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# Polytene chromosome analysis of a population of *Simulium pertinax* (Diptera: Simuliidae)

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## ABSTRACT

Cytological studies were performed on a semi-natural population of *Simulium pertinax* from Morungaba, State of São Paulo, Brazil. This species has 2n = 6 chromosomes, being one pair of metacentric and two pairs of submetacentric chromosomes. Polytene chromosome maps of the three autosomes from larval salivary gland cells were obtained. The chromosomes of this population have conspicuous centromeric regions and high morphological stability. Asynaptic regions were rare. Inversions and morphological altered bands were not detected in the population (N = 102). A comparison of the band patterns of *S. pertinax* maps with the standard for the subgenus *Simulium* showed many similarities in all chromosome arms.

## INTRODUCTION

*Simulium* (*Chirostilbia*) *pertinax* Kollar, 1832, is a widespread black fly species occurring throughout Brazil, Northern Argentina and Paraguay (Coscarón, 1987). This anthropophylic species is normally a pest in many regions of Brazil, and efforts to control it have been made over the last decades. Organophosphate resistance has been detected in this species in Brazil (Ruas Neto *et al.*, 1984; Andrade and Castello Branco Jr., 1990) and, in the culicid *Anopheles albimanus*, such resistance has been correlated with gene translocations in polytene chromosomes (Kaiser *et al.*, 1979; Hemingway, 1992).

Cytological studies of simuliid populations have been carried out for many purposes. Larval polytene chromosome analysis has been used to determine intra and interspecific variability (Brockhouse *et al.*, 1989a,b; Feraday *et al.*, 1989; Leonhardt and Ferady, 1989), and to identify sibling species (Rothfels *et al.*, 1978; Rothfels, 1987), as well as for phylogenetic relationships among species (Hunter, 1989), and the biosystematics of economically important species (Weber and Grunewald, 1989). These studies have identified vectorial species and cytotypes (Procunier, 1989; Charalambous *et al.*, 1993), and have established the development of resistance for vector or pest-control strategies (Post, 1986).

In spite of the importance of *S. pertinax* for public health, cytological studies have not yet been reported. This report presents the first cytogenetic observation on *S. pertinax* giving a description of the mitotic chromosomes and the polytene chromosome maps of a population from Morungaba, State of São Paulo, that has not been exposed to chemical control programs. The banding patterns of *S. pertinax* polytene chromosomes are also compared to the standard published map for the subgenus *Simulium* (Rothfels *et al.*, 1978).

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# MATERIAL AND METHODS

This study was carried out on a population of *S. pertinax* from Morungaba, State of São Paulo. The larvae were collected between October 1993 and July 1994 in a tributary stream of the Jaguari river of the Serra das Cabras. The larvae were fixed in Carnoy's fixative (75% absolute ethanol, 25% glacial acetic acid) with changes within the first hour and then storage at 4°C until slide preparation.

Metaphase chromosomes were analyzed in neuroblast preparations of sixth or seventh instar larvae. The neural ganglia were dissected and maintained for 20 min in a hypotonic solution of 1% sodium citrate plus 0.1% colchicine (19:1) and fixed for three to five minutes in 45% acetic acid. The preparations were stained with 2% orcein for 20 min and then squashed (Imai *et al.*, 1988).

Polytene chromosomes were analyzed in salivary gland squashes (N = 102). A modified Feulgen staining technique was used as described by Charalambous *et al.* (1993). Fifth to seventh instar larvae were dissected in fresh Carnoy's fixative to expose the salivary glands, which were then transferred to distilled water for 30 to 60 min. Acid hydrolysis was carried out at room temperature in 5N HCl, for 30 to 60 min. Following hydrolysis the larvae were washed twice for two minutes in distilled water and then stained for one-two hours in Schiff's reagent. After 10 min in sulfurous water and washing with tapwater the glands were removed and transferred to a drop of 2% lacto-acetic orcein. After five to 20 min of staining, the tissue was mounted in 60% acetic acid. The shape of the gonads reveals the sex of the larva (Puri, 1925 *apud* Charalambous *et al.*, 1993). Males have small spherical testes, while females have a thin ribbon-like ovary. Map nomenclature was based on that adopted by Rothfels (1987). The entire polytene complement is divided into 100 sections: 42 for the first chromosome, 29 for the second and 29 for the third.

# RESULTS

## Mitotic chromosomes

*S. pertinax* has three pairs of chromosomes, as in most simuliid species. Chromosome I stands out as the longest metacentric. Pairs II and III are submetacentric and have a similar length, but they can be distinguished by the short arms of the third pair that are slightly smaller than those of the second pair (Figure 1).

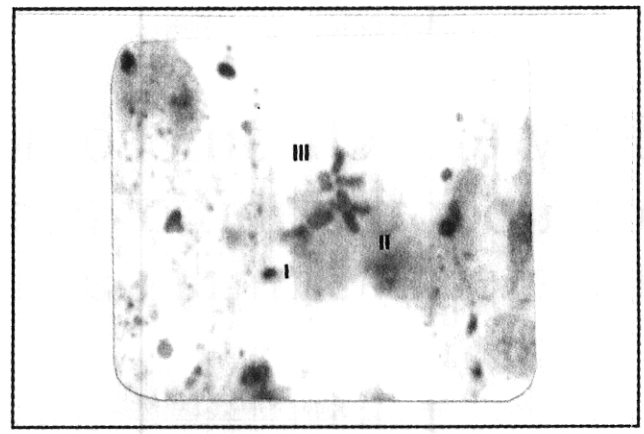


**Figure 1** - Mitotic metaphase chromosomes prepared from ganglia of *Simulium pertinax* larvae. Chromosome I is metacentric and chromosomes II and III are submetacentric. The centromeric regions are conspicuous. (1750X).

## Gross morphology of the polytene chromosomes

The two homologous chromosomes pair tightly, and thus, there appears to be only three chromosomes, I, II, III, each having a short arm, S, and a long arm, L (Figure 2-4). All six chromosome arms of *S. pertinax* are homologous to those of the standard map of the subgenus *Simulium* (Rothfels *et al.*, 1978).

There are conspicuous centromeric regions in each chromosome, but there is no chromocenter. The highest degree of polytenization was observed in salivary glands from the 5th and 6th larval instars. Lack of pairing between the homologues was not frequent. Also, B chromosomes were not observed, and undifferentiated sex chromosomes characterized this population.

## Chromosome bands

### IS (Figure 2)

The IS arm is divided into 20 sections and is most easily recognized by the banding pattern of the telomeric chromosome (section 1). It can be also recognized by the following markers: "two blocks" in section 5, the "crack" in section 11A, the nucleolar organizer region (NOR) between sections 11 and 12, the "Cup & Saucer" between sections 16 and 17, and the basal "3" in section 18.

### IL (Figure 2)

The IL arm is separated into 22 sections (21-42). The most distinctive characteristics for the

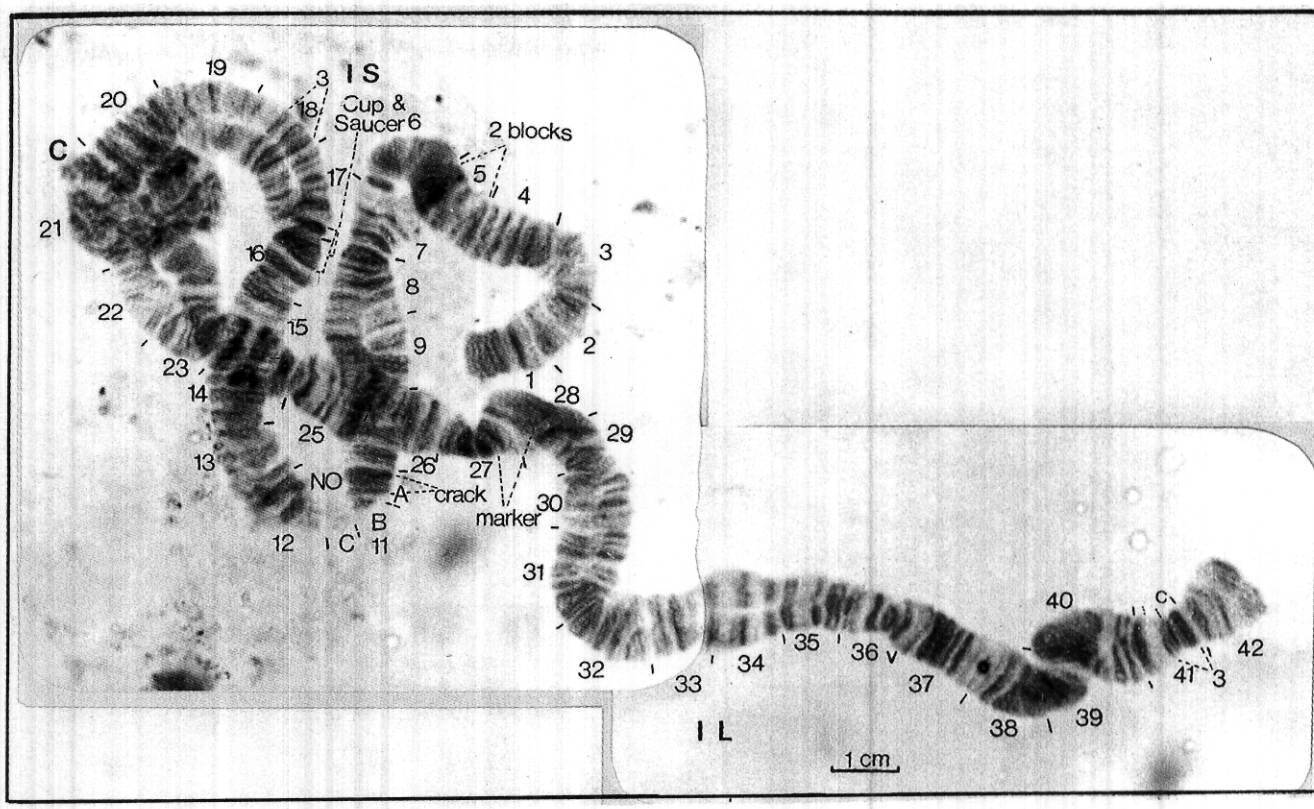


**Figure 2** - Photocomposite chromosome map of *Simulium pertinax* polytene chromosome I. Note in the IS arm (sections 1-20) the markers "2 blocks" (section 5), the "crack" (section 11), the nucleolar organizer region (NOR) (sections 11 and 12), the "cup & saucer" (sections 16 and 17) and the "3" (section 18); C, Centromere (section 21). The IL arm (sections 21-42) has the "marker" (section 29) and the subterminal "3" (section 41). (1250X).

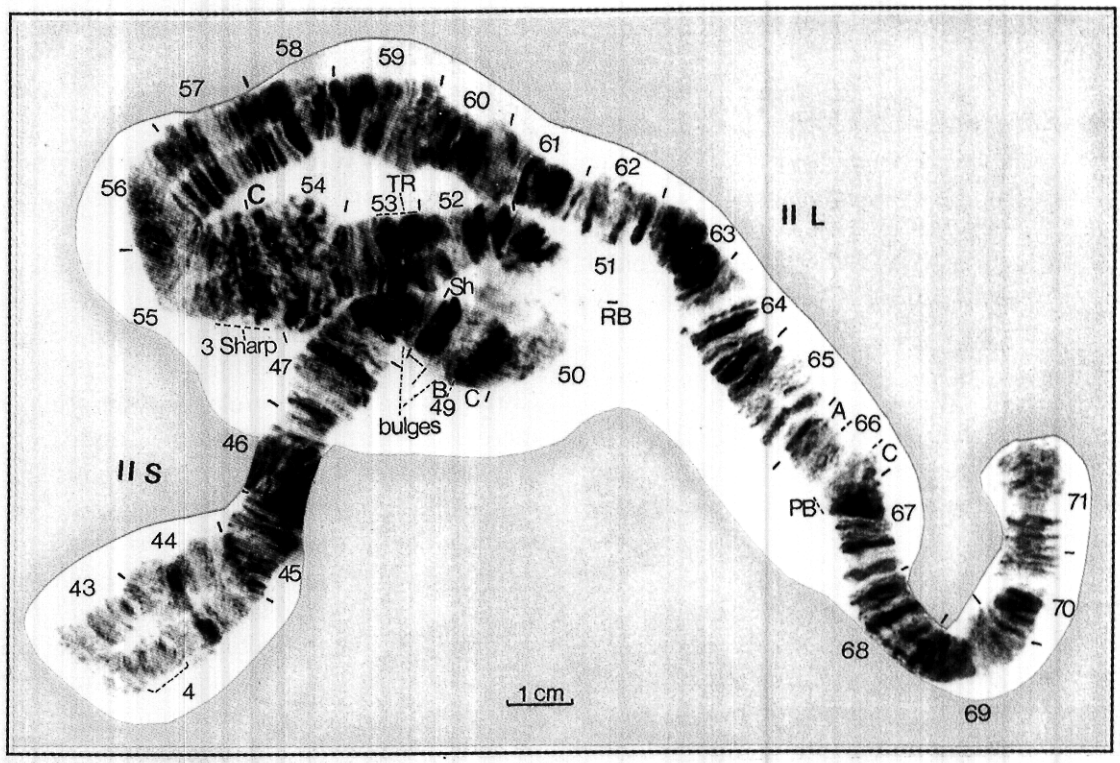


**Figure 3** - Photocomposite chromosome map of *Simulium pertinax* polytene chromosome II. Note in the IIS arm (sections 43-54B) the markers "4" (section 43), the "shoestring" (Sh) central to the "bulges" (sections 48 and 49), the Balbiani ring (RB) (section 51) and the "trapezoidal" (TR) (section 53). C, Centromere (section 54C). The IIL arm (sections 54C-71) has the "3 sharp" (section 55) and the parabalbiani (PB) (section 66). (1250X).

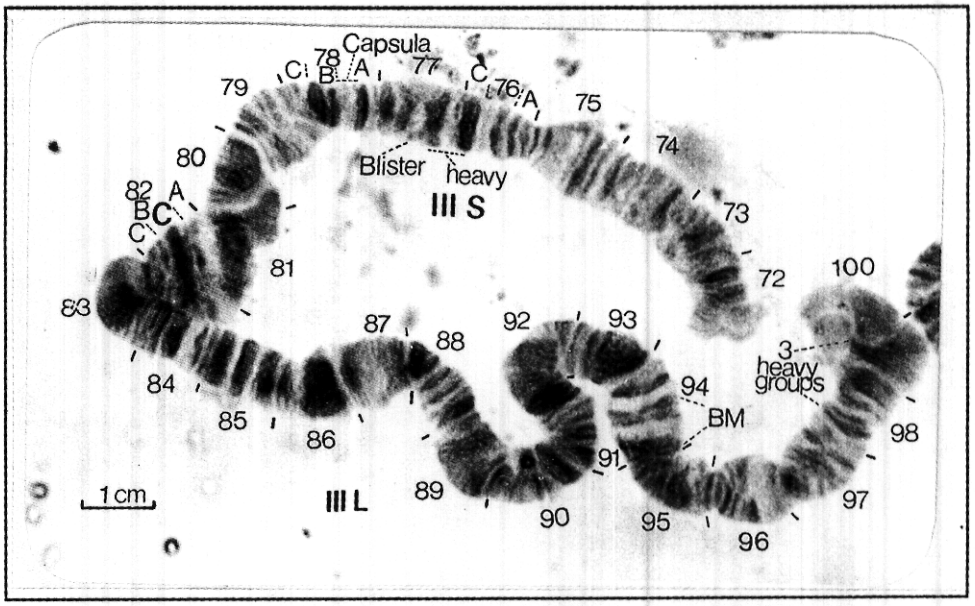


**Figure 4** - Photocomposite chromosome map of *Simulium pertinax* polytene chromosome III. Note in the arm IIIS (sections 72-82A) the markers "heavy" and "blister" (sections 76C-77), and the marker "capsula" (section 78A). C, Centromere (section 82B). The IIIL arm (sections 82C-100) has the "basal marker" (BM) (section 94) and the subterminal "3 heavy groups" (sections 98-99). (1250X).

identification of this arm are the expanded centromeric region (section 21), and the markers: "marker" in section 28 and subterminal "3" in section 41C close to the terminal pattern (section 42).

## IIS (Figure 3)

This arm includes 12 sections (43-54B) and is identified by two universal markers, the Balbiani ring (RB) in section 51 and the bulge in sections 48C to 49B, beyond the "shoestring" (Sh) in section 49A, the terminal "4" in section 43 and the basal trapezoidal marker (TR) between sections 52C and 53B.

## IIL (Figure 3)

The IIL arm has 17 sections (54C-71) and is recognized by the universal mark "3 sharp" band adjacent to the centromere and the parabalbiani marker (PB) in section 66C.

## IIIS (Figure 4)

The IIIS is divided into 10.5 sections (72-82A) and is universally marked by a group of "heavy" bands in sections 76C-77, distally bounded by two conspicuous bands and basally by one expanded region, the marker group blister. Also, the telomere is slightly fan-shaped and the basal "capsula" in section 78A is a good marker.

## IIIL (Figure 4)

The IIIL can be subdivided into 18.5 sections (82B-100) and is recognized by a "basal" marker (BM) in section 94 and the subterminal "3 heavy groups" between sections 98 and 99.

## Comparison with the subgenus *Simulium* standard map

The polytene chromosome banding patterns of *S. pertinax*, subgenus *Chirostilbia*, showed some similarities to those of the standard map of the subgenus *Simulium* (Rothfels *et al.*, 1978) (Figure 5). Chromosome I is very similar between sections A-E, but there is a re-orientation of B and C. In addition, the NOR is located in arm IS while in the subgenus *Simulium* it is in the IIIL arm. Another difference is the centromeric region which is expanded in *S. pertinax*. Chromosome II with homology in segments A-G showed re-oriented B and C segments in relation to the *Simulium* standard, in such a way that the Balbiani ring is basal in *S. pertinax* and distal in the *Simulium* standard map, when considered in relation to the centromere. Chromosome III had less similarities to the standard chromosomes of the subgenus *Simulium* than the others, with homology only in segments A-I.

The population studied (N = 102) was very homogeneous, except for rare asynaptic regions not

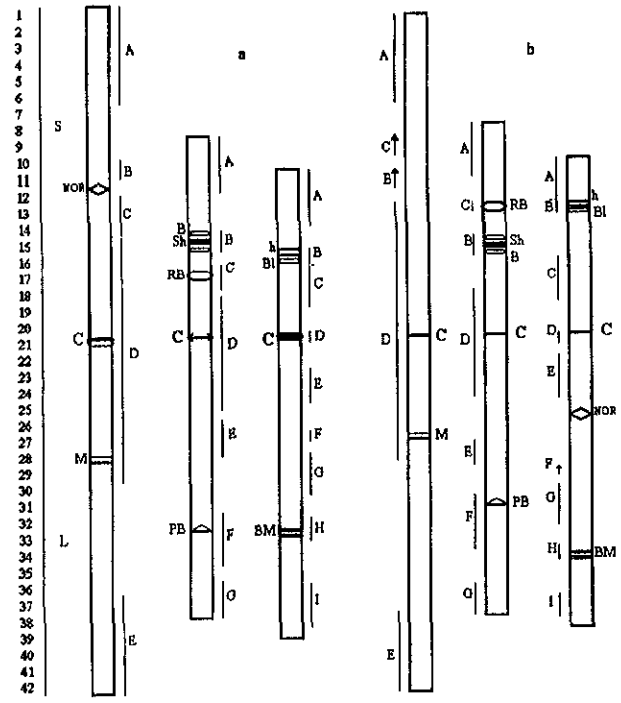


**Figure 5** - Idiogram of (a) *Simulium (Chirostilbia) pertinax* and (b) *S. (Simulium)* standard. The chromosomes are arranged from the longest (I) to the shortest (III); short arms (S) are above, long arms (L) are below the centromere. The relative positions of the main markers are shown. C, Centromere; NOR, nucleolar organizer region; M, "marker"; BM, "basal marker". The lines indicated by letters correspond to the homologous sections in the two species. The arrows indicate reorientation of chromosome sections; C and B in the IS arm, and F in the IIIS arm of *S. (Simulium)*. The complete complement of I to III has 100 sections.

associated to any inversion. Neither polymorphic inversions nor polymorphic bands were observed in the 102 larvae analyzed.

## DISCUSSION

*S. pertinax*, a neotropical black fly species, like most species in the Simuliidae family so far studied, has a karyotype of 2n = 6. This is the characteristic number also found in many species of Culicidae (Ramirez and Dessen, 1994). Asynaptic regions or lack of pairing was not frequently observed for this population although this used to be the natural condition in some species (Rothfels and Featherston, 1981). Chromosomes B are not present in *S. pertinax*, although they are present in four genera of Simuliidae (Rothfels, 1979 *apud* Brockhouse *et al.*, 1989a). Cytological undifferentiated sex chromosomes with identical polytene band patterns as in *S. pertinax* are also present in many species (Feraday *et al.*, 1989).

The population of *S. pertinax* studied did not exhibit any intraspecific polymorphism either in band form or inversion. Since the frequency of chromosomal polymorphism in species of Simuliidae can vary with season, altitude or geographic region (Rothfels, 1980), and polymorphisms have also been detected in some neotropical species (Duque, 1980; Moreno, 1982; Duque *et al.*, 1988; Conn *et al.*, 1989; Hirai *et al.*, 1994) even when a small number of specimens are analyzed (Campos, 1989), it would now be interesting to undertake seasonal studies to confirm whether the *S. pertinax* population is homogeneous in terms of chromosome variability. In the case of *Wilhelmia lineata* and *W. equina*, collected from 13 sites in the southwestern part of Germany, many chromosomic polymorphisms were found to occur in the former (N = 45) while the latter (N = 203) was more homogenous (Wever and Grunewald, 1989).

In addition to the band pattern, *S. pertinax* can also be characterized by the conspicuous centromeric regions in chromosomes I and III and the position of the RB and the bulges in the IIS arm, both good markers for these chromosomes. Although NOR is not a universal marker, it can be useful to identify chromosome I, considering that it is constant in the IS arm.

The majority of the segments found in the six arms of *S. (Chirostilbia) pertinax* are homologous to the standard chromosomes of the subgenus *Simulium* and also to some species of the subgenus *Ectemnaspis* (Duque *et al.*, 1988; Campos, 1989; Campos and Muñoz de Hoyos, 1990). This morphological comparison seems to agree with the position of these three groups in the supraspecific cladogram of the genus *Simulium* in the neotropical region (Coscarón, 1987).

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## RESUMO

Foram realizados estudos citológicos em uma população semi-natural de *Simulium pertinax* de Morungaba, Estado de São Paulo, Brasil. Esta espécie apresenta 2n = 6 cromossomos, sendo um par metacêntrico e dois submetacêntricos. O trabalho apresenta o mapa dos cromossomos politênicos obtidos de glândulas salivares larvais. Os cromossomos desta população apresentam conspícuas regiões centroméricas e alta estabilidade

morfológica. Regiões assinápticas foram raras. Não foram observadas inversões nem bandas morfologicamente diferentes na população (N = 102). A comparação do padrão de bandamento dos mapas de *S. pertinax* com aquele padrão do subgênero *Simulium* mostrou semelhança em várias regiões nos seis braços cromossômicos.

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