Close chromosomal congruence in two species of ground squirrel: *Xerus inauris* and *X. princeps* (Rodentia: Sciuridae)

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Chromosomes from cultured fibroblasts of two southern African ground squirrel species, *Xerus inauris* (2n = 38) and *X. princeps* (2n = 38), were compared using G-banding, C-banding and silver nitrate staining for the detection of NORs (nucleolar organizer regions). The karyoptypes of the two species, whose taxonomic status is the subject of some uncertainty, are largely identical except for a subtle heterochromatic difference which affects a single autosomal chromosome pair in *X. inauris*. The species specificity of this marker in their zone of contact indicates an absence of gene flow between these taxa and gives credence to the recognition of *X. inauris* and *X. princeps* as two biologically distinct species.

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G- en C-band gekleurde chromosome afkomstig van fibroblaskulture van twee Suid-Afrikaanse grondeekhoringspesies *Xerus inauris* (2n = 38) en *X. princeps* (2n = 38) is vergelyk. Daar is ook gebruik gemaak van 'n silvernitraatkleuringstegniek vir die identifikasie van die nukleolus organiserende gebiede van die chromosome. Die kariotipes van die twee spesies, waarvan die taksonomiese status onseker is, is byna identies behalwe vir geringe verskille in heterochromatien ten opsigte van een outosomale chromosoompaar in *X. inauris*. Die spesiespesifisiteit van hierdie chromosoommerker dui op die afwesigheid van geenuitruiling tussen die taksa in hulle geografiese kontakgebied. Hierdie data ondersteun dus die huidige klassifikasie wat *X. inauris* en *X. princeps* as twee afsonderlike spesies beskou.

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The family Sciuridae to which the terrestrial, arborial and flying squirrels belong comprises two subfamilies, the Sciurinae and the Petauristinae. In view of an almost cosmopolitan distribution and the wide variety of habitat types associated with the family, squirrels are particularly suited to studies of speciation processes and adaptations involving contrasting environments.

Two terrestrial squirrel species (subfamily Sciurinae) occur in the southern African subregion. The more widespread of the two, the Cape ground squirrel, *Xerus inauris*, has the greater part of its distributional range confined to the south-western arid zone (Figure 1A), while the mountain ground squirrel, *X. princeps*, is associated primarily with the Namibian western escarpment and the south-western parts of Angola (Figure 1B; Smithers 1983). Although both taxa are found exclusively in arid areas, they rarely occur sympatrically, *X. inauris* being replaced in extreme north-west Namibia by *X. princeps*. Moreover, casual observations tend to support Shortridge's (1934) claim that the former taxon shows a preference for open terrain associated with sparse bush, while *X. princeps* is a rock-dwelling species.

Although generally regarded as being morphologically distinct (Amtmann 1975; Smithers 1983), the recognition of the taxa as separate species is accepted with reservation by De Graaff (1981). In an attempt to provide definitive data on the taxonomic status of these species and to illustrate factors possibly contributing to the maintenance of species integrity in their contact zones, a multidisciplinary investigation employing karyotypic, craniometric, behavioural and ecophysiological parameters was initiated. The cytosystematic data form the basis of this report.

Material and Methods

The species, number of specimens studied and grid reference to the collection localities are as follows:

X. inauris: 3 ♂♂, 2 ♀♀; Farm Klein Spitzkopp 45 km west of Usakos (22°00'S/15°33'E), Namibia.

X. princeps: 2 ♂♂, 2 ♀♀; Farm David Ost 18 km north of Usakos, Namibia.

All animals examined in the present study were deposited as voucher specimens in the mammal collection of the Transvaal Museum, Pretoria.

Metaphase cells were obtained from fibroblast cultures initiated from skin biopsies and disaggregated kidney tissue using standard procedures. Air-dried slides were prepared from cultured cells and subsequently G- and C-banded using

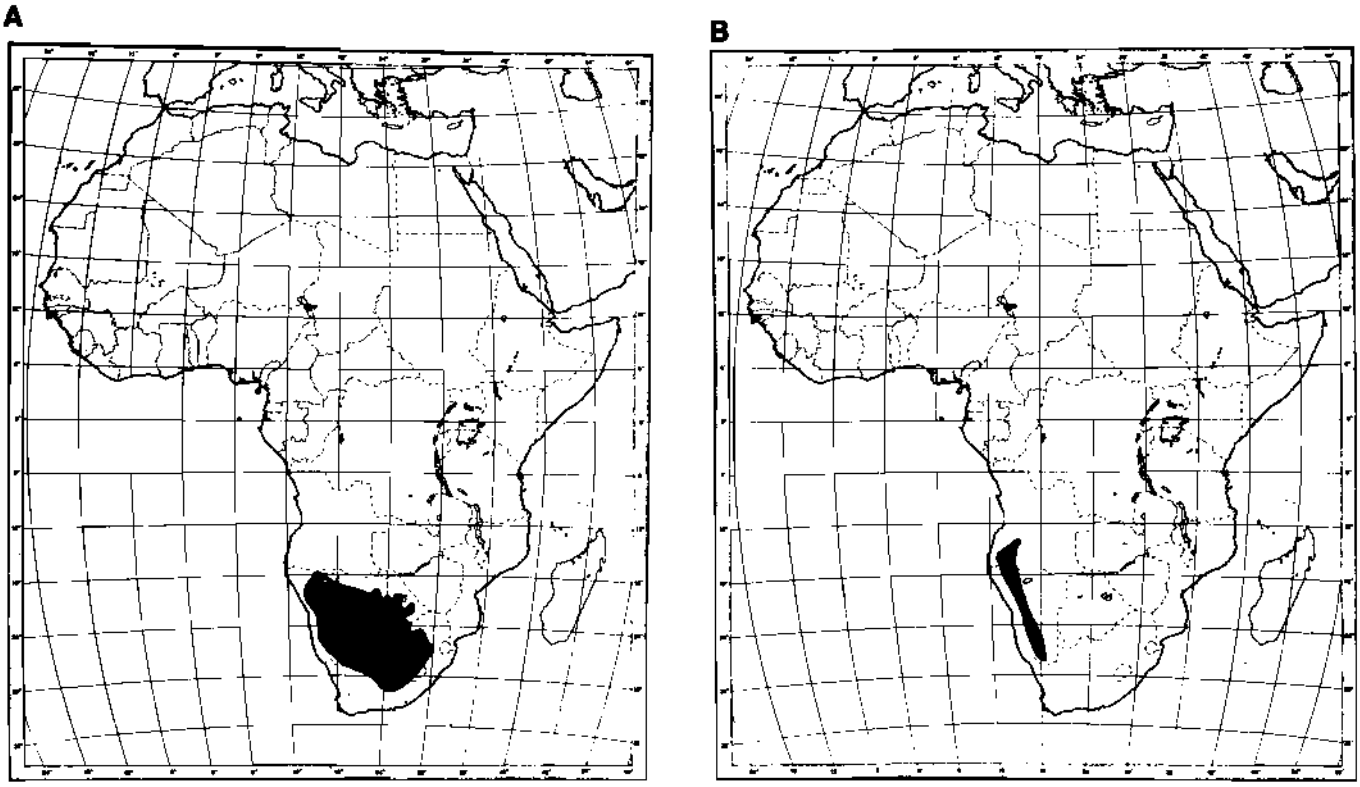

Figure 1 Distribution of (A) the Cape ground squirrel, *X. inauris* and (B) the mountain ground squirrel, *X. princeps* in the southern African subregion (Smithers 1983).

the methods of Wang & Fedoroff (1972) and Sumner (1972) respectively. Silver nitrate staining for nucleolar organizer regions (Ag-NORs) followed the Ag–I procedure of Bloom & Goodpasture (1976).

The grouping of the chromosomes in both *X. inauris* and *X. princeps* follows the format adopted by Nadler & Hoffman (1974) for the East African ground squirrel, *X. rutilus*. The designation of chromosomes as metacentric, submetacentric and acrocentric follows these authors but is, to some extent, arbitrary. The ranking of chromosomes in each group was determined by the relative chromosome lengths expressed as a percentage of the haploid female karyotype (Lee & Martin 1980). For this, homologous chromosomes were identified by their G-band patterns and length measurements of each chromosome made directly from photographs using Vernier calipers. The precise measurement of the short arms of the NOR-bearing chromosomes was often confounded by the stalks. Consequently, measurements of this autosomal pair reflect only the relative lengths of the long arms.

For reference purposes, representative cell cultures from both species were protected with dimethylsulfoxide (DMSO) and deep frozen in liquid nitrogen. Accession numbers are: *X. inauris* A6 FD 1985; *X. princeps* A5 FD 1985.

Results

Cape ground squirrel, *X. inauris* (2n = 38)

The G-banded chromosomes of this species are shown in Figure 2A. The chromosomes have been numbered and the karyotypes standardized based on the percentage contribution of each chromosome to the genome (Table 1). The autosomal complement comprises seven metacentric and 10 submetacentric chromosome pairs, as well as one acrocentric pair with distinct satellites. The X chromosome, which is submetacentric in morphology constitutes on average, 5,19 + 0,12% of the genome. The Y chromosome, in turn, is a small metacentric and comprises 2,09 + 0,08% of the

Table 1 Relative chromosome lengths of the Cape ground squirrel, *X. inauris*, expressed as a percentage of the haploid karyotype (A + X). Measurements were taken from G-banded chromosomes derived from eight male cells. $\bar{X}$ = Arithmetic mean; *SE* = Standard error.

	Relative length % of (A + X)	
Chromosome	$\bar{X}$	*SE*
1	8,48	0,16
2	7,80	0,20
3	6,96	0,11
4	6,21	0,04
5	4,28	0,08
6	3,04	0,09
7	2,44	0,07
8	7,78	0,10
9	6,55	0,08
10	6,02	0,06
11	5,91	0,10
12	5,88	0,14
13	5,16	0,09
14	5,01	0,10
15	4,51	0,07
16	3,80	0,28
17	2,03	0,09
18	2,52	0,04
X	5,19	0,12
Y	2,09	0,08

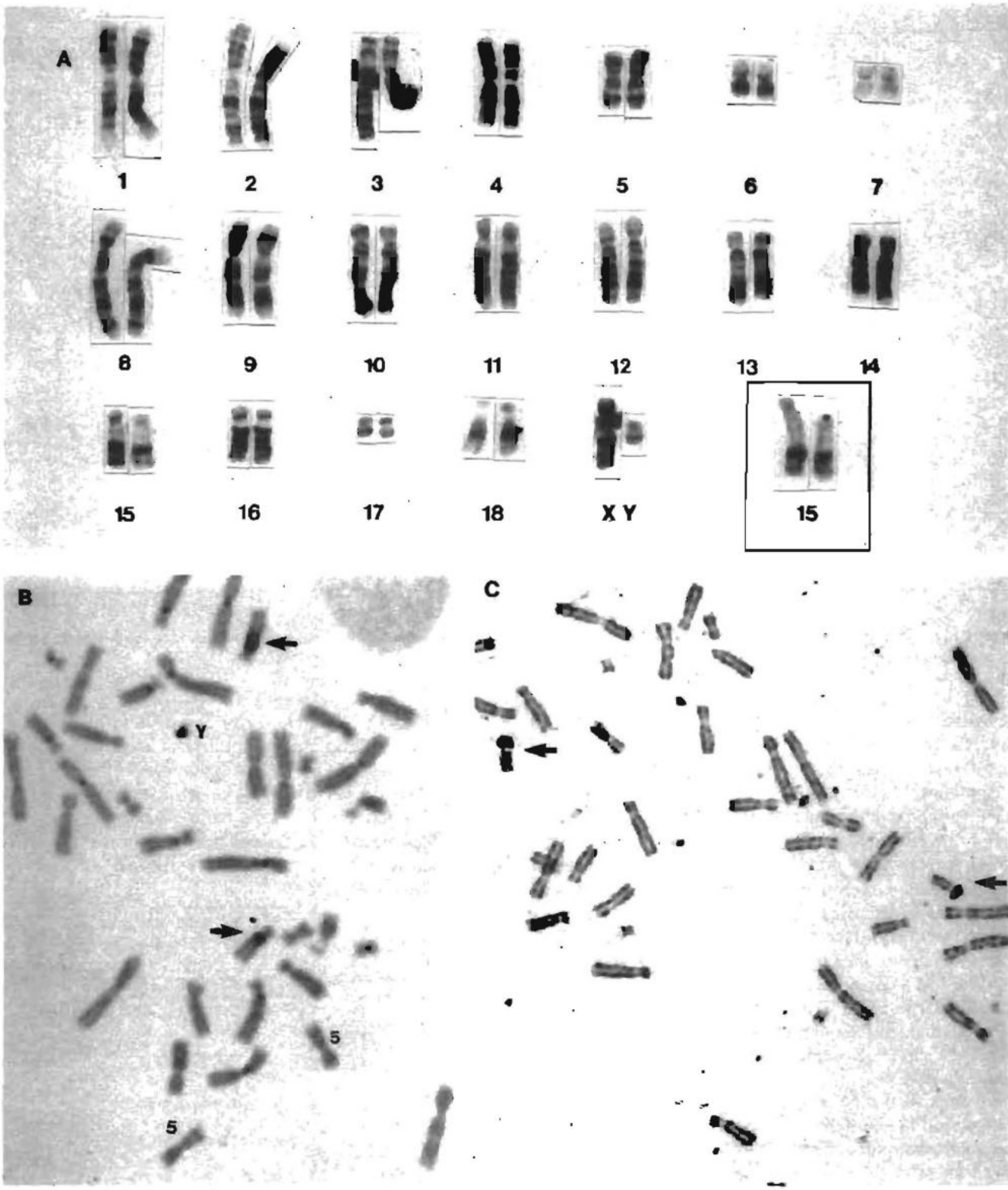

Figure 2 (A) G-banded karyotype of the Cape ground squirrel, *X. inauris* (2n = 38). Inset illustrates the heteromorphic nature of pair 15 observed in a single *X. inauris* specimen. (B) Partial C-banded metaphase cell illustrating the paucity of heterochromatin in this species. Note in particular the absence of a C-band positive centromeric region in pair 5 and the heterochromatic nature of the long arm of the Y chromosome. Arrows illustrate the differing quantities of heterochromatin in pair 15 responsible for the heteromorphism referred to above. (C) Silver-stained metaphase chromosomes showing the presence of two NOR-bearing autosomes (arrows).

haploid complement.

A striking feature of the G-banded analysis of *X. inauris* was the evidence of a marked heteromorphism in pair 15 of one of the specimens studied (Figure 2A). The discrepancy in the length of this heteromorphic pair was found, following C-banding (Figure 2B arrows), to be attributable to the addition of juxtacentromeric heterochromatin in the longer variant. However, since this anomaly was not noted in the remainder of the *X. inauris* study material, it is not possible to determine whether the heteromorphism is indicative of a widespread polymorphism or, alternatively, that it merely reflects a *de novo* structural change in this specimen.

With the exception of the homologs constituting pair 15, C-banding revealed minimal amounts of pericentromeric heterochromatin in this species's chromosomes with only four or five pairs possessing easily observable quantities

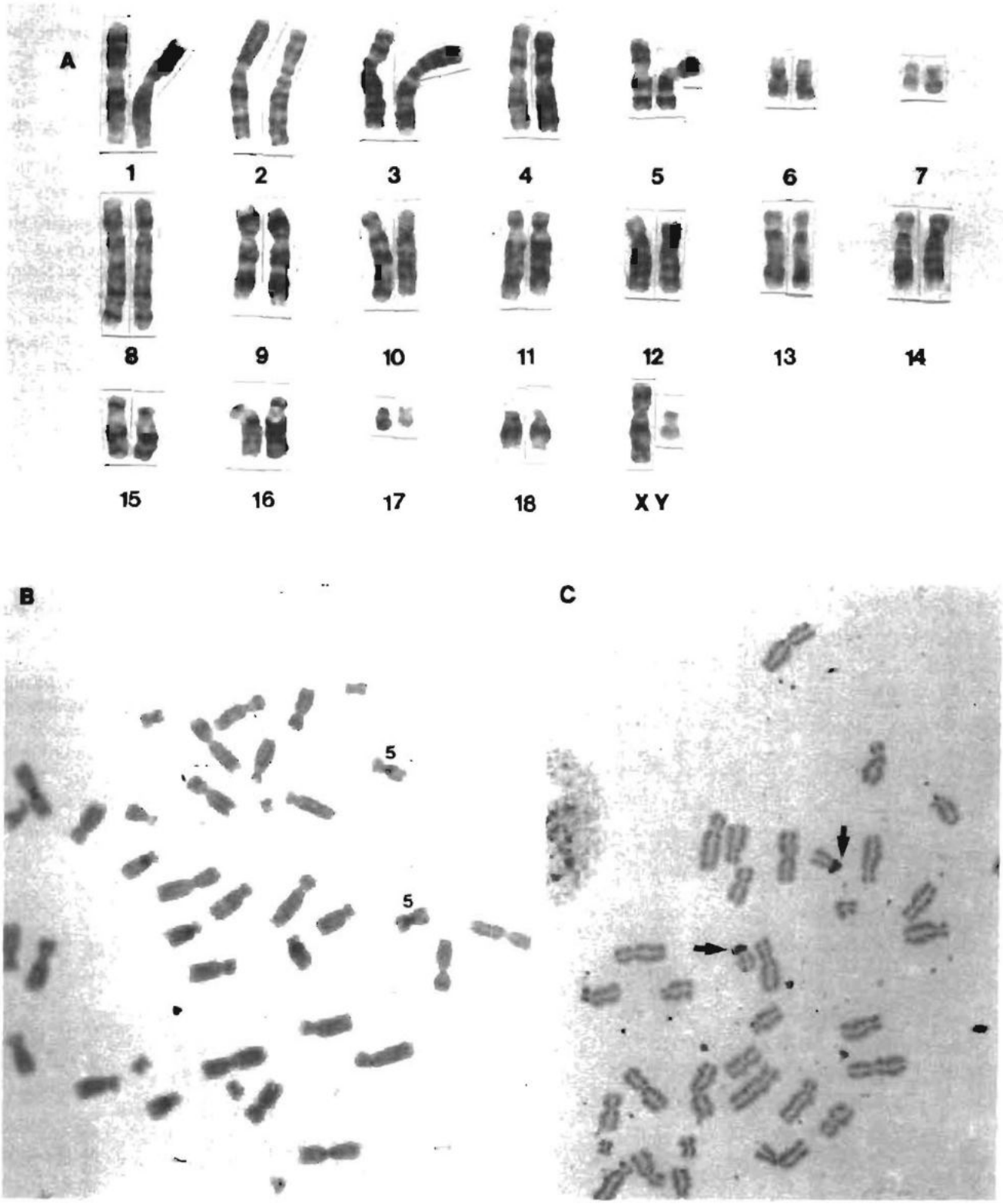

Figure 3 (A) G-banded karyotype of the mountain ground squirrel, *X. princeps* (2n = 38). (B) C-banded metaphase cell illustrating the paucity of heterochromatin in this species. Note however, the presence of prominent centromeric heterochromatin in the autosomes constituting pair 5. (C) Partial silver-stained metaphase cell showing the presence of two NOR-bearing autosomes (arrows).

(Figure 2B). Although most of the chromosomes of the complement cannot be distinguished by C-banding alone, the small metacentric Y is an exception. The long arm of this chromosome is almost entirely heterochromatic which facilitates its identification.

A representative AgNOR-stained metaphase cell (Figure 2C) shows clearly the presence of two NOR-bearing chromosomes and confirms our initial observations of two 'satellited' chromosomes (pair 18) visible after conventional staining and, in many cases, following G-banding.

Mountain ground squirrel, *X. princeps* (2n = 38)

Since the chromosomes of *X. princeps* are, with minor exceptions, essentially the same as those of *X. inauris* (see below), no attempt will be made to describe the karyotype in any detail. The G- and C-banded chromosomes are shown respectively in Figures 3A and B, while the relative chromosome lengths used in the standardization of the species's

karyotype are presented in Table 2. The X chromosome constitutes 5,03 ± 0,09% of the genome and the Y 2,21 ± 0,07%; these values resemble very closely, the situation in the previous species.

Table 2 Relative chromosome lengths of the mountain ground squirrel, *X. princeps*, expressed as a percentage of the haploid karyotype (A + X). Measurements were taken from G-banded chromosomes derived from 10 male cells. $\bar{X}$ = Arithmetic mean; *SE* = Standard error.

	Relative lengths % of (A + X)	
Chromosome	$\bar{X}$	*SE*
1	8,45	0,12
2	7,96	0,09
3	7,04	0,19
4	6,84	0,12
5	4,57	0,07
6	2,97	0,10
7	2,48	0,09
8	8,22	0,13
9	6,62	0,08
10	6,20	0,05
11	6,13	0,10
12	5,63	0,08
13	5,32	0,08
14	5,11	0,11
15	4,22	0,07
16	3,95	0,09
17	1,89	0,08
18	2,59	0,05
X	5,03	0,09
Y	2,21	0,07

Silver nitrate staining (Figure 3C) regularly revealed prominent silver deposits at the terminal ends of the short arms of two acrocentric chromosomes which correspond to the satellited autosomes, pair 18.

Comparison of *X. inauris* and *X. princeps* karyotypes

The G-banded chromosomes of *X. inauris* are compared to those of *X. princeps* in Figure 4. The chromosomes of the species can be convincingly matched and except for subtle heterochromatic differences, appear to be very similar, if not identical. The only consistent interspecific character involves the centromeric regions of autosomal pair 7, which are routinely G-positive following trypsin treatment in *X. inauris* and strikingly G-negative in *X. princeps* (Figure 4).

Discussion

The remarkable diversity of karyotypes found in vertebrate species suggests that speciation events may sometimes be associated with karyotypic change. This certainly holds for many of the North American terrestrial squirrels as is evidenced by the interspecific variation in diploid numbers encountered within the genera *Spermophilus* and *Marmota* where diploid numbers vary between 2n = 30–46 in the former and 2n = 36–42 in the latter (see Nadler & Hoffmann 1970 and references therein).

In contrast, the African species appear karyotypically highly conservative. In addition to the cytogenetic data on the two xerids contained in the present report, conventionally stained karyotypes as well as a schematic representation of the G-band patterns are available for the East African *X. rutilus* (Nadler & Hoffmann 1974). While lack of sufficient

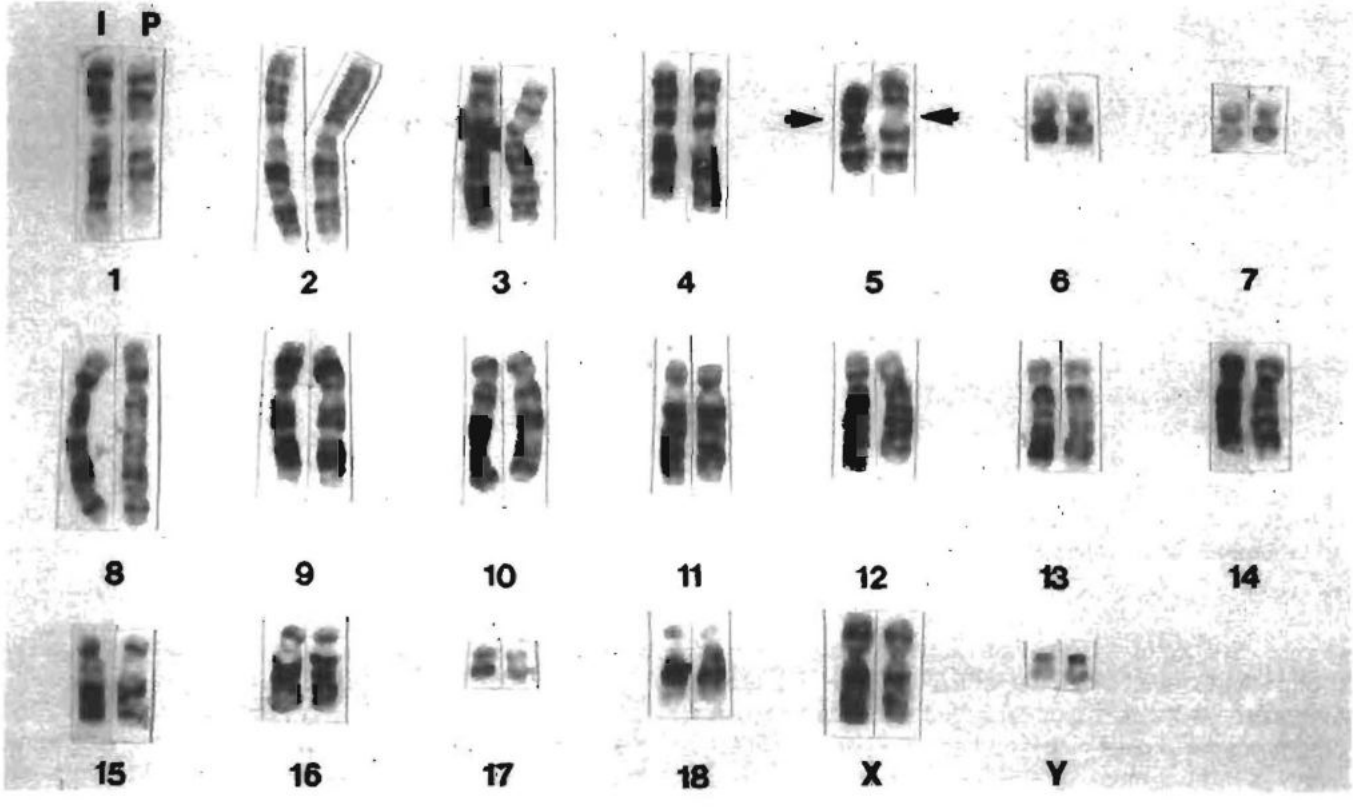

Figure 4 Comparison of the G-banded chromosomes of *X. inauris* (I) and *X. princeps* (P). The chromosomes have, with the exception of the sex chromosomes, been taken from the karyotypes presented in Figures 1A and 2A. The arrows indicate the species specific differences in respect of the centromeric regions of pair 5.

detail in the G-band idiogram precludes meaningful comparisons with the G-banded karyotypes of either *X. inauris* or *X. princeps*, it is nevertheless apparent that the gross chromosomal morphology and diploid numbers of all three species are remarkably similar. However, conclusions based on conventionally stained chromosomes have been shown to be misleading (Baker & Bickham 1980; Haiduk, Baker, Robbins & Schlitter 1981) since there is a tendency to underestimate the degree of chromosomal divergence between related taxa. Our results on *X. inauris* and *X. princeps* further support this observation.

Consequently although appearing indistinguishable on gross morphology, the banded chromosomes of *X. inauris* and *X. princeps* nevertheless differ, though subtly, in respect of autosomal pair 5. The difference can be found in the centromeric regions of these chromosomes, their euchromatic portions showing excellent concordance. Following G-banding this region is clearly giemsa negative and more attenuated in *X. princeps* than the corresponding giemsa positive area in *X. inauris* (Figure 4). Staining reversal of this nature is not unusual and may be associated with heterochromatin; centromeres with intense C-banding often show lack of G-banding (Buckland & Evans 1978; Stock 1981). This is reflected in the present study where *X. princeps* is characterized by distinct pericentromeric heterochromatin in pair 5 (Figure 3B) which is absent in *X. inauris* (Figure 2B).

While this difference is certainly consistent in our material, minor variation in heterochromatin has, due to its genetically inert characteristics (John & Miklos 1979), not traditionally been accorded much weight in interspecific comparisons (Stock & Hsu 1973). However, more recently this technique has been used to distinguish species independently of other banding methods (Stock 1981) and, in cases of unusual heterochromatic distribution, also in assessing phylogenetic relationships (Van Tuinen & Ledbetter 1983).

Although the heterochromatic difference between *X. princeps* and *X. inauris* is undeniably slight, the contrasting staining behaviour of the centromeric regions of pair 5 nevertheless provides an unequivocal means of species identification and supports their recognition as two distinct biological species. Although such minor karyotypic modification is unlikely to have any effect in checking potential introgression between the taxa, it is our contention that it provides a useful marker for the detection of possible interspecific mating in their zones of contact. In this regard, the cytogenetic data will be particularly valuable should further study show an intergradation of the phenotypic characters currently used in species identification.

One further aspect of the xerid karyotypes presented here that deserves special comment is the shared presence of satellites on the autosomal chromosomes constituting pair 18. In addition to *X. inauris* and *X. princeps*, satellited chromosomes have also been observed in East African ground squirrels *X. rutilus* (Nadler & Hoffman 1974; Nadler, Hoffmann & Hight 1975), long-toed ground squirrels of middle Asia *Spermophilopsis leptodactylis* (Nadler & Hoffmann 1974) and certain of the Asian tree squirrels genus *Callosciurus* (Nadler *et al.* 1975).

A comparison of the G-banded satellited chromosomes of *X. rutilus* and *C. notatus* presented by Nadler *et al.* (1975), and those of both *X. inauris* and *X. princeps*, reveal excellent concordance. While similarities in banding patterns do not necessarily imply total homology it seems likely that the hypothesis that the xerine ground squirrels and certain Asian tree squirrel species have evolved from a common ancestor is correct (Nadler *et al.* 1975). However, the inclusion of a greater number of species and, importantly, the use of several banding techniques in addition to other systematic parameters would do much to elucidate the phylogenetic relationships of the African and Asian sciurids.

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