5 min to precipitate the DNA which settled at the bottom. The DNA was removed by centrifugation, dissolved in saline-citrate (NaCl 0.15 *M*, Na-citrate 0.015 *M*, at pH 7.0) and the protein reprecipitated by chloroform. The subsequent purification of DNA from protein was done by alternate precipitation of DNA with alcohol and protein with chloroform as long as no protein precipitate was obtained at the interface of DNA solution in saline-citrate and chloroform layer. RNA was removed by addition of RNase at a concentration of 50 µg/ml and incubated at 37 °C for 60 min, followed by addition of 1 ml acetate-EDTA (3 *M* Na-acetate, EDTA 0.001 *M*). Final treatment was done twice with isopropanol and the DNA was removed by centrifugation. The DNA so obtained was dissolved in saline-citrate, precipitated with absolute alcohol, centrifuged and stored in absolute alcohol. The concentration of the preparation was checked from E_{260} nm in saline-citrate solution.

1 ml of the DNA solution (165 µg) in sterile citrate solution was added to 4 ml of meiospores (haploid zoospores) suspension of *A. macrogynus* and allowed to react for 30 min. The meiospores were then plated on Petri plates containing solid GCY medium. The control consisted of 4 ml meiospores added with 1 ml of sterile saline-citrate solution and allowed to react for the same period. The transformation scores were made after 3–4 days by microscope.

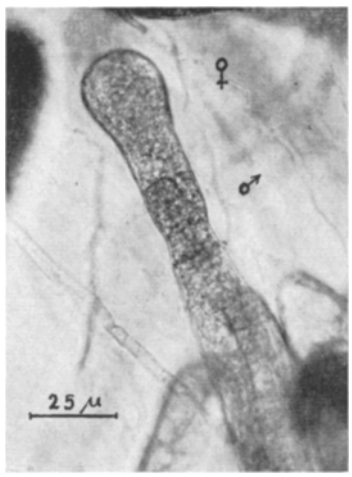

Fig. 4. A case of inverted sexual polarity in *Allomyces macrogynus* with orange yellow, hypogynous male compartment.

Fewer colonies appeared on the plates from DNA-treated meiospores and, like in controls, their size was unequal. Screening was made on all colonies. We found that, among some of the colonies from DNA-treated meiospores, sectors appeared showing consistantly modified characters. The most consistant change concerned the shape, as illustrated by the round arbusculate-type gametangia formed on *A. macrogynus* colonies (Figure 2). A tendency towards the shortening of the length of *A. macrogynus* gametangia, with a more or less roundish shape, was only exceptionally observed in control plates (Figure 3).

In some cases we could observe a clear inversion of the sexual polarity, i.e. hypogyny, in our *A. macrogynus* treated with DNA from *A. arbusculus* (Figure 4). Under such conditions, the hypogynous male was containing unambiguously its characteristic orange yellow pigment due to carotenoids. The modified sectors were isolated and subcultured. Their stability was checked for few subcultivations and no major reversion was observed.

Résumé. Les caractères de forme arrondie des gamétanges et, dans quelques cas, de position réciproque de leurs sexes (épigynie versus hypogynie) ont pu être transférés à l'*Allomyces macrogynus* par de l'ADN extrait d'*Allomyces arbusculus*.

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Sex Determining Mechanism XY_1Y_2 in *Artibeus lituratus lituratus* (Chiroptera-Phyllostomidae)[1]

Occurrence of a XX/XY_1Y_2 mechanism of sex determination has been described in a few mammal species. The first report of this mechanism refers to the insectivore *Sorex araneus*[2], being later described in the marsupials *Potorus tridactylus*[3] and *Protemnodon bicolor*[4] and in the rodent *Gerbillus gerbillus*[5].

Recently, in an extensive study on Chiroptera, the same mechanism has been found in the species *Choeroniscus godmani, Carollia perspicillata, Artibeus jamaicensis, A. toltecus* and *A. lituratus*[6,7].

The present paper reports our observations concerning the karyotype and meiosis of the species *A. lituratus lituratus* Lichtenstein, family Phyllostomidae. Comparative analysis with the meiotic behaviour of the species *Noctilio leporinus*, family Noctilionidae, is also presented.

The specimens under study, 2 males and 2 females, were collected in São José do Rio Preto, São Paulo, Brazil, where this species is abundant.

Mitotic and meiotic cells, used for this study, were obtained by squashing of spleen and gonads of animals previously inoculated with a 1% colchicine solution, in the dosage of 0.1 ml/10 g of body weight. Small tissue fragments, obtained after sacrifice and dissection of the

[1] This work was supported by Public Health Service Research Grant No. GM-14577-03 from the National Institute of General Medical Sciences and by the Fundo de Pesquisas do Instituto Butantan. R. F. B. holds a FAPESP training fellowship.

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[3] G. B. Sharman, A. J. McIntosh and H. N. Barber, Nature *166*, 996 (1950).

[4] G. B. Sharman, Aust. J. Zool. *9*, 38 (1961).

[5] R. Matthey, Experientia *10*, 464 (1964).

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specimen were treated in cold distilled water for 15 min, fixed in 50% glacial acetic acid for 15 min, and squashed. Permanent slides were obtained by the dry-ice method. The preparations were stained with Giemsa after hydrolysis in 1 N HCl at 60 °C for 10 min.

The diploid number found in the female of *A. lituratus lituratus* is 30 chromosomes while the male presents $2n = 31$, thus confirming the observations of HSU et al.[7].

The chromosomes in the karyotype are distributed in 5 groups. Group A contains 4 pairs of large metacentrics. Group B, in the female exhibits 5 pairs of large submetacentrics including a XX pair; in the male, group B shows 4 large submetacentric pairs and 1 X. Group C contains 4 median size metacentric pairs. Group D exhibits 2 small metacentric pairs (Figure 1). Group E, found only in the male, presents a small submetacentric chromosome (Y_2) and 1 acrocentric chromosome (Y_1), the smallest of the complement (Figure 2).

Through study of male meiosis, it was shown that its extra chromosome is related to the sex complement, which is of type XY_1Y_2. The spermatocytes present 14 bivalents and 1 trivalent formed by the X, the Y_1 and the Y_2. The X chromosome is connected with the Y_1 at one of the extremities, and at the other one with the Y_2. This aspect probably results from the translocation of an autosome to the X. The original Y chromosome was designated as Y_1 and the neo-Y as Y_2.

Sex chromosome-autosome translocations envolving the Y chromosome as in *Herpestes auropunctatus*[8] and *Mus minutoides*[9] reduces the male diploid number. However, when the X chromosome is involved as in the present case, the female diploid number is the smaller one.

Meiotic analysis revealed the presence, in the pachytene, of 2 vesicles, a larger and a smaller one. Frequently, a connection of both vesicles by a filament may be seen. In several pachytene configurations, the filament continues beyond the smaller vesicle, distending itself more or less, depending on the stage. Furthermore, in other cells, besides the filament attached to the smaller vesicle, another protrusion deriving from the large vesicle was observed.

Comparative analysis between these aspects and the meiotic ones of *N. leporinus* was performed. This species has a diploid number of 34 chromosomes in both sexes. The sex complement is similar to that of most mammals,

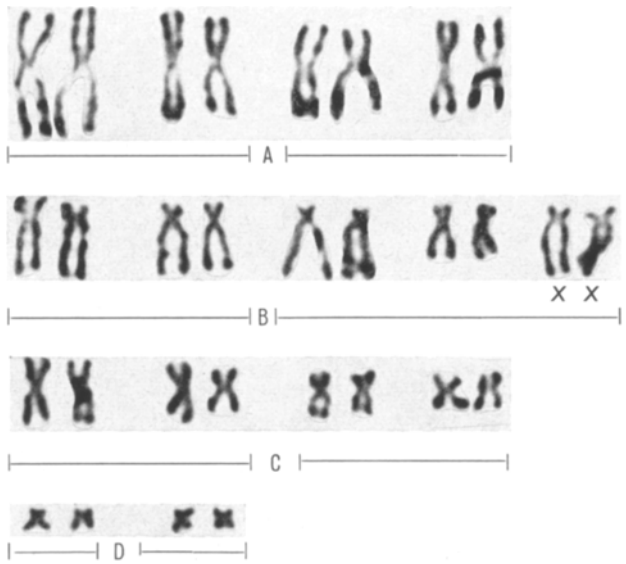

Fig. 1. Female karyotype from spleen metaphase, showing 30 chromosomes with XX complement.

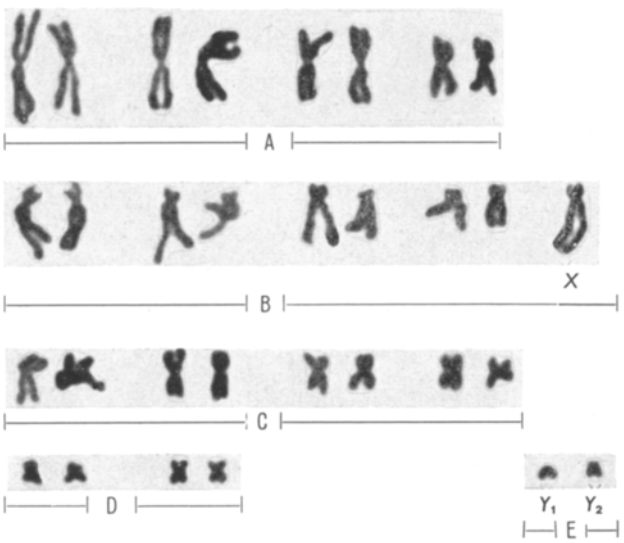

Fig. 2. Male karyotype from spleen metaphase showing 31 chromosomes with XY_1Y_2 complement.

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[9] R. MATTHEY, Chromosoma *16*, 351 (1965).

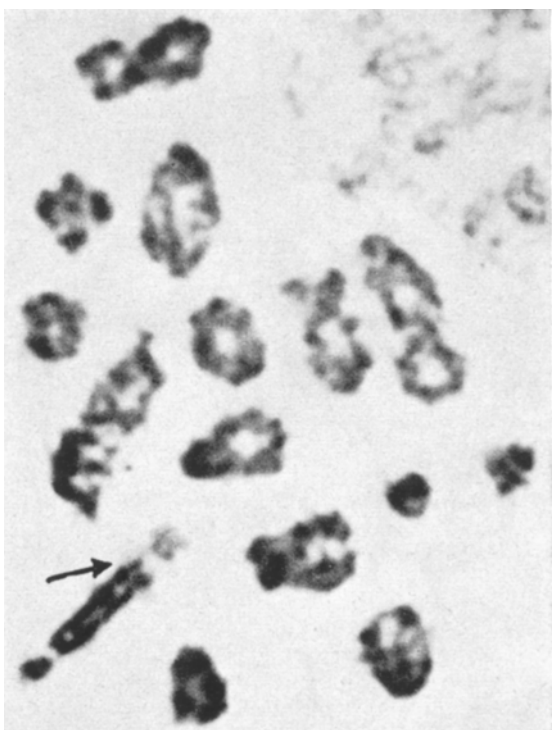

Fig. 3. Diakinesis showing 14 bivalents and the sex trivalent. Both Y_1 and Y_2 are longitudinally disposed in relation to the X extremities (× 3600).

XX in the female and XY in the male. In meiosis of the latter, a large and a small vesicle could also be seen in severalp achytene configurations. The larger one was interpreted as the X chromosome and the smaller one as the Y. By comparison, the vesicles in *A. lituratus lituratus* may be interpreted, the smaller one as the Y_1, corresponding to the original Y, and the larger one as containing the remainder of the sex complement. The second protrusion observed in some pachytene figures probably corresponds to the Y_2.

In diplotene of *A. lituratus lituratus*, the sex vesicle was no longer observed. In diakinesis the trivalent is longitudinally disposed, both the Y connected end-to-end with the extremities of the X; the original Y (Y_1) with the short arm, and the Y_2, which shows negative heteropycnosis, with the long arm of the X (Figure 3). In *N. leporinus* the submetacentric X is distended, showing negative heteropycnosis; the Y is connected end-to-end with the short arm of the X.

It is interesting to emphasize that, while in meiotic prophase of *C. perspicillata*[7] the Y and 1 of the arms of the X synapse, in *A. lituratus lituratus* no Y shows synapsis behaviour with the X chromosome.

In late metaphase I the trivalent is folded in an arch-like configuration with both the Y side-by-side, but still connected end-to-end with the X. Apparently this configuration warrants the distribution of both Y to the pole opposite to the X, assuring the production of balanced gametes.

Two kinds of metaphases I were found in *A. lituratus lituratus*, with $n = 15$ containing 1 sex chromosome, the X and with $n = 16$ containing 2 sex chromosomes, the Y_1 and the Y_2.

Resumen. En el murciélago *Artibeus lituratus lituratus* el número de cromosomas es de $2n = 30$ en la hembra y de $2n = 31$ en el macho. El mecanismo de determinación del sexo es XX/XY_1Y_2. La translocación de un autosoma al X resultó en la diferenciación del neo-Y. Son descritos los aspectos del comportamiento del trivalente sexual durante la meiosis. Estos son comparados a los que presenta la especie *Noctilio leporinus* ($2n = 34$) cuyo mecanismo es XX/XY.

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Chromosome Preparations of Bovine Leucocytes

The recent development of refined techniques has enabled the determination of somatic karyotypes of a variety of animals, either directly from bone marrow or other tissues established in culture. Unfortunately, the reliability of these various techniques is unsatisfactory since, the problems often encountered when utilizing such techniques have not been discussed nor mentioned in the literature. The purpose of this paper is to present a sufficiently reliable technique for bovine chromosome preparations from cultured peripheral-blood leucocytes and to comment on some of the technique's inherent problems. The technique is essentially similar to the ones suggested by Hungerford et al.[1] and Basrur and Gilman[2].

Materials and methods. Blood samples were collected in sterile, 15 ml graduated conical centrifuge tubes, containing 0.5 ml of an anticoagulant (heparin, 0.4 mg/10 ml blood) and 0.25 ml of an antibiotic mixture (penicillin-streptomycin), centrifuged at 800 rpm for 5–7 min (at room temperature) and then stored in the refrigerator at 4 °C for 40–48 h. This procedure was found to yield a high mitotic index.

The culturing procedure was carried out under ethylene glycol conditions as a precaution against contamination. All equipment used in the procedure was autoclaved for 20 min.

Approximately 1.5 ml plasma along with the buffy coat (layer of lymphocytes) was withdrawn with a sterile pipette, from the stored samples and diluted, in sterile, plastic T-flasks (Falcon), with a solution made up as follows: To 5 ml TC medium 199 were added 2 ml bovine serum (heat inactivated at 58 °C for 30 min), 0.25 ml penicillin-streptomycin (100 U and 100 µg respectively) and 0.25 ml bacto-phytohemagglutinin (M or P), Difco Laboratories, Detroit.

The cultures were swirled at room temperature to ensure thorough mixing and then horizontally incubated at 38 C° ($\pm$ 0.5 °C). Swirling of the cultures was continued periodically for about 2 h. Temperature constancy was found to be an absolute prerequisite for achieving desired results and therefore maintained throughout the incubation period.

Following 70½ h of incubation, colchicine (1 ml of 0.008 g + 100 ml water) was added to the cultures. Sterile precautions beyond this stage of the procedure were found unnecessary. The procedure of hypotonic pretreatment was begun immediately following the period of incubation in colchicine (4–5 h at 38 $\pm$ 0.5 °C). The contents of each flask were transferred to 15 ml graduated conical centrifuge tubes and centrifuged at 800 rpm for 15 min (room temperature approximately).

Hypotonic pretreatment in synergy with colchicine treatment is necessary for the swelling of cells and the dispersion of metaphase chromosomes. Therefore a fresh solution of 0.8% of sodium citrate was used as the hypotonic solution.

The supernatant was decanted from each centrifuge tube with a pipette. The 'button' of cells at the bottom of each tube was resuspended by tapping the outside wall of the tube vigorously and by a gradual addition of 4–5 ml of hypotonic solution. After 30 min in hypotonic solution the cells were centrifuged at 800 rpm for 15 min.

Since optimal fixation was achieved when the amount of hypotonic surrounding the cells was minimal, most of

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