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Comparative cytogenetics of the Hyracoidea: chromosomes of two *Hyrax* species from South Africa

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Abstract

Described the chromosomes of the tree hyrax (*Dendrohyrax arboreus*: 2n = 54) and the yellow-spotted hyrax (*Heterohyrax brucei:* 2n = 54) from South Africa. Metaphase chromosomes were obtained from tissue culture and G-banding and C-banding patterns studied. Karyotypic comparisons between the species allowed for the identification of G-band homologies for most of the autosomes. The X chormosomes were identical in morphology and banding patterns, while the *D. arboreus* submetacentric Y chromosome differed from the acrocentric *H. brucei* counterpart by the addition of a heterochromatic short arm. While the C-band material is strictly centromeric in *H. brucei*, the tree hyrax, *D. arboreus,* is characterized by pronounced heterochromatic short arms in several of the chromosomes; terminal C-bands were also noted in some of the autosomes.

Introduction

The Hyracoidea is a distinctive order containing a single family, the Procaviidae, which is confined to Africa and the Arabian region (Corbet 1978). Although disputed since 1892, the consensus now appears that the family includes three distinct genera – *Procavia, Heterohyrax* and *Dendrohyrax* (Meester et al. 1986). In South Africa the rock hyrax, *P. capensis,* occurs widely though limited to suitable rocky habitat. In contrast, the distribution patterns of the two species which form the subject of this report, *D. arboreus* and *H. brucei*, are restricted in South Africa. The yellow-spotted hyrax, *H. brucei,* occurs only in the extreme northeastern parts of the country but extends through Zimbabwe, parts of Mozambique and Botswana, northwards through central and eastern Zambia, Malawi, Tanzania, Kenya, Uganda, Somalia and southern and southwestern Sudan. The tree hyrax, *D. arboreus,* classified in the South African Red Data Book as rare due to habitat destruction (Smithers 1986), is limited to pockets of indigenous forest in the south-eastern Cape Province and the Natal midlands and has been recorded from parts of Mozambique, Malawi, Zambia, Zaire, Tanzania, Kenya and Uganda. The species is replaced in eastern Tanzania and its offshore islands by *D. validus* and, in the forests of the Congo Basin and west Africa, by *D. dorsalis*, both of which are closely allied species (Smithers 1983).

Published karyotypic data on the Hyracoidea are restricted to unbanded preparations of the ubiquitous *P. capensis* (2n = 54; Hungerford and Snyder 1969; Hsu and Benirschke 1971b). In the present report, we detail the G-banding and C-banding patterns of two, hitherto unreported, hyrax species. Our data, together with those published on *Procavia*, suggest an ancestral diploid number of 2n = 54 for the family. Furthermore, at least in respect of the species covered by this report, karyotypic evolution has proceeded largely through complex chromosomal rearrangements that appear to have disrupted G-band homology in some autosomes and through changes in the distribution of constitutive heterochromatin.

Material and methods

Number of specimens studied from each species, the sample localities and their approximate grid references are presented below.

Dendrohyrax arboreus: 1 male – Pirie Forest, eastern Cape Province (32° 46′ S, 29° 21′ E).

Heterohyrax brucei: 2 males – Blyde River Nature Reserve (24° 39′ S 30° 50′ E) and Vhembi Nature Reserve (22° 12′ S, 29° 21′ E).

Skins and skulls from each study animal (excluding the *H. brucei* specimen from Blyde River which was badly damaged) have been deposited as voucher specimens in the mammal collections of the Kaffrarian and Transvaal Museums.

Metaphase chromosomes were obtained from fibroblast cultures which were initiated from ear biopsies and maintained in McCoy 5A medium supplemented with 10% fetal calf serum. Chromosomes were G-banded and C-banded following the methods of Wang and Fedoroff (1972) and Sumner (1972) respectively. The diploid chromosome number was determined by counting 25 cells from each specimen while chromosome measurements were taken directly from photographs using a Quantimet 520 Image Analyzer (Cambridge Instruments, UK). The G-banded karyotype of each species was standardized based on the percentage contribution of each chromosome to the female genome (Lee and Martin 1980). Chromosomes were grouped by establishing chromosome arm ratios (metacentric 1.0–1.1; submetacentric 1.1–1.9; subtelocentric > 2; acrocentric with no visible short arm; Levan et al. 1964) and ordered in decreasing size. In the case of *D. arboreus*, one autosomal pair, pair 6, was characterized by marked heteromorphism. The submetacentric morph differed from its acrocentric homolog by the presence of additional heterochromatin in the short arm. Consequently, in standardizing this species karyotype, we have chosen to exclude the heterochromatic short arm of the submetacentric morph; therefore length measurements of this pair merely represent the acrocentric morphology.

Results

Both *D. arboreus* and *H. brucei* are characterized by 2n = 54 which, when taken together with the published data on *P. capensis*, reflect the conserved nature of the diploid number across the three extant hyrax genera. The G-banded karyotype of *D. arboreus* is shown in Figure 1 and the chromosomes numbered and standardized based on the percentage contribution of each to the genome (Tab. 1).

The autosomal chromosome complement of *D. arboreus* comprises 15 acrocentric pairs (1–15, one of which, pair 6, shows pronounced heteromorphism), five pairs of subtelocentric autosomes (16–20), five pairs of submetacentric (21–25) and one pair of metacentric

Table 1. **Relative chromosome lengths of *Dendrohyrax arboreus* expressed as a percentage of the haploid female karyotype (n=4)**

Chromosome number	Relative length % of (A+X) x	SE	Chromosome number	Relative length % of (A+X) x	SE
1	5.212	0.076	15	2.056	0.069
2	5.038	0.112	16	6.417	0.154
3	4.944	0.127	17	4.896	0.129
4	4.424	0.171	18	4.598	0.059
5	4.292	0.159	19	3.658	0.042
6	3.238	0.057	20	3.541	0.071
7	3.587	0.114	21	3.475	0.086
8	3.572	0.212	22	3.345	0.098
9	3.428	0.037	23	2.712	0.030
10	3.332	0.091	24	2.705	0.065
11	3.322	0.092	25	2.249	0.100
12	2.822	0.049	26	1.985	0.054
13	2.529	0.163	X	4.938	0.145
14	2.351	0.045	Y	1.955	0.108

x = arithmetic mean; SE = standard error.

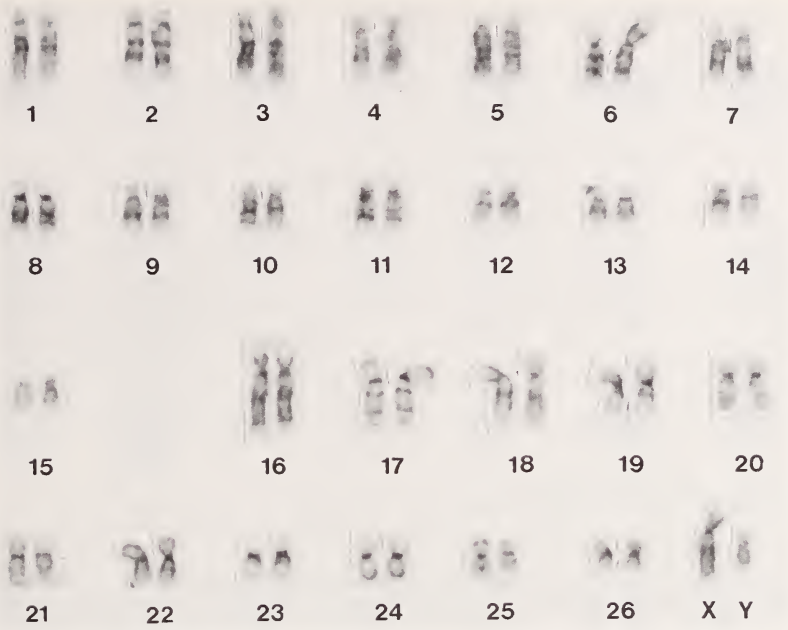

Fig. 1. G-banded karyotype of a male tree hyrax, *Dendrohyrax arboreus* (2n = 54). The heteromorphism evident in the lengths of the p arms of the homologs constituting pair 6 is attributable to differences in heterochromatin

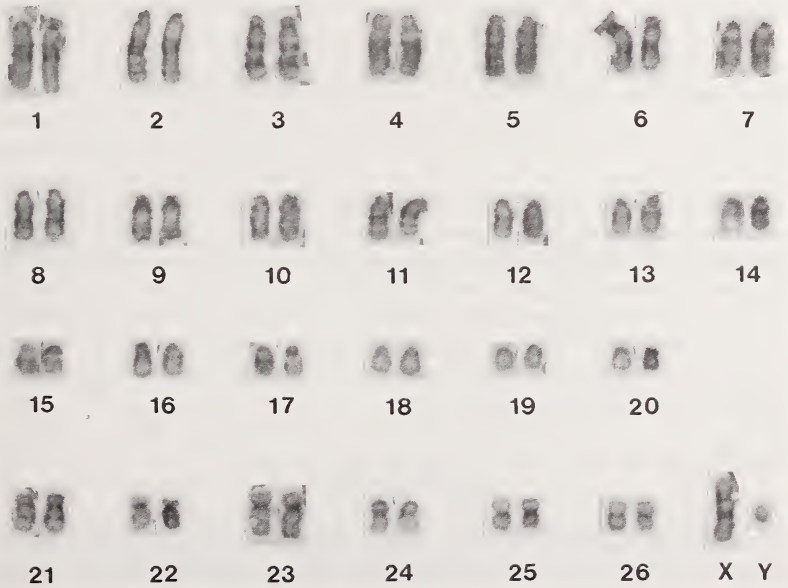

Fig. 2. G-banded karyotype of a male yellow-spotted hyrax, *Heterohyrax brucei* (2n = 54)

Table 2. **Relative chromosome lengths of *Heterohyrax brucei* expressed as a percentage of the haploid female karyotype (n=4)**

Chromosome number	Relative length % of (A+X) x	SE	Chromosome number	Relative length % of (A+X) x	SE
1	6.588	0.166	15	2.770	0.151
2	6.010	0.200	16	2.560	0.073
3	5.732	0.159	17	2.449	0.083
4	5.360	0.279	18	2.145	0.104
5	5.226	0.120	19	2.119	0.038
6	4.748	0.157	20	1.972	0.091
7	4.400	0.115	21	3.353	0.085
8	4.257	0.066	22	2.796	0.058
9	3.916	0.108	23	4.027	0.047
10	3.865	0.097	24	2.713	0.052
11	3.774	0.067	25	2.548	0.061
12	3.240	0.030	26	2.327	0.084
13	3.034	0.041	X	5.199	0.130
14	2.884	0.054	Y	1.350	0.166

x = arithmetic mean; SE = standard error.

chromosomes (26). The X chromosome is the largest submetacentric chromosome and constitutes 5 % of the female haploid complement; the submetacentric Y is intermediate in size between pairs 25–26 forming approximately 2 % of the female genome (Tab. 1).

The *H. brucei* karyotype (Fig. 2) has 20 acrocentric autosomal pairs (1–20), two subtelocentric autosomal pairs (21–22), two submetacentric (pairs 23–24) and two metacentric autosomal pairs (25–26). The X chromosome is the largest submetacentric chromosome in the complement and contributes 5.2 % to the female genome, while the acrocentric Y, due to its small size, is readily distinguishable even in unbanded preparations and constitutes 1.4 % of the haploid genome (Tab. 2).

A comparison of the G-banded chromosomes of the two hyrax species is shown in Figure 3. Convincing G-band homology is clearly evident for the euchromatic portions of the majority of chromosomes including the X which shows two dark bands in the middle of the long arm which are characteristic for most mammals (Pathak and Stock 1974). The only exceptions to this are chromosomes 4, 9, 17–19, and 21 in the *Dendrohyrax* karyotype and 9–10, 15, 17, 19, and 20 in *Heterohyrax*. In determining homologies between these species it is also informative to compare the relative amounts and distribution of constitutive heterochromatin present in their genomes. In *H. brucei* the distribution of C-band material is strictly centromeric while the *D. arboreus* karyotype is characterized by several of the autosomes having pronounced heterochromatic short arms and blocks of terminal heterochromatin (Fig. 4A and B). This difference has led to the length discrepancies evident in the comparison of the G-banded chromosomes of the species and is most pronounced in respect of their Y chromosomes as well as *Dendrohyrax* chromosome 6 cf *Heterohyrax* 7, *Dendrohyrax* 7 cf *Heterohyrax* 8, *Dendrohyrax* 16 cf *Heterohyrax* 2 and *Dendrohyrax* 22 cf *Heterohyrax* 16.

Discussion

The Hyracoidea are a group of phenotypically similar herbivorous mammals (also known as "dassies" and sometimes referred to as "conies") whose distribution is Afro-Arabian and whose origins, based on the fossil record, are strictly African (De Blaise and Martin 1982). The terrestrial forms (*Procavia* and *Heterohyrax*) live in colonies while *Dendro-*

Fig. 3. Comparison of the G-banded chromosomes of *Dendrohyrax arboreus* (D) and *Heterohyrax brucei* (H). The first chromosome in each pair is that of *Dendrohyrax*. Both chromosome 6 morphs detected in the *D. arboreus* specimen are matched to the *H. brucei* counterpart. Unmatched chromosomes in the *D. arboreus* karyotype are indicated by (?), while the acrocentric elements at the bottom of the figure are of *H. brucei*

hyrax, although not showing any obvious limb modifications, is arborial and not gregarious (Smithers 1983). Since cytogenetic rearrangements which characterize chromosomal evolution are thought to reach fixation through small effective population size (Bentsson and Bodmer 1976; Bush et al. 1977; Lande 1979), it is not unreasonable to anticipate that those species whose habitat specificity predisposes them to population fragmentation may be characterized by variation in karyotype – a situation which could hold for the Hyracoidea. For example, *Heterohyrax* like *Procavia* inhabits "koppie" habitat typically comprising jumbled boulders and rocky outcrops which is often discontinuous, resembling terrestrial islands, with limited opportunity for gene flow between populations. Likewise, the tree hyrax's (*Dendrohyrax*) dependency on developed woodland and forest could similarly predispose it to population fragmentation.

Given evidence of a conserved diploid number of 2n = 54 in representative species of all three extant genera, we propose that the ancestral Hyracoidea was characterized by this chromosome number although, it should be noted, commonality does not always necessarily imply the primitive condition (Qumsiyeh and Baker 1988). However, should this hypothesis hold, karyotypic evolution in the Hyracoidea has obviously not proceeded by

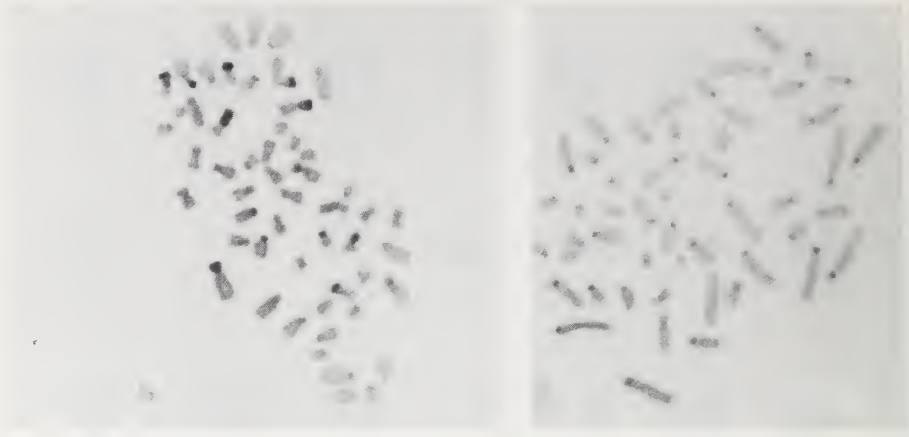

Fig. 4. C-banded metaphase cells of *Dendrohyrax arboreus* (A) and *Heterohyrax brucei* (B). Note the presence of heterochromatic short arms in several of the *D. arboreus* chromosomes (as well as the telomeric blocks of C-positive material in certain instances)

structural changes which involve fusions of chromosomes or chromosome arms. As is evident from the comparative cytogenetic data contained herein, the failure to identify corresponding homologs for several autosomes of the *D. arboreus* and *H. brucei* karyotypes would seem to suggest that, instead, karyotypic evolution has probably progressed through paracentric inversions and reciprocal translocations, both categories of chromosomal change that would disrupt G-band sequence homology.

Of particular interest are the marked differences that exist in the distribution of constitutive heterochromatin in the *H. brucei* and *D. arboreus* karyotypes. While the former has small amounts of strictly pericentromeric heterochromatin, the *D. arboreus* genome is characterized both by the presence of entirely heterochromatic short arms (for example pair 6) as well as terminal bands of heterochromatin in several of the autosomal pairs (Fig. 4 A). C-banding is thought to stain genetically inert blocks of DNA (John and Miklos 1979) and, for this reason, often shows intraspecific and intra-individual variability (for example Ward et al. 1987; Matayoshi et al. 1987; Robinson and Elder 1987). However, chromosomal position (terminal, interstitial or centromeric) of C-positive material occasionally differs between species and has been used in a cladistic framework (van Tuinen and Ledbetter 1983; van Tuinen and Valentine 1986). This contrast in C-band patterns between *D. arboreus* and *H. brucei* raises the possibility that, following analysis of other hyrax species, C-banding may be a particularly useful cytogenetic adjunct in ascertaining synapomorphies for this group.

In conclusion, although cytogenetic data are currently sparse for the Hyracoidea, it is quite possible that interspecific variation in the euchromatic and heterochromatic portions of the genome may exist beyond the confines of the species analyzed in this study. Future comparisons both within the Hyracoidea, as well as with a representative Subungulate outgroup (for example the elephant, *Loxodonta africana* 2n = 56; Hsu and Benirschke 1971a), may shed light on the phylogenetic relationships of this primitive mammalian assemblage.

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Zusammenfassung

Vergleichende Cytogenetik der Hyracoidea: Chromosomen von zwei Schliefer-Arten aus Südafrika

Die Karyogramme der beiden Schliefer-Arten (Procaviidae) *Dendrohyrax arboreus* (2n = 54) und *Heterohyrax brucei* (2n = 54) zeigten weitgehende Übereinstimmung in den G-Banden der Autosomen und der X-Chromosomen. Offenbar infolge zu großer struktureller Veränderungen ließen sich aber einige Chromosomen nicht homologisieren. Die Lokalisation des konstitutiven Heterochromatins auf den Chromosomen ist bei den beiden Arten unterschiedlich: bei *H. brucei* sitzt es am Zentromer, während bei *D. arboreus* ganze kurze Arme von Chromosomen heterochromatisch sind oder das C-positive Material endständig ist.

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