CYTOLOGICAL CHARACTERISATION OF AUTOSOMES AND SEX CHROMOSOMES IN TEPHRITID FRUIT FLIES *BACTROCERA ZONATA* AND *ZEUGODACUS TAU* (DIPTERA, TEPHRITIDAE)

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Cytological Characterisation of Autosomes and Sex Chromosomes in Tephritid Fruit Flies *Bactrocera zonata* and *Zeugodacus tau* (Diptera, Tephritidae). Yesmin, F., Haymer, D., Uddin, M. N. & Hasanuzzaman, M. — Two fruit fly species, *Bactrocera zonata* (Saunders, 1842) and *Zeugodacus tau* (Walker, 1849) (Diptera, Tephritidae) were studied with regard to their autosomes and sex chromosomesw from mitotic metaphase spreads. Both species are native to South and South-East Asia and have major economic impacts in agriculture sectors of these areas. They are polyphagous, can easily adapt and spread. Both of them have the same diploid chromosome number of 2n = 12 (10 autosomes + heteromorphic sex chromosome pair XX/ XY). The male is heterogametic (XY) and the female is homogametic (XX). Chromosomes were classified into groups on the basis of centromere position and arranged in order of decreasing size. For *B. zonata*, chromosome formula is (3sm + 2m + Xm + Ydot-shape) and for *Z. tau*, the formula is (1sm + 4m + Xm + Ydot-shape). The results are analyzed and compared to chromosome studies done for other species of Tephritid fruit flies using chromosome morphometrics. Detailed images of mitotic chromosomes and measurements of various parameters relating to these chromosomes are provided. These cytological data are useful for systematics and contribute to a better understanding of the chromosomal evolution of these fruit fly species.

K e y   w o r d s: peach fruit fly, pumpkin fruit fly, metaphase spreads, chromosome morphology.

Introduction

Dipteran insects, particularly fruit flies (nearly 5,000 species in the family Tephritidae), are notable for their diversity within Southeast Asia. Approximately 800 species have been recognized and described from the Asia-Pacific-Australian and Oriental Regions alone (Drew, 1989; White & Evenhuis, 1999). Several of these species are considered to be major agricultural pests that pose enormous threats to the production of different fruits and vegetables throughout the world. Furthermore, increasing international trade has facilitated the risk of further infestation of these pests into new localities (Bonizzoni et al., 2001). Due to their invasiveness, fruit producing countries have had to adopt extensive quarantine treatments before export of their products (Vargas et al., 2008).

In South-East Asian region, fifteen species of *Bactrocera* and twelve species of *Zeugodacus* have been recorded in Bangladesh (Leblanc et al., 2019). Among these, the pumpkin fruit fly, *Zeugodacus tau* (Walker, 1849) is a serious pest of melon, cucumber, giant pumpkin, angled luffa, sapodilla, gourds and guava. Previously, this species had been known as *Bactrocera* (*Zeugodacus*) *tau*, but as the *Zeugodacus* was shown to be a separate monophyletic genus by Virgilio et et al. (2015), it has now been reclassified as *Zeugodacus tau*. As a pest, it is already widespread and economically important throughout South and Southeast Asia (Li et al., 2020). It is known to be distributed throughout India, Sri Lanka, Bhutan, Vietnam, Southern China, Taiwan, Thailand, Peninsular Malaysia, Singapore, East Malaysia, Brunei, Indonesia, Bangladesh (Drew & Romig, 2013; Leblanc et al., 2014). Estimates of harvest losses caused by *Z. tau* run as high as 40% (Hasyim et al., 2007) depending on climatic conditions and fruit species (Dillon et al., 2005).

The peach fruit fly, *Bactrocera zonata* (Saunders, 1842) is found in numerous other countries of tropical Asia including India, Bangladesh, Indonesia (Sumatra, Moluccas), Laos, Sri Lanka, Vietnam, Thailand, Burma, and Nepal (Duyck et al., 2004). This species causes severe damage to commercial crops such as mango, guava, peach, apple, fig, tomato, pepper, avocado and many other citrus fruits (Mosleh et al., 2011). Upon introduction into a new region, it is known to adapt and spread as a polyphagous pest with high reproductive potential (>300 eggs in a lifetime, Duyck et al., 2007) and high biotic potential (a number of generations of progeny per year). It is also known to be a strong flier and active whole year. Commercial impacts of *B. zonata* result mostly from the loss of export markets and the expensive requirements of quarantine constraints and eradication measures. Additionally, like other Tephritid pests, its establishment may have a severe effect on the environment following the application of chemical and/or biological control programs. *B. zonata* is of quarantine significance. For these and other reasons, countries within the EPPO (European and Mediterranean Plant Protection Organization) have it classified on the A1 List of Pests recommended for regulation as quarantine pests (EPPO 2020).

Considering their potential importance in agriculture sectors, it is necessary to know as much as possible about the biology of these species. Studies of chromosome makeup and evolution of these pest species can help in many areas such as identification and resolution of boundaries between closely related species (Zacharopoulou et

al., 2017). Cytogenetic studies of this types have been done for a number of Diptera including anopheline mosquitoes (Baimai et al., 1981, 1984; Green & Baimai, 1985), carrot rust fly, black flies (Simuliidae) (Robertson, 1957; Rothfels, 1979), drosophilid fruit flies (Lemeunier & Ashburner, 1976) and tephritids (Zacharopoulou et al., 2017). In the tephritids, these studies have been especially helpful in distinguishing taxa within complexes of closely related species such as those found in the genus *Anastrepha* (*A. fraterculus* (Wiedemann, 1830) *A. sororcula* Zucchi, 1979, *A. striata* Schiner, 1868, *A. bistrigata* Bezzi, 1919) based on karyotypic differences in sex chromosomes. (Morgante et al., 1993). The Oriental fruit fly, *Bactrocera dorsalis* (Hendel, 1912) is also recognized to be a member of the *B. dorsalis* species complex, a group of extremely closely related taxa. Here, the cytological work of Baimai et al. (1995, 1999 a, b) and others was critical in a multidisciplinary approach to resolve long standing controversies over the inclusion of certain species in this complex (Schutze et al., 2015). *Zeugodacus tau* is also a group member of closely related species, the *Z. tau* complex. Some karyotype studies have been done previously on taxa found within this complex in Thailand (Baimai et al., 2000).

In this study, we present a detailed cytological analysis of the mitotic metaphase chromosomes for *B. zonata* and *Z. tau*, both of which are native to South and South-East Asia and have major economic impacts in these areas. Our purpose here is to provide a more detailed characterization of the morphology of mitotic chromosomes in these species. In addition, we discuss their affinities in relations to metaphase chromosomes known for other Tephritid species in order to gain a better understanding of chromosomal morphology and evolution within these important insect pests. We accomplished this by providing detailed images of mitotic chromosomes and measurements of various parameters relating to these chromosomes.

Material and Methods

Fruit fly culture. Laboratory cultures of *Bactrocera zonata* and *Zeugodacus tau* have been maintained as a routine practice. Adult flies are reared in steel frame cages (12×10×8 cm) covered with mosquito netting. Laboratory temperature and humidity are maintained at 25 ± 2 °C and 65–70%, respectively, with 14 : 10 light and dark photoperiod. A mixture of Sugar and Yeast extract (3 : 1 ratio by weight) provides as adult food and soaked cotton with tap water in 50 ml conical flaxes are served as water in the rearing cages. Larvae of *B. zonata* and *Z. tau* are reared on ripe bananas and pumpkins, respectively.

Mitotic metaphase chromosome preparation. Neural ganglia of third-instar larvae of *B. zonata* and *Z. tau* were used for mitotic metaphase chromosome preparations following the methods of Zacharopoulou et al. (2011) and Yesmin (2013). Healthy larvae were dissected in the depression well of a slide with a drop of Ringer's solution. Cleaned brain tissues were transferred to a hypotonic solution (1% sodium citrate) for 10–15 minutes, and then the tissues were fixed in freshly prepared methanol-acetic acid (3 : 1) for 2 minutes, with several changes for complete removal of water. The tissues were then transferred to a small drop of 60% acetic acid and macerated by moving the tissue in and out of a micropipette several times to make a cell suspension. The cell suspension was then put on previously heated clean slides and

dried at 45 °C on a hot plate. The dried slides were stained for at least 30 minutes in 5% Giemsa with 10 mM phosphate buffer, pH 6.8.

Well prepared slides were used to take chromosome photos using Carl Zeiss microscope (AXIO Lab A1) with ZEN (blue edition) software. The nomenclature for chromosome morphology and the methodology for determining the centromeric index was done following Levan et al. (1964). Briefly, chromosome lengths (long arm and short arms) are measured, and the relative length (RL) is calculated by expressing the length of each chromosome as a percent of the summed length of all chromosomes. The centromeric index (CI) and the relative length (RL) of chromosomes are calculated mean values of measurements from multiple metaphase preparations (43 for *B. zonata* and 37 for *Z. tau*). Chromosome pairs are identified based on their relative length and morphology. Autosomes are numbered and labelled according to the system followed by Radu et al. (1975). This system was used for *Ceratitis capitata* (Wiedemann, 1824) and *Bactrocera oleae* (Rossi, 1790) (Canovai et al., 1996; Mavragani-Tsipidou et al., 1992), it labels the sex pair as the first and the autosomes from 2 to 6 in order of descending size. Successively, this system has been used commonly for other tephritid fruit fly species.

Abbreviations: m — Metacentric; sm — Submetacentric; TCL — total chromosome length; AR — arm ratio; RL — relative length; CI — centromeric index; a — acrocentric; st — sub telocentric.

Results

Analysis of chromosome preparations from *Bactrocera zonata* and *Zeugodacus tau* show that in both cases the metaphase spread consists of six pairs of chromosomes. Among these, five pairs are autosomes (numbered 2–6) with different size and shape, and one pair represents the heteromorphic sex chromosomes (XY in males and XX in females). In both species, the sex chromosomes are remarkable well differentiated

Table 1. **Mean ± S.E. of total chromosome lengths (in micrometers) and percentages of total complement lengths of mitotic metaphase chromosomes of the two tephritid species, *Bactrocera zonata* and *Zeugodacus tau***

Chromosome	*Bactrocera zonata*	*Zeugodacus tau*
No. metaphase spreads	43	37
TCL*, μm	33.35 ± 0.67	40.65 ± 1.15
Percentages of total chromosome complement lengths		
2	22.78 ± 0.16	20.39 ± 0.17
3	20.60 ± 0.16	18.41 ± 0.15
4	17.91 ± 0.15	17.36 ± 0.14
5	15.75 ± 0.13	15.95 ± 0.13
6	14.63 ± 0.16	14.91 ± 0.15
X	8.33 ± 0.18	12.99 ± 0.26

* TCL = total lengths of mitotic metaphase chromosomes in a single cell, dot shaped Y chromosomes were not considered in theses measurement.
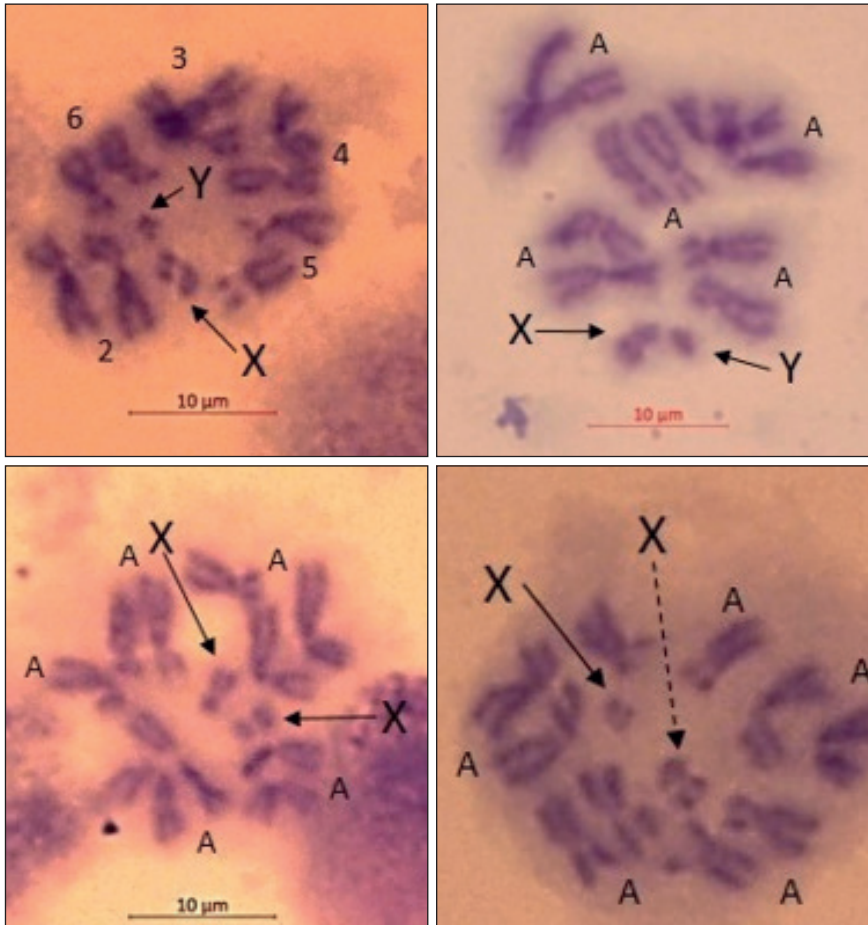
Fig. 1. Mitotic metaphase chromosomes of *Bactrocera zonata*: A — autosomes (2–6); XY — sex chromosomes. XX for female and XY for male fly

based on their heterochromatin content (deeply stained). The autosomes are usually associated in pairs (Figs 1, 2). Autosomes of the two species are submetacentric or metacentric, and the homomorphic X chromosomes are metacentric (Tables 2 and 3). Chromosome formula for *Z. tau* is $1sm + 4m + Xm + Y_{dot\text{-}shape}$ and for *B. zonata*, $3sm + 2m + Xm + Y_{dot\text{-}shape}$.

The relative lengths (RL) of the mitotic metaphase chromosomes indicate differences between all six chromosomes. By measuring the arm ratio (AR) and centromeric index (CI), the autosomes of *B. zonata* can be grouped into three pairs of submetacentric and two pairs of metacentric chromosomes. The autosomes of *Z. tau* consist of one pair of submetacentric and four pairs of metacentric chromosomes. In both species the X chromosomes are metacentric and the Y chromosomes appeared as dot shaped. In somatic cells, the homologous chromosomes of both species are typically found in paired association (Figs 1, 2).

Some exceptions to this chromosome behaviour also observed in some preparations where one or two pair were found separately from each other, this occurred probably during slide preparations.

Chromosomes of *Bactrocera zonata*. Specific results for chromosome lengths, arms ratios, centromeric indices and morphological classification of the mitotic metaphase chromosomes of *Bactrocera zonata* are presented in Table 1. The X chromosome is metacentric with deeply stained region in the short arm. Autosome pairs 2, 5 and 6 are submetacentric while pairs 3 and 4 are metacentric (Table 2). Table 1 shows that the mean of the total length of this mitotic complement is 33.35 μm. Chromosome 2 carries the largest percentage (23%) of this metaphase complement (Table 1) while the X chromosome contains the smallest (8%). In the autosome sets (Table 2), chromosome 2 is also the longest overall (7.60 μm) with a larger long arm (5.02 μm). Chromosome 6 is the smallest overall (4.85 μm). Chromosomes 5 and 6 are almost similar in size (5.25 and 4.85 μm, respectively), making it difficult to differentiate in the karyotypes. The X chromosome is the shortest generally (2.77 μm) among the chromosomes. The Y chromosome is dot shaped and appears to be com-

Table 2. **Morphometric characteristics of the chromosomes of *Bactrocera zonata* based on forty three metaphase preparations**

Chromosome Pair No	Long arm Length, μm	Short arm Length, μm	Relative Length, μm	Difference	Arm Ratio	Centromeric Index	Chromosome Morphology
	l	s	c	d	r	i	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
2	5.02 ± 0.12	2.58 ± 0.07	7.60 ± 0.17	3.21 ± 0.11	1.98 ± 0.05	33.96 ± 0.55	sm
3	3.82 ± 0.11	3.07 ± 0.08	6.89 ± 0.17	1.07 ± 0.11	1.25 ± 0.03	44.65 ± 0.54	m
4	3.21 ± 0.09	2.77 ± 0.07	5.97 ± 0.15	0.71 ± 0.09	1.17 ± 0.02	46.42 ± 0.48	m
5	3.54 ± 0.08	1.70 ± 0.04	5.25 ± 0.11	3.49 ± 0.09	2.10 ± 0.05	32.55 ± 0.49	sm
6	3.26 ± 0.08	1.60 ± 0.03	4.85 ± 0.08	3.38 ± 0.10	2.05 ± 0.05	33.08 ± 0.63	sm
X	1.46 ± 0.04	1.31 ± 0.04	2.77 ± 0.08	0.50 ± 0.05	1.11 ± 0.01	47.49 ± 0.27	m

* m = metacentric; sm = submetacentric; X = female sex chromosome.

Table 3. **Morphometric characteristics of the chromosomes of *Zeugodacus tau* based on thirty seven metaphase preparations**

Chromosome Pair No	Long arm Length, μm	Short arm Length, μm	Relative Length, μm	Difference	Arm Ratio	Centromeric Index	Chromosome Morphology
	l	s	c	d	r	i	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
2	5.02 ± 0.12	2.58 ± 0.07	7.60 ± 0.17	3.21 ± 0.11	1.98 ± 0.05	33.96 ± 0.55	sm
3	3.82 ± 0.11	3.07 ± 0.08	6.89 ± 0.17	1.07 ± 0.11	1.25 ± 0.03	44.65 ± 0.54	m
4	3.21 ± 0.09	2.77 ± 0.07	5.97 ± 0.15	0.71 ± 0.09	1.17 ± 0.02	46.42 ± 0.48	m
5	3.54 ± 0.08	1.70 ± 0.04	5.25 ± 0.11	3.49 ± 0.09	2.10 ± 0.05	32.55 ± 0.49	sm
6	3.26 ± 0.08	1.60 ± 0.03	4.85 ± 0.08	3.38 ± 0.10	2.05 ± 0.05	33.08 ± 0.63	sm
X	1.46 ± 0.04	1.31 ± 0.04	2.77 ± 0.08	0.50 ± 0.05	1.11 ± 0.01	47.49 ± 0.27	m

* m = metacentric; sm = submetacentric; X = female sex chromosome.
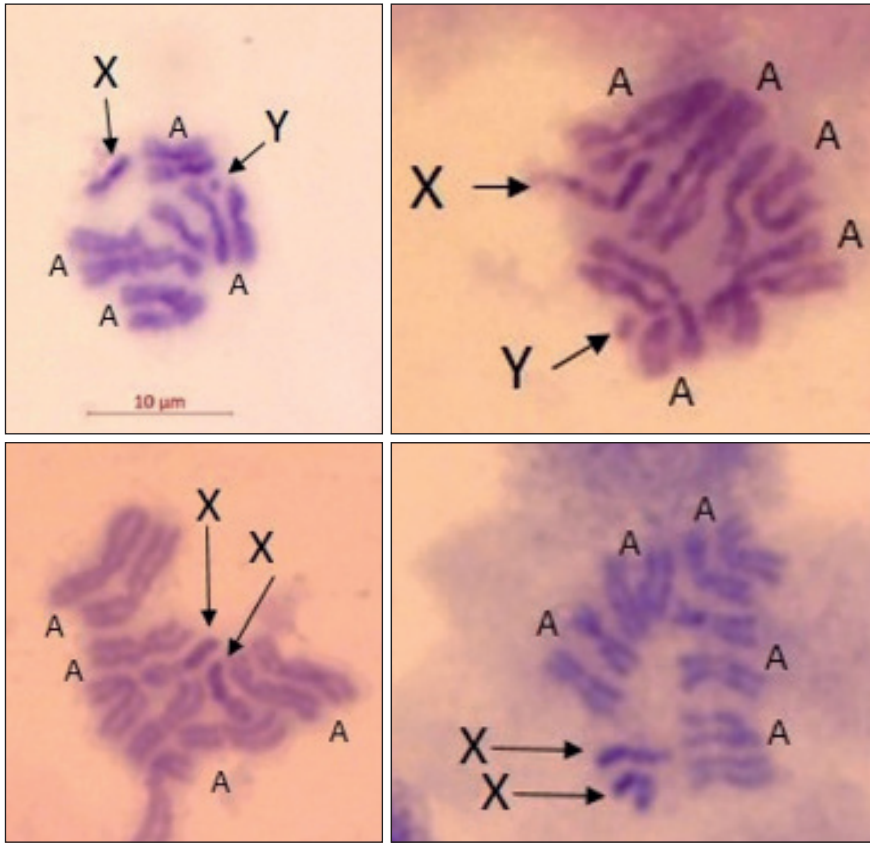
Fig. 2. Mitotic metaphase chromosomes of *Zeugodacus tau*: A — autosomes (2–6); XY — sex chromosomes. XX for female and XY for male fly

posed of entirely heterochromatic material (Fig. 1). The summary formulation of the chromosomes of *B. zonata* is 3sm + 2m + Xm + Ydot-shape (Table 2).

Chromosomes of *Zeugodacus tau*. Results of the mitotic metaphase chromosomes of Zeugodacus tau for chromosome length, arm ratio, centromeric index, and morphometric classification are shown in Table 3. The autosomes are numbered as 2 through 6 from the longest (8.26 µm) to the shortest (6.03 µm). The X chromosome is smaller than any of the autosomes (5.25 µm), showing a metacentric configuration where the long arm appears to be totally heterochromatic (deeply stained) and short arm is euchromatic (lightly stained) (Fig. 2). The chromatids of the sex chromosomes are not separated in these metaphase preparations, consistent with the sex chromosomes being somewhat heterochromatic. Table 1 shows that mean of total length of the complement of these chromosomes is 40.65 µm, with the highest percentage (20%) from chromosome 2 compared to the X (13%). The Y chromosome is very small and dot-like. The present findings reveal that chromosomes 2, 3, 4 and 6 are metacentric while chromosome 5 has a submetacentric appearance (Table 3). The relative lengths of chromosomes 3 and 4 (7.48 and 7.05 µm), as well as those of chromosomes 5 and 6 are almost similar (6.43 and 6.03 µm). The chromosome formula for *Z. tau* is 1sm + 4m + Xm + Ydot-shape (Table 3).

Table 4. **Configuration of the chromosome formula of fruit fly species of *Bactrocera*, *Dacus* and *Zeugodacus* genera from different localities (Diptera, Tephritidae)***

Pest species	Chromosome formula (Autosomes + X + Y)	Locality	References
Bactrocera oleae	$4sm + 1m + Xsm + Y_{dot-shape}$	Italy	Canovai et al., 1996
Dacus cucurbitae	$4m + 1sm + Xm + Y_{dot-shape}$	India	Bhatnagar et al., 1980
B. cucurbitae	$3m + 2sm + Xm + Ysm$	Thailand	Hunwattanakul & Baimai 1994
B. cucurbitae	$5sm + Xsm + Y_{dot-shape}$	Bangladesh	Shahjahan & Yesmin 2002
B. cucurbitae	$3m + 2sm + Xsm + Y_{dot-shape}$	»	Zacharopoulou et al., 2011
B. cucurbitae (GSS)	$3m + 2sm + Xsm + Y_{dot-shape}$	Hawaii	Zacharopoulou et al., 2011
B. dorsalis	$2m + 3sm + Xm + Ysm$	Thailand	Hunwattanakul & Baimai 1994
B. dorsalis	$2m + 3sm + Xm + Y_{dot-shape}$	»	Baimai et al., 1995
B. dorsalis	$4sm + 1m + Xm + Y_{dot-shape}$	Seibersdorf, IAEA	Zacharopoulou et al., 2017
B. dorsalis (GSS)	$4sm + 1m + Xm + Y_{dot-shape}$	» »	Zacharopoulou et al., 2017
B. dorsalis	$4sm + 1m + Xm + Y_{dot-shape}$	Malaysia	Yesmin & Clyde 2011
B. papayae	$2sm + 1m + 2a + Xm + Y_{dot-shape}$	»	Yesmin 2013; Yesmin & Clyde 2014
B. papayae	$4sm + 1m + Xm + Y_{dot-shape}$	Malaysia	Augustinos et al., 2014
B. carambolae	$2sm + 1m + 2a + Xm + Y_{dot-shape}$	»	Yesmin & Clyde, 2012
B. carambolae	$2sm + 1m + 2a + Xm + Y_{dot-shape}$	»	Yesmin, 2013
B. carambolae	$4sm + 1m + Xm + Y_{dot-shape}$	Suriname	Augustinos et al., 2014
B. carambolae	$3m + 2sm + Xsm + Ysm$	Thailand	Baimai et al., 1999 a
Dacuszonatas	$3m + 2sm + X_{dot-shape} + Y_{dot-shape}$	India	Bhatnagar et al., 1980
B. zonata	$3sm + 2m + Xm + Y_{dot-shape}$	Bangladeshi sample	Present study
B. tau	$4m + 1sm + Xm + Ysm$	Thailand	Hunwattanakul & Baimai 1994; Baimai et al., 2000
Zeugodacus tau	$4m + 1sm + Xm + Y_{dot-shape}$	Bangladeshi sample	Present study
Dacus diversus	$4m + 1sm + X_{dot-shape} + Y_{dot-shape}$	India	Bhatnagar et al., 1980
B. correcta	$3m + 2sm + Xm + Ysm$	Thailand	Hunwattanakul & Baimai 1994
B. kanchanaburi	$2m + 3sm + Xsm + Ysm$	»	Baimai et al., 1995
B. raiensis	$2sm + 3m + Xa + Y_{dot-shape}$	»	Baimai et al., 1995
B. verbascifoliae	$2sm + 3m + Xst + Y_{dot-shape}$	»	Baimai et al., 1995
B. diversa	$4m + 1sm + Xsm + Ysm$	»	Baimai et al., 1996
B. rubella	$4m + 1sm + Xst + Y_{dot-shape}$	»	Baimai et al., 1996
B. scutellaris	$4m + 1sm + Xsm + Ysm$	»	Baimai et al., 1996
B. latifrons	$4m + 1sm + Xm + Y_{dot-shape}$	»	Baimai et al., 1996
B. modica	$4m + 1sm + Xm + Y_{dot-shape}$	»	Baimai et al., 1996
B. tryoni	$3sm + 2a + Xm + Ym$	Australia (eastern)	Zhao et al., 1998
B. propinqua	$3m + 2sm + Xsm + Ysm$	Thailand	Baimai et al., 1999 a
B. irvingiae	$3m + 2sm + Xm + Y_{dot-shape}$	»	Baimai et al., 1999 a
B. pyrifolae	$3m + 2sm + Xsm + Y_{dot-shape}$	»	Baimai et al., 1999 a
B. arecae	$3m + 2sm + Xsm + Ysm$	»	Baimai et al., 1999 a
B. melastomatos	$3m + 2sm + Xm + Ym$	»	Baimai et al., 1999 a
Dacusciliatus	$5m + X_{dot-shpae} + Y_{dot-shape}$	Israel	Drosopoulou et al., 2011 b
B. philippinensis	$4sm + 1m + Xm + Y_{dot-shape}$	Philippines	Augustinos et al., 2014
B. invadens	$4sm + 1m + Xm + Y_{dot-shape}$	Kenya	Augustinos et al., 2014

* All using chromosome formula method of Levan et al., 1964.

Cytogenetic comparisons to other tephritid species. A summary of information available for the mitotic metaphase spreads of other species of the Tephritidae is shown in Table 4. All of the species listed here exhibit a basic complement of 5 autosomes and heteromorphic sex chromosomes. However, it several cases where multiple studies have been conducted on the same species, different configurations have been described in different publications. For example, *Zeugodacus cucurbitae* (Coquillett, 1899) chromosomes range from all submetacentric to various combinations of metacentric and submetacentric autosomes. Even the Y chromosome appearance varies from dot-shaped to submetacentric. In *Z. tau*, Y chromosome was found as dot shaped in every case whereas *B. zonata* Y chromosome somewhat varies from dot shaped to submetacentric in few cases observed in this study. Similar variation can be observed in *B. dorsalis* chromosomes including differing numbers of metacentric and submetacentric autosomes. However, no differences are reported for the sex chromosome in B. dorsalis (all metacentric X and dot-shaped Y). Some differences are also indicated in the multiple reports for *B. dorsalis* complex (*B. papayae* Drew & Hancock, 1994 and *B. carambolae* Drew & Hancock, 1994). An overall configuration of the chromosome formula of Tephritid fruit fly species studied so far was presented in the Table 4.

Discussion

The study of mitotic metaphase chromosomes has helped as a simple and suitable tool for cytotaxonomic study in clusters of closely related species and cryptic species in dipteran insects as demonstrated by *Drosophila* (Drosophilidae), *Bactrocera* (Tephritidae), *Anastrepha* (Tephritidae) and *Anopheles* (Culicidae). Present study deals with the mitotic metaphase chromosomes of *Bactrocera zonata* and *Zeugodacus tau* configured with five pairs of autosomes and one pair of sex chromosome (XX in female and XY in male). The chromosome counts of 2n = 12 observed here have been the most reported for other Tephritid species. The overall pattern of autosomes with heteromorphic sex chromosomes (X and Y) is similar to the findings of *Chamaepsila rosae* (Schutze et al., 2015) and *Ceratitis capitata* (Rothfels, 1979). Our results also support the characteristic features of sex chromosomes being mostly heterochromatic in different fruit fly genera including *Anastrepha*, *Bactrocera dorsalis* (Augustinos et al., 2014), *Ceratitis capitata* (Bedo 1987), *Dacus ciliatus* Loew 1862 (Drosopoulou et al., 2011 b), *Rhagoletis completa* Cresson, 1929 (Drosopoulou et al., 2010), *Rhagoletis cingulata* (Loew, 1862) (Drosopoulou et al., 2011 a), *Bactrocera carambolae* (Yesmin & Clyde, 2012), and *Zeugodacus cucurbitae* (Zacharopoulou et al., 2011).

For *B. zonata*, the autosomes designated as 2, 5 and 6 were submetacentric while autosomes designated as 3 and 4 were metacentric (Table 2), consistent with general patterns seen throughout the Tephritidae (Zacharopoulou et al., 2017). The sex chromosome in this species, metacentric X and dot shaped Y chromosome, also a very common combination found in most Tephritid species. For *Z. tau*, autosomal chromosomes designated as 2, 3 and 4 were metacentric while autosomes 5 and 6 were submetacentric; our findings are similar reported by Hunwattanakul & Baimai (1994) where they found the X chromosome of *B. tau* to be medium in size and metacentric in appearance. The autosomal chromosomes 2, 3, 4 and 6 are metacen-

tric while chromosome 5 was submetacentric. Metaphase chromosomes of five sibling species of the *B. dorsalis* complex were also studied by Baimai et al (1995). They observed five autosome pairs and one heteromorphic sex chromosome pair in all five species, and our results are consistent with these findings.

Baimai et al. (1999 a) also described the chromosomes of six other *Bactrocera* species (*B. propinqua* (Hardy & Adachi, 1954), *B. irvingiae* Drew & Hancock, 1994, *B. carambolae*, *B. pyrifoliae* Drew & Hancock, 1994, *B. arecae* (Hardy & Adachi, 1954), and *B. melastomatos* Drew & Hancock, 1994) within *B. dorsalis* complex. Metaphase chromosomes of those species also revealed five pairs of autosomes. The Y chromosomes of all six species, except *B. arecae*, were dot-like appearance while the X chromosomes were different in size. Baimai et al. (2000) studied cytological evidence of seven taxa with undefined species status (Form A-G) within the *B. tau* complex. This study showed seven distinct chromosomal patterns representing these seven closely related taxa. Metaphase chromosomes here also consisted of five pairs of autosomes and one pair of heteromorphic sex chromosome (XX/XY). In form A, the X chromosome was medium sized with metacentric appearance while the Y chromosome appeared to have a dot shaped. This was denoted as the *B. tau* type specimen, and this finding is completely consistent with our findings for *Z. tau*.

The information presented in table 4 shows that overall, the autosomes of all the *Bactrocera*, *Dacus* and *Zeugodacus* species listed are a combination of metacentric, submetacentric or acrocentric based on the arms ratio and the centromeric index value measurements. It is of importance to note that this table also shows some potentially important differences in the configuration of autosomal chromosomes for the same species (notably *B. cucurbitae* and *B. dorsalis*) when reported by different authors from collections made in different localities. All of the studies, mentioned above, are mainly composed of metacentric and submetacentric chromosomes accompanied by a lower proportion of acrocentric and subtelocentric chromosomes. Because of the prevalence of metacentric and submetacentric chromosomes, the chromosomes almost present a high degree of symmetry. So, it can be assumed that the diversification of the genus Bactrocera has been accompanied by small changes in the structure of the chromosomes.

In the case of *B. dorsalis*, these differences may not be surprising given that this species is known to be a member of a complex of closely related species that can be very difficult to distinguish morphologically. Recently, multiple taxa within this complex have been synonymised under the umbrella of *B. dorsalis*, including *B. papayae* and *B. invadens* Drew, Tsuruta & White, 2005 (Schutze et al., 2015). It is worth noting here that the chromosome formulae listed here for these species are identical to the *B. dorsalis* (Table 4). The discrepancies for *B. cucurbitae* are more different to explain. Using other markers, some recent studies of different populations of this species from around the world have been reported (Boontop et al., 2017), but not to the extent of other tephritids such as *B. dorsalis* (Schutze et al., 2015). Whether these differences are due to methodological errors or real polymorphic variation in the chromosome composition of this species from various localities will require further study. Finally, since there is already some evidence for the existence of a species complex within *Z. tau* (Baimai et al., 2000), it will be critical to consider the possibility of regional differences in chromosomes and other genetic markers here as well. There

are several species to *Z. tau*, but most of them are morphologically different, there is certain variation within *Z. tau*, but recent molecular studies showing that actual complex may be simpler than it seems.

Conclusions

The results of this study show that there is no variation in chromosome number, but chromosome formula and chromosome morphometrics are different between *Bactrocera zonata* and *Zeugodacus tau*. Obtained information is valuable as they may reveal polymorphic forms when specimens of these species are collected from different geographic areas. In addition, it will be desirable to develop maps derived from polytene chromosomes from same species for better understanding of the systematics and chromosome evolution with the species of these genera. Furthermore, the baseline set of chromosome results developed here may provide important information for the use of future control strategies directed at these pest species.

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