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Homology of polytene elements between *Drosophila* and *Zaprionus* determined by *in situ* hybridization in *Zaprionus indianus*

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ABSTRACT. The drosophilid *Zaprionus indianus* due to its economical importance as an insect pest in Brazil deserves more investigation into its genetics. Its mitotic karyotype and a line-drawing map of its polytene chromosomes are already available. This paper presents a photomap of *Z. indianus* polytene chromosomes, which was used as the reference map for identification of sections marked by *in situ* hybridization with gene probes. Hybridization signals for *Hsp70* and *Hsr-ω* were detected, respectively, in sections 34B and 32C of chromosome V of *Z. indianus*, which indicates its homology to the chromosomal arm 3R of *Drosophila melanogaster* and, therefore, to Muller's element E. The main signal for *Hsp83* gene probe hybridization was in section 17C of *Z. indianus* chromosome III, suggesting its homology to arm 3L of *D. melanogaster* and to element D of Muller. The *Ubi* probe hybridized in sections 10C of chromosome II and 17A of chromosome III. Probably the 17A is the polyubiquitin locus, with homology to arm 3L of *D. melanogaster* and to the mullerian D element, as suggested also by *Hsp83* gene location. The *Br-C* gene was mapped in section 1D, near the tip of

the X chromosome, indicating its homology to the X chromosome of *D. melanogaster* and to mullerian element A. The *Dpp* gene probe hybridized mainly in the section 32A of chromosome V and, at lower frequencies to other sections, although no signal was observed as expected in the correspondent mullerian B element. This result led to the suggestion of a rearrangement including the *Dpp* locus in *Z. indianus*, the secondary signals possibly pointing to related genes of the TGF-β family. In conclusion, the results indicate that chromosomes X, III, V of *Z. indianus* are respectively correspondents to elements A, D, and E of Muller. At least chromosome V of *Z. indianus* seems to share synteny with the 3R arm of *D. melanogaster*, as indicated by the relative positions of *Hsp70* and *Hsr-ω*, although the *Dpp* gene indicates a disruption of synteny in its distal region.

Key words: Polytene chromosomes, Gene mapping, Drosophilids, Chromosome homology, *Zaprionus indianus*

INTRODUCTION

The family Drosophilidae is one of the most diverse and widely distributed families of the Diptera order, consisting of about 2500 species that has been highlighted in studies of ecology, genetics, development, and evolution (Wheeler, 1981, 1986). Even though it has been widely studied, the phylogeny and taxonomy of drosophilids have shown controversial conclusions. Phylogenetic trees based on morphological or molecular characters are sometimes contradictory, arranging some branches of the *Drosophila* subgenus closer to the *Zaprionus* genus than to other branches of *Drosophila* genus (Thomas and Hunt, 1993; Kwiatowski and Ayala, 1999).

The genus *Zaprionus*, which currently consists of 56 species (Tsacas and Chassagnard, 1990; Chassagnard and Tsacas, 1993), was initially thought to be related to the *immigrans* group of the *Drosophila* genus, due to the white-silver-plated stripes on its mesonotum (Throckmorton, 1975). This relationship was later contested (Pasteur, 1978), since all species of the *Zaprionus* genus studied have a karyotype configuration considered as ancestral, with five pairs of acrocentric and a pair of dot chromosomes, differing from the composition of one metacentric pair, one acrocentric pair and two telocentric pairs present in the *immigrans* group. After new analysis of morphological characters, Grimaldi (1990) considered a new classification of the Drosophilidae family, based on monophyletic relationships between the groups and placed the *Zaprionus* genus in the phylogenetic tree as a divergent branch before the spreading of the *Drosophila* genus. However, later studies based on the molecular sequences of different genes, such as dopa decarboxylase (*Ddc*), alcohol dehydrogenase (*Adh*), superoxide dismutase (*Sod*), and glycerol-3-phosphate-dehydrogenase (*Gpdh*) supported the idea that the *Sophophora* subgenus had split prematurely from the Drosophilidae family and pointed to the *Zaprionus* genus as being closer to the *immigrans* group and to other species of the distinct genus, as first suggested by Throckmorton (1975).

Cytogenetic studies of the polytene chromosomes in the genus *Zaprionus* have shown that the pattern of five long rod chromosomes and a shorter sixth one correlating with the five pairs of acrocentric and one pair of dot chromosomes is a common characteristic of all species in the genus (Sciandra et al., 1973; Tsacas et al., 1977; Su et al., 1992; Hatch and Jeffery, 1992). The first polytene chromosome map of this genus was a line-drawing map of the complement of *Z. indianus* that was published together with the localization of the nucleolar organizer regions in the X chromosome and in the dot pair, besides the identification of a paracentric inversion in chromosome II (Gupta and Kumar, 1987). Also, photomaps for polytene complements were reported for *Z. inermis* (Hatch and Jeffery, 1992) and *Z. tuberculatus* (Su et al., 1992). In this last study, the first genes were mapped by *in situ* hybridization, establishing the correlation of the polytene chromosomes of *Z. tuberculatus* with the ones of *D. melanogaster*.

Several studies using *in situ* hybridization were performed for the Drosophilidae family, mainly in the species of the *obscura* (Segarra and Aguadé, 1992; Segarra et al., 1995, 1996), *virilis* (Whiting Jr. et al., 1989; Vieira et al., 1997; Evgen'ev et al., 2004), *repleta* (Ranz et al., 1997; Ruiz et al., 1997; González et al., 2002), *willistoni* (Bonorino et al., 1993; Rohde et al., 1995), and *melanogaster* groups (Drosopoulou et al., 1996). The data obtained by *in situ* hybridization, along with that of banding pattern analysis, were fundamental in establishing the homology between the polytene complements and also in estimating the number of paracentric inversions and detecting rare pericentric inversions that gave rise to karyotype divergence during the groups' evolution (Segarra and Aguadé, 1992; Ranz et al., 1997; González et al., 2002). This approach can also be applied to other genera, such as *Zaprionus*.

The most common *Zaprionus* species, *Z. indianus*, has received special attention due to its invasion of the Neotropical region in the last years (Vilela, 1999; Tidón et al., 2003; Silva et al., 2005). In Brazil, *Z. indianus* adopted a new behavior never before observed among drosophilids, colonizing fruits before their maturation, making them unsuitable for consumption and causing economic damage in the commercialization of figs and other fruits cultivated in Southeastern Brazil (Vilela et al., 2001). In its migration to Northeastern Brazil, *Z. indianus* was found colonizing native fruits, representing a possible threat to fruit-producing regions such as the São Francisco River Valley (Santos et al., 2003). The same concern is now being considered for Central and North America (van der Linde et al., 2006).

In the present study, we prepared a formal photographic map of the polytene karyotype of *Z. indianus* to allow the localization of *in situ* hybridization signals. It is also useful in defining precise inversion breakpoints. We used six probes obtained from the *D. melanogaster* genome to localize on the polytene chromosomes of *Z. indianus* the heat shock genes *Hsp70*, *Hsp83*, *Hsr-ω*, and *Ubi* (ubiquitin), and the developmental genes *Br-C* (broad-complex) and *Dpp* (decapentaplegic). The mapping of these genes suggests the homology among the chromosomes of species of the genera *Zaprionus* and *Drosophila*, contributing to the clarification of the evolutionary relationships among drosophilids.

MATERIAL AND METHODS

Identification and maintenance of fly stocks

We used the VVI strain of *Z. indianus* Gupta 1970 which was collected in Vila Velha (Itamaracá, PE, 07° 41' 15" S, 34° 48' 45" W) and established since 2001 in the Laboratório de

Genética Animal of the Departamento de Genética of Universidade Federal de Pernambuco. The specimens had been captured with banana baits and by collecting fruits in the fermentation state with the presence of eggs and larvae of drosophilids. The collected specimens were identified in accordance with the dichotomous keys of Tsacas and Chassagnard (1990) and Chassagnard and Tsacas (1993) and maintained in the laboratory stock on standard medium at 23 ± 1°C.

Cytological preparations

Salivary glands of third-instar larvae were dissected in 0.67% saline, treated by acid hydrolysis with 1 N HCl for 30 s (Hartmann-Goldstein, 1961; Gupta and Kumar, 1987) and fixed in a solution of acetic acid, water and lactic acid (3:2:1 ratio). For the preparation of the polytene chromosome photomap, the material was stained with 1% lacto-aceto-orcein and squashed (Ashburner, 1967). For *in situ* hybridization the cytological preparations were treated as described by Engels et al. (1986).

In situ hybridization

The plasmids containing clones of the *D. melanogaster* genes *Hsp70* (Livak et al., 1978), *Hsp83* (Holmgren et al., 1981), *Hsr-ω* (Rysek et al., 1987), *Ubi* (Izquierdo et al., 1984), *Br-C* (Chao and Guild, 1986), and *Dpp* (St. Johnston et al., 1990) were transformed into *Escherichia coli* DH5α and extracted by alkaline lysis (Sambrook et al., 1989). The plasmids were biotin-labeled by nick translation using the BioNick DNA system (Gibco/BRL, Paisley Scotland) to be used as probes. The *in situ* hybridization was performed in 50% formamide at 37°C for 40 h, using 500 ng DNA probe for each slide and detected by BCIP, SAP and NBT (Gibco/BRL). Chromosomes were counterstained with 0.1% lacto-aceto-orcein and mounted in Entellan (Merck).

Microscopic analysis

The chromosomes for the photomap preparation were photographed with a digital camera, Sony Cyber-Shot Dsc-p73, programmed for a 3.2-mp resolution, connected to a Leitz Orthotoplan photomicroscope. To determine the place and the number of *in situ* hybridization signals on the polytene chromosomes, the material was analyzed and photographed under phase contrast microscopy with 100X magnification. The frequency of hybridization signals was quantified by direct counting. Although this quantification is not common in the literature, we decided to perform it in order to improve the reliability of the results, since we used heterologous sequences as probes.

RESULTS

Polytene chromosome photomap

The polytene chromosome photomap of *Z. indianus* is presented in Figure 1. The complement consists of five long euchromatic chromosomes which correspond to acrocentric

pairs I (chromosome X) to V, and a small chromosome which corresponds to the microchromosome pair VI. Based on the main landmarks of the first line-drawing map for the species (Gupta and Kumar, 1987), there are 38 sections and 152 subsections in this photomap.

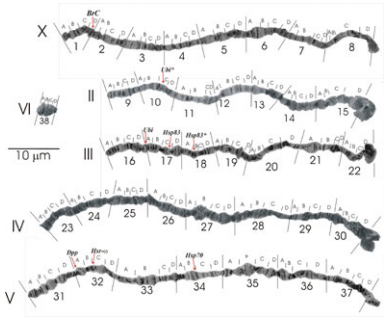

Figure 1 is available at http://www.funpecrp.com.br/gmr/year2007/vol2-6/pdf/gmr0291fig1.pdf

Figure 1. Photomap of polytene chromosomes of *Zaprionus indianus*. Section subdivision was based on Gupta and Kumar (1987) with the centromeres on the left. The genes mapped are pointed out by arrows, and asterisks denote the secondary marks.

Chromosome X is easily recognized in males, being lighter and thinner due to its hemizygous condition. Some marks detailed on the map drawn by Gupta and Kumar (1987) were identified on this chromosome, such as its characteristic narrow tip and the bulb-like structure with two strong bands in section 3A. A doublet puff was detected in section 6AB.

Chromosome II is one of the minor acrocentric chromosomes, whose size is similar to chromosome III. Few marks are recognizable on this chromosome, but it can be identified by its softly rounded tip followed by two strong bands in the initial section 9A.

Chromosome III is the one of the more easily identified chromosomes, since its thin tip shows a set of four prominent bands in subsection 16A-B, followed by a puff at the end of the subsection 16B, four other bands in subsection 16C and a characteristic bulb in subsection 16D. Another evident signal in the map of Gupta and Kumar (1987) is a bulb-like structure in section 19C, also present in our preparations, frequently in the form of a large puff delimited by strong bands.

Chromosome IV showed in our material a clearly opened tip in a flat straight line and a small puff in subsection 23B, which is markedly different from the pointed free tip shown by Gupta and Kumar (1987), without puffs and with several thin bands. Another distinctive feature was the bulb clearly formed between darker bands in subsection 24B.

Chromosome V is similar in size to chromosome X and recognized by its tip in a spindle form, with two puffs separated by two strong bands in subsection 31B. Throughout some sections it was possible to find other recognizable bulb-like structures, such as those clearly demonstrated in subsections 33B and 35B.

Chromosome VI is the minor polytene element of the complement, comprising only one section subdivided in four subsections, consisting of four dark bands, the widest one being next to the base.

Localization of genes by *in situ* hybridization

The frequencies of hybridization signals of the *Hsp70*, *Hsp83*, *Hsr-ω*, *Ubi*, *Br-C*, and *Dpp* gene probes in the polytene chromosomes of *Z. indianus* are summarized in Table 1. The analysis of 16 polytene nuclei hybridized *in situ* with the *Hsp70* gene probe showed 11 signals in section 34B of chromosome V, representing 68.7% of the detected signals (Figure 2). In some of these nuclei, we observed signals that were very strong and apparently a duplicated band.

Table 1. Frequency of hybridization signals of *Hsp70*, *Hsp83*, *Hsr-ω*, *Ubi*, *Br-C*, and *Dpp* gene probes in polytene chromosomes of *Zaprionus indianus*.

Gene	Chromosome	Section	Frequency
Hsp70	V	34B	68.75%
N = 11			
Hsp83	III	17C	44.4%
N = 18	III	18B	38.8%
	III	21A	17.0%
Hsr-ω	V	32C	71.4%
N = 21	II	9CD	9.5%
Ubi	II	10C	41.9%
N = 31	III	17A	38.7%
Br-C	X	1D	48.5%
N = 33	V	32C	27.7%
Dpp	V	32A	33.3%
N = 36	X	2BC	16.6%
	III	16D	16.6%
	III	17B	11.1%

N = number of marked nuclei.

For the *Hsr-ω* gene mapping, 21 polytene nuclei with hybridization signals were analyzed. Of these, 15 nuclei showed signals in section 32C of chromosome V (Figure 3), representing 71.4% of the nuclei analyzed. Another signal was found at a lesser frequency on section 9CD, consisting of 9.5% of the nuclei analyzed.

The hybridization of the *Hsp83* gene was analyzed in 18 nuclei and two evident signals appeared in two distinct sections of chromosome III. Eight signals were situated in section 17C, representing about 44.4% of the signals, and seven appeared in section 18B, representing 38.8% (Figure 4a). Another signal appeared at a lesser frequency in section 21A of the same chromosome, meaning 17% of the signals (Figure 4b).

After the hybridization with *Ubi* probe, 31 polytene nuclei were selected. Of these, two regions of the chromosomes of *Z. indianus* were significantly marked. The mark on section 10C of chromosome II (Figure 5a) was detected in 13 nuclei, which represents 41.9% of the total. The other signal, in section 17A of chromosome III (Figure 5b), was observed in 12 nuclei, representing 38.7% of the nuclei analyzed.

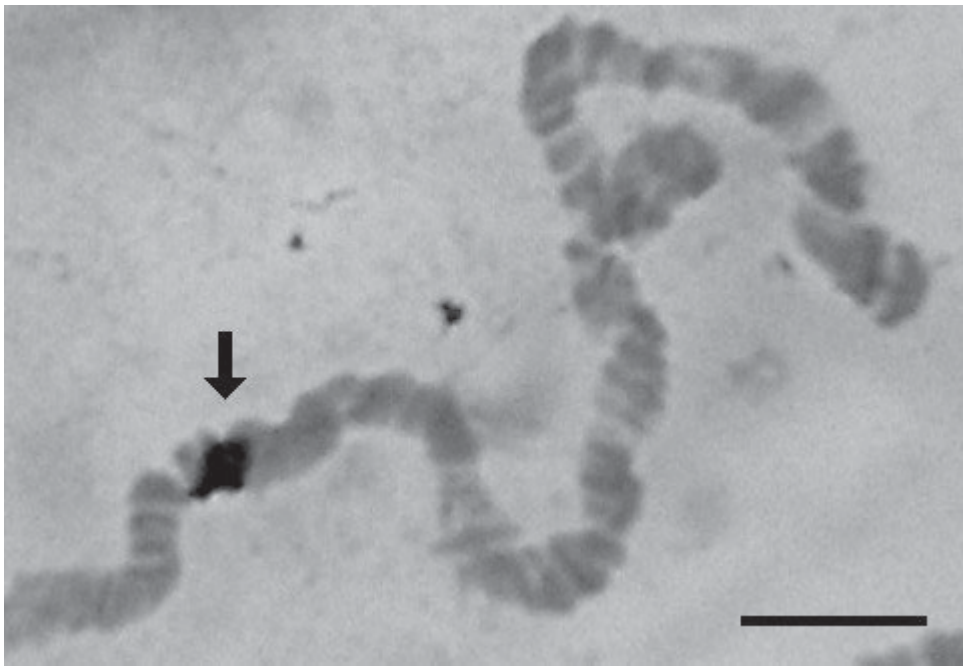

Figure 2. Signal of *Hsp70* gene probe hybridization (arrow) in section 34B of chromosome **V** of *Zaprionus indianus*. Bar represents 10 µm.

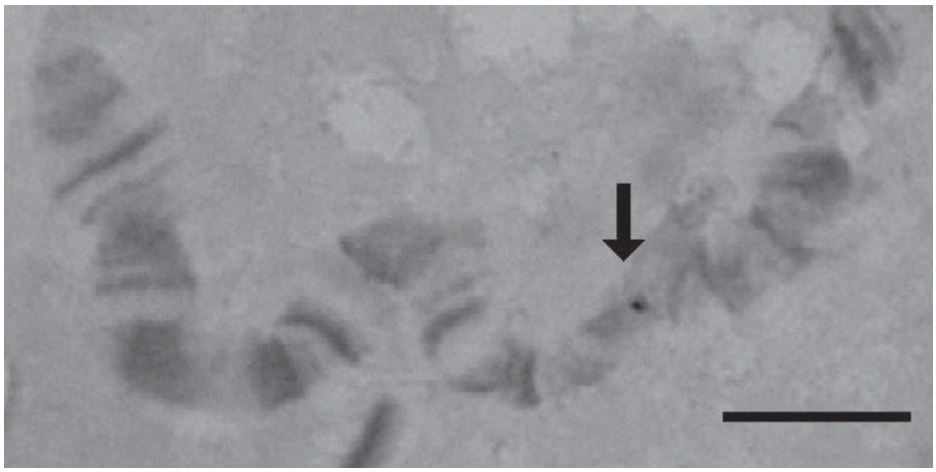

Figure 3. Signal of *Hsr-ω* gene probe hybridization (arrow) in section 32C of chromosome **V** of *Zaprionus indianus*. Bar represents 10 µm.

The analysis of *Br-C* gene hybridization was performed in 33 polytene nuclei. Only section 1D of chromosome X (Figure 6) had significant signals, appearing in 16 nuclei and representing 48.5% of the total. At a lower frequency, a signal in section 32C of chromosome V appeared in 9 nuclei, representing 27.7% of the nuclei analyzed.

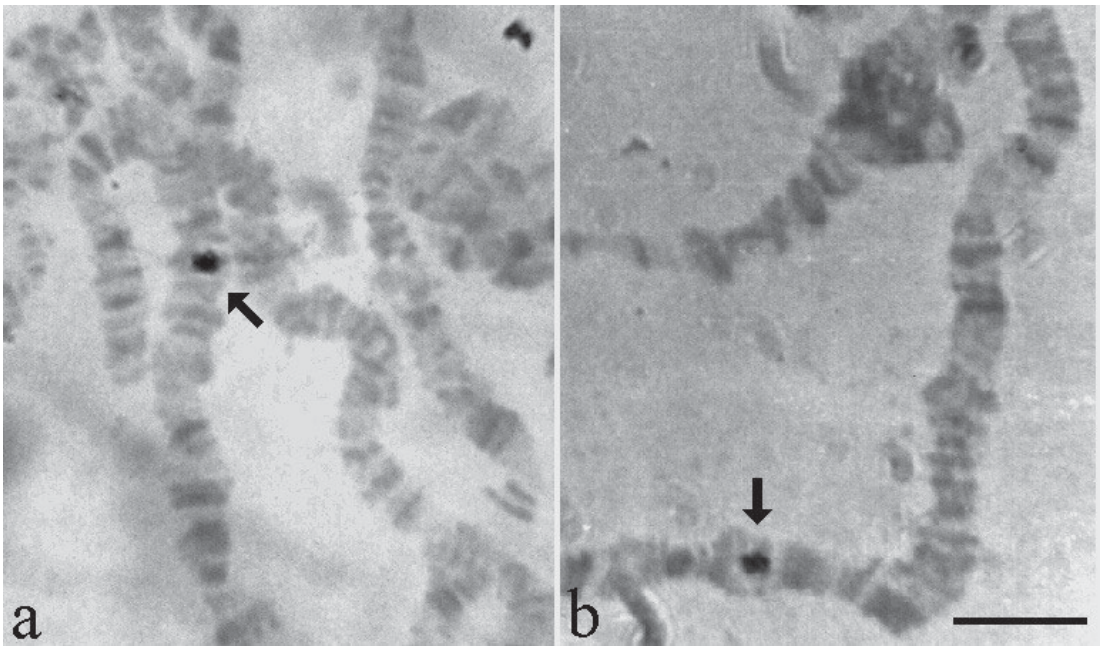

Figure 4. Signals of *Hsp83* gene probe hybridization (arrows) in sections 17C (a) and 18B (b) of chromosome **III** of *Zaprionus indianus*. Bar represents 10 µm in both panels.

The *Dpp* gene hybridization was analyzed in 36 polytene nuclei with distinct signals. Only region 32A of chromosome V (Figure 7a) had a significant frequency of 33.3%, appearing in 12 nuclei. Other *Dpp* signals appeared as well, with minor frequencies, in section 2BC of chromosome X (Figure 7a and b) and sections 16D (Figure 7c) and 17B (Figure 7d) of chromosome III.

DISCUSSION

Photomap

The polytene chromosomes of the *Zaprionus* genus have been described in the literature as being difficult to study due to their highly twisted state, high affinity of the cytoplasm for the chromosomal stains and low degree of polyteny (Hartmann-Goldstein, 1961; Gupta and Kumar, 1987). Another difficulty reported was the relative fragility of the chromosomes, which tend to break up easily (Hatch and Jeffery, 1992). Despite these difficulties, the chromosome complement of some species of this genus has been already studied. The polytene karyotype of *Z. multistriatus* (Sciandra et al., 1973), *Z. sepsoides* (Tsacas et al., 1977) and *Z. indianus* (Gupta and Kumar, 1987) has been described, and photomaps have been prepared for *Z. inermis* (Hatch and Jeffery, 1992) and *Z. tuberculatus* (Su et al., 1992). Based on these studies, the pattern of five long arms and one sixth short chromosome seemed to be a characteristic common to all the species of the *Zaprionus* genus.

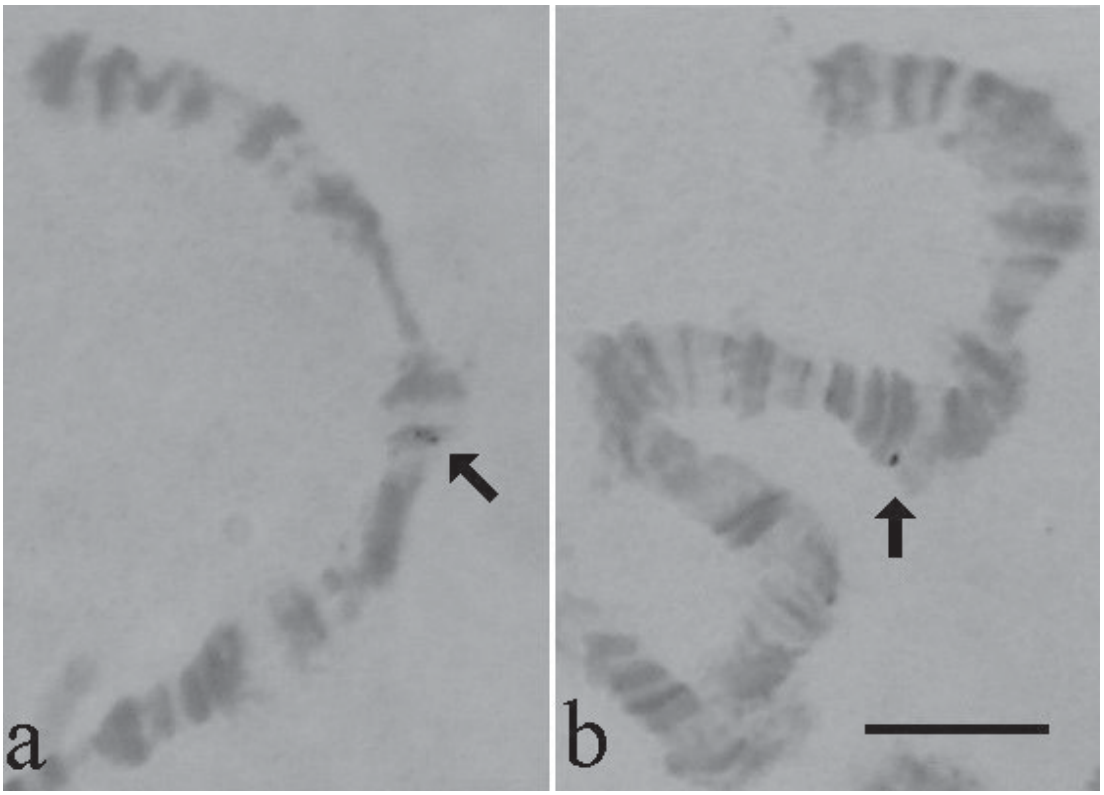

Figure 5. Signals of *Ubi* gene probe hybridization (arrows) in section 10C of chromosome **II** (a) and in section 17A of chromosome **III** (b) of *Zaprionus indianus*. Bar represents 10 µm in both panels.

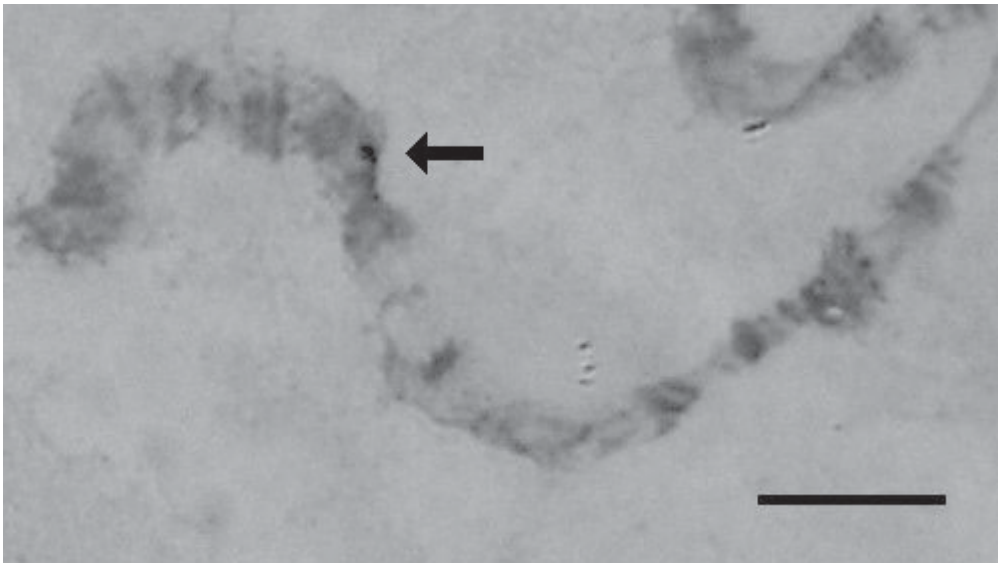

Figure 6. Signal of *Br-C* gene probe hybridization (arrow) in section 1D of chromosome **X** of *Zaprionus indianus*. Bar represents 10 µm.

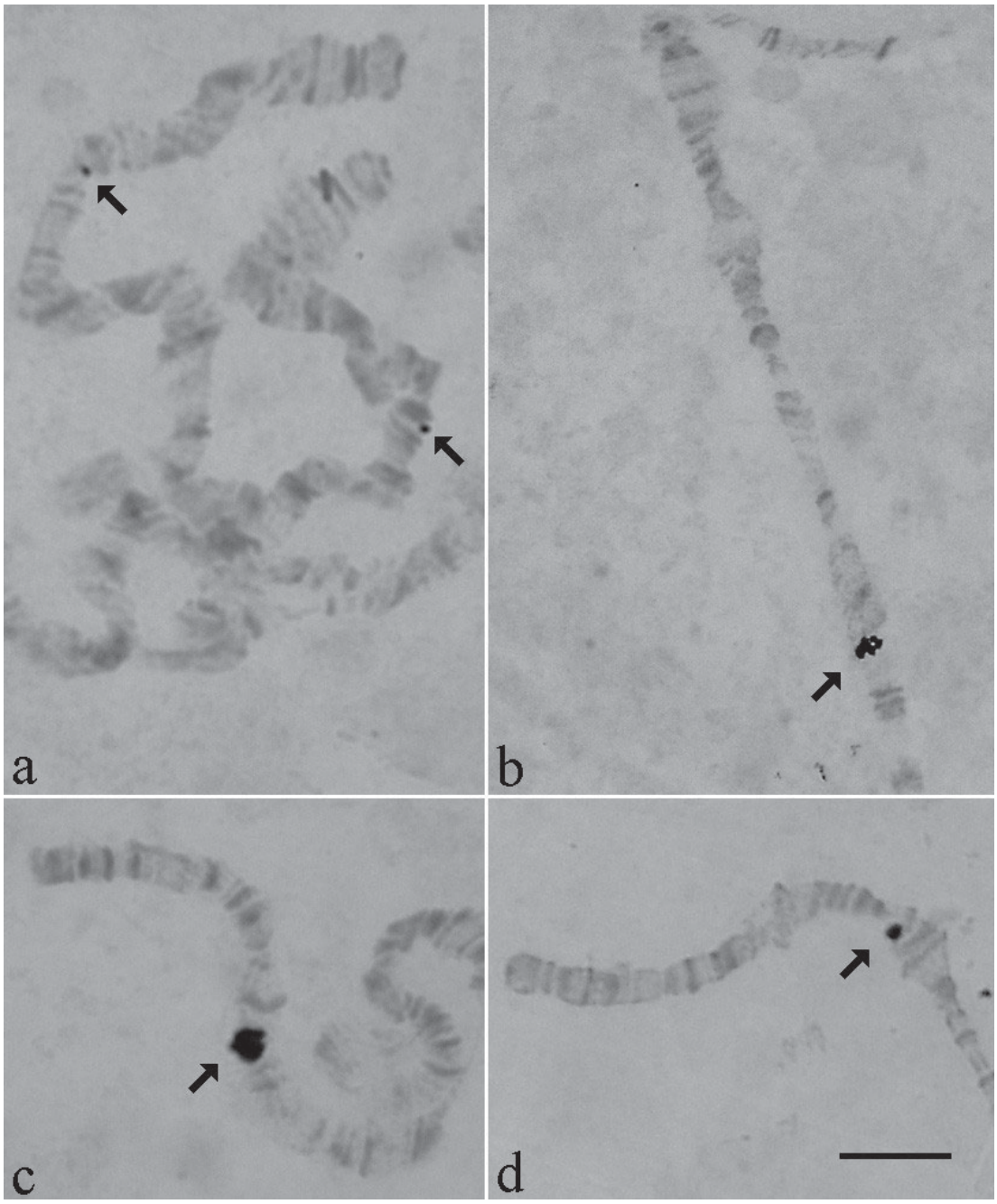

Figure 7. Signals of *Dpp* gene probe hybridization (arrows) in section 32A of chromosome **V** (a), section 2BC of chromosome **X** (a and b) and sections 16D (c) and 17B (d) of chromosome **III** of *Zaprionus indianus*. Bar represents 10 µm in all panels.

Many difficulties have been found in the use of the line-drawing map of *Z. indianus* made by Gupta and Kumar (1987). Due to its low resolution, it was difficult to identify the distinct polytene elements, mainly based on its tips. Additional difficulties arose by some differ-

ential band activation in the material studied. We prepared a photomap from the strain VVI derived from a local population, where the elements and the division of the sections and subsections could be easily recognized. A maximum effort was carried out to accurately distribute the sections and subsections in a similar position as seen in the line-drawing map of Gupta and Kumar (1987). This should facilitate its use for the definition of breakpoints of inversions or localization of differential gene activation during larval development.

Localization of genes by *in situ* hybridization

According to Muller's (1940) hypothesis, the drosophilids share chromosomal homology which can be identified by their bands and interband pattern and puffing activity. Gene localization by *in situ* hybridization in the polytene chromosomes of *Z. tuberculatus* performed by Su et al. (1992) resulted in the proposition of homology among its chromosomes A, B, C, D, E, and F and the chromosomal arms X, 2L, 2R, 3L, 3R, and 4 of *D. melanogaster*, respectively.

In *Z. indianus* the *Hsp70* gene was localized in section 34B of the polytene chromosome V pointing out its homology with the 3R arm of *D. melanogaster* and consequently to element E of Muller. It is also possible to propose the homology between chromosome V of *Z. indianus* and chromosome E of *Z. tuberculatus*, since the localization of tubulin genes in chromosome E of the last species (Su et al., 1992) also indicates its homology to arm 3R of *D. melanogaster*. The hybridization signal of *Hsp70* in *Z. indianus* encompasses two consecutive bands of 34B section, raising the possibility of gene duplication in very close regions as seen in arm 3R of the *melanogaster* species group and in arm II of the *obscura* group (Segarra et al., 1996). This duplication, however, was not observed in *D. virilis* and in the *willistoni* group, where the gene is located, respectively, in chromosomes 2 (Evgen'ev et al., 2004) and III (Bonorino et al., 1993), also equivalents to Muller's element E.

The localization of the *Hsr-ω* gene in the region 32C of chromosome V of *Z. indianus* indicates its homology with arm 3R of *D. melanogaster*, which corresponds to the Muller element E, as was indicated above by the *Hsp70* gene probe hybridization. The *Hsr-ω* gene position in element E of Muller is conserved also in other species of the *Drosophila* genus. In *D. hydei* it is localized in section 48B of chromosome 2 (Garbe and Pardue, 1986; Ryseck et al., 1987), and in six species of the *montium* subgroup (*melanogaster* group) it is localized in the same chromosomal element corresponding to the 3R arm of *D. melanogaster* (Drosopoulou et al., 1996). It is interesting to note that the genes *Hsr-ω* (32C), *Hsp70* (34B) and the centromere are arranged in *Z. indianus* in the same order as in *D. melanogaster*, showing gene synteny for element E between *Drosophila* and *Zaprionus* genera.

The main signal for *Hsp83* gene probe hybridization was in section 17C of *Z. indianus* chromosome III, suggesting its homology to the arm 3L of *D. melanogaster* and, therefore, to element D of Muller. This finding is in agreement with the homology between chromosome III of *Z. indianus* and chromosome D of *Z. tuberculatus*, where the *Hsp83* gene is located (Su et al., 1992). A second hybridization signal was detected in section 18B of the same chromosome III of *Z. indianus*. Since the *Hsp83* gene is not duplicated in *D. melanogaster* (Holmgren et al., 1981; Blackman and Meselson, 1986; Konstantopoulou and Scouras, 1998) and other insects (Landais et al., 2001), this second signal probably represents partial homology to an unknown gene. Although the *Hsr-ω* gene is in the same family as *Hsp83*, as stated above it was localized in chromosome V of *Z. indianus*.

The *Ubi* gene signal present in subsection 17A of chromosome III of *Z. indianus* indicates the locus for polyubiquitin and corroborates its homology with the 3L arm of *D. melanogaster*, both corresponding to element D of Muller as also indicated above by the localization of the *Hsp83* gene in the same chromosome. However, a second significant signal of *Ubi* hybridization in section 10C of chromosome II of *Z. indianus* points to another homologous ubiquitin locus. The polyubiquitin gene, thus, seems to be duplicated in *Z. indianus*, as observed in some strains of *D. melanogaster* where it is localized in section 63F of the arm 3L and also in region 5F of chromosome X (Izquierdo, 1994). This second hybridization signal for ubiquitin gene in *Z. indianus* could also be explained by a gene coding for a protein fused with ubiquitin monomer. In *D. melanogaster*, loci in region 97A of the arm 3R and in the region 31E of arm 2L code for ubiquitin-ribosomal protein fusion genes (Izquierdo, 1994), which could be identified by the *in situ* hybridization probes. Probes produced from these ubiquitin-fused genes hybridized to chromosomes of two species of the *repleta* group, and three signals were detected besides that of the B1d region of chromosome arm 4 of *D. repleta* and *D. buzzati*, which corresponds to the polyubiquitin loci of section 63F of *D. melanogaster* (Ranz et al., 1997). Based on this evidence from the literature, the second signals of ubiquitin in chromosome II of *Z. indianus* could indicate its possible homology with element B of Muller.

The most significant signal of the *Br-C* gene, localized in section 1D of chromosome X of *Z. indianus*, demonstrates its homology to chromosome X of *D. melanogaster* where this gene is in section 2B5 (Chao and Guild, 1986). Previously, the largest chromosome of the polytene complement of *Z. indianus* was identified as the sex chromosome, due to its light staining in males, a common characteristic in the majority of the drosophilids (Gupta and Kumar, 1987). The localization of the *Br-C* gene in the chromosome X of *Z. indianus* points to a conservation also in the genes of the sex chromosome homologous arms between *Drosophila* and *Zaprionus*. Similar homology was previously indicated by Su et al. (1992) for chromosome A of *Z. tuberculatus* and the X of *D. melanogaster*, although they noticed an apparent rearrangement with the probe 548 that they used. Chromosome A of *Z. tuberculatus* and the X of *Z. indianus* should then correspond to element A of Muller. On the other hand, the second signal for *Br-C* gene in section 32C of chromosome V of *Z. indianus* could indicate a locus duplication in this species. After hybridization with a probe consisting of a cluster formed by the genes *Br-C*, *dor* and *swi* (region 2B3-8 of *D. melanogaster*) in several species of the *obscura*, *repleta* and *virilis* groups, the majority of them showed a single-hybridization signal in chromosome X. However, *D. hydei* (*repleta* group) has shown more than twelve hybridization sites in different chromosomes, which raised the possibility of duplications of region 2B in this species (Kokoza et al., 1992).

The most significant hybridization signal of the *Dpp* gene in *Z. indianus* was localized in subsection 32A of chromosome V, which corresponds to arm IIIR of *D. melanogaster* in accordance with the localization of the genes *Hsr-ω* and *Hsp70* discussed above. However, the *Dpp* gene is localized in the section 22F1-3 of IIR arm in *D. melanogaster*, which can indicate that a complex rearrangement such as a translocation probably occurred involving the *Dpp* gene during the evolutionary divergence between *D. melanogaster* and *Z. indianus*. Since the genes *Hsr-ω* and *Hsp70* also localized in chromosome V of *Z. indianus* are in synteny with arm IIIR of *D. melanogaster*, the rearrangement should involve only the distal part of chromosome V of *Z. indianus*. To uncover the chromosomal events that separate *Drosophila* and *Zaprionus*, it will be necessary to compare the localization of *Dpp* and neighbor genes in

Zaprionus species and closely related drosophilids, mainly the Hirtodrosophila clade and the *immigrans* group species of *Drosophila* (Throckmorton, 1975; Kwiatowski and Ayala, 1999). On the other hand, the minor signals for Dpp probe detected in chromosomes X and III of *Z. indianus* could result from partial sequence homology to other genes with high conservation in the family TGF-β. It should be noted that none of these signals correspond to the expected location in Muller's element C.

Finally, the data from the *in situ* hybridization of the genes *Hsp70*, *Hsp83*, *Hsr-ω*, *Ubi*, *Br-C*, and *Dpp* in the polytene chromosomes of *Z. indianus* presented in this study indicate that this species maintains homology with the mullerian elements, mainly the elements A, D and E. The second signal of ubiquitin can also be considered an indirect indication of homology of chromosome II of this species with element B of Muller. In this way, chromosome IV would correspond to element C by exclusion. These data when compared with the literature implies that the Muller (1940) hypothesis of homology between the main polytene elements of *Drosophila* can be extended for the *Zaprionus* genus, and can also indicate that synteny in large chromosomal segments has been preserved between these two drosophilid genera.

An important approach in further analyses will be to extend the localization of these and other genes to the *immigrans* (*Drosophila* subgenus) group of species, a close clade of the *Zaprionus* genus, which may indicate the extension of the rearrangements that separate them. It will also be interesting to use gene probes that are originally located close to *Dpp* gene in *D. melanogaster* for *in situ* hybridization in the chromosomes of *Z. indianus*, to verify the extension of the suggested rearrangement that had occurred. The localization of other conserved genes in the polytene chromosomes of *Z. indianus* still remains to be done to confirm the relationhips with mullerian elements B and C. Together with the comparison of the banding patterns, this will determine which types of rearrangements had occurred in the divergence of *Zaprionus* and *Drosophila* genera.

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