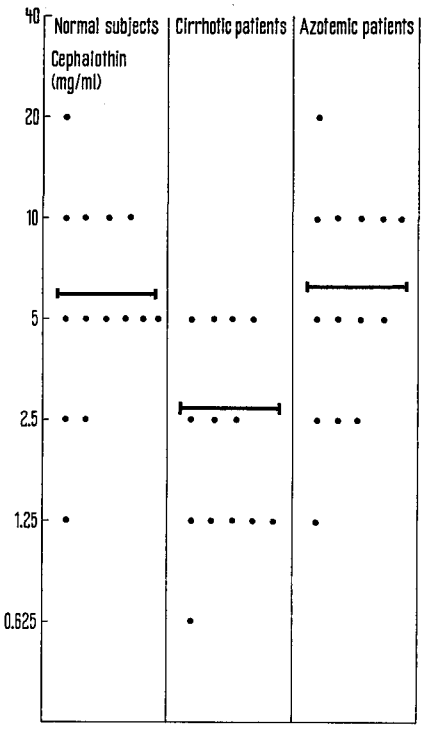

Fig. 1. Minimum concentration of cephalothin sufficient to produce a positive direct Coombs test in normal subjects and in cirrhotic and azotemic patients. Each dot represents a single case. The horizontal bar represents the mean of values.

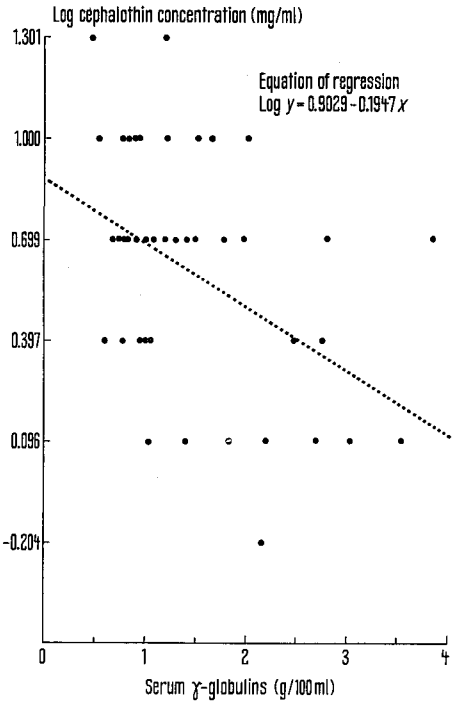

Fig. 2. Relationship between the log of the minimum concentration of cephalothin sufficient to produce a positive direct Coombs test (y) and the level of serum γ-globulins (x). Each dot represents a single case.

stromatic proteins become reactive with the anti-α and anti-β antibodies present in the antiglobulin reagent.

Further evidence that the drug alters the red cell membrane is given by the finding that cephalothin-treated red cells are susceptible to acid lysis[4] and display a low acetylcholinesterase activity[5].

From all this, it seems reasonable to conclude that the drug causes an alteration of the red cell surface with subsequent uptake of serum globulins[6].

Riassunto. Il test di Coombs diretto da cefalotina si produce a più bassa concentrazione dell'antibiotico se il sangue impiegato per l'esperimento in vitro è quello di pazienti affetti da cirrosi epatica classica piuttosto che quello di pazienti nefropatici o di soggetti normali. L'analisi statistica dei risultati ottenuti esaminando 41 differenti campioni di sangue dimostra che esiste una relazione inversa tra concentrazione minima di cefalotina sufficiente a determinare la positività del Coombs diretto e tasso di γ-globuline del siero. Pertanto, nel determinismo del Coombs diretto da cefalotina, oltre ad un'alterazione della membrana eritrocitaria, giocano un ruolo anche le caratteristiche proteiche del plasma.

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Indications of an Interspecific Transformation in *Allomyces*

The attempts of genetic transformations in moulds, *Neurospora crassa*[1,2] and *Penicillium chrysogenum*[3] have not been successful. We are unaware of examples of heterospecific transformation in fungi of the type reported in bacteria[4–6]. The resistance of fungi to the action of heterologous DNA may be due to the difficulty of transfer and passage across the thick cell wall, rich in chitin and of poor permeability. In this connection it is interesting to add that certain amino acids which are major components of the cell wall can impair the development of competence in *Bacillus subtilis*[7].

We think that *Allomyces* with its naked zoospores (plasmic membrane only) permits to overcome the difficulty of transfer. Additionally, at the time of encystment, the zoospores, in a fashion similar to the other Blastocladiales[8], loose their flagella by retraction, creating that way a solution of continuity or at least a weak point in the plasmic periphery. This could present a particularly

favourable site for the introduction of heterologous DNA in the direction of a nucleus still close to the point of retraction of the flagellum.

We tried to transfer some characters of *A. arbusculus* to *A. macrogynus*. In the *Allomyces*, female gametangia are colourless, normally bigger in size than the male gametangia which are orange coloured when mature. The two gametangia lie in close proximity to each other; in *A. arbusculus* (Figure 1, A), where the gametangia have a round shape, the male gametangium is hypogynous (i.e. male immediately below the female gametangium) and in *A. macrogynus* with rather elongated gametangia, it is epigynous (i.e. above the female gametangium, Figure 1, M). The shape and the position of the gametangia, both genetically controlled characters[9], were therefore selected as markers for the transformation experiments.

A. arbusculus Burma LD was used as the donor and *A. macrogynus* Emerson and Wilson as the recipient. The resistant sporangia of *A. arbusculus* were incubated in sterile distilled water at room temperature to liberate the meiospores which were inoculated in Fernbachs containing 150 ml of sterile GCY medium[10] and incubated at 25 °C for 4 days. The resulting gametophytic mycelial mats were harvested and used for DNA extraction.

The usual procedure of MARMUR[11] was followed with some variations for the preparation of native DNA. The fresh mycelial mat was ground in the cold, suspended in saline-EDTA (NaCl 0.15 *M*, EDTA 0.015 *M* at pH 8.0) and the coarse debris removed by filtration. The homogenate was then spunned at 10,000 *g* for 10 min, the resulting pellet was suspended in saline-EDTA and added with 25% sodium lauryl sulphate solution. The mixture was then incubated for 30 min at 60 °C. 1–2 drops of chloroform were added and the mixture was shaken for 2–3 min to precipitate the proteins. The suspension was centrifuged at 12,000 *g* for 10 min and the supernatant collected. Two volumes of ethanol was carefully layered on the surface of the supernatant and placed in the cold for

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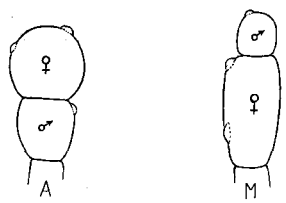

Fig. 1. The gametophytic stage of *Allomyces arbusculus* (A) and *A. macrogynus* (M) showing hypogynous (A) and epigynous (M) positions of the male gametangia on the 2 species.

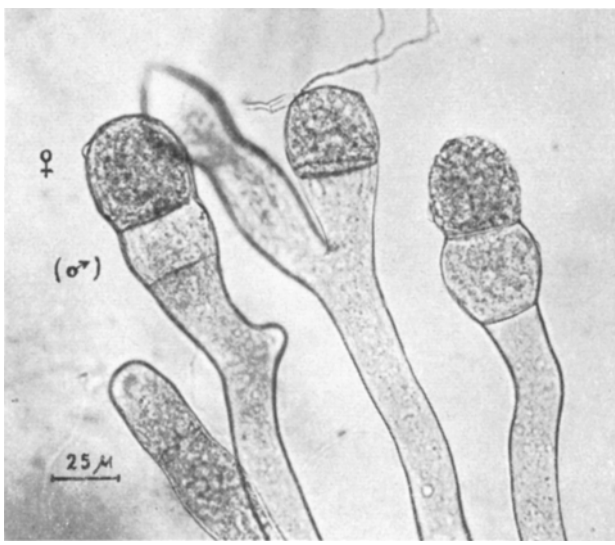

Fig. 2. *Allomyces macrogynus* from a sector of a colony which ensued from an *A. arbusculus* DNA-treated meiospore. Apical gametangium round, with granular, greyish cytoplasmic content (♀), subapical compartment with more homogenous, pale yellowish content (♂).

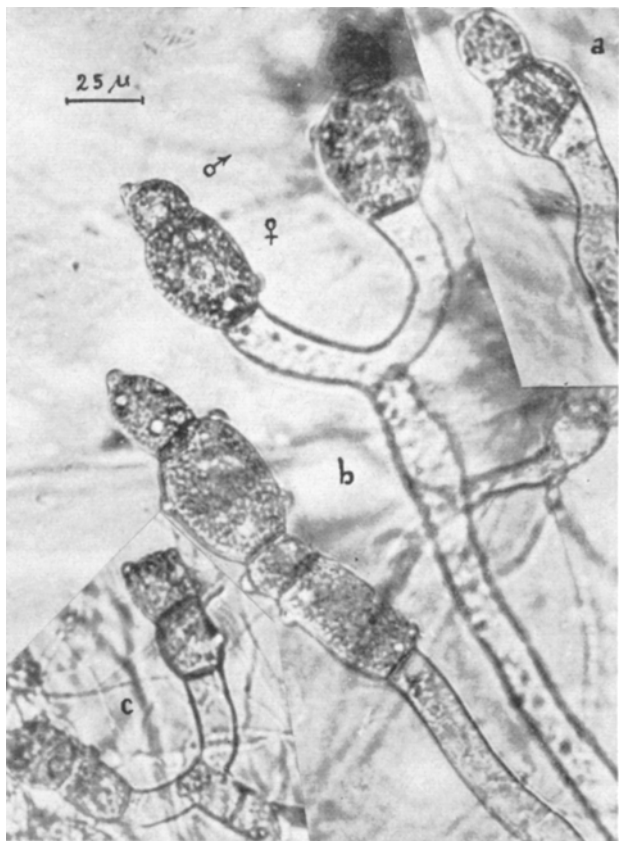

Fig. 3. (a) and (b), control cultures of *Allomyces macrogynus* grown for 4 days on GCY medium; a, extreme case of environmental shortening of female gametangium; b, normal, unequal-sized gametangia. (c) *A. arbusculus*-shaped gametangia of *A. macrogynus* treated with heterologous DNA.

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.

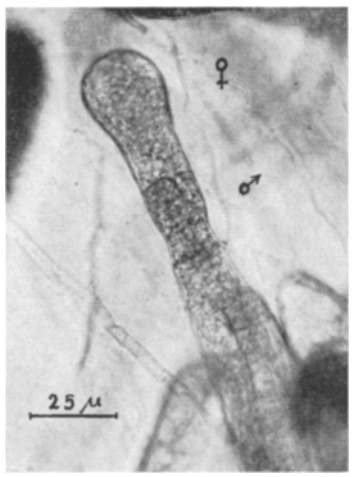

Fig. 4. A case of inverted sexual polarity in *Allomyces macrogynus* with orange yellow, hypogynous male compartment.

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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