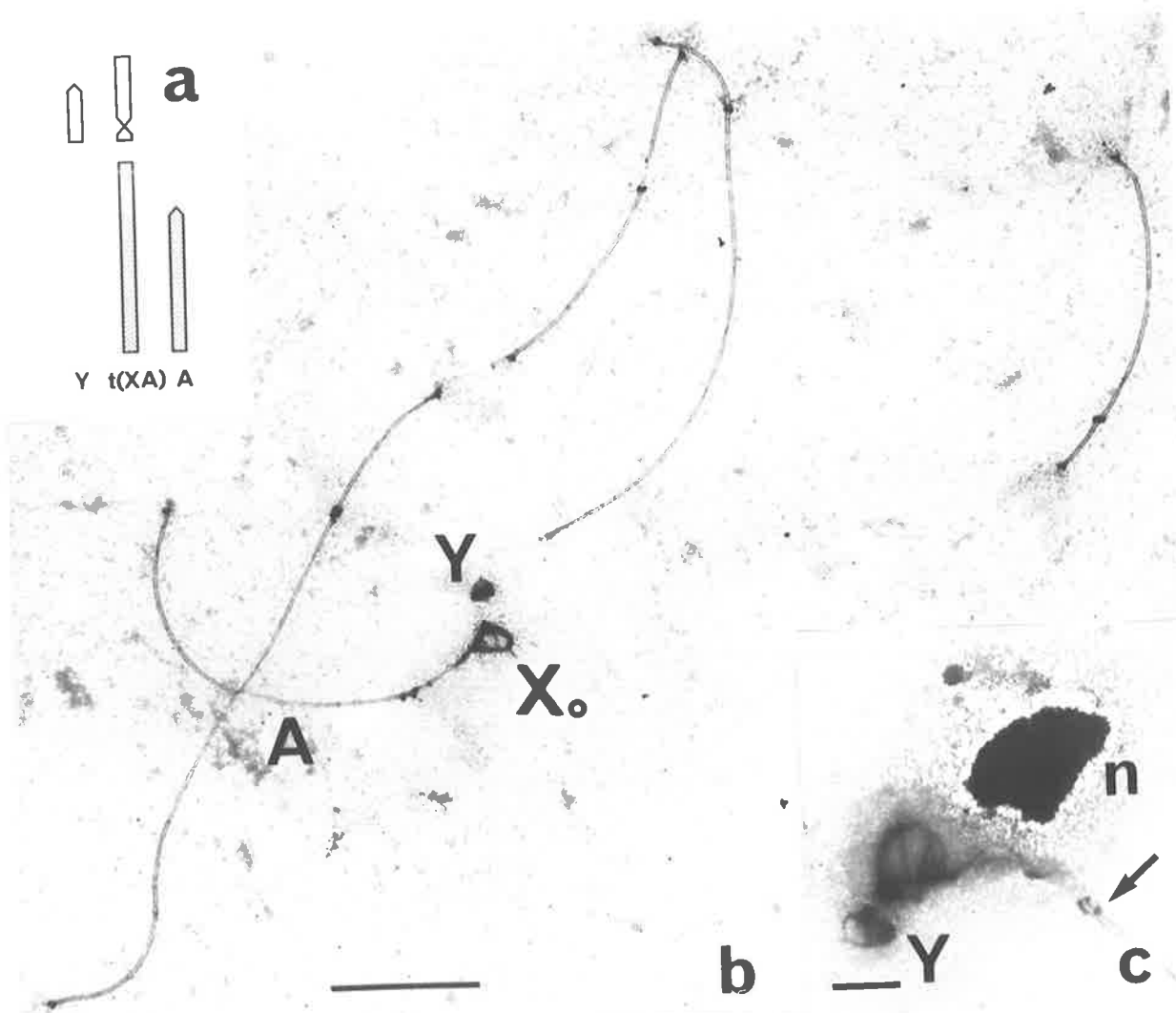
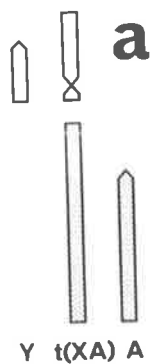
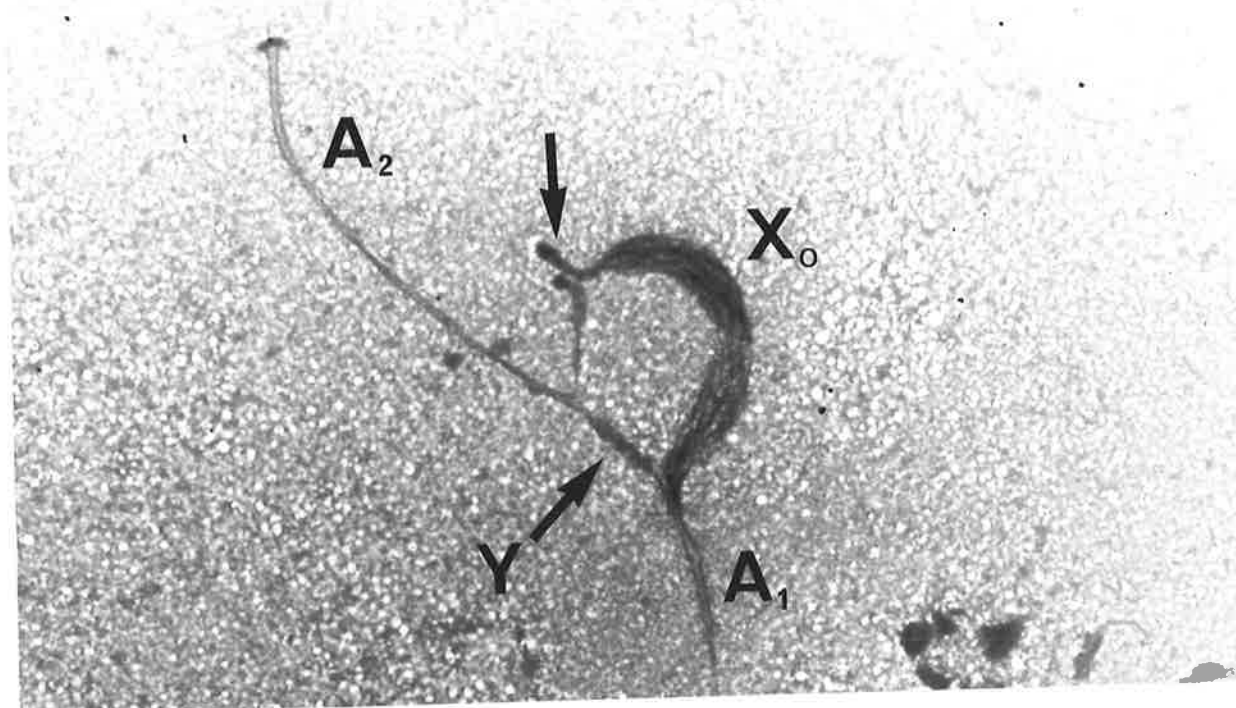


Figure 12.11: *Lagorchestes conspicillatus*.

Detail of the sex trivalent showing the *cis* arrangement of the $t(XA_1)$ and A_2 chromosomes, and the association of the free end of the $t(XA_1)$ chromosome with the end of the A_2 chromosome. The position of the Y in the $t(A_2YA_1)$ axis is indicated by the thickening of the axis (arrowed). EM. Bar = 2 μ m.



obtained allows two more observations to be made on the structure of the $t(XA_1), t(A_2YA_1), A_2$ trivalent. Firstly, in forty spermatocytes examined the axes of the $t(XA_1)$ and A_2 chromosomes always pair on the same side of the $t(A_2YA_1)$ chromosome, i.e. they are in a *cis* arrangement (Figure 12.11), even though the $t(A_2YA_1)$ chromosome has the original Y chromosome inserted between the two autosomal elements (Hayman and Sharp, 1981b). Secondly, the free end of the original X chromosome occasionally associates loosely with the end of the A_2 chromosome (Figure 12.11).

Discussion

- (1) Absence of synaptonemal complex between the X and Y axes.

The pairing of homologous chromosomes or regions during meiosis enables both the disjunction of these elements at anaphase I and the exchange of material by crossing-over as visualized by chiasma formation. Homologous pairing usually involves synaptonemal complex formation (Moses, 1968) but not all synaptonemal complexes are between homologous regions (Gillies, 1974) and synaptonemal complex formation does not invariably lead to crossing over.

The major finding of this study is that in all twenty-two marsupial species examined, no segment of synaptonemal complex forms between the axes of the X and Y chromosomes during meiosis. These species are representative of the four major superfamilies of Australian marsupials; both the other Australian superfamilies are monospecific (Kirsch and Calaby, 1977). These observations in conjunction with the absence of synaptonemal complex in two American marsupial species suggest that the absence of a regular pairing region between the sex chromosomes is characteristic of all marsupials. In contrast, it is present in

the majority of eutherian mammals, which separated from the marsupials approximately 130 million years ago (Air et al., 1971).

The absence of synaptonemal complex between the sex chromosomes of previously described mammalian species has been suggested to be the result of either displacement of the homologous pairing regions by C banding heterochromatin (in *Psammomys*, Solari and Ashley, 1977; and *Didelphis*, Pathak et al., 1980) or by the region becoming non-functional or nonexistent (in *Monodelphis*, Solari and Bianchi, 1975; and *Baiomys*, Pathak et al., 1980). Since all marsupial species examined lack this feature, inactivation of the region is an unlikely explanation. Displacement of the pairing region is also unlikely as the dasyurids discussed all have very small C bands on their sex chromosomes (Rofe, unpublished). One explanation for the absence of the synaptonemal complex in marsupials is that either or both of the X and Y chromosomes have lost the pairing segment which is present in all eutherian mammals, with sporadic exceptions. There is some evidence that the X chromosome of marsupials is indeed smaller than the eutherian X. The smallest X chromosome of eutherian species comprises approximately 5% of the haploid genome (Ohno, 1967, 1974), and no exceptions to Ohno's hypothesis of conservation of the genetic content of the X chromosome in mammalian (both eutherian and marsupial) evolution has been found (Pearson and Roderick, 1979; Donald and Hope, 1981). However, Hayman and Martin (1974) estimated the basic unit of the marsupial X chromosome as 2-3% of the haploid genome on the basis of chromosome length, a conclusion supported by measuring the DNA contents of the individual chromosomes of *Sminthopsis crassicaudata* (Hayman et al., 1982). Unfortunately, no quantitative measurements are available to compare the size of the Y chromosomes in the two groups,

but the Y chromosome of dasyurid species is minute (Hayman and Martin, 1974).

It had been suggested that the metaphase pairing between the sex chromosomes in male marsupials is the result of a chiasma (McIntosh and Sharman, 1953; Sharman, 1970), but the lack of side-by-side pairing of the sex chromosome axes and the absence of synaptonemal complex would argue strongly that no chiasma forms between them, as Koller (1936) indicated. There must be some other mechanism to ensure the regular disjunction of the sex chromosomes.

On the basis of their observations of the pairing of the X and Y in *Psammomys*, Solari and Ashley (1977) and Ashley and Moses (1980) proposed that end-to-end associations of the X and Y axes resulted in these chromosomes being associated together at metaphase I and then disjoining normally at anaphase I. Similarly, in the marsupial species studied here the only associations which develop between the sex chromosome axes involve their ends. In *Trichosurus vulpecula* however, the sex chromosome axes do not associate, but the sex chromosomes do disjoin normally at anaphase I. Hence in this species, neither pairing as defined by synaptonemal complex formation, nor end-to-end associations of the axes are required for regular disjunction of the sex chromosomes. This is not a unique situation as meiosis in male *Drosophila* involves association of homologous chromosomes without synaptonemal complexes or crossing-over (Rasmussen, 1973) though here homologous^{ue} are parallelly aligned throughout their entire lengths. In *Trichosurus*, the association which results in the sex vesicle must ensure normal disjunction of the X and Y chromosomes.

The sex chromosomes of *Macropus rufus*, *M. rufogriseus* and *M. parryi* have large amounts of C-banding material additional to the

basic X and Y (Hayman and Martin, 1974; Hayman and Rofe, 1977). There is a degree of sequence similarity in the heterochromatin of these chromosomes in each species since they are almost the only chromosomes to be affected by Hoechst 33258 (Hayman and Sharp, 1981a). Further, the X and Y chromosomes of *M. rufogriseus* have a common satellite sequence (Dunsmuir, 1976; Venolia and Peacock, 1981), and all the *Macropus* species examined have Hoechst 33258 sensitive material on both their X and Y chromosomes (Hayman and Sharp, 1981a). Yet this similarity does not lead either to the formation of synaptonemal complex or to chiasma formation. It would seem that this is a further example of the general proscription against chiasmata forming in heterochromatic regions (John and Miklos, 1979). In the eutherians the only convincing examples of chiasma formation between the X and Y chromosomes are in the hamsters (Vistorin *et al.*, 1977; Allen, 1979), which have large sex chromosomes. The chiasma formed there are unusual in that they occur between C-banding regions that do not contain highly repeated DNA sequences (Vistorin *et al.*, 1977; Arrighi *et al.*, 1974).

(2) Complex sex chromosomes.

In the species with complex sex chromosome systems, the axes of the original sex chromosome regions show typical marsupial sex chromosome behaviour, while the autosomal portions of the complex sex chromosomes behave as autosomes. This parallels the division of the complex X chromosomes revealed in these species by the asynchrony in the DNA replication in females (Hayman and Martin, 1965).

Moses *et al.*, (1979) and Elder and Pathak (1980) report that in heterozygotes for autosomal Robertsonian translocations the lateral elements (and hence the centromeres) of the nontranslocated chromosomes

occupy a *cis* position in the trivalent. That is, there is a single pairing face between the axes. They suggest that this one-sided pairing would predispose the trivalent to balanced disjunction. Additionally, Pathak and Lin (1981) have suggested that a *cis* orientation of the centromeres invariably occurs in the $t(XA), Y, A$ sex trivalent in the Indian muntjac, leading to the regular disjunction of the $t(XA)$ chromosome from the Y and A chromosomes. This conclusion seems to be based on the orientation of the Y axis with respect to the unpaired end of the A axis as the orientations of the centromeres involved are not clear in their preparations, which are silver-stained light microscope spreads.

As it is impossible to distinguish the centromere of the Y chromosome in the comparable $t(XA), Y, A$ sex chromosome systems examined here the relative orientations of the centromeres of the Y and A chromosomes cannot be determined. It can be seen, however, that the region of the original X with which the Y associates bears no relationship to the orientation of the A centromere. In these trivalents correct disjunction is therefore determined by a mechanism other than a *cis* arrangement of the co-segregating elements. A possible mechanism is that the precocious disjunction of the original X and Y in the trivalent determines the direction of the subsequent disjunction of the $t(XA)$ and A chromosomes.

In the $t(XA_1), t(A_2YA_1), A_2$ sex trivalent of *Lagorchestes conspicillatus* the $t(XA_1)$ and A_2 axes always synapse in a *cis* arrangement with the axis of the $t(A_2YA_1)$ chromosome. The original X and Y axes do not form a sex vesicle, the pairing of the chromosomes in the trivalent being dominated by the synapsis of the autosomal regions. This trivalent therefore behaves like the autosomal trivalents of

Lemurs, *Mus* and *Sigmodon* (Moses et al., 1974; Elder and Pathak, 1980) in that it appears that the lateral elements have a single pairing face. The *cis* arrangement seen in the *Lagorchestes* sex trivalent would presumably favour the disjunction of the $t(XA_1)$ and A_2 chromosomes from the $t(A_2YA_1)$ chromosome at anaphase I. The loose association of the free end of the $t(XA_1)$ with the end of the A_2 would also tend to ensure correct co-segregation of these elements. The difference between this trivalent and those of the $t(XA), Y, A$ species where the association of the Y with the original X is independent of the orientation of pairing between the A and the autosomal region of the $t(XA)$ chromosome is a further indication of the special nature of the pairing between the sex chromosomes.

(3) Modifications of the sex chromosome axes.

The species studied here reveal a diversity in the modifications of their unpaired sex chromosome axes, ranging over a slight general thickening as in *Pseudocheirus peregrinus*, the massive general thickening of the *Macropus* species, to the extensive localized splitting, excrescences and cross-connections of *Perameles nasuta*. There is no correlation between the ~~amount~~ ^{amount} or location of the structural modifications of the sex chromosome axes and the amount of location of either the C-banding material or of the Hoechst 33258 sensitive material (Hayman and Sharp, 1981a, b; Sharp and Hayman, 1981; Murray, 1977; Murray et al., 1980; Rofe, 1979).

The nature of the modifications does, however, tend to be conserved within taxonomic groupings. For example, the three dasyurids examined all have the balloon-like structure enclosing the two X axis ends and the Y axis, while the *Macropus* species all have massive thickenings

and splitting of the sex chromosome axes, as do the representatives of the related genera (*Potorous*, *Lagorchestes*, *Thylogale* and *Wallabia*) of the superfamily Phalangerioidea. Similarly in the eutherians, the two *Cricetulus* species have almost identical modifications of the sex chromosome axes (Solari, 1974b; Moses, 1977b), and the related hamster *Mesocricetus auratus* has similar modifications (Moses and Counce, 1974). This parallelism between related species indicates the operation of selective forces during evolution - although the bases for the selective differentials are unknown.

12.2: Further material on sex chromosome pairing

Since Chapter 12.1 was written the synaptonemal complexes of three further species of Australian marsupials have been examined with the electron microscope. In addition, a number of studies of sex chromosome pairing in both marsupials and eutherians have been published.

Vombatus ursinus

Figure 12.12 shows the pairing of X and Y chromosomes in this species. The X and Y axes approach each other (Figures 12.12a, b), and form a sex vesicle (Figures 12.12c, d). The axes of the X and Y do not associate within the sex vesicle, but there are associations between the two ends of the X and the two ends of the Y axis (Figure 12.12d), although the ends of the X and Y appear to interact during the formation of the sex vesicle (Figure 12.12b). The X axis of the sex vesicle has an attached nucleolus (Figures 12.12c, d). *Vombatus ursinus* therefore does not form synaptonemal complex between the X and Y, and the type of association is different than that observed in the other wombat *Lasiorhinus latifrons* (see Chapter 12.1) where the X and Y axes either form a ring, or all four axis ends associate.

Petaurus norfolcensis

The pairing of the sex chromosomes in this species is similar to that occurring in *Petaurus breviceps* as described in Chapter 12.1. Figure 12.12e shows the X and Y axes approaching each other, and Figure 12.12f shows the X axis forming a ring, with the Y axes associated with one of the X axis ends, but not forming a segment of synaptonemal

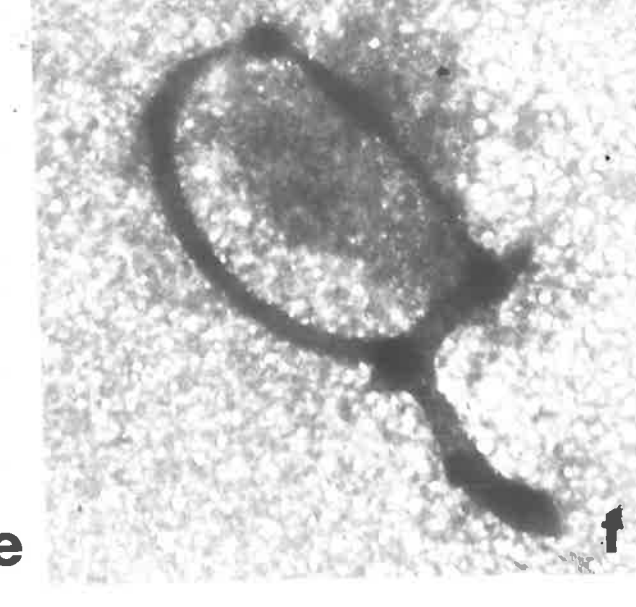
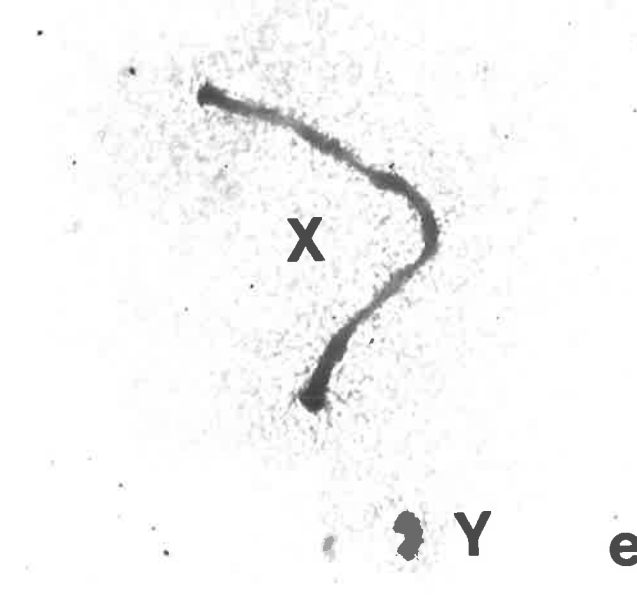
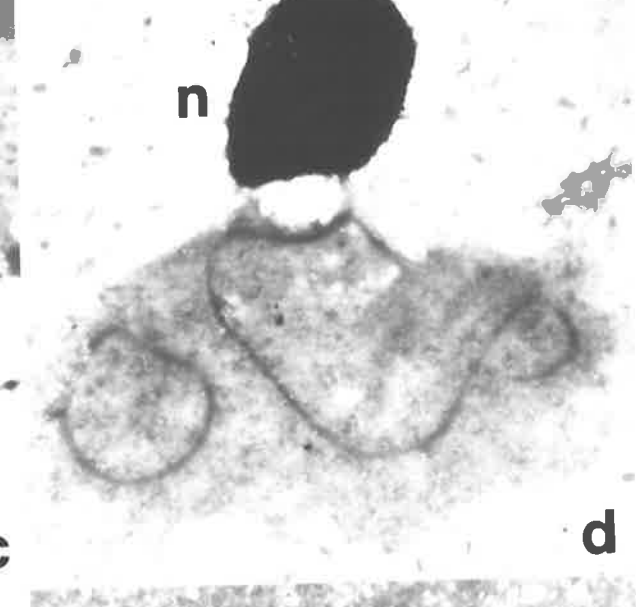
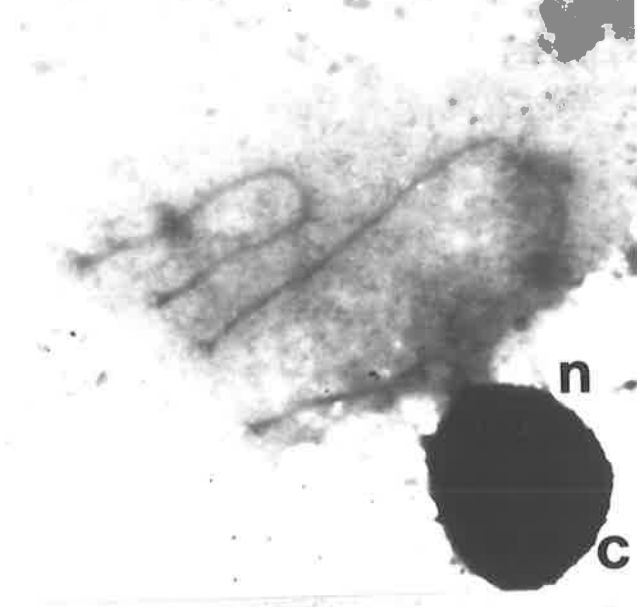
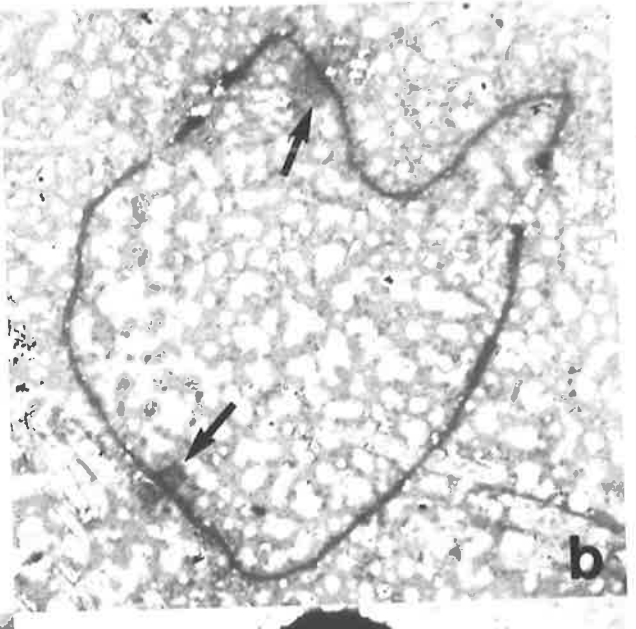
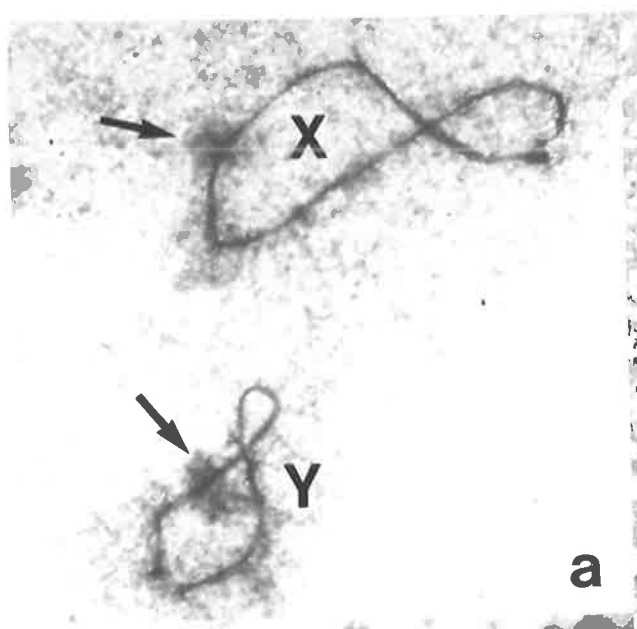
Figure 12.12: Sex chromosome pairing in *Vombatus ursinus* and *Petaurus norfolcensis* (EM)

Vombatus ursinus

- a. The X and Y axes are approaching each other. The ends of the X, and the ends of the Y are associated. The centromeres are arrowed.
- b. The sex chromosome axes are interacting by their telomeres.
- c,d. The sex vesicle has formed, and a nucleolus (n) is associated with the X axis. In c the telomeres are not closely associated, whereas in d both the X and Y axes form rings due to associations of their respective telomeres.

Petaurus norfolcensis

- e. The X and Y axes are approaching each other.
- f. One end of the Y axis is associated with an end of the X axis, which forms a ring due to the association of its ends.



complex. The only difference between *P. norfolcensis* and *P. breviceps* is that the latter has a "balloon-like" structure enclosing the X axis ends and the Y (Figure 12.7e), whereas this was not observed in *P. norfolcensis*.

Macropus robustus

Macropus robustus has essentially the same type of sex chromosome pairing seen in the other *Macropus* species described in Chapter 12.1. After the X and Y axes approach each other they begin to associate, usually by their ends, but there is an occasional connection between interstitial regions of the axes of the two chromosomes (Figure 13.6a). The sex chromosome axes then undergo splitting and modification to produce sex vesicles in which the individual axes cannot be traced (Figures 13.7a, b). No segment of synaptonemal complex is formed. The sex vesicle has either one (Figure 13.7a) or two (Figure 13.7b) nucleoli attached to it. Both the X and Y have nucleolus organizers in this species (Hayman, Rofe and Sharp, in preparation).

Three more Australian marsupials can therefore be added to Table 12.2, making a total of twenty-five which have been examined, all of which are without synaptonemal complex formation between the X and Y during meiosis in the male. In addition, sex chromosome pairing has been analysed in two more American marsupials not discussed in Chapter 12.1. Wettstein et al., (1974) found no synaptonemal complex between the X and Y in *Marmosa elegans*, and Merry et al., (1983) have described a similar situation in *Monodelphis domestica*. Four American marsupials have therefore been shown not to have synaptonemal complex formation between their X and Y chromosomes. Consequently, the evidence suggests that this feature is a characteristic of the marsupials, as considered in Chapter 12.1.

Further information has also accumulated concerning X-Y chromosome pairing during meiosis in eutherian mammals. In addition to the species listed in Table 12.1, the dog (Pathak et al., 1982), and two species of hamster, *Phodopus roborovskii* and *P. sungorus* (Spyropoulos et al., 1982) have been shown to form a segment of synaptonemal complex between their X and Y chromosomes, so that at least 16 eutherian species have this feature. One other species, *Mus setulosus* (Jotterand-Bellomo, 1981) lacks this feature, so that three eutherian species have now been found which do not form synaptonemal complex between the X and Y. Synaptonemal complex formation between the X and Y therefore appears to be a characteristic of eutherians, but in a few isolated species it has either been lost, or is not expressed.

The behaviour of the sex chromosome axes in two gerbil species, *Gerbillus pyramidum* and *G. gerbillus* with unusual sex chromosomes has been reported recently (Wahrman et al., 1983). Extensive cytological evidence shows that *G. pyramidum* has a $t(A_1X), t(A_1Y)$ sex chromosome system, while *G. gerbillus* has a $t(A_1Y), t(A_1XA_2), (A_2+het)$ sex chromosome system in the terminology suggested by Hayman and Sharp (1981b). The *G. gerbillus* system is postulated to have arisen from a *G. pyramidum*-like system by translocation of the A_2 autosomal element, and inversion of the original X, since the X is in different orientations relative to the A_1 element in the two species. Because of the autosomes translocated to the original X and Y in both cases it is unknown whether the original sex chromosomes had a pairing region; e.g. the long apparent pairing segment in the *G. pyramidum* sex bivalent could be entirely of autosomal origin, or it could be synaptonemal complex of autosomal origin together with an ancestral

XY pairing segment. In both species, however, the pairing is largely determined by the autosomal segments.

The synaptonemal complex trivalent in *G. gerbillus* shows many similarities to that observed in *Lagorhynchus conspicillatus* (Hayman and Sharp, 1981b; Chapter 12.1). The two clear cells illustrated have a *cis* arrangement of the $t(A_1Y)$ and (A_2+het) axes, and the unpaired ends of these axes are often near each other. *G. gerbillus* differs from *Lagorhynchus*, however, in that a sex vesicle is clearly present at pachytene.

A number of theories and experimental results concerning both the structure of the sex chromosomes and the nature of the pairing between the X and Y during meiosis have recently been published. Both Polani (1982) and Burgoyne (1982) have reviewed the evidence from eutherians (mainly Man and mouse) on these points and have presented almost identical speculations. The main evidence considered was (1) there is synaptonemal complex formation between the X and Y to form a pairing region, (2) the presence of a "recombination nodule" in this segment, (3) the existence in Man of two genes, located on the short arm of the X, determining steroid sulphatase (*STS*) and the Xg blood group (*Xg*) both of which do not undergo X inactivation in the female, and (4) the genetic behaviour of the sex-reversing mutation *Sxr* in the mouse. Both authors have proposed that the X and Y pair partially due to homology in the pairing region, and that there is a localized chiasma between the X and Y close to the telomere (of Xp and Yp in man, and Xq and Yq in the mouse which has acrocentric sex chromosomes). Under this hypothesis loci within the region of homology proximal to the chiasma will still be either X linked, but may not be subject to

X-inactivation (*STS* and *Xg* in man), or Y linked (the gene determining H-Y antigen). It is further postulated that the "homologous" loci ("*STS*-related" and "*Yg*" on the Y, and "*H-Y* related" on the X) are either silent, or have taken on other functions. Loci distal to the chiasma will be inherited as autosomal genes, although they will not be linked to any true autosomal loci. They are therefore called "pseudoautosomal" loci.

Experimental results from a number of sources bear on the different inter-related parts of this hypothesis. Evidence of homology between regions of the human X and Y chromosomes independent from meiotic behaviour has been found at three different levels. At the cytological level Müller and Schempp (1982) have demonstrated that there are homologous early DNA replication patterns on the distal parts of the short arms of the X and Y using BUdR labelling. At the genetic level evidence of a Y-linked locus determining a cell surface antigen homologous to an antigen coded by a locus located on the tip of the short arm of the X has been presented by Goodfellow *et al.*, (1983). Finally, at the molecular level, a single-copy DNA sequence has been cloned and found to be located on both the X and Y chromosomes, although regional mapping of this sequence excluded it from the very tip of Xp (Page *et al.*, 1982).

Evidence of a chiasma between the X and Y in the mouse has come from a cytological study of XY *Sxr* carrying male mice, which Evans *et al.*, (1982) found had an additional dark G-band on the end of the long arm of the Y chromosome. During meiosis in these males, this additional dark G-band was transferred to the distal tip of the X chromosome, thereby producing progeny that are XX♀♀, XY♂♂, XX* *Sxr*♂♂

(sterile), and XY* Sxr♂♂ like their fathers (* indicates the dark G-band which they postulate contains the Sxr locus, and which Burgoyne (1982) suggests is an additional copy of the normal male determining gene). Sxr consequently appears to be an autosomal dominant, but is in fact "pseudo-autosomal" with the dark G-band being distal to an obligate chiasma between the X and Y. They further postulated that this Sxr-associated dark G-band was revealing an obligate chiasma normally present between the X and Y, but which cannot be visualized in normal males as it is too close to the telomere. A scheme such as this would also explain some of the *in situ* hybridization results obtained by Singh and Jones (1982) in their study of Sxr mice.

As a consequence of this evidence Burgoyne (1982) postulated that the X and Y chromosomes are associated via a single localized chiasma in all mammals where no chiasma can be seen. Under this scheme species in which a chiasma is visible (e.g. the hamsters, Vistorin *et al.*, 1977; Murer-Orlando and Richer, 1983) have chromosomal modifications such that the localized chiasma has been moved to an interstitial region. The evidence of both Chapter 12.1 and this chapter suggest very strongly, however, that there is no such chiasma between the X and Y in marsupials. As indicated in Chapter 12.1 there is evidence that the X chromosome of marsupials is smaller than that of eutherians (Hayman *et al.*, 1982), so that it is possible they do not have regions on their X and Y homologous to that in the eutherians. Future studies of the chromosomal location in marsupials of genes that are thought to be in or near the pairing segment in eutherians (such as steroid sulphatase) will be of great interest with respect to this difference between the two groups.

Ohno (1967, 1974) has postulated that the X-chromosome linkage group has been conserved throughout mammalian evolution, and that this extreme conservatism is related to the necessity of a mechanism of dosage compensation of X-linked genes. In all the studied X-linked genes (of which the genes controlling the enzymes HPRT, PGK-A, G6PD, and α GAL are the most studied), no exception to Ohno's hypothesis has been found in both a wide range of eutherian mammals, and in the few marsupial species that have been studied (Pearson *et al.*, 1982). At present it is not known whether this conservation of the X-linked genes will also apply to those X-linked genes in man that do not undergo dosage compensation, such as Xg and steroid sulphatase. Preliminary evidence suggests that steroid sulphatase is X-linked in the wood lemming and in *Microtus agrestis* (Ropers *et al.*, 1982), but that steroid sulphatase deficiency is inherited as an autosomal gene in the mouse (Balazs *et al.*, 1979), although it is not known if this mutation is homologous to the X-linked one in Man. However, studies on steroid sulphatase level in oocytes of XX and XO mice suggest that this enzyme is X-linked (Gartler and Rivest, 1983).

Matsuda *et al.*, (1983) have studied the frequency of early X-Y dissociation in hybrids and backcrosses between different subspecies of *Mus*, and their results suggest that the pairing regions of the different subspecies have diverged so that pairing between the sex chromosomes of the different subspecies is less effective than within subspecies pairing. The pairing region can evidently adopt different ways of ensuring high X-Y pairing in different subspecies.

As mentioned in Chapter 12.1, some *Macropus* species have large amounts of C-banding material on their sex chromosomes, and this material has been shown to have a degree of homology by its similar sensitivity to Hoechst 33258 (Hayman and Sharp, 1981a), and in one species through these chromosomes sharing a particular satellite DNA sequence (Dunsmuir, 1976; Venolia and Peacock, 1981). This homology does not lead to synaptonemal complex formation between the X and Y chromosomes. Similarly, the presence of nucleolus organizer regions on the X and Y does not lead to such synaptonemal complex, since *Trichosurus vulpecula* (Stock and Mengden, 1982), *Macropus parryi*, *Pseudocheirus peregrinus*, and *Lasiiorhinus latifrons* (Hayman and Rofe, 1977), and *Macropus robustus* (Hayman, Rofe and Sharp, in preparation) have nucleolus organizer regions on both the X and Y, yet have no synaptonemal complex between these chromosomes. Clearly, the homology needed to produce synaptonemal complex between the X and Y is not provided by either the Hoechst 33258 sensitive material or the nucleolus organizer regions.

The nucleolus organizer regions on the X chromosomes do not behave as expected under the hypothesis that the X chromosome is inactivated during meiosis (Lifschytz and Lindsley, 1972). There are prominent nucleoli attached to the X chromosome axes during pachytene in the species examined (Chapter 12.1 and this chapter) with X-linked nucleolus organizers. This is possibly due to two factors; the nucleolus organizers are not part of the "original" X chromosome of Hayman and Rofe (1977) and are therefore not subject to the same controls, and the large amount of nucleolar activity which seems necessary during meiotic prophase (Monesi, 1971).

CHAPTER 13: SYNAPTIC ADJUSTMENT OF C-BAND HETEROZYGOSITY

In a number of individual animals synaptonemal complex spreads showed that the centromeres in a particular bivalent were not aligned, i.e. the bivalent was heterozygous for centromere position. This situation was found in a number of specimens of *Trichosurus vulpecula*, and in one specimen of *Macropus rufus*.

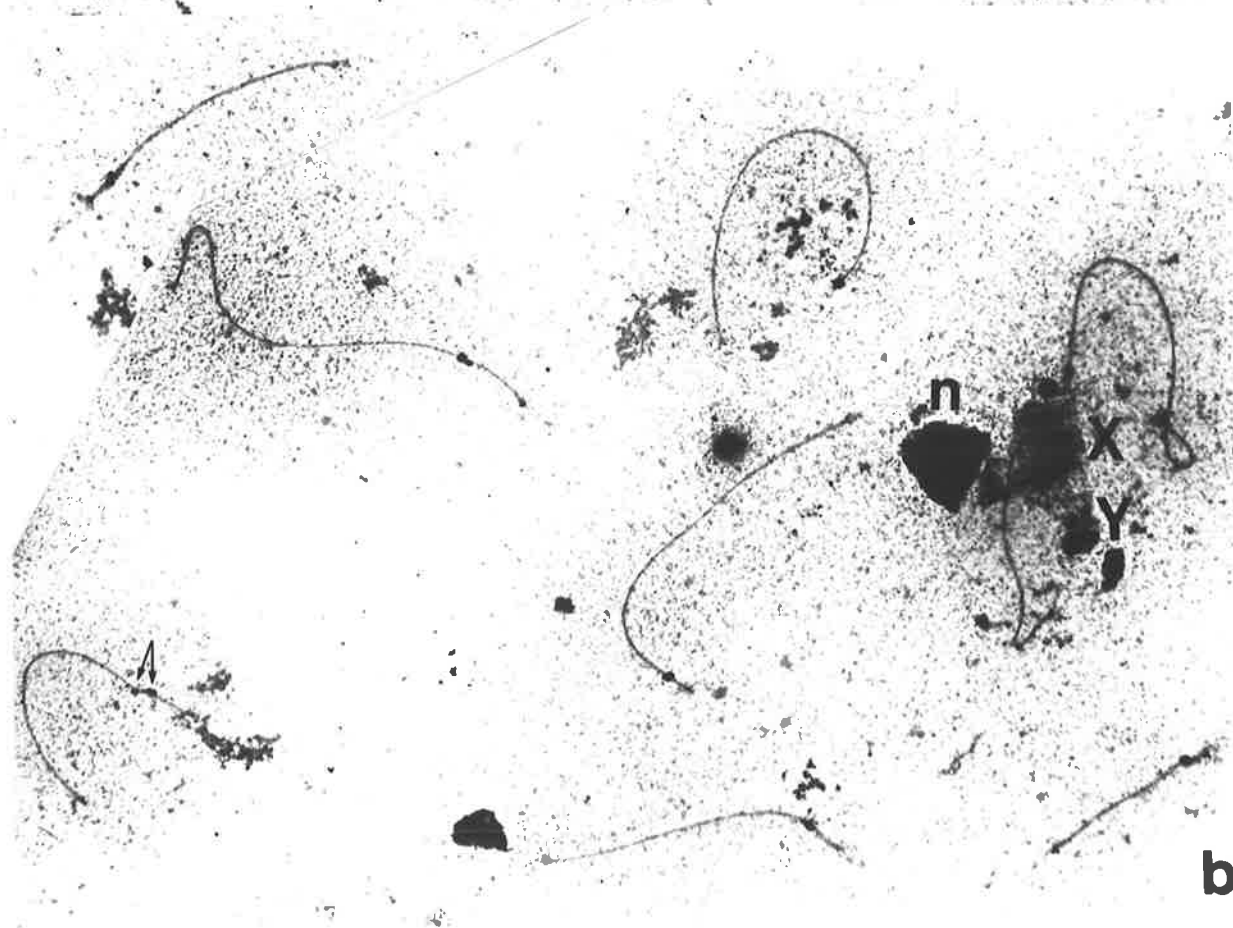
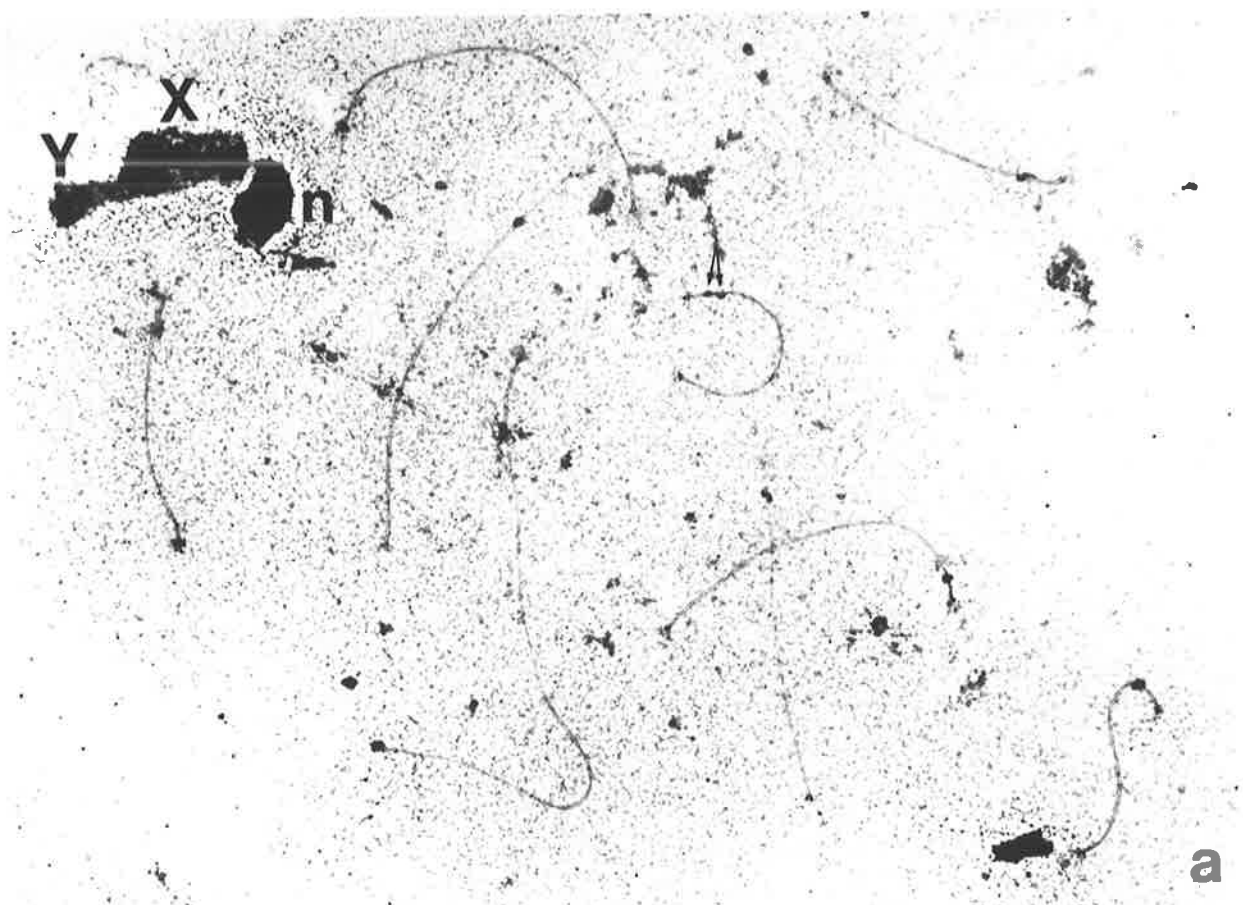
Trichosurus vulpecula

Synaptonemal complex spread preparations were examined with the electron microscope from six *T. vulpecula* individuals. Four of these had synaptonemal complexes in which there were no detectable differences in centromere position in all of the autosomal bivalents. The centromeres on each lateral element were directly opposite each other (Figure 12.2a). The other two animals each had one bivalent in which the centromeres were clearly out of alignment (see Figure 13.2b). In the first individual found with this feature, the heterozygosity was in the shortest bivalent (Figure 13.1a) in most cells, whereas in the other case, most cells showed centromere heterozygosity in a bivalent that usually had two bivalents shorter than it (Figure 13.1b). The occasional cells in each animal in which the rank length of the heterozygous bivalent did not conform to the majority were most likely due to differential stretching of the bivalents during cell preparation.

In these animals the heterozygous bivalent was present in almost all cells examined (Figures 13.1a, b). Such bivalents could arise in animals heterozygous for a pericentric inversion that did not form an inversion loop at pachytene. Ashley *et al* (1981) have described a pericentric inversion in the sand rat *Psammomys* where no inversion loop forms. However, the cell illustrated in Figure 13.2a suggests

Figure 13.1: *Trichosurus vulpecula* synaptonemal complexes.

a, b. Complete cells from two different individuals heterozygous for centromere position. This is indicated by double arrows. In a the heterozygosity is on the shortest synaptonemal complex, while in b it is on a synaptonemal complex which has two shorter than it. In both a and b the sex vesicle (X-Y) and nucleoli (n) are indicated. EM.

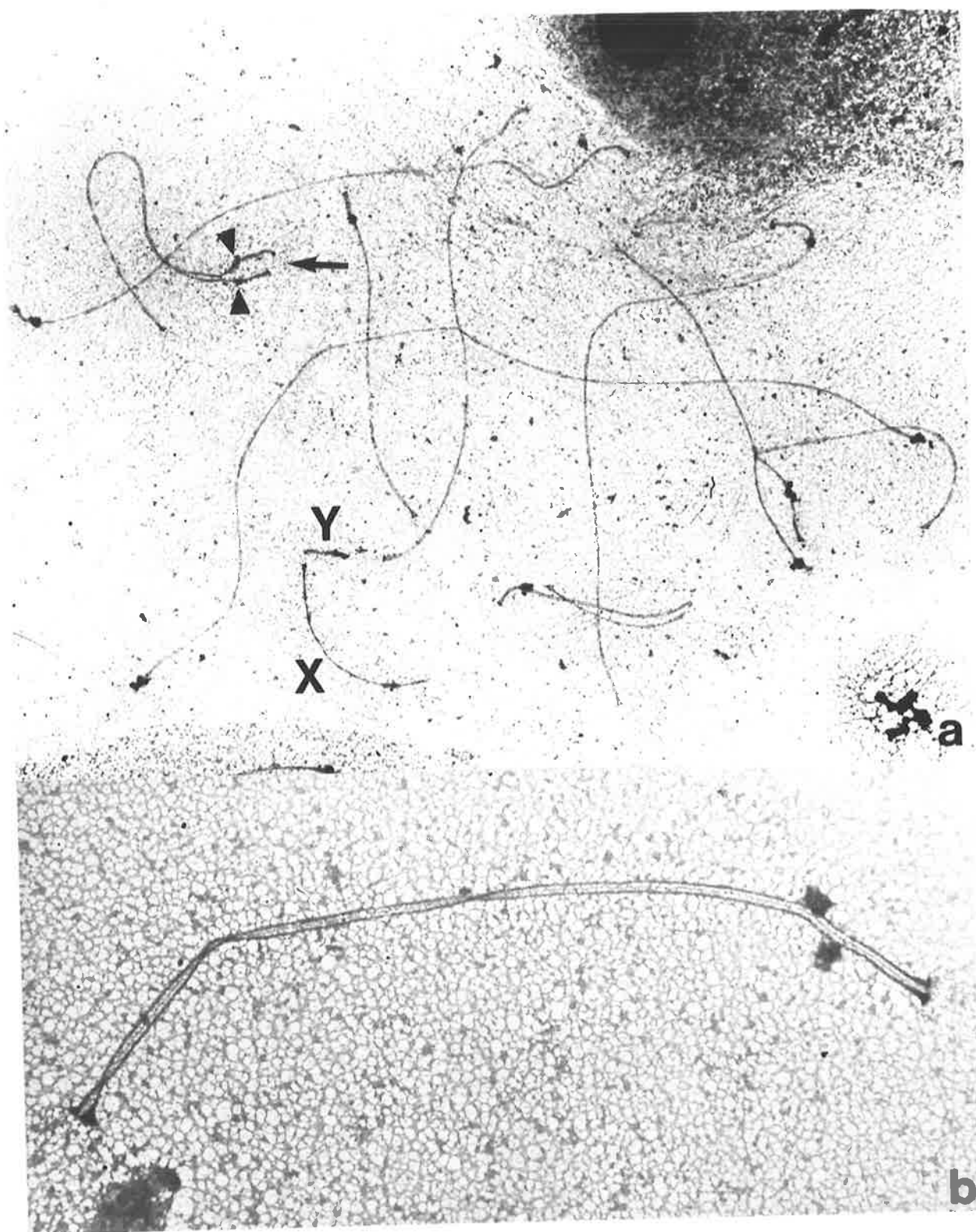


that this is not the case in *Trichosurus*. This cell, from the same animal as Figure 13.3b, was in early pachytene since the sex chromosome axes were approaching each other, but they had not formed the sex vesicle. The heterozygous bivalent had not completely paired, and the unpaired peri-centromeric telomere regions were of unequal length (Figure 13.2a). This suggests that the animal was not heterozygous for a pericentric inversion, but was heterozygous for additional chromosomal material, and the homologous positions of the centromeres with respect to the long arm of the bivalent (Figure 13.2a) suggests that the additional material was on the short arm. Subsequent pairing in this region, followed by synaptic adjustment to equalize the lengths of the lateral elements involved might then result in the non-alignment of the centromeres. This cell was the only one found from over 150 cells examined from both animals which had a region of asynapsis around the heterozygous centromere. If the explanation given above holds, then the synaptic adjustment must occur very quickly, and usually very early.

An obvious candidate for this additional chromatin would be C-banding material, but attempts to confirm this by C-banding mitotic chromosomes of lymphocyte cultures derived from these animals were unsuccessful. The *T. vulpecula* karyotype (Figure 5.1a) consists of twenty acrocentric chromosomes which gradually decrease in length from the largest to the smallest autosome, each with a small C-band at the centromere (Murray, 1977). Consequently, homologous chromosomes cannot be detected with certainty to allow a search either for possible differences in short arm length, or for differences in the amount of C-banding material between homologues. The relative differences in centromere position on the synaptonemal complexes suggests that any differences in the mitotic chromosomes would be small in both animals studied.

Figure 13.2: *Trichosurus vulpecula* synaptonemal complexes.

- a. A complete cell at early pachytene. The X and Y axes are approaching each other. There is a region of asynapsis (arrowed) at the centromeric end of one of the autosomes, and the short arms are of unequal length. The centromeres are indicated by arrowheads. EM.
- b. An autosomal synaptonemal complex showing heterozygosity for centromere position. EM.



Macropus rufus

A single *M. rufus* examined was also found to be heterozygous for centromere position. In all cells from this animal where the synaptonemal complexes were fully paired the centromeres of the largest autosome (chromosome 1) were out of alignment. Figure 13.3a is an example of this. The karyotype of *M. rufus* is such that chromosome 1 can always be distinguished on the basis of length and arm ratio (Hayman and Martin, 1974; Figure 3b in Appendix 3). The centromeres in the fully paired chromosome 1 bivalents were so far apart that in many cells the lateral elements of the synaptonemal complex were twisted in the interval between the two centromeres.

One cell (out of 85) was found in which there was a degree of asynapsis in the chromosome 1 bivalent, but where all the other synaptonemal complexes were fully paired. Here, the long arm of chromosome 1 was fully paired right up to the centromeres which were in homologous positions, but the region of the short arm near the centromere was unpaired (Figure 13.3b). The unpaired regions in the two homologues were clearly different in length. Based on this cell, then, this animal was heterozygous for extra chromatin at the centromeric end of the short arm of chromosome 1.

C-banding was therefore performed on mitotic chromosomes from a fibroblast cell culture derived from this animal. Chromosome 1 was clearly heterozygous for additional C-banding material on the short arm. Figure 13.4 shows chromosome 1 homologues from eight C-banded cells. One of the homologues appears to have a single C-band on the short arm slightly away from the centromeric C-band, while in the other homolog this appears to be either larger, or duplicated. In all the other chromosomes there were no detectable differences between

Figure 13.3: *Macropus rufus* synaptonemal complexes.

- a. A montage of a complete cell displaying unaligned centromeres. The individual bivalents are numbered and the XY pair and nucleolus are indicated. The heterozygous centromeres on the synaptonemal complex of chromosome 1 are arrowed. This is enlarged in the lower left panel; there is a twist of the lateral elements around each other between the two centromeres. EM.
- b. Bivalent 1 from another cell. Here the centromeres are aligned, but there is asynapsis in the short arm near the centromere (arrowed), and the asynapsed lateral elements are of unequal length. EM.

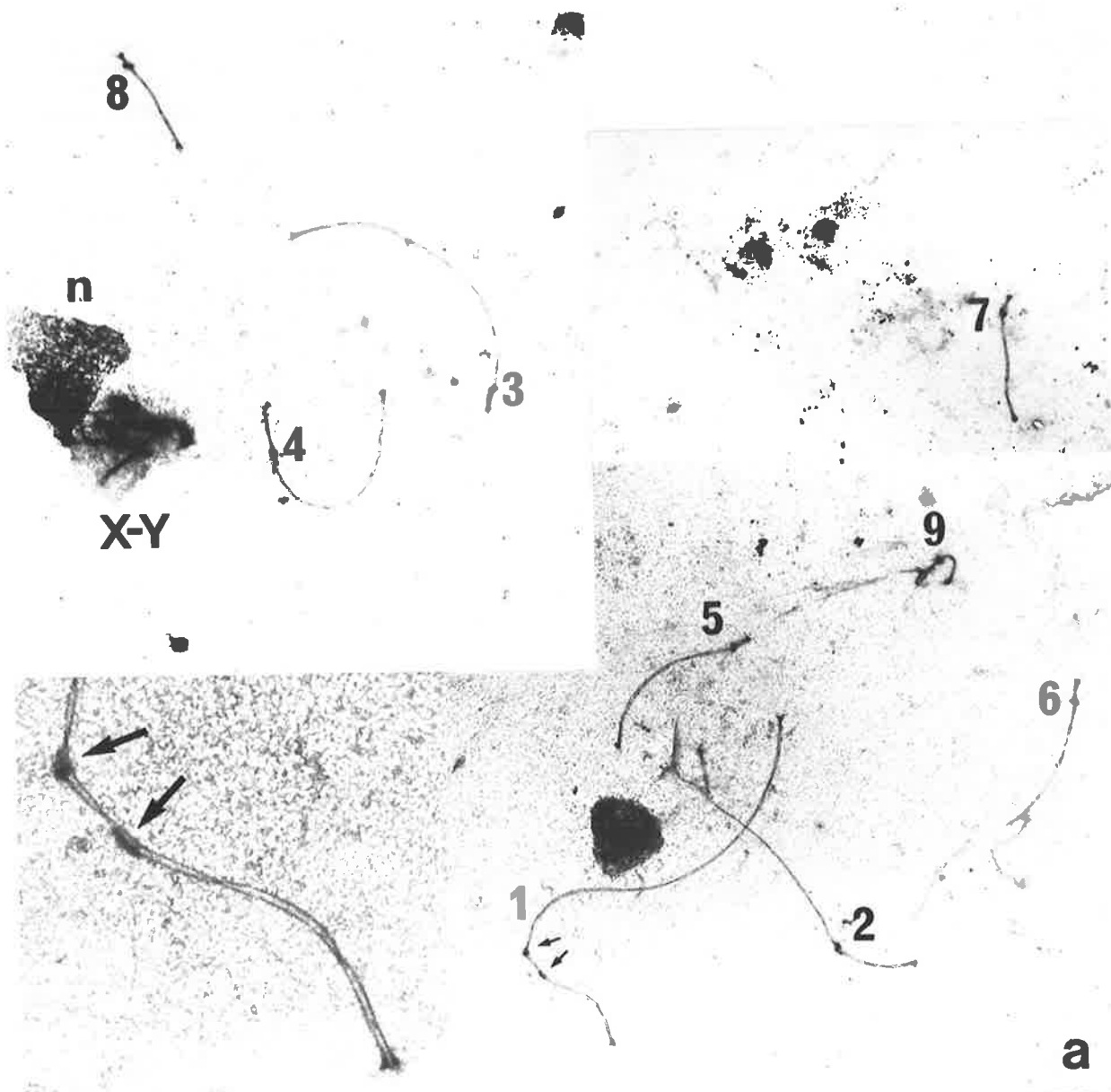


Figure 13.4: C-banding of the chromosome 1 homologues from the *Macropus rufus*. Chromosome 1 homologues from eight cells are shown, with the homolog with the larger C-band on the right in each case.



the homologues. Based on synaptonemal complexes and the C-banding this animal was heterozygous for extra C-banding material on the short arm of chromosome 1.

The conversion of a synaptonemal complex with a buckle (Figure 13.3b) into a fully paired synaptonemal complex in which the centromeres are out of alignment would appear to be an example of synaptic adjustment (Moses and Poorman, 1981). The evidence from the single unadjusted synaptonemal complex and the C-banding suggests that the heterozygosity is for extra chromatin in the short arm, yet in the adjusted synaptonemal complexes the centromeres are not aligned, leading to apparent heterozygosity in both chromosome arms.

Moses and Poorman (1981) have concluded from their study of a mouse tandem duplication that synaptic adjustment involved not only the buckle region, but also the adjacent homosynapsed regions, so that these regions became heterosynapsed. The behaviour of the chromosome 1 bivalent described here would support this; the heterozygosity involves extra chromatin on the short arm near the centromere, but the process of forming a fully paired synaptonemal complex results in heterosynapsis in the long arm, which is visualized as heterozygosity for centromere position. The proximity of the centromere to the extra chromatin allows the heterosynapsis of the regions adjacent to the heterozygosity to be observed directly, as the centromeres provide visual markers on the lateral elements.

Macropus robustus

Because of the behaviour of the heterozygous centromeres in *T. vulpecula* and in the *M. rufus* considered above, a search was made to find other animals with C-banding polymorphisms in order to examine the behaviour of the synaptonemal complexes in heterozygous individuals.

Figure 13.5: C-banding of the lymphocyte chromosomes of *Macropus robustus* 034. The chromosomes from one cell are arranged into a karyotype in the centre, and examples of chromosome 1 and 4 from another cell are included in the boxes. 034 is clearly heterozygous for C-band size on chromosomes 1 and 4. From C-banded preparations supplied by Dr. P. Johnston, Macquarie University.



1



2



3



4



5



6



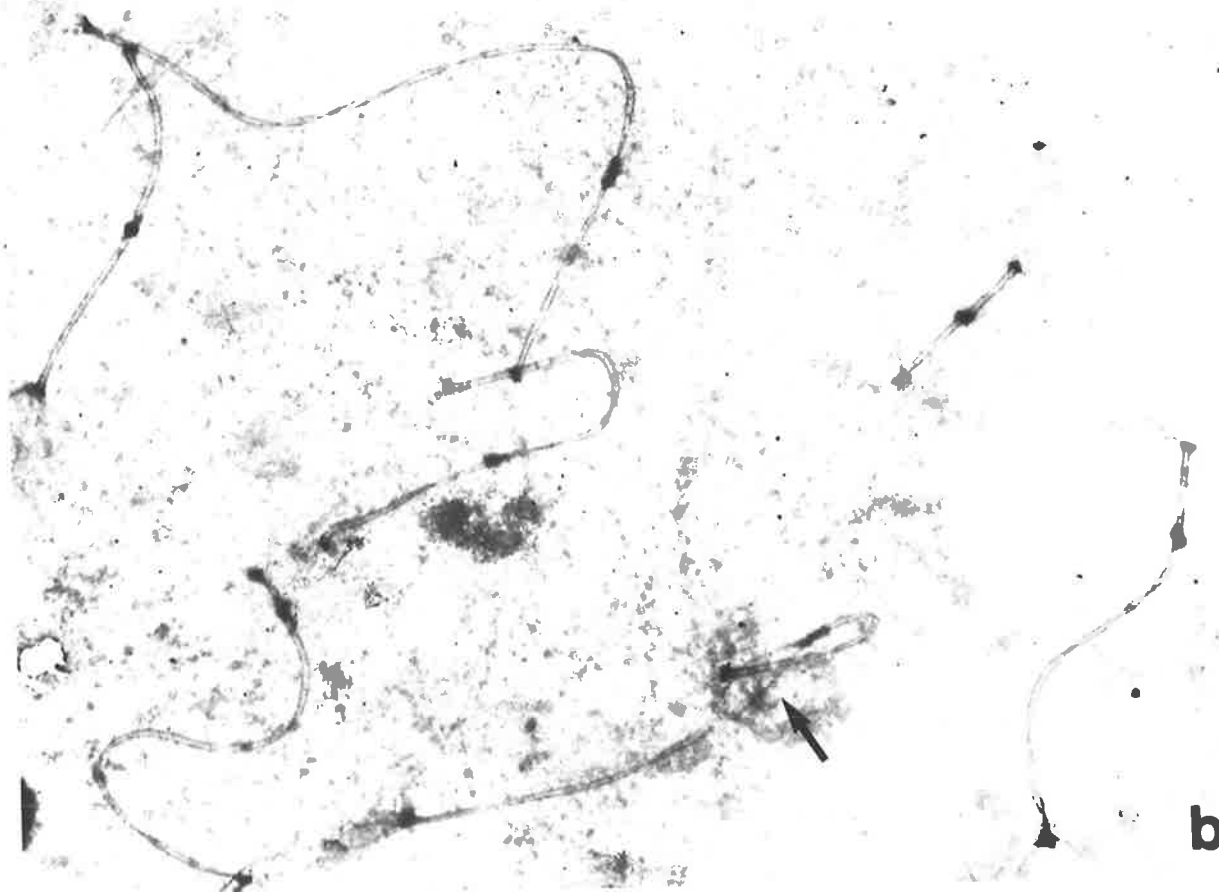
7



XY

Figure 13.6: *Macropus robustus* synaptonemal complexes.

- a. A complete cell displaying the centromeres of all the autosomal bivalents closely aligned. The X and Y axes have moved close together, and have a connection, but have no segment of synaptonemal complex. EM.
- b. A montage showing the autosomal synaptonemal complexes of a cell with closely aligned centromeres. There is a region of asynapsis near the centromere of one autosome (arrowed), but the lateral elements in this region are not clearly of unequal length. This bivalent is possibly interlocked with itself through the region of asynapsis. The sex chromosomes were under a grid bar. EM.



Only one animal could be analysed. This animal was from the Macquarie University (Sydney) colony of *Macropus robustus* which has animals, and their offspring, from various areas of the range of the species, and it was known that some of the animals were heterozygous for the size of particular C-bands (Sharman and Muntagh, personal communication).

Synaptonemal complex preparations were made from a testis biopsy taken from "Blackie" (animal 034 in the Macquarie colony), and lymphocyte cultures were grown from blood taken at the same time.

C-banding of the lymphocyte chromosomes revealed that 034 was clearly heterozygous for C-band size on chromosome 1, and on chromosome 4 (Figure 13.5). The difference between the chromosome 4 homologues was particularly large. The animal was also possibly heterozygous for C-band size on chromosomes 2 and 3, but in these cases the differences, if any, were much smaller.

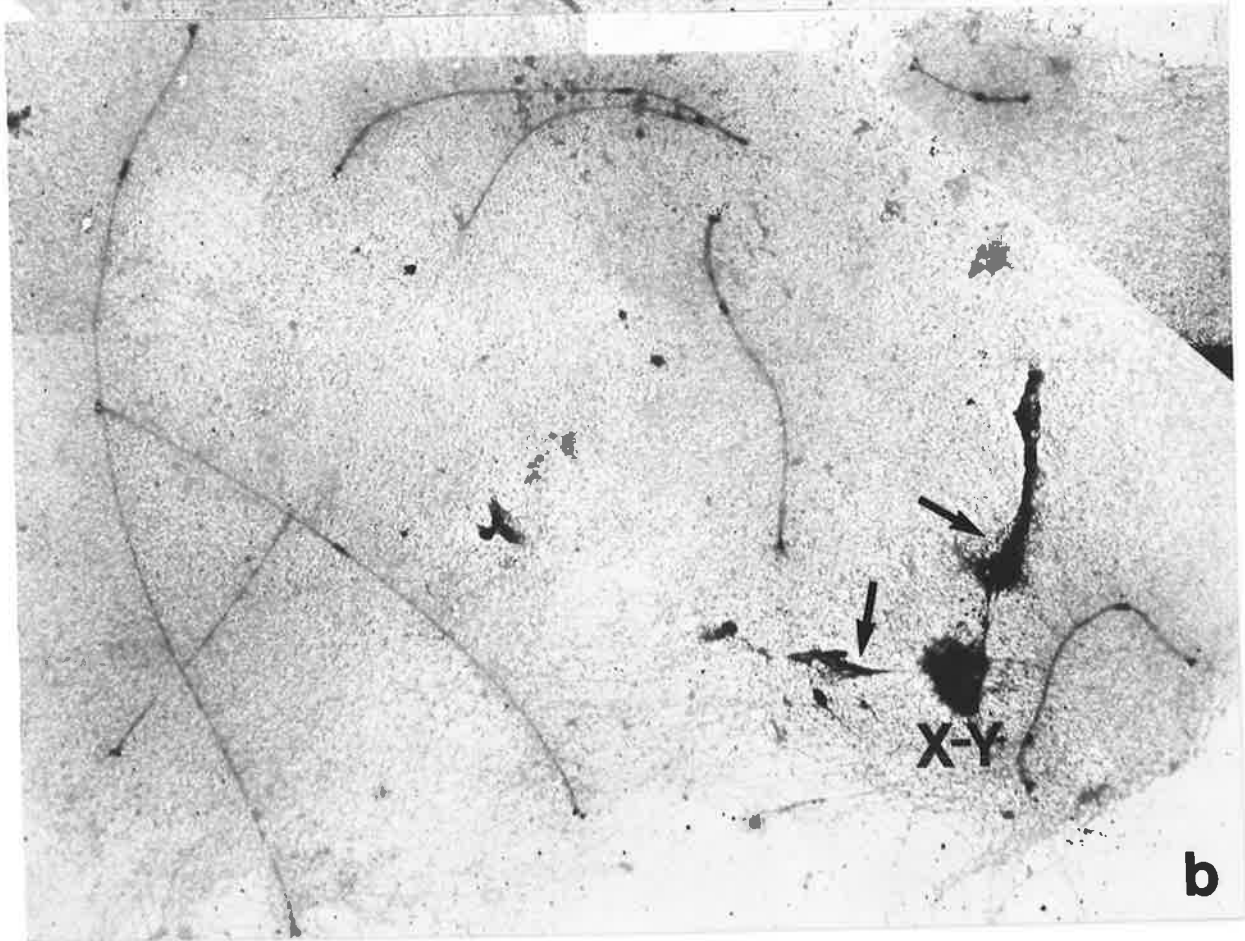
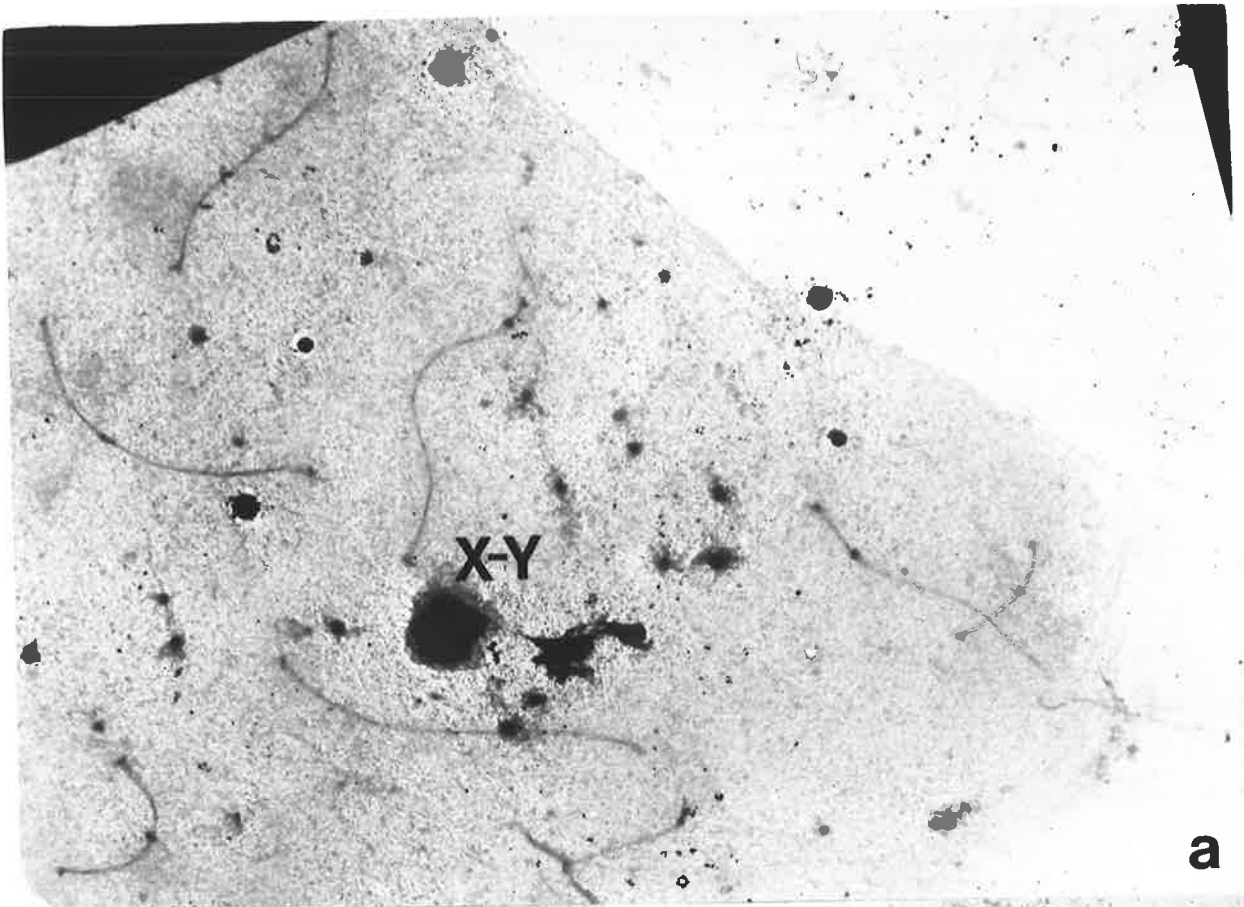
However, when the synaptonemal complex spreads from this animal were examined there was no evidence of heterozygosity for centromere position on any synaptonemal complex in the 173 cells observed. Figures 13.6 and 13.7 show four cells that display no heterozygosity for centromere position. Additionally, in each of the five synaptonemal complexes found that had regions of asynapsis, as in Figure 13.6b, there was no evidence of differences between the homologues in the length of non-synapsed lateral elements.

Closer examination of the C-banding patterns of the lymphocyte chromosomes revealed that the additional C-banding material on the homolog with the larger C-band appeared to be added symmetrically on both sides of the centromere in both chromosomes 1 and 4 (Figure 13.5).

Based on the *M. rufus* and *M. robustus* considered here, asymmetrical C-band heterozygosity around the centromere is associated with centromere

Figure 13.7: *Macropus robustus* synaptonemal complexes.

- a. A complete cell showing the centromeres of all the autosomal bivalents closely aligned. The sex vesicle (X-Y) has little internal detail visible, and has a single nucleolus attached.
- b. A montage of a complete cell. The centromeres of all the autosomal bivalents are closely aligned. The sex vesicle (X-Y) has little internal detail visible, and has two nucleoli attached (arrowed).



position heterozygosity on the synaptonemal complex, whereas symmetrical C-band heterozygosity around the centromere leads to synaptonemal complexes with both centromeres aligned, as in homomorphic bivalents. Overall differences between the homologues in chromosome length and arm ratio do not appear to result in unaligned centromeres, since these differences arise with both asymmetrical and symmetrical (in non-metacentric chromosomes, as in the *M. robustus*) C-banding heterozygosity.

This suggests that the synaptic adjustment responsible for the unaligned centromeres in the *M. rufus* chromosome 1 is not a response to whole chromosome heterozygosity (for length and arm ratio), but a response to heterozygosity of a more local nature. This must be because pairing is not a whole chromosome but a more local process, and the synaptic adjustment takes place after homologous pairing. Synaptic adjustment of the asymmetric heterozygosity in the *M. rufus* would then cause heterozygosity for centromere position in the adjusted synaptonemal complex as the heterozygosity is close to the centromere, whereas adjustment of the symmetric heterozygosities in the *M. robustus* 034 would not result in centromere position heterozygosity as the adjustment is balanced on both sides of the centromere, resulting in no net effect on relative centromere positions. Studies of other instances of asymmetric C-banding heterozygosity should throw light on how far the effects of synaptic adjustment extend along the bivalent, and how differences in the size of the heterozygosity might influence this.

The very low frequency of unadjusted synaptonemal complexes observed (1 cell out of over 300 from the two *T. vulpecula*, 1 cell from the 85 from the *M. rufus*, and none out of 173 cells from the

M. robustus) indicates that the synaptic adjustment in these C-band heterozygosities must occur very early during chromosome pairing.

APPENDIX ONE

Hayman, D., & Sharp, P. (1981). Hoechst 33258 induced uncondensed sites in marsupial chromosomes. *Chromosoma*, 83(2), 249-262.

NOTE:

This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<https://doi.org/10.1007/bf00286793>

APPENDIX TWO

Hayman, D., & Sharp, P. (1981). Verification of the structure of the complex sex chromosome system in *Lagorchestes conspicillatus* Gould (Marsupialia: Mammalia). *Chromosoma*, 83(2), 263-274.

NOTE:

This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<https://doi.org/10.1007/bf00286794>

APPENDIX THREE

This paper was written during enrolment for the degree of Ph.D. Data from the hybrid wallaby were collected and analysed during my Honours year (Unpublished Honours thesis, Genetics Department, University of Adelaide), but the data from the X chromosomes of *Macropus parryi* and *M. rufus* were collected during my enrolment for the degree of Ph.D.

Sharp, P., & Hayman, D. (1981). The distribution of SCEs within and between the genomes of an interspecific hybrid. *Chromosoma*, 84(2), 231-242.

NOTE:

This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

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APPENDIX FOUR

The species called *Tarsipes spencerae* in this paper should now be referred to as *Tarsipes rostratus* (Mahoney, 1981).

Hayman, D., & Sharp, P. J. (1982). The Chromosomes of *Tarsipes spencerae* Gray (Marsupialia). *Australian Journal of Zoology*, 30(5), 799-803. doi:

NOTE:

This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

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REFERENCES:

- Air, G.M., Thompson, E.O.P., Richardson, B.J. and Sharman, G.B.
(1971) Amino-acid sequences of kangaroo myoglobin and haemoglobin and the date of marsupial-eutherian divergence. *Nature* 229, 391-394.
- Allen, J.W. (1979) BrdU-dye characterization of late replication and meiotic recombination in Armenian hamster germ cells. *Chromosome* 74, 189-207.
- Altmann, S.C.A. and Ellery, M.E.W. (1925) The chromosomes of four species of marsupials. *Quart. J. Micro. Sci.* 69, 463-470.
- Andrews, J.H. and Rouse, D.I. (1982) Plant pathogens and the theory of r- and K-selection. *Amer. Nat.* 120, 283-296.
- Arrayo, M.T.K. (1973) Chiasma frequency evidence on the evolution of autogamy in *Limnathes floccosa*. *Evolution* 27, 679-688.
- Arrighi, F.E., Hsu, T.C., Pathak, S. and Sawada, H. (1974) The sex chromosomes of the Chinese hamster: Constitutive heterochromatin deficient in repetitive DNA sequences. *Cytogenet. Cell Genet.* 13, 268-274.
- Ashley, T. and Moses, M.J. (1980) End association and segregation of the achiasmatic X and Y chromosomes of the sand rat, *Psammomys obesus*. *Chromosoma* 78, 203-210.
- Ashley, T., Moses, M.J. and Solari, A.J. (1981) Fine structure and behaviour of a pericentric inversion in the sand rat, *Psammomys obesus*. *J. Cell Sci.* 50, 105-119.
- Ayonoadu, U.W.U. and Rees, H. (1968) The influence of B chromosomes on chiasma frequencies in Black Mexican Sweet Corn. *Genetica* 39, 75-81.

Balazs, I., Filippi, G., Rinaldi, A, Grzeschik, K.H. and Siniscales, M.

(1979) Studies on human X-linked ichthyosis and steroid sulphatase in man, mice, and their hybrids. Cytogenet. Cell Genet. 25, 133

Baker, T.G. (1972) Oogenesis and ovulation. In: Reproduction in Mammals I Germ Cells and Fertilization (C.R. Astin and R.V. Short, eds.) Cambridge University Press.

Baverstock, P.R., Gelder, M. and Jahnke, A. (1982) Cytogenetic studies of the Australian rodent, *Chomys caudimaculatus*, a species showing extensive heterochromatin variation. Chromosoma 84, 517-533.

Belling, J. (1933) Critical notes on C.D. Darlington's "Recent Advances in Cytology". University of California Publications in Botany 17, 75-110.

Bengtsson, B.O. (1980) Rates of karyotype evolution in placental mammals. Hereditas 92, 37-47.

Bennett, J.H., Smith, M.J., Hope, R.M. and Chesson, C.M. (1982) Fat-tailed Dunnart *Sminthopsis crassicaudata*: establishment and maintenance of a laboratory colony. In: "The Management of Australian Mammals in Captivity" (D.D. Evans, ed.) Zoological Board of Victoria, Melbourne.

Bennett, M.D., Finch, R.A. Smith, J.B. and Rao, M.K. (1973) The time and duration of female meiosis in rye, wheat and barley. Proc. R. Soc. Lond. B. 183, 301-319.

Bishop, O.N. (1981) Statistics for Biology: A practical guide for the experimental biologist. 3rd Ed., Longman.

- deBoer, R. and Branje, H.E.B. (1979) Association of the extra chromosome of tertiary trisomic male mice with the sex chromosomes during first meiotic prophase, and its significance for impairment of spermatogenesis. *Chromosoma* 73, 369-379.
- deBoer, P. and Searle, A.G. (1980) Summary and synthesis. In: Workshop on chromosomal aspects of male sterility in mammals. *J. Reprod. Fert.* 60, 257-265.
- deBoer, P. and Van den Hoeven, F.A. (1977) Son sire regression based heritability estimates of chiasma frequency using T70H mouse translocation heterozygotes, and the relation between univalence, chiasma frequency and sperm produced. *Heredity* 39, 335-343.
- Burgoyne, P.S. (1982) Genetic homology and crossing over in the X and Y chromosomes of mammals. *Hum. Genet.* 61, 85-90.
- Bush, G.L., Case, S.M. Wilson, A.C. and Patton, J.L. (1977) Rapid speciation and chromosomal evolution in mammals. *Proc. Nat. Acad. Sci.* 74, 3942-3946.
- Callan, H.G. and Perry, P.E. (1977) Recombination in male and female meiocytes contrasted. *Phil. Trans. R. Soc. Lond. B.* 277, 227-233.
- Cameron, F.M. and Rees, H. (1967) The influence of B chromosomes on meiosis in *Lolium*. *Heredity* 22, 446-450.
- Carpenter, A.T.C. (1975) Electron microscopy of meiosis in *Drosophila melanogaster* II. The recombination nodule - a recombination associated structure at pachytene? *Proc. Nat. Acad. Sci.* 72, 3186-3189.

- Carpenter, A.T.C. (1979) Synaptonemal complex and recombination nodules in recombination - deficient mutants of *Drosophila melanogaster*. *Chromosoma* 75, 259-292.
- Carpenter, A.T.C. (1981) EM autoradiographic evidence that DNA synthesis occurs at recombination nodules in *Drosophila melanogaster*. *Chromosoma* 83, 59-80.
- Carson, H.L. (1955a) Variation in genetic recombination in natural populations. *J. Comp. Cell Physiol.* 45, Suppl. 2, 221-236.
- Carson, H.L. (1955b) The genetic characteristics of marginal populations of *Drosophila*. *Cold Spring Harbor Symp. Quant. Biol.* 20, 276-287.
- Carson, H.L. (1957) The species as a field for gene recombination. In: *The Species Problem* (Mayr, E. ed.) *Am. Ass. Adv. of Sci. Publ.* 50.
- Carson, H.L. (1958) Response to selection under different conditions of recombination in *Drosophila*. *Cold Spring Harbor Symp. Quant. Biol.* 23, 291-306.
- Carson H.L. and Heed, W.B. (1964) Structural homozygosity in marginal populations of nearctic and neotropical species of *Drosophila* in Florida. *Proc. Nat. Acad. Sci. U.S.A.* 52, 427-430.
- Catcheside, D.G. (1977) "The Genetics of Recombination", Edward Arnold.
- Chandley, A.C. (1982) A pachytene analysis of two male-fertile paracentric inversions in chromosome 1 of the mouse and in the male-sterile double heterozygote. *Chromosoma* 85, 127-135.
- Charlesworth, B. (1976) Recombination modification in a fluctuating environment. *Genetics* 83, 181-195.

- Chinnici, J.P. (1971a) Modification of recombination frequency in *Drosophila* I. Selection for increased and decreased crossing over. *Genetics* 69, 71-83.
- Chinnici, J.P. (1971b) Modification of recombination frequency in *Drosophila* II. The polygenic control of crossing over. *Genetics* 69, 85-96.
- Church, K. and Wimber D.E. (1969) Meiosis in the grasshopper: Chiasma frequency after elevated temperature and X-rays. *Can. J. Genet. Cytol.* 11, 209-216.
- Clarke, B.C. (1979) The evolution of genetic diversity. *Proc. R. Soc. Lond. B.* 205, 453-474.
- Clemens, W.A. (1977) Phylogeny of the marsupials. In: *The Biology of Marsupials*. (Stonehouse B. and Gilmore, D. eds.) Macmillan Press.
- Cockburn, A., Lee, A.F. and Martin, R.W. (1983) Macrogeographic variation in litter size in *Antechinus* (Marsupialia: Dasyuridae) *Evolution* 37, 86-95.
- Collins, L.R. (1973) "Monotremes and Marsupials - A reference for Zoological Institutions". Smithsonian Institution Press, Washington.
- Counce, S.T. and Meyer, G.F. (1973) Differentiation of the synaptonemal complex and kinetochore in *Locusta* spermatocytes studied by whole mount electron microscopy. *Chromosoma* 44, 231-253.
- Couzin, D.A. and Fox, D.P. (1974) Variation in chiasma frequency during tulip anther development. *Chromosoma* 46, 173-179.
- Crowley, J.G. (1969) Chiasma frequency and longevity in *Lolium multiflorum*. *Heredity* 24, 181-188.

- Darlington, C.D. (1932) "Recent Advances in Cytology", J. and A. Churchill, London.
- Darlington, C.D. (1932) The origin and behaviour of chiasmata
V. *Chorthippus elegans*. Biol. Bull. Woods. Hole 63, 357-367.
- Darlington, C.D. (1939) "The Evolution of Genetic Systems",
Cambridge University Press.
- Darlington, C.D. (1965) "Cytology", J. and A. Churchill, London.
- Darlington, C.D. (1973) The place of the chromosomes in the
genetic system. Chromosomes Today 4, 1-13.
- Darlington, C.D. (1978) A diagram of evolution. Nature 276,
447-452.
- Davies, E.D.G. and Jones, G.H. (1974) Chiasma variation and control
in pollen mother cells and embryo-sac mother cells of rye.
Genet. Res. 23, 185-190.
- Davisson, M.T., Poorman, P.A., Roderick, T.H. and Moses, M.J.
(1981) A pericentric inversion in the mouse. Cytogenet.
Cell Genet. 30, 70-76.
- Davisson, M. and Roderick, T.H. (1981) Linkage map of the mouse.
In: Biology of the House Mouse (Berry, R.J. ed.) 47th Symposia
of the Zoological Society of London.
- Donald, J.A. and Hope, R.M. (1981) Mapping a marsupial X chromosome
using kangaroo-mouse somatic cell hybrids. Cytogenet. Cell
Genet. 29, 127-137.
- Douglas, L.T., Cupedo, D.F. and Douglas, R.M. (1978) Are terminal
chiasmata in male *Schistocerca gregaria* produced by telomere
stickiness or by crossover processes? Genetica 49, 127-130.
- Dresser, M.E. and Moses, M.J. (1980) Synaptonemal complex karyo-
typing in spermatocytes of the Chinese hamster (*Cricetulus*
griseus) IV. Light and electron microscopy of synapsis and
nucleolar development by silver staining. Chromosoma 76, 1-22.

- Drummond, F.H. (1938) Meiosis in *Dasyurus viverrinus*. *Cytologia* 8, 343-352.
- Dunsmuir, P. (1976) Satellite DNA in the kangaroo *Macropus rufogriseus*. *Chromosoma* 56, 111-156.
- Eisenberg, J.F. (1981) "The Mammalian Radiations". University of Chicago Press.
- Elder, F.F.B. and Pathak, S. (1980) Light microscopic observations on the behaviour of silver-stained trivalents in pachytene cells of *Sigrodon fulviventer* (Rodentia, Muridae) heterozygous for centric fusion. *Cytogenet. Cell Genet.* 27, 31-38.
- Elliot, C.G. (1955) The effect of temperature on meiosis. *Heredity* 11, 37-49.
- Evans, E.P. (1979) Cytological methods for the study of meiotic properties in mice. *Genetics* 92, s97-s103.
- Evans, E.P., Breckon, G. and Ford, C.E. (1964) An air drying method for meiotic preparations from mammalian testes. *Cytogenetics* 3, 289-294.
- Evans, E.P., Burtenshaw, M.D. and Cattanaach, B.M. (1982) Meiotic crossing-over between the X and Y chromosomes of male mice carrying the sex-reversing (Sxr) factor. *Nature* 300, 443-445.
- Fawcett, D.W. (1956) The fine structure of chromosomes in the meiotic prophase of vertebrate spermatocytes. *J. Biophys. Biochem. Cytol.* 2, 403-406.
- Felsenstein, J. (1974) The evolutionary advantage of recombination. *Genetics* 78, 737-756.
- Felsenstein, J. and Yokoyama, S. (1976) The evolutionary advantage of recombination II. Individual selection for recombination. *Genetics* 83, 845-859.

- Fisher, R.A. (1930) "The Genetical Theory of Natural Selection".
Oxford University Press, revised (1958), 2nd edition. Dover
Publications.
- Fletcher, H.L. and Hewitt, G.M. (1978) Non-homologous synaptonemal
complex formation in a heteromorphic bivalent in *Keyaeris
scura* (Morabinae, Othoptera). *Chromosoma* 65, 271-287.
- Fletcher, H.L. and Hewitt, G.M. (1980a) A comparison of chiasma
frequency and distribution between sexes in three species
of grasshoppers. *Chromosoma* 77, 129-144.
- Fletcher, H.L. and Hewitt, G.M. (1980b) Effect of a B chromosome
on chiasma localization and frequency in male *Euthystira*
brachyptera. *Heredity* 44, 341-347.
- Fletcher, J.M. (1979) Light microscope analysis of meiotic
prophase chromosomes by silver staining. *Chromosoma* 72,
241-248.
- Flexon, P.B. and Rodell, C.F. (1982) Genetic recombination and
directional selection for DDT resistance in *Drosophila
melanogaster*. *Nature* 298, 672-673.
- Fontana, P.G. and Vickery, V.R. (1973) Segregation distortion in
the B-chromosome system of *Tettigidea lateralis* (Say)
(Orthoptera: Tettigidae). *Chromosoma* 43, 75-100.
- Forejt, J. and Gregorová, S. (1977) Meiotic studies of trans-
locations causing male sterility in the mouse I. Autosomal
reciprocal translocations. *Cytogenet. Cell Genet.* 19, 159-179.
- Forejt, J., Gregorová, S. and Goetz, P. (1981) XY pair associates
with the synaptonemal complex of autosomal male-sterile
translocations in pachytene spermatocytes of the mouse (*Mus
musculus*). *Chromosoma* 82, 41-53.

- Fox, D.P. (1973) The control of chiasma distribution in the locust, *Schistocerca gregaria* (Forsk.) . *Chromosoma* 43, 289-328.
- Gale, M.D. and Rees, H. (1970) Genes controlling chiasma frequency in *Hordeum*. *Heredity* 25, 393-410.
- Gartler, S.M. and Rivest, M. (1983) Evidence for X-linkage of steroid sulfatase in the mouse: Steroid sulfatase levels in oocytes of XX and XO mice. *Genetics* 103, 137-141.
- Gemmell, G.T. (1982) Breeding bandicoots in Brisbane (*Isodon macrourus*; Marsupialia, Peramelidae). *Aust. Mammal.* 5, 187-193.
- Gillies, C.B. (1972) Reconstruction of the *Neurospora crassa* pachytene karyotype from serial sections of synaptonemal complexes. *Chromosoma* 36, 117-130.
- Gillies, C.B. (1974) The nature and extent of synaptonemal complex formation in haploid barley. *Chromosoma* 48, 441-453.
- Gillies, C.B. (1975) Synaptonemal complex and chromosome structure. *Ann. Rev. Genet.* 9, 91-109.
- Gillies, C.B. (1981) Electron microscopy of spread maize pachytene synaptonemal complexes. *Chromosoma* 83, 575-591.
- Glesener, R.P. and Tilman, D. (1978) Sexuality and the components of environmental uncertainty: clues from geographic parthenogenesis. *Amer. Natur.* 112, 659-673.
- Gondos, B. (1978) Oogonia and oocytes in mammals. In: *The Vertebrate Ovary. Comparative Biology and Evolution* (Jones, R.E. ed.) Plenum Press: New York.
- Goodfellow, P., Banting, G., Sheer, D., Ropers, H.H., Caine, A., Ferguson-Smith, M.A., Povey, S. and Voss, R. (1983) Genetic evidence that a Y-linked gene in man is homologous to a gene on the X chromosome. *Nature* 302, 346-349.

- Grant, V. (1958) The regulation of recombination in plants.
Cold Spring Harbor Symposium on Quantitative Biology 23,
337-363.
- Grant, V. (1975) "The Genetics of Flowering Plants". Columbia
University Press.
- Greenslade, P.J.M. (1982) Selection processes in arid Australia.
In: "Evolution of the Flora and Fauna of Arid Australia"
Barker, W.R. and Greenslade, P.J.M. eds.) Peacock Publications,
A.S.B.S., A.N.Z.A.A.S., S.A.Inc.
- Grell, R.F. (1962) A new hypothesis on the nature and sequence
of meiotic events in the female of *Drosophila melanogaster*.
Proc. Nat. Acad. Sci. 48, 165-172.
- Gunson, M.M., Sharman, G.B. and Thomson, J.A. (1968) The affinities
of *Burramys* (Marsupialia: Phalangerioidea) as revealed by a
study of its chromosomes. Aust. J. Sci. 31, 40-41.
- Haldane, J.B.S. (1931) The cytological basis of genetical inter-
ference. Cytologia 3, 54-65.
- Hartung, M., Mirre, C. and Stahl, A. (1979) Nucleolar organizers
in human oocytes at meiotic prophase I, studied by the Silver-
NOR method and electron microscopy. Hum. Genet. 52, 295-308.
- Hartung, M. and Stahl, A. (1977) Preleptotene chromosome condensation
in mouse oogenesis. Cytogenet. Cell Genet. 18, 309-319.
- Hayinarayana, G. and Murty, B.R. (1973) Cytological regulation of
recombination in *Pennisetum* and *Brassica*. Cytologia 36,
435-448.
- Hayman, D.L. (1981) Components of genetic systems in mammals.
In: "Evolution and Speciation: Essays in Honor of M.J.D. White".
(Atchley, W.R. and Woodruff, D. eds.) Cambridge University
Press.

- Hayman, D.L., Ashworth, L.K. and Carrano, A.V. (1982) The relative DNA contents of the eutherian and marsupial X chromosomes. *Cytogenet. Cell Genet.* 34, 265-270.
- Hayman, D.L. and Martin, P.G. (1965) An autoradiographic study of DNA synthesis in the sex chromosomes of two marsupials with an XX/XY_1Y_2 sex chromosome mechanism. *Cytogenetics* 4, 209-218.
- Hayman, D.L. and Martin, P.G. (1974) Mammalia I: Monotremata and Marsupialia. Vol. 4: Chordata 4. In: *Animal Cytogenetics* (John, B. ed.) Gebrüder Borntraeger, Berlin-Stuttgart.
- Hayman, D.L., Martin, P.G. and Waller, P.F. (1969) Parallel mosaicism of supernumerary chromosomes and sex chromosomes in *Echymipera kalabu* (Marsupialia). *Chromosoma* 27, 371-380.
- Hayman, D.L. and Rofe, R.H. (1977) Marsupial sex chromosomes. In: *Reproduction and Evolution* (Calaby, J.A. and Tyndale-Biscoe, C.H. eds.) Australian Academy of Sciences.
- Hayman, D. and Sharp, P. (1981a) Hoechst 33258 induced uncondensed sites in marsupial chromosomes. *Chromosoma* 83, 249-262.
- Hayman, D. and Sharp, P. (1981b) Verification of the structure of the complex sex chromosome system in *Lagorchestes conspicillatus* Gould (Marsupialia: Mammalia). *Chromosoma* 83, 263-274.
- Henderson, S.A. (1963) Chiasma distribution at diplotene in a locust. *Heredity* 18, 173-190.
- Hewitt, G.M. (1973) The structure and role of B-chromosomes in the Mottled Grasshopper. *Chromosomes Today* 3, 208-222.
- Hewitt, G.M. and John, B. (1967) The B-chromosome system of *Myrmeleotettix maculatus* (Thumb) III. The statistics. *Chromosoma* 21, 140-162.

- Hobolth, P. (1981) Chromosome pairing in allohexaploid wheat var. Chinese Spring. Transformation of multivalents into bivalents, a mechanism for exclusive bivalent formation. Carlsberg Res. Commun. 46, 129-173.
- Holm, P.B. and Rasmussen, S.W. (1978) Human meiosis III. Electron microscopical analysis of chromosome pairing in a individual with a balanced translocation 46, XY, t(5p-; 22p+). Carlsberg Res. Commun. 43, 329-350.
- Holm, P.B., Rasmussen, S.W. and von Wettstein, D. (1982) Ultra-structural characterization of the meiotic prophase. A tool in the assessment of radiation damage in man. Mutation Research, 95, 45-59.
- Holm, P.B., Rasmussen, S.W., Zickler, D., Lu, B.C. and Sage, J. (1981) Chromosome pairing, recombination nodules and chiasma formation in the Basidiomycete *Coprinus cinereus*. Carlsberg Res. Commun. 46, 305-346.
- Hotta, Y. and Chandley, A.C. (1982) Activities of X-linked enzymes in spermatocytes of mice rendered sterile by chromosomal alterations. Gamete Res. 6, 65-72.
- How, R.A. (1978) Population strategies of four species of Australian "possum". In: "The Ecology of Arboreal Folivores (Montgomery, G.G. ed.) Smithsonian Institute Press, Washington.
- Hsu, T.C., Spirits, E. and Pardne, M.L. (1975) Distribution of 18 + 285 ribosomal genes in mammalian genomes. Chromosoma 53, 25-36.
- Hulten, M. (1974) Chiasma distribution at diakinesis in the normal human male. Hereditas 76, 55-78.

Hulten, M., Luciani, J.M., Kirton, V. and Devietor-Vuillet, M.

(1978) The use and limitations of chiasma scoring with reference to human genetic mapping. *Cytogenet. Cell Genet.* 22, 37-58.

Hungerford, D.A., Ashton, F.T., Balaban, G.B., LuBadie, G.V.,

Messatzya, L.R., Haller, G. and Miller, A.E. (1972) The C-group bivalent with a locus characteristic for parachromosomally situated particulate bodies (parameres): a provisional map in human males. *Proc. Nat. Acad. Sci.* 69, 2165-2168.

Hutson, V. and Law, R. (1981) Evolution of recombination in populations experiencing frequency dependent selection with time delay. *Proc. R. Soc. Lond. B.* 213, 345-359.

Hyett, J. (1980) "Australian Mammals - A field guide for New South Wales, South Australia, Victoria and Tasmania". Nelson, Melbourne.

Imai, H.T. and Moriwaki, K. (1982) A re-examination of chiasma terminalization and chiasma frequency in male mice. *Chromosoma* 85, 439-452.

Inns, R.W. (1982) Life-spans of the Tammar wallaby *Macropus eugenii* (Marsupialia: Macropodidae) in wild populations. *Aust. Mammal.* 5, 283-284.

Jaenike, J. (1978) An hypothesis to account for the maintenance of sex within populations. *Evolut. Theory* 3, 191-194.

Jagiello, G., Ducayen, M., Fang, J.-S. and Graffeo, J. (1975) Cytogenetic observations in mammalian oocytes. *Chromosomes Today* 5, 43-63.

Jagiello, G. and Fang, J.-S. (1980) A pachytene map of the mouse oocyte. *Chromosoma* 77, 113-121.

- Johannison, R., Gropp, A., Winking, H., Coerdts, W., Rehder, H.
and Schwinger (1983) Down's syndrome in the male. Reproductive
pathology and meiotic studies. Hum. Genet. 63, 132-138.
- John, B. (1976) Myths and mechanisms of meiosis. Chromosoma 54,
295-325.
- John, B. and Hewitt, G.H. (1965) The B chromosome system of
Myrmecotettix maculatus (Thumb). I. The mechanics.
Chromosoma 16, 548-578.
- John, B. and Lewis, K.R. (1965) "The meiotic system". Protoplasma-
tologia 6, 1-335.
- John, B. and Lewis, K.R. (1975) "Chromosome Hierarchy: An
Introduction to the biology of the chromosome".
- John, B. and Miklos, G.L.G. (1979) Functional aspects of satellite
DNA and heterochromatin. Int. Rev. Cytol. 58, 1-114.
- Jones, G.H. (1967) The control of chiasma distribution in rye.
Chromosoma 22, 69-90.
- Jones, G.H. (1974a) Is chiasma determination sequential? Nature 250,
146-147.
- Jones, G.H. (1974b) Correlated components of chiasma variation and
the control of chiasma distribution in rye. Heredity 32,
375-387.
- Jones, G.H. (1978) Giemsa C-banding of rye meiotic chromosomes
and the nature of "terminal" chiasmata. Chromosoma 66,
45-57.
- Jones, G.H., Stamford, W.K. and Perry, P.E. (1975) Male and
female meiosis in grasshoppers. II. *Chorthippus brunneus*.
Chromosoma 51, 381-389.

- Jones, G.H. and Tease, C. (1981) Moietic exchange analysis by molecular labelling. *Chromosomes Today* 7, 114-125.
- Jones, G.H. and Wallace, B.M.N. (1980) Meiotic chromosome pairing in *Stethophyma grossum* spermatocytes studied by a surface-spreading and silver-staining technique. *Chromosoma* 78, 187-201.
- Jones, R.N. and Rees, H. (1967) Genotypic control of chromosome behaviour in rye XI. The influence of B-chromosomes on meiosis. *Heredity* 22, 333-347.
- Jones, R.N. and Rees, H. (1982) B. Chromosomes. Academic Press, London-New York.
- Jotterand-Bellomo, M. (1981) The karyotype and the spermatogenesis of *Mus setulosus*. (G, Q, C bands and Ag-staining). *Genetica* 56, 217-227.
- Kanda, N. and Kato, H. (1980) Analysis of crossing over in mouse meiotic cells by BrdU labelling technique. *Chromosoma* 78, 113-122.
- Kaufmann, J.H. (1974) The ecology and evolution of social organization in the kangaroo family (Macropodidae) *Amer. Zool.* 14, 51-62.
- Keast, A. (1977) Historical biogeography of the marsupials. In: *The Biology of Marsupials* (Stonehouse, B. and Gilmore, D. eds.) Macmillan Press.
- King, M. and Hayman, D. (1978) Seasonal variation of chiasma frequency in *Phyllodactylus marmoratus* (Gray) (Gekkonidae - Reptilia). *Chromosoma* 69, 131-154.
- King, M. and John, B. (1980) Regularities and restrictions governing C-band variation in Acridoid grasshoppers. *Chromosoma* 76, 123-150.

- Kirsch, J.A.W. and Calaby, J.A. (1977) The species of living marsupials - an annotated list. In: The biology of marsupials (Stonehouse, B. and Gilmore, D. eds.) Macmillan Press.
- Koller, P.C. (1936a) The genetical and mechanical properties of the sex chromosomes. II. Marsupials. J. Genet. 32, 451-472.
- Koller, P.C. (1936b) The origin and behaviour of chiasmata XI. *Dasyurus* and *Sarcophilus*. Cytologia 7, 82-103.
- Kyslikova, L. and Forejt, J. (1972) Chiasma frequency in three inbred strains of mice. Folia Biol. 18, 216-218.
- Lange, K., Page, B.M. and Elston, R.C. (1975) Age trends in human chiasma frequencies and recombination fractions. I. Chiasma frequencies. Am. J. Hum. Genet. 27, 410-418.
- Laurie, D.A., Hultén, M. and Jones, G.H. (1981) Chiasma frequency and distribution in a sample of human males: chromosomes 1, 2 and 9. Cytogenet. Cell Genet. 31, 153-166.
- Laurie, D.A. and Jones, G.H. (1981) Inter-individual variation in chiasma distribution in *Chorthippus brunneus* (Orthoptera: Acrididae). Heredity 47, 409-416.
- Lee, A.K., Woolley, P. and Braithwaite, R.W. (1982) Life history strategies of dasyurid marsupials. In: Carnivorous Marsupials (Archer, M. ed.) Roy. Zool. Soc. N.S.W., Sydney, 1-11.
- Levin, D.A. (1975) Pest pressure and recombination systems in plants. Amer. Natur. 109, 437-451.
- Lewis, K.R. and John, B. (1963) "Chromosome Marker". I. and A. Churchill.
- Lewontin, R.C. (1974) "The Genetic Basis of Evolutionary Change". Columbia University Press.

- Lifschytz, E. and Lindsley, D.L. (1972) The role of X-chromosome inactivation during spermatogenesis. *Proc. Nat. Acad. Sci.* 69, 182-186.
- Lindsley, D.L. and Grell, E.H. (1967) "Genetic Variations of *Drosophila melanogaster*". Carnegie Institution of Washington.
- Lindstedt, S.L. and Calder, W.A. (1981) Body size, physiological time, and longevity of homeothermic animals. *Q. Rev. Biol.* 56, 1-16.
- Lyne, A.G. and Verhagen, A.M.W. (1957) Growth of the marsupial *Trichosurus vulpecula* and a comparison with some higher mammals. *Growth* 21, 167-195.
- Lyon, M.F. (1976) Distribution of cross-over in mouse chromosomes. *Genet. Res.* 28, 291-299.
- McIntosh, A.J. and Sharman, G.B. (1953) The chromosomes of some species of marsupials. *J. Morph.* 93, 509-528.
- MacArthur, R.H. and Wilson, E.O. (1967) "The Theory of Island Biogeography". Princeton University Press.
- Maguire, M.P. (1974) The need for a chiasma binder. *J. Theor. Biol.* 48, 485-487.
- Maguire, M.P. (1979) Direct cytological evidence for true terminalization of chiasmata in maize. *Chromosoma* 71, 283-287.
- Maguire, M.P. (1981) A search for the synapic adjustment phenomenon in maize. *Chromosoma* 81, 717-725.
- Mahoney, J.A. (1981) The specific name of the honey possum (Marsupialia: Tarsipedidae: *Tarsipes rostratus* Gervais and Verreaux, 1842). *Aust. Mammal.* 4, 135-138.

Martin, J. (1967) Meiosis in inversion heterozygotes in Chironomidae.

Can. J. Genet. Cytol. 9, 255-268.

Martin, P.G. and Hayman, D.L. (1967) Quantitative comparisons

between karyotypes of Australian marsupials from three

different superfamilies. Chromosoma 20, 290-310.

Mather, K. (1935) Meiosis in Liliaceae. Cytologia 6, 354-380.

Mather, K. (1936) Competition between bivalents during chiasma

formation. Proc. Roy. Soc. Lond. B. 120, 208-227.

Mather, K. (1943) Polygenic inheritance and natural selection.

Biol. Rev. 18, 32-64.

Mather, K. (1953) The genetical structure of populations. Symp.

Soc. Exp. Biol. 7, 66-95.

Matsuda, Y., Imai, H.T., Moriwaki, K. and Kondo, K. (1983)

Modes of inheritance of X-Y dissociation in intersubspecies

hybrids between BALB/c mice and Mus musculus molossineus.

Cytogenet. Cell Genet. 35, 209-215.

Matsuda, Y., Imai, H.T., Moriwaki, K., Kondo, K. and Bonhomme, F.

(1982) X-Y chromosome dissociation in wild derived Mus musculus

subspecies, laboratory mice, and their F₁ hybrids. Cytogenet.

Cell Genet. 34, 241-252.

Maudlin, I. and Evans, E.P. (1980) Chiasma distribution in mouse

oocytes during diakinesis. Chromosoma 80, 49-56.

Maynard Smith, J. (1971) What use is sex? J. Theor. Biol. 30,

319-335.

Maynard Smith, J. (1976) A short term advantage for sex and

recombination through sib-competition. J. Theor. Biol. 63.

245-258.

Maynard Smith, J. (1977) Why the genome does not congeal.

Nature 268, 693-696.

- Maynard Smith, J. (1978) "The Evolution of Sex". Cambridge University Press.
- Maynard Smith, J. (1980) Selection for recombination in a polygenic model. Genet. Res. 35, 269-277.
- Merry, D.E., Pathak, S. and Van de Berg, J.L. (1983) Differential NOR activities in somatic germ cells of *Monodelphis domestica* (Marsupialia, Mammalia). Cytogenet. Cell Genet. 35, 244-251.
- Moeas, P.B. (1978a) Ultrastructural studies of chiasma distribution. Ann. Rev. Genet. 12, 433-450.
- Moeas, P.B. (1978b) Lateral element cross connections of the synaptonemal complex and their relationship to chiasmata in rat spermatocytes. Can. J. Genet. Cytol. 20, 567-579.
- Morton, S.R. (1978) An ecological study of *Sminthopsis crassicaudata* (Marsupialia: Dasyuridae) III. Reproduction and life history. Aust. Wildl. Res. 5, 183-211.
- Monesi, V. (1971) Chromosome activities during meiosis and spermiogenesis. J. Reprod. Fertil. Suppl. 13, 1-14.
- Mortimer, R.K. and Schild, D. (1981) Genetic map of *Saccharomyces cerevisiae*. In: The Molecular Biology of the Yeast *Saccharomyces*. Life cycle and inheritance. (Strathern, J.N., Jones, E.W. and Broach, J.R. eds.) Cold Spring Harbor.
- Morton, S.R. (1982) Dasyurid marsupials of the Australian Arid Zone: An ecological review. In: Carnivorous Marsupials. (Archer, M. ed.) Roy. Zool. Soc. N.S.W., Sydney, pp. 117-130.
- Moses, M.J. (1956) Chromosomal structures in crayfish spermatocytes. J. Biophys. Biochem. Cytol. 2, 215-218.
- Moses, M.J. (1968) Synaptonemal complex. Ann. Rev. Genet. 2, 363-412.

- Moses, M.J. (1977a) Synaptonemal complex karyotyping in spermatocytes of the Chinese hamster (*Cricetulus griseus*). I. Morphology of the autosomal complement in spread preparations. *Chromosoma* 60, 127-137.
- Moses, M.J. (1977b) Synaptonemal complex karyotyping in spermatocytes of the Chinese hamster (*Cricetulus griseus*). II. Morphology of the XY pair in spread preparations. *Chromosoma* 60, 127-137.
- Moses, M.J. (1977c) The synaptonemal complex and meiosis. In: *Molecular Human Cytogenetics*. (Sparkes, K.S., Cornings, D. and Fox, C.F. eds.) Academic Press, New York.
- Moses, M.J. and Counce, S.J. (1974) Synaptonemal complex karyotyping in spreads of mammalian spermatocytes. In: *Symp. Mechanisms in Recombination* (Grell, R. ed.) Plenum Press, New York.
- Moses, M.J., Counce, S.J. and Paulson, D.F. (1975) Synaptonemal complex complement of man in spreads of spermatocytes, with details of the sex chromosome pair. *Science* 187, 363-365.
- Moses, M.J., Karatisis, P.A. and Hamilton, A.E. (1979) Synaptonemal complex analysis of the heteromorphic trivalents in Lemur hybrids. *Chromosoma* 70, 141-160.
- Moses, M.J. and Poorman, P.A. (1981) Synaptonemal complex analysis of mouse chromosomal rearrangements II. Synaptonemal adjustment in a tandem duplication. *Chromosoma* 81, 519-535.
- Moses, M.J., Poorman, P.A., Roderick, T.H. and Davisson, M.T. (1982) Synaptonemal complex analysis of mouse chromosomal rearrangements IV. Synapsis and synaptic adjustment in two paracentric inversions. *Chromosoma* 84, 457-474.

- Moses, M.J., Russell, L.B. and Cacheiro, N.L.A. (1977) Mouse chromosome translocations: visualization and analysis by electron microscopy of the synaptonemal complex. *Science* 196, 892-894.
- Moses, M.J., Slatton, G.H., Gambling, T.M. and Starmer, C.F. (1977) Synaptonemal complex karyotyping in spermatocytes of the Chinese hamster (*Cricetulus griseus*) III. Quantitative evaluation. *Chromosoma* 60, 345-375.
- Muller, H.J. (1932) Some genetic aspects of sex. *Amer. Natur.* 66, 118-138.
- Muller, H.J. (1964) The relation of recombination to mutational advance. *Mutat. Res.* 1, 2-9.
- Müller, U. and Schempp, W. (1982) Homologous early replication patterns of the distal short arms of prometaphasic X and Y chromosomes. *Hum. Genet.* 60, 274-275.
- Murer-Orlando, M. and Richer, C.-L. (1983) Heterochromatin heterogeneity in Chinese hamster sex chromosomes. *Cytogenet. Cell Genet.* 35, 195-199.
- Murray, J.D. (1977) Non-random sex - chromosome association and constitutive heterochromatin in the brush-tailed possum *Trichosurus vulpecula* (Marsupialia: Phalangeridae). *Cytogenet. Cell Genet.* 18, 90-96.
- Murray, J.D., Sharman, G.B., McKay, G.M. and Calaby, J.H. (1980) Karyotypes, constitutive heterochromatin and taxonomy of ringtail opossums of the genus *Pseudocheirus* (Marsupialia: Petauridae). *Cytogenet. Cell Genet.* 27, 73-81.
- Nanda, E. and Raman, R. (1981) Cytological similarity between the heterochromatin of the large X and Y chromosomes of the soft-furred field rat, *Millardia meltada* (family: Muridae). *Cytogenet. Cell Genet.* 30, 77-82.

- Natarajan, A.T. and Gropp, A. (1971) The meiotic behaviour of autosomal heterochromatic segments in hedgehogs. *Chromosoma* 35, 143-152.
- Navarro, J., Vidal, F., Guitant, M. and Egozcue, J. (1981) A method for the sequential study of synaptonemal complexes by light and electron microscopy. *Hum. Genet.* 59, 419-421.
- Navotna, B. and Forejt, J. (1975) Polymorphism of centromeric heter^ochromatin and its relation to chiasma formation in the paracentromeric regions of murine chromosomes. *Folia Biol.* 21, 1-7.
- Neuffer, M.G. and Coe, E.H. (1974) Corn (maize). In: (King, R.C. ed.) *Handbook of Genetics*, Vol. 2. Plenum, New York.
- Nur, U. (1968) Synapsis and crossing over within a paracentric inversion in the grasshopper (*Carnnula pellucida*). *Chromosoma* 25, 198-214.
- Oakley, H.A. and Jones, G.H. (1982) Meiosis in *Merostoma ehrenbergii ehrenbergii* (Turbellaria, Rhabdocoela). I. Chromosome pairing, synaptonemal complexes and chiasma localization in spermatogenesis. *Chromosoma* 85, 311-322.
- Ohno, S. (1967) Sex chromosomes and sex-linked genes. Springer Verlag, Berlin.
- Ohno, S. (1974) Conservation of ancient linkage groups in evolution and some insights into the genetic regulatory mechanisms of X inactivation. *Cold Spring Harbor Symp. Quantit. Biol.* 38, 155-164.
- Oliveira, D., Semina, G., Solari, A.J. and Bianchi, N.O. (1979) Synaptonemal complex in the XY pair of *Akodon azarae*. *Cytologia* 44, 353-357.

- Page, D., de Martinvill, B., Barker, D., Wyman, A., White, R., Franke, U. and Botstein, D. (1982) Single-copy sequence hybridizes to polymorphic and homologous loci on human X and Y chromosomes. *Proc. Nat. Acad. Sci.* 79, 5352-5356.
- Page, B.M. (1973) Identification of chromosome 9 in human male meiosis. *Cytogenet. Cell Genet.* 12, 254-263.
- Pathak, S., Elder, F.F.B. and Maxwell, B.L. (1980) Asynaptic behaviour of X and Y chromosomes in the Virginia opossum and the southern pygmy mouse. *Cytogenet. Cell Genet.* 26, 142-149.
- Pathak, S. and Hsu, T.C. (1979) Silver stained structures in mammalian meiotic prophase. *Chromosoma* 70, 195-203.
- Pathak, S. and Lin, C.C. (1981) Synaptonemal complex of the sex-autosome trivalent in a male Indian muntjac. *Chromosoma* 82, 367-376.
- Pathak, S., Van Tuinen, P. and Merry, D.E. (1982) Heterochromatin, synaptonemal complex, and NOR activity in somatic and germ cells of a male domestic dog, *Canis familiaris* (Mammalia, Canidae) *Cytogenet. Cell Genet.* 34, 112-118.
- Patton, J.L. (1977) B chromosome systems in the pocket mouse *Perognathus baileyi* Merriam: Meiosis and C-band studies. *Chromosoma* 60, 1-14.
- Peacock, W.J. (1970) Replication, recombination and chiasmata in *Goniaea anstraliasiae* (Orthoptera: Acrididae). *Genetics* 65, 593-617.
- Pearson, P.L. and Roderick, T.H. (1979) Report of the committee on comparative mapping. *Cytogenet. Cell Genet.* 25, 85-95.
- Pearson, P.L., Roderick, T.H., Davisson, M.T., Lalby, P.A. and O'Brien, S.J. (1982) Report of the committee on comparative mapping. *Cytogenet. Cell Genet.* 32, 208-220.

- Perry, P.E. and Jones, G.H. (1974) Male and female meiosis in grasshoppers I. *Stethophyma grossum*. *Chromosoma* 47, 227-236.
- Pekelharing, C.J. (1970) Cementum deposition as an age indicator in the Brush-tailed Possum, *Trichosurus vulpecula* Kerr (Marsupialia). *Aust. J. Zool.* 18, 71-76.
- Pianka, E.R. (1970) On r- and K-selection. *Amer. Nat.* 104, 592-597.
- Polani, P.E. (1972) Centromere localization at meiosis and the position of chiasmata in the ~~A~~ male and female mouse. *Chromosoma* 36, 343-374.
- Polani, P.E. (1982) Pairing of X and Y chromosomes, non-inactivation of X-linked genes and the maleness factor. *Hum. Genet.* 60, 207-211.
- Polani, P.E., Crolla, J.A. Sellar, M.J. and Moir, F. (1979) Meiotic crossing over exchange in the female mouse visualized by BUdR substitution. *Nature* 278, 348-349.
- Poole, W.E. (1983) Breeding in the Grey Kangaroo, *Macropus giganteus*, from widespread locations in Eastern Australia. *Aust. Wildl. Res.* 10, 453-466.
- Poole, W.E., Carpenter, S.M. and Wood, J.T. (1982) Growth of Grey Kangaroos and the Reliability of age determinations from body measurements. I. The Eastern Grey Kangaroo, *Macropus giganteus*. *Aust. Wildl. Res.* 9, 9-20.
- Poorman, P.A., Moses, M.J., Russell, L.B. and Cacheiro, N.L.A. (1981a) Synaptonemal complex analysis of mouse chromosomal rearrangements I. Cytogenetic observations on a tandem duplication. *Chromosoma* 81, 507-518.

- Poorman, P.A., Moses, M.J., Davisson, M.T., Roderick, T.H. (1981b) Synaptonemal complex analysis of mouse chromosomal rearrangements III. Cytogenetic observations on two paracentric inversions. *Chromosoma* 83, 419-429.
- Price, D.J. (1975) Chiasma frequency variation in *Cepaea hortensis* (Müll.) and a comparison with *C. nemoralis* (L.). *Genetica* 45, 497-508.
- Price, D.J. and Bantock, C.R. (1975) Marginal populations of *Cepaea nemoralis* (L.) on the Brendon Hills, England. II. Variation in chiasma frequency. *Evolution* 29, 278-286.
- Price, S. (1959) Chiasma terminalization in a structural heterozygote in *Secale*. *Genetics* 44, 705-712.
- Rasmussen, S.W. (1973) Ultrastructural studies of spermatogenesis in *Drosophila melanogaster* Meigen. *Z. Zellforsch. Mikrosk. Anat.* 140, 125-144.
- Rasmussen, S.W. (1975) Ultrastructural studies of meiosis in males and females of the $c(3)G^{17}$ mutant of *Drosophila melanogaster* Meigen. *C.R. Trav. Lab. Carlsberg* 40, 163-173.
- Rasmussen, S.W. (1977) Chromosome pairing in triploid females of *Bombyx mori* analysed by three-dimensional reconstructions of synaptonemal complexes. *Carlsberg Res. Commun.* 42, 163-197.
- Rasmussen, S.W. and Holm, P.B. (1979) Chromosome pairing in autotetraploid *Bombyx* females. Mechanism for exclusive bivalent formation. *Carlsberg Res. Commun.* 44, 101-125.
- Rasmussen, S.W. and Holm, P.B. (1980) Mechanics of meiosis. *Hereditas* 93, 187-216.
- Rees, H. and Ahmad, K. (1963) Chiasma frequencies in *Lolium* populations. *Evolution* 17, 575-579.

- Rees, H. and Dale, P.J. (1974) Chiasmata and variability in *Lolium* and *Festuca* populations. *Chromosoma* 47, 335-351.
- Rees, H. and Jones, R.N. (1977) "Chromosome Genetics". Edward Arnold.
- Rees, H. and Naylor, B. (1960) Developmental variation in chromosome behaviour. *Heredity* 15, 17-27.
- Rees, H. and Thompson, J.B. (1956) Genotypic control of chromosome behaviour in rye. 3. Chiasma frequency in homozygotes and heterozygotes. *Heredity* 10, 409-424.
- Rhoades, M.M. (1950) Meiosis in maize. *J. Heredity* 41, 58-67.
- Richardson, B.J. (1975) r and K selection in kangaroos. *Nature* 255, 323-324.
- Ride, W.D.L. (1970) "A Guide to the Native Mammals of Australia". Oxford University Press, Melbourne.
- Robinson, E.S., Johnston, P.G. and Sharman, G.B. (1977) X chromosome activity in germ cells of female kangaroos. In: *Reproduction and Evolution* (Calaby, J.H. and Tyndale-Biscoe, C.H. eds.) Australian Academy of Sciences.
- Rofe, R.H. (1978) G-banded chromosomes and the evolution of *Macropodidae*. *Aust. Mam.* 2, 52-63.
- Rofe, R.H. (1979) G-banding and chromosomal evolution in Australian marsupials. Ph.D. Thesis, University of Adelaide.
- Röhrborn, G., Hansmann, I. and Buckel, U. (1977) Cytogenetic analysis of pre- and postovulatory oocytes and pre-implantation embryos in mutagenesis of mammals. In: *Handbook of Mutagenicity Test Procedures* (Kilbey, B.J., Legator, M., Nichols, W. and Ramel C. eds.) Elsevier.

- Ropers, H.H., Wiberg, U., Migl, B., Sperling, K. and Fredga, K.
(1982) X-linkage of STS in rodent species: evolutionary conservation of the non-inactivated X chromosome segment. *Cytogenet. Cell Genet.* 32, 312.
- Rose, R.W. (1978) Reproduction and evolution in female Macropodidae. *Aust. Mammal.* 2, 65-72.
- Rose, R.W. and McCartney, D.J. (1982) Growth of the Red-bellied Pademelon, *Thylogale billiardierii*, and age estimation of pouch young. *Aust. Wildl. Res.* 9, 33-38.
- Roughgarden, J. (1979) "Theory of Population Genetics and Evolutionary Ecology". Macmillan.
- Russell, E.M. (1982) Patterns of parental care and parental investment in marsupials. *Biol. Rev.*, 57, 423-486.
- Sachs, L. (1954) Sex linkage and sex chromosomes in man. *Ann. Eugen.* 18, 255-261.
- Selwood, L. (1982) A review of maturation and fertilization in marsupials with special reference to the dasyurid: *Antechinus stuartii*. In: *Carnivorous Marsupials* (Archer, M. ed.) Roy. Zool. Soc. N.S.W., Sydney.
- Sharman, G.B. (1970) Reproductive physiology of marsupials. *Science* 167, 1221-1228.
- Sharman, G.B. (1973) The chromosomes of non-eutherian mammals. In: *Cytotaxonomy and Vertebrate Evolution* (Chiarelli, A.B. and Capanna, E. eds.) Academic Press, London/New York.
- Sharman, G.B. and Barber, H.N. (1952) Multiple sex chromosomes in the marsupial, Potorous. *Heredity* 6, 345-355.
- Sharman, G.B., McIntosh, A.J. and Barber, H.N. (1950) Multiple sex chromosomes in the marsupials. *Nature* 166, 996.

- Sharp, P. and Hayman, D. (1981) The distribution of SCEs within and between the genomes of an interspecific hybrid. *Chromosoma* 84, 231-242.
- Shaw, D.D. (1971) Genetic and environmental components of chiasma control I. Spatial and temporal variation in *Schistocerca* and *Stethophyma*. *Chromosoma* 34, 281-301.
- Shaw, D.D. (1972) Genetic and environmental components of chiasma control II. The response to selection in *Schistocerca*. *Chromosoma* 37, 297-308.
- Shaw, D.D. (1974) Genetic and environmental components of chiasma control III. Genetic analysis of chiasma frequency variation in two selected lines of *Schistocerca gregaria* Forsk. *Chromosoma* 46, 365-374.
- Shaw, D.D. and Knowles, G.R. (1976) Comparative chiasma analysis using a computerized optical digitiser. *Chromosoma* 59, 103-127.
- Singh, L. and Jones, K.W. (1982) Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent non-reciprocal crossover involving the X and aberrant Y chromosome. *Cell* 28, 205-216.
- Slatkin, M. (1975) Gene flow and selection in a two locus system. *Genetics* 81, 787-802.
- Smith, M.J. and Godfrey, G.K. (1970) Ovulation induced by gonadotrophins in the marsupial, *Sminthopsis crassicaudata* (Gould). *J. Reprod. Fert.* 22, 41-47.
- Sokal, R.R. and Rohlf, F.J. (1969) "Biometry. The principles and practice of statistics in biological research". Freeman, San Francisco.

- Sokal, R.R. and Rohlf, F.J. (1973) "Introduction to Biostatistics".
Freeman, San Francisco.
- Solari, A.J. (1974a) The behaviour of the XY pair in mammals.
Int. Rev. Cytol. 38, 273-317.
- Solari, A.J. (1974b) The relationship between chromosomes and
axes in the chiasmatic XY pair of the Armenian hamster
(*Cricetulus migratorius*). *Chromosoma* 48, 89-106.
- Solari, A.J. (1980) Synaptonemal complexes and associated structures
in microspread human spermatocytes. *Chromosoma* 81, 315-337.
- Solari, A.J. and Ashley, T. (1977) Ultrastructure and behaviour of
the achiasmatic telosynaptic XY pair of the sand rat
(*Psammomys obesus*). *Chromosoma* 62, 319-336.
- Solari, A.J. and Bianchi, N.O. (1975) The synaptic behaviour of
the X and Y chromosomes in the marsupial *Monodelphis dimidiata*.
Chromosoma 52, 11-25.
- Sournia, A. (1982) Form and function in marine phytoplankton.
Biol. Rev. 57, 347-394.
- Southern, D.I. (1967) Chiasma distribution in Truxaline grass-
hoppers. *Chromosoma* 22, 164-191.
- Speed, R.M. (1977) The effects of aging on the meiotic chromosomes
of male and female mice. *Chromosoma* 64, 241-254.
- Speed, R.M. (1982) Meiosis in the foetal mouse ovary I. An
analysis at the light microscope level using surface-spreading.
Chromosoma 85, 427-437.
- Spyropoulos, B., Ross, P.D., Moens, P.B. and Cameron, D.M. (1982)
The synaptonemal complex karyotypes of Palearctic Hamsters,
Phodopus roborouskii Satunin and *P. sungorus* Pallas.
Chromosoma 86, 397-408.

- Stahl, A., Luciani, J.M., Devictor, M., Capodano, A.M. and Gagné, R.
(1975) Constitutive heterochromatin and micronucleoli in
the human oocyte at the diplotene stage. *Humangenetik* 26,
315-327.
- Stearns, S.C. (1976) Life-history tactics: A review of the ideas.
Q. Rev. Biol. 51, 3-47.
- Stearns, S.C. (1977) The evolution of life history traits: A
critique of the theory and a review of the data. *Ann. Rev.
Ecol. Syst.* 8, 145-171.
- Stebbins, G.L. (1958) Longevity, habitat and release of genetic
variability in the higher plants. Cold Spring Harbor
Symposium on Quantitative Biology 23, 365-378.
- Stern, H. and Hotta, Y. (1978) Regulatory mechanisms in meiotic
crossing-over. *Ann. Rev. Plant Physiol.* 29, 415-436.
- Stock, A.D. and Mengden, G.A. (1982) The origin of mitotic sex-
chromosome association in the brush-tailed possum, *Trichosurus
vulpecula* (Marsupalia: Phalangeridae). *Cytogenet. Cell
Genet.* 34, 305-309.
- Stonehouse, B. and Gilmore, D. (1977) "The Biology of Marsupials".
Macmillan, London.
- Strahan, R. (ed.) (1983) "Complete Book of Australian Mammals -
The National Photographic Index of Australian Wildlife".
Angus and Robertson, The Australian Museum, Melbourne.
- Strobeck, G., Maynard Smith, J. and Charlesworth, R. (1976) The
effects of hitch-hiking on a gene for recombination.
Genetics 82, 547-558.
- Sun, S. and Rees, H. (1964) Genotypic control of chromosome
behaviour in rye VII. Unadaptive heterozygotes. *Heredity* 19,
357-367.

- Sung, W.K. and Jagiello, G. (1982) A technique for light and electron microscopy of the synaptonemal complex of the mouse oocyte. *Can. J. Genet. Cytol.* 24, 675-680.
- Tarkowski, A.K. (1966) An air-drying method for chromosome preparations from mouse eggs. *Cytogenetics* 5, 394-400.
- Tease, C. (1978) Cytological detection of crossing-over in BUdR substituted meiotic chromosomes using the fluorescent plus Giemsa technique. *Nature* 272, 823-824.
- Tease, C. and Jones, G.H. (1978) Analysis of exchanges in differentially stained meiotic chromosomes of *Locusta migratoria* after BrdU-substitution and FPG staining I. Crossover exchanges in monochiasmate bivalents. *Chromosoma* 69, 163-178.
- Therman, E. and Sarto, G.E. (1977) Premeiotic and early meiotic stages in the pollen mother cells of *Eremurus* and in human embryonic oocytes. *Hum. Genet.* 35, 137-151.
- Ting, Y.C. (1973) Synaptonemal complex of haploid maize. *Cytologica* 38, 447-500.
- Tsafriri, A. (1978) Oocyte maturation in mammals. In: *The Vertebrate Ovary. Comparative Biology and Evolution* (Jones, R.E. ed.) Plenum Press, New York.
- Turner, J.R.G. (1967) Why does the genome not congeal? *Evolution* 21, 646-656.
- Turner, J.R.G. (1979) Genetic control of recombination in the silkworm I. Multigenic control of chromosome 2. *Heredity* 43, 273-293.
- Tyndale-Biscoe, H. (1973) "Life of Marsupials". Edward Arnold, London.

- Tyndale-Biscoe, C.H. (1978) Ecology of small marsupials. In:
"The Ecology of Small Mammals" (Stoddart, D.M. ed.) Chapman
and Hall, London.
- Venolia, L. and Peacock, W.J. (1981) A highly repeated DNA from
the genome of the Wallaroo (*Macropus robustus robustus*)
Aust. J. Biol. Sci. 34, 97-113.
- Vistorin, G., Gamperi, R. and Rosenkranz, W. (1977) Studies on
sex chromosomes of four hamster species: *Cricetus cricetus*,
Cricetulus griseus, *Mesocricetus auratus*, and *Phodopus*
sungorus. *Cytogenet. Cell Genet.* 18, 24-32.
- Wahrman, J., Richler, C., Neufeld, E. and Friedmann, A. (1983)
The origin of multiple sex chromosomes in *Gerbillus gerbillus*
(Rodentia: Gerbillinae) *Cytogenet. Cell Genet.* 35, 161-180.
- Walters, M.S. (1976) Variation in preleptotene chromosome contraction
among three cultivars of *Lilium longiflorum*. *Chromosoma* 57,
51-80.
- Wells, R.T. (1978) Field observations of the Hairy-nosed Wombat,
Lasiiorhinus latifrons (Owen). *Aust. Wildl. Res.* 5, 299-303.
- Westergaard, M. and Wettstein, D. Von (1972) The synaptonemal
complex. *Ann. Rev. Genet.* 6, 71-110.
- Western, D. (1979) Size, life history and ecology in mammals.
Afr. J. Ecol. 17, 185-204.
- Wettstein, R., Fernández-Donoso, R. and Sotelo, J.R. (1974)
Sex chromosomes of *Marmosa elegans* spermatocytes. *Rev.*
Microsc. Elect. 2, 136-137.
- Wettstein, R. and Sotelo, J.R. (1967) Electron microscopic serial
reconstruction of the spermatocytes. I. Nuclei at pachytene.
J. Microscopie 6, 557-576.

- White, M.J.D. (1973) "Animal Cytology and Evolution", 3rd ed.
Cambridge University Press.
- White, M.J.D. (1978) "Modes of Speciation". W.H. Freeman and Co.
- Williams, G.C. (1975) "Sex and Evolution". Princeton University Press.
- Wood Jones, F. (1923-25) "The Mammals of South Australia - Parts I-III". Government Printer, Adelaide; Reprinted 1968.
- Whitehouse, C., Edgar, L.A., Jones, G.H. and Parker, J.S. (1981)
The population cytogenetics of *Crepis capillaris* I. Chiasma variation. *Heredity* 47, 95-103.
- Wilson, A.C., Bush, G.L., Case, S.M. and King, M.-C. (1975)
Social structuring of mammalian populations and rate of chromosomal evolution. *Proc. Nat. Acad. Sci.* 72, 5061-5065.
- Young, G.J., Marshall Graves, J.A., Barbieri, I., Woolley, P.A., Cooper, D.W. and Westerman, M. (1982) The chromosomes of Dasyurids (Marsupialia). In: Carnivorous Marsupials, (Archer, M. ed.) Roy. Zool. Soc. N.S.W., Sydney.
- Zardii, Y., Simchen, G., Hillel, J. and Schaap, T. (1972)
Chiasmata and the breeding system in wild populations of diploid wheats. *Chromosoma* 38, 77-94.
- Zickler, D. and Sage, J. (1981) Synaptonemal complexes with modified lateral elements in *Sordaria humana*: Development of and relationship to the "Recombination Nodules". *Chromosoma* 84, 205-318.