Comparative karyology of three species of Elephant-shrew (Insectivora: Macroscelididae)

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Abstract

Compared the chromosomes of three southern African species of elephant-shrew (subfamily Macroscelidinae) (*Elephantulus rupestris*, *Macroscelides proboscideus* and *Petrodromus tetradactylus*) by means of G-banding, C-banding and silver-nitrate staining for the detection of nucleolar organizer regions. Extensive G-band homologies were evident in the chromosomes constituting the genomes of the three species. The difference in diploid number between *P. tetradactylus* (2n = 28) and *E. rupestris* and *M. proboscideus* (2n = 26) is attributable to a tandem fusion of two chromosomes in the lineage common to the 2n = 26 species. The distribution of heterochromatin differed between the three species studied; although telomeric C-bands occurred in both *E. rupestris* and *P. tetradactylus*, the staining patterns were not identical. The distribution of NOR's in the species' genomes was, except for the common occurrence of one pair, species specific.

Introduction

Elephant-shrews, members of the family Macroscelididae, are endemic to the African continent. The family, which comprises two subfamilies, the Macroscelidinae and Rhynchocyoninae, stands as a distinctive and relatively isolated group whose ordinal classification is the subject of some uncertainty. PATTERSON (1968) and RATHBUN (1979) are of the opinion that the Macroscelididae should be elevated to a separate order, the Macroscelidea, as members of the taxon are considered to have a more specialised morphology and life history than other Insectivora, a view supported by blood protein studies (GOODMAN 1974). These data suggest a monophyletic origin for the Macroscelididae which shares only primitive traits with the other insectivores. However, most taxonomic treatments of the group persist in the retention of the elephant-shrews within the family Macroscelididae, order Insectivora (CORBET 1974; BUTLER 1978; SMITHERS 1983).

Of the two subfamilies the Macroscelidinae is the more widely represented in the southern African subregion. It comprises the monotypic genera *Macroscelides* (A. Smith, 1829) and *Petrodromus* (Peters, 1846), and the relatively speciose *Elephantulus* (Thomas and Schwann, 1906) which has five species occurring in the subregion. The latter genus has a wide distribution in other parts of Africa and includes four extralimital species in East and North Africa and the Somali (SMITHERS 1983).

Little cytogenetic information is available for elephant-shrews in general and, in particular, the southern African taxa. What information exists is restricted to unbanded karyotypes or the reports of diploid numbers of *E. myurus* (2n = 30; FORD and HAMERTON 1956), *E. (nasilio) brachyrhynchus* (2n = 26; STIMSON and GOODMAN 1966) and the extralimital *E. rufescens* (2n = 34; CHU and BENDER 1962) and *E. rozeti* (2n = 28; MATTHEY 1954). Unfortunately, these data are of limited value in phylogenetic assessments since the absence of differential staining precludes unequivocable identification of shared derived chromosomal traits (synapomorphies) and, as has been pointed out previously

(BAKER and BICKHAM 1980; HAIDUK et al. 1981; ROBINSON et al. 1986), grossly underestimates the degree of chromosomal divergence among species.

As part of an extensive investigation into the chromosomal relationships of the Macroscelididae we present chromosome banding data on three previously unreported taxa: *M. proboscideus, E. rupestris* and *P. tetradactylus*. By determining the extent of chromosomal concordance between these three genera we hope to provide preliminary information on their phylogenetic relationships.

Material and methods

Fibroblast cultures were established from skin biopsies or disaggregated kidney tissue. Air dried slides were routinely prepared from the fibroblast cultures and, occasionally, from bone marrow preparations following a yeast dextrose pretreatment (LEE and ELDER 1980). The methods of WANG and FEDOROFF (1972) and SUMNER (1972) were used respectively for G-banding and C-banding. Nucleolar organizer regions (NOR's) were visualized using a modified silver-staining technique (BLOOM and GOODPASTURE 1976).

The numbers of specimens analyzed and the collection localities with their corresponding grid references are presented:

M. proboscideus: 1 ♂, van Wyksvlei (3021 BD), 1 ♂, Sutherland (3220 BC), 1 ♂, Vaalputs (3021 BA)

E. rupestris: 1 ♀, Beaufort West (3222 BD)

P. tetradactylus: 2 ♂♂, Sordwana Bay (2732 DA)

Results

Round-eared elephant-shrew, *M. proboscideus* (2n = 26)

The G-banded chromosomes of this species are shown in Fig. 1A. The autosomal male karyotype comprises seven pairs of metacentric and submetacentric chromosomes and five pairs of subtelocentric and acrocentric chromosomes. The X is a small metacentric chromosome while the minute Y is acrocentric.

A typical C-banded cell is presented in Fig. 1B and shows the small amount of heterochromatin present in the genome of this species. Even in the best of preparations only approximately 50 % of the chromosomes regularly showed positive C-banding, this generally being concentrated in the proximal portion of the long arms immediately adjacent to the primary constrictions (for example pair 9-arrows). While the X chromosome routinely appeared devoid of heterochromatin, the Y chromosome, in contrast, is almost entirely heterochromatic.

Two nucleolar organizer regions (NOR's), which are located at the terminal ends of autosomal pair 6, were visible following silver staining (Fig. 1C – arrows). Non-specific centromeric staining unrelated to NOR activity was observed infrequently.

Smith's rock elephant shrew, *E. rupestris* (2n = 26)

The G-banded chromosomes of a female *E. rupestris* are presented in Fig. 2A. The karyotype comprises eight pairs of metacentric and submetacentric chromosomes and four pairs of subtelocentric and acrocentric chromosomes. The X chromosome is metacentric in morphology; due to the absence of males in the test material the morphology of the Y remains unknown.

A C-banded metaphase cell is presented in Fig. 2B. A striking feature of the heterochromatic distribution of this species is the presence of telomeric C-bands in many of the larger autosomes (Fig. 2B– arrowheads). In most preparations the telomeric heterochromatin stains less intensely than the centromeric C-bands.
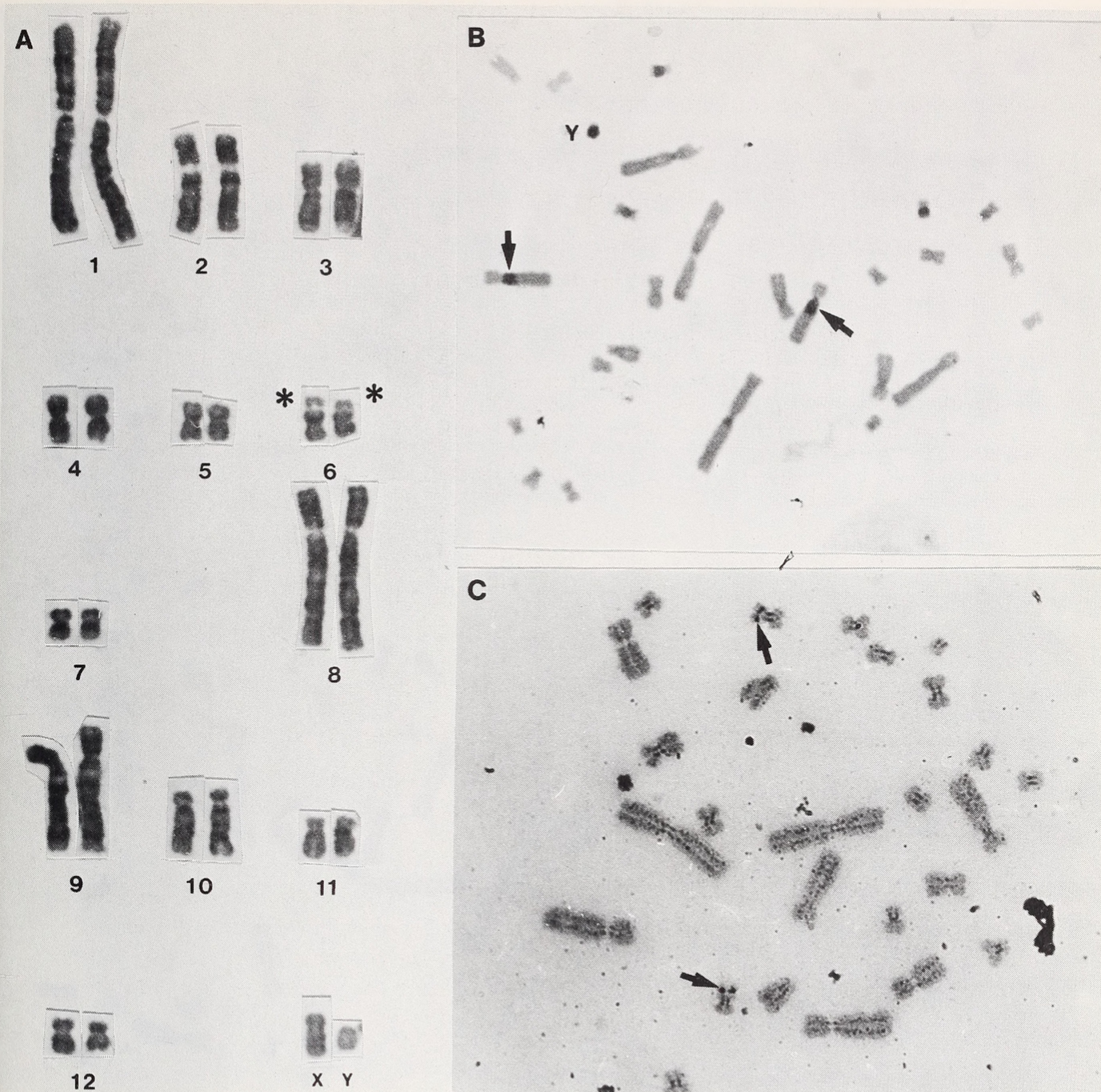
Fig. 1. A: G-banded karyotype of a male *Macroscelides proboscideus* (2n = 26). Asterisks indicate the positions of NOR's. B: C-banded cell showing the large blocks of heterochromatin present on chromosome 9 (arrows). The heterochromatic Y is indicated. C: Silver-stained cell showing one pair of NOR's identified as being located on autosomal pair 6 (arrows)

Evidence of two NOR bearing autosomal pairs was found in *E. rupestris* following silver staining (Fig. 2C). These were tentatively identified as being situated at the terminal ends of pairs 5 and 7 although invariably, silver deposit was found only on one homolog of each pair.

Four toed elephant shrew, *P. tetradactylus* (2n = 28)

The G-banded chromosomes of this species are illustrated in Fig. 3A. The male karyotype consists of eight pairs of metacentric and submetacentric chromosomes and five pairs of subtelocentric and acrocentric chromosomes. The sex chromosomes comprise a small

Fig. 2. A: G-banded karyotype of a female *Elephantulus rupestris* (2n = 26). Asterisks indicate the positions of NOR's. B: C-banded cell showing terminal (arrowheads) and centromeric (arrows) heterochromatin. C: Silver-stained cell showing two of the four NOR bearing chromosomes (arrowheads), the homologs in each case being free of silver deposit

submetacentric X and an acrocentric Y which is the smallest chromosome of the complement.

A typical C-banded metaphase cell is presented in Fig. 3B. As with the preceding species the distribution of heterochromatin is both telomeric (arrowheads) and centromeric (arrows).

A representative silver stained metaphase cell (Fig. 3C) shows clearly the presence of two NOR bearing chromosomes corresponding to pair 7. The NOR's are situated at the terminal ends of the short arms of this autosomal pair.
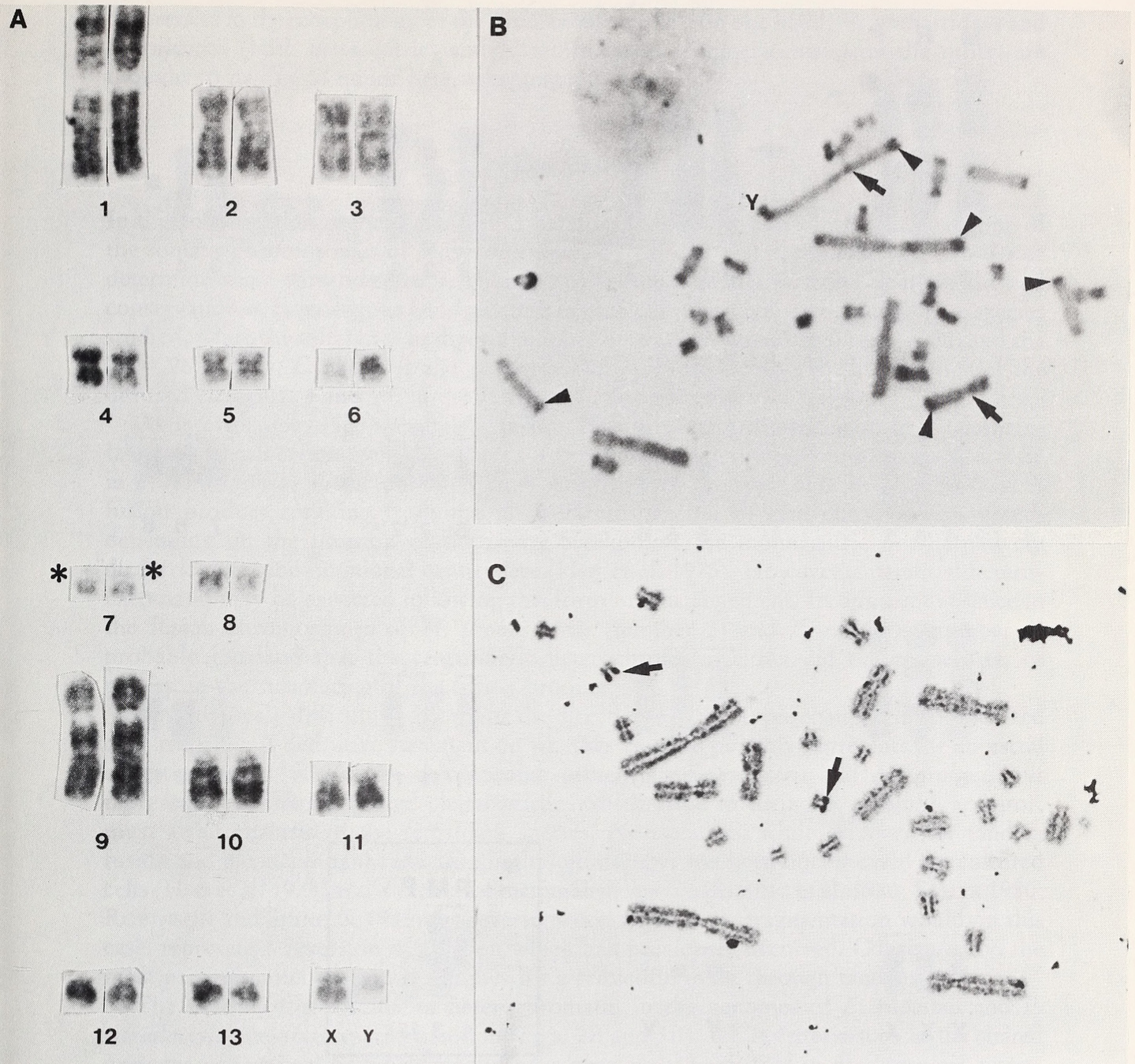
Fig. 3. A: G-banded karyotype of a male *Petrodromus tetradactylus* (2n = 28). Asterisks indicate the positions of NOR's. B: C-banded cell showing differential staining of terminal (arrowheads) and centromeric (arrows) heterochromatin. The heterochromatic Y abutting on chromosome 1 is indicated. C: Silver-stained cell showing a pair of NOR bearing chromosomes identified as being autosomal pair 7 (arrows)

Interspecific comparison

A comparison of the G-banded chromosomes of *M. proboscideus*, *E. rupestris* and *P. tetradactylus* is presented in Fig. 4. The euchromatic portions of all the chromosomes have banding equivalents in their specific genomes. The difference in diploid number between the two former species, each with 2n = 26, and *P. tetradactylus* (2n = 28), is due to the presence of two unfused chromosomal elements in the *P. tetradactylus* genome (chromosomes 4 and 13), which show banding homologies with the submetacentric chromosomes constituting pairs 3 and 4 in the respective 2n = 26 karyotypes (Fig. 4).
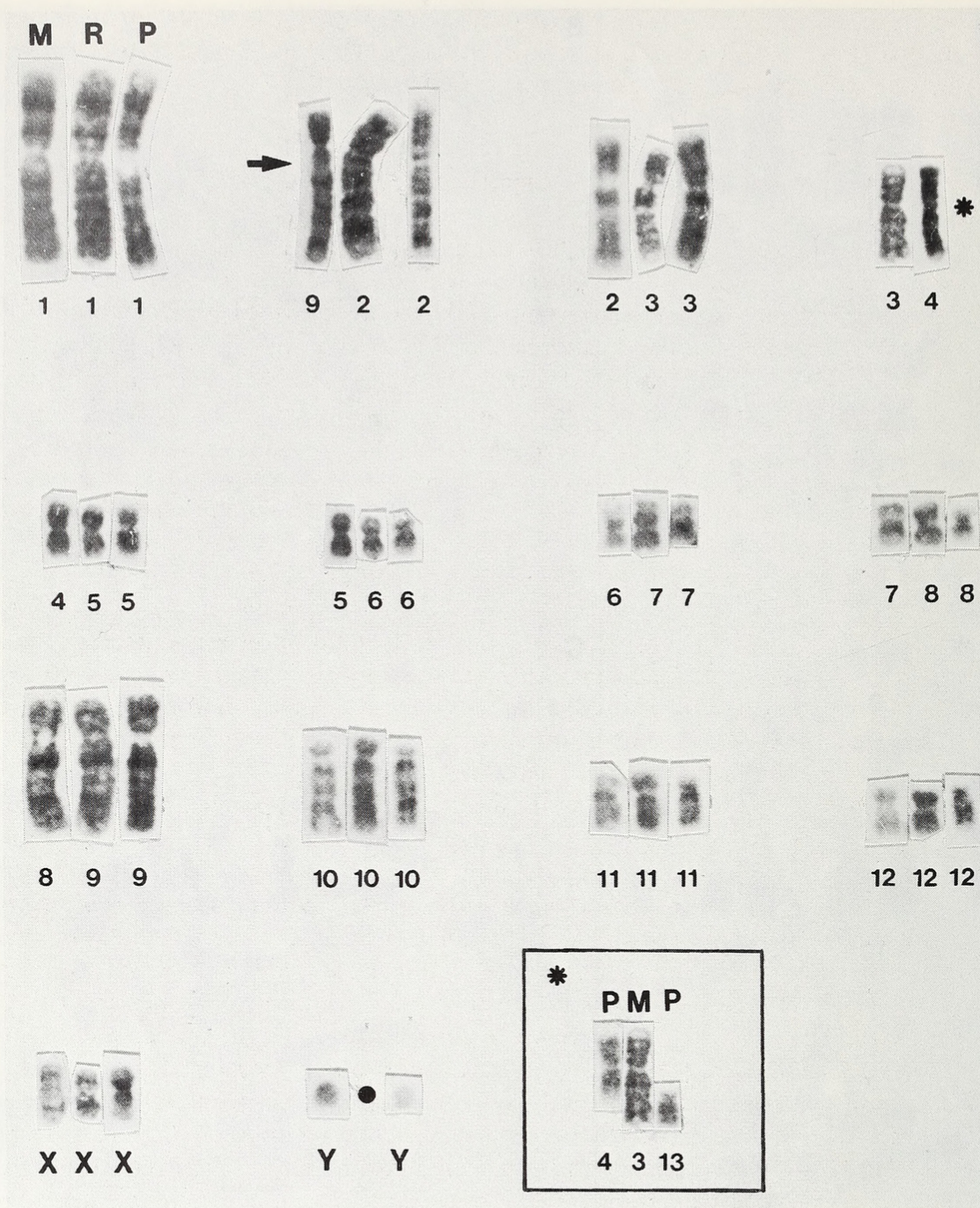
Fig. 4. Comparison of the half karyotypes of three species. M = *M. proboscideus*, R = *E. rupestris* and P = *P. tetradactylus*. Dot indicates the absence of the Y of *E. rupestris* in the study material. The box contains the two chromosomes of *P. tetradactylus* involved in the tandem fusion, as well as the corresponding submetacentric fusion chromosome responsible for the difference in diploid numbers observed between *P. tetradactylus* (2n = 28) and the 2n = 26 species *M. proboscideus* and *E. rupestris*. The corresponding position of the two unfused elements in the half karyotype comparison illustrated here is shown by an asterisk

Subtle interspecific differences in chromosome morphology due to the presence or absence of heterochromatin were noted for several pairs, the most dramatic difference being evident in pair 9 of *M. proboscideus*. This chromosome corresponds in banding pattern to the autosomes constituting pair 2 of the other species. However, the addition of juxtacentromeric heterochromatin in the long arm of this chromosome (Fig. 4 – arrow) has resulted in a change in its gross morphology from submetacentric in *E. rupestris* and *P. tetradactylus* to subtelocentric in *M. proboscideus*. This is responsible for the differential placement of this chromosome in the *M. proboscideus* karyotype. Likewise, the slight

differences in the morphology of the X chromosomes of, on one hand, *M. proboscideus* and *E. rupestris* (both metacentric) and *P. tetradactylus* (submetacentric) on the other, are thought to be due to minor heterochromatic differences.

Discussion

In this investigation use was made of G-banding, C-banding and silver-nitrate staining of the somatic chromosomes of *M. proboscideus, E. rupestris* and *P. tetradactylus* in order to determine their chromosomal relationships. The comparative G-band analysis showed conservation of homologous band patterns in each pair of chromosomes in all three species studied, while the difference in diploid number between *P. tetradactylus* (2n = 28) and the 2n = 26 species, *E. rupestris* and *M. proboscideus,* is attributable to the presence of the unfused elements, 4 and 13, in the *P. tetradactylus* genome.

As is evident in Fig. 4 a tandem fusion, be it a centromere-telomere or a telomere-telomere translocation, would account for the reduction in diploid number from 2n = 28 in *P. tetradactylus* to the observed 2n = 26 in both *E. rupestris* and *M. proboscideus*. A fusion product resulting from the tandem translocation of two chromosomes could, depending on the position of the initial breakpoints, be monocentric or alternatively dicentric with one functional centromere (Hsu et al. 1975). However, interstitial hetero-chromatin may be expected following this form of rearrangement. Its apparent absence in the fusion chromosomes of *M. proboscideus* (number 3) and *E. rupestris* (number 4) probably indicates that the centromeric heterochromatin either did not participate, or persist, in the structuring of the fusion product.

It is obviously difficult to determine the direction of karyotypic change from the limited data available. A definitive statement on whether 2n = 28 possibly represents the ancestral condition, with the fusion chromosome present in *E. rupestris* and *M. proboscideus* representing a shared derived characteristic, which arose in their last common ancestor, must await analysis of the remaining species. Nevertheless, while evidence for tandem fusion and a concomitant reduction in diploid number has been documented for cultured cells (Hsu et al. 1975) and a variety of mammalian species (LIMING et al. 1980; ELDER 1980; ROBINSON and SKINNER 1983) the reverse process, fission or fragmentation would, in this case, represent a reversion of a fusion which had previously occurred. Consequently, the most parsimonious explanation argues for a reduction in 2n through tandem fusion.

The unusual distribution of heterochromatin in the genomes of *E. rupestris* and *P. tetradactylus* deserves special mention (Figs. 2B and 3B). While criteria such as the shared presence of terminal heterochromatin have previously been used in phylogenetic assess-ments (van TUINEN and LEDBETTER 1983), in this study distinct differences in both the staining intensity and the number of chromosomes showing the material in *E. rupestris* and *P. tetradactylus* negate its usefulness as a synapomorphic trait. Likewise, the locations of NOR's are, with a single exception, species specific and of no cladistic value. The common occurrence of a pair of NOR's on homologous chromosomes in all three species (chromo-some 6 of *M. proboscideus* and 7 of the other species) may however prove useful in future investigations incorporating the remaining taxa.

The relative conservativeness of the karyotypic change exhibited by the three species investigated in this study is to some extent unexpected, in view of the social system of elephant-shrews. RATHBUN (1979) is of the opinion that elephant-shrews form monoga-mous pairs occupying small territories, a situation which may predispose to the fixation of structural rearrangements through inbreeding; a situation not dissimilar to foxes which show extensive karyotypic variability (BUSH 1975; MAKINEN and GUSTAVSSON 1982).

When interspecific variation in diploid number is not great, this often indicates that the euchromatic segments of chromosomes are conservative in their structural arrangements,

which is borne out by the present investigation. Should the conservative nature of karyotypic change evident from the three genera analysed in this study hold for the other species in the order, comparative cytogenetic data may prove particularly useful in the assessment of the groups' phylogenetic relationships.

Acknowledgements

Zusammenfassung

Vergleichende Untersuchungen an Chromosomen von drei Arten der Elefantenspitzmäuse
(Insectivora: Macroscelididae)

Die Chromosomen der drei in Südafrika lebenden Elefantenspitzmausarten (Macroscelididae) *Elephantulus rupestris*, *Macroscelides proboscideus* und *Petrodromus tetradactylus* wurden in ihren G- und C-Banden sowie nach Silbernitratfärbung (Lage von NOR = nucleolar organizer region) verglichen. In den G-Banden stimmen die drei Arten weitgehend überein. Die gegenüber *Petrodromus* um ein Paar verminderte Chromosomenzahl bei den beiden anderen Arten ist das Ergebnis einer Verschmelzung der Chromosomen 4 und 13 von *Petrodromus*. *Petrodromus* und *Elephantulus rupestris* haben im Gegensatz zu *Macroscelides* endständiges Heterochromatin, unterscheiden sich aber in der Stärke der C-Banden und der Anzahl betroffener Chromosomen. Ein NOR fand sich bei allen drei Arten auf homologen Chromosomen, bei *Elephantulus rupestris* zusätzlich auf einem weiteren.

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Aktionsräume und Verteilung erwachsener Luchse, *Lynx lynx* (L.), im Hinteren Bayerischen Wald

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Abstract

Home ranges and dispersion of adult lynxes, Lynx lynx (L.), in the Bayerischer Wald, West Germany

Studied movements and habitat use of three adult lynxes in the eastern Bayerischer Wald. Information on activities was obtained from tracking in snow, occasional traces during summer, kills and prey residues, scats, urine marks, and dens frequently used for rest; direct sightings occurred rarely. Individuals were identified by measuring footprints and toothmarks on prey bones and by traces of sex-related behaviour. Freshly found prey objects were inoculated with polystyrene granulate to obtain marked scats at distant localities. Home ranges consisted of a core area of ca. 30 km^2 and a more extended peripheral zone. Ranges of two resident females overlapped with that of one resident male. Each core area included vast rocky sites with dens providing shelter under various weather conditions. These prerequisites had mainly determined the choice of habitat by the lynxes. They lived there for at least 7 years. Some studies from different countries support the concept of a core area as part of the home range. Other literature data, however, diverge extremely on the spatial requirements of the lynx. The relation between methods and results is discussed.

Einleitung

Zu den Ansprüchen des Luchses an Raum und Landschaftsstrukturen liegen wenige und widersprüchliche Angaben vor. Ursachen dafür sind die Schwierigkeiten der Freilandforschung und erhebliche Unterschiede in den eingesetzten Methoden. Der Luchs hält sich vor dem Beobachter verborgen, bewegt sich vorwiegend bei Nacht und über weite Entfernungen. Darum müssen seine Aktivitäten aus Spuren erschlossen und die Untersuchungen auf überschaubare Gebiete und damit auf einzelne Individuen konzentriert