



## RESEARCH NOTE

# First detection of chromosomal inversions in a natural population of the invasive pest species *Drosophila suzukii*

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**Abstract.** *Drosophila suzukii* is native to East and Southeast Asia and spread very fast around the world being considered an invasive pest species. Many demographic, population genetics and genomic studies have been recently developed, but so far no analysis has been carried out regarding the presence of chromosomal inversions in *D. suzukii* natural populations. In this research, we studied polytene chromosomes of flies collected from the Font Gropa (Barcelona) population. The chromosomes and many of their segments were characterized for their similarity with those from *D. melanogaster*. This is the report of one paracentric inversion (in heterozygous condition) in the right arm of the third chromosome (3R). As far as we know, it is the first time that an inversion has been observed in a *D. suzukii* natural population. Finally, the evolutionary significance of the finding of inversions in this species is discussed.

**Keywords.** invasive species; polytene chromosomes; chromosomal inversions; adaptation; *D. suzukii*.

## Introduction

*Drosophila suzukii* is an invasive pest species native to East and Southeast Asia that spread very fast around the world (Walsh *et al.* 2011). For instance, it was first detected in Europe in 2008 (Calabria *et al.* 2012) and showed a rapid expansion across the continent (Cini *et al.* 2012; Asplen *et al.* 2015; Arnó *et al.* 2016; Lavrinienko *et al.* 2017). In this species, males are characterized by a dark spot on the leading edge close to the tip of the wings and females present a serrated ovipositor which is able to cut the fruits skin and lay their eggs inside them. From a systematic point of view and based on the morphology of male external genital apparatus, *D. suzukii* was classified in the group *melanogaster* and subgroup *suzukii* (Hsu 1949).

The metaphase chromosomes also confirmed its connection to the *melanogaster* group, with a karyotype composed of four chromosomes: two metacentric, one acrocentric and one dot (Lemenieur *et al.* 1986). This result has been

recently confirmed by Drosopoulou *et al.* (2019) who also developed polytene chromosomal maps of this species. Using *in situ* hybridization of 12 gene markers, they detected regions of synteny with *D. melanogaster*. Further, genomic analysis of *D. suzukii* allowed comparing the similarities at molecular level with *D. melanogaster* (Chiu *et al.* 2013; Ometto *et al.* 2013).

The aim of our research was to analyse the presence of chromosomal inversions in a natural population of *D. suzukii*. Chromosomal inversions have been described as fundamental genetic elements to the success of invasive *Drosophila* species (Dobzhansky 1970; Krimbas and Powell 1992, 2000; Powell 1997; Hoffmann *et al.* 2004; Singh 2019). To achieve our goal, we trapped *D. suzukii* flies in the Font Gropa (Barcelona) site, a place where a small but well-established population has been monitored over time (Galudo *et al.* 2020). From many isofemale lines generated, the salivary glands from third instar larvae were obtained and polytene chromosome preparations were observed in search of inversions.

Carla Esteve and Clàudia Lagares have contributed equally to this work.

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## Materials and methods

### *D. suzukii* collection

*D. suzukii* flies were trapped at the Font Groga site on the foothills of the Tibidabo mountain, a well-characterized location for collecting different species of *Drosophila* genus. It is located on the limit of Barcelona city and about 400 m above the sea level. It presents typical Mediterranean vegetation composed mainly of pine forest (*Pinus pinea*), some ilexes (*Quercus ilex*) and brushwood. During autumn, in this place, the presence of red fruits (for instance *Rubus*, *Arbutus*, *Ruscus* or *Smilax*), which are the preferred breeding site of *D. suzukii*, would favour the permanence of a stable population (Galludo et al. 2020). Flies were trapped in two periods: on 6 October 2014 (Esteve and Mestres 2015) and on 4 October 2016 (Madrenas et al. 2017). A total of 12 fermenting banana baits were placed along a trail and separated about 10 m apart. Flies were netted from 4 pm to dusk (about 7.30 pm). Samples were classified next day in the laboratory and isofemale lines of *D. suzukii* were established. Flies were maintained in vials with 25 mL of standard corn-meal-sugar-agar-yeast medium and were kept at 17°C, with 60% humidity and 12 h / 12 h light/dark cycle. The isofemale lines were changed to fresh medium every three weeks.

### Polytene chromosomes preparation method

To obtain the karyotypes, two females and two males from isofemale lines were put in new vials with 25 mL of standard corn-meal-sugar-agar-yeast medium, but supplemented with 10 mL of strawberry jam (Eroski basic) added after the medium was solidified. These vials were maintained at 25°C to improve the larvae development. Large third instar F<sub>1</sub> larvae were selected and dissected in a saline solution (NaCl 0.9%) to obtain their salivary glands. They were transferred to new slides for staining during 15 min and smoothly squashed in acetic/lactic orcein solution (orcein in glacial acetic acid / lactic acid in 3:2 rate). This solution was prepared in two steps: first, the acetic orcein was obtained dissolving 2 g of orcein (Fluka) in 100 mL of 60% glacial acetic acid (Merck). This process was carried out at high temperature, but preventing to reach the boiling point. Once dissolved, it has to be filtered to remove precipitates. This solution was stored at room temperature avoiding light. In a second step, just before dissecting the larvae, the acetic orcein is mixed with the lactic acid (Merck). It is important to store the acid lactic at 4°C before using it. The glacial acetic acid / lactic acid in 3:2 rate allowed a good balance of stiffness/plasticity of chromosomes during the squash. Finally, the obtained slides were observed using an Olympus BX41 photo microscope using phase-contrast at 400×.

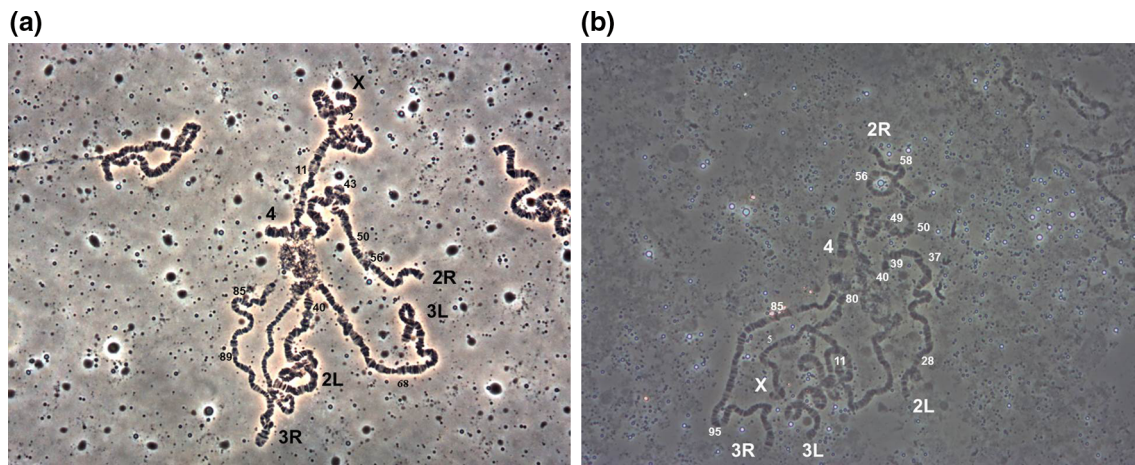
Pictures and schematic drawings of *D. melanogaster* were used from Painter (1934), Lindsley and Zimm (1992) and

Lemenieur and Aulard (1992) to identify the correspondence in chromosomes and several chromosomal fragments between *D. melanogaster* and *D. suzukii*.

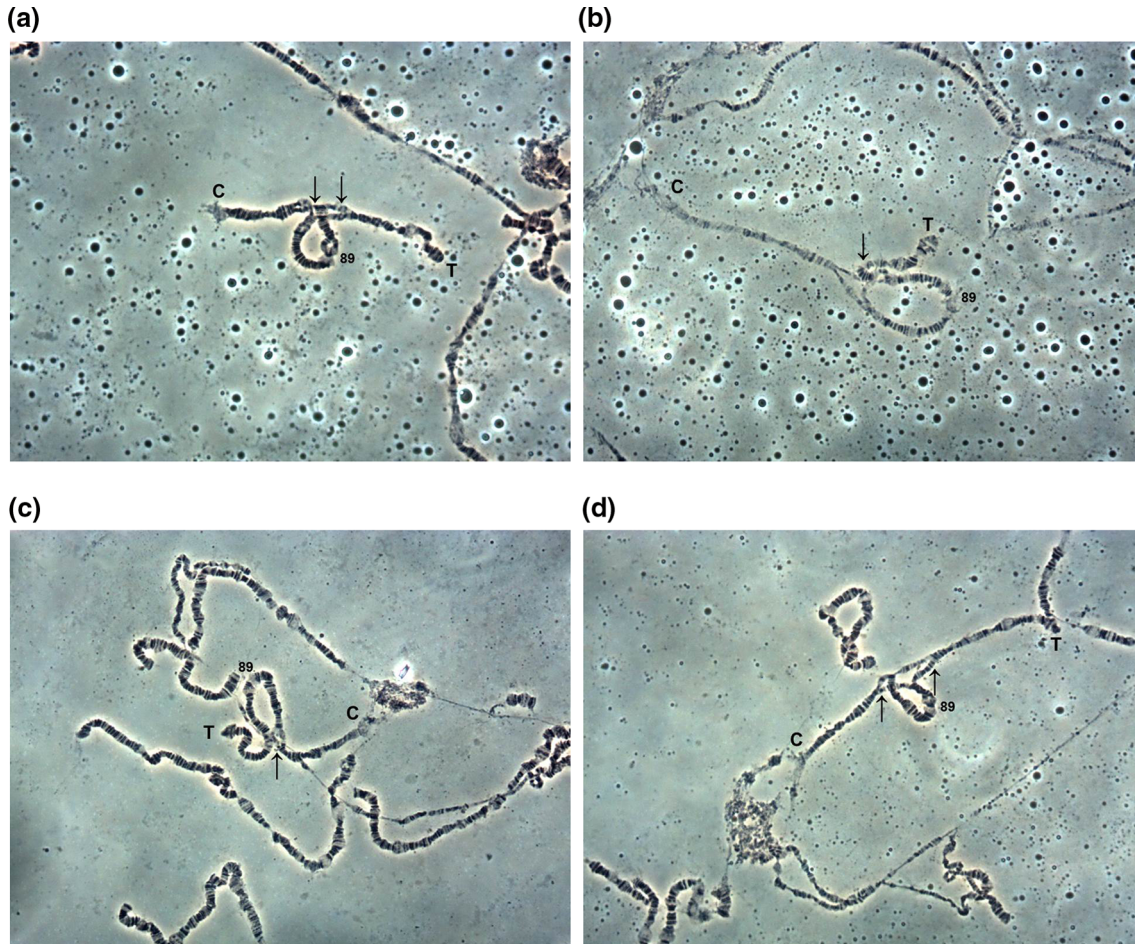
## Results and discussion

It was not difficult to trap *D. suzukii* at the Font Groga site and to generate isofemale lines. However, although this species was maintained at 17°C to obtain large third instar larvae, it was better to use 25°C and to supplement the medium with strawberry jam. Large larvae are essential to obtain polytene chromosomes slides with enough quality to identify particular chromosomes and their segments. Two images of the complete karyotype are presented in figure 1. It is possible to recognize the chromocenter and the chromosomal arms. The karyotype fits with previous descriptions (Lemenieur et al. 1986; Drosopoulou et al. 2019) and the chromosomal arms have been named according to *D. melanogaster* nomenclature: X, 2R, 2L, 3R, 3L and 4 (dot). Several chromosomal arm sections of *D. suzukii* looked similar to those of *D. melanogaster*. They were named according to *D. melanogaster* segments and were useful as recognition points of the chromosomes (figure 1). However, the most outstanding result was the repeated observation of a particular paracentric inversion in heterokaryotypic condition in the samples from 2014 and 2016 (figure 2). It was located in the 3R chromosome arm and the reference segment 89 is situated approximately in the middle of the inverted region. It can be considered a large inversion covering approximately a third of the 3R chromosome arm length. The pattern of bands in both breakpoints can be easily recognized (figure 2, a&d), whereas the inner bands of the inversion are clearly visible in figure 2, b&c. As far as we know, this is the first time that an inversion has been observed in a *D. suzukii* natural population.

Most probably, this inversion was included in the initial sample of colonizers that reached Europe for the first time in 2008 (Calabria et al. 2012). We observed *D. suzukii* in the Font Groga site for the first time in autumn 2011, being the second most abundant species (40.84%), only overcome by *D. subobscura* (57.63) (Galludo et al. 2020). The population of *D. suzukii* is stable in that location, because every time we trapped flies the species was found there either in spring or autumn, although with fluctuations in its frequency (Lagares and Mestres 2018; Rojo et al. 2019; Galludo et al. 2020). If we assume that in nature *D. suzukii* presents the same number of generations than *D. melanogaster*, this value was estimated as 15 in this latter species (Pool 2015). Thus, from our detection of *D. suzukii* in the Font Groga for the first time (2011) until our karyotype studies (2014 and 2016), the number of generations elapsed for this species was 45 (until 2014) and 75 generations (until 2016). For this reason and considering that the isofemale lines were also maintained for some generations in the laboratory, although we cannot rule out the genetic drift, natural selection is suggested as the



**Figure 1.** Two images at 400x of the whole karyotype of *D. sukuzii* polytene chromosomes. Each chromosomal arm is indicated using the equivalent name of *D. melanogaster*. Numbers indicate particular segments that look similar to those from *D. melanogaster*. (a) The pattern of bands in the centromere and the telomere tips can be clearly identifiable. (b) A better extension of chromosomes allows identifying more segments.



**Figure 2.** Four pictures at 400x of heterokaryotypic individuals from the Font Gropa population of *D. sukuzii* with the inversion located in the 3R chromosome. C and T indicate the centromere and the telomere tips, respectively. The number 89 identifies a characteristic segment of the 3R chromosome. Arrows show the breakpoints of the chromosomal inversions: (a) it is possible to observe the band pattern of both breakpoints. (b) The pattern of bands of the whole inversion can be easily recognized. (c) A general view of the inversion with an excellent visualization of the bands inside it. (d) The pattern of bands outside both breakpoints is clearly visible and could be useful to properly identify this inversion.

most likely evolutionary factor to explain the persistence of the observed inversion. For instance, in the well documented colonization of America (North and South) by *D. subobscura*, several inversions were included in the original small sample of colonizers. They have persisted over time due to their adaptive values (Prevosti et al. 1988), even if they were associated with lethal genes (Mestres et al. 2001). To properly understand the adaptation to new environments of the invasive pest species *D. suzukii*, it would be essential to analyse in depth the chromosomal inversions of this species. This is a preliminary research, but this topic could be very valuable to understand the expansion potential of the species and to design new strategies to control it.

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