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METHODOLOGY

Preparation of mitotic chromosomes of leaf-cutting ants from the genera *Atta* and *Acromyrmex*

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ABSTRACT

Some modifications were made to the methodology of Imai *et al.* (*Jpn. J. Genet. 63*: 159-185, 1988) for cytogenetic analysis of the leaf-cutting ants *Atta sexdens piriventris* and *Acromyrmex heyeri* (Hymenoptera, Formicidae), shortening preparation time and improving chromosomal preparations. The brain ganglia of prepupae were dissected in a 0.0025% hypotonic solution of colchicine, placed on a glass slide on a cold plate (4 ± 1°C) for 20 min. The material was fixed directly on the cold slide (with cold fixative I), macerated with a histological needle and fixed again with fixative I, followed by fixatives II and III, all of them cold. The slide was flame-dried right after the use of fixative III, and it was allowed to air-dry at room temperature for 2 h. The resulting metaphases presented less contracted chromosomes, with separated and well defined sister chromatids at a high frequency, when the material was processed in the manner described and stained with 3% Giemsa in phosphate buffer (pH 6.8) for 15 min.

INTRODUCTION

The genus *Acromyrmex* was studied by Goñi *et al.* (1983), who determined the chromosome number of *A. heyeri*, *A. hispidus*, and *A. ambiguus*, from Uruguay. The karyotype of the genus *Atta* was studied by Santos *et al.* (1993) and Fadini and Pompolo (1993, 1996).

Imai *et al.* (1988) related in some metaphases, a pattern of bands they called C bands. This pattern became evident even without pretreatment with a strong base followed by a saline-citrate solution. These authors used a solution of Giemsa at 3% (pH 6.8) for staining.

MATERIAL AND METHODS

Prepupae of the species *Atta sexdens piriventris* and *Acromyrmex heyeri* were identified by Nádia Brancher and Luciana Gusmão. These came from the Laboratório de Entomologia do Departamento de Fitossanidade da Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, and from wild anthills, respectively. The material collected was processed according to the technique of Imai *et al.* (1988), with the changes described below.

The brain ganglia (BG) of the prepupae were dissected in a hypotonic solution of colchicine at 0.0025% (HSC) on a glass slide, under a stereoscopic

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microscope. The BG were then transferred to a new slide, previously washed and put on a cold plate (4 ± 1°C). Two drops of HSC were dropped on the BG, and then it was allowed to rest for 20 min, at room temperature. The excess HSC was removed by tilting the slide. Fixatives were also kept on the cold plate, so that they would be at 4 ± 1°C, ready to be used.

A drop of fixative I (1:1 ethanol/acetic acid at 60%) was dropped on the tilted slide so that it fell on the BG. After removal of excess fixative I, another two drops of fixative I were put on the material. Removal of excess fixative was followed by maceration of the BG, with a histological needle, to spread it well on the slide.

One drop of fixative I was poured on the BG and, after retraction of the fixative, two drops of fixative II (1:1 ethanol/acetic acid at 100%) were added and allowed to act for about 1 min. After evaporation of fixative II, two drops of fixative III (PA glacial acetic acid at 100%) were dropped and then the slide was quickly flame-dried. This material was allowed to air-dry for 2 h. The slides were stained with a solution of Giemsa at 3% in phosphate buffer (pH 6.8) for 15 min.

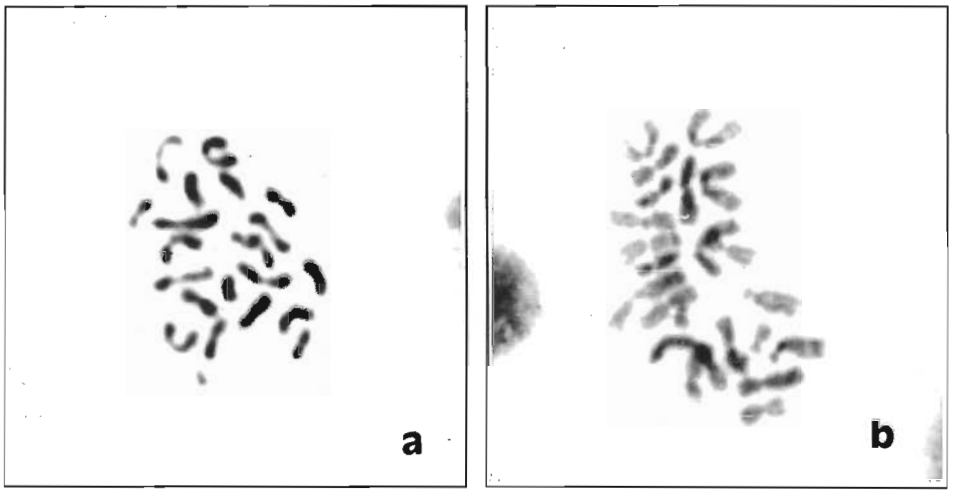

Figure 1 - Metaphase chromosomes of *Atta sexdens piriventris* (1000X). **a**: Obtained with the method of Imai *et al.* (1988). **b**: Prepared at low temperature.

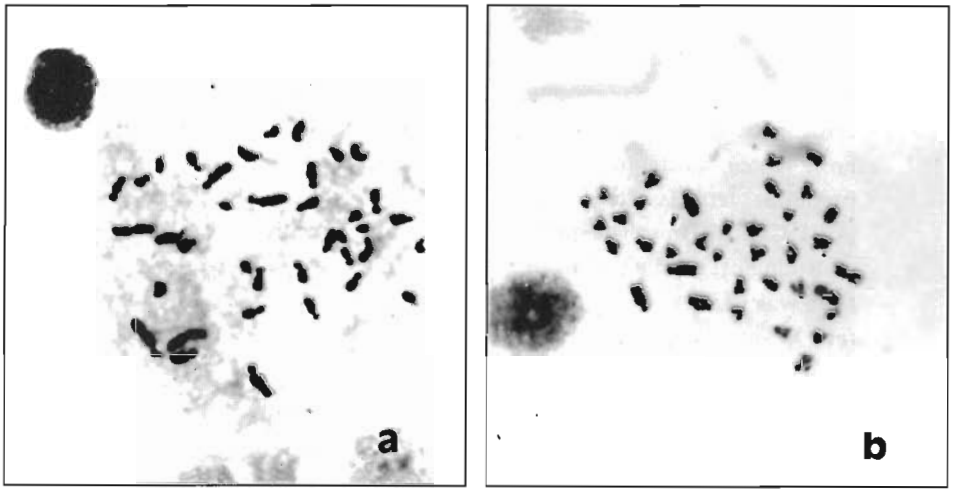

Figure 2 - Metaphase chromosomes of *Acromyrmex heyeri* (1000X). **a:** Obtained with the method of Imai *et al.* (1988). **b:** Prepared at low temperature.

RESULTS AND DISCUSSION

The slides prepared with the changes described in the present paper (decreased concentration of colchicine, slides prepared at low temperature, flamed and air-dried for 2 h) presented metaphases with chromosomes scattered and less contracted, with sister chromatids separated and well defined and at a higher frequency than with the method of Imai *et al.* (1988) (Figures 1 and 2). The pattern of C bands mentioned by Imai *et al.* (1988) was not found in the chromosomes of the two species studied.

Ten metaphases per individual were observed in a total of 10 females of *Atta* and 10 females of *A. heyeri*. The mitotic plates of *A. sexdens piriventris* showed 2n = 22, presenting metacentric, submetacentric, and acrocentric chromosomes. This number was also found by Fadini and Pompolo (1993, 1996) for *Atta sexdens rubropilosa*, *A. laevigata* and *A. bisphaerica*.

A. heyeri had 2n = 38, with metacentric, submetacentric, acrocentric and telocentric chromosomes. Goñi *et al.* (1983) found no telocentric, but described subtelocentric chromosomes for *A. heyeri*, *A. hispidus*, and *A. ambigus*.

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RESUMO

Objetivando uma melhor análise citogenética das formigas cortadeiras *Atta sexdens piriventris* e *Acromyrmex heyeri*, algumas modificações foram feitas no sentido de otimizar a metodologia de Imai *et al.* (*Jpn. J. Genet. 63*: 159-185, 1988), tendo-se conseguido a diminuição do tempo de preparo do material e melhor qualidade da preparação. O gânglio cerebral de pré-pupa foi dissecado em solução de colchicina hipotônica 0,0025% e colocado sobre lâmina de vidro (nova e previamente limpa para ser corada com Giemsa) com colchicina hipotônica. A lâmina foi colocada sobre placa de gelo (4 ± 1°C) por 20 min. O material foi fixado diretamente na lâmina (com fixador I gelado), macerado com agulha histológica e fixado novamente com fixador I, seguido dos fixadores II e III, todos gelados. A lâmina foi rapidamente flambada após a última fixação e foi deixada secar à temperatura ambiente por 2 h. As metáfases resultantes apresentaram, com maior freqüência, cromossomos menos contraídos, com cromátides irmãs separadas e bem definidas, quando o material foi processado como descrito acima e corado com solução de Giemsa 3% em tampão fosfato pH 6,8, por 15 min.

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