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.

1 Acknowledgments. Supported by NCI contract N01-CP-43251. Technical assistance of Mr Jorge Rodriguez and Mr Norman Schmidt is gratefully appreciated.

2 H.A. Lubs and F.H. Ruddle, Nature *233*, 134 (1971).
3 R.S. Verma, H. Dosik and H.A. Lubs, Ann. human Genet. *41*, 257 (1977).
4 Paris conference 1971. Standardization in Human Cytogenetics. Birth Defects: Original Article Series, XI, *9*, The National Foundation, New York.
5 Paris conference 1971. Standardization in Human Cytogenetics. Birth Defects: Original Article Series, XI, 9, The National Foundation, New York; Supplement 1975.
6 ISCN 1978. An International System for Human Cytogenetics Nomenclature. Birth Defects: Original Article Series, Vol. XIV, No. 8. The National Foundation, New York.
7 R.S. Verma and H.A. Lubs, Am. J. human Genet. *27*, 110 (1975).
8 R.S. Verma, H. Dosik and H.A. Lubs, Human Genet. *38*, 231 (1977).
9 R.S. Verma, C. Rubenstein and H. Dosik, in Vitro *13*, 806 (1977).
10 R.S. Verma and H.A. Lubs, Can. J. Genet. Cytol. *18*, 45 (1976).
11 R.S. Verma and H. Dosik, J. Microsc. *108*, 339 (1976).
12 R.S. Verma and H.A. Lubs, Humangenetik *30*, 225 (1975).
13 R.S. Verma, H. Dosik and H.A. Lubs, J. Hered. *68*, 262 (1977).
14 H.A. Lubs and F.H. Ruddle, in: Human Population Cytogenetics. Ed. P. Jacobs, W.H. Price and P.J. Law. Univ. Edinburgh Press, Edinburgh 1970.
15 R.S. Verma and H.A. Lubs, Int. Rev. Cytol. *62*, 361 (1980).

A frog with highly evolved sex chromosomes[1]

M. Schmid and K. Bachmann

Institut für Humangenetik, University of Göttingen, Nikolausberger Weg 5a, D-3400 Göttingen (Federal Republic of Germany), and Fakultät für Biologie, University of Heidelberg, Im Neuenheimer Feld, Postfach 105760, D-6900 Heidelberg (Federal Republic of Germany), 9 July 1980

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

sex of the animals and must be the result of unequal crossing-over or paracentric inversions.

The chromosomes No. 8 of all the male animals are always homomorphic (figure 1a). In the female animals, 1 of the 2 chromosomes No. 8 has the same length and centromere position as in the male animals, whereas the other is almost half its size (figure 1b). The chromosomes No. 8 must therefore be sex-specific chromosomes of the ZZ ♂/ZW ♀-type. The female animals are heterogametic. The W-chromosomes possess a completely heterochromatic short arm and a very intensely stained centric heterochromatin. The Z-chromosomes exhibit a heterochromatic centromere and a somewhat fainter heterochromatic region extending from the centromere to approximately the middle of the long arm. The fluorescence of the heterochromatic regions after staining with the AT-specific fluorochrome quinacrine mustard is comparatively weak. Conversely, they show a very enhanced fluorescence intensity with the GC-specific fluorochromes mithramycin and chromomycin A_3 (figure 2a, b). It can therefore be concluded that the constitutive heterochromatin of the autosomes and sex chromosomes consists of GC-rich DNA sequences[12,13]. As in other species of Anura examined, the staining with quinacrine mustard did not reveal such multiple banding patterns in the euchromatic regions of the chromosomes of *P. adspersus* as are known from the chromosomes of the more highly evolved classes of vertebrates (mammals, birds and reptiles)[8,9].

Staining the chromosomes according to the ammoniacal $AgNO_3$-method[8] showed the nucleolus organizer regions to be localised in the short arms of the chromosomes No. 6 (figure 1c, d). In both sexes, the short arms of the chromosomes No. 6 consist of constitutive heterochromatin and nucleolus organizer region (figure 2c).

The total length of the haploid karyotype (12 autosomes + Z-chromosome) amounts to only 48 µm. This value is remarkably low compared with the chromosome lengths of other species from the family Ranidae (e.g. in *Rana* between 80 and 100 µm)[9]. The short length of the chromosomes in *P. adspersus* is paralleled by the very small volumes and low DNA-contents of the erythrocyte nuclei. The volume of the nuclei was calculated to be 22.3 μm^3 (in *Rana* it averages 100 μm^3). The Feulgen cytophotometric measurements yielded a value of only 4.00 ± 0.56 pg DNA per diploid nucleus (between 6 pg and 13 pg in *Rana*)[14]. This genome size is among the lowest found for Anura and it represents the smallest genome size hitherto determined for a species of the family Ranidae.

The ZZ/ZW-sex chromosomes of *P. adspersus* are the first unequivocal example that heteromorphic sex chromosomes actually have evolved in the order Anura[7,10]. As in the highly evolved snakes and the birds[2], the morphological differentiation between the Z- and W-chromosomes in *P. adspersus* has taken place entirely at the expense of the W-chromosome. It is considerably smaller than the Z-chromosome and most of it consists of constitutive heterochromatin.

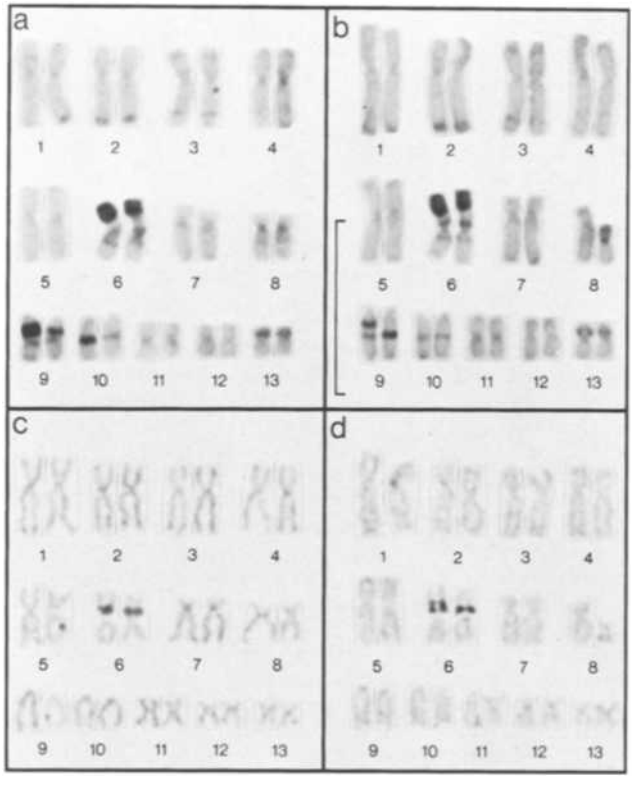

Fig. 1. Karyotypes of male (a,c) and female (b,d) animals of *Pyxicephalus adspersus* showing the highly heteromorphic ZZ/ZW-sex chromosome pair No. 8. *a,b* Constitutive heterochromatin stained according to a modified C-band technique[8,9]. *c,d* Nucleolus organizer regions stained with ammoniacal $AgNO_3$. The karyotypes shown were prepared from metaphases obtained from the bone marrow. The bar in the figure represents 10 µm.

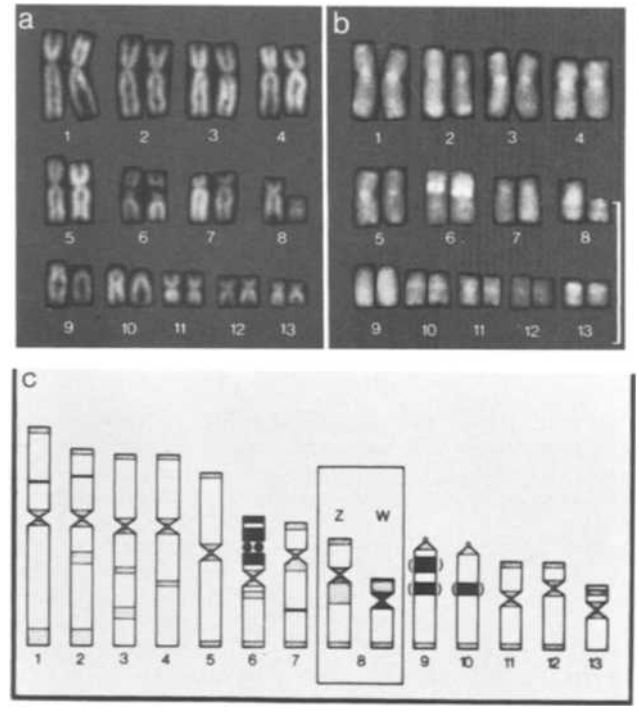

Fig. 2. Karyotypes of female *Pyxicephalus adspersus* stained with *a* quinacrine mustard and *b* mithramycin[13], showing the weak quinacrine fluorescence and the bright mithramycin fluorescence of the heterochromatic regions and the nucleolus organizer regions. *c* Diagrammatic representation of the maximum number of constitutively heterochromatic regions determined in the metaphase chromosomes of *P. adspersus* (compare with figures 1a-d and 2a,b). Dark sectors: intensively stained C-bands (mithramycin positive and quinacrine negative). Spotted sectors: faintly C-band positive heterochromatin. White sectors: euchromatin. Dark circles: nucleolus organizer regions. The heterochromatic regions exhibiting inter-individual variability are enclosed in brackets. The bar in the figures represents 10 µm.

It should be noted with regard to the exceptionally small genome size of this species, that those frogs living in extremely arid habitats generally have a reduced DNA content[15,16]. The low genome sizes presumably exert an influence on the cell volume, the minimum mitotic cycle and the rate of oxidative metabolism, among other things[5,14]. This again shortens the duration of the embryonic and larval development, an essential adaptation for survival in temporary waters. Comparative investigations with the technique of DNA reassociation kinetics must reveal which of the DNA sequences in *Pyxicephalus* is present in lesser quantity than in other species of the family Ranidae.

1 This study was supported by a grant from the Deutsche Forschungsgemeinschaft. We thank Professor W. Engel for his support and interest.

2 S. Ohno, Sex chromosomes and sex-linked genes. Springer, Berlin-Heidelberg-New York 1967.
3 N.N. Vorontsov, in: Cytotaxonomy and vertebrate evolution, p.619. Ed. A.B. Chiarelli and E. Capanna. Academic Press, London-New York 1973.
4 M.J.D. White, Animal cytology and evolution. University Press, Cambridge 1973.
5 A. Morescalchi, in: Cytotaxonomy and vertebrate evolution, p.233. Ed. A.B. Chiarelli and E. Capanna. Academic Press, London-New York 1973.
6 S. Ohno, in: Animal cytogenetics, vol.4, p.1. Ed. B. John Borntraeger, Berlin-Stuttgart 1974.
7 L. Singh, Nucleus *17*, 17 (1974).
8 M. Schmid, Chromosoma *66*, 361 (1978).
9 M. Schmid, Chromosoma *68*, 131 (1978).
10 M. Schmid, J. Olert and C. Klett, Chromosoma *71*, 29 (1979).
11 N.I. Passmore and V.C. Carruthers, South African frogs. Witwatersrand Univ. Press, Johannesburg 1979.
12 D. Schweizer, Chromosoma *58*, 307 (1976).
13 M. Schmid, Chromosoma *77*, 83 (1980).
14 E. Olmo, Caryologia *26*, 43 (1973).
15 K. Bachmann and R. Blommers-Schlösser, Zool. Anz. *194*, 13 (1975).
16 K. Bachmann, J. Florida Acad. Sci. *35*, 225 (1972).

Effect of *Lantana camara* L. extract on fern spore germination

C. Wadhwani and T.N. Bhardwaja

Pteridophyte Biology Lab, Department of Botany, Government College, Ajmer (India), 29 January 1980

Summary. Extracts of root, stem, leaf and inflorescence of *Lantana camara* Linn., (Verbenaceae) inhibited exine bursting, rhizoid initiation and protonemal initiation of spores of the fern *Cyclosorus dentatus* (Forsk.) Ching. The leaf extract was found to be qualitatively and quantitatively most potent in its inhibitory effect. Attention is drawn to the fact that further spread of *Lantana camara* at Mt. Abu, Rajasthan, India may lead to the complete destruction of the sizeable pteridophytic component in this locality, the richest vegetationally in Rajasthan.

The phenomenon of allelopathy, has been known for a long time[1,2]. However, it has been investigated exclusively with reference to flowering plants, there being no account so far of the allelopathic potential of flowering plants on spore germination and the gametophytic phase of ferns. Our attention was drawn to this by the continuing depletion of the pteridophytic component in the vegetation of Mt. Abu, a hill station in the south west of Rajasthan, India, which coincided with the spread of *Lantana camara* L. (Verbenaceae) introduced at this station during the last decade. The fact that the dense spread of this robust shrub did not alter materially such environmental parameters as moisture, light, temperature etc. with regard to the growth of ferns at Mt. Abu indicated that perhaps an allelopathic effect on spore germination and the gametophytic phase was responsible for the continuing decline in density of the ferns, since otherwise pteridophytes are well adapted ecologically to growing here [3-7]. Subsequent experiments conducted in this laboratory have confirmed this.

Material and method. Fresh material of *Lantana camara* was collected locally and after separation roots, stems, leaves and inflorescences of this plant were kept in an oven and dried at 80 °C for 24 h. These plant parts were then chopped into small pieces and 10 g of each of these was soaked in 100 ml of distilled water. This extract of each organ was autoclaved at 15 lb pressure and diluted by the addition of sterile Knop's basal medium to give the desired concentration of the extract (80%, 50%, 25%, 10%, and 1%). Spores of *Cyclosorus dentatus* (Forsk.) Ching, (Thelypteridaceae), a fern occurring at many localities in Rajasthan, were collected from fronds of plants under cultivation at the Botanic Garden, Government College, Ajmer, India. These were sprinkled in separate 7.5 cm petri-dishes containing 15 ml of Knop's basal medium for control and

Allelopathic effect of *Lantana camara* Linn. extract on germination of spores of *Cyclosorus dentatus* (Forsk.) Ching

Plant part extract	Control (Knop's basal medium)			Dilution percentage of the extract by addition of Knop's basal medium														
				1			10			25			50			80		
	EB	RI	PI	EB	RI	PI	EB	RI	PI	EB	RI	PI	EB	RI	PI	EB	RI	PI
Root				44.83 ±3.46	44.83 ±2.39	39.85 ±1.85	40.44 ±3.15	37.57 ±2.20	33.88 ±2.68	37.86 ±2.86	29.32 ±2.68	22.17 ±2.12	25.53 ±4.30	15.92 ±1.89	13.46 ±2.40	03.26 ±1.90	-	-
Stem				50.75 ±4.74	49.35 ±5.23	47.82 ±4.45	48.30 ±2.77	44.56 ±2.84	40.82 ±2.13	37.31 ±5.10	25.02 ±5.46	19.69 ±2.53	25.31 ±4.45	05.72 ±1.00	01.43 ±0.91	-	-	-
	55.35 ±3.52	54.18 ±3.46	52.48 ±2.45															
Leaf				43.43 ±2.00	39.61 ±2.92	37.43 ±3.09	37.45 ±5.53	26.14 ±3.19	19.61 ±4.78	26.16 ±2.05	12.53 ±3.97	06.61 ±2.57	00.54 ±0.28	-	-	-	-	-
Inflorescence				51.04 ±1.49	48.84 ±2.00	46.74 ±4.73	47.92 ±4.16	45.98 ±4.30	44.72 ±4.28	31.12 ±4.20	23.15 ±4.25	14.55 ±3.76	13.05 ±2.74	07.23 ±3.31	04.54 ±3.00	-	-	-

EB, percent spores with bursted exine; RI, percent spores with rhizoid initials; PI, percent spores with protonema initials.