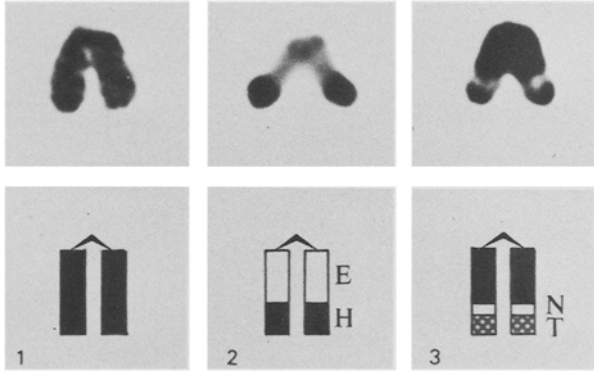
several changes of distilled water to remove scum that has formed. Preparations are incubated in 2×SSC (0.3 M sodium chloride containing 0.03 M trisodium citrate, adjusted to pH 7 with 0.1 citric acid) at 60 °C for 30 min, rinsed in distilled water and stained in buffered Giemsa (G.T. Gurr) 3% solution for 3–5 min. Finally they are rinsed briefly in deionised water and dried in air. Mount in 'De Pex' or a similar mountant.

A. fusca presents a male chromosome complement 2n=22+X. The autosomes (L_1-L_2, M_3-M_8, S_9-S_{11}) and the X chromosome are acrocentric. The pattern of C-banding shows 2 principal categories defined by their localization within the karyotype: paracentromeric, in the vicinity of the centromere of all the chromosomes and distal in M_4 to S_{11} chromosomes (Gonsálvez et al., in preparation). S_{10} autosome particularly shows 2 big distal blocks of constitutive heterochromatin clearly visible when the C-banding procedure is used (figure, 2). This pattern of pycnosis differs from that obtained just with orcein stain (figure, 1)



S_{10} selected univalents and diagrams of *Arcyptera fusca;* metaphase II. *1* A selected univalent with orcein staining. *2* Same univalent with C-banding. *3* Same univalent with orcein staining and C-banding. E, euchromatin; H, heterochromatin; N, non-staining gap; T, terminal heterochromatin.

or when an orcein stain is applied before the C-banding technique. This latter method gives rise to a banding pattern which shows 2 bands within the zone occupied by the constitutive heterochromatin obtained with the C-banding procedure (figure, 3). We have studied about 150 males from different populations and all of them were homozygous for the 2 big heterochromatic blocks in chromosome S_{10}. 20 of them were processed to unravel the band and we obtained good results in 12 of them. Mitotic chromosomes in sufficient numbers for a comparison have not been available so far.

It is interesting to note the similarity between the non-staining gaps (figure, N in 3) and those obtained in the grasshopper *Warramaba virgo* with the G-banding method[6]. But while in *W. virgo* all these gaps correspond well with the bands that were darkly stained by the C-banding technique, in *A. fusca* just half of the C-positive band shows a non-staining gap which means that the supposed homogeneous heterochromatic band (figure, H in 2) has been unfolded. 2 different bands can be observed within this region; one of them is a telomeric positively stained (T) and the other a negatively stained band (N). One might speculate that saturated barium hydroxide causes a different effect in the DNA when the orcein is present in the structure of the chromosome, but a role for base composition in the resistance of heterochromatin bands has not yet been ruled out[7].

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Human chromosomal heteromorphism in American blacks. V. Racial differences in size variation of the short arm of acrocentric chromosomes[1]

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Summary. Normal American blacks were studied by the RFA (R-bands by fluorescence using acridine orange) technique to estimate the frequency of size variation of the short arm of acrocentric chromosomes and to compare the data on Caucasians.

Heteromorphisms of human chromosomes have been recognized since the early 1960s. Racial differences in the size and morphology of the short arms of human chromosomes in D and G groups have been recognized without banding techniques[2]. Improved banding techniques have permitted recognition of greater numbers of chromosomal heteromorphisms[3]. To our knowledge, there is only one study where the size of D and G group chromosomes have been examined in American blacks and Caucasians using conventional staining. Individual chromosomes of D (13, 14 and 15) and G (21 and 21) groups could not be indentified in their study. Consequently, they could not demonstrate racial differences for each acrocentric chromosome but reported by group (D and G). Recently, we developed a RFA technique (R-bands by fluorescence using acridine orange as suggested by the Paris conference[4–6]) which distinguishes each human chromosome with absolute certainty[7]. One of the most important advantages of this banding technique is that the short arms of human acrocentric chromosomes are well delineated and minor differences can easily be detected. Consequently, we utilized the RFA technique to demonstrate racial differences in the size of human acrocentric chromosomes in 100 American blacks and 100 Caucasians. To our knowledge, this is the first

reported study where the morphology of the short arms of human acrocentric chromosomes have been examined in 2 races by RFA technique.

The 100 normal American blacks (50 females and 50 males) were all healthy and unrelated between the ages of 25 and 65. The data on 100 Caucasians were taken from our earlier survey[8]. All subjects had negative medical histories and were unrelated. The presence or absence of chromosomal heteromorphisms was not known at the time of selection for the study. All chromosome preparations were made from cultured peripheral blood as described earlier[9]. Chromosomes were banded by the RFA technique as described earlier[7,10,11]. Cells were photographed on Kodachrome 64 colour transparency film using a Zeiss Photomicroscope II. At least 20–30 cells were photographed from each individual with more than 2500 cells from each population.

In order to establish a method for reliable characterization of short arm variation, it has proven necessary to have within-cell standards; otherwise, if homologues differ, it is difficult to determine whether one is small, or the other large, or whether both are variants. The short arm of chromosome 18 (18p) was chosen as the most useful standard because it was intermediate in length in respect to the D and G group short arms. The details of the criteria with pictorial examples for scoring RFA size heteromorphisms have been described earlier[12,13]. Size levels 1, 2, 3, 4, and 5 represent very small, small, average, large, and very large, respectively (table 1).

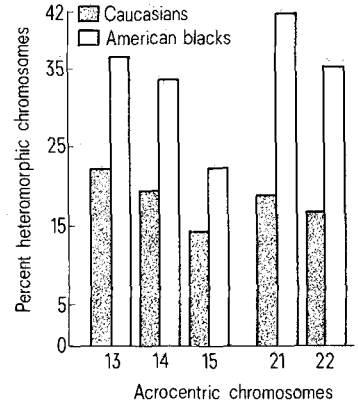
At least 5–6 of the best differentiated cells were utilized for recording RFA size heteromorphisms in each individual. Mounted colour slides were projected by a 35-mm projector onto an opaque Carroll Rear Projection Screen (Kalt Corp. Santa Monica, CA). RFA size heteromorphisms were recorded directly from the screen.

The present study describes the size variation on the short arm of human acrocentric chromosomes and divides it into 5 classes. The short arm of chromosome 18 (18p) was chosen as a reference standard for comparison and the entire short arm of D and G group chromosomes i.e. band p11 to p13 have been taken into consideration for recording the size. Frequencies for different levels in American blacks are presented in table 2. It can be seen that the most frequent size level was 3, therefore, this was regarded as the 'average' size. If one excludes average size, the frequency of size heteromorphism for chromosome 13, 14, 15, 21 and 22 would be 36.5, 33.5, 22.5, 42.0, and 35.0, respectively. Generally, the extreme sizes (i.e. very small and very large) are uncommon. There was no significant difference for overall frequencies of the size heteromorphisms between sexes (table 2). Comparison of size heteromorphisms be-

Table 1. Criteria for short arm size (area)

Code	Size	13–15	21–22
1	Very small	Virtually absent	Virtually absent
2	Small	$<0.5\times 18$ p	$\leq 0.25\times 18$ p
3	Average	≥ 0.5 to 1.5×18 p	$>0.25\times 18$ p
4	Large	>1.5 to 2.0×18 p	$=18$ p
5	Very large	$>2.0\times 18$ p	>18 p

See Verma and Lubs[12].



Comparison of size heteromorphisms of human acrocentric chromosomes in American blacks and Caucasians using the RFA technique. Pictorial demonstration (colour) of different sizes has been reported elsewhere[2,13].

Table 2. Size distribution between males and females by RFA in American blacks

Size (level)	Acrocentric chromosomes																			
	13				14				15				21				22			
	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%
Very small (1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Small (2)	35	29	64	32.0	33	21	54	27.0	15	11	26	13.0	27	29	56	28.0	7	9	16	8.0
Average (3)	62	65	127	63.5	59	74	133	66.5	76	79	155	77.5	57	59	116	58.0	64	66	130	65.0
Large (4)	3	6	9	4.5	8	4	12	6.0	9	10	19	9.5	15	11	26	13.0	26	24	50	25.0
Very large (5)	0	0	0	0	0	1	1	0.5	0	0	0	0	1	1	2	1.0	3	1	4	2.0

M, male; F, female; T, total.

Table 3. Comparison of size heteromorphisms among Caucasian and American blacks

Size (level)	Acrocentric chromosomes									
	13		14		15		21		22	
	C	B	C	B	C	B	C	B	C	B
Very small (1)	0	0	0	0	0	0	0	0	0	0
Small (2)	21.0	32.0	16.0	27.0	7.5	13.0	9.0	28.0	5.5	8.0
Average (3)*	77.5	63.5	80.5	66.5	85.5	77.5	81.0	58.0	83.0	65.0
Large (4)	1.5	4.5	3.5	6.0	6.5	9.5	10.0	13.0	10.0	25.0
Very large (5)	0	0		0.5	0.5	0	0	1.0	1.5	2.0
% heteromorphism	22.5	36.5	19.5	33.5	14.5	22.5	19.0	42.0	17.0	35.0

* Average (3) is the most common class and regarded normal. B, American blacks; C, Caucasians.

tween Caucasians and American blacks is presented in table 3. Size heteromorphisms occurred 1.5 times as often in blacks for chromosomes 13, 14 and 15 and more than twice for chromosomes 21 and 22 (figure 1).

Our data cannot be compared with those of Lubs and Ruddle[2] because of lack of objectivity in their study. 1st, chromosomes were not banded and 2ndly, they classify the heteromorphisms into 2 classes using 18p (i.e. increased length; $=18$ p and >18 p). In such a system, many chromosomes would be placed in the same category although they differed in their sizes. 3rdly, chromosomes were classified in groups i.e. individual chromosomes could not be identified. Based on conventional staining, Lubs and Ruddle found that minor variants were twice as common in black children as in Caucasians. We propose that these differences can be classified into at least 5 categories. It is quite evident from these findings that RFA technique detects more heteromorphisms in the size of human acrocentric chromosomes than any other banding procedure. It would be interesting to employ an annealing technique to different racial and ethnic groups to demonstrate these differences with greater precision.

The biological and clinical implications of length heteromorphisms of human chromosomes are poorly understood. The clinical significance of these minor heteromorphisms is also under study in several laboratories. It has been suggested that Gp^+ (enlarged short arm) variant in Caucasians was associated with a 2-fold increase in the frequency of low birth weight[14]. Certain of the rare heteromorphisms may also carry an increased risk (i.e. mental retardation, infertility, and fetal wastage etc., see review by Verma and Dosik[15]). Racial heteromorphisms seem to be the most common correlate of chromosomal variation. Racial differences have anthropological interest and have great value in linkage and population studies. The present study provides base line data in a normal population for comparing length heteromorphisms with abnormal populations.

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A frog with highly evolved sex chromosomes[1]

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Summary. Highly differentiated ZZ/ZW sex chromosomes and an exceptionally low genome size were found in the karyotypes of *Pyxicephalus adspersus* (Anura, Ranidae). The W-chromosome is considerably smaller than the Z-chromosome and consists to a very great proportion of constitutive heterochromatin. The DNA content of this species and the chromosome length have the lowest values determined in the Ranidae to date.

Detailed cytogenetic analyses and genetic breeding experiments have shown, that the sex chromosomes of the overwhelming majority of primitive vertebrates are still in an initial evolutionary stage[2-6]. The 2 sex chromosomes in the heterogametic sex (XY or ZW) are still morphologically identical and have, with the probable exception of the few opposing sex-determining genes, maintained the same linkage groups as well. It has not yet been possible to determine heteromorphic sex chromosomes with either the classical cytogenetic methods or with the new improved techniques of chromosome banding specifically in the evolutionarily important order of the Anura (frogs and toads)[7-10]. In the present study, highly differentiated heteromorphic ZZ/ZW-sex chromosomes and an exceptionally low genome size were determined in the frog *Pyxicephalus adspersus*.

The bull frog *P. adspersus* belongs to the highly evolved anuran family Ranidae. This species is the largest frog in South Africa, inhabiting most of the sub-Saharan region. The animals burrow in the dry periods and spawn in temporary rain-filled depressions during the rainy season[11]. 8 male and 8 female specimens from Transvaal were available for this investigation. The chromosomes were prepared from bone marrow and testes and from leucocyte cultures. The cells were processed by the conventional air-drying technique as previously described[8,9]. The diploid chromosome number of the species is $2n=26$. After staining the constitutive heterochromatin according to the C-band method[8], every chromosome pair is clearly identifiable (figures 1a, b, 2c). 2 chromosome pairs are telocentric (Nos 9 and 10), all others are metacentric or submetacentric. The constitutive heterochromatin is localised in the centromeric region as well as in the interstitial and terminal regions of the chromosomes. The largest and most intensely stained heterochromatic region is localised in the short arms of the metacentric chromosomes No. 6. The heterochromatic bands in the long arms of the telocentric chromosomes Nos 9 and 10 exhibit a high degree of interindividual variability. These variations are independent of the