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Newly recorded inversion and re-annotation of inversion breakpoints in *Drosophila cardini* species.

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Abstract

This report presents a new paracentric inversion in the middle of the chromosome X in *Drosophila neocardini*. Also, a careful analysis was carried out of chromosomal inversion breakpoints that were previously described for *Drosophila polymorpha*. Photo comparison, and the release of a newly designed photomap, allowed us to introduce changes to the breakpoints of In(2R)A and In(2R)D. All individuals analyzed were collected in two conserved areas in Santa Catarina/south of Brazil. Key words: chromosomal polymorphism, cytogenetic, polytene chromosomes.

Introduction

Chromosomal inversions were first discovered by Sturtevant in 1917 as the recombination modifier in *Drosophila melanogaster*, that occurs when a chromosome breaks at two points and the segment bounded by the breakpoints is reinserted in the reversed orientation (Navarro *et al*., 2000). This inversion mutation that reordered genes in the genome became a powerful genetic marker to assess naturally occurring genetic variation (Wallace *et al*., 2013). Also, the ability to identify inversions with simple cytological analysis proved to be very important in these early studies (Painter, 1933).

The cytological analysis involves a technique with polytene chromosomes present in the nuclei of salivary glands of *Drosophila* species. These chromosomes allow easy identification of the arrangements in heterozygotes, since they in this case result in an inversion loop. Different types of inversion are classified into two basic categories: pericentric inversions that include a centromere region, and paracentric inversions, that do not. Paracentric inversions are also the more common type of chromosomal inversion (Krimbas and Powell, 1992).

Natural populations of *Drosophila* species show a wealth of gene arrangement polymorphisms and may play a significant role in adaptation, speciation, and sex chromosome evolution (Kirkpatrick, 2010). The variation of chromosomal inversions in a population allows us to estimate the genetic diversity of the species, while the comparison between populations provides an overview of the gene flow and habitat fragmentation with regard to where these populations reside (Feder *et al*., 2011).

The *cardini* group belongs to the subgenus *Drosophila* and was established by Sturtevant (1942). It currently includes 16 species of a large geographical distribution in Neotropical America (Heed, 1962). The group comprises two subgroups: *dunni* (*D. antillea, D. arawakana, D. belladunni, D. caribiana, D. dunni, D. nigrodunni, D. similis*) and *cardini* (*D. bedichecki, D. acutilabella, D. cardini, D. cardinoides, D. neocardini, D. neomorpha, D. parthenogenetica, D. polymorpha, D. procardinoides*).

Drosophila neocardini, described by Streisinger (1946), and *Drosophila polymorpha*, described by Dobzhansky and Pavan (1943), from *cardini* subgroup, are extremely similar with respect to their morphology and ecological requirements (Rohde and Valente, 1996; Medeiros and Klaczko, 2004). Both species are common in Neotropical forests and the specific differentiation between them is made through the analysis of the internal male genitalia and the pattern of abdominal pigmentation. Their nuclei consist of four chromosome pairs: submetacentric chromosomes 2 (chr2R, chr2L) and 3 (chr3R, chr3L), the sexual pair composed of the acrocentric XX, (chrX) and the Y chromosome (chrY), which is heterochromatic and not distinguishable from the chromocenter, and a dot pair, being chromosome 4 (chr4) (Rohde and Valente, 1996; Cordeiro *et al*., 2014).

Studies involving chromosomal inversions in the *cardini* group indicate that *D. neocardini* does not have many arrangements described so far, whereas *D. polymorpha* shows the highest number of polymorphisms of the group (Da Cunha *et al*., 1953; De Toni *et al*., 2001a; Cordeiro *et al*., 2014). Thus far, 19 rearrangements have been described for *D. polymorpha* and three for *D. neocardini* (see Table 1).

The current study reports a new paracentric inversion in *D. neocardini* and also a careful re-annotation of the breakpoints of two inversions previously described for *D. polymorpha.* These remarks were noticed in the course of a major work involving inversion polymorphisms in the *cardini* group species. Data presented here are considered relevant in order to avoid further misinterpretation and, thus, guarantee effective results regarding the *cardini* group's genetic research.

Table 1. Arrangements described for *Drosophila polymorpha* and *Drosophila neocardini.*

Species	Heterozygous rearrangements	First description
Drosophila polymorpha	In(2R)A	Rohde and Valente, 1996
	In(X)A, In(X)B, In(2L)A, In(2L)B, In(2R)B, In(2R)C, In(2R)D, In(3R)A	De Toni *et al.,* 2001b
	In(X)C, In(3L)A, In(3L)B, In(3L)C, In(3R)B, In(3R)C, In(3R)D, In(3R)E, In(2R)A+C, In(2R)A+D	Cordeiro *et al.*, 2014
Drosophila neocardini	In(3L)A	De Toni *et al.,* 2001a
	In(3L)B, In(3L)C	Cordeiro *et al.*, 2014

Material and Methods

Adult *Drosophila* population samples were obtained through fermented banana bait traps (Roque *et al*., 2011) from conserved areas in Santa Catarina, south of Brazil during the year 2013. Isofemale lines of flies from *cardini* group were established and samples were analyzed by observing the banding patterns of polytene chromosomes. Slides with salivary glands were processed according to Ashburner (1967). Images were captured using a digital camera attached to a binocular microscope connected to a computer, and processed with the Adobe® Photoshop® program (Adobe® Photoshop® CS5 extended, v 12.0 x32).

The newly discovered inversion and the correction of inversion breakpoints were established using the reference photomaps for *D. polymorpha* and *D. neocardini* proposed by Cordeiro *et al*. (2014). At least 10 nuclei where analyzed in order to achieve a conclusive notation for the breakpoints.

Results and Dicussion

Cytological analyses of inversion breakpoints in polytene chromosomes formed the first step for further investigation on the regions of gene rearrangements. Also, registering inversions and defining their breakpoints has led to studies such as inversion polymorphisms and genetic variations in populations.

We report here the first record of an inversion in the chrX in *D. neocardini*. According to the sections established in the photomap of this species (Cordeiro *et al*., 2014), the new paracentric inversion, now named In(X)A, comprises sections 13a proximal and 9b distal (Figure 1).

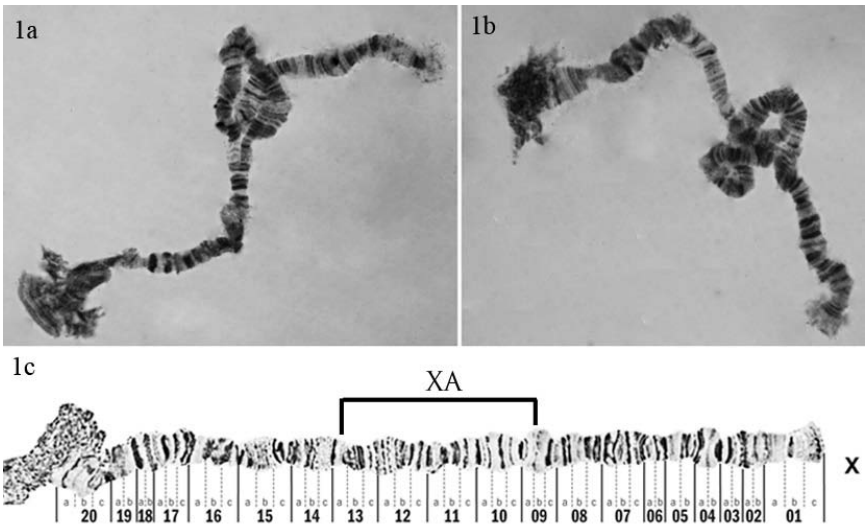

Figure 1. 1a, 1b) Polytene chrX of *Drosophila neocardini* and its new paracentric inversion, In(X)A, in heterozygous state showing the inversion loop. 1c) Photomap of chrX of *D. neocardini* designed by Cordeiro *et al*. (2014). Brackets point to the sections that comprise the new inversion.

Studies involving inversion polymorphisms in *D. polymorpha* reveal that the chromosome arm 2R is the most polymorphic and In(2R)A is the most commonly recorded in the populations investigated (De toni, *et al*., 2001b; Rohde and Valente, 1996; Cordeiro *et al*., 2014). Rohde and Valente (1996) were the first to describe the In(2R)A in *D. polymorpha*, comprising of sections 53 proximal and 48 distal. Wildemann and De Toni (2011) described a new inversion in the chr2R in the same species, and named it In(2R)E (sections 50a proximal and 48c distal). Moreover, the frequent appearance of In(2R)A (Rohde and Valente, 1996) and In(2R)E (Wildemann and De Toni, 2011) during this study, has led us to compare and re-evaluate the breakpoints to those described previously, since they are located in very close regions of the chromosome. Photographic comparison between In(2R)A, In(2R)E, and the 2R inversions found in this study has allowed us to confirm that, in fact, In(2R)A and In(2R)E are the same. Therefore, we are suggesting a new proximal breakpoint for In(2R)A (In(2R)E), which comprises of the sections 52b proximal (instead of 53 or 50a, respectively), and distal sections 48b (Figure 2).

A similar misinterpretation occurred for In(2R)D in *D. polymorpha*. This inversion was first described by De Toni *et al*. (2001b), involving section 45 distal and 47 proximal. Photographic comparison allowed us to confirm that in the case of In(2R)D, in fact, its distal breakpoint comprises of the section 45b, instead of 44, and its proximal breakpoint is 47a (Figure 3).

Detecting inversions and determining their breakpoints allows us to focus studies on the importance of arrangements such as described above, based on the fact that it has been proposed that inversions persist in natural populations as recombination-protected co-adapted gene complexes (Dobzhansky, 1970). Moreover, if two subsequent inversions with similar or identical breakpoints are overlooked, a phylogenetic link could be missed as a result. Thus, when two different inversions are mistakenly identified as the same one, it creates a false phylogenetic link between two unrelated species (Rohde and Valente, 2012).

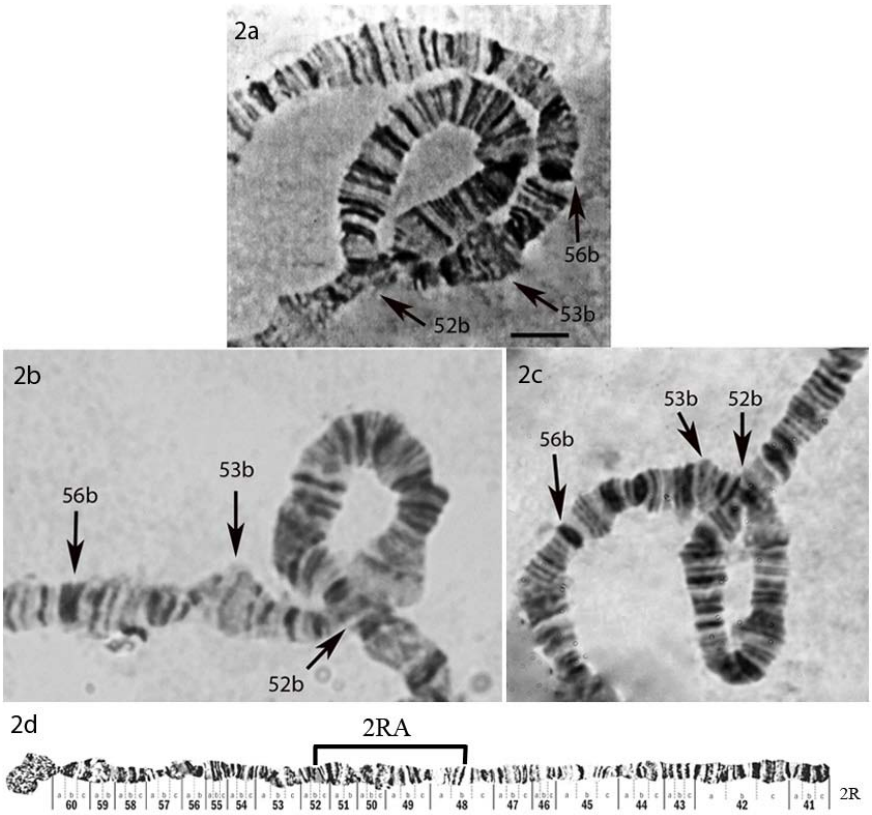

Figure 2. Comparison of In(2R)A in *Drosophila polymorpha*. 2a) In(2R)A (Rohde and Valente, 1996). 2b) In(2R)E (Wildemann and De Toni, 2011). 2c) Inversion 2R from current work. Arrows indicate sections that are key features for the identification of its chromosome regions. 2d) Photo-map of chr2R of *D. polymorpha* designed by Cordeiro *et al*. (2014). Brackets point to the re-evaluated sections that comprise In(2R)A. Bar represents 10µm.

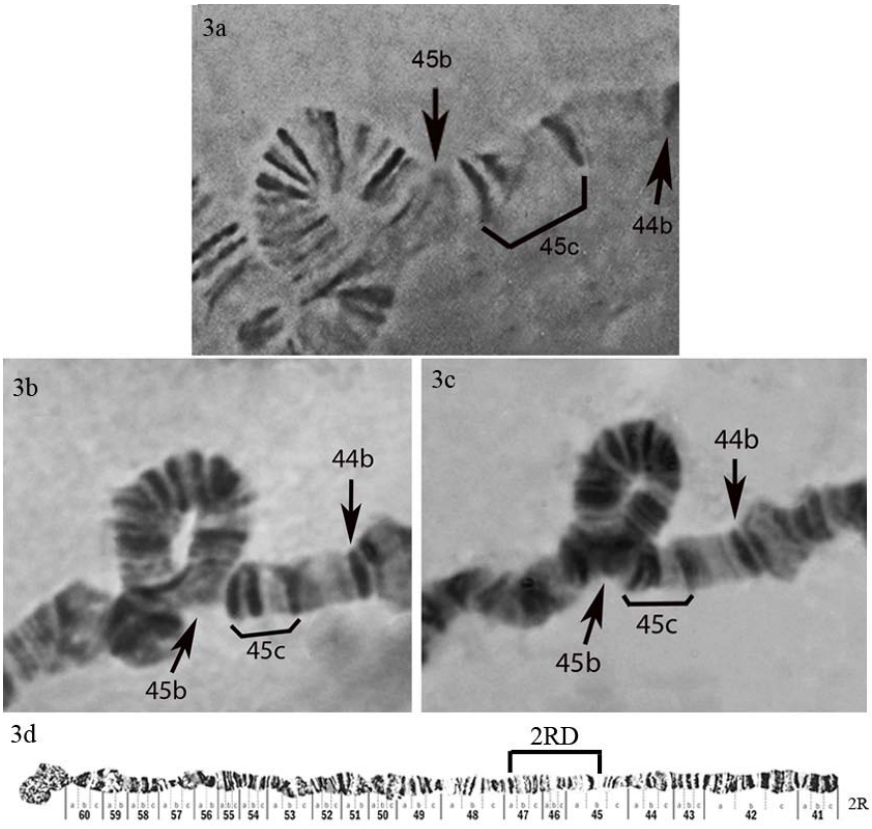

Figure 3. Comparison of In(2R)D in *Drosophila polymorpha*. 3a) In(2R)D (Cordeiro *et al*., 2014). 3b, 3c) In(2R)D from current work. Arrows and brackets indicate sections that are key features in the identification of its chromosome region. 3d) Photo-map of chr2R of *D. polymorpha* designed by Cordeiro *et al*. (2014). Brackets point to the re-evaluated sections that comprise In(2R)D.

The misinterpretation confirmed here with regard to the first records of breakpoints in case of In(2R)A suggests that it was a newly discovered inversion. However, we confirmed by means of photographic comparison and a careful analysis of the new version of *D. polymorpha*'s photomap (Cordeiro *et al*., 2014) that it was only necessary to re-evaluate In(2R)A breakpoints. The corrections were only possible due to the improvement in the photomap that shows the chromosome regions in larger detail.

Although in total *D. polymorpha* present 19 documented rearrangements (Table 1), this work only reevaluated two inversion breakpoints present in the chr2R, since they were common inversions observed in collections of populations from the south of Brazil.

All these results and observations show that chromosomal polymorphisms deserve further investigation. Detailed descriptions of inversions, molecular isolation and the analysis of chromosomal breakpoint sequences can provide an overview of the gene content in the region of the inversion. Also, molecular analysis can detect if any gene expressions have been altered due to the new arrangement that is formed (Wesley and Eanes, 1994).

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First register of *Drosophila carcinophila* at South America, Brazil.

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This academic work describes the occurrence of *Drosophila carcinophila* in Southern Brazil, in Southern Brazilian coastal strand vegetation, in the neighborhood of Rio Vermelho, Florianópolis, Santa Catarina State (Figure 1).

For the monthly gathering was utilized 6 traps made out of PET bottles, with the volume of 2 litters**,** as adaption of the Ferreira's **(**1978) traps. As a bait, it was utilized chicken liver and bovine meat placed in each trap, in a portion of approximately 25 grams of each (total of 50 grams of bait). Before being put in the traps, the baits are kept in an average environment temperature in a period of 48 hours, inside a closed plastic container, to avoid contact with insects. The traps were exposed to the environment for a 4-day period in each month, during 12 months. The collections were made between November 2012 and October 2013. The