chromosomes were also observed. These dicentric ring chromosomes also remained in the center of the cell at anaphase with the 2 centromeres stretched towards the 2 poles (fig. 3). In all of these cells, in addition to the ring chromosomes, some chromosome fragments were also invariably observed. The ring chromosomes, centric and acentric, as well as fragments formed laggards at mitotic anaphase, leading to the deletion of some chromosome segments. The laggards formed micronuclei at telophase.

Since the formation of ring chromosomes involves breakage-reunion (erratic) cycle and the hard rays (gamma) are known for their chromosome-breaking properties, the relationship between the formation of ring chromosomes and exposure to gamma-rays is quite clear. The present investigations have revealed that, like gamma-rays, diethyl sulphate too can induce breakages in the chromosomes resulting in the production of ring chromosomes and fragments.

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Unusual heteromorphic sex chromosomes in a marsupial frog[1]

M. Schmid, T. Haaf, Beatrix Geile and S. Sims

Institut für Humangenetik, University of Würzburg, Koellikerstr. 2, D-8700 Würzburg (Federal Republic of Germany), and Department of Zoology, University of Leicester, Leicester (England), April 11, 1983

Summary. Heteromorphic sex chromosomes of the XY/XX-type were found in the karyotypes of the South American marsupial frog *Gastrotheca riobambae* (Anura, Hylidae). The Y chromosome is considerably larger than the X chromosome and almost completely heterochromatic. The only nucleolus organizer region is localized in the X chromosome; this leads to a sex-specific difference in the number of nucleolus organizers. In the male meiosis, X- and Y chromosomes form a sex bivalent which can be readily distinguished from the autosomal bivalents.

Cytogenetic analyses have been performed in about half of the extant species of the Amphibia[2-4]. The chromosomes of salamanders, frogs and toads are very attractive for cytogenetic studies, because most of the species are distinguished by very long chromosomes and a low diploid chromosome number. Furthermore, the pairing arrangements of the chromosomes can be studied not only in the male stages of meiosis, but also in the fine-structured lampbrush chromo-

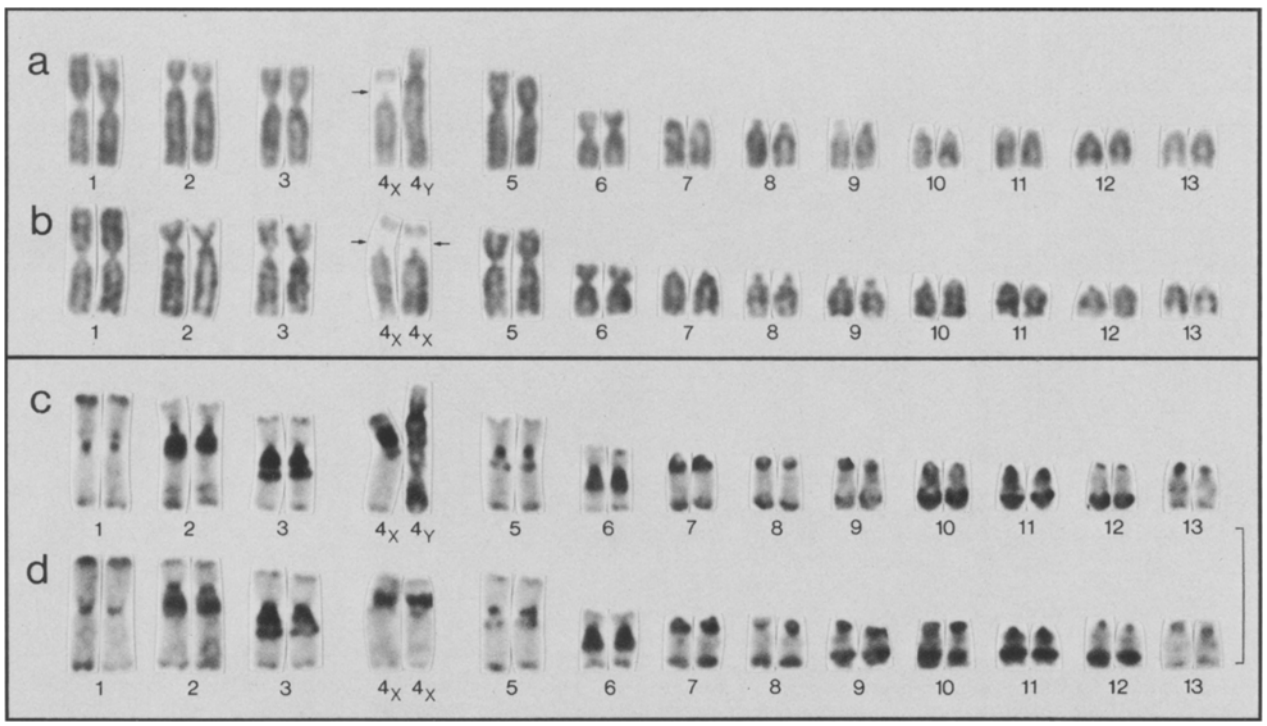

Figure 1. Karyotypes of male (a, c) and female (b, d) animals of *Gastrotheca riobambae*. *a* and *b* Conventional orcein staining demonstrating the highly heteromorphic XY sex chromosome pair No. 4 in the male (a), and the homomorphic XX pair in the female (b). The arrows indicate the conspicuous secondary constriction (nucleolus organizer region) in the short arm of the X chromosome. *c* and *d* Constitutive heterochromatin stained according to the C-band-technique[9]. Note the large number of constitutively heterochromatic regions in the karyotype and the almost completely heterochromatic Y chromosome (c). The bar in the figure represents 10 µm.

somes of the oocytes. Nonetheless, heteromorphic sex chromosomes have only been demonstrated in 5 species of the Anura to date[5,6]. In the majority of the species, the sex chromosomes are still in an initial stage of morphological differentiation and cannot be distinguished microscopically. In the present study, unusually highly heteromorphic XY/XX-sex chromosomes were demonstrated in a further amphibian species, the South American marsupial frog *Gastrotheca riobambae*.

The marsupial frog *G. riobambae* belongs to the highly evolved anuran family Hylidae. This genus occurs exclusively in Central and South America and possesses an exceptional mode of reproduction: the back skin of the female animals is folded to a pocket, into which the fertilized eggs are deposited; here the embryos spend a large part of their total larval development[7,8]. 12 male and 7 female specimens from Ecuador were available for this study. The chromosomes were prepared from bone marrow and testes by conventional techniques[9,10]. The diploid chromosome number of *G. riobambae* is 2n = 26. After staining with orcein, the chromosomes can be arranged in 13 pairs (fig. 1,a and b). The chromosome pairs 1–6 are meta- to submetacentric, the pairs 7–13 are acro- to telocentric. The chromosomes 4 of all male animals are always distinctly heteromorphic (fig. 1, a), whereas they are homomorphic in the female sex (fig. 1, b). Therefore the chromosomes 4 must be sex-specific chromosomes of the XY♂/XX♀-type. The X chromosome has a very distinct secondary constriction in the pericentromeric region of the short arm. The Y chromosome is longer than the X chromosome and presents no secondary constriction.

The chromosomes are distinguished by large amounts of constitutive heterochromatin as demonstrated by the C-band technique[9] (fig. 1,c and d). The C-band-positive heterochromatic regions permit a clear characterization of every chromosome pair. The X chromosomes exhibit pericentromeric and telomeric heterochromatin in both arms; the secondary constriction in the short arm is only faintly stained (fig. 1,c). The large Y chromosome is almost completely heterochromatic; several heterochromatic bands can be recognized in the long Y chromosomes of the prometaphases (fig. 1,c).

After staining with the AT-specific fluorochrome quinacrine mustard, 10 heterochromatic regions are found to fluoresce considerably more strongly than the euchromatin. 8 of these quinacrine-bright regions are localized in the autosomes (pairs Nos 1, 2 and 7–12), and 2 in the distal third of the long arm of the Y chromosome (fig. 2,a). Several of the heterochromatic regions fluoresce somewhat more brightly than the euchromatin following staining with the GC-specific fluorochrome mithramycin (fig. 2,b). This can be observed especially well on the large heterochromatic segments in the long arms of the autosomes 2, 3 and 6. In the Y chromosome 4 heterochromatic regions are mithramycin-positive. The secondary constriction in the short arm of the X chromosome exhibits the brightest mithramycin fluorescence of the karyotype; it can be concluded from this – in accordance with the results obtained in many other

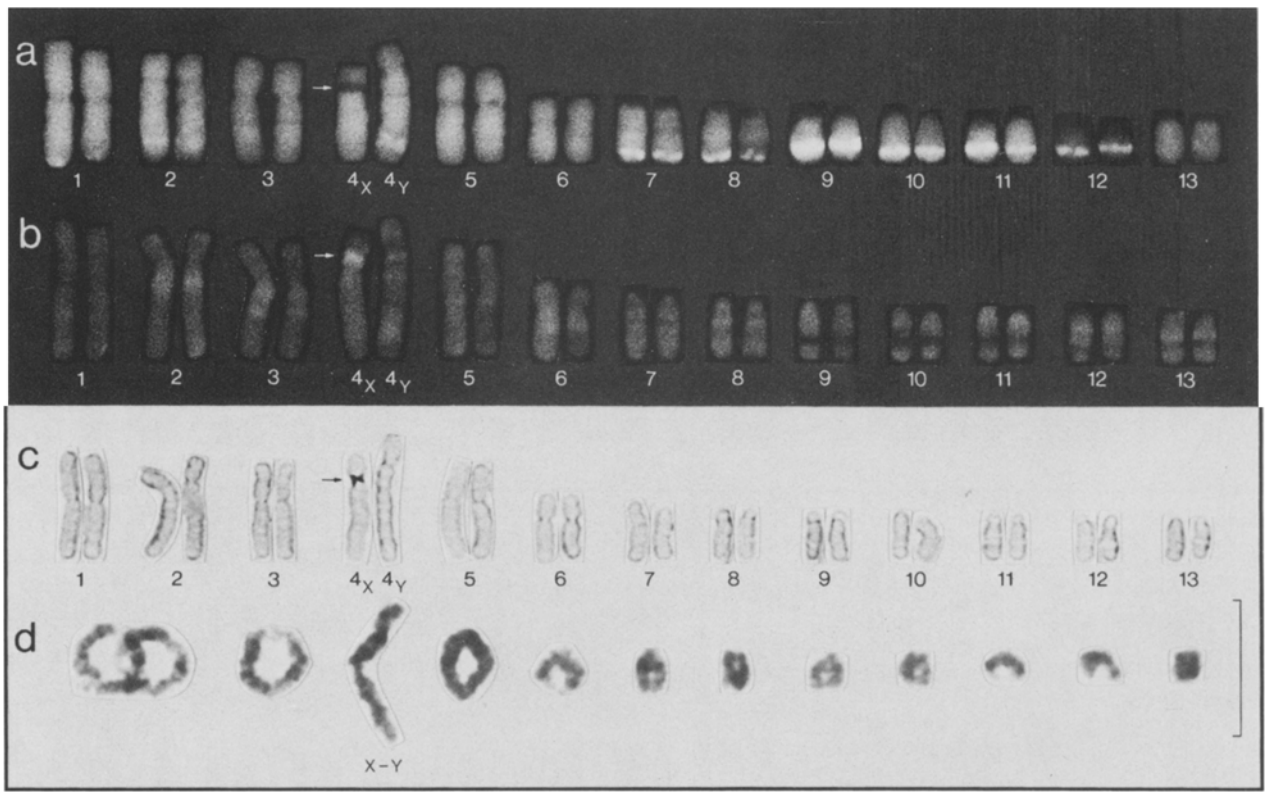

Figure 2. Mitotic karyotypes of male *Gastrotheca riobambae* stained with *a* quinacrine[9], *b* mithramycin[11] and *c* $AgNO_3$[14], and *d* diakinetic bivalents from male meiosis stained with orcein. The arrows indicate the nucleolus organizers. *a* Note the extremely bright quinacrine fluorescence of the heterochromatin in the autosomes Nos 7–12 and in the long arm of the Y chromosome; the nucleolus organizer shows no quinacrine fluorescence at all. *b* Note the bright mithramycin fluorescence of the nucleolus organizer and several heterochromatic regions; those heterochromatic regions showing a mithramycin-dull fluorescence are distinguished by a quinacrine-bright fluorescence. *c* The only Ag-positive nucleolus organizer region of the karyotype is localized in the secondary constriction in the short arm of the X chromosome. *d* All autosomal bivalents possess 2 terminal chiasmata, thereby obtaining a ring-like form, whereas the heteromorphic XY sex chromosomes only show an end-to-end association. The bar in the figure represents 10 µm.

species of Anura[11] – that the nucleolus organizer region is localized in this position. The fluorochrome staining permits the conclusion that the heterochromatic regions in the karyotype of *G. riobambae* differ greatly with regard to their DNA-base composition. The quinacrine-bright heterochromatin is AT-rich, and the heterochromatin with a mithramycin-bright fluorescence consists of GC-rich DNA-sequences[11,12]. The comparison of the quinacrine- and mithramycin-stained karyotypes reveals complementary banding patterns (fig. 2,a and b).

The specific staining of the nucleolus organizer regions according to the $AgNO_3$-technique[9,14] confirms the result obtained with mithramycin fluorescence. The only nucleolus organizer is localized in the secondary constriction of the short arm of the X chromosome (fig. 2,c). Since nucleolus organizers are located neither on the Y chromosome nor on any autosome, there is a dosage ratio of 1:2 between male and female animals with regard to the number of 18s and 28s ribosomal RNA genes. Such a sex-specific number of nucleolus organizer regions is very rare among vertebrates[13,14].

In the diakinetic stages of male meiosis of *G. riobambae* all autosomal bivalents possess 2 terminal chiasmata, which gives them a ring-like configuration (fig. 2,d). This exclusive occurrence of terminal chiasmata in diakinetic bivalents is characteristic of male meiosis in the highly evolved families of the Anura[2,15]. In contrast to the autosomal bivalents, the heteromorphic XY sex chromosomes of *G. riobambae* exhibit an end-to-end arrangement in the diakinetic stage (fig. 2,d). This is the first species found in the Anura in which the existence of a sex bivalent can be demonstrated, as in the male meiosis of mammals. The conclusion from this is that there is almost no homology any longer between the heteromorphic XY sex chromosomes of *G. riobambae*.

There are several reasons why the XY sex chromosomes of *G. riobambae* are attractive for cytogenetic studies: 1. The Y chromosome is larger than the X chromosome; this is the first example of a vertebrate species whose Y chromosome is larger than the X chromosome. 2. An X-linked nucleolus organizer region (NOR), and, consequently, a sex-specific difference in the number of NORs (females 2 NORs: males 1 NOR). 3. An unequivocal XY sex bivalent in male meiosis. A comparative cytogenetic investigation on the 32 further species of the genus *Gastrotheca*[16] is necessary in order to determine whether these unusual XY sex chromosomes are an exception or whether they are present in other species.

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A Feulgen technique for identification of cucumber chromosomes

C. Ramachandran[1], R. A. Pai and V. S. Seshadri

Division of Vegetable Crops and Floriculture, Indian Agricultural Research Institute, New Delhi-110012 (India), February 28, 1983

Summary. A Feulgen procedure was followed for identifying the somatic chromosomes of cucumber (*C. sativus* L.). The chromosomes were differentiated into dark and light banded regions facilitating the precise karyotyping of somatic complement. Pre-treatment temperature was found to be not critical for producing Feulgen bands in cucumber.

One of the pre-requisites of assigning genes to specific chromosomes is the unmistakable and easy identification of the individual chromosomes. The introduction of the banding techniques has to a great extent helped gene mapping even in organisms where controlled breeding is not possible. While these techniques have been extensively used in animal cytology, their success in plant cytogenetics has been very limited. Plants with small chromosomes pose further difficulties in responding to these new techniques, hampering even identification of individual chromosomes. In cucumber (*Cucumis sativus* L.), even though 83 genes have been identified[2] it has not been possible to assign them to different chromosomes which are not easily distinguishable. In view of this, attempts were made to improve on the staining technique to overcome this difficulty. The present paper communicates a simple staining method which has yielded better clarity and easier identification of chromosomes compared to the previous report[3].

For the present investigation, root tips of *Cucumis sativus* L. variety Japanese Long Green were pretreated with 0.002 M 8-hydroxyquinoline for 2 h and fixed in Carnoy's II fluid (6 alcohol : 3 chloroform : 1 acetic acid) for 48 h. The root tips were then hydrolyzed in 1 N HCl at 60 °C for 15 min and stained with leuco-basic fuchsin for 10–15 min. For softening, the stained root tips were macerated in 2.5% pectinase + 2.5% cellulase solution for 30 min at 20 °C. The root tips were washed in water, and squashed in 0.5% acetocarmine.

Following this technique the chromosome differentiated into distinct dark and light banded regions facilitating easy