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Polytene Chromosome Analysis of *Bactrocera carambolae* (Diptera: Tephritidae)

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Abstract: The present investigation constitutes a first effort to study the polytene chromosomes of *Bactrocera carambolae* Drew and Hancock (Diptera: Tephritidae). It is a serious pest of the *Bactrocera dorsalis* complex group, infesting various types of fruits and vegetables in Southeast Asia, Australia and the Pacific. The aim of this study was to determine and analyse each arm of the salivary gland polytene chromosomes of this species individually. The tips, distinguishing characteristics as well as significant landmarks are recognized in each chromosome arm. Photographic illustrations of the chromosomes is presented and discussed. The information can be used for comparative studies among species of the tephritid genera which facilitate the development of novel control methods.

Keywords: Agricultural pest, carambola fruit fly, characteristic landmarks, identifying tips, salivary gland, tephritidae

INTRODUCTION

Fruit flies are among the world's most serious pests causing enormous yield losses. Tephritid flies of the genus *Bactrocera* are of particular concern throughout Asia and Australia. There are about 500 described species of *Bactrocera* that are grouped into 28 subgenera (Drew and Hancock, 2000; Kim *et al*., 1999; Asokan *et al*., 2011). The majority attack the seed bearing organs of plants, with many species inflicting significant economic losses to commercial agriculture through fruit destruction (White, 1996). Under this genus, the *Bactrocera dorsalis* complex group contains 75 described species which are largely endemic to Southeast Asia. Within this complex, a small number of polyphagous pests are of international significance, including *B. dorsalis s.s.*, *B. papayae*, *B. carambolae* and *B. philippinensis* (Drew and Hancock, 1994; Clarke *et al*., 2005).

The carambola fruit fly, *Bactrocera carambolae* has been recorded on more than 151 kinds of fruits and vegetables including cashew (*Anacardium occidentale*), mango (*Mangifera indica*), sugar palm (*Arenga pinnata*), avocado *(Persea americana*), breadfruit, jackfruit (*Artocarpus heterophyllus*), guava (*Psidium guajava*), lemon, grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), orange (*Citrus sinensis*), tomato, sapodilla (*Manilkara zapota*), West-Indian cherry (*Malpighia punicifolia*), tropical almond (*Terminalia cattapa*) and chilli pepper. It is a serious pest of carambola (*Averrhoa carambola* L. Family: Oxalidaceae), which can be attacked while the fruit is still very young (Van Sauers-Muller, 1991). The fruit fly infestations reduce production of host crops, increase the use of insecticides in the systems and often have other secondary effects such as toxicity on worker exposed to agrochemicals, food safety issues etc. (Jeyasankar, 2009). Moreover, the invasive movement of certain species within the *B. dorsalis* complex, especially the spread of *B. carambolae* into South America during the mid 1970s (Van Sauers-Muller, 1991) renders this group a truly global problem (Schutze *et al*., 2011).

Considering the economic importance as an insect pest, it is necessary to know the genetic and cytogenetic information aiming at control measures of this species. However, little work has been done on the cytotaxonomy of *B. carambolae* by Baimai *et al*. (1999), focusing on amounts and distribution of constitutive heterochromatin in sex chromosomes and autosomes. But, detailed information on polytene chromosomes of this important species is unavailable. Dipteran polytene chromosomes provide an excellent model for understanding in species complexes, as well as for structural and functional cytogenetics (Campos *et al*., 2003).

Previously, we have reported the mitotic metaphase karyotype from larval neural ganglion and polytene nuclei from larval salivary gland of *B. carambolae* (Yesmin and Clyde, 2012a, b). The karyotypic complements consist of five autosomal pairs and one

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pair of sex chromosomes (females XX/males XY). The investigation on polytene nuclei revealed that the species has five long polytene chromosomes, i.e., ten polytene arms. Sex chromosomes were not found in polytene chromosomes which indicate that five polytene chromosomes are corresponding to the five autosomes of the mitotic nuclei. As a continuation of the study, in this study, we have described the five banded salivary gland polytene chromosomes of this species as an entity. Hence, the objective is to determine and analyze each polytene chromosome arm of *B. carambolae* separately.

MATERIALS AND METHODS

Fly stock: The initial culture of *Bactrocera carambolae* were obtained from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia and reared in the school laboratory of Universiti Kebangsaan Malaysia (UKM), Bangi. The rearing laboratory was maintained at 25±1°C, 70±5% Relative Humidity (RH) and a 10 h light: 14 h dark cycle. The adult food consisted of yeast and sugar (1:3). Water was supplied as soaked cottons. Fresh star fruits were used for oviposition (2 days/week). Larvae were reared on star fruit medium.

The study was carried out in 2010 at the Cytogenetics Laboratory of the School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, UKM, Bangi, Malaysia.

Polytene chromosome preparations: Third-instar larvae (7-8 days old) of *Bactrocera carambolae* were used for the salivary gland polytene chromosome preparations following the method described by Shahjahan and Yesmin (2002) and Zacharopoulou *et al*. (2011a). Salivary glands of larvae were dissected in 45% acetic acid and the tissues were transferred to 3N HCl on a depression slide for 2-3 min. Then the tissues were fixed in a drop of glacial acetic acid: water: lactic acid (3:2:1) for about 5 min. After fixation, the tissues were stained with lacto-acetic-orcein for about 30 min. Excess stains were removed by washing the glands two or three times in a drop of lacto-acetic acid before squashing. Well spread chromosomes were examined and photographed with Soft Imaging System GmbH 5.0.1054 using an Olympus BX41 microscope with 40x magnification. Selected photographs, which showed the clear morphology, were used for the construction of composite chromosomes using Adobe Photoshop CS6.

RESULTS AND DISCUSSION

The salivary gland polytene chromosomes of *Bactrocera carambolae* were identified, numbered (2 to 6) and analyzed as it is commonly done for the polytene chromosomes of tephritid species. The numbering system indicates only the relative size of polytene chromosomes (Drosopoulou *et al*., 2010; Shahjahan and Yesmin, 2002; Zacharopoulou, 1987). According to the centromere position, the two arms of each polytene chromosome of *B. carambolae* are of unequal length. The centromere positions were determined using the criteria described by Bedo (1987), Zacharopoulou (1990) and Zhao *et al*. (1998):

- Regions that show heterochromatic character, such as darkly stained bands or bands with a disperse structure, are likely to be centromeric regions. Chromosomes 2 and 3 showed this characteristic. In chromosome 4, the centromere region is likely heterochromatic.
- Centromeric regions tend to be weak points that are easily broken during slide preparation. This was case for chromosome 5.
- The centromere often presents as a form of constriction. This was noted for chromosome 6. The longer part of each chromosome arm is assigned as Left arm (L) and the shorter part as Right arm (R). It was already reported that this species has five long banded chromosomes in the salivary gland polytene nuclei (Yesmin and Clyde, 2012a, b). Here, an account of each polytene chromosome arm with photographic illustration is described below.

Chromosome 2 (Fig. 1): The chromosome 2 is identifiable by its characteristic two tips at 2L (a) and 2R (e). Three well-known puffs (one in left arm at region b and two in right arm at region c) serve as indicative landmarks of chromosome 2. Distinctive look of region d is another trait of right arm. Several weak points are found along the length of the chromosome. Centromere region of the chromosome is connected with heterochromatic mass.

Chromosome 3 (Fig. 2): The left end of the chromosome 3 is characterized by dotted band at the tip. The presence of several darkly stained bands makes this end as a unique shape (3L, a) which was apparent during preparation. A prominent puff with clear internal structure at b and a series of bands at c, giving these regions a typical form. Banding patterns at region d make the right arm easily recognizable; two pairs of prominent double bands and one dotted band together with two lightly stained bands are unique in the right arm of chromosome 3. The composition of telomere region (e) helps to identify the right tip of this chromosome. Like chromosome 2, the centromeric region of this chromosome is also connected with heterochromatic mass.

Chromosome 4 (Fig. 3): This chromosome is easy to identify by its characteristic band pattern at regions 4L (a) and 4R (f). The tip of left arm is square shaped with alternate expression of dark and light bands. The

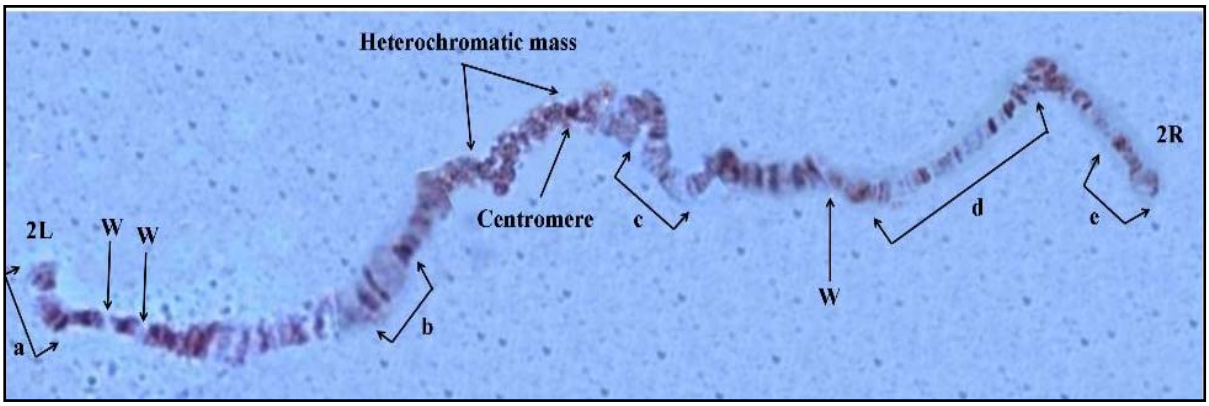

Fig. 1: Salivary gland polytene chromosome 2 of *Bactrocera carambolae*
L: Left arm; R: Right arm; W: Weak point; a-e: Most important distinguishing characteristics and landmarks

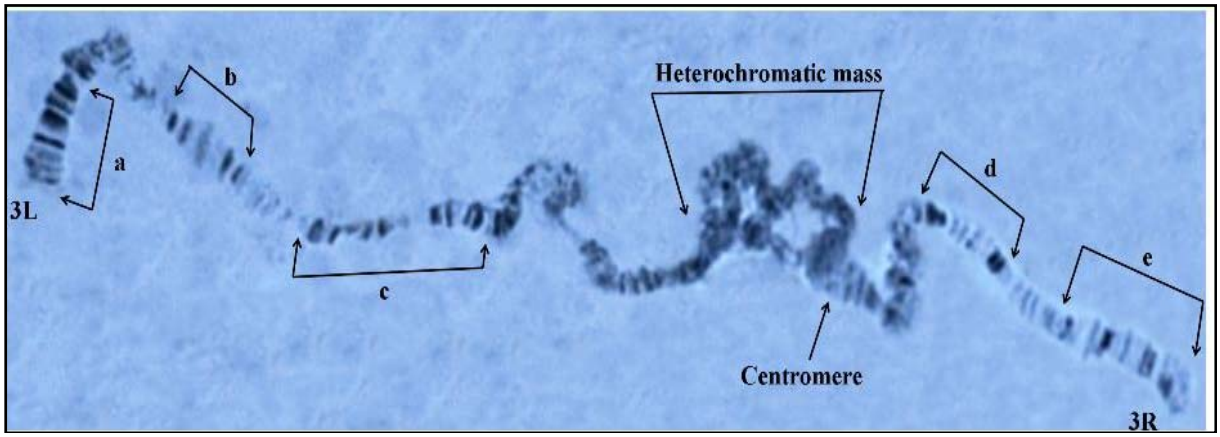

Fig. 2: Salivary gland polytene chromosome 3 of *Bactrocera carambolae*
L: Left arm; R: Right arm; a-e: Most important distinguishing characteristics and landmarks

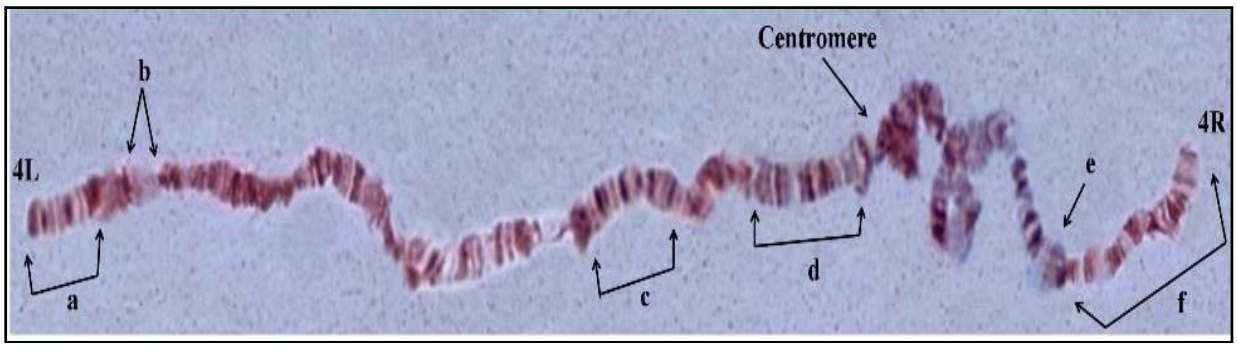

Fig. 3: Salivary gland polytene chromosome 4 of *Bactrocera carambolae*
L: Left arm; R: Right arm; a-f: Most important distinguishing characteristics and landmarks

expanded region at 4L (b), a characteristic puffed area showing composite structure at c and a group of clear bands at region d close to the centromere are the distinguishing landmarks of right arm of chromosome 4. The right end (region f) shows a unique morphology together with a conspicuous puff at region e of right arm. The centromeric region is characterized by an attenuated thread, by diffused heterochromatic bands that connect the two arms.

Chromosome 5 (Fig. 4): The left arm of chromosome 5 has regions with a distinctive tip (a) and a prominent puff (b) along with regions of poor banding pattern, whereas the right arm has the best banding pattern

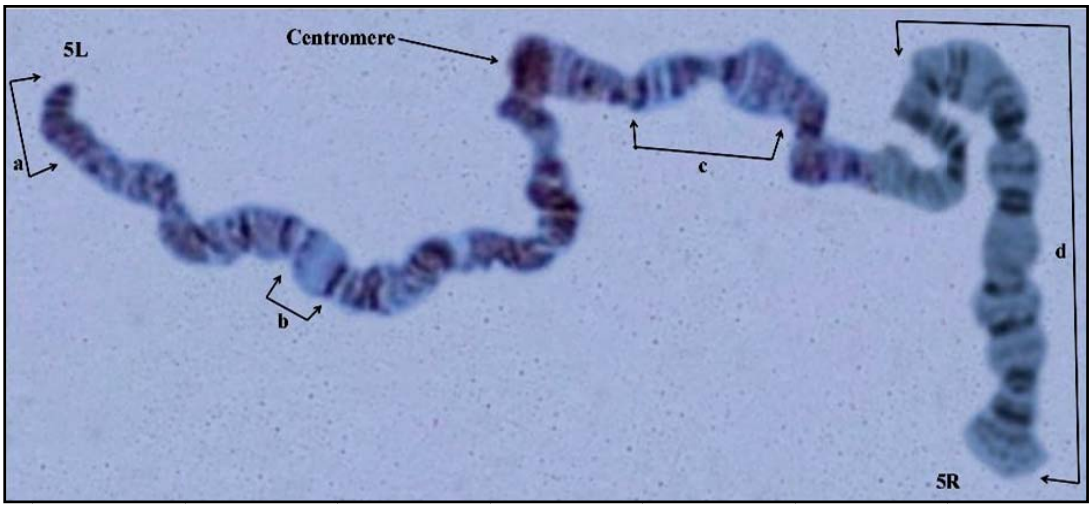

Fig. 4: Salivary gland polytene chromosome 5 of *Bactrocera carambolae*
L: Left arm; R: Right arm; a-c: Most important distinguishing characteristics and landmarks; d: Easily identifiable portion of right arm

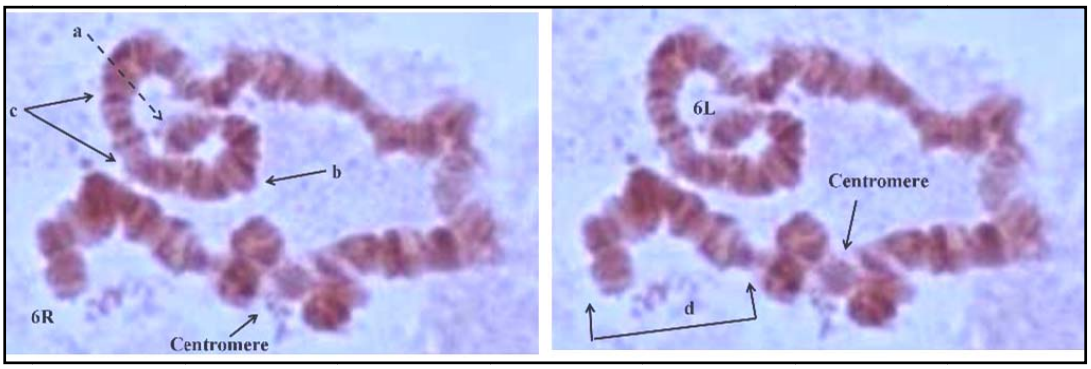

Fig. 5: Salivary gland polytene chromosome 6 of *Bactrocera carambolae*
L: Left arm; R: Right arm; a-d: Most important distinguishing characteristics and landmarks

(region d) of the polytene complement. The prominent region at c provides an additional aid for recognition of this arm. The centromere position is defined by a frequent break during slide preparation.

Chromosome 6 (Fig. 5): This chromosome is easily recognized by the characteristics free ends but it is difficult to follow the linear arrangement of band pattern along the chromosome length due to its folding or coiling nature. The left tip is swollen with a deeply stained band at region 6L (a) and followed by a prominent puff at region b, making this arm identifiable. Another key landmark is the particular structure at region c. Complex puffing morphology (region d) can aid the detection of right arm of chromosome 6. This telomere is characterized by different bulbs and swellings. Centromeric region is in the form of a constriction with a disperse nature.

The tephritid polytene chromosomes have been studied by many authors: the Med fly, *Ceratitis capitata* (Bedo, 1986, 1987; Zacharopoulou, 1987), the olive fruit fly, *Bactrocera oleae* (Zambetaki *et al*., 1995), the Queensland fruit fly, *Bactrocera tryoni* (Zhao *et al*., 1998), the melon fly, *Bactrocera cucurbitae* (Shahjahan *et al*., 2000; Shahjahan and Yesmin, 2002; Zacharopoulou *et al*., 2011a), *Rhagoletis cerasi* (Kounatidis *et al*., 2008), the Mexican fruit fly, *Anastrepha ludens* (Garcia-Martinez *et al*., 2009), the Walnut-Husk fly, *Rhagoletis completa* (Drosopoulou *et al*., 2010), the Oriental fruit fly, *Bactrocera dorsalis* (Zacharopoulou *et al*., 2011b), the Ethiopian fruit fly *Dacus ciliatus* (Drosopoulou *et al*., 2011), the American cherry fruit fly, *Rhagoletis cingulata* (Drosopoulou *et al*., 2012), *Bactrocera papayae* (Yesmin and Clyde, 2012c,d). In all of the above species, authors reported five long polytene chromosomes, i.e., ten polytene arms and non-polytenization of sex chromosomes in the polytene nuclei. This information is in full agreement with the present study on *B. carambolae*. Also, we have

confirmed that sex chromosomes were not found in the salivary gland polytene chromosomes of *B. carambolae*, i.e., the sex chromosomes are not polytenized (Yesmin and Clyde, 2012a, b). Mavragani-Tsipidou *et al*. (1992) observed an extra very short polytene element together with five long chromosomes in *Dacus oleae*. The authors described this element as sections 101-102, although they did not observe this extra element obviously in all polytene chromosomes of *D. oleae*.

The non-polytenized XX and XY chromosomes may be represented by the heterochromatic mass, observed in polytene chromosomes of *B. carambolae*, where the centromeric regions of chromosomes 2-4 are connected (Fig. 1 to 3). The correspondence of the heterochromatic mass of the polytene nuclei to the sex chromosomes of the mitotic complement has also been described for other Diptera: *Sarcophaga bulata* (Whitten, 1968), *Lucilia cuprina* (Bedo, 1982; Childress, 1969), *Lucillia cuprina dorsalis* (Foster *et al*., 1980), *Ceratitis capitata* (Bedo, 1987), *Dacus oleae* (Mavragani-Tsipidou *et al*., 1992), *Bactrocera cucurbitae* (Shahjahan and Yesmin, 2002), *Rhagoletis cerasi* (Kounatidis *et al*., 2008), *Rhagoletis completa* (Drosopoulou *et al*., 2010), *Dacus ciliatus* (Drosopoulou *et al*., 2011), *Rhagoletis cingulata* (Drosopoulou *et al*., 2012) and *Bactrocera papayae* (Yesmin and Clyde, 2012c).

In our ongoing project of the cytogenetics of fruit flies in Malaysia, the main purpose is to investigate the sibling species of the *Bactrocera dorsalis* complex in order to gain a better understanding of chromosomal characteristics of these important insect pests in the region. Results of the present study indicate that a detailed comparative analysis of the salivary gland polytene chromosomes in the genus *Bactrocera* may be of significant help in establishing phylogenetic relationships among different species. Additionally, the information will provide more insight into the problem of speciation in the *Bactrocera dorsalis* complex group which may lead to more effective and environmentally safe control practices of these agricultural pests.

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