



**CYTOGENETICAL STUDIES IN AUSTRALIAN SCORPIONS**

**by**

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Female *Urodacus manicatus* with young.



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## SUMMARY

This thesis is divided into three chapters each considering a different aspect of cytogenetics in Australian scorpions.

The first chapter is an analysis of the chromosomal systems of the two most species-rich scorpion families in Australia: Buthidae and Scorpionidae. Individuals from a number of species in each family were analysed and it was found that although males from all species exhibit achiasmate meiosis and no distinguishable sex chromosomes, other aspects of their cytogenetic systems differ greatly. Scorpionids have high chromosome numbers ( $2n=29 \rightarrow 2n=175$ ) and exhibit both inter- and intra-population variation in chromosome number due to fusion/fission polymorphism. One species, *Urodacus manicatus*, also exhibits inversion polymorphism.

In contrast, scorpions belonging to the family Buthidae have a conserved, low chromosome number of  $2n=14$  although some individuals may have  $2n=12$  or 16 chromosomes. Many individuals exhibit interchange heterozygosity resulting in a high degree of intra- and inter-population polymorphism. Associations at metaphase I of up to a ring of 12 chromosomes have been observed.

Studies of females in both families revealed similar chromosome numbers to the males, however meiotic divisions could not be found to determine whether females also exhibited an achiasmate meiosis.

Another striking difference between the chromosome systems of each family is that Buthids have holocentric chromosomes while Scorpionids possess monocentric chromosomes. The evolution of the chromosome polymorphism shown by each family is considered in relation to centromere type.

Chapter 2 contains a description of the synaptonemal complexes during male meiosis from both Buthid and Scorpionid individuals. The process of synaptic adjustment was observed in Scorpionid individuals heterozygous for inversions, however not all inversions showed identical synaptic behaviour. Additionally, the two-stage pairing cycle as described by Rasmussen and Holm (1979) in which multivalents resolved to bivalents in the absence of chiasmata, was not observed in Buthid individuals heterozygous for interchanges. The processes of two-stage pairing and synaptic adjustment are discussed in the context of achiasmate meiosis and complex pairing arrangements.

Chapter 3 describes a number of observations and experiments that led to the elucidation of the nature of the centromere in each family, including an analysis of X-ray breakage of chromosomes from selected species.

## 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.

C.M. Shanahan



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## GENERAL INTRODUCTION

The small amount of literature available that examines scorpion cytogenetics presents a rather sketchy and confused account of many aspects of their cytogenetic systems. However, the literature does suggest that scorpions exhibit diverse and unique combinations of generally uncommon cytogenetic features. These include holocentric chromosomes, achiasmatic meiosis, and complex interchange heterozygosity. The aim of this project was to examine the species-rich scorpion fauna of Australia to clarify and extend our knowledge of scorpion cytogenetics. Additionally, their unique cytogenetic features make scorpions ideal systems in which to test a number of recent theories (describing chromosome behaviour at meiotic prophase) that are widely accepted but essentially unproven in many species.

The adaptive and evolutionary significance of such diverse cytogenetic systems are tantalizing problems. Like any cytogenetic study, the most difficult problem to address because of its complexity is the role of chromosome change in evolution. The variety and extent of information necessary to perform such studies was perhaps best summarized by Muller in 1922:

"Must we evolutionists become cell physiologists, electron microscopists and molecular biologists, as well as ecologists, cytogeneticists and population geneticists. Let us hope so."

CHAPTER 1

CHROMOSOMAL SYSTEMS IN AUSTRALIAN SCORPIONS



## CHAPTER 1

### SECTION 1: LITERATURE REVIEW

Present day scorpions belong to an ancient group of terrestrial arthropods (Sub-Phylum: Chelicerata, Class: Arachnida, Order: Scorpionida. The earliest known scorpion fossils date from the Silurian (350-320 million years ago) and these are the earliest known terrestrial animals (Marshall and Williams, 1974).

There are seven recognized extant families of scorpions of which Australia has representatives of three: Bothriuridae, Scorpionidae and Buthidae. Representatives of these same three families are also widespread in South America, the Mediterranean, Africa, Madagascar, parts of Asia and the Pacific (Koch, 1979). There is some geographic and taxonomic evidence suggesting that the large family Buthidae (42 genera) diverged from the main phylogenetic line, which includes the remaining families, very early in the evolution of the order (Kraepelin, 1905). Scorpion cytological data, although incomplete, supports this theory. The cytogenetic system that is apparently common to all species in the family Buthidae differs markedly from those of all other families studied. The most striking difference is that Buthids have holocentric chromosomes in contrast to the monocentric systems exhibited by species from all other families. This difference is discussed in detail in Chapter 3 and is considered here in relation to the types of chromosome

changes that can occur and be maintained in holocentric compared with monocentric systems. Despite this centromeric difference, however, all scorpion species have in common the unusual cytological feature of achiasmate male meiosis.

#### **Achiasmate Meiosis.**

Achiasmate meiosis is characterized by the lack of any diplotene or diakinesis stages (Ullerich, 1961). The chromosomes undergo a contraction phase with the paired homologues of each bivalent maintaining a parallel alignment until the spindle forms. At metaphase I the homologues are often described as 'distance-paired'. To date, achiasmate meiosis has been described in the pollen mother cells of six species from the plant group *Fritillaria japonica* (Noda, 1975) and in a number of diverse animal species including protozoans, turbellarians, annelids and molluscs, with the largest number of species found in the order Arthropoda (see Table 1.1.1 modified from John and Lewis, 1965).

Achiasmate meiosis is usually restricted to one sex. An exception to this observation occurs in some of the hermaphroditic Enchytraeids (e.g. *Buchholzia fallax* and *Marionina subterranea*) where chiasmata appear to be absent during both male and female meiosis (Christensen, 1961). The hermaphroditic nature of these annelids may make them an exceptional case amongst animals, however, in *Fritillaria* species with hermaphrodite flowers achiasmate meiosis is confined to the male sporocytes within a single genotype; that is, there is intra-individual differentiation in the

meiotic system (Noda, 1975). There is no reason to suppose that such intra-individual variation should be confined to plants. Thus in the Enchytraeids selection may have favoured a total loss of chiasmata. In many achiasmate organisms both sexes have not been studied cytologically, so it is possible that other examples of achiasmate meiosis in both sexes of a dioecious species may exist.

Where achiasmate meiosis is restricted to one sex either the male or female may lack chiasmata. However, it has been observed in organisms possessing differentiated sex chromosomes that it is always the heterogametic sex that is achiasmate (Nokkala and Nokkala, 1984). It has been suggested that in these organisms achiasmate meiosis is coupled to the sex chromosomes with controlling elements situated on the X-chromosome (Nokkala and Nokkala, 1983). It is not possible to judge whether this is a secondary association or a primary causal effect as it is unknown whether the achiasmate meiosis or the sex chromosomes evolved first.

The adaptive significance of achiasmate meiosis remains obscure. White (1973) suggested that it may act either as a mechanism to reduce recombination (and thus preserve co-adapted gene complexes) or to facilitate paracentric inversion heterozygosity. There is very little evidence for the latter of these hypotheses. One might expect to observe a reduced frequency of recombination in the chiasmate sex on the basis of the first hypothesis; that is, the species is tending towards an overall reduction in chiasmata frequency.

The most extreme example of this selection may have occurred in the Enchytraeids. Additionally, *Mesostoma ehrenbergii ehrenbergii*, a turbellarian with achiasmate female meiosis, has very reduced and localized chiasmata formation in the male such that there is virtually no recombination (Oakley and Jones, 1982; Oakley, 1982). Similarly, the grasshopper *Thericles whitei* has no chiasmata in the male and a very low chiasmata frequency in the female (White, 1973). Although these few examples tend to suggest that in achiasmate species selection is tending towards an overall reduction in chiasmata frequency, information on chiasmata frequencies in both sexes of many more species is required before the adaptive significance is fully understood.

Achiasmate meiosis is assumed to have evolved from the chiasmate process. In Diptera (White, 1949), mantids (Hughes-Schrader, 1950, 1953; White, 1965) and Enchytraeid worms (Christensen, 1961) the phylogenetically primitive groups have chiasmate meiosis while the higher groups have established an achiasmate meiotic system. In order to achieve achiasmate meiosis an alternate mechanism for maintaining homologue association through to anaphase I must exist. White (1965) suggested that species were pre-adapted to the change due to the establishment of prolongation of pachytene synapsis. Evidence for this comes from the Thericleinae grasshoppers where males are either chiasmate, achiasmate or show an intermediate meiosis termed *cryptochiasmate* (White, 1965). In these species numerous chiasmata are concealed between closely synapsed homologues

until anaphase I separation occurs. Clearly the concealed chiasmata are not responsible for maintaining the bivalents until anaphase I. An additional mechanism contributing to homologue association exists. When the prolongation of lateral association coincides with the suppression of chiasmata formation the conversion of a crytochiasmate meiosis into an achiasmate one becomes possible (White, 1965; Noda, 1975). The diversity of plant and animal groups showing achiasmate meiosis suggests that it has evolved independently in a number of distinct evolutionary radiations. Thus, a diversity of mechanisms may exist to maintain lateral associations of bivalents. Recent ultrastructural analysis of structures such as the synaptonemal complex suggests this to be correct (see Chapter 2).

#### **Achiasmate Meiosis in Scorpions.**

Achiasmate meiosis occurs in species with both holocentric and monocentric chromosomes (as shown in Table 1.1.1). Achiasmate meiosis in scorpions was first described during male meiosis in holocentric Buthid species by Piza (1939). He found no evidence of a diplotene stage. Brieger and Graner (1942) disputed Piza's claim and published photographs of what they considered were diplotene stages showing chiasmata. However, this evidence was not convincing and evidence from other workers studying Buthid species (Sharma and Joneja, 1959; Guenin, 1961; Ashish and Sanat 1965) led White (1973) to concur with Piza's original



Table 1.1.1

List of species with achiasmatic meiosis.

(modified from John and Lewis, 1965).

Species	Sex	Reference
Heliozoa - <i>Actinophrys sol</i> Coccidia - <i>Aggregata eberthi</i> Foraminifera - <i>Rubratella intermedia</i> Gregarina - <i>Stylocephalus longicollis</i> Flagellata - <i>Trichonympha</i>		Belar, 1923) Belar, 1926) see Bauer, 1946 Grell, 1958, Le Calvez, 1950 Grell, 1940 Cleveland, 1949
<u>Turbellaria</u> - <i>Mesostoma</i> <u>Annelida</u> - <i>Enchytraeidae</i> <u>Mollusca</u> - <i>Sphaerium corneum</i> <u>Arthropoda</u>	♀ ♀(± ♂)	Oakley, 1982 Christensen, 1961 Keyl, 1956
a) <u>Copepoda</u> <i>Ectocyclops strenzkei</i> <i>Cyclops strenuus</i> <i>Tigriopus californicus</i> , <i>japonicus</i> and <i>brevicornis</i> <i>Acanthocyclops vernalis</i> <i>Diacyclops bicuspidatus</i> <i>Eucyclops serrulatus</i> <i>Macrocyclops ater</i> and <i>albidus</i> <i>Mesocyclops leuckarti</i> <i>Tropocyclops prasinus</i>	♀ ♀	Beerman, W., 1954 Beerman, S., 1959 Ar-Rushdi, 1963 Chinnappa and Victor, 1979
b) <u>Acarina</u> * <i>Eylais setosa</i> <i>Hydrodroma despicens</i>	♂	Keyl, 1957
c) <u>Scorpionida</u> *Buthidae - <i>Tityus</i> <i>Isometrus</i> <i>Rhopalurus</i> <i>Buthus</i> Scorpionidae - <i>Palamnaeus</i>	♂ ♂ ♂	Piza, 1943, 1947c, 1957 Ashish and Sanat, 1965 Srivastava and Agrawal, 1961
d) <u>Insecta</u> 1. <u>Orthoptera</u> Eumastacidae - Thericleinae <i>Thericles</i> (3 sp)	♂	White, 1965
2. <u>Dictyoptera</u> Manteidae - <i>Callimantis</i> <i>Acanthiothespis</i> <i>Pseudiomiopteryx</i> <i>Miomantis</i> <i>Bolbe pallida</i> <i>Bolbe nigra</i>	♂ ♂	Hughes-Schrader, 1943, 1950 White, 1965 Gassner, 1969

\* species with holocentric chromosomes.

<sup>1</sup> genetical studies have confirmed absence of crossing-over.

Table 1.1.1 (cont.)

Species	Sex	Reference
3. <u>Mecoptera</u> - <i>Panorpa communis</i> , <i>germanica</i> <i>cognata</i> <sup>1</sup>	♂	Atchley and Jackson, 1970, Ullerich, 1961
4. * <u>Lepidoptera</u> - <i>Cidaria</i>		
<i>Galleria</i>	♀	Suomalainen, 1953
<i>Erebia</i>	♀	Bauer, 1939
<i>Bombyx mori</i> <sup>1</sup>	♀	Tazima, 1964; Robinson, 1971
<i>Agraulis vanillae</i>		
<i>Dryadula phaetusa</i>		
<i>Heliconius</i> (8 sp.)	♀	Suomalainen <i>et al.</i> , 1973
5. <u>Diptera</u>		
a) Nematocera-Phryneidae <sup>1</sup>	All	Bauer, 1946b; Wolf, 1950
<i>Bibionidae</i>	in ♂	)
<i>Scatopsidae</i>		) Wolf, 1941
<i>Thaumaleidae</i>		)
<i>Blepharoceridae</i>		Wolf, 1946
<i>Mycetophilidae</i>		Le Calvez, 1947; Fahmy, 1949
<i>Chironomidae</i>		Bauer and Beermann, 1952
<i>Metriocnemus</i> <i>cavicola</i>		
<i>Tipulidae</i>		Bauer and Beermann, 1952
<i>Tipula caesia</i> and <i>pruinosa</i>		Bayreuther, 1956
<i>Sciaridae</i>	♂	Metz, 1933
<i>Cecidomyiidae</i>		White, 1973
b) Orthorrhapha - <i>Asilus seri-</i> <i>ceus</i> and <i>notatus</i>		Metz and Nonidez, 1921, 1923
c) Cyclorrhapha - many species incl. <i>Drosophila</i> <sup>1</sup>		
6. <u>Tricoptera</u>		
* <i>Limophilus decipiens</i> and <i>borealis</i>	♀	Suomalainen, 1966
7. <u>Hemiptera</u>		
*Homoptera - <i>Protortonia</i> <i>primitiva</i> <i>Parlatoria oleae</i>	♂	Schrader, 1931 Nur, 1965
Heteroptera -		
* <i>Saldula orthochila</i> and <i>saltatoria</i>	♂	Nokkala and Nokkala, 1983
* <i>Nabis brevis</i> , <i>flavomarginatus</i> and <i>limbatus</i>	♂	Nokkala and Nokkala, 1984
8. <u>Coleoptera</u> - <i>Bembidiidae</i> (48 sp.)	All	
<i>Trechidae</i> ( 3 " )	♂	
<i>Pogonidae</i> ( 1 " )		
<i>Harpalidae</i> ( 1 " )		Serrano, 1981
<u>Plants</u>		
<i>Fritillaria japonica</i> (6 spec.)	PMC ♂	Noda, 1975

interpretation.

Srivastava and Agrawal (1961) have reported achiasmate male meiosis in a number of monocentric Scorpionid species. Again controversy exists due to the misinterpretation by some authors of metaphase II stages as representing a chiasmate diplotene (Rajasekarasetty *et al.*, 1979). This confusion illustrates the importance of accurately assigning division stages at meiosis before making cytological interpretations.

To conclusively show that meiosis is achiasmate (assuming that crossing-over does not occur in the absence of chiasmata) genetical data are required. This is available for very few organisms (e.g. *Drosophila* ♂ and *Bombyx* ♀). Scorpions and many other achiasmate species have not been adequately studied. There is no information as to the nature of female meiosis in scorpions and there are no genetical markers available for linkage analysis. The application of a more sophisticated cytological technique, such as BUdR substitution, to identify sister chromatids could be used to determine the presence or absence of chiasmata in putative achiasmate species. This technique has been used successfully in revealing chiasmata in *Locusta* (Tease, 1978). However, the small size of the chromosomes in many achiasmate species would probably make application of this technique difficult.

#### Centric Nature and Chromosome Change.

The laws and principles of chromosome rearrangements in holocentric systems are not fully understood but it is possible that they differ in some respects from those

governing monocentric chromosome rearrangements. In monocentric systems the possibilities for karyotypic change are limited by the fact that only chromosomes with single centromeres, or in some cases fragmented centromeres, can survive. John (1976) argues that stable telocentric chromosomes - chromosomes that have arisen by a single break event resulting in the subdivision of a centromere - have been shown to exist in species of the genera *Mus*, *Melanoplus*, *Microtus* (Fredga and Bergstrom, 1970) and *Nigella* (references in John, 1976).

Theoretically, karyotypic change via single break fragmentation events should be easier in holocentric systems as each fragment should be able to attach to the spindle. Although fragmentation requires that telomeres arise *de novo*, evidence from X-ray fragmentation of holocentric chromosomes suggests this is not totally improbable (see Chapter 3). Interstitial palindromic sequences may exist or alternatively, physiological conditions may exist (similar to those described by McClintock (1941) in embryonic maize) that allow spontaneous repair. Despite this, White (1973) suggested that chromosome numbers in holocentric groups are less divergent than might be expected.

#### **Karyotypic Change in Holocentric Species.**

White (1978), using the Hemiptera as an example, suggested that the magnitude and types of karyotypic differences found in this holocentric order were similar to those found in insect orders with monocentric chromosomes.

This is generally true although there are examples within the hemiptera where chromosome fragmentation appears to have occurred. Generally there appear to be forces operating within many holocentric groups such that chromosome numbers remain relatively low and conserved.

Manna (1984) reviewed chromosomal evolution in the Heteropterans. He observed that chromosome numbers were low with little variation. Most of the observable changes could be attributed to fusion or fission events. DNA content differences between species suggested that other changes such as multiple duplications and deletions must also have been involved in evolution (White, 1978; Manna, 1984). Interestingly, structural heterozygotes for inversions and reciprocal translocations have not been reported in natural populations. Similarly in a number of families of Australian leafhoppers (Homoptera) chromosome numbers are low and conservative (Whitten, 1965). Differences are due to fusions with more advanced species having lower but more variable chromosome numbers when compared to the less advanced species. The hemipteran group *Oncopsis* exhibits low conserved chromosome numbers, however some individuals heterozygous for reciprocal translocations or having a chromosome fragment as a consistent karyotypic element have been reported (John and Claridge, 1974).

A strong case for chromosome fragmentation as a major contributor to chromosome change exists in other holocentric species. Schrader and Hughes-Schrader (1956) showed that in (Hemiptera: Pentatomidae) where DNA contents were similar,

the chromosome numbers were  $2n=14$  and  $2n=28$  respectively. They suggested a possible alternative to fragmentation which implies that each chromosome has a particular 'weak region' (based on their observation the chromosomes of the  $2n=28$  species were half the size of the  $2n=14$  species). In holocentric species which show pre-reduction and independent sister chromatid movement at meiosis, 'chromatid autonomy' may have been achieved. Chromatids become wholly independent and are able to function as chromosomes. Nur (1980) observed chromosome variation up to a factor of 8 in some coccid families (Homoptera: Pseudococcidea). Nevertheless, in most of the families most species shared the same (modal) chromosome number. Nur concluded that in spite of the holocentric nature of the chromosomes, forces were operating that tended to select against an increase in chromosome number. Whether these forces are due to mechanical restrictions or are an adaptive response is unknown. Detailed studies of those species that have evolved chromosome number increases may prove informative.

Species from the plant families Cyperaceae and Juncaceae have generally low diploid chromosome numbers. True polyploid series based on the haploid number of six ( $n=6$ ) from diploids up to octoploids ( $8n=48$ ) occur in the *Luzula campestris* - *multiflora* complex (Nordenskiöld, 1956). *L. campestris* also exhibits another form of chromosome change. Plants may exhibit a  $2n=12$  karyotype or a  $2n=24$  karyotype where the chromosomes are half the size of those with  $2n=12$  (Nordenskiöld, 1951). Plants with intermediate chromosome

numbers exist, with combinations of small and large chromosomes; these are thought to be hybrid in origin. Pairing at metaphase I in these hybrids involves the single long chromosome pairing homologously with two or more small chromosomes presumably the products of fragmentation. The genus *Carex* exhibits a similar phenomenon where an aneuploid series from  $n=6$  to  $n=56$  occurs with every number from 12 to 43 being represented (Davies, 1956).

Malheiros-Garde and Garde (1951) used the term *agmatoploidy* to describe increases in chromosome number due to fragmentation of holocentric chromosomes. However, the mechanisms involved and the evolutionary significance of this process remain enigmatic.

Although chromosome evolution in the Lepidoptera seems to have been via fragmentation resulting in very high chromosome numbers, the holocentric nature of the chromosomes in many species from this group has yet to be confirmed (White, 1973). Thus, they are not considered here as a further example of *agmatoploidy*.

#### **Karyotypic Evolution of Scorpions.**

As shown in Table 1.1.2, there is a striking range of chromosome numbers in scorpions; with Buthid species tending to have low, conserved chromosome numbers when compared to species from non-Buthid families where chromosome numbers are often very high.

The very small sample of non-Buthid scorpions karyotyped makes it impossible to comment on chromosome evolution in

Table 1.1.2

List of chromosome numbers for Order Scorpionida.

Species	Diploid chromosome number 2n	Reference
<b>BUTHIDAE:</b>		
<u>South America</u>		
<i>Tityus bahiensis</i>	6 <sup>1,2,3</sup> , 7, 9, 10, 18 <sup>4</sup>	Piza 1939, 1940, 1943a, 1944, 1946, 1947b.
<i>Tityus serralatus</i>	12	Piza 1947c.
<i>Tityus mattogrossensis</i>	20 <sup>2,3</sup>	Piza 1947c.
<i>Tityus trivittatus</i>	14 <sup>2</sup>	Piza 1948.
<i>Tityus neglectus</i>	26, 27 <sup>3</sup>	Piza 1950.
<i>Tityus stigmurus</i>	16	Piza 1950.
<i>Tityus metuendus</i>	15, 16 <sup>2,3</sup>	Piza 1952.
<i>Isometrus maculatus</i>	12 <sup>2</sup>	Piza 1947a.
<i>Ananteris balzani</i>	12	Piza 1947c.
<i>Rhopalurus rochae</i>	28 <sup>2</sup>	Piza 1957.
<u>Africa</u>		
<i>Buthotus trilineatus</i>	24	Newlands and Martindale 1981.
<i>Parabuthus mosambicensis</i>	36 <sup>4</sup>	" "
<i>Parabuthus raudus</i>	18	" "
<i>Parabuthus transvaalicus</i>	20	" "
<i>Uroplectes carinatus</i>	20, 48 <sup>5</sup>	" "
<i>Uroplectes chubbi</i>	20	" "
<i>Uroplectes flavoviridis</i>	26	" "
<i>Uroplectes olivaceus</i>	24	" "
<i>Uroplectes planimanus</i>	22	" "
<i>Uroplectes vittatus</i>	24	" "
<u>India</u>		
<i>Buthus martensii</i>	24	Sato 1936.
<i>Buthus eupeus</i>	22	Sokolo 1913.
<i>Buthus tamulus</i>	20, 22, 24 <sup>1,2,3</sup> 28	Ashish and Sanat 1965; Sharma <i>et al.</i> 1959.
<i>Buthus macmahoni</i>	24	Sharma <i>et al.</i> 1959.
<i>Buthus doriae</i>	22 <sup>2</sup>	Sharma <i>et al.</i> 1959.
<i>Buthus occitanus</i>	22	Guénin 1961.
<u>North America</u>		
<i>Centruroides vittatus</i>	26	Riess <i>et al.</i> 1978.
<i>Centrurus exilicauda</i>	26	Wilson 1931; Makino 1951.



Table 1.1.2 (cont.)

Species	Diploid chromosome number 2n	Reference
<u>VEJOVIDAE</u>		
<u>North America</u>		
<i>Opisthacanthus elatus</i>	~100	Wilson 1931.
<i>Vejovis borcus</i>	~100	Wilson 1931.
<u>SCORPIONIDAE</u>		
<u>India</u>		
<i>Palammaeus gravimanus</i>	112	Venkatanarasimhaiah and Rajesekarasetty 1964.
<i>Palammaeus logimanus</i>	64	Srivastave and Agrawal 1961.
<i>Palammaeus swammerdami</i>	86	Venkatanarasimhaiah and Rajesekarasetty 1965.
<i>Palammaeus</i> sp.	62	Rajesekarasetty <i>et al.</i> 1979.
<i>Heterometrus scaber</i>	96	Venkatanarasimhaiah and Rajesekarasetty 1965.
<i>Heterometrus fulvipes</i>	86, 88	Venkatanarasimhaiah and Rajesekarasetty 1965.
<u>Africa</u>		
<i>Pandinus imperator</i>	120	Guénin 1957.
<u>BOTHRIURIDAE</u>		
<u>South America</u>		
<i>Bothriurus</i> sp.	36	Piza 1947c.

- <sup>1</sup> most common karyotype.
- <sup>2</sup> translocations occur.
- <sup>3</sup> fusion/fission occurs.
- <sup>4</sup> possible polyploidy.
- <sup>5</sup> origin unknown.

these families. The cause of chromosome number variation between two populations of *Heterometrus fulvipes* ( $2n=88$  North India and  $2n=86$  South India) has not been determined (Venkatanarasimhaiah and Rajesekarasetty, 1965). However, a quadrivalent and chains of six or eight chromosomes have been reported in species of the genus *Palamnaeus* (Rajesekarasetty et al., 1979; Srivastava and Agrawal, 1961). While these authors suggest these are due to reciprocal translocations they appear very similar to the chains of 4 observed in *Urodacus manicatus* resultant from centric fusions. Whether these are regular features at meiosis in non-Buthid scorpions is unknown.

The conserved chromosome numbers observed in the holocentric Buthid species are consistent with observations in other holocentric groups despite the fact that theoretically these species should be tolerant to chromosome fragmentation. Apparently selection operates to maintain low chromosome numbers. Most of the observed numerical variation can be attributed to fusion/fission events. Individuals in some populations of *Tityus bahiensis* are variously heterozygous for fusion/fission products which show characteristic trivalents at metaphase I (Piza, 1947a). These are possibly due to single break fission events (i.e. fragmentation). Only one species, *Uroplectes carinatus* shows an increase in chromosome number of unknown origin that could be due to extensive chromosome fragmentation (i.e. agmatoploidy) (Newlands and Martindale, 1980). Unfortunately no information from this species on chromosome size or DNA

content is available from published material.

The most singular feature of male meiosis in Buthid scorpions is the presence at metaphase I of complex rings and chains of chromosomes resulting from interchange heterozygosity, i.e. reciprocal translocations (Piza, 1940, 1947; and illustrated in White, 1973, as shown in Fig. 1.1.1; Sharma *et al.*, 1959). White (1978) made the following comment:

"Clearly, the chance of translocations establishing themselves in phylogeny is somewhat greater in species with holocentric chromosomes, since all the products of reciprocal translocations are transmissible at meiosis instead of half of them being acentric or dicentric chromosomes."

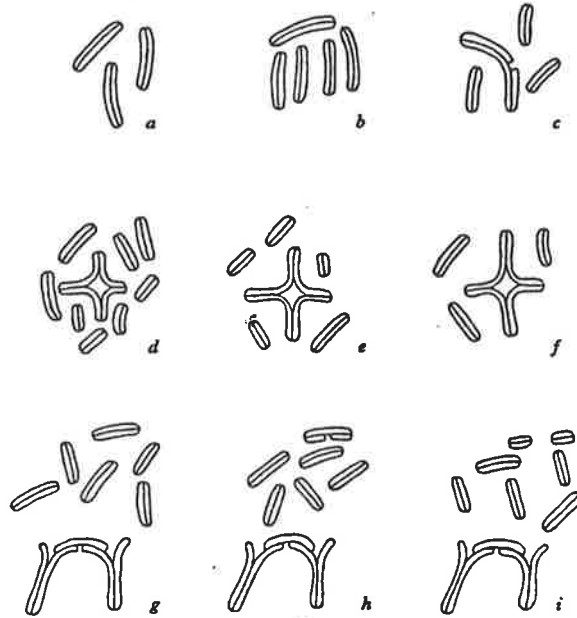
It appears, however, that Buthid scorpions are the only holocentric group to have evolved such a chromosome system. All other species with significant interchange heterozygosity are monocentric: *Oenothera* (Catcheside, 1940; Cleland, 1972), *Isotoma* (James, 1965, 1970), *Rhoeo* (Belling, 1927; Lin, 1979), *Viscum* (Weins and Barlow, 1979), *Periplaneta* (John and Lewis, 1957, 1958; John and Quraishi, 1964), *Blaberus* (John and Lewis, 1959) and *Mesocyclops* (Chinnappa and Victor, 1979).

#### Interchange Heterozygosity.

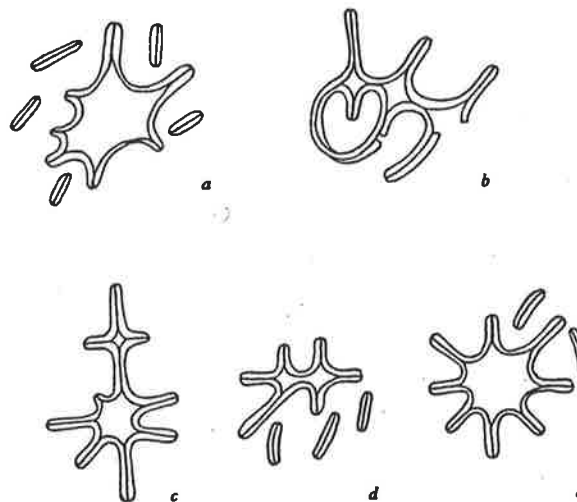
The most detailed study of complex interchange heterozygosity has been carried out in the plant genus *Oenothera* (reviewed by Cleland, 1972; White, 1978). Species in this genus can be divided into three groups on the basis of their cytogenetic strategies:

Figure 1.1.1

Metaphase I chromosome configurations of South American  
Buthid scorpions. (from White, 1973)



Chromosome sets, as seen at male first metaphase, of different individuals of the scorpion genus *Tityus*. (a) *T. bahiensis*, 'normal' set ( $2n = 6$ , three bivalents); (b) *T. bahiensis* ( $2n = 10$ , five bivalents); (c) *T. bahiensis* ( $2n = 9$ , three bivalents and a trivalent); (d) *T. mattogrossensis* ( $2n = 20$ , eight bivalents and an association of four); (e) *T. trivittatus* ( $2n = 14$ , five bivalents and an association of four); (f) *T. bahiensis* ( $2n = 10$ , three bivalents and an association of four); (g) (h) (i) three individuals of *T. bahiensis* from the same locality, with  $2n = 17$ , 18 and 19 (each with an association of five chromosomes). From Piza, redrawn and modified.



Chromosome sets, as seen at male first metaphase, of various species of scorpions. (a) an individual of *Tityus bahiensis* ( $2n = 17$ , five bivalents and a ring of seven chromosomes); (b) *T. bahiensis* ( $2n = 7$ , all chromosomes associated in a complex configuration); (c) *T. bahiensis* ( $2n = 9$ , all chromosomes associated in a complex configuration); (d) and (e) *Isometrus maculatus* ( $2n = 12$  in both individuals, but there is a complex configuration of six in (d) and a ring of eight in (e)). From Piza, redrawn and modified.

- 1) The most primitive group cytogenetically contains the species groups *Oenothera hookeri*, *Oe. grandiflora* and *Oe. argillicola*. These species are outcrossing and usually have seven bivalents at meiosis although rings of 4 and 6 chromosomes are present in some individuals as a result of translocation heterozygosity (floating polymorphism).
- 2) *Oe. strigosa*, *Oe. biennis* and *Oe. parviflora* are species groups that are self-fertilizing (autogamous). These species have fixed heterozygosity for a ring of 14 chromosomes at metaphase I with a system of balanced lethals that act either in the gametophyte or zygote and prevent the occurrence of structural homozygotes.
- 3) Finally, the *Oe. irrigua* alliance is cross-pollinating but structurally heterozygous forming rings of 4, 6 and 8 chromosomes at meiosis. However, this group lacks a balanced lethal system and consequently is chromosomally polymorphic with some individuals showing structural homozygosity (i.e. 7 bivalents).

Two proposals for the evolutionary mechanism by which interchange heterozygosity was established in *Oenothera* have been made:

- 1) Cleland (1936, 1960, 1962) suggested it was established by hybridization of open-pollinating, structurally homozygous races which differed from each other by at least six interchanges resulting in progeny that

exhibited a ring of 14 chromosomes. These rings subsequently became stabilized by the evolution of autogamy and balanced lethal systems.

- 2) Alternatively, Darlington (1931, 1958) proposed that the complex heterozygosity had arisen gradually in a step-wise fashion, as a response to imposed inbreeding (especially in marginal populations) as a system to preserve genetic heterozygosity.

Evidence from *Oenothera* favours the simpler hypothesis of Darlington that requires only inbreeding for the evolution and maintenance of the interchange heterozygosity. Evidence from the mainly autogamous species *Isotoma petraea*, which exhibits a similar interchange system and associated balanced lethals, is also compatible with Darlington's proposal (James, 1965, 1970).

Inbreeding may also account for the high incidence of interchange heterozygotes (rings of 4, 6 and occasionally 8) in populations of *Periplaneta americana* in Wales and Pakistan (Lewis and John, 1957; John and Lewis, 1958; John and Quraishi, 1964). However, no animal species appears to have evolved the sophisticated system of balanced lethals to maintain fixed heterozygosity as found in plants. The only examples of fixed interchange heterozygosity in animals occur when a sex chromosome is incorporated in the translocation series: *Otocryptops sexspinosus* (Ogawa, 1954), *Kaloterms approximatus* (Syren and Luykx, 1981), *Incisitermes schwarzi* (Syren and Luykx, 1977), *Delena cancerides* (Rowell, 1985) and in the monotremes (Murtagh, 1977). Selection for this type

of reciprocal translocation heterozygosity differs from that in species where the sex chromosomes are not involved.

Concomitant with the establishment of interchange heterozygosity must be a system that ensures regular disjunction of the multiples at anaphase I. Consistent with regular disjunction is a high frequency of alternate orientation of multiples. Apart from genetic and perhaps environmental effects, three main cytogenetic factors influence this orientation (John, 1976; Rickards, 1983):

- 1) The frequency and distribution of chiasmata.

Terminal chiasmata at a frequency that ensures regular multivalent formation results in a high frequency of alternate orientation.

- 2) The symmetry of the interchange.

Interchanges where the chromosome arms exchanged are of equal length preserves the symmetry of the multivalent (i.e. centromeres roughly equidistant) and contributes to alternate disjunction.

- 3) In many species, the larger the number of chromosomes involved in interchange events the lower the frequency of regular disjunction.

The only two animal groups where most of the chromosomes are regularly involved in multiple formation both exhibit achiasmate meiosis and lack distinguishable sex chromosomes; scorpions, *Tityus bahiensis* and *Isometrus maculatus* (Piza, 1939, 1947; White, 1973) and the copepod *Mesocyclops edax* (Chinnappa and Victor, 1979). Regular disjunction is

ensured in *Mesocyclops* by a unique zig-zag arrangement of adjacent small and large chromosomes.

The nature of multivalent disjunction in scorpions is poorly understood. However, where rings and other configurations consisting of odd numbers of chromosomes exist (e.g. 7 and 9), duplications and deficiencies must occur. (However, evidence presented from Australian Buthids suggests these may be aberrant cells resultant from synaptic failure.) White (1973) suggested that a large amount of redundant heterochromatic material may be present in the genome. Alternatively, he suggested that males may be functionless and reproduction pseudogamous (i.e. a form of reproduction where sperm initiate the development of the egg but do not contribute any chromosomes to the offspring). Evidence for parthenogenesis comes also from the discovery by Mathieson (1962) of what he claims is a totally female parthenogenetic species (*Tityus serrulatus*). Related species *Tityus bahiensis* and *T. trivittatus* appear to have a disparity in the number of males per population. John and Quraishi (1964) suggested this is in keeping with parthenogenetic reproduction of the ameiotic type. Females retain the advantages of interchange heterozygosity without the meiotic disadvantages, while any balanced sperm produced by males would result in both males and females. They suggested the process is akin to accumulation of chromosomal mutations in asexually reproducing species. These chromosomal mutations would be fertility affecting in species that reproduce sexually. However, evidence for parthenogenetic



reproduction is somewhat tenuous and no information on cytogenetic aspects of female meiosis is known.

Considering that interchange heterozygosity in most species has arisen as a response to inbreeding (to hold adapted gene arrays together and maintain heterozygosity), it seems premature to exclude this selective possibility from scorpion evolution. Very little is known of the ecological aspects of Buthid and other scorpion species' lifestyles. This chapter considers chromosomal aspects of the scorpion fauna of Australia in an attempt to elucidate some of the factors that have influenced chromosome evolution in this group. However, one of the greatest unsolved problems in biology is the role of chromosome change in evolution. Studies in one particular group cannot hope to reveal more than a fraction of the many factors that come into play during evolution.

## SECTION 2

### MATERIALS AND METHODS

#### Ecological Data.

Table 1.2.1 lists the 29 extant species of scorpions in Australia (Koch, 1979).

Australian scorpions have been rarely studied in the field and consequently very little ecological data is available. Most of the information presented here is from personal field observations. All species are nocturnal and are usually solitary. Most are opportunistic predators although some have evolved more specialized food requirements. In general, Buthid species are much smaller than the larger burrowing Scorpionids.

#### Buthid Species.

*Lychas marmoreus*, the most abundant of the Buthid species, was collected mainly under rocks or bark. Individuals of this species tended to congregate under the same piece of tree bark during certain times of the year (autumn/winter) which greatly facilitated collection.

*Lychas variatus*, a rarer species, was also found under bark on large Eucalypts or occasionally under rocks. Generally, populations of this species were small and isolated.

*Lychas alexandrinus* is an extremely rare cryptozoic desert dweller. Similarly, *Isometroides vescus* is cryptozoic

Table 1.2.1

Scorpion species of the Australo-Papuan region.

Family	Genus	Species
BOTHRIURIDAE	<i>Cercophonius</i>	* <i>C. squama</i>
BUTHIDAE	<i>Lychas</i>	* <i>L. marmoreus</i>
		* <i>L. variatus</i>
		* <i>L. alexandrinus</i>
	<i>Isometroides</i>	* <i>I. vescus</i>
	<i>Isometrus</i>	* <i>I. maculatus</i>
		* <i>I. melanodactylus</i>
SCORPIONIDAE	<i>Liocheles</i>	<i>L. australasiae</i>
		<i>L. waigiensis</i>
		<i>L. karschii</i>
	<i>Urodacus</i>	( * <i>U. manicatus</i>
		( * <i>U. elongatus</i>
		( * <i>U. novaehollandiae</i>
		( * <i>U. planimanus</i>
		( <i>U. centralis</i>
		( * <i>U. armatus</i>
		1. Species group <i>armatus</i> ( <i>U. koolanensis</i>
		2. Species group <i>megamastigus</i> <i>U. megamastigus</i>
		( <i>U. varians</i>
		( <i>U. hoplurus</i>
		( <i>U. giuliani</i>
		( <i>U. carinatus</i>
( <i>U. macrurus</i>		
( <i>U. excellens</i>		
( <i>U. spinatus</i>		
( <i>U. lowei</i>		
3. Species group <i>hoplurus</i> ( <i>U. similis</i>		
4. Species group <i>hartmeyeri</i> <i>U. hartmeyeri</i>		
5. Species group <i>yaschenkoi</i> * <i>U. yaschenkoi</i>		

\* Species examined in this study.

by day. This species preys exclusively on burrowing megalomorph (trap door) and lycosid spiders and can be found in the burrows of its prey. However, the rarity of this species was demonstrated by Main (1956) who collected only 18 specimens of *I. vescus* from 3,200 spider burrows examined. Main's collecting procedure included habitats under rocks and logs which yielded only three additional specimens.

Specimens of these two species were collected in this study using a drift net system. A small mesh net 20-100m long and 0.3m high was positioned vertically, flush with the ground. Plastic collection tubs were dug into the ground under the net. Scorpions foraging at night run along the length of the net, fall into the tubs and are collected the next morning.

*Isometrus maculatus* and *Isometrus melanodactylus* were also not particularly abundant and were collected as individuals under rocks.

#### Scorpionid Species.

Specimens of *Urodacus manicatus*, *Urodacus elongatus*, *Urodacus planimanus* and occasionally *Urodacus novaehollandiae* were found under rocks, often in short burrows from which they could be removed with forceps.

*Urodacus yaschenkoi*, *Urodacus armatus* and *U. novaehollandiae* construct distinctive burrows, often in sandy soil. Burrows of *U. yaschenkoi* are in open ground in localized dense populations. The other two species often burrow at the base of bushes or under rocks. To collect

specimens from burrows plastic drinking cups were dug into the ground at the mouth of the characteristic crescent-shaped burrows. During the night most individuals come to the mouth of the burrow for housekeeping or hunting. They leave the borrow backwards, drop into the cup and can be collected the next morning.

It was difficult to obtain large numbers of adult males from Scorpionid populations as there was often a bias towards females and juveniles. Shorthouse and Marples (1982) observed this in *U. yaschenkoi* and Smith (1966) observed the same phenomenon in *U. manicatus*. The bias is only present at the 6th instar (adult stage) as there is a 1:1 sex ratio at birth and in juvenile stages. It appears to be due to greater female longevity and the more sedentary nature of females that tend to live under the same rock (or burrow) especially during pregnancy. Thus, they are protected from predators and environmental extremes and are more likely to be sampled by collectors.

#### **Bothriuridae.**

*Cercophonius squama* is found in moist habitats in detritus or under bark and rocks. During winter males are often found on house walls, however, all specimens collected at this time and examined cytologically were found to contain only stored sperm.

#### **Maintenance in the Laboratory.**

Adult Scorpionid specimens were maintained for up to 18

months in the laboratory while Buthid specimens lived for a maximum of 3 months. The environment for all species was kept continually moist: desiccation appeared to be the main cause of death. Food sources consisted of *Drosophila*, mealworms and, occasionally, moths.

Juveniles of *U. manicatus* have been maintained for over four years in the laboratory. During this period, specimens underwent three moults and were thus in their 4th instar at the end of four years. This is a much slower development rate than that obtained by Smith (1966) who suggested scorpions of this species were 32 months when they first mated (i.e. 6th instar). However, the difference could be attributed to inadequate laboratory conditions or alternatively there are inter-population differences in development rate. The juveniles that were maintained in the laboratory came from a population in one of the most arid regions of the distribution range of the species, while Smith studied specimens from a very wet environment where he observed that if conditions were unfavourable individuals would delay moulting. Shorthouse and Marples (1982) showed that the minimum generation time for *U. yaschenkoi*, an arid region species, was six years.

Although young from already pregnant females were born in the laboratory, breeding experiments could not be carried out due to the long maturing period of scorpions and the inability to produce conditions conducive to mating in the laboratory.

## CYTOLOGICAL TECHNIQUES

### Meiotic Chromosome Preparations.

Live scorpions were dissected in Insect Saline (0.7% Sodium Chloride, 0.3% Calcium Chloride) by the removal of the ventral surface. Testis from males and oviducts and embryos from females were removed and directly fixed in two changes of cold 3:1 methanol:acetic acid. Some were treated with colchicine before fixation:

- Testis - 30 min in 0.02% colchicine in Insect Saline.  
Oviducts - 2-3 hrs in 0.1% colchicine in Insect Saline.  
and Embryos

After overnight fixation, chromosome preparations were made either by the squash (A) or surface spreading (B) techniques.

#### A. Squashes.

1. A small piece of tissue was placed on a slide in a drop of 50% acetic acid and macerated with a blunt probe.
2. A coverslip was placed over the now suspended material and pressure applied for 30 secs with the palm of the hand through 3 layers of filter paper.
3. The coverslip was removed by immersion in liquid nitrogen and the slide immediately placed in absolute alcohol for 1-2 mins.
4. After drying, the slide was stained for 6-12 mins in 2% Giemsa in Sorenson's buffer, pH 6.8.
5. After rinsing in ddH<sub>2</sub>O, the slide was dried and a coverslip mounted with Xam.

## B. Air Drying.

This technique proved more successful for embryos and oviducts where large amounts of non-dividing tissue were present.

1. The tissue was placed on a slide and macerated in 3 drops of 50% acetic acid.
2. The slide was placed on a hot plate at 37 -40 C and the drops with the tissue suspended were continually moved about on the surface of the slide until the liquid had completely evaporated.
3. Stained and mounted as described in A.

Numerous unsuccessful attempts were made to induce high resolution banding of both Buthid and Scorpionid chromosomes (to enable a more detailed analysis of the types of rearrangements occurring both within and between populations and species). Techniques used included G-banding (Seabright, 1971), Insect G-banding (Maudlin, 1974), fluorescence banding (Schweizer, 1971), restriction endonuclease banding (Kaelbling *et al.*, 1984) and *in situ* hybridization (Harper and Saunders, 1981) of a repeated sequence isolated from *U. manicatus*, to the metaphase I chromosomes of males from various populations. The repeated sequence (a 1.8 kilobase Bgl II repeat cloned into the *E. coli* plasmid vector pBR 322) did not differentiate particular chromosomes or chromosome regions but had a dispersed distribution on all chromosomes of all individuals examined. Thus, it could not be used as a marker for chromosomes involved in rearrangements. C-banding was the only



technique successfully applied.

#### **C-Banding of Meiotic Preparations.**

C-banding was carried out using a modification of the technique of Sumner (1972).

Scorpionid slides were aged for one week prior to C-banding while it was necessary to use freshly made slides to induce C-banding in Buthid chromosomes. Slides were:

1. Placed in saturated (5%) Barium Hydroxide ( $\text{BaOH}_2$ ) at  $50^\circ\text{C}$  for 45 secs + 2 mins.
2. Rinsed in  $\text{ddH}_2\text{O}$  and placed in 2X SSC at  $65^\circ\text{C}$  for 45 mins - 1 hr.
3. Rinsed in  $\text{ddH}_2\text{O}$  and stained for 12-20 mins in 4% Giemsa in Sorenson's buffer, pH 6.8.
4. Rinsed, dried and coverslip mounted in Xam.

#### **Preparations from Somatic Tissue.**

Juvenile and adult scorpions are very poor in somatic divisions. However, a few divisions were obtained from intestine, haemotube and haemolymph.

The spreading technique used to obtain intestinal and haemotubal divisions was the same as that described for meiotic preparations (B) with the same colchicine treatment as for oviducts and embryos.

Haemolymph preparations were as follows:

1. 4-20 hrs before haemolymph removal, animal was pre-injected with 0.1 ml of 0.02% colchicine in Insect Saline.

2. Haemolymph was removed by piercing the exoskeleton with a needle and the droplets obtained were collected in a capillary tube (<0.1 ml collected).
3. An equal volume of ddH<sub>2</sub>O was added and the solution left for 15-20 mins at room temperature (hypot<sup>o</sup>nic treatment).
4. Cells were centrifuged for 10 mins at 500 r.p.m. and the supernatant removed.
5. Cells were resuspended in 3:1 methanol:acetic acid. Repeat fixation procedure 2X before dropping cells on to slide.
6. Stain and mount as for A.

#### Tissue Culture.

The very slow growth rate of embryonic scorpions (16 months gestation) resulted in very poor yields of divisions from most embryo preparations. Consequently embryos were cultured *in vitro* to try and improve the number of divisions obtainable. Although some divisions were found, culturing the cells did not improve the growth rate to any useful extent.

1. Embryos, either macerated whole, or embryonic cells separated from fatty tissue by centrifugation were placed in six well plates.
2. Coverslips and <sup>Media</sup>~~medium~~ were placed over these cells. Media used was L15 supplemented with 15% FCS, 1% Lobster Haemolymph, 1% w.v. glutamine, 60 µg/ml penicillin and 50 µg/ml streptomycin sulphate.

3. Cells were incubated at 24°C-26°C.
4. Chromosome preparations were obtained by scraping the cells from the surface of the well and treating them as per haemolymph preparations.

### Electrophoresis.

Electrophoresis of fat body extracts from an adult female *U. manicatus* and whole body extracts from her 25 young was carried out as follows:

Extracts were run on 'cellogel 250' for 1½ hrs at 200v in Tris Maleate Buffer (pH 7.8) or Sodium Veranol (pH 9.9).

Gels were stained for either GPI (phosphoglucoisomerase) PGM (phosphoglucomutase), or General Protein (Brown and Knudson, 1980).

### Nomenclature.

Each individual collected was given either a one or two letter locality code. Additionally, Scorpionids were identified by a letter corresponding to the first letter of their species name, while Buthids were identified by the first letter of their generic name:

e.g. BM I - Belair, *manicatus*, individual I.

OCL II - Overland Corner, *Lychas*, individual II.

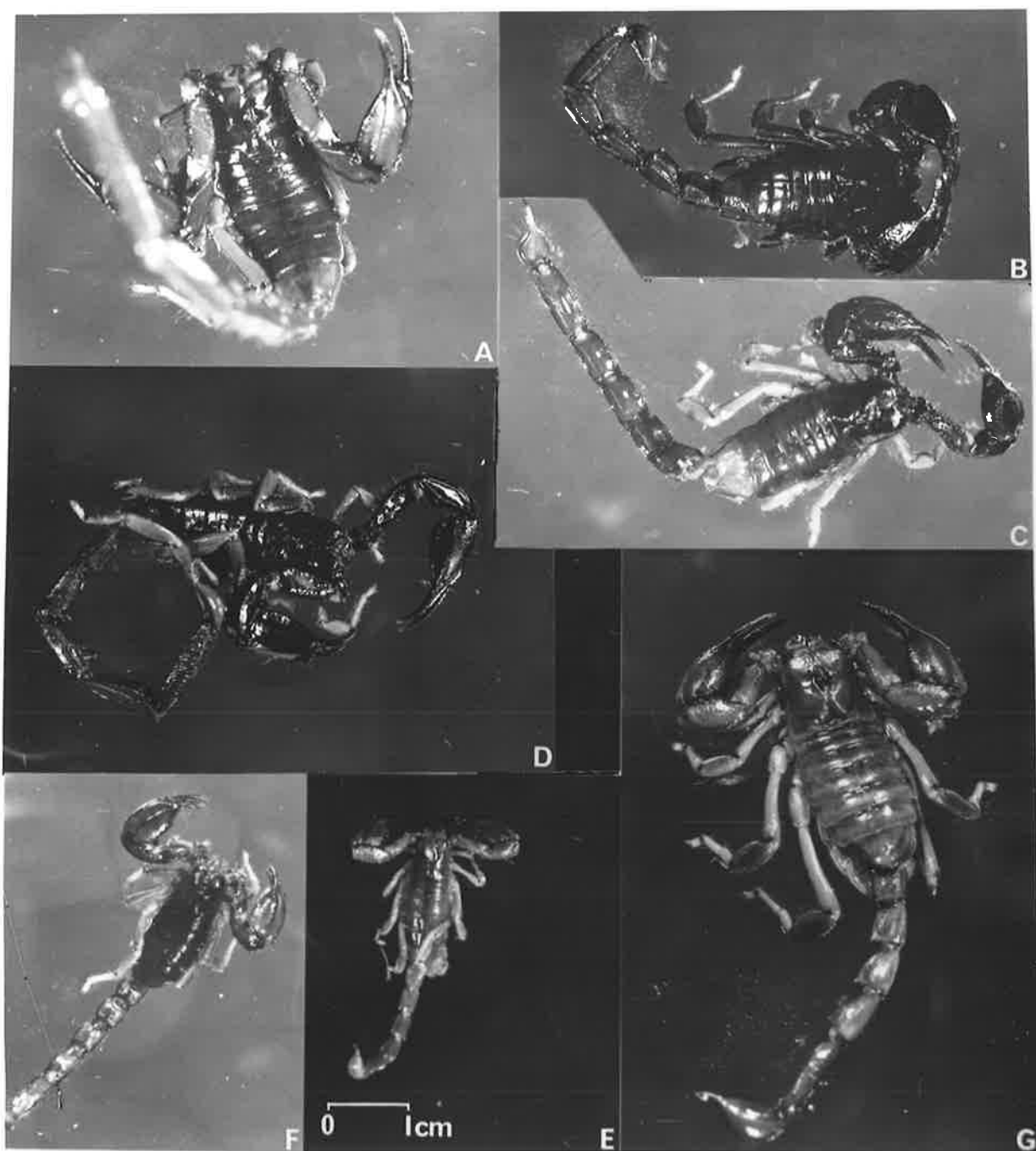
The Roman numeral corresponds to the number of the individual collected.

All specimens have been lodged at the South Australian Museum. Figures 1.2.1 and 1.2.2 show the species that were examined in this study.

**Figure 1.2.1**

**Adult male specimens of species from the family  
Scorpionidae examined during this study.**

- A. *Urodacus elongatus.*
- B. *Urodacus novaehollandiae.* (S. Australia)
- C. *Urodacus novaehollandiae.* (W. Australia)
- D. *Urodacus planimanus.*
- E. *Urodacus armatus.*
- F. *Urodacus manicatus.*
- G. *Urodacus yaschenkoi.*

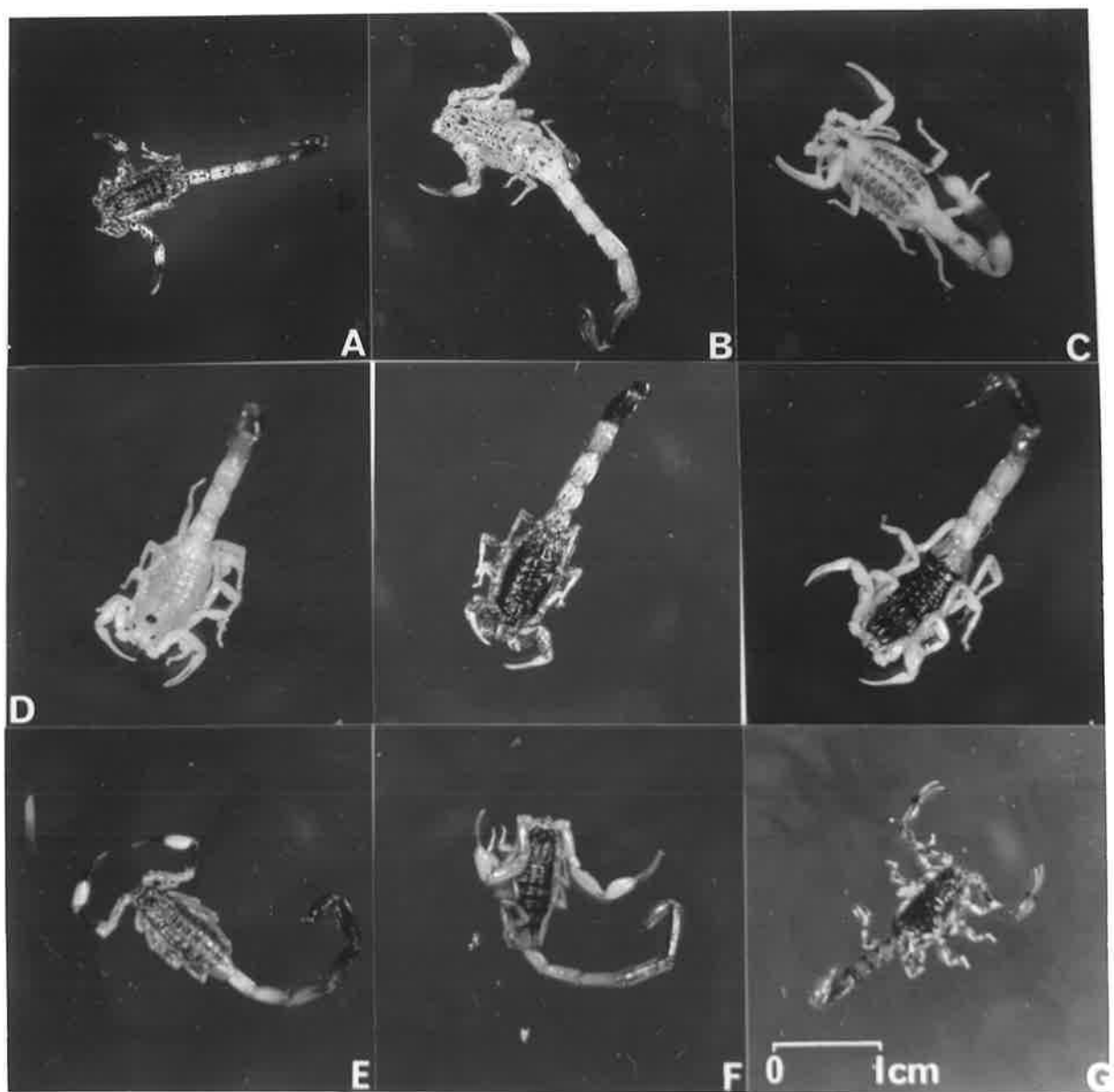


**Figure 1.2.2**

Adult male specimens of species from the family Buthidae examined during this study.

- A. *Lychas marmoreus.*
- B. *Lychas variatus.*
- C. *Lychas alexandrinus.*
- D. *Isometroides vescus.*
- E. *Isometrus melanodactylus.*
- F. *Isometrus maculatus.*
  
- G. *Cercophonius squama.*

Family Bothriuridae



## SECTION 3

## CHROMOSOMAL STUDIES IN THE FAMILY BUTHIDAE

## INTRODUCTION

Australia has six species of scorpion from the family Buthidae (sub-family Buthinae); three species of *Lychas*, one species of *Isometroides* and two species of *Isometrus*. Of these genera, *Isometroides* evolved within Australia and is monospecific containing only *I. vescus*. There are twenty seven species of *Lychas* outside Australo-Papua, but of the three species in Australia *L. variatus* is found mainly in Australo-Papua while *L. marmoreus* and *L. alexandrinus* are confined to Australia. Similarly there are eleven species of *Isometrus* outside Australo-Papua. Of the two species within Australo-Papua, *I. melanodactylus* is confined to the region whereas *I. maculatus* is cosmopolitan (Koch, 1977).

None of these species, until this study, had been karyotypically studied within Australia. However, Piza (1947a) examined cytologically male specimens of *Isometrus maculatus* from South America. He found this species to have a diploid chromosome number of  $2n=12$  with evidence of translocation heterozygosity at metaphase I.

This section presents the results of karyotypic analysis of all species of Australian Buthid scorpions. Table 1.3.1 summarizes the cytogenetic features of the Australian Buthid fauna. Australian species exhibit holocentric chromosomes, achiasmatic meiosis and interchange heterozygosity, features



Table 1.3.1

Cytogenetic features of the Australian Buthid scorpion fauna.

Species	Chromosome ( $\sigma$ ) number 2n	Male meiosis	Centric nature	Polymorphism	
				intra	inter
<i>Lychas marmoreus</i>	14, 12	Achiasmate	Holocentric	Interchange	Interchange fusion/fission
<i>Lychas variatus</i>	14, 16 <sup>+</sup>	Achiasmate	Holocentric	Interchange	Interchange fusion/fission
<i>Lychas alexandrinus</i>	14	Achiasmate	Holocentric	n.i.	n.i.
<i>Isometroides vesicus</i>	14	Achiasmate	Holocentric	Interchange	Interchange
<i>Isometrus maculatus</i>	14	Achiasmate	Holocentric	Interchange	n.i.
<i>Isometrus melanodactylus</i>	14	n.i.	Holocentric	n.i.	n.i.

+ = only one individual found.

n.i. = no information.

common to almost all Buthids examined.

The interpretation of the chromosomes as holocentric will be discussed in detail in Chapter 3. The interpretation of achiasmate meiosis was based on cytological observations. No evidence for chiasmata could be found either at early meiotic stages where the bivalents and ring multiples exhibited gradual contraction, or, at later metaphase/anaphase stages where homologous chromosome regions remained parallel and distance paired. There was evidence in some chromosome preparations of fine cross-connecting strands between paired homologous chromosomes, however, these connections were clearly not chiasmata in origin. Whether they are involved in pairing maintenance at metaphase I is discussed in Chapter 2.3.

## RESULTS

### Chromosome Polymorphism in Species from the Genus *Lychas*.

#### *Lychas marmoreus*.

*Lychas marmoreus* is the most common of the *Lychas* species and occurs mainly in cooler, wetter Southern habitats, although its range does extend into arid regions. Figure 1.3.1 and Table 1.3.2 show locality and karyotypic data for this species.

The diploid chromosome number ( $2n=14$ ) is highly conserved in males with only two out of eighty nine individuals karyotyped differing due to homozygosity for a tandem fusion resulting in  $2n=12$  (Fig. 1.3.2a). Most populations showed evidence for interchange heterozygosity

**Figure 1.3.1**

**A. Locality and karyotypic data for *Lychas marmoreus* collected in this study.**

- 2n=12 (6 bivalents)
- 7 bivalents
- △ 04 + 5 bivalents, 06 + 4 bivalents
- ▲ Mixed populations, structural homozygotes (7 biv.) and heterozygotes (04, 06, 08 and 010)
- 08 + 3 bivalents
- 010 + 2 bivalents
- ☆ 04 + 5 bivalents
- ★ 2n=14 (Metaphase I data unknown)

**B. Museum localities of *L. marmoreus* (Koch, 1979).**

(small black squares)

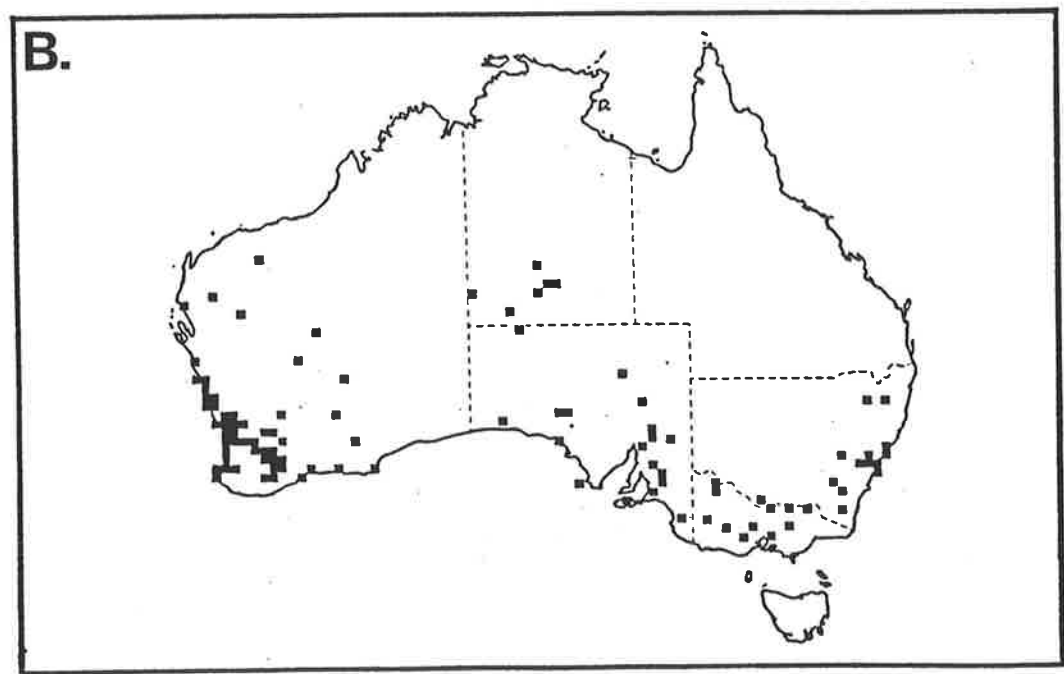
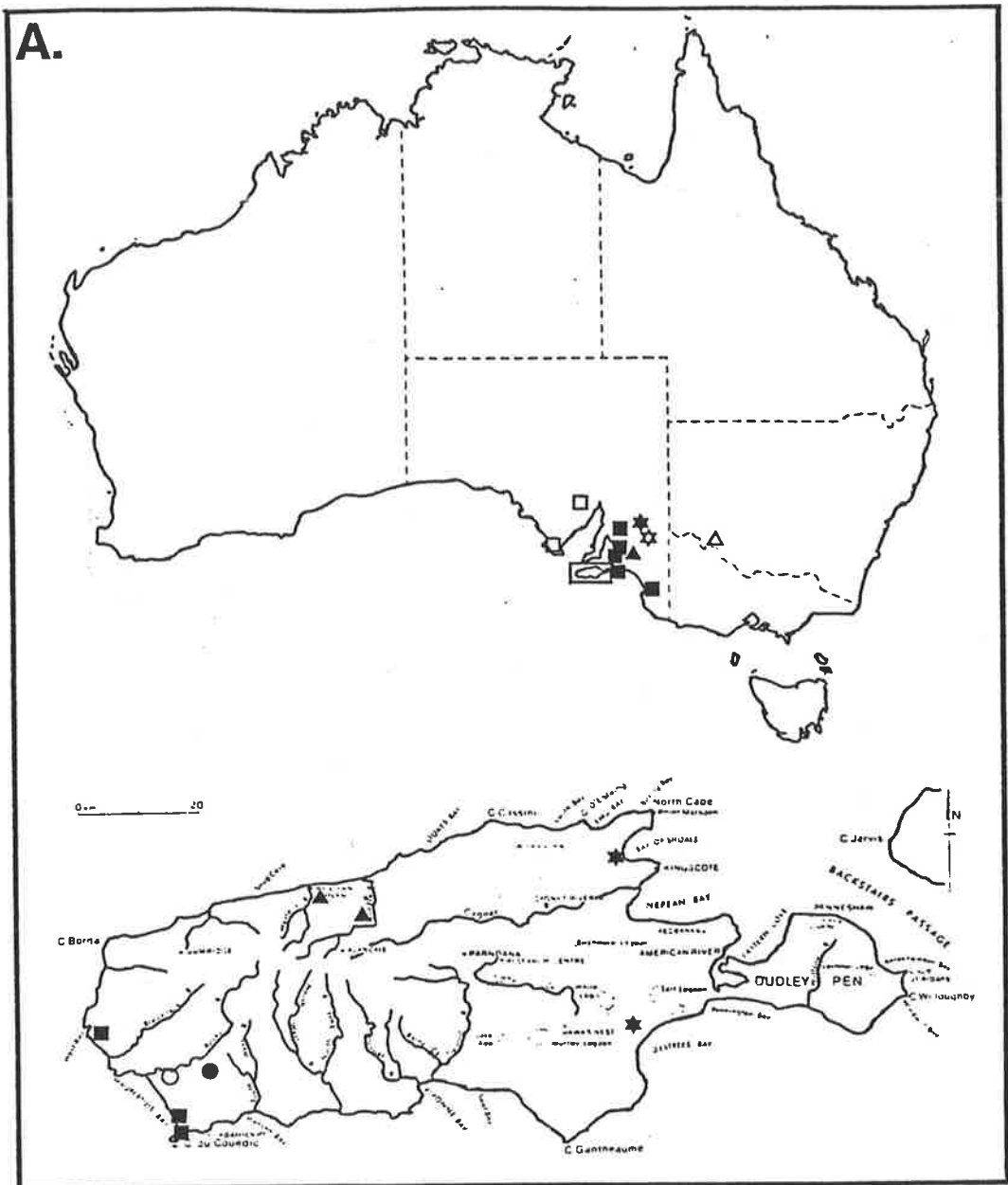


Table 1.3.2

Locality and karyotypic data for *L. marmoratus*.

Locality	Code	2n	<sup>1</sup> N	Metaphase I	<sup>2</sup> No. ♂ animals
Bald Hill, S.A.	BHL	14	( 1)	7 bivalents	1
Belair, S.A.	BL	14	(12)	7 bivalents	4
				04 + 5 biv.	1
Clare, S.A.	CL	14	( 3)	7 bivalents	1
Happy Valley, S.A.	HVL	14	( 1)	7 bivalents	1
Middleback, S.A.	MBL	12	( 1)	6 bivalents	1
Onkaparinga Gorge, S.A.	OGL	14	(11)	7 bivalents	5
Waikerie, S.A.	WL	14	( 1)	04 + 5 biv.	1
Cape de Couedic, K.I.	CCL	14	( 3)	7 bivalents	1
Flinders Chase, K.I.	FCL	14	( 1)	010 + 2 biv.	1
Rocky River, K.I.	RRL	14	( 1)	08 + 3 biv.	1
Western Cove, K.I.	WCL	14	( 1)	7 bivalents	1
Western River, K.I.	WRL	14	( 3)	7 bivalents	2
				08 + 3 biv.	1
Western River, Conservation Pk. K.I.	WPL	14	(40)	7 bivalents	24
				04 + 5 biv.	9(1)*
				06 + 4 biv.	1
				010 + 2 biv.	2*
Balranald, N.S.W.	BRL	14	( 4)	04 + 5 biv.	1
				06 + 4 biv.	2
				06 + 4 biv. + fragment	1
Overland Corner, S.A.	OCL	14	( 1)	-	-
Sultana Point, S.A.	SPL	12	( 1)	-	-
D'estrees Bay, K.I.	DBL	14	( 3)	-	-
Emu Bay, K.I.	EBL	14	( 2)	-	-
T o t a l			90		62

<sup>1</sup>N = number ♂ animals karyotyped.

<sup>2</sup>N = number ♂ animals where metaphase I data was available.

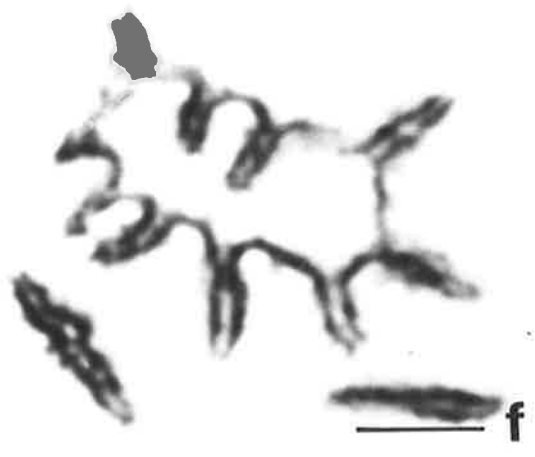
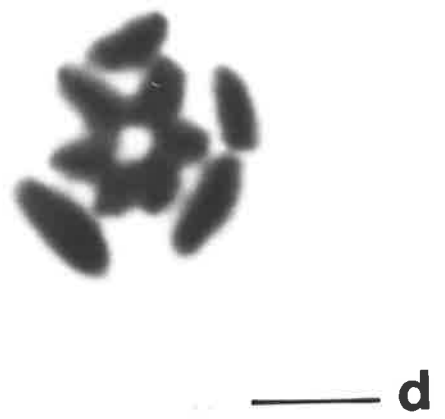
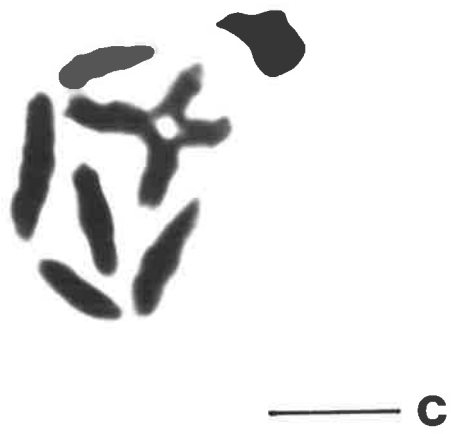
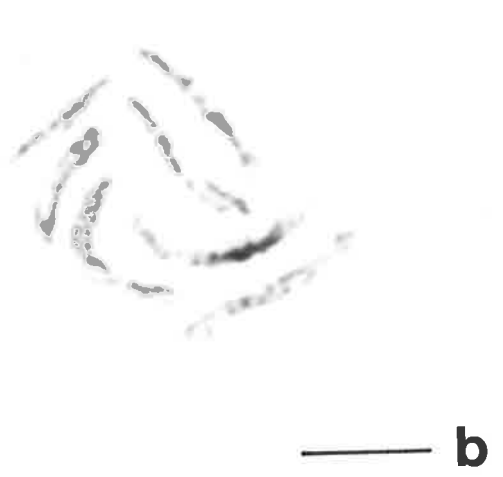
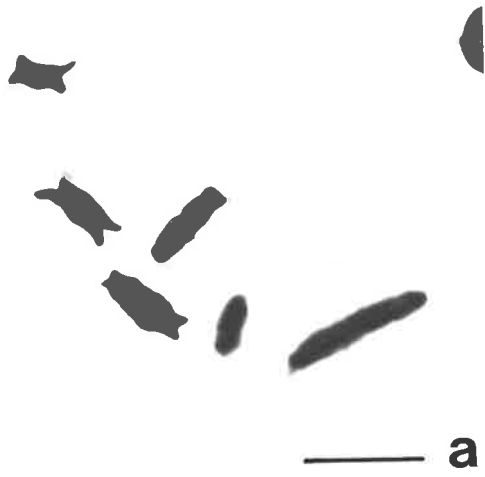
\* synaptic failure in many cells.

0 metaphase I ring configuration.

**Figure 1.3.2**

**Representative metaphase I karyotypes for *Lychas marmoreus*  
structural homozygotes and interchange heterozygotes.**

<b>a</b>	<b>6 bivalents</b>	<b>(MBL)</b>
<b>b</b>	<b>7 bivalents</b>	<b>(OGL)</b>
<b>c</b>	<b>04 + 5 bivalents</b>	<b>(WPL)</b>
<b>d</b>	<b>06 + 4 bivalents</b>	<b>(WPL)</b>
<b>e</b>	<b>08 + 3 bivalents</b>	<b>(RRL)</b>
<b>f</b>	<b>010 + 2 bivalents</b>	<b>(FCL)</b>



manifest by ring formation at metaphase I with either 4, 6, 8 or 10 chromosomes involved (Fig. 1.3.2). Populations where a large sample size was obtained were polymorphic containing both structural homozygotes (7 bivalents) and often a number of different structural heterozygotes (rings of 4, 6 or 8).

#### Description of the $2n=14$ Karyotypes.

##### 7 Bivalents.

Due to the holocentric nature of the chromosomes, there were no morphological features excepting size, that could be used to differentiate the members of a karyotype. Compounding this problem was the very contracted state of the chromosomes at both mitotic and meiotic metaphase which reduced further the size differential. C-banding produced telomeric bands, however, they were not distinctive enough to allow the clear identification of particular chromosomes or bivalents.

However, at early metaphase I (before the chromosomes had completed contraction) it was possible to identify 3 size classes. These consisted of 2 small bivalents (S), 3 medium bivalents (M), and 2 large bivalents (L), one ( $L^+$ ) being obviously larger than any other bivalent in the karyotype. Thus the karyotype could be abbreviated as  $L^+LMMMSS$  (see Fig. 1.3.3a-d). These bivalent sizes were consistent in all populations karyotyped. Correspondingly, these size classes were present (but usually not clearly definable) as homologous pairs at mitosis.



**Figure 1.3.3**

**Interpopulation comparison of metaphase I karyotypes  
from *Lychas marmoreus*.**

**7 bivalents**

- a Onkaparinga Gorge (OGL)
- b Western River Conservation Park (WPL)
- c Western Cove (WCL)
- d Clare (CL) C-banding

Size classes of bivalents ( $L^+LMMMSS$ ) same for all populations.

*Association*

~~Ring~~ of 4 and 5 bivalents

- e Belair (BL) C-banding
- f Western River Conservation Park (WPL)  
C-banding
- g Waikerie (WL)
- h Balranald (BRL)

Bivalents are the same size classes for each population.  
Breakpoints of interchange differ between populations.

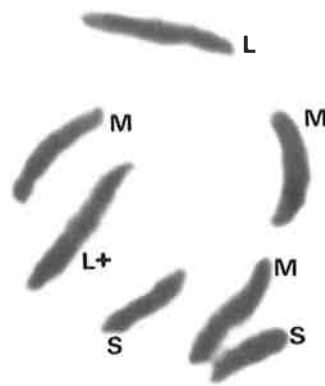
Bar = 5  $\mu$ m.



**a**



**b**



**c**



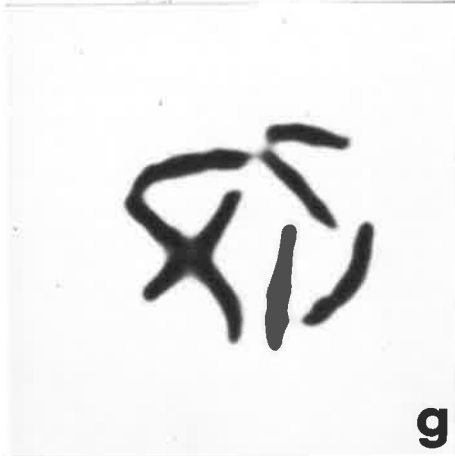
**d**



**e**



**f**



**g**



**h**

### Ring of 4 and 5 Bivalents.

Figure 1.3.3e-h shows examples of the ring of 4 and 5 bivalents karyotype from four different localities. Again, size was the only feature that could be used to distinguish the chromosomes. An analysis of the rings of four showed that all individuals in a particular population showed identical rings, e.g. WRL showed rings of four with a small, small, medium, large (SSML) conformation of arm sizes. However, rings differed between populations. WL and BL rings had SLMM arm sizes. As far as could be determined the chromosomes not involved in ring formation were the same for all populations, i.e. the bivalents always consisted of the size classes L<sup>+</sup>LMSS. This suggested that differences between populations involved different interchange breakpoints. The differences in sizes of mitotic chromosomes between populations also reflected breakpoint differences.

### Ring of 6 and 4 Bivalents.

Metaphase I cells from individuals (from two separate populations) with this karyotype are shown in Figure 1.3.4a, b, c. The arm sizes of the ring conformations were indistinguishable between the two populations, i.e. WPL and BRL II (LL M/S M/S M/S M/S). However, the remaining bivalents differed; WPL (LMSS) and BRL II (L M/L SS). Additionally, there was an intrapopulation difference in chromosome size in the Balranald population. BRL IV was either homozygous for a fusion event involving part of a

**Figure 1.3.4**

**Complex interchange heterozygotes of *Lychas marmoreus*.  
Interpopulation comparison of metaphase I karyotypes.**

**Ring of 6 and 4 bivalents**

**a Western River Conservation Park (WPL)**

**b,c Balranald (BRL)  
(Note difference in ring conformation  
within this population)**

**Same bivalents (not involved in interchange).**

**Ring of 8 and 3 bivalents**

**d Rocky River (RRL)**

**e,f Western River Conservation Park (WPL)**

**Same bivalents (not involved in interchange).**

**Ring of 10 and 2 bivalents**

**g Flinders Chase (FCL)**

**h Western River Conservation Park (WPL)**

**Same bivalents (not involved in interchange).**

**Bar = 5  $\mu$ m.**



**a**



**b**



**c**



**d**



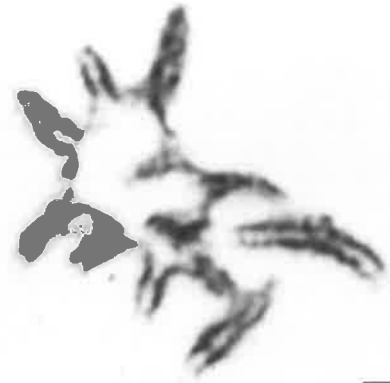
**e**



**f**



**g**



**h**

large bivalent and a small chromosome (also involved in the interchange) or, the interchange involved different breakpoints and chromosomes (cf. BRL II). Mitotic data could not be obtained to distinguish between these two possibilities.

#### Ring of 8 and 3 Bivalents.

Figure 1.3.4d,e shows metaphase I cells from the two individuals with this karyotype (RRL I and WRL III). The three bivalents in both individuals could be characterized as LMS. It was difficult to characterize the ring conformations in these larger multiples, however mitotic data clearly showed differences in interchange breakpoints. RRL I appeared to require twisting to facilitate homologous pairing in the ring which did not occur in WRL. This pairing in RRL I made many rings appear to only involve 7 chromosomes, however, mitotic data (Fig. 1.3.5) showed that an additional small chromosome was involved in the ring.

#### Ring 10 and 3 Bivalents.

This karyotype was found in only three individuals; one from Kangaroo Island (FCL) and two from Western River (WPL) where the synaptic failure phenomena (described later) resulted in very few cells showing a ring of 10 chromosomes. Figure 1.3.4f,g shows again that the chromosomes involved in ring formation have different interchange breakpoints between populations, while the two bivalents (LS) not involved in interchanges were the same size in both populations.

### Mitotic Karyotypes.

Due to the condensed nature of the chromosomes at metaphase of mitosis many mitotic karyotypes show very little size variation. However, as shown in Figure 1.3.5, individuals showing ring formation at metaphase tend to show greater variation in chromosome size (e.g. RRL). Thus interchanges do not result in preservation of the conformity of chromosome size present in the 7 bivalent individuals.

### Population Structure in *Lychas marmoreus*.

The scarcity of animals in many localities resulted in only single individuals being collected and karyotyped. Consequently no data on the abundance of particular interchange heterozygotes in many populations could be obtained. For example, the individuals collected at Rocky River (RRL) and Flinders Chase (FCL) showed a ring of 8 (Ø8) and a ring of 10 (Ø10) respectively. Whether these ring configurations were fixed or at a high frequency in these populations is unknown.

However, a large sample of 36 males were karyotyped from an isolated, dense population of *Lychas marmoreus* at Western River Conservation Park, Kangaroo Island (WPL). These individuals were found under the bark of 19 large, isolated Eucalypts as shown in Figure 1.3.6 and Table 1.3.3. The karyotypes of the male individuals collected from each tree are shown in Table 1.3.4. This was a mixed population showing mainly structural homozygotes 23/36 (7 bivalents) and heterozygotes for a single interchange 10/36 (Ø4 and 5

**Figure 1.3.5**

**Mitotic karyotypes from *Lychas marmoreus*.**

SPL	2n = 12	Do not pair to form bivalents.
MBL	2n = 12	6 bivalents.
T1 I	2n = 14	7 bivalents.
T3 I	2n = 14	04 + 5 bivalents.
WL I	2n = 14	04 + 5 bivalents.
BRL	2n = 14	06 + 4 bivalents.
WRL	2n = 14	08 + 3 bivalents.
RRL	2n = 14	08 + 3 bivalents - note uneven sizes of chromosomes.
T4 II	2n = 14	010 + 2 bivalents.



SPL      ) ( 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

MBL      ) > 0 0 0 0 0 0 0 0

T1 I      ) ) ) < ( 0 1 0 1 0 1 0 1

T3 I      ( ) ) ) ( < < > < > > > > > > > > >

WL I      ) ) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

BRL      ( ( ) ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )

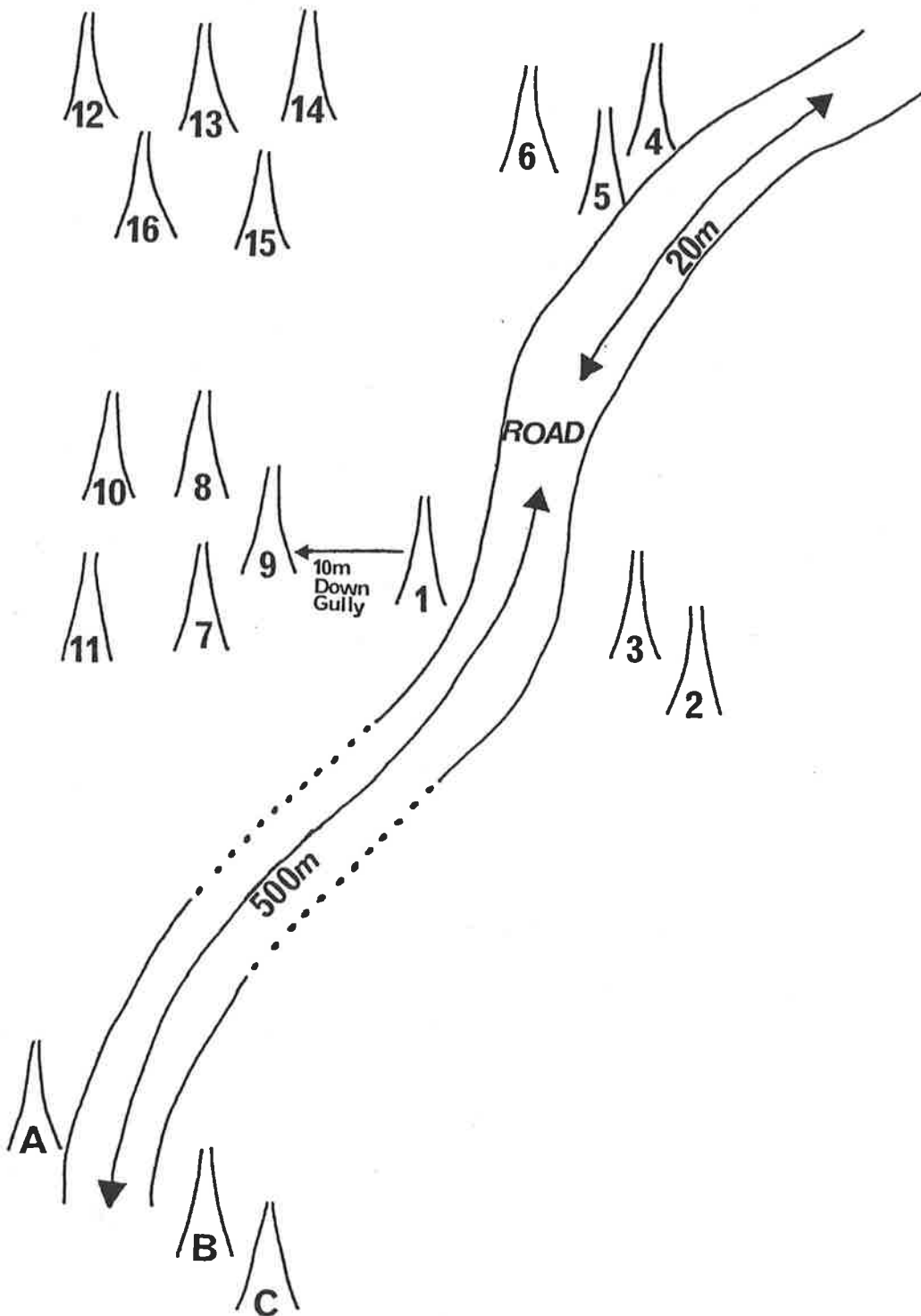
WRL      ( ( ) ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )

RRL      ( ( ) ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )

T4 II      ) ( 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

Figure 1.3.6

Diagrammatic representation of the trees from which WPL (*Lychas marmoreus*) individuals were collected.



Synaptic failure observed in individuals collected from tree 4 ( $T_4$ ).

Table 1.3.3

Population collection data for Western River.

Specimens		Adult		Juvenile		Total	
		♂	♀	♂	♀	♂	♀
I	1	10	3	4	3	14	6
	2	6	2	0	1	6	3
	3	8	4	0	7	8	11
	4	4	0	0	3	4	3
	5	3	2	3	2	6	4
	6	5	3	0	3	5	6
	7	0	3	0	3	0	6
	8	0	0	1	3	1	3
	9	1	0	0	0	1	0
	10	0	0	1	0	1	0
	11	1	1	1	1	2	2
	12	0	1	2	1	2	2
	13	0	0	1	0	1	0
	14	0	3	3	2	3	5
	15	2	0	1	2	3	2
	16	2	3	1	2	3	5
II	A	0	1	0	0	0	1
	B	0	1	0	0	0	1
	C	0	1	0	1	0	2
Total		42	28	18	34	60	62

Some females that were classed as juveniles (initially on size) were found to be adult non-gravid females when dissected.

Table 1.3.4

Adult male chromosome numbers and metaphase I configurations  
for Western River population (WPL).

Tree	No.	Metaphase I	N
1	9	7 bivalents	5
		04 + 5 bivalents	3
		06 + 4 bivalents	1
2	4	7 bivalents	4
3	7	7 bivalents	6
4	3	04 + 5 bivalents	1
		04 + 5 bivalents	1*
5	2	010 + 2 bivalents	2*
		7 bivalents	1
6	1	04 + 5 bivalents	1
		7 bivalents	1
7	-	-	-
8	1	04 + 5 bivalents	1
9	-	-	-
10	-	-	-
11	1	7 bivalents	1
12	1	7 bivalents	1
13	-	-	-
14	2	7 bivalents	2
15	2	7 bivalents	1
		04 + 5 bivalents	1
16	3	7 bivalents	1
		04 + 5 bivalents	2
Total		7 bivalents	23
		04 + 5 bivalents	10
		06 + 4 bivalents	1
		010 + 2 bivalents	2
			<u>36</u>

\* show synaptic failure phenomena.

No. = number of animals karyotyped from each tree.

N = number of animals with particular metaphase I karyotype.

bivalents). These ten individuals all showed the same SSML ring conformation suggesting they were heterozygous for the same interchange. There was a single individual heterozygous for an additional interchange (06 and 4 bivalents). The bivalents not involved in ring formation in this individual were of the size classes LMSS, while the bivalents in the individuals heterozygous for a single interchange were  $L^+LMSS$ . This suggests that the 06 individual may have resulted from an additional, sequential interchange event.

The two 010 + 2 bivalent individuals were found on the same tree, i.e (T4 II and T4 III). Both exhibited a synaptic failure condition resulting in decondensation and aberrant pairing configurations at metaphase I. Interestingly the only other individual from this tree karyotyped (T4 I) showed a 04 and 5 bivalents but also exhibited similar synaptic failure at metaphase I. These were the only individuals in this WPL population to exhibit synaptic failure.

#### Synaptic Failure in *Lychas marmoreus*.

While most individuals showed regular ring formation and pairing at metaphase I, individuals of *Lychas marmoreus* from two populations (OGL and WPL) showed extensive synaptic failure and decondensation at metaphase I. Examples of these cells are shown in Figure 1.3.7. Up to 40% of metaphase I cells in Onkaparinga Gorge (OGL) individuals (that normally showed 7 bivalents at metaphase I) were

**Figure 1.3.7**

**Examples of metaphase I cells from individuals exhibiting  
the synaptic failure phenomenon.**

<b>a,b</b>	<b>OGL</b>	<b>7 bivalents</b>
<b>c,d</b>	<b>WPL</b>	<b>04 + 5 bivalents</b>
<b>e,f</b>	<b>WPL</b>	<b>010 + 2 bivalents</b>

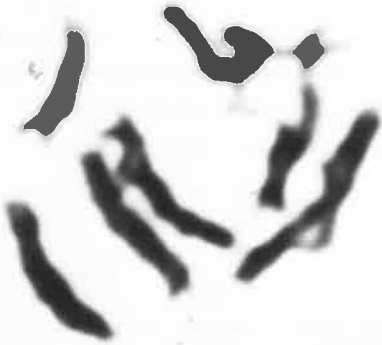
**Note decondensation and interstitial synaptic  
failure.**



**a**



**b**



**c**



**d**



**e**



**f**



affected to some degree. A similar percentage of cells in the Western River, Kangaroo Island (WPL) individuals that exhibit either a @ 10 and 2 bivalents or a @ 4 and 5 bivalents at metaphase I were also affected. Details of synaptonemal complex analysis in these ring of ten individuals are presented in Chapter 2.3.

The presence of this phenomena in individuals that are either homozygous or structural heterozygotes suggests that it is not associated with mechanical difficulties in homologous pairing of, for example, a ring of 10. Nor does it appear to be associated with chiasmata failure (as is usually postulated for failure of pairing at metaphase I) as it was often interstitial regions that were not paired, and decondensed to such an extent they often appeared broken. Thus, these mutants provide additional evidence that meiosis is achiasmata because the results of pairing failure do not correspond to those observed in other organisms with chiasmata failure.

Whether this phenomena is associated with an environmental influence could not be determined. The micro-habitat of a single tree (as in T4 (WPL)) seems unlikely to be responsible for such an effect. Takahashi (1976) observed a similar phenomena in the South American scorpion *Tityus bahiensis*. However, she was able to positively correlate the degree of metaphase I 'damage' with the levels of ionizing radiation that were naturally present in the areas she studied. Clearly, this was not the case in *L. marmoreus*.



An alternative explanation is that these animals are mutants for a particular gene involved in meiotic pairing or chromosome condensation; although this does not account clearly for why only some cells are affected. The isolation of the phenomena to particular populations or at WPL to a particular tree suggests these individuals are related. Inbreeding in scorpion populations may act to increase the frequency of the phenotype. Data (anaphase I, metaphase II) to score the frequency of non-disjunction was not found. However, the holocentric nature of the chromosomes may result in greater viability of these mutants than would be expected from a monocentric system. This may account for the relatively high frequency of such mutants in the Onkaparinga Gorge population where all animals karyotyped exhibited the phenomenon to some extent.

This synaptic failure phenomenon may also account for the rings of 5 and 7 Piza (1947) observed in *Tityus bahiensis*. Although decondensation was a feature of most cells with synaptic failure some cells (see later Fig. 2.3.7) exhibited regularly condensed chromosomes that were paired to form rings with odd numbers of chromosomes. These were either non-homologously paired or possibly homologously paired if additional spontaneous interchanges had occurred within the cell to form rings with odd chromosome numbers (e.g. 7 and 3).

*Lychas variatus* and *Lychas alexandrinus*.

The locality and karyotypic data for the remaining Australo-Papuan *Lychas* species are shown in Table 1.3.5 and Figure 1.3.8. Both these species are relatively rare and difficult to collect in large numbers. Karyotypically they are very similar to *L. marmoreus* with most individuals having a diploid chromosome number of  $2n=14$ . *L. variatus* also exhibited a high degree of interchange heterozygosity with up to 12 chromosomes involved in ring formation.

*Lychas variatus*.

7 Bivalent Karyotype. (JL I) (Fig. 1.3.9a, b)

As shown in Fig. 1.3.9 this karyotype was very similar to that of *L. marmoreus*. There were two small (S), one medium (M) and one large ( $L^+$ ) bivalent that were clearly recognisable. The remaining three bivalents were approximately the same size and were classed as large. Thus, the karyotype could be represented as  $L^+LLMSS$ .

C-banding in this species differed from *L. marmoreus*. Although telomeric bands were an obvious feature there were large interstitial blocks of heterochromatin in *L. variatus* which were not present in *L. marmoreus* (Fig. 1.3.9b). These large interstitial blocks may account for the chromosome size differences between the two species.

Table 1.3.5

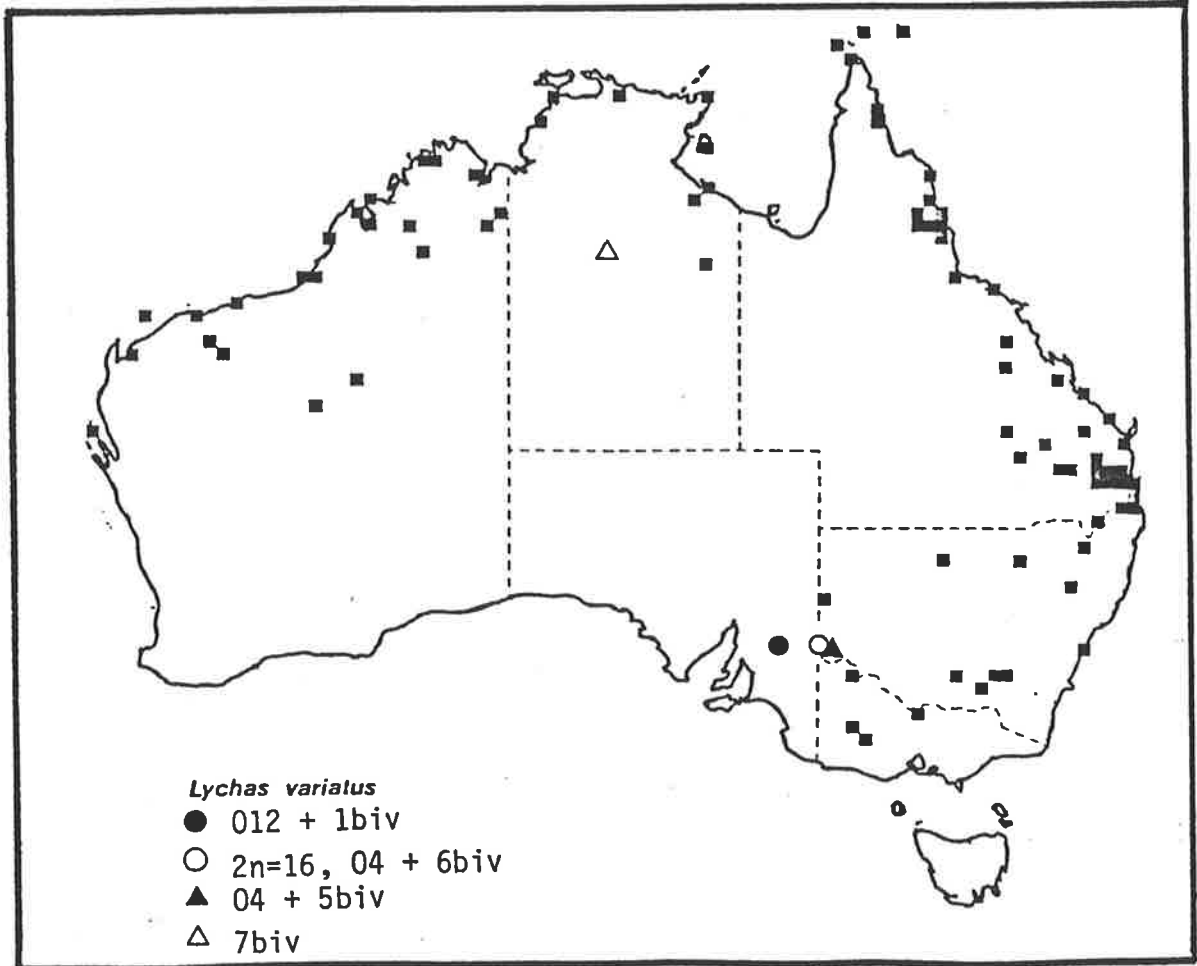
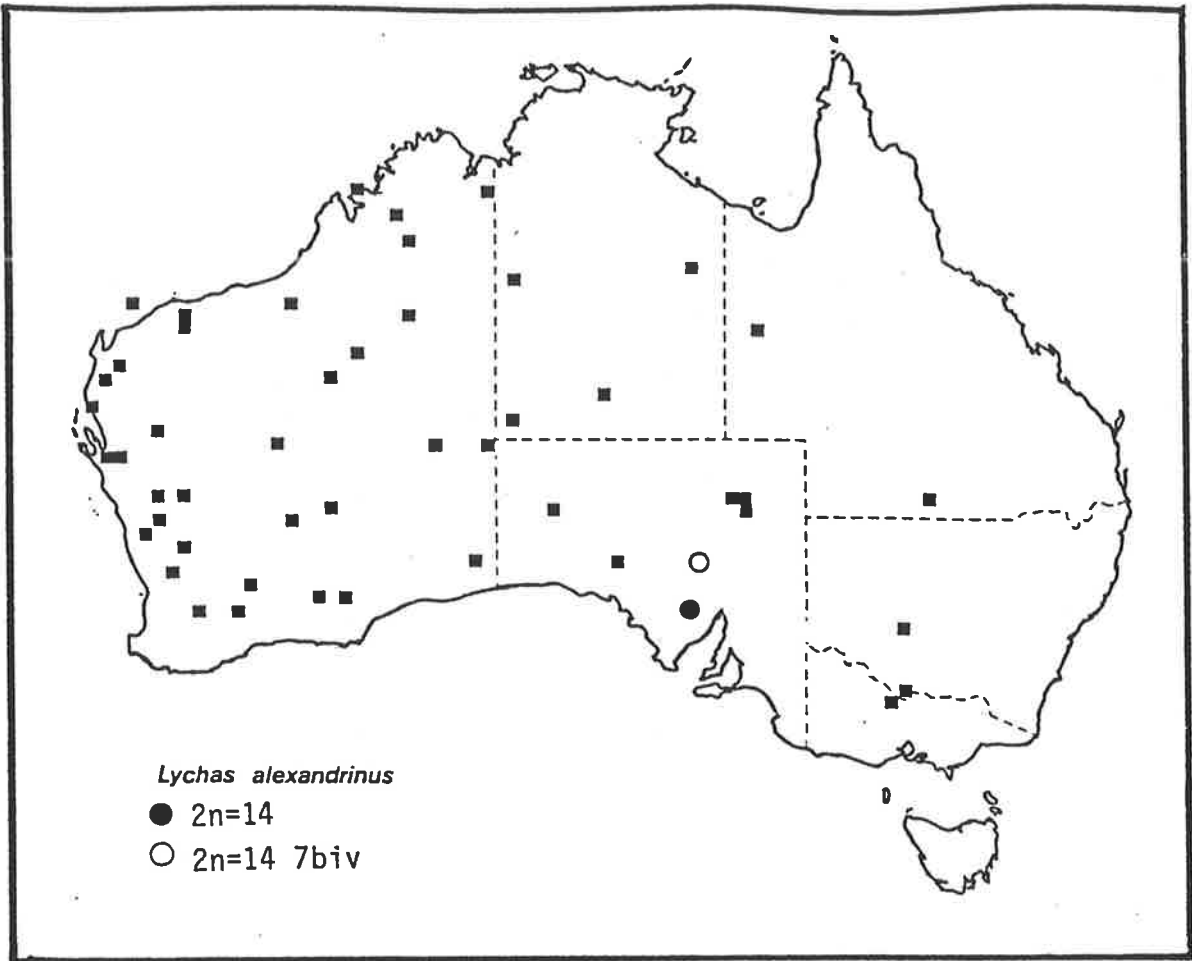
Karyotypic and locality data for *L. variatus*  
and *L. alexandrinus*.

Locality	Code	2n	N	Metaphase I	N
<i>L. variatus</i>					
Overland Corner, S.A.	OCL	14	12	012 + 1 biv.	7
Murtho Forest, S.A.	MFL	16	1	04 + 6 biv.	1
Lake Victoria, N.S.W.	LVL	14	1	04 + 5 biv.	1
Jabiluka Billabong, N.T.	JL	14	1	7 bivalents	1
<i>L. alexandrinus</i>					
Roxby Downs, S.A.	RDL	14	1	-	-
Lake Gillies, S.A.	LGL	14	1	-	-

**Figure 1.3.8**

**Maps showing locality and karyotypic data for  
*Lychas alexandrinus* and *Lychas variatus*.**

**Small black squares represent museum localities  
(Koch, 1979).**



**Figure 1.3.9**

**Metaphase I karyotypes from *Lychas variatus*  
populations.**

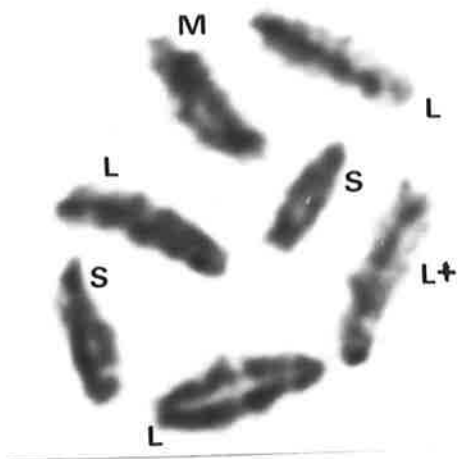
**a,b      Jabiluka Billabong (JL)  
          7 bivalents  
          Giemsa stain and C-banding.**

**c         Lake Victoria (LVL)  
          04 + 5 bivalents.**

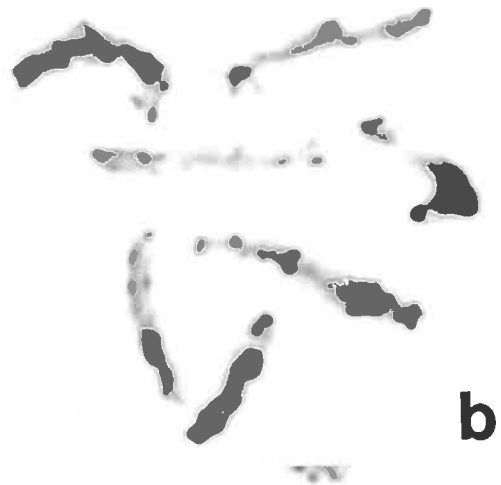
**d         Murtho Forest (MFL)  
          04 + 6 bivalents.**

**e,f       Overland Corner (OCL)  
          012 + 1 bivalent  
          Giemsa stain and C-banding.**

**Bar = 5  $\mu$ m.**



**a**



**b**



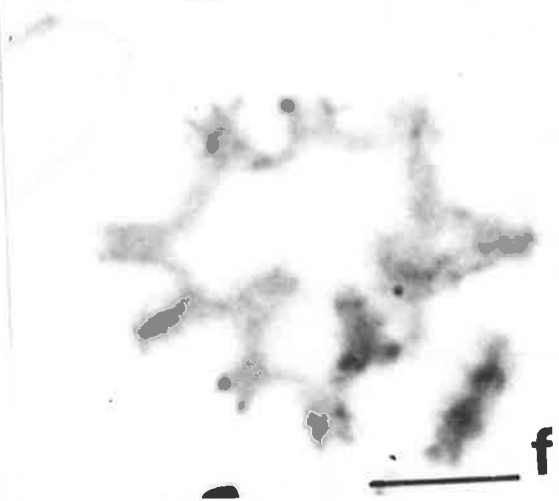
**c**



**d**



**e**



**f**

**Ring of 4 and 5 Bivalents Karyotype. (LVL I) (Fig. 1.3.9c)**

In this single interchange heterozygote the two smallest chromosome pairs of the complement were involved in ring formation leaving L<sup>+</sup>LLLM bivalents.

**Ring of 4 and 6 Bivalents Karyotype. (MFL I) (Fig. 1.3.9d)**

This individual showed the synaptic failure phenomenon common to some *L. marmoreus* individuals together with a high frequency of chromosome breakage in some cells. The basic karyotype consisted of bivalents in the size classes L<sup>+</sup>LLMS<sup>-</sup> and a ring of 4 chromosomes. In this animal one of the small bivalents (from LVL karyotype) had become disassociated to form two smaller products. One of these products together with the small chromosome pair (S) were involved in ring formation. Only a single individual from this population was obtained so it was not possible to determine if this represented an aberrant individual or if the normal diploid chromosome number in this population was 2n=16.

**Ring of 12 and 1 Bivalent Karyotype. (OCL) (Fig. 1.3.9e, f)**

In this karyotype the L<sup>+</sup> chromosome pair were the only elements not involved in ring formation. All males karyotyped in this population (N=7) were heterozygous for at least six interchange events. The lack of other metaphase I karyotypes in this isolated population suggests that this ring may have become fixed (i.e. this population shows fixed structural heterozygosity). C-banding in these individuals



showed both telomeric and interstitial bands.

*Lychas alexandrinus*. (LGL, RDL)

The mitotic karyotypes ( $2n=14$ ) of the two *Lychas alexandrinus* individuals sampled are compared with those from *Lychas variatus* in Figure 1.3.10. The mitotic chromosomes from LGL I could be paired and arranged in a regular descending size order. This mitotic karyotype is similar to that of *L. variatus* individual JL I (7 bivalents at metaphase I). However, the chromosomes of RDL could not be paired and showed greater size variation. This mitotic karyotype is indicative of interchange heterozygosity. Thus, it appears that all *Lychas* species in Australia exhibit interchange heterozygosity.

Chromosome Variation in *Isometroides vescus*.

*Isometroides vescus* is derived from the genus *Lychas* within Australia and is closely related to *L. alexandrinus* (Koch, 1979). Figures 1.3.11, 1.3.12 and Table 1.3.6 show locality and karyotypic data for this rare species. As with all species of *Lychas* the basic chromosome number is 14. The size classes of the bivalents (in the 7 bivalents karyotype) were LL M/L SSSS which differs from that in *L. variatus* and *L. marmoreus*. One of the three individuals collected at Middleback (MBI) was heterozygous for an interchange resulting in a very asymmetrical ring of 4 at metaphase I.

Figure 1.3.10

Mitotic karyotypes of *Lychas variatus*  
and *Lychas alexandrinus*.

*Lychas variatus*

JL I	2n = 14	7 bivalents.
LVL I	2n = 14	④4 + 5 bivalents.
MFL I	2n = 16	④4 + 6 bivalents.
OCL	2n = 14	④12 + 1 bivalent. (Chromosomes similar sized.)

*Lychas alexandrinus*

RDL	2n = 14	Chromosomes do not pair to form bivalents .'. possibly interchange heterozygote.
LGL	2n = 14	Similar sized chromosomes.

JL I



LVL I



MFL I



OCL



RDL



LGL

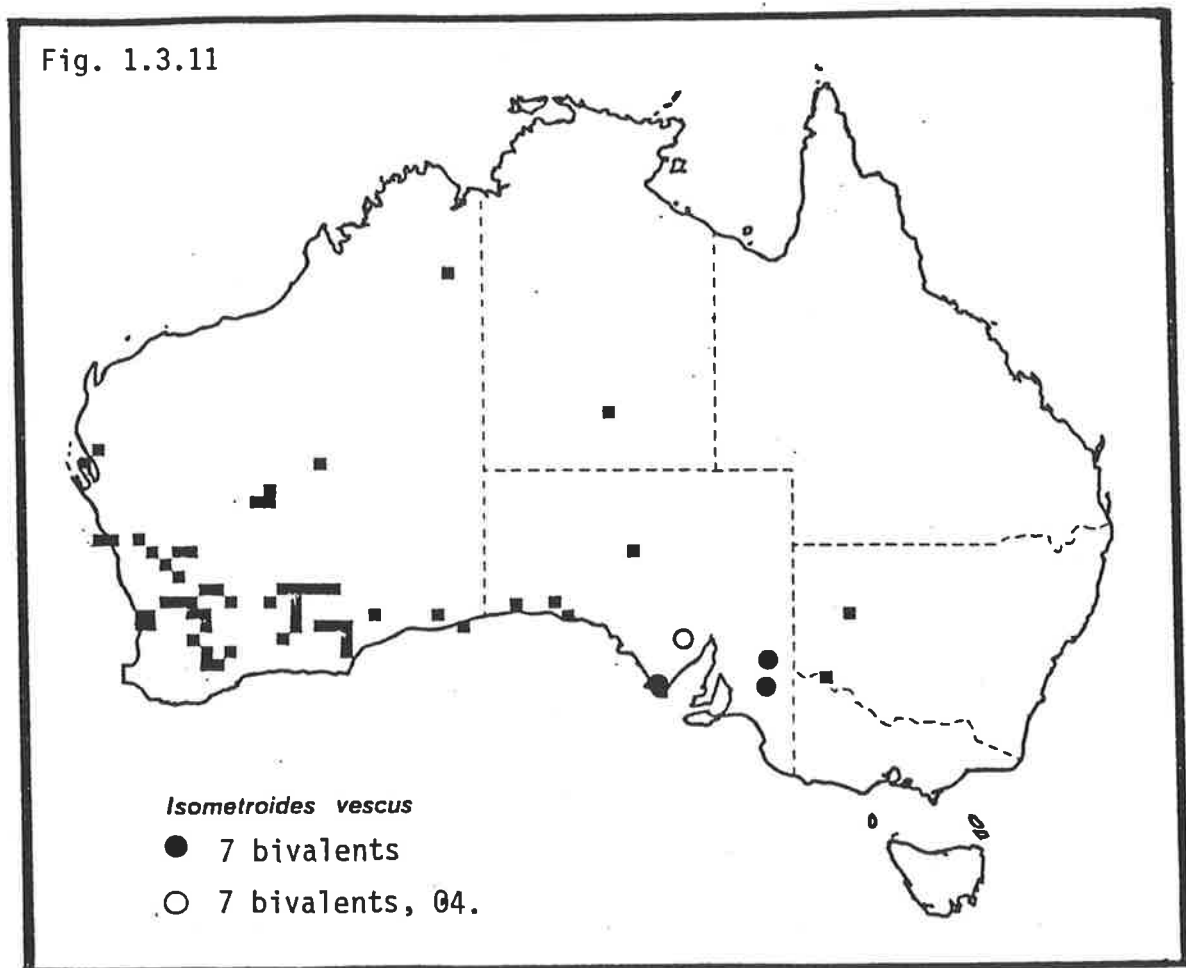


Table 1.3.6

Locality and karyotypic data for *Isometroides vescus*.

	Code	2N	N	Metaphase I	N
Bow Hill, S.A.	BHI	14	1	04 + 5 biv.*	-
Middleback Station, S.A.	MBI	14	3	7 bivalents 04 + 5 biv.	1 2
Coffin Bay, S.A.	CBI	14	1	7 bivalents*	-
Calperum Station, S.A.	CPI	14	1	7 bivalents*	-

\* meiotic karyotype estimated from mitotic chromosome size.



Small squares show museum localities.

**Figure 1.3.12**

***Isometroides vescus***

a - d            **Metaphase I**  
                  **7 bivalents**  
                  **04 + 5 bivalents.**

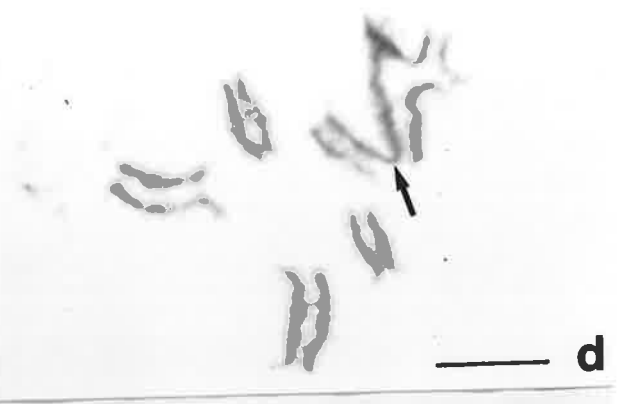
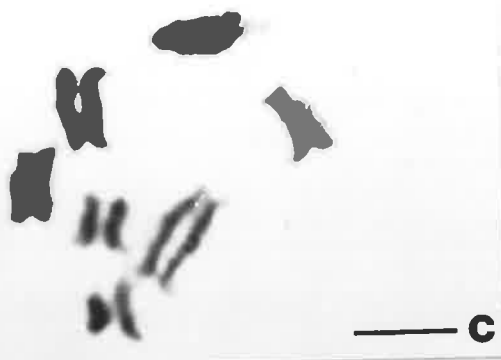
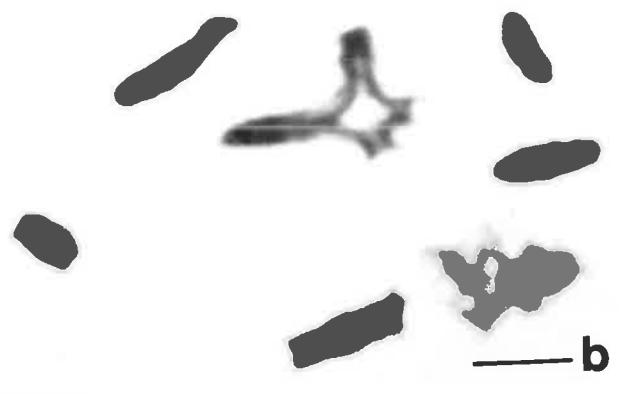
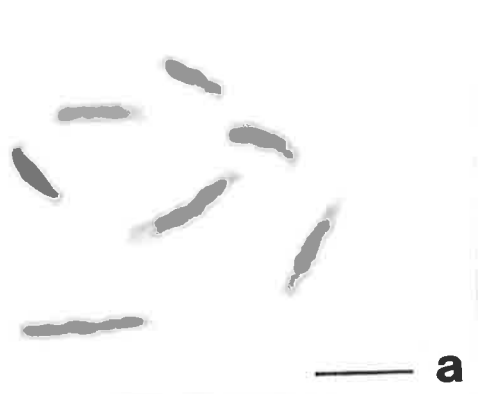
d                **Spontaneous aberration.**  
                  **Chromosome end-end fusion.**

**Mitotic karyotypes:**

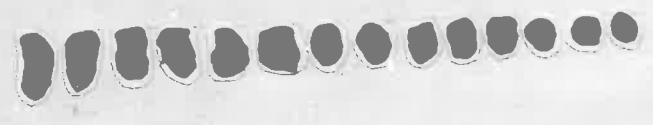
**MBM I            04 + 5 bivalents**  
**MBM III         04 + 5 bivalents**

**CBI              Possibly pair to form 7 bivalents.**

**BHI              Chromosomes do not pair to form**  
**7 bivalents.**



**MBM I**  
⊙4



**MBM II**  
⊙4



**CBI**



**BH I**



The mitotic karyotypes of the single individuals collected from isolated habitats within South Australia suggest that CBI I and CPI I would exhibit 7 bivalents at metaphase I, while BHI may also be heterozygous for a single interchange as its karyotype was similar to that of MBI interchange heterozygotes (Fig. 1.3.12).

#### Chromosome Evolution in the Genus *Isometrus*.

Only four specimens of *I. melanodactylus* and one specimen of *I. maculatus* were obtained (see Fig. 1.3.13 and Table 1.3.7). All animals had  $2n=14$  chromosomes. Although no metaphase I data was obtained for *I. melanodactylus*, mitotic chromosomes could not be readily paired suggesting interchange heterozygosity also occurred in this species.

The single specimen examined of the cosmopolitan species *I. maculatus* (thought to have been transported from South America on ships) showed a ring of 4 chromosomes plus 5 bivalents at metaphase I. Piza (1947a) studied this species in South America where the chromosome number was  $2n=12$ . This suggests that there may be some adaptive significance associated with  $2n=14$  chromosomes within Australia as this was the basic diploid number of all Buthid species within Australia.

The C-band pattern of *I. maculatus* showed mainly sub-telocentric and interstitial bands (Fig. 1.3.14).

Table 1.3.7

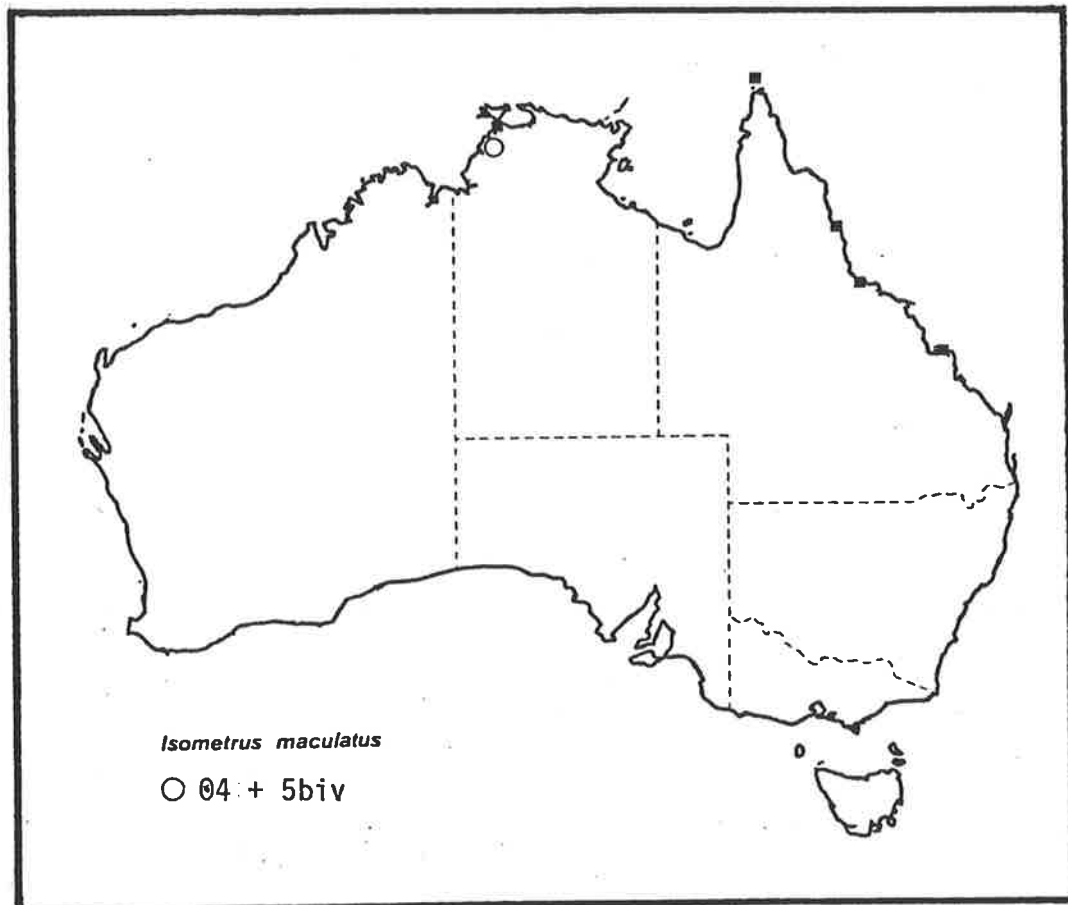
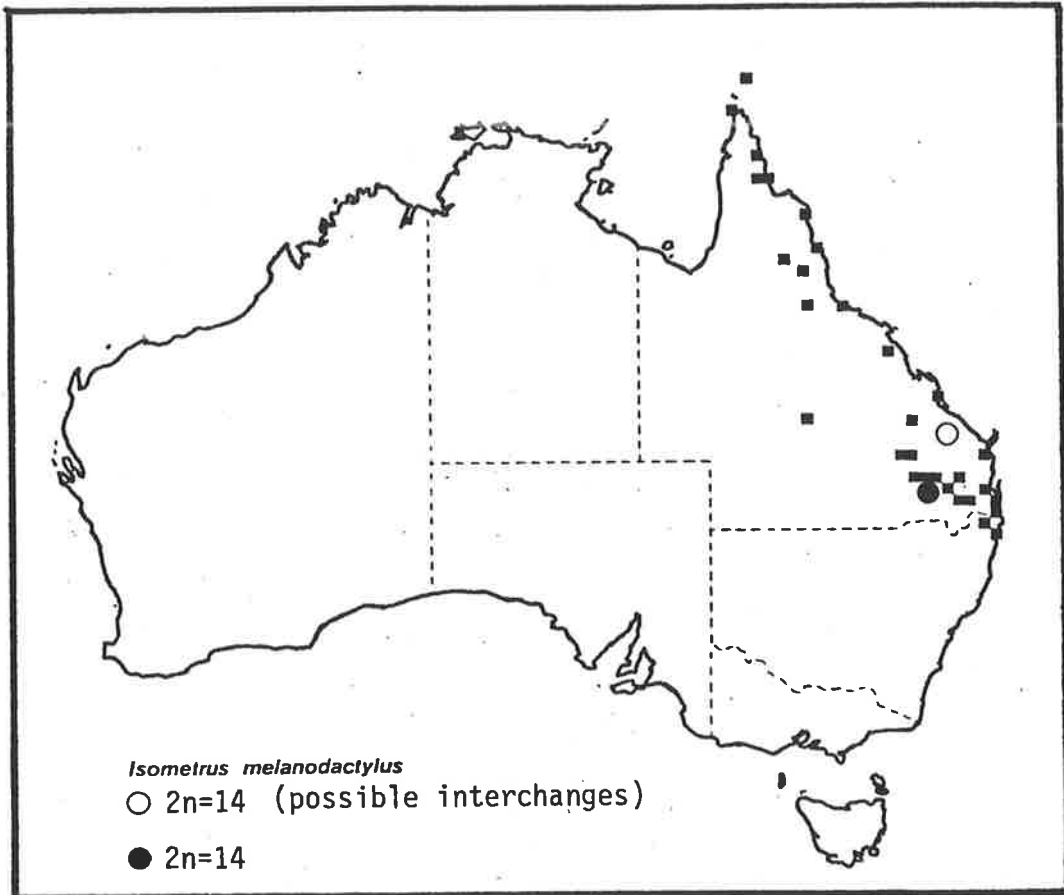
Locality and karyotypic data for *Isometrus melanodactylus*  
and *Isometrus maculatus*.

Locality	Code	2n	N	Metaphase I	N
<i>I. melanodactylus</i>					
10 km Nth. Gin Gin, Qld.	GGI	14	3	-	-
25 km S.W. Dalby, Qld.	DI	14	1	-	-
<i>I. maculatus</i>					
78 km W. Red Bank, Dill Doll Rock, N.T.	RBI	14	1	04 + 5 biv	1



Fig. 1.3.13

Locality and karyotypic data for *Isometrus*.



Small squares show museum localities.

**Figure 1.3.14**

*Isometrus melanodactylus* and *Isometrus maculatus*  
karyotypes.

GGI Mitosis  $2n=14$  *Isometrus melanodactylus*  
- chromosomes do not pair to form 7 bivalents.

RBI Mitosis  $2n=14$  *Isometrus maculatus*  
04 + 5 bivalents.


*Isometrus maculatus* (RBI)


a,b C-banded early metaphase I.

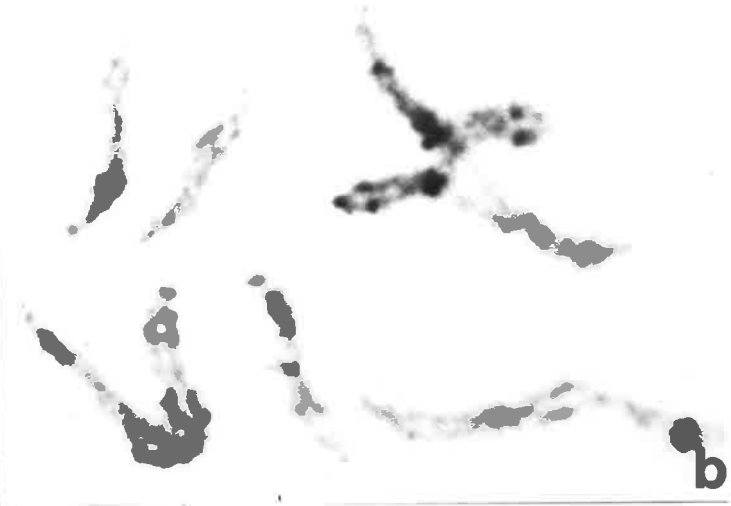
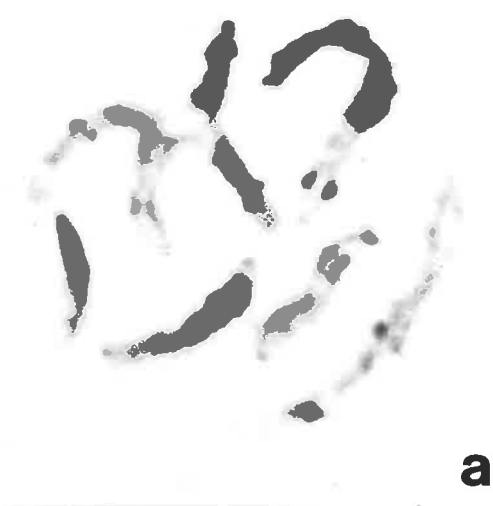
c C-banded late metaphase I.

d Metaphase II.

Large interstitial blocks of heterochromatin.

**GG I** 

**RBI** 



### Disjunction in Interchange Heterozygotes.

Piza (1940, 1941) suggested that disjunction of ring multiples in *T. bahiensis* involved half the chromosomes of each ring moving to opposite poles. However, he envisaged that adjacent chromosomes would regularly move to the same pole. Clearly this would always result in unbalanced gametes. Observations from Australian Buthid species show that chromosomes in rings segregate at anaphase I, however there is no evidence that adjacent orientation is the rule. Ring multiples may orientate at 90° to the spindle axis rather than 180° as implied by Piza. As explained in Chapter 2.3, alternate orientation may be maximized in this holocentric achiasmate system.

Achiasmate meiosis and holocentric chromosomes result in metaphase I ring configurations that observed under the light microscope are not comparative to those in monocentric interchange heterozygotes. Metaphase I data from Buthid scorpions where the cells have not been colchicine treated is difficult to obtain due to the very contracted nature of the chromosomes. Anaphase I cells are very rarely observed. Thus metaphase II data was the only indicator of whether disjunction of rings at anaphase I regularly produced balanced gametes.

The asymmetrical ring of 4 in Middleback *Isometroides vescus* individuals enabled positive identification of all segregation products (see Fig. 1.3.15). Sixteen metaphase II cells were analysed and all showed that disjunction was regular, i.e. balanced gametes would be formed. Although

**Figure 1.3.15**

**Segregation of a ring of 4 interchange heterozygote**

*Isometroides vescus*

a,b Early metaphase I cells where size classes of bivalents and interchange chromosomes can be clearly identified.

Interchange      S<sup>-</sup>S M/L L<sup>+</sup>

Bivalents        L M/L SSS

Regular disjunction:

Contribution of bivalents            L M/L SSS

Contribution of quadrivalent      L<sup>+</sup>S<sup>-</sup> or M/L S

possible balanced gametes

1            L<sup>+</sup>L M/L SSSS<sup>-</sup>

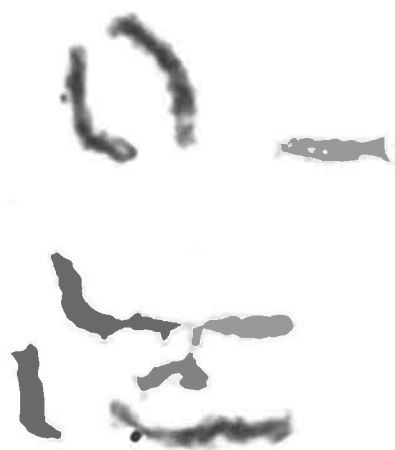
2            L M/L M/L SSSS

c and f      Daughter cells showing gametes

1 and 2

d and g      Gamete 2

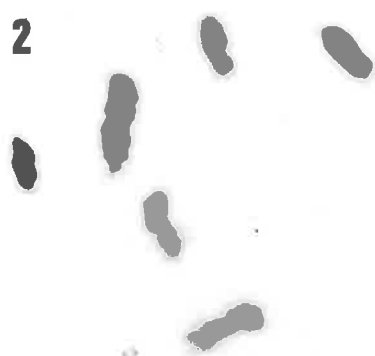
e and h      Gamete 1



**a**

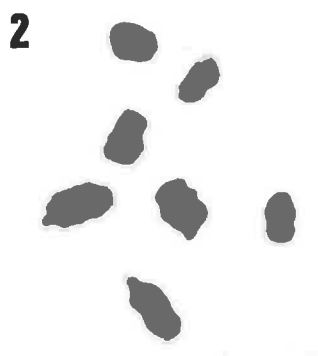
**b**

**2**



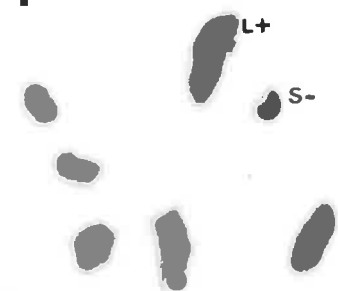
**c**

**2**



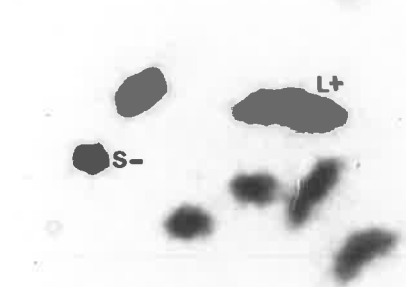
**d**

**1**



**e**

**1**



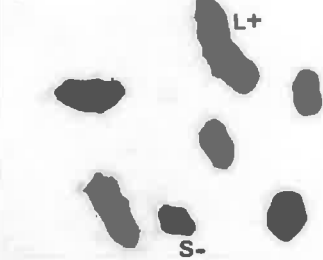
**f**

**2**



**g**

**1**



**h**

very few cells were scored (due to the scarcity of divisions in most testis), the results suggest that a mechanism exists in Buthids that ensures regular disjunction of rings at anaphase I. The asymmetry of the ring does not appear to effect regular disjunction which is contrary to what is found in monocentric interchange heterozygotes where the greater the symmetry the more regular the disjunction. Whether regular 'alternate' disjunction is also achieved in multiples involving more than four chromosomes could not be determined. However, considering the frequency of such multiples in some populations it seems likely.

## DISCUSSION

### The Evolution of Interchange Heterozygosity in Buthid Scorpions.

Organisms that evolve interchange heterozygosity are usually 'pre-adapted' to tolerate such a system (John and Lewis, 1957, 1958; White, 1973). 'Pre-adapted' monocentric species exhibit similar sized metacentric chromosomes and distal localization of chiasmata. Male Buthid scorpions exhibit similar sized holocentric chromosomes and achiasmate meiosis. This unusual combination of chromosomal features results in the extensive, disjunctionally efficient interchange heterozygosity found in male Buthids. White (1973) predicted that holocentric species should theoretically be more likely to evolve interchange heterozygosity due to the viability of all products of interchange events. Thus, the question arises as to whether

interchange heterozygosity is of any adaptive significance in holocentric species or whether its presence is due to the high level of 'pre-adaptation' of the chromosomal system. There are a number of lines of evidence that suggest interchange heterozygosity is of adaptive significance in Buthid scorpions.

#### **Adaptive Significance of Interchange Heterozygosity.**

The adaptive significance of interchange heterozygosity is that it operates to preserve genetic heterozygosity usually within inbred populations. John and Lewis (1957, 1958) postulated that selection favoured interchange heterozygotes in inbred populations of the cockroach *Periplaneta*. The level of inbreeding correlated with the degree of structural heterozygosity. Similarly, Darlington (1958) proposed that inbreeding led to selection for interchange heterozygosity in *Oenothera*.

The karyotypic population structure in Buthid scorpions is similar to that of *Periplaneta*. Most populations are polymorphic containing both structural homozygotes and single interchange heterozygotes (e.g. WPL, *Lychas marmoreus*). However, there were some populations where most individuals were heterozygous for a large number of interchanges. These populations were those with the highest levels of inbreeding.

Ecological observations of Buthid scorpions suggest that most species are of low vagility. Many populations were composed of a small number of individuals, occupying isolated (island) habitats. Two such populations were sampled on



Kangaroo Is.; FCL and RRL. The two males karyotyped from these populations exhibited a ring of 10 and a ring of 8 respectively. The frequency of individuals heterozygous for more than a single interchange was relatively low in larger (probably less inbred) populations (e.g. WPL, OGL, BL). Thus, these ring of 10 and ring of 8 individuals may be representative of the degree of heterozygosity within these smaller populations.

Additionally, there was a population of *Lychas variatus* (OCL) where all males karyotyped (N=7) exhibited a ring of 12 chromosomes at metaphase I. This population was small (less than 10 individuals were observed at any one time during 4 years of extensive sampling) and isolated. It occupied a region of river bank approximately 30-50m wide and 750m long and was isolated by water from any other population. The high level of inbreeding likely to be occurring in this population could lead to selection for large numbers of interchanges to maintain heterozygosity. Whether this ring of 12 chromosomes has become fixed within this population is unknown. However, for fixation to occur it would be necessary to evolve a system of balanced lethals as in *Oenothera* and *Isotoma*, to prevent structural homozygotes from surviving. No animal has been found that has a lethal system probably because self-fertilization (as occurs in plants) is not possible in dioecious species.

Although it has been postulated (John and Quraishi, 1964) that the South American Buthid *Tityus bahiensis* may be parthenogenetic with few males and ameiotic meiosis in the

female, there is no evidence from Australian Buthids that reproduction can occur parthenogenetically (see Section 5). Populations of Australian Buthids where a number of individuals were obtained showed a 1:1 sex ratio, whereas *T. bahiensis* populations were mainly female. Additionally, the complex configurations (due to interchanges and fusion/fission events) observed in *T. bahiensis* (Piza, 1944, 1946) (that would most likely result in inviable gametes) were not equivalent to those observed in Australian species. Evidence suggests males of Australian species produce a high frequency of balanced gametes. However (as presented in Section 5), studies on female meiosis to determine if females also showed interchange heterozygosity proved inconclusive.

Further evidence that interchange heterozygosity is adaptive comes from the observation that within a population individuals were heterozygous for identical interchanges. This suggests that maintenance of heterozygosity for particular gene arrays is advantageous. This is especially significant in terms of the holocentric nature of the chromosomes (where all interchanges should be viable) and the inbred nature of the populations. If random events (drift not selection) were responsible for the interchange heterozygosity it might be expected that a number of different interchanges could occur at a high frequency within a population.

Finally, evidence presented in Chapters 1 and 3 suggest that not all chromosome breakage events in

holocentric chromosomes necessarily lead to reunion. Broken chromosomes can spontaneously repair and be maintained such that chromosome fragmentation becomes a viable process. Despite this, the diploid chromosome number in Buthid scorpions is highly conserved. Most breakage events appear to lead to reunion that results in interchange events.

## SECTION 4

## CYTOGENETIC SYSTEMS IN NON-BUTHID AUSTRALIAN SCORPIONS

Table 1.4.1 shows a summary of the chromosomal features of species from the families Bothriuridae and Scorpionidae analysed in this study. All species exhibit monocentric chromosomes and achiasmate male meiosis. These appear to be universal features of all non-Buthid scorpions. Details of the centric nature of these chromosomes are presented in Chapter 3. The evidence for achiasmate meiosis comes solely from cytological observations. In all species there was no evidence for a diplotene-diakinesis stage and homologues appeared 'distance paired' at metaphase I. Attempts were made to induce BrdU differential sister chromatid staining to verify achiasmate meiosis in specimens of the Scorpionid *U. manicatus*. However, these proved unsuccessful.

**Chromosome Evolution in the Family Bothriuridae.*****Cercophonius squama*.**

*Cercophonius squama* is the only representative of the family Bothriuridae in Australia and is widely distributed.

Chromosome preparations were obtained from only one male of this species. The diploid chromosome number was  $2n=114$  represented by 57 bivalents at metaphase I (see Fig. 1.4.1). In contrast, haemolymph preparations from a female from a different locality showed  $2n=107$ . These high chromosome

Table 1.4.1

Cytogenetic features of non-Buthid Australian scorpions.

Species	Chromosome ( $\sigma$ ) number 2n	Male meiosis	Centric nature	Polymorphism	
				intra	inter
<u>BOTHRIURIDAE FAMILY</u>					
<i>Cercophonius squama</i>	107♀ 114♂	Achiasmata	Monocentric	n.i.	p*
<u>SCORPIONIDAE FAMILY</u>					
<i>Urodacus manicatus</i>	29+64	Achiasmata	Monocentric	fusion/ fission B chromosomes	fusion/ fission B chromosomes
<i>Urodacus elongatus</i>	56	Achiasmata	Monocentric	n.i.	n.i.
<i>Urodacus novaehollandiae</i>	66,68,72,130, 175	Achiasmata	Monocentric	fusion/ fission	p*
<i>Urodacus planimanus</i>	72	Achiasmata	Monocentric	n.i.	n.i.
<i>Urodacus armatus</i>	124,144	Achiasmata	Monocentric	n.i.	p*
<i>Urodacus yaschenkoi</i>	94,114,116	Achiasmata	Monocentric	fusion/ fission	fusion/ fission

n.i. = no information.

p\* = polymorphic, origin unknown.

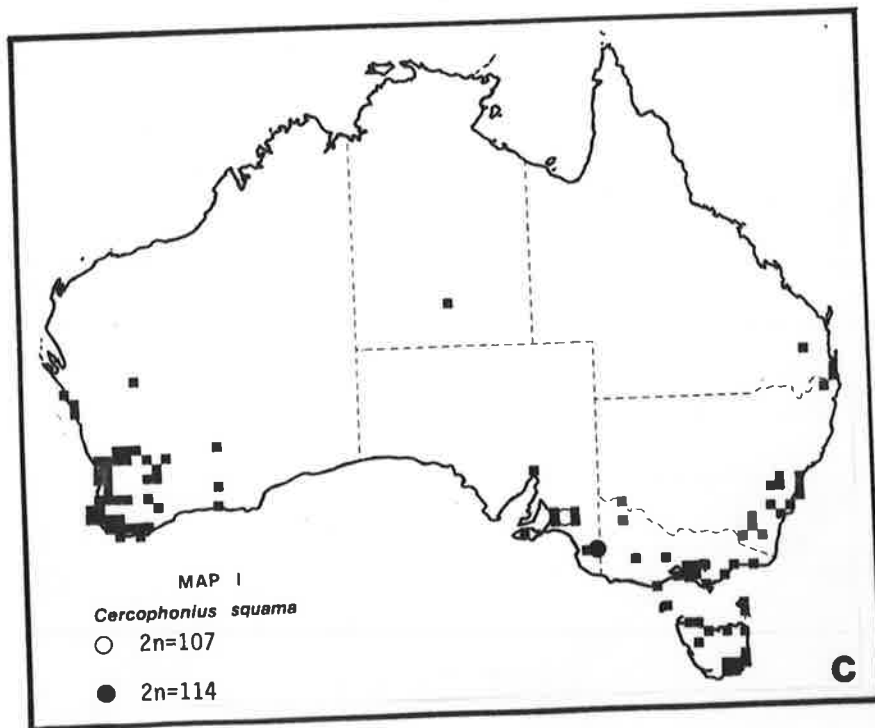
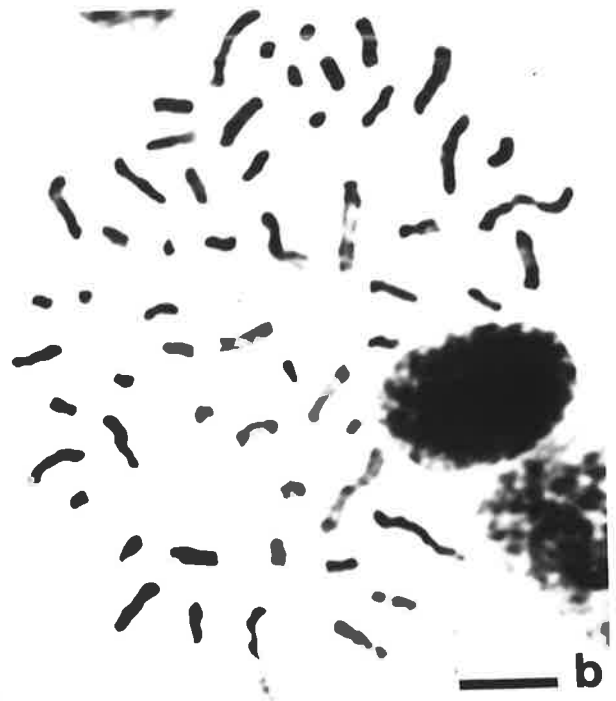
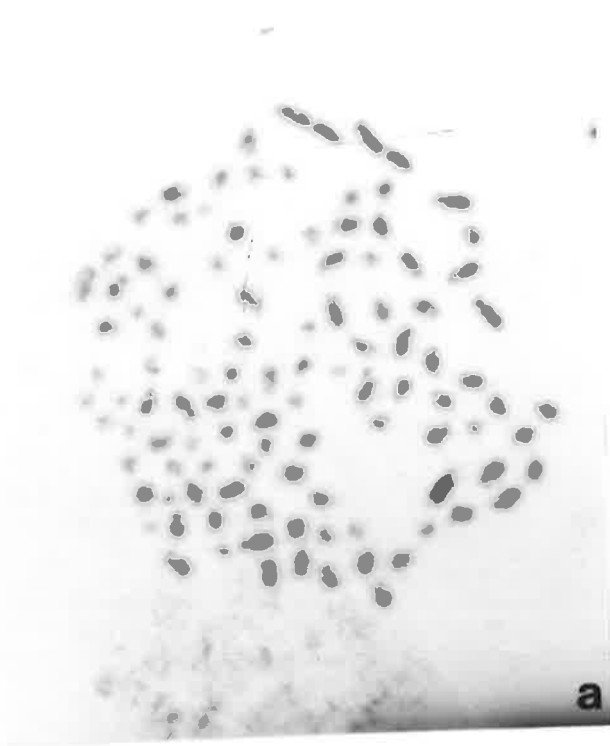
**Figure 1.4.1**

**Distribution and karyotypic data for  
*Cercophonius squama*.**

- a. 2n=107 mitotic cell (♀).
- b. 2n=114 57 bivalents (metaphase I ♂).
- c. Map showing the localities of specimens collected.

Small black squares show museum localities.

(Koch, 1979)



numbers contrast with those reported by White (1973) and suggest that his statement that the Bothriurids have the lowest chromosome numbers of the non-Buthid species is no longer correct.

This study has verified that Bothriurids have monocentric chromosomes and achiasmate meiosis in common with all other non-Buthid scorpions studied.



## Chromosome Evolution in the Family Scorpionidae.

### INTRODUCTION

There are twenty two extant species of Scorpionidae in Australia, nineteen of which belong to the genus *Urodacus* which evolved in and is confined to Australia (Koch, 1979).

Within Australia the genus *Urodacus* has several distinct evolutionary radiations which are represented by five species-groups based on external morphology and male genitalia (see Table 1.2.1). Due to the scarcity and remote distribution of many of these species, this study has concentrated on cytogenetic aspects of two species-groups; species-group *armatus* which has seven species and includes two of the most morphologically primitive species (*U. manicatus* and *U. novaehollandiae*) and species-group *yaschenkoi* which is monospecific containing only *U. yaschenkoi*.

Five of the seven species from the *armatus* group have been analysed cytogenetically - *U. manicatus*, *U. armatus*, *U. novaehollandiae*, *U. elongatus* and *U. planimanus*.

This is the first detailed study of chromosome polymorphism in a family of non-Buthid scorpions.

### RESULTS

#### Species-group *armatus*.

##### Chromosome Polymorphism in *Urodacus manicatus*.

*Urodacus manicatus* is a common member of the *armatus* species-group and shows a typical Bassian distribution

occurring mainly along the southern coast in areas of winter rainfall. Morphologically it is a conservative species with no significant quantitative or qualitative differences between populations. Its basic habitat is under rocks, and it has low vagility such that many populations could be considered inbred isolates.

Extensive collection of this species was undertaken in South Australia (including Kangaroo Island). Karyotypic analysis of male specimens revealed that this species was highly polymorphic for chromosome numbers. Table 1.4.2 summarizes the collection and karyotypic data which is shown diagrammatically on Fig. 1.4.2.

Most of the numerical variation is due to Robertsonian fusion/fission polymorphism manifest as trivalents and occasional quadrivalents at metaphase I. (There were two exceptional populations (BM and EBM) that were highly polymorphic for a large number of Robertsonian rearrangements.) However, some variation was also attributable to the variable presence of small supernumerary or B chromosomes. Additionally, individuals in the Belair population (BM) exhibited heterozygosity for both pericentric inversions (identified by loops or C-band heterozygosity at metaphase I) and paracentric inversions identified only by synaptonemal complex analysis (see Chapter 2.4).

High resolution chromosome banding was not inducible and primary constrictions were not obvious at mitosis in this species. This made it difficult to distinguish metacentric and acrocentric chromosomes. Therefore, C-banded metaphase

Table 1.4.2

Locality data and chromosome numbers for *U. manicatus* males.

Locality	Code	2n	No. of specimens	Polymorphic
Belair, S.A.	BM	29→41	17	+*i
Bridgewater, S.A.	BWM	56	1	(-)
Greenhills, S.A.	BM	50	1	(+)*
Heathfield, S.A.	HM	55	1	(+)
Middleback, S.A.	MBM	41	1	(+)
Overland Corner, S.A.	OCM	62,63	16	+
Onkaparinga Gorge, S.A.	OGM	50,51	3	+
Pernong, S.A.	PGM	32	1	(-)
Scrubby Gully, S.A.	SGM	29,30	2	+(*i)
Swan Reach, S.A.	SRM	64	1	(-)
Taylor's Landing, S.A.	TLM	58	1	n.i.
Waikerie, S.A.	WM	61,63	2	+
Amen Corner, K.I.	ACM	60	1	(-)
Brownlow, K.I.	BRM	54	1	(-)
Cape du Couedic, K.I.	CCM	59,60,62	4	+
Coast Rd.(Flinders Chase),K.I.	CRM	60,64	6	+
Cape Torrens, K.I.	CTM	54,56	3	+
Emu Bay, K.I.	EBM	41→51	36	+*
Flinders Chase (H.Q.), K.I.	FCM	60	4	-
King Goerge Beach, K.I.	GBM	59	1	(+)
Lake Ada, K.I.	LAM	59,60	4	+
Line Road, K.I.	LRM	58,59,64	6	+
Ravine de Casoars, K.I.	RCM	58,60	2	+
Rocky River, K.I.	RRM	57,59,60	3	+
Stokes Bay, K.I.	SBM	59,60	3	+
Shackle Road, K.I.	SKM	60	1	(-)
Tandanya, K.I.	TDM	59	1	(+)
Vivonne Bay, K.I.	VBM	60,62	2	+
West Bay, K.I.	WBM	59,60	2	+
Western River, K.I.	WRM	60	2	-
Black Mountain, A.C.T.	CM	44,45	3	+

+ = polymorphic

\* = highly polymorphic

- = no polymorphism evident

i = inversion polymorphism

(+) = trivalents observed at metaphase I

n.i. = no information

(-) = all bivalents at metaphase I

When only one animal from a population could be karyotyped the symbol in brackets indicates the likelihood of the population being polymorphic based on metaphase I data.

**Figure 1.4.2**

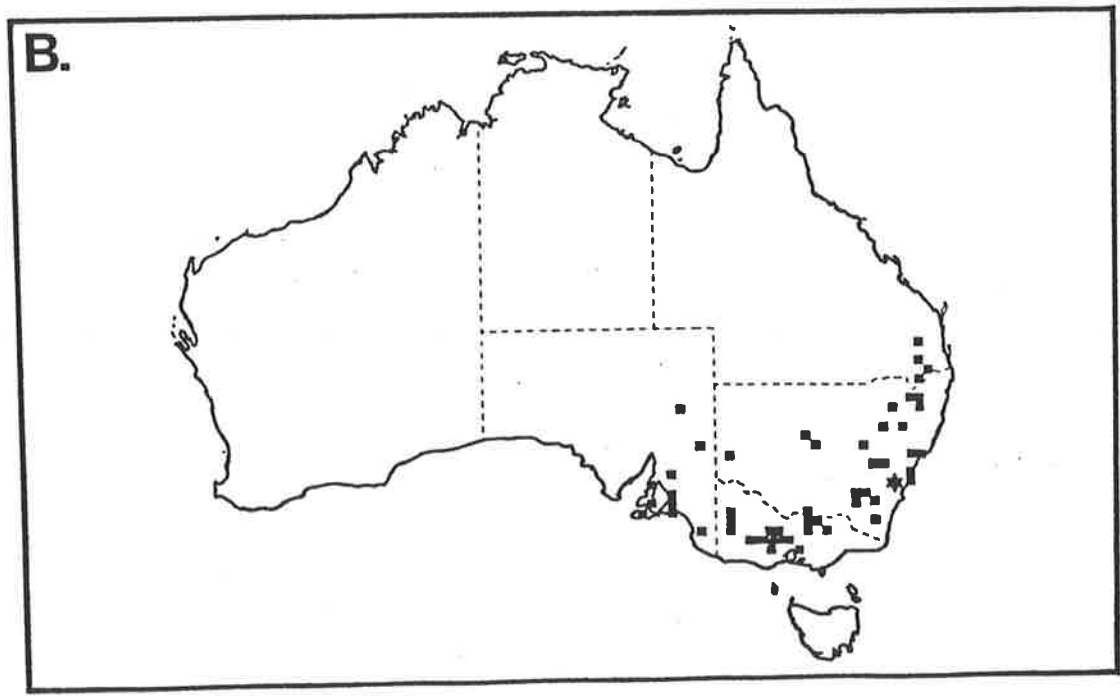
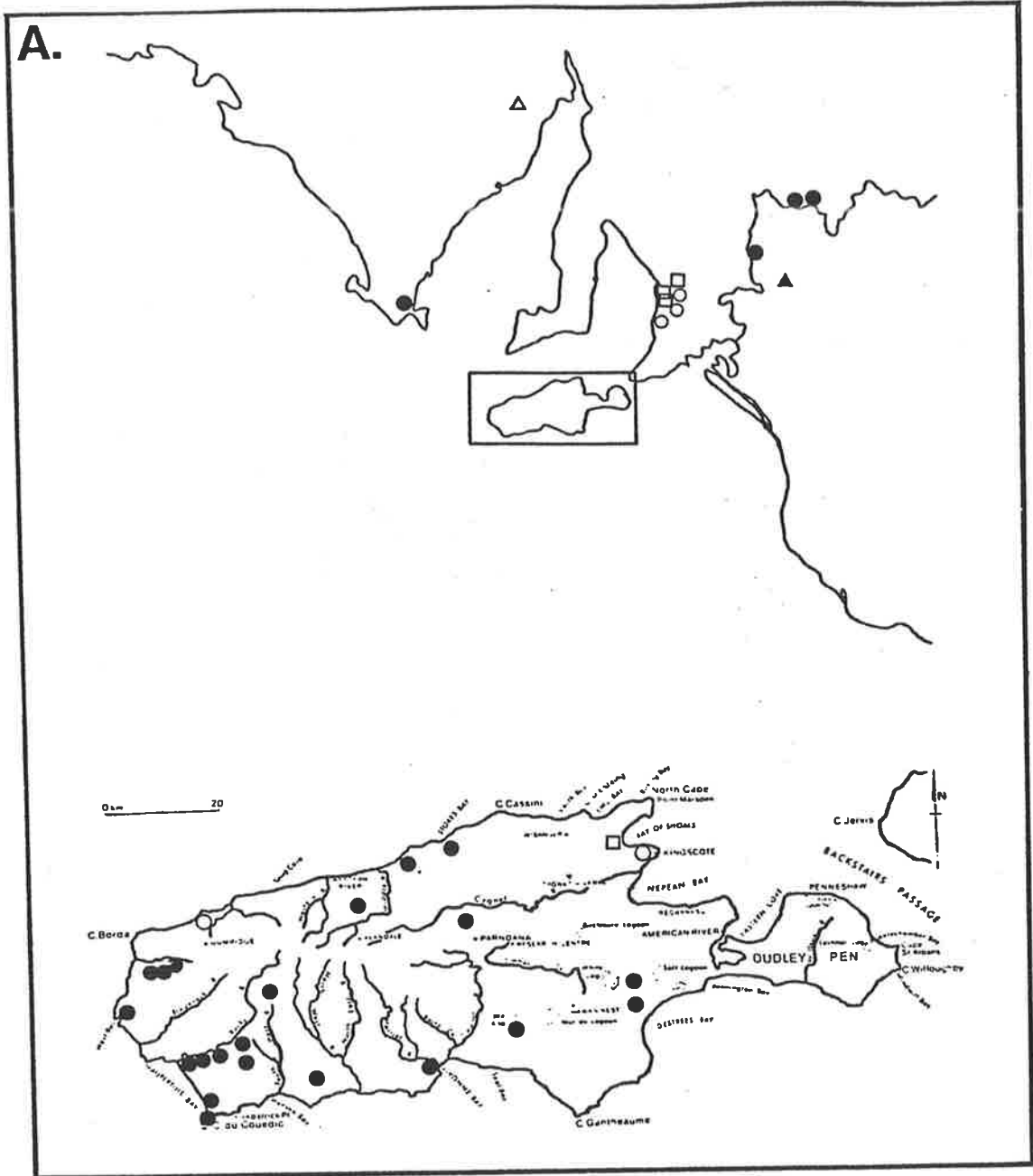
**A. Locality and karyotypic data for *Urodacus manicatus*.**

- Telocentric populations.  $2n > 58$ .
- Telocentric populations.  $2n < 58$ .
- Highly polymorphic populations.
- ▲ Pernong population.  $2n = 32$  (metacentrics)
- △ Middleback population.  $2n = 41$  (acrocentrics)

**B. Museum localities for *U. manicatus*.**

(Koch, 1979)

- ★ Canberra population.  $2n = 44, 45$ .



I preparations and metaphase II chromosomes which exhibited constrictions proved the most informative.

#### Metaphase I Karyotypes in *U. manicatus*.

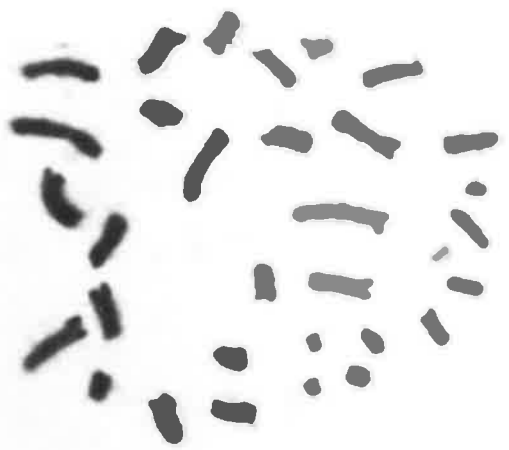
These karyotypes could be divided into four (or possibly five) main groups (cytotypes) (Fig. 1.4.3):

1. **Telocentric.** These karyotypes were the most common and were found in all but one Kangaroo Is. population and in all the N.E. Riverland populations of the mainland. All but one (or occasionally two) chromosome pairs were telocentric. At metaphase I most chromosomes paired to form bivalents. Telocentric karyotypes could be divided into two groups on the basis of chromosome number;  $2n \geq 58$  and  $2n < 58$ .
2. **Pernong.** This karyotype (from a single individual from an isolated and sparse population) showed homozygosity for a large number of centric fusions. This resulted in all metacentric chromosomes excepting the smallest pair which was telocentric.
3. **Highly Polymorphic.** All animals from these populations (one on Kangaroo Is. (EBM) and a few in the Adelaide Hills (BM, SGM, GM)) showed structural heterozygosity and homozygosity for a large number of Robertsonian changes. These were manifest at metaphase I as trivalents and quadrivalents. The Belair population (BM) was also polymorphic for inversions.
4. **Middleback.** A single individual from this isolated population showed a mainly telocentric karyotype with

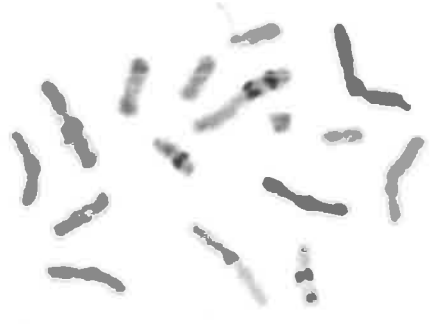
**Figure 1.4.3**

**Metaphase I cells from individuals representative  
of the main cytotypes observed in  
*Urodacus manicatus*.**

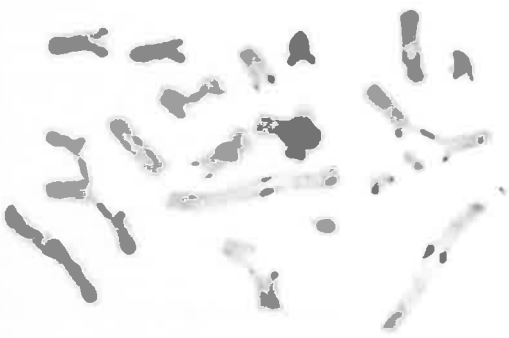
- a. Telocentric karyotype.
- b. Pernong karyotype.
- c. Highly polymorphic. (Emu Bay)
- d. Highly polymorphic. (Belair)
- e. Middleback karyotype.
- f. Canberra karyotype.



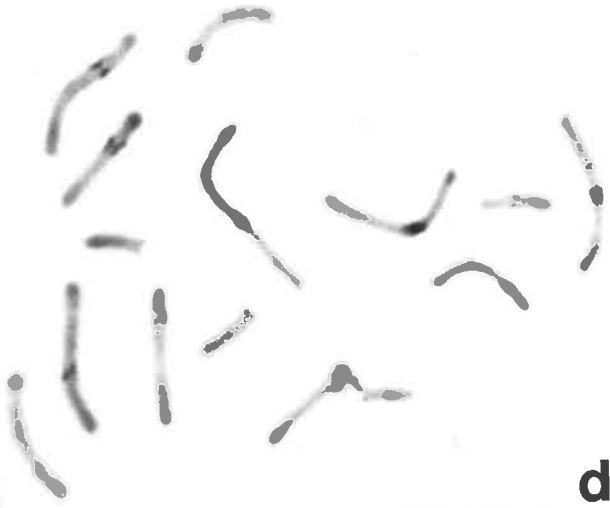
a



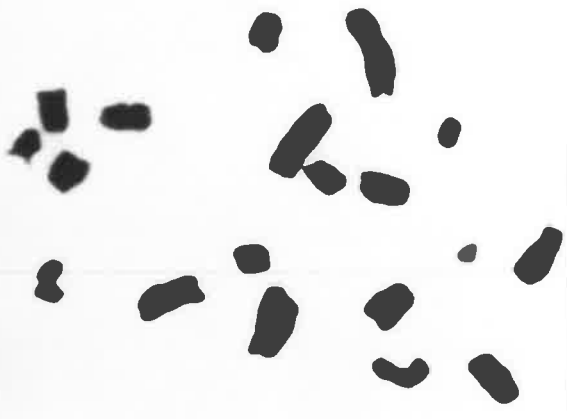
b



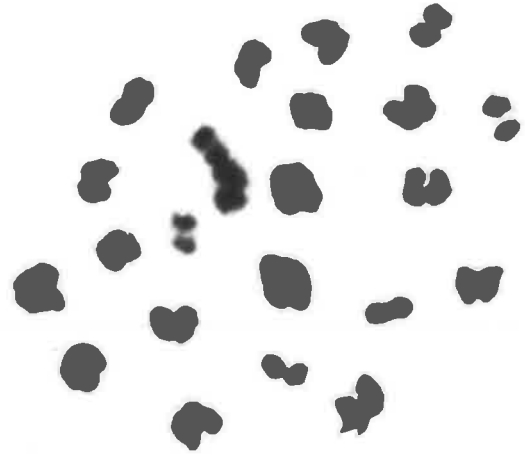
c



d



e



f



three distinctive acrocentric pairs not observed in other *U. manicatus* karyotypes.

5. Canberra. A population from the Eastern Range of *U. manicatus* was also sampled. Three male individuals from Canberra, A.C.T., had diploid chromosome numbers of  $2n=44$  or 45. At metaphase I, one individual exhibited 21 bivalents and 1 trivalent (Fig. 1.4.3f). The chromosome number and the presence of a single trivalent suggest this population may represent another cytotype of *U. manicatus*.

#### Description of Metaphase I Karyotypes.

##### 1. Telocentric Populations.

###### $2n \geq 58$

The most common karyotype found in populations of *U. manicatus* was comprised mainly of telocentric chromosomes. However each karyotype possessed at least one metacentric chromosome (e.g. OCM XX) or chromosome pair. No entirely telocentric karyotype was observed (see Table 1.4.3).

The chromosome number in most of these populations was  $2n \geq 58$  with the maximum number  $2n=64$  (SRM I). Chromosome number variation was due in some animals to Robertsonian fusion/fission events and in others variation in the number of small telocentric chromosome pairs which appeared to be behaving as B chromosomes.

Table 1.4.3

Populations of *U. manicatus* showing mainly telocentric bivalents.

Population	Metaphase I karyotype	2n	Total metaphase I arm count	No metacentric pairs at metaphase I	N
<u>Mainland</u>					
SRM	32b	64	33	1	1
OCM	31b	62	32	1	12
	30b 1t	63	32	1	4
WM	30b 1t	63	32	1	1
	29b 1t	61	32	2	1
<u>Kangaroo Is.</u>					
CRM	32b	64	33	1	1
	30b	60	32	2	5
LRM	32b	64	33	1	1
	28b 1t	59	31	2	3
	29b	58	31	2	2
CCM	31b	62	32	1	1
	30b	60	31	1	2
	28b 1t	59	31	2	1
VBM	31b	62	33	2	1
	30b	60	32	2	1
FCM	30b	60	31	1	4
WRM	30b	60	32	2	2
SKM	30b	60	31	1	1
ACM	30b	60	32	2	1
LAM	30b	60	32	2	2
	28b 1t	59	31	2	1
RRM	30b	60	32	2	1
	28b 1t	59	31	2	1
	27b 1t	57	31	3	1
RCM	30b	60	31	1	1
	29b	58	31	2	1
SBM	30b	60	32	2	1
	28b 1t	59	31	2	2
WBM	28b 1t 1uni	60	32	3	1
	28b 1t	59	31	2	1
TDM	28b 1t	59	31	2	1
GBM	28b 1t	59	31	2	1
Total					61

b = bivalent

t = trivalent

uni - univalent

### Evidence for B-Chromosomes.

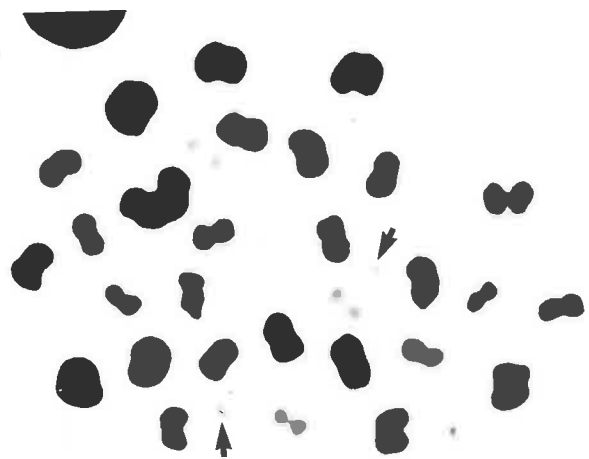
The almost continuous size range of chromosomes in these karyotypes made it difficult to positively identify any element that was behaving as a supernumerary or B chromosome. However, at metaphase I in many cells the smallest telocentric elements were often present as univalents rather than bivalents (see Fig. 1.4.4a). This is likely to lead to non-disjunction for these elements resulting in the presence of additional copies in some gametes. If these gametes were involved in fertilization the resultant zygote would have additional B chromosomes. Some individuals (e.g. VBM I) showed intra-testicular variation for the number of these B chromosomes present at metaphase I. Most of the variation involved the gain or loss of a pair of B chromosomes suggesting single univalents are inherently unstable. Of the metaphase I cells scored in VBM I, 3/30 → 29 bivalents, 16/30 → 30 bivalents, 10/30 → 31 bivalents, while 1/30 showed 30 bivalents and one univalent. Only two of the 163 individuals karyotyped (WBM, EBM XXVII) showed a consistent univalent present at metaphase I. It was not possible to assign any positive or negative heterotic effects to the presence or absence of these B chromosomes. Although interestingly Jones and Rees (1982) observed differential effects of even numbers of B chromosomes compared with odd numbers in maize. The distribution of B chromosomes was not clinal but varied both within and between populations (i.e. individuals collected from beneath the same rock may possess different numbers of B chromosomes).

**Figure 1.4.4**

**B chromosomes in *U. manicatus*.**

- a. Metaphase I cell from Overland Corner  
'telocentric' population showing  
univalent B chromosomes (arrowed).
  
- b. Metaphase I cell from Pernong  
'metacentric' population showing  
univalent B chromosomes (arrowed).

**VBM I Metaphase I karyotypes from two  
testicular cells of an individual (VBM I)  
exhibiting B chromosome variation.  
Top karyotype (31 bivalents) shows 8  
possible B chromosome pairs (in brackets).  
Bottom karyotype (30 bivalents) shows 7  
possible B chromosome pairs (in brackets).**

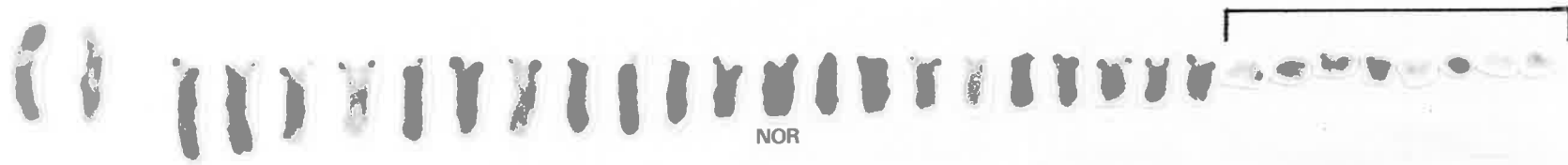


a

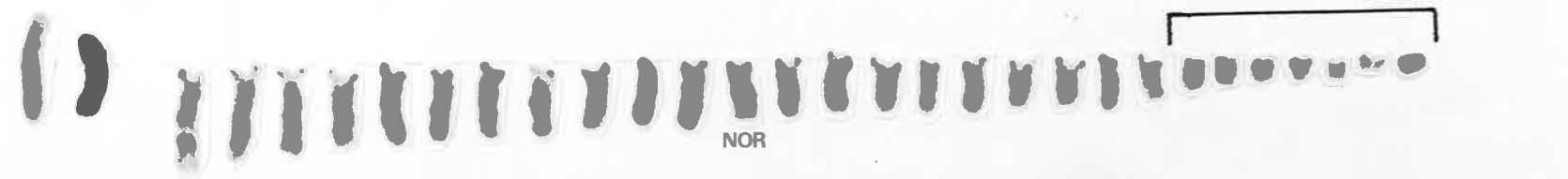


b

### VBMI



NOR



NOR



Besides variation in B chromosomes, there was also inter- and intra-population variation for Robertsonian fusion/fission events. Figure 1.4.5 shows the karyotypes for the mainland telocentric populations. Each karyotype showed at least one metacentric element; either in a trivalent at metaphase I (heterozygote) or as a metacentric pair involved in a bivalent (homozygote). WM II was heterozygous for an additional fusion and showed two metacentrics at metaphase I (bivalent pair and trivalent heterozygote).

The telocentric populations on Kangaroo Is. were very similar to those on the mainland except they showed greater variation in the number of B chromosomes. Most Kangaroo Is. populations also showed fixation for an additional Robertsonian fusion resulting in the presence of two metacentric bivalents at metaphase I. Figure 1.4.6 shows a number of karyotypes from different Kangaroo Is. populations where  $2n \geq 58$ . Examples of intra-population Robertsonian variation are shown in Figure 1.4.7. There is also B chromosome variation in many of these examples.

A comparison of the Mainland and Kangaroo Is. populations suggests that the fundamental arm number is 31. The NF (fundamental arm number) was calculated at metaphase I and consequently represents the haploid arm count. Any individual with an arm number greater than 31 is carrying B chromosomes.

**Figure 1.4.5**

**Metaphase I karyotypes from mainland telocentric  
populations.**

- SRM I - 32 bivalents (one metacentric pair)  
NF = 33
- OCM XI - 31 bivalents (one metacentric pair)  
NF = 32
- OCM XX - 30 bivalents, 1 trivalent  
(one metacentric, fusion/fission  
heterozygote)  
NF = 32
- WM II - 30 bivalents, 1 trivalent  
(one metacentric, fusion/fission  
heterozygote)  
NF = 32
- WM I - 20 bivalents, 1 trivalent  
(two metacentrics, trivalent  
resultant from fusion of two  
telocentric elements)  
NF = 32

NF = haploid arm count.

Bar = 5  $\mu$ m.

**SRMI**



**OCM XI**



**OCM XX**



**WM II**



**WMI**





Figure 1.4.6

Representative karyotypes of Kangaroo Island telocentric populations showing interpopulation variation in B chromosome numbers and Robertsonian fusions.

- CRM II - 32 bivalents (one metacentric pair)  
note two large telocentric pairs  
(brackets)  
NF = 33
- FCM II - 30 bivalents (one metacentric pair)  
two large telocentric pairs  
NF = 31
- WRM II - 30 bivalents (two metacentric pairs)  
two large telocentric pairs not  
observable  
NF = 32
- LAM I - 29 bivalents (two metacentric pairs)  
only one large telocentric  
NF = 31
- WBM I - 28 bivalents, 1 trivalent, 1 univalent  
(arrowed) (three metacentrics)

The trivalent may have resulted from fusion of one long telocentric pair (open triangle) with a smaller telocentric i.e. only one large telocentric pair.  
NF = 32

Bar = 5  $\mu$ m.

CRM II



FCM II



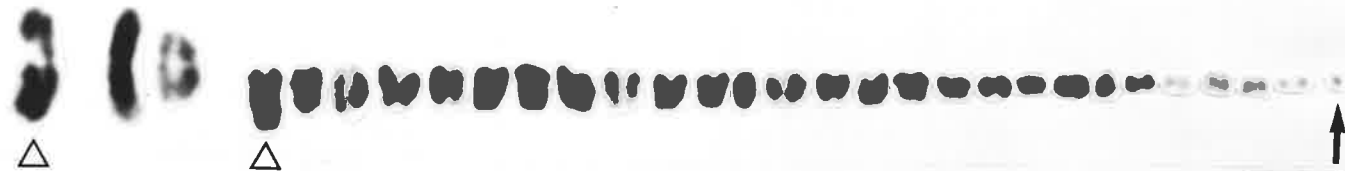
WRM II



LAM I



WBM I

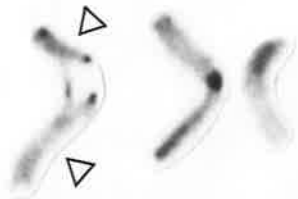




RRM I



II



III



RCM I



II



### C-band Differentiation.

Although chromosome differentiation was minimal, the metacentric element appeared to be the same in all karyotypes. This bivalent (occasionally trivalent) showed very little C-band material and was of large-medium size. No totally telocentric karyotype was found suggesting this element must be present as a metacentric (even if in a heterozygous state) for males to be viable. In the OCM population 12/16 were structural homozygotes while 4/16 were heterozygous for a fusion or fission event involving this chromosome. The probability of not observing a fission homozygote in this population is 17%. Although this is not statistically significant, the absence of heterozygotes for this chromosome pair in any other population suggests selection is operating to maintain it as a metacentric. Whether this metacentric is a sex chromosome and thus resistant to fragmentation is unknown.

Frequently B chromosomes are heterochromatic, however, there was no evidence for C-band positive small chromosomes in any karyotype. Thus, the total number of B chromosomes in any karyotype could not be determined. It is unknown whether B chromosomes are involved in fusion events to become permanent members of the karyotype.

### $2n < 58$

The telocentric populations with  $2n < 58$  are shown in Table 1.4.4 and Figure 1.4.8. The karyotypic differences between these populations and those described previously

Table 1.4.4

Telocentric populations of *U. manicatus* where  $2n < 58$ .

Population	Karyotype	2n	NF	No. metacentrics	N
Mainland					
BWM	28b	56	30	2	1
HM	26b, 1t	55	30	3	1
OGM	24b, 1t	51	26	1	2
	25b	50	26	1	1
Kangaroo Is.					
CTM	28b	56	30	2	1
	27b	54	29	2	2
BRM	27b	54	29	2	1
<b>Total</b>					<b>9</b>

NF = haploid number of chromosome arms.

(total metaphase I arm count)

**Figure 1.4.8**

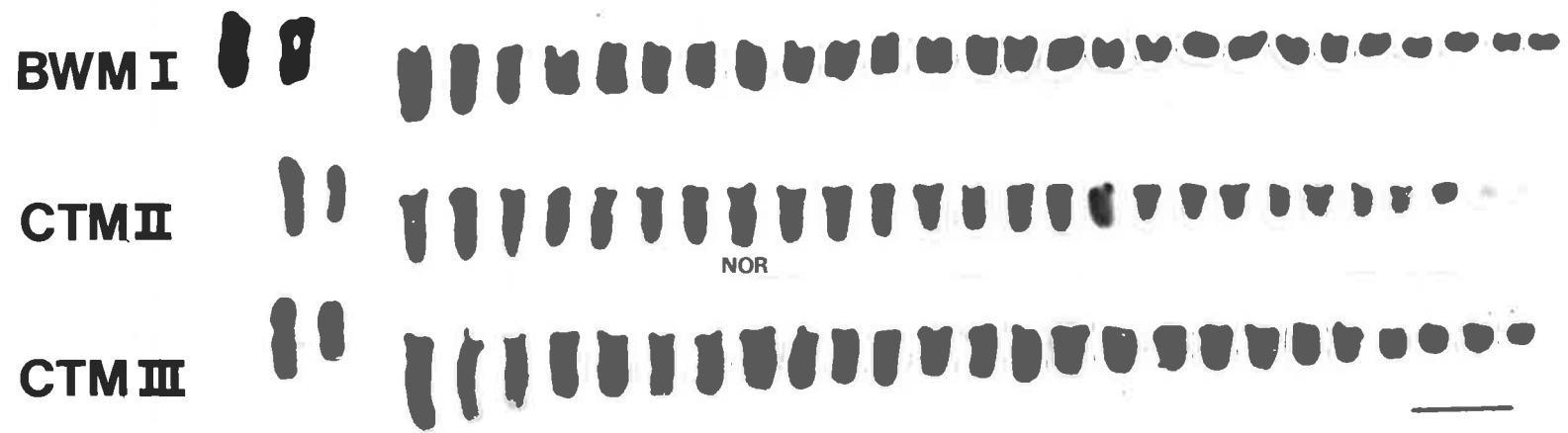
**Mainland and Kangaroo Island telocentric karyotypes  
where  $2n < 58$ .**

**BWM I      28 bivalents (2 metacentric pairs)  
            NF = 30**

**CTM II     28 bivalents (2 metacentric pairs)  
            NF = 30**

**CTM III    27 bivalents (2 metacentric pairs)  
            NF = 29  
            (B chromosome variation)**

**Bar = 5  $\mu$ m.**





where  $2n \gg 58$  can be attributed to polymorphism for small chromosome pairs (i.e. B elements). Thus the fundamental arm number of this species is further reduced to 29. However, B chromosomes may still constitute a portion of the karyotype for these populations. If so, the haploid arm count could be reduced still further.

## 2. Pernong Karyotype.

The population of *U. manicatus* in this area was very sparse and scattered. Extensive collection resulted in only one adult male although females and juveniles were also found. The mitotic, metaphase I and II karyotypes of this male are shown in Figure 1.4.9. All the chromosomes except the smallest pair are metacentrics and only bivalents are formed at metaphase I. The smallest telocentric pair were observed as univalents in some metaphase I cells suggesting these may be a pair of B chromosomes (Fig. 1.4.4a). The total haploid arm count of this karyotype was 31, however exclusion of the telocentric bivalent would give a fundamental number of 30. To reduce this number further to the 29 derived from the CTM and BWM populations, either inversions have occurred in these populations or in PGM I B chromosomes have been involved in fusion events with stable chromosomes of the karyotype, or with other B chromosomes to become stable metacentric elements.

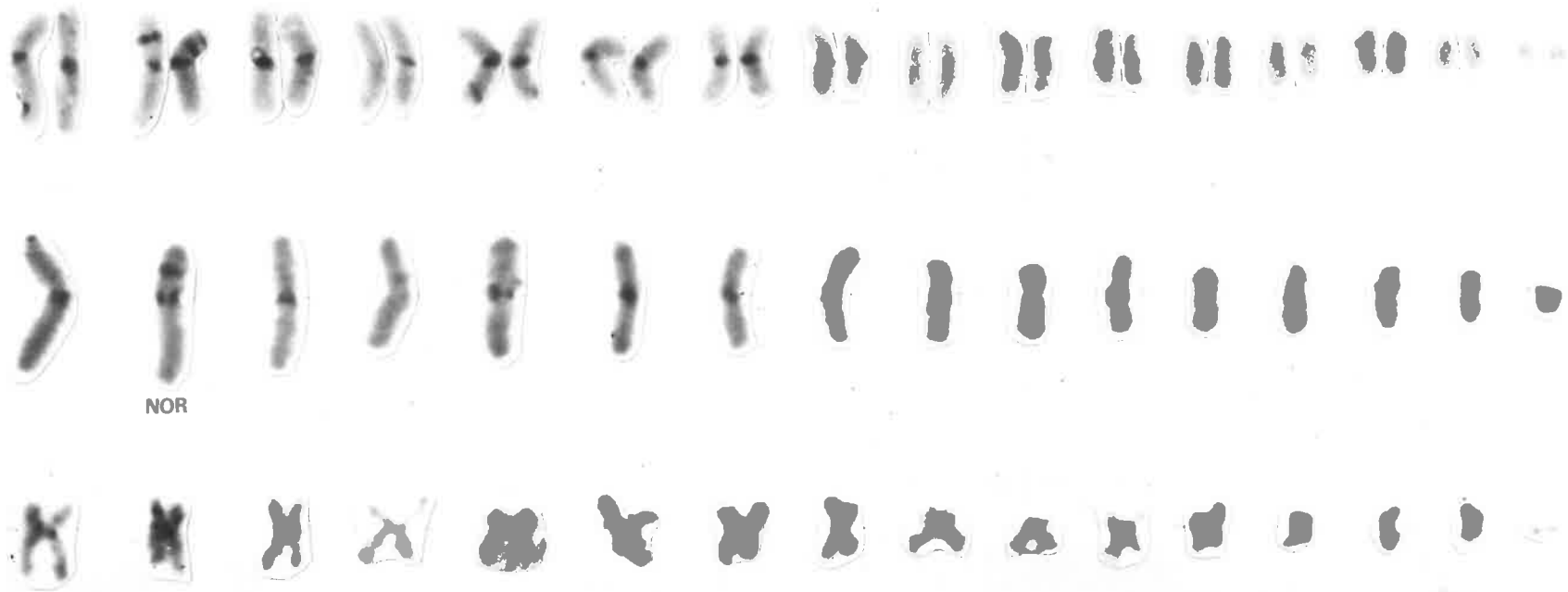
**Figure 1.4.9**

**Pernong karyotype, *U. manicatus*.**

<b>Top</b>	<b>Mitotic karyotype (C-banded) 2n=32</b>
<b>Middle</b>	<b>Metaphase I (16 bivalents) 15 metacentric bivalents 1 telocentric bivalent</b>
<b>Bottom</b>	<b>Metaphase II</b>

**Bar = 5  $\mu$ m.**

**PGM I**



### 3. Highly Polymorphic Populations.

Three populations in the Adelaide Hills and a single population near Emu Bay on Kangaroo Island showed highly polymorphic karyotypes. Two of these populations were studied in detail; BM and EBM.

#### Emu Bay: (EBM)

Table 1.4.5 shows the metaphase I karyotypes and chromosome arm counts for EBM individuals. Where C-banding was not clear arm counts could not be calculated. All individuals were heterozygous for a number of Robertsonian changes resulting in trivalents and quadrivalents at metaphase I. In this population the fundamental arm numbers were very similar to those of the telocentric Kangaroo Is. and mainland populations. Figure 1.4.10 shows a number of metaphase I karyotypes from EBM individuals. The chromosomes are either telocentric or metacentric/submetacentric and the karyotypes appear to be derived from the telocentric karyotype by a series of Robertsonian fusions. Clearly, individuals are heterozygous or homozygous for a large number of different fusion events.

The quadrivalents found in individuals EBM I, EBM IX and EBM X must be derived from fusions while an additional fusion would be required to establish a closed quadrivalent in EBM XXXI.

The variation in fundamental arm numbers at metaphase I between animals in this population suggests that the small elements of some karyotypes may be B chromosomes (similar to

Table 1.4.5

Karyotypes of male specimens of *U. manicatus*  
collected at Emu Bay, Kangaroo Island.

Animal	Metaphase I	2n	Haploid arm count (MI)
EBM I	16b 3t 1q	45	31
II	21b 2t	48	31
III			
IV	12b 7t	45	-
V	16b 3t	41	-
VI	15b 5t	45	-
VII	18b 4t	48	-
VIII	12b 8t	48	31
IX	13b 4t 1q	42	30
X	15b 4t	42	31
XI	15b 4t	42	-
XII	21b 3t	51	30
XIII	13b 7t	47	-
XIV	17b 4t	46	-
XV	12b 7t	45	32
XVI	11b 8t	46	-
XVII	16b 5t	47	-
XVIII	13b 7t	47	-
XIX	19b 3t	47	-
XX	12b 6t	42	-
XXI	16b 5t	47	-
XXIII	17b 3t	43	-
XXIV	14b 5t 1q	47	-
XXVII	17b 3t	43	-
XXVIII	17b 2t 1q 1uni	45	-
XXIX	13b 7t	47	-
XXX	16b 6t	50	32
XXXI	16b 4t 1q	48	31
XXXII	17b 4t	46	-
XXVIII	-	47	-
EB'M I	-	47	-
II	-	45	-
III	-	44	-
IV	13b 5t	41	31
Total	35		

b = bivalent  
t = trivalent  
q = quadrivalent  
uni = univalent

Figure 1.4.10

Emu Bay (highly polymorphic) metaphase I karyotypes.

EMU XII	21 bivalents, 3 trivalents 2n=51 <u>6</u> metacentric pairs, 18 telocentric pairs NF = 30
II	21 bivalents, 2 trivalents 2n=48 <u>8</u> metacentric pairs, 15 telocentric pairs NF = 31
VIII	12 bivalents, 8 trivalents 2n=48 <u>10</u> metacentric pairs, 11 telocentric pairs NF = 31
XXX	16 bivalents, 6 trivalents 2n=50 <u>10</u> metacentric pairs, 12 telocentric pairs NF = 32
X	15 bivalents, 4 trivalents 2n=42 <u>12</u> metacentric pairs, 7 telocentric pairs NF = 31

- ascending order of metacentrics.

Bar = 5  $\mu$ m.

EBM VII.



II



VIII



XXX



X



Figure 1.4.10 (cont.)

EBM XV    12 bivalents, 7 trivalents     $2n=45$   
          13 metacentric pairs, 6 telocentric pairs  
                                  NF = 32

IV        13 bivalents, 5 trivalents     $2n=41$   
          13 metacentric pairs, 5 telocentric pairs  
                                  NF = 31

Karyotypes showing quadrivalents:

EBM I     16 bivalents, 3 trivalents, 1 quadrivalent  
                                   $2n=45$   
          9 metacentric pairs + quadrivalent  
          10 telocentric pairs  
                                  NF = 31

IX        13 bivalents, 4 trivalents, 1 quadrivalent  
                                   $2n=42$   
          10 metacentric pairs + quadrivalent  
          7 telocentric pairs  
                                  NF = 30

XXXI     16 bivalents, 4 trivalents, 1 quadrivalent  
                                   $2n=48$   
          7 metacentric pairs + quadrivalent  
                                  NF = 31

Bar = 5  $\mu$ m.



EBM XV

} | } = ||| ||| < > ||| ||| ||| |||

EB'M IV

| < | | > | | | | | | | | | | | | | | | |

EBM I



| | | | | | | | | | | | | | | | | | | | | |

NOR

IX



| | | | | | | | | | | | | | | | | | | | | |

XXXI



| | | | | | | | | | | | | | | | | | | | | |

those found in telocentric populations of *U. manicatus*). There was no evidence from this population that inversions had occurred to effect arm number.

#### Belair Population: (BM)

The Belair population (and nearby populations) showed a high degree of heterozygosity for Robertsonian fusions manifest as in EBM as trivalents and quadrivalents at metaphase I. However, these populations also showed inversion heterozygosity. C-banding resulted in the identification of pericentric inversions in mitotic and metaphase I cells. Table 1.4.6 shows the metaphase I data for this population. Inversion heterozygotes are identified and total haploid arm counts at metaphase I have been calculated.

The diploid chromosome numbers are generally lower than those for EBM, and as Figure 1.4.11 shows this is due to homozygosity for a large number of centric fusions. The metaphase I chromosomes are mainly metacentric, submetacentric or acrocentric in these karyotypes, i.e. there is a reduction in the number of telocentrics.

The total arm count for individuals is lower than that observed in other populations. This may be due to the total or partial loss of B chromosomes in this population (although BM XVII, for example, appears to have a small telocentric B) or pericentric inversion events in fusion product metacentrics resulting in reformation of telocentric chromosomes (e.g. INV B1).

Table 1.4.6a

Metaphase I configurations and haploid arm counts  
for specimens of *U. manicatus* from Belair (S.A.).

Animal	Metaphase I	2n	Arm count MI	Inversions
BM III	13b 3t	35	-	-
VI	14b 4t	40	25	-
VII	8b 7t 1q	41	24	-
VIII	12b 4t	36	25	-
IX	8b 7t	37	-	-
X	13b 3t	35	26	INV T1
XI	9b 6t	36	26	-
XII	12b 2t	30	26	No inversions
XIII	10b 4t	32	25	INV T1
XIV	13b 1t	29	24	No inversions
XV	8b 5t	31	26	INV T1, INV B1
XVI	9b 4t	30	24(26)	INV T1
XVII	9b 5t	33	27	INV T1, INV B1
XVIII	11b 3t	31	25	No inversions
XIX	12b 2t	30	24	INV T1
XX	13b 1t	29	24(26)	INV T1
XXI	9b 4t	30	24	INV T1
Total	17			

Table 1.4.6b

Metaphase I configurations in Adelaide Hills  
populations of *U. manicatus*.

Animal	Metaphase I	2n	NF	Inversions
SGM I	12b 2t	30	25	No inversions
II	13b 1t	29	24	INV T1
GM I	17b 4t 1q	50	28	No inversions

b = bivalents  
t = trivalents  
q = quadrivalents

INV T1 = pericentric inversion trivalent 1.  
INV B1 = pericentric inversion bivalent 1  
- = could not be scored

NF = numbers in brackets indicate maximum haploid arm count if  
very short acrocentric arms of some chromosomes are included.

**Figure 1.4.11**

**Belair (highly polymorphic) metaphase I karyotypes.**

BM XX	13 bivalents, 1 trivalent <u>2</u> telocentric pairs NF = 24 (26)	2n=29
XV	8 bivalents, 5 trivalents <u>0</u> telocentric pairs NF = 26	2n=31
XVII	9 bivalents, 5 trivalents <u>1</u> telocentric pair NF = 27	2n=33
XVI	9 bivalents, 4 trivalents <u>2</u> telocentric pairs NF = 24	2n=30

Stars indicate inversions.

(INV T1, INV B1)

Bar = 5  $\mu$ m.

BM XX



XV



XVII



XVI



Figure 1.4.11 (cont.)

BM XXI	9 bivalents, 4 trivalents <u>2</u> telocentric pairs NF = 24	2n=30
XIX	12 bivalents, 2 trivalents <u>2</u> telocentric pairs NF = 24	2n=30
XVIII	11 bivalents, 3 trivalents <u>3</u> telocentric pairs NF = 25	2n=31
XIII	10 bivalents, 4 trivalents <u>5</u> telocentric pairs NF = 25	2n=32
VII	9 bivalents, 7 trivalents 1 quadrivalent <u>7</u> telocentric pairs NF = 24	2n=41

Stars indicate INV T1

Bar = 5  $\mu$ m.

BM XXI



XIX



XVIII



XIII



VII



The Belair population exhibits a more extreme form of chromosome change via centric fusions (when compared to the Emu Bay population). This is coupled with multiple inversion events. Paracentric inversions which could only be identified by synaptonemal complex analysis also occur in this population. The frequency of heterozygotes of the INV T1 was greater than expected on the basis of Hardy-Weinberg equilibrium. There were nine animals heterozygous for INV T1 out of a total of thirteen animals scored (i.e. the frequency of heterozygotes was greater than 50%).

#### 4. Middleback: (MBM)

Figure 1.4.12 shows mitotic metaphase I and II chromosomes of the single male specimen from Middleback. Although superficially the chromosomes appear similar to that of the telocentric populations, the fundamental arm number for MBM is 23. This is much lower than that of other wholly telocentric populations (e.g. SRM=33, CTM=28). Additionally, the general features of the karyotype differ. There is a single medium-small metacentric chromosome that forms a trivalent at metaphase I. The remainder of the karyotype is comprised of telocentric and acrocentric elements. These acrocentric elements, with obvious primary constrictions, are not common features of any other karyotypes observed.

No C-banding differentiation was observed so it was not possible to determine further the nature of chromosome change in this population since it is not known whether the acrocentric short arms are heterochromatic. The low



**Figure 1.4.12**

**Karyotypes of Middleback *U. manicatus* individual.**

- Top**      **Mitotic karyotype 2n=43**
- Middle**      **Metaphase I karyotype.  
20 bivalents, 1 trivalent.  
All acrocentric pairs and telocentric  
pairs except trivalent.**
- Bottom**      **Metaphase II.**

**Closed triangle indicates heteromorphic pair.**

**Arrows indicate acrocentric pairs.**

**Bar = 5  $\mu$ m.**

**MBM I**



fundamental number suggests that tandem fusions may have been involved in chromosome change. There was no evidence for inversion heterozygosity.

#### Segregation in Robertsonian Fusion Heterozygotes.

Two animals were analysed to determine the frequency of non-disjunction in fusion heterozygotes. Trivalent formation at metaphase I in this achiasmate system was highly efficient, i.e. univalents due to pairing failure of trivalents were never observed. Additionally, a mechanism existed by which the two telocentric chromosomes always paired on the same side of the metacentric fusion product. This pairing behaviour has been shown by Moses *et al.* (1979) to facilitate regular disjunction in chiasmate *Lemur* hybrids.

The animals analysed were OCM XX which have a single metacentric chromosome and which formed a trivalent with two telocentric elements at metaphase I. BM XVI was a complex heterozygote and exhibited 4 trivalents, one of which included INV T1 at metaphase I. Metaphase II cells of OCM XX were scored for the presence or absence of a single metacentric chromosome. Data in Table 1.4.7a show that in non-aneuploid cells the frequencies of the two spermatocyte types do not differ significantly from 1:1 expectations. Individual metaphase II cells of BM XVI were karyotyped as shown in Figure 1.4.13. In this individual the segregation types as observed at metaphase II were as expected on the basis of the binomial expansion. No aneuploid cells were observed in OCM XX while one cell out of 16 analysed was

Table 1.4.7a

Segregation of the trivalent in  $OCM_{XX}$ .  
(30 bivalents, 1 trivalent)

Metaphase II cells	Observed	Expected	$\chi^2$
Metacentric present (n=31)	18	20	0.2
Metacentric absent (n=32)	22	20	0.2
Total	40	40	0.4

All chi-squared values non-significant (at 5% level).

Table 1.4.7b

Segregation data for  $BM_{XVI}$ .  
(9 bivalents, 4 trivalents)

Chromosome number at metaphase II	Observed	Expected ratio
13	1	1
14	3	4
15	6	6
16	4	4
17	1	1
Total	15	(16)

**Figure 1.4.13**

Some of the possible metaphase II segregants  
observed in BM XVI.

Metaphase I → 9 bivalents, 4 trivalents

Possible metaphase II configurations:

Biv. +	Triv. =	Total	Reciprocal	Exp. ratio
9 + 4	=	13	9 + 8 = 17	1
9 + 3 + 2	=	14	9 + 6 + 1 = 16	4
9 + 2 + 4	=	15	9 + 4 + 2 = 15	6
9 + 1 + 6	=	16	9 + 2 + 3 = 14	4
9 + 0 + 8	=	17	9 + 0 + 4 = 13	1

A and B → only possible 13-17 segregants.

C and D → only one of 6 possible 15-15 segregants.

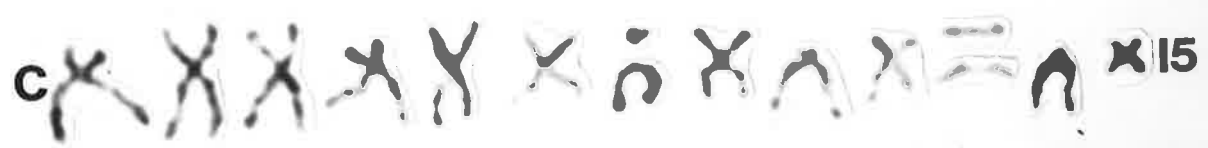
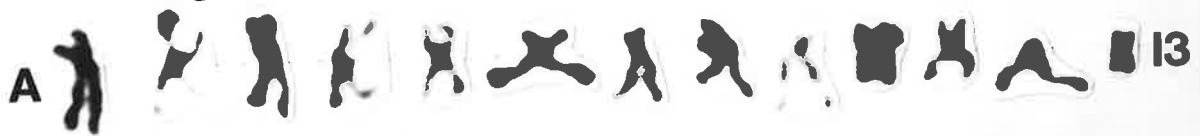
E and F → only one of 4 possible 16-14 segregants.

BM XVI

MI.



MII Segregants.



aneuploid in BM XVI. However, the aneuploidy was not necessarily due to non-disjunction from a trivalent but may have been due to some other disjunctional error.

It was not possible to analyse the disjunctional properties of the chain quadrivalents present in 14% of individuals. However, the high frequency of such quadrivalents and the similar late metaphase I-anaphase I behaviour of the quadrivalents compared with the trivalents suggests that disjunction in these was also regular.

## DISCUSSION

### Chromosome Polymorphism in *Urodacus manicatus*.

The most common karyotype in *U. manicatus* consisted of telocentric chromosomes with a single metacentric pair. Whether the primitive karyotype consisted of purely telocentric chromosomes is unknown, however the lack of totally telocentric karyotypes in any population suggests that in males this karyotype is no longer viable. Considering the preponderance of karyotypes with a single metacentric it is probable that the Robertsonian heterozygotes in the OCM populations were derived from a fission event involving this metacentric. However, all other Robertsonian changes appear to be fusion events. The origin of the chain quadrivalents (in EBM and BM populations) must be the result of fusion events. The presence of the nucleolar organiser region (NOR-identified as a single C-band on metaphase I chromosomes) on various metacentric and submetacentric bivalents and trivalents at metaphase I in the

EBM population suggests a number of different fusion events have occurred with the original telocentric chromosome. If fission was the mechanism, only a particular metacentric as well as a particular telocentric would carry the NOR (this is assuming the NOR is a stable region in this species). Whether secondary fission events are also occurring in the highly polymorphic populations cannot be determined.

Almost all populations of *U. manicatus* showed some degree of polymorphism for B chromosome number or Robertsonian fusions or both. There were few definable fixed differences between most populations and nothing could be determined of the adaptive significance of these B chromosomes and fusions. Most of the intrapopulation fusion/fission chromosome variation appeared to be due to individual aberrations, however, this explanation cannot account for the high frequency of heterozygotes in the Emu Bay (EBM) and Belair (BM) populations.

#### Origin of the Highly Polymorphic Populations.

Extensive chromosome heterozygosity could have arisen either through hybridization between different cytotypes in a relatively narrow zone (*U. manicatus* shows low vagility) or 'intrapopulation polymorphism'.

The extensive karyotypic data from Kangaroo Is. and the position of the Emu Bay population with respect to other cytotypes suggests that recent hybridization has not occurred. Additionally, there are no two widely divergent cytotypes on Kangaroo Is. whose hybridization would result in



the EBM karyotypes. The Emu Bay population is isolated totally from other populations by farmland. It is located in a relatively small area of uncleared scrub within an established farming area (i.e. >100 years).

The most likely explanation for the highly polymorphic populations is intrapopulational polymorphism due either to random drift or selection. The inbred nature of the populations would facilitate the increase in frequency of unique chromosome rearrangements. Additionally, in *U. manicatus*, achiasmate meiosis leads to regular disjunction of trivalents at meiosis. Thus, there is no selection against heterozygotes due to mechanical problems at meiosis. The effect of fusion (and inversion) events on an achiasmate species suggests selection may not be involved in the process of chromosome change.

Achiasmate meiosis results in each chromosome representing a fixed linkage group. Presumably the evolution of achiasmate meiosis served to preserve co-adapted gene arrays. The only effect of fusion and inversion events in an achiasmate system would be position effects resultant from either expanding or rearranging the order of genes within a linkage group. If these effects were neutral and there were no mechanical problems associated with disjunction of rearranged chromosomes the high level of structural heterozygosity could be considered analogous to the situation in vegetatively reproducing plants and parthenogenetic insects which aggregate and tolerate numerous chromosomal aberrations (John and Quraishi, 1964; White, 1980).

Evidence (section 5) suggests *U. manicatus* is not a parthenogenetic species, however, if achiasmate meiosis occurs in both sexes there should be no barrier to the accumulation of structural changes. Achiasmate meiosis in both sexes poses the question of how such a species would generate variability. This in turn leads back to a somewhat circular argument that these structural changes replace recombination as a source of variation (White, 1973).

It is difficult however, to answer why some scorpion populations have maintained a relatively stable karyotype while others exhibit highly polymorphic karyotypes, without invoking selection. There do not appear to be any common environmental or habitat similarities between the Belair and Emu Bay populations compared with other populations of *U. manicatus* that would suggest these populations are undergoing differential selection. Many chiasmate species (i.e. species that could not tolerate aggregation of mechanically disadvantageous rearrangements) show some highly polymorphic populations (not resultant from hybridization) where the rearrangements are presumably of adaptive significance. However, it is rare that any specific influence of chromosome rearrangements on the level of adaptation can be determined.

Perhaps highly polymorphic populations are resultant from simultaneous multiple chromosome rearrangements (King, 1982) that have led to rapid karyotypic change.

### The Role of Chromosomal Change in Speciation.

Most models describing the role of chromosomal changes in speciation assume that unique chromosome rearrangements will decrease the fitness of the heterozygote (White, 1978). In view of this argument it is unlikely that the fusion differences observed between populations of *U. manicatus* would be a prime speciation factor. Hybridization between most populations would most probably be mechanically viable. The only karyotype with which hybridization may prove problematical is the Middleback karyotype. Chromosomal changes in this individual have not involved simple fusion events (unlike the obviously karyotypic orthoselective changes in other populations). Selection or random genetic drift has played a role in establishing the Middleback karyotype in an isolated, inbred population on the edge of the species distribution.

**Karyotypic Analysis of *Urodacus elongatus*, *Urodacus armatus*,  
*Urodacus planimanus* and *Urodacus novaehollandiae*.**

The locality and karyotypic data for the remaining species in the *armatus* group are shown in Table 1.4.8 and Figure 1.4.14. Generally, these species have higher chromosome numbers than *U. manicatus*. There was inter-population variation in chromosome number in most species however, there was no evidence for the extreme intra-population fusion/fission variation as found in *U. manicatus*.

**Description of Karyotypes.**

***Urodacus elongatus*.**

*Urodacus elongatus* is most closely related to *U. manicatus* and has a restricted distribution within, but confined to, the drier regions of its range (Koch, 1979). The diploid chromosome number in the single individual karyotyped was  $2n=56$  manifest as 28 bivalents at metaphase I (see Fig. 1.4.15). Bivalents were either metacentric or acrocentric/telocentric with one chromosome pair exhibiting a distinctive secondary constriction.

***Urodacus armatus*.**

*Urodacus armatus* is a widely distributed dry adapted species. Specimens from two localities showed widely varying chromosome numbers. The  $2n=124$  male showed 62 bivalents at metaphase I. Metaphase II data showed these to be mainly metacentric with some acrocentric chromosomes. Mitotic data only was available for the  $2n=144$  specimen where

Table 1.4.8

Locality and karyotypic data for four species in the *armatus* group.

Species	Locality	Code	Metaphase I	2n	No. animals
<i>Urodacus elongatus</i>	Mambray Creek, S.A.	MCE	28b	56	1
<i>Urodacus armatus</i>	Middleback, S.A. Toolache Waterhole, S.A.	MBA	62b	124	4
		TA	-	144	1
<i>Urodacus planimanus</i>	Kalamunda, W.A. Mundaring Weir, W.A. (Koch 1977)	WAP	35b	70	2*
		MWP	34b	68	1
<i>Urodacus novaehollandiae</i>	Moonta, S.A.	MN	-	175	3
	Cleve, S.A.	CN	-	126	1
				65b	130
	John Forrest, National Park, W.A.	WAN	34b	68	1
	Mundaring Weir, W.A.	MWN	33b	66	1
	Mundaring Weir, W.A. (Koch 1977)		34b	68	1
Esperance, W.A.	EN	36b	72	1	

\* High frequency of polyploid cells found in the testis of some animals.

Figure 1.4.14

Maps showing the karyotypic and locality data  
for four species of *Urodacus*.  
Museum localities (Koch, 1977).

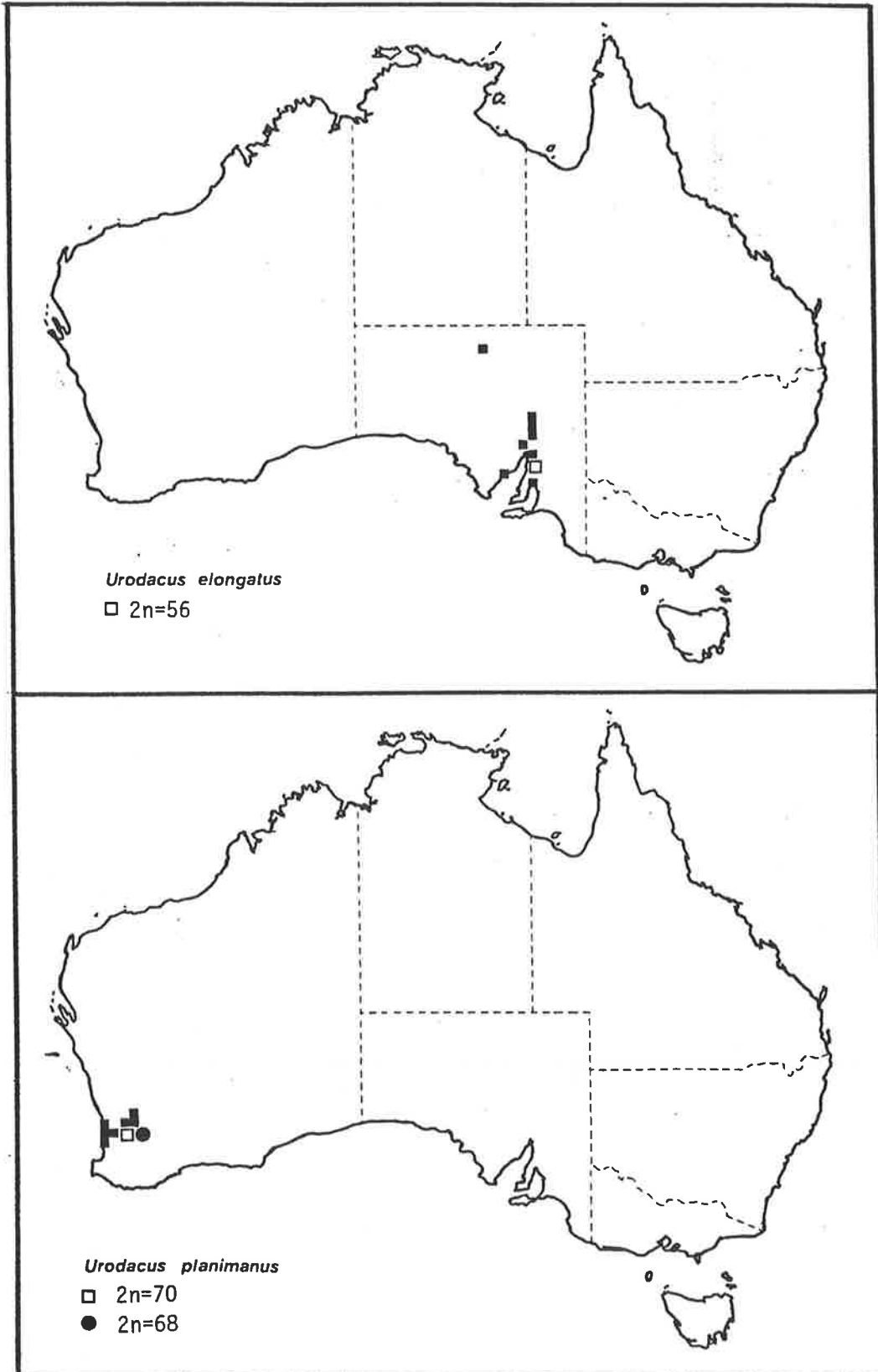
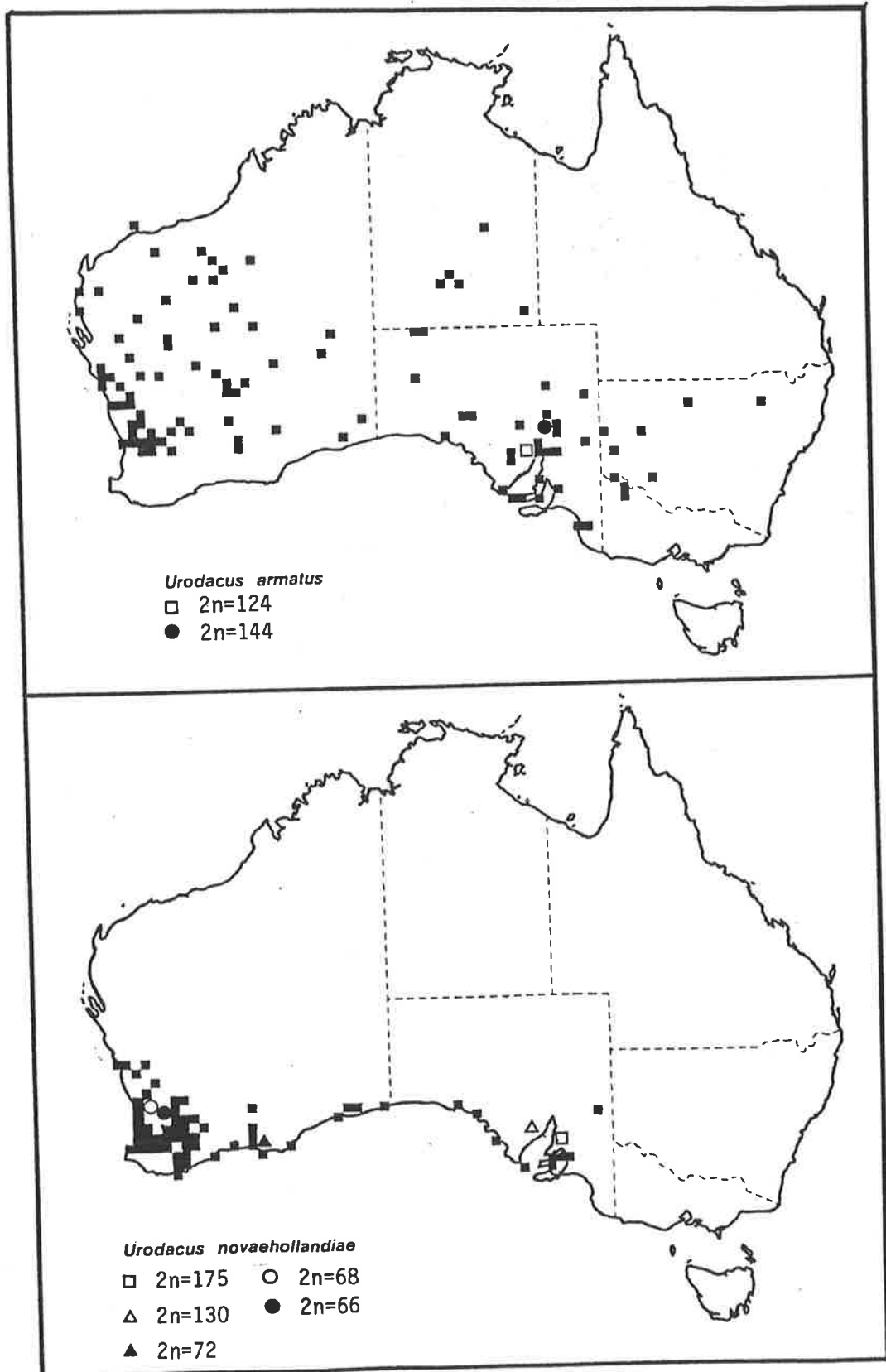


Figure 1.4.14 (cont.)







all except six chromosome pairs were small and dot-like (see Fig. 1.4.15).

The most probable mode of chromosome change between the two populations is Robertsonian fusion/fission events.

*Urodacus planimanus*.

*Urodacus planimanus* occupies a very small region within the extensive distribution range of *U. novaehollandiae*. These two species share the same localized habitats and morphologically are very similar. Koch (1979) used metaphase I chromosome morphology as a taxonomic tool to differentiate between them: Although both males collected from the same locality exhibited 34 bivalents at meiosis ( $2n=68$ ) the karyotypes were clearly distinguishable. *U. novaehollandiae* had smaller equal-sized chromosomes when compared to *U. planimanus* which had chromosomes of varying size including seven characteristically large bivalents.

In this study two specimens of *U. planimanus* from a nearby locality (cf. those karyotyped by Koch (1979)) were karyotyped (as shown in Fig. 1.4.15). These exhibited 35 bivalents at metaphase I ( $2n=70$ ) including the 7 large bivalents and a smaller metacentric bivalent. Thus, the additional bivalent in these individuals is not due to a fusion/fission difference in any of the large elements of the karyotype. The extra bivalent is a small telocentric element that may have arisen from fusion/fission of a smaller element (or is perhaps a B chromosome pair similar to those in *U. manicatus*).

*Urodacus novaehollandiae*.

This species showed extreme interpopulation variation in chromosome number (Fig. 1.4.16). The Western Australian populations had similar chromosome numbers and morphology to those described by Koch (1979) (i.e. 34 bivalents). The individual with 33 bivalents appeared homozygous for a centric fusion resulting in a large metacentric bivalent while the Esperance individual (36 bivalents) differed by two Robertsonian changes.

The South Australian populations had much higher chromosome numbers. Individuals from the Cleve population exhibited 65 bivalents at metaphase I which is almost double that of the Western Australian populations. A comparison of the mitotic chromosomes of specimens from the two South Australian populations suggests that numerical variation has occurred via a process of fragmentation (Fig. 1.4.16). The Cleve population exhibits a few large metacentric pairs while the mitotic chromosomes of the Moonta individuals are small and dot-like.

Assuming the Western Australian and South Australian populations are the same species - Dr. L.E. Koch verified the identifications as *U. novaehollandiae* on morphological criteria - karyotypic evolution is difficult to explain. Morphologically the chromosomes differ. Western Australian populations exhibit similar sized metacentric and acrocentric elements, while chromosomes from the Cleve population consisted of relatively large metacentrics, acrocentric and small telocentric elements. Clearly fragmentation (via

**Figure 1.4.16**

**Mitotic, metaphase I and metaphase II karyotypes  
of *U. novaehollandiae* from Western Australia  
and South Australia.**

- a Mitotic,  $2n=68$  (East Perth, W.A.)
- b Metaphase I, 33 bivalents (Mundaring Weir, W.A.)  
Arrow indicates large metacentric bivalent,  
possible fusion homozygote.
- c Metaphase I, 36 bivalents (Esperance, W.A.)
- d Metaphase II ( $n=\overset{36}{\cancel{72}}$ )
- e Metaphase I, 65 bivalents  $2n=130$  (Cleve, S.A.)
- f Metaphase II ( $n=65$ )
- g Mitotic,  $2n=126$  (Cleve, S.A.)
- h Mitotic,  $2n=175$  (Moonta, S.A.)

Bar = 5  $\mu$ m.



a



b



c



d



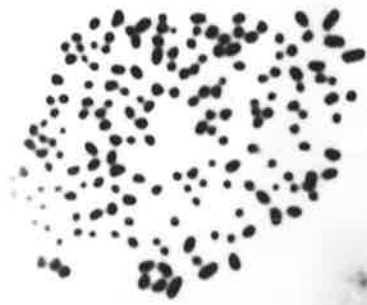
e



f



g



h

centric fission) has not occurred and although DNA content data is unavailable it appears that simple polyploidy can also be excluded. Thus, on the basis of chromosomal evidence, it appears that Western Australian and South Australian populations of *U. novaehollandiae* in reality represent two separate species.

#### Species-group *yaschenkoi*.

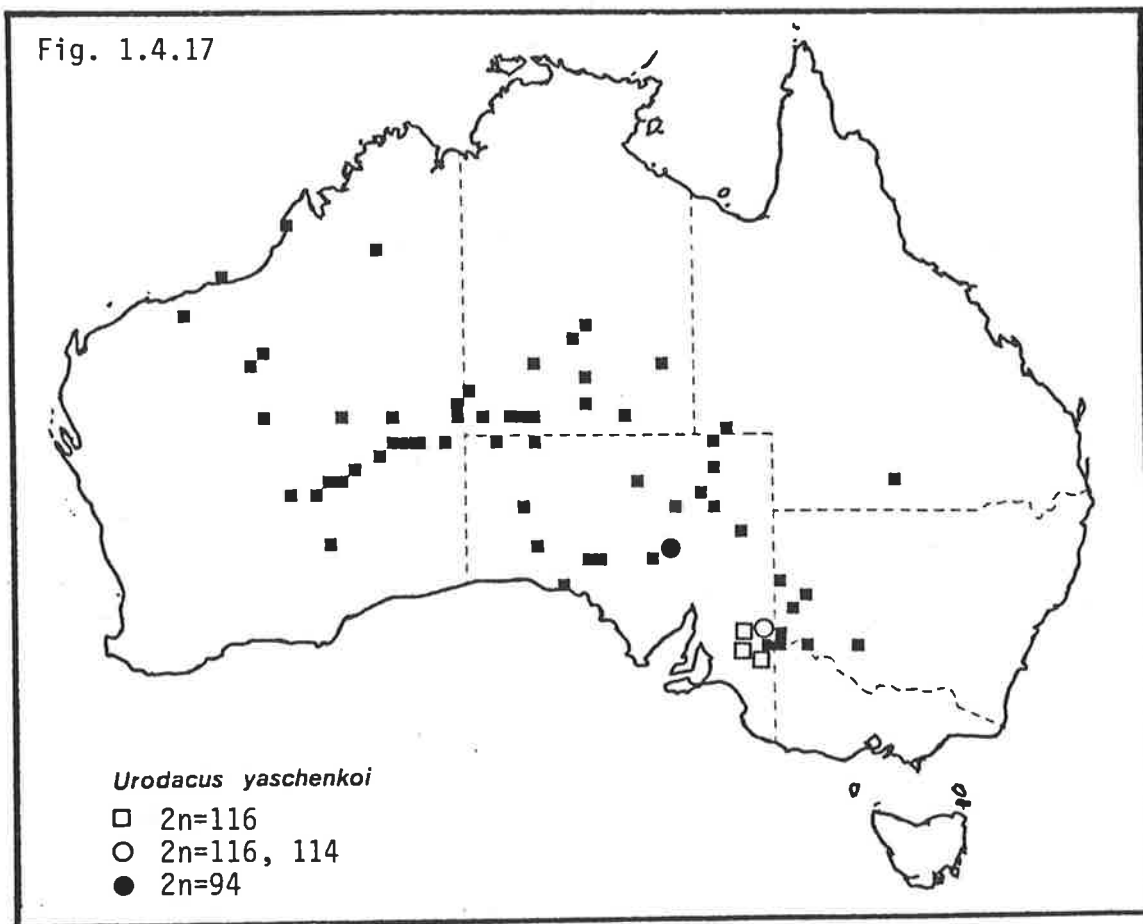
#### Karyotypic Analysis of *Urodacus yaschenkoi*.

*Urodacus yaschenkoi* is a widely distributed desert dwelling species (see Fig. 1.4.17). Table 1.4.9 shows the karyotypic data for specimens collected in the Riverland and a more arid northern region. At metaphase I, Riverland specimens generally exhibited 58 bivalents although one individual was homozygous for a Robertsonian fusion and correspondingly exhibited 57 bivalents at meiosis. Chromosomes were metacentric, acrocentric or telocentric. In contrast, the individual from Roxby Downs exhibited 47 bivalents at metaphase I. The chromosomes were mainly metacentric with a few smaller acrocentric/telocentric elements. Karyotypic change between the two populations appears to have involved fixation for a number of Robertsonian fusions. Thus, the Riverland populations have a larger number of small telocentric bivalents when compared to the Roxby Downs karyotype (Fig. 1.4.18).

Table 1.4.9

Locality and karyotypic data for *U. yaschenkoi*.

Locality	Metaphase I	2n	No. specimens ♂
Berri, S.A.	58b	116	6
Overland Corner, S.A.	58b	116	1
Waikerie 22kmN, S.A.	58b	116	1
Calperum, S.A.	57b	114	1
	58b	116	3
Roxby Downs, S.A.	47b	94	1



Small squares show museum localities.

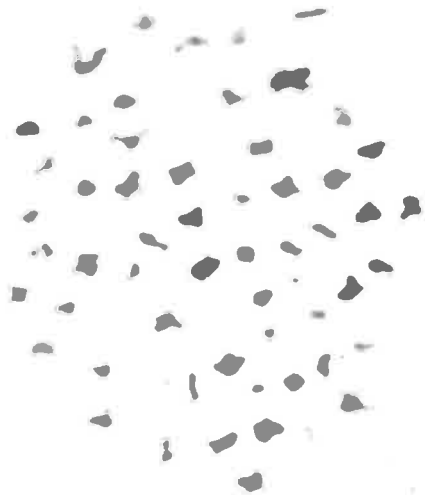
**Figure 1.4.18**

*U. yaschenkoi* metaphase I and metaphase II karyotypes.

- a Overland Corner, S.A.  $2n=116$   
58 bivalents, metaphase I
- b Metaphase II,  $n=58$
- c Calperum, S.A.  $2n=116$   
58 bivalents, metaphase I
- d Berri, S.A.  
57 bivalents, metaphase I
- e Roxby Downs, S.A. ( $2n=94$ )  
47 bivalents, metaphase I
- f Metaphase II,  $n=47$



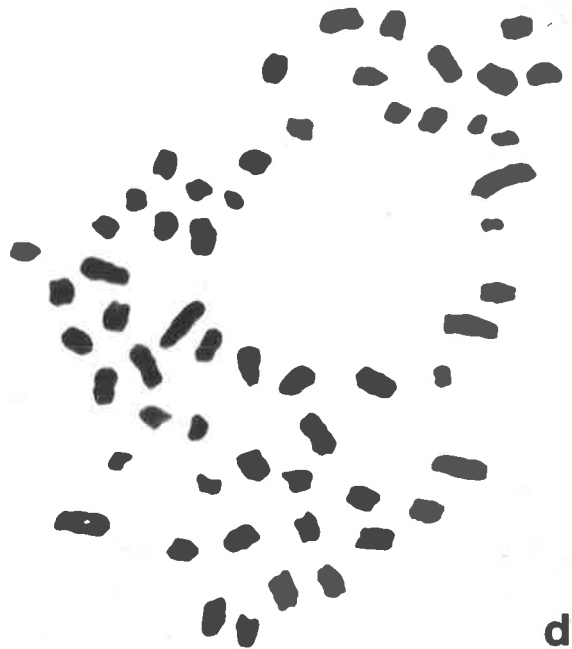
a



b



c



d



e



f



## DISCUSSION

### Mechanisms of Chromosome Change in the Genus *Urodacus*.

The predominant mechanism of chromosome change in species of the genus *Urodacus* is centric fusions. Extreme examples of interpopulation variation in chromosome number exist in *U. novaehollandiae* and to a lesser extent in *U. yaschenkoi*. The mechanism of fusion is more parsimonious than fission as it does not require the *de novo* production of centromeres as would be necessary to account for the change from  $2n=66$  to  $2n=175$  in *U. novaehollandiae*. (However, if, as suggested, the S.A. populations of *U. novaehollandiae* represent a separate species from the W.A. populations, fusion or fission in the S.A. populations are equally likely.)

*U. yaschenkoi* shows evidence of fixation for fusion differences between populations as does *U. planimanus*. The large chromosomes of *U. elongatus* suggest that chromosome fusion has occurred during the evolution of this highly specialized species. *U. manicatus* is polymorphic for a number of centric fusions, however, due to small sample sizes from many populations the level of fixation for fusion differences between populations is unknown.

The general opinion of evolutionists is that allopatric speciation has occurred in Australian Scorpionids (Koch, 1979). The Australian ancestral species was originally widely distributed, then due to a number of environmental changes a series of localised climatic and habitat zones

arose. Isolation of components of the proto-species in these zones led to speciation subsequent to which contracting, spreading and overlapping has resulted in the present day distribution of extant species.

In the *armatus* species-group *U. elongatus* is considered to have evolved from an isolate of *U. manicatus* and subsequently overlapping of the two species has recurred. On the basis of chromosome morphology this explanation does not appear unlikely (*U. elongatus*  $2n=56$ , *U. manicatus*  $2n=29-64$ ). However, more karyotypic data is required before the role of chromosome change in speciation can be discussed.

Karyotypic evidence does support however the existence of one proto-species (possibly similar to *U. novaehollandiae* (Koch, 1979)) leading to the extant *Urodacus* species. All *Urodacus* species thus far studied show very similar chromosome morphology and behaviour that would be expected if the ancestral species was achiasmate with a high chromosome number - like all other Scorpionid species world wide that have been examined.

#### Chromosomes as a Taxonomic Tool in Australian Species of *Urodacus*.

Australian scorpions from the genus *Urodacus* are taxonomically difficult to differentiate using morphological criteria. Morphology of male paraxial organs is the most useful taxonomic criteria. This makes identification of females (except by association with males of a particular species at a locality) difficult. Chromosomal morphology

and number, however, could not be considered a powerful taxonomic tool at the species level, due to the high levels of intraspecific chromosome polymorphism observed in most species.

## SECTION 5

### FEMALE MEIOSIS IN BUTHID AND SCORPIONID SPECIES

#### INTRODUCTION

In view of the achiasmate meiosis and extensive structural heterozygosity exhibited by Australian male scorpions questions arise as to the mode of meiosis in females. If reproduction in Australian scorpions is not parthenogenetic, is female meiosis achiasmate? If not, how do females cope with the various chromosomal rearrangements present at metaphase I?

The extended reproductive cycle of scorpions complicates the study of meiotic events in females. Scorpions are viviparous. They give birth to larval young after a gestation period of between 6 and 16 months, dependent on the species (Millot and Vachon, 1949). Smith (1966) calculated the gestation period for *Urodacus manicatus* to be 16 months. However, there is very little detailed information on reproduction in scorpions after fertilization has occurred.

#### Observations During This Study.

Observations made during this study on female *Lychas* showed that motile sperm were present in the oviducts from April to at least December. Young were born during March which resulted in the oviducts being cleared of sperm. Mating probably occurs soon after the young leave the

mother 7-14 days after birth. However, females dissected immediately after giving birth to young had small underdeveloped 'embryos' associated with the oviduct. It could not be determined if these were unfertilized eggs/yolk or true embryos whose development had been suppressed by the previous brood of young. If this is so (as suggested also by Piza, 1950), two rounds of young may be possible from only a single mating.

Nevertheless, there was no evidence to suggest that any scorpion species (Buthid or Scorpionid) were parthenogenetic as had been suggested for some South American Buthids (Mathieson, 1962; White, 1973). Overall the sex ratios in each species were equal with the apparent shortage of males during certain months of the year due to behavioural and ecological factors rather than an unequal sex ratio at birth (Shorthouse and Marples, 1982 and this study WPL, *L. marmoreus* data). Additionally, electrophoretic data showed that a female, *U. manicatus*, was homozygous while some of her young were heterozygous for the enzyme GPI suggesting that male gametes were involved in reproduction.

## RESULTS AND DISCUSSION

Table 1.5.1 lists the locality and species data for female scorpions examined. Usually only a few divisions could be obtained from any individual because the embryos were slow growing (as reflected in the long gestation period). Females were sampled at many different times during the year as it was unknown when female meiosis

Table 1.5.1

Locality and karyotypic data for female scorpions.

Species	Locality	Code	N	2n
<u>BUTHIDAE</u>				
<i>Isometrus melanodactylus</i>	Whitsunday Is., Qld.	QI	1	14
<i>Lychas variatus</i>	Waikerie, S.A.	WL	2	14
	Overland Corner, S.A.	OCL	11	14 (intestine and oviduct)
	80km E. Renmark, S.A.	EKL	1	-
	Whitsunday Is., Qld.	WIL	2	14
	Murtho Forest, S.A.	MFL	1	-
<i>Lychas marmoreus</i>	Ballina, N.S.W.	NSWL	1	14
	Western River Conservation Park, K.I.	WPL	13	14/15
	Emu Bay, K.I.	EBL	1	14 + 1f
	Cape de Couedic, K.I.	CCL	4	15*
	Western River, K.I.	WRL	3	14/15*
	Western Cove, K.I.	WCL	1	14
	Onkaparinga Gorge, S.A.	OGL	10	14 + 1f*
	Overland Corner, S.A.	OCL <sub>M</sub>	1	14
	Waikerie, S.A.	WL	1	14
	Belair, S.A.	BL	5	14
<u>SCORPIONIDAE</u>				
<i>Urodacus manicatus</i>	Overland Corner, S.A.	OCM	6	58+62
	Belair, S.A.	BM	10	27+29
	Emu Bay, K.I.	EBM	10	41+46
	Coast Road, K.I.	CRM	1	~60
	Pernong, S.A.	PGM	1	34
<i>Urodacus elongatus</i>	Blacks Gap, S.A.	BGE	2	~56
T o t a l			88	

N = number of specimens examined.

\* some embryos have 2n=14.

f = fragment.

occurred. There was often difficulty in separating purely oviduct material from embryonic cells which may account for some of the variation in chromosome number.

#### Family Buthidae.

Many female specimens of *L. marmoreus* contained an extra chromosome or fragment in mitotic cells obtained from the oviduct (see Fig. 1.5.1). In many of these individuals there were both 15 chromosome cells and 14 chromosome cells, probably due to the scoring of some embryonic divisions in the oviduct (i.e. all males of *L. marmoreus* have  $2n=14$  therefore male embryos will have this chromosome number).

The morphology of this additional chromosome varied between individuals from fragment sized (OGL, EBL) to a medium sized chromosome element (CCL, WRL). Whether this additional element plays a role in sex determination is unknown. However, additional elements were not identified in female specimens of *L. variatus*. These fragments may represent spontaneous aberrations that have become established in the germ-line of some females due to the holocentric nature of the chromosomes. The greater longevity of females compared to males may account for the differences between sexes in the presence of these fragments, however this does not adequately explain why they are present in *L. marmoreus* but not *L. variatus*.

**Figure 1.5.1**

**Mitotic cells from female (oviduct) *Lychas marmoreus*  
and *Lychas variatus***

***L. marmoreus***

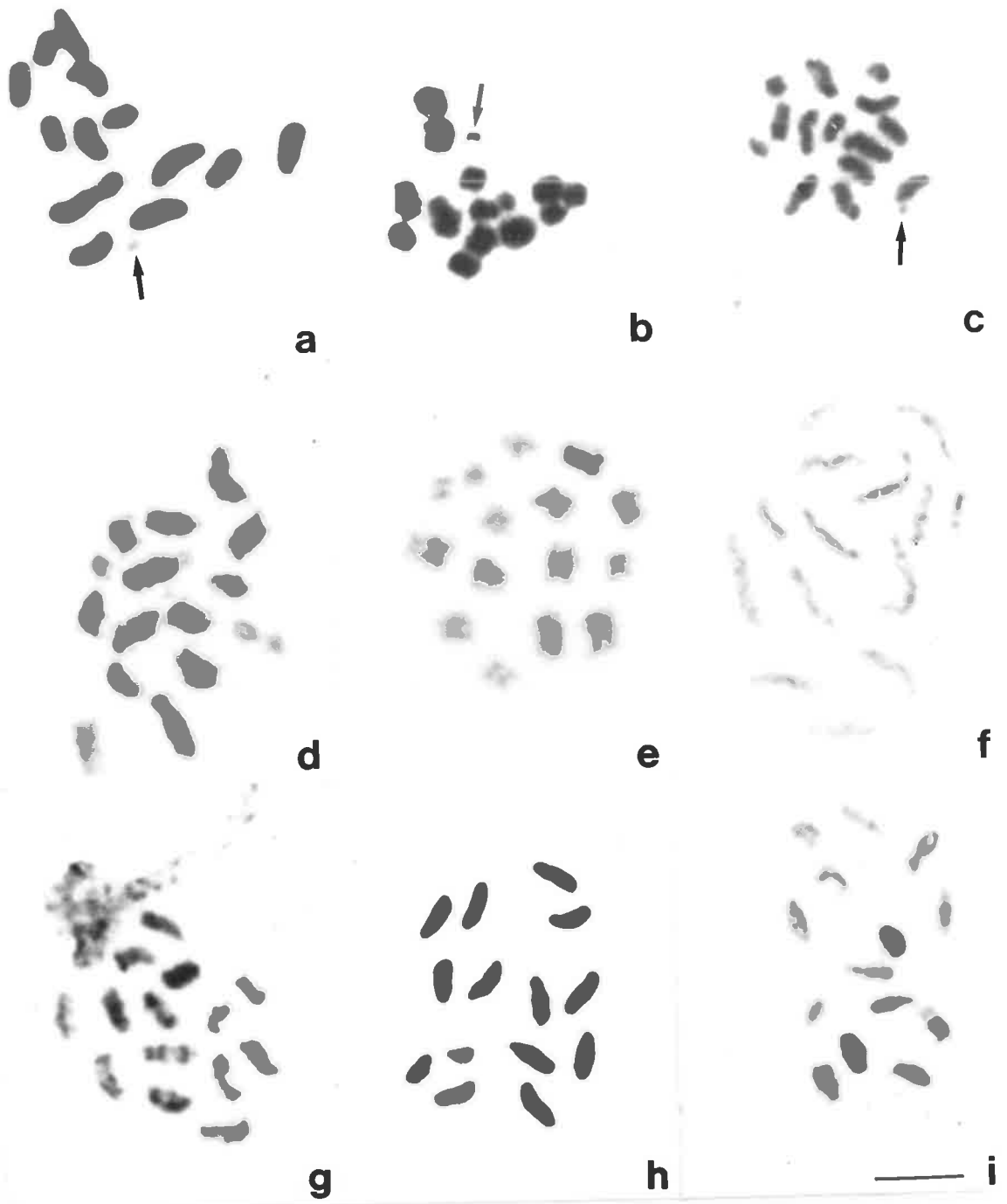
a	2n=15	(1 fragment)	OGL
b	2n=15	(1 fragment)	OGL
c	2n=15	(1 fragment)	EBL
d	2n=15	WRL	
e	2n=15	CCL	
f	2n=14	CCL - possible embryonic cell	

(fragments arrowed)

***L. variatus***

g	2n=14 (intestine)	OCL
h,i	2n=14 (oviduct)	OCL
j	mitotic karyotype	OCL♀ (not indicative of 7 bivalents)
k	mitotic karyotype	NSWL♀ (indicative of 7 bivalents)





OCL



NSWL



### Meiosis in *Lychas* Females.

No cell that could be positively identified as a metaphase I division (i.e. no cell showing interchange heterozygosity ring formation) was observed. However, Fig. 1.5.2 shows some examples of cells that could be interpreted as metaphase I of meiosis. The seven chromosomal bodies, 'putative bivalents', present in these cells were in the same size range as found in males of each species. A female (OGL) dissected a short time after she gave birth to young showed L<sup>+</sup>LMMMSS bivalents as expected for a male from this population of *L. marmoreus*. Similarly, cells from a female of the same species collected at Overland Corner showed L<sup>+</sup>LMMMSS 'bivalents'. However, the homologues in these bivalents were more closely paired than in the bivalents observed in males. They had more the appearance of metaphase II cells in males except they were larger in size. The fragment observed at mitosis in some mitotic cells were not observable in these cells.

A cell obtained from a *L. variatus* female from Overland Corner (Fig. 1.5.2) had a division that could be interpreted as anaphase I. If this interpretation is correct the chromosome sizes and conformation suggest it may have been derived from a ring of 12. It is to be expected that females will exhibit the same interchange heterozygosity as males if reproduction is not parthenogenetic and the interchanges are not involved in sex determination.

Whether the putative 'bivalents' observed represent achiasmate meiosis could not be determined. The close

**Figure 1.5.2**

**Divisions that could be interpreted as meiotic  
in female *Lychas*.**

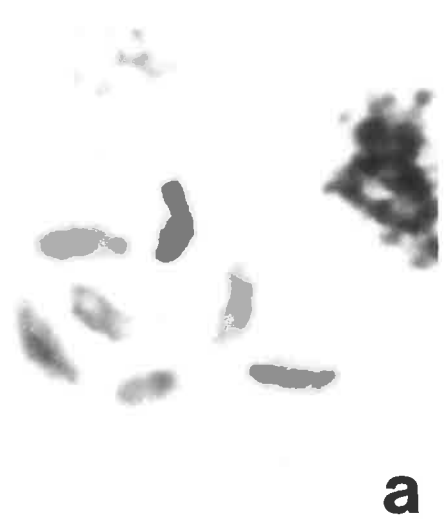
***Lychas marmoratus***

- a-d      **Metaphase I showing 7 bivalents (OGL).  
The size classes of these bodies in  
all cells was L<sup>+</sup>LMMMSS,  
i.e. the same as in males with 7  
bivalents.**

***Lychas variatus***

- e      **NSWL female showing seven bodies in  
same size classes as males L<sup>+</sup>LLLMSS.**
- f      **OCL.    Thirteen anaphase bodies.  
The size classes are not as expected  
for 7 bivalents.**

**Bar = 5  $\mu$ m.**



homologous pairing would not enable the resolution of chiasmata if they were present. Nevertheless, if these are true bivalents they provide no indication of chiasmata, leaving the possibility that meiosis may be achiasmate in both sexes of Buthid scorpions.

#### Family Scorpionidae.

Chromosome numbers in females of *U. manicatus* corresponded with the numbers found in males from the same population. Females from polymorphic populations (EBM, BM) showed intra-individual variation in chromosome number, probably the result of differences in embryonic karyotypes due to fertilization by different gametes from a (fusion/fission) heterozygous male.

A single cell was obtained from a female dissected in October (not long after fertilization) that appeared to be a metaphase I division (Fig. 1.5.3). It contained 12 bodies, 11 of which were very similar to the achiasmate bivalents found in males while one appeared to be a trivalent. The diploid chromosome number corresponding to this cell was  $2n=25$  which is lower than that found in any male (min.  $2n=29$ ). Assuming this is a complete cell, the karyotype found in the female is not impossible considering the large amount of fusion/fission heterozygosity in this population. Gametes from this female would be compatible with gametes from individual BM XVI for example.

The mitotic cells in this female ranged in number from  $2n=24+31$  so it was not possible to positively identify female

**Figure 1.5.3**

**Divisions obtained from the oviduct of *U. manicatus* females.**

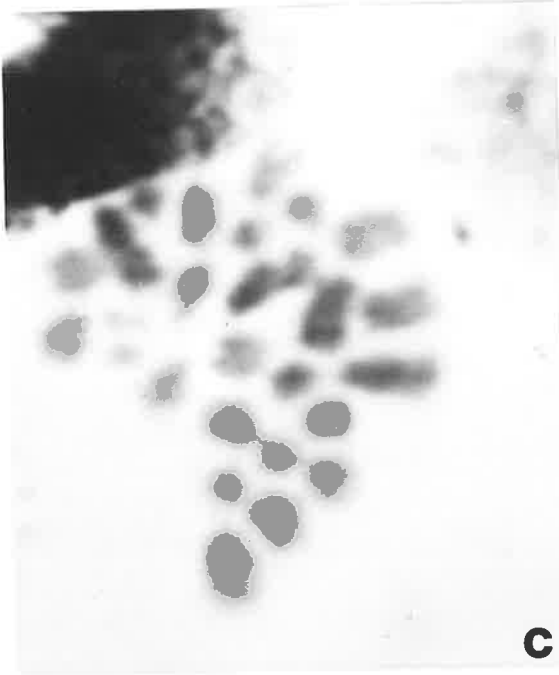
- a** Possible metaphase I cell (BMVII ♀)  
11 bivalents, 1 trivalent (arrowed) 2n=25  
Achiasmate meiosis.
- b** Mitotic cell from same *U. manicatus*  
female. 2n=29
- c** Possible metaphase I cell (OCM ♀)  
29-30 bivalents.
- d** Mitotic cell from same (OCM) female, 2n=62



**a**



**b**



**c**



**d**

cells from embryonic mitotic divisions. There is no reason to exclude this cell as a representative of achiasmate meiosis in a female. An additional cell, found in an Overland Corner female of *U. manicatus*, contained approximately 29 bodies that appeared to be bivalents. Although the preparation was 'fuzzy', again there was no evidence for chiasmata.

Therefore, in Scorpionids, evidence suggests that both males and females exhibit achiasmate meiosis. This is the first report of achiasmate meiosis in both sexes of a dioecious species. The implications of achiasmate meiosis in both sexes have been discussed briefly in the previous section dealing with the extreme chromosome polymorphism in *U. manicatus*. This problem will be discussed further in the next section.



## SECTION 6

## GENERAL DISCUSSION

## Chromosome Evolution in Achiasmate Scorpions.

Scorpion species from the families Buthidae and Scorpionidae show extreme karyotypic orthoselection; Buthids in their propensity to form interchanges and Scorpionids with their high degree of centric fusions and inversions. It appears that the interchange heterozygosity observed in Buthids is of adaptive significance. Interchanges maintain heterozygosity in inbred populations. The achiasmate meiosis further preserves heterozygosity while simultaneously ensuring regular pairing and disjunction of multiples. Although evidence from females was inconclusive, it is likely that females possess a very similar system to the males as the presence of anything but distal chiasmata would serve to disrupt the effect of interchange heterozygosity. Darlington (1958) proposed that species that evolve complex (interchange) heterozygosity sacrifice flexibility to fitness, future variability to present variation. As the system becomes more highly specialized, gametically and zygotically, crossing-over and mutation are more severely restricted and the species finds itself in an evolutionary blind alley. He suggested that the advantageous changes a species undergoes initially prove irreversible and fatal in the last, and he <sup>c</sup>ited *Rhoco discolor* (ring of 12 heterozygote) as a restricted and invariable species that

had undergone this process. Although Buthid scorpions regularly exhibit interchange heterozygosity they are an ancient, highly speciated and widespread group. Interchange heterozygosity in this holocentric group does not appear to be an evolutionary "dead end". In species where chromosomal change has been massive (e.g. South American *Tityus bahiensis*) parthenogenesis may be an alternate evolutionary pathway. However, this does not appear to have occurred in Australian species.

Conversely, the adaptive significance of centric fusion and inversion heterozygosity in Scorpionids remains unclear. Evidence suggests that females of at least one species (*U. manicatus*) also exhibit achiasmate meiosis. Thus, the only mechanism for generating variability in this species involves mutation and perhaps position effects caused by fusions and inversions. The argument that these fusions serve to enlarge linkage groups (i.e. adaptive gene arrays) does not seem appropriate as fusions do not always involve the same two chromosomes. Random fusions involving many different chromosomes have become polymorphic (perhaps due to random drift, facilitated by inbreeding) in many populations. Similarly, unless the effects of chromosome rearrangements have been vastly underestimated, White's (1973, 1978) argument that chromosome rearrangements compensate for the lack of chiasmata seems inappropriate. The amount of variation generated by centric fusions would be much lower than that generated by recombination. (The role of B chromosomes in generating variation in Scorpionid species

would also not equal that of recombination.)

Perhaps Scorpionids have reached an evolutionary dead end. Karyotypic orthoselective events have occurred and adaptively neutral rearrangements have become established by random drift. The establishment of these rearrangements is not hindered by selection against heterozygotes. Again, the achiasmate meiosis ensures regular pairing and disjunction of fusion heterozygotes while the absence of any chiasmata ensures that both paracentric and pericentric inversion heterozygotes are fully viable. Bridges and deletion/duplication gametes will not occur. The entire system (especially that in *U. manicatus*) is analogous to vegetative reproduction. Chromosomal changes may be virtually unrestricted.

#### Holocentric and Monocentric Chromosomes.

The chromosome systems found in Buthids and Scorpionids are related to the holocentric and monocentric nature of the chromosomes. Typical of most holocentric species, Buthids show low, conservative chromosome numbers. There was no evidence for agmatoploidy within or between any Australian Buthid species. No other holocentric group has evolved interchange heterozygosity. However, as a chromosomal strategy it is rare. It appears that specific events (namely inbreeding and a 'pre-adapted' chromosomal system, i.e. achiasmate meiosis) are necessary for its establishment. As suggested by White (1973), holocentric species can tolerate any breakpoints within an interchange as dicentric

chromosomes cannot be produced. This and different orientation properties may account for the very asymmetrical nature of some multiples in Buthids compared to monocentric systems.

Scorpionid species show centric fusion and inversion heterozygosity, features common in monocentric chromosome systems. Additionally, some species exhibit a large amount of interpopulational chromosome number variation. This variation has been generated by fusion/fission events and there appear to be fixed differences between populations of some species. Whether fragmentation via centric fission events has occurred cannot be determined. However, the chromosome morphology (small, dot-like) bears strong resemblance to some Lepidopteran species where agmatoploidy (as opposed to centric fission) has been proposed. Chromosome morphology is not necessarily an indicator of holocentricity, thus chromosome change in the controversially holocentric Lepidopteran groups may be similar to that in Scorpionids. In monocentric Scorpionids, if both sexes exhibit achiasmate meiosis there should be no mechanical or pairing barriers (assuming each chromosome has a centromere) to prevent chromosome fragmentation. Thus, this process may not necessarily be exclusive to holocentric species but may be tolerated in a number of monocentric species (e.g. Scorpions and Lepidoptera). In both these groups at least one sex exhibits achiasmate meiosis.

This study reveals the complexities involved in any analysis of chromosome change and the unique and complex

properties and events that influence the evolution of a chromosomal system in any group. However, it has also shown that the evolution of chromosome change in achiasmate groups differs vastly from that in chiasmate groups. Clearly, achiasmate species are worthy of greater study.

CHAPTER 2

SYNAPTONEMAL COMPLEXES DURING MALE MEIOSIS  
IN AUSTRALIAN BUTHIDS AND SCORPIONIDS

## CHAPTER 2

### SECTION 1: LITERATURE REVIEW

#### General Features of the Synaptonemal Complex.

Synaptonemal complexes, visible in electron microscope sections as filamentous tripartite structures that follow the axes of meiotic prophase chromosomes were first described in 1956 by Moses in primary spermatocytes of crayfish and by Fawcett in the spermatocytes of pigeon, cat and man. Subsequently, they have been recognised in a variety of eukaryotes including protozoa, fungi, algae, angiosperms, invertebrates, birds and mammals (see von Wettstein *et al.*, 1984 for comprehensive list). The synaptonemal complex is usually only present during the synaptic phase of the first meiotic division (i.e. zygotene, pachytene and part of diplotene) and is intimately involved in chromosome pairing, crossing over and disjunction.

The structural organization of the synaptonemal complex (i.e. its tripartite nature) shows a high degree of evolutionary stability. The higher order structure of the synaptonemal complex however, shows some variation between various taxonomic groups (Moses, 1968). For example, the ascomyceteous fungi are characterized by banded lateral elements while the insects tend to have a lattice-like central element. Other unrelated species show various abnormalities of the synaptonemal complex visualized as doubling (Moses, 1968) or thickenings of one or both lateral

elements (Gillies, 1984; Zickler and Sage, 1981). The function of most of these modifications remains enigmatic.

The complete 0.1  $\mu$ m wide pachytene synaptonemal complex composed of two lateral elements and a central element was shown to be gradually assembled during leptotene and zygotene in species where the meiotic stages are spatially ordered within the testis (reviewed by Moses, 1968). Leptotene is characterized by the formation of the lateral components (of the synaptonemal complex) between the sister chromatids of each chromosome. Most of the lateral components become attached by both ends to the inner membrane of the nuclear envelope. Completion of lateral component formation may (e.g. *Lilium*, maize) or may not (e.g. human, *Bombyx*) precede zygotene synaptonemal complex formation. At zygotene pairing of the lateral elements is initiated either at the telomeres (facilitated by their often polar association with the nuclear envelope) or in some organisms at multiple sites (Gillies, 1984; Gillies 1975 review). After completion of pairing (early pachytene) the prominent 'bouquet' (polar association of telomeres) is maintained until mid-late pachytene when the synaptonemal complexes become evenly distributed in the nucleus (review - see Westergaard and von Wettstein, 1972; von Wettstein et al., 1984). The details of the subsequent disaggregation and elimination of synaptonemal complex material through diplotene and metaphase varies markedly between species. Polycomplexes, multiple arrays of stacked complexes, usually present at diplotene and thought to be remnants of discarded synaptonemal complexes,



have been described in a number of unrelated species: mosquito oocytes (Fiil and Moens, 1973), gooseneck barnacle (Dudley, 1973), arachnids (Wettstein and Sotelo, 1965), *Drosophila* oocytes (Rasmussen, 1975), *Mesostoma* (Oakley and Jones, 1982), yeast (Zickler and Olson, 1975). However, the presence of polycomplexes during pre-pachytene stages in *Ascaris* suggests that in some species they may not just represent discarded complexes, but may act as transient storage sites for synaptonemal complex material (Fiil et al., 1977; Fuge, 1979).

#### Role in Crossing Over.

The synaptonemal complex is a pre-requisite for efficient meiotic recombination but its presence does not guarantee the occurrence of crossing over. Evidence for this comes from species where chiasmata are confined to only one sex. In *Drosophila melanogaster* males, where recombination (chiasmata) does not occur, synaptonemal complexes are absent while in the chiasmate female synaptonemal complexes are present (Rasmussen, 1973; Moens, 1978). Furthermore, the homozygous C(3)G mutant in female *Drosophila* prevents recombination by causing suppression of synaptonemal complex formation (Rasmussen, 1975). However, in the silkworm *Bombyx mori*, synaptonemal complexes are present at pachytene in both the chiasmate male and the achiasmate female (Rasmussen and Holm, 1980). In the achiasmate *Glossina austeni* synaptonemal complexes are absent, while in *Glossina morsitans morsitans*, a species where occasional

chiasma-like configurations occur, synaptonemal complexes of restricted length are present (Craig-Cameron *et al.*, 1973). Additionally, in *Stethophyma grossum* and *Mesostoma ehrenbergii ehrenbergii* spermatocytes, chiasmata and synaptonemal complex formation seem to be restricted to specific regions of the chromosomes (Fletcher, 1978; Wallace and Jones, 1978; Oakley and Jones, 1982). Using a causal argument, localized synaptonemal complex formation has the effect of localizing chiasmata.

The 'recombination nodule' described by Carpenter in 1975 is a modification of the synaptonemal complex thought to represent the site of crossing over. These electron dense nodes are usually found associated with the central element. The number and position of these nodules has been correlated with the number and position of chiasmata in species studied. They are not present or are present in a modified form in achiasmate species (von Wettstein *et al.*, 1984). Recombination nodules have also been shown to be sites of pachytene DNA synthesis (Carpenter, 1981). The method by which these nodules facilitate recombination is unknown, however, Moens (1978) has suggested that they allow DNA cross-connections between the lateral elements of the synaptonemal complex. The presence in many species of remnants of the synaptonemal complex between metaphase I bivalents at the site of chiasmata is well documented. This also implies a direct role for the synaptonemal complex in recombination (Moens, 1978). Stack (1984) has studied the structure of the synaptonemal complex in euchromatin and

heterochromatin in two plant species and mouse. He suggested that slight structural differences may be related to the observed lack of chiasmata in heterochromatic regions. Carpenter (1975) previously reported the absence of recombination nodules in heterochromatic regions.

#### Role in Disjunction.

Chiasmata which are also sites of synaptonemal complex remnants at metaphase I, are thought to maintain pairing of homologous chromosomes in bivalents until anaphase I segregation. In species without chiasmata some other mechanism to maintain pairing up to anaphase I must operate. The mechanism in achiasmate *Bombyx* oocytes has been described in detail by Rasmussen (1977). A modified synaptonemal complex maintains the co-orientation of the homologues up to anaphase I. These modified complexes are left on the metaphase plate as the homologues separate, to be later expelled from the cell as 'elimination chromatin'. Synaptonemal complexes are maintained up to anaphase I in other species with achiasmate meiosis: *Bolbe* (Gassner, 1969), Lepidoptera (Sorsa and Soumalainen, 1975), and Carob moth (Morag *et al.*, 1982). However, this mechanism of pairing maintenance is not universal. In achiasmate *Mesostoma* females (Oakley, 1982) and in *Panorpa communis* (Welsch, 1973) synaptonemal complexes are lost before metaphase. Oakley has proposed that the observed twisting of synaptonemal complexes may account for the maintenance of pairing in *Mesostoma*.

Moens and Church (1979), in *Locusta*, report the presence of synaptonemal complex material at metaphase I, not only at chiasma sites but also between sister chromatids. They proposed that the synaptonemal complex functions in sister chromatid cohesion and thereby regulates bivalent structure and disjunction. This view is shared by Maguire (1974, 1978, 1979) who had previously proposed it to account for the pairing behaviour in desynaptic maize strains. In the hemipteran *Triatoma infestans*, the autosomes form synaptonemal complexes and co-orientate while the sex chromosomes are achiasmate, do not form axes and divide equationally at anaphase I. Solari (1979) suggested that synaptonemal complexes do not form between the sex chromosomes as they would be likely to cause sister chromatid cohesiveness which would inhibit separation of the sister chromatids at anaphase I. Contrary to this, in achiasmate *Mesostoma ehrenbergii ehrenbergii* oocytes sister chromatid cohesiveness is maintained until metaphase II in the absence of synaptonemal complex material (Oakley, 1982). Thus while synaptonemal complexes may be involved in sister chromatid cohesiveness, their presence is not essential.

#### **Methodology.**

There are two methods for visualizing the synaptonemal complex at meiotic prophase; either by 3D reconstructions of serial sections observed under the electron microscope (Gillies, 1972) or by the surface spreading of whole nuclei on an air-liquid interface (hypophase) and subsequent

observation under the light or electron microscopes (Counce and Meyer, 1973; Fletcher, 1979; Dresser and Moses, 1980).

The method of sectioning enables the three dimensional arrangement of the chromosomes to be studied and consequently the various stages of prophase can be sequentially differentiated. However, this method is time consuming and allows only a limited number of cells to be studied. Conversely, the spreading technique is quick and although three dimensionality is lost the synaptonemal complexes are preserved whole, there is very little background material and a large number of cells can be analysed. The sectioning technique has provided most of the high resolution ultra-structural information. It was originally used by Wettstein and Sotelo (1967) to show that the number of synaptonemal complexes equalled the haploid number of autosomes and that there was a length correspondence between synaptonemal complex and mitotic chromosome relative lengths (i.e. the first synaptonemal complex karyogram). However, the surface spreading technique now enables the quantitative analysis of large numbers of cells and is especially useful in studying individuals heterozygous for chromosome re-arrangements as the lateral elements show the pairing relationships of the homologues with diagrammatic clarity (Poorman *et al.*, 1981).

#### **Synaptic Behaviour of Chromosome Rearrangements.**

The behaviour of chromosomal axes during synapsis in translocation heterozygotes has been demonstrated in the mouse by serial section reconstruction of Searle's

translocation (Solari, 1971) and by surface microspreading of three reciprocal translocations (Moses *et al.*, 1977a). Breakpoint analyses of these autosome/autosome and two X/autosomes translocations in microspread preparations show reasonable agreement with banded chromosome data. These analyses have been extended to other cytologically and genetically defined rearrangements in the mouse; inversions (Moses *et al.*, 1978), duplications (Poorman *et al.*, 1981), and to heteromorphic trivalents derived from Robertsonian fusion/fission differences in *Lemur* hybrids (Moses *et al.*, 1979). These studies have revealed additional properties of the synaptonemal complexes as well as information on the origin of chromosome rearrangements.

Moses *et al.* (1979) made the following observations of the synaptonemal complex trivalents expected (from G-band analysis of chromosomes) and observed in spermatocytes of the *Lemur* hybrids:

- a) Each of the acrocentric long axes is synapsed with an arm of the metacentric axis, confirming the homology predicted from banding similarities.
- b) At late zygotene, the acrocentric short arms which are heterochromatic and presumably non-homologous are the last to pair producing at late pachytene a short synaptonemal complex side arm. Moses took this non-homologous pairing as an example of synaptic adjustment (described later).

c) The kinetochores of the acrocentrics always lie together on the same side of the metacentric kinetochore (*cis* configuration), implying a single pairing face on the metacentric axis. This observed *cis* pairing configuration would predispose the trivalent to correct meiotic disjunction which was consistent with the fertility regularly observed in such hybrid Lemurs.

The pachytene analysis of tandem duplications, pericentric and paracentric inversions in the mouse has revealed in detail another aspect of pairing control in synaptonemal complexes termed 'synaptic adjustment' (Moses *et al.*, 1979).

Moses and Poorman (1981) carried out a detailed analysis of prophase using surface spread cells in a mouse heterozygous for a tandem duplication. A method first described in Chinese hamster by Moses *et al.*, (1977b), using sex chromosome pairing and nucleolus size, enabled them to identify the different stages of prophase such that they could identify zygotene and five stages of pachytene accounting for approximately 50% of the time scale of prophase. They observed a number of events which bore remarkable similarities to the two-stage pairing hypothesis of Grell (1962): exchange and distributive pairing the latter of which may lead to non-homologous pairing in the absence of crossing over.

At zygotene Moses and Poorman observed a heteromorphic synaptonemal complex containing a lateral element that buckled out into an unpaired loop as a consequence of the

added length of the duplication. This is exactly what was expected if synaptonemal complex formation at this stage was restricted to homologous regions, hence they termed it homosynapsis. In the last half of pachytene however, no buckles were observed only simple synaptonemal complexes with lateral elements of equal length. Intermediate stages (i.e. buckles of different size and conformation) were found throughout the first half of pachytene. They attributed this change in pairing to synaptic adjustment; i.e. shortly after homosynapsis is complete synaptic adjustment begins. Through a process of desynapsis of regions close to the duplication end points and twisting of the longer lateral element, the lateral elements equalize as the long axis shortens, leading to complete synaptonemal complex formation and non-homologous pairing (heterosynapsis) for the duplicated region. Thus synaptic adjustment in this instance was initiated by a localized instability of the homosynapsed condition leading to desynapsis then restoration of the synaptonemal complex by heterosynapsis. Moses *et al.* (1982) studied the same phenomenon in two paracentric inversions also in mouse. Synaptic adjustment was found to occur but the process differed slightly from that found in the duplication in that there was no necessity to equalize unequal lateral elements and no observable desynapsis occurred ahead of heterosynapsis. In the inversion heterosynapsis seemed to result from a process akin to gradual stretching of the loop by pulling at either end. Although synaptic adjustment is an obvious feature in these two mouse



systems, there are exceptions.

Ashley *et al.* (1981) studied a small pericentric inversion in the sand rat *Psammomys obesus*. Rather than form a loop during early pachytene, the inverted region remained unsynapsed until later in pachytene when the region was observed to be heterosynapsed (straight pairing with unaligned centromeres characteristic for an adjusted pericentric inversion). Ashley postulated that due to the small size of the inversion topological considerations prevented loop formation.

Chandley (1982), studying two overlapping paracentric inversions in the mouse, observed that different inversions in the mouse behave differently. In mice heterozygous for either one of the inversions, synaptic adjustment is complete, however, in the more complex double heterozygote, 90% of cells exhibited loop formation, suggesting the loop was not eliminated as pachytene progressed. There is no definitive reason why some inversions are adjusted while others seem not to be, but variously the size, position and complexity of the loops and the position of chiasmata have been suggested as causal mechanisms (Chandley, 1982; Moses *et al.*, 1982).

In maize, there is very little evidence that synaptic adjustment occurs. Maguire (1981), using light microscope preparations, studied a paracentric inversion from pachytene through to diplotene. In all stages she observed three pairing types: homosynapsis (loop formation), heterosynapsis (straight pairing), and synaptic failure. Gillies (1981)

studying maize synaptonemal complexes found similar results. There was no correlation between chromosome length (a measure of pachytene stage) and degree of homologous synapsis; he observed synaptic failure and heterosynapsis. These two pairing types (heterosynapsis and synaptic failure) are also common to some grasshoppers and chironomids heterozygous for inversions (Martin, 1967; Nur, 1968; White, 1973; Fletcher and Hewitt, 1978; John, 1983). As yet detailed pachytene synaptonemal complex analyses have not been carried out to determine whether these pairing types are the result of synaptic adjustment. However, in grasshoppers, they appear to prevent chiasmata formation in the inverted regions.

The role of chiasmata, if indeed it has a role in synaptic adjustment, is unclear in the cases discussed so far. However, in cases that involve pairing and synaptonemal complex formation between more than two homologous or partially homologous chromosomes, correction of pairing is dependent almost entirely on timing and positioning of chiasmata.

Rasmussen and Holm (1979) have carried out a number of experiments involving three dimensional reconstructions of nuclei from the silkworm *Bombyx mori*, including comparisons between male and female (chiasmate and achiasmate) autotetraploids. They demonstrated that in the achiasmatic female the mean number of bivalents (36.7) and quadrivalents (8.4) present at early pachytene was reduced (to 51.9 bivalents and 0.9 quadrivalents) by late pachytene due to homologous synaptonemal complex formation followed by a

correction phase (i.e. two stage pairing). Thus, there seems to be a drive towards two by two or bivalent associations (in many cases independent of homology) so that by metaphase I only bivalents are observed. This same phenomenon is observed in triploid oocytes where only bivalents and univalents (often non-homologously paired) are found at metaphase I (Rasmussen, 1977). The situation differs in the tetraploid chiasmate male which has a mean frequency of 6.7 quadrivalents and occasional trivalents and univalents at metaphase I. Reconstructions have also confirmed the presence of quadrivalents at pachytene in these spermatocytes (referenced in von Wettstein *et al.*, 1984). Thus, the occurrence of crossing over in the male prevents the transformation of multivalents into bivalents during pachytene and the chromosome associations formed during the homologous pairing phase at zygotene are preserved up to metaphase I.

An analysis of the preservation from pachytene to metaphase of a translocation quadrivalent in man has also been carried out by Holm and Rasmussen (1978). Again, the frequency and position of crossovers appeared to determine whether the quadrivalent is maintained or lapses into two theoretically heteromorphic bivalents. The crossover data has also been correlated with the position of transient recombination nodules in the quadrivalent. Similarly, translocation heterozygotes in the fungus *Coprinus* (Holm *et al.*, 1981) and the mouse (Ashley *et al.*, 1982; von Wettstein *et al.*, 1984) show a correlation between presence of

crossovers and absence of synaptic adjustment. However, many of these correlations between expected crossover frequency and quadrivalent formation are based on only a small amount of data. The relevance of this is discussed further in Section 3.

Extensive studies have also been carried out by a number of workers in the allohexaploid bread wheat *Triticum aestivum* (Riley and Kempanna, 1963; Hobolth, 1981). Hobolth (1981) showed that in *Triticum* extensive multivalent formation, including univalent foldback pairing, occurred at zygotene while pachytene showed only bivalents, a requirement for disomic inheritance in polyploids. This behaviour requires crossing over to be delayed until the completion of pairing correction. Hobolth postulated that the *Ph* gene on the long arm of chromosome 5B served this function. A similar type of two stage pairing has been observed in triploid *Lolium* hybrids where pairing appeared to be influenced by the presence of a B chromosome, thought to have a function similar to the *Ph* gene in wheat (Jenkins, 1985).

The presence of multivalents at zygotene demonstrates that the formation of synaptonemal complex between non-homologous or homeologous chromosome regions is a common occurrence. Non-homologous fold back pairing has been shown to be of common occurrence in haploids: maize (Ting, 1973), tomato (Menzel and Price, 1966), barley (Gillies, 1974), *Physarum* and many other species reviewed by Lie and Laane (1982). Thus, in species where the chromosome complement is rearranged in some way or is aneuploid for particular

segments, a common feature of synaptonemal complex formation and synapsis is the ability of non-homologous regions to pair (heterosynapsis) during pachytene whether or not there is a preceding visible period of homologous pairing (homosynapsis) at zygotene. The evolutionary significance of this process remains unclear.

## SECTION 2

### MATERIALS AND METHODS

A number of different techniques and modifications were used to obtain synaptonemal complex spreads from scorpion testicular material for both the light and electron microscopes. The larger Scorpionid species provided enough material to allow routine use of the air/water interface spreading technique. However, many of the smaller Buthid species provided so little material (one drop of testicular cell suspension) that this method was not often successful. Therefore various modifications of Speeds (1982) or Gillies' (1981) settling techniques were utilized.

#### Light microscope preparations.

The technique described by Fletcher (1979) was used except the spreading solution was that of Jones and Wallace (1980) developed for grasshopper spermatocytes.

The solutions used were as follows:

#### 1. Spreading Solution.

0.3M Sucrose in ddH<sub>2</sub>O. Freshly prepared and filtered before use.

#### 2. Fixative

4% paraformaldehyde with 3.4% Sucrose in ddH<sub>2</sub>O.

After dissolving the Sucrose in 70 mls of ddH<sub>2</sub>O, the paraformaldehyde was added and the solution made up to a

final volume of 100 mls with ddH<sub>2</sub>O . This solution was then slowly heated (with stirring) to approximately 65°C. Six drops of NaOH were then added and stirring continued until all the paraformaldehyde had dissolved. After cooling to room temperature, the fixative was adjusted to pH 8.5-9 with sodium tetraborate buffer.

### 3. Photoflo. (Rinsing Solution)

0.4% photoflo, freshly prepared and filtered  
(pH adjusted to 8.5-9 using the borate buffer).

Stock Solution:- 4% Kodak "Photoflo 600" in ddH<sub>2</sub>O.

Suspensions of testis cells were made by macerating at least one half of the tubule present in 1-3 drops of medium (RPMI 1640 culture medium supplemented with 20% foetal calf serum). Using a pasteur pipette, one drop of this suspension was gently touched onto the surface of the spreading solution which had been poured into watchglasses previously sprayed with matte black enamel paint.

After approximately 30" - 1 min. the cells were picked up on to a clean statically charged microscope slide. Static was achieved by rubbing the surface of the slide with a piece of silk.

Slides were left on a flat surface for 10 min. to allow the cells to settle, then fixed for 10 min. in a Coplin jar.

Slides were then rinsed in photoflo for 30 sec. and allowed to air dry.

### Staining.

Slides were stained overnight in 70% silver nitrate in ddH<sub>2</sub>O at 60°C in a moist chamber. 2-3 drops of silver were placed on each slide and coverslips were sealed with rubber gum for overnight incubation. After staining, coverslips were removed and the slides were washed well in ddH<sub>2</sub>O, air dried and mounted in depex.

### Electron Microscope Preparation.

Spreads for electron microscopy were made by a modification of the method of Solari (1980).

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

1. The spreading solution used was 0.5% NaCl in ddH<sub>2</sub>O.
2. The fixative did not contain Sucrose and for some preparations 0.05% SDS (sodium dodecyl sulphate) was added.
3. The microscope slides were coated with a plastic film; Slides were dipped in a solution of xylene and Gurr's depex and hung on clips to dry. To obtain the necessary film thickness (ideally one producing a silver interference colour), approximately 8 drops of depex were added to 50-60 mls of xylene.
4. The plastic coating is charged, thus it was not necessary to rub the slides to produce a static charge. The slides could be transferred directly to fixative immediately after the cells had been picked up.





5. Staining was with Millipore (0.22  $\mu\text{m}$ ) filtered ethanolic phosphotungstic acid (PTA). This was prepared by mixing a 3:1 solution of 95% ethanol : 4% PTA in ddH<sub>2</sub>O. Slides were stained for 10 mins, rinsed well in 95% ethanol and air dried.

The plastic film was removed from the slide by scraping around the edges with a scalpel blade and floating the plastic film off on to ddH<sub>2</sub>O. Copper-rubidium EM grids (100 mesh) were then placed on the floating film and the film and grids lifted from the water with a lens tissue. After air-drying the grids could be examined with the electron microscope.

#### **Air-Drying (Settling) Technique.**

This method was modified from Speed's (1980) technique for mouse ovary cells and Gillies (1981) settling technique for maize.

The technique is the same for both the light and electron microscopes except that plastic coated slides were used for electron microscope analysis.

A suspension of testis cells was made as described for the light microscope. One drop of this suspension was placed on a clean or plastic coated slide with one drop of 0.2M Sucrose. This mixture was gently spread over the slide and allowed to settle and dry for at least 30 minutes. The slide was then fixed, rinsed in photoflo, stained and prepared for either the EM or light microscopes as described in the previous section.

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

#### Species Examined.

The species examined, their location and the method used are listed in Table 2.2.1. In total, over 100 specimens were examined, however, only those that yielded clear results are listed.

Table 2.2.1

Locality data for species examined and methods used  
for synaptonemal complex analysis.

Species	Location	Code	Method
<b>BUTHIDAE</b>			
<i>Lychas marmoreus</i>	Flinders Chase, K.I.	FCL	EM <sup>1</sup>
	Belair, S.A.*	BL	LM <sup>2</sup> EM <sup>2</sup>
	Onkaparinga Gorge, S.A.*	OGL	LM <sup>1,2</sup> EM <sup>1,2</sup>
	Western River, K.I.*	WRL	LM <sup>2</sup> EM <sup>2</sup>
<i>Lychas variatus</i>	Overland Corner, S.A.*	OCL	EM <sup>1</sup>
	Jabiluka Billabong, N.T.	JL	EM <sup>2</sup>
	Murtho Forest, S.A.	MFL	EM <sup>1</sup>
<b>SCORPIONIDAE</b>			
<i>Urodacus manicatus</i>	Belair, S.A.*	BM	LM <sup>1</sup> EM <sup>1</sup>
	Overland Corner, S.A.*	OCM	LM <sup>1</sup> EM <sup>1</sup>
	Swan Reach, S.A.	SRM	EM <sup>1</sup>
<i>Urodacus novaehollandiae</i>	Experence <sup>a</sup> , W.A.	EN <sub>I</sub>	EM <sup>1</sup>
<i>Urodacus planimanus</i>	Perth, W.A.	WAP <sub>I</sub>	EM <sup>1</sup>

\* = More than one animal studies.

<sup>1</sup> = Spread.

<sup>2</sup> = Settled.

## SECTION 3

## SYNAPTONEMAL COMPLEXES IN BUTHID INTERCHANGE HETEROZYGOTES

Synaptonemal complexes were obtained from males of two species of Buthid scorpions; *Lychas marmoreus* and *Lychas variatus*. The metaphase I karyotypes and the individuals studied are listed on Table 2.3.1. The results in this section are presented in increasing order of complexity with respect to the number of interchanges in each animal and the degree of synaptic failure they exhibited.

## RESULTS

## General Morphology of Buthid Synaptonemal Complexes.

The complete synaptonemal complex of Buthid species is composed of two lateral elements and a central element - common for almost all synaptonemal complexes. Although lateral elements have a lumpy appearance at pachytene, there are no localized kinetochore proteins visible, in keeping with the holocentric nature of the chromosomes. Additionally, there are no obvious telomeric nuclear membrane attachment plaques which are a common feature of mammalian synaptonemal complexes. The nucleolus is not a localized darkly staining region showing a consistent location on a particular synaptonemal complex. Rather, it is diffuse, composed of ribonuclear proteins visible with PTA staining as a granular lattice usually associated with the clumped ends of pachytene synaptonemal complexes.

Table 2.3.1

Individuals of *L. marmoreus* and *L. variatus* investigated using synaptonemal complex analysis.

Species	Code	2n	Karyotype
<i>Lychas marmoreus</i>	BL <sub>VI</sub>	14	7 bivalents
	OGL <sub>X</sub>	14	7 bivalents*
	FCL <sub>I</sub>	14	Ring 10 + 2 bivalents
	WPL T <sub>4</sub> II	14	Ring 10 + 2 bivalents*
	T <sub>4</sub> III		" " " "
<i>Lychas variatus</i>	JL <sub>I</sub>	14	7 bivalents
	MFL <sub>I</sub>	16	Ring 4 + 6 bivalents*
	OCL <sub>I</sub> , II,IV,VI	14	Ring 12 + 1 bivalent

\* exhibit synaptic failure (as described in Chapter 1).

### Meiotic Stages Observed.

It was not possible to assign accurately stages of prophase to the cells observed. However, variation in synaptonemal complex morphology and development indicated that a range of prophase stages were present. To simplify analysis the following broad categories were assigned:

#### Stage 1. Pre/Early Zygotene. (Figure 2.3.1a)

In these cells the synaptonemal complexes were polarized i.e. clumped in a particular region of the cell with their ends associated. Occasionally, a remnant nuclear membrane was visible. The synaptonemal complexes were usually incomplete with only the ends showing the typical tripartite structure. The remainder of the complex was diffuse or often not visible suggesting that at this stage either pairing or lateral element formation was incomplete. The most obvious feature of these cells was the large amount of darkly staining material associated with the ends of the complexes giving them a dark, thickened appearance. If all ends are paired the number of thickened regions should correspond to the diploid chromosome number.

#### Stage 2. Zygotene-Pachytene. (Figure 2.3.1<sup>c,d</sup>)

At this stage the ends of the synaptonemal complexes were no longer polarized and there was no darkly staining material associated with the ends, i.e. the lateral thickenings had disappeared. Pairing and synaptonemal complex formation was almost or totally complete such that bivalent and multivalent associations were clearly visible.

**Figure 2.3.1**

**Stages of meiotic prophase identified using  
synaptonemal complex analysis.**

**a,b** Stage 1 cells.  
Polarized thickened ends.

**c,d** Stage 2 cells.

(c) Complete usually separate synaptonemal complexes.

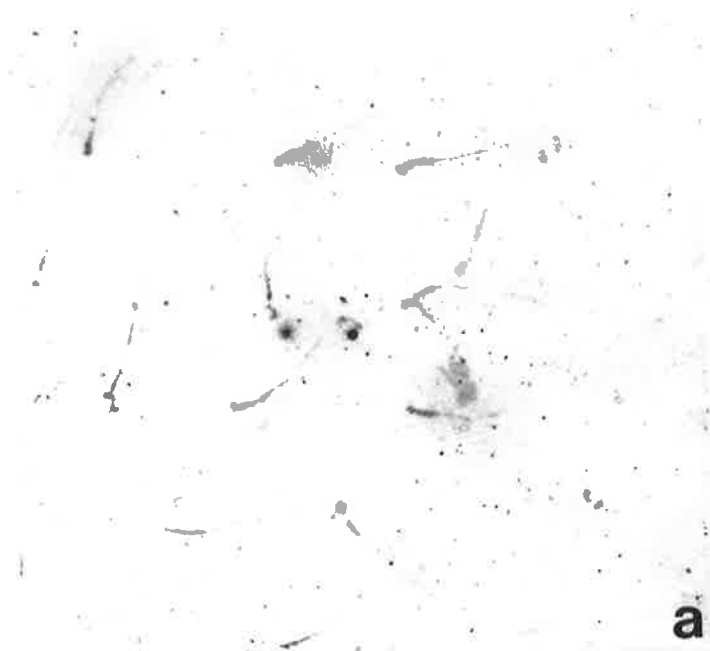
(d) Earlier stage where interstitial pairing not yet complete.

**e,f** Stage 3 cells.

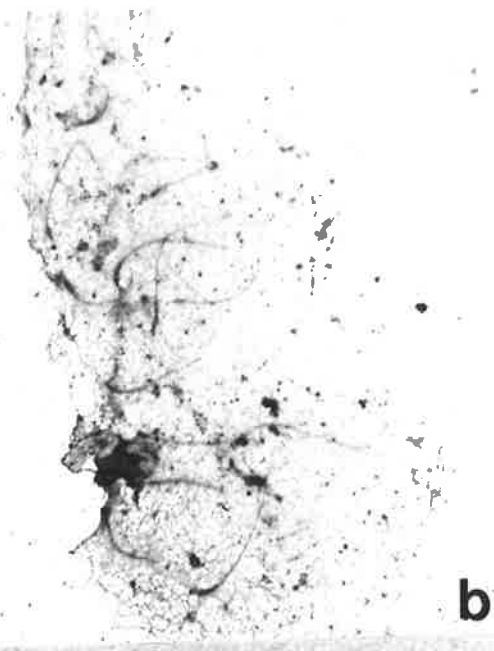
Parallel thickened elements with cross-connections.

Configuration consistent with metaphase I (4 + 6 bivalents).

(f) enlargement of quadrivalent and bivalent.



**a**



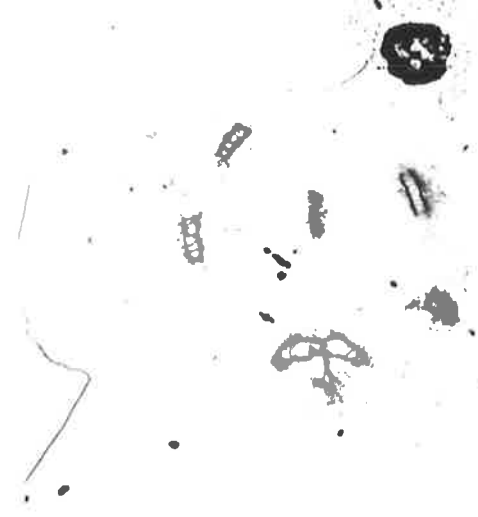
**b**



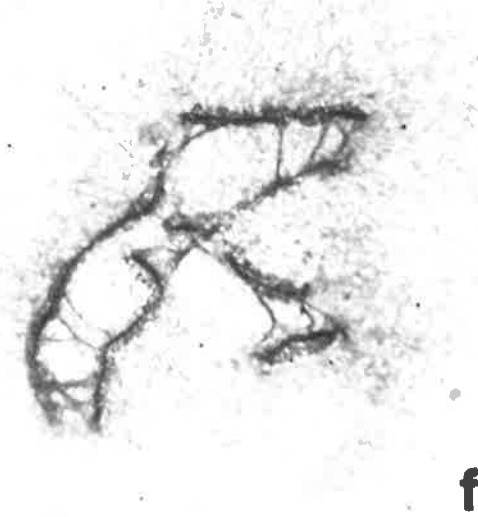
**c**



**d**



**e**



**f**



Any unpaired lateral elements were also visible.

The presence of paired end segments in pre/early zygotene cells (Stage 1) and the diffuse nature of the remainder of the synaptonemal complex/lateral elements suggests that pairing is initiated at the ends of the chromosomes. Figure 2.3.1<sup>d</sup> shows an example of a synaptonemal complex where only the interstitial region is unpaired. This observation and the absence of short segments of synaptonemal complex (apart from the end segments) in later stages suggests that pairing is accomplished by gradual "zippering" from both ends.

Stage 3. Metaphase. (Figure 2.3.1<sup>e,f</sup>)

Occasionally cells exhibiting thick, PTA positive, doubled structures that could be interpreted as modified lateral elements with filamentous cross-connections were observed. The number and configuration of these structures were consistent with the metaphase I karyotype of the individual from which the cell was obtained.

The meiotic cells of the animals listed on Table 2.3.1 were categorized into the stages as described and analysed with respect to the following features:

Stage 1 Cells: The number of thickened ends per cell were scored.

Stage 2 Cells: The number of whole synaptonemal complexes present were scored. These cells were also scored for the presence of unpaired lateral elements and regions indicative of pairing partner exchange.

Stage 3 Cells: These were compared with conventional, metaphase I, light microscope preparations.

### 7 Bivalent Karyotypes.

*Lychas variatus* (JL I)

*Lychas marmoreus* (BL VI)

Both these animals exhibited 7 bivalents in all metaphase I cells of conventional Giemsa stained cytological preparations. Only stage 2 (zygotene-pachytene) cells were observed in these animals. The synaptonemal complexes were completely paired (i.e. 7 entire complexes) with nucleolar material associated with their end regions (Fig. 2.3.2). No changes of pairing partner or unpaired lateral elements were observed.

### Ring 10 and 2 Bivalent Karyotype.

*Lychas marmoreus* (FCL I)

The metaphase I configuration in this animal was consistently a ring of 10 chromosomes plus two bivalents. Very few cells were obtained for synaptonemal complex analysis. However, stage 1 cells exhibited 14 thickened ends while in stage 2 cells there were 11-12 separate synaptonemal complexes with no evidence of partner exchange.

### Ring 12 and 1 Bivalent Karyotype.

*Lychas variatus* (OCL I, II, IV, VI)

Table 2.3.2 gives the results for the 22 prophase cells analysed from individuals that consistently exhibited a ring

**Figure 2.3.2**

*Lychas marmoreus*, Belair (BL VI).

(7 bivalents)

Stage 2 cell (and interpretation) showing 7  
complete synaptonemal complexes.

Dotted region (ribonucleolar proteins).

Bar = 5  $\mu$ m.

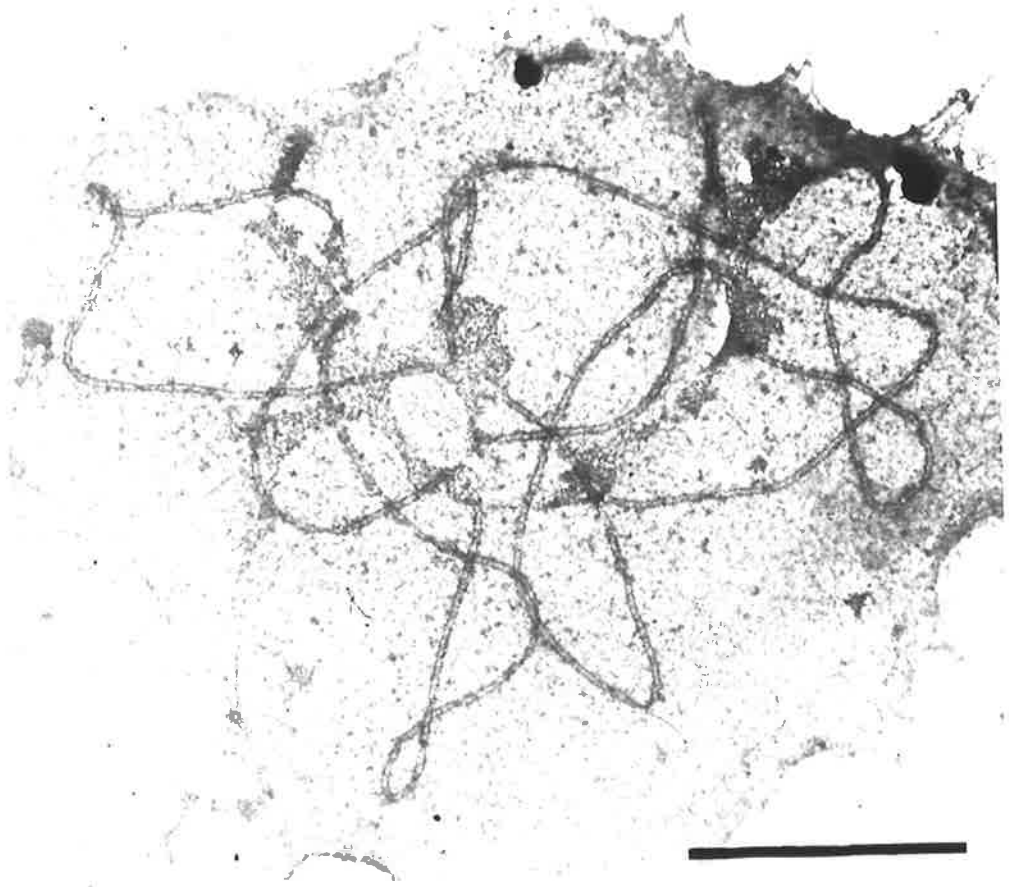
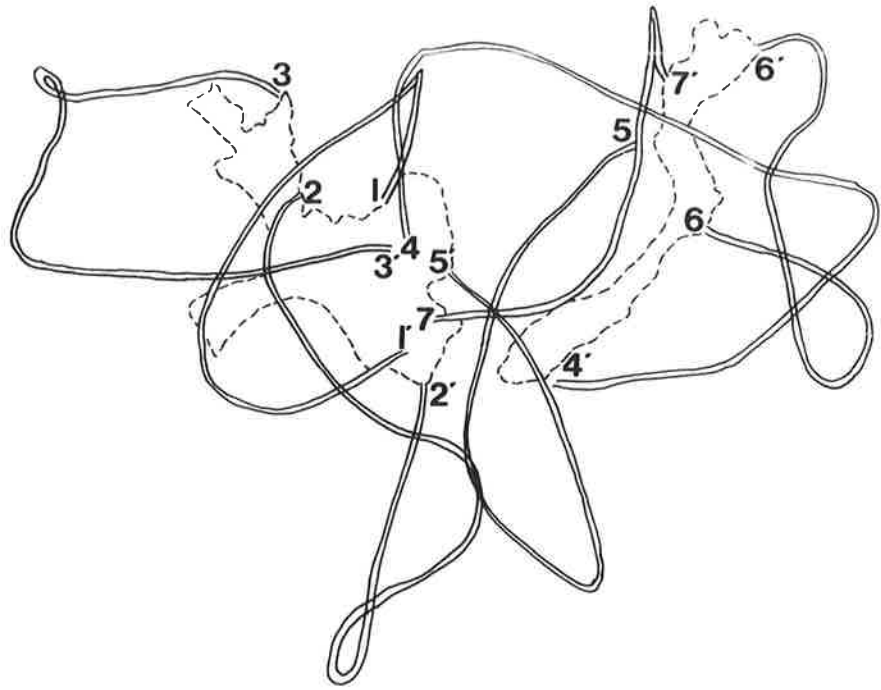


Table 2.3.2

Synaptonemal complex analysis of 22 prophase cells  
from *L. variatus* individuals exhibiting a ring  
of 12 and 1 bivalent at metaphase I. (2n=14)

Cell stage	No. synaptonemal complexes or 'thickened ends'	Changes of pairing partner	Unpaired lateral elements
Stage 1	14	-	+(e)
	14	-	-
	14	-	-
	14	-	-
	14	-	-
	13 + LE + 2 SC	-	+
	12	-	+(e)
	13*	-	-
	14	-	-
	14	-	+(e)
Stage 2	13	+	+(cpp)
	13	+	-
	13	+(3)	+(cpp)
	13	+	+(cpp)
	12	-	-
	12	-	-
	12	-	-
	12	-	-
	10	-	-
	11*	-	-
	13	+	-
12+	+(3)	+(cpp)	

LE = lateral elements.

SC = synaptonemal complexes.

+ = observed (numbers in brackets → no. of regions observed).

- = none observed.

+(e) = unpaired lateral elements at ends.

+(cpp) = unpaired lateral elements at region of pairing exchange.

\* = incomplete.

of 12 plus one bivalent in conventional metaphase I preparations. In a total of 20 metaphase I cells scored, only a single cell exhibited a chain of 12 instead of a ring of 12. This chain configuration may have been a result of the preparative techniques rather than pairing failure.

Stage 1 cells mostly exhibited 14 thickened ends as expected if pairing is initiated at both ends of each chromosome. These ends were usually polarized towards a particular region of the cell (Fig. 2.3.3a<sup>-c</sup><sub>h</sub>). In some cells the lateral elements associated with each chromosome were present but had not completed pairing. Even at this early unpaired stage the lateral elements were typically dark and thickened. In most stage 1 cells it was only the dark, distal regions of the synaptonemal complexes that were visible.

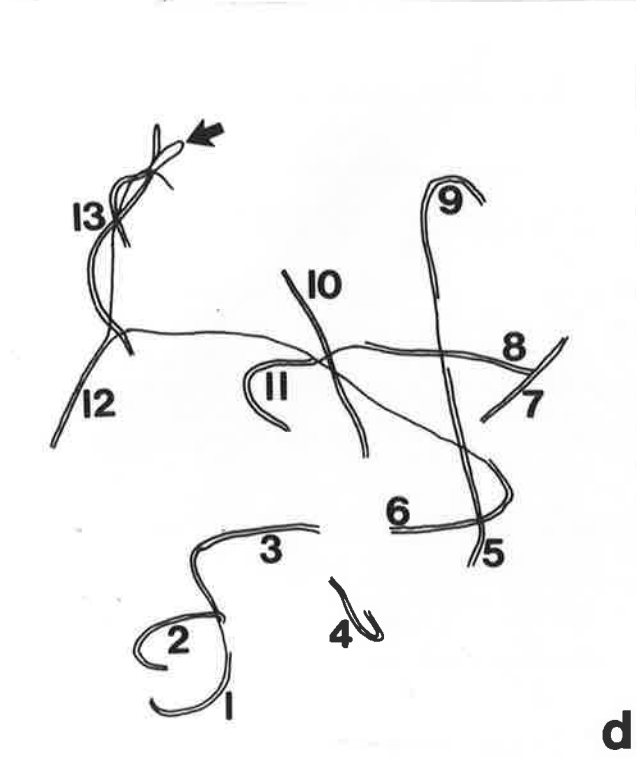
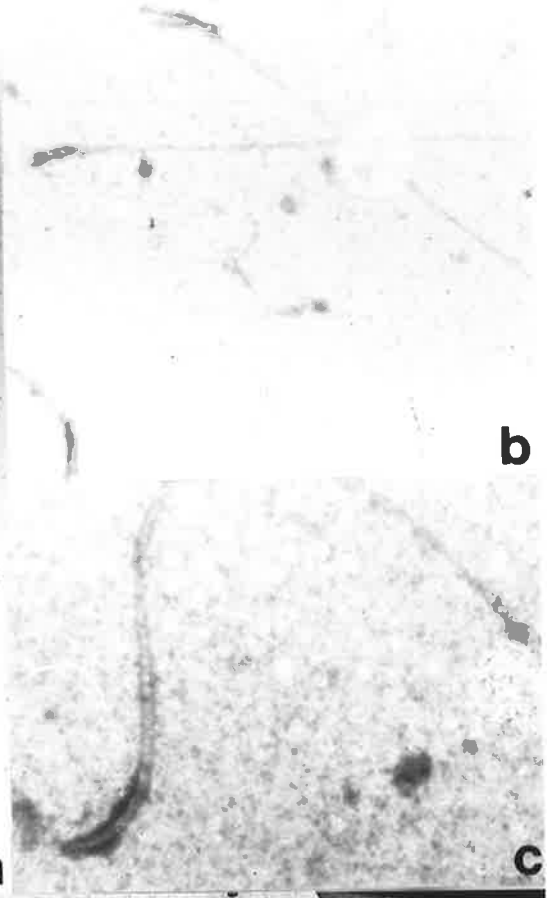
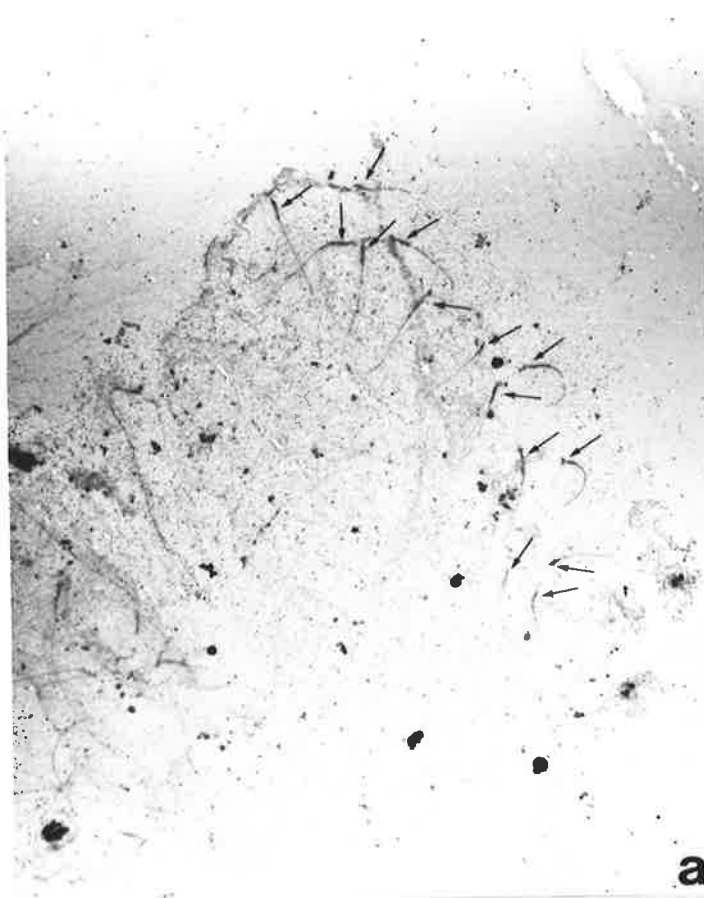
Stage 2 cells exhibited a modal number of 13 complete, uninterrupted synaptonemal complexes. In early stage 2 cells (Fig. 2.3.3b<sup>de</sup>) where pairing was not yet complete there were obvious regions of changes of pairing partner. These were characterized by a single lateral element joining two regions of complete synaptonemal complex, and represented the region of interchange.

In later stage 2 cells, where pairing was complete, 13 discreet, often totally separated synaptonemal complexes were observed. The separate nature of the synaptonemal complexes in these cells was probably due to the preparative technique; the delicate nature of the single axes at regions of pairing partner change results in their breakage when cells are

**Figure 2.3.3**

**Overland Corner, *Lychas variatus* (OCL)  
(Ring of 12 and one bivalent) synaptonemal  
complexes.**

- a**      **Stage 1 cell.**  
**14 thickened ends (arrowed) polarized  
in cell.**
  
- b,c**     **Details of thickened lateral elements  
at ends of synaptonemal complexes.**
  
- d,e**     **Early stage 2 cell showing 13 regions of  
synaptonemal complex formation, regions  
of pairing partner change, and a single  
interstitial unpaired region (arrowed).**





dispersed on a hypophase. Thus, at late pachytene most single axes were broken and lost with only fully formed synaptonemal complexes remaining.

It is possible to predict from the metaphase I configuration how many fully formed synaptonemal complexes should be observed. For example, for a ring of 12 and one bivalent there are 12 regions of homology and synaptonemal complex formation in the ring and one full synaptonemal complex corresponding to the single bivalent, i.e. 13 synaptonemal complexes as observed in OCL animals (Figs. 2.3.4 and 2.3.5). Further evidence that breakage of single axes resulted in the separate synaptonemal complexes observed was found in cells where not all single axes had broken (these were often cells that had been settled rather than spread on a hypophase). Due to straightening of two synaptonemal complexes still joined by a single axis the complex would appear as a single element interrupted by a 'gap' in one lateral element (Figs. 2.3.4b, 2.3.5b).

The lack of unpaired lateral elements, either as 'background' in the cell or associated with fully paired regions of synaptonemal complex, suggests that pairing in the interchange ring is complete, i.e. all regions of homology form synaptonemal complex with only minimal regions of single axes at pairing partner exchanges.

**Figure 2.3.4**

**Stage 2 cells from Overland Corner *Lychas variatus***  
**(Ring 12 and 1 bivalent).**

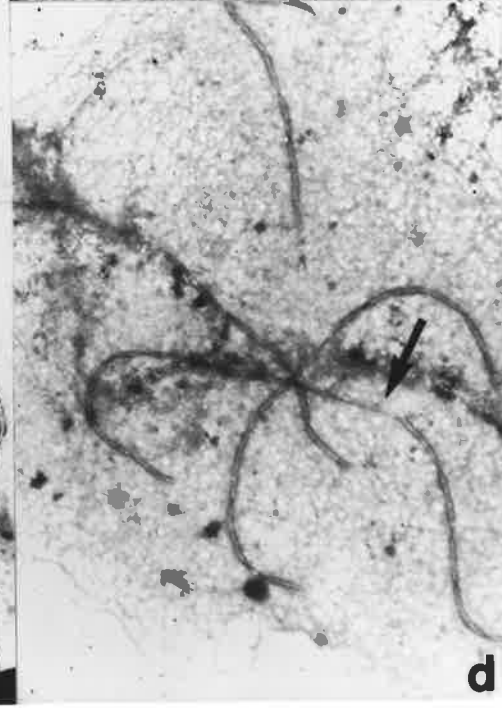
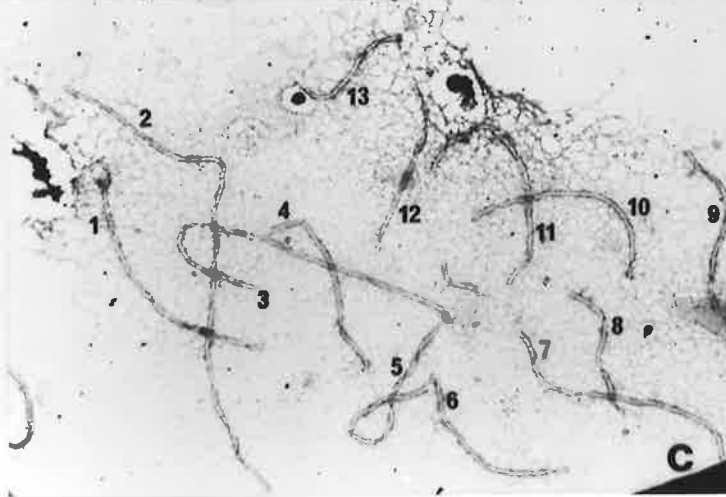
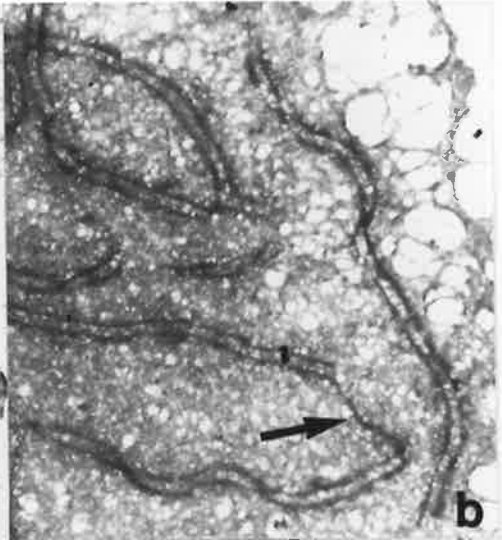
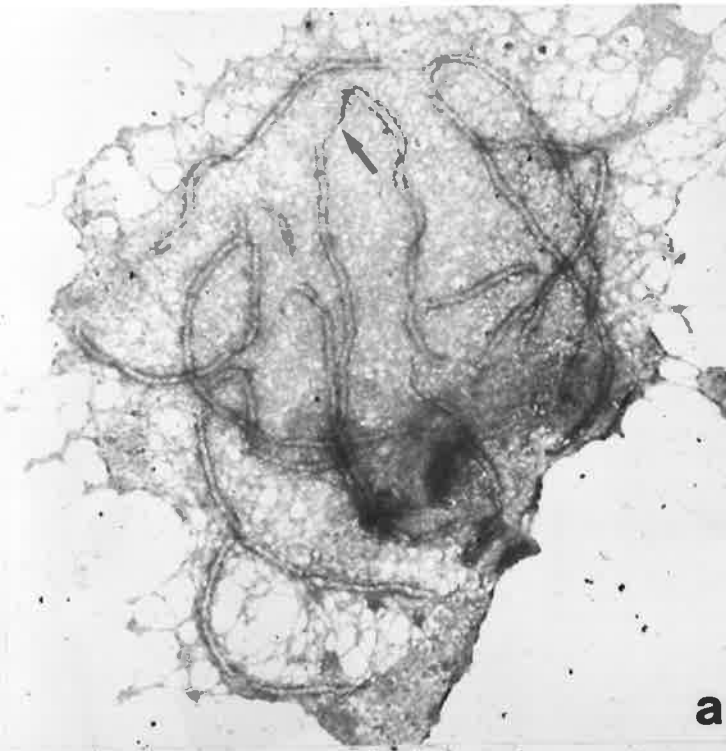
- a** Cell not disrupted by excessive spreading showing almost continuous synaptonemal complex formation.

Gaps arrowed (b,d) show regions of pairing partner change where a single axis has been disrupted.

(see Fig. 2.3.5)

- c** Cell that has been disrupted by spreading. All single axes have been broken leading to 13 separate synaptonemal complexes.

Note lack of single lateral elements as 'background' in cell.

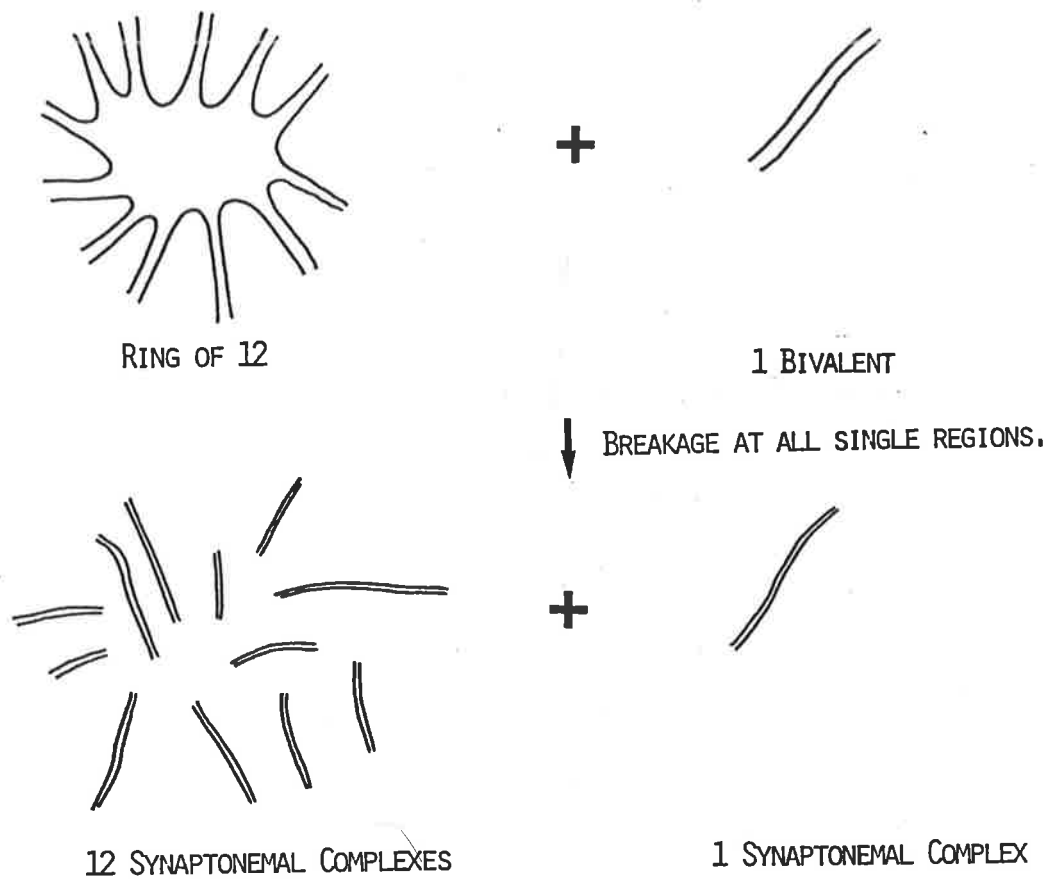


**Figure 2.3.5**

- A. Schematic diagram showing the result of mechanical breakage during preparation of all single regions in pachytene multiple pairing.
- B. Synaptonemal complexes showing 'gap' resultant from mechanical breakage of two alternate single regions during pachytene multiple pairing.

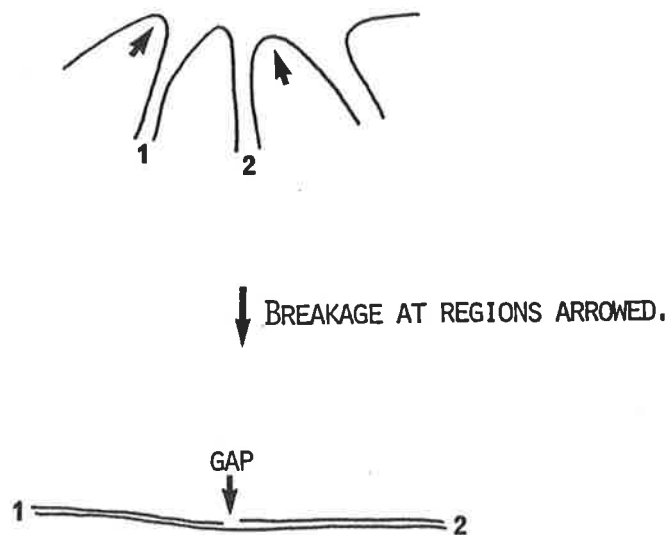
(Stack and Soulliere (1984), when studying ring 12 multiples in *Rhoeo* postulated only 6 synaptonemal complexes with gaps should be observed if mechanical breakage of single regions occurred. However, they observed 12 separate synaptonemal complex regions. This is because all single regions have the same probability of breaking - alternate regions are not more susceptible to breakage (which must be postulated for their theory to be correct).)

**A. Breakage at all single regions.**



PACHYTENE CELL CONTAINS 13 SYNAPTONEMAL COMPLEXES IN TOTAL.

**B. Breakage at some single regions.**



### Individuals Exhibiting Synaptic Failure.

#### 7 Bivalent Karyotype.

##### *Lychas marmoreus* (OGL X)

This individual exhibited synaptic failure and associated decondensation in 84% (31/37) of metaphase I cells. Of the 67% of cells that recognisably showed 7 bivalents, 51% of these showed some form of decondensation at metaphase I.

The stage 2 synaptonemal complexes in this individual were very fuzzy and difficult to interpret. The two stage 1 cells observed had 14 and 11 thickened ends respectively. Whether the cell with 11 was incomplete or reflected a failure in early pairing in this cell is unknown.

#### Ring of 4 and 6 Bivalents Karyotype. (2n=16)

##### *Lychas variatus* (MFL I)

At metaphase I this animal usually exhibited a ring of 4 plus 6 bivalents (69% of cells). However, in many cells, especially those exhibiting decondensation, one bivalent (that exhibiting a palely staining gap in normal cells) appeared dissociated into two smaller bivalents. Thus 27% of cells exhibited a ring of 4 plus 7 bivalents. The presence of this additional bivalent was also reflected at metaphase II where 25% of cells showed n=9 rather than n=8 as expected.

Table 2.3.3 shows the synaptonemal complex data for this animal. The number of thickened ends expected at stage 1 (assuming pairing is initiated at end regions) was 16 and

Table 2.3.3

Synaptonemal complex data for MFL<sub>I</sub>.

(Ring 4 + 6 bivalents, 2n=16)

Cell stage	No. synaptonemal complexes or 'thickened ends'	Changes of pairing partner	Unpaired lateral elements
Stage 1	16	-	+(e)
	15	-	-
	16	-	+(e)
	15	-	-
	16	-	-
	16	-	-
	16	-	+(e)
	16	-	+(e)
	15	-	+(e)
	16	-	+(e)
	16	Trivalent association observed	-
Stage 2	9	-	+
	8	-	-
	11	+(4)	+(cpp)
	10	-	-
	10	-	-
	10	-	-
Stage 3	6 (modified SC bivalents)+	-	-
	1 (modified SC quadrivalent)	+(4)	-

- = none observed.

+(e) = observed at ends.

+(cpp) = observed at changes in pairing partner.

+ = observed. (numbers in brackets indicate number of regions).

this number was observed in the majority of cells (Fig. 2.3.6a, b). The lack of cells showing 18 ends suggests that breakage of the bivalent does not occur until after pairing is initiated. The breakage may be associated with the decondensation phenomenon also observed in this animal.

Stage 2 cells were broken by the preparative procedure and showed 10 discrete synaptonemal complexes. However, one cell was obtained where the quadrivalent was still intact (Fig. 2.3.6c, d). The quadrivalent is fully paired with only small stretches of single lateral elements in regions of pairing partner change. This cell showed 7 rather than 6 synaptonemal complexes not involved in the quadrivalent. This was either due to breakage of the synaptonemal complexes during preparation (which is unlikely as the quadrivalent is intact) or due to real breakage of the bivalent with the palely staining region. The lack of stage 2 cells made it impossible to positively identify each synaptonemal complex/bivalent through comparative measurements so the exact origin of this synaptonemal complex could not be determined.

The two stage 2 cells that exhibited only 8 and 9 complete synaptonemal complexes probably represent cells where synaptic failure has occurred. For example, in a cell where pairing failure occurs in the ring to result in a chain of 4 and 6 bivalents, the expected number of synaptonemal complexes would be 9 instead of 10. The single unpaired lateral elements in the chromosome regions where pairing had failed may be visible associated with a synaptonemal complex or as background due to breakage on preparation (as observed



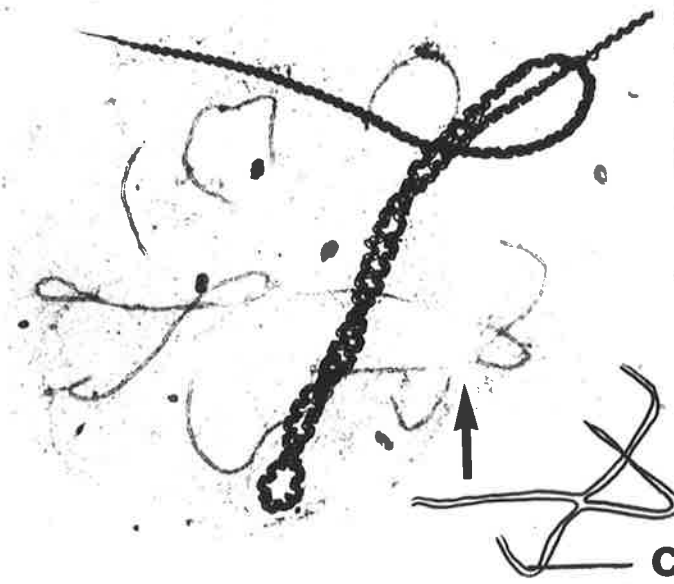
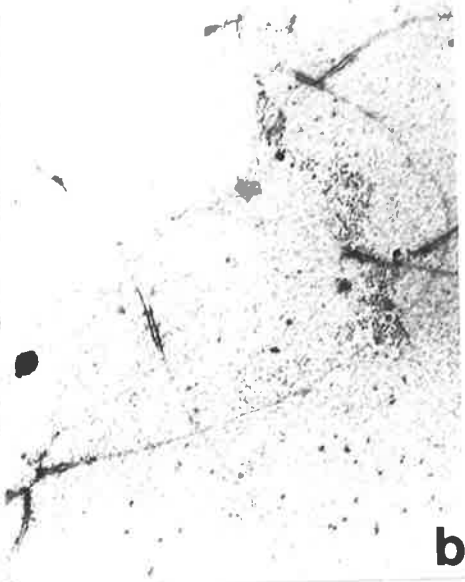
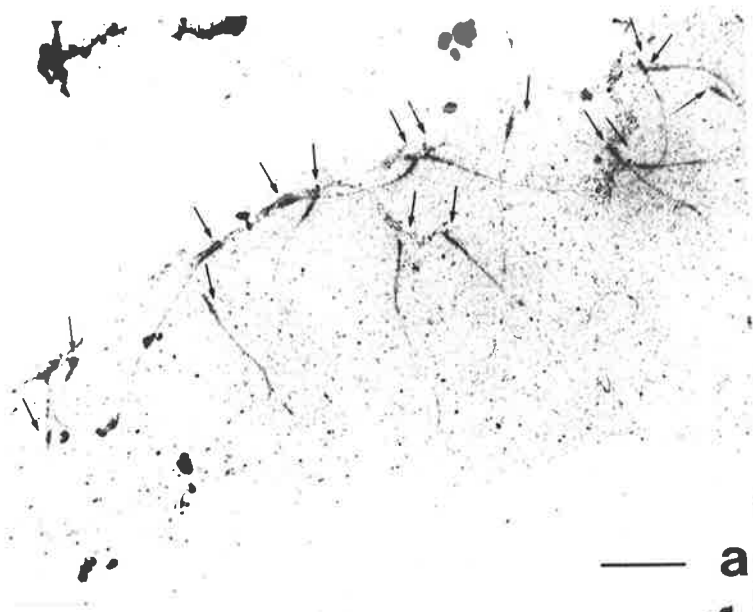
**Figure 2.3.6**

**Synaptonemal complexes of MFL I *Lychnis variatus*.**

**(Ring of 4 and 6 bivalents)**

- a** Stage 1 cell. Note 16 thickened ends (arrowed).
  
- b** Enlargement showing continuity of a synaptonemal complex from one polarized thickened end to the next.
  
- c** Stage 2 cell. Quadrivalent (arrowed) fully paired and clearly visible.  
  
6-7 separate synaptonemal complexes representing bivalents.
  
- d** Enlargement of quadrivalent showing short single axes.
  
- e** Stage 3 cell. Thickened protein 'cores' with cross-connections. (quadrivalent arrowed)
  
- f** Corresponding metaphase I cell.

Bar = 5  $\mu$ m.



in the MFL cell with 9 synaptonemal complexes).

### Ring of 10 and 2 Bivalents Karyotype.

*Lychnis marmoratus*. (WPL T4 II, T4 III)

Table 2.3.4 lists the metaphase I pairing configurations observed in these animals. Although a ring of 10 and 2 bivalents occurs a chain of 10 with 2 bivalents was the most common configuration. The cause of pairing failure is unknown although it did appear that only a very short region of homology existed between two chromosomes in the ring. Examples of metaphase I cells are shown in Fig. 2.3.7. For some cells chromosomes exhibit decondensation which appears to result in non-homologous associations of chromosomes. Other cells do not exhibit decondensation, however, aberrant pairing occurs resulting in odd numbers of chromosomes being involved in ring formation (Fig. 2.3.7d).

Table 2.3.5 shows the synaptonemal complex data for these animals. No stage 1 cells were observed probably due to the 'settling' preparative technique used which did not adequately preserve these cells. However, this technique was less disruptive on the synaptonemal complexes and enabled intact single lateral elements to be observed.

Figure 2.3.8 shows stage 2 cells that are representative of the most common pairing configuration observed; a chain of 10 chromosomes plus two bivalents. Both cells contain eleven synaptonemal complexes some of which are connected by single axes that represent regions of pairing partner exchange. Besides these, there were no other regions where

Table 2.3.4

Metaphase I pairing configuration observed in WPL, T<sub>4</sub> II.

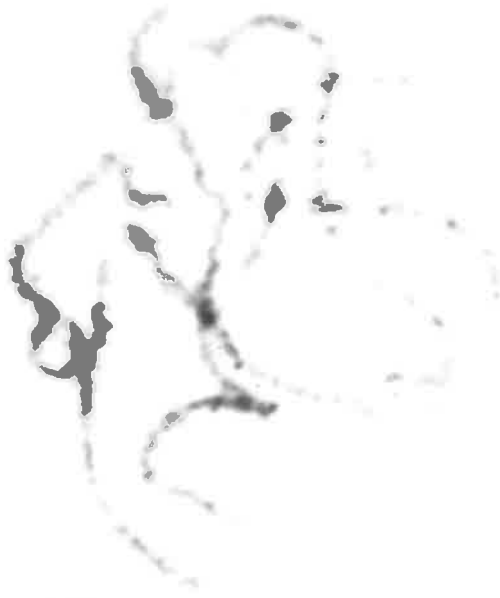
Pairing configuration	N	Condensed	Decondensed
Chain 10 + 2 bivalents	48	39	9
Ring 10 + 2 bivalents	9	8	1
Ass. 10 + 2 bivalents	21	13	8
Chain 12 + 1 bivalent	6	2	4
Ass. 12 + 1 bivalent	8	1	7
Ass. 10 + Ass. 2 bivalents	1	-	1
Ring 7 + Chain 3 + 2 bivalents	1	1	-
Ring 7 + Ring 3 + 2 bivalents	1	1	-
Ass. 7 + Ass. 3 + 2 biv.	2	2	-
Ring 4 + Ring 6 + 2 bivalents	1	1	-
Chain 4 + Chain 6 + 2 bivalents	1	-	1
Ass. 6 + Ass. 3 + 2 biv. + univ.	1	-	1
7 bivalents	1	-	1
2X (Ass. 3 + Ass. 4)	1	-	1
2X (unclassified assoc.) + 2 biv.	2	-	2
Ass. 8 + 3 bivalents	2	1	1
Ass. 5 + Ass. 3 + 3 bivalents	1	1	-
2X (unclassified assoc.)	1	-	1
2X (unclassified assoc.) + 1 biv.	2	-	2
All associated	2	-	2

Ass. = association - exact pairing relationships between chromosomes could not be determined.

**Figure 2.3.7**

**Metaphase I cells from T4 II (Ring of 10 and 2 bivalents)  
*Lychas marmoreus* that exhibits synaptic failure  
phenomenon.**

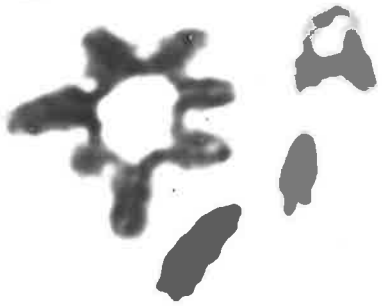
- a LM pachytene cell.
- b Ring of 10 and 2 bivalents.  
Chain of 10 and 2 bivalents.
- c Ring of 7, Ring of 3 and 2 bivalents.  
(no decondensation)
- d Chain of 10 and 2 bivalents.
- e Ring of 6, Ring of 4 and 2 bivalents.  
(no decondensation)
- f-j All divisions show some degree of  
decondensation:
  - f 7 bivalents.
  - g Association of 14.
  - h Chain 10 and 2 bivalents.
  - i Association of 10 and 2 bivalents.
  - j Unclassified association.



a



b



c



d



e



f



g



h



i



j

Table 2.3.5

Synaptonemal complex data for WPL, T<sub>4</sub> II and T<sub>4</sub> III.

Cell stage	No. synaptonemal complexes or 'thickened ends'	Changes of pairing partner	Unpaired lateral elements
Stage 2	11 + LE	+	+
	12 + LE	+(trivalent)	+*
	8 + LE	-	+
	11	+	-
	10	+	+(cpp)
	11	+	+(cpp)
	9	-	+
	11	+	+(cpp)
	12 + LE	-	+
	11	+	+(cpp)
	11	+	+(cpp)
	11	+	+(cpp)
	11	+	+(cpp)
	12 + LE	-	+
	10	+	+(cpp)
	8	-	-
	10	-	-
	11	-	-
	13 + LE	-	+
	11 + LE	-	+
11	+	+(cpp)	
11	+	+(cpp)	
15 + LE	-	+	
Stage 3	Chain 10 + 2 biv.	+	+(cpp)
	Ass 4 + Ass 4 + 1 biv.	+	+(cpp)
	Ass 6 + 4 bivalents	+	-
	Ass 10 + 2 bivalents	+	+(cpp)
	Ass 10 + 2 bivalents	+	+(cpp)

\* = wide SC regions observed (Fig. 2.3.9).

- = not observed.

LE = lateral elements.

cpp = observed at change of pairing partner.

**Figure 2.3.8**

*Lychas marmoreus*, T4 II.

Stage 2 cells (and interpretations) showing regular synaptonemal complex formation as expected for a chain of 10 and 2 bivalents.

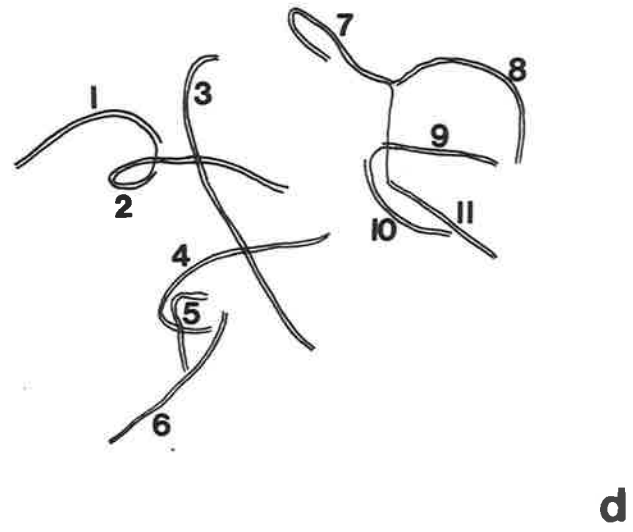
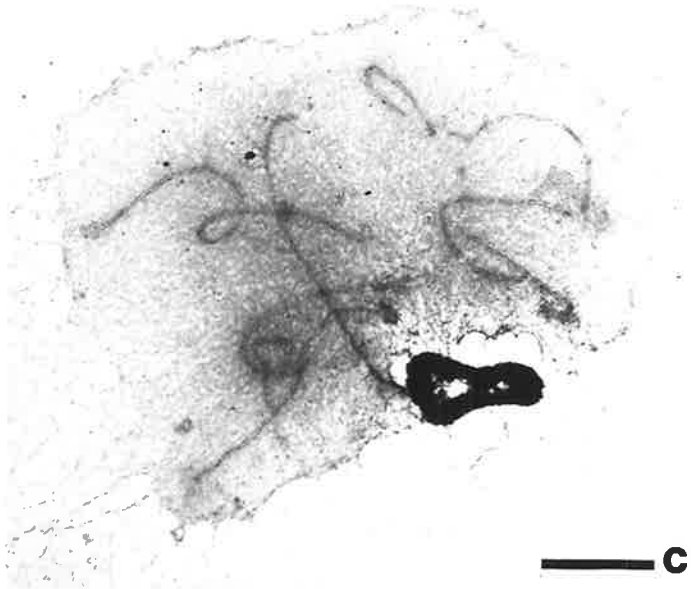
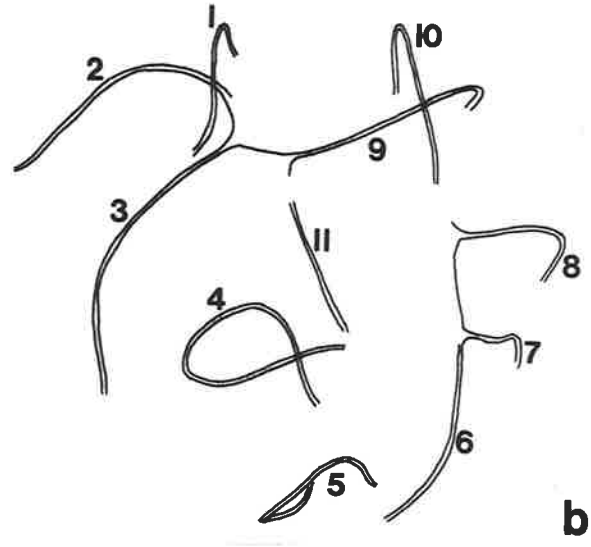
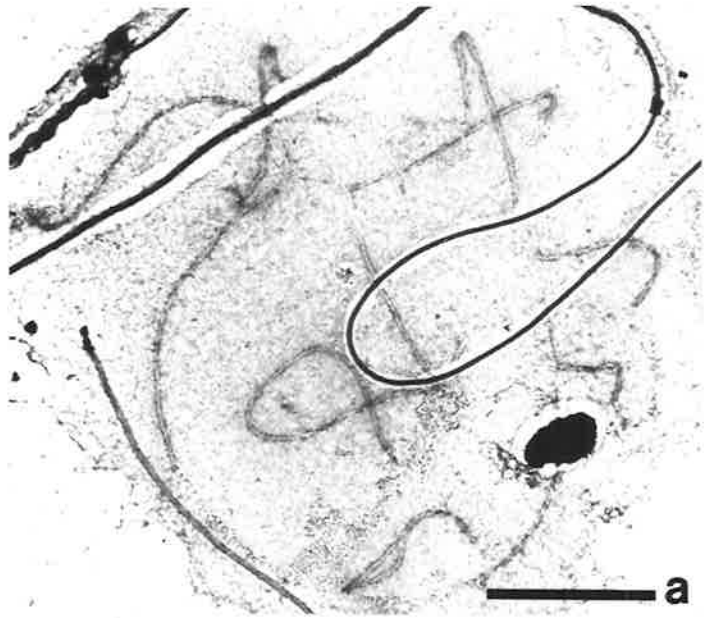
a,b Eleven regions of synaptonemal complex formation and change in pairing partners.

c,d Eleven regions of synaptonemal complex formation and change in pairing partners.

Note lack of single lateral elements as 'background' in cell.

Bar = 5  $\mu$ m.





single lateral elements occurred. Conversely, Fig. 2.3.9 shows cells with varying numbers of synaptonemal complexes and showing large amounts of broken lateral elements in the background of the cell. Cells of this kind were only observed in individuals exhibiting the synaptic failure phenomenon. Additionally, these cells contained aberrant synaptonemal complexes that were wider than normal synaptonemal complexes and without a central element, and, pairing configurations that were not typical of interchange heterozygotes (e.g. triradial structure). This suggests that these cells do not represent early pachytene stages where pairing was not yet completed. It is more likely that they represent the synaptonemal complex configurations of cells that at metaphase show decondensation, synaptic failure and aberrant pairing. Thus the pairing failure at metaphase I is resultant from failure of synaptonemal complex formation at pachytene. This is more likely than complete synapsis followed by desynapsis as very few paired pachytene cells were observed.

A number of metaphase I cells were observed in EM preparations. Figure 2.3.10 shows an example of two of these cells. They are similar to the MFL I cell except for the absence of fibrous cross-connections (probably due to preparative differences). However, the laterally paired protein structures are visible. A cell intermediate between a pachytene cell and a metaphase cell is also shown. Whether the proteins observed are modified synaptonemal complexes or are due to other chromosomal proteins or

**Figure 2.3.9**

*Lychas marmoreus*, T4 II.

Stage 2 cells showing synaptic failure and abnormal pairing behaviour.

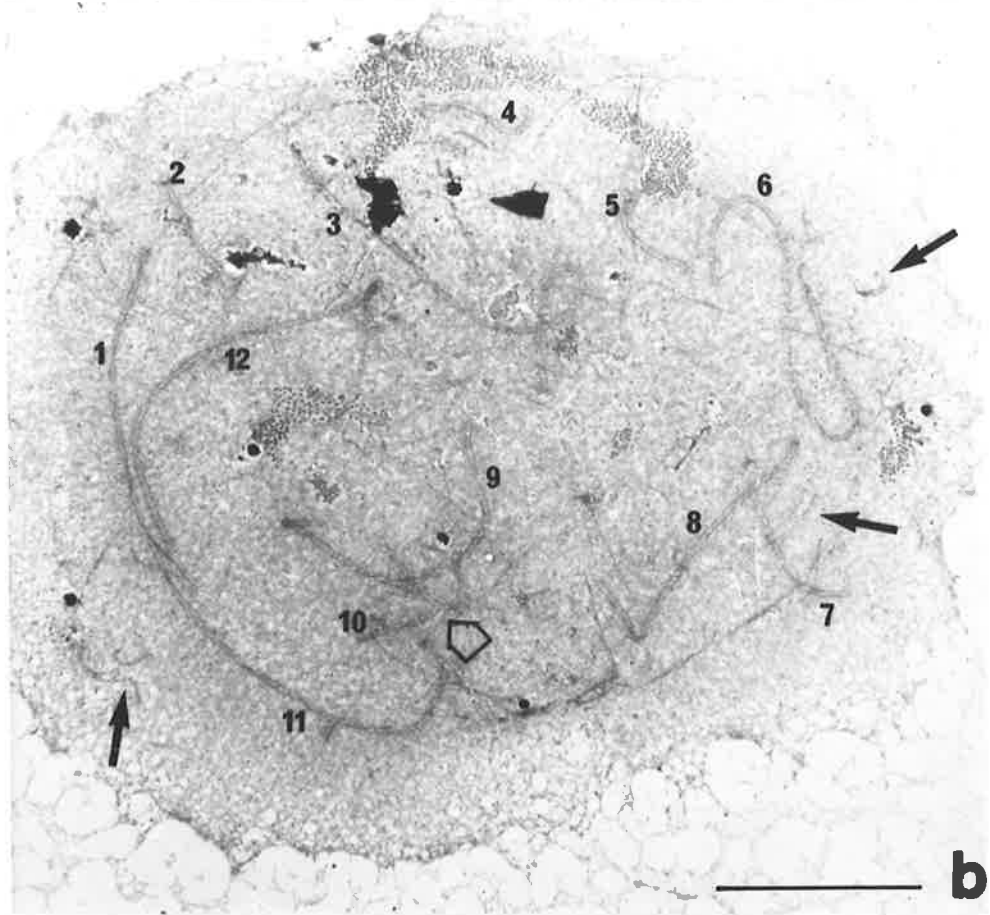
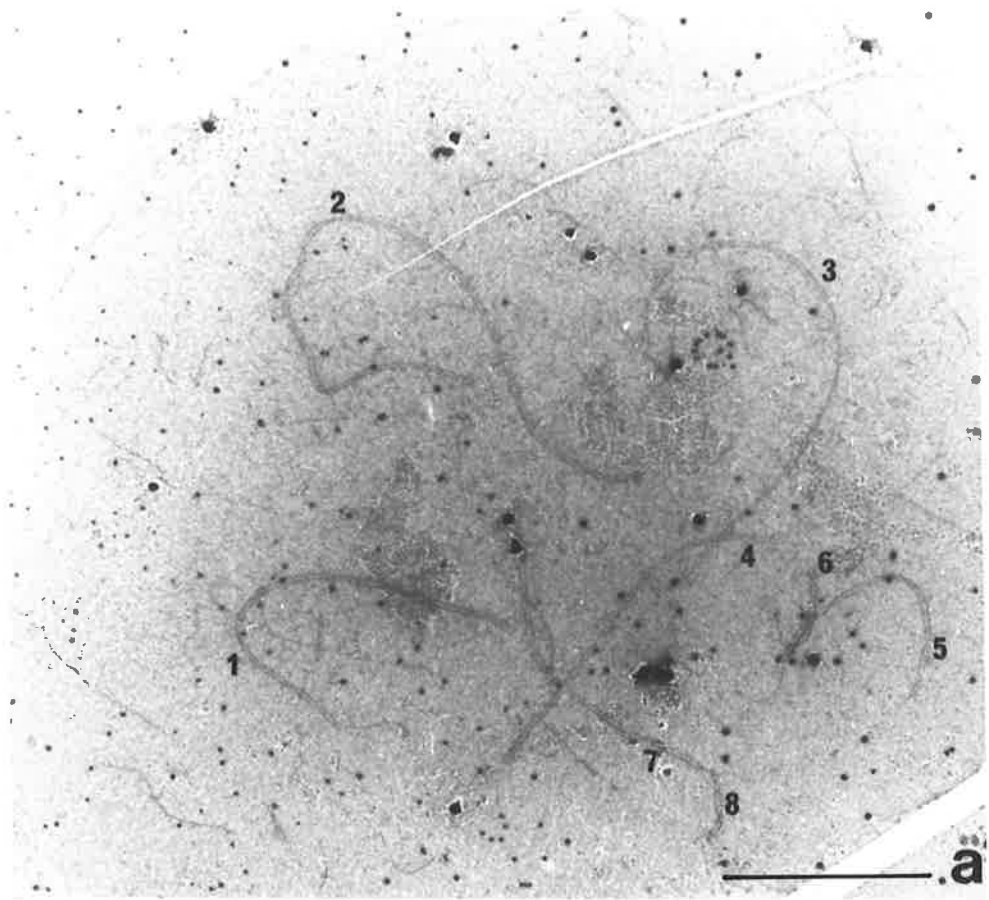
a Eight regions of synaptonemal complex formation with a high background of single axes.

b Twelve regions of synaptonemal complex formation with high background of single axes.

Dark arrows indicate abnormal synaptonemal complex formation.

Hollow arrow shows a triradial structure not expected in an interchange heterozygote.

Bar = 5  $\mu$ m.



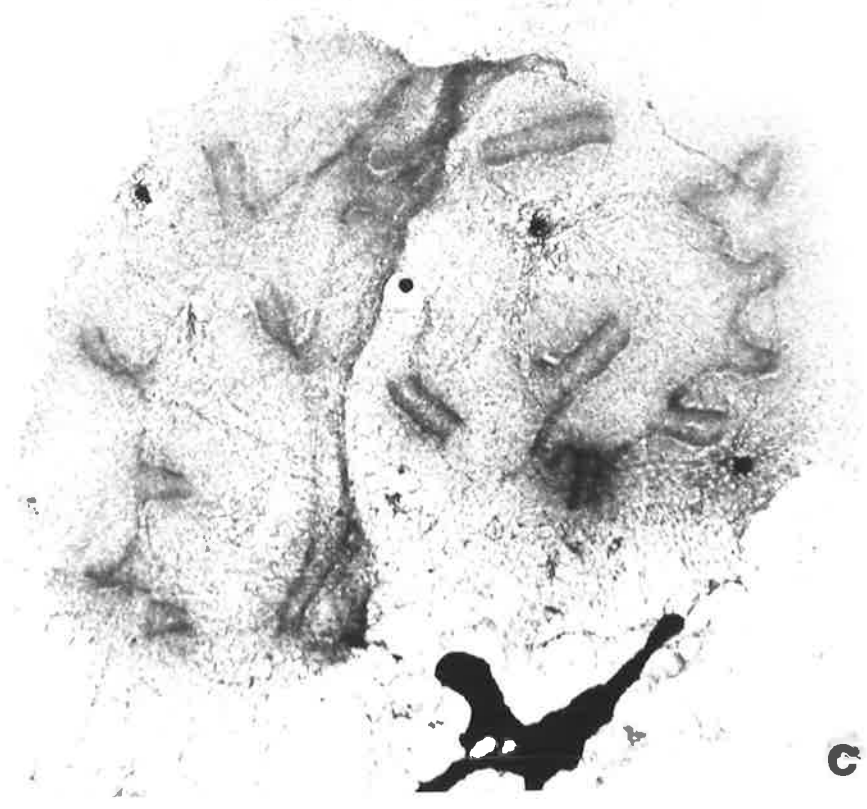
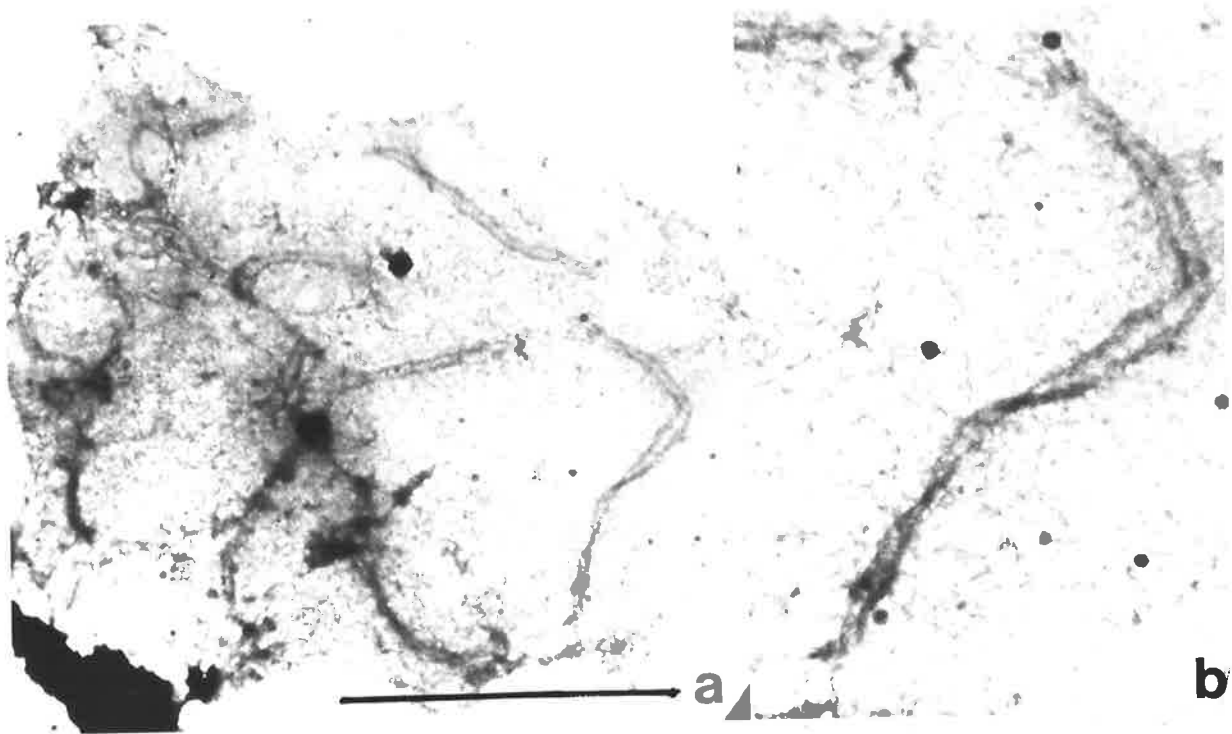
**Figure 2.3.10**

**a,b Possible modified synaptonemal complexes  
that lead to the structures observable  
under the EM at metaphase I (c).**

*Lychas marmoreus* (T4 III) chain of 10 and 2 bivalents.

Modified synaptonemal complexes may maintain pairing in  
achiasmate *Lychas*.

Bar = 5  $\mu$ m.



chromatin contraction could not be determined in spread preparations.

## DISCUSSION

The study of meiotic chromosome behaviour in Buthid scorpions provides a unique system in which to test the universality of the hypothesis of Rasmussen and Holm (1979) that chiasmata stabilize multivalents:

"Homologous pairing and synaptonemal complex formation during zygotene apparently leads to stable associations if a continuous synaptonemal complex is formed. If synaptonemal complex continuity is interrupted by a change of pairing partners, the subsequent correction phase transforms multivalents into bivalents by turnover of the central region of the complex as long as crossing-over is absent. In normal diploid organisms, the correction process is of little importance, as initial pairing at zygotene results in regular bivalent formation in most cases."

von Wettstein, Rasmussen and Holm (1984)

The observations which gave rise to this hypothesis were made on autotetraploids and triploids in *Bombyx* (Rasmussen, 1977; Rasmussen and Holm, 1979), the homeologous chromosomes of *Triticum* (Hobolth, 1981), and reciprocal translocation heterozygotes in mouse, man, and *Coprinus* (Holm *et al.*, 1981; Ashley *et al.*, 1982; von Wettstein *et al.*, 1984; Holm and Rasmussen, 1978). There is no indication in this hypothesis that the same resolution of multivalents would not occur in individuals heterozygous for interchanges.

Many Buthid scorpions are heterozygous for various numbers of interchange chromosomes. However, meiosis in the

male is achiasmate. Thus, although it should be possible to observe multivalent synaptonemal complex associations at zygotene/early pachytene these multivalents should, if the model of Rasmussen and Holm is correct, be resolved to bivalents by metaphase I. Obviously this was not the case as consistent multivalent associations at metaphase I were found in most individuals. The question thus arises as to what are the differences between the situations studied by Rasmussen and Holm (namely *Bombyx*, *Coprinus*, man and mouse) and Buthid scorpions.

Consider the example of *Bombyx*. The female exhibits achiasmate meiosis yet clearly shows resolution of multivalents to bivalents. Inhibition of this process occurs in the chiasmate male. Resolution of multivalents to bivalents occurs in the females despite the presence of a mechanism (modified synaptonemal complexes remain until anaphase) other than chiasmata to maintain pairing. Obviously this mechanism is not functional until after the second synaptic stage that allows non-homologous pairing. Multivalent synaptonemal complexes in Buthid scorpions must be stabilized directly after the initial homologous pairing stage. (Whether a second pairing stage allowing non-homologous pairing follows cannot be determined. However, synaptic failure mutants suggest non-homologous association can occur at pachytene in Buthids.) The mechanism of pairing maintenance in Buthids is unclear. Nevertheless it is apparent that it is not solely the absence of chiasmata that inhibits multivalent resolution (as described by



Rasmussen and Holm).

Both *Bombyx* and Buthids possess holocentric chromosomes. However, one distinction between *Bombyx* and Buthids that may influence pairing is that the multivalents studied by Rasmussen and Holm in *Bombyx* were the result of polyploidy and not due to interchange heterozygosity. In a tetraploid cell all four chromosomes involved in multivalent association are homologous (i.e. there are a large number of strictly homologous pairing options available), conversely in an interchange heterozygote homology is exclusive such that each chromosome or chromosome region has only one homologous pairing partner or region (i.e. there is only one homologous pairing option). As an alternative to Rasmussen and Holm's hypothesis it may be the 'competitive' pairing situation in a polyploid quadrivalent that results in an inherently unstable structure rather than just a region of pairing partner change as they suggested. In interchange heterozygotes pairing is initiated due to end homology and is 'non-competitive' and exclusive to two chromosome regions as in bivalents - which according to Rasmussen and Holm are stable regardless of the presence or absence of chiasmata.

In the light of this argument, how can the apparent resolution of quadrivalents (due to reciprocal translocations) to bivalents in man and mouse be explained? Firstly, these translocations (and polyploid *Bombyx*) are the result of either spontaneous or induced mutations. They are not part of the organism's adaptive chromosome system. Australian species of Buthid scorpions have evolved a genetic

system that without exception involves a large degree of heterozygosity for interchanges. This implies that the system must be of some adaptive significance and logically an efficient mechanical means of ensuring correct pairing and disjunction must also exist. Resolution of multivalents into heteromorphic bivalents in interchange heterozygotes would result in a high frequency of unbalanced gamete formation. The translocation heterozygotes in man and mouse studied are usually sterile due to meiotic failure soon after pachytene, consequently metaphase I data is unavailable (Ashley *et al.*, 1982).

Secondly, the translocations studied in mouse by Ashley *et al.* (1982) and interpreted as favourable to their hypothesis by von Wettstein, Rasmussen and Holm (1984) involved an autosome and the X chromosome (R2 and R5). This results in the X and Y pairing to form part of the quadrivalent. Thus the pairing in these quadrivalents could not be considered directly analogous to a purely autosomal rearrangement. Although a reasonably large number of spread cells were examined the data deviated considerably from expected. Additionally, this study did not involve a direct comparison of early and late pachytene cells but rather a comparison of late pachytene pairing types in two translocation heterozygotes involving the same chromosomes but different breakpoints. There is no *a priori* reason to expect that the timing and types of early pachytene pairing to be identical for these two translocations. Heteromorphic bivalents may be formed initially at early pachytene or

alternatively pairing may be delayed until late pachytene when again bivalents may be formed.

Thirdly, the evidence for multivalent resolution from the reciprocal translocation between chromosomes 5 and 22 in man presented by Holm and Rasmussen (1978) is somewhat tenuous. The difficulty in reconstructing sectioned cells means that very few cells can be analysed. Thus, only eight early pachytene nuclei and four mid-pachytene nuclei have been analysed and it is observations of quadrivalent formation and presence of transitory recombination nodules from these few cells on which they base their conclusions. Clearly more data are required before an unequivocal statement can be made as to whether chiasmata have an inhibitory effect on the resolution of quadrivalents (formed at early pachytene) into heteromorphic bivalents observed at mid-late pachytene.

An analysis of a naturally occurring interchange heterozygote may prove informative (e.g. *Periplaneta americana*). Does chiasma failure in these multivalents result in two by two pairing at late pachytene? There are no examples where the synaptonemal complexes of a simple, single interchange (quadrivalent forming) heterozygote has been studied. However, species of the plant genus *Rhoeo* which exhibit up to a ring of 12 chromosomes at metaphase I have been studied in detail (Moens, 1972; McQuade and Wells, 1975; Stack and Soulliere, 1984). These species have evolved modifications to their synaptonemal complexes that ensure distal localization of chiasmata (a necessary

condition for regular disjunction of these multivalents). Whether this is a modification to prevent pairing reorganisation in the absence of chiasmata is unknown. However, evidence suggests that synaptic failure results in chiasmata failure rather than the reverse as hypothesized by the model of Rasmussen and Holm (1979). However, only a very detailed comparison of early pachytene with late pachytene nuclei would clarify this interpretation of the *Rhoeo* data.

#### Synaptonemal Complexes of Chiasmate and Achiasmate Interchange Heterozygotes.

Evidence suggests that in the achiasmate *Buthid* interchange heterozygotes normal pairing is associated with complete synaptonemal complex formation. Very short single axes (lateral elements) exist only in regions of pairing partner change. Failure of complete synaptonemal complex formation appears to result in synaptic failure and aberrant metaphase I configurations. In contrast, evidence from the chiasmate interchange heterozygotes of *Rhoeo spathacea* suggests that synaptonemal complex formation involves only the distal 15-30% of total lateral element length. Interstitial regions form lateral elements, however these are not involved in pairing and thus do not form synaptonemal complex (Stack and Soulliere, 1984). This mechanism of restricted synaptonemal complex formation ensures distal localization of chiasmata and has been observed in other species with strong chiasmata localization (e.g. Oakley and

Jones, 1982, in *Mesostoma*). Complete synaptonemal complex formation in Buthids may be a pre-requisite for pairing maintenance and regular disjunction, especially in the knowledge that Buthids possess holocentric chromosomes. This probably results in different forces during orientation at metaphase I compared with monocentric chiasmata forming interchange heterozygotes.

Buthids and *Rhoeo* share the common feature of localized darkly staining material on distal synaptonemal complex regions during early pachytene. (In *Rhoeo* there is also distal coiling of synaptonemal complex which was not observed in Buthids.) Assuming this material does not represent modified attachment plaques, its presence may have a role in ensuring or enhancing at early prophase, strong distal localization of pairing. This is clearly necessary in chiasmate *Rhoeo*, however, its significance is not obvious in Buthids. However, Buthids exhibit telocentric activity of both chromosome ends at metaphase I. (see Chapter 3.2) For alternate segregation and hence balanced gametes, it is necessary to postulate that both ends (i.e. the entire chromosome) are orientated to the same pole and adjacent chromosomes are orientated to opposite poles. Strong distal chromosome pairing must necessarily be maintained until metaphase I to ensure that chromosome ends co-orientate. To ensure that both chromosome ends are aligned to the same pole without twisting it could be postulated that each synaptonemal complex has only one pairing face (as postulated by Moses (1979) from evidence in *Lemur* hybrids). If this

were so, both chromosome ends (which are the first regions to pair at zygotene) are committed to pair on the same side of their respective adjacent homologous regions. Thus, adjacent chromosomes will pair on opposite sides and orientate to opposite poles facilitating regular alternate disjunction. If the ring multiples orientated at  $90^\circ$  to the spindle with adjacent chromosomes lying above or below each other alternately, regular disjunction would be ensured (see Fig. 2.3.11).

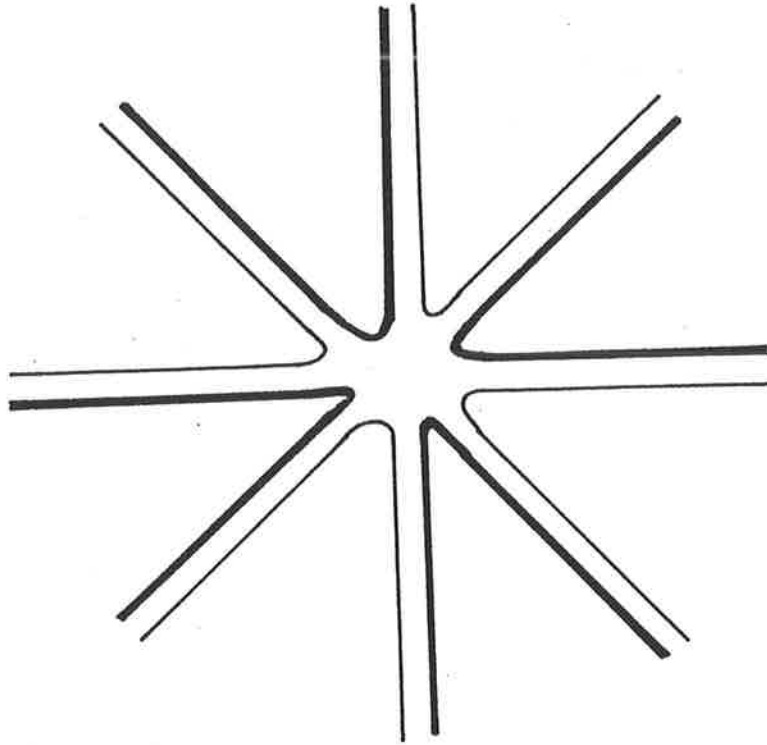
**Figure 2.3.11**

- A. A single pairing face for each lateral element would ensure regular pairing of multiples.**
- B. Hypothetical orientation of ring multiples on the spindle at metaphase I.**

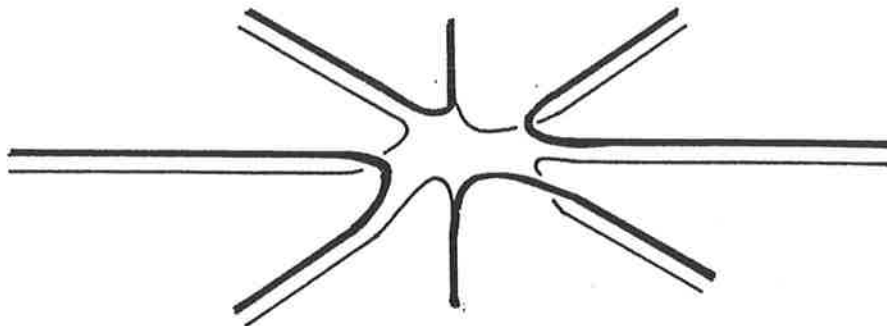
**A single pairing face for each lateral element results in alternate orientation if the multiple is at  $90^\circ$  to the spindle axis.**

**Alternate "above and below" orientation.**

A. SINGLE PAIRING FACE FOR EACH CHROMOSOME



B. HYPOTHETICAL SPINDLE ORIENTATION OF MULTIPLES AT METAPHASE I.





## SECTION 4

SYNAPTONEMAL COMPLEXES IN SCORPIONID FUSION/FISSION  
AND INVERSION HETEROZYGOTES

Synaptic adjustment, a process akin to the two-stage pairing of Rasmussen and Holm (1979) (and considered to be part of the same phenomenon by Moses *et al.*, 1982), was first described in detail in a mouse tandem duplication (Moses and Poorman, 1981). It has since been observed in mouse, rat and maize inversion heterozygotes; (a rearrangement where only two lateral elements are involved in pairing) (Moses *et al.*, 1982; Ashley *et al.*, 1981; Chandley, 1982). Similarly, in the process of synaptic adjustment, an initial pairing stage that is restricted to only homologous synapsis (homosynapsis; inversion loop formation) is followed by a second unrestricted pairing stage where non-homologous synapsis (heterosynapsis; straight-pairing of inversion) may occur. The pairing of the trivalent side arm in hybrid Lemurs (heterosynapsis) was also considered an example of synaptic adjustment (Moses *et al.*, 1979). Unlike the two-stage pairing of Rasmussen and Holm, the role or effect of chiasmata on synaptic adjustment remains enigmatic.

Achiasmate Scorpionids, heterozygous for both inversions and fusion/fission rearrangements were investigated to determine if the process of synaptic adjustment was operating. The pachytene synaptonemal complex data was analysed by the same method used by Chandley (1982) in

unstaged mouse pachytene cells. She used the per cent loop formation (homosynapsis) at pachytene as an indicator of synaptic adjustment; the lower the frequency of pachytene loop formation for a particular inversion the greater the synaptic adjustment. The difficulty in using this technique in organisms where unlike mouse there is no *a priori* evidence that adjustment does occur is discussed later. Additionally, the ratio of inversion loop formation to straight pairing (L:S, homosynapsis:heterosynapsis) was calculated at both pachytene and metaphase I. Moses *et al.* (1982), when analysing Maguires' (1981) maize data (where synaptic adjustment was not complete), considered a decrease in this ratio from pachytene to diplotene as indicative of an increase in heterosynapsis as expected with synaptic adjustment.

The pairing behaviour of trivalents and quadrivalents was analysed and compared to the structures described by Moses *et al.* (1979) in *Lemur* hybrids.

Table 2.4.1 lists the locality data, species and individual karyotypes of the animals used in this study. Most were *U. manicatus* from the Belair population where individuals exhibited both inversion and fusion/fission heterozygosity. Due to the large numbers of inversions and trivalents in some animals, where possible, the pairing behaviour of these rearrangements are presented separately.

Table 2.4.1

Locality data, species and karyotypes of individuals  
studied using synaptonemal complex analysis.

Species	Code	Karyotype (metaphase I)	2n	
<i>Urodacus manicatus</i>	BM <sub>VII</sub>	10 biv, 7 triv, 1 quad.	45	
	BM <sub>XIII</sub>	10 biv, 4 triv.	32	
	BM <sub>XVI</sub>	9 biv, 4 triv.	30	
	BM <sub>XVII</sub>	9 biv, 5 triv.	33	
	OCM <sub>III</sub>	31 bivalents	62	
	OCM <sub>XI</sub>	31 bivalents	62	
	OCM <sub>XX</sub>	31 bivalents, 1 trivalent	63	
	SRM <sub>I</sub>	32 bivalents	64	
	<i>Urodacus novaehollandiae</i>	EN <sub>I</sub>	36 bivalents	72
	<i>Urodacus planimanus</i>	WAP <sub>II</sub>	35 bivalents*	70

\* polyploid testicular cells common in this individual.

## RESULTS

### General Morphology of Scorpionid Synaptonemal Complexes.

Phosphotungstic Acid (PTA) stained synaptonemal complexes obtained from Scorpionid testicular cells show a typical tripartite structure. Kinetochores were observed in many preparations as PTA positive proteinaceous lumps associated with the synaptonemal complex. Telomeric attachment plaques were often visible although they were not as pronounced as those observable in mammalian preparations. The nucleolar organiser (NO) region could be identified by a darkly staining spherical structure attached to a particular synaptonemal complex region.

Although surface spreading techniques resulted in a loss of the three dimensional aspect of the cell it was possible (as observed in Buthids) to discern some polarity of the synaptonemal complex ends. Scorpionid synaptonemal complexes, however, did not exhibit a pronounced bouquet arrangement during prophase.

In many cells representative of early prophase stages (early-late zygotene), single lateral elements were not visible until they paired to form a complete synaptonemal complex. This suggests that the process of pairing does not involve zippering but may involve a number of pairing initiation sites. There was no evidence that the telomeres were the first to pair; some cells exhibited complete pairing except for some telomeric regions. The fate of the synaptonemal complexes prior to or at metaphase I is unknown, although possible remnant synaptonemal complexes associated

with metaphase I cells were observed under the electron microscope, (~~see later Fig. 2.4.13~~).

The exact stage of prophase could not be determined from synaptonemal complex preparations of Scorpionid cells. Cells were arbitrarily classified as either early-pachytene or pachytene; cells where synaptonemal complex formation was complete were classed as pachytene, while cells exhibiting unpaired lateral elements associated with more than one region of synaptonemal complex were classed as early pachytene.

#### Synaptic Behaviour in Inversion Heterozygotes.

##### *Urodacus manicatus*. (BM XVII)

Figure 2.4.1 shows C-banded mitotic and metaphase I karyotypes for this animal. The inversions are clearly identifiable due to the centromeric shift induced in one homologue of a bivalent and a trivalent. These have been previously classified as INV T1 (pericentric inversion in trivalent) and INV B1 (pericentric inversion in bivalent). Table 2.4.2 presents the synaptonemal complex data. Cells were classified, and scored for total number of synaptonemal complexes, presence of inversion loops and regions of synaptic failure.

Two cells exhibited three loops at pachytene (corresponding to homosynapsis of inverted regions) while other cells exhibited no inversion loops. (Fig 2.4.2a, b shows examples of these two cell types.) In both cell types pachytene synapsis was complete suggesting the inversions were showing straight pairing (heterosynapsis) in some

**Figure 2.4.1**

**a Mitotic karyotype of BM XVII.**

**i = inversion**

**b,c Metaphase I karyotypes.**

**9 bivalents, 5 trivalents  
including INV B1 and INV T1**

**b INV T1, INV B1 both homosynapsed**

**c INV T1, INV B1 both heterosynapsed**

**U. manicatus**

**BM XVII Male**

**2n=33**

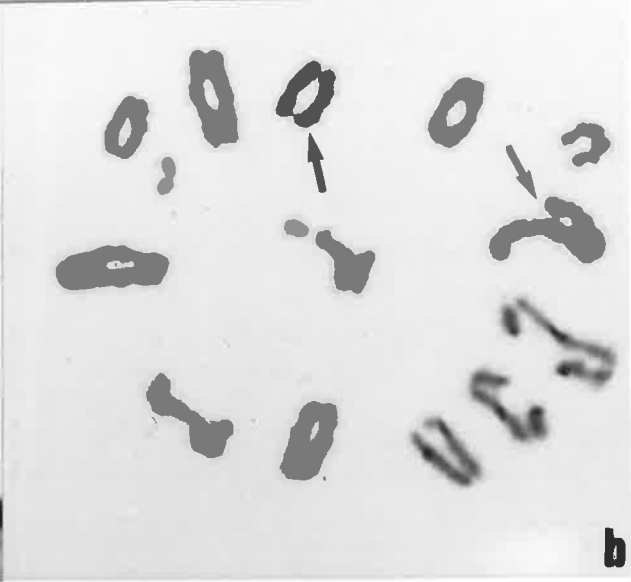
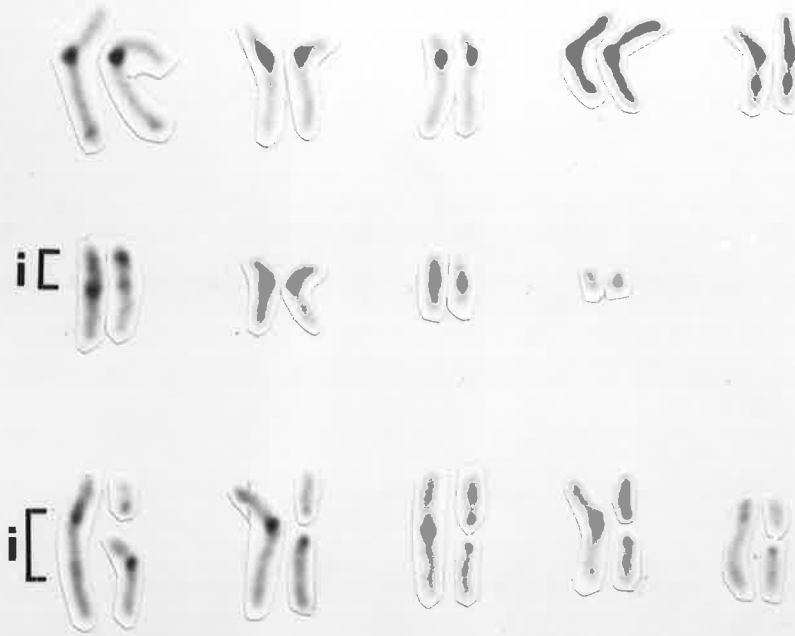


Table 2.4.2

Pachytene synaptonemal complex data for BM<sub>XVII</sub>.

Cell no.	No. synaptonemal complexes	Pairing stage	No. inversion loops	Regions of asynapsis
1	14	pachytene	3	-
2	14	pachytene	0	-
3	14	early pachytene	1 INV B1	+
4	15	pachytene	1 INV T1	-
5	14	pachytene	2 INV T1 INV ?	-
6	14	early pachytene	0	-
7	14	pachytene	1 INV B1	INV T1
8	14	early pachytene	0	INV T1, +
9	14	pachytene	3	-
10	14	early pachytene	0	INV T1, +
11	14	pachytene	1 INV B1	-
12	~13	early pachytene	2 INV B1 INV B2	+
13	15	early pachytene	0	INV T1, INV B1
14	14	pachytene	0	-
15	incomplete ~12	early pachytene	0	+
16	incomplete	zygotene	0	+
17	14	pachytene	2 INV B1 INV B2	-
18	14	early pachytene	1 INV B1	INV T1, +
19	14	early pachytene	1 INV B2	INV T1, +
20	14	pachytene	0	INV T1

+ = observed

- = not observed

The inversion loops or regions of synaptic failure have been identified by:

INV T1 pericentric inversion trivalent  
 INV B1 pericentric inversion bivalent  
 INV B2 paracentric inversion bivalent



**Figure 2.4.2**

**Two pachytene cells from BM XVII showing  
synaptonemal complexes.**

**a Three inversion loops (arrowed) corresponding  
to homosynapsis of INV T1, INV B1 and INV B2.**

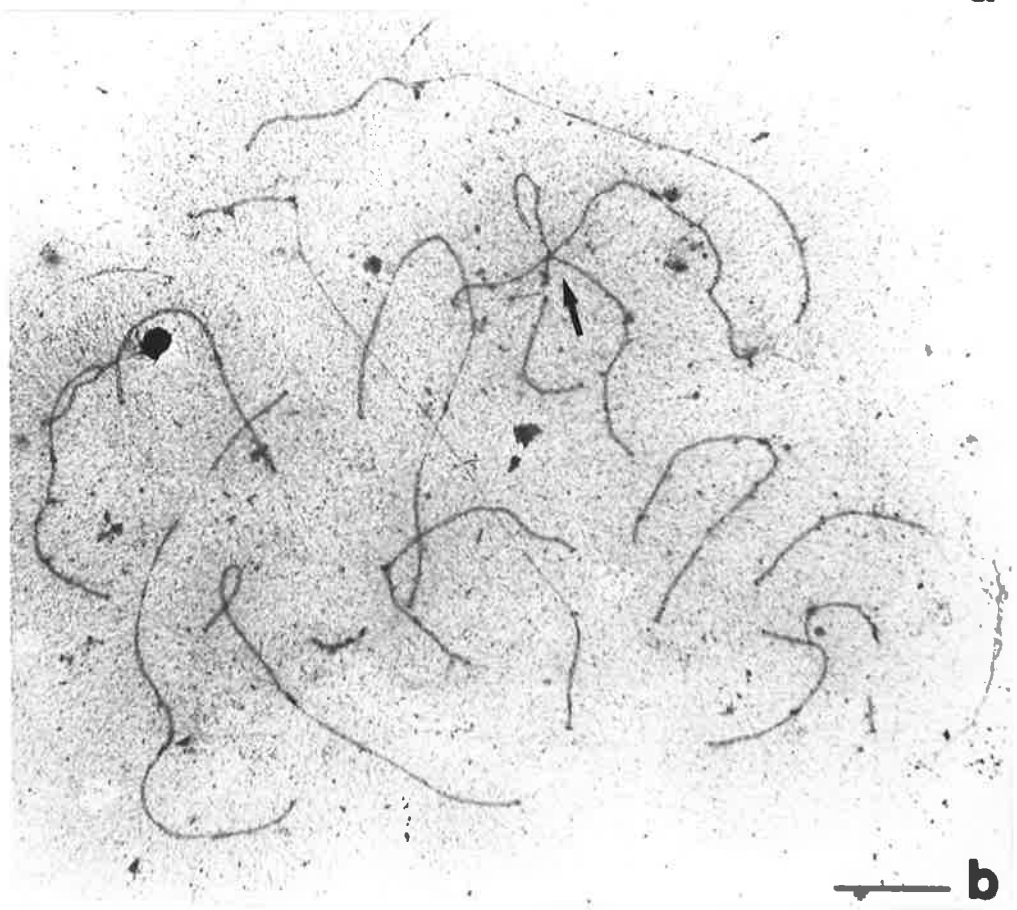
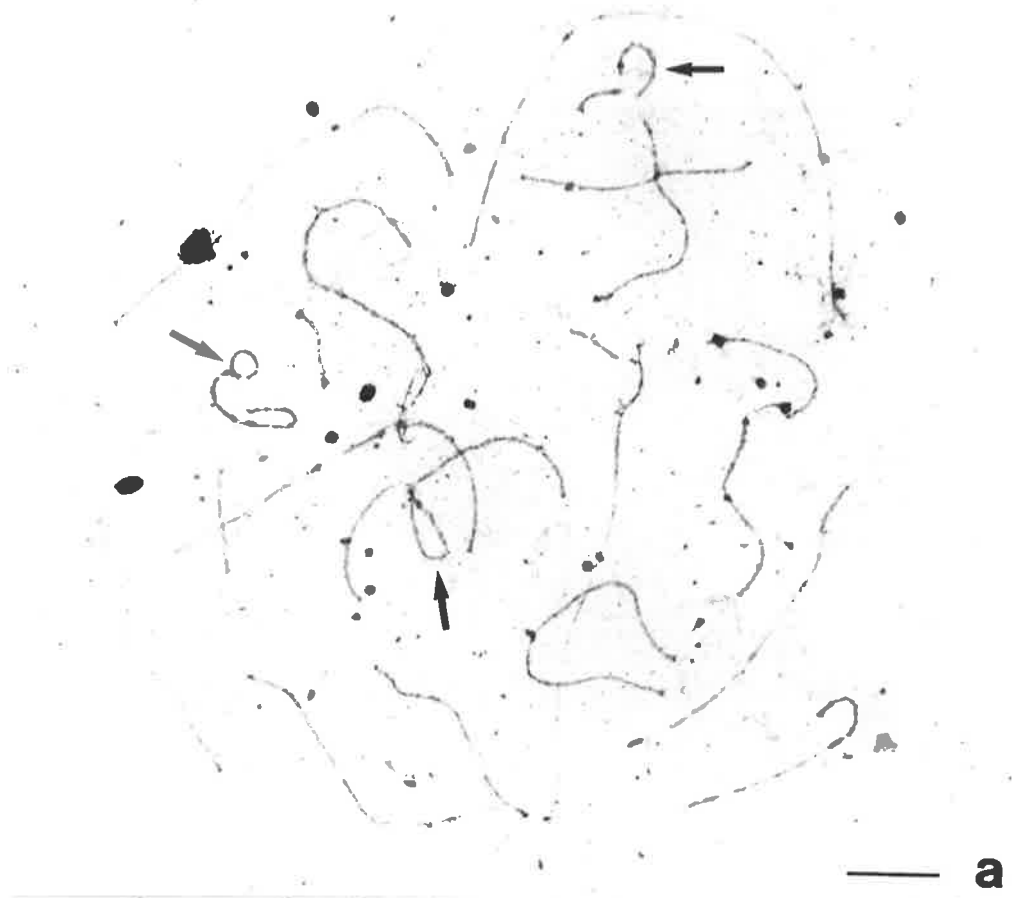
**b No inversion loops present.**

**All inversions heterosynapsed.**

**Arrow shows short single side arm on INV T1.**

**Note stretching of synaptonemal complexes in both  
cells (probably trivalents).**

**Bar = 5  $\mu$ m.**



cells. Figure 2.4.3a, b shows that the inversion loops corresponding to INV T1 and INV B1 were clearly distinguishable on the basis of relative position of the loop with respect to the kinetochores and telomeres. The additional loop found in two cells corresponded to a small paracentric inversion in an acrocentric bivalent (INV B2) and was only identified by synaptonemal complex analysis (Fig. 2.4.3c). Figure 2.4.4 shows the synaptonemal complex karyotypes from a cell showing complete homosynapsis compared with a cell showing complete heterosynapsis and their corresponding metaphase I bivalents and trivalents.

A further observation was that in many cells pairing was complete except for a single interstitial unpaired region (Fig. 2.4.5). Although individual identification of all synaptonemal complexes was difficult in many cells, it was the inverted region of INV T1 that often corresponded to this interstitial unpaired region. This may reflect that this region was often late to pair or did not pair at all in many cells. Analysis of the pairing arrangements for the INV T1 at metaphase I would distinguish between these two proposals.

Figure 2.4.6 shows the types of pairing exhibited by all three inversions at metaphase I. The INV T1 exhibited three types of pairing; homosynapsis (loop formation), heterosynapsis (straight pairing), and synaptic failure (probably asynapsis (failure to pair) as opposed to desynapsis (synaptic failure subsequent to pairing)). Similarly, the INV B1 exhibited both homosynapsis and

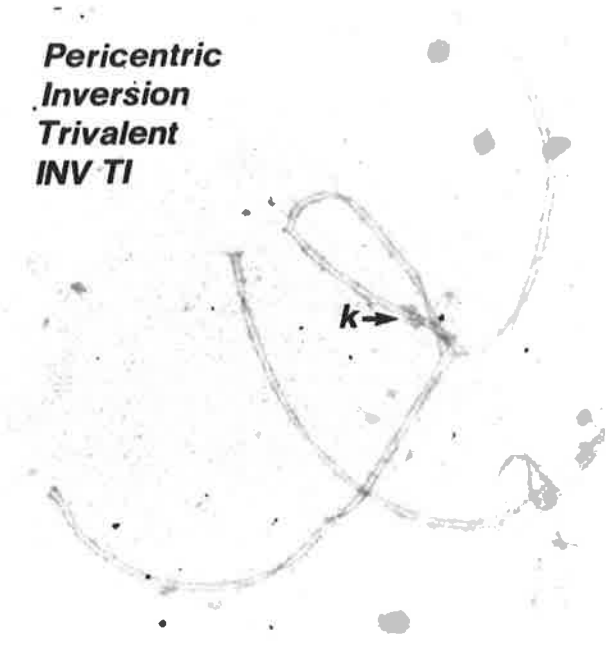
**Figure 2.4.3**

**Details of the three inversions observed in BM XVII.**

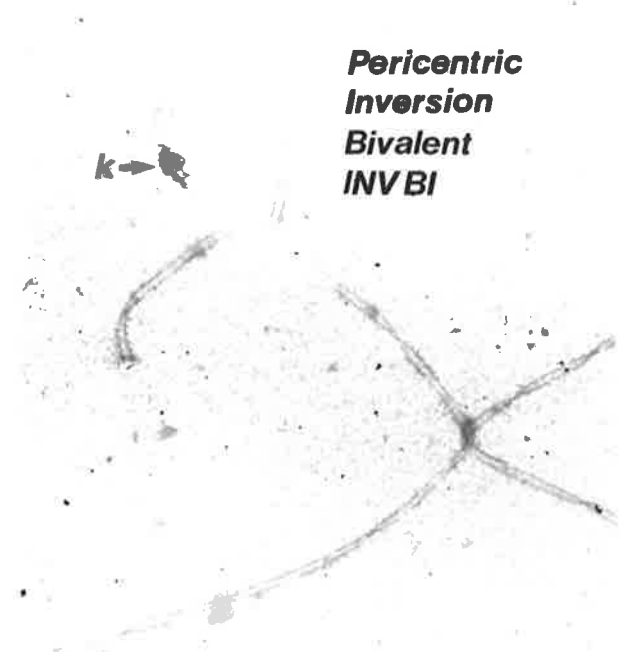
**k = kinetochore**

**INV B2 shows breakage of the synaptonemal complex just  
beyond the inverted region.**

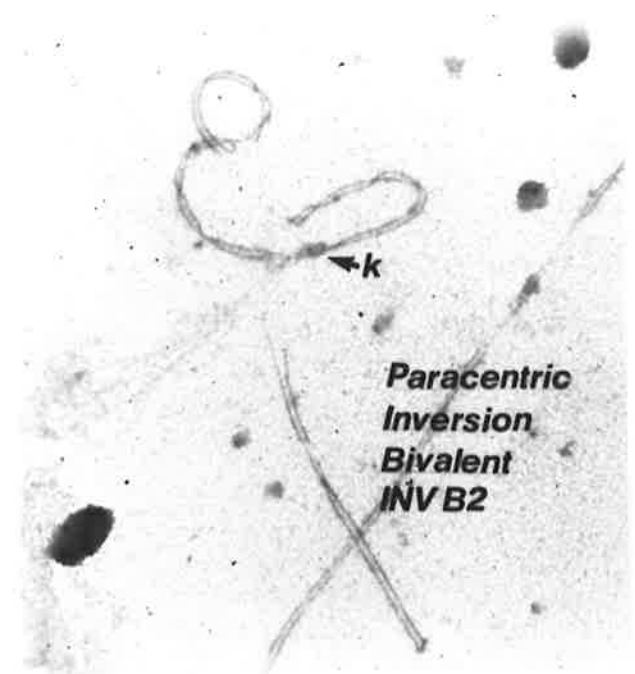
**Pericentric  
Inversion  
Trivalent  
INV T1**



**Pericentric  
Inversion  
Bivalent  
INV B1**



**Paracentric  
Inversion  
Bivalent  
INV B2**



**Figure 2.4.4**

**Details of synaptonemal complexes and their  
corresponding metaphase I configurations.**

- 1 Homosynapsed cell.**
- 2 Heterosynapsed cell.**

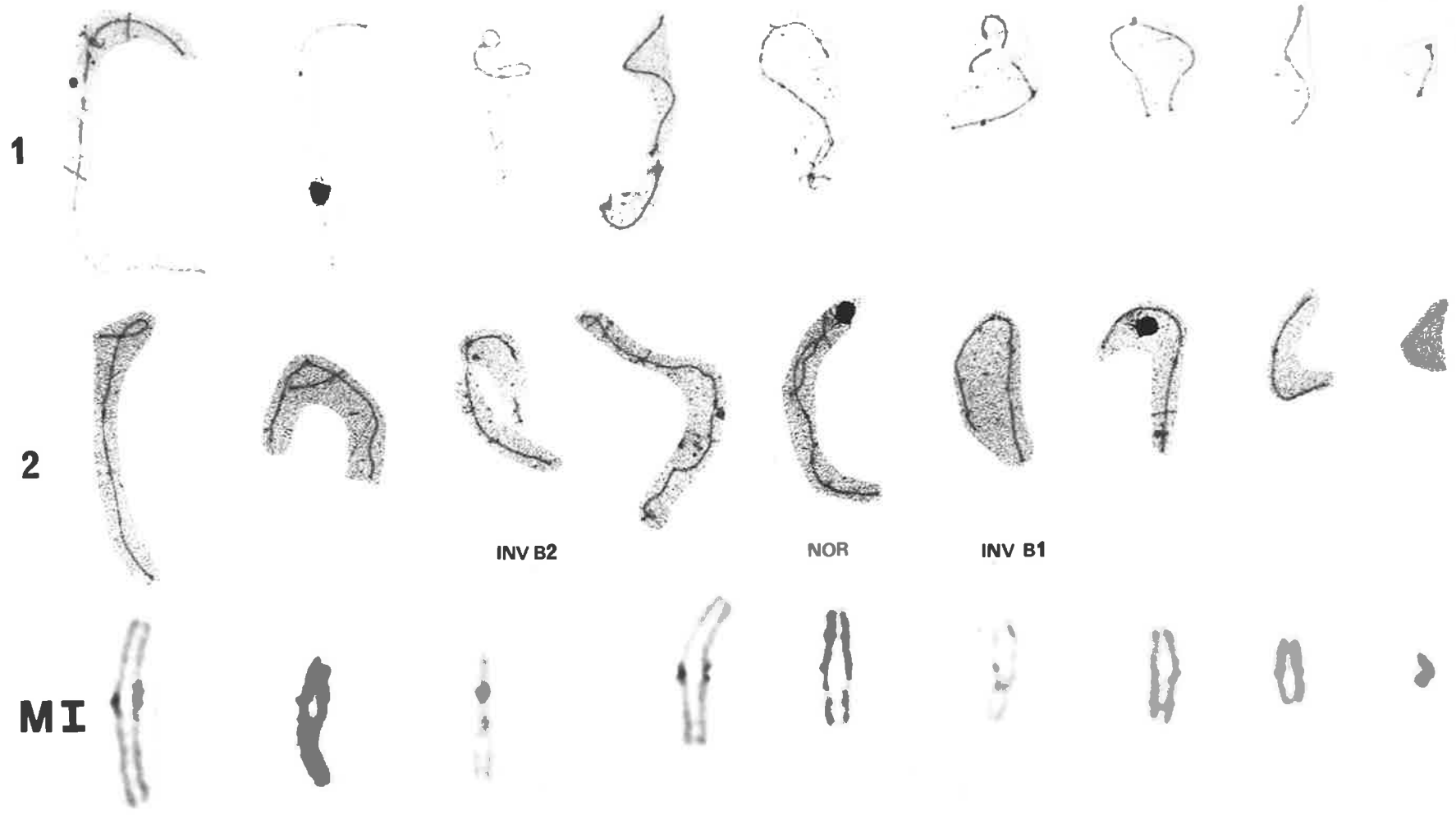
**The inversions are marked.**

**Opposite page shows bivalents.**

**Trivalents shown overpage.**

**Note lack of side arm formation in trivalent  
synaptonemal complexes.**

**BM XVII Bivalents**



# BM XVII Trivalents

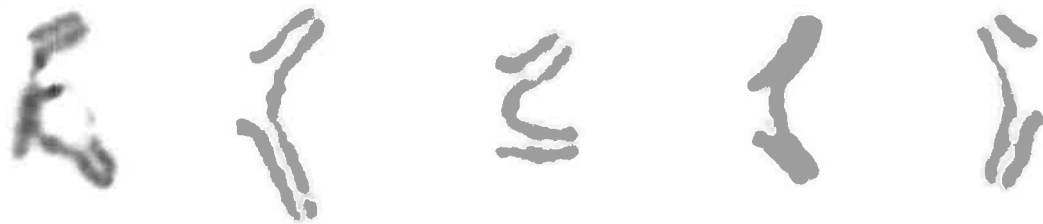
1



2



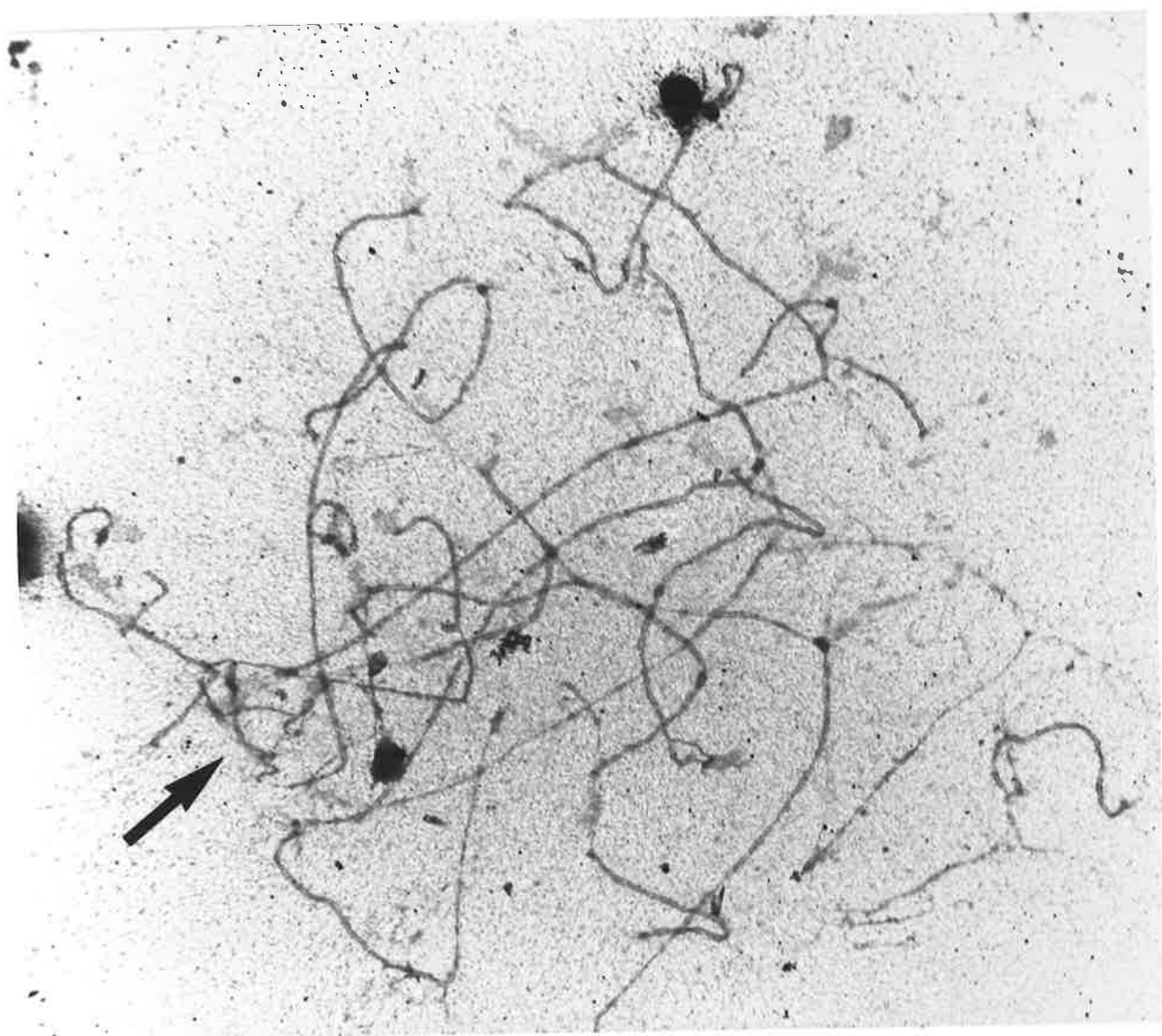
MI





**Figure 2.4.5**

**Pachytene cell (BM XVII) showing single region  
of asynapsis. (arrowed)**



**Figure 2.4.6**

**Details of the pairing arrangements exhibited  
at metaphase I by INV T1, INV B1 and INV B2.**

HOMOSYNAPSIS

HETEROSYNAPSIS

SYNAPTIC FAILURE

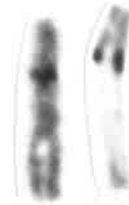
PERICENTRIC INVERSION  
TRIVALENT  
INV T1



PERICENTRIC INVERSION  
BIVALENT  
INV B1



PARACENTRIC INVERSION  
BIVALENT  
INV B2



heterosynapsis and occasionally synaptic failure, while the paracentric inversion (INV B2) exhibited only heterosynapsis at metaphase I. Table 2.4.3 compares the frequencies of the pairing arrangements exhibited by each inversion at both pachytene and metaphase I. Additionally, the ratio of loops (homosynapsis) to straight pairing (heterosynapsis) has been calculated.

On the basis of both Chandley's (1982) and Moses' *et al.* (1982) criteria (i.e. per cent loop formation <100% at pachytene and decrease in L:S ratio from pachytene to metaphase I), all three inversions apparently exhibit synaptic adjustment. However, the synaptic behaviour of INV T1 and INV B1 is clearly not analogous to the situation in some mouse inversions where synaptic adjustment results in only heterosynapsis at metaphase I (Moses *et al.*, 1982).

Direct evidence of synaptic adjustment, i.e. inversion loops that were reduced in size during the transition from homosynapsis to heterosynapsis, could not be positively identified. This was due to difficulty in identifying particular synaptonemal complexes. Differential contraction at metaphase, stretching of synaptonemal complexes, particularly trivalents, at pachytene and difficulty in identifying kinetochores made comparisons of relative lengths of synaptonemal complexes and metaphase I chromosomes, both within and between cells, virtually impossible.

Table 2.4.3

Pairing behaviour exhibited by three different inversions  
in BM<sub>XVII</sub> at pachytene and metaphase I.  
(shown as per cent of total cells scored)

Inversion	N	Division stage	Pairing type			L:S
			homo-synapsis	hetero-synapsis	synaptic failure	
Pericentric inversion trivalent INV T1	17	P	24%	35%	41%	0.68
	89	M	12%	71%	17%	0.17
Pericentric inversion bivalent INV B1	18	P	44%	50%	6%	0.88
	89	M	15%	84%	1%	0.18
Paracentric inversion bivalent INV B2	18	P	28%	72%	-	0.39
	89	M	-	100%	-	0.0

N = number of cells scored

P = pachytene

M = metaphase I

L:S = ratio of loop formation to straight pairing

(homosynapsis : heterosynapsis)

### Comparison of Metaphase I Pairing of INV T1 and INV B1.

An intracellular comparison was made in this animal (BM XVII) of the pairing arrangements exhibited by the two pericentric inversions at metaphase I. Table 2.4.4 shows the frequency of each pairing type exhibited by INV T1 and INV B1 in 89 metaphase I cells, compared with the expected frequencies based on independence. A chi-squared test for independence (i.e. pairing in INV T1 is independent of pairing in INV B1) was highly significant. The data showed that INV T1 and INV B1 exhibited the same pairing type within a cell at a higher frequency than expected. Assuming synaptic adjustment occurred in some cells to convert homosynapsis to heterosynapsis, this result suggests synaptic adjustment is cell specific. If synaptic adjustment does not occur this suggests that cellular conditions influence pairing behaviour.

A further two animals from the Belair population (BM XVI and BM XIII) heterozygous for inversions (including INV T1) were analysed to determine if synaptic adjustment was operating.

### *Urodacus manicatus*. (BM XVI)

This animal was heterozygous for only INV T1. Figure 2.4.7 shows a LM pachytene cell exhibiting homosynapsis for this inversion and a metaphase I cell where the inversion was heterosynapsed. The pachytene synaptonemal complex data is presented in Table 2.4.5. Examples of pachytene synaptonemal complex cells exhibiting homosynapsis,

Table 2.4.4

- a) Observed numbers of pairing arrangements for INV T1 and INV B1 at metaphase I in  $BM_{XVII}$ .

	Observed			Total
	homo	hetero	asyn	
INV T1	11	63	15	89
INV B1	13	75	1	89

- b) Observed frequencies of pairing arrangements.

	Observed		
	homo	hetero	asyn
INV T1	$\frac{11}{89}$	$\frac{63}{89}$	$\frac{15}{89}$
	= 0.124	= 0.708	= 0.168
INV B1	$\frac{13}{89}$	$\frac{75}{89}$	$\frac{1}{89}$
	= 0.146	= 0.843	= 0.011

Expected frequencies calculated on the basis of pairing in INV T1 being independent of pairing in INV B1.

homo = homosynapsis (loop)

hetero = heterosynapsis (straight pairing)

asyn = synaptic failure



Table 2.4.4 (cont.)

c) Pairing configurations within cells for INV T1 and INV B1 at metaphase I.

Pairing type	Observed nos.	Expected nos.
<u>Same Pairing</u>		
<u>INV T1 - INV B1</u>		
homo - homo	9	1.6
hetero - hetero	62	53.1
asyn - asyn	1	0.1
<u>Different Pairing</u>		
<u>INV T1 - INV B1</u>		
homo - hetero	2	9.3
homo - asyn	0	0.1
hetero - homo	1	9.2
hetero - asyn	0	0.8
asyn - homo	3	2.2
asyn - hetero	11	12.6

d) \* Pooled data.

	Pairing type		
	Same	Different	
Observed	72	17	89
Expected	54.8	34.2	89
	126.8	51.2	178

$$\chi^2_1 = 13.95 \quad P < 0.001$$

\* Some classes showed expected numbers <5 therefore data was pooled for statistical analysis.

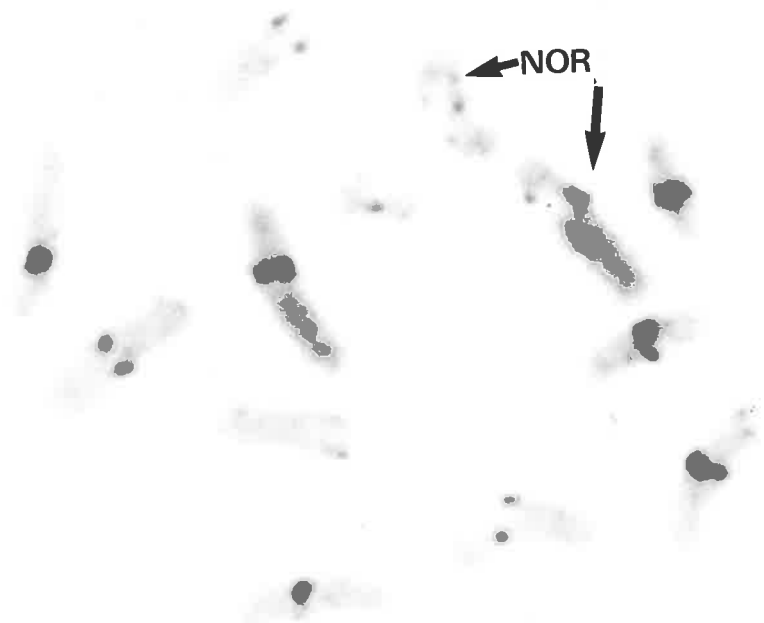
**Figure 2.4.7**

***Urodacus manicatus*, BM XVI**

- a** C-banded (LM) pachytene cell showing homosynapsis (loop formation) (arrowed).
  
- b** C-banded metaphase I cell showing heterosynapsis of INV T1 (arrowed). NOR present on trivalent.



**a**



**b**

Table 2.4.5

Pachytene synaptonemal complex data for INV T1 in BM<sub>XVI</sub>.

Cell no.	No. synaptonemal complexes	Pairing stage	No. inversion loops	Regions of asynapsis
1	13	pachytene	0	-
2	13 <sup>+</sup>	early pachytene	0	+ (INV T1)
3	13	pachytene	0	-
4	13 <sup>+</sup>	early pachytene	0	+ (NOR region)
5	13	pachytene	0	-
6	13	pachytene	1 INV T1	-
7	13	pachytene	0	-
8	13	pachytene	0	-
9	13	early pachytene	0	+
10	13	pachytene	0	-
11	13	early pachytene	1 INV T1	+
12	13	pachytene	0	-
13	13	pachytene	0	-
14	13	pachytene	1 INV T1	-
15	13	pachytene	0	-
16	13 <sup>+</sup>	pachytene	0	-
17	13 <sup>+</sup>	early pachytene	0	+ (INV T1)
18	* 13 <sup>+</sup>	early pachytene	0	+
19	* 13 <sup>+</sup>	early pachytene	0	-
20	* -	early pachytene	0	+
21	* 13 <sup>+</sup>	early pachytene	0	+

\* early cells not included in analysis.

+ exact number of synaptonemal complexes not clear.

heterosynapsis and synaptic failure for INV T1 are shown in Fig. 2.4.8. Table 2.4.6 gives the comparative data for pachytene and metaphase I for this inversion. Again INV T1 does not exhibit 100% homosynapsis at pachytene while the L:S ratio is consistent with the operation of synaptic adjustment. However, the difference between the L:S ratio at pachytene compared with metaphase I is less than that recorded for the same inversion in BM XVII. This suggests there are either differences between animals in the frequency of synaptic adjustment or, that the sample of pachytene cells scored from BM XVI were generally at a later stage than those scored in BM XVII. That is, the BM XVII L:S ratio compares a mixture of early (unadjusted) and late (adjusted) pachytene cells with metaphase I cells while the BM XVI ratio compares late pachytene (adjusted) with metaphase I cells. This is a possibility in *U. manicatus* where the testis is small (few cells can be scored) and coordinated division occurs in particular regions, i.e. all the cells scored in BM XVI may be from the same testicular region and in the same pachytene stage.

***Urodacus manicatus*. (BM XIII)**

BM XIII exhibited 14 synaptonemal complexes at pachytene corresponding to 10 bivalents and 4 trivalents, including the pericentric inversion trivalent (INV T1) as in BM XVI and BM XVII. Pachytene synaptonemal complex analysis revealed the presence of two additional chromosomal rearrangements that were not observed in conventional cytological preparations;

**Figure 2.4.8**

**BM XVI, pachytene cells showing synaptonemal complexes.**

- a      INV T1 - homosynapsed**
- b      INV T1 - heterosynapsed**
- c      INV T1 - asynapsed**

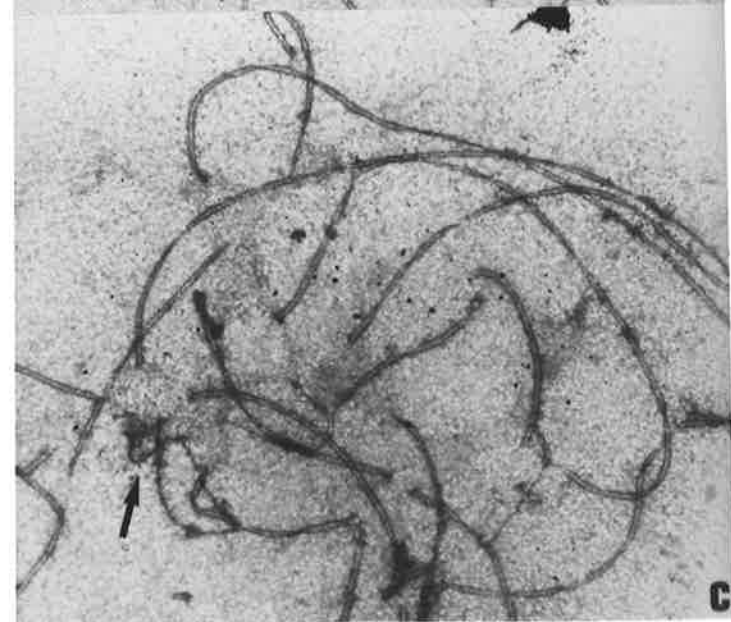
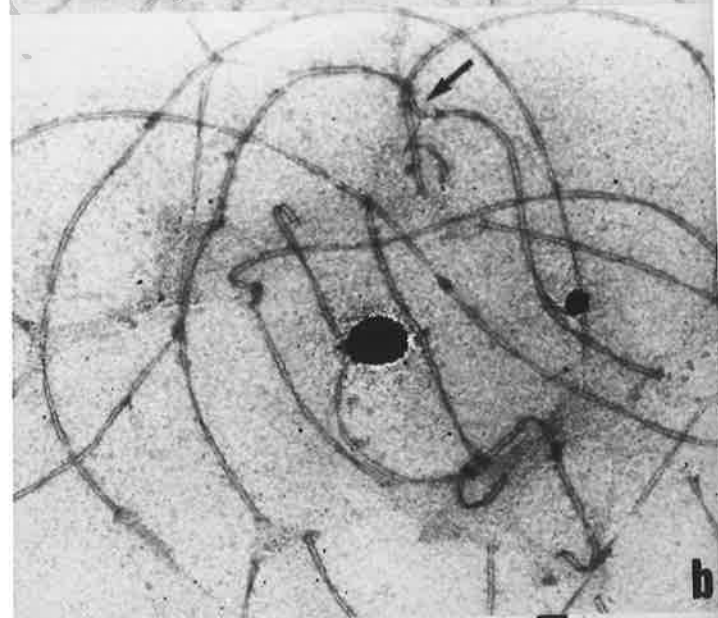
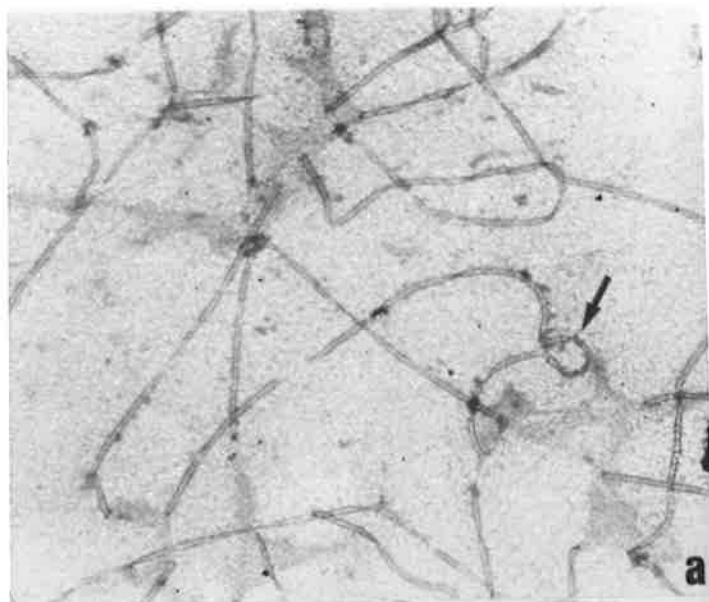


Table 2.4.6

Pairing behaviour at pachytene and metaphase I  
for INV T1 in BM<sub>XVI</sub>.

Inversion	N	Division stage	Pairing type			L:S
			homo-synapsis	hetero-synapsis	synaptic failure	
Pericentric inversion trivalent INV T1	17	P	18%	70%	12%	0.26
	19	M	16%	74%	10%	0.21

P = pachytene

M = metaphase I

N = no. cells scored



INV T2 was a pericentric inversion involving the short arm of a submetacentric fusion heterozygote, and INV TSA which appeared to be a very small paracentric inversion involving only the short side arms of a trivalent. This trivalent was clearly identifiable in most cells as it also exhibited a centric shift; the kinetochore proteins of one or two elements were not located at the fusion (side-arm) position.

The pachytene synaptonemal complex data for these three inversions is presented in Table 2.4.7. Figure 2.4.9 shows a fully paired pachytene cell with INV T2 and INV TSA clearly identifiable. INV T2 shows almost terminal loop formation with a small region of desynapsis (Fig. 2.4.9b). Table 2.4.8 shows a comparison of the pachytene and metaphase I pairing data for the three inversions. Only 17 metaphase I cells from this animal could be scored. There was no evidence for synaptic adjustment for INV T1 in this animal, although, again, this may be due to the scoring of late pachytene cells. However, INV T2 and INV TSA showed almost complete heterosynapsis at metaphase I suggesting synaptic adjustment had occurred.

Direct evidence for synaptic adjustment in INV TSA is shown in Figure 2.4.10. Although the cells could not be adequately staged there were a number of different pairing configurations exhibited by this region that could be interpreted as different stages of synaptic adjustment. The final pairing arrangement (heterosynapsis) was a small side-arm identical to those observed by Moses *et al.* (1979) in *Lemur* hybrids excepting the kinetochore position. Synaptic

Table 2.4.7

Pachytene synaptonemal complex data for BM<sub>XIII</sub>.

Cell no.	No. synaptonemal complexes	Pairing stage	No. loops	Regions of asynapsis
1	14	pachytene	0	-
2	14	pachytene	2 INV TSA INV T2	+ (INV T2)
3	14	pachytene	1 INV T2	+ (INV T2)
4	14	early pachytene	1 INV TSA	+
5	14	pachytene	1 INV T1	-
6	14	pachytene	0	-
7	14	pachytene	2 INV TSA INV T2	-
8	14	early pachytene	0	+ (INV T1)
9	14	pachytene	1 INV T1	-
10	14	pachytene	0	-
11	14	early pachytene	1 INV T2	+
12	14	pachytene	1 INV T1	-
13	14	pachytene	1 INV TSA	-
14	14	pachytene	1 INV TSA	-
15	14	pachytene	0	-
16	14	pachytene	1 INV TSA	-
17	14	early pachytene	1 INV T1	+
18	14	pachytene	1 INV T2	-
19	14	pachytene	1 INV T2	-

INV T1 - pericentric inversion trivalent.

INV T2 - pericentric (small, acrocentric) inversion trivalent.

INV TSA - inversion trivalent, side-arms.

**Figure 2.4.9**

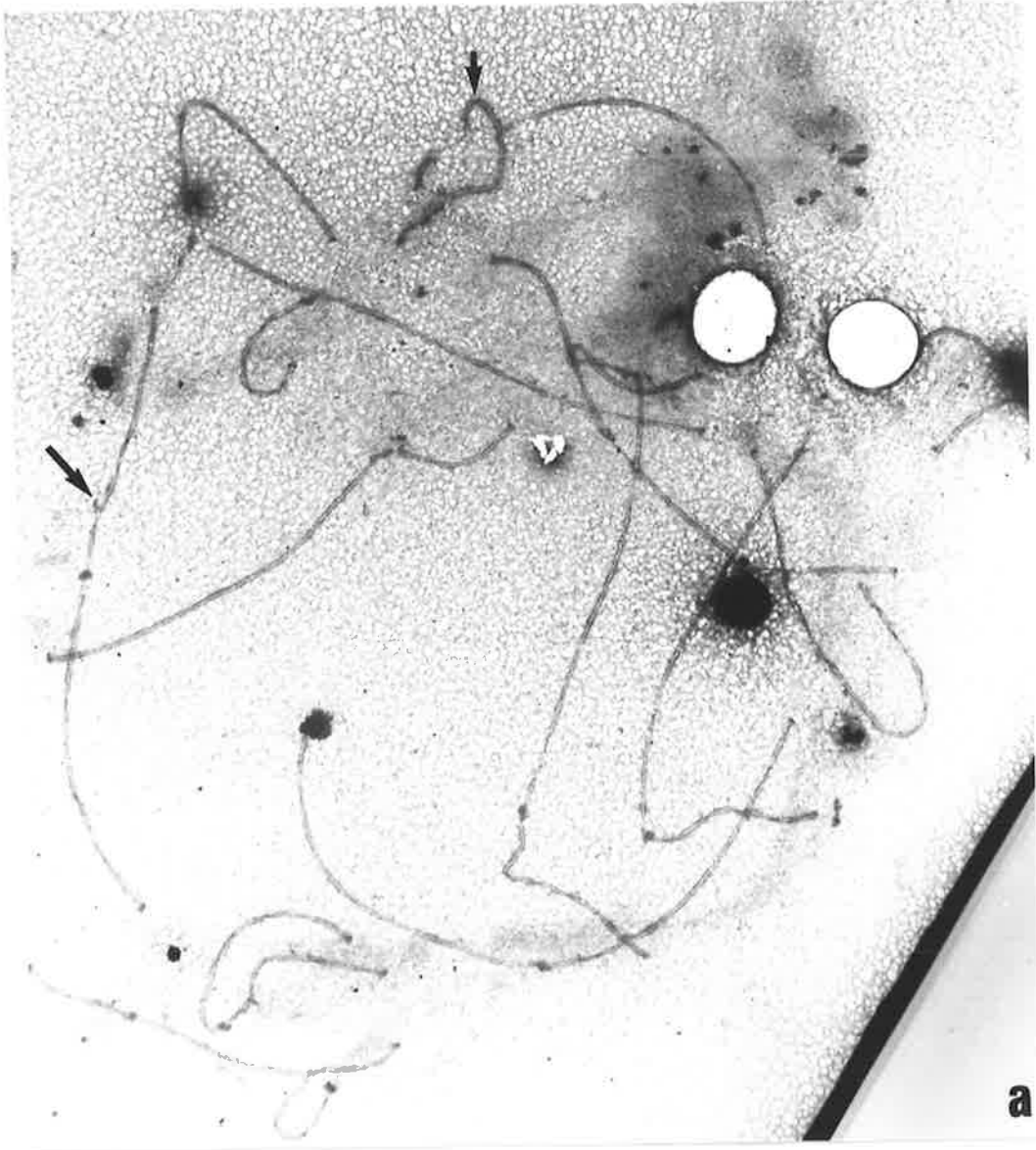
*U. manicatus*, BM XIII

**a. Pachytene cell showing synaptonemal complexes.**

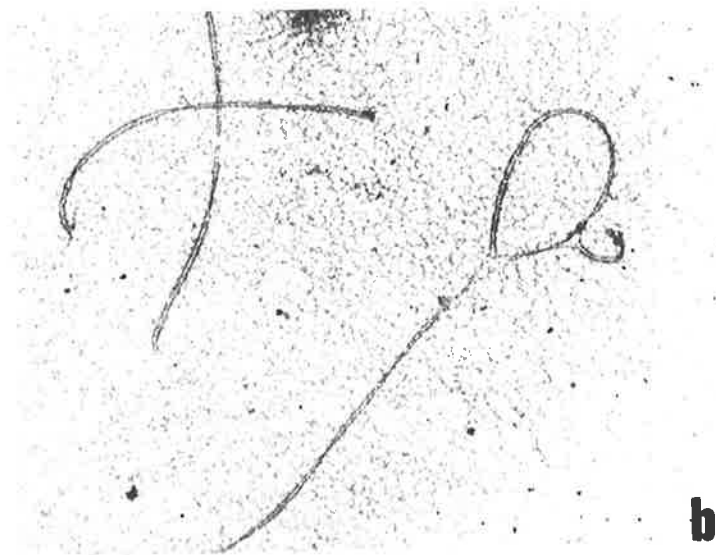
**INV TSA - short side arm (arrowed).  
Note kinetochore position.**

**INV T2 - loop region showing some  
asynapsis (arrowed).**

**b. Detail of INV T2 from a cell showing homosynapsis  
for the inverted region.**



**a**



**b**

Table 2.4.8

Pairing behaviour at pachytene and metaphase I  
for INV T1, INV T2 and INV TSA in BM<sub>XIII</sub>.

Inversion	N	Division stage	Pairing type			L:S
			homo-synapsis	hetero-synapsis	synaptic failure	
INV T1 pericentric inversion trivalent	19	P	21%	74%	1%	0.29
	17	M	18%	59%	23%	0.30
INV T2 pericentric inversion trivalent (small, acrocentric)	19	P	32%	68%	-	0.46
	17	M	-	*88%	12%	0.0
INV TSA inversion in trivalent side-arm	19	P	32%	68%	-	0.46
	17	M	-	100%	-	0.0

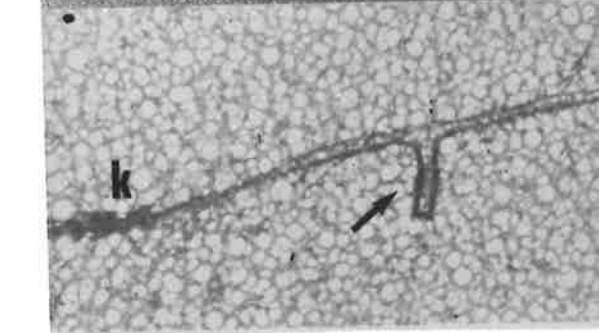
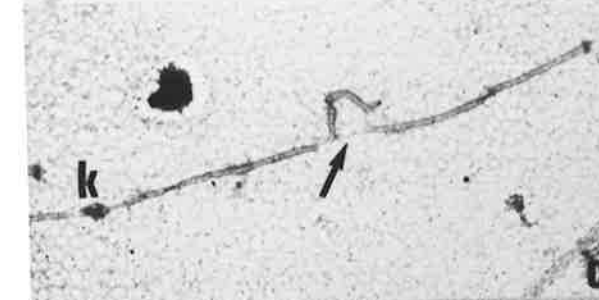
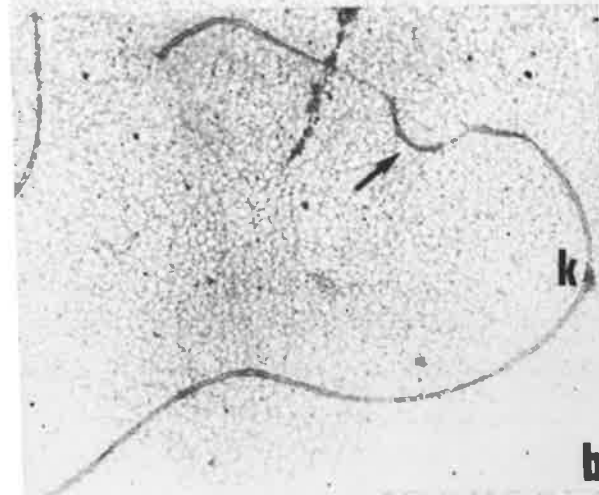
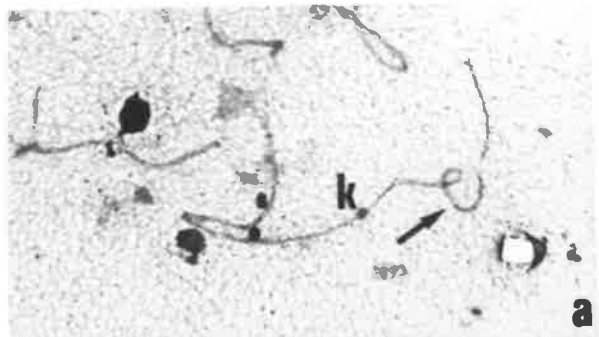
\* Due to the small size of this inversion it may have been possible that in some cells this inversion was still homosynapsed at metaphase I.

**Figure 2.4.10**

**Stages of homosynapsis and heterosynapsis  
observed for INV TSA.**

**d,e - complete heterosynapsis**

**k = kinetochore**



adjustment in INV TSA clearly involves homosynapsis followed by heterosynapsis contrary to that observed in *Lemur* where asynapsis preceded heterosynapsis.

No other trivalents in BM XIII, BM XVI or BM XVII exhibited obvious asynapsis followed by late pairing to form a heterosynapsed side-arm as observed in Lemurs. Many trivalents in *U. manicatus* appeared not to form side-arms.

## DISCUSSION

### Synaptic Adjustment.

### Inversion Data.

The data presented in this section show that in achiasmate *U. manicatus* individuals heterozygous for various inversions, homosynapsis, heterosynapsis and synaptic failure are found at both pachytene and metaphase I. These data argue that there is no obligatory synaptic adjustment and that different rearrangements behave differently (based on the different frequencies of these three pairing types for different inversions at metaphase I). Additionally, chiasmata do not play a major role in determining whether synaptic adjustment of an inversion will occur. The three pairing arrangements observed are identical to those observed by Maguire (1981) in chiasmate maize, where she postulated presence/absence of chiasmata determined pairing type. Are then the pairing arrangements observed at metaphase I resultant from synaptic adjustment, or, are they representative of the original pairing initiated at pachytene?



### Evidence for Synaptic Adjustment in *U. manicatus*.

As stressed by Moses and others who have investigated synaptic adjustment, only a comparison of accurately staged early and late pachytene cells can provide a clear, temporal display of pairing change from homosynapsis to heterosynapsis. However, in many organisms pachytene cannot be accurately staged and indirect methods (as used in this study) are the only means by which data can be accumulated.

Indirect evidence presented in this study suggests synaptic adjustment does occur to a certain extent in *U. manicatus*. All inversions (except INV T1) consistently showed a decrease in homosynapsis from pachytene to metaphase I. Most of the smaller, simpler inversions (i.e. INV B2, INV T2, INV TSA) showed loop formation at pachytene but complete heterosynapsis at metaphase I, consistent with synaptic adjustment having operated. However, pairing in INV T1 was more complex. When the pachytene and metaphase I data from the three animals studied is pooled the L:S ratio shows a decrease from 0.35 at pachytene to 0.21 at metaphase I. It is difficult to determine if this small decrease is consistent with synaptic adjustment, whereas the data from a single animal (BM XVII) where the L:S ratio decreased from 0.68 to 0.17 is clearly consistent with synaptic adjustment. The differences observed between animals could represent chance differences in the proportion of early and late pachytene cells scored or, could be due to real differences in synaptic adjustment between animals. However, the lack of evidence for synaptic adjustment in INV T1 does not

preclude other inversions from exhibiting synaptic adjustment. Chandley (1982) described a complex inversion in mouse which did not appear to undergo synaptic adjustment, whereas, most other rearrangements studied in mouse show complete synaptic adjustment (Moses *et al.*, 1982).

#### **Evidence from Co-ordinated Pairing at INV T1 and INV B1.**

The observation that co-ordinated pairing seemed to be occurring for INV T1 and INV B1 in BM XVII suggests physiological differences between cells influence whether synaptic adjustment occurs or not (i.e. synaptic adjustment is possibly cell specific in some species). The excess of both similar classes at metaphase I (i.e. both homosynapsed and both heterosynapsed) suggests that synaptic adjustment was operating (at least in some cells) or the implication of this observation would be that inversions simultaneously paired in particular cells. There was no evidence at pachytene that pairing of INV T1 and INV B1 was associated or co-ordinated.

Due to lack of pachytene staging, this observation of co-ordinated pairing in BM XVII could not be adequately analysed. It would be of interest to analyse synaptic adjustment for two inversions within cells in mouse to determine if synaptic adjustment is in any way co-ordinated. Of special interest would be an intracellular analysis of two inversions one of which does not necessarily show synaptic adjustment such as In(1)1rk/In(1)12rk studied by Chandley (1982). Such a study would determine if two large

inversions present in the same cell interact to effect the level of synaptic adjustment.

#### **Possible Influences on Synaptic Adjustment.**

Physiological differences that affect synaptonemal complex behaviour have been invoked to account for differences observed between male and female mice. For example, Mahadevaiah *et al.* (1984) observed that the end-to-end associations of bivalents at prophase were more frequent in oocytes than spermatocytes perhaps due to physiological differences between the sexes.

The association of purely autosomal rearrangements with the XY pair during pachytene has also been well documented and may play a role in presence/absence of synaptic adjustment (Forejt *et al.*, 1981; Chandley, 1982; Searle *et al.*, 1983; Mahadevaiah, 1984). This association is partly responsible for male sterility for rearrangements that are fertile in the female. The association of the X and Y with a rearrangement may inhibit synaptic adjustment in the male (e.g. Chandley (1982)  $\text{In}(1)1rk/\text{In}(1)12rk$ ).

Mahadevaiah *et al.* (1984) observed differential pairing behaviour of the  $\text{Is}(7;1)40H$  ( $\text{Is}40H$ ) insertion at pachytene between male and female mice. The proportion of quadrivalents scored at pachytene was higher in spermatocytes compared with oocytes.  $\text{Is}(7;1)40H$  is a male sterile (female semi-sterile) rearrangement that results in a high frequency of XY association with the rearranged pachytene chromosomes. They considered that observed pairing differences between the

sexes to be due to the higher proportion of late pachytene cells scored in oocytes and to be consistent with synaptic adjustment in the female. However, there was no consistent evidence that the proportion of bivalents in oocytes increased with advancing stages of pachytene; although they considered their data compatible with such a trend. Even when only early pachytene stages are compared the approximate frequency of bivalents to quadrivalents in males and females is 0.5:0.5 and 0.66:0.33 respectively. The data is also compatible with physiological or XY association effecting the frequency of synaptic adjustment resulting in different frequencies of unadjusted rearrangements in males and females. Additionally, Searle *et al.* (1983), when examining Is40H in oocytes found a frequency of 76% quadrivalent formation at metaphase I. This contrasts with the 50% quadrivalent formation observed by Mahadevaiah *et al.* (1984) for the same insertion in late pachytene cells. They suggested that differential survival of cells (with quadrivalents compared with bivalents) could account for the frequency differences. However, other explanations such as differences in pairing types or synaptic adjustment between animals could also be invoked.

The programmed pairing stages of the X and Y chromosomes (used in part to determine pachytene stages) may also influence autosomal pairing (and synaptic adjustment) in rearrangements that are male fertile. It would be of great interest to carry out a detailed study of early and late pachytene cells to determine whether the process of synaptic

adjustment can be observed in female mice heterozygous for inversions. The less defined and incomplete synaptic adjustment in maize and *U. manicatus* (species which lack sex chromosomes) suggests there may be some association between X and Y pairing and synaptic adjustment. If this is so, synaptic adjustment in the female may differ from that observed in male mice.

However, there is also evidence from *U. manicatus* that suggests small, simple rearrangements are adjusted while larger, more complex rearrangements may not necessarily show synaptic adjustment. Chandley (1982) suggested complexity of the inversion may influence adjustment in mouse. In *U. manicatus* it appears that delayed pairing in INV T1 results in asynapsis. Simpler rearrangements may be easily adjusted while more complex rearrangements do not have time to complete pairing and adjustment before the end of pachytene. Thus there are possibly a large number of factors mechanical, physiological and genetical (i.e. XY) that determine if synaptic adjustment occurs.

#### **Two Phases of Synapsis: a Real Phenomenon?**

There appear to be two lines of argument for two phases of synapsis:

- (1) that of Rasmussen and Holm where 'synaptic adjustment' of quadrivalents in tetraploids and translocation heterozygotes is chiasmata dependent;
- (2) the *synaptic adjustment* of inversions and duplications in mouse that are chiasmata independent (Moses *et al.*,

1982).

Moses *et al.* (1982) considered both these arguments to be part of the same phenomenon. Evidence from achiasmate Buthids and Scorpionids supports the view that chiasmata do not influence 'synaptic adjustment'. Additional evidence from polyploid testicular cells in Scorpionids further suggests adjustment of quadrivalents is not chiasmata dependent. Although pachytene data is unavailable, Figure 2.4.11 shows polyploid testicular cells exhibiting a high frequency of quadrivalent formation. Similarly both chain quadrivalents and a ring (translocation) quadrivalent in *U. manicatus* were never resolved to heteromorphic bivalents despite the absence of chiasmata. This data suggests that differences between male and female *Bombyx* with respect to the observed frequency of 'synaptic adjustment' may be due to physiological differences between the sexes (as may the differences in Is(7;1)40H pairing in mouse). These differences may, in part, be related to chiasmata versus achiasmata meiosis in *Bombyx*, but the position of chiasmata may not be the sole influencing factor.

Additionally, returning to the example of Is(7;1)40H in mouse, the different frequencies of quadrivalents in males and females could be accounted for by possible differences in chiasmata frequencies between the sexes (on the basis of Rasmussen and Holms' hypothesis). However, some authors (Mahadevaiah *et al.*, 1984) tend to ignore chiasmata when examining synaptic adjustment. Yet, if two-stage pairing and synaptic adjustment are the same phenomenon some

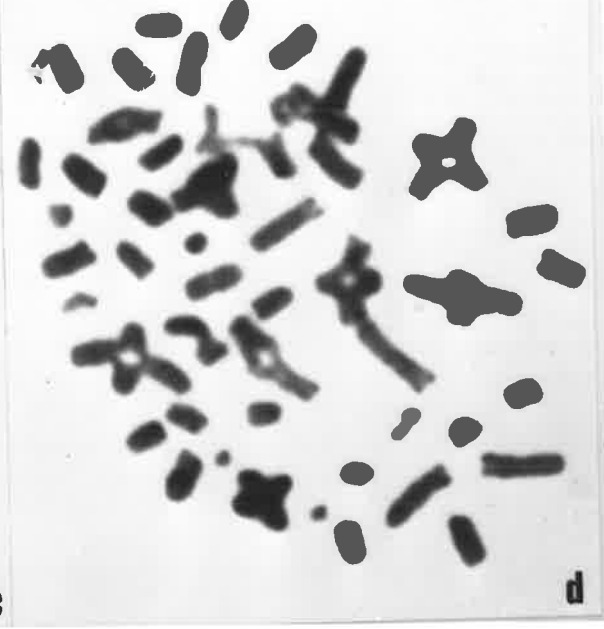
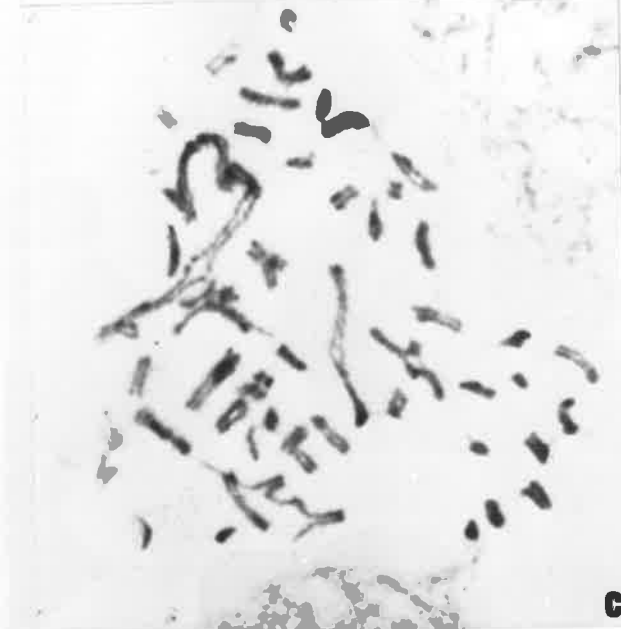
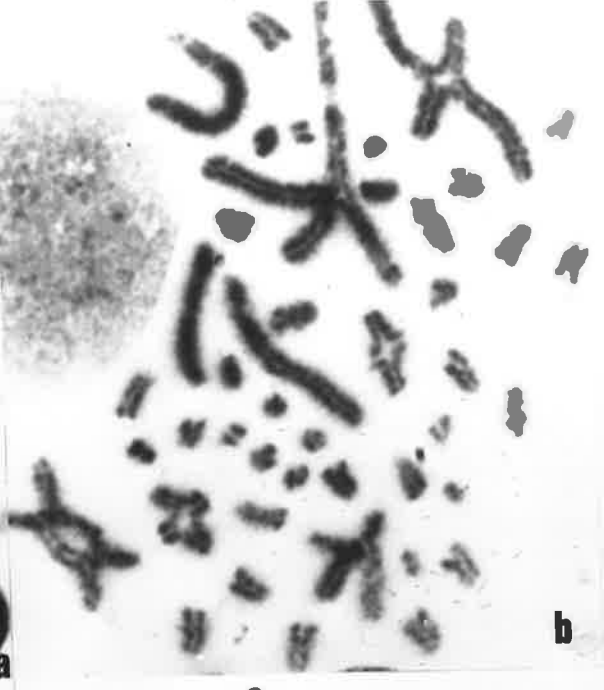
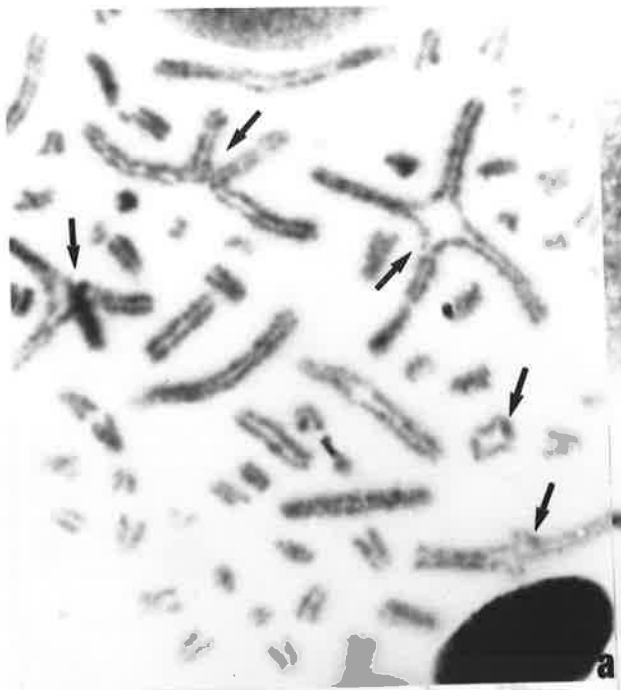
**Figure 2.4.11**

**Polyploid testicular cells from achiasmate  
*Urodacus* species exhibiting a high frequency  
of multivalent formation.**

**a,b    *Urodacus planimanus***

**c        *Urodacus novaehollandiae***

**e        *Urodacus manicatus***



a

b

c

d



consistency in examining the data must be found. What are the criteria that determines if synaptic adjustment of a chromosomal rearrangement will be inhibited by chiasmata or not?

There is also difficulty in accommodating two-stage pairing as a specific switch from exclusive homosynapsis to pairing that allows heterosynapsis; especially as in many cells in *U. manicatus* homosynapsis and heterosynapsis obviously occur together (e.g. comparing INV T1 and smaller inversions). Moses and Poorman (1981) in mouse found that the transition from homo- to heterosynapsis was occasionally observed in nuclei in which homosynapsis was still incomplete in some bivalents. Moses *et al.* (1982) suggested that metabolic events specifically related to recombination (e.g. pachytene DNA repair synthesis) may mark the end of homology dependent pairing and allow homology-indifferent synapsis. This scheme suggests that in haploids where homology is lacking there may be no restriction and indifferent synapsis could occur at any time. Clearly two phases of synapsis has yet to be adequately explained. The universality of the phenomenon (especially in haploids and some achiasmate species) has not been shown.

#### **Unstable vs. Stable Pairing Arrangements.**

Another enigmatic feature of synaptic adjustment phenomena is what determines that a particular pairing arrangement is unstable? Two-by-two synapsis can be invoked as the force leading to adjustment of some quadrivalents,

however this is not so for homosynapsed inversion loops which are already paired two-by-two. One consistent factor is that all the rearrangements that show synaptic adjustment are characterized by a switch of pairing partners which are eliminated by synaptic adjustment. However, this does not account for the stability of trivalents in *Lemur* hybrids where pairing partner switches appear stable (Moses *et al.*, 1982) or interchange heterozygotes in Buthids. It may be possible to invoke competitive pairing as the influence towards adjustment. Duplications involve two-by-two synapsis and change of pairing partners, however there may be some competition for pairing (as in polyploid quadrivalents) which does not exist in the trivalents of *Lemur* hybrids. Synaptic adjustment operates to stabilize these regions. However, whether or not synaptic adjustment occurs may be influenced by a multiplicity of events that may differ between cells (as in *U. manicatus*) between individuals and between species. As yet, these 'events' have yet to be adequately explained.

## SECTION 5

CHROMOSOMAL FEATURES REVEALED BY SYNAPTONEMAL COMPLEX  
ANALYSIS IN SCORPIONIDS

Synaptonemal complex analysis in *U. manicatus* revealed that many individuals from the Belair population were heterozygous for a large number of inversions that were not revealed by conventional cytological preparations (see Section 2.4). In the absence of high resolution banding synaptonemal complexes have revealed that karyotypic change is much greater in this species than previously thought.

The telocentric nature of the chromosomes was revealed in EM preparations of synaptonemal complexes (Fig. 2.5.1). The position of the NOR region at the single C-band in the karyotypes of all individuals was shown. This C-band represents the site of a meiotically active nucleolar organiser. Figure 2.5.1 also shows heterosynapsis of a univalent which probably corresponds to a B chromosome that has failed to pair.

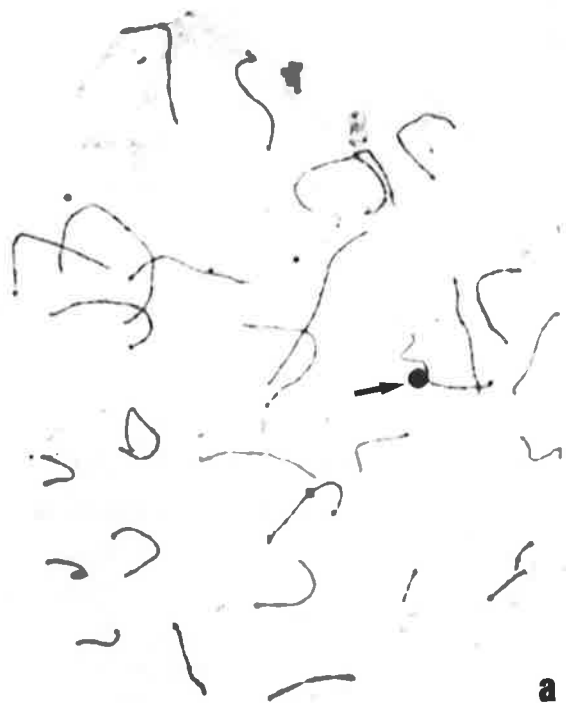
**Trivalent Pairing in *Urodacus manicatus*.**

With the exception of INV TSA where the side-arm of the trivalent was clearly identifiable in 8/19 pachytene cells, the remaining trivalents in *U. manicatus* individuals did not, or only rarely, formed side-arms. Table 2.5.1 shows the frequency of pachytene side-arms observed for all trivalents in BM XVII, XVI and XIII.

**Figure 2.5.1**

***Urodacus manicatus***

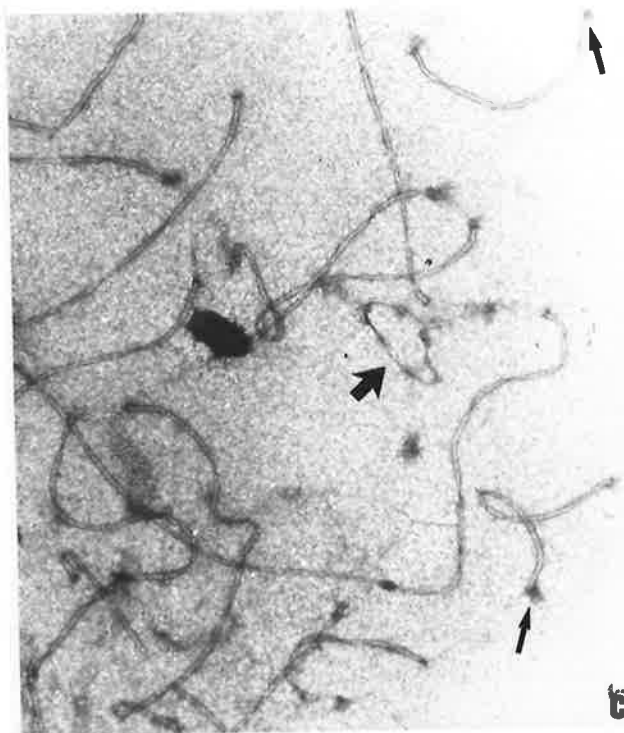
- a LM synaptonemal complex preparation showing telocentric bivalents and NOR (OCM)
- b Metaphase I cell from same animal (above). Correspondance of C-band and NOR.
- c Electron microscope preparation of synaptonemal complexes showing telocentric bivalents (kinetochores arrowed).  
Univalent B (large arrow).
- d Enlargement of univalent showing asynapsis and heterosynapsis.



a



b



c



d

Table 2.5.1

The frequency of trivalent side arms observed at pachytene  
in *U. manicatus*.

Animal	Trivalents	No. cells side arms observed	Cells scored	Frequency
BM <sub>XVII</sub> <sup>a</sup> (5 trivalents)	1. INV T1	3	20	0.15
	2. Large submetacentric	0	20	0
	3. Large metacentric	0	20	0
	4. Small submetacentric	0	20	0
	5. Submetacentric- unequal fusion arms	3	20	0.15
BM <sub>XVI</sub> (4 trivalents)	1. INV T1	2	20	0.1
	2. Large acrocentric	2	20	0.1
	3. Large metacentric (NOR)	0	20	0
	4. Small submetacentric	5	20	0.25
BM <sub>XIII</sub> (4 trivalents)	1. INV T1	1	19	0.052
	2. INV T2	3	19	0.16
	3. INV TSA	8	19	0.42
	4. Large metacentric	1	19	0.052

<sup>a</sup> Figures 2.4.2, 2.4.4

INV T1 did not form a paired side-arm but could be identified in cells where it showed heterosynapsis as a single unpaired lateral element (see Figs. 2.4.2, 2.4.4). Of the remaining 4 trivalents in BM XVII (see Fig. 2.4.4 where all trivalents are identified)  $\chi$  only one could be identified by the presence of a side arm. Again this side arm was not a short region of paired synaptonemal complex but often manifest as a short unpaired lateral element due to the unequal length of the short-arm of the fusion submetacentric and its telocentric homologue. The other trivalents were never observed to form side arms and often showed stretching of a single lateral element in the region of centric fusion.

Two BM XVI trivalents (Fig. 2.5.2) occasionally formed side-arms similar to those observed in Lemurs. However, one metacentric trivalent which could be clearly identified at pachytene (as it possessed a nucleolar organiser region (NOR)) was never observed to form a side-arm. Occasionally the centromeres appeared split at the fusion region.

All BM XIII trivalents exhibited side-arms in at least one pachytene cell (Fig. 2.5.2). However, the region of side-arm formation was often very small (when compared with INV TSA).

A general feature of all trivalents that formed side-arms was the observation that both small chromosomes of the trivalent paired on the same side of the fusion product metacentric. This was also evident at metaphase I where each fusion chromosome exhibited only one pairing face on which both homologous telocentrics paired. This feature was

**Figure 2.5.2**

**Trivalent side-arms.**

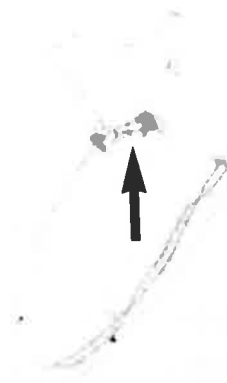
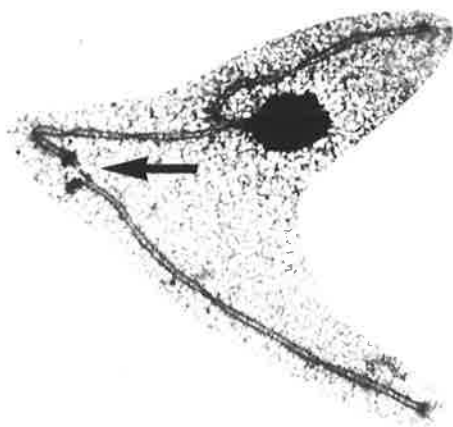
**BM XVI**

- a      Trivalent with NOR - not observed  
to form a side arm at pachytene.
  
- b      Small trivalent - side arm formation.

**BM XIII**

- c      Metaphase I - remnants of trivalents  
observed under the EM after  
synaptonemal complex preparation.
- Note lack of side arm formation by  
some trivalents.

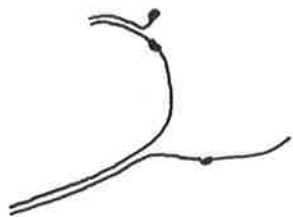




**a**

**b**

**c**



Bivalent

INV TI  
asynapsis

Trivalent  
no side arm

INV T2  
loop remnant

INV TSA  
possible side  
arm

also observed in hybrid Lemurs which, like *U. manicatus*, show a high degree of regular gamete formation (Moses *et al.*, 1979).

The lack of side-arms in some trivalents probably reflects the totally telocentric nature of these chromosomes. Centric fusion of two telocentric chromosomes has resulted in heterozygotes which do not form trivalent side-arms. In those trivalents where side-arm formation occurs the telocentric elements have been converted to acrocentrics. This may have been via small inversions, a rearrangement very common in this species (a small inversion in one arm could have resulted in trivalent 5 in BM XVII), or alternatively, the side-arm may be short 'added on' heterochromatic segments or short arms remaining if fusion occurred via a Mullerian two break process (Fig. 2.5.3). Clearly, synaptonemal complex analysis in *U. manicatus* has revealed that besides the gross forms of chromosome change visible under the LM many more subtle changes have occurred (i.e. small inversions) that could not be revealed under the LM without high resolution banding.

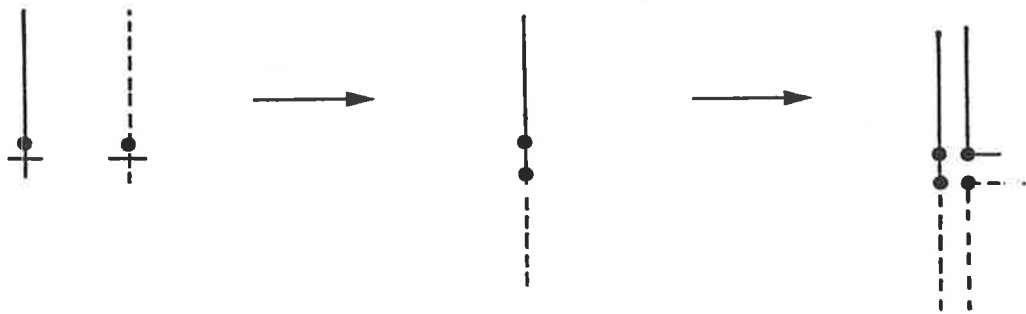
Figure 2.5.3

MECHANISMS OF CHROMOSOME FUSION/FISSION.

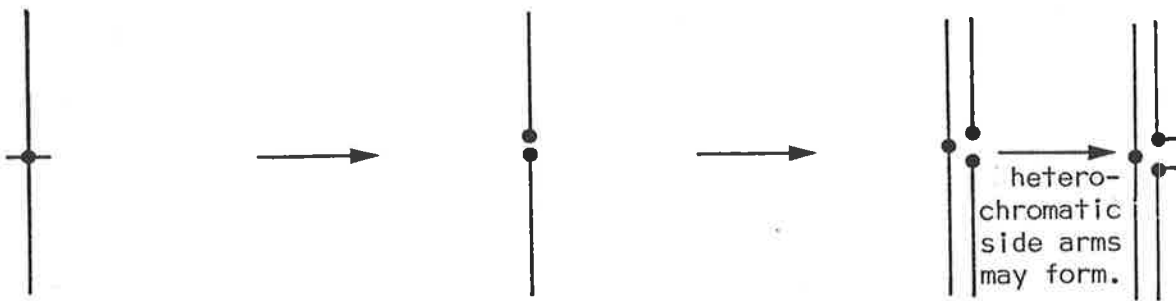
TRANSLOCATION FUSION.



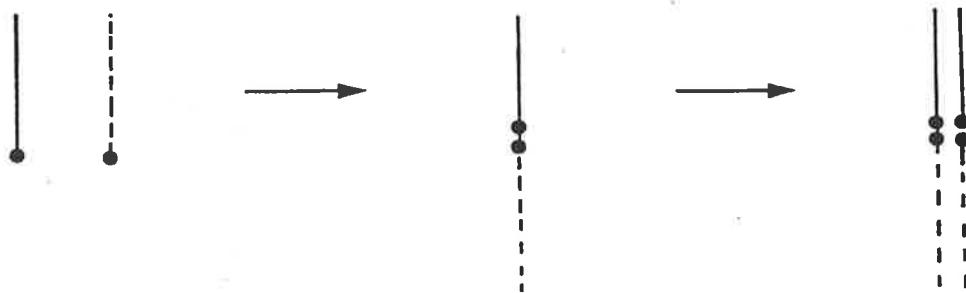
CENTRIC FUSION.



CENTRIC FISSION.



CENTRIC FUSION (TELOCENTRICS).



CHAPTER 3

EVIDENCE FOR THE NATURE OF THE CENTROMERE  
IN AUSTRALIAN SCORPIONS

## CHAPTER 3

### SECTION 1: LITERATURE REVIEW

#### The Nature of the Centromere/Kinetochores.

Originally the terms 'centromere' (Darlington, 1937) and 'kinetochores' (Sharp, 1934) were used as synonyms to describe the primary constriction of chromosomes that attached to microtubules during cell division. Ris and Wit (1981) argue that these terms should remain synonymous. However, as knowledge of this region of the chromosome has increased it seems appropriate to differentiate between these two terms. Rieder (1982) suggested the term kinetochores should refer at the ultrastructural level to the precise region of the chromosome that becomes attached to spindle microtubules. In mammalian cells this region differentiates into a trilaminar disc structure during mitosis and appears to contain unique components. Centromere, should be a less precise term referring to the region on the chromosome (e.g. primary constriction, pericentromeric heterochromatin etc.) with which the kinetochores is associated.

Under both definitions this means that chromosomes that exhibit neocentric activity (i.e. a region of microtubule attachment) must be described as having more than one kinetochores which again is confusing. Thus, it is perhaps more appropriate to use centromere as a general term to describe the region of primary constriction (and associated heterochromatin) responsible for microtubule attachment which

can be differentiated at the light microscope level. Kinetochore should be an even more precise term than Rieder suggests and refer to ultrastructurally visible differentiated chromosome regions (i.e. protein plates associated with the chromosome) that are involved in microtubule attachment. This enables electron microscopists to use a separate term when considering ultrastructural details of the chromosomes. It also avoids confusion between LM and EM observations. For example, Rieder describes multiple sites of microtubule association along the chromosome length in the absence of a kinetochore plate as denoting polycentric chromosomes. Under the terms of my definition these chromosomes would exhibit holocentric behaviour (i.e. non-localized microtubule attachment) while lacking an ultrastructurally visible kinetochore.

#### Centric Properties of Chromosomes.

Two main chromosome types can be identified based on cytological observations of their centromeric/kinetochore structure and behaviour (i.e. anaphase movement) namely, monocentric chromosomes and holocentric chromosomes.

Monocentric chromosomes are characterized by the following cytological properties:

1. An observable primary constriction.
2. Localized kinetic activity (monokinetic) resulting in J and V shaped elements at mitotic and meiotic anaphase due to the orientation of the centromeric region towards

the poles.

3. Localized regions of heterochromatin are often associated with the centromeric constriction (i.e. pericentromeric heterochromatin). The original technique used to differentiate this region is known as C-banding, i.e. Centromeric banding (Sumner, 1972).
4. Bivalents (homologous centromeres) co-orientate at metaphase I which leads to pre-reduction of non-crossover regions (i.e. at anaphase I paternal and maternal strands separate).

In contrast, the features indicative of holocentric chromosomes are as follows:-

1. The chromosomes do not show a primary constriction.
2. Kinetic activity is non-localized (holokinetic) such that chromatids separate almost simultaneously along their entire length and tend to move in a parallel fashion towards the poles. Whilst this is always the case at mitosis, centric activity may appear to be telomeric at meiosis (e.g. Hemiptera, Hughes-Schrader and Schrader, 1961).
3. Chromosomes do not have a single localized region of heterochromatin.
4. Meiosis in some species is inverted. In these species bivalents (homologous centromeres) auto-orientate at

metaphase I and co-orientate at metaphase II resulting in post-reduction (i.e. maternal and paternal strands do not separate until anaphase II). The meiotic products of both pre- and post-reduction are identical (see Fig. 3.1.1a from Rhoades 1961, and Fig. 3.1.1b).

Most plants and animals possess monocentric chromosomes. Only a few diverse species of plants and invertebrates have evolved holocentric chromosome systems. Table 3.1.1 modified from John and Lewis (1965) lists all the species that have been shown to possess holocentric chromosomes.

Besides holocentric and monocentric chromosomes there are two other rare centromeric forms termed polycentric and neocentric characterized by peculiar centric structure/activity. Boveri (1887) showed that in *Ascaris megalocephala* the two large chromosomes present at meiosis appeared as many minute chromosomes during mitosis in the early embryo. This process of chromosome diminution has since been verified and characterized in *A. megalocephala* (Lin, 1954) and *A. lumbricoides* var. *suum* (Goldstein, 197<sup>7</sup>~~6~~) where it was concluded that the gametic chromosomes are compound polycentric units while the small somatic chromosomes produced by diminution have a single functional centromere and behave as stable units. However, recent ultrastructural evidence has shown that the composite germ-line chromosomes have a thin continuous trilaminar plate along their length, a feature indicative of holocentric chromosomes (Goday *et al.*, 1985). Thus, at the ultrastructural level, holocentric and polycentric



Figure 3.1.1

a) Rhoades (1961)

Schematic diagrams comparing the meiotic behavior of a bivalent having localized centromeres with one having nonlocalized centromeres as in the coccid *Puto* and in *Luzula purpurea*. The chiasma in the localized type has become terminalized by metaphase I; this by no means occurs in all forms with localized centromeres but is the rule in forms with nonlocalized centromeres. Note that there is no difference between the basic coccid type and the *Luzula* type, but both differ markedly in behavior from the localized type. Sketch prepared by Tristão de Mello-Sampayo.

Localized centromere - co-orientation and pre-reduction  
for non-crossover regions.

Coccids and *Luzula* - auto-orientation and post-reduction  
for non-crossover regions.

b) Possible division cycles in an hypothetical organism  
showing holocentric chromosomes and achiasmatic meiosis.

Non-sister chromatids show terminal associations if  
auto-orientated.

Buthid scorpions show co-orientation.

Diakinesis	Metaphase I	Anaphase I	Telophase I	Prophase II	Metaphase II	Anaphase II	Telophase II
Localized centromere type							
Basic type in coccids							
Luzula type							

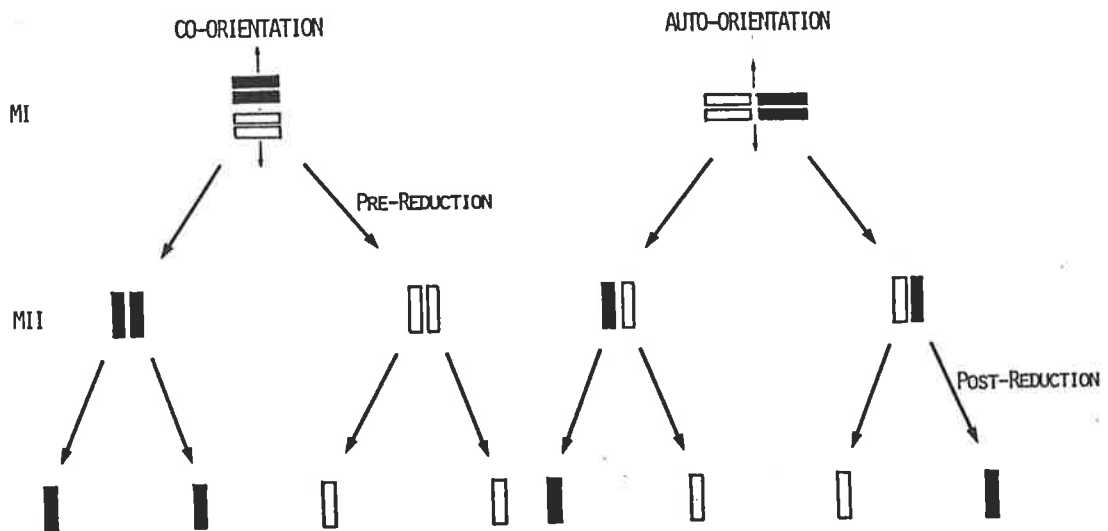


Table 3.1.1

List of species with holocentric chromosomes.

Type	Species	Reference
P L A N T S	Algae - Conjugales - Zygnemataceae	Godward, 1961*
	<i>Spirogyra crassa</i>	King, 1960
	Desmidiaceae	
	Angiosperms - Monocotyledons	
	Juncales - Juncaceae (probably all)	Castro <i>et al.</i> , 1949
A N I M A L S	<i>Luzula purpurea</i>	Nordenskiöld, 1961, 1962
	<i>Luzula campestris</i>	Nordenskiöld, 1961
	Cyperales - Cyperaceae (probably all)	
	<i>Eleocharis palustris</i>	Hakansson, 1954, 1958
	Protozoa	
Ciliata - Heterotricha		
<i>Nyctotherus oralis</i>	Eichenlaub-Ritter and Ruthman, 1982 <sup>1</sup>	
Radiolaria		
<i>Aulacantha scolymantha</i>	Lecher, 1973	
Metazoa		
Aschelminthus		
Nematoda		
<i>Meloidogyne hapla</i>	Goldstein and Triantophyllou, 1978	
<i>Caenahabditis elegans</i>	Albertson and Thomson, 1982	
Arthropoda		
Scorpionida - Tityus		
(many include all Buthidae)	Piza, 1943 (this study)	
Acarina		
<i>Pediculopsis gramineum</i>	Cooper, 1939	
<i>Pediculoides rentricosis</i>	Hughes-Schrader and Ris, 1941	
<i>Eylais setosa</i>	Keyl, 1957	
<i>Tetranychus urticae</i>	Tempelaar, 1979; Feiertag-Koppen, 1976 Tempelaar, 1979	
Araneae		
<i>Dysdera crocata</i>	Benavente and Wettstein, 1980	
<i>Segestria florentia</i>		
Chilopoda		
<i>Thereuonema hilgondorfi</i> <sup>2</sup>	Ogawa, 1962	
Insecta		
a) Hemiptera - Homoptera	Hughes-Schrader, 1948	
- Heteroptera	Hughes-Schrader and Schrader, 1962	
	Nokkala, 1985	
b) Anoplura -		
<i>Pediculus corporis</i>	Bayreuther, 1955	
<i>Haematopinus suis</i>		
c) Mallophaga -		
<i>Gyropus ovalis</i>	Scholl, 1955	
d) Odonata <sup>2</sup>	Oksala, 1943 Kiauta, 1967, 1968, 1969	
e) Lepidoptera <sup>3</sup>	Suomalainen, 1953	
<i>Bombyx mori</i>	Murakami and Imai, 1974	
f) Dermaptera	Ortiz, 1969; Webb and White, 1970; White, 1971	
g) Trichoptera <sup>2</sup>	Suomalainen, 1966	

\* Later refuted - see Rieder (1982, p.7).

<sup>1</sup> Continuous kinetochore plate on chromosomes of micronucleus. Chromosomes formed by lateral association of telokinetic chromosomes, i.e. composite chromosomes.<sup>2</sup> Confirmation required.<sup>3</sup> May not include all species (White, 1973; Bigger, 1975).

chromosomes appear identical so that distinguishing between them solely on differences at the behavioural level (i.e. presence/absence of chromosome diminution at mitosis) may be unjustified.

Holocentric systems also exhibit differences in chromosome behaviour due to apparent shifts in centric activity (between mitosis and meiosis) influenced by physiological or genetic factors that may effect kinetochore expression or organisation. Neocentricity is a further example of this phenomenon.

Neocentric activity occurs in a few plant species when telomeres and other chromosome regions manifest active mobility on the spindle at either mitosis or meiosis. Neocentric activity at meiosis is not comparable to the short-lived mitotic phenomena which involves only one of two sister chromatids (Bajer and Ostergren, 1961). Neocentromeres are only associated with chromosome complements exhibiting heterochromatic segments, although neocentric activity may occur in euchromatic regions of such complements. It is usually only manifest in the presence of the main centromere and details of its mechanism are unknown. However, it appears to involve direct insertion of microtubules into the chromosome which is also a common feature of holocentric chromosomes at meiosis (reviewed by John and Lewis, 1965).

Examples of variable expression and mechanisms of centric activity between mitosis and meiosis pose the question of whether all chromosomes in a single nucleus are

alike with respect to centromere type. Ultrastructural evidence suggests that the microchromosomes of birds may have a simpler kinetochore structure than the macrochromosomes as they do not show any trilaminar structure (Brinkley et al., 1974). Kubai and Wise (1981) studying non-random segregation of sex chromosomes ( $X_1X_2Y$ ) in the mole cricket *Neocurtilla* at anaphase I observed massive aggregates of kinetochore-like material associated with the sex chromosomes and which affected their segregation. Perhaps a more extreme example of this occurs in centipedes where the chromosomes are monocentric, however, the X and Y in *Theracupoda clunifera* and *Teuonema lilgendorfi* and two outstandingly large autosomes in *Esastigmatobius longtarisis* were considered to have holocentric chromosomes (Ogawa, 1952, 1955a, b). Additional studies are needed to verify these observations.

#### Differentiating Between Holocentric and Monocentric Chromosomes.

##### 1. EM Ultrastructural Studies.

Lima-de-Faria (1956) gave the first detailed description of the centromere using light microscopy. He interpreted the chromosome arrangement he observed as evidence that the centromeric DNA exhibited a reverse tandem repeat structure with the differences in number and position of chromomeres as evidence for telocentric and compound metacentric chromosomes. Detailed ultrastructural data on the kinetochore is now almost exclusively from electron

microscopy using transmission electron microscopy (TEM) of serial sections and scanning electron microscopy (SEM) of whole mount chromosomes.

Using TEM analysis two main classes of kinetochores have been identified: the trilaminar structure found in many animals and a few lower plants and the ball and cup structure typical of higher plants (Bajer, 1968; Mole-Bajer, 1969; reviews by Kubai, 1975; Bostock and Sumner, 1978).

Detailed accounts of the mammalian trilaminar kinetochore have been presented in Chinese hamster (Brinkley and Stubblefield, 1966), foetal rat cells (Jokelainen, 1967), rat kangaroo (Roos, 1973), and in the Indian muntjac (Comings and Okada, 1971). It consists of a plate or disc-like structure present on the poleward side of each sister chromatid at metaphase. The electron dense inner layer is contiguous with the chromosomal material and separated from the dense outer layer by a lighter middle layer. The outer layer is covered by a corona of electron opaque substances. Spindle micro-tubules attach to the outer plate which is thought to contain DNA (Pepper and Brinkley, 1980; Ris and Witt, 1981). The role of the kinetochore in associating microtubules and the mechanism by which spindle microtubules facilitate chromosome movement are complex and unresolved issues (reviewed by Rieder, 1982; Pickett-Heaps *et al.*, 1982).

In species with monocentric chromosomes the kinetochore can be identified on both mitotic and meiotic chromosomes. This is often not so with holocentric chromosomes. Serial

sectioning and TEM analysis has revealed a single, continuous kinetochore plate extending over at least 75% of the mitotic chromosome length. However, at meiosis, in many species microtubules appear to insert directly into the chromatin. These species include the hemipterans *Oncopeltus fasciatus* (Comings and Okada, 1972; Gassner and Klemetson, 1974), *Rhodnius prolixus* (Buck, 1967), *Philaenus* (Ris and Kubai, 1970), *Dysdercus intermedius* (Ruthmann and Permantier, 1973), the protozoan *Aulacantha* (Lecher, 1973), and the primitive spider *Dysdera crocata*, where kinetochores reappear at metaphase II (Benavente, 1982).

Recent ultrastructural studies in *Luzula* have shown that the initial notion that chromosomes of *Luzula* were polycentric with a number of ball and cup kinetochores distributed along their length is incorrect (Braselton, 1971, 1981; Lambert, 1971; Bokhari and Godward, 1980). This interpretation was an artifact of chromosome clumping. Bokhari and Godward (1980) have subsequently identified the mitotic chromosomes of *Luzula* as holocentric with a palely staining kinetochore extending along the entire chromosome. Braselton (1981) identified four distinct kinetochore regions, similar in structure to the mitotic kinetochores, on metaphase I bivalents of *Luzula*, each associated with the poleward surface of a chromatid. This is consistent with the observation that in *Luzula* homologous centromeres show auto-orientation at metaphase I and subsequent post-reduction at anaphase II (i.e. inverted meiosis).

Continuous trilaminar plates, present at both meiosis

and mitosis, have also been reported in the nematode *Caenorhabditis elegans* (Albertson and Thompson, 1982). Similarly in the Lepidoptera, continuous mitotic kinetochore plates have been observed (Bauer, 1967; Maeki, 1981). However, there is some contention over the nature of the kinetochore in Lepidoptera (White, 1973). Evidence suggests there may be some species with holocentric and others with monocentric chromosomes in this group. *Bombyx mori* exhibits holocentric activity at mitosis, however, Holm and Rasmussen (1980) reported the presence of localized kinetochore plates at metaphase I. This is contrary to the report of Friedlander and Wahrmann (1970) who found no evidence for a kinetochore at metaphase I and the X-ray data of Murakami and Imai (1974) who observed stable transmission of radiation induced aberrant chromosomes. Localized kinetochore plates at mitosis have been reported in other species; *Ephestia kuehniella* and *Trichoplausia ni* (Gassner and Klemetson, 1974). The very small size of the chromosomes of many Lepidopteran species, making them technically difficult to study, has probably contributed to the contradictory nature of the evidence.

## 2. X-ray Breakage of Chromosomes.

The indirect method of X-ray breakage of chromosomes has been used extensively to determine whether chromosomes are monocentric or holocentric. This method relies on X-ray breakage of monocentric chromosomes producing acentric fragments which are lost during subsequent divisions;



fragments of holocentric chromosomes retain their ability to divide and are not lost during division.

Analyses of X-irradiated chromosomes are made complex due to the numerous physiological effects of radiation on cells, difficulty in scoring aberrations and the differential effects of radiation caused by numerous nuclear and physical factors.

Lea (1955) presented a comprehensive summary of the physiological changes exhibited by cells as a result of irradiation. These included a delay in the continuation of cell division and the tendency for chromosomes to exhibit a generalized 'stickiness' leading to clumping at metaphase.

The stage of division at the time of irradiation, the dosage, duration of exposure, and the type of radiation (i.e. X-rays,  $\gamma$ -rays), and the nature of the tissue irradiated, are some of the factors that determine the type and extent of radiation effects. Sparrow and Evans (1961) have determined the various nuclear factors that influence radiation sensitivity in plants. These include the DNA content, the nuclear/nucleolar volume ratio, the amount of heterochromatin present, the size of the chromosomes, chromosome number and ploidy, the rate of cell division and the nature of the centromere.

The bases or mechanisms responsible for the manner in which these factors modify the effects of radiation are unknown. (For review see Evans, 1972)











































### Effects of Irradiation on Monocentric Chromosomes.

The most obvious effect of irradiation is its capacity to induce chromosome breakage. Breakage of monocentric chromosomes has been studied extensively at both mitosis and meiosis in plant and animal species (see reviews by Lea, 1955; Evans, 1962; Brinkley and Hittelman, 1975; Comings, 1974; Evans, 1974).


























Following chromosome breakage and the subsequent rejoining of broken ends (often described as "sticky ends"), a number of cytological effects can be observed and scored as a measure of chromosome damage: micronuclei at interphase, bridges at anaphase and fragments, ring chromosomes and chromosomes that have undergone translocations or interchanges observable at metaphase, are among these features. Micronuclei represent acentric chromosome fragments excluded from the daughter nuclei after cell division. Anaphase bridges are produced when dicentric chromosomes (formed by the rejoining of broken chromosome or chromatid ends) orientate at metaphase with their two centromeres aligned to opposite poles. The chromatin connecting the centromeres is stretched across the spindle at anaphase resulting in breakage and fragment formation. This may lead to the bridge-breakage-fusion cycle first described by McClintock (1941) in maize. Fig. 3.1.2 from Lea (1955) illustrates some of the chromosome aberrations observed after irradiation of cells. Other effects of irradiation on chromosomes include decondensation of heterochromatic regions, the formation of diplochromosomes (White, 1937) and achromatic

Figure 3.1.2

Structural changes induced by X-irradiation of chromosomes. (from Lea, 1955)

	SYMMETRICAL INTRACHANGES			ASYMMETRICAL INTRACHANGES			
	Chromosome break	Inter-arm inversion	Intra-arm inversion	Inter-arm deletion	Intra-arm deletion	Symmetrical interchange	Asymmetrical interchange
Unbroken	 A1	 B1	 C1	 D1	 E1	 F1	 G1
Broken	 A2	 B2	 C2	 D2	 E2	 F2	 G2
Rearranged		 B3	 C3	 D3	 E3	 F3	 G3
Metaphase configuration	 A3 or  A4	 B4	 C4	 D4	 E4	 F4	 G4
Anaphase configuration	 A5 or  A6	 B5	 C5	 D5 or  D6 or  D7	 E5	 F5 or  F6 or  F7	 G5 or  G6 or  G7

Structural changes induced in unsplit chromosomes.

	Chromatid break	Isochromatid break	Symmetrical intrachange	Asymmetrical intrachange	Symmetrical interchange	Asymmetrical interchange
Unbroken	 A1	 B1	 C1	 D1	 E1	 F1
Broken	 A2	 B2	 C2	 D2	 E2	 F2
Rearranged		 B3 Sister-union NUp NUd NUps	 C3	 D3	 E3	 F3
Anaphase configuration	 A4	 B4 Sister-union	 C4	 D4	 E4 or  E5	 F4 or  F5

Structural changes induced in split chromosomes.

gaps (Lea, 1955). Whether achromatic gaps represent true chromosome breaks is enigmatic (Comings, 1974; Brinkley and Hittleman, 1975).

Studies of the effects of irradiation on meiotic chromosomes are complicated by difficulties in determining the exact stage at which irradiation occurred. Mitra (195~~8~~<sup>7</sup>) was able to overcome this problem in *Lilium* where the length of the anther corresponded to a particular stage of meiosis. She showed that the effects of radiation at meiosis were similar to those at mitosis. However, interpretation of structural aberrations was more difficult due to the added complications of homologous chromosome pairing and chiasmata formation (review Evans, 1962).

#### Effects of Irradiation on Holocentric Chromosomes.

X-irradiation of holocentric chromosomes also results in chromosome breakage. However, the observable cytological features are not identical to those of monocentric chromosomes due to the fact that all products of breakage are able to divide autonomously in the nucleus. Thus, fragments can be observed many divisions after irradiation, micronuclei are uncommon, and chromosome bridges are rarely observed as dicentric chromosomes cannot be formed (Bauer, 1967). Only sister chromatid fusion can result in bridge formation.

The effect of X-rays on holocentric chromosomes has been studied in both plant and animal species. Hughes-Schrader and Ris (1941) verified the diffuse spindle attachment of coccid chromosomes by showing that induced fragments could be

maintained through mitotic divisions. Brown and Nelson-Rees (1961) demonstrated the same survival of fragments in the coccid, *Planococcus citri*, and also reported the absence of bridge-breakage-fusion cycles. Similarly, survival of fragments (indicating the holocentric nature of the chromosomes) has been demonstrated in the Hemipterans (Hughes-Schrader and Schrader, 1961), *Limotettix*, Homoptera (Halkka, 1965), *Oncopeltus* (La Chance and Degrugillier, 1969), *Rhodnius* (Maudlin, 1976), the Lepidopterans (Bauer, 1967), *Bombyx mori* (Murakami and Imai, 1974), and the Acarina *Tetranychus urticae* (Feiertag-Koppen, 1976), *Siteroptes graminum* (Cooper, 1972), *Eylais setosa* (Keyl, 1957).

Tempelaar (1979a, 1979b, 1980) gave a detailed account of the fate of induced fragments in *Tetranychus urticae*. After irradiation of mature sperm, he scored the chromosome fragments in early cleavage stages and found that 94.29% of fragments passed through mitosis while only 4.76% were lost and 0.95% affected by non-disjunction. Irradiation of unfertilized eggs showed differential sensitivity of various meiotic stages (metaphase I and telophase I being the most sensitive); bridges, translocation heterozygosity and micronuclei were not observed. Tempelaar and Drenth-Diephuis (1983) supplemented this radiation data with TEM of sectioned chromosomes which showed association of microtubules along the entire length of each chromosome, thus verifying that these were characteristics of holocentric chromosomes.

Cooper (1972), when reporting the survival of X-ray

induced fragments in the grass mite *Siteroptes*, observed ring chromosomes, translocations and sticky decondensed bridges (thought to be due to decondensation of chromatin after irradiation), which do not seem to be features common to all holocentric species studied (see Table 3.1.2). The lack of observations of ring chromosomes, bridges and translocations may reflect problems in scoring these aberrations in holocentric species which often have small, condensed chromosomes. Studies on holocentric species with larger chromosomes may prove informative. However, it may be a general property of holocentric chromosomes for ends to spontaneously repair to form functional telomeres rather than tending to rejoin with other broken ends.

After irradiation of *Luzula*, fragments are maintained and micronuclei are very rare (Evans and Sparrow, 1961; Evans and Pond, 1964). Bridge formation is also rare and huge doses of radiation are necessary to induce chromosome repatterning (Nordenskiöld, 1957, 1963, 1964). Evans and Pond (1964) used *Luzula* in a comparative study with *Vicia faba*, a monocentric species with similar DNA content and nuclear volume. The results indicated that *Luzula*, unlike *Vicia*, rarely forms micronuclei and under chronic radiation the number of fragments per cell increased with time in *Luzula* but remained constant in *Vicia*. This was due to the retention of fragments through division in *Luzula*, although ultimately, the cells with relatively high frequencies of fragments were selectively eliminated from the cell population.

Table 3.1.2

Comparison of the effects of irradiation on mitotic/meiotic cells of holocentric and monocentric chromosomes.

	Micronuclei	Anaphase bridges	Rings/ Translocation	Surviving fragments	References
<u>HOLOCENTRIC SPECIES</u>					
Coccids	*	*	*	+	Brown and Nelson Rees, 1961
Hemiptera	*	+ "sticky"	*	+	Hughes-Schrader and Schrader, 1961
<i>Oncopeltus</i>	*	-	+	+	LaChance and Rieman, 1971
<i>Rhodnius</i>	-	*	+	+	Maudlin, 1976
<i>Limotettix</i>	*	*	*	+	Halkka, 1965
<i>Tetranychus urticae</i>	*	-	+ $\sigma$ , - $\varphi$	+	Tempelaar, 1978, 1979a, b
<i>Siteroptes graminiae</i>	-	+ "sticky"	+	+	Cooper, 1972
<i>Bombyx mori</i>	-	-	+	+	Murakami and Imai, 1974
<i>Luzula</i>	-	-	$\pm$	+	Evans and Sparrow, 1963; Evans and Pond, 1964; Nordenskiold, 1964
<u>MONOCENTRIC SPECIES</u>					
<i>Locusta</i>	+	+	+	-	White, 1935
<i>Vicia</i>	+	+	+	-	Sparrow and Evans, 1961
<i>Lilium</i>	+	+	+	-	Mitra, 1957

\* not scored

- not observed

+ observed

$\pm$  observed but infrequent

### 3. Recent Techniques Using Light Microscopy.

C-banding identifies specific heterochromatic regions including the pericentromeric heterochromatin on monocentric chromosomes making it useful in differentiating the centromere from other chromosomal regions (Sumner, 1972). Few examples of C-banded holocentric chromosomes exist due to difficulties in applying this technique to small condensed metaphase chromosomes. When mitotic chromosomes of *Tetraynchus urticae* were C-banded they failed to show any blocks of heterochromatin (Pijnacker and Ferwerda, 1976). The interstitial and telomeric bands induced during early meiotic prophase in the earwig were no longer discernible at metaphase (Giles and Webb, 1972). Muramoto (1980) induced C-banding in Heteropteran species but considered the techniques and results unreliable as they could not be standardized.

Ray and Venkateswaran (1978) demonstrated the presence of both telomeric and interstitial blocks of heterochromatin within the genomes of *Luzula purpurea* and *L. multiflora*. Collet and Westerman (1984) extended this study by C-banding *Luzula flaccida* and identified blocks of heterochromatin interspersed between euchromatic regions. This C-band pattern also corresponded to *in situ* hybridization patterns of a 127 base pair repeat sequence isolated from *L. flaccida* (Collet, 1984). Previously Bernardini and Lima-de-Faria (1967) showed late replicating regions were distributed along the length of *L. purpurea* mitotic chromosomes. They and other workers (Ray and Venkateswaran, 1978) interpreted the



*Luzula* data as denoting a polycentric system. However, ultrastructural evidence has shown the chromosomes as holocentric. There is not a direct relationship between the C-banded pericentromeric heterochromatin and the extent of the kinetochore plate even in monocentric systems, and not all C-band regions indicate a centromere. Thus, it is impossible to extrapolate fully from C-band data to centromeric type.

Recently, techniques using silver nitrate as a stain (on chromosomes that have or have not been subjected to various pretreatments) have been devised as a means of detecting the kinetochore regions on metaphase, monocentric chromosomes (Denton *et al.*, 1977; Brat *et al.*, 1979). Silver is thought to stain residual or acidic proteins common to both nucleolar organizer regions and kinetochores (Goodpasture and Bloom, 1975). These techniques have not been applied to holocentric chromosomes.

Nokkala and Nokkala (1984) have developed an N-banding (silver staining) technique to reveal the somewhat controversial 'axial cores' of chromosomes. They proposed that organization of these cores differed; they are situated laterally on holocentric mitotic chromosomes while being coiled inside the chromatid in mitotic monocentric chromosomes. They concluded that their technique could be used as a diagnostic tool for holocentric chromosomes and suggested that an observed shift in core organization between mitotic and meiotic holocentric chromosomes accounted for the differences in centric activity exhibited at different stages.

### CREST Scleroderma Antibodies.

The discovery of antibodies showing specificity for the mammalian kinetochore in the sera of individuals showing the CREST symptoms of Scleroderma has led to the identification of specific kinetochore associated proteins (Moroi *et al.*, 1980). Immunofluorescence has shown these proteins in the interphase pre-kinetochores as well as fully formed metaphase kinetochores (Brenner *et al.*, 1981).

Cox *et al.* (1983) identified four antigens that reacted with antibodies from Scleroderma sera, three of which have subsequently been localized at the centromere (Earnshaw and Rothfield, 1985). Whether these are specific for different regions of the trilaminar plate is unknown.

Immunofluorescence has been used to study the evolution of the centromere in Indian ( $2n=6♀, 7♂$ ) and Chinese ( $2n=46$ ) muntjacs. Ultrastructural evidence has shown the kinetochore plate to be much larger in the Indian muntjac. Immunofluorescence revealed that it was a compound structure comprised of a number of fluorescing subunits. Both the compound kinetochores of the Indian muntjac and the single kinetochores of the Chinese muntjac showed clustering at interphase. Brinkley *et al.*, 1984) suggested that this clustering had facilitated centric fusion during the evolution of these two species.

Chromosomes known to be dicentric have been studied using immunofluorescence. A dicentric chromosome must have one centromere inactivated if it is to be stable at mitosis. Eiberg (1974) developed a technique called Cd-staining which

identified only active centromeres. Hsu *et al.* (1975) suggested inactivation might be reversible and regarded inactivated centromeres as 'latent'. Immunofluorescence revealed very faint fluorescence for a human 11p:9q telomere-telomere fusion chromosome and no detectible fluorescence for the inactive centromere in an isodicentric X-chromosome (Merry *et al.*, 1985; Earnshaw and Migeon, 1985). This suggests that inactivation involves the loss of essential kinetochore proteins. Earnshaw and Migeon (1985) have presented two hypotheses on possible modes of inactivation involving mutation of the DNA or chromatin conformation at the centromere, however, they could not determine if the inactivation is reversible.

A number of human auto-antibodies have been found to react with *Drosophila* salivary gland chromosomes, including one which appeared specific for the chromocentre (Will *et al.*, 1981). In yeast, where the DNA sequence of the centromere has been defined antibodies from Scleroderma patients have failed to recognize any antigens (Blackburn and Szostak, 1984).

#### **Molecular Analysis of the Centromere.**

Holmquist and Dancis (1979) proposed that a gene, the kinetochore organizer, situated at the primary constriction on the chromosome acted to organize the proteins of the kinetochore plate into a functional structure. Recently, the DNA sequences of a number of functional yeast centromeres have been defined (Clark and Carbon, 1980; reviews by

Struhl, 1983; Carbon, 1984; Blackburn and Szostak, 1984). They consist of an approximately 3.5 kilobase sequence of DNA with a core region of 82-89 base pairs of highly AT rich DNA flanked by two conserved regions. The data from yeast are consistent with the centromere as a protein-binding region with only 370-375 base pairs (of the 3.5 kb region) sufficient to define a functional centromere.

Little progress has been made in isolating the specific centromeric sequences responsible for kinetochore organization on mammalian chromosomes. A number of DNA probes exist which are specific for centromeric regions - probably sequences located in pericentromeric heterochromatin - as shown by *in situ* hybridization (Pardue and Gall, 1970; Mitchell *et al.*, 1985). This lack of information is probably due to the complexity of the mammalian kinetochore which has the ability to bind large numbers of microtubules (compared to yeast kin<sup>e</sup>t<sup>h</sup>ochores which only attach <sup>to</sup> a single microtubule). Further, the masking effect of the large amount of repeated sequence DNA associated with the centromere region makes it difficult to isolate and localize specific sequences.

#### The Nature of the Centromere/Kinetochore in Scorpions.

The evidence available on the centric nature of scorpion chromosomes is very confused. In reviewing the matter, White (1973) suggested that Buthids possessed holocentric chromosomes while non-Buthid species have monocentric chromosomes. The data presented in this thesis supports his

view.

Most interpretations of the centric nature of scorpion chromosomes have been based on light microscope observations of chromosome morphology and movement (see Table 3.1.3 for summary). Non-Buthid species often have variable expression of primary constrictions at metaphase, however, typical J and V shaped elements at anaphase have led many authors to consider the chromosomes as monocentric (Wilson, 1931; Venkatanarasimhaiah and Rajasekarasetty, 1964, 1965). However, this absence of primary constrictions and the parallel alignment of bivalents at metaphase I (due to achiasmatic meiosis not the centric nature of the chromosomes) has confused some authors and led them to postulate the chromosomes are monocentric but behave as if holocentric (Rajasekarasetty *et al.*, 1979).

The chromosomes of Buthid scorpions do not show primary constrictions, however, their behaviour at anaphase where both ends of each chromosome appear to lead to the poles has led a number of authors to suggest the chromosomes are dicentric (Piza, 1939; Gupta and Sarker, 1965). This interpretation has been maintained even though it is a common feature of species with holocentric chromosomes to exhibit telocentric activity at meiosis (Hughes-Schrader and Schrader 1961). Additionally, Rhoades and Kerr (1949) carried out an X-ray experiment on *Tityus bahiensis* a scorpion considered by Piza to possess dicentric chromosomes. They observed fragment survival through divisions subsequent to irradiation with some fragments showing the typical telocentric activity

Table 3.1.3

Evidence presented for the centric nature of scorpion chromosomes.

Species	Centric nature	Evidence	Reference
<u>BUTHIDAE:</u>			
<u>South America</u>			
<i>Tityus bahiensis</i>	dicentric	LM sections	Piza 1939, 1943a, 1943b
<i>Tityus bahiensis</i>	diffuse	LM squash	Brieger and Graner, 1943 Hughes-Schrader and Ris, 1941
<i>Tityus bahiensis</i>	polycentric/diffuse	X-rays fragment survival	Rhoades and Kerr, 1949
<i>Tityus bahiensis</i>	holocentric	EM sections	Benavente, 1982
<i>Tityus meteundus</i>	dicentric	EM sections	Piza, 1952
<i>Tityus mattagrossensis</i>	dicentric	LM sections	Piza, 1947c
<i>Tityus trivittatus</i>	dicentric	LM sections	Piza, 1948
<i>Isometrus maculatus</i>	dicentric	LM sections	Piza, 1947a
<i>Rhopalurus rochai</i>	dicentric	LM sections	Piza, 1957
<u>Indian</u>			
<i>Buthus tamulus</i>	dicentric	LM	Gupta and Sarker, 1965
<i>Buthus tamulus</i>	monocentric/ bicentric/ holokinetic	LM	Venkatanarasimhaiah and Rajasekarasetty, 1965
<i>Buthus tamulus</i>	dicentric	LM	Sharma <i>et al.</i> , 1959
<i>Buthus doricae</i>	dicentric	LM	Sharma <i>et al.</i> , 1959
<i>Buthus macmahoni</i>	dicentric	LM	Sharma <i>et al.</i> , 1959
<u>North America</u>			
<i>Centroides vittatus</i>	holocentric	EM sections	Riess <i>et al.</i> , 1978
<u>European</u>			
<i>Buthus occitanus</i>	monocentric	LM squash constrictions	Guenin, 1961
<u>VEJOVIDAE:</u>			
<u>North America</u>			
<i>Opisthacanthus elatus</i>	monocentric	LM sections	Wilson, 1931
<u>SCORPIONIDAE:</u>			
<u>Indian</u>			
<i>Palamnaeus gravimanus</i>	monocentric	LM	(Venkatanarasimhaiah and Rajasekarasetty, 1964, 1965)
<i>Palamnaeus logimanus</i>	monocentric	LM	(1965)
<i>Palamnaeus swammerdami</i>	monocentric	LM	Rajasekarasetty <i>et al.</i> , 1979
<i>Palamnaeus sp.</i>	monocentric/ holocentric	LM	
<i>Heterometrus scaber</i>	monocentric	LM	Venkatanarasimhaiah and Rajasekarasetty, 1965
<i>Heterometrus fulvipes</i>	monocentric	LM	
<u>BOTHRURIDAE:</u>			
<u>South America</u>			
<i>Bothriurus sp.</i>	monocentric	LM sections	Piza, 1947c

of unbroken chromosomes. These observations are indicative of holocentric chromosomes. Furthermore, Benavente (1982), using TEM analysis showed that the kinetochore plate of *T. bahiensis* extends along the entire length of mitotic and meiotic (metaphase I and II) chromosomes. The presence of a kinetochore plate at meiosis needs to be verified as there are problems in accurately staging divisions in sectioned material, however, the kinetochores observed indicate the chromosomes are holocentric.

Thus, in all scorpion species studied White's interpretation stands; Buthids possess holocentric chromosomes, non-Buthid species have monocentric chromosomes. Observations and experiments presented in this Chapter test the validity of this hypothesis with respect to the Buthid and non-Buthid scorpion fauna of Australia.

## SECTION 2

### LIGHT MICROSCOPE (LM) ANALYSIS OF BUTHID AND SCORPIONID CHROMOSOMES

#### INTRODUCTION

The following section describes a number of techniques used to differentiate the centromeric region of monocentric chromosomes. The techniques have been applied to chromosome preparations from Australian Scorpionid species (genus *Urodacus*) and Australian Buthid species. Morphological evidence suggests that these scorpion groups possess monocentric and holocentric chromosomes respectively.

The results from the application of these techniques emphasize that the use of morphological criteria alone (i.e. presence or absence of primary constrictions) is insufficient to assign a centric nature to chromosomes.

#### MATERIALS AND METHODS

##### General Stain and C-Banding.

As described in Chapter 1.2.

##### Kinetochores Staining.

1. Squash preparations of testicular material were made 48-76 hrs after initial fixation.
2. 3 drops of 50% Ag (in distilled H<sub>2</sub>O previously adjusted to pH 3-3.5 with formic acid) were placed on slides and a coverslip added.



3. Slides were incubated at 60°C for 5-15 mins.
4. After staining was complete (as adjudged by microscopic examination) slides were washed in distilled H<sub>2</sub>O, dried and fresh coverslips mounted with XAM.

#### **Indirect Immunofluorescence Using CREST Scleroderma Antibodies.**

Serum (from an individual with scleroderma) known to be positive for anti-kinetochore antibodies was a gift from Professor Eng. M. Tan of the W.M. Keck Foundation, Autoimmune Disease Centre, Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.

#### **Immunofluorescence of Cells.**

Cells were obtained from either the testis or the haemolymph of *U. manicatus* and *L. marmoreus* individuals. Testis cells were macerated to form a cell suspension in one drop of RPMI 1640 containing 10% v/v Foetal Calf Serum. Control cells were from mouse testis (meiotic control) or a transformed human cell line Heb 7 (mitotic control).

Heb 7 and haemolymph cells were centrifuged on to poly-L-lysine coated coverslips by placing 10  $\mu$ l of cell suspension on to 9 mm diameter circular coverslips. These were placed on to filter paper disks in a plastic 24 well plate that had been cut into two pieces to facilitate centrifugation. The plate was centrifuged in a Beckman TJ6-R centrifuge for 2.5 mins at 1500g.

Cells on coverslips were then:-

1. Rinsed in PBS for 10 mins.
2. Fixed in 3% paraformaldehyde in PBS for 20 mins.
3. Rinsed in PBS (2X) for 10 mins.
4. Placed in 0.05% Triton-X in PBS for 1.5 mins.
5. Rinsed in PBS.
6. Incubated in 1/200 dilution of CREST sera for 1 hr at 37°C.
7. Rinsed for 1 hr in 3 changes of PBS.
8. Incubated in 1/20 dilution of FITC - coupled goat anti-human IgG for 1 hr at 37°C.
9. Rinsed for 1 hr in 3 changes of PBS.
10. Mounted in a 9:1 dilution of glycerol:PBS.

Cells were optionally stained with propidium iodide before mounting. All washes were carried out at 4°C.

During all experiments, positive and negative controls were performed:

- +ve control - Heb 7 cells incubated in CREST serum;
- ve control - Heb 7 and scorpion cells incubated in either PBS or normal human serum (i.e. containing no anti-kinetochore antibodies).

When testis cells were used they were either:-

- a) centrifuged on to coverslips as previously outlined, or
- b) picked up on to slides using a hypophase as described in Chapter 2.2 for LM preparations of synaptonemal complexes.

Mounted cells were observed under a Leitz fluorescence microscope and photographed using Tri-X pan film.

## RESULTS AND DISCUSSION

### 1. General Chromosome Morphology and Anaphase Movement.

#### Family Buthidae.

The mitotic and meiotic division stages of Australian Buthid scorpions show identical features to those observed by Piza (1939, 1944, 1945) in the South American Buthids *Tityus* and *Isometrus* (Fig. 3.2.1). The chromosomes are small (<5  $\mu\text{m}$ ) and highly contracted at metaphase with no visible primary constrictions. During anaphase the chromosomes move in a parallel fashion towards the poles, often with one or both ends of the chromosome leading slightly. These characteristically upturned ends are also observable at late metaphase.

Meiotic division in Buthid species is not inverted as shown by the behaviour of ring multiples at anaphase I; the multiples separate with half the chromosome complement moving to each pole rather than a chromatid ring association moving to each pole (as observed in *Luzula* (Nordenskiöld, 1963)). Thus, bivalents co-orientate and show prereluction. At metaphase II the chromatids are separated and lie parallel. However, at earlier stages (late anaphase/ telophase/early metaphase II) the chromatids are not visibly separated and the chromosomes are often convoluted and bean- or crescent-shaped.

These unusual conformations may be related to the apparent telocentric behaviour of the chromosomes at anaphase. The more convoluted of the crescent shaped chromosomes observed at anaphase I/metaphase II are usually

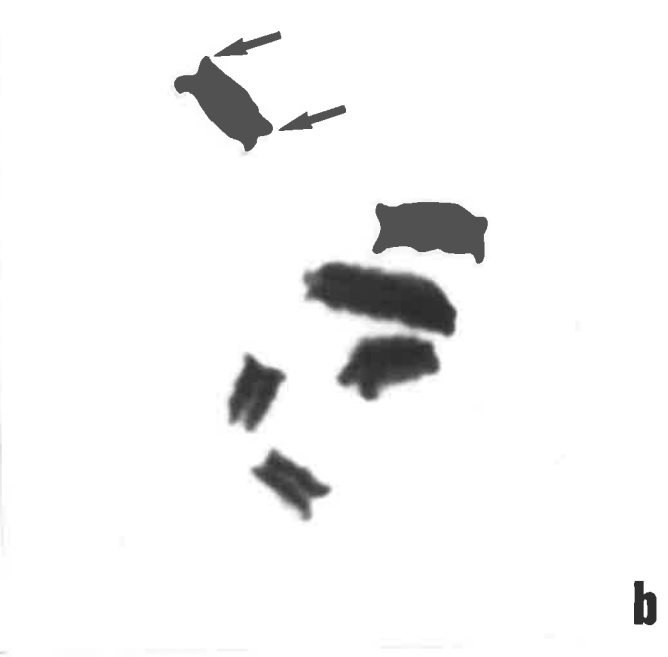
**Figure 3.2.1**

**Family Buthidae.**

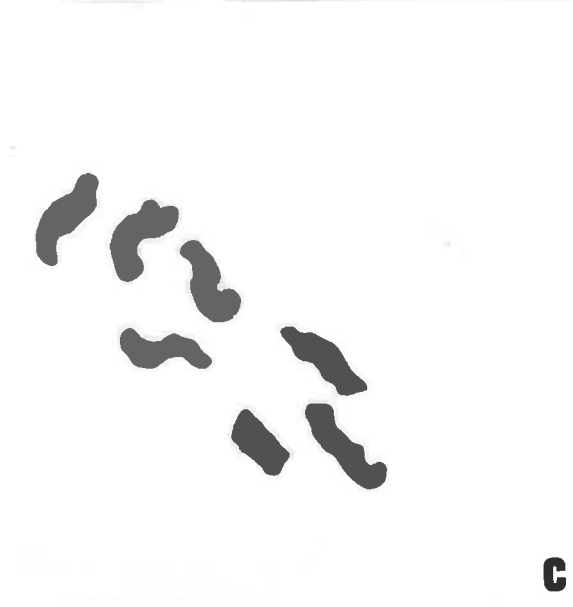
- a**    mitosis - early anaphase showing upturned ends (arrowed).
- b**    metaphase I - ends upturned (arrowed).
- c**    late telophase-early metaphase II.  
Note convoluted appearance of chromosomes.
- d**    early anaphase II - ends show centric activity.



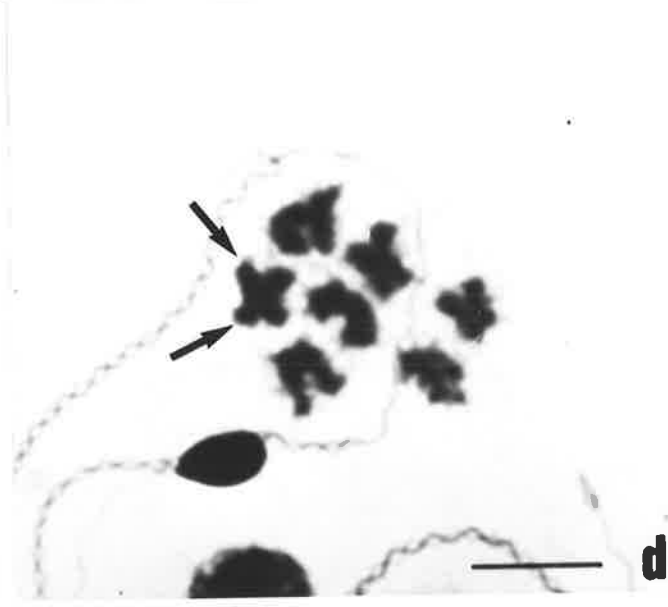
**a**



**b**



**c**



**d**

those segregating from a ring. Clearly, the arrangement of the multiple on the spindle and the telocentric activity of both ends contributes to chromosome conformation and may be necessary for regular disjunction.

It has been well documented that many holocentric species exhibit an apparent shift to telocentric activity (one telomere only apparently active) at anaphase I (Hughes-Schrader and Schrader, 1961). However, the Buthids are unusual in that the telocentric activity is apparent at both chromosome ends at both mitosis and meiosis. Various theories have been advanced to account for telocentric behaviour. Hughes-Schrader and Ris (1941) and Rhoades and Kerr (1949) considered it an adaptation permitting chromosomes, whose size are large relative to that of the spindle, to converge at the poles. However, this does not account for the expression of precocious end-movement at metaphase. Comings and Okada (1972) suggested the centric change allowed for co-orientation where terminalization of chiasmata had occurred. This may hold for other holocentric species that exhibit centric shift from mitosis to meiosis, however in achiasmate Buthid species this is clearly unnecessary. John and Lewis (1965) regarded the telocentric behaviour in terms of neocentricity suggesting a structural and functional relationship between telomeres and centromeres. The C-band evidence from Buthid species supports this theory as both telomeres in all species were C-band positive indicating the presence of heterochromatin which is a necessary karyotypic component for neocentric

activity. However, LM observations indicate a centric shift, ultrastructural studies of other holocentric species (Buck, 1967) do not support this as there is no evidence that microtubules become redistributed or are restricted at metaphase I.

Telocentric activity may also have a role in ensuring regular disjunction in the ring multiples common to Buthid species. Pairing at metaphase I is generally greatest at the ends of homologous chromosomes (excepting the upturned extremities at late metaphase I). Due to the close proximity of centromeric regions at the ends, spindle orientation may operate via the telomeres and this is manifest as telocentric activity.

Although Buthids show a slightly atypical anaphase behaviour when compared to other holocentric species, morphological evidence suggests the chromosomes are holocentric. The modifications at anaphase are probably associated with the achiasmate meiosis and ring multiple formation.

#### **Family Scorpionidae.**

Figure 3.2.2 shows the chromosome morphology and behaviour during mitosis and meiosis typical for a Scorpionid species. Mitotic metaphase chromosomes do not have well defined primary constrictions. Exposure of cells to colchicine for longer periods (>1 hour) however, did induce chromatid separation making primary constrictions visible. At anaphase centric activity is restricted to a single region

**Figure 3.2.2**

**LM examination of the Scorpionid species**

***Urodacus manicatus***

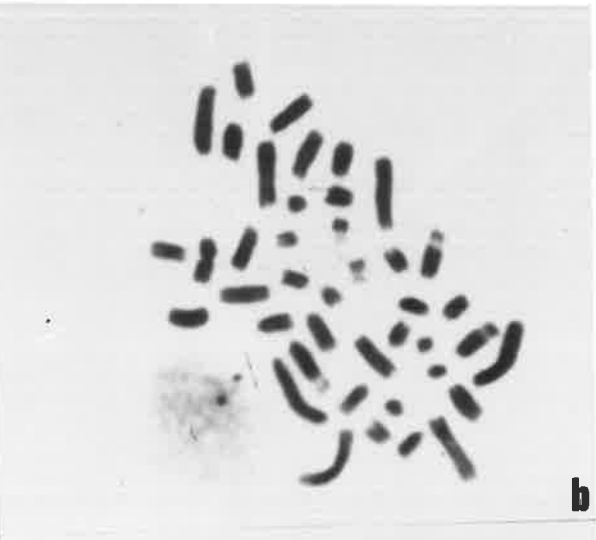
- a mitosis - note lack of primary constrictions.
- b mitosis - constrictions visible in some preparations after colchicine treatment.
- c mitotic anaphase.
- d metaphase I - the parallel alignment of the chromosomes (because of the achiasmate meiosis) led some authors to suggest the chromosomes are holocentric.
- e anaphase I - arms dragging as expected for monocentric chromosomes.
- f metaphase II - constrictions and arm repulsion; metacentrics and telocentrics.

Bar = 5  $\mu$ m.





a



b



c



d



e



f

of each chromosome resulting in classical monocentric J- and V-shaped elements.

Similarly, at metaphase I primary constrictions and single chromatids cannot be observed. The homologous chromosome pairs associate in a parallel fashion in each achiasmate bivalent. By late metaphase this pairing is disrupted due to the opening out of one end of the telocentric bivalents resulting in end-to-end pairing. Again, at anaphase (I) centric activity is restricted to a single chromosome region (either telomeric or interstitial). Metaphase II chromosomes show sister chromatid repulsion resulting in V- and cross-shaped elements typical of acrocentric and metacentric chromosomes.

Thus, excepting the absence of primary constrictions at metaphase, Scorpionid chromosomes show morphological characteristics typical of monocentric chromosomes. This illustrates the necessity of using more than the single criterion of 'lack of primary constrictions' to assign a holocentric nature to any chromosome system. In the Lepidoptera, where the chromosomes are small and dot-like and do not show primary constrictions (similar to many Scorpionid chromosomes) this practice may have contributed to the confusion associated with the centric nature of many species in this group. Like Scorpionids, primary constrictions can be induced in some species of Lepidoptera by colchicine treatment and air-dried rather than squash preparations (Bigger, 1975; Rishi and Rishi, 1979). Therefore, in some Lepidopteran families as in the scorpion family, Scorpionidae

chromosomes may be truly monocentric despite the absence of morphological evidence at metaphase.

## 2. C-banding and Kinetochore Staining.

### Family Buthidae.

As shown previously (Chapter 1.3) and in Fig. 3.2.3a, C-banding of Buthid chromosomes does not identify a specific region of pericentromeric heterochromatin. Dependent on the state of contraction of the chromosomes, a number of interstitial regions can be identified, while most species also show terminal C-bands at both telomeres. This C-band pattern is not consistent with the chromosomes being monocentric but is similar to the pattern observed by Collet and Westerman (1984) in holocentric *Luzula*.

Kinetochore staining was difficult to induce. However, Fig. 3.2.3<sup>e,f</sup> shows that no specific silver staining region that could be interpreted as a localized kinetochore was observed. Some scattered chromosome regions (more than one per chromosome) did stain comparatively darker, however it was not possible to determine if these were due to non-specific binding of silver or whether these regions represented sites of kinetochore proteins. Nevertheless, the pattern observed was not that typical of monocentric chromosomes.

### Family Scorpionidae.

The distribution of C-band material on Scorpionid chromosomes further predicts their monocentric nature (Fig.

**Figure 3.2.3**

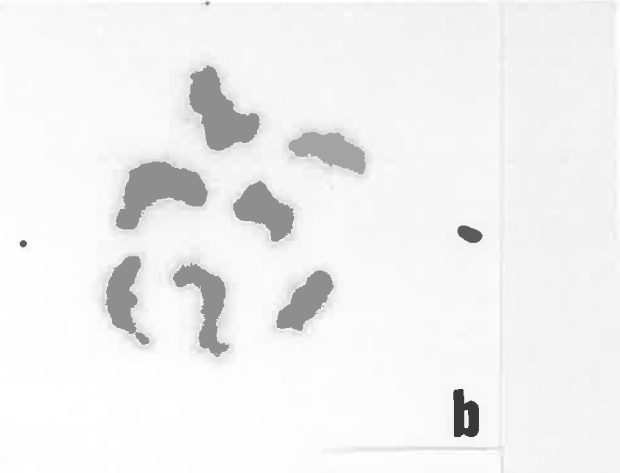
**C-banding and kinetochore staining in Buthids.**

- a** C-banded mitosis - diffuse bands.
- b** C-banded metaphase II - interstitial and telomeric bands.
- c,d** C-banded metaphase I - interstitial and telomeric bands.

The contracted state of the chromosomes makes band definition poor.

- e** K-staining of mitosis - no localized Ag staining.
- f** K-staining of metaphase I - non-specific binding of silver.

Bar = 5  $\mu$ m.



3.2.4a). The C-band material present on each chromosome was confined to either a single interstitial or telomeric band indicative of metacentric and telocentric chromosomes. Similarly, the silver binding induced by kinetochore staining was confined to these same regions (Fig. 3.2.4<sup>ef</sup>~~b~~).

On the basis of C-banding pattern homologues could be identified. At anaphase the C-band regions led to the poles and at metaphase II the chromatin at the constrictions was C-band positive. Additionally, in Robertsonian fusion heterozygotes a double C-band (indicating that centric fusion had occurred) could often be observed.

Thus, the lack of primary constrictions at metaphase in Scorpionids is not associated with any lack of pericentromeric heterochromatin.

### 3. CREST Sera: Indirect Immunofluorescence.

The results of indirect immunofluorescence using anti-kinetochore antibodies to the human cell line (Heb 7) and mouse meiotic cells are shown on Fig. 3.2.5. A positive reaction gives specific fluorescence of kinetochores. In cells where the chromosomes and kinetochores have replicated two dots representing the kinetochores of each sister chromatid can be identified.

No such brightly fluorescent dots could be observed in preparations of either Buthid or Scorpionid cells. In Buthids, there was a general enhancement of overall fluorescence when compared to negative controls. However, this was most likely due to non-specific binding of the

**Figure 3.2.4**

**C-banding and kinetochore staining, Scorpionid**

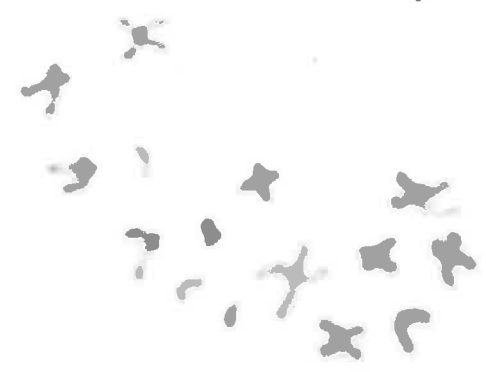
*Urodacus manicatus*.

- a      Localized C-bands at mitosis.
- b      Localized C-bands at metaphase II.
- c      Localized C-bands at metaphase I.
- d      Early anaphase I showing poleward orientation of C-band regions.
- e, f    Kinetochore staining at metaphase I. Pattern comparative to C-banding with mainly telomeric (i.e. telocentric chromosomes) binding at silver.

Bar = 5  $\mu$ m.



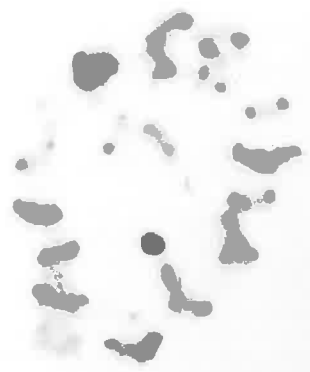
a



b



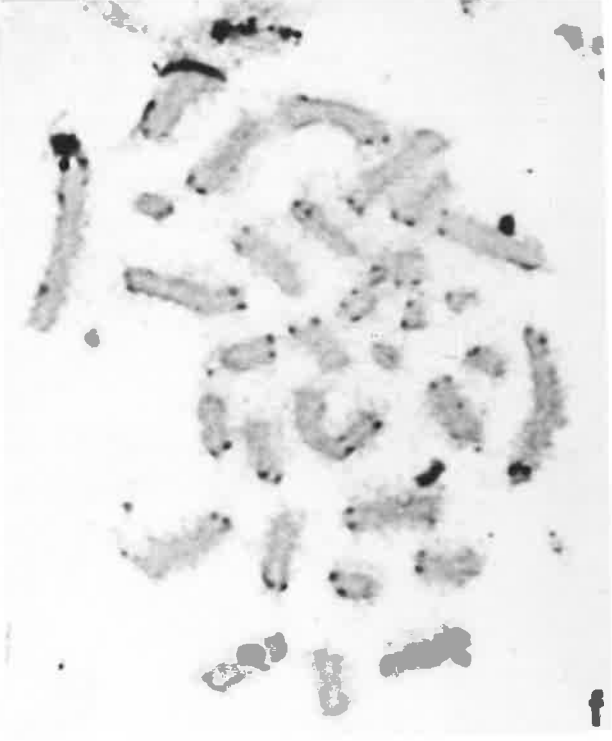
c



d



e



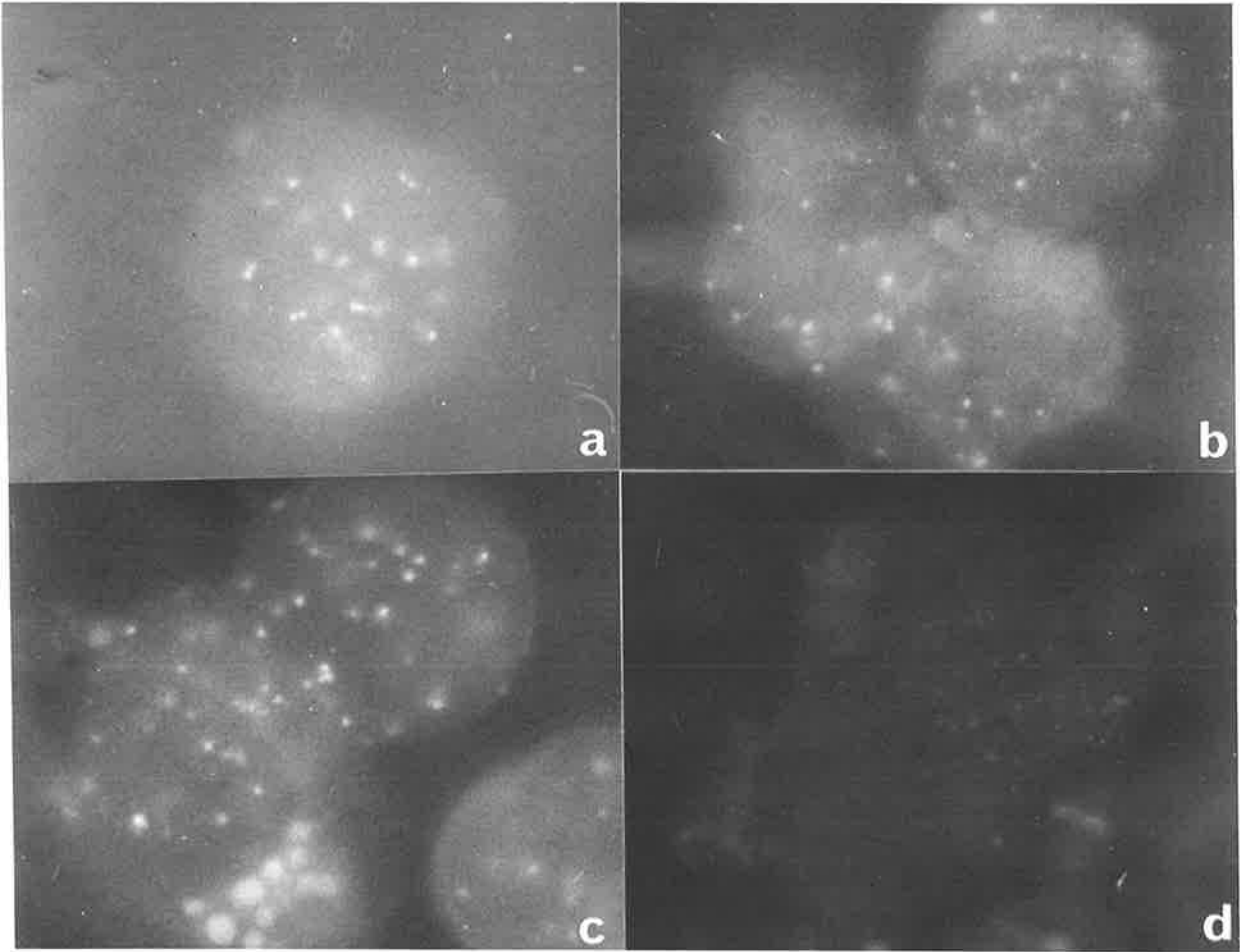
f



**Figure 3.2.5**

**Indirect immunofluorescence of anti-kinetochore  
antibodies to interphase cells.**

- a**      **Heb 7 +ve control.**
- b**      ***U. manicatus* +ve with antibody.**
- c**      **Mouse +ve control.**
- d**      **Mouse (meiotic) cell +ve control.**



antibody to the cells.

In the Scorpionid, although the positive reaction was weak compared to that of positive controls, there was some indication, especially in the surface spread meiotic material, that the antibody had bound to a specific kinetochore region (Fig. 3.2.5<sup>b</sup>). Further experiments are necessary to confirm this observation. However, this result is similar to those of Earnshaw and Migeon (1985) and Merry *et al.* (1985) who observed very faint fluorescence or no fluorescence respectively for inactive centromeres on two dicentric human chromosomes. The inactive centromere studied by Earnshaw and Migeon (1985) also lacked expression of a primary constriction (a feature common to all Scorpionid chromosomes). They suggested that the loss of an essential kinetochore protein during the process of inactivation resulted in both the loss of fluorescence and the primary constriction: like Scorpionid centromeres, inactive centromeres have pericentromeric heterochromatin. Perhaps the active centromeres of Scorpionids lack or have a modified form of some of the proteins common to active mammalian kinetochores, resulting in features similar to those of some inactive mammalian centromeres. Obviously further characterization of the antigens recognized by anti-kinetochore antibodies is required.

**SUMMARY**

A summary of the results of LM experiments and observations of Buthid and Scorpionid chromosomes indicates that Scorpionids have monocentric chromosomes (see Table 3.2.1). The LM evidence strongly suggests that Buthids have holocentric chromosomes, however, further evidence (as presented in the following section) is required to support this conclusion.

Table 3.2.1

Summary of LM observations of Buthid and Scorpionid chromosomes.

Observation	Buthidae	Scorpionidae
Mitosis - primary constrictions	None observed	Usually none observed
Metaphase II	Chromatids remain parallel	Repulsion of chromatid arms
Anaphase	Parallel movement with ends leading	Single region leading-arms dragging
C-banding	Interstitial and distal bands	Localized region of heterochromatin
K-staining	No regions differentiated	Region at C-band darkly stained
CREST antibody	No localized fluorescence	Weak fluorescence
Synaptonemal complexes	No localized proteins stained	A localized protein region/SC stained
	possibly → HOLOCENTRIC	→ MONOCENTRIC

### SECTION 3

#### X-RAY BREAKAGE OF BUTHID AND SCORPIONID CHROMOSOMES

##### INTRODUCTION

This section describes the use of X-irradiation on Buthid and Scorpionid chromosomes in order to deduce their centric nature. The previous section has provided evidence from light microscope preparations that Scorpionids possess monocentric chromosomes while the behaviour of Buthid chromosomes was not indicative of monocentricity. Caution must be used in assigning a holocentric organisation to any chromosome system as evidence from the light microscope is often inconclusive (e.g. like Lepidopteran chromosomes, Scorpionid chromosomes appear to have variable expression of primary constrictions which are used as indicators of monocentricity in L.M. preparations). Thus, a more definitive test such as X-ray breakage of chromosomes must be applied to identify the centric nature of chromosomes.

##### MATERIALS AND METHODS

###### Selection of Material.

Species were chosen primarily on their availability.

*Urodacus manicatus* is a relatively common species with the lowest chromosome number so far recorded in a Scorpionid species ( $2n=29-64$ ). To minimize the variation due to fusion/fission polymorphism specimens were chosen from FCM

(Flinders Chase, Kangaroo Is.) where all male specimens studied had a chromosome complement of  $2n=60$  represented by 30 bivalents at metaphase I. Additionally, polymorphic populations RRM (Rocky River, Kangaroo Is.),  $2n=57-60$  and EBM (Emu Bay, Kangaroo Is.),  $2n=41-51$  (bivalents, trivalents and quadrivalents at metaphase I) were chosen for comparative purposes because, in general, these chromosomes are larger and easier to score.

Two Buthid species each with  $2n=14$  were selected. They came from a population of *Lychas marmoreus* OGL (Onkaparinga Gorge, S. Aust.) where all animals have been found to have 7 bivalents at metaphase I (although some individuals do exhibit the synaptic failure phenomenon - see Chapters 1.3 and 2.3), and a population of *Lychas variatus* OCL (Overland Corner, S. Aust.) where males exhibit a ring of 12 and 1 bivalent at metaphase I.

#### Tissue Irradiated.

Scorpions have no rapidly dividing somatic tissues. A few mitoses can be obtained from haemolymph, intestine and embryonic tissues but the number of divisions are too few to allow quantitative analysis. The testis is the only suitably dividing tissue. However, using a tissue undergoing meiosis complicates analysis as many different stages of division are present during and subsequent to irradiation. Unlike locusts (White, 1937) and *Lilium* (Mitra, 1958), scorpion testes do not show an ordered array of divisions. Thus, when scoring a division for radiation

effects it is very difficult to determine accurately at what stage of division the particular cell was when irradiation occurred. This must be inferred from results and thus complicates analysis.

#### Irradiation.

All scorpions were irradiated with a DXRT (X-ray) unit, HVT = 10 mm Cu (200 KV and 19 mA). To ensure the doses given were accurate, a specially designed lead cut out applicator was used so that only the body of each scorpion was irradiated.

Scorpions were immobilized with masking tape in plastic petri dishes which were placed on 10 cm of bolus for irradiation. Incident distance was 25 cm and the accuracy of dosage received at 0.5 cm was approximately 90%.

Scorpions received doses of either 330r, 500r or 1,000r and the exposure times for each of these doses is shown in Table 3.3.1.

Table 3.3.1

Exposure time for each X-ray dosage. (r=rads)

Species	Dose (r)	Exposure time (min.)
<i>U. manicatus</i>	330	1.93
	500	2.92
	1,000	5.85
<i>Lychas</i> sp.	500	3.09
	1,000	6.17



Testis and tissue samples were taken at times ranging from 33 hours to 45 days after irradiation.

#### Sampling of Testis Material.

The smaller *Lychnis* animals were killed and the entire testis removed at each sampling time, i.e. it was possible to score these animals only once after irradiation. However, it was possible, without killing the larger *U. manicatus* animals, to remove a portion of the testis. This enabled these animals to be scored either before and after irradiation or at two separate times after irradiation. To obtain a testis sample, animals were immobilized and a small cut was made in the soft tissue on the lateral region of the animal's body. A portion of the testis could then be removed with fine forceps and the cut resealed with warm beeswax. Control animals survived up to three months after this procedure with no noticeable effect on meiotic division.

After removal, testes were immediately fixed in 3:1 methanol:acetic acid and chromosome preparations were subsequently made, either by the squash or air-dry techniques described in Chapter 1.2.

#### Controls.

Due to the small number of animals available for study, unirradiated controls could not be sampled at all times after irradiation. However, animals that could be sampled before irradiation and then tested after irradiation gave some measure of the types of divisions present in the testis

at the time of irradiation. Generally, although the testis did not present an ordered array of divisions there was a tendency for animals to have a particular division stage predominating; some testes contained only spermatogonial mitotic divisions while others contained mainly pachytene/metaphase I division stages.

Buthid controls were killed at time zero, i.e. the day on which experimental animals from the same population were irradiated.

#### Scoring and Analysis.

The entire testis from each animal was analysed. In addition an investigation was made of intestinal tissue from some animals and embryonic tissue from gravid females. Cells were scored for micronuclei, anaphase bridges, chromosome aberrations (rings and translocations) and fragments.

## RESULTS AND DISCUSSION

#### Dosage.

An initial experiment was carried out to determine the dosage necessary to induce scorable effects without causing immediate cell death. On the basis of this initial experiment 1,000r was the dose used in all subsequent experiments. Results from all experiments are presented in the following sections.

### **Irradiation of Buthid Species.**

Results from all the Buthid individuals irradiated are presented in Table 3.3.2.

### **Micronuclei.**

There appeared to be no difference between irradiated and control individuals with respect to the presence of micronuclei. They were of rare occurrence in the cells of all testes examined (i.e. they were not produced as a result of radiation damage).

### **Anaphase Bridges.**

Anaphase cells were not common and in those scorable it was often difficult to distinguish between mitotic and meiotic (anaphase I) divisions. Although there appeared to be connections between some anaphase cells, it was not possible to determine if these were true bridges (due, for example, to sister chromatid fusion) or 'sticky bridges' due to irradiation effects other than chromosome breakage. However, the testis of one animal, OGL B, did contain a number of analysable anaphase I/telophase I cells. The number of chromosomes present and their segregation behaviour into each daughter cell could be determined. These results are presented in Table 3.3.3, while examples of these anaphase cells as well as the metaphase cells from which they were probably derived are presented in Fig. 3.3.1.

In this animal cells with a chromosome number of greater than 14 showed anaphase segregation without any obvious

Table 3.3.2

Summary of the effects of radiation on Buthid species.

Animal	Dose	Time after irradiation	Micronuclei/cell*	% Anaphase bridges	N	Fragments/ <sup>+</sup> cell	N	% Aneuploidy at metaphase
OGL A	500r	7 days	0.0	7.6	(26)	1.33	(21)	95
OGL B	1000r	14 "	0.005	3.1	(14)	2.05	(21)	91
OCL A	1000r	14 "	0.01	0.0	(9)	2.8	(85)	96
OCL B	1000r	21 "	0.01	11.1	(9)	1.25	(103)	92
OCL C	1000r	37 "	0.012	11.7	(17)	1.5	(102)	93
OCL 1 ♀	1000r	45 "	0.015	-		0.162	(37)	78
Control	-	-	0.005	0.0	(20)	0.05	(20)	5

\* = Micronuclei counts based on at least 200 interphase cells.

N = Numbers in brackets represent the number of scorable cells in each testis.

+ = Mitotic cells (metaphase).

Table 3.3.3

Anaphase I segregants OGL B.  
(1000r, 14 days)

Chromosome No.	Segregation type	No. of cells scored for each segregation type
12	6-6	1
13	5-8	1
14	7-7	3
15	7-8, 6-9	5
16	8-8, 7-9	6
17	8-9, 7-10	7
18	12-6, 8-10, 11-7, 9-9	7
19	9-10, 8-11	5
20	-	-
21	11-10	2
22	11-11, 10-12	2
23	11-12	2
Total		41

**Figure 3.3.1**

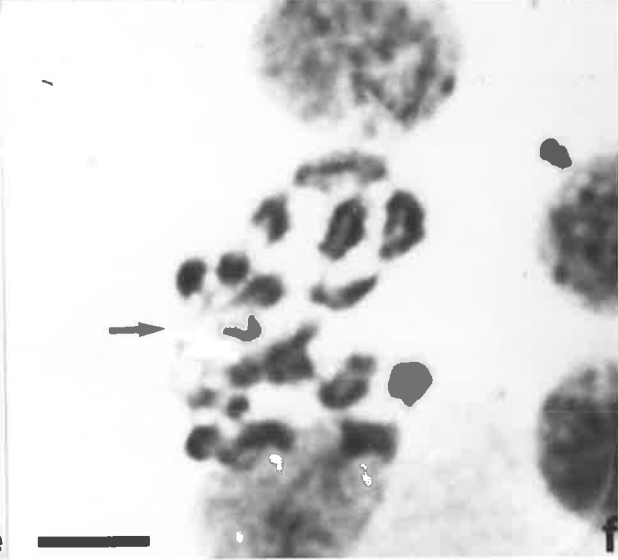
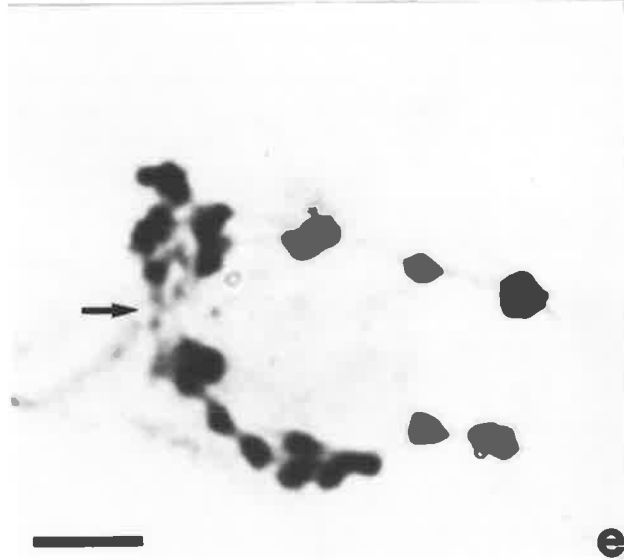
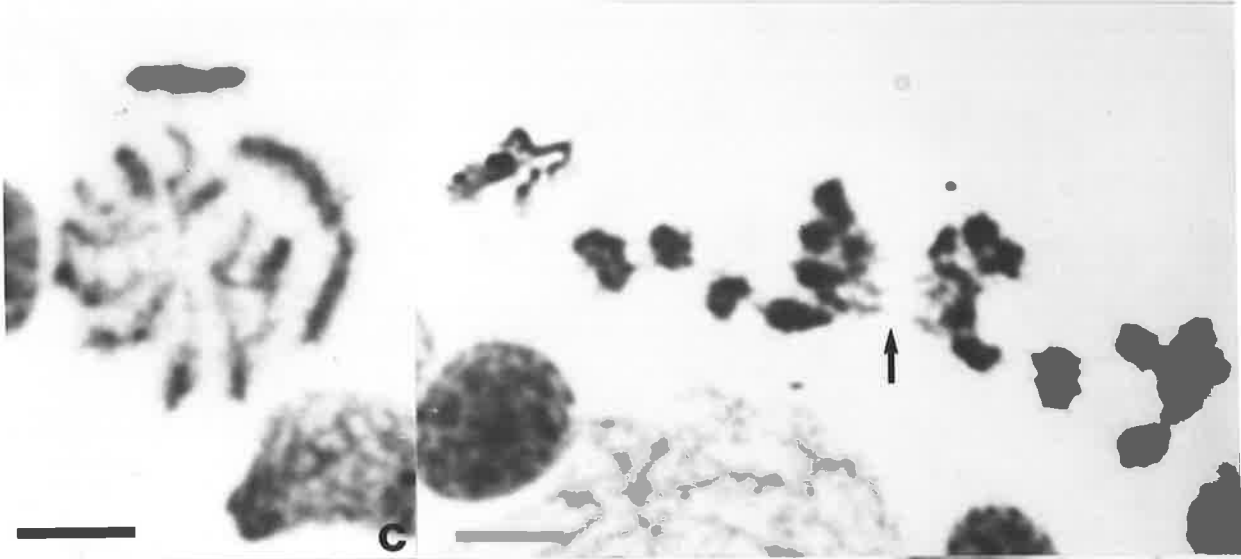
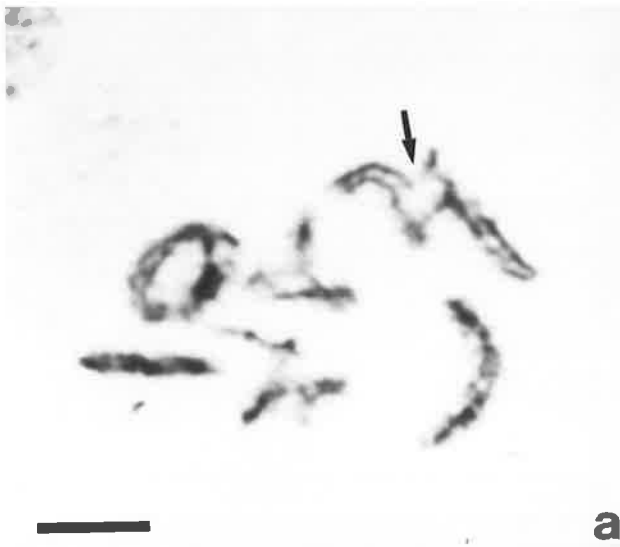
**a-c** Metaphase cells after irradiation showing decondensation and regions of synaptic failure.

Arrows indicate interstitial regions of pairing failure.

**d-f** Anaphase/telophase cells from OGLB showing 'sticky bridges' due to dragging of decondensed regions (arrowed).

- d) 8-9 segregation (+ fragments)
- e) 7-8 segregation
- f) 8-9 segregation.

Bar = 5  $\mu$ m.



lagging of fragments (see following page for definition of fragments). This observation suggests these chromosomes are not behaving in a strict monocentric fashion. The anaphase/telophase cells present however, did show fine strands of what appeared to be decondensed chromatin connecting the two daughter nuclei. These connections did not seem to represent true bridges (due to breakage and reunion) but rather appeared analogous to the 'sticky bridges' reported by Cooper (1972) in mites. Decondensation of chromatin (particularly heterochromatic regions) is a common feature of cells that have been exposed to X-rays (White, 1937). However, the situation in the OGL population was further complicated by the fact that synaptic failure and associated decondensation is a natural feature in up to 40% of metaphase I cells in some animals studied (see Chapters 1.3 and 2.3). After irradiation approximately 90% of metaphase I cells showed decondensation and synaptic failure (often interstitial with the ends paired) suggesting that irradiation either enhanced this phenomenon or produced a phenotypically similar effect. There was very little anaphase I/metaphase II data from unirradiated individuals where synaptic failure and decondensation spontaneously occurs. However, in the irradiated individuals it appeared that centric activity was confined to regions of the chromosomes that were not greatly decondensed (often the paired end regions of presumptive bivalents) such that their movement apart caused the decondensed regions to be dragged behind resulting in the connections of fine strands of



chromatin. The number of condensed regions exhibiting centric activity was often greater than 14, suggesting these chromosomes may not be monocentric.

#### Fragments.

The definition of a fragment used by White (1937) was a portion of a chromosome lacking a spindle attachment. In the context of holocentric chromosomes this is not applicable as theoretically all chromosome segments produced as a result of breakage induced by X-irradiation should be able to attach to the spindle. Thus, in the framework of this experiment fragments are defined on the basis of their size. Any categorization on the basis of size is arbitrary, but as long as it can be consistently scored the data should be reliable. The differential condensation of chromosomes observed between different cells, the small and similar size range of Buthid chromosomes and complications due to the possibility of translocation (i.e. the largest chromosome in a given cell may not necessarily be the largest chromosome in the normal complement but may be a result of a translocation) make it difficult to use just relative size as the criterion. However, as can be seen from Fig. 3.3.2 there was a class of breakage products in Buthid mitotic cells that were usually very much smaller than most of the other chromosomes in any cell, but additionally showed a tendency to be palely staining compared to the other chromosomes in the complement. These products were easily and consistently scorable as fragments. Although scoring only these chromosomes as

**Figure 3.3.2**

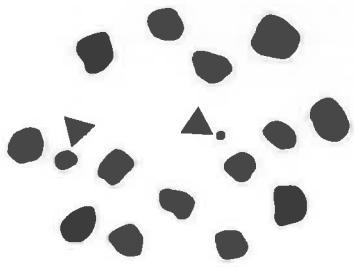
**a-f** Fragments at mitotic metaphase in irradiated OCL individuals.

Closed triangles represent fragments scored.

Open triangles show probable fragments that were not scored.

- a) 2n=16 (including 2 fragments)
- b) 2n=14 (including 3 fragments)
- c) 2n=19 (including 7 fragments)
- d) 2n=19 (including 5 fragments)
- e) 2n=21 (including 4 fragments)
- f) 2n=28 (including 9 fragments)

Bar = 5  $\mu$ m.



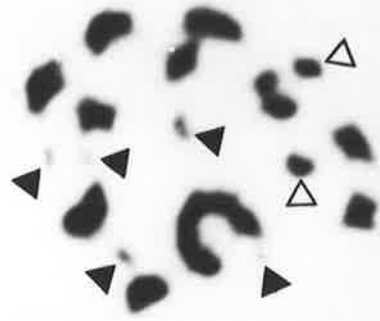
a



b



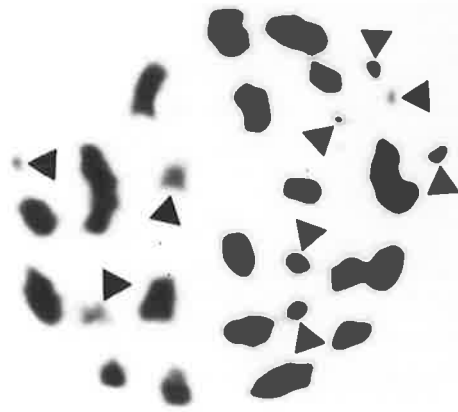
c



d



e



f



fragments probably results in an underestimate of the number of breakage products in a cell as some small chromosomes (obviously smaller than any chromosome in the normal complement) will not be scored as being the result of breakage, it is the behaviour of fragments that is definitive in differentiating between holocentric and monocentric chromosomes. Thus, stringency in defining a fragment should further validate any conclusion pertaining to fragment survival.

The number of fragments per cell was scored for all countable mitotic cells. The chromosome number of each cell included the number of fragments present. Figure 3.3.3 shows graphically the average number of fragments per cell for each chromosome number for animals sampled at various times after irradiation. The lower chromosome numbers (i.e.  $2n < 14$ ) may represent incomplete cells or alternatively these cells may contain fusion products (translocations) due to rejoining of broken ends (see later section on frequency of chromosome rearrangements).

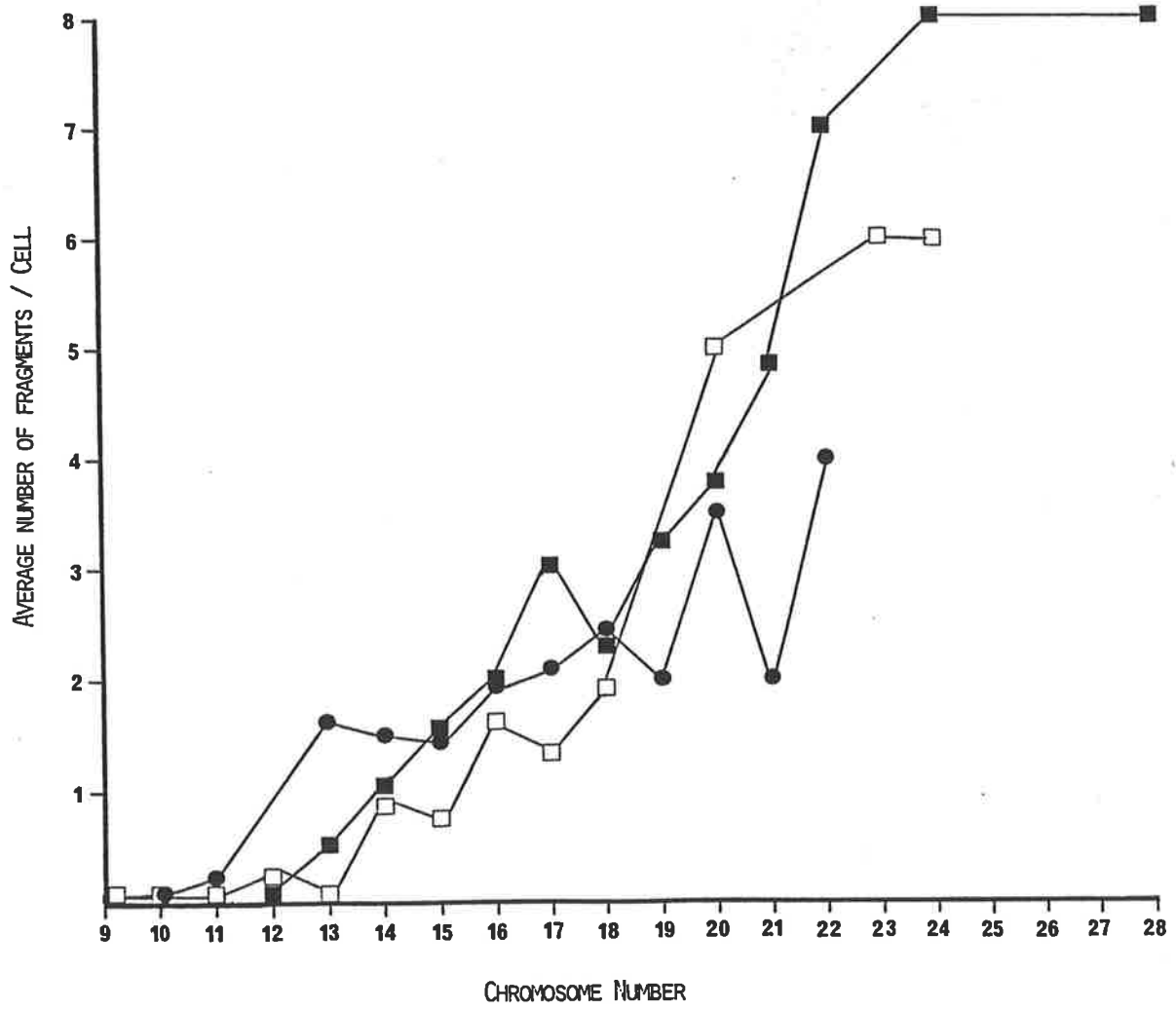
Assuming the cells did not contain translocations it would be expected that for each cell containing fragments the number of fragments would be equal to the chromosome number of each irradiated cell (this includes all bodies in the cell regardless of size) minus 14 which is the normal diploid complement. As can be seen from Figs. 3.3.2 and 3.3.3, this was not always the case, suggesting that either translocations have occurred (Fig. 3.3.2b) or, as previously suggested, the number of fragments per cell was generally

### Figure 3.3.3

The average number of fragments per cell for each chromosome number in *Lychus variatus* individuals after irradiation.

- OCL A scored 14 days after irradiation.
- OCL B scored 21 days after irradiation.
- OCL C scored 37 days after irradiation.

Some of the lower chromosome numbers (i.e. <14) may represent incomplete cells or, alternatively, cells containing fusion products.



underestimated (Fig. 3.3.2c, d, e).

Table 3.3.4a summarizes the fragment data for the three animals from the OCL population killed at three different times after irradiation, while Table 3.3.4b shows tests of significance for the number of fragments per cell between the three animals and controls.

Assuming that the initial radiation effects were similar in all three animals and that at least one mitotic division had occurred between the time of irradiation and the first sampling time (at 14 days) and at least one mitotic division had occurred between each subsequent sampling time ((i.e. between 14 and 21 days and between 21 and 37 days), results can be interpreted as follows: There was not a significant difference in the number of fragments/cell between 21 days (B) and 37 days (C) after irradiation, suggesting that fragments surviving to 21 days can be maintained through subsequent divisions. This was also reflected in the non-significant result for average chromosome number between B and C. However, both B and C are highly significant when compared to A (14 days after irradiation). This suggests either that some fragments are unstable and are lost in early divisions subsequent to irradiation or there was selection against cells with high fragment numbers (i.e. cells showing a higher degree of radiation damage). The latter explanation was supported by the observation that the reduction in fragment number per cell was most likely due to the loss of cells with high chromosome numbers and high fragment numbers (see Fig. 3.3.3). In these cells mitotic

Table 3.3.4a

Average number of fragments/cell scored at mitotic metaphase for *L. variatus* individuals sampled at 14 days (OCL A), 21 days (OCC B), and 37 days (OCL C) after irradiation.

Table 3.3.4b

Tests of significance for the average number of fragments/cell comparing pairwise combinations of *L. variatus* animals from the table above.



Table 3.3.4a

Code	N	Average chromosome number	S <sup>2</sup>	Average number fragments/cell
OCL A	85	17.225	6.41	2.8
OCL B	103	15.728	6.70	1.29
OCL C	102	15.378	4.86	1.5
Control	20	13.95	0.05	0.05

Table 3.3.4b

Test	Z (1.96)	Poisson (SND)
A,B	3.99*	7.08*
A,C	5.26*	5.96*
B,C	1.04	1.27
Control, A	11.72*	14.6*
Control, B	6.83*	10.12*
Control, C	6.36*	11.05*

N = Number of cells scored (metaphase mitotic).

\* = Significant at 5% level.

Test denotes what two animals were compared in pairwise combinations, e.g. A,B → OCL A cf. OCL B.

Z (1.96): testing for a significant difference (at 5% level) between the chromosome numbers of each animal sampled (assuming chromosome numbers are normally distributed).

Poisson (SND): testing whether there is a significant difference (at 5% level) between the number of fragments per cell for each animal sampled assuming a Poisson distribution for fragment numbers in irradiated cells.

breakdown may occur due to difficulty in accommodating high chromosome numbers on the spindle (e.g.  $2n=24-28$  cf.  $2n=14$ ).

#### Chromosome Aberrations.

Figure 3.3.4 illustrates the types of ring chromosomes and translocation chromosomes visible in mitotic cells, while Table 3.3.5 gives the frequency of these aberrations. Both ring chromosomes and translocations were difficult to score due to the contracted state of the chromosomes at metaphase making it impossible to distinguish small ring chromosomes. Only very large translocation chromosomes resulting from the fusion of two breakage products could be scored. Translocations that did not result in a size difference would not have been scored. Thus, it is probable that the frequency of translocations obtained was an under-estimate of the true frequency.

#### Evidence for Division.

In order to make valid conclusions about observations made after irradiation (especially concerning fragment behaviour), it is necessary to show that division has occurred. There are a number of qualitative observations that suggest cells continued to divide after irradiation and that at least one nuclear division had occurred in the cells scored:

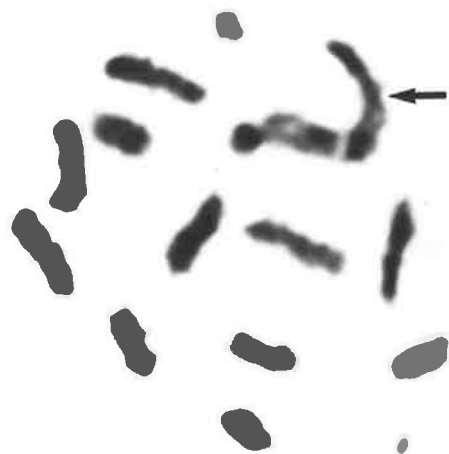
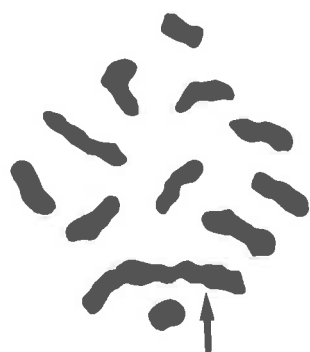
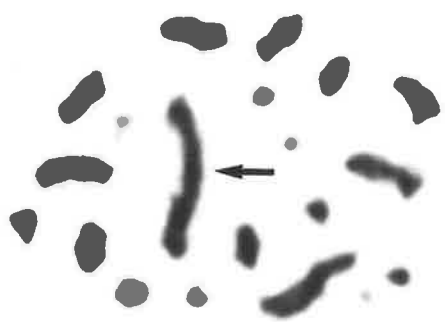
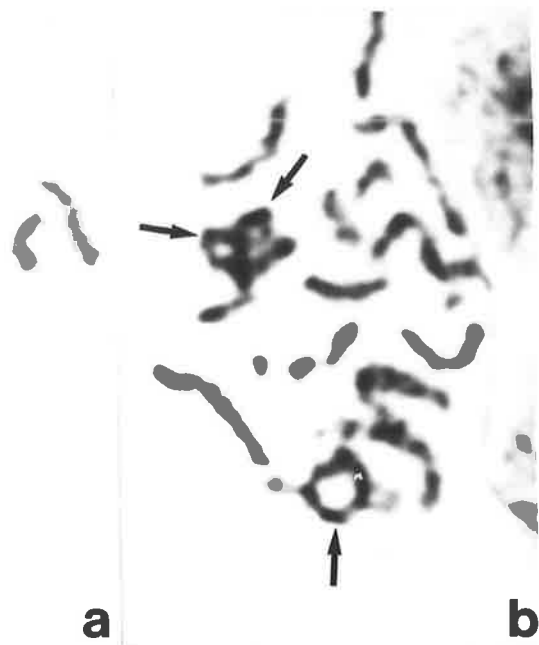
1. Many mitotic cells were in an early anaphase stage where sister chromatids could be seen separating in the typical parallel fashion of these species. In fragment

**Figure 3.3.4**

- a** Mitotic cell irradiated *Lychas* individual  
(OCL B) showing one ring chromosome.
  
- b** Mitotic cell showing three ring chromosomes  
(arrowed).
  
- c-f** Cells showing probable translocation products  
(arrowed).

- c**  $2n=20$
- d**  $2n=12$
- e**  $2n=13$
- f**  $2n=14$

Bar = 5  $\mu$ m.



e



f

Table 3.3.5

Data showing the number of chromosome aberrations  
in mitotic cells of Buthid species.

Animal	N	No. rings	No. translocations
OGL A	21	0	0
OGL B	21	0	2
OCL A	85	1	3
OCL B	103	4*	1
OCL C	102	2	3
OCL 1 ♀	37	1	0
Total	369	8	9
Control	50	0	0
% cells with aberrations		2.2%	2.5%

N = no. of cells scored.

\* = 3 ring chromosomes observed in the same cell.

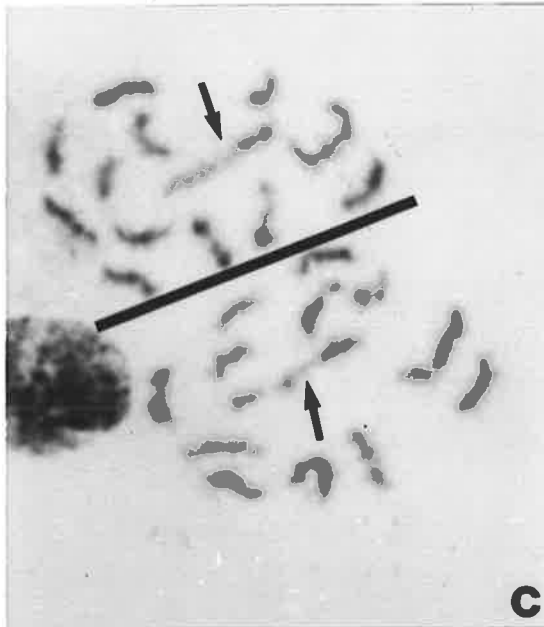
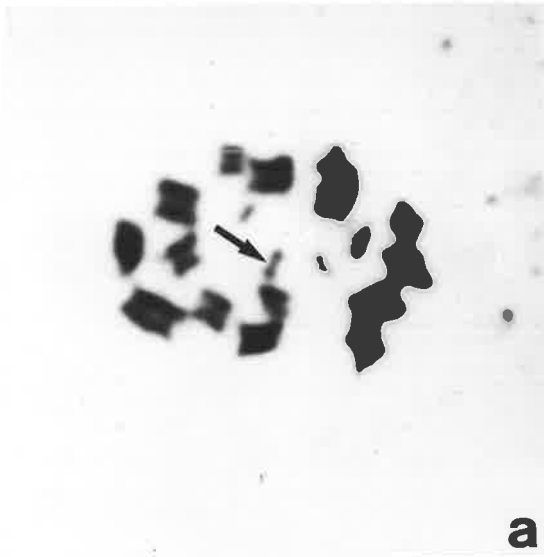
containing cells at this stage even the smallest fragments could be observed as double dividing units (Fig. 3.3.5a, b). Although this is evidence that irradiation occurred at the G1 stage (i.e. prior to chromosome replication), the fact that doubled fragments could be observed dividing up to 37 days after irradiation suggests the cell division must have been continuing subsequent to irradiation as it is unlikely that the delay in mitotic division caused by irradiation would be effective for this period of time.

2. The difference in the number of fragments per cell between 14 days compared with 21 and 37 days also suggests that more than one cycle of cell division has occurred resulting in the probable loss of cells with high fragment numbers. If this difference was not due to loss during division but differential repair time it would be expected that cells scored at 21 days and 37 days would show some differences in fragments per cell, with those scored at 21 days having more fragments per cell (i.e. less repair time before re-entering division cycle). The data showed no significant difference between fragments per cell at 21 and 37 days with the 37 day cells showing slightly more fragments per cell.
3. Groups of mitotic cells were observed in which at least two of the cells showed the same number of chromosomes and fragments suggesting a damaged cell had divided subsequent to irradiation (Fig. 3.3.5c, d, e). Individual OGL B had few scorable mitotic cells (N=21) at 14 days, however,

**Figure 3.3.5**

- a,b** Early mitotic anaphase cells showing fragments as double dividing units (arrowed).
- c,d** Telophase cells showing no lagging of fragments and equal division of cells.
- c** Shows a translocation product (arrowed) clearly dividing with the resulting cells having  $2n=13$ .
- d** Each daughter cell has  $2n=16$  including fragments that have divided and are not lagging.
- e** Two cells at metaphase each showing the same chromosome constitution of  $2n=17$  including three fragments (arrowed).

Bar = 5  $\mu$ m.





most cells had either  $2n=13$ , 15 or 18, suggesting that the few cells present at the time of irradiation had divided producing cysts with similar damage - Fig. 3.3.6 shows this graphically.

4. The series of anaphase I cells in OGL B show that more than 14 bodies are capable of movement at anaphase. Comparisons revealed that the chromosomes in different cells showing anaphase movement were not of the same size, suggesting that breakage anywhere along a chromosome results in a stably dividing product. The few mitotic anaphase cells observed showed no evidence of lagging fragments (Fig. 3.3.5c, d).

#### Irradiation of the Scorpionid, *U. manicatus*.

The individual results for all doses and animals irradiated are presented in Tables 3.3.6 and 3.3.7. These data have been summarized in Table 3.3.8 so that a simplified picture of the overall effect of irradiation with time can be analysed.

#### Micronuclei.

Figure 3.3.7 shows the incidence of micronuclei per cell against time after irradiation for EBM, FCM and RRM animals and their controls. There was a general trend for micronuclei to increase as time after irradiation increased. At 45 days there was a drop in the incidence of micronuclei. However, at this stage, cell division in the testis seemed to have almost entirely ceased leading to a decrease in the

### Figure 3.3.6

Effect of irradiation on chromosome number 14 days  
after irradiation (1000r).

■ OCL A (148 mitotic cells scored).

△ OGL B (41 meiotic [MII] cells scored).

▲ OGL B (21 mitotic cells scored).

OCL A and OGL B meiotic cells show similar distributions of chromosome numbers suggesting the damage incurred in these two individuals was comparable. The peaks shown by the few OGL B mitotic cells scored suggests cysts of cells with the same chromosome damage have been produced due to division of an original irradiated cell.

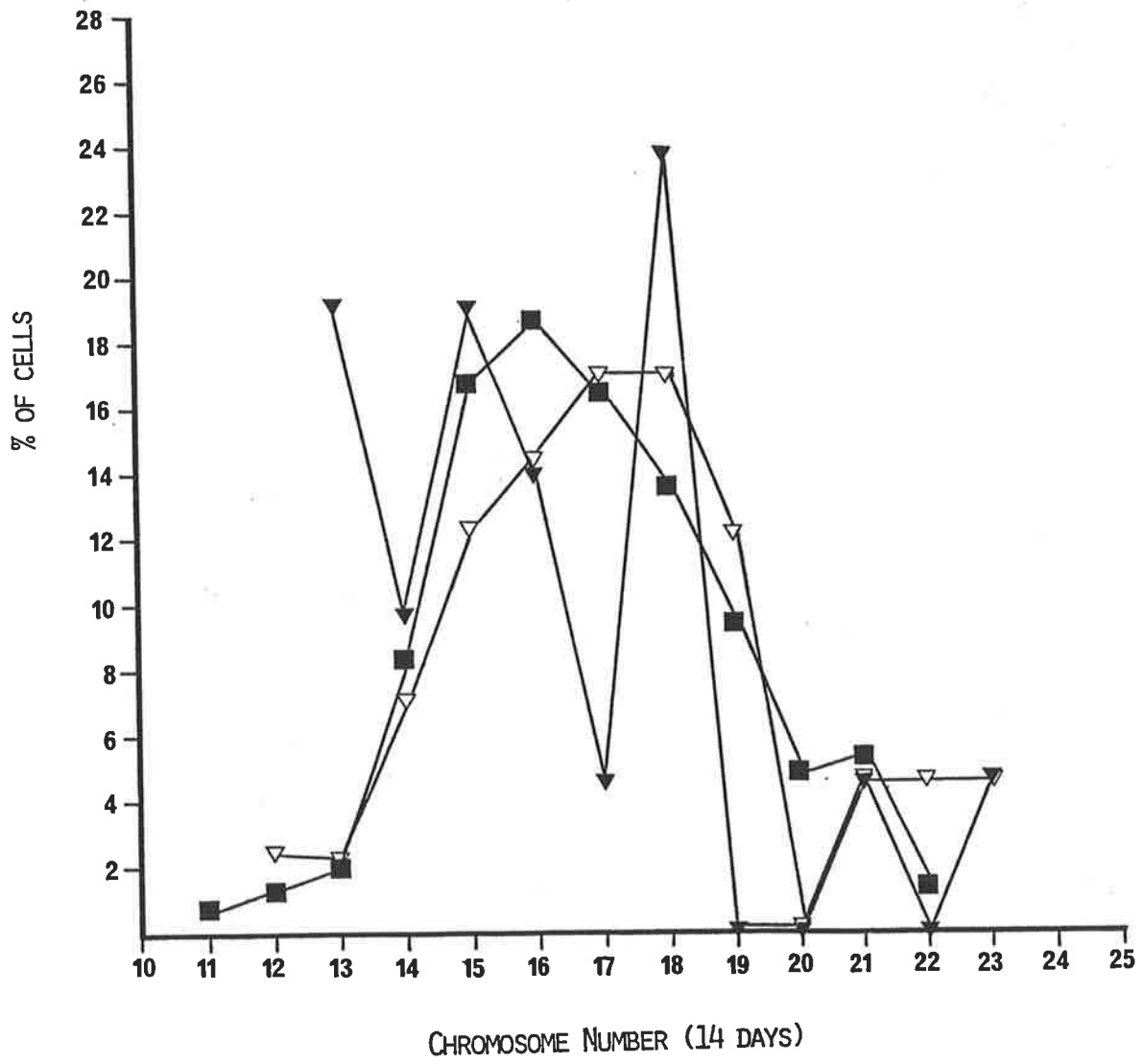


Table 3.3.6

The effects of irradiation in *U. manicatus* scored at interphase and anaphase for all doses and times after irradiation.

Animal and dose	Time of sampling	Micronuclei/cell	N	% Bridges at anaphase/telophase	N
330r					
EBM A	33 hrs.	0.0	200	no anaphases	
EBM A	100 hrs.	0.035	200	no anaphases	
500r					
EBM B	54 hrs.	0.11	200	70%	47
EBM B	6.9 days	0.06	600	48%	46
1000r					
EBM 1	8.25 days	0.12	200	100%	2
EBM 2	8.25 days	0.145	200	96%	25
EBM 5	8.25 days	0.395	200	90%	30
EBM C	7.3 days	0.16	200	no anaphases	
EBM C	10.2 days	0.095	200	85%	13
EBM 3	14 days	0.31	200	94%	34
EBM 4	21 days	0.195	200	98%	45
EBM 5	31 days	0.46	200	92.5%	40
EBM 6	45 days	0.075	200	no anaphases	
EBM 7	45 days	0.125	200	0%	1
330r					
FCM A	54 hrs.	0.0	200	no anaphases	
FCM A	107 hrs.	0.04	200	no anaphases	
500r					
FCM B1	54 hrs.	0.24	200	100%	2
FCM B1	99 hrs.	0.19	200	no anaphases	
FCM B2	6.375 days	0.085	200	75%	8
FCM B2	13 days	0.12	200	47%	17
1000r					
FCM C1	54 hrs.	0.085	200	92%	13
FCM C2	7.4 days	0.06	200	89.5%	37
FCM 1	7.8 days	0.25	200	89%	46
FCM C1	14 days	0.09	200	93%	28
FCM 2	14 days	0.055	200	100%	7
FCM 3	21 days	0.445	200	100%	3
FCM 3	29 days	0.5375	400	100%	28
RRM 6	21 days	0.475	200	89%	45
RRM 5	28 days	0.605	200	86%	64
Controls					
FCM	0.0 days	0.01	200	none observed	10
	7 days	0.0	200	"	8
	10.5 days	0.008	200	"	10
	14 days	0.005	200	"	10
	21 days	0.01	200	"	8
	30 days	0.005	200	"	9
	45 days	0.005	200	"	12
EBM	0.0 days	0.015	200	"	2
	8 days	0.02	200	"	7
	14 days	0.03	200	"	4
	21 days	0.025	200	"	6
	30 days	0.02	200	"	9

Table 3.3.7

The effects of irradiation (1000r) scored at metaphase I  
in *U. manicatus* individuals at various times after irradiation.

Code	Cell stage	Fragments/ cell	Achromatic gaps/cell	Average chromosome number	Average number bodies	N
EBM 2 8.6d  2n=50	early/mid MI	1.8	13.0	61.9	22.8	10
	late MI	6.3	4.7	54.3	22.3	3
	Total MI	2.85	11.1	60.15	22.7	13
EBM 3 14d  2n=48	early/mid MI	1.0	4.8	48.6	21.5	12
	late MI	3.375	4.6	52.5	20.0	8
	Total MI	1.95	4.75	50.2	20.9	20
EBM 4 21d  2n=46	early/mid MI	0.64	4.07	52.6	23.8	14
	late MI	2.2	4.3	51.3	20.3	6
	Total MI	1.1	4.15	52.2	22.75	20
Controls	Total MI	0.1	1.67	-	-	27
FCM C1 54hrs	early/mid MI	2.82	13.8	64.4	30.5	11
	late MI	9.0	9.0	76.0	-	1
	Total MI	3.33	13.4	65.3	30.7	12
	Mitotic	26.5	-	86.5	-	2
FCM C2 7-10d	early/mid MI	1.18	12.23	63.7	29.85	13
	late MI	1.875	4.25	61.3	28.25	8
	Total	1.67	9.19	62.8	29.24	21
	Mitotic	3.286	-	53.7	-	7
FCM 1 7-20d Pooled	mid MI	1.5	4.8	62.3	29.8	10
	early/mid MI	1.52	9.0	63.1	29.8	23
	Total MI	1.61	7.77	62.65	29.4	31
FCM C1 14d	early/mid MI	0.33	8.67	65.0	29.8	6
	late MI	1.8	2.8	60.6	28.0	5
	Total MI	1.0	3.27	63.0	29	11
FCM 2 14d	early/mid MI	0.86	2.57	60.7	29.3	7
	late MI	1.67	1.67	68.3	30.0	3
	Total MI	1.1	2.3	63.0	29.5	10
Pooled	early/mid MI	0.62	3.07	62.7	29.5	13
	late MI	1.75	2.375	63.5	28.75	8
	Total MI	1.05	2.81	63.0	29.2	21
FCM 3 21d	early/mid MI	0.92	7.8	66.67	31.6	12
	late	0.875	0.0	64.5	29.6	20
	Total MI	0.9	5.45	65.8	30.8	20
FCM 3 29d	early/mid MI	0.46	3.15	60.85	28.0	13
	late	1.0	0.0	56.0	27.0	1
	Total	0.5	2.9	60.5	27.9	14
Control	Total	0.0	1.5	60.5	30.5	20

Table 3.3.8

Pooled results for damage after irradiation of all individuals  
of *U. manicatus*.  
(i.e. populations RRM, FCM and EBM) Dose 1000r.

Code/sampling time range	Micronuclei/cell <sup>3</sup>	% Anaphase <sup>2</sup> bridges	N	Fragments/cell <sup>1</sup>	N
FCM 2.25d	0.085	92%	13	3.33	12
EBM/FCM 7→8d	0.188	89%	140	2.1	27
EBM/FCM 14d	0.1516	96.5%	69	1.5	41
EBM/FCM/RRM 21d	0.37	98%	48	1.0	40
EBM/FCM/RRM 29.31d	0.53	94%	68	0.5	14
EBM 45d	0.1	0%	1	no metaphases	
Controls	0.015	0%	20	0.0	200

N = Number of cells scored.

<sup>1</sup> Fragments/cell based on counts of metaphase I cells.

<sup>2</sup> Anaphase counts based on mitotic and meiotic cells.

<sup>3</sup> Micronuclei/cell based on at least 200 cells per animal.

Figure 3.3.7

The number of micronuclei/cell for *U. manicatus* individuals sampled at various times after irradiation (1000r).

▲ EBM control.

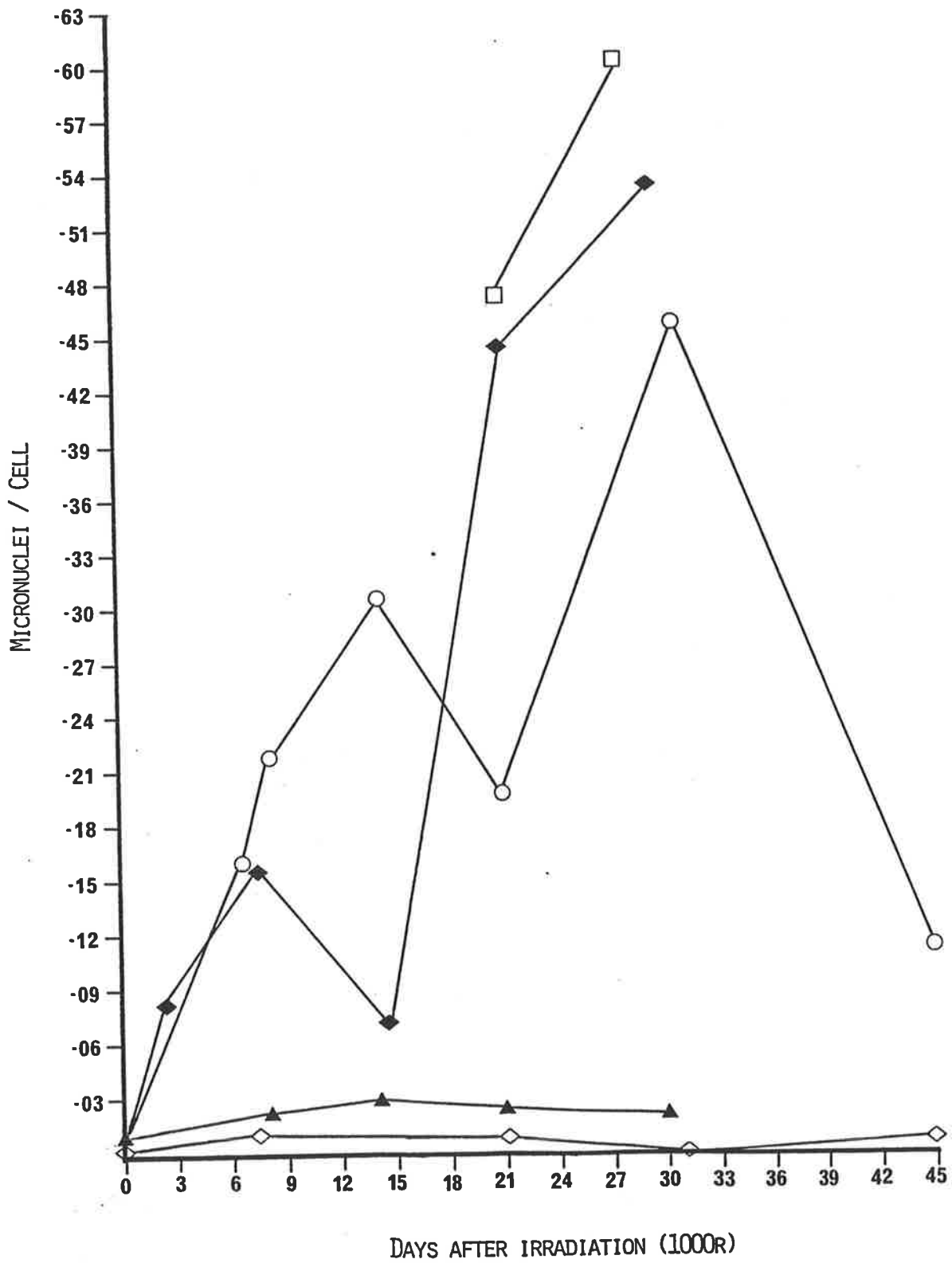
◇ FCM control.

◆ FCM.

○ EBM.

□ RRM.

The drop-off in the number of micronuclei at 45 days was probably due to cessation of division and breakdown of any already existing micronuclei.





production of micronuclei with those micronuclei already existing possibly having broken down.

#### **Anaphase Bridges.**

Figure 3.3.8 illustrates graphically the percent of anaphase cells showing bridges or lagging fragments. The frequency of anaphase bridges was very high at all times after irradiation for those individuals given a dose of 1,000r. However, for lower doses (500r) anaphase bridge frequency decreased as time after irradiation increased, probably due to the scoring of slightly damaged or undamaged cells as they entered a division cycle. In the testis of the two animals scored after 45 days, only one anaphase cell was observed. This cell was normal (i.e. no bridges), however, it was the only obviously dividing cell scored in two entire testes. Most of the bridges were observed at anaphase I of meiosis with a small proportion scored at mitosis, however, both types of anaphase were often indistinguishable as the chromosomes in telomeric nuclei could not be accurately counted. Figure 3.3.9 shows examples of micronuclei, anaphase bridges and fragments, and telophase cells showing obvious chromatin connections or lagging fragments.

#### **Fragments.**

Due to the almost total absence of mitotic cells in the testes of irradiated individuals, fragment counts were performed at metaphase I of meiosis. As shown previously in

Figure 3.3.8

Effect of irradiation on the incidence of anaphase bridges.

◆FCM (500r).

◇FCM (1000r).

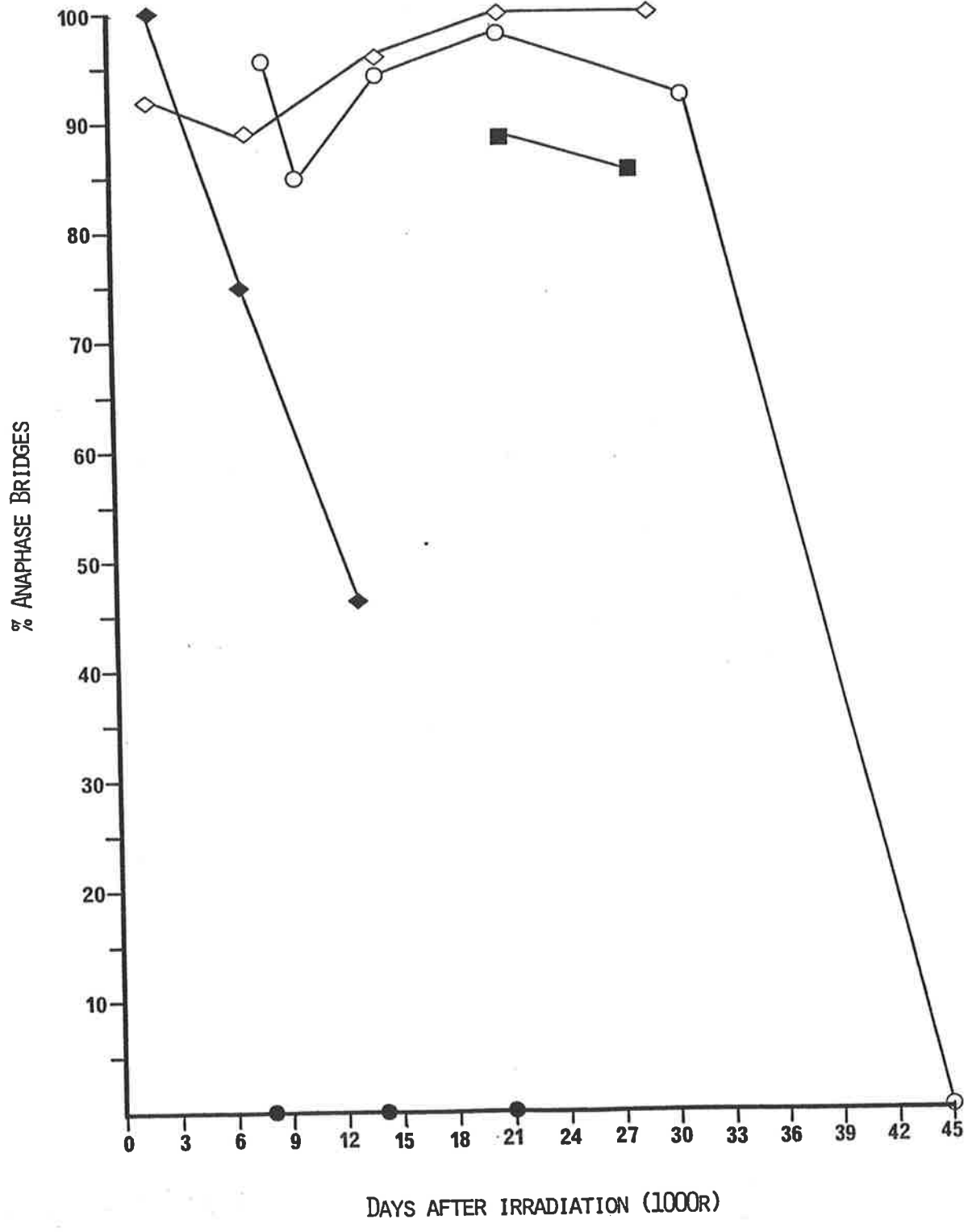
○EBM (1000r).

■RRM (1000r).

●EBM control. (No bridges observed.)

The frequency of anaphase bridges remained high until 45 days when division in the testis ceased in those animals receiving 1000r.

The animals given 500r showed a rapid fall-off in bridge frequency as repaired or undamaged cells entered division after irradiation.



**Figure 3.3.9**

- a. *U. manicatus* showing micronuclei (m) and bridges (b) still remaining after cell division has occurred.
- b,c Cells showing bridges (b) stretching between telophase cells and fragments (f) remaining on the metaphase plate due to their acentric nature.

Bar = 5  $\mu$ m.

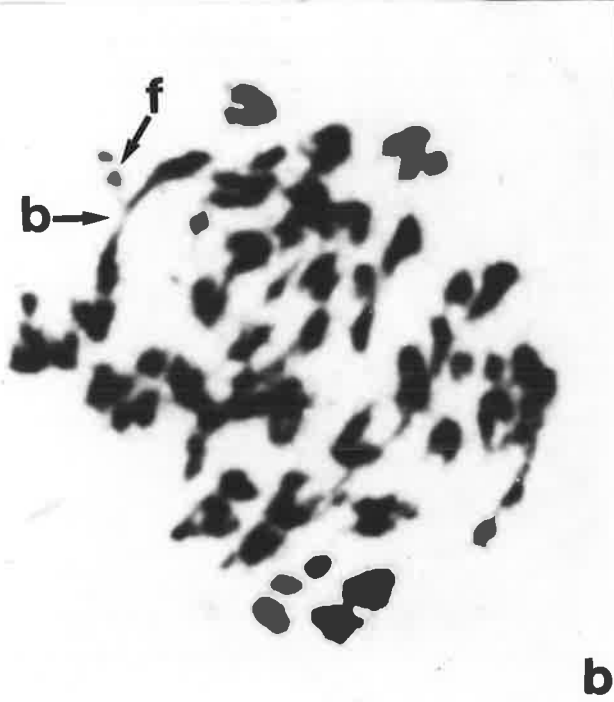
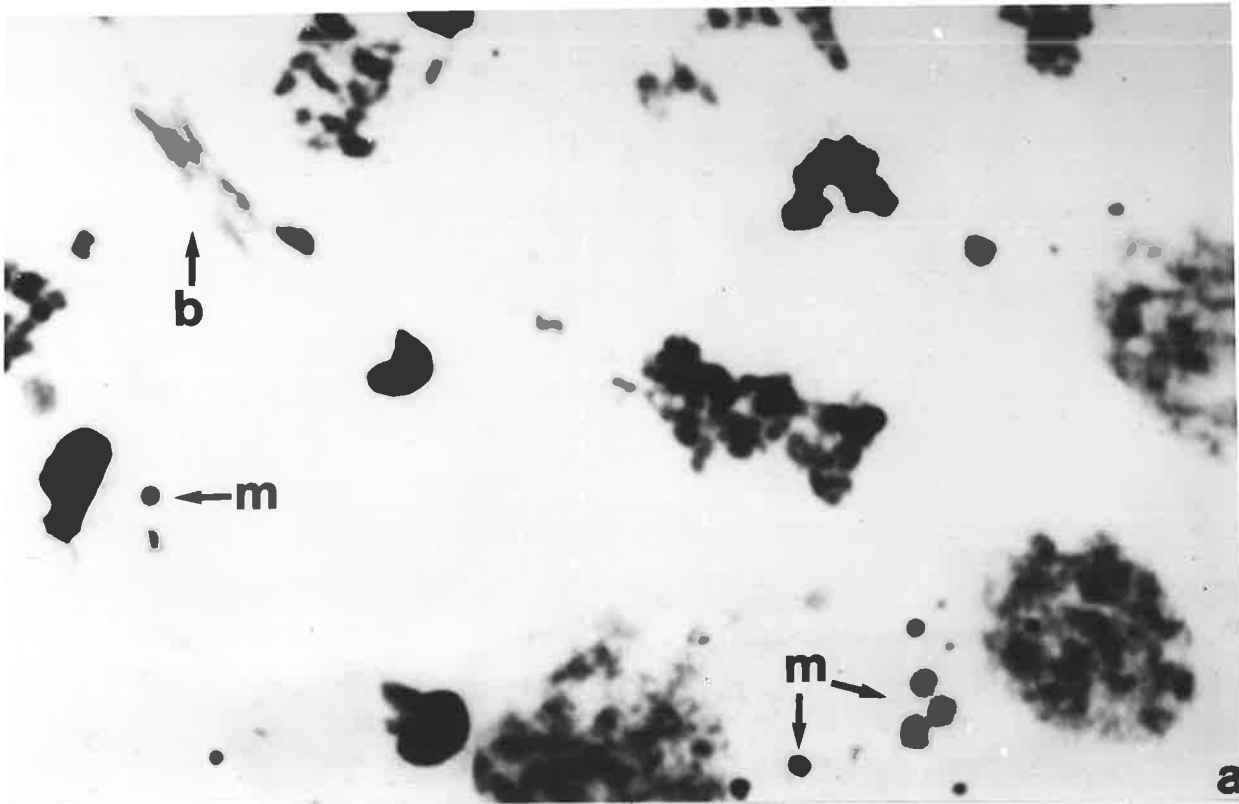


Table 3.3.7, cells were categorized as either early to mid metaphase or late metaphase-early anaphase. Close pairing of homologous regions meant that some fragments could not be observed until these later stages. As shown in Fig. 3.3.10, the frequency of fragments/cell shows a continuing trend to decrease as time after irradiation increases. However, as these are meiotic cells the interpretation of this trend is more complex than with mitotic cells as explained later in this section. The few mitotic cells that could be scored (FCM C1 and FCM C2 - Table 3.3.7) showed a very marked decrease with time for fragments per cell. Figure 3.3.11 gives some examples of early - mid metaphase cells with fragments, compared with unirradiated control cells from the same animals. Figure 3.3.12 shows mitotic cells with fragments.

#### **Evidence for Division.**

Again, all the previous evidence must be interpreted in the light of whether nuclear division has or has not occurred between the time of irradiation and the time of sampling.

The presence of micronuclei in the testicular cells indicates that nuclear division has occurred in some cells after irradiation as micronuclei correspond to acentric fragments that have been excluded from the nucleus during cell division. Correspondingly, the decrease in fragments per cell with time could perhaps be taken to indicate that division has occurred and consequently acentric fragments have been lost. This is probably true for the mitotic

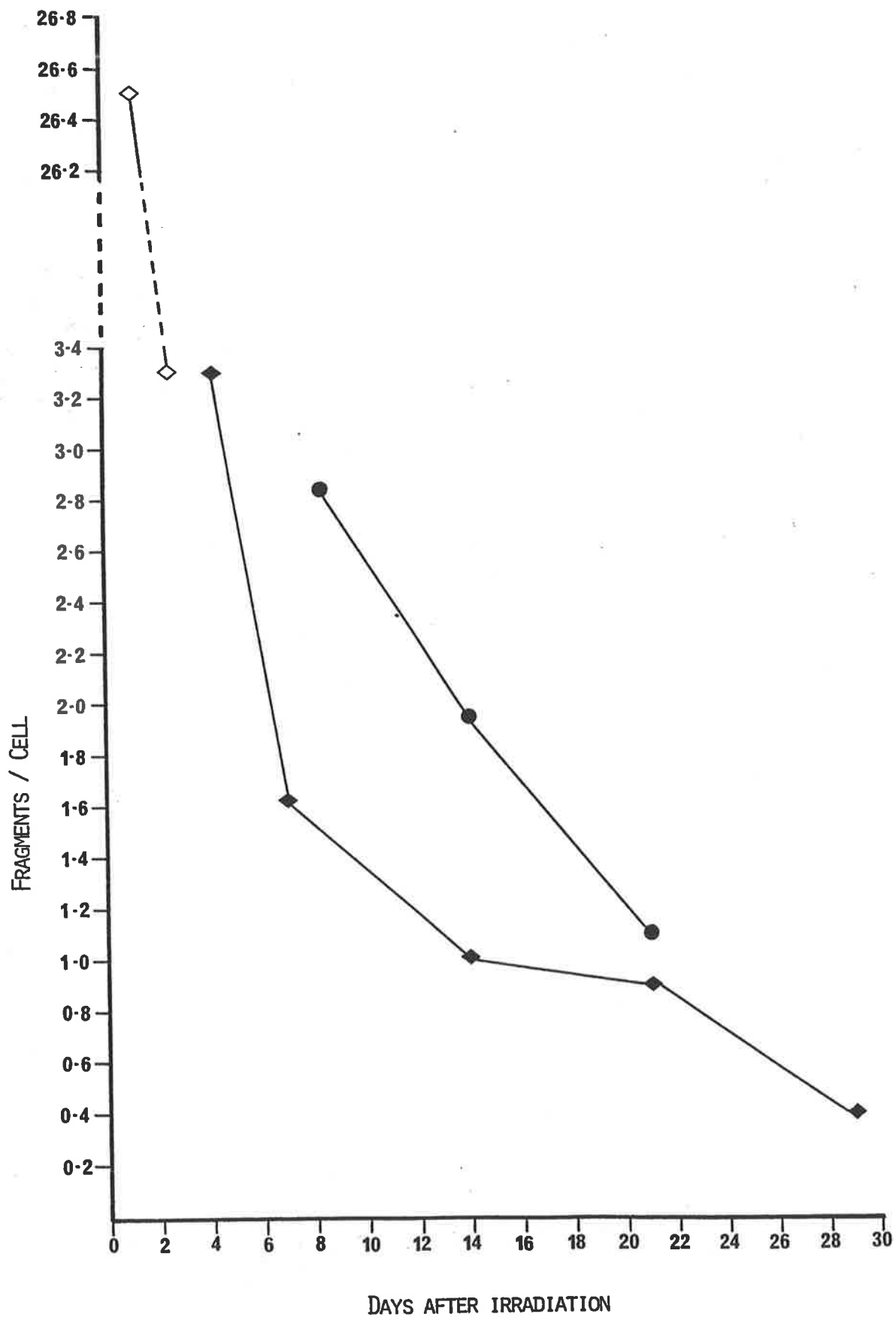
**Figure 3.3.10**

The number of fragments per cell scored at various  
times after irradiation in *U. manicatus*  
individuals.

◆FCM (metaphase I).

●EBM (metaphase I).

◇FCM (mitotic).



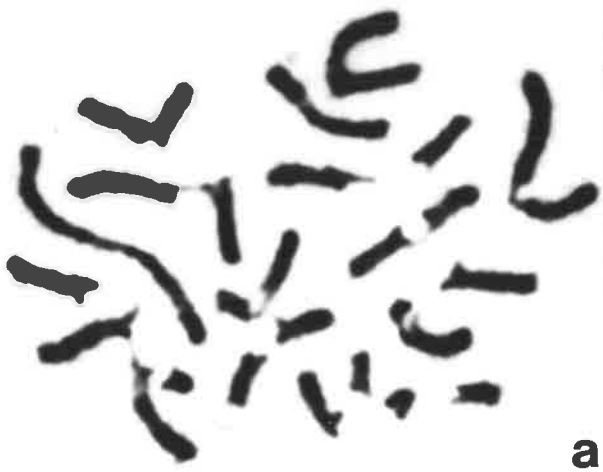


**Figure 3.3.11**

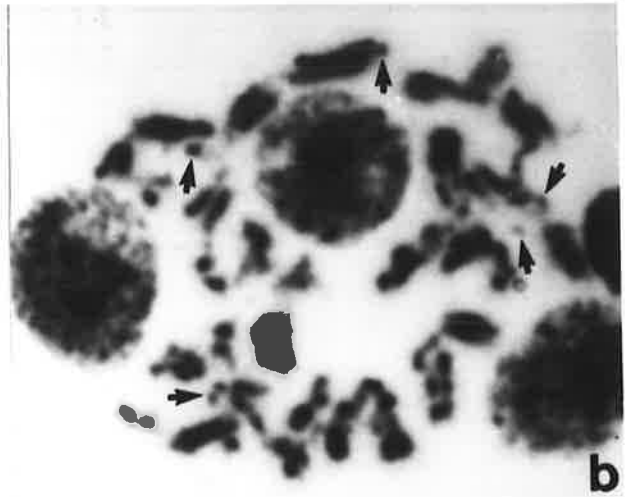
**Early-mid metaphase I cells of *U. manicatus*  
before and after irradiation.**

- a EBM 2 control cell showing 16 bivalents and 6 trivalents. ( $2n=50$ )
- b EBM 2 8.6 days after irradiation (1000r).  
Fragments are arrowed.  
  
Note also achromatic gaps and regions of decondensation.
- c EBM 3 control cell showing 16 bivalents, 4 trivalents and a quadrivalent (q). ( $2n=48$ )
- d EBM 3 scored 14 days after irradiation.  
Fragments are arrowed.  
  
Note distorted appearance of quadrivalent.
- e EBM 4 control cell showing 17 bivalents and 4 trivalents. ( $2n=46$ )
- f EBM 4 scored 21 days after irradiation.  
Fragments arrowed.

Bar = 5  $\mu$ m.



a

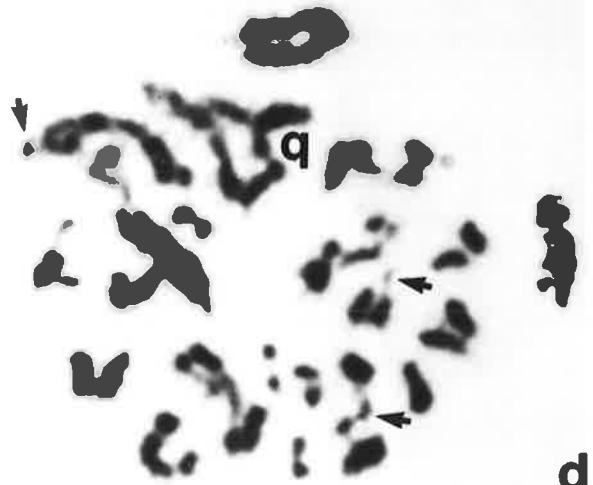


b

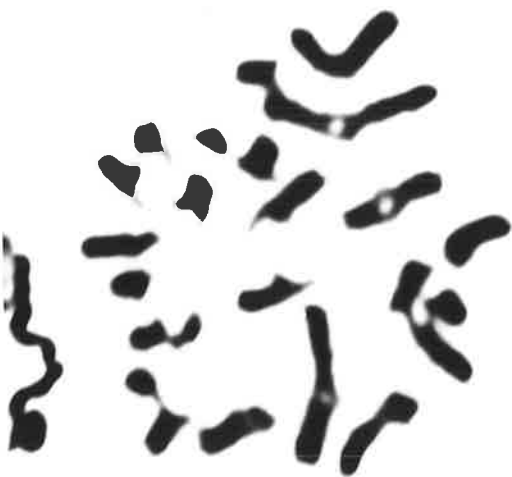


q

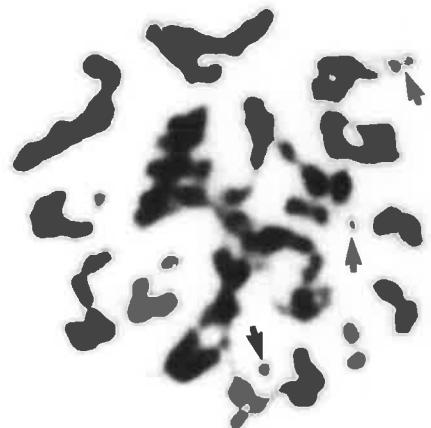
c



d



e



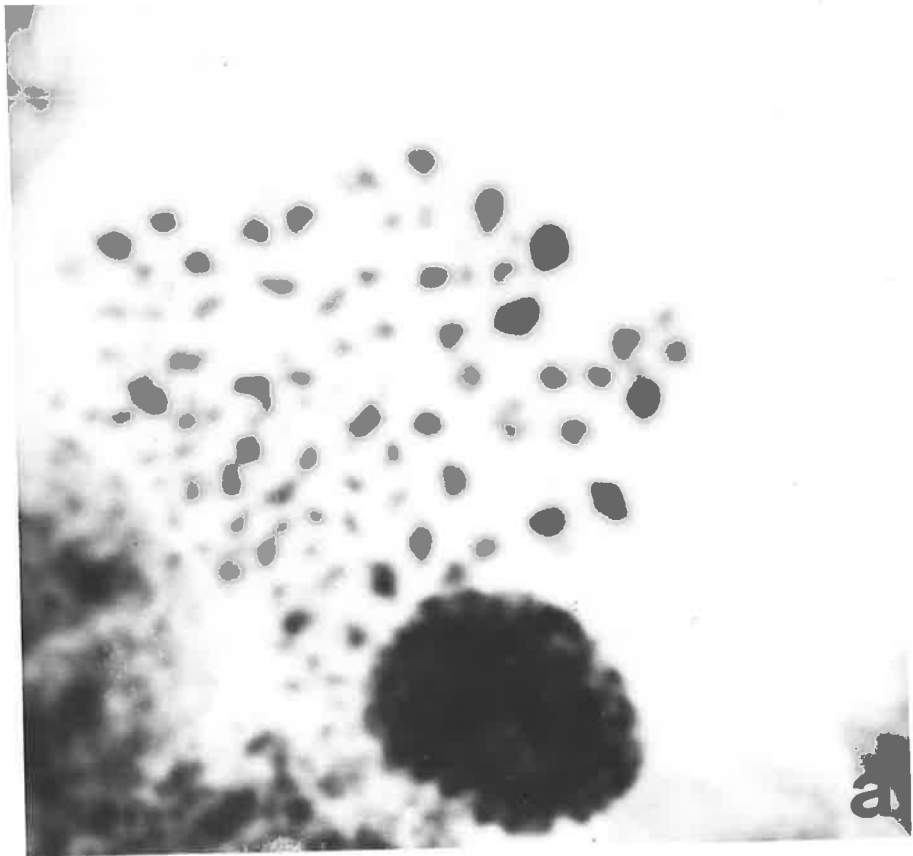
f

**Figure 3.3.12**

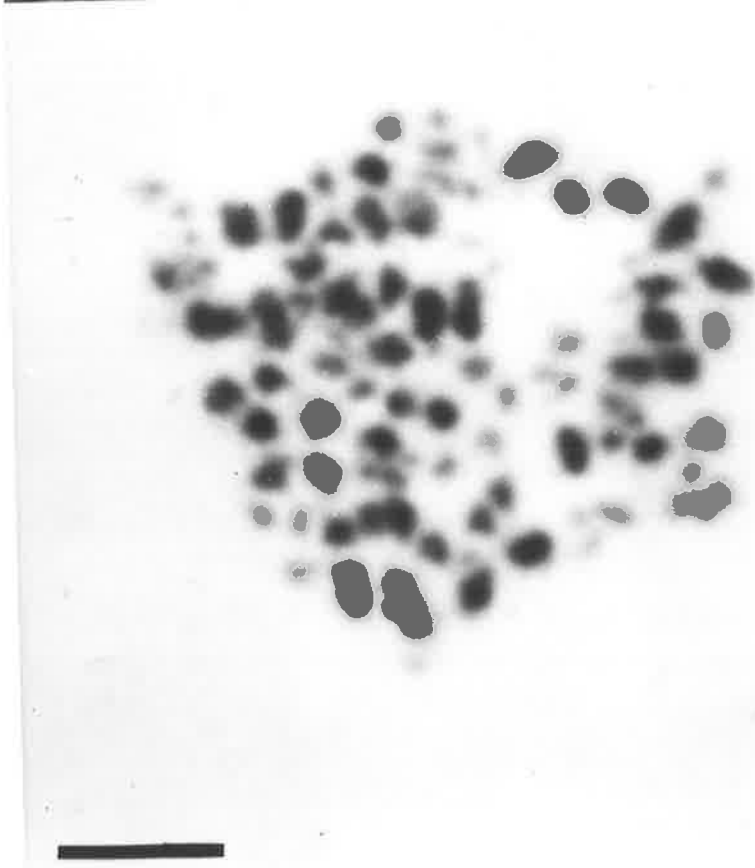
**Mitotic cells of FCM individuals showing large  
numbers of fragments.**

- a FCM C1 54 hours after irradiation (1000r)  
2n=86.**
- b FCM C1 54 hours after irradiation (1000r)  
2n=94.**

**Bar = 5  $\mu$ m.**



**a**



**b**

cells scored. However, most of the fragment counts were done at metaphase I, a stage which is preceded by a long series of pairing and replication stages. Thus it is possible that the differences in fragment number were due to differential sensitivity of different stages of meiosis to irradiation rather than loss of fragments in mitotic divisions leading up to metaphase I. A way of distinguishing between these two possibilities is to look at the types of aberrations present at metaphase I. The presence of mainly chromatid aberrations would suggest that irradiation occurred before the chromosomes underwent replication, while chromosome aberrations would indicate replication occurred subsequent to irradiation. Table 3.3.9 shows the distribution of chromosome and chromatid aberrations present in metaphase I cells at various times after irradiation, while Fig. 3.3.13 shows some examples of these aberrations.

Chromatid aberrations predominate in animals scored early after irradiation but are replaced by chromosome aberrations in those scored later (i.e. after about 21d). This suggests that the cells have not gone through mitotic divisions before entering metaphase I but rather that all cells were in some stage of meiosis at the time of irradiation. Cells with only chromatid aberrations were irradiated at pachytene/diplotene stages (i.e. after DNA replication) while those showing only chromosome aberrations were irradiated preleptotene (i.e. before replication). Thus, the reduction in fragments per cell with time reflects

Table 3.3.9

Number of chromosome and chromatid aberrations present  
in metaphase I cells at various times after irradiation.

Animal	Sampling time	N	No. chromatid aberrations	No. chromosome aberrations
FCM C1	54 hours	12	17	-
FCM C2	7.4 days	20	8	2
FCM 1	7.8 days	10	4	-
FCM C1, 2	14 days	21	7	-
FCM 3	21 days	20	2	10
FCM 3	29 days	14	-	16
EBM 2	8.25 days	13	19	-
EBM 3	14 days	20	7	-
EBM 4	21 days	20	-	5
EBM 5	31 days	6	-	4
Control		50	-	2

N = Number of cells.

**Figure 3.3.13**

**Chromosome and chromatid aberrations.**

**a,b FCM C1 54 hours after irradiation (1000r).**

**Arrows indicate chromatid aberrations.**

**c FCM C2 7.4 days after irradiation (1000r).**

**Arrows indicate chromosome aberrations. Chromosome aberrations did not usually appear until 21 days after irradiation making the origin of these aberrations obscure. This cell may have precociously entered division or was spontaneously heterozygous for two fusion events.**

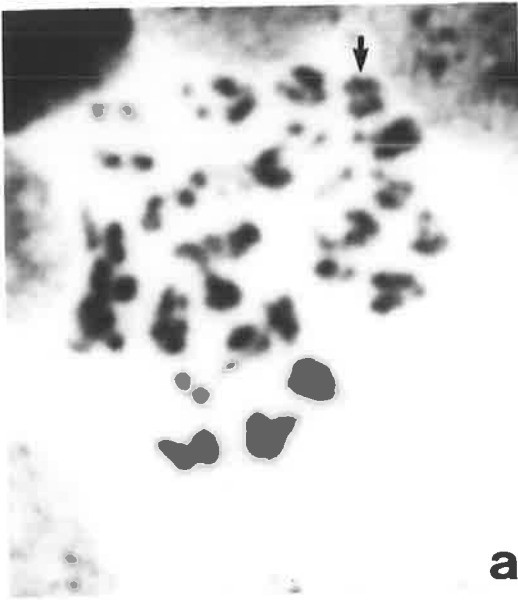
**d FCM 3 scored 21 days after irradiation.**

**Chromosome aberrations arrowed.**

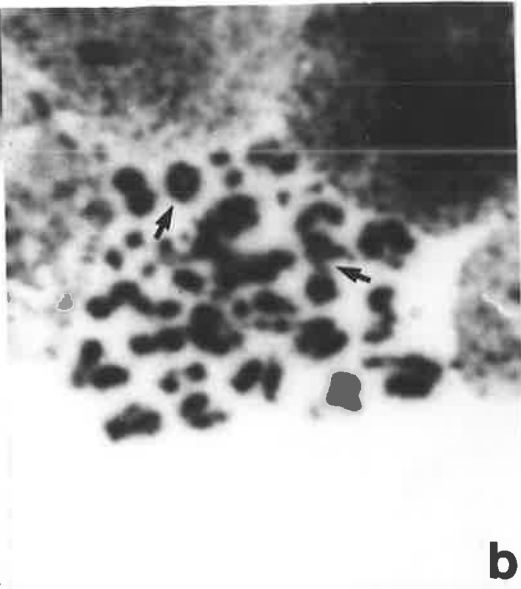
**e,f FCM 3 scored 29 days after irradiation.**

**Chromosome aberrations arrowed.**

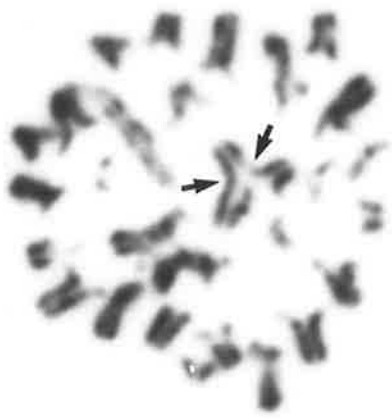
**Bar = 5  $\mu$ m.**



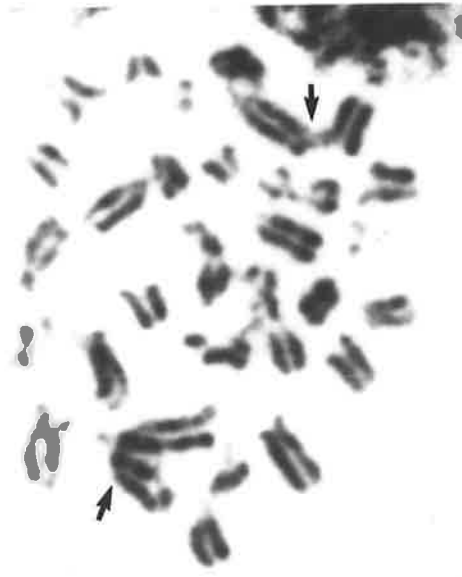
a



b



c



d



e



f



differential sensitivity of different stages of division to irradiation and differences perhaps in the time available and efficiency of repair between cells at different stages rather than the loss of fragments through division.

Additionally, achromatic gaps (see Fig. 3.3.14 and Table 3.3.7), usually considered a direct effect of radiation, show a reduction in frequency as time after irradiation increases. Although the exact nature of the lesion that produces an achromatic gap is still contentious (i.e. whether or not it represents the site of a true chromatid break), the reduction in frequency further supports the idea that the differences observed between metaphase I cells through time are due to differential sensitivity and repair. However, it is possible that some of the cells scored late (i.e. >29 days) may have undergone mitotic division before entering meiosis.

The presence of micronuclei and anaphase cells in most testes showed that many cells were able to complete at least one nuclear division after irradiation. However, 45 days after irradiation almost all division had ceased and the frequency of what appeared to be dead cells was about 70% of all testicular cells. This indicates that damage resulting in anaphase bridges and other radiation effects, had led to cell death as division progressed.

#### **Ring Chromosomes and Chromatid Interchanges.**

Figure 3.3.15 illustrates the types of chromosome aberrations that could be scored in the few mitotic cells observed; ring chromosomes and cross-shaped chromosomes

**Figure 3.3.14**

**Achromatic gaps in *U. manicatus*.**

**a,b** FCM C2 scored 7.4 days after irradiation (1000r).

Arrows indicate achromatic gaps.

**c** FCM C2 scored 14 days after irradiation (1000r).

Arrows indicate achromatic gaps.

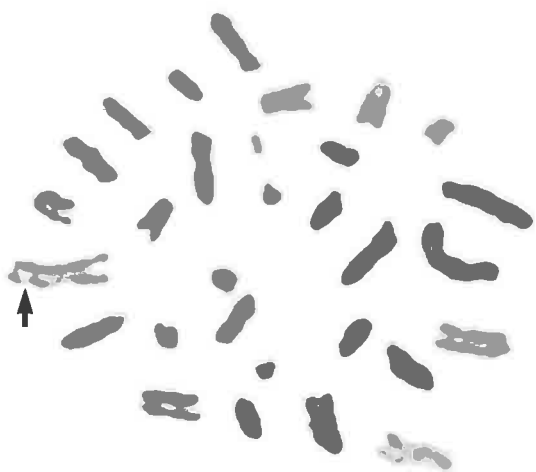
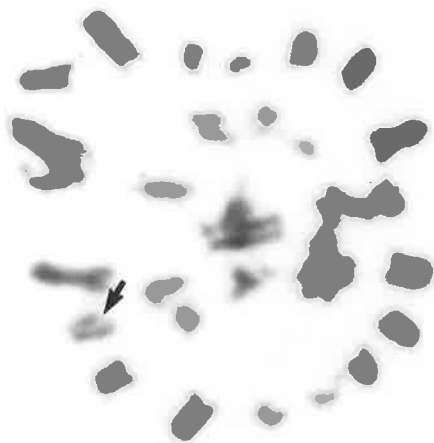
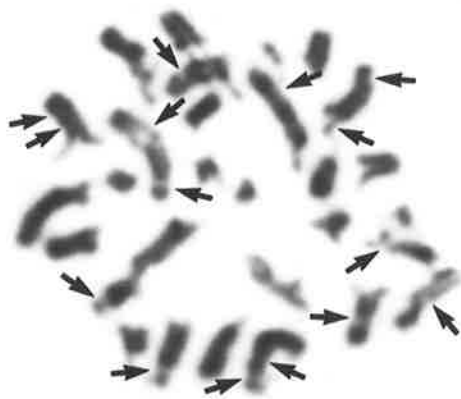
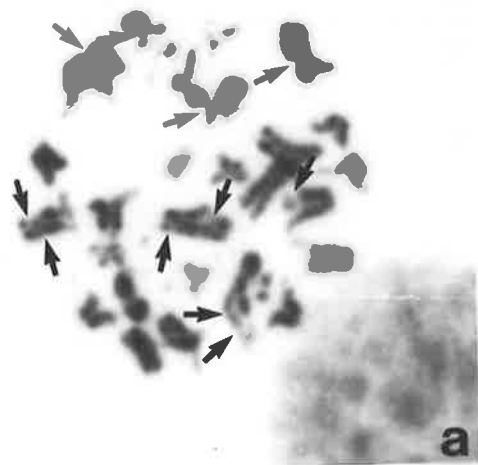
**d** FCM 3 scored 29 days after irradiation.

Note lack of achromatic gaps.

**e** Control cell of FCM showing 30 bivalents.

The 'achromatic gap' arrowed in this cell probably represents a pale region due to differential staining and is not a true gap.

Bar = 5  $\mu$ m.



a

b

c

d

e

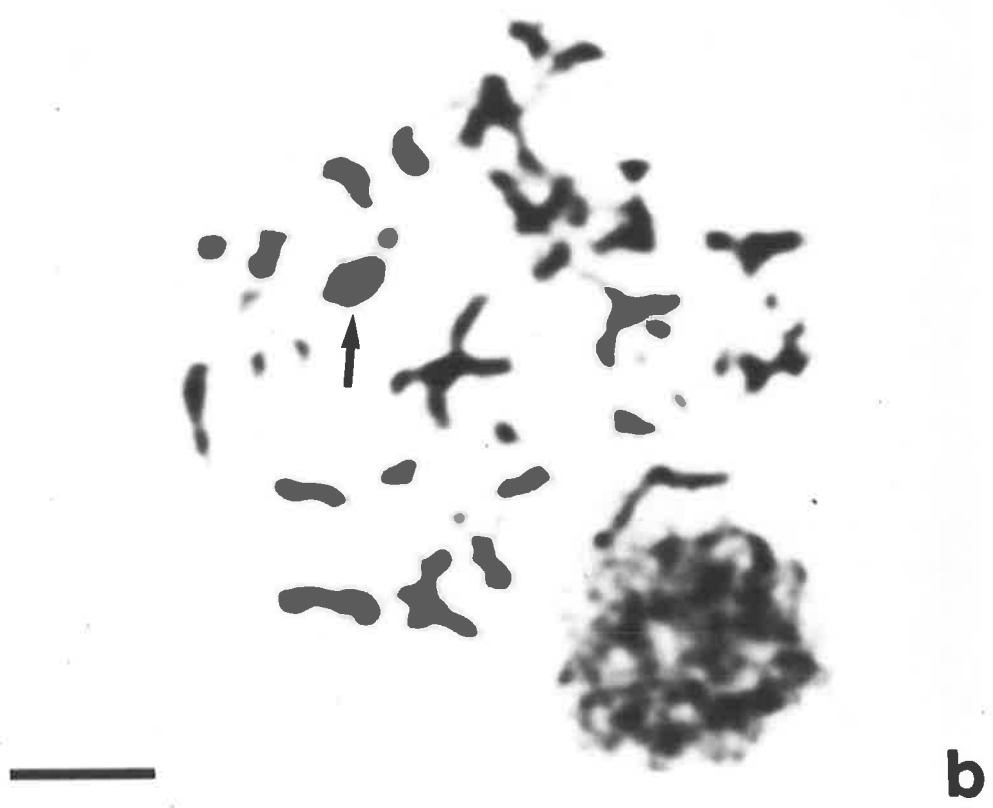
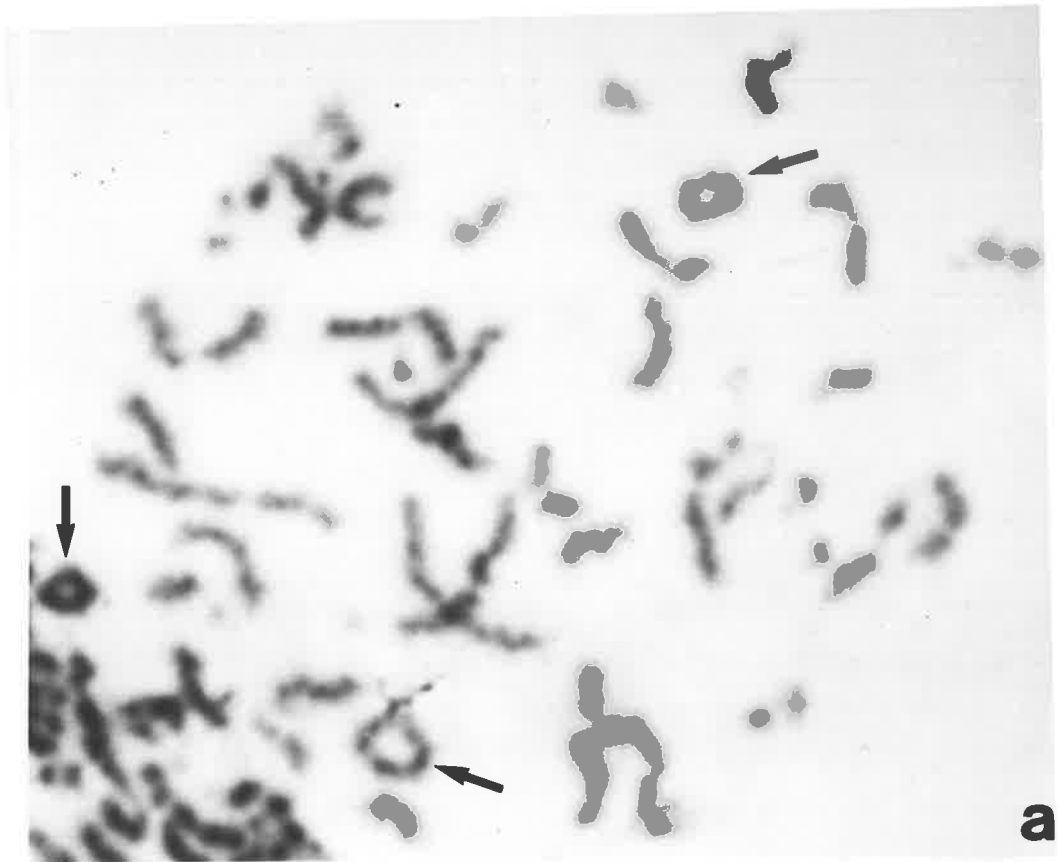
**Figure 3.3.15**

**Mitotic cells of *U. manicatus* showing ring  
chromosomes (arrowed).**

- a) FCM C2 scored 7.4 days after irradiation (1000r).
- b) EBM C scored 10.2 days after irradiation (1000r).

**Note stickiness of chromosomes.**

**Bar = 5  $\mu$ m.**



resulting from reciprocal chromatid fusion. The frequencies of these chromosome changes are shown in Table 3.3.10. In many cells both types or multiples of each aberration were present. Although chromosome rearrangements were difficult to score, the frequency of anaphase bridges suggest they were of common occurrence.

Table 3.3.10

Number of chromosome aberrations scored in mitotic cells at various times after irradiation.

Animal	Time of sampling	N	No. ring chromosomes	No. chromatid interchanges
FCM C1	54 hours	3	-	-
FCM C2	7.4 days	8	6*	2
FCM 1	7.8 days	2	1	-
FCM C1, 2	14 days	-	-	-
FCM 3	21 days	3	-	-
FCM 3	29 days	1	1	-
EBM 2	8.25 days	6	2	-
EBM C	10.2 days	1	1	2
EBM 3	14 days	-	-	-
EBM 4	21 days	8	2	2
Total		32	13	6
Control		50	-	-

N = Number mitotic cells scored.

\* = 3 ring chromosomes observed in one cell.

## DISCUSSION

### Comparison of the Effects of Irradiation on the Chromosomes of Buthid and Scorpionid Species.

#### Qualitative Observations.

Assuming that in general the testes of both Buthid and Scorpionid individuals irradiated contained a range of both meiotic and mitotic divisions, the general effects of irradiation appeared different in each. Buthid mitotic cells appeared resistant and were able to continue division despite the presence of numerous fragments while after irradiation mitotic divisions in *U. manicatus* were rare with those present appearing greatly damaged. Conversely, although metaphase I cells in *U. manicatus* showed varying degrees of damage they were of common occurrence while in the Buthid species metaphase I cells were rarely observed. If these observations truly reflect differential sensitivity to irradiation between the two groups, a number of both nuclear and physiological factors could be contributing to these differences.

#### Micronuclei.

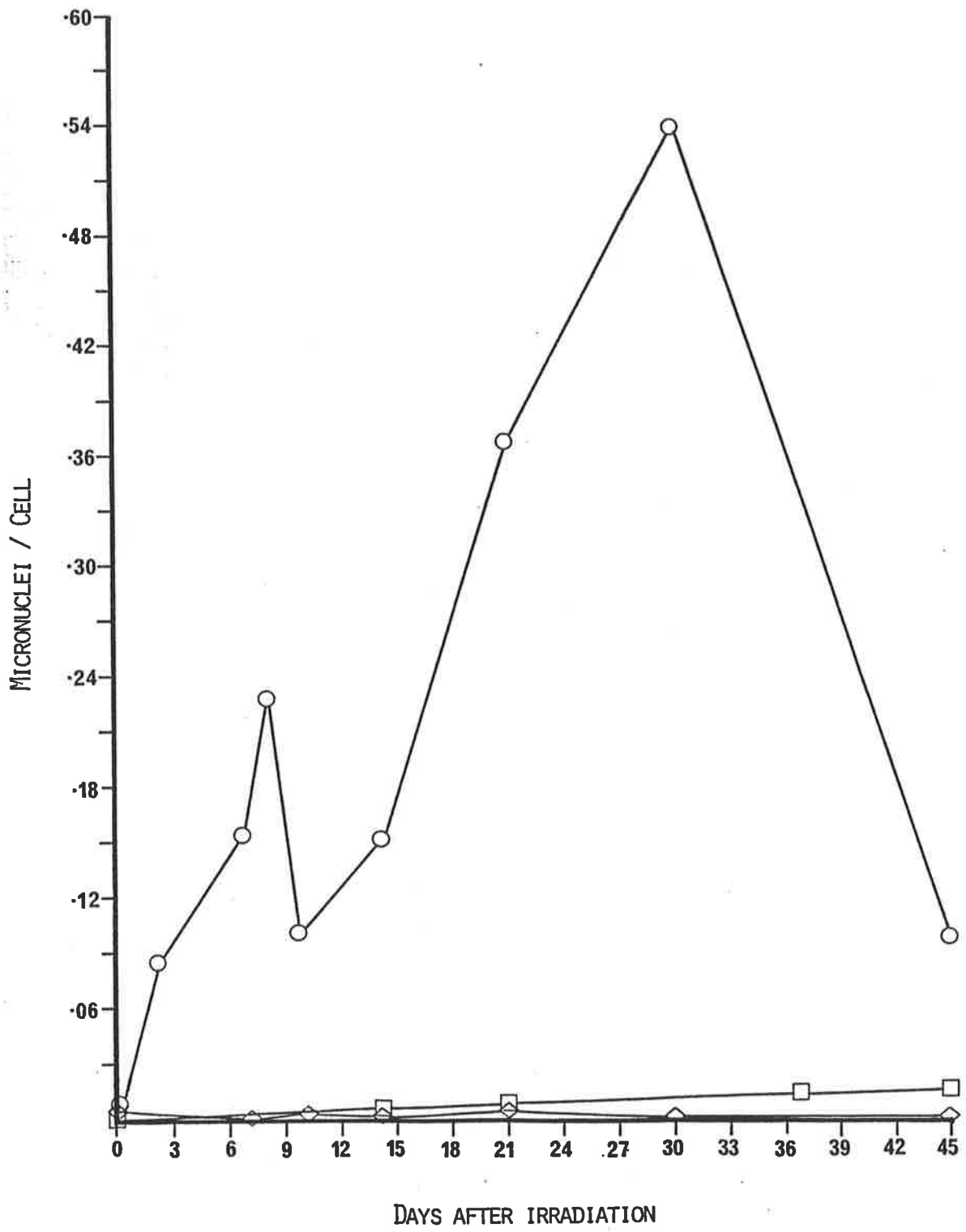
Figure 3.3.16 shows a comparison of the number of micronuclei per cell in the testes of Buthids and Scorpionids after irradiation. The data from the Buthids show no significant deviation from control values, while in *U. manicatus* micronuclei values are greatly elevated when compared with controls. As micronuclei are formed from



Figure 3.3.16

The average number of micronuclei per cell in  
Buthids and Scorpionids at various times after  
irradiation (1000r).

- ◇ *U. manicatus* FCM control.
  
- *U. manicatus* Pooled results from  
all individuals  
scored (RRM, FCM,  
EBM).
  
- *L. variatus* OCL A, B, C and  
OCL 1 female.  
Value at time zero  
is control value for  
OCL.



acentric chromosome fragments, and fragments were observed in both Buthids and Scorpionids, this difference between Buthids and Scorpionids may reflect a basic difference in chromosome structure.

#### Fragments.

As shown in Fig. 3.3.17, after an initial loss of fragments or cells with large numbers of fragments, in Buthids, those remaining were maintained for many days and presumably many divisions after irradiation. Scorpionids show a decrease in the number of fragments per cell as time after irradiation increases, for fragments scored at mitosis and metaphase I. However, the metaphase I results may not reflect a loss due to division but rather a differential sensitivity at the time of irradiation. Thus, although the frequencies between the two groups may not be directly comparable, the behaviour of fragments in Buthids is typical for a species with holocentric chromosomes.

Evans and Pond (1964) when comparing the effect of chronic irradiation on monocentric *Vicia* with holocentric *Luzula*, compared the ratio of micronuclei to fragments which theoretically should approximate one if each acentric fragment produced a single micronucleus. When this ratio is calculated for Buthids and Scorpionids, as shown in Table 3.3.11, the very high ratio observed in Buthids is similar to the ratios observed in *Luzula*, while the lower ratio for the Scorpionid species is similar to that of *Vicia*. This indicates that while in the Scorpionid most fragments result

Figure 3.3.17

The number of fragments per cell scored at various times after irradiation (1000r) for Buthid and Scorpionid individuals.

- ◊ *U. manicatus* FCM mitotic cells.
- ◆ *U. manicatus* FCM metaphase I cells.
- *U. manicatus* EBM metaphase I cells.
- ▲ *L. variatus* OCL mitotic cells.

A comparison of mitotic fragments shows that in *U. manicatus* there is a rapid loss of fragments, while in *L. variatus*, after an initial loss of fragments, the number remains stable for a long period after irradiation.

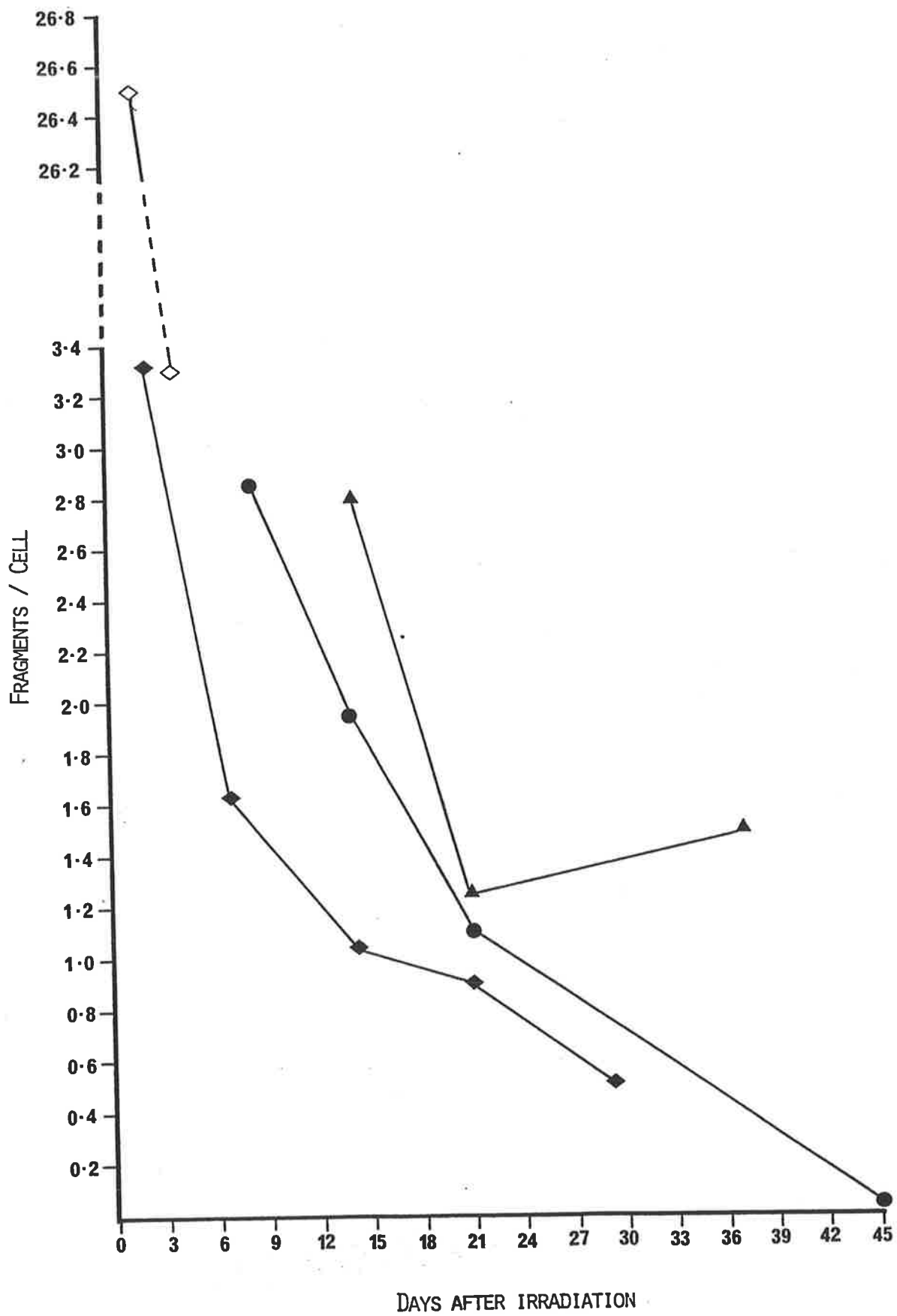


Table 3.3.11

Ratio of micronuclei to fragments in irradiated Buthid  
and Scorpionid individuals.

BUTHIDS		SCORPIONIDS	
Time after irradiation	MN : fragment	Time after irradiation	MN : fragment
14 days		2.25 days	1 : 39.18
OGL B	1 : 410		
OCL A	1 : 280	7 - 8 days	1 : 11.17
21 days			
OCL B	1 : 125	14 days	1 : 9.89
37 days			
OCL C	1 : 125	21 days	1 : 2.7
45 days			
OCL 1 ♀	1 : 10.8	29-31 days	1 : 0.94
		45 days	0.1 : 0

in micronuclei formation, in Buthids, fragments undergo normal division and do not form micronuclei. (The mixed nature of the cellular divisions in the testes probably accounts for the deviation of the earlier counts from 1:1 in *U. manicatus*.)

#### **Anaphase Bridges.**

Figure 3.3.18 shows that the frequency of anaphase bridges in Buthids was relatively low while in Scorpionids their frequency was highly elevated when compared to controls. This either reflects a difference between Buthids and Scorpionids in the frequency of irradiation induced rearrangements capable of forming bridges at anaphase or differences in the behaviour of similar rearrangements at anaphase, i.e. do Buthid chromosomes form dicentrics as a result of rearrangement?

#### **Rings and Translocations.**

Table 3.3.12 compares the numbers of ring chromosomes and other chromosome rearrangements (end to end fusion in Buthids and chromatid interchanges in the Scorpionid) for all mitotic cells scored.

A large number of mitotic cells could be scored in Buthids but the frequency of rings and translocations was low ( $F = 2\%$  for each). In the Scorpionid, although very few mitotic cells were observed, the frequency of rings was high in comparison ( $f(\text{rings}) = 40\%$ ,  $f(\text{chromatid interchanges}) = 18\%$ ). This could be due to the difficulty in scoring

**Figure 3.3.18**

**The effect of irradiation on the incidence of  
anaphase bridges in Buthids and Scorpionids.**

**□ Pooled results for all *U. manicatus*  
individuals.**

**■ Frequency of anaphase bridges in OCL  
individuals at 14, 21 and 37 days after  
irradiation.**

**Control cells from both Buthid and Scorpionid  
individuals showed no evidence of bridge formation.**



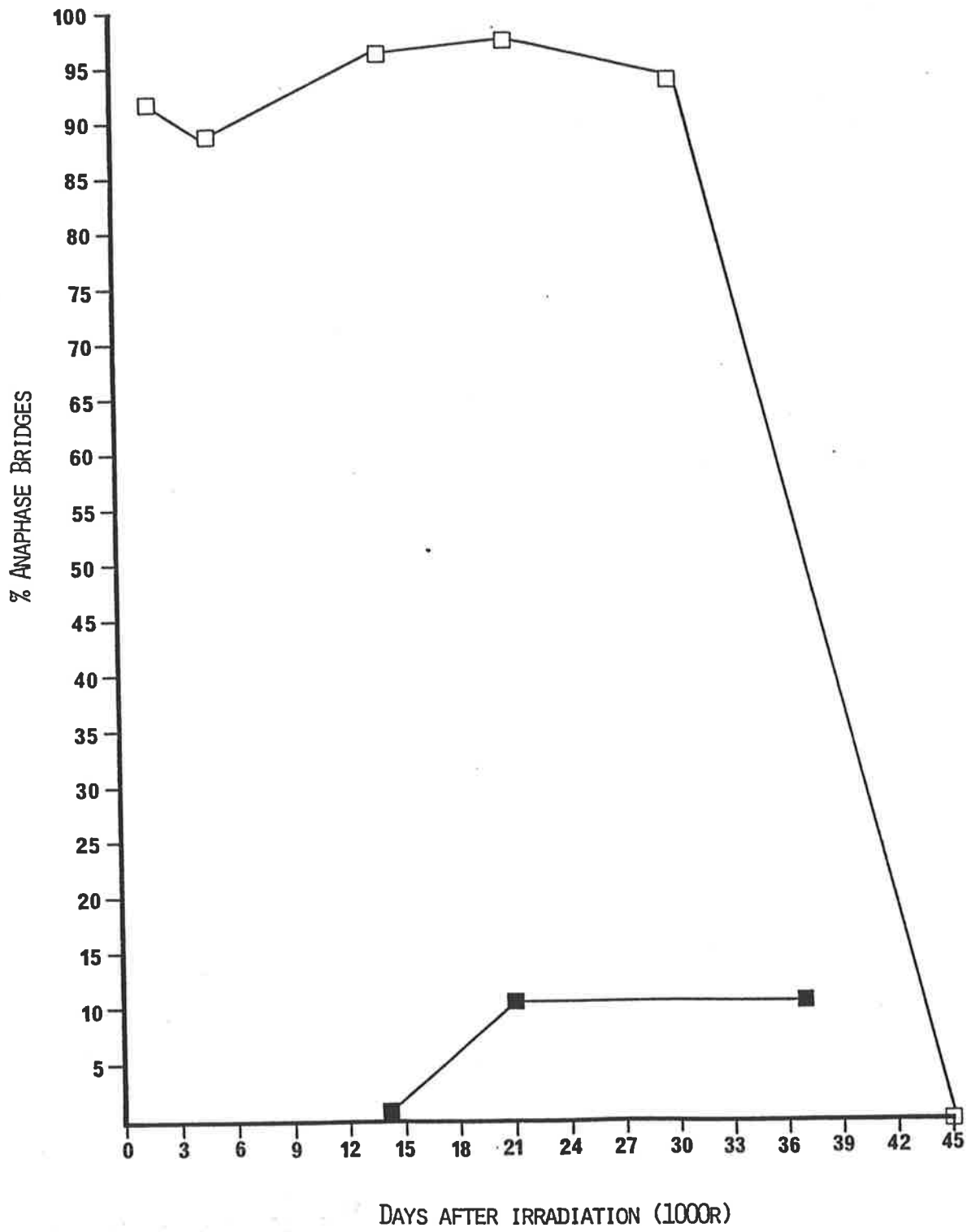


Table 3.3.12

Numbers and frequency of chromosome re-arrangements  
in Buthid and Scorpionid mitotic cells.

Time after irradiation	No. cells scored	No. ring chromosomes	F	No. other re-arrangements	F
7-10 days					
B	21	0	0.0	0	0.0
S	20	10	0.5	4	0.2
14 days					
B	85	1	0.0117	3	0.0365
S	0	0	0.0	0	0.0
21 days					
B	103	4	0.0388	1	0.0097
S	11	2	0.1818	2	0.1818
>30 days					
B	102	2	0.0196	3	0.294
S	1	1	1.0	0	0.0
Total					
B	369	8	0.0216	9	0.0244
S	32	13	0.406	6	0.1875

B = Buthids

S = Scorpionids

F = frequency

rearrangements in Buthids, lack of scorable cells in Scorpionids, or, alternatively, could reflect a difference in the propensity for the chromosomes of these two groups to rejoin after irradiation induced breakage. This difference could be attributed to physiological differences between the species or to differences in chromosome organization including centric nature.

#### SUMMARY

The results of the effects of irradiation on both Buthids and Scorpionids are presented in Table 3.3.13. The results are in accordance with the light microscope studies which indicated that Buthids possess holocentric chromosomes and the Scorpionid *U. manicatus* has monocentric chromosomes. The results from the single Scorpionid species could reasonably be used to extrapolate to other Scorpionid species, especially those of the genus *Urodacus*, as the studies in Chapter 1.4 show that all species in this genus show similar chromosome morphology and behaviour.

Table 3.3.13

Summary of all radiation effects on Buthid and Scorpionid chromosomes.

Radiation effect	Buthid	Scorpionid
Micronuclei	Absent	Present
Anaphase bridges	Low frequency	High frequency
Fragments	Persist	Decrease in frequency
Anaphase movement of fragments	Normal	Lagging
No. of chromosomes capable of division (maximum)	23 ( $\gg 2n$ )	60 (2n)
Rings, translocations	Rare	Common
Cell death	None observable	Few cells survived to 45 days
Conclusion	Radiation resistant → HOLOCENTRIC	Radiation sensitive → MONOCENTRIC

## SECTION 4

## GENERAL DISCUSSION

**X-ray Breakage of Holocentric and Monocentric Chromosomes.**

Excluding the ability for fragments to divide autonomously in holocentric organisms, X-ray breakage has revealed what is perhaps another basic difference between holocentric and monocentric chromosomes. This difference involves the ability or tendency of broken chromosome ends to rejoin. Breakage of holocentric chromosomes yields functional, heritable products (fragments) possessing what in monocentric chromosomes would be unstable 'sticky ends'. Apparently stable telomeres are formed *de novo*. Additionally, the frequency of chromosome rearrangements formed after X-irradiation of holocentric and monocentric chromosomes differ substantially (see Tables 3.1.2 and 3.3.12). The differences in rearrangement frequencies may be due to difficulties or bias in scoring aberrations or alternatively, it may reflect further the differences in properties of chromosome ends in holocentric and monocentric chromosomes. Physiological or structural differences (including centric nature) between the two chromosome types could determine these differences in end properties.

McClintock (1941) described a situation in monocentric maize where broken-ends healed spontaneously to form functional telomeres. However, 'healing' was found to occur in only one tissue (embryonic) suggesting that physiological

differences between cells affect whether healing can occur. Physiological differences may exist between the cells of species with holocentric and monocentric chromosomes such that spontaneous healing is a permanent feature of cells with holocentric chromosomes.

There is another situation in monocentric chromosomes where broken ends appear to spontaneously heal; that being, Robertsonian fission events. A metacentric chromosome can split at the centromere to produce two stable telocentric chromosomes. (These may generate short heterochromatic arms to become acrocentric.) Thus, breakage within a centromere results in the *de novo* production of telomeres.

Centromeres and telomeres are functionally related in some plants (i.e. neocentric activity). Recent molecular evidence suggests that centromeres and telomeres may share common DNA sequences. Fitzgerald-Hayes *et al.* (1982) found that both the CEN 3 and CEN 11 yeast centromere DNAs contained one region of dyad symmetry that could form a hairpin loop with theoretically significant stability; a hairpin loop being the proposed DNA structure for a telomere (Blackburn and Szostak, 1984). Young *et al.* (1983) using *in situ* hybridization found that a cloned *Drosophila* telomeric sequence also hybridized to a specific region of the pericentromeric heterochromatin. Although this evidence is preliminary it does suggest there is a sequence association between centromeres and telomeres. Thus, if chromosome breakage occurs at a centromere, sequences related to telomeric sequences present at the centromere may be able to

function as stable telomeres. In holocentric chromosomes, if centromeric sequences are distributed along the entire length of the chromosome, many breaks have the potential to be stabilized by centromere associated telomeric sequences. This would account for the lower frequency of chromosome aberrations due to rejoining of broken ends. Variation between holocentric species could be accounted for by differences in the number of centromeric sequences per chromosome. However, it would be simplistic to ignore physiological and other differences in chromosome organisation as determining the fate of chromosome breaks in both monocentric and holocentric systems.

#### Centromere Evolution.

Holocentric chromosomes appear to have evolved independently in a number of groups. The evolution of holocentric chromosomes has not been clearly explained in any group. Scorpions appear to be the only animal group where one family (Buthidae) has evolved holocentric chromosomes while all other families have monocentric chromosomes. However, an analysis of the centromeres of Australian Buthid and Scorpionid species has not revealed any new information as to how holocentric chromosomes have evolved. Nevertheless, it has revealed that in some groups the centric system has a certain plasticity.

Centromeres can no longer be considered as immutable or invariable as previously thought. Even within the monocentric species *U. manicatus*, centromeres have been

gained or lost due to chromosome change resulting only in inter-populational chromosome polymorphism. The Chinese muntjac and the Indian muntjac have vastly different chromosome numbers due to centric fusions that have resulted in the large compound kinetochores of the Indian muntjac. However this fusion process has not led to holocentric chromosomes. Even amongst holocentric species variation exists in the level of expression of kinetochore plates to the extent that mitotic and meiotic chromosomes differ in some species.

Greater characterisation of the kinetochore and the DNA sequences that are responsible for its organisation at both the chromosomal and molecular level are required before centromere evolution can be clearly understood. The isolation using CREST sera of mammalian kinetochore proteins has provided a new base from which further research can extend.



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