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# Cytogenetics of the neotropical flesh fly *Pattonella intermutans* (Diptera, Sarcophagidae)

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*Abstract*

*Pattonella intermutans* has 2n = 12 chromosomes including three metacentric and two submetacentric pairs of autosomes and an XX/XY sex chromosome pair. The autosomes are characterized by the presence of a C band in the pericentromeric region while sex chromosomes are totally heterochromatic. The FISH technique showed a nucleolar organizer region (NOR) in autosome IV.

## INTRODUCTION

Flies of the family Sarcophagidae are extremely common insects of worldwide distribution. These flies have an extensive variety of alimentary habits during the larval phase, the most common of which involves animal carcasses (James, 1947).

Most reports on the Sarcophagidae deal with their medical importance and role in the degradation of the carrion larval substrate (Cornaby, 1974; James, 1947; Jirón and Marin, 1982).

Members of the Sarcophagidae and Calliphoridae are the main invertebrate consumers of vertebrate carcasses (Braack, 1987). *Pattonella intermutans* is reported to be a good forensic indicator in carcasses in the region of Campinas, Brazil (Carvalho, 1996). The distribution of this species is Neotropical (Brazil, Costa Rica, Equator, Guatemala, Guyana, Honduras, Mexico, Panama, Paraguay, Peru, Sta. Lucia, Trinidad & Tobago) (Pape, 1996).

Previous investigations on the karyotype of some species of Sarcophagidae have revealed that the size of their sex chromosomes varies greatly from species to species (Stevens, 1908; Metz, 1916, 1922; Keneuke, 1924; Boyes, 1953, 1963; Boyes and Van Brink, 1965; Kaul *et al*., 1978; Tewari *et al*., 1983). In contrast, the gross morphology of the autosomes is rather uniform throughout the family.

Our investigation of the chromosomal morphology of *P. intermutans* is part of a more comprehensive study of the cytogenetics of Muscoidea, comparing the general morphology and number of their chromosomes, particularly the sex chromosomes and their nucleolar organizing regions (NORs).

## MATERIAL AND METHODS

### Fly rearing

The fly colony was started with flies collected from rat carcasses found in the neighborhood of the Biology Institute and maintained in the Entomology section of the Department of Parasitology of the State University of Campinas (UNICAMP). Adult flies were kept in nylon cages (30 x 30 x 48 cm), at 24 ± 2°C, 40-50% relative humidity and a 12-h light/dark cycle, in the continuous presence of cane sugar.

### Chromosome preparation

Mitotic chromosomes were obtained from the brains of L3 larvae. Meiotic chromosomes were obtained from the testis cells of young males. Hypotonic treatment and fixation were performed as described by Imai *et al*. (1988).

### Chromosome morphology

For morphological studies, the slides were mainly stained with 10% Giemsa. Mean descriptive values of the karyotype were calculated from information obtained from at least one well-spread mitotic metaphase plate obtained from each of 10 individuals. The nomenclature of Levan *et al*. (1964) was used to describe chromosome morphology.

### C-banding

Sumner's (1972) technique, with a slight modification to allow for the localization of constitutive heterochromatin regions, was employed.

### FISH

*In situ* hybridization was performed on mitotic and meiotic cells, using a 12-kb rDNA probe (pDm 238-*Drosophila melanogaster*). Chromosome preparations were pretreated with RNase, dehydrated in an ethanol series, air-dried, and denatured in 70% formamide (in 20% 10X SSC) at 70°C for 2 min and immediately de-

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hydrated in a succession of cold 50%, 75% and absolute ethanol. Hybridization was performed for at least 16 h in a humid chamber at 37°C.

Slides were washed twice in 50% formamide (in 2X SSC) and twice in 2X SSC, for 5 min each. The slides were incubated with the first antibody (antibiotin) for 45 min in a humid chamber at 37°C. After washing in PBT (PBS, 0.1% Tween 20 and 0.4% BSA 30% w/v), slides were incubated for 45 min with the second antibody (RAG-FITC) in a humid chamber at 37°C. Following a final wash in PBT, they were stained with propidium iodide and mounted with anti-fading (vectashild).

The probe was labeled using the Bionik kit (Gibco BRL-nick translation), and denatured for 10 min at 100°C immediately prior to *in situ* hybridization. For some slides this was followed by washing in water for 2 h and staining with Giemsa for better morphological identification of each chromosome and to ascertain the exact location of the signal (Viegas-Pequignot, 1992). Slides were examined under an Olympus fluorescence microscope and photographed using 400-ASA color negative film.

## RESULTS

### Karyotype morphology

The *P. intermutans* karyotypic complement consisted of five autosomal and one pair of sex chromosomes XX/XY (females XX/males XY) (Figures 1, 2). Pairs I, III and V are metacentric and pairs II and IV are submetacentric (Table I).

Pair I is the longest autosome of the complement and is just slightly larger than pair II. Chromosome I is the only one of the complement with a distinct space in the centromeric region (Figure 1: arrow). Pairs III and IV are similar in length, making it difficult to distinguish between them. Pair V is smaller than any other pair, and corresponds to 11% of the total complement length (TCL).

Chromosome X is subtelocentric and the longest of the whole complement; the Y chromosome is submetacentric and just half the size of X. Chromosomes X and Y appear heteropycnotic after conventional staining (Figures 1, 2).

### C Band

All autosomes had large blocks of heterochromatin in the pericentromeric region (Figures 3-6). After C-banding, a large pericentromeric block was very noticeable in pair I, an unmistakable characteristic of this chromosome, which usually exhibited the most prominent band of the complement and had an asymmetric distribution around the centromere (Figures 3, 6, arrows).

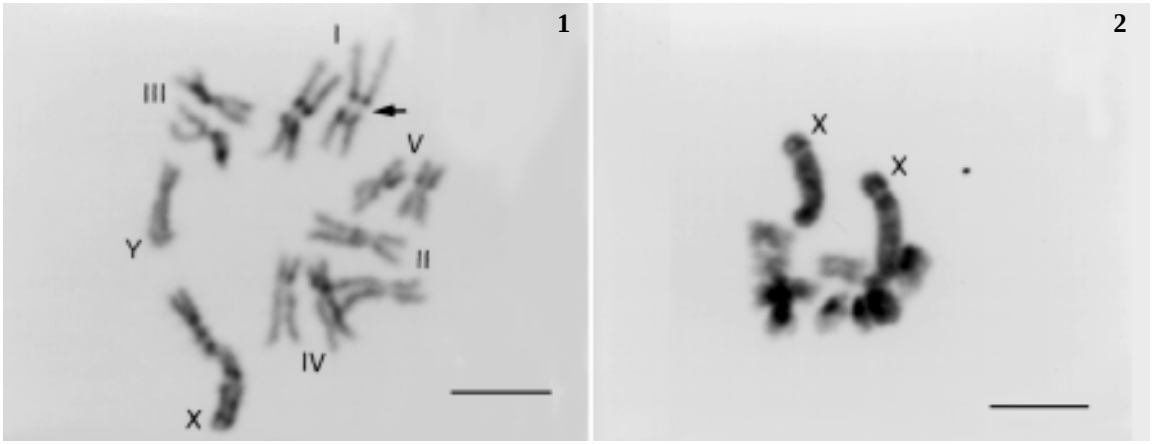
In addition to the pericentromeric band, pair II had an intercalary band on the long arm which was shorter and lighter than the pericentromeric band (Figures 4, 5: arrows). There was a large pericentromeric band on the small arm of pair IV and a minute pericentromeric band on the long arm (Figure 6).

The sex chromosomes were totally heterochromatic. In some metaphases, the X (Figure 3) and Y (Figures 3, 5) chromosomes had different band intensities. Previous analysis using restriction endonucleases had also shown bands with different intensities, possibly indicating different classes of heterochromatin (data not shown).

C-banding in meiotic chromosomes (Figure 6) showed the same bands seen in mitotic chromosomes. X and Y chromosomes showed an allocyclic behavior, typical of heterochromatic chromosomes at meiosis (Figure 6: arrowhead).

### *In situ* hybridization

*In situ* hybridization with an rDNA probe showed that the NOR is located on autosome pair IV (Figure 7). The intense signal, seen in both homologs, coincided with




**Figures 1, 2** - Mitotic chromosomes in *Pattonella intermutans*. **1:** Mitotic male metaphase showing 12 chromosomes. **2:** Mitotic female metaphase. Arrow in Figure 1 shows the space in the centromeric region of pair I. Scale bar = 10 μm.

the location of the large block of heterochromatin present in this chromosome pair, as described above (Figure 6).

## DISCUSSION

The modal number of chromosomes among species of the family Sarcophagidae is 2n = 12. Only *Pseudosarcophaga affinis* is exceptional in having 2n = 19/20 and chromosomes of unusual morphology (Boyes, 1953).

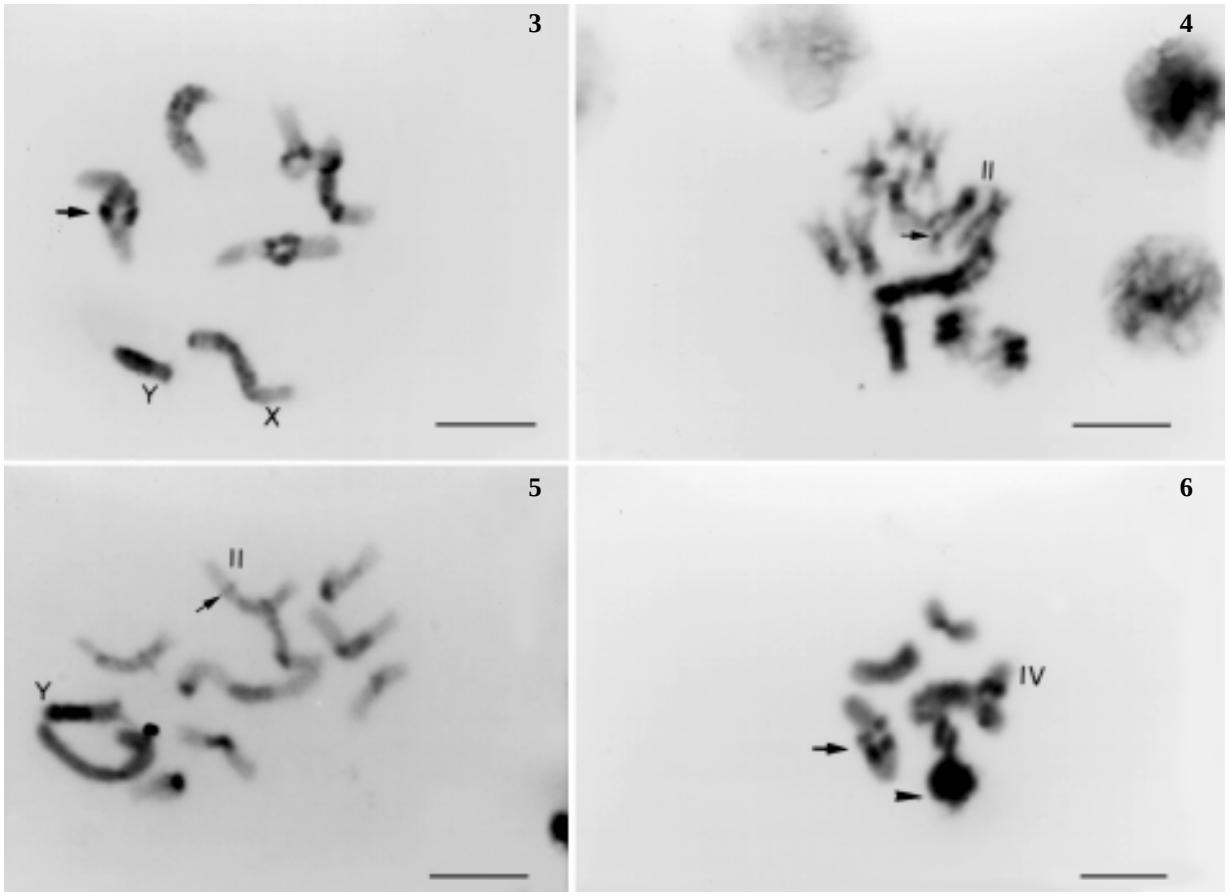
**Table I** - Analysis of the somatic complements of *Pattonella intermutans*. The relative length of Y was expressed as a function of the length of X. N = 10.

| Chromosome | I | II | III | IV | V | X | Y |
|---|---|---|---|---|---|---|---|
| Length (μm) | 5.3 | 4.5 | 3.9 | 3.8 | 2.9 | 7.7 | 3.4 |
| Arm ratio | 1.23 | 1.91 | 1.45 | 1.75 | 1.16 | 3.32 | 2.1 |
| Relative length | 0.19 | 0.16 | 0.14 | 0.14 | 0.11 | 0.26 | 0.12 |
| Designation | M | Sb | M | Sb | M | St | Sb |

M: Metacentric; Sb: submetacentric; St: subtelocentric.

Previous studies of the karyotypes in this family (Metz, 1916; Boyes, 1953, 1963; Kaul *et al*., 1978) have shown that the size of the sex chromosomes varies considerably among species, as also occurs for other dipteran groups (Boyes and Boyes, 1975; Kaul and Tewari, 1979; Parise, P.P.M., unpublished data). The results of our analysis of the karyotype of *P. intermutans* fit this pattern of autosomal and sex chromosomic organization.

In agreement with reports (Boyes, 1953, 1963) for the subfamily Sarcophaginae (*Helicobia* sp., *Helicobia rapax*, *Neobelliera bullata*, *Sarcophaga carnaria* and *Sarcophaga exuberans*, among others) there was little difference in the relative lengths and arm ratios among the autosomes. This feature was first noted by Boyes and Van Brink (1965), Kaul *et al*. (1978) and Gaur *et al*. (1984). This similarity supports the argument that the autosomes of the Sarcophagidae retain a high degree of structural integrity and may have differentiated by structural rearrangements, which did not affect the gross morphology of the chromosomes (Kaul *et al*., 1978). In contrast, there is considerable variation in the size and morphology of the sex chromosomes and their length does not correlate with total




**Figures 3-6** - C-banded chromosomes of *Pattonella intermutans*. **3-5:** Mitotic chromosomes. **6:** Meiotic chromosomes. Arrow in Figure 3 and in Figure 6 shows a large pericentromeric band in pair I and in Figures 4 and 5 shows a intercalary band in pair II. Arrowhead in Figure 6 shows an allocyclic behavior for heterochromatic chromosomes. Scale bar = 10 μm.

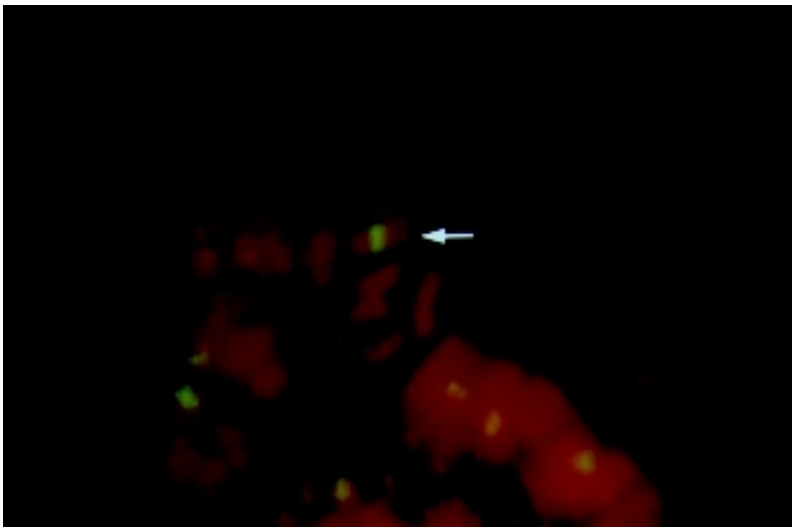


**Figure 7** - FISH of *Pattonella intermutans* chromosomes using a *Drosophila* rDNA probe. White arrow indicates the FISH signal in pair IV. The hybridization signal is yellow, whereas the chromosomes and nuclei are counterstained with propidium iodide (red).

autosome length. The constancy on the total autosome length and the variability of the sex chromosomes strengthen the hypothesis that substantial modifications in sex chromosomes do not result in either loss or gains in autosomal material.

Large blocks of constitutive heterochromatin characterize the chromosomes of *P. intermutans*, whose sex chromosomes are totally heterochromatic. C-banding revealed variably stained segments along the length of these chromosomes. Kaul *et al*. (1978) reported differential staining in the sex chromosomes of *Parasarcophaga* spp. and suggested that large changes in the size of the sex chromosomes must have taken place by the accumulation or deletion of heterochromatin. This variation was both quantitative and qualitative since there were some segments which stained differentially. Euchromatic segments have not been as well studied as heterochromatic segments. Euchromatic segments have played a relatively minor role in chromosome events and the karyotype has evolved mainly through changes in the amount, nature and distribution of heterochromatic segments.

Heterochromatin has a significant role in the evolution of sex chromosomes. The incorporation of inert heterochromatin may represent an initial phenomenon in the specialization of sex chromosomes (John, 1988; for review, see Jablonka and Lamb, 1990). According to the model described by Muller (1932), morphologically distinct sex chromosomes would have evolved from normal, homomorphic autosomes. One typical process during the progression to heteromorphic sex chromosomes is the heterochromatinization of one of the two sex chromosomes in the heterogametic sex (Ohno, 1967; for review, see John, 1988).

During heterochromatinization, the original sequences undergo dramatic changes, genes degenerate to pseudogenes, the chromosomal region acquires many kinds of transposable elements and DNA segments are duplicated (Steinemann and Steinemann, 1992). At the end of the process, one of the two sex chromosomes may become heterochromatic, the number of functional genes may be drastically reduced and, in the extreme case, the only genes that remain functional are those involved in fertility, as is the case in *Drosophila* (for review, see Hennig, 1986).

With sex chromosomes of different sizes (big, as in *Pattonella intermutans*, or small, as in *Parasarcophaga* spp.) there may be a move towards total loss of the sex chromosomes, as occurs in some species of the Muscidae family (Parise, P.P.M. and Avancini, R.M.P., unpublished data), or to accumulation of parts of autosomes, thereby increasing their size.

NORs are markedly conserved among diptera species and are therefore good markers for studying karyotypic evolution. In most species studied so far, the NOR(s) are located in the sex chromosomes (Bedo and Howells, 1987; Bedo and Webb, 1989; Willhoeft and Franz, 1996; Willhoeft, 1997). Kaul and collaborators (1989) applied the N-banding technique to six species of *Parasarcophaga* and obtained similar results to C-banding for all chromosomes; however, they were not able to distinguish between C bands and NORs. The detection of NORs by conventional techniques can be difficult, mainly because of the lack of specificity of such techniques. The use of *in situ* hybridization can circumvent these limitations becoming useful for detecting the location of the NOR in *P. intermutans*. In this case, the NOR was not located on the sex chromosomes, but in autosome IV, close to a large block of constitutive heterochromatin. This may be evidence of an intermediary stage in the evolution of these chromosomes, with important sequences of the genome moving to other sites to avoid drastic consequences, associated with the loss of part of the sex chromosomes.

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## RESUMO

Neste trabalho está descrito o cariótipo detalhado de *Pattonella intermutans*. Esta espécie apresenta 2n = 12 cromossomos: três pares de autossômos metacêntricos e dois submetacêntricos e um par de cromossomos sexuais XX/XY. Os autossomos são caracterizados pela presença de bandas C nas regiões pericentroméricas e os cromossomos sexuais são totalmente heterocromáticos. A técnica de FISH detectou a NOR no autossomo IV.

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