UNIVERSITY OF LONDON

CHROMOSOMAL INVESTIGATIONS ON SOME BRITISH WILD RODENTS.

A thesis submitted for the degree of
Doctor of Philosophy

by

NAGWA HASSAN ALI

Royal Holloway College

October 1984

ABSTRACT

The chromosomes of the two species of field mouse occurring in Britain, A. sylvaticus (L.) and A. flavicollis (Melchior) have not been widely studied. An investigation was therefore initiated to analyze the chromosomes of these species from different localities on the mainland of Britain.

The chromosome characteristics studied at mitotic metaphase include the relative length of the chromosomes, G-banding, the presence and pattern of heterochromatic regions in the chromosomes (C-banding) and the position of the nucleolus organizers.

In meiosis the type of association between the x and y and the mode of their segregation has been discussed.

Species comparisons based on these chromosomal data showed a chromosomal difference between A. sylvaticus and A. flavicollis which support the separate identities of these two species.

Intraspecific variation between different populations of A. sylvaticus has been reported. Chromosomal polymorphism due to B chromosomes has been detected in all the populations studied. Four classes of B chromosome have been identified in nine populations of A. sylvaticus from the mainland of Britain. They are distinguished by shape and size and according to their euchromatic or heterochromatic composition. Inter- and intra-individual variation (mosaicism) as well as interpopulational variation of the number and the nature of B chromosomes has been studied.

Polymorphism resulting by a deletion, that is the loss of chromosomes or parts of chromosomes, has been found in the X chromosome of two females from different populations; one of them had XO and the other Xx.

Intraspecific karyotype homology, as judged by G- and C-banding has been demonstrated between populations of the bank vole Clethrionomys glareolus (Schreber) from different localities on the British mainland and from Skomer Island.

In conclusion, the results of this investigation clearly indicate that studying karyotype evolution and variation between species, G- and C-banding and nucleolar organizer regions (NORs) are essential tools without which erroneous conclusions may be reached.

* * * * *

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. R. Angus for his diligent effort in programming the work, his direct and serious supervision and for his instructive discussion, critical reading of the manuscript and presentation of this thesis.

I am grateful to Professor C.T. Lewis, Head of the Department, for the use of the facilities and technical staff of his department.

Thanks also due to Professor R.J. Berry and his student P. Brooker for valuable advice with the technique, Mr. T.D. Healing for collecting the Skomer fieldmice and voles, and to the Nature Conservancy Council for permission to use Skomer mice. To Mr. A. Wroot for his advice on statistical analysis, Mrs. S. Wroot for drawing Fig. 1 and to Mr. Z. Podhorodecki for photographic assistance.

I would like to thank all the staff members and technicians at the Zoology Department, Royal Holloway College (University of London) for excellent working facilities.

I am indebted to the Egyptian Government for financial support. Also the support of an ORS award is gratefully acknowledged.

* * * * *

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GENERAL INTRODUCTION

The present study is an investigation of chromosomal variation within and between members of two rodent genera inhabiting the mainland of Britain, Apodemus Kaup and Clethrionomys Tilesius.

The genus Apodemus contains the dominant mice of the Palaearctic Region, with about ten species, only two of which occur in Britain: the wood mouse, A. sylvaticus (L.) and the yellow-necked mouse, A. flavicollis (Melchior). A. sylvaticus is the common field-mouse of Britain and is found everywhere. It is also commonly found in Central Europe (Brohmer, 1929; Heinrich, 1929). The yellow-necked mouse is not so abundant and is associated more with deciduous wood, copses and hedgerows, living and competing with wood mice quite successfully (Warn er, 1978). In Britain the two species are readily distinguished - the yellow collar in A. flavicollis appears to be consistently well developed and allows easy separation from the wood mouse (and other mice). The upper parts are a rather richer reddish brown than in the wood mouse and the tail is usually longer than the head and body (Saint-Girons, 1957). However, in parts of continental Europe the two species are so similar that they have been suspected of interbreeding, although this has not been proved. On the continent of Europe, Zimmermann (1957) and Saint-Girons (1962) have failed to get these species to interbreed, as have Jewell and Fullagar (1965) and Niethammer (1969) in Britain.

The genus Clethrionomys contains about five species in Eurasia and North America, in woodland and tundra habitats. The only species in Britain, the bank vole Clethrionomys glareolus (Schreber) is similar to the common North American species, Clethrionomys gapperi Vigors, and may

perhaps be conspecific, as is suggested by cytological evidence (Matthey, 1956). Miller (1900 & 1912) separated the Clethrionomys of England from those of the adjacent parts of Europe on the basis of colour and size, his Clethrionomys glareolus britannicus being characterized by less intensive colour and smaller size. On the Islands off the west coast of Britain (Jersey, Skomer, Mull and Raasay) there are isolated populations of the bank vole. Many years ago they were described as separate species of Evotomys, namely E. caesarius Miller (1908) from Jersey, Channel Islands; E.skomerensis Barrett-Hamilton (1903)from Skomer, Wales; E. alstoni Barrett-Hamilton and Hinton (1913 a) from Mull, Inner Hebrides; and E. erica Barrett-Hamilton and Hinton (1913 b) from Raasay, Inner Hebrides. Recent authors (Steven, 1955; Godfrey, 1959; Corbet,1964) have considered them to be subspecies.

A diploid number of 48 chromosomes has been shown to exist in both A. sylvaticus and A. flavicollis by Matthey (1936 a and b), and Raynaud (1936), and also in field mice from the outer Hebrides by Koller (1941). All the chromosomes are acrocentric or telocentric and the karyotypes of the two species are indistinguishable (Kral, 1970). Clethrionomys glareolus species and subspecies have 2n = 56 (Renaud, 1938), comprising 26 pairs of acrocentrics, one pair of small metacentrics, a large acrocentric x chromosome and a small nearly metacentric y chromosome. Kral (1971) reported a distinctly acrocentric to feebly subtelocentric y chromosome in C.g. garganicus Hagen from Southern Italy.

A number of techniques has been developed that produce banding patterns on mitotic chromosomes and greatly facilitate the identification of individual chromosomes as well as the different types of chromatin.

Chromosome bands were first discovered when Cassperson and his collaborators (1969 a & b) found that certain fluorochromes (e.g. quinacrine mustard) produce characteristically bright and dark bands (Q or G-bands) when applied to cytological preparations and observed under ultra violet light. Many reports have been published and knowledge continues to accumulate about the mechanism of these bands and whether the banding procedures are inducing the G-bands or simply enhancing bands that are already present.

There has been a great deal of confusion in the literature regarding the conditions by which G-banding is elicited. Various pretreatments of the chromosomes have been recommended to show different bands. Sumner et al. (1971) found that the reactions of human chromosomes to Giemsa stain depend upon the severity of the treatment to which they are subjected before staining. Treatment with strong alkali produces C-bands at the centromeres which indicates the presence of highly repetitive DNA in this region. However, mild treatment and incubation in 2x SSC, generates a pattern of bands along the arms of each chromosome with little or no reaction at the centromere (G-banding). Other pretreatments have been recommended to show G-bands including proteolytic enzymes (Seabright, 1971; Wang et al., 1972), urea (Kato and Yosida, 1972; Shiraishi and Yosida, 1972), detergent (Lee et al., 1973). Since the above mentioned treatments can be used to extract proteins from chromatin and since untreated chromosomes bind Giemsa intensely in all regions, Lewin (1974) reported that G-banding is caused by the removal of proteins from those regions of the chromosome which do not bind the stain (interbands) and he added that the patterns produced by G- and Q-banding are the result of the state of DNA in its local interaction with chromosomal protein. Similar results are obtained using

electron microscopy. Burkholder (1974 and 1975) has shown that unstained, methanol acetic acid-fixed chromosomes are uniformly electron-dense and show no banding pattern. However, after treatment with trypsin, as in trypsin banding method, electron-dense bands and less dense interbands can be seen. The same result is obtained with the scanning electron-microscopy (Bath, 1976).

Yunis and Sanchez (1973) have reported that such pretreatment is not essential for the visualization of G-bands and they succeeded in obtaining G-banded preparations by exposing the slides to very dilute solutions of the stain (1/80 or 1/100 dilution of Giemsa stain) for prolonged periods. This has been confirmed by the fact that G-bands can be observed by either contrast or U.V. microscopy (McKay, 1973) and electron microscopy (Bahr et al., 1973) without any post fixation treatment or staining.

There is a school of thought that argues that the mechanism of G-banding is an enhancement of the basic chromomere pattern. Okada and Comings (1974), working with Chinese hamsters, proved that the chromosomal band patterns of pachytene chromosomes in meiosis are the same as the Q and G-bands of mitotic chromosomes. Similar studies by Hungerford et al. (1971 a & b); Ferguson-Smith and Page (1973); Luciani et al. (1975) suggested such a possibility for human chromosomes. The equivalence of chromomeres and G-banding patterns indicates that the latter correspond to the content and arrangement of nucleoprotein fibres in the native state (Luciani et al., 1975). This interpretation supports the hypothesis formulated by Yunis and Sanchez (1973) and McKay (1973) which interprets mitotic chromosomal G-banding as the result of differen-

tial chromatin condensation.

Restriction enzymes break DNA at specific sequences of base pairs, and some of these enzymes produce bands similar to those obtained by usual G-banding methods. Production of banding patterns varies with different restriction enzymes (Jones, 1977), but indicates that, in some cases at least, there is a difference between the DNA of band and interband regions. The bands may possess sequences of DNA which are different from those of the non-banding regions (Lewin, 1974). Yunis and Sanchez (1973) reported that the difference in the two regions may result from differences in the type of DNA (repetitive and non repetitive) and/or proteins (basic or acidic) resulting in differences in the concentration of nucleoproteins along the chromatid.

In the field of mammalian cytogenetics, the study of chromosome banding is very useful, permitting the identification of sections of individual chromosomes and is thus helpful in attempts to identify homologous chromosomes in different species (Pearson, 1977) and for the study of chromosome polymorphism. Two different types of chromosomal polymorphism have been encountered in the present study: the presence of supernumerary chromosomes (B chromosomes) and deletions of either whole or part of the x chromosome in the females.

B chromosomes are chromosomes additional to the normal karyotype and not homologous with members of the regular set (A chromosomes). They represent one of the many causes of variation in chromosome number which exist in higher plants and animals.

Concerning the nomenclature, different names have been used in the literature. B's were first described in the insects as supernumeraries (Wilson, 1907 ; Stevens, 1908). Lutz (1916) found supernumeraries in the evening primrose Oenothera lamarchiana, which she called diminutive chromosomes. Cleland (1951) called them extra diminutive chromosomes. It was Randolph (1928) who called them B's, to distinguish them from the normal A chromosomes of the complement. Melander (1950) has used the term accessory chromosomes. The terms supernumerary chromosomes and B chromosomes are in current use. The term B chromosomes (B's) has been used extensively in this account.

A number of characters that sharply differentiate B chromosomes from the basic chromosome complement as well as from other types of chromosomal polymorphism have been reported by Battaglia (1964), Jones (1975), Jones and Rees (1982). The more important characters are:

1. B chromosomes are dispensable and non homologous with A chromosomes.

2. They may be present or absent within individuals of a species. In case of their presence they may exhibit numerical variability between cells, tissues, individuals or populations due to abnormal behaviour in mitosis and meiosis.

3. They display non-Mendelian inheritance. They are unstable during somatic cell division and meiosis. Their maintenance in a population depends on equilibrium between forces of elimination and accumulation.

4. They depress fertility and reduce growth, when present in high numbers.

5. They carry no genes with major effects, so their presence or absence does not affect the appearance of the individual carrying them.

B chromosomes have been reported in various species of plants, (Darlington and Wylie, 1955; Federov, 1969) and animals, particularly insects (Melander, 1950; Makino, 1951a; White, 1973). Battaglia (1964), Jones (1975) and Jones & Rees (1982) reported B chromosomes in both animals and plants. Two hundred and sixty animal species containing B's have been reported by Jones and Rees (1982). Eighty-one per cent of this number are insects and most of them are from the orders Coleoptera, Diptera and Orthoptera. The occurrence of these supernumeraries is apparently rather rare in mammals. Among about 1,300 karyologically studied mammalian species, only 14 have been found to have B chromosomes (Volobuev, 1980b). More recent data report nineteen species (Jones and Rees, 1982). At least it has been found in a number of species from various mammalian groups: Marsupials - Schoinobates volans (Kerr) (Hayman and Martin, 1965b) and Echymipera kalabu ((Peramelidae) (Hayman et al., 1969); Rodents - Reithrodontomys megalotis Baird (Blanks & Shellhammer 1968; Shellhammer, 1969), Perognathus baileyi Merriam (Patton, 1972), Uromys caudimaculatus Krefft. (Baverstock et al., 1976), three species of the genus Rattus (Fischer) from Australia (Baverstock et al., 1977), three subspecies of Rattus rattus, (L.)(Gropp et al., 1970; Satya Prakash and Aswathanarayana, 1972; Yong & Dhaliwal, 1972; Raman and Sharma

1974; Yosida, 1975 & 1977), Apodemus (Hayata et al., 1970; Kral, 1971; Bekasova and Vorontsov, 1974 & 1975) and in carnivores, Vulpes fulva (L.) (Gustavsson and Sundt, 1967; Buckton and Cunningham, 1971; Renzoni and Omodeo, 1972; Low and Benirschke, 1972; Belyaev et al., 1974b).

Polymorphism of supernumerary chromosomes has been reported in the Asiatic forest mice of the genus Apodemus. The systematics and nomenclature of this group are very confused. Hayata et al., (1970); Hayata (1973) have described a wide individual variation of chromosome number, ranging from 48 to 59 in mice of this genus under the name Apodemus giliacus (Thomas); Kral (1971) described it under the name Apodemus speciosus (Temminck); Bekasova et al. (1980)have reported supernumerary chromosomes in A. peninsulae (Thomas) and they established that in all cases polymorphism of B chromosomes in the genus Apodemus is characteristic of only A. peninsulae.

The effect of B chromosomes upon the growth and development of the external phenotype are slight and difficult to detect. Some exceptions have been reported especially in plants. In Haplopappus gracilis A. Gray Jackson (1960) noticed that the normally brownish-red colour of the achenes is changed in the presence of B chromosomes to dark purple. El-Nahas (1983) has reported that the presence of low numbers of euchromatic B's in Allium cernum Roth produces brown spots and split edges on the scape while the presence of a high numbersof metacentric heterochromatic B's gave the scape pinkish colour rather than green. Also she noticed a delay in the flower formation in plants with B's. Conversely, leek plants Allium porrum Linnaeus have been found to fruit earlier in the presence

of B's (Vosa, 1966).

In animals there is less information about the effect of B's on the external phenotype of the individuals. White (1973), Jones (1975) have reported that the presence of low numbers of B's is not serious and may even be beneficial for some characters and confirm an adaptive advantage on the individual, but that higher numbers of them are deleterious. In the fresh water turbellarian worm Polycelis tenuis Ijima B's delay the hatching of eggs, they increase susceptibility to disease and reduce vigour (Melander, 1950). B's cause a reduction in the size of larvae of the black flies Crephia dacotensis (Dyar and Shannon) and Crephia ornithophilia Davies, Peterson and Wood (Procunier, 1975). Nur (1966 and 1969) reported that B's have little or negligible effect on either the development or the fertility of the female mealy bug Pseudococcus obscurus Effig. Bigger (1976)noticed no phenotypic differences between individuals of two species of butterfly with and without B's. The data on the effect of B chromosomes on the mammalian phenotype are scanty. The only attempt to reveal a correlation between B's number and body and cranial measurements of Californian "harvest mouse" Reithrodontomys megalotis has not yielded a pos itive result (Shellhammer, 1969).

B chromosomes play a role in the adaptation of the animals to the conditions of the environment. Hewitt and John (1967, 1970) found the frequency of B chromosomes in populations of Myrmeleotettix maculatus (Thunb.) (Orthoptera) was higher in warm dry places and that they gradually disappear when it spreads into more extreme conditions. The adaptive role of B chromosomes has also been reported in mammals.

In Reithrodontomys megalotis, it was found that there was a greater variation in chromosome numbers in the geographically peripheral areas (Shellhammer, 1969). Belyaev et al. (1974 a) came to the conclusion that groups of silver foxes (Alopex) (L.) selected for behaviour (domestication and lesser degree of agressiveness) exhibit significant differences in the nature of the variation of the supernumeraries in comparison with the groups of unselected animals.

At the level of the endophenotype, important results have been obtained concerning the effects of B's on A chromosome behaviour at meiosis. Barker (1960) was the first to discover the effects of B's on A chromosome chiasma frequency in the grasshopper Myrmeleottix maculatus. The presence of B's raises the mean chiasma frequency in certain plant species e.g. in maize (Zea mays) (Ayonoadu and Rees, 1968), and in wild populations of rye (Zecevic and Paunović, 1969) while in Najas marina Linnaeus (Viinikka, 1973) they decrease the mean chiasma frequency.

The behaviour of B chromosomes in meiosis of different mammalian species has not been widely studied. Shellhammer (1969) reported that the B's in Reithrodontomys megalotis did not associate with one another during meiosis. Also Raman and Sharma (1974) failed to detect pairing between B chromosomes of the black rat. In Crocidura suaveolens Pallas and in the Arctic lemming Dicrostonyx torquatus torquatus Pallas both univalents and bivalents are found (Meylan & Hausser, 1974; Gileva, 1973). Besides univalents and bivalents, Hayata (1973) has reported an association of non homologous B's differing in size and morphology in Apodemus peninsulae. In silver foxes B's may stay as univalents, but

bi- and tri-valents are also found and they may associate with bivalents of the basic set (Gustavsson, 1970; Radzhably et al., 1978).

There is no doubt that B chromosomes exist in many species from which they have not yet been recorded, for example, in species not yet investigated cytologically and, as well, in species where the number of individuals or populations investigated is smaller because in certain cases B's are confined to a few individuals in only some populations within a species (White, 1951). B's are sometimes restricted to certain tissue, or even to a few cells so that they can be easily overlooked.

Although Koller (1941) studied the karyotype of the British field mouse Apodemus sylvaticus from the Outer Hebrides and from the mainland of Britain, as did Berry and Parrington, unpublished (cited in Berry, 1970) from Edinburgh, Rhum, St. Kilda and Fair Isle, there is no record of B chromosomes in this species.

The present work has revealed the presence of B chromosomes in the British field mouse from a number of different populations.

Polymorphism caused by deletions has been found in a number of species, but only involving the x chromosome. Many xo human females have been reported since the first discovery by Ford et al., (1959) and Fraccaro et al., (1959). Similar sex chromosome variation has also been found in other mammals. In the laboratory mouse Mus musculus L. (2n : 40) animals with 39 chromosome and an xo complement were described (Russel et al., 1959; Welshons and Russell, 1959); these animals were phenotypically normal, fertile females.

Among wild populations, Bianchi and Contreras (1967) reported the occurrence of an individual with Xx/XO chromosome mosaic and another with an XO sex chromosome complement among a small sample of the Argentinian field mouse, Akodon azarae (Fisher). Sharma and Raman (1971) reported an XO female in the Indian mole rat, Bandicota bengalensis bengalensis (Gray) while Gadi, Sharma and Raman (1982) reported an individual with XX/XO mosaicism in the great bandicoot-rat, Bandicota indica nemorivaga (Bechstein). The present work reports the occurrence of Xx and XO females in the field mouse Apodemus sylvaticus.

Little work on cytologically detectable genetic variation has been undertaken on British mice. Koller (1941) reported a difference between Hebridean and mainland mice in respect of the y chromosome, this being at least twice as large in A. sylvaticus as in A.s. hebridensis. He also found differences in the course of segregation of the sex chromosomes during meiosis. Berry and Parrington, unpublished, (cited in Berry, 1970) have been unable to detect any differences in karyotypes from mitosis of A. sylvaticus from Edinburgh, Rhum, St. Kilda and Fair Isle.

From the foregoing it can be seen that very little work has been done on the chromosomes of British mice and voles. The present investigation was undertaken in an attempt to produce a more detailed study of the chromosomal differences between British field mouse and vole species and subspecies, and of intraspecific variation. Particular attention has been paid to the study of the morphology and nature of the B chromosomes in mitosis and meiosis as well as their frequency in different populations of the field mouse A. sylvaticus.

CHAPTER 2

MATERIALS & METHODS

I Materials:

The materials used in the present investigation consisted of a total of 294 animals including 203 Apodemus sylvaticus, 42 Apodemus flavicollis and 49 Clethrionomys glareolus.

During the period July to November, 1982 and July to early November 1983, animals were live-trapped from nine different localities on the mainland of Britain. In addition, 5 skomer field mice and 10 skomer voles have been investigated. The numbers and sexes of individuals from each locality are given in table 1. The locations of the collecting sites are shown in Fig 1.

II Methods:

1. Culture of lymphocytes from peripheral blood

Good quality chromosome preparations were made from peripheral blood lymphocytes cultured in vitro. An adaption of the method of Triman, Davisson and Roderick (1975) was used, with concanavalin A as the mitrogen, as recommended by Brooker (1982).

Solutions required:

Antibiotics: Penicillin - streptomycin solution containing 5,000 units of penecillin and 5,000 µg of streptomycin per millilitre, available from Grand Island Biological Company (GIBCO, Cat. No. 240).

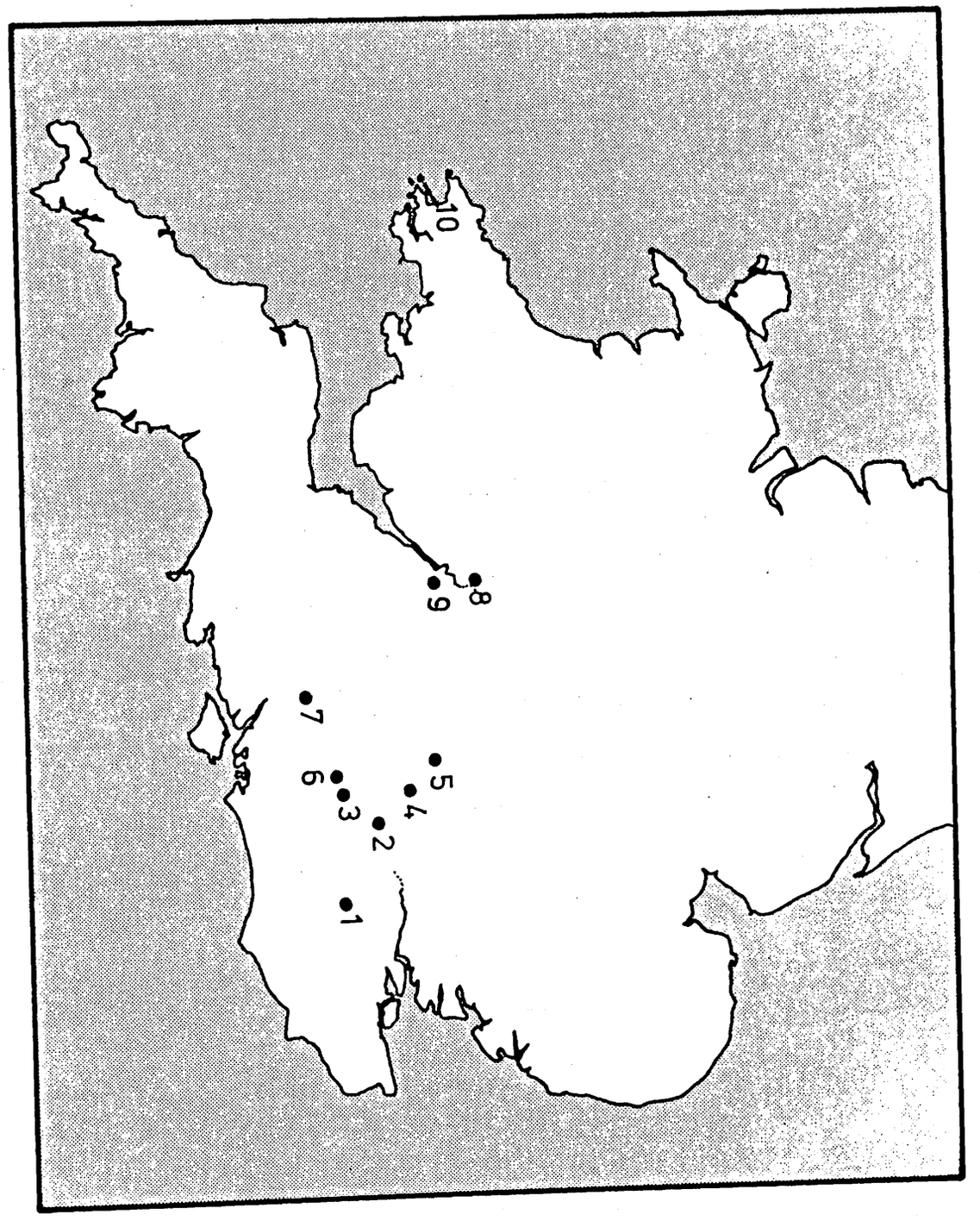
Heparin solution (5,000 units/ml) available from Flow Laboratories.

Concanavalin A stock solution: 20 mg concanavalin A are dissolved in

Table 1: Numbers and sexes of individuals from the different localities sampled.

Locality	A. sylvaticus		A. flavicollis		Clethrionomys glareolus	
	♂	♀	♂	♀	♂	♀
1. East Grinstead (Kent)	4	4	-	4	-	-
2. Englefield Green Surrey (Alderhurst)	19	24	4	1	4	6
3. Tilford (Surrey)	22	20	3	2	2	1
4. Marlow (Bucks)	8	5	1	2	1	1
5. Aston Rowant (Oxon)	5	5	-	-	3	2
6. Alton (Hants)	8	13	4	2	4	3
7. Stockbridge (Hants)	11	8	6	3	5	3
8. Tirley (Gloucestershire)	15	14	2	1	1	3
9. Woodchester Pk. (Gloucestershire)	8	5	3	4	-	-
10. Skomer Island	4	1	-	-	5	5
T O T A L	104	99	23	19	25	24
	203		42		49	

Fig. 1: Location of the collecting sites. (For explanation of numbers, see Table 1).



100ml in the culture medium RPMI 1640 without L. Glutamine (i.e. 200 ug/ml) and stored at -20°C in 5 ml aliquots to avoid frequent thawing and refreezing.

Incomplete medium: 30ml 1 X RPMI 1640 (with sodium carbonate, without L. Glutamine, available from Flow Laboratories) supplemented with 1.5ml penecillin-streptomycin and 0.25ml heparin.

Medium for microculture (complete medium): 20ml of RPMI 1640 (1X) with 25m M Hepes buffer and L. Glutamine available from GIBCO (cat. No. 041 2400) supplemented as follows: 6ml of foetal calf serum (GIBCO Cat.No. 013 6290), 0.25 ml penecillin-streptomycin solution, 0.25ml heparin solution and 1.1ml Concanavalin A stock solution.

Collection of blood

Blood was collected through orbital plexus puncture, using 1 x 75mm heparinized micro-haematocrit tubes. Three tubes of blood (0.2ml) can be collected from each animal without losing the animal.

Procedure for culturing

1. Drop the blood as soon as it is collected, into 5ml of incomplete medium in a bijou. Screw the bijou cap down tightly and centrifuge at 2000 r.p.m. (450g) for 10 minutes.

2. Pipette off the supernatant to get rid of the mouse serum and resuspend the cells in a further 5ml incomplete medium.

3. Recentrifuge as before, remove the supernatant and add 4-5 ml of complete medium. Mix the cells gently with a sterile Pasteur pipette, cap tightly and incubate in a tilted position at 37°C for 48-72 hours.

In the case of 72 hour incubation, the culture medium is exchanged for an equivalent amount of fresh medium after 48 hours. Following this change, the culture is continued as before for another 24 hours. Arakaki and Sparkes (1963) and Triman et al. (1975) also recommended replenishment of the medium during the culture period but at different times.

Treatment with colchicine:

At the end of the incubation period (48 or 72) add 0.1ml colcemid solution (20ug/ml) to each culture using a syringe and hypodermic needle and mix gently and thoroughly with Pasteur pipette. Incubate at 37°C for an additional 1.5 - 2 hours to allow accumulation of metaphases.

Harvesting of cells:

Divide each culture into two 5ml centrifuge tubes and centrifuge at 1500 r.p.m. (250g) for 5 minutes. Remove supernatant and replace with 0.56% KCl for 10 minutes. Centrifuge again, remove supernatant completely and gently run 3:1 absolute alcohol: acetic acid fixative very slowly down the inside of the tube to avoid clumping of the lysed red blood cells. Fix as a pellet for 2 minutes in 1ml of the fixative, then flick pellet up into the fixative, mix hard and add more fixative.

Centrifuge and resuspend twice more in fresh fixative.

Preparations are made by the air-drying method of Rothfels and Siminovitch(1958)on slides previously cleaned in acid alcohol using a micro-pipette for spreading the cell suspension.

This method has only occasionally been successful, but when it does work it consistently gives numerous clear and well-spread figures of metaphases. Macario (1966) and Buckton and Nettesheim (1968) pointed out that the occasional successes seem to have been limited to only certain strains of mice; the latter reported that the response to the mitogenic agent within some strains seemed to be poor and the cultures seemed to degenerate during the second day in culture, that is, one day before the maximal blastogenic response occurs. In the present investigation, some of the animals showed different degrees of success while others showed complete degeneration of the cells in the culture. The frequency of success in different populations is given in table 2. Unlike those of A. sylvaticus, cultures of A. flavicollis peripheral blood have been totally unsuccessful.

2. Bone marrow preparations

For preparing mitotic chromosomesfrombone marrow, the procedure of Hsu and Patton (1969) was used. Animals were injected intraperitoneally with 0.5ml of 0.04% colchicine as reported by Bianchi and Contreras (1967). Two to three hours after injection the animals were killed either by cervical dislocation or anaesthesia. The method of preparation is as follows:

Table 2: Frequency of success in culturing peripheral blood

Locality	♂♂	Number succeeded	Number failed	% succeeded	♀♀	Number succeeded	Number failed	% succeeded
Englefield Green	18	18	-	100	16	5	11	31.2
Tilford	16	11	5	68.8	12	4	8	33.3
Marlow	8	4	4	50	5	2	3	40
Aston Rowant	5	2	3	40	5	1	4	20
Alton	7	3	4	42	8	4	4	50
Stockbridge	9	3	6	33.5	8	2	6	25
Tirley	8	8	-	100	6	-	6	0
TOTAL	71	49	22	69	60	18	42	30

1. Flush out the marrow plug from the two femora with a small syringe (5ml capacity) equipped with a fine needle and loaded with a hypotonic solution (1%) of sodium citrate. Leave for 5 minutes in the citrate solution.

2. Place the bone marrow solution in 5ml centrifuge tube and spin at 1500 r.p.m. (250g) for 5 minutes.

3. Remove the supernatant and replace with 0.56% Kcl for 10 minutes then complete as in the previous method.

3. Meiotic chromosome preparation:

The procedure of Clendenin (1969) was used. Remove the testis from colchicine injected animals and place it in isotonic sodium citrate solution (2.2%) in a small petri dish. Open the tunica and swirl for 3 - 5 seconds. Cut the testis into 3 portions, swirl again, drain and transfer to isotonic KCl (1.125%) in a second small petri dish. Under a dissecting microscope mince as finely as possible, then tease out the tubular contents with fine forceps and a dissecting needle. When the tubules appear straight and opaque, allow them to settle momentarily. Transfer the cloudy overlying fluid to a centrifuge tube and spin at 1500 r.p.m. for 5 minutes. Pipette off and discard the supernatant. Add 5ml of 0.56% KCl (half isotonic) to the centrifuge tube. Disperse the cells thoroughly by gentle pipetting, let stand for 15 minutes then complete as before.

4. Orcein staining

For immediate examination of the slides, aceto-lactic orcein stain

was used. The staining solution was prepared by dissolving 2gm of synthetic orcein in 50ml of hot glacial acetic acid, adding 50ml of 85% lactic acid and filtering. The staining solution was pipetted on to a freshly prepared slide, covered with a cover glass and left for 20 to 30 minutes at room temperature. The Cover slip was washed off with absolute alcohol, the preparation soaked for 5 minutes in absolute alcohol, then mounted in Euparal.

5. Giemsa banding techniques

(a) G-banding

Various procedures have been reported for G-banding mammalian chromosomes, but treatment with SSC followed by trypsin appears to be the most useful one. Incubate 7-day-old slides prepared by the air drying method as mentioned previously in preheated 2 X SSC (0.3 M sodium chloride and 0.03 M trisodium citrate) at 60°C for $1\frac{1}{2}$ hours. Rinse in distilled water buffered at PH 6.8 with Sörensen, then treat with 0.01 % solution of trypsin (Difco , 1 : 250) in 0.75% NaCl buffered to PH 7.6 with Sörensen for 15 seconds at 10°C. Rinse the slides three times in distilled water buffered at PH 6.8 with Sörensen, and stain for about 15 minutes in 2% Giemsa at PH 6.8.

(b) C. Banding

The procedure for C-banding is that of Sumner (1972) with slight modifications in the time and temperature of the Barium hydroxide treatment. Seven-day-old slides were treated with 0.2N hydrochloric acid for one hour at room temperature (about 20°C), rinsed with

distilled water, and placed in a freshly prepared saturated aqueous solution of barium hydroxide for 10 minutes at 25°C. After thorough rinsing in several changes of distilled water, the slides were incubated for 1 hour at 60°C in 2 X SSC (0.3 M sodium chloride and 0.03 M trisodium citrate) then rinsed three times with distilled water and stained for 1 - 1½ hours in 2% Giemsa at pH 6.8. The barium hydroxide treatment is the most critical step in this technique. Slides more than 10-days-old need more time in barium hydroxide at higher temperatures (30-35°C).

6. Staining the nucleolus organiser

The nucleolus organiser has been silver stained using the method of Goodpasture and Bloom (1975). Three solutions were prepared (1) A pretreatment (A g) solution of 50% aqueous silver nitrate (2) an ammonical silver (AS)solution prepared by dissolving 4gm silver nitrate in a solution of 5ml distilled water and 5ml concentrated ammonium hydroxide (PH 12-13) (3) a developing solution of 3% formalin first neutralized with sodium acetate crystals and then adjusted to a PH of 5-6 with formic acid. The procedure is as follows:

Pipette the Ag solution on to a slide, cover with a cover glass and place the slide about 10 cm below a photoflood (275w bulb)for 10 minutes. Rinse off the cover glass in distilled water and develop the slide by adding 4 drops of AS solution followed by 4 drops of developing solution. Cover the slide immediately with a cover glass and monitor the staining under the microscope. When the chromosomes reach a golden yellow colour, rinse the slide in distilled water, dehydrate in an ethanol series and mount

in Euparal.

7. Ag - G-banding

Silver nitrate treated slides, as in the method mentioned above, were rinsed in distilled water and then processed as in G-banding technique.

8. Mounting the slides

All Giemsa banding preparations (G, C and Ag-G-banding) were washed thoroughly with distilled water after staining and placed vertically in racks and left for at least 12 hours to dry. Half an hour before mounting, the slides were put into a 37°C incubator to ensure dryness. Loctite 358 polymerizing resin was used as a mounting medium as recommended by Angus (1982). It keeps the preparation as good as new for a longer time than any other mounting medium does.

9. Photography

All microphotographs were taken on a Leitz Orthophan photomicroscope, using a Leitz Fluortar 100/1.32 oil immersion lens, and a Zeiss precision interference filter No. 467 808 to give a monochromatic green light. Initially, preparations were photographed on to Ilford Ilfodata HS23 type J 500 film, with the exposure meter set to 10 or 11 DIN (8-10 ASA); the film was developed for 1.5 minutes in 1 part Ilfodata CP developer in 5 parts water at 20°C. When the Ilfodata film was discontinued, Agfa-Gevaert film Copex Pan A.H.U. has been used with the exposure meter set to 9 DIN (6ASA) and developed for 2 minutes in 1 part Agfa G. G.141C developer in 5 parts water at 20°C. Photographs were printed on Ilford Ilfobrom No. 3 and 4 paper.

10. Measurements

Measurements were made from photographs printed at 3000
A ruler can be easily used to measure the length of the straight chromosomes, but it was found to be difficult and not accurate in curved ones. A micrometer made by fastening a cog wheel to the winder of an old wrist watch was used for measurements in the case of curved chromosomes, as mentioned by Angus (1982). Running the cog wheel along the chromosomes and using the minutes of the clock face as units of measurement enables curved chromosomes to be measured accurately. For each chromosome the relative chromosome length (the length of the chromosome divided by the total haploid autosome length and multiplied by 100) was calculated.

CHAPTER 3

MITOTIC & MEIOTIC CHROMOSOMES OF A. SYLVATICUS & A. FLAVICOLLIS & COMPARISON OF THEIR KARYOTYPES

I Apodemus Sylvaticus

Karyotype and G-banding

Nine samples of A. sylvaticus from localities in the mainland of Britain listed in table 1 (p.27) were examined cytologically to establish the karyotype and look for intraspecific variation. At least 6 well spread, G-banded metaphases were karyotyped and examined for each animal. Chromosome measurements were performed on 47 males and 9 females karyotypes. The relative chromosome lengths (the length of each chromosome expressed as a percentage of the total haploid autosome length) are given in table 3. B chromosomes are not reported here, but are discussed in Chapter 4.

All karyotypes were identical (apart from the B chromosomes) containing 23 pairs of telocentric autosomes and a sex chromosome pair which is xy in males and xx in females (Fig. 2, A and B). Frequently a single karyotype does not display all bands, so an idiogram was constructed after compa ring many G-banded karyotypes (Fig. 3).

G-banding permits the identification of all chromosome pairs. Homologous chromosomes can be readily identified since the bands are identical in size and position for each chromosome pair. By comparing the karyotypes of males and females, sex chromosomes can be easily identified. The x chromosome has a relative chromosome length of 7.24 intermediate between those of autosomes No. 1 and 2. It has three distinct deeply staining bands, one near the centromere and the other two near the distal end of the chromosome. There are also three paler bands between the centromeric and distal dark bands. At the distal end

Table 3: Relative chromosome length of the chromosomes of A. sylvaticus

Chromosome	Relative chromosome length and 95% confidence limits (t-test)	Chromosome	Relative chromosome length and 95% confidence limits (t-test)
1	7.96 (7.86-8.10) N=112	13	3.79 (3.73-3.86) N=112
2	6.77 (6.68-6.87) N=112	14	3.64 (3.59-3.70) N=112
3	6.21 (6.13-6.29) N=112	15	3.58 (3.52-3.64) N=112
4	5.85 (5.77-5.94) N=112	16	3.46 (3.41-3.51) N=112
5	5.58 (5.50-5.67) N=112	17	3.37 (3.33-3.41) N=112
6	5.29 (5.20-5.38) N=112	18	3.26 (3.21-3.31) N=112
7	5.04 (4.96-5.13) N=112	19	3.22 (3.17-3.26) N=112
8	4.60 (4.53-4.67) N=112	20	3.19 (3.13-3.24) N=112
9	4.26 (4.19-4.32) N=112	21	3.09 (3.04-3.14) N=112
10	4.17 (4.12-4.22) N=112	22	2.95 (2.90-2.99) N=112
11	4.00 (3.99-4.06) N=112	23	2.76 (2.70-2.83) N=112
12	3.87 (3.81-3.92) N=112	x	7.84 (7.53-8.14) N= 65
		y	2.96 (2.78-3.14) N= 47

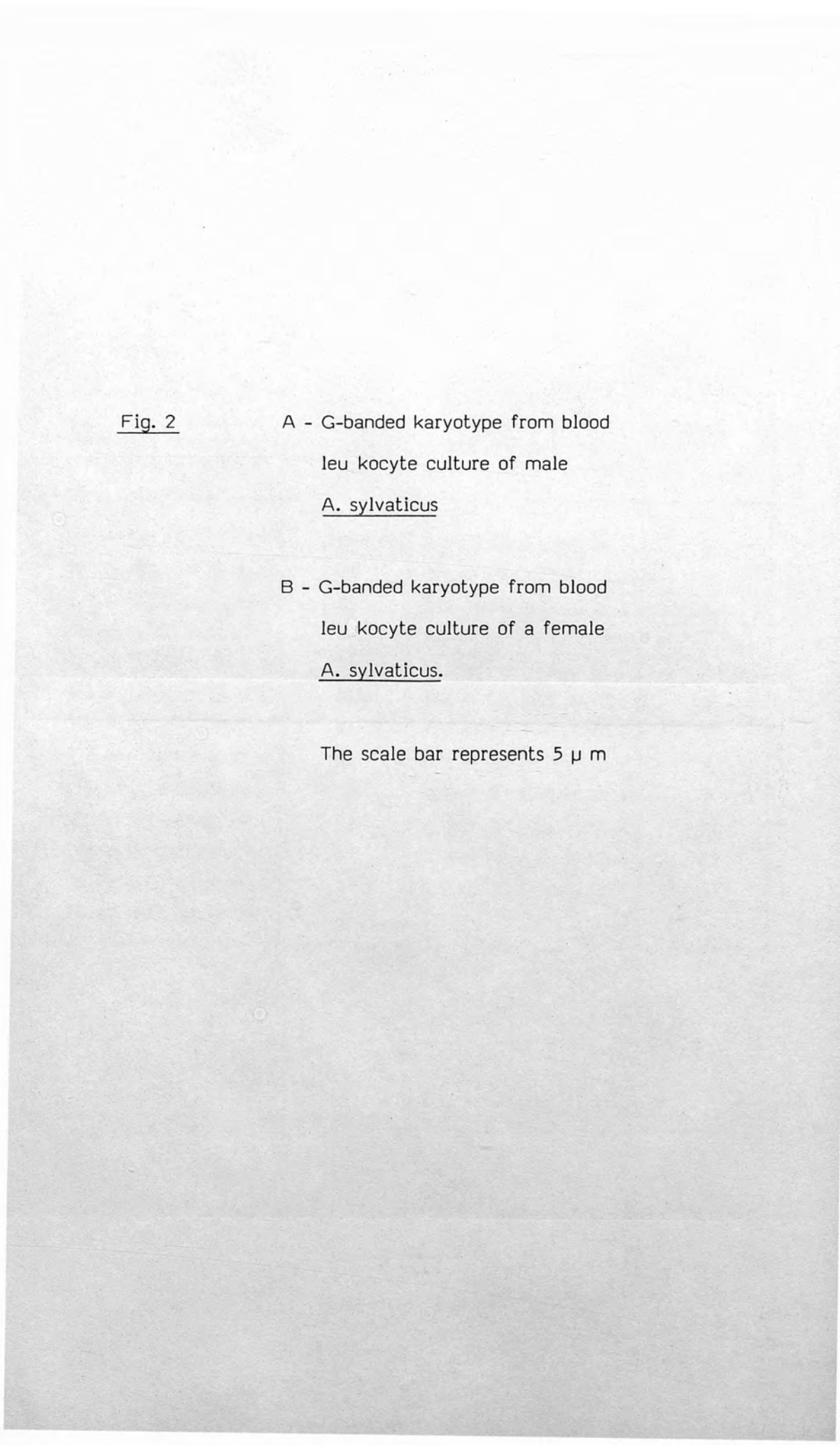
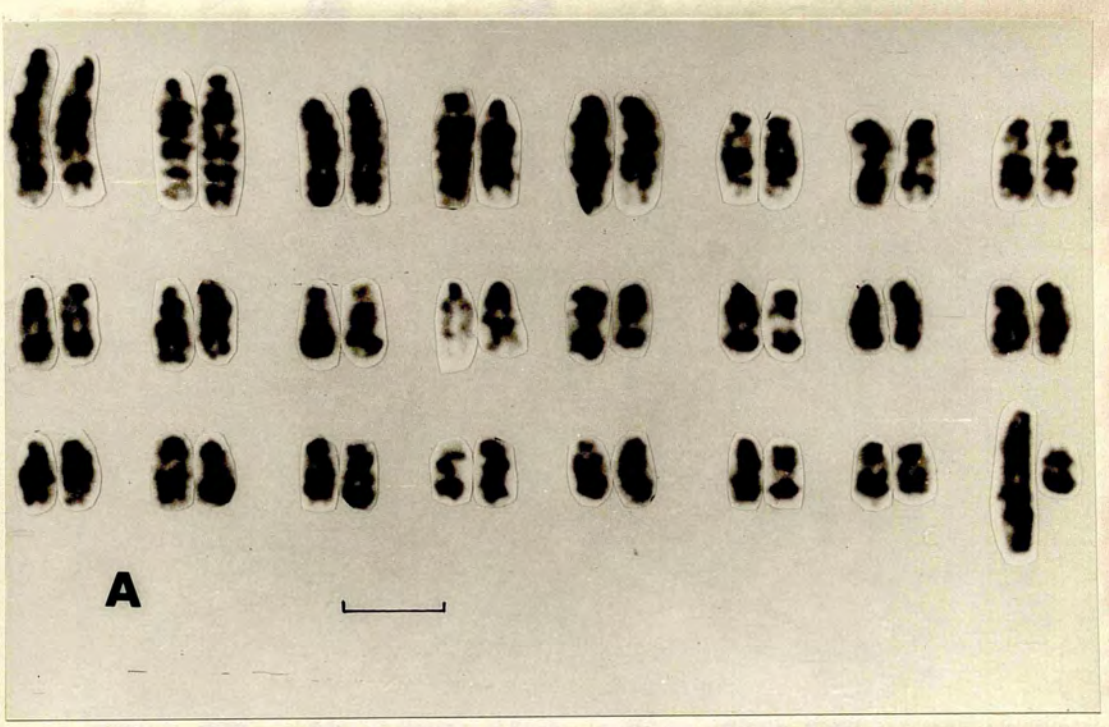


Fig. 2 A - G-banded karyotype from blood leu kocyte culture of male *A. sylvaticus*

B - G-banded karyotype from blood leu kocyte culture of a female *A. sylvaticus*.

The scale bar represents 5 μ m



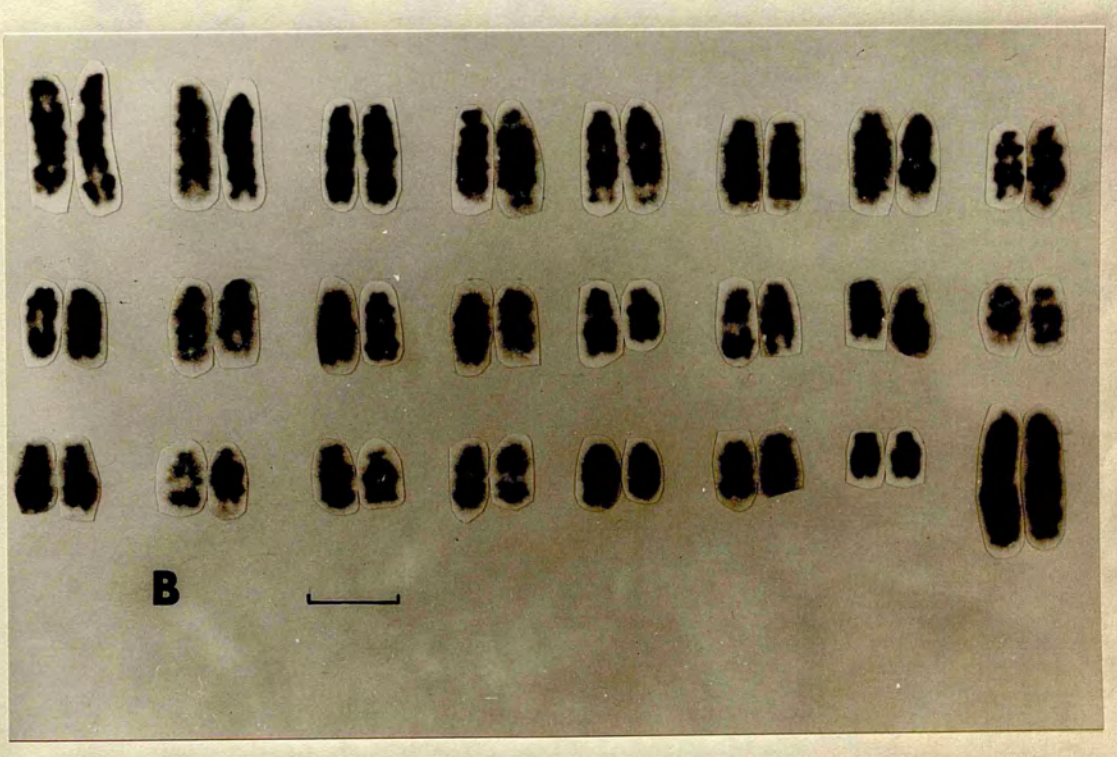


Fig. 2

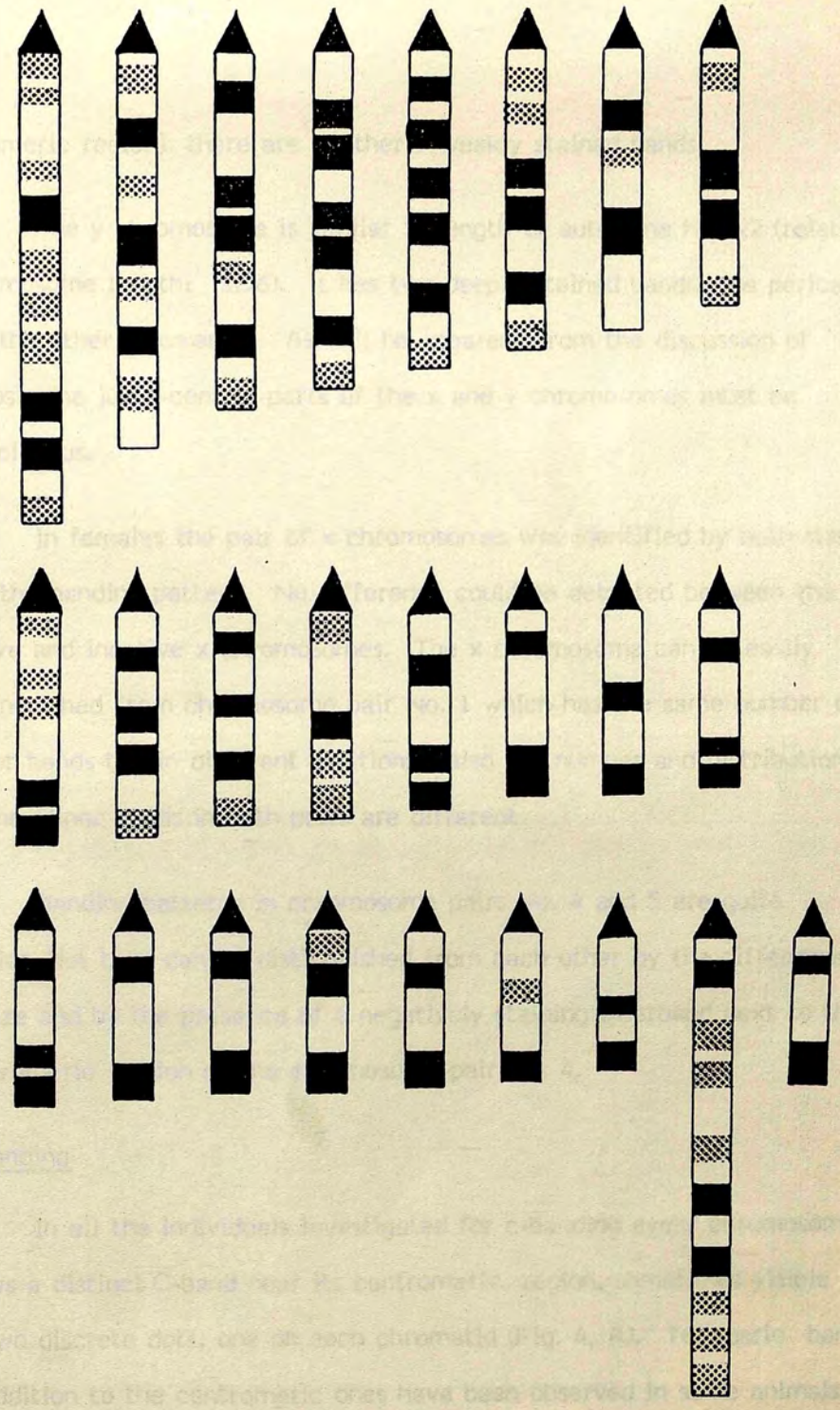


Fig. 3 G-band idiogram of A. sylvaticus metaphase chromosomes showing the characteristic bands observed in most metaphases. Major bands are shown in black, minor bands stippled.

(telomeric region) there are another 3 weakly stained bands.

The y chromosome is similar in length to autosome No. 22 (relative chromosome length: 2.96). It has two deeply stained bands, one pericentric and the other telomeric. As will be apparent from the discussion of meiosis the juxta-centric parts of the x and y chromosomes must be homologous.

In females the pair of x chromosomes was identified by both size and the banding pattern. No difference could be detected between the active and inactive x chromosomes. The x chromosome can be easily distinguished from chromosome pair No. 1 which has the same number of major bands but in different positions; also the number and distribution of the minor bands in both pairs are different.

Banding patterns in chromosome pairs No. 4 and 5 are quite similar, but they can be distinguished from each other by the difference in size and by the presence of a negatively staining interband next to the centromeric region of the chromosome pair No. 4.

C-banding

In all the individuals investigated for c-banding every chromosome shows a distinct C-band near its centromeric region, sometimes visible as two discrete dots, one on each chromatid (Fig. 4, A). Telomeric bands in addition to the centromeric ones have been observed in some animals, but this is not consistent even in the cells of the same animal (Fig. 4, B).

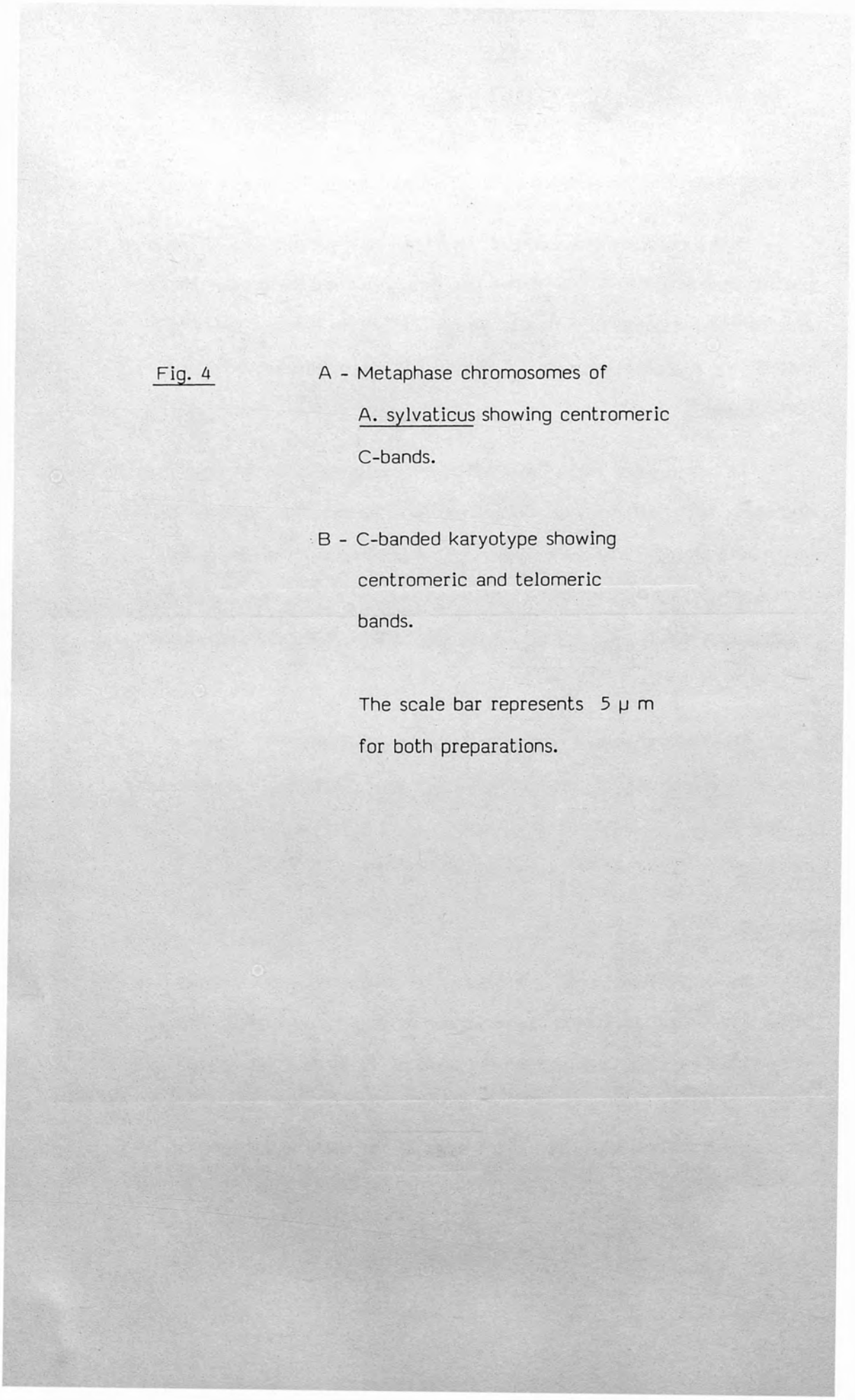
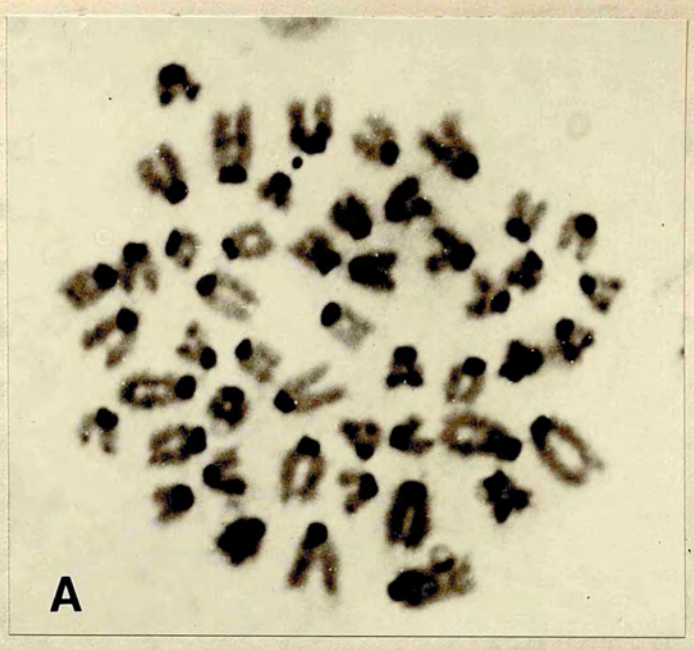


Fig. 4 A - Metaphase chromosomes of *A. sylvaticus* showing centromeric C-bands.

B - C-banded karyotype showing centromeric and telomeric bands.

The scale bar represents 5 μ m for both preparations.



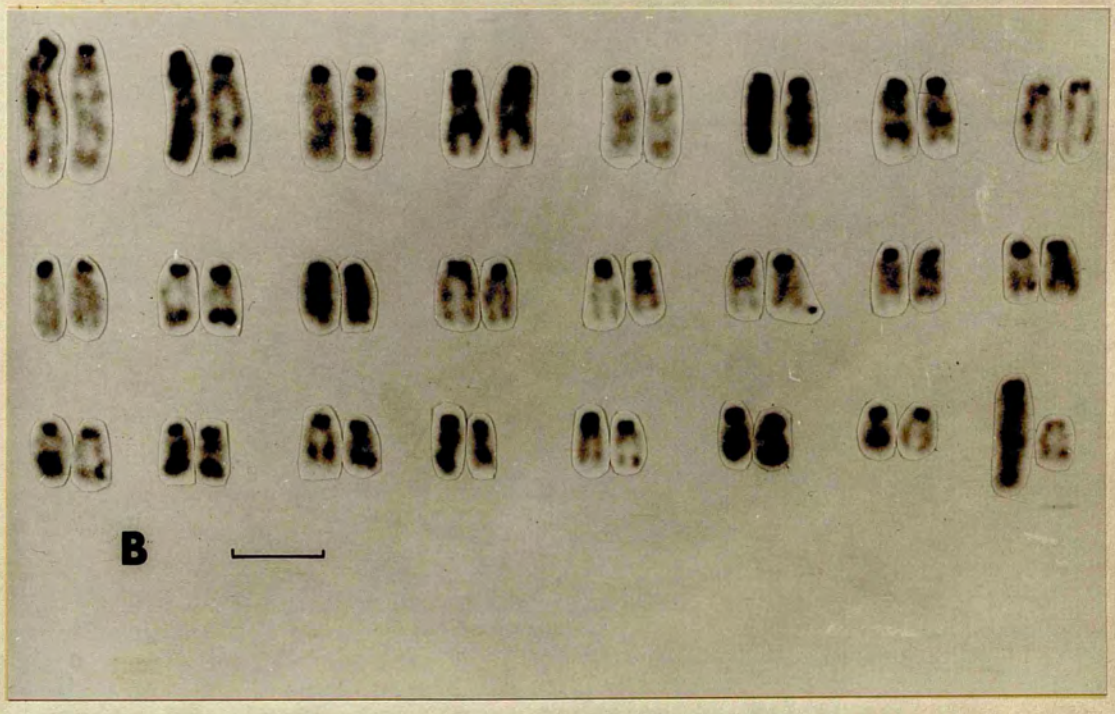


Fig. 4

Nucleolus Organizer Regions (NORs)

Silver stained metaphase plates from different animals were examined for the presence of NORs. As shown in Fig. 5, A a maximum of 10 NORs bearing chromosomes could be observed. The NORs were represented by a black dot at the extreme tip of the 2 chromatids of each chromosome. Other preparations showed lower numbers of NORs (Fig. 5, B). This variation can be attributed to the state of contraction of the chromosomes. Goodpasture and Bloom (1975) reported that the silver stained NORs were usually most conspicuous in highly contracted metaphase chromosomes. Unfortunately, the chromosome pair numbers for those bearing the NORs could not be determined as the chromosomes were too contracted to be karyotyped. Besides the main sites of silver stained NORs, silver frequently precipitates as a distinct dot at the centromeric region of some chromosomes (Fig. 6, A). Silver staining of interphase nuclei revealed no more than five nucleoli (Fig. 6, B), thus confirming that no more than five pairs of chromosomes organize them.

Meiosis

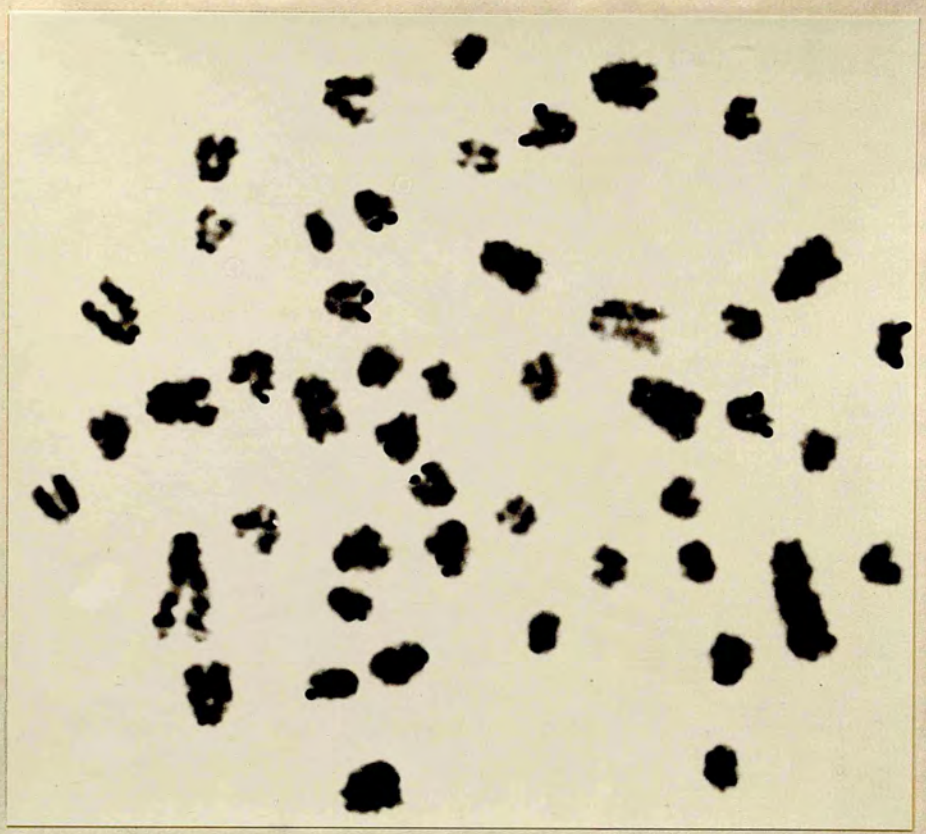
Meiosis was analysed in 92 males from the nine different localities already mentioned (Table 1). Examination of the meiotic chromosomes at diakinesis and metaphase 1 has demonstrated that the autosomes pair regularly forming 23 bivalents as well as the sex chromosomes which always occupy a peripheral position in the metaphase arrangement.

The sex chromosomes are largely non-homologous and show two different types of association, occurring with almost equal frequency in different preparations. In the first type the x and y associate end to

Fig. 5 A & B Ag - G-banding preparations of A. sylvaticus.

A - Metaphase plate showing 10 chromosomes with Ag - NORs x 3000.

B - Metaphase plate showing a smaller number of chromosomes with Ag - NORs x 3000.



A

B

Fig. 5

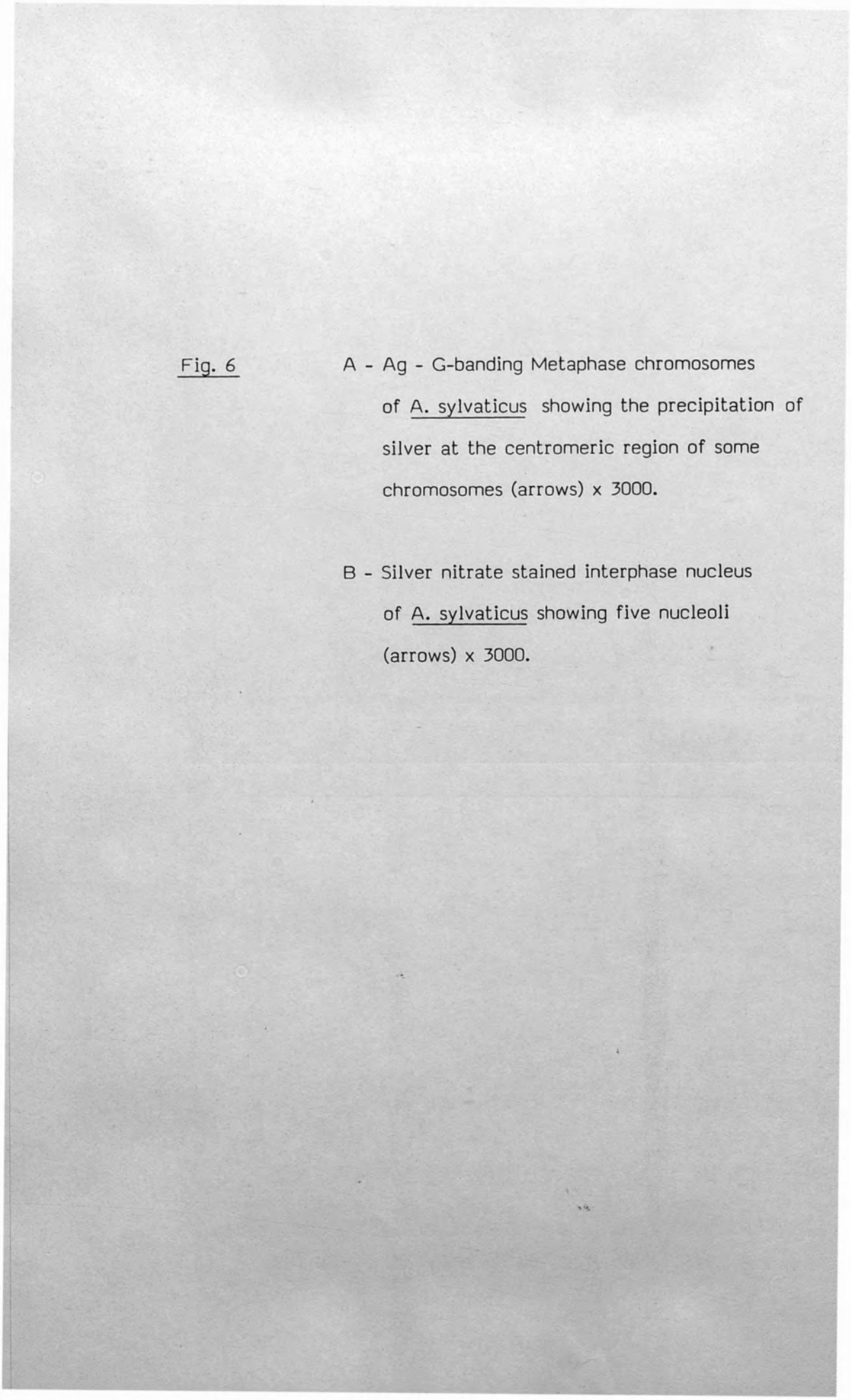
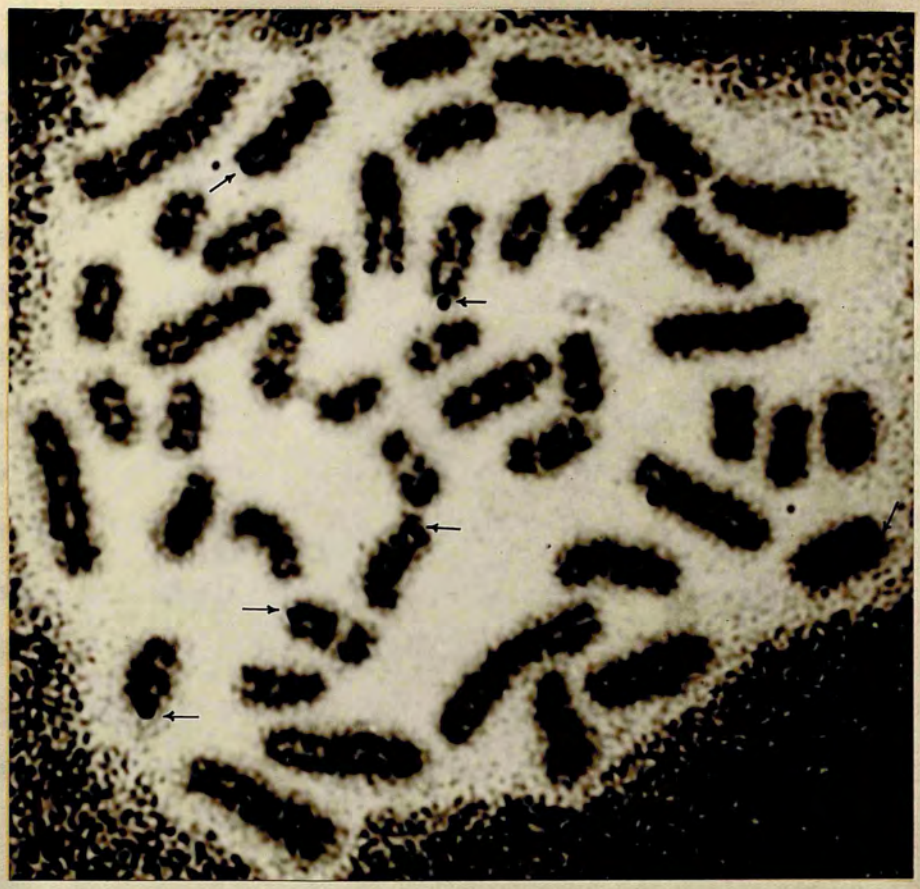


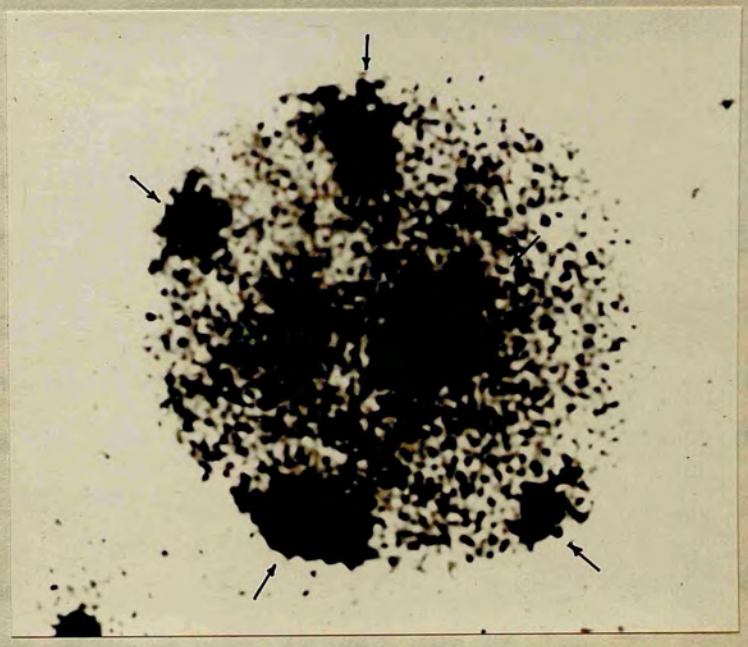
Fig. 6

A - Ag - G-banding Metaphase chromosomes of A. sylvaticus showing the precipitation of silver at the centromeric region of some chromosomes (arrows) x 3000.

B - Silver nitrate stained interphase nucleus of A. sylvaticus showing five nucleoli (arrows) x 3000.



A



B

Fig. 6

end forming a characteristic heteromorphic asymmetrical structure comprising two unequal components (Fig. 7, A and B). In this structure the x chromosome appears to consist of a prominent, deeply stained proximal body and a lightly stained, thin arm of considerable length. The y element is represented by a similar compact proximal body, but with a very short, lightly stained arm. The two elements thus differentiated come into contact with each other through the tips of their heteropycnotic proximal parts, with their arms free. This end to end association was first described in man by Painter (1923 and 1924). It has also been demonstrated in many mammals (Koller, 1934; Makino, 1951; Matthey, 1951; Ohno et al., 1959). Sachs (1954, 1955) has described such an association in man and also in Mus musculus. This type of association does not require any homology between parts of the main arms of the x and y chromosomes.

Koller (1937) considered this association to be a classic chiasma-type association, but it has been suggested by Makino (1941) and Sachs (1955) that at this stage the x and y chromosomes are held together by means other than chiasmata; for example, by the adhesive action of the sex vesicle remnant. This association cannot be broken by ribonuclease digestion and it resists very severe hypotonic treatment (Ohno et al., 1957).

Ohno et al. (1959) studied the end to end association of the x and y chromosomes of Mus musculus and concluded that it was very probably due to a chiasma formed between the extremely short second arms of the x and y chromosomes.

In the present investigation it was found that in a few cases the x and y chromosomes were somewhat separated from each other, but still

showed their characteristic configuration, as if there was some attachment pulling them towards each other (Fig. 7, C and D). This sort of gap is also occasionally found between autosomal bivalents (Fig. 7, C and E), so that it does not throw any light on the nature of the attachment and consequently a chiasma could be involved.

Ohno et al. (1959) reported that this asymmetrical association in Mus musculus leads to pre-reduction segregation of x and y (at first meiotic anaphase). This gives two secondary spermatocytes, each with either x or y, and is the normal pattern for mammals. Figure 7, F shows such a secondary spermatocyte in A. sylvaticus.

In the second type (Fig. 8) the sex chromosomes associate with each other side-by-side forming a symmetrical chiasmate sex bivalent, quite distinct amongst the autosomal bivalents of ordinary structure. It is composed of two distinct regions, a proximal massive section and a distal elongated section, differing sharply in staining properties. The proximal section is very prominent as a condensed massive body, stained as deeply as the autosomal bivalents, while the distal section is represented by a free thread-like arm, lightly stained and showing diffuse texture (Fig. 8, A). Sometimes the pale region of the x chromosome appears as two distinct chromatids closely associated with each other (Fig. 8, B). This symmetrical association leads to post-reduction segregation of x and y (Koller, 1941). The xy complex divides equationally along the longitudinal split as shown in Fig. 8, C and D, thus resulting in two equal xy dyads in the first division. Examination of the secondary spermatocytes showed some with the xy dyad appearing very distinct from the v-shaped autosomal

dyads (Fig.8, E).

For this type of pairing between x and y, it is necessary that the basal parts of the arms of the two chromosomes are homologous, to allow chiasma formation.

In contrast with the foregoing, a sex chromosome complex having an appearance as shown in Fig. 9 was detected in 61 cells out of 712 (8.57%). In analyzing its structure it appears as two long chromatids clearly distinguished from each other, and at one end, two darkly stained dots, one on each chromatid (Fig. 9, A). Special note should be made here of the fact that it may be such a chromosome that Matthey (1936b) interpreted as a sex chromosome pair of the pre-reduction type, because his figures 3, A and B, (p. 506, 36b) show a sex chromosome closely resembling that just described and shown in Fig. 9. According to Matthey the dark end of this structure does not correspond only to the point of insertion, but it has the y chromosome placed perpendicularly to the x and in consequence parallel to the axis of the spindle. Makino (1951) described such a structure and the interpretation given by him is that this appearance is attributed to an irregular orientation with the x chromosome lying vertical to the equatorial plane, whereas the y element is hidden by the superimposed x.

These problems of orientation could account for the arrangements seen by Matthey and Makino (loc. cit.), as they were working with sectional material. However, since the preparations in the present study were made from disaggregated cells, which had been inflated with hypotonic saline and then fixed, before being air-dried on slides, all the

chromosomes lie flat (or crumpled) on the slide and there is thus no possibility of any chromosome being viewed end on.

It thus seems that this structure represents the x chromosome without any attached y. It is not possible, on the information at present available, to account for this arrangement. It seems unlikely that it is entirely due to artifact as no unattached y chromosomes have been found in meiotic metaphase preparations showing the isolated x. Some preparations of spermatogonial mitosis lack one small chromosome (possibly y), but otherwise appear intact (Fig 9, D), again suggesting a genuine absence rather than a faulty preparation.

Finally, it will be noted that if these meiotic metaphases do contain x and no y, they would result in offspring being either xx - normal females, or xo. One such mouse, female and apparently healthy, has been encountered in the present study (p.120).

Fig. 7 First metaphase of meiosis in germinal cells of male A.sylvaticus.

A & B Showing end to end association between x and y, forming asymmetrical bivalent.

C & D Asymmetrical sex bivalent with the x and y somewhat separated from each other.

C & E Showing separation between the chromosomes of some autosomal bivalents as well as between the x and y (arrows).

F Secondary spermatocyte with only one sex-chromosome (either x or y).

Scale bar represents 5 μ m

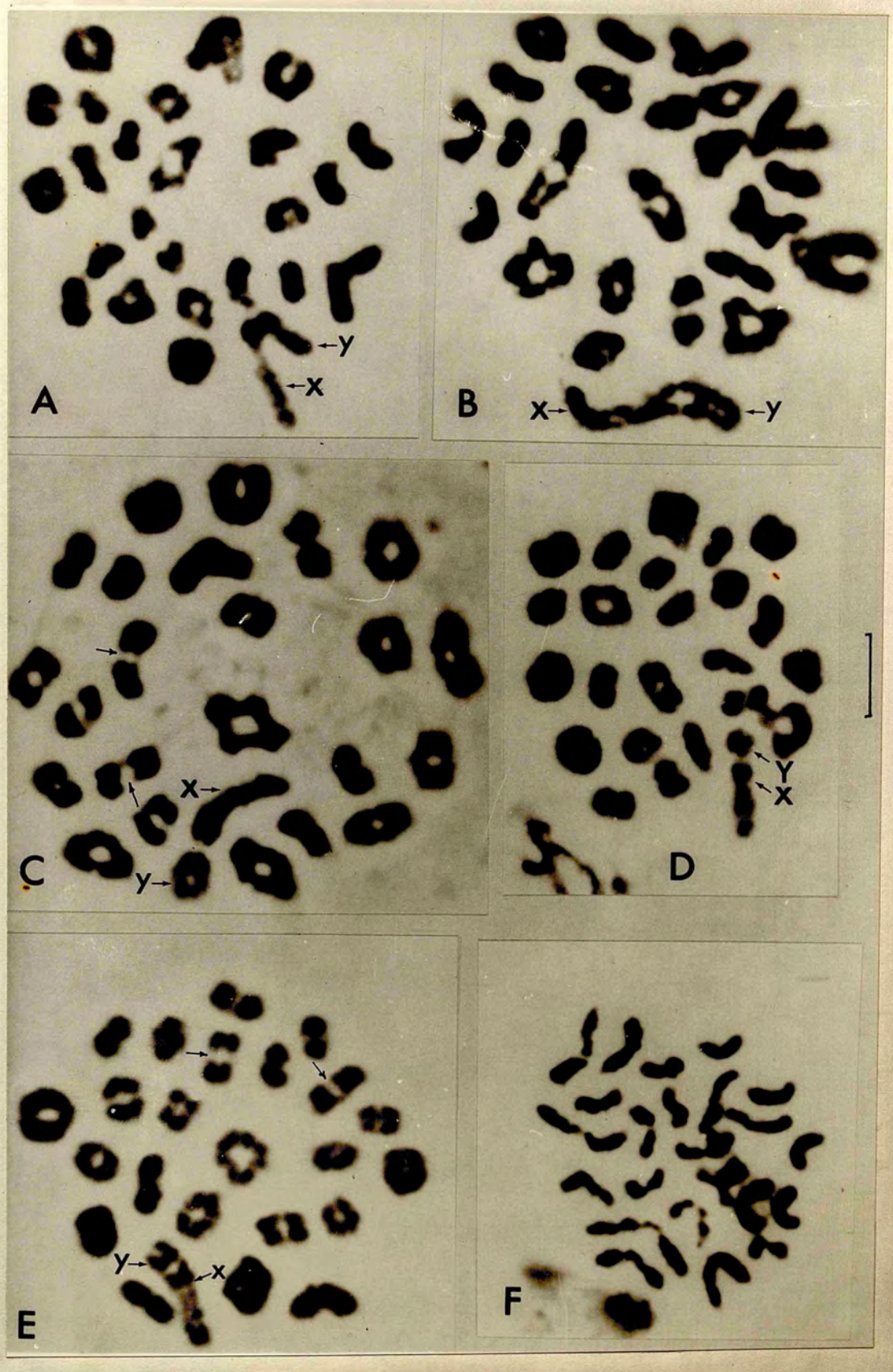


Fig. 7

Fig. 8 First metaphase of meiosis in germinal cells of male A. sylvaticus.

A, B, C & D showing symmetrical sex bivalent (xy)

C & D The xy complex divides equationally along the longitudinal split (arrows).

F Secondary spermatocyte showing xy dyad.

Scale bar represents 5 μ m

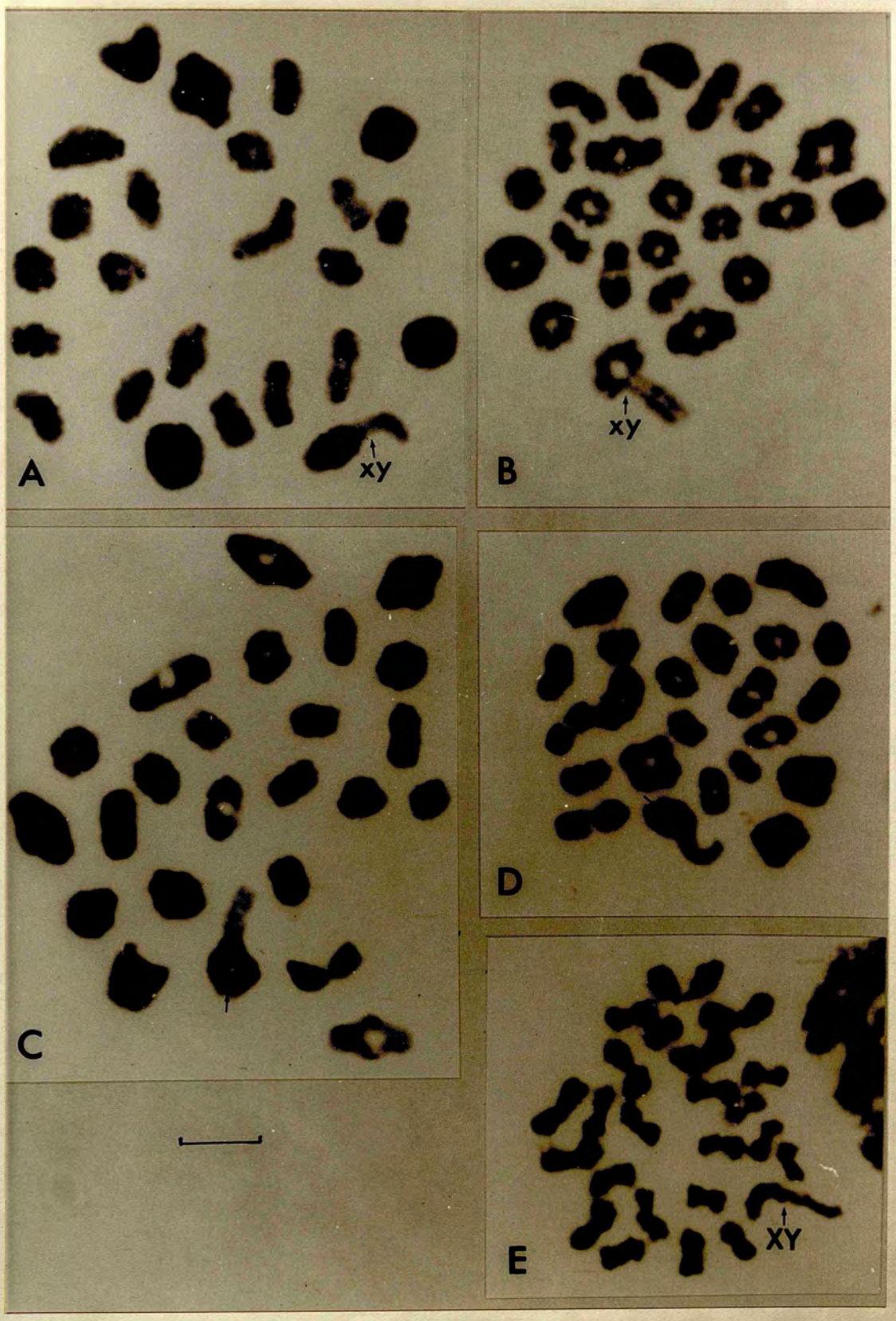


Fig. 8

Fig 9 A, B & C Meiotic metaphases of *A. sylvaticus* showing only the x chromosome and the absence of the y.

D Spermatogonial mitosis with 47 chromosomes.

Scale bar represents 5 μ m

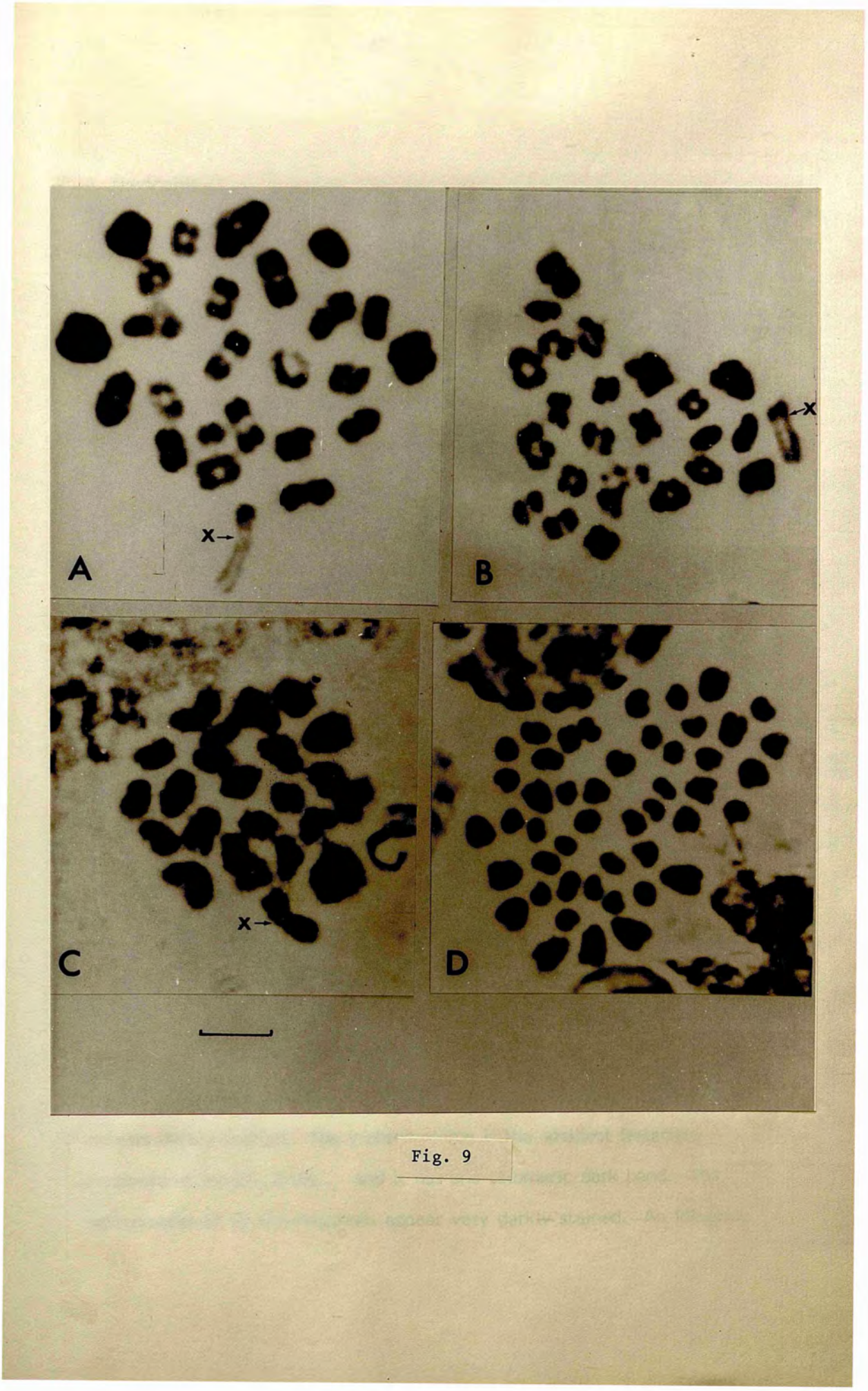


Fig. 9

II A. flavicollis

Karyotype

The chromosomes of the yellow-necked mouse, A. flavicollis, have been investigated to establish the karyotype and to compare it with that of the wood mouse A. sylvaticus.

Forty-two animals have been used for the present investigation. The animals were trapped from eight different localities in the mainland of Britain already mentioned in table 1. All karyotypes were identical containing 23 pairs of telocentric autosomes and a pair of sex chromosomes which are xy in males and xx in females. The x chromosome is one of the largest telocentrics of the entire complement and the y is the smallest one. The relative chromosome lengths are given in table 4.

G-banding

G-banding stains the chromosomes heavily, so that it is difficult to determine the number and size of the bands. Contracted chromosomes appear totally darkly stained without any conspicuous bands. Relatively elongated chromosomes reveal the presence of darker bands which were found to be characteristic for each autosome pair as well as for the x and y (Fig. 10, A and B). The x chromosome is the second largest chromosome in the normal complement (relative chromosome length:7.77). It has seven dark bands distributed along its length, and the area below the centromere appears darkly stained. The y chromosome is the smallest (relative chromosome length: 2.10), and it has one telomeric dark band. The centromeres of all chromosomes appear very darkly stained. An idiogram

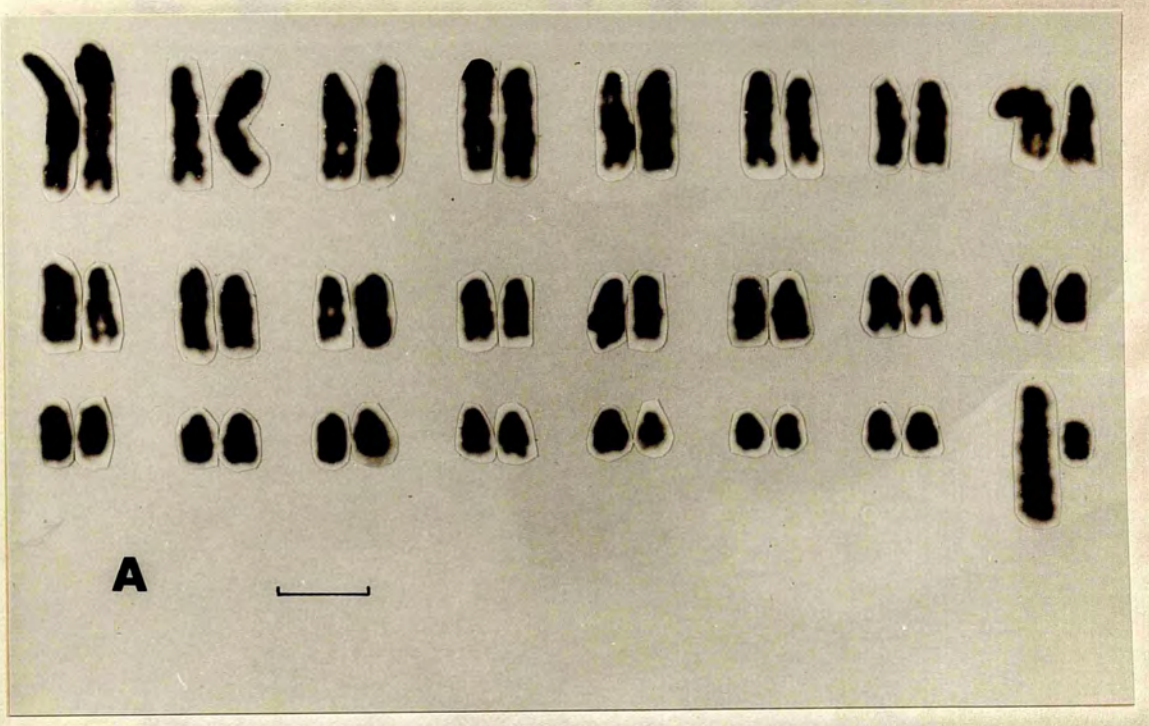
Table 4: Relative chromosome length of the chromosomes of A. flavicollis

Chromosome	Relative chromosome length and 95% confidence limits (t-test)	Chromosome	Relative chromosome length and 95% confidence limits (t-test)
1	8.68 (8.32-9.05) N=16	13	3.78 (3.58-3.97) N=16
2	6.92 (6.74-7.10) N=16	14	3.59 (3.44-3.74) N=16
3	6.28 (6.03-6.54) N=16	15	3.36 (3.24-3.48) N=16
4	6.14 (5.98-6.30) N=16	16	3.33 (3.14-3.51) N=16
5	5.92 (5.70-6.13) N=16	17	3.28 (3.14-3.43) N=16
6	5.43 (5.23-5.63) N=16	18	3.01 (2.89-3.13) N=16
7	5.08 (4.93-5.22) N=16	19	2.99 (2.78-3.20) N=16
8	4.92 (4.73-5.11) N=16	20	2.71 (2.59-2.83) N=16
9	4.66 (4.45-4.86) N=16	21	2.72 (2.56-2.87) N=16
10	4.50 (4.41-4.59) N=16	22	2.55 (2.39-2.71) N=16
11	4.08 (3.94-4.22) N=16	23	2.33 (2.10-2.57) N=16
12	3.98 (3.83-4.12) N=16	x	7.77 (7.32-8.22) N=13
		y	2.10 (1.85-2.35) N= 3

Fig 10 A - G-banded karyotype of bone marrow cells of male A. flavicollis.

B - G-banded karyotype of bone marrow cells of female A. flavicollis.

The scale bar represents 5 μ m



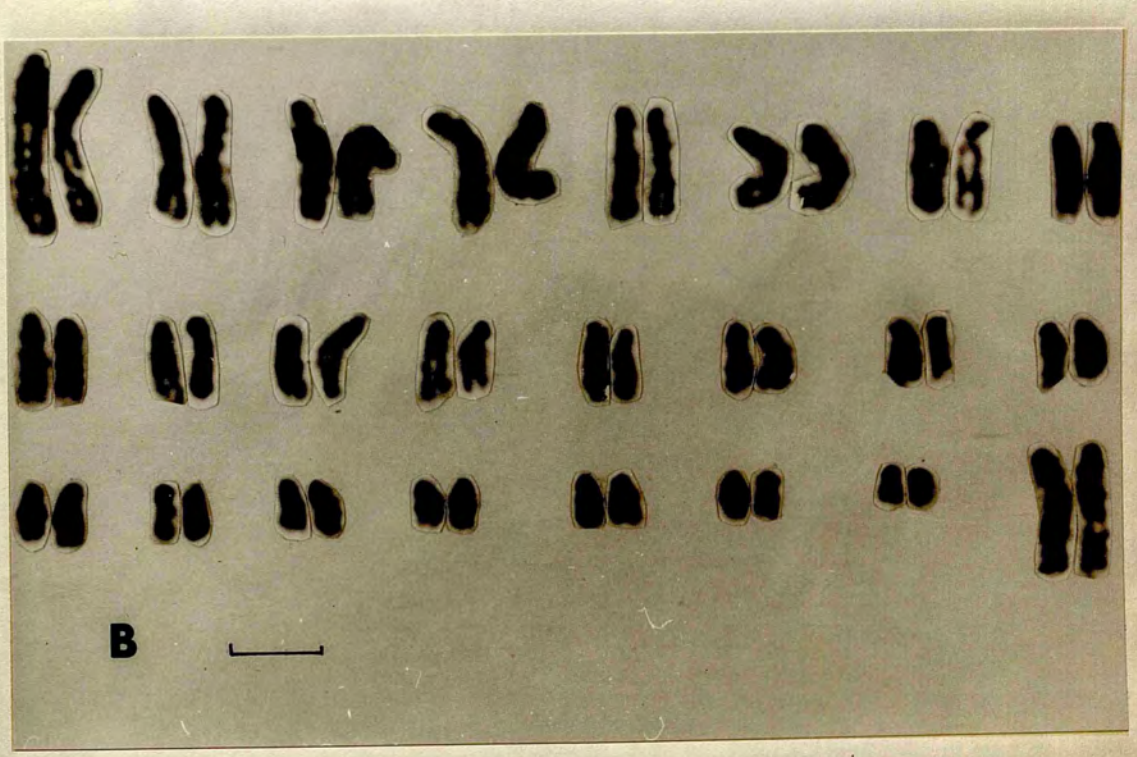


Fig. 10

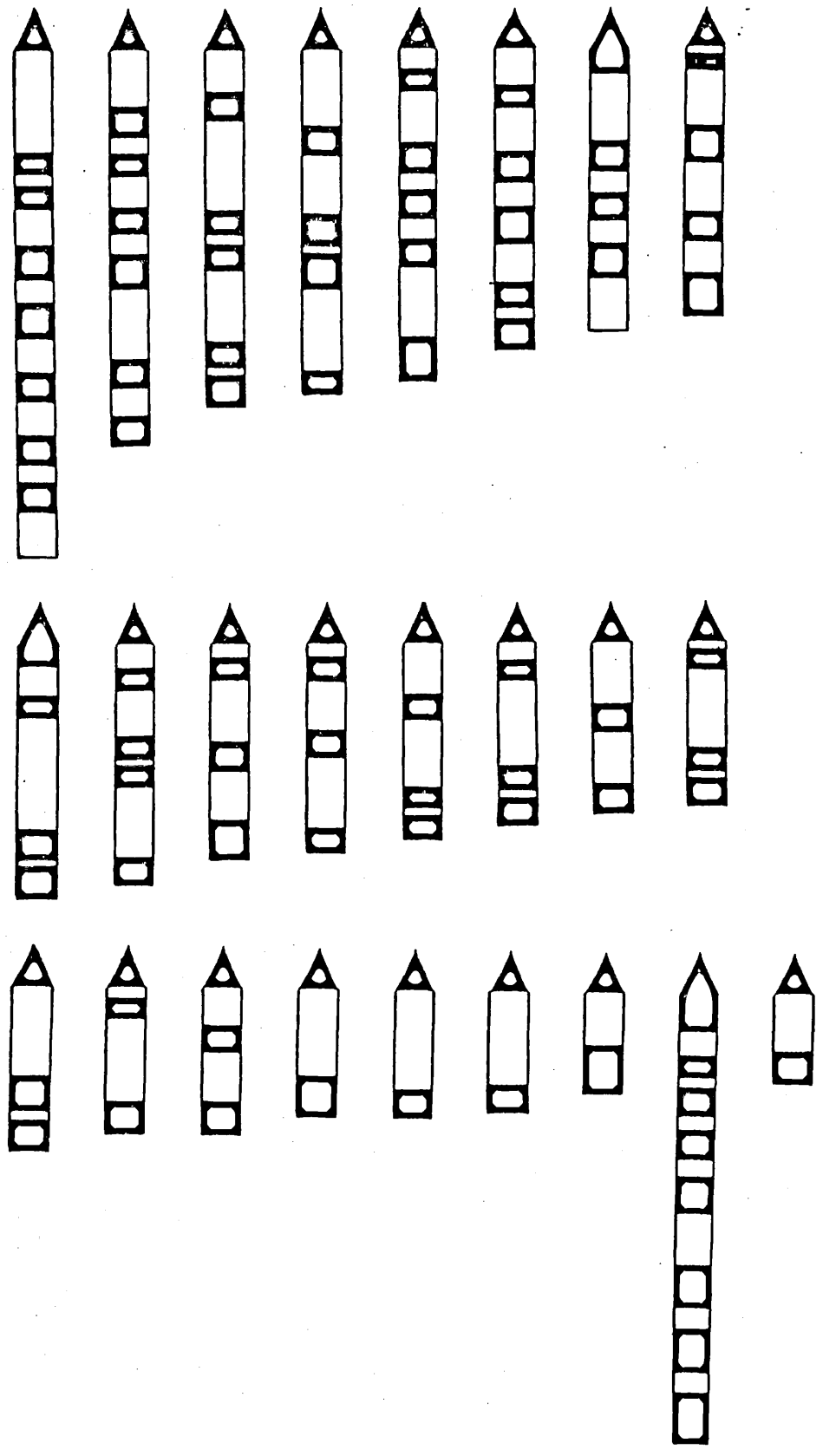


Fig 11 G-band idiogram of A.flavicollis metaphase chromosomes showing the characteristic bands observed in most metaphases.

of G-banded chromosomes is illustrated in Fig. 11.

C-banding

Constitutive heterochromatin in the form of positive C-banding is largely restricted to the centromeric regions. These centromeric blocks are dense and very conspicuous in all chromosomes of the complement. The x chromosome pair shows a small block of heterochromatin adjacent to the centromeric region (Fig. 12).

Nucleolar Organizer Regions (NORs)

Silver stained NORs were stained as black spherical bodies at the telomeric regions of the chromosomes. As shown in Fig. 13, A a maximum of 12 NOR bearing chromosomes could be observed. Silver staining of interphase nuclei showed six nucleoli (Fig. 13, B) thus confirming that no more than six pairs of chromosomes organize them.

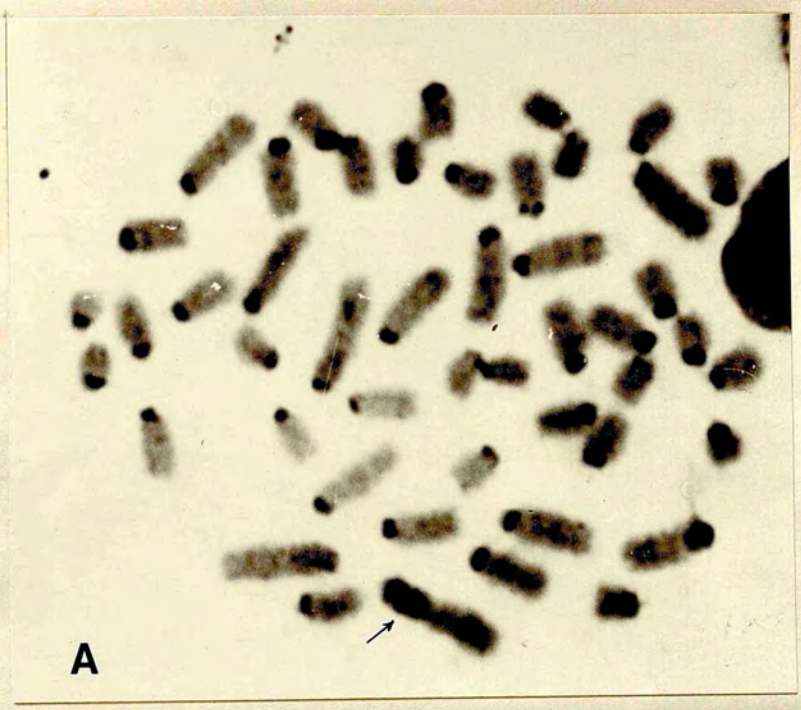
Meiosis

The behaviour of chromosomes in meiosis was investigated in 23 males. It was observed that the 46 telocentric autosomes formed 23 bivalents at metaphase 1. The sex bivalent was clearly detected by its characteristic configuration and connection of the x and y at the proximal ends (Fig. 14). Unlike the xy bivalent in A. sylvaticus, that of A. flavicollis showed only one sort of association; 269 cells have been investigated and all of them showed terminal association between x and y chromosomes. This is inevitable as the y chromosome lacks the juxta-centric heterochromatin block found in the x chromosome. This asymmetrical type of association leads to prereduction segregation as

Fig 12 A & B Mitotic metaphase of A. flavicollis chromosomes showing C-bands. x 3000

A - Male. One x chromosome showing juxta centromeric block of heterochromatin (arrow).

B - Female. The sex chromosome (XX) showing juxta centromeric blocks of heterochromatin (arrows).



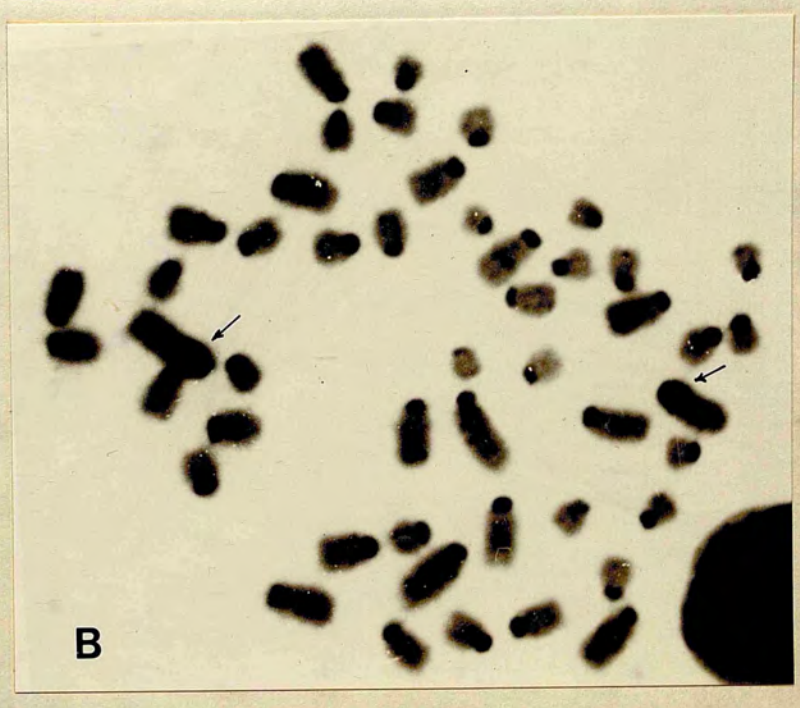
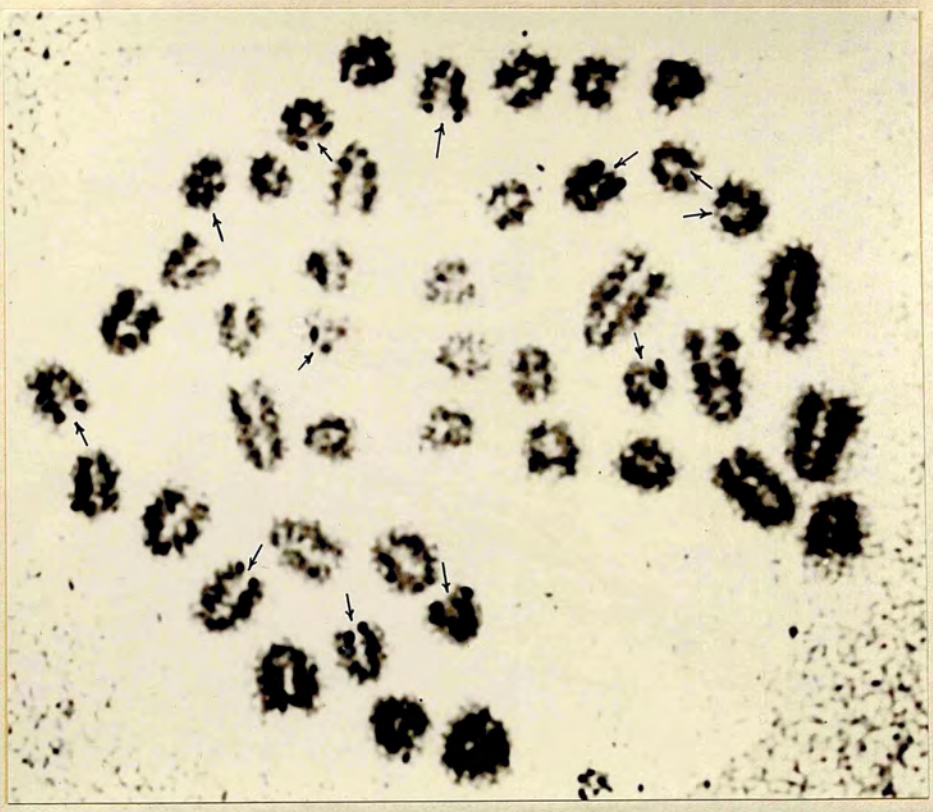


Fig. 12

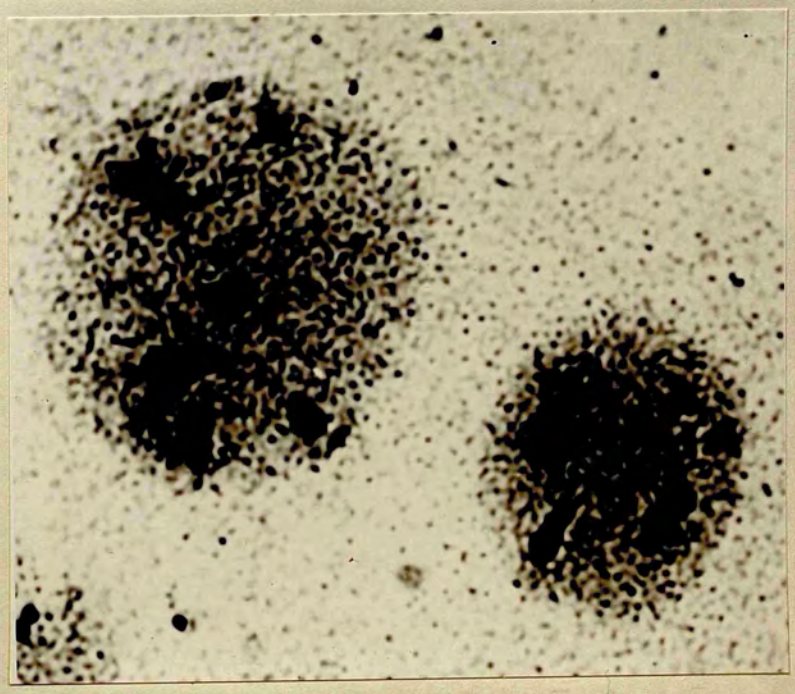
Fig 13

A - Ag - G-banding metaphase chromosomes of A. flavicollis showing 12 chromosomes with Ag - NORs (arrows). x 3000

B - Silver nitrate stained interphase nucleus of A. flavicollis showing 6 nucleoli x 3000.



A



B

Fig. 13

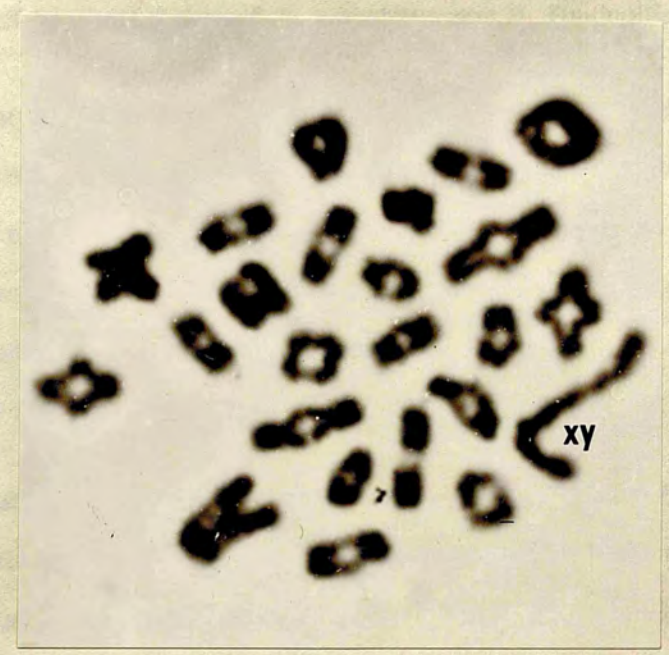


Fig 14 First metaphase of meiosis in germinal cells of male A. flavicollis showing asymmetrical sex bivalent (xy).

already discussed under A. sylvaticus.

Comparison of the karyotypes of A. sylvaticus and A. flavicollis

The karyotypes of A. sylvaticus and A. flavicollis are similar in the number and morphology of the chromosomes. From tables 3 and 4 it is obvious that the relative chromosome lengths are different in the two species. Chromosome pair Nos. 1 to 12 are larger in flavicollis than in sylvaticus and the reverse is true for the rest of the autosomes as well as the x and the y, these being larger in sylvaticus than in flavicollis.

Comparison of the G-banding in the two species is not helped by the heavy overall staining of the A.flavicollis chromosomes. This made it difficult to get good clear G-banded preparations of this species, and precludes classification of the G-bands into major and minor ones. I have tested various alterations of the technique including changes in time and concentration of both trypsin and staining solutions in order to improve the quality of bands; it was found that these changes made no difference to the result. Furthermore, the same technique held good for the other species, A. sylvaticus .

Figs. 15 and 16 show the idiograms prepared for the two species placed together for comparison. It may be seen that the banding patterns of all chromosome pairs in the two species are broadly similar, although there are some exceptions:

Pair No. 1: The seven bands in the A.flavicollis chromosome appear to be matched by 3 major and 4 minor bands in

A. sylvaticus . This leaves two juxta centric minor bands and one telomeric one in the sylvaticus chromosome unmatched in flavicollis. They could be undetected as a result of the difficulties already mentioned in banding of flavicollis chromosomes.

Pair No. 2: Three pairs of bands are found in both species. Each pair is differentiated into minor and major band in A. sylvaticus but not in A. flavicollis in which all bands stained deeply. An extra minor band has been found in the centromeric region of A. sylvaticus .

Pair No. 3: The number and position of bands are quite similar in the two species, except for one minor band in between the telomeric pair of bands and the middle one in A. sylvaticus.

Pair No. 4:
Pair No. 5: Chromosome pair Nos. 4 and 5 are quite similar in A. sylvaticus and are most easily distinguished by the conspicuous juxta-centric interband in No. 4. Each of them shows three pairs of bands, and only the telomeric one has been differentiated into minor and major bands. In A. flavicollis the banding pattern of these two chromosome pairs is different from each other as well as from those of A. sylvaticus. Four bands have been detected in pair No. 4, and five bands in pair No. 5.

Pair Nos. 6 - 16 These show similar banding patterns in the two species , except for three minor bands, two of which are in the

centromeric region of pair Nos. 6 and 9 and the third in the telomeric region of pair No. 11.

Pair Nos. 17 - 23: Recognition of banding patterns in these chromosome pairs in A. flavicollis is difficult, because the chromosomes are small and the banding patterns are generally obscure. Only the telomeric bands can be detected, and these are similar to those of A. sylvaticus. Chromosome pair Nos. 18 and 19 showed the centromeric bands which are quite similar to those of A. sylvaticus.

Sex Chromosomes: In comparing the x chromosomes it must be noted that the x chromosome of A. flavicollis is (relatively) slightly shorter than that of A. sylvaticus, and that its euchromatic region is shorter still, there being a heterochromatic (C-banding) region just behind the centromere. If it is assumed that the total chromosome lengths of the two species are the same, then comparison of Figs. 15 and 16 suggests that the two major G-bands just distal to the centre of the chromosome which are so characteristic of the A. sylvaticus chromosome (Bekasova et al, 1980) may be matched by the two subapical ones of A. flavicollis. Basal to these bands the sylvaticus chromosome has three minor and one major band, which may be homologous with the four bands which in flavicollis lie between the subapical band and the heterochromatic block. The three apical minor bands in A. sylvaticus would appear to represent more chromosome material than the single apical band in A. flavicollis.

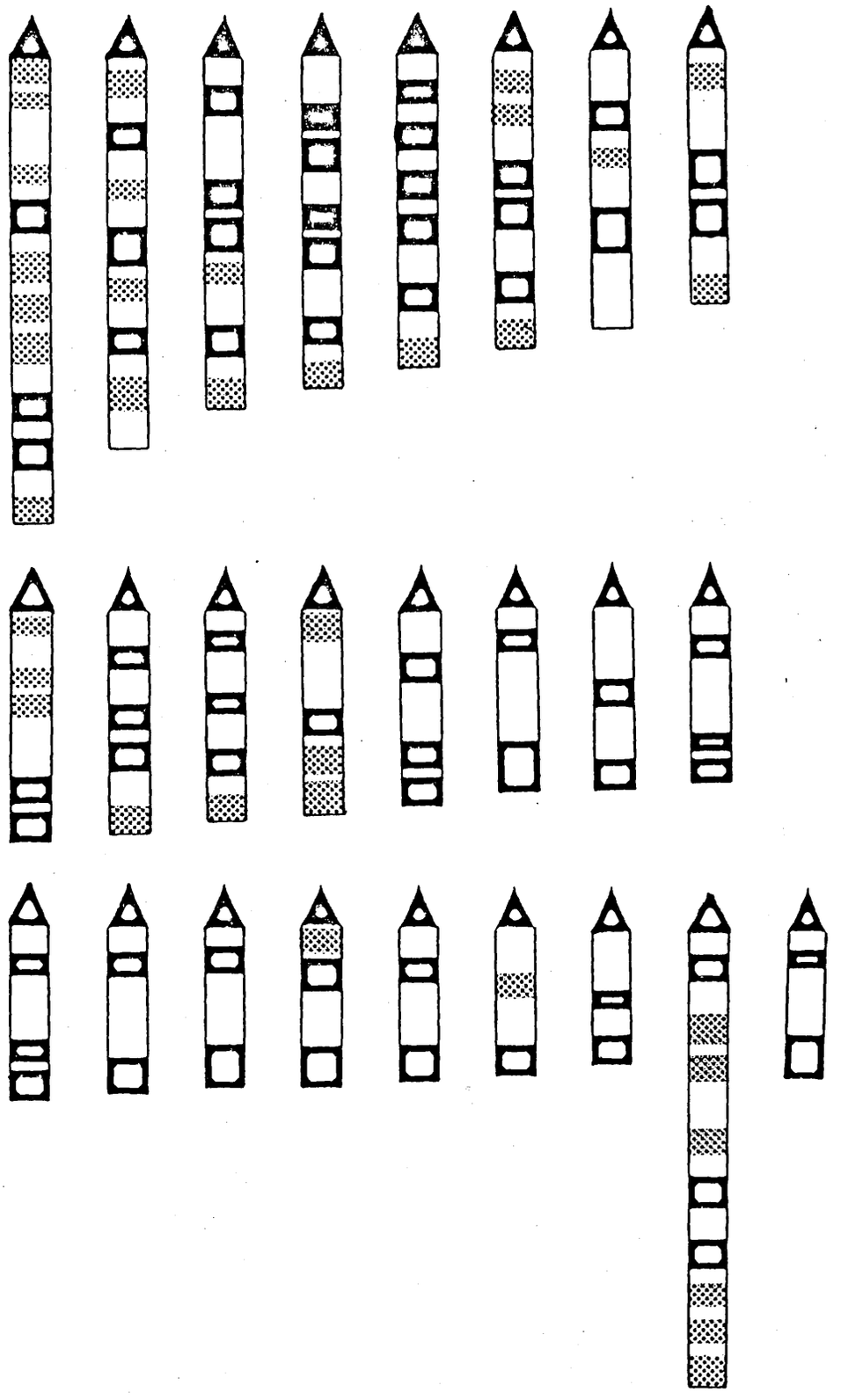


Fig 15 An idiogram of G-banded karyotype of A. sylvaticus

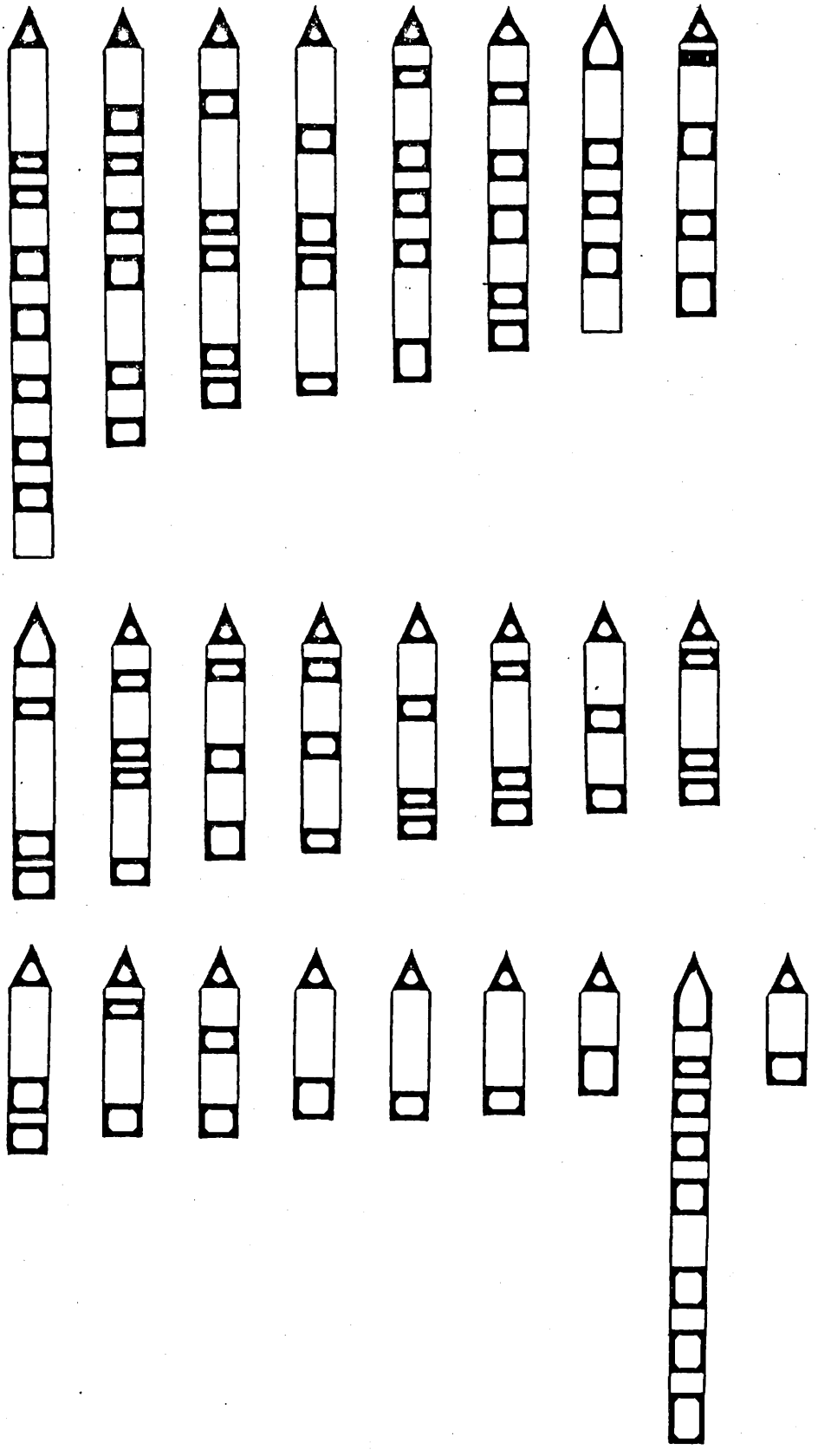


Fig. 16 An idiogram of G-banded karyotype of A. flavicollis

Two distinct bands are recognised in the y chromosome of A. sylvaticus, one telomeric and the other near the centromeric region. In A. flavicollis the y chromosome is smaller, without any conspicuous bands except for one telomeric band.

The general similarity of the banding of the larger chromosomes of the two species suggests that they are largely homologous, and hence approximately the same size in the two species. This suggests that these species both have approximately the same amount of chromosomal material.

Examination of the chromosomes of the two species suggests that the comparisons listed above are the most appropriate. There is no indication either that a chromosome of one species is homologous with a chromosome having a different pair number in the other species. There is also no evidence of the translocation of large segments of chromosome when the two karyotypes are compared. However, the differences in the relative chromosome lengths shown by the chromosomes of the two species strongly suggest extensive translocation of small chromosomal elements.

The numbers of nucleoli and NORs provides a further difference between the karyotypes of these species, 5 pairs of NORs giving 5 nucleoli in sylvaticus and 6 pairs NORs giving 6 nucleoli in flavicollis. This chromosomal data, as well as the morphological differences fully support the separate identities of these two species.

CHAPTER 4

B CHROMOSOMES IN A. SYLVATICUS

Number and distribution of B's

The chromosome complements of individuals within a species usually exhibit a remarkable degree of constancy both in respect of numbers and form of the chromosomes. B chromosomes are a notable exception to this rule. Generally they are less stable than A chromosomes and frequently show numerical and morphological variability between populations, individuals, various tissues in the same individual and moreover between cells in the same tissue.

An analysis of chromosome complements of the British field mouse A. sylvaticus from different populations showed that most of the animals studied (61.6%) had a stable karyotype consisting of 48 telocentric chromosomes which represent the normal diploid complement of the species. Mosaic karyotype has been detected in the rest of the animals (38.4%) due to the presence of B chromosomes.

B chromosomes were found in all the populations studied. Inter-populational variation in the number of animals with B's has been detected, as well as differences in the frequency of B's in males and females in the same population (Table 5). In most of the populations the percentage of males carrying B's is higher than that of females. Studying B chromosomes in meiosis in males raises this frequency to include all the populations.

From table 6 it becomes obvious that in all mosaic individuals most of the cells show the normal diploid number of the species (48) except that one female from Woodchester Park had 47 chromosomes as a result of one x deletion. B chromosomes are found in a very few cells in all the

TABLE 5: FREQUENCY OF B CHROMOSOMES IN DIFFERENT POPULATIONS

Locality	No. of animals	No. ♀♀	No. & (%) with B's in somatic tissues	No. ♂♂	No. & (%) with B's in somatic tissues	No. & (%) with B's in germinal tissues	Total No. & (%) with B's
East Grinstead	8	4	1 (25)	4	-	1 (25)	2 (25)
Englefield Green (Alderhurst)	43	24	5 (20.8)	19	4 (21.1)	8 (42.1)	13 (30.2)
Tilford	42	20	6 (30)	22	10 (45.4)	14 (63.6)	20 (47.6)
Marlow	13	5	-	8	1 (12.5)	3 (37.5)	3 (23.1)
Aston Rowant	10	5	1 (20)	5	2 (40)	2 (40)	3 (30)
Alton	21	13	3 (23)	8	2 (25)	3 (37.5)	6 (28.6)
Stockbridge	19	8	-	11	2 (18.1)	5 (45.5)	5 (26.3)
Tirley	29	14	6 (42.9)	15	8 (53.3)	10 (66.6)	16 (55.2)
Woodchester Park	13	5	3 (60)	8	4 (50)	5 (62.5)	8 (61.5)
Skomer Island	5	1	-	4	-	2 (50)	2 (40)

TABLE 6: FREQUENCY OF B CHROMOSOMES IN DIFFERENT INDIVIDUALS

Locality	sex	MITOSIS						MEIOSIS	
		No of chromosomes				Total No. of examined cells	No. & (%) with B's	Total No. of examined cells	No. & (%) with B's
		48 OB	49 1B	50 2B	51 3B				
East Grinstead	♂	50	-	-	-	50	-	33	3 (9.1)
	♀	49	-	1	-	50	1 (2)		
Englefield Green (Alderhurst)	♂	50	-	-	-	50	-	25	2 (4)
	♂	50	-	-	-	50	-	30	4 (13.3)
	♂	62	5	1	2	70	8 (11.4)	(died)	
	♂	50	-	-	-	50	-	47	4 (8.5)
	♂	48	1	1	-	50	2 (4)	39	6 (15.4)
	♂	42	1	1	1	45	3 (6.7)	23	4 (17.4)
	♂	50	-	-	-	50	-	30	5 (16.7)
	♂	49	1	-	-	50	1 (2)	33	3 (9.1)
	♀	49	1	-	-	50	1 (2)		
	♀	49	1	-	-	50	1 (2)		
	♀	49	1	-	-	50	1 (2)		
	♀	49	1	-	-	50	1 (2)		
	♀	32	2	-	-	34	2 (5.7)		
Tilford	♂	37	3	-	-	40	3 (7.5)	29	5 (17.2)
	♂	50	-	-	-	50	-	26	3 (11.5)
	♂	82	2	1	-	85	3 (3.5)	22	3 (13.6)
	♂	48	2	-	-	50	2 (4)	25	2 (8)
	♂	51	3	-	-	54	3 (5.6)	18	3 (16.7)
	♂	53	2	-	-	55	2 (3.6)	25	4 (16)
	♂	50	-	-	-	50	-	22	3 (13.6)

(cont'd.....

(Table 6: Frequency of B chromosomes in different individuals - continued (2))

Locality	sex	MITOSIS						MEIOSIS	
		No of chromosomes				Total No. of examined cells	No. & (%) with B's	Total No. of examined cells	No. & (%) with B's
		48 OB	49 1B	50 2B	51 3B				
Tilford (cont'd)	♂	51	3	-	-	54	3 (5.6)	24	3 (12.5)
	♂	46	2	-	-	48	2 (4.2)	35	4 (11.4)
	♂	50	-	-	-	50	-	26	2 (7.7)
	♂	56	3	1	-	60	4 (6.7)	18	2 (11.1)
	♂	47	2	1	-	50	3 (6)	24	4 (16.7)
	♂	31	1	-	-	32	1 (3.1)	23	2 (8.7)
	♂	50	-	-	-	50	-	28	2 (7.1)
	♀	45	2	-	-	47	2 (4.3)		
	♀	37	3	-	-	40	3 (7.5)		
	♀	50	1	1	-	52	2 (3.8)		
	♀	37	1	-	-	38	1 (2.6)		
	♀	33	2	-	-	35	2 (5.7)		
	♀	45	1	-	-	46	1 (2.2)		
Marlow	♂	50	-	-	-	50	-	21	1 (4.8)
	♂	45	-	-	-	45	-	20	2 (10)
	♂	46	1	-	-	47	1 (2.1)	30	4 (13.3)
Aston Rowant	♂	49	1	-	-	50	1 (2)	20	2 (10)
	♂	45	1	-	-	46	1 (2.2)	32	4 (12.5)
	♀	50	2	-	-	52	2 (3.8)		
Alton	♂	48	2	-	-	50	2 (4)	25	3 (12)
	♂	42	3	-	-	45	3 (6.7)	died	
	♂	50	-	-	-	50	-	22	2 (9.1)
	♀	45	3	-	-	48	3 (6.3)		
	♀	43	2	-	-	45	2 (4.4)		

(cont'd....

(Table 6: Frequency of B chromosomes in different individuals - continued (3))

Locality	sex	MITOSIS						MEIOSIS	
		No of chromosomes				Total No. of examined cells	No. & (%) with B's	Total No. of examined cells	No. & (%) with B's
		48 OB	49 1B	50 2B	51 3B				
Alton (continued)	♀	52	4	-	-	56	4 (7.1)		
Stockbridge	♂	49	1	-	-	50	1 (2)	20	2 (10)
	♂	50	-	-	-	50	-	25	3 (12)
	♂	50	-	-	-	50	-	60	4 (6.7)
	♂	50	-	-	-	50	-	35	3 (8.6)
	♂	54	2	-	-	56	2 (3.6)	29	3 (10.3)
Tirley	♂	53	3	-	-	56	3 (5.4)	died	
	♂	48	2	-	-	50	2 (4)	died	
	♂	39	1	-	-	40	1 (2.5)	died	
	♂	56	4	-	-	60	4 (6.7)	25	2 (8)
	♂	52	3	-	-	55	3 (5.5)	32	3 (9.4)
	♂	50	-	-	-	50	-	20	3 (15)
	♂	39	1	-	-	40	1 (2.5)	20	2 (10)
	♂	62	1	-	-	63	1 (1.6)	32	4 (12.5)
	♂	50	-	-	-	50	-	20	2 (10)
	♂	56	2	-	-	58	2 (3.4)	25	3 (12)
	♀	56	1	-	-	57	1 (1.8)		
	♀	46	4	-	-	50	4 (8)		
	♀	54	8	-	-	62	8 (12.9)		
	♀	47	3	-	-	50	3 (6)		
	♀	43	2	-	-	45	2 (4.4)		
	♀	55	2	1	-	58	3 (5.2)		

(cont'd....

(Table 6: Frequency of B chromosomes in different individuals - continued (4))

Locality	sex	MITOSIS						MEIOSIS	
		No of chromosomes				Total No of examined cells	No. & (%) with B's	Total No.of examined cells	No. & (%) with B's
		48 OB	49 1B	50 2B	51 3B				
Woodchester Park	♂	70	4	2	-	76	6 (7.9)	22	3 (13.6)
	♂	40	2	-	-	42	2 (4.8)	30	3 (10)
	♂	50	2	-	-	52	2 (3.8)	35	2 (5.7)
	♂	50	-	-	-	50	-	30	1 (3.3)
	♂	56	4	-	-	60	4 (6.6)	25	3 (12)
	♀	66	3	1	-	70	4 (5.7)		
	♀	74	1	-	-	75	1 (1.3)		
	♀	91	2	2	-	95	4 (4.3)		
Skomer	♂	55	-	-	-	55	-	25	3 (12)
	♂	50	-	-	-	50	-	35	7 (20)

individuals and in different numbers (1-3). Cells with 3B chromosomes have been detected in two individuals from Englefield Green (Alderhurst) and cells with 2 B's have been found in few individuals from five different populations. A single B is the usual representation in all the populations.

Size and Morphological character of B's

The B chromosomes are a heterogeneous group both in size and morphology. Different types of B's have been detected in the different populations. Telocentric B's which vary in size from large, as long as the second chromosome pair of the A chromosomes, to small, similar in size to the twenty-second chromosome pair, have been found in seven populations (Fig 17 and 18).

Metacentric B's have been found in two individuals from the Alderhurst population. The sizes of these metacentrics are correlated with the size of the 6th, 8th, 11th, 17th and 22nd of the A chromosomes (Fig. 19). These metacentrics have been found in various combinations with each other (Fig. 19, A 1) and/or with telocentric B (Fig. 19, A 2 & 3).

The 3rd type is a minute B chromosome which is several times smaller than the smallest chromosome of the basic complement. The position of the centromere being uncertain, they appear as acentric fragments. This type of B has been found in only two populations (Fig.20,B).

Dot-like B's have been found only in the Tilford population in combination with the telocentric ones (Fig. 20,A).

G-banding

By means of G-banding it was possible to identify all autosomes and

also the x and y chromosomes. Banding patterns, in combination with the size, increase the reliability of matching homologous chromosomes and identifying the B's. The various populations containing B chromosomes may now be discussed.

Alderhurst:

An investigation of 43 individuals revealed the presence of two classes of B chromosomes, telocentric and metacentric. The telocentrics have been subdivided into three categories according to the size of the B chromosome: large (L), ranging in relative chromosome length from 10 to 13.6 (5B); medium (M), relative chromosome length 6.4 - 8.6 (6B); and small (S), quite similar in size to the smallest chromosome pair of the normal complement, relative chromosome length 5.6 (3B). Because of the numbers of B chromosomes available for measurement is so small, no statistical analysis is possible. A range of banding patterns for the different chromosomes is illustrated (Fig. 18, A). Among the five large chromosomes, three different banding patterns have been identified (L_1, L_2, L_3) and among the six medium sized B's two different banding patterns appear to be present (M_1, M_2). The three small B's are identical both in relative chromosome length and banding patterns (S). Minor variations between similar chromosomes could be attributed to the state of contraction of the chromosome since the number of distinct bands increases with increasing the length of the chromosome (Rowley,1974).

The five metacentric chromosomes have relative chromosome lengths of 6, 6.8, 8, 9.2 and 10.6 and are illustrated in Fig. 19, B. Recognition of banding patterns in three of them was difficult as they are completely

stained (a, b&c). The other two showed one band on each arm. One of them shows the band near the middle of each arm (d), while in the other one, a thin, faintly stained band appears at the distal end of each arm (e). The centromeric regions are deeply stained and clearly recognizable in all chromosomes. These metacentrics are suggested to be isochromosomes, since the two arms of each chromosome are identical. These isologous arms formed from the two sister chromatids of the intermediate unstable telocentric, result from transverse division of the centromere instead of the usual longitudinal one (Hamerton, 1971).

Tilford

Forty-two animals were investigated and showed two classes of B chromosomes. The first consists of telocentric chromosomes of three different sizes (relative chromosome lengths: 5.6, 8.4 and 10); the banding pattern for each size is illustrated in Fig. 18, B. The second class is a dot-like B chromosome which has been found to be heterochromatic by C-banding (Fig. 22, B).

Alton:

A sample of 21 animals revealed three telocentric B's of different sizes (relative chromosome lengths: 5.6, 6.4 and 9.2). Illustrations of the banding patterns for each of them are shown in Fig. 18, C. It is clear that the small B in this population is quite similar both in size and banding pattern to the small B in the Tilford population (Fig. 18, B).

Small telocentric B's have been detected in populations from

Stockbridge, East Grinstead, Marlow and Aston Rowant but no G-banded preparations are available.

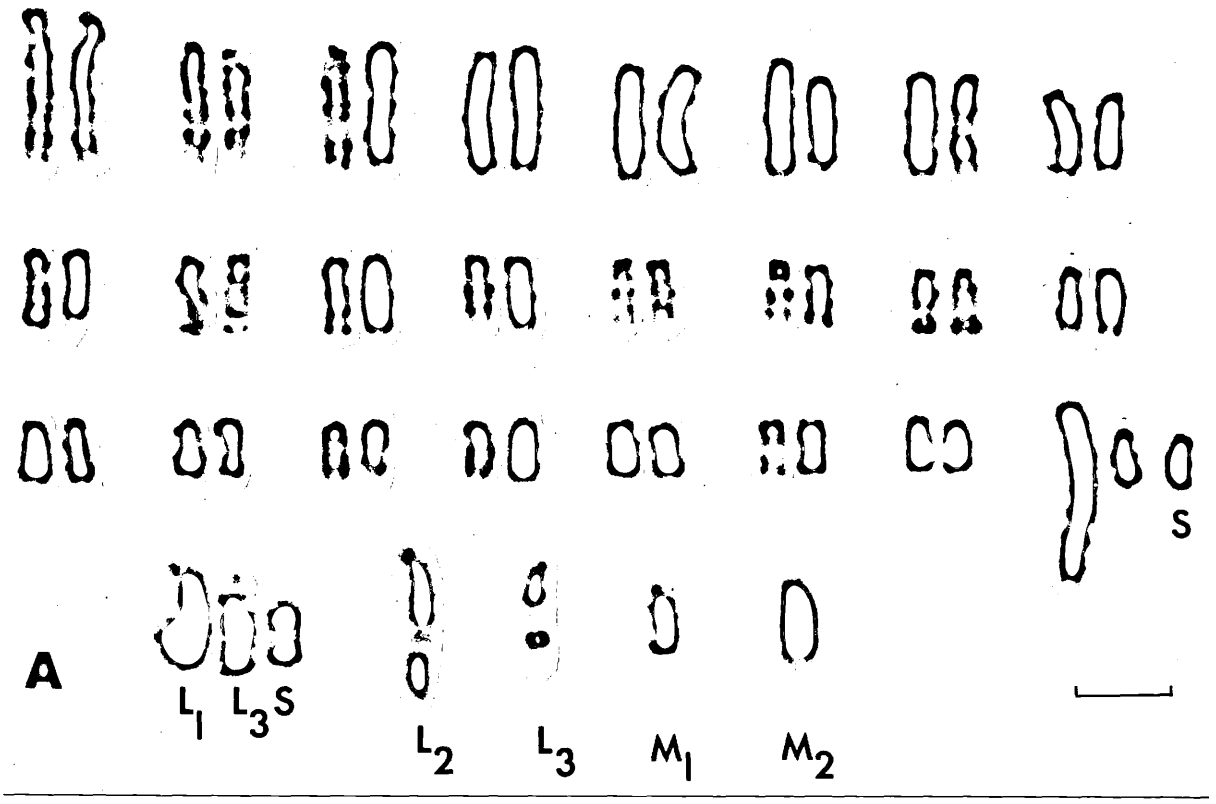
A different class of B chromosome has been detected in populations from Tirley and Woodchester Park in Gloucestershire. The B's characteristic of these populations are minute and heterochromatic, appearing darkly stained in both G- and C-banded preparations (Fig. 21, A and B). Although the two chromatids are usually distinct, it is extremely difficult to detect the centromere in this class of B.

C-banding

In investigating the nature of the B chromosomes, the C-banding technique has been considered the most useful one. Jones and Rees (1982) have stated that the majority of B's in animals are heterochromatic. Bekasova et al. (1980)reported that all the B's found in the Siberian field mouse Apodemus peninsulae were heterochromatic. Application of C-banding technique in the present investigation indicates that the small B's from Gloucestershire and the dot-like B from Tilford consist entirely of heterochromatin (Fig. 22, A and B). Telocentric B's appear to be euchromatic as they are negatively stained with C-banding (Fig. 22, C). Centromeric heterochromatin which appears very clear and darkly stained in all A chromosomes is difficult to recognize in the B chromosomes. The telocentric B chromosome in Fig. 22, B show a very small centromeric heterochromatic area in comparison with that of A chromosomes.

B chromosomes in meiosis

To verify the presence of B chromosomes in A.sylvaticus, meiotic



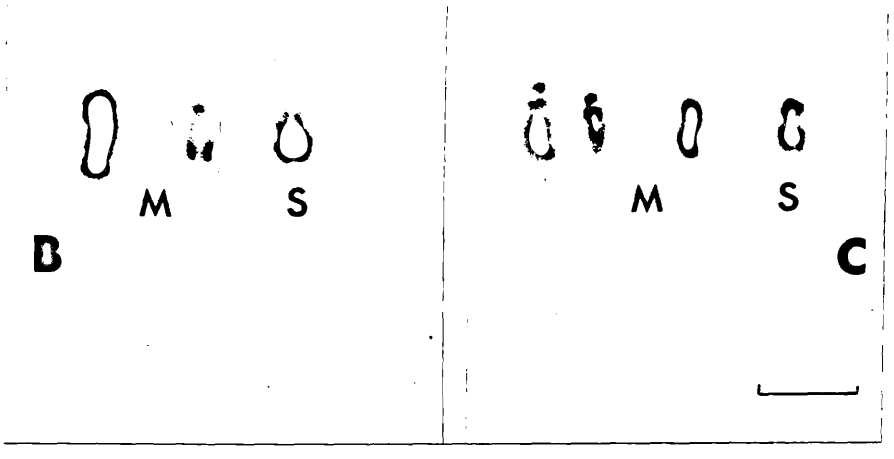


Fig. 17

Fig 17

A - 1st 3 rows: G-banded karyotype of male A. sylvaticus from Alderhurst. 2N = 48 + 1B (small)

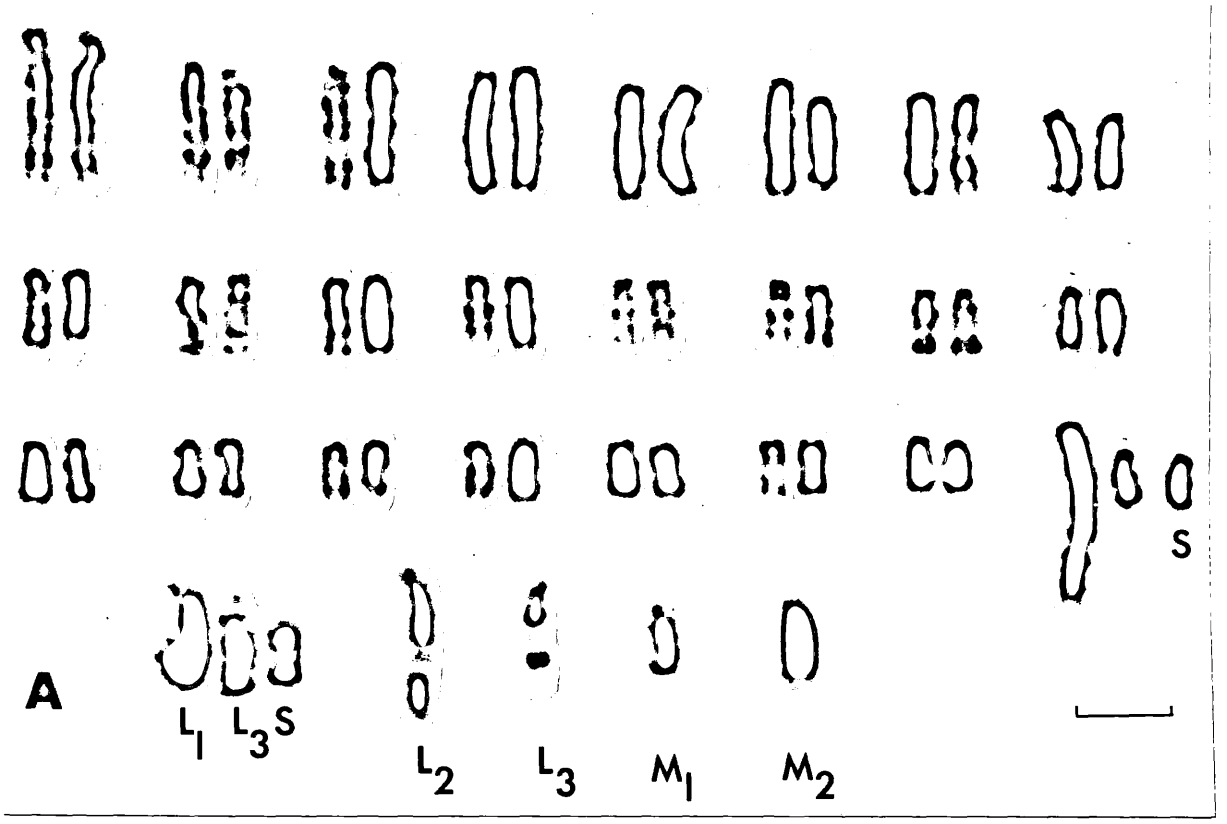
Bottom row: Different telocentric B chromosomes from different animals of the same population, at approximately the same degree of contraction.

B - G-banded telocentric B chromosomes found in the Tilford population.

C - G-banded telocentric B chromosomes found in the Alton population.

For explanation of the lettering of the B chromosomes see Page 87.

Scale bar represents 5 μ m



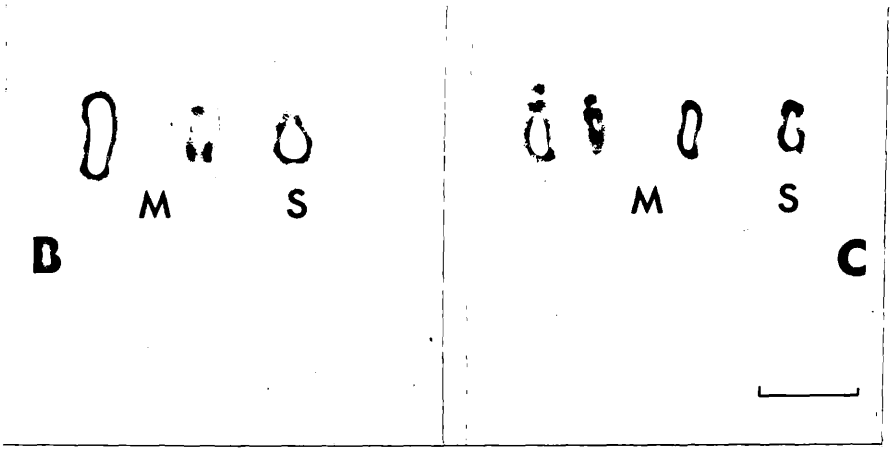


Fig. 17

Fig 18 Idiogram showing the distribution of G-bands in three different sizes of telocentric B chromosomes from different populations.

A - Englefield Green (Alderhurst)

B - Tilford

C - Alton

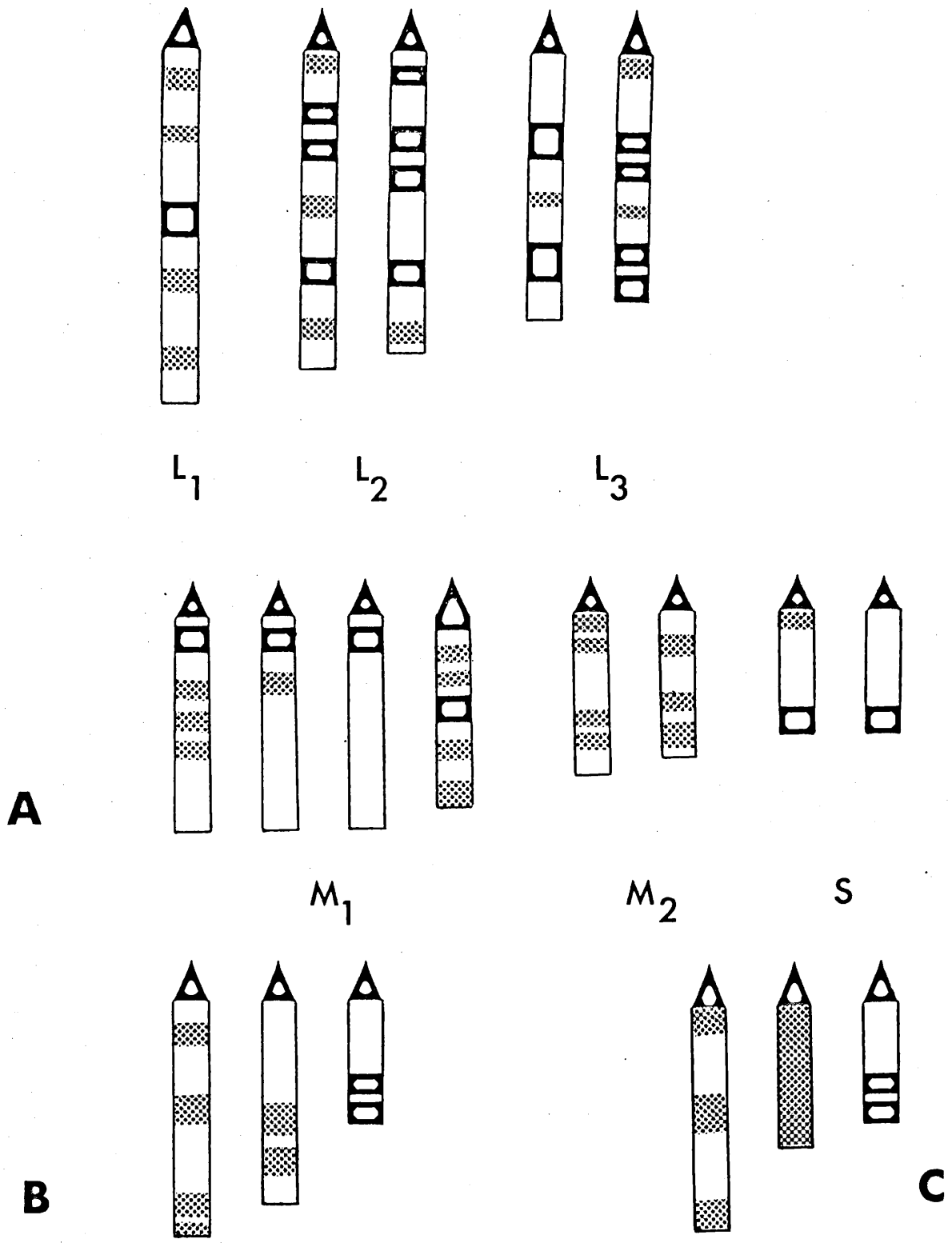


Fig. 18

Fig 19

A - 1st 3 rows: G-banded karyotype of male *A. sylvaticus* from the Alderhurst population. 2N = 48 + 2B (isochromosomes) (1a,d)

Bottom row: B chromosomes from different animals at approximately the same degree of contraction.

2 - 2N = 48 + 3B (1 telocentric + 2 isochromosomes)

3 - 2N = 48 + 3B (2 telocentrics + 1 isochromosome)

Scale bar represents 5 μ m

B - Diagrams of G-banded isochromosomes of two *A. sylvaticus* from the Alderhurst population.

For explanation of the lettering of the B chromosomes, see P. 86 - 87.

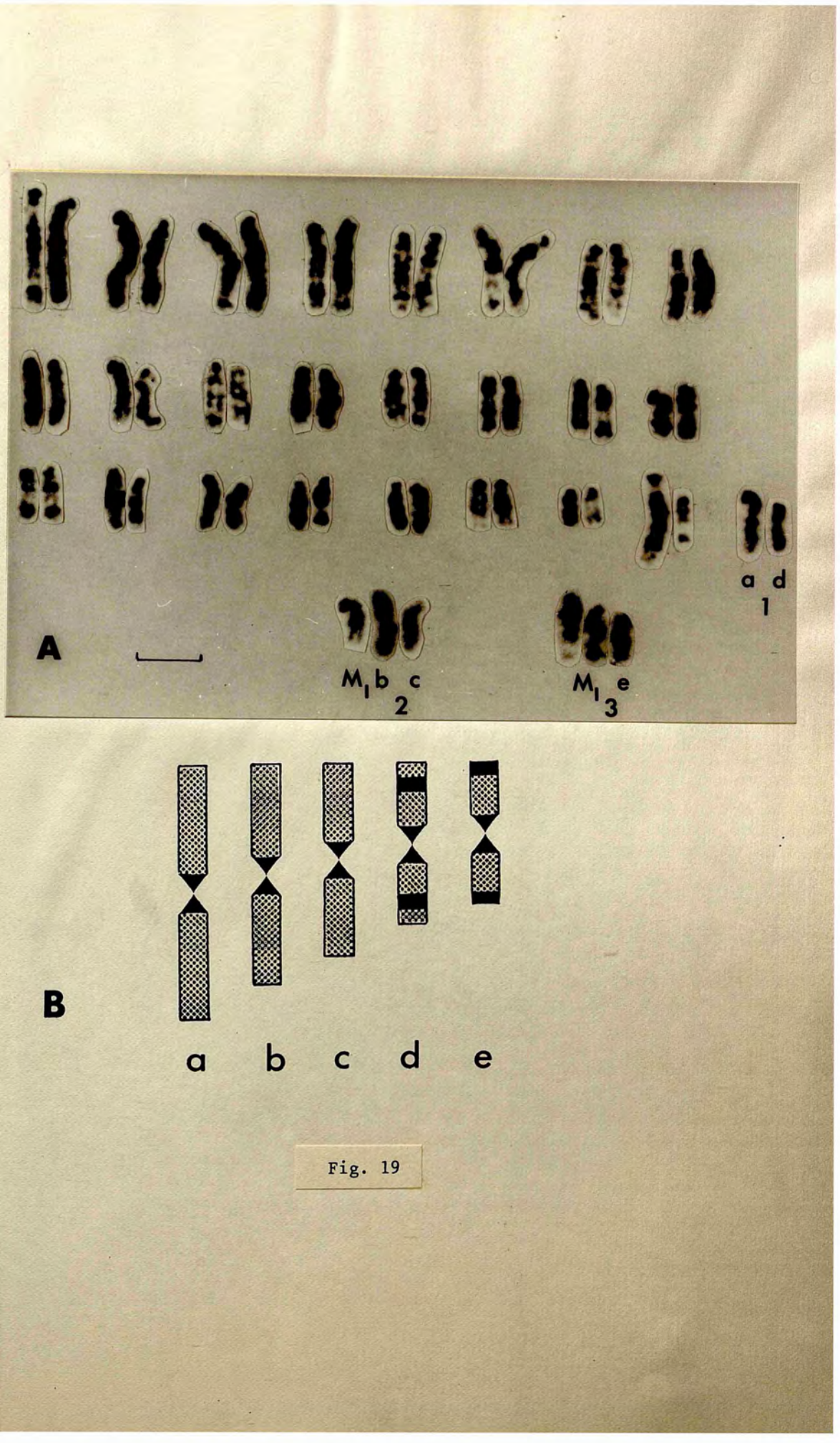
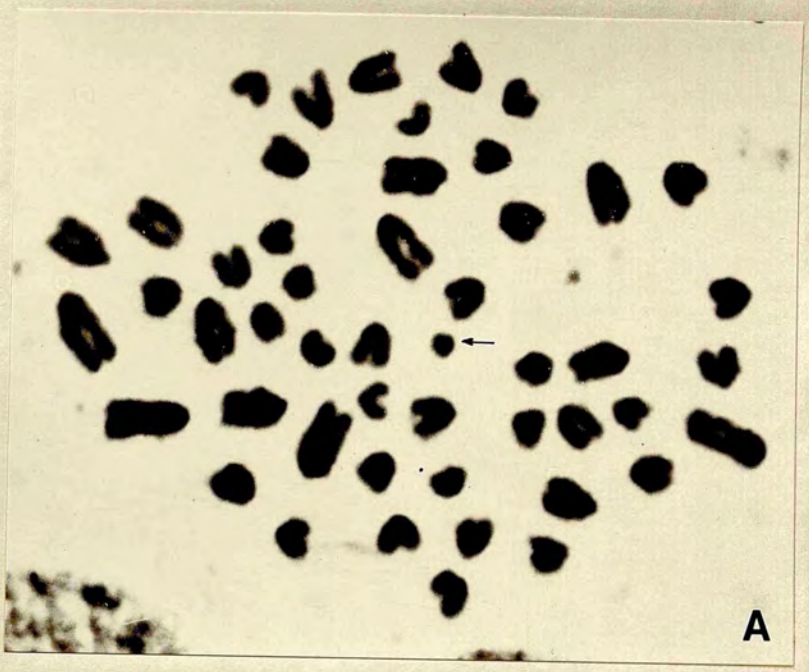


Fig. 19

Fig 20

A - Metaphase plate of A. sylvaticus from the Tilford population, showing a dot-like B (arrowed) (Orcein stained) x 3000.

B - Metaphase plate of A. sylvaticus from Woodchester Park showing a minute B chromosome (Orcein stained) x 3000. (2 nuclei, both with 49 chromosomes, B arrowed in the right hand nucleus).



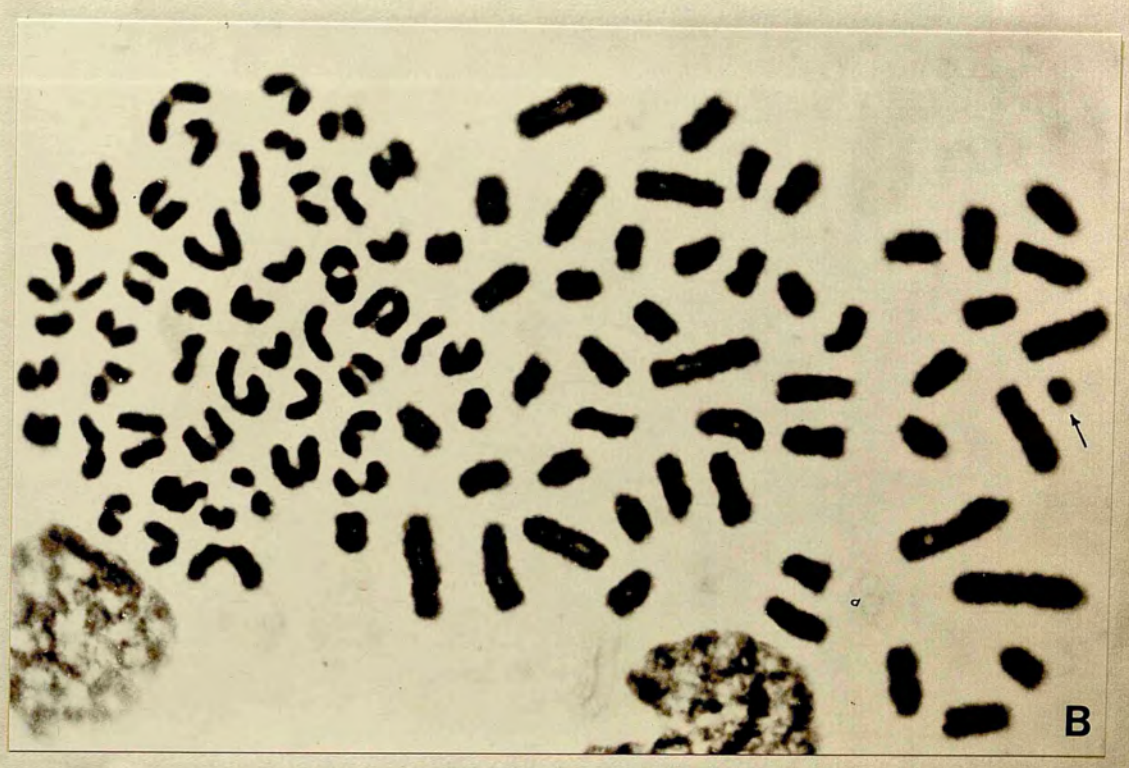
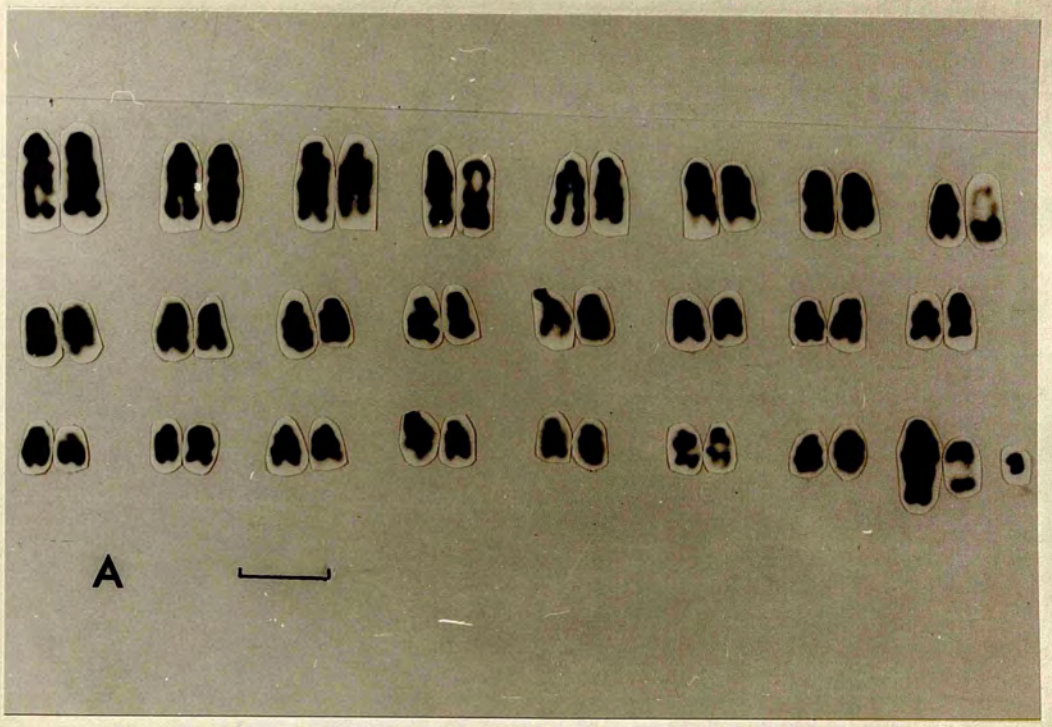


Fig. 20

Fig 21 A - G-banded karyotype of A. sylvaticus from Tirley. 2N = 48 + 1B

B - C-banded karyotype of A. sylvaticus from Tirley with one heterochromatic B chromosome.

Scale bar represents 5 μ m



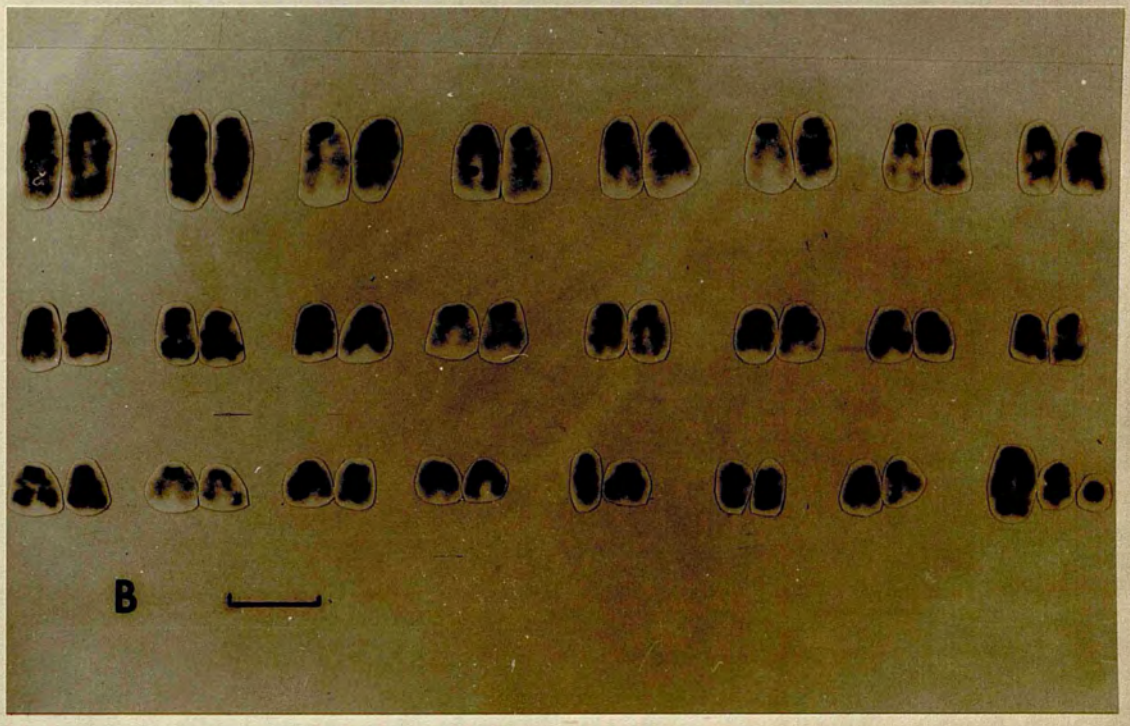


Fig. 21

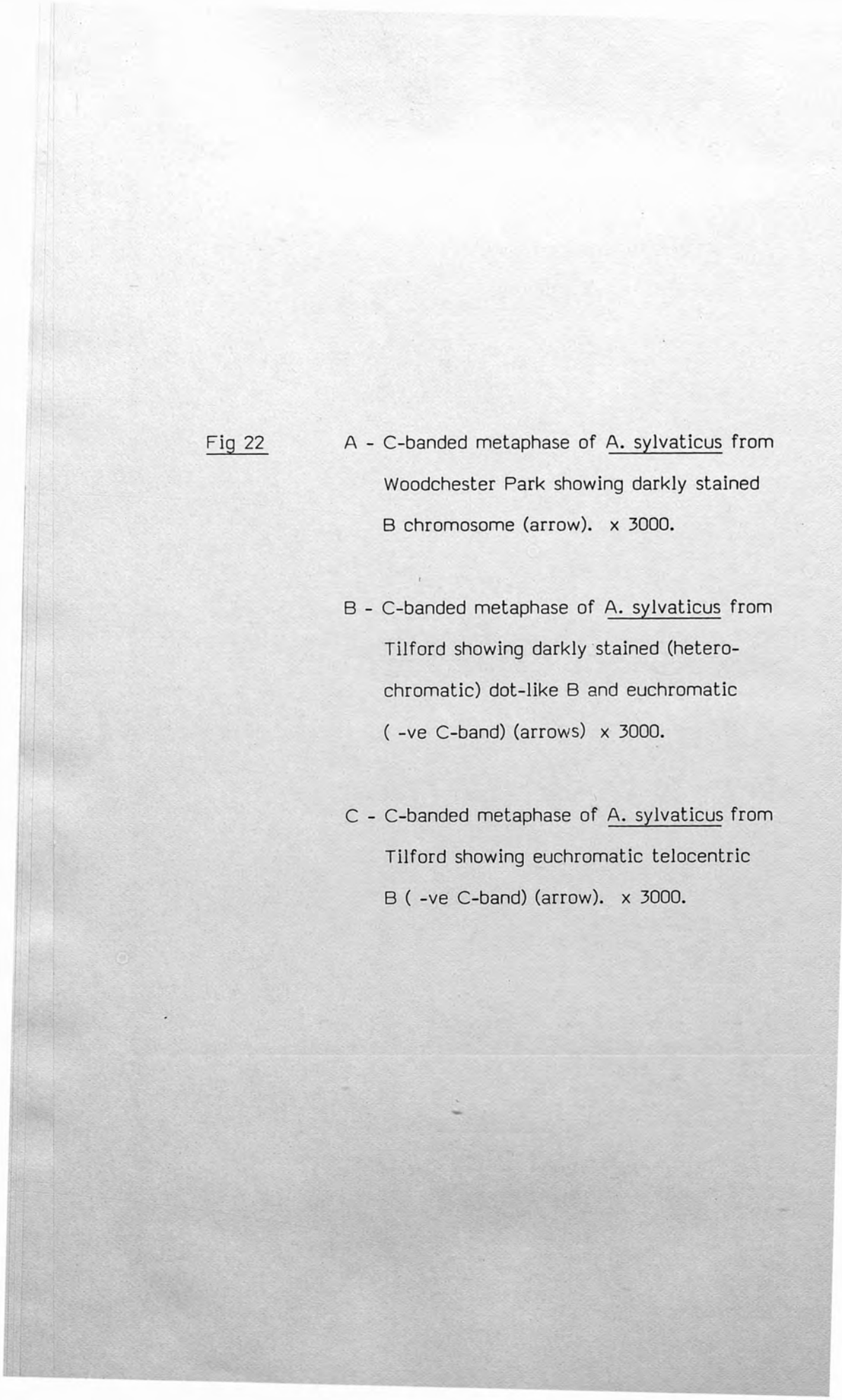


Fig 22 A - C-banded metaphase of A. sylvaticus from Woodchester Park showing darkly stained B chromosome (arrow). x 3000.

B - C-banded metaphase of A. sylvaticus from Tilford showing darkly stained (heterochromatic) dot-like B and euchromatic (-ve C-band) (arrows) x 3000.

C - C-banded metaphase of A. sylvaticus from Tilford showing euchromatic telocentric B (-ve C-band) (arrow). x 3000.

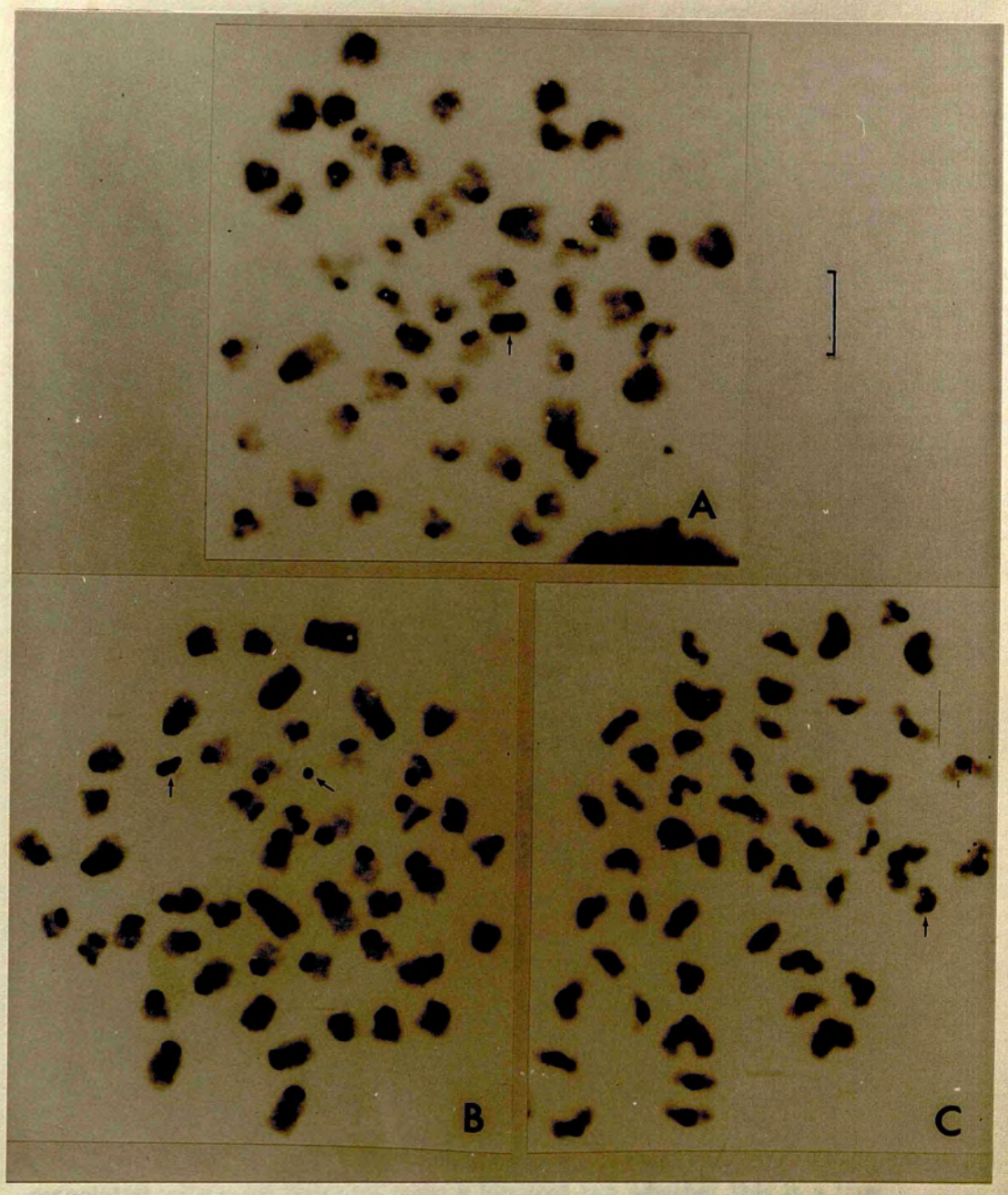


Fig. 22

preparations from the testes of all the males have been investigated. Analysis of the chromosome number in somatic mitosis and spermatogonial meiosis has suggested that the number of B's in germinal tissues is higher than in somatic tissues (table 5) although on the present data this is not statistically significant. In some individuals, B chromosomes have been found in the germinal tissues but not in the somatic tissues. Such accumulations have been reported in the silver fox (Volobuev , 1975) and in the pocket mouse, Perognathus baileyi Merriam (Patton, 1977).

In diakinesis - metaphase 1, the chromosomes of the main set form 23 autosomal bivalents and an xy bivalent which is clearly detected by its characteristic configuration (chapter 3). B chromosomes can be easily detected either as separate bodies or associated with other B's forming bivalents. In cells with a single telocentric B chromosome, the latter stays as a univalent (Fig. 23, A, B and C) while in cells with two homologous telocentric B's, these invariably form a bivalent which is difficult to differentiate from the telocentric bivalents of the basic set (Fig. 23, D).

Single metacentric isochromosomes appear as rings in diakinesis, because chiasmata are regularly formed between the distal regions of the two limbs (White, 1973). As shown in Fig. 23, E and F, two homologous isochromosomes, apparently in the form of highly condensed rings, are associated with each other to form a bivalent but whether this is a true chiasmate bivalent or false bivalent due to a degree of stickiness remains open to question.

As shown in Fig. 23, G, non homologous B chromosomes do not associate with each other. One of them appears lying free in the

cytoplasm while the other is attached to a bivalent of the basic set.

Minute heterochromatic B chromosomes are extremely small and their meiotic behaviour is rather irregular. Some of them do not show pairing at metaphase 1, being left as univalents, usually attached to a bivalent of the basic set (Fig. 24, A, B and C), while others do show association among themselves forming bivalents (Fig. 24, C, D and E), trivalents (Fig. 24, E) and quadrivalents (Fig. 24, F and G).

Five individuals from Skomer Island have been investigated but none of them showed B chromosomes in somatic tissue mitosis. Meiotic preparations from the testes of four males showed B chromosomes, probably representing more than one type (Fig. 25).

Association of B chromosomes with the A chromosomes has been reported by Henriques-Gid, et al. (1982) in Eyprepocnemis plorans (Acrididae; Orthoptera). In two mice from Alderhurst and two from Tilford, B chromosomes have been found associated with the xy bivalent in different positions (Fig. 26). It is difficult to be certain of the nature of the association , either it is chiasmatic or due to a certain degree of stickiness.

Irregularities at meiosis due to the presence of B chromosomes have been reported in many different plant and insect species. Such irregularities include changing the frequency and position of the chiasmata, either by increasing the chiasma frequency (Barker, 1960; John and Hewitt, 1965) or by decreasing it (Simchen , et al. 1971). In rye plants with many B's, Håkansson (1957) found irregularities in the form of

Fig 23 First metaphase of meiosis in germinal cells of A.sylvaticus from Central and Eastern English populations.

A, B & C with one univalent telocentric B chromosome.

D with two telocentric B chromosomes forming a bivalent which is not easily distinguished from other bivalents of the basic set.

E & F with two homologous isochromosomes forming bivalent (double arrowed).

The single arrow in Fig. E indicates a univalent telocentric B.

G Two different telocentric B chromosomes remaining as univalents, one lying free and the other attached to a bivalent of the basic set.

A, B & C of animals from Tilford
D of animal from Stockbridge
E & F of animals from Alderhurst
G of animal from Alton

(→) = univalent B chromosome
(⇉) = bivalent B chromosome

Scale bar represents 5 μ m

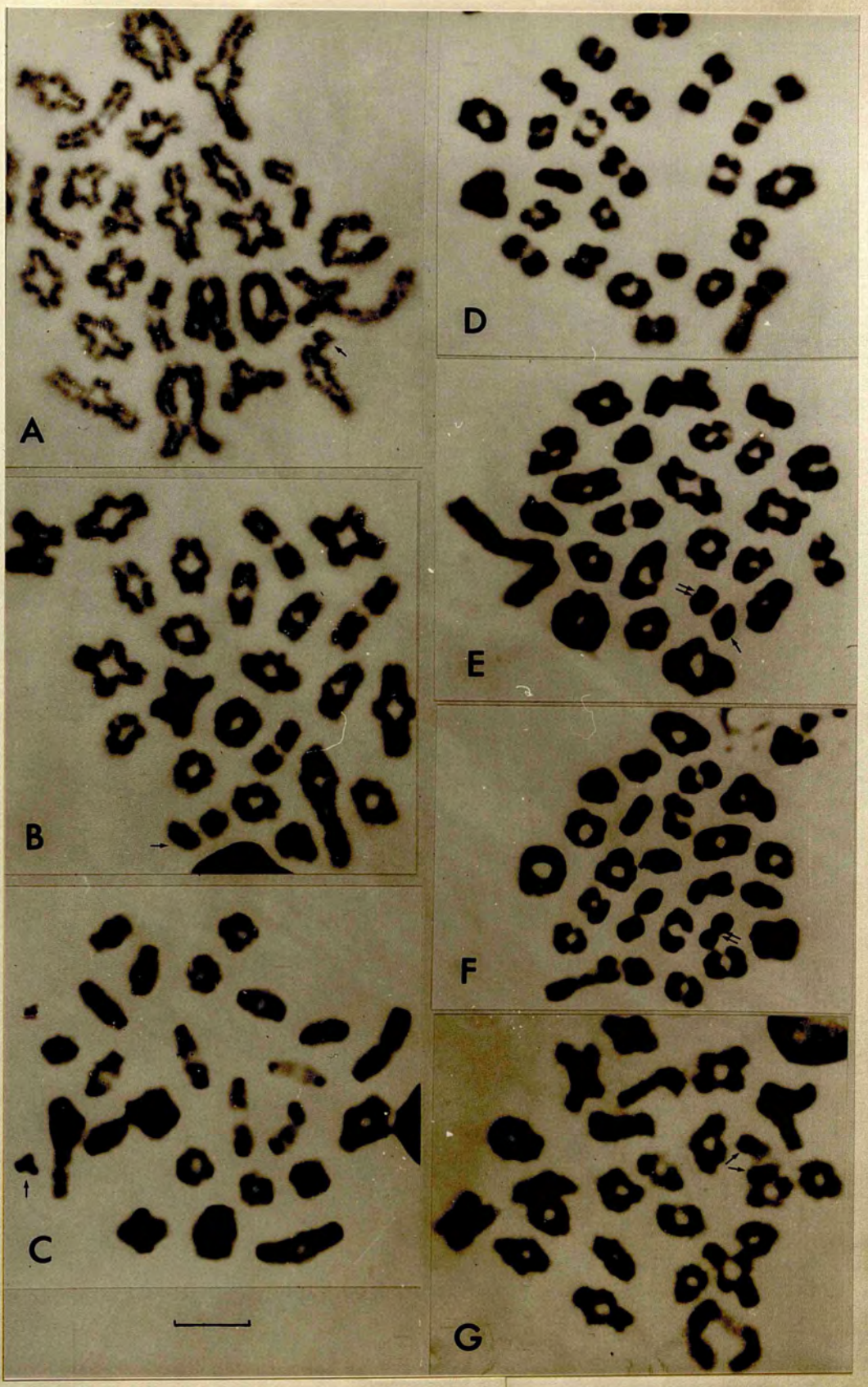


Fig. 23

Fig 24 First metaphase of meiosis in germinal cells of A. sylvaticus from Western English populations, showing the presence and the behaviour of the minute heterochromatin B chromosomes.

A & B each show a minute B chromosome left as univalent attached to a bivalent of A chromosomes.

C 1 bivalent and 1 univalent attached to A chromosome bivalent.

D 1 bivalent

E 1 trivalent and 1 bivalent.

F & G each show 1 quadrivalent which is not easily distinguished from the bivalent of the basic set in G.

A, C, D, F & G of animals from Tirley
B & E of animals from Woodchester Pk.

(→) = univalent B chromosome
(⇉) = bivalent B chromosome
(⇶) = trivalent B chromosome
(⇶) = quadrivalent B chromosome

Scale bar represents 5 μ m

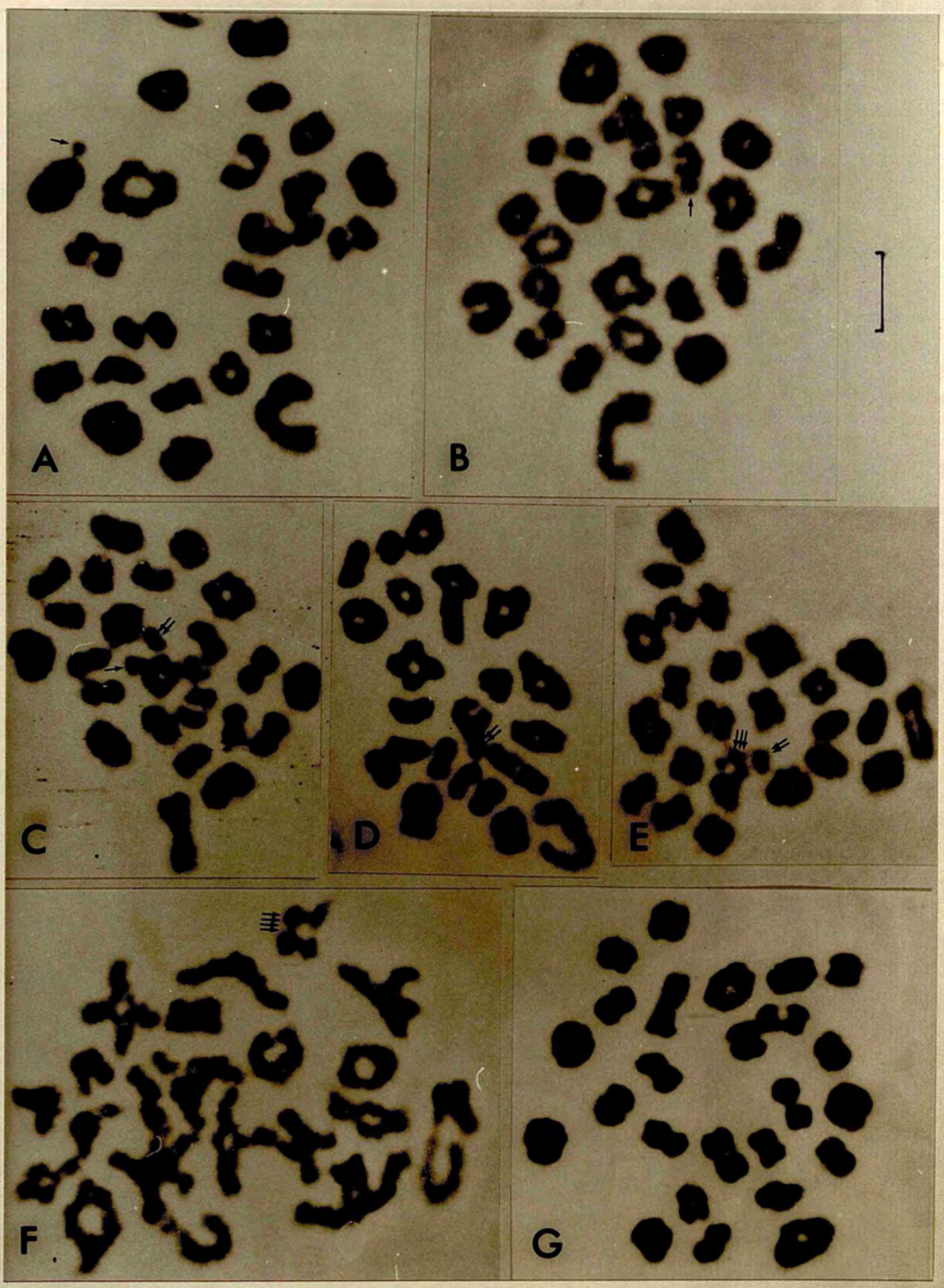


Fig. 24

Fig.25 First metaphase of meiosis in germinal cells of A. sylvaticus from Skomer Island showing different B chromosomes.

A - minute B chromosome left as univalent

B - minute B chromosome attached to a bivalent of the basic set.

C - 1 quadrivalent and 1 univalent

D - 1 bivalent

(→) = univalent

(⇉) = bivalent

(⇶) = quadrivalent

The scale bar represents 5 μ m

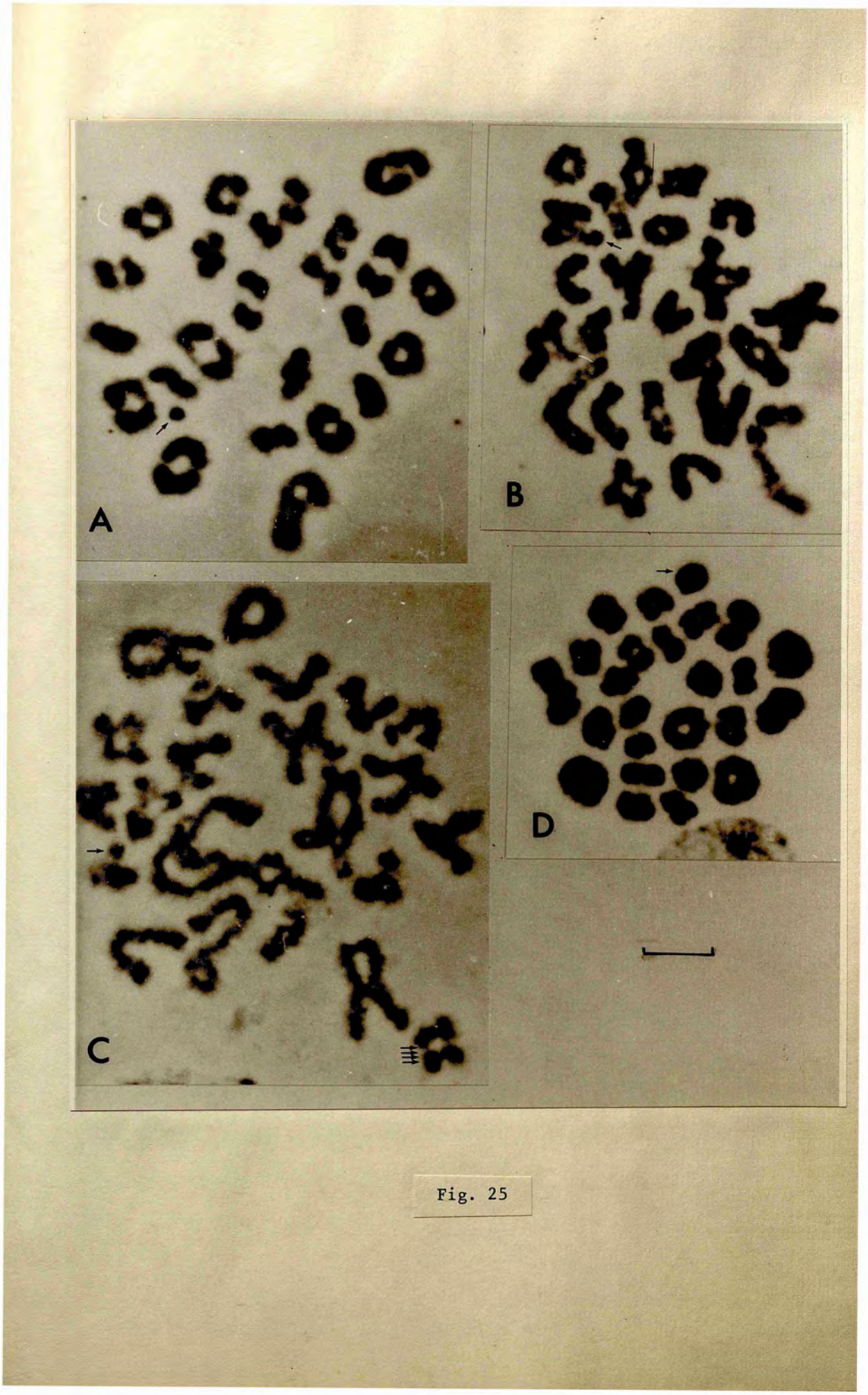


Fig. 25

Fig. 26 First metaphase of meiosis in germinal cells of A. sylvaticus showing the association of B chromosomes with the xy bivalent.

A & B - of animals from Tilford

C & D - of animals from Alderhurst

Scale bar represents 5 μ m

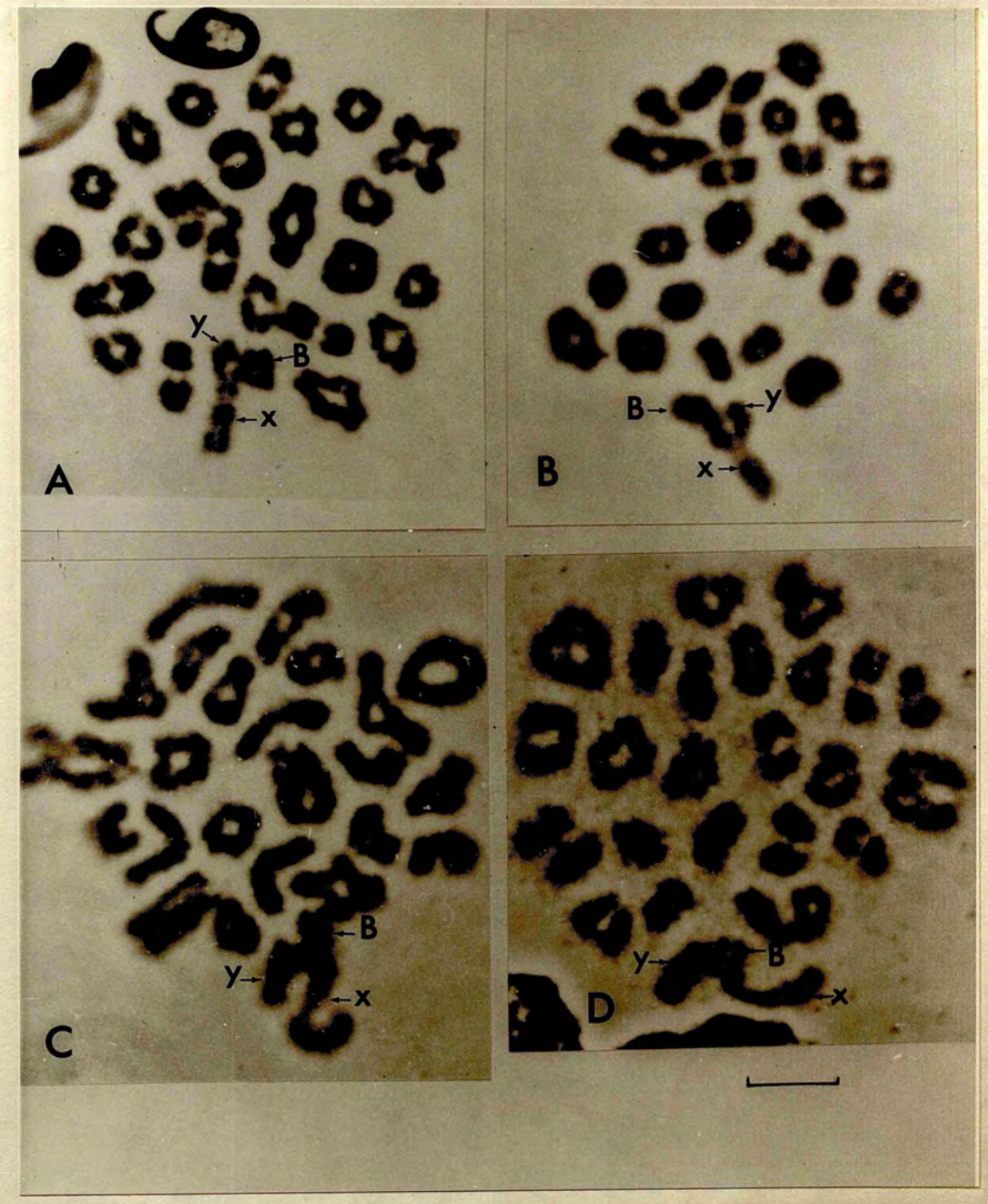


Fig. 26

bridges and lagging at anaphase. In Puschkinia libanotica (Barlow and Vosa, 1970) it was found that B chromosomes disturb the control of cell division.

In mammals, the study of B chromosomes has been largely restricted to karyotyping the diploid metaphase. Analysis of meiosis has been slight and merely concerned with the behaviour of B's. In the present investigation irregularities in the behaviour of the bivalents of the basic set have been detected in animals with B chromosomes but not in those without them. These irregularities occur regardless of whether the nucleus concerned has B's. Thus of 470 preparations from animals with B's 187 (39.87%) had irregularities while none of the 272 preparations from animals without B's showed irregularities.

As shown in Fig. 27, A-D, association between bivalents of the normal basic set is either by tenuous strands that connect different numbers of bivalents together, i.e. 2 or 4, or by direct sticking to each other. In some cases (Fig. 28, A, B and D), there appears to be chiasma formation between the bivalents (giving quadrivalents), but in others (Fig. 28, A, B and C) the association appears to result from stickiness.

The frequency of B chromosomes in different populations

The heterogeneity of the B chromosomes hinders useful comparisons of their frequencies in different populations, because it is difficult to judge to what extent like is being compared with like. The large euchromatic telocentric chromosomes appear sufficiently similar to be grouped together, and the metacentrics, if isochromosomes derived from telocentrics, may also be included here. These chromosomes are

Fig. 27 Irregularities in the behaviour of A chromosomes in meiosis in mice with B chromosomes.

A - D showing association between bivalents of the normal basic set.

(→) by tenuous strands

(≺) by sticking

A & D of animals from Stockbridge

B & C of animals from Alderhurst

Scale bar represents 5 μ m

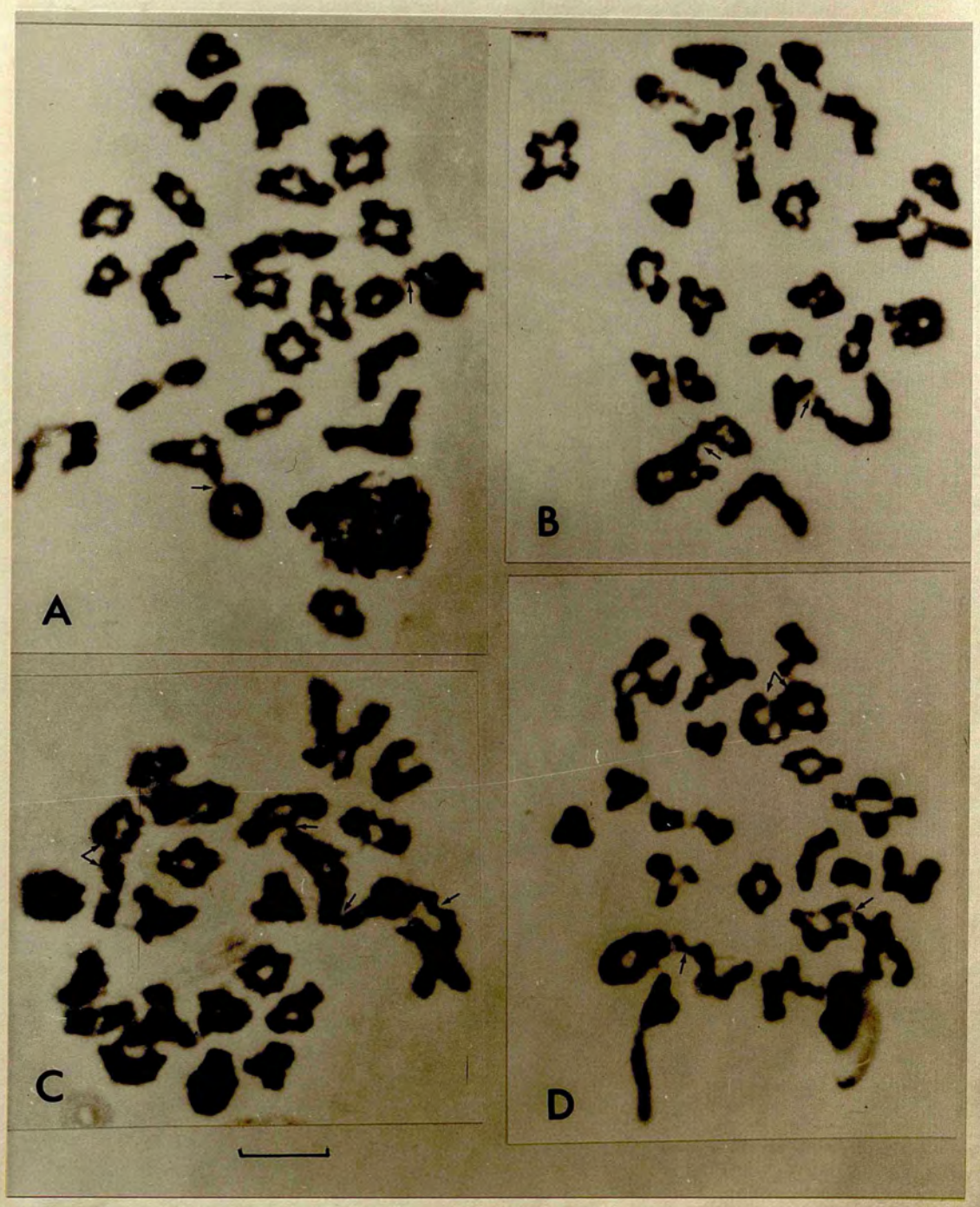


Fig. 27

Fig 28 Irregularities in the behaviour of A chromosomes in meiosis in mice with B chromosomes.

A, B & D showing chiasma formation between 2 bivalents giving quadrivalents (⇌)

A, B & C showing sticking between 2 bivalents (≺) or three bivalents (⋦)

A, C & D showing association between bivalent by tenuous strands (→)

A of animal from Stockbridge

B & D of animals from Tilford

C of animal from Alton

Scale bar represents 5 μ m

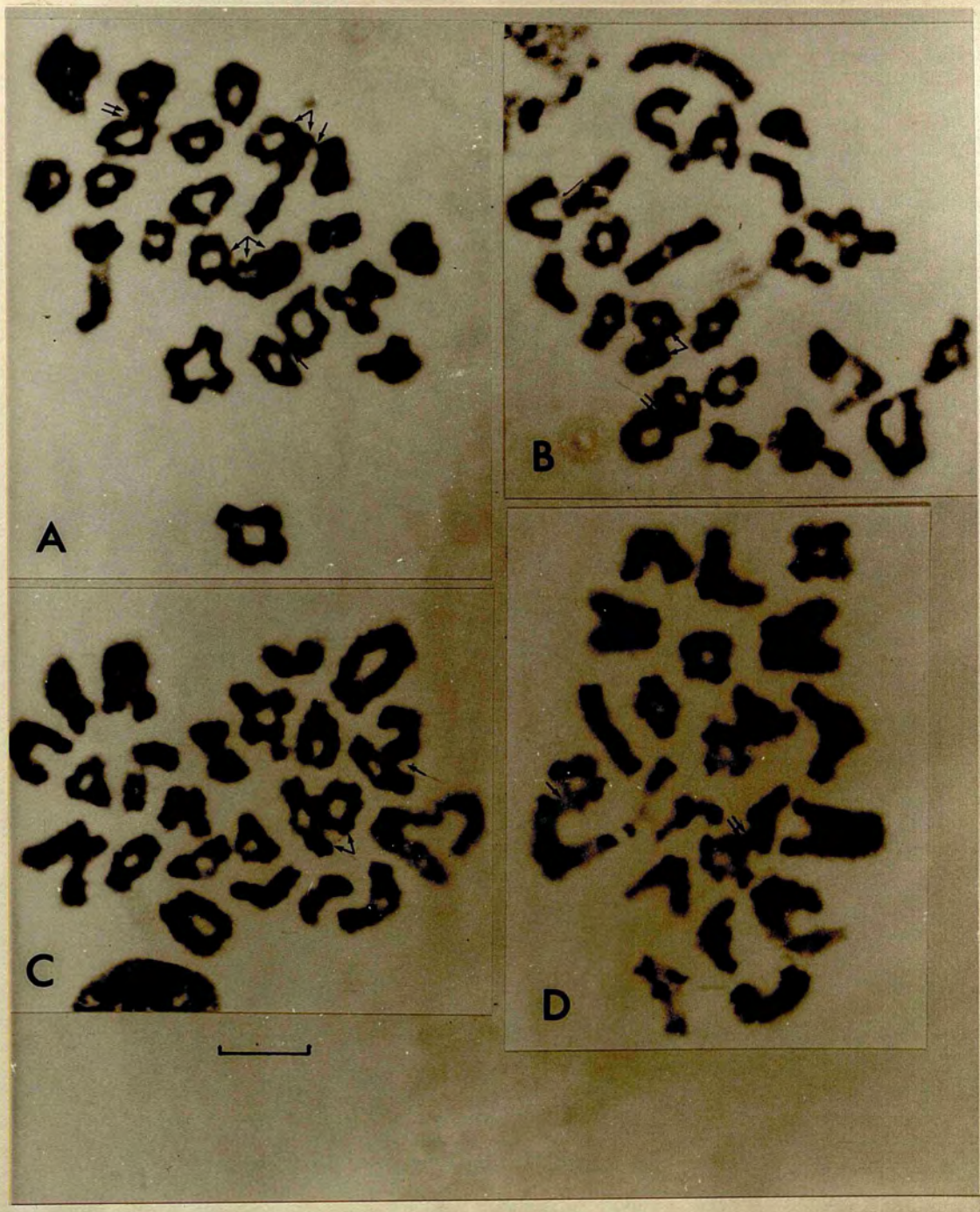


Fig. 28

characteristic of the eastern and central English populations.

The small heterochromatic B chromosomes of the Tirley and Woodchester Park populations appear comparable with each other, but are clearly more different from the euchromatic B chromosomes.

However, it seems wisest to begin with a blanket 9 x 2 contingency Chi^2 comparison of all populations, using the frequency of B's in somatic mitosis to avoid any bias due to the proportion of males in the different samples.

Table 7 shows the various comparisons. Comparison of all the populations show that they all show significant differences from their "expected" values. Examination of the individual contributions to the X^2 shows that the largest inputs are from the Tirley and Woodchester Park populations. If these populations are set aside, comparison of the remaining 7 central and eastern populations shows no significant differences. Similarly, comparison of the Tirley and Woodchester Park populations shows no difference. On the other hand, comparison of the central and eastern populations with the western (Tirley and Woodchester Park) ones shows a difference significant at the 99.9% level. Thus the small heterochromatin B chromosomes of the western populations occur at significantly higher frequencies than the larger euchromatic ones of the central and eastern populations.

Table 7: Comparisons of frequency of B chromosomes in different populations of Apodemus sylvaticus

(a) All Mainland populations:

x^2 18.223 Df = 8 P = 0.0196

Contribution to x^2 :		
	East Grinstead	0.940
	Englefield Green	1.034
	Tilford	0.215
	Marlow	2.637
	Aston Rowant	0.021
	Alton	0.175
	Stockbridge	2.849
	Tirley	5.987
	Woodchester Park	4.346

(b) Eastern and Central populations:

x^2 6.135 Df = 6 P = 0.408

(c) Tirley with Woodchester Park (Western populations)

x^2 0.111 Df = 1 P = 0.738

(d) Eastern and Central with Western populations:

x^2 11.036 Df = 8 P = 0.0009

CHAPTER 5

SEX CHROMOSOME ABNORMALITIES

Result

A total of 203 individuals of A. sylvaticus from ten different populations were studied. Only two females, one from Alton and the other from Woodchester Park, have been found to show sex chromosome abnormalities. In the Alton female most of the cells showed the normal diploid number of chromosomes while a very small number of cells had extra chromosomes (B chromosomes). G-banded karyotypes revealed a deletion in one of the sex chromosomes (Fig. 29, A). Chromosomes of ten well spread, G-banded metaphase plates were karyotyped. All of them showed unequal sex chromosomes (Xx). The relative chromosome length for the normal X is 7.84 while, as a result of a deletion in the other x chromosome, its relative chromosome length is found to be 4.56. As shown in Fig. 29, B, it is a terminal deletion which results in loss of a distal portion of the chromosome.

In a female from Woodchester Park, the chromosomes of 95 well spread metaphase plates were counted. Of these, 92 cells had 47 chromosomes, 2 had 47 + 1B and one had 47 + 2B (Fig. 30, B and C). G-banded karyotypes showed that all the autosomes are in pairs while the sex chromosome is left unpaired (Fig. 30, A).

Morphologically, both the Xx and the Xo females did not differ from the normal individuals. The Xx female was kept in captivity for almost a year. Attempts to breed from this female all failed. This might be usual as some wild-caught pairs refused to breed (Jewell and Fullagar, 1965).

Histological investigation of the ovaries of the Xx female revealed a normal histological structure although the size is smaller than normal (Fig. 29, C). The cross sectional area is about two thirds that of a control

preparation.

In the XO female, both ovaries were considerably smaller than normal, with a cross-sectional area rather less than half that of a control preparation. The right ovary (Fig. 31, A) has a normal histological structure, but the left one is entirely abnormal. As shown in Fig. 31, B, it is made up of two highly cellular masses and therein characterised by many round or oval spaces with sometimes a content of degenerated material. Polyhedral cells are clearly distinct (Fig. 31, C) the cytoplasm is feebly acidophilic and enclosed by a delicate membrane. The nuclei are relatively large and centrally located. This shows complete resemblance to Granulosa-cell tumour of ovary (Ogilvie, 1962).

Fig. 29 A - G-banded karyotype from a blood leu kocyte culture of a female A. sylvaticus from Alton, showing a deletion in one of the x chromosomes.

The scale bar represents 5 μ m

B - Enlarged photograph of the Xx of the same female showing the deleted part (dotted line).

The scale bar represents 23 μ m

C - T.S. of the ovary of the same female showing a normal histological structure although the size is smaller than normal. x 70.

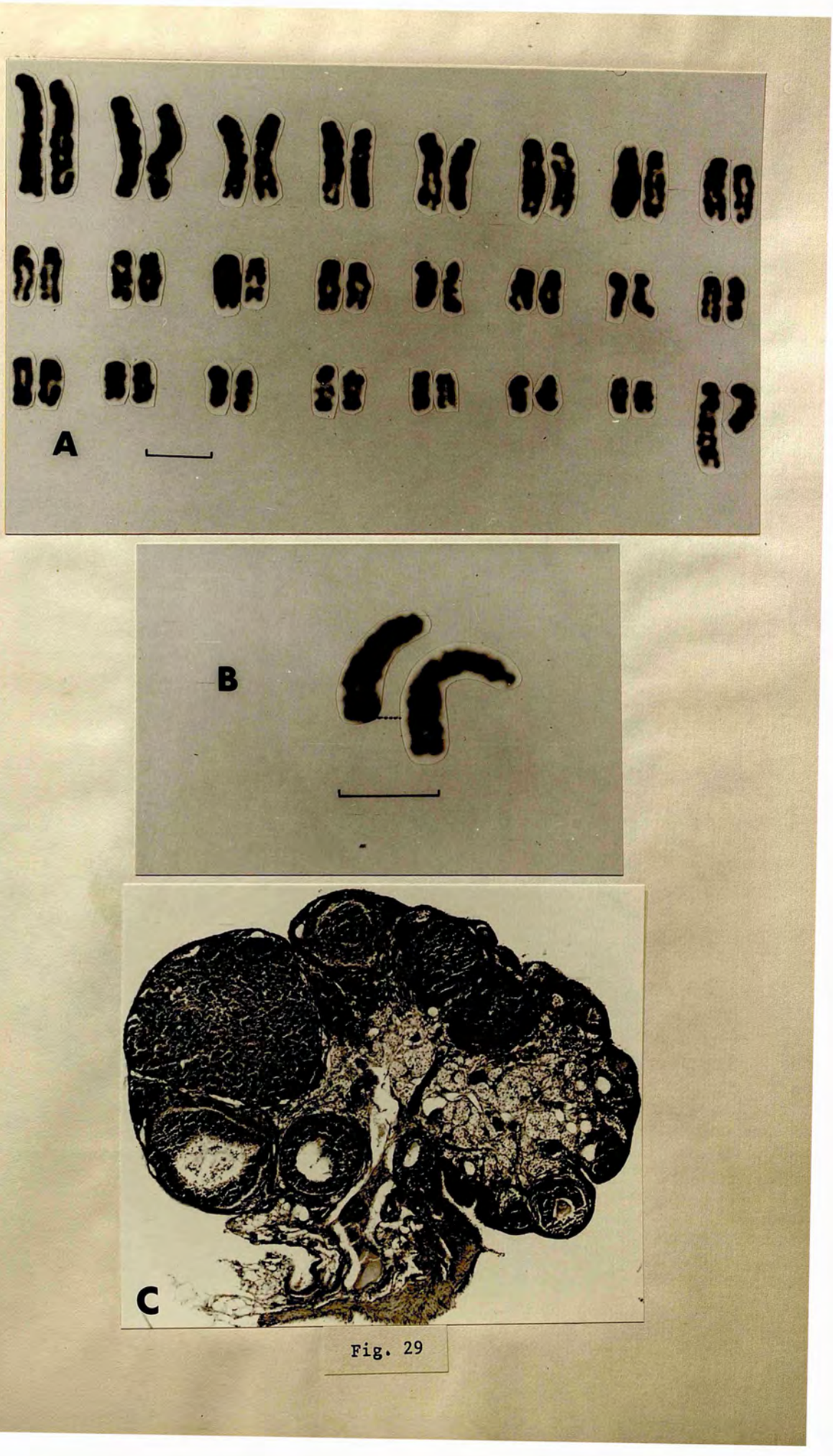


Fig. 29

Fig. 30 A - G-banded karyotype from bone marrow of a female A. sylvaticus from Woodchester Park showing a deletion of one of the sex chromosomes.

The scale bar represents 5 μ m.

A & B - Mitotic metaphase in bone marrow cells of the same female showing different No. of B's (arrows) (Orcein stained). x 3000

B - 47 + 1B

C - 47 + 2B

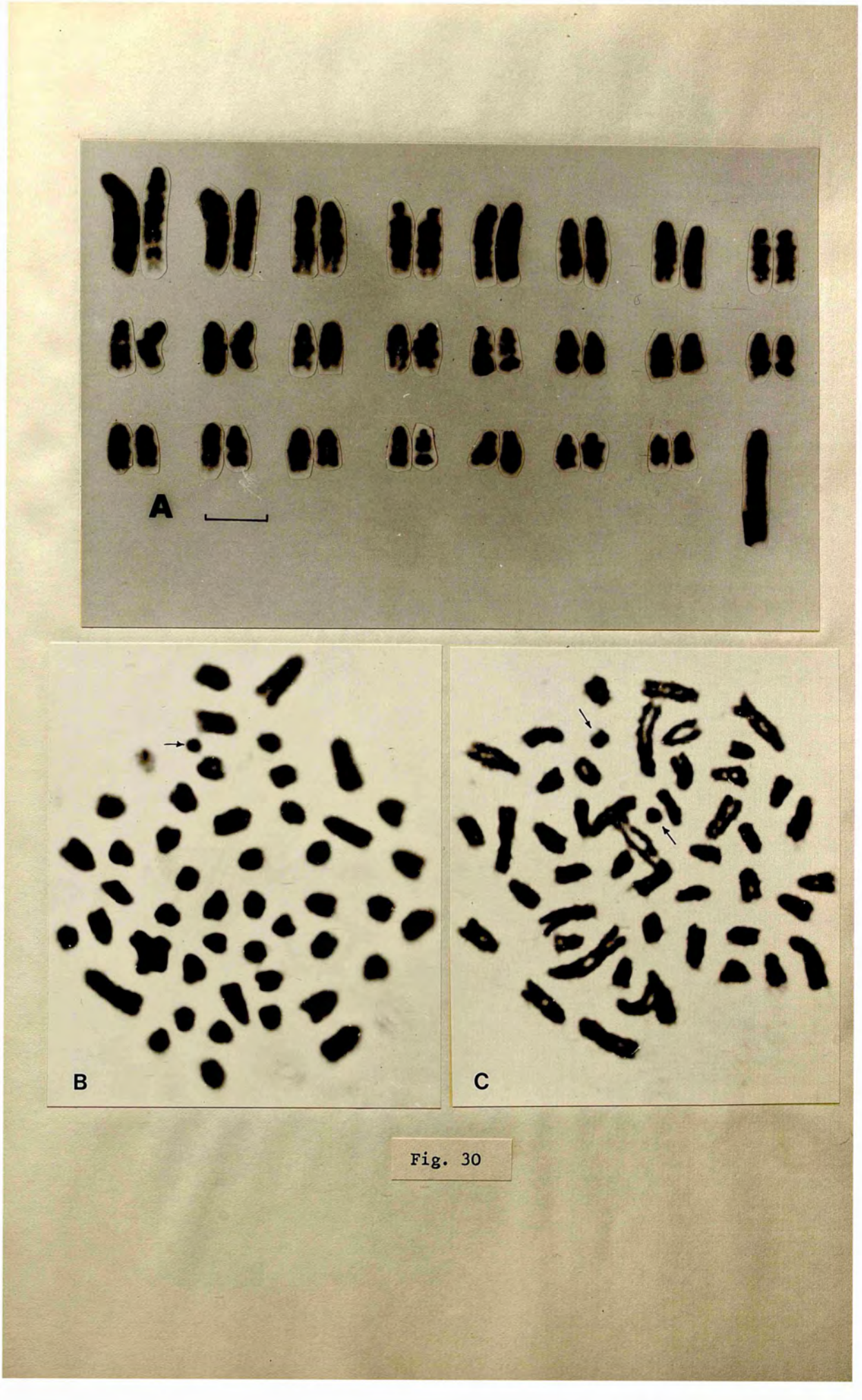
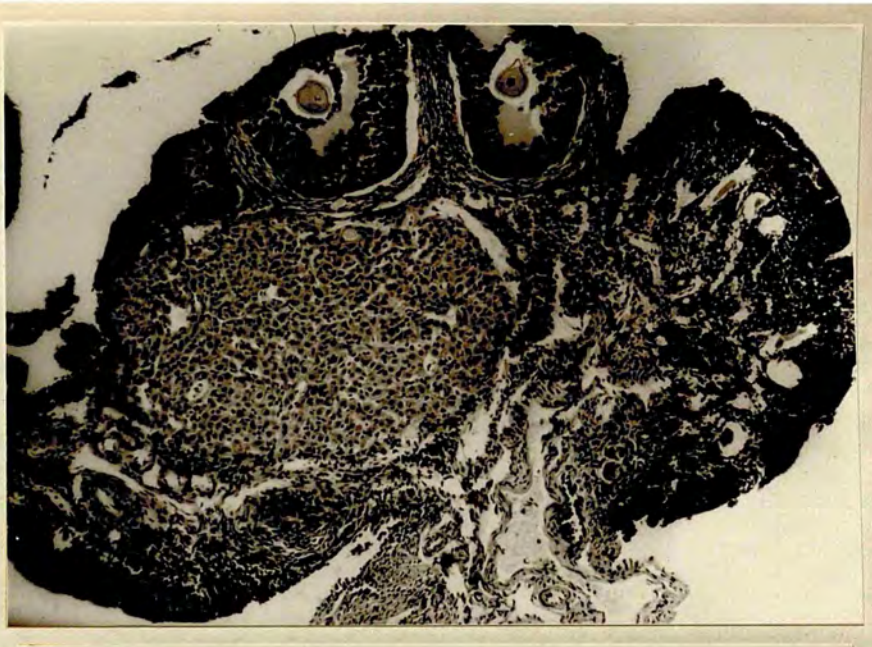


Fig. 30

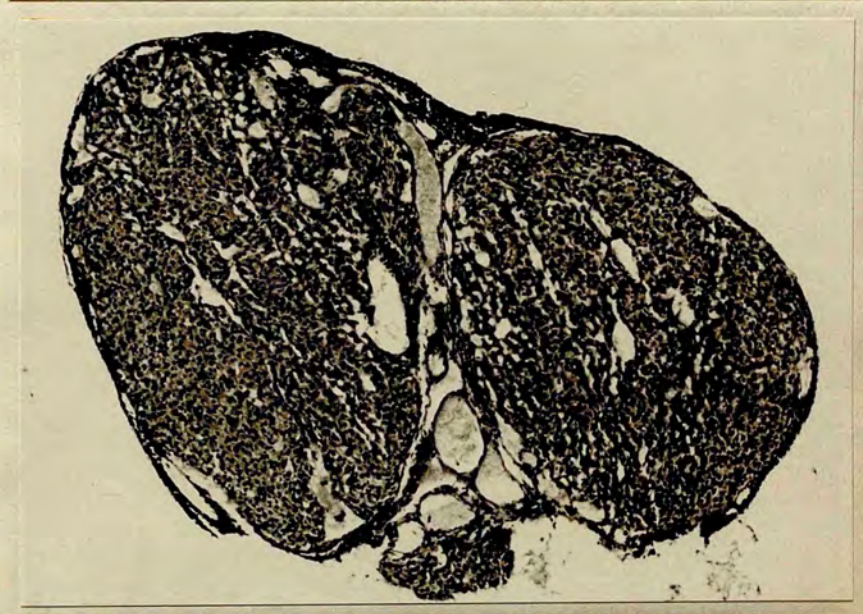
Fig. 31 A - T.S. of the right ovary of xo female from Woodchester Park showing a normal histological structure. x 125.

B - T.S. of the left ovary of the same female showing abnormal histological structure. x 95.

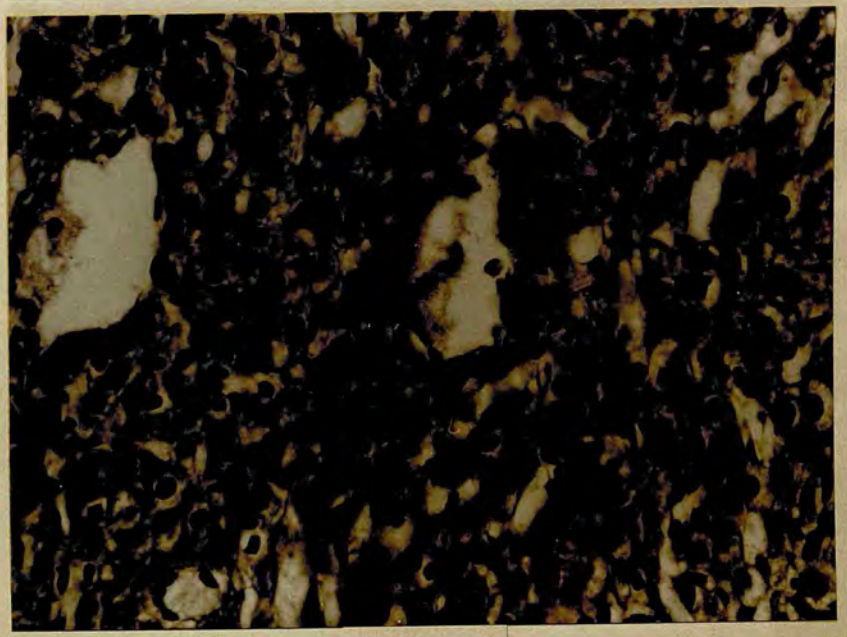
C - Enlarged part of the left ovary. x 500



A



B



C

Fig. 31

CHAPTER 6

KARYOTYPES, G- & C-BANDING OF THE BANK VOLE & SKOMER VOLE

Karyotype and G-banding

Forty-nine mainland bank voles Clethrionomys glareolus and ten Skomer voles (Clethrionomys g. skomerensis) have been cytologically investigated. The diploid chromosome complement is 56. Repeated observations of large numbers of cells in each group have revealed that all karyotypes are identical containing 26 pairs of telocentric and one pair of small metacentric autosomes. The x chromosome was identified as one of the largest telocentrics while the y is a small metacentric. These data agree with those of Renaud (1938); Kral (1971) and Nadler et al. (1976). The relative chromosome lengths of the bank vole and the Skomer vole are given in tables 8 and 9. Differences in the relative chromosome length between mainland and Skomer voles appear to be due to the different state of contraction of the chromosomes in preparations from the two subspecies, rather than to addition, deletion or translocation.

Comparison of G-band patterns of mainland bank voles and Skomer voles yielded similar banding patterns that together were utilized for preparation of the idiogram illustrated in Fig. 32. The preparations from Skomer voles, being of less condensed chromosomes than the mainland ones, show a number of subsidiary bands (Fig. 33, A). It is quite obvious that the G-band pattern of each chromosome is characteristic and specific, so that individual pairs of autosomes can be easily recognised. The x chromosome can be identified by its large size and unique band pattern. Two distinct deeply staining bands are prominent in the middle region of the chromosome, as well as four paler bands, 3 in the telomeric region and one near the centromere. The y chromosome stained completely without any conspicuous banding pattern so that it can be easily distinguished from the small metacentric pair which showed one band on each arm.

TABLE 8: RELATIVE CHROMOSOME LENGTH OF THE CHROMOSOMES OF BANK VOLE CLETHRIONOMYS GLAREOLUS

Chromosome	Relative chromosome length and 95% confidence limits (T-test)		Chromosome	Relative chromosome length and 95% confidence limits (T-test)	
1	5.92 (5.57 - 6.27)	N = 12	15	3.21 (3.03 - 3.39)	N = 12
2	5.69 (5.52 - 5.86)	N = 12	16	3.19 (3.08 - 3.30)	N = 12
3	5.45 (5.04 - 5.86)	N = 12	17	3.02 (2.85 - 3.19)	N = 12
4	5.42 (5.31 - 5.53)	N = 12	18	3 (2.95 - 3.21)	N = 12
5	5.11 (4.91 - 5.31)	N = 12	19	2.97 (2.80 - 3.14)	N = 12
6	5.02 (4.87 - 5.17)	N = 12	20	2.95 (2.82 - 3.08)	N = 12
7	4.85 (4.68 - 5.02)	N = 12	21	2.88 (2.68 - 3.08)	N = 12
8	4.53 (4.41 - 4.65)	N = 12	22	2.63 (2.47 - 2.79)	N = 12
9	4.44 (4.31 - 4.57)	N = 12	23	2.62 (2.36 - 2.88)	N = 12
10	4.10 (3.80 - 4.40)	N = 12	24	2.42 (2.21 - 2.63)	N = 12
11	3.68 (3.53 - 3.83)	N = 12	25	2.28 (2.08 - 2.48)	N = 12
12	3.58 (3.48 - 3.68)	N = 12	26	2.04 (1.88 - 2.20)	N = 12
13	3.46 (3.30 - 3.62)	N = 12	27	1.86 (1.76 - 1.96)	N = 12
14	3.38 (3.24 - 3.52)	N = 12	x	6 (5.67 - 6.33)	N = 8
			y	1.40 (1.15 - 1.65)	N = 4

TABLE 9: RELATIVE CHROMOSOME LENGTH OF THE CHROMOSOMES OF SKOMER VOLE (C.g. skomerensis)

Chromosome	Relative chromosome length and 95% confidence limits (T-test)	Chromosome	Relative chromosome length and 95% confidence limits (T-test)
1	6.36 (6.09 - 6.63) N = 12	15	3.31 (3.19 - 3.43) N = 12
2	6.24 (6 - 6.48) N = 12	16	3.17 (3.05 - 3.29) N = 12
3	5.56 (5.33 - 5.79) N = 12	17	3.12 (3.05 - 3.19) N = 12
4	5.54 (5.29 - 5.79) N = 12	18	3.05 (2.94 - 3.16) N = 12
5	5.26 (5.08 - 5.44) N = 12	19	2.82 (2.60 - 3.04) N = 12
6	5.31 (5.06 - 5.56) N = 12	20	2.77 (2.63 - 2.91) N = 12
7	4.92 (4.76 - 5.08) N = 12	21	2.65 (2.55 - 2.75) N = 12
8	4.62 (4.43 - 4.81) N = 12	22	2.63 (2.49 - 2.77) N = 12
9	4.43 (4.27 - 4.59) N = 12	23	2.31 (2.14 - 2.48) N = 12
10	4.01 (3.85 - 4.17) N = 12	24	2.17 (1.99 - 2.35) N = 12
11	3.56 (3.39 - 3.73) N = 12	25	2.15 (1.99 - 2.31) N = 12
12	3.56 (3.42 - 3.70) N = 12	26	1.91 (1.70 - 2.12) N = 12
13	3.46 (3.27 - 3.65) N = 12	27	1.78 (1.65 - 1.91) N = 12
14	3.35 (3.21 - 3.49) N = 12	x	6.26 (5.54 - 6.98) N = 7
		y	1.44 (0.95 - 1.93) N = 5

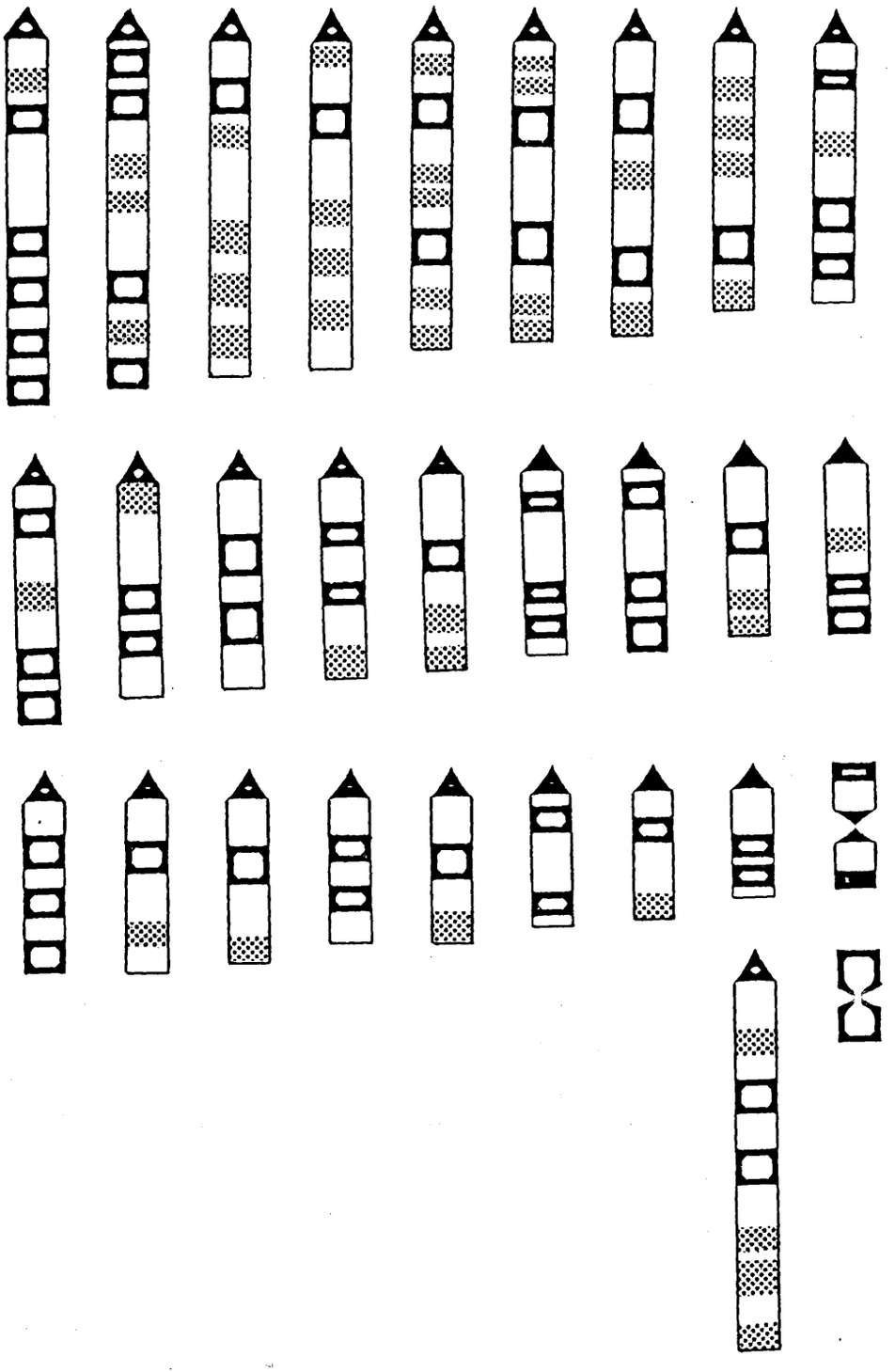


Fig. 32 G-banded idiogram of Clethrionomys glareolus metaphase chromosomes showing the characteristic bands observed in most metaphases of both bank vole and Skomer vole. Major bands are shown in black, minor ones stippled.

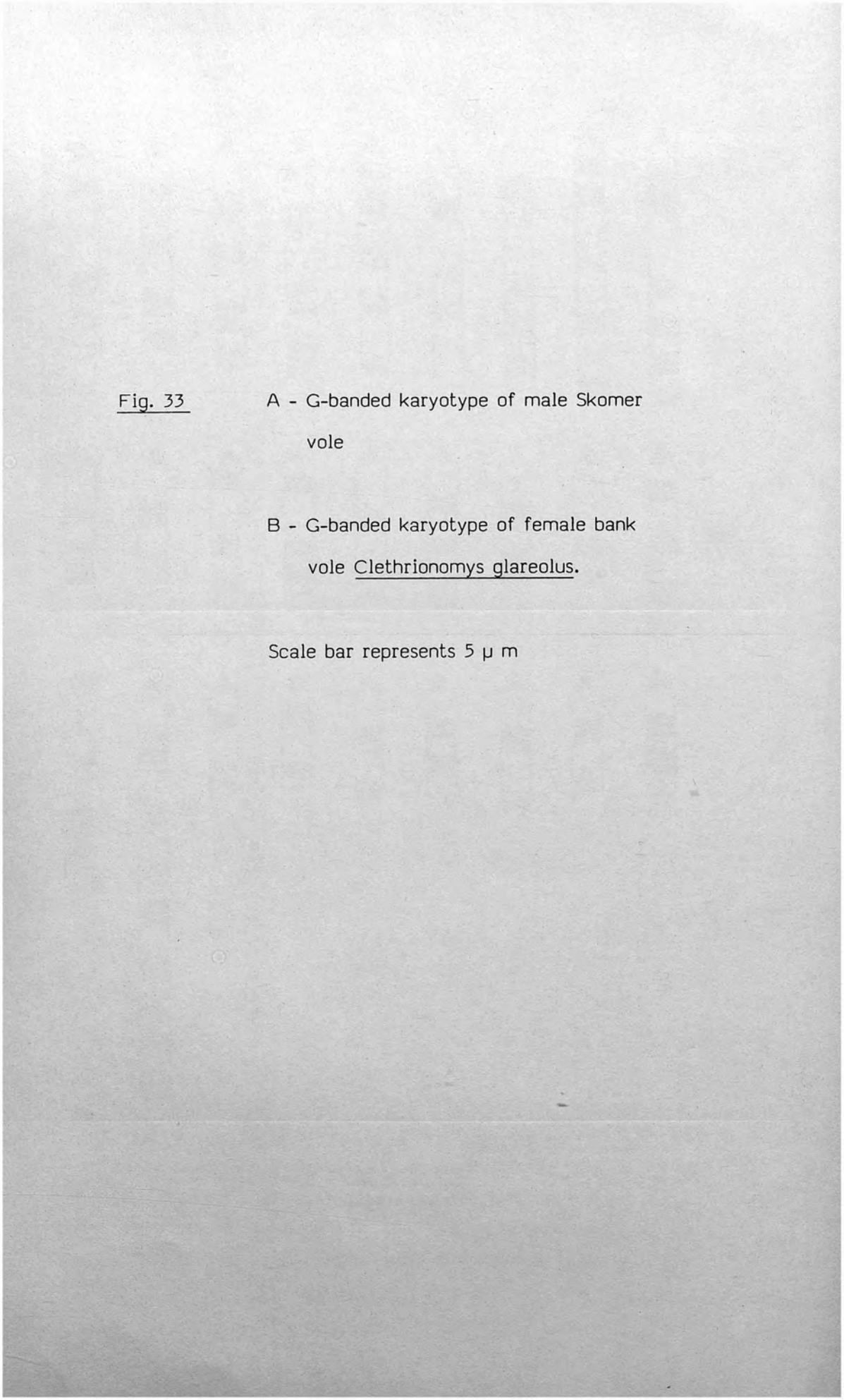
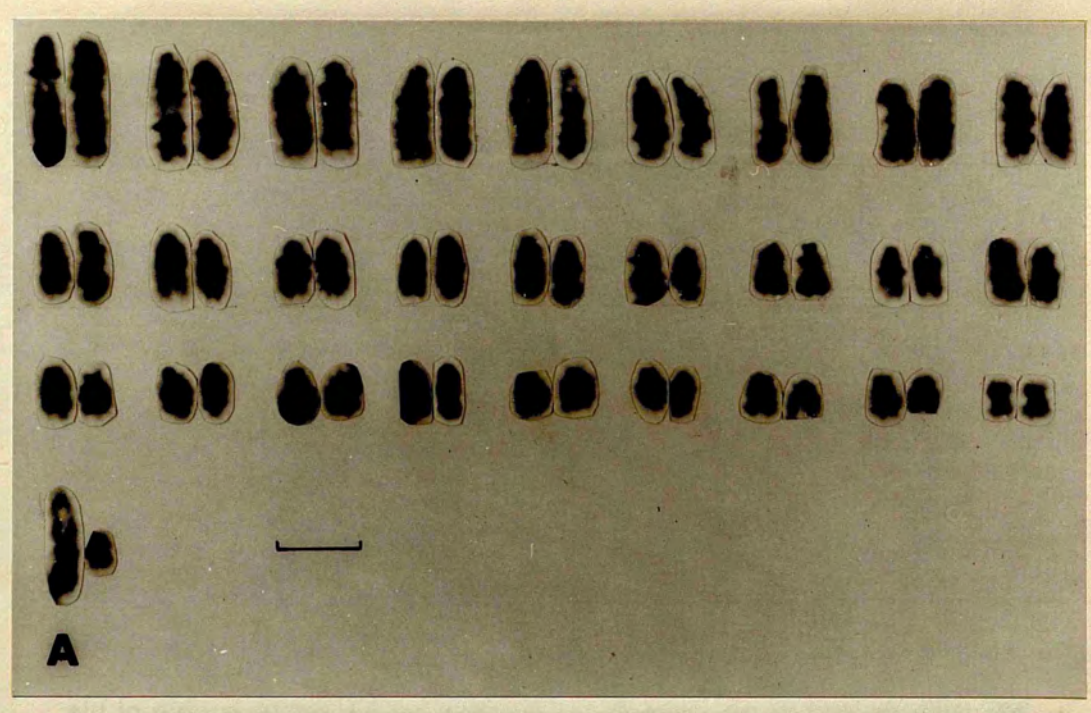


Fig. 33 A - G-banded karyotype of male Skomer vole

B - G-banded karyotype of female bank vole Clethrionomys glareolus.

Scale bar represents 5 μ m



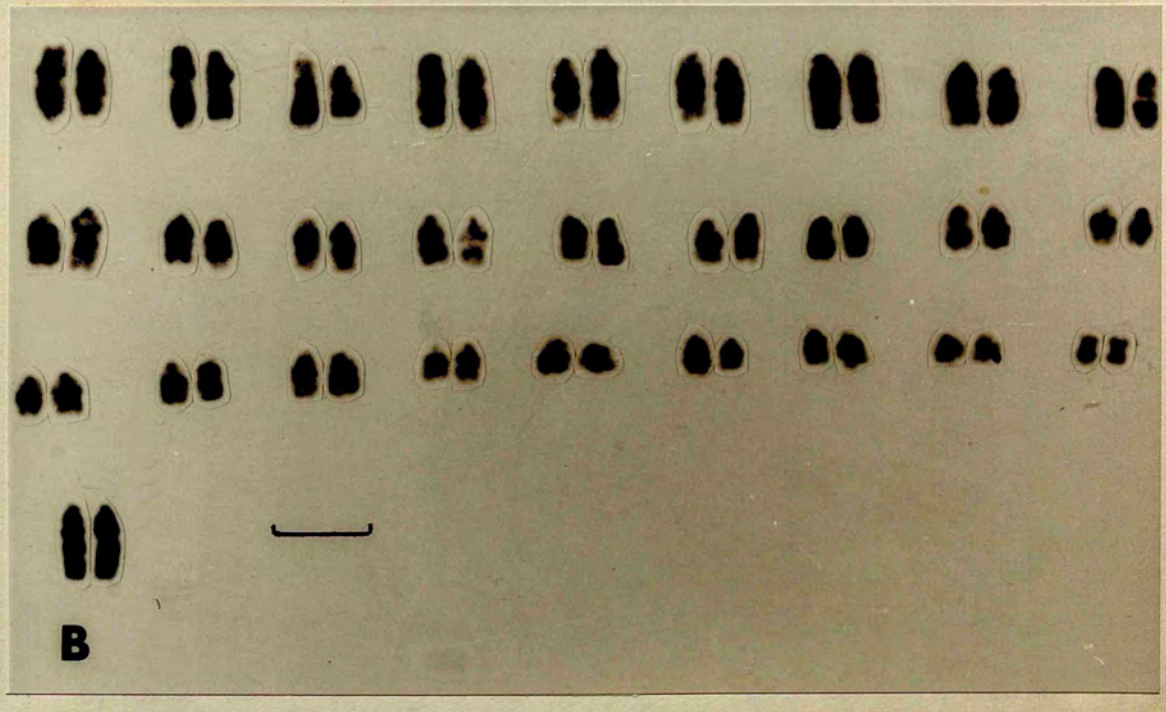


Fig. 33

The use of relatively elongated chromosome results in a greater number of discernible bands (Fig. 33, A). In contracted chromosomes, apparent fusion of bands occurs, resulting in fewer, thicker bands (Fig. 33, B). Most of the minor bands (stippled) are generally obscured as in the idiogram given by Nadler et al. (1976).

C-banding

Figure 34, A and B shows the distribution of constitutive heterochromatin as revealed by C-banding in mitotic metaphase of male Skomer and mainland voles. Every autosome possesses only a small C-band at its centromeric region as does the x chromosome and therefore C-banding cannot aid in distinguishing the autosomal pairs or even the x chromosome. The y chromosome is most easily recognized as it is completely heterochromatic and appears darkly stained with C-banding.

Discussion

The observations given in the present study on the size of the diploid number of chromosomes in the Clethrionomys glareolus from different localities of Britain confirm those described by Matthey and Renaud (1935); Renaud (1938); Shimba et al. (1969); Kral et al. (1971) and Nadler et al. (1976).

The grouping of species of Clethrionomys into two groups based on the presence of telocentric or metacentric y chromosomes was discussed by Rausch and Rausch (1975). Vorontsov et al. (1978) studied the chromosomes of four species of Clethrionomys from 43 localities of Eurasia and North America and reported an interpopulation parallelism of variability of the y chromosome structure in Clethrionomys glareolus, Clethrionomys

rutilus Pallas and Clethrionomys rufocanus Sundevall. Peripheral populations with acrocentric y chromosomes were found in these species whereas the major part of these areas is inhabited by voles with metacentric y chromosomes. In the present investigation a heterochromatic metacentric y chromosome has been found in all the populations studied.

The complete intraspecific homology between G-banding patterns of the chromosomes of the mainland voles and the Skomer island ones reported in the present study supports Godfrey (1959) who reported that the island forms have been derived from the ancestors of the present Clethrionomys glareolus britannicus. Corbet(1964) reported that the principal character distinguishing the island and mainland populations is size and the only character common to all island forms is the large size. This, however, is such a common attribute of island races of small mammals in general that an environmental cause is strongly suggested. In particular the absence of competitive and predatory mammals must constitute a very profound difference in the species environment. Berry (1977) reported that lack of gene-flow into island forms from the same species living under slightly different conditions on the mainland makes local adaptation easier and more precise. Also he added that the presence of few competing species provides increased opportunities for adaptation to a wider variety of environmental opportunities.

Fig 34 C-banding at metaphase of mitosis of *Clethrionomys glareolus*

A - Male Skomer vole

B - Male bank vole

NOTE: y chromosomes (arrows) completely stained

(x 3000)

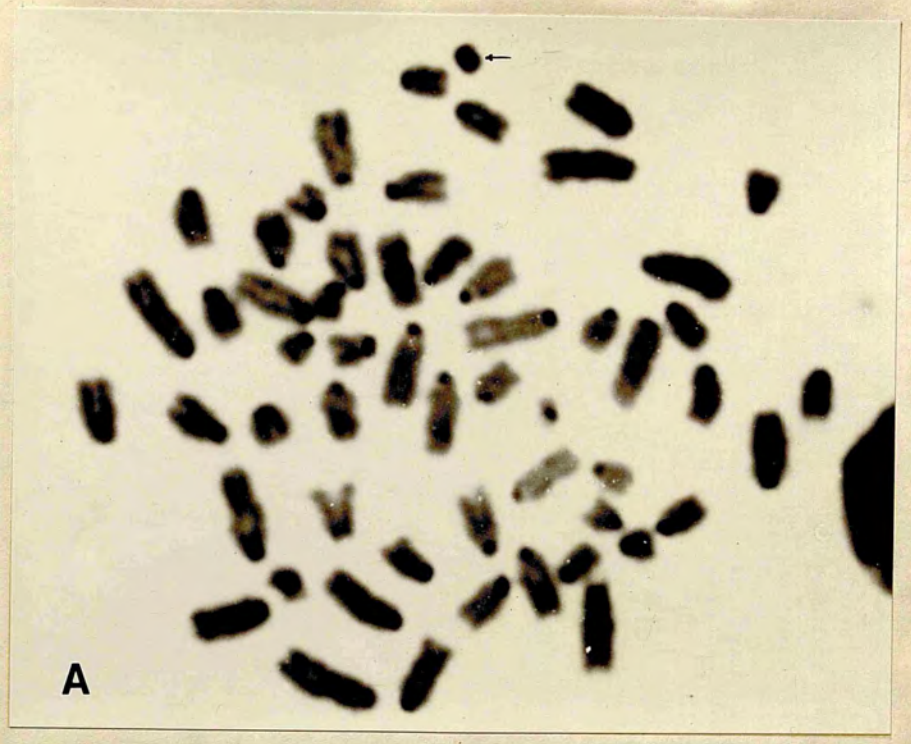


Fig. 34

CHAPTER 7

GENERAL DISCUSSION

Introduction:

Karyotypes of species within a particular genus may either be very similar or vary greatly. Karyotypic variation may occur not only within and between populations but also between races within a species. In the present study, the precise analysis of chromosome banding patterns has shown both inter and intraspecific variation in two species of the genus Apodemus from different localities in Britain.

Discussion of the results must be divided into the following topics:

I Interspecific variation (species comparison)
(a) Karyotypes, (b) Banding and (c) Meiosis

II Intraspecific variation
(a) B chromosomes (b) Sex chromosome deletion

I Interspecific variation (species comparison)

(a) Karyotypes

The standard karyotype of the two species of field mouse occurring in Britain, the wood mouse, A. sylvaticus and the yellow-necked mouse, A. flavicollis consists of 48 telocentric chromosomes, 46 autosomes gradually decreasing in size and a pair of sex chromosomes. These data agree with those of Federley (1919), Raynaud (1936), Matthey (1936 a & b), Zivkovic et al. (1966), Bishun (1968) and Kral (1970) in A. sylvaticus and Matthey (1936 a & b) and Kral (1970) in A. flavicollis. The papers of the above authors, however, give no measurements of the chromosomal length. It is only Kral who gave detailed measurements of the autosomes and the sex chromosomes of both species as well as other species of the

genus Apodemus. The measurements given in the present study agree with those of Kral (1970) in giving the lengths of the autosomes of A. flavicollis as larger than those of A. sylvaticus for the first twelve chromosome pairs, and smaller for the rest of the autosomes. However, the relative chromosome lengths for the sex chromosomes do not agree at all with those given by Kral. The x chromosome is not the largest one in the set but in both species is second to largest. In A. sylvaticus, however, this sex chromosome is markedly larger in comparison with the same chromosome in A. flavicollis. Also the y chromosome is not a medium sized one, ranking in the 13th place in the idiogram , but one of the smallest chromosomes, being similar in length to the 22nd autosome of A. sylvaticus and the smallest one in A. flavicollis. Kral's measurements were based on unbanded karyotypes which might lead to mistaken identification of the sex chromosomes. Koller (1941) arranged the different species of Apodemus according to the size of the y chromosome, as follows:- A. speciosus ainu Thomas, (with the smallest y), A. agrarius ningpoensis Swinhoe, A. geisha Thomas, A. speciosus speciosus Temminck, A.hebridensis (generally regarded as a form of A. sylvaticus), A. agrarius Pallas, A. flavicollis and A. sylvaticus (with the largest y), and he assumed that differentiation of the sex chromosomes is a gradual evolutionary process. My results support Koller in showing A. flavicollis to have a smaller y chromosome than A. sylvaticus.

The above mentioned chromosome characteristics, however, do not offer sufficiently distinct criteria for reliable karyological separation of individual species. Kral (1970) put them into a chromosomally uniform

subgenus (subgenus Sylvimus) and stated that apart from fine differences in the phenotype of these closely related Apodemus species, still finer differences can be found in their chromosome sets.

(b) Banding

Examination of G-banded preparations of the two British species has demonstrated an overall difference in staining reaction and in the degree of differentiation between bands and interbands. Despite this difference the identification of individual chromosomes is relatively easy and the major bands characteristic for most of the larger chromosome pairs are quite similar in both species, suggesting a high degree of homology between the chromosomes of the two species. Only chromosome pairs No. 4 and 5 appear different. In the smaller chromosome pairs (Nos. 17 - 23) the banding patterns are too limited to be useful.

In view of the fact that different stains used for G-banding are believed to stain different components of chromatin (namely, chromatin, DNA and histones) Yunis and Sanchez (1973) suggested two possibilities with respect to the nature of the band and interband regions: in the first, the two regions are possibly reflections of the concentration and arrangement of nucleoprotein fibres within specific chromosome regions, associated with earlier or later condensation at mitosis as suggested later by the electron micrographs of human chromosomes by Bahr (1972&1973). In the second, the two regions may result from differences in the type of DNA (repetitive and non repetitive) and/or proteins (basic or acidic) resulting in differences in the concentration of nucleoproteins along the chromatid. Thus the difference in the staining reaction of the chromosomes of the 2 species

could be attributed to a difference in the protein structure, not the gene sequence.

It has been found that the C-banding technique is the most suitable for the recognition and location of heterochromatin in somatic chromosomes. The amount and distribution of constitutive heterochromatin varies even between closely related species e.g. Microtus agrestis Linnaeus possesses about 7 times as much constitutive heterochromatin as Microtus pennsylvanicus Ord (Schmid, 1967). In different species of hedgehog (Hemiechinus & Erinaceus), the amount of constitutive heterochromatin is largely the same, but the distribution in the karyotypes varies (Gropp, et al., 1969; Gropp and Natanajan, 1972). Usually, mammals seem to have no large blocks of heterochromatin, but have it distributed in the majority of autosomes and located in the centromeric areas (Hsu and Arrighi, 1971; Yunis and Yasmineh, 1972) although suggestion of its presence in telomeres has been made. In some individuals of the Australian rodent Uromys caudimaculatus Krefft, Baverstock et al.(1976) reported the presence of telomeric C-bands in 13 of 23 chromosome pairs which make up the standard diploid set while two pairs had an interstitial band. Also, in the pocket mouse, Perognathus baileyi Merriam, Patton (1977) reported telomeric C-bands in the smallest pair of acrocentric autosomes in two of the six individuals investigated from Baja, California. In A. sylvaticus Bekasova et al. (1980) reported telomeric C-bands in some pairs of chromosomes (6-8) and a slightly stained interstitial and telomeric C-band in the x chromosome. Telomeric C-bands encountered in the present investigation are not consistent even in the cells of the same animal, so the significance of this variation is not clear. It may be that the DNA

of these regions is to some extent serially repeated, but not completely so, so that it is on the threshold of the type needed for the C-banding reaction. This suggests that A. sylvaticus may be labile in heterochromatin characteristics. Unlike Bekasova's result, no C-bands have been detected along the length of the x chromosome of A. sylvaticus, while that of A. flavicollis showed a prominent juxta-centric C-band. The significance of heterochromatin in relation to karyological evolution has been discussed by several authors (Yunis and Yasmineh, 1971; Gropp and Natarajan, 1972). According to Hsu and Arrighi (1971), an increase of heterochromatin has occurred as one type of karyotype evolution. On the other hand, a diminution or decrease of C-band heterochromatin has been recognised in closely related subspecies of the black rat as one of the possible causes of karyotype evolution (Yosida and Sagai, 1975). Also difference in nucleolus organizers (NORs) such as is found between the two Apodemus species has been considered to be important in the evolution of species. This is supported by the findings of Yosida (1978 & 1979) in Rattus norvegicus Berkenhout, and Rattus rattus flavipectus Milne-Edwards, both species showing similar karyotypes but with differences in the number of NORs. Interestingly, individuals of these species could not be mated, either in nature or in the laboratory, as shown in a foregoing experiment (Yosida and Taya, 1977). Artificial insemination led to a hybrid embryo which degenerated completely on the 13th day of development. Two explanations have been considered by the authors, first immunological reactions, and second genic imbalance, induced by differences in the structure and function of the NORs and the heterochromatin.

The chromosomal differences between A. sylvaticus and A. flavicollis include a difference in the number of NORs, the presence in the

A. flavicollis x chromosome of a juxta centromeric heterochromatin block and its absence in A. sylvaticus, as well as other differences between individual chromosomes (especially clear in the x chromosome). The degree of karyotype difference between these species is entirely consistent with the findings of Jewell and Fullager (1965) and Niethammer (1969) that these species are reproductively isolated, and must cast considerable doubt on suggestions that they hybridise in other parts of Europe (Zimmermann, 1962).

(c) Meiosis

Contrary to all known cases in mammals , the sex chromosomes of Apodemus are sometimes divided postreductionally at the second division of meiosis. Even in the vast field of the animal kingdom it is rather rare for the sex chromosomes to divide equally at the first division and reductionally at the second one. Such examples are known only in insects belonging to the orders Hemiptera and Odonata (Oguma, 1934). Koller (1941) reported the presence of both types of segregation (post and prereduction segregation) in A. sylvaticus and A. flavicollis and he mentioned that no visible difference was detected in the configuration of the sex bivalent in each type of segregation, describing it as a symmetrical sex bivalent in all cases. The present study supports the presence of the two types of segregation only in A. sylvaticus but with differences in the configuration of the sex bivalent, this being symmetrical in the case of postreduction and asymmetrical in prereduction. In A. flavicollis only the asymmetrical sex bivalent can be detected, which leads to prereduction segregation. The juxta centric heterochromatic block, present in the A. flavicollis x chromosome, but not the y, would in any event preclude the formation of

a symmetrical bivalent necessary for postreduction division.

II Intraspecific variation

According to Rood (1965); Delany and Healy (1967 a & b); Berry et al. (1967); Delany and Whittaker (1969), differences in size, colour of belly fur, length of pectoral stripe and colour of dorso-lateral pelage are described in populations of A. sylvaticus from the Hebrides, Scilly and Channel Islands as well as several localities on the British mainland. On the basis of skull measurements, Berry (1973) has reported a fairly clear distinction between two mainland races of A. sylvaticus: western and central populations, and eastern ones (which have closer affinities to French mice than western British ones).

From the cytological point of view, Berry and Parington, unpublished, (cited in Berry, 1979) have been unable to detect any differences in karyotype from mitosis of A. sylvaticus from Edinburgh, Rhum, St. Kilda and Fair Isle. Berry (1970) has reported that the variation in wild populations of certain species may affect the number of the chromosomes and their structure, or it may take the form of gene changes which are cytologically indetectable, revealing their occurrence only by their effects on the phenotypes of individuals carrying them.

(a) B chromosomes

Thorough examination of the karyotypes of 203 A. sylvaticus proved the presence of interindividual variation (polymorphism) as well as intrainidvidual variation (mosaicism) in all the populations studied. This polymorphism was due to the presence of B chromosomes.

B chromosome polymorphism has been reported in only one species of the genus Apodemus , the Asian Wood mouse, A. peninsulae, by Hayata et al., (1970) who described this species as A. giliacus and by Kral (1971) who described it as A. speciosus. Bekasova and Vorontsov (1974 & 1975), Vorontsov et al., (1977) and Bekasova et al., (1980) established that in all cases polymorphism of B chromosomes in the genus Apodemus is characteristic of only A. peninsulae Thomas and they reported high stability of karyotypes of A. agrarius and A. sylvaticus throughout their area. The present study reports that 38.4% of the animals had a mosaic karyotype due to the presence of B chromosomes while 61.6% had a stable karyotype which represents the normal karyotype of the species. These estimates of the number of individuals with B chromosomes must be regarded as a minimum estimate as the presence of B chromosomes was generally restricted to a few cells which could easily pass undetected. This also might be the reason why although many authors have studied the karyotype of A. sylvaticus, the presence of B's in this species has been overlooked.

On the basis of size and composition, B chromosomes of the two species of the genus Apodemus are quite different. Although Volobuev (1980) and Bekasova et al., (1980) found two groups of B's in A. peninsulae, one comprising biarmed B's of two different sizes, and the second dot-like B's, both types being fully heterochromatic, in A. sylvaticus, four types of B chromsomes, both euchromatic and heterochromatic, have been found and recorded in the present study.

The distribution of different types of B's in the different populations is quite different. The telocentric euchromatic B's are the most widespread

ones in Central and East Southern England. Isochromosomes have been detected in combination with the telocentrics in one of these populations. The dot-like heterochromatic B has been detected in combination with the telocentrics in another of these populations. Two populations, both from South West England were found to be characterized by the presence of a different type of B, a heterochromatic minute B larger than the dot, which is found in higher frequencies than the other types of B.

As mentioned before, B chromosomes vary in frequency between populations exposed to different conditions of climate, soil, cultivation, etc. (Rees and Hutchinson, 1973). In the grass Phleum phleoides B's were most frequent in populations in soils low in organic matter (Bosemark, 1956). In another grass, Festuca pratensis, the frequency of B's was directly correlated with the clay content of the soil. The conclusion in both cases was that B chromosomes attained the highest frequency under conditions most favourable for the growth of the species concerned. In grasshoppers Myrmelotettix maculatus (Thunb.) Barker (1966) was the first to point out that the proportion of grasshoppers of this species carrying B's was higher in environmental conditions approaching the optimum for the species. Detailed studies by Hewitt and John (1967, 1970) on the same species confirmed Barker's observations as they found that B's were more abundant in British populations occupying warm, dry habitats, conditions considered ideal for the species.

In A. sylvaticus , the present study showed a correlation between the type of B's and the frequency of individuals carrying B's in different populations. Populations carrying minute heterochromatic B's have a

significantly higher proportion of animals with B chromosomes than occurred in populations where euchromatic B's were present.

As to intraindividual variation, all the individuals with B's had only a few of them. This supports Gadi et al. (1982) who reported that more than a certain number of B's lower the fecundity, and also Jones and Rees (1982) who report that the effect of B's in high frequency are generally deleterious. Accumulation mechanisms of various kinds have been proposed to explain the maintenance of B's in a population (Hewitt, 1973 a & b; Müntzing, 1974). The data given earlier in the present study (Table 5) suggest the presence of higher frequency of B chromosomes in the male germinal tissues rather than in the somatic tissues, though this is not statistically significant on the present data.

The origin of B chromosomes is a matter of conflicting opinions. White (1954); Gustavsson and Sundt (1967) considered mammalian B's to be the remnant of structural rearrangements taking place in the karyotypic evolution of ancestral forms. John (1973) assumed that supernumeraries are derived from the regular complements by nondisjunction followed by heterochromatinization or by amplification of deleted autosomal heterochromatin. Yosida (1977) has assumed nondisjunction as the probable mechanism of origin of supernumeraries in Rattus because of their morphological similarity to the small metacentrics of the regular complement. Raman and Sharma (1974) have, however, concluded from meiotic studies that supernumeraries of R. rattus (L.) have not arisen by polysomy of any chromosome of the regular complement as they do not associate with any autosome. In fact, there is no reason to suppose that supernumeraries have

all evolved along the same pathway, because B's are a heterogeneous group so that each type might originate by its own mechanism. Volobuev (1978&1980a) classified mammalian B chromosomes according to size into three groups and he suggested that each of his dimensional types of B chromosomes originated by its own particular mechanism. According to him the dot-like B's (the first dimensional type) which are smaller than the smallest chromosomes of the basic set, arise as a result of structural rearrangements of A chromosomes. B chromosomes of the second dimensional type, similar in size to small A chromosomes in the complement, result from nondisjunction of the latter and subsequent genetic inactivation. The third dimensional type, comprising B's as large as or larger than the largest A chromosomes, arises by one of the two abovementioned ways but afterwards these chromosomes have undergone structural rearrangements such as translocation and centromere division leading to isochromosomes and in this way increased their size.

The origin of different types of B chromosomes in A. sylvaticus may not differ greatly from the foregoing hypotheses. The only striking thing is the association of the small telocentric B's with the sex bivalent in meiosis. Morphologically this type of B chromosome is quite similar to the y chromosome in mitosis, so it is more probable that it arose from the y chromosome rather than from the smaller A chromosomes. As has already been mentioned, numerical changes in the frequencies of x and y chromosomes, certainly in mammals including man, are tolerable to a far greater degree than in other chromosomes of the complement (Jones and Rees, 1982). An x or y chromosome frag ment which might become established as a B chromosome is likely therefore to be more tolerable to the organism and a cause of less genic imbalance than would be the case for fragments of autosomes. From

the foregoing it seems possible to attribute the absence of y chromosomes from 8.57% of the primary spermatocytes to the presence of B chromosomes. Hayman et al., (1969) have considered the B chromosomes to be responsible for the elimination of the sex chromosomes in Echymiptera kalabu (Peramelidae : Marsupialia). In this species an instability in the number of B chromosomes parallels an instability in the A chromosomes themselves in which the somatic tissues do not have the full complement. The chromosomes missing are the y in the male and an x in the female. The full complement is present only in the corneal epithelium and the reproductive tissue. This confirms the active role of B chromosomes although it has been reported that B's are genetically inert. Mather (1944) was among the first to suggest that the inertness was illusive and that B chromosomes possess genetic activity of a kind forming an internally balanced system (Darlington and Mather, 1949).

(b) Sex chromosome deletion

Partial and total deletion of one x chromosome have been found in two phenotypically normal females. According to the hypothesis of Lyon (Lyon, 1961), originally derived from observation on mice, and later to cover mammals generally (Lyon, 1962), one of the x chromosomes in mammalian females is genetically inactivated. Mittwoch (1964); Thompson (1965) described this phenomenon as a dosage compensation mechanism in which the inactive x changes from isochromatin to heterochromatin in early embryogenesis, and forms the sex chromatin body in interphase nuclei. This dosage compensation mechanism may manifest itself in a different way in other mammalian species. For example, inactivation of a sex chromosome has led to its elimination in the Marsupial genera Isoodon

and Perameles. As a consequence, the somatic cells of both sexes are xo, while the germinal cells still contain xy and xx sex chromosomes (Hayman and Martin, 1965a). In the rodent Microtus oregoni Bachman, the x chromosome is eliminated only in the male germinal cells (Ohno et al., 1963). Hence, male individuals result from the fertilization of x ova by y sperm and xo females occur when ova are fertilized by o sperm. In the present study, y-deficient spermatocytes have been found, suggesting that o sperm may be produced. However, because there is no proven xo male in mammals, White (1960) concluded that the y is an indispensible part of the mammalian sex determining mechanism because it is necessary for the development of the male. It is more reasonable to say that o sperm resulting from elimination of either x or y will give xo females on fertilization of x ova. Russell (1961) reported that the female determiners are not suspected to be located in the x, but possibly in autosomes, since addition of extra x chromosomes or deletion of the second x does not grossly alter the female sex phenotype. The high incidence of females with x chromosome anomalies within a limited number of individuals studied from wild populations of Akodon azarae Fisher (Bianchi and Contreras, 1967) and Mus (Leggada) triton Thomas and Mus (Leggada) minutoides musculoides Temminck (Matthey 1967a&b) is explained on the basis of variability in the dosage compensation mechanism. The presence only of one xo female in the present study makes it more likely to be due to the presence of o sperm rather than on the basis of variability in the dosage compensation mechanism.

In man 45, x females almost invariably results in sterile girls with ovarian dysgenesis; the gonads are streaks and lack germ cells. However,

45, x fetuses have relatively normal gonads containing germ cells in similar numbers to 46, xx gonads up to the third month of gestation. After this time the germ cells begin to degenerate and the ovarian stromal tissue increases in the 45, x relative to 46, xx ovaries.

On the contrary, in the mouse, 39, x females are fertile although a reduced litter size may indicate fewer germ cells or resorption of 39, x or y fetuses (Hamerton, 1971). In A. sylvaticus the ovarian structure of xo female appears normal in one of the ovaries while the other is made up of highly cellular masses and lack of any germ cells. Morris (1968) in an extensive study of breeding data of 39, x compared to 40, xx female mice, concluded that the reduced litter size could be accounted for by complete pre-implantation loss of 39, y zygotes and both pre and post implantation loss of a proportion of the 39, x zygotes. This is supported by the infrequent occurrence of xo female and the complete absence of oy individuals, and proves that the presence of at least one x chromosome is necessary for survival (Chu, 1964).

SUMMARY

1. The standard karyotype of the two species of field mouse occurring in Britain, the wood mouse, A. sylvaticus and the yellow-necked mouse, A. flavicollis consists of 48 telocentric chromosomes.

2. Comparison of the relative chromosome lengths of the two species shows that the lengths of the autosomes of A. flavicollis are larger than those of A. sylvaticus for the first twelve chromosome pairs and smaller for the rest of the autosomes and the sex chromosomes.

3. G-banding comparison has demonstrated an overall difference in staining reaction and in the degree of differentiation between bands and inter-bands which could be attributed to a difference in the protein structure, not the gene sequence. There are also differences in the banding patterns of the two species.

4. C-banding technique showed a prominent juxta centromeric C-band in the x chromosome of A-flavicollis but not in A. sylvaticus.

5. The number of nucleoli and NORs provides a further difference between the karyotype of these species, 5 nucleoli and 5 pairs of NORs in A. sylvaticus, and 6 nucleoli with 6 pairs of NORs in A. flavicollis.

6. In meiosis of A. sylvaticus the sex bivalent shows two different types of association, occurring with almost equal frequency. These are an asymmetrical sex bivalent which leads to prereduction segregation and a symmetrical one which leads to post reduction segregation.

7. In A. flavicollis the sex bivalent showed only one sort of association (asymmetrical) which leads to pre reduction segregation.

8. These chromosomal data support the separate identities of these two species.

9. Intraspecific variation between different populations of A. sylvaticus has been reported due to the presence of B chromosomes.

10. Interindividual variation (polymorphism) as well as intra individual variation (mosaicism) has been found in all the populations studied.

11. Four classes of B chromosome have been identified in nine populations of A. sylvaticus from the mainland of Britain.

12. The distribution of different types of B's in different populations is quite different. Euchromatic telocentric B chromosomes are the most widespread ones in Central and East Southern England. Heterochromatic minute B's have been found in higher frequencies in two populations, both from South West England.

13. Irregularities in the behaviour of the bivalents of the basic set during meiosis have been detected in animals with B chromosomes but not in those without them.

14. Sex chromosome deletion has been found in two females from different populations; one of them had Xx and the other XO.

15. Intraspecific karyotype homology, as judged by C- and G-banding has been demonstrated between populations of the bank vole Clethrionomys

glareolus from different localities on the British mainland and from Skomer Island.

REFERENCES

REFERENCES

ANGUS, R.B. (1982). Separation of two species standing as Helophorus aquaticus (L.) (Coleoptera, Hydrophilidae) by banded chromosome analysis. Systematic Entomology, 7: 265-281.

ARAKAKI, D.T. and SPARKES, R.S. (1963). Microtechnique for culturing leukocytes from whole blood. Cytogenetics, 2: 57-60.

AYONOADU, U. and REES, H. (1968). The influence of B chromosomes on chiasma frequences in Black Mexican sweetcorn. Genetica, 39: 75-81

BAHR, G.F. (1972). The ultrastructure basis for chromosomal banding patterns observed after renaturation and Giemsa staining. 10th Ann. Somat. Genet. Conf., Aspen, Colorado.

BAHR, G.F. (1973). Correlates of chromosomal banding at the level of ultrastructure. Tutorial Proceedings of the Int. Acad. of Cytology, 2: 58-73.

BAHR, G.F., MIKEL, U. and ENGLER, W.F. (1973). Correlates of chromosomal banding at the level of ultrastructure. In:"Chromosome Identification". (T. Caspersson and L. Zech, Eds.) Academic Press, New York.

BARKER, J.F. (1960). Variation of chiasma frequency in and between natural populations of Acrididae. Heredity, 14: 211-214.

BARKER, J.F. (1966). Climatological distribution of a grasshopper supernumerary chromosomes. Evolution, 20: 665-667.

BARLOW, P.W. and VOSA, C.G. (1970). The effect of supernumerary chromosomes on meiosis in Puschkinia libanotica (Liliaceae). Chromosoma, (Berl.), 30: 344-355.

BARRETT-HAMILTON, G.E.H. (1903). An addition to the list of British boreal mammals. Proc. R. Irish Acad., 24B: 315

BARRET-HAMILTON, G.E.H. and HINTON, M.A.C. (1913a). On a collection of mammals from the Inner Hebrides. Proc. Zool. Soc. London., 1913: 821-839.

BARRET-HAMILTON, G.E.H. and HINTON, M.A.C. (1913b). Three new voles from the Inner Hebrides, Scotland. Ann. Mag. nat. Hist., 12: 361.

BATH, D.W. (1976). Surface ultrastructure of trypsin-banded chromosomes. Expl. Cell Res., 98: 262-268.

BATTAGLIA, E. (1964). Cytogenetics of B chromosomes. Caryologia, 17: 245-299.

BAVERSTOCK, P.R., WATTS, C.H.S. and HOGARTH, J.T. (1976). Heterochromatin variation in the Australian rodent Uromys caudimaculatus. Chromosoma (Berl.), 57: 397-403.

BAVERSTOCK, P.R., WATTS, C.H.S., HOGARTH, J.T., ROBINSON, A.C. and ROBINSON, J.F. (1977). Chromosome evolution in Australian Rodents. II. The Rattus group. Chromosoma (Berl), 61: 227-241.

BEKASOVA, T.S. and VORONTSOV, N.N. (1974). Taxonomic position of Asian Wood mouse of the genus Apodemus (Rodentia) inhabiting the territory of Far East and Siberia. Abstr. First int. Theriol. Congr., publ. house VINITI, Moscow: 52-53.

BEKASOVA, T.S. and VORONTSOV, N.N. (1975). Populational chromosome polymorphism in the Asiatic forest mouse Apodemus peninsulae. Genetika (Moscow), 11 (6): 89-94.

BEKASOVA, T.S., VORONTSOV, N.N., KOROBITSYNA, K.V. and KORABLEV, V.P. (1980). B chromosomes and comparative karyology of the genus Apodemus. Genetica 52/53: 33-43.

BELYAEV, D.K., VOLOBUEV, V.T., RADZHABLI, S.I. and TRUT, L.N. (1974a). Investigation of the nature and role of the supernumerary chromosomes of silver foxes. Communication II. Supernumerary chromosomes in the selection of animals according to behaviour. Genetika (Moscow), 10 (8) : 83-91.

BELYAEV, D.K., VOLOBUEV, V.T., RADZHABLI, S.I. and TRUT, L.N. (1974b). Polymorphism and mosaicism for additional chromosomes in silver foxes. Genetika (Moscow), 10: 58-67.

BERRY, R.J. (1970). Covert and overt variation as exemplified by British mouse populations. In: "Variation in mammalian populations" (Berry, R.J. and Southern, H.N. eds). Academic Press, London.

BERRY, R.J. (1973). Chance and change in British long-tailed field mice (Apodemus sylvaticus). J. Zool. (Lond), 170: 351-366.

BERRY, R.J. (1977). "Inheritance and Natural History." Collins, London.

BERRY, R.J., EVANS, I.M. and SENNITT, B.F.C. (1967). The relationship and ecology of Apodemus sylvaticus from the small Isles of the Inner Hebrides, Scotland. J. Zool. (Lond.) , 152: 333-346.

BIANCHI, N.O. and CONTRERAS, J.R. (1967). The chromosomes of the field mouse Akodon azarae (Cricetidae, Rodentia) with special reference to sex chromosome anomalies. Cytogenetics, 6: 306-313.

BIGGER, T.R.L. (1976). Karyotypes of three species of Lepidoptera including an investigation of B chromosomes in Pieris. Cytologia, 41: 261-282.

BISHUN, N.P. (1968). Karyological analysis in the field mouse, Apodemus sylvaticus. Mammal. Chromos. Newsletter, 9: 19.

BLANKS, G.A. and SHELLHAMMER, H.S. (1968). Chromosome polymorphism in California populations of Harvest mice. J. Mammal., 49: 726-731

BOSEMARK, N.O. (1956). On accessory chromosomes in Festuca pratensis III. Frequency and geographical distribution of plants with accessory chromosomes. Hereditas, 42: 189-210.

BROHMER, P. (1929). Die Tierwelt Mitteleuropas, Wirbeltiere Leipzig.

BROOKER, P.C. (1982). Robertsonian translocation in Mus musculus from N.E. Scotland and Orkney. Heredity, 48 (2): 305-309.

BUCKTON, K.E. and NETTESHEIM, P. (1968). In vitro and in vivo culture of mouse peripheral blood for chromosome preparations. Proc. Soc. exp. Biol. Med., 128: 1106-1110.

BUCKTON, K.E. and CUNNINGHAM, C. (1971). Variations of the chromosome number in the Red Fox. (Vulpes vulpes). Chromosoma (Berl.), 33: 268-272.

BURKHOLDER, G.D. (1974). Electron microscopic visualization of chromosomes banded with trypsin. Nature, 247: 292-294.

BURKHOLDER, G.D. (1975). The ultrastructure of G- and C- banded chromosomes. Expl. Cell Res., 90: 269-278.

CASPERSSON, T., ZECH, L., MODEST, E.J., FOLEY, G.E., WAGH, U. and SIMONSSON, E. (1969a). Chemical differentiation with fluorescent alkylating agents in Vicia faba metaphase chromosomes. Expl. Cell Res., 58: 128-140

CASPERSSON, T., ZECH, L., MODEST, E.J., FOLEY, G.E., WAGH, U. and SIMONSSON, E. (1969b). DNA binding fluorochromes for the study of the organization of the metaphase nucleus. Expl. Cell Res. 58: 141-152

CHU, E.H.Y. (1964). Abnormalities of sex chromosomes. In: "Mammalian cytogenetics and related problems in radiobiology." (C. Pavan, et al., eds). Pergamon Press, Oxford.

CLELAND, R.E. (1951). Extra diminutive chromosomes in Oenothera. Evolution, 5: 165-176

CLENDENIN, T.M.(1969). Intraperitoneal colchicine and hypotonic KCl for enhancement of abundance and quality of meiotic chromosome spreads from Hamster testes. Stain Technol., 44(2): 63-69.

CORBET, G.B. (1964). Regional variation in the Bank-vole Clethrionomys glareolus in the British Isles. Proc. Zool. Soc. (Lond.), 143: 191-219

DARLINGTON, C.D. and MATHER, K. (1949). The elements of genetics. Allen and Unwin, London.

DARLINGTON, C.D. and WYLIE, A.P. (1955). "Chromosome Atlas of Flowering Plants." Allen and Unwin, London.

DELANY, M.J. and HEALY, M.J.R. (1967a). Variation in the long-tailed field mouse (A. sylvaticus (L.)) in the Channel Isles. Proc. R. Soc (B), 166: 408-421.

DELANY, M.J. and HEALY, M.J.R. (1967b). Variation in the long-tailed field mouse (Apodemus sylvaticus) in South-West England. J. Zool. (Lond.), 152: 319-332

DELANY, M.J. and WHITTAKER, H.M. (1969). Variation in the skull of the long-tailed field mouse Apodemus sylvaticus in mainland Britain. J. Zool. (Lond.), 157: 147-157

EL-NAHAS, A. (1983). Cytogenetics of B chromosomes in Allium cernuum Roth. PhD Thesis, Univ. Reading, England.

FEDERLEY, H. (1919). Beiträge Zur Kenntnis der Säugetiergametogenese 1. Die spermatogenese von Mus sylvaticus L. Acta. Soc. Sc. Fenn. 48

FEDEROV, A.A. (1969). "Chromosome numbers of Flowering plants." Acad. Sci. USSR, Leningrad.

FERGUSON-SMITH, M.A. and PAGE, B.H. (1973). Pachytene analysis in human reciprocal (10:11) translocation. J. Med. Genet., 10: 283-286

FORD, C.E., JONES, K.W., POLANI, P.E., DE ALMEIDA, J.C. and BRIGGS, J.H. (1959). A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet, 1: 711-713

FRACCARO, M., KAIJSER, K. and LINDSTEIN, J. (1959). Chromosome complement in gonadal dysgenesis. Lancet, 1: 886

GADI, I.K., SHARMA, T. and RAMAN, R. (1982). Supernumerary chromosomes in Bandicota indica nemorivaga and a female individual with XX/XO mosaicism. Genetica, 58: 103-108

GILEVA, E.A. (1973). B chromosomes, unusual inheritance of sex chromosomes, and sex ratio in the Arctic lemming (Dicrostonyx torquatus torquatus Pall (1779). Proc. Acad. Sci. USSR, 213: 952-955

GODFREY, J. (1959). The origin of sexual isolation between bank voles (Clethrionomys). Proc. int. Congr. Zool., 15: 203-204

GOODPASTURE, C. and BLOOM, S.E. (1975). Visualization of nucleolar regions in mammalian chromosomes using silver staining. Chromosoma (Berl.), 53: 37-50.

GROPP, A., CITOLER, P. and GEISLER, M. (1969). Karyotype variation and heterochromatin pattern in hedgehogs (Erinaceus and Hemiechinus). Chromosoma (Berl.), 27(3): 288-307

GROPP, A., MARSHALL, J., FLATZ, G., OLBRICH, M., MANYANONDHA, K. and SANTADUTSI, A. (1970). Chromosomen-polymorphismus durch überzählige Autosomen. Z. Säugetierk, 35: 363-371.

GROPP, A. and NATARAJAN, A.T. (1972). Karyotype and heterochromatin pattern of the Algerian hedgehog. Cytogenetics, 11(4): 259-269.

GUSTAVSSON, I. (1970). Mitotic and meiotic behaviour of the B chromosomes in the silver fox. Giessener Beitr. Erbpath. Züchtung, Suppl. 1: 34-42

GUSTAVSSON, I. and SUNDT, C.O. (1967). Chromosome elimination in the evolution of the silver fox. J. Hered., 58: 75-78.

HÅKANSSON, A. (1957). Meiosis and pollen mitosis in rye plants with many accessory chromosomes. Hereditas, 43: 603-620

HAMERTON, J.L. (1971). "Human Cytogenetics" General cytogenetics Volume 1. Academic Press, New York/London.

HAYATA, I. (1973). Chromosome polymorphism caused by supernumerary chromosomes in the field mouse, Apodemus giliacus. Chromosoma (Berl.), 42: 403-414.

HAYATA, I., SHIMBA, H., KOBAYASHI, T. and MAKINO, S. (1970). Preliminary accounts on the chromosomal polymorphism in the field mouse, Apodemus giliacus, a new form from HOKKAIDO. Proc. Japan Acad., 46: 567-571.

HAYMAN, D.L. and MARTIN, P.G. (1965a). Sex chromosome mosaicism in the marsupial genera Isoodon and Perameles. Genetica, 52: 1201-1206

HAYMAN, D.L. and MARTIN, P.G. (1965b). Supernumerary chromosomes in the marsupial Schoinobates volans (Kerr.), Aust. J. biol. Sci., 18: 1081-1082

HAYMAN, D.L., MARTIN, P.G. and WALLER, P.F. (1969). Parallel mosaicism supernumerary chromosomes and sec chromosomes in Echymipera kolabu (Marsupialia). Chromosoma (Berl.), 27: 371-380.

HEINRICH, G. (1929). Über Sylvamus sylvaticus L. and flavicollis Melchior. Z. Säugetierkde, 2.

HENRIQUES-GIL, N., SANTOS, J.L. and GIRALDEZ, R. (1982). B chromosome polymorphism and interchromosomal chiasma interference in Eyprepoc nemis plorans (Acrididae, Orthoptera). Chromosoma (Berl.), 85: 349-359.

HEWITT, G.M. (1973a). Evolution and maintenance of B chromosomes. Chromosomes Today, 4: 351-369.

HEWITT, G.M. (1973b). The integration of supernumerary chromosomes into the orthopteran genome. Cold Spring Harb. Symp. quant. Biol., 38: 183-194

HEWITT, G.M. and JOHN, B. (1967). The B Chromosome system of Myrmeleotettix maculatus (Thunb.) III. The statistics. Chromosoma (Berl.), 21: 140-162

HEWITT, G.M. and JOHN, B. (1970). The B Chromosome system of Myrmeleotettix maculatus (Thunb.). IV. The dynamics. Evolution, 24: 169-180.

HSU, T.C. and ARRIGHI, E. (1971). Distribution of constitutive heterochromatin in mammalian chromosomes. Chromosoma (Berl.), 34: 243-253

HSU, T.S. and PATTON, J.L. (1969). Bone marrow preparations for chromosome studies. In: "Comparative Mammalian Cytogenetics." (K. Benirshke ed.) Berlin, Heidelberg and New York.

HUNGERFORD, D.A., La BADIE, G.U. and BALABAN, G.B. (1971a). Chromosome structure and function in man. II. Provisional maps of the two smallest autosomes (chromosomes 21 and 22) at pachytene in the male. Cytogenetics, 10: 33-37.

HUNGERFORD, D.A., La BADIE, G.U., BALABAN, G.B., MESSATZZIA, L.R., HALLER, G. and MILLER, A.E. (1971b). Chromosome structure and function in man. IV. Provisional maps of the three long acrocentric autosomes (chromosomes 13, 14 and 15) at pachytene in the male. Annls. Génét., 14: 257-260

JACKSON, R.C. (1960). Supernumerary chromosomes in Haplopappus gracilis Evolution, 14: 135.

JEWELL, P.A. and FULLAGAR, P.J. (1965). Fertility among races of the field mouse (Apodemus sylvaticus) and their failure to form hybrids with the yellow-necked mouse (Apodemus flavicollis). Evolution, 19: 175-181.

JOHN, B. (1973). The cytogenetic systems of grasshoppers and locusts. II. The origin and evolution of supernumerary segments. Chromosoma (Berl.), 44: 123-146

JOHN, B. and HEWITT, G.M. (1965a). The B chromosome system of Myrmeleotettix maculatus (Thunb.). I. The mechanics. Chromosoma (Berl.), 16: 548-578.

JONES, K.W. (1977). Repetitive DNA and Primate evolution. In: "Molecular Structure of Human Chromosomes." (ed. by J.J. Yunis). Academic Press, New York.

JONES, R.N. (1975). B chromosome systems in flowering plants and animal species. Int. Rev. Cytol., 40: 1-100.

JONES, R.N. and REES, H. (1982). "B chromosomes." Academic Press Inc. (London).

KATO, H. and YOSIDA, T.H. (1972). Banding patterns of Chinese hamster chromosomes revealed by new techniques. Chromosoma (Berl.), 36: 272-280.

KOLLER, P.C. (1937). The genetical and mechanical properties of sex chromosomes. III. Man. Proc. R. Soc. Edinb., 57: 194-214.

KOLLER, P.C. (1941). The genetical and mechanical properties of the sex chromosomes. VII Apodemus sylvaticus and A. hebridensis. J. Genet., 41: 375-389.

KOLLER, P.C. and DARLINGTON, C.D. (1934). The genetical and mechanical properties of the sex chromosomes. I. Rattus norvegicus, male. J. Genet., 29: 159-173.

KRÁL, B. (1970). Chromosome studies in two subgenera of the genus Apodemus. Zool. Listy., 19: 119-134.

KRÁL, B. (1971). Chromosome characteristics of certain Murine rodents (Muridae), of the Asiatic part of the USSR. Zool. Listy., 20: 331-347.

LEE, C.L.Y., WELCH, J.P. and LEE, S.H.S. (1973). Banding of human chromosomes by protein denaturation. Nature New Biol. (Lond.), 241: 142-143.

LEWIN, B. (1974). "Gene Expression" Vol.II. Eucaryotic chromosomes. John Wiley & Sons Ltd.

LOW, R.J. and BENIRSCHKE, K. (1972). Microchromosomes in the American red fox Vulpes fulva. Cytologia, 37: 1-11.

LUCIANI, J.M., MORAZZANI, M.R. and STAHL, A. (1975). Identification of pachytene bivalents in human male meiosis using G-banding technique. Chromosoma (Berl.), 52: 275-282.

LUTZ, A.M. (1916). Oenothera mutants with diminutive chromosomes. Am. J. Bot., 3: 502-526.

LYON, M.F. (1961). Gene action in the x chromosome of the mouse. Nature (Lond)., 190: 372-373.

LYON, M.F. (1962). Sex chromatin and gene action in the mammalian x chromosome. Am. J. Hum. Genet., 14: 135-148.

MACARIO, A.J.L. (1966). Phytohaemagglutinin and mouse lymphocytes. Lancet, 1: 823.

MAKINO, S. (1941). Studies on the murine chromosomes. I. Cytological investigations of mice included in the genus Mus. J. Fac. Sci. Hokkaido Univ. Ser. 6, 7(4): 305-380.

MAKINO, S. (1951a). An atlas of chromosome numbers in Animals. 2nd Ed. Iowa State Coll. Press, America.

MAKINO, S. (1951b). Studies on the murine chromosomes. V. A study of the chromosomes in Apodemus, especially with reference to the sex chromosome in meiosis. J. Morph., 88: 93-126.

MATHER, K. (1944). The genetical activity of heterochromatin. Proc. R. Soc. (B), 132: 308-332.

MATTHEY, R. (1936a). Le probléme des hétérochromosomes chez les mammiféres. Archs. Biol. Paris, 46: 319.

MATTHEY, R. (1936b). La formule chromosomiale et les hétérochromosomes chez les Apodemus Européens. Z.F. Zellforschung u. mikr. Anatomie. Bd., 25: 501-515.

MATTHEY, R. (1951). The chromosomes of the vertebrates. Adv. Genet., 4: 159-180.

MATTHEY, R. (1956). Cytologie chromosomique comparée et systematique des Muridae. Mammalia, 20: 93-123.

MATTHEY, R. (1967a) Cytogénétique de Leggada: I. La formula chromosomique de Mus (Leggada) bufo. Th. 2. Nouvelles dormées sur la délétion portant sur la bras coust d'un x chez Mus (Leggada)triton Th. Experientia, 24: 1160-1161.

MATTHEY, R. (1967b). Etude de deux femelles hétér ozygotes pour une délétion portant sur un bras du chromosome x chez Mus (Leggada) minutoides musculoides Temminck Cytogenetics, 6: 168-177

MATTHEY, R. and RENAUD, P. (1935). Le type de digametie male et les chromosomes chez deux Campagnols. C. r. Seanc. Soc. Biol., 120: 595.

McKAY, R.D.G. (1973). The mechanism of G- and C-banding in mammalian metaphase chromosomes. Chromosoma (Berl.), 44: 1-14.

MELANDER, Y. (1950). Accessory chromosomes in animals, especially in Polycelis tenuis. Hereditas, 36: 19-38.

MEYLAN, A. and HAUSSER, J. (1974). Position cytotaxonomique de quelques musaraignes du genre Crocidura au Tessin. Mammal. Chromos. Newsletter, 11 (3): 105

MILLER, G.S. (1900). Preliminary revision of the European redbacked mice. Proc. Wash. Acad. Sci., 2: 83.

MILLER, G.S. (1908). Eighteen new European voles. Ann. Mag. nat. Hist., (8) 1: 194

MILLER, G.S. (1912). Catalogue of the mammals of Western Europe. London: British Museum (Natural History).

MITTWOCH, U. (1964). Sex chromatin. J. Med. Genet., 1: 50-76.

MORRIS, T. (1968). The XO and OY chromosome constitutions in the mouse. Genet. Res. 12: 125-137.

MÜNTZING, A. (1974). Accessory chromosomes. Annual Rev. Genet., 8: 243-266.

NADLER, C.F., RAUSCH, V.R., LYAPUNOVA, E.A., HOFFMAN, R.S. and VORONTSOV, N.N. (1976). Chromosomal banding patterns of the Holarctic rodents, Clethrionomys rutilus and microtus oeconomus. Z. Säugetierk., Bd. 41(3): 137-146.

NIETHAMMER, J. (1969). Zur frage der introgression bei den Waldmäusen Apodemus sylvaticus und A. flavicollis (Mammalia, Rodentia). Z. zool. Syst. Evolutions. f ., 7: 77-127.

NUR, U. (1966). The effect of supernumerary chromosomes on the development of mealy bugs. Genetics, 54: 1239-1249.

NUR, U. (1969). Harmful B chromosomes in a mealy bug: additional evidence. Chromosoma (Berl.), 28: 280-297

OGILVIE, R.F. (1947). "Pathological Histology." E & S Livingstone Ltd., Edinburgh.

OGUMA, K. (1934). A new type of the mammalian sex chromosome, found in a field mouse, Apodemus speciosus. Cytologia, 5: 460-471.

OHNO, S., KAPLAN, W.D. and KINOSITA, R. (1957). Note on non-chiasma type association between the x and y chromosomes of Drosophila melanogaster and Mus musculus. Expl. Cell Res., 13: 422-424.

OHNO, S., KAPLAN, W.D. and KONISOTA, R. (1959). On the end to end

association of the x and y chromosomes of Mus musculus. Expl . Cell Res., 18: 282-290.

OHNO, S., JAINCHILL, J. and STENIUS, C. (1963). The creeping vole (Microtus oregoni) as a gonosomic mosaic I. The oy/xy constitutions of the male. Cytogenetics, 2: 232-239.

OKADA, T.A. amd COMINGS, D.E. (1974). Mechanisms of chromosome banding. III. Similarity between G-bands of mitotic chromosomes and chromomeres of meiotic chromosomes. Chromosoma (Berl.), 48: 65-71.

PAINTER, T.S. (1923). Studies in mammalian spermatogenesis. II. The spermatogenesis of man. J. exp. Zool., 37: 291-335.

PAINTER, T.S. (1924). The sex chromosomes of man. Am. Naturalist, 58: 506-524.

PATTON, J.L. (1972). A complex system of chromosomal variation in the Pocket Mouse, Perognathus baileyi Merriam. Chromosoma (Berl.), 36: 241-255.

PATTON, J.L. (1977). B chromosome systems in the Pocket Mouse, Perognathus baileyi: Meiosis and C-band studies. Chromosoma (Berl.), 60: 1-14

PEARSON, P.L. (1977). Banding patterns, chromosome polymorphism and primate evolution. In: "Molecular structure of Human Chromosomes." (ed. by J.J. Yunis) Academic Press, New York

PROCUNIER, W.S. (1975). B chromosomes of Cnephia dacotensis and C. ornithophilia (Diptera : Simuliidae). Can. J. Zool., 53: 1638-1647.

RADZHABLI, S.I., IASENKO, A.A. and VOLOBUEV, V.T. (1978). Investigation of the nature and role of the supernumerary chromosomes in the silver fox. IV. Behaviour of the supernumerary chromosomes in meiosis. Genetika (Moscow), 14 (3): 438-443

RAMAN, R. and SHARMA, T. (1974). DNA replication, G- and C-bands and meiotic behaviour of supernumerary chromosomes of Rattus rattus. Chromosoma (Berl.), 45: 111-119.

RANDOLPH, L.F. (1928). Types of supernumerary chromosomes in maize. Anat. Rec., 41: 102.

RAUSCH, R.L. and RAUSCH, V.R. (1975). Relationships of the red-backed vole, Clethrionomys rutilus (Pallas), in North America: Karyotypes of the subspecies dawsoni and albiventer. Syst. Zool., 24: 163-170

RAYNAUD, A. (1936). Les hétérochromosomes du Mulot (Apodemus) C.R. Acad. Sci., 202: 1942-1944

REES, H. and HUTCHINSON, J. (1973). Nuclear DNA variation due to B chromosomes. Cold. Spring Harb. Symp. quant. Biol., 38: 175-182

RENAUD, P. (1938). La formule chromosomiale chez sept espéces de Muscardinidae et de Microtinae. Revue suisse Zool., 45: 349-383.

RENZONI, A. and OMODEO, P. (1972). Polymorphic chromosome system in the fox. Caryologia, 25(2): 173-187.

ROOD, J.P. (1965). Observation on the life cycle and variation of the long-tailed field mouse Apodemus sylvaticus on the Isles of Scilly and Cornwall. J. Zool. (Lond.), 147: 99-107.

ROTHFELS, K.H. and SIMINOVITCH, L. (1958). An air-drying technique for flattening chromosomes in mammalian cells grown in vitro. Stain. Technol., 33: 73.

ROWLEY, J.D. (1974). Identification of Human chromosomes. In: "Human Chromosome Methodology" (ed by J.J. Yunis). Academic Press, New York.

RUSSELL, L.B. (1961). Genetics of mammalian sex chromosomes. Science, 133: 1795-1803.

RUSSELL, W.L., RUSSELL, L.B. and GOWER, J.S. (1959). Exceptional inheritance of a sex linked gene in the mouse explained on the basis that the X/O sex chromosome constitution is female. Proc. natn. Acad. Sci. U.S.A., 45: 554-560.

SACHS, L. (1954). Sex-linkage and the sex chromosomes in man. Ann.Eugen. (Lond.), 18: 255-261.

SACHS, L. (1955). The possibilities of crossing-over between the sex chromosomes of the House mouse. Genetica, 27: 309-322.

SAINT-GIRONS, M.C. (1957). Les Mammiféres des pyrénées orientales. I. Observations sur quelques mammiféres recueillis dans la région de Banyals et plus particuliérement le mulot Apodemus flavicollis Vie et Milieu, 8: 287-296.

SAINT-GIRONS, M.C. (1962). A propos de L'hybridation eventuelle entre mulot gris Apodemus sylvaticus (Linnaeus, 1758) et mulot fauve Apodemus flavicollis (Melchior, 1934). Säugetierk. Mitt., 10: 25

SATYA PRAKASH, K.L. and ASWATHANARAYANA, N.V. (1972). A preliminary note on the occurrence of supernumerary (B) chromosome in the 38 chromosomes taxon of Rattus rattus. Mammal.chromos. Newsl., 13: 117-118.

SCHMID, W. (1967). Heterochromatin in mammals. Arch. J. Klaus-Stift., Verebungs - Forsch. 42: 1

SEABRIGHT, M. (1971). A rapid banding technique for human chromosomes. Lancet, 30: 971-972.

SHARMA, T., RAMAN, R.(1971). An XO female in the Indian mole rat. J. Hered., 62: 384-387.

SHELLHAMMER, H.S. (1969). Supernumerary chromosomes of the Harvest mouse, Reithrodontomys megalotis. Chromosoma (Berl.), 27: 102-108.

SHIRAISHI, Y. and YOSIDA, T.H. (1972). Banding pattern analysis of human chromosomes by use of a urea treatment technique. Chromosoma (Berl.), 37: 75-83.

SHIMBA, H., ITOH, M., OBARA, Y. and KOHNO, S. (1969). A preliminary survey of the chromosomes in field mice, Apodemus and Clethrionomys. J. Fac. Sci. Hokkaido Univ. Ser. VI, 17: 257-262.

SIMCHEN, G., ZARCHI, Y. and HILLEL, J.(1971). Supernumerary chromosomes in the second outbreeding species of the wheat groups. Chromosoma (Berl)., 33: 63-69

STEVENS, N.M. (1968). The chromosomes in Diabrotica Vittata, Diabrotica soror and Diabrotica 12-punctata a contribution to the literature on heterochromosomes and sex determination. J. exp. Zool., 5: 453-470.

STEVEN, D.M. (1955). Untersuchungen über die britischen formen von Clethrionomys. Z. Sängetierk., 20: 70.

SUMNER, A.T. (1972). A simple technique for demonstrating centromeric heterochromatin. Expl. Cell Res., 75(2): 304-306.

SUMNER, A.T., EVANS, H.J. and BUCKLAND, R.A. (1971). A new technique for distinguishing between human chromosomes. Nature, New Biol. (Lond.),232: 31-32.

THOMPSON, M.W. (1965). Genetic implications of heteropycnosis of the x chromosome. Can. J. Genet. Cytol., 7: 202-213.

TRIMAN, K.L., DAVISSON, M.T. and RODERICK, T.H. (1975). A method for preparing chromosomes from peripheral blood in the mouse. Cytogenet. Cell Genet., 15: 166-176.

VIINIKKA, Y. (1973). The occurrence of B chromosomes and their effect on meiosis in Najas marina. Hereditase, 75: 207-212.

VOLOBUEV, V.T. (1975). The additional chromosomes of silver foxes. Synopsis of thesis, Moscow: 1-26.

VOLOBUEV, V.T. (1978). Mammalian B chromosomes. USP. Sovrem. Biol., 86: 387-398.

VOLOBUEV, V.T. (1980a). The B chromosome system of the Asiatic Wood Mouse Apodemus peninsulae (Rodentia, Muridae). 1. Karyotype, G- and C-band structure and the variation of the B chromosome number. Genetika (Moscow), 16: (7) 1277-1284

VOLOBUEV, V.T. (1980b). The B chromosome system of mammals. Genetica, 52/53: 333-337.

VORONTSOV, N.N., BEKASOVA, T.S., KRÁL, B., KOROBITSYNA, K.V. and IVANITSKAYA, E.Y. (1977). On specific status of Asian Wood Mice of the genus Apodemus (Rodentia, Muridae) from Siberia and Far East. Zool. Zh., 56: 437-450

VORONTSOV, N.N., LYAPUNOVA, E.A., IVANITSKAYA, E.Y., NADLER, Ch. F., KRÁL, B., KOZLOVSKY, A.I. and HOFFMAN, R.S. (1978). Variability of sex chromosomes in mammals. 1. Geographical variability of y-chromosome in voles of genera Clethrionomys (Rodentia, Microtinae). Genetika (Moscow), 14 (8): 1432-1446

VOSA, C.G. (1966). Seed germination and B chromosomes in the leek (Allium porrum). Chromosomes today, 2: 189-191.

WANG, H.C. and FEDOROFF, S. (1972). Banding in human chromosomes treated with trypsin. Nature New Biol. (Lond.), 235: 52-53

WARNER, L.J. (1978). "Mammal photography and observation" (A practical field guide). Academic Press.

WELSHONS, W.J. and RUSSELL, L.B. (1959). The y-chromosome as the bearer of the male determining factors in the mouse. Proc. natn. Acad. Sci. U.S.A., 45: 560-566.

WHITE, M.J.D. (1951). Evolution of cytogenetic mechanisms in animals. In: "Genetics in the Twentieth Century". New York. Macmillan.

WHITE, M.J.D. (1960). Are there no mammal species with XO males - and if not, why not? Am. Naturalist, 94: 301-304.

WHITE, M.J.D. (1973). "Animal cytology and evolution". 3rd ed. Cambridge University Press.

WILSON, E.B. (1907). The supernumerary chromosomes of Hemiptera. Science, N.Y., 26: 870-871.

YONG, H.S. and DHALIWAL, S.S. (1972) Supernumerary (B-) chromosomes in the Malayan house rat, Rattus rattus diardii (Rodentia, Muridae) Chromosoma (Berl.), 36 (3): 256-262.

YOSIDA, T.H. (1975). Population survey of B chromosomes in black rats. Rep. natn. Inst. Genet., Misima N. 26: 33-34.

YOSIDA, T.H. (1977). Supernumerary chromosomes in the black rat (Rattus rattus) and their distribution in three geographic variants. Cytogenet. Cell Genet., 18: 149-159.

YOSIDA, T.H. (1978). A preliminary note on silver stained nucleolar organized regions in the black and Norway rats. Proc. Japan. Acad., Ser. B, 54: 353-358.

YOSIDA, T.H. (1979). A comparative study on nucleolus organizer regions (NORs) in 7 Rattus species with special emphasis on the organizer differentiation and species evolution. Proc. Japan. Acad., Ser.B, 10: 481-486.

YOSIDA, T.H. and SAGAI, T. (1975). Variation of C-bands in the chromosomes of several subspecies of Rattus rattus. Chromosoma (Berl.), 50: 283-300.

YOSIDA, T.H. and TAYA, C. (1977). Studies on interspecific hybridization in Rodents. II. Histological observations on the hybrid embryos developed by artificial insemination between Norway rats and black rats. Jap. J. Genet., 52: 289-299.

YUNIS, J.J. and YASMINEH, W.G. (1971). Heterochromatin, satellite DNA and cell function. Science, 174: 1200-1209.

YUNIS, J.J. and YASMINEH, W.G. (1972). Model for mammalian constitutive heterochromatin. Adv. Cell Molec. Biol., 2: 1-46

YUNIS, J.J. and SANCHEZ, O. (1973). G-banding and chromosome structure. Chromosoma (Berl.), 44: 15-23.

ZECEVIC, L.J. and PAUNOVIC, D. (1969). The effect of B-chromosomes on chiasma Frequency in wild populations of Rye. Chromosoma (Berl.), 27: 198-200.

ZIMMERMANN, K. (1957). Sind Gelbhalsmaus und Waldmaus miteinander Kreuzbar. Z. Säugetierk., 22: 214-217.

ZIMMERMANN, K. (1962). Die untergattungen der Gattung Apodemus Kaup. Bonn. Zool. Beitr., 13: 198-208.

ZIVKOVIC, S., SOLDATOVIC, B., MILOSEVIC, M., MIKES, M. and SAVIC, I. (1966). Chromosome analysis in three populations of Apodemus sylvaticus from the Banat (Voivodina). Arch. Biol. Sci. (Belgrade), 18: 35-36.