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ORIGINAL ARTICLE

The chromosomes of *Drosophila suzukii* (Diptera: Drosophilidae): detailed photographic polytene chromosomal maps and in situ hybridization data

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Abstract

The spotted wing drosophila, *D. suzukii*, is a serious agricultural pest attacking a variety of soft fruits and vegetables. Although originating from East Asia it has recently invaded America and Europe raising major concern about its expansion potential and the consequent economic losses. Since cytogenetic information on the species is scarce, we report here the mitotic karyotype and detailed photographic maps of the salivary gland polytene chromosomes of *D. suzukii*. The mitotic metaphase complement contains three pairs of autosomes, one of which is dot-like, and one pair of heteromorphic (XX/XY) sex chromosomes. The salivary gland polytene complement consists of five long polytene arms, representing the two metacentric autosomes and the acrocentric X chromosome, and one very short polytene element, which corresponds to the dot-like autosome. Banding pattern as well as the most characteristic features and prominent landmarks of each polytene chromosome arm are presented and discussed. Furthermore, twelve gene markers have been mapped on the polytene chromosomes of *D. suzukii* by in situ hybridization. Their distribution pattern was found quite similar to that of *D. melanogaster* revealing conservation of synteny although the relative position within each chromosome arm for most of the genes differed significantly between *D. suzukii* and *D. melanogaster*. The chromosome information presented here is suitable for comparative cytogenetic studies and phylogenetic exploration, while it could also facilitate the assembly of the genome sequence and support the development of genetic tools for species-specific and environment-friendly biological control applications such as the sterile insect technique.

Keywords Spotted wing drosophila · Agricultural pest · Molecular markers · Gene mapping · Chromosome organization · Genome evolution

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Introduction

Drosophila suzukii (Diptera: Drosophilidae), also known as the spotted wing drosophila, was first described by Matsumura in Japan (Matsumura 1931). It belongs to the *suzukii* subgroup and is closely related to *D. melanogaster* and other *Drosophila* species of the *melanogaster* species group (Yang et al. 2004, 2012). The species is considered native to Southeast Asia. However, it was reported in California and Spain in 2008 (Hauser 2011; Calabria et al. 2012) and has been expanding across America (north and south) and Europe ever since (Hauser 2011; Burrack et al. 2012; Cini et al. 2012; Deprá et al. 2014; Asplen et al. 2015), proving to be highly invasive. The rapid expansion of *D. suzukii* in a wide temperature and climate range has raised serious concern since it is one of the very few

Drosophila species that infests healthy, marketable fruits. Females of *D. suzukii* possess a non-typical serrated ovipositor that allows them to pierce and lay eggs inside ripening fruits (Atallah et al. 2014). The developing larvae feed on the fruit pulp, while the wounds on the fruit skin serve as entrance to bacterial and fungal pathogens, both decreasing fruit quality and value (Walsh et al. 2011; Cini et al. 2012; Hamby et al. 2012; Ioriatti et al. 2015; Asplen et al. 2015). *D. suzukii* is a polyphagous pest known to infest a variety of berries (blueberries, blackberries, raspberries, and strawberries), and other soft skin fruits as well, such as cherries, apricots, peaches, pears, grapes, and kiwis (Grassi et al. 2011; Lee et al. 2011; Walsh et al. 2011; Bellamy et al. 2013; Haye et al. 2016). This insect is at present causing substantial crop losses and economical damage in USA and Europe (Bolda et al. 2010; Goodhue et al. 2011; Walsh et al. 2011; Cini et al. 2012), while the danger of expanding to new territories in the future renders it a major insect pest threat worldwide. Therefore, there is an intensive need for effective management and population control of the species. Innovative biological control methods such as the Sterile Insect Technique (SIT) could be a promising practice for the efficient management of *D. suzukii*, as it has been successfully implemented at a large scale for other insect pests, requiring, nevertheless, extensive knowledge on the biology of the insect (Dyck et al. 2005).

Cytogenetic analysis can provide significant information on the genetics and the genomic organization of insect species. Dipteran polytene chromosomes are extremely useful for studying chromosome structure and function, as well as temporal gene activities (Zhimulev et al. 2004 and references therein). Their characteristic species-specific banding pattern is used to assess phylogenetic relationships among closely related species (Krimbas and Powell 1992; Zhao et al. 1998; Gariou-Papalexiou et al. 2007, 2016; Drosopoulou et al. 2011a, b, 2017; Zacharopoulou et al. 2011a, b, 2017; Mavragani-Tsipidou et al. 2014; Augustinos et al. 2015) or even to distinguish among members of species complexes (Lemeunier and Ashburner 1984; Mavragani-Tsipidou et al. 1992; Coluzzi et al. 2002; Gariou-Papalexiou et al. 2007; Caceres et al. 2009; Augustinos et al. 2014). They also enable the construction of detailed physical genetic maps through in situ hybridization and precise localization of genomic sequences, revealing homologies of chromosomes or chromosome segments even among more distantly related species (Zacharopoulou et al. 1992, 2017; Drosopoulou et al. 1997, 2015, 2017; Zhao et al. 1998; Zambetaki et al. 1999; Gariou-Papalexiou et al. 2002; Holt et al. 2002; Mavragani-Tsipidou 2002; Campos et al. 2007; Sharakhova et al. 2007; Tsoumani et al. 2011; Stocker et al. 2012; Garcia et al. 2015). Furthermore, cytogenetic analysis has supported the development, characterization, and improvement of genetic sexing strains (GSSs) used in effective SIT control of important pest species (Zacharopoulou and Franz 2013; Zacharopoulou et al. 2017).

In the current study, we present the mitotic karyotype and detailed photographic polytene chromosome maps of the spotted wing drosophila, *D. suzukii*. We also compare the chromosome organization of the above species to *D. melanogaster* based on in situ hybridization data. Our data are expected to contribute to the assembly of the *D. suzukii* genome and to the better understanding of the species phylogenetic relationships within the melanogaster subgroup. Moreover, it could prove useful for the construction and characterization of GSSs in support to the efforts for the development and application of effective SIT for the population control of this major agricultural pest.

Materials and methods

Flies

Third instar larvae of *D. suzukii* used in the present study came from a laboratory colony maintained at the Joint FAO/IAEA Insect Pest Control Laboratory (IPCL), Seibersdorf, Austria. The IPCL colony was established in 2014 using pupae originated from the Agricultural Entomology Unit of the Edmund Mach Foundation in San Michele All'Adige, Trento Province, Italy. Larvae and adult flies of *D. melanogaster*, strain Canton-S, maintained at the Department of Genetics, Development and Molecular Biology of Aristotle University of Thessaloniki, were also used.

Mitotic chromosome preparations

Mitotic chromosome preparations were made from nerve ganglia of third instar larvae, following the air-drying technique without the use of colchicine described by Mavragani-Tsipidou et al. (2014). Brain tissue was dissected in Ringer's solution and transferred to a well slide containing 1% sodium citrate hypotonic solution for at least 15 min. Subsequently, it was transferred to fresh methanol/acetic acid 3:1 fixative solution which was replaced with new solution every minute to ensure the complete removal of the water. After 3 min, the fixative solution was removed, and 60% acetic acid was added. The material was pipetted through a thin micropipette tip several times for dispersal and dried on a clean slide placed on a pre-warmed hot plate (40–45 °C). Chromosomes were stained with 5% Giemsa in 10 mM phosphate buffer, pH 6.8. More than 20 chromosome preparations, each from an individual larva, were analyzed and about 50 well-spread metaphases were photographed using a phase contrast microscope (LEIKA DMR) and a

CCD camera (ProgResCFcool; Jenoptik Jena Optical Systems, Jena, Germany).

Polytene chromosome preparations

Polytene chromosome preparations were made from salivary glands of well-fed third instar larvae following the procedure described by Mavragani-Tsipidou et al. (2014), with some modifications. Larvae were dissected in Ringer's solution and the glands were transferred to 45% acetic acid for 2-3 min where the adhering fat body was removed. Tissue was transferred to 3 M HCl for 1 min and to lacto-acetic acid (glacial acetic acid:water:lactic acid in 3:2:1 ratio) for about 5 min before staining in lacto-aceto-orcein for 5–7 min. Excess stain was removed by washing the material in a drop of lacto-acetic acid before squashing.

Construction of photographic chromosome maps

Chromosome slides were observed with 60 × and 100 × objectives on a phase contrast microscope (LEIKA DMR) and at least 100 well-spread nuclei or isolated chromosomes were photographed using a CCD camera (ProgResCFcool; Jenoptik Jena Optical Systems, Jena, Germany). Selected chromosomal regions, providing a clear banding pattern and demonstrating the continuity of each polytene element, were assembled using the Adobe Photoshop CS6 Extended Software, to construct the composite photographic map for each chromosomal element.

In situ hybridization procedures

Polytene chromosome preparations for in situ hybridization were made from salivary glands of late third instar larvae following the protocol described by Pardue (1986) with some modifications. Larvae were dissected in Ringer's solution and the glands were transferred to 45% acetic acid for 2–3 min where the adhering fat body was removed. Then the material was transferred into a small volume (about 15 µl) of lacto-acetic acid on an 18X18 coverslip for about 5 min. The preparation was squashed after a slide had been laid on the coverslip and turned over. The slide was placed horizontally at − 20 °C for 24 h and the coverslip was removed by a razor blade after the preparation was dipped in liquid nitrogen. Slides were dehydrated in 95% ethanol and stored at room temperature (RT).

Six of the gene probes used for in situ hybridization represented genomic or cDNA fragments previously cloned from *D. melanogaster* or *D. auraria,* while the remaining six were generated in the present study (Table 1). Primers (Table 2) were designed on selected gene sequences of *D. melanogaster* and *D. suzukii* genomes available in FlyBase, release FB2018_04 (Thurmond et al. 2019) and SpottedWingFlyBase, v 1.0 (Chiu et al. 2013), respectively. PCR amplifications on *D. melanogaster* DNA were performed using BIOTAQ DNA Polymerase (BIOLINE, UK). Amplification products, after purification with Exonuclease I and Shrimp Alkaline Phosphatase (NEB, USA), were cloned using the QIAGEN PCR cloning kit (QIAGEN, Germany) and sequenced by Eurofins Genomics (Germany). All sequences were confirmed by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Labeling and detection was performed using the DIG-DNA labeling and Detection kit (ROCHE, Germany). Hybridization was performed at 62–65 °C as previously described (Drosopoulou and Scouras 1995). Parallel hybridization of *D. melanogaster* polytene chromosome preparations was performed for each probe as positive control. Four to five preparations were hybridized with each probe, and at least ten well-spread nuclei per preparation were observed at 63 × or 100 × magnification with a Nikon Eclipse 80i or a Leica DMR phase contrast microscope. Photographs were captured using a Nikon DS-5M-U1 (63 ×) or a JenoptikProgRes (100 ×) CCD camera.

Results and discussion

Mitotic chromosomes

The mitotic karyotype of the *D. suzukii* strain analyzed consists of four pairs of chromosomes: (a) one pair of sex chromosomes, (b) two pairs of meta- or sub-metacentric autosomes and (c) one pair of dot-like autosomes (Fig. 1). The sex chromosomes are designated as the first pair of the mitotic karyotype, while the three autosomes are labeled from 2 to 4, in order of descending size. The first autosome pair (chromosome 2) has about twice the size of the second one (chromosome 3), while the third autosome (chromosome 4) is very small like a dot. The X chromosome is an acrocentric of medium size (about half the size of pair 3), and the Y is a short, rod-shaped chromosome that is heavily stained (Fig. 1).

The above-described mitotic complement is in agreement with previous descriptions of the *D. suzukii* mitotic karyotype (Lemeunier et al. 1986; Deng et al. 2007) and very similar to the karyotype of *D. melanogaster* and other species of the *melanogaster* species group (Lemeunier et al. 1986).

Polytene chromosomes

The analysis of the salivary gland polytene chromosomes of *D. suzukii* showed that the polytene complement consists of five long and one very short well-banded polytene elements (Fig. 2). The polytene chromosome arms were named X (the sex chromosome), 2L, 2R, 3L, 3R, and 4, based on the

Table 1 The hybridization probes used in the present study and their hybridization sites on the polytene chromosomes of *Drosophila suzukii and D. melanogaster*

Gene symbol[a]	Description	Species of origin	References	Hybridization site(s) in *D. suzukii*	Hybridization site(s) in *D. melanogaster*
w	PCR fragment of the *white* gene	*Drosophila melanogaster*	Present study	**13-X**	**3B-X**
Sxl	PCR fragment of the *sex lethal* gene	*Drosophila melanogaster*	Present study	**7-X**	**6F-X**
if	PCR fragment of the *inflated* gene (integrin alpha subunit)	*Drosophila melanogaster*	Present study	**16-X**	**15A-X**
CG17652	PCR fragment of a putative small ribosomal subunit rRNA binding protein	*Drosophila melanogaster*	Present study	**29-2L**	**22B-2L**
Adh	Genomic clone of the *alcohol dehydrogenase* gene	*Drosophila melanogaster*	Goldberg (1980)	**22-2L**	**35B-2L**
Opa1	PCR fragment of the *Optic atrophy 1* gene	*Drosophila melanogaster*	Present study	**53-2R**	**50E − 2R**
βtub56D	cDNA clone of the *β-tubulin* gene located at 56D	*Drosophila melanogaster*	Drosopoulou and Scouras (1995)	**50-2R** 49-2R[b]	**56D-2R** 60D-2R[b]
βTub60D	cDNA clone of the *β-tubulin* gene located at 60D	*Drosophila melanogaster*	Drosopoulou and Scouras (1995)	**49-2R** 50-2R[b]	**60D-2R** 56D-2R[b]
hsp83	cDNA clone of the *heat-shock 83* protein gene	*Drosophila auraria*	Konstantopoulou and Scouras (1998)	**70-3L**	**63B-3L**
Toll-9	PCR fragment of the *Toll-9* gene	*Drosophila melanogaster*	Present study	**71-3L**	**77B-3L**
aTub84D	Genomic clone of the *α-tubulin* gene located at 84D	*Drosophila melanogaster*	Kalfayan and Wensink (1981)	**84-3R** 87-3R[b]	**84D-3R** 85E-3R[b]
hsp70	cDNA clone of the *heat-shock 70* protein gene	*Drosophila auraria*	Konstantopoulou et al. (1995)	**86-3R** 92-3R[b] 94-3R[b]	**87A, 87B-3R** 95D-3R[b] 88E-3R[b]

[a]Gene symbols as in FlyBase, release FB2018_04

[b]Secondary hybridization signal

Table 2 The primer sequences, the annealing temperature and the amplicon size for the PCR-generated gene fragments used as probes in the present study

Gene	Primer sequence	Amplicon size (bp)	Annealing, *T* (°C)
w	Forward: 5′-AAGACGACCCTGCTGAATGC-3′	958	58
	Reverse: 5′-CGCACTTTTACGAGGAGTGG-3′		
Sxl	Forward: 5′-CCTATTCAGAGCCATTGGACC-3′	994	56
	Reverse: 5′-CCATCTGCGACATAAAGTGGG-3′		
if	Forward: 5′-CGAGGAGAAGCGTTGGACG-3′	1064	50
	Reverse: 5′-GTGACCATGTAGAAGGCAGC-3′		
CG17652	Forward: 5′-GAAGTCGCACAAAACTCTGG-3′	957	52
	Reverse: 5′-GCATATTACACTCAGGTGGC-3′		
Opa1	Forward:5′-GCTGGAGAAGATGGAGAAGG-3′	1036	56
	Reverse: 5′-CCTGATGGGGCATGATAACC-3′		
Toll-9	Forward: 5′-CGAGTACGTGAGCAAGGAGG-3′	1000	56
	Reverse: 5′-CGGAGCACTTGGCTACCATG-3′		

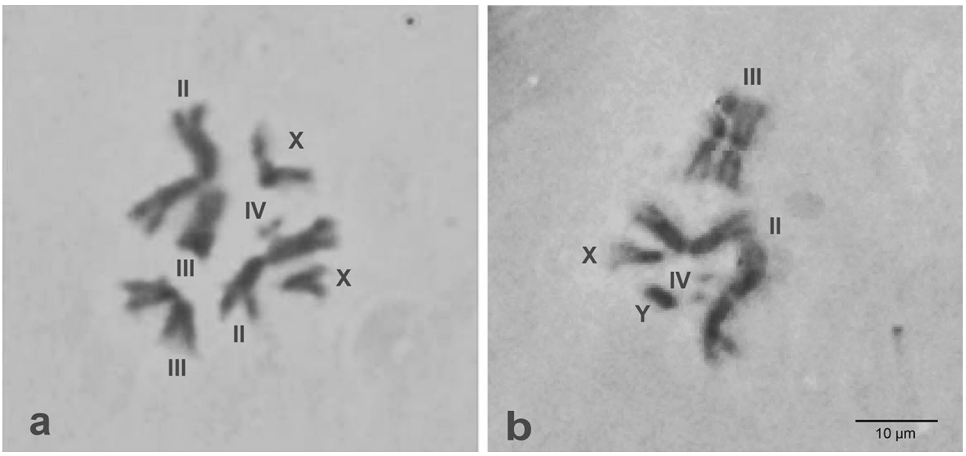

Fig. 1 Mitotic karyotype of *Drosophila suzukii.* **a** Female karyotype. **b** Male karyotype. The autosome pairs II, III and IV and the X and Y chromosomes are indicated. Chromosomes were stained with Giemsa

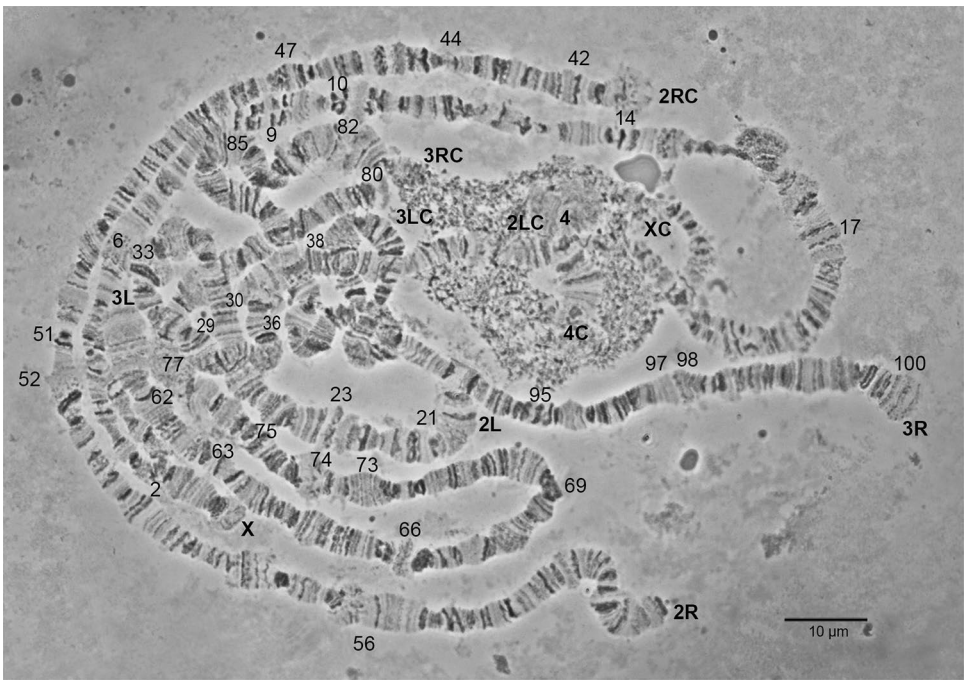

Fig. 2 Polytene nucleus of *Drosophila suzukii.* The telomeres (X, 2L, 2R, 3L, 3R, 4) and the centromeres (XC, 2LC, 2RC, 3LC, 3RC, 4C) of each polytene arm are indicated. Numbers indicate the sections of the characteristic landmarks described in the text

similarities of the telomeric regions and the chromosomal banding pattern to *D. melanogaster* and were divided into sections from 1 to 102. The detailed photographic maps of the polytene chromosomes of *D. suzukii* third instar larvae are shown in Fig. 3. A short description of the most prominent diagnostic landmarks for each element is given below.

X chromosome (sections 1–20, Fig. 3)

In the polytene complement of *D. suzukii*, the X chromosome is represented by one long polytene arm with a very characteristic torus-shaped tip and an easily recognizable proximal end. Prominent landmarks of this polytene arm are the three intense bands in section 2, the puffed structure in section 6, the dense banding pattern in section 9, the two bands in section 10 surrounded by two puffs, the three bands in section 14 followed by three puffs at sections 14–16 and the two zones at section 17.

2L chromosome arm (sections 21–40, Fig. 3)

The tip of this chromosome arm is followed by two dark bands separated by a bright interband region (section 21). Section 23 has a characteristic banding pattern. Other landmarks are the dense banding pattern in sections 29–30, the puff at section 33, the series of bands at section 36 as well as at the beginning of section 38.

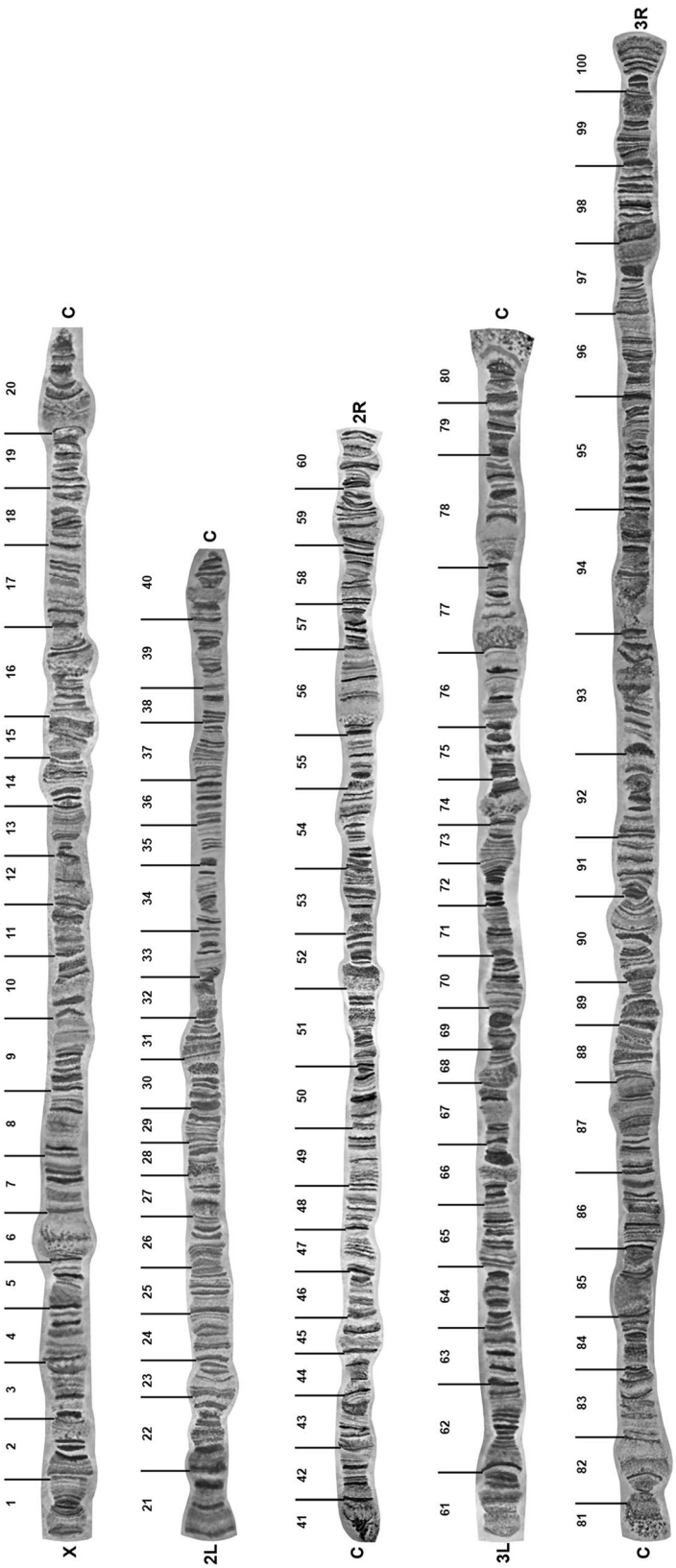

Fig. 3 Photographic polytene chromosome map of *Drosophila suzukii*. C indicates the centromere

2R chromosome arm (sections 41–60, Fig. 3)

The 2R chromosome arm is easily identified by its characteristic tip. Prominent landmarks of this chromosome are section 56 with poor banding pattern followed by two intense bands and the puff at section 52 also preceding two intense and clear bands in section 51. Section 47 consists of thin bands followed by a diffused area and two dark bands (the second belongs to section 46). A prominent landmark is the thin area of section 44.

3L chromosome arm (sections 61–80, Fig. 3)

Apart from its characteristic tip, the 3L polytene arm is identified by the presence of a very prominent band at the beginning of section 62, a series of three dark bands at section 63, the section 66 with two sharp bands followed by a puff and a very thick band, another thick band at section 69, the section 73 followed by the puff at section 74, the series of tree bands at 75, the puff at 77 and the fan-shaped proximal end (80).

3R chromosome arm (sections 81–100, Fig. 3)

This is the longest polytene element of the complement. It is recognized not only by its tip but also by its characteristic slightly puffed proximal end (82). The region at the borders of sections 98 and 97 is easily recognized. Other landmarks of this arm are the series of seven intense bands in section 95 and the characteristic structure of section 85. The chromosome was often found broken or stretched between sections 93 and 90.

Chromosome 4 (sections 101–102, Fig. 3)

Chromosome 4 forms a very short polytenized element tightly joined with the heterochromatic mass of the chromocenter.

Chromosome localization of molecular markers

Twelve gene markers selected to represent all five long polytene elements of *D. melanogaster* were localized on the polytene chromosomes of *D. suzukii* by in situ hybridization (Table 1). Eight of the probes gave unique hybridization signals. In detail, the *Sxl*, *w,* and *if* genes were mapped on the X chromosome of *D. suzukii* at sections 7, 13 and 16, respectively (Fig. 4); the *Adh* and the *CG17652* probes were localized at sections 22 and 29 of the 2L chromosome arm, respectively (Fig. 5a, b); *Opa1* mapped at section 53 of the 2R arm (Fig. 5e) and the *hsp83* and *Toll-9* markers were localized on 3L chromosome arm at sections 70 and 71, respectively (Fig. 6a, b). For *βtub 56D, βtub60D, aTub*

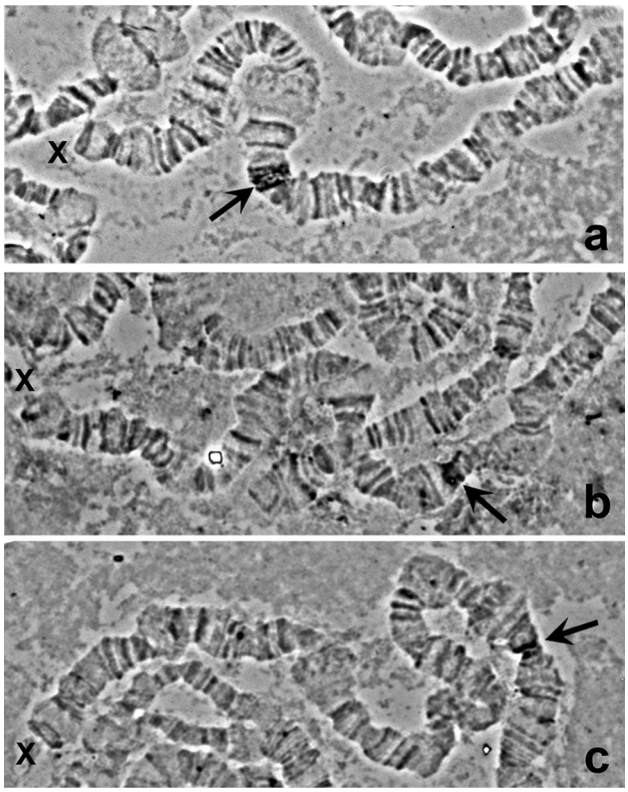

Fig. 4 In situ hybridization on the X polytene chromosome of *Drosophila suzukii.* **a** Hybridization site of the *Sxl* probe; **b** hybridization site of the *w* probe; **c** hybridization site of the *if* probe. Arrows indicate the hybridization signals

84D and *hsp70* probes, which represent members of the *β-tubulin*, *α-tubulin* and *Hsp70* multigene families, respectively, apart from the main hybridization signals, additional secondary signals presenting lower frequency and intensity were observed both in *D. suzukii* and *D. melanogaster.* Specifically, the main hybridization signals for *βtub60D* and *βTub56D* were identified at sections 49 and 50 of the *D. suzukii* 2R polytene arm, respectively (Fig. 5c, d), while both probes presented weaker hybridization at each other's main hybridization sites in several nuclei of *D. suzukii* (Fig. 5d) similar to *D. melanogaster*. The *αTub84D* probe gave a main hybridization signal at section 84 and a secondary signal at section 87 of the *D. suzukii* 3R chromosome arm (Fig. 6c). This pattern was similar to the one observed in *D. melanogaster*, where the main signal was identified at the locus of the *αTub84D* gene and a secondary one at the locus of the *aTub85E* gene, suggesting that the hybridization sites at 84 and 87 in *D. suzukii* is where the putative orthologues of the *αTub84D and aTub85E* genes are located, respectively (Fig. 7). Similarly, the *hsp70* probe hybridized mainly at section 86 but also gave secondary signals in sections 92 and 94 of the *D. suzukii* 3R polytene arm (Fig. 6d). Comparing the above hybridization pattern (number, relative

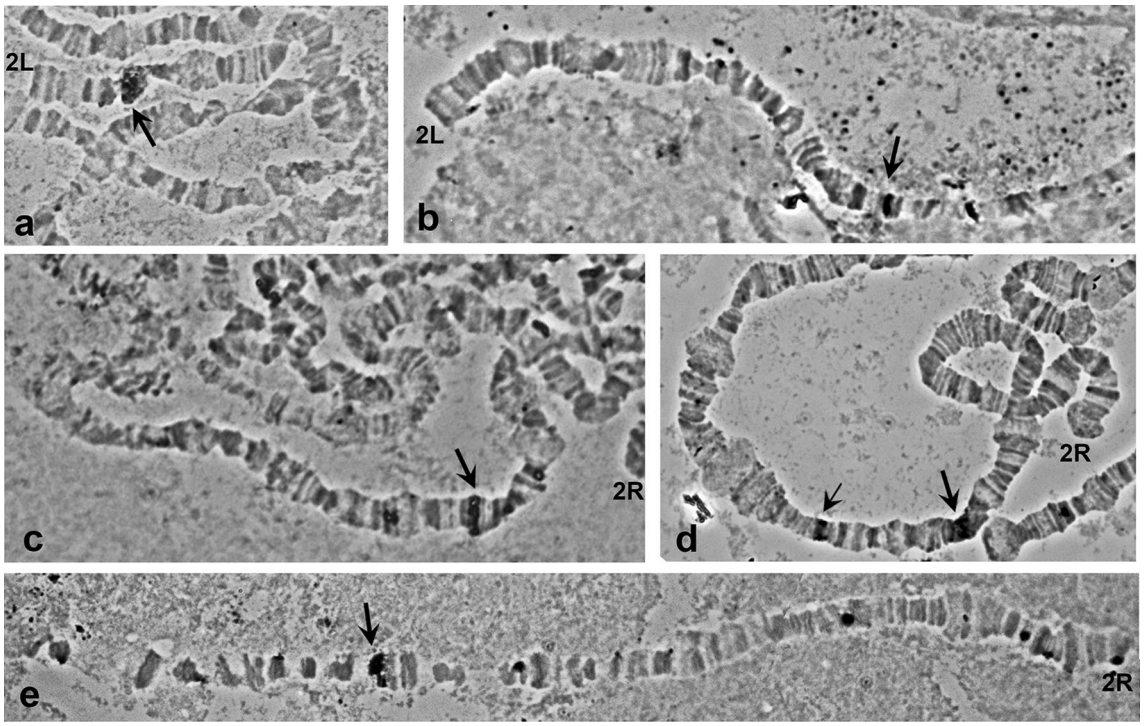

Fig. 5 In situ hybridization on polytene chromosome 2 of *Drosophila suzukii.* **a** Hybridization site of the *Adh* probe; **b** hybridization site of the *CG17652* probe; **c** hybridization site of the *βtub60D* probe; **d** hybridization site of the *βtub56D* probe; **e** hybridization site of the *Opa1* probe. Thick arrows indicate the main hybridization signals. Thin arrow on **d** indicates secondary hybridization

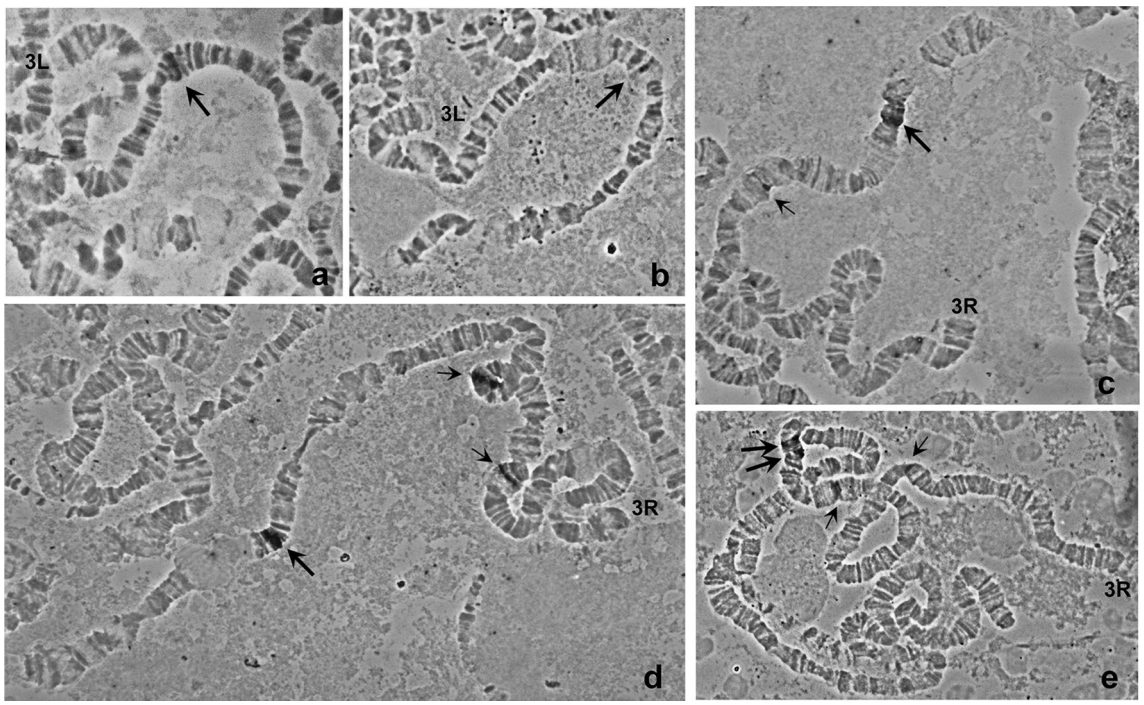

Fig. 6 In situ hybridization on polytene chromosome 3 of *Drosophila suzukii* (**a**–**d**) and *Drosophila melanogaster* (**e**). **a** Hybridization site of the *hsp83* probe; **b** hybridization site of the *Toll-9* probe; **c** hybridization sites of the *aTub84D* probe; **d**, **e** hybridization sites of the *hsp70* probe. Thick arrows indicate the main hybridization signals. Thin arrows on **c**–**e** indicate secondary hybridization

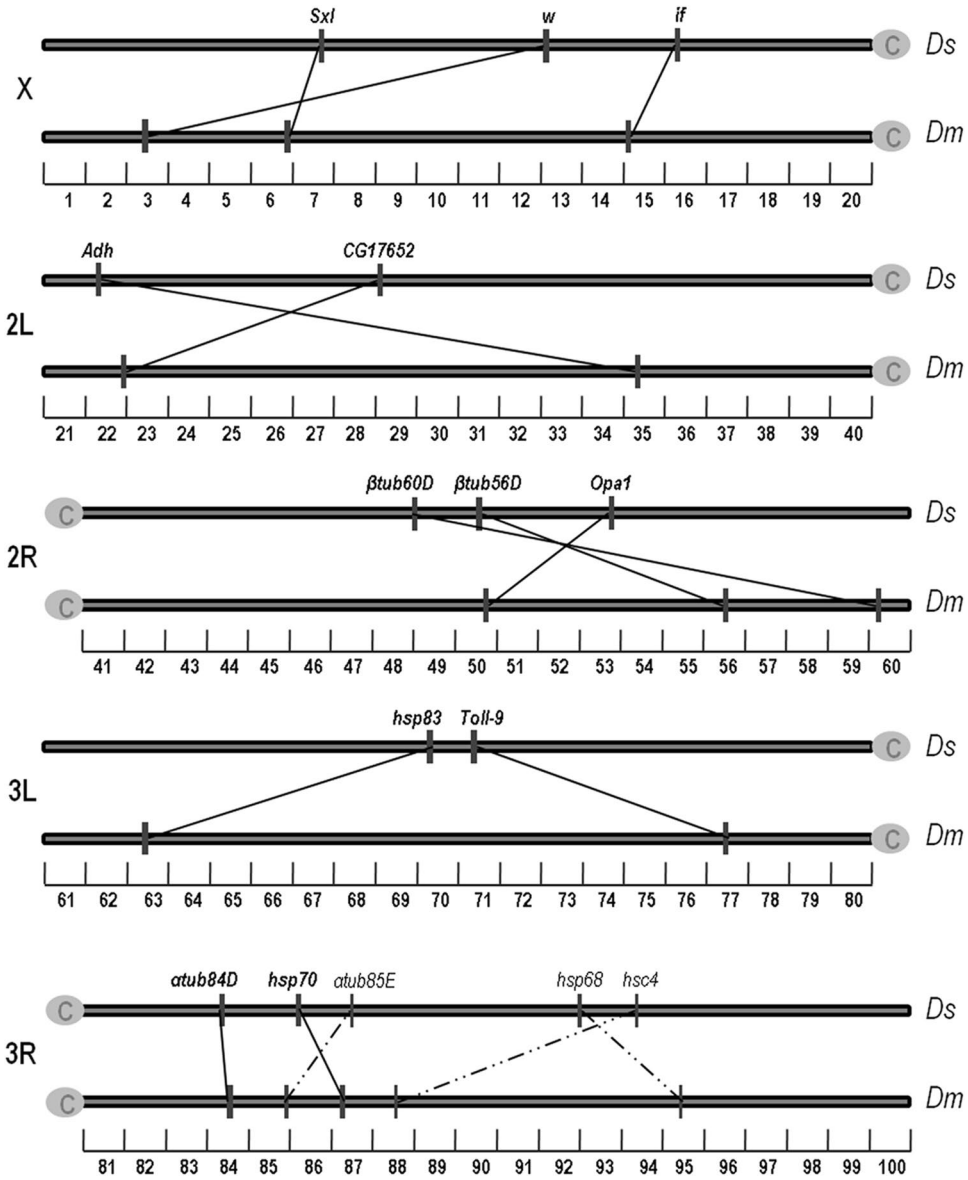

Fig. 7 Schematic comparative representation of *Drosophila suzukii* (Ds) and *Drosophila melanogaster* (Dm) polytene chromosomes. Solid lines link the relative positions of the orthologue genes revealed by main hybridization signals. Dashed lines link the relative positions of putative orthologue genes revealed by secondary hybridization. C in grey circles indicates the centromeres

intensity and frequency of the hybridization signals) with the one observed in *D. melanogaster*, i.e. main signal at 87A and 87B at the loci of the *hsp70* genes and secondary hybridization at the loci of the *hsp68* and the *hsc70-4* genes (sections 95D and 88E, respectively; Fig. 6e), it could be suggested that the hybridization sites in sections 86, 92 and 94 in *D. suzukii* indicate the location of the *hsp70*, *hsp68* and the *hsc70-4* putative orthologues in this species, respectively (Fig. 7).

The distribution of the gene loci on the chromosomes of *D. suzukii* is very similar to that of *D. melanogaster* as diagrammatically shown in Fig. 7. The same set of genes appears to be linked on the respective chromosome arms suggesting conservation of chromosomal gene content between the two species. The above is also supported by the *D. suzukii* genome assembly (Chiu et al. 2013). Chromosomal arm-level synteny has been previously shown by physical mapping and genome sequencing among numerous closely or distantly related *Drosophila* species (Drosopoulou and Scouras 1995, 1998; Pardali et al. 1996; Drosopoulou et al. 1996, 1997, 2002; Clark et al. 2007; Bhutkar et al. 2008; Schaeffer et al. 2008; Stocker et al. 2012) proving true Muller's hypothesis that during *Drosophila* evolution the six chromosomal elements A–F, maintained their structure and identity (Sturtevant and Novitski 1941). Similarly, cytogenetic and genomic studies outside the *Drosophila* genus were able to reveal synteny of genetic loci and correspondence of chromosome elements among species of different dipteran families (Foster et al. 1981; Zacharopoulou et al. 1992, 2017; Zhao et al. 1998; Zambetaki et al. 1999; Gariou-Papalexiou et al. 2002; Mavragani-Tsipidou 2002; Campos et al. 2007; Tsoumani et al. 2011; Drosopoulou et al. 2015,

2017; Sved et al. 2016), suggesting that the overall organization and content of chromosome elements has been conserved throughout Schizophora evolution (Sved et al. 2016).

However, within each chromosome arm the relative positions of the majority of the gene loci mapped are significantly different between *D. suzukii* and *D. melanogaster* (Fig. 7). This is not surprising since 58 out of the 160 synteny blocks identified by the *D. suzukii* genome assembly presented inverted direction between the two species (Chiu et al. 2013). Extensive reshuffling of genes within chromosome arms has been also revealed from comparisons among a number of *Drosophila* (Drosopoulou and Scouras 1995, 1998; Pardali et al. 1996; Drosopoulou et al. 1996, 1997, 2002; Clark et al. 2007; Bhutkar et al. 2008; Schaeffer et al. 2008; Stocker et al. 2012) and non drosophilid species (Foster et al. 1981; Zacharopoulou et al. 1992, 2017; Zhao et al. 1998; Zambetaki et al. 1999; Gariou-Papalexiou et al. 2002; Mavragani-Tsipidou 2002; Campos et al. 2007; Tsoumani et al. 2011; Drosopoulou et al. 2015, 2017; Sved et al. 2016). The above observations support that, unlike inter chromosomal arm rearrangements, intra-chromosomal events, such as within arm inversions, have been a common phenomena playing an important role during Diptera evolution (Ashburner et al. 1982; Ashburner 1989; Krimbas and Powell 1992; Rieseberg 2001; Schaeffer et al.2008; Stocker et al. 2012; Lee et al. 2013; Sharakhov et al. 2016; Sved et al. 2016; Zacharopoulou et al. 2017).

In summary, the first high-quality polytene chromosome maps for *D. suzukii* presented here could be used in comparative cytogenetic studies providing information on the phylogenetic status of the species within the *melanogaster* species group, while they enable the physical mapping of additional gene markers that should prove particularly useful for the assignment of scaffolds to chromosomal loci and the assembly of the genome sequence. Furthermore, linking cytogenetic with molecular knowledge could also assist the development and characterization of stable GSSs for their potential use in SIT applications against this destructive pest.

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