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HETEROCHROMATIN DISTRIBUTION IN MITOTIC METAPHASE KARYOTYPES OF THE PEACH FRUIT FLY, *BACTROCERA ZONATA* AND THE PUMPKIN FRUIT FLY, *ZEUGODACUS TAU* (DIPTERA, TEPHRITIDAE), WITH C-BANDING TECHNIQUE

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Heterochromatin Distribution in Mitotic Metaphase Karyotypes of the Peach Fruit Fly, *Bactrocera zonata* and the Pumpkin Fruit Fly, *Zeugodacus tau* (Diptera, Tephritidae), with C-Banding Technique. Yesmin, F., Haymer, D., Uddin, M. N. & Hasanuzzaman, M. — Mitotic metaphase karyotypes of the two economically important fruit fly species, *Bactrocera zonata* and *Zeugodacus tau*, with C-banding technique were studied to understand their genomic organization and distribution patterns. Both species consist of a diploid set with 12 biarmed (2n = 12) chromosomes including one pair of heteromorphic (XX/XY) sex chromosomes. We found a characteristic distribution pattern in both species with positive C^+ bands in all chromosome sets in their centromere region. Chromosome 2 in both species consisted of minimal amount of heterochromatin while two C^+ bands decorated the X chromosome of male *Z. tau* in their centromeric and telomeric region and X chromosome in female had no C-band in their telomeric region in short arm. Long arms of the X chromosome in both sexes were totally heterochromatic. In both species, the Y chromosome is dot-shaped with almost fully heterochromatic and deeply stained. Moreover, metacentric X chromosomes in both sexes of *B. zonata* contained one

thin telomeric C^+ band in their short arm with one deep band in the centromeric region. The present findings revealed the knowledge of genomic structure as well as contribute to the understanding of interference of heterochromatin in ontogeny and chromosomal evolution of these two fruit fly species.

Key words: fruit fly, mitotic chromosomes, C-heterochromatin, karyotypic evolution, C-bands.

Introduction

Tephritid fruit flies constitute one of the major dipteran pest groups regarding their species abundance and economic importance. Sex chromosomes and their chromosomal variability at heterochromatin level play a key role to identify the species of a complex group. Constitutive heterochromatin (C-heterochromatin) deflated light on the study of origin and evolution of fruit flies. Larval morphology, C-heterochromatin characteristics as well as chromosomal variability at heterochromatin level can be used for species identification (Frias, 2002; Sullivan, 2013). Metaphase karyotypes along with C-banding demonstrated the cytotaxonomy, inter and intraspecific differences, separation of cryptic or isomaric species, genetic differentiation, sex chromosome identification and karyotypic evolution of Dipteran insects (Baimai, 1998; Buonomo, 2010). In tephritid flies, the sex chromosome differences in mitotic karyotypes have been used to differentiate the members of species complexes within a genus (Cáceres et al., 2009; Hernández-Ortiz et al., 2012; Giardini et al., 2015). It is induced to show different degrees of heterochromatin content involved in genetic differentiation.

Heterochromatin has been thoroughly studied since the term first used by the Botanist Emil Heitz (Heitz, 1928). This part of chromosomes was more compacted than the euchromatin. After that heterochromatin was recognised as a common phenomenon in general biology; appearing in both animals and plants. Heterochromatin normally carries highly repetitive DNA sequences (repDNA) (Yasuhara & Wakimoto, 2008) that are characterised by restriction endonucleases and it separates in satellite bands during density-gradient sedimentation (Lohe et al., 1993). It interferes with DNA replication that contributes to chromosome structure, gene expression, genome organization, chromosomal evolution and speciation (Baimai, 1998; Wichman et al., 1990; Elder & Turner, 1995). Cytogenetically it can be detected as homogeneously staining after G-banding or as an extra C-band after C-banding (Sumner, 1990). The C-banding technique spotlighted the distribution of heterochromatin that comprises entire parental complement i. e. sex-linked chromosome of mosses (Heitz, 1928), Y chromosome in *Drosophila,* autosomes and Xs chromosome of *Drosophila* (Ranganath & Hagele, 1982). Giemsa C-banding stains the centromeres including interstitial and telomere chromosome bands. It is a powerful strategy to stain C-heterochromatin that has been successfully applied to identify individual chromosomes in many species and to establish genomic relationships among different species (Falistocco et al., 1995; Bauchan & Hossain, 1999; Tuna et al., 2004). In molecular biology, C-heterochromatin is regarded as an alignment of highly repetitive non-protein-encoding sequences (Pardue & Hennig, 1990). It coincides with the delay-replicating fragments of karyotypes and forms a more condensed part of interphase chromatin that is conceived as the inactive end of chromosome.

In the fruit fly genus, there are some cryptic or isomorphic species that cause great problems in taxonomic identification because of their similarity in external morphology, for example *Bactrocera dorsalis* species complex (Schutze et al., 2015). It creates more ambiguity when such sibling (cryptic) species exhibit different behaviours in mating, feeding and ovipositing on specific host plants (Baimai, 1998; Baimai et al., 2000). Sibling species can easily be separated based on the knowledge of mitotic karyotype analysis. Despite overall significance, the understanding of their genome organization is still inadequate. Therefore, the present study was carried out to enlighten the distribution and localization of C-heterochromatin on chromosomes and compared C-heterochromatin distribution patterns between these two species. Such specific patterns of heterochromatin in mitotic karyotypes are applied as diagnostic characters for the differentiation of affinities in closely related species. It could reveal certain genomic similarities/dissimilarities between these two economically important tephritid species, *B. zonata* and *Z. tau*. *Bactrocera zonata* is an important invasive species creating significant losses to the horticulture industry worldwide including Bangladesh. Among the *Bactrocera* species, *B. zonata* has high interest due to their

highly invasive nature. It attacks more than 50 commercial and wild host plants including peach, guava, mango, apricot, citrus, prickly pear and fig. Moreover, it is considered as A1 quarantine pest in the European and Mediterranean Plant Protection Organization (EPPO) countries, affecting export markets (Zingore, et al., 2020). On the other hand, *Z. tau* is an important worldwide quarantine pest. It is a phytophagous insect, and its larva can damage many parts of the host. This species also has a wide range of hosts and strong fecundity and adaptability, and many countries have listed *Z. tau* as a key quarantine species (Liu & Ji, 2024).

Material and Methods

Species culture:
Adult colonies (*Bactrocera zonata* and *Zeugodacus tau*) were maintained according to Yesmin et al. (2019). Yeast and Sugar, 1 : 3 and tap water with soaked cotton were served as adult food sources. Flies were reared in 25 ± 2 °C temperature and 65–70% relative humidity condition with 14 : 10 h light and dark cycle. Larvae were reared in ripe banana for *B. zonata* and pumpkin for *Z. tau*.

C-banding technique:
Mitotic metaphase chromosomes were prepared according to the procedure followed by Zacharopoulou et al. (2011 b) and Yesmin (2013). The technique (C-banding) described by Selivon and Perondini (1997) with minor modifications was used for detecting C-heterochromatin. Slides of the metaphase plates were left at least one week as C-banding demands aged/old slides. Slides were immersed in 0.2N HCl solution for 10 minutes at room temperature, rinsed in distilled water and then transferred into saturated Ba $(OH)_2$ solution at 50 °C for two minutes. The materials were then rinsed in acid water thoroughly in order to remove additional Ba $(OH)_2$ crystals and treated with 2X SSC (0.3M NaCl, 0.03M trisodium citrate; pH 7.00) saline solution at 60 °C for 30 minutes. Subsequently the treated slides were rinsed with distilled water and stained with 5% Giemsa solution with phosphate buffer (0.01 M). Finally, the slides were rinsed with enough distilled water and air dried at room temperature.

Chromosome numbering procedure:
The chromosomes of each karyotype set were labeled as it is commonly done for other tephritid species. Autosomes are numbered from 2 to 6 in order of their descending length, where 2 are the longest and 6 is the smallest chromosome. Sex chromosomes were labeled as XX/XY. Morphology of the karyotypes was done according to centromeric index and arm ratio followed by Levan et al. (1964).

Microscopy and Image processing:
Preparations were observed in Carl Zeiss AXIO Lab A1 microscope (Carl Zeiss Jena, Germany). Well spread images were captured and recorded with the software Carl Zeiss Zen Blue Version. Heterochromatin distribution patterns were measured with the same software. Heterochromatin percentages and ranges (Tables 1 and 2) were evaluated by calculating fifteen male and fifteen female karyotype sets of each species following Kuvangkadilok et al. (1998) and Ata (2005). Statistical analysis was done by using IBM SPSS software version 24. The relative length of the chromosomes, arm ratios and centromere index data were used for preparing ideograms of the karyotype sets of both species of our previous research article (Yesmin, et al., 2025).

Results

Giemsa stained C-banding patterns of mitotic metaphase karyotypes of *Bactrocera zonata* and *Zeugodacus tau* are shown in Fig. 1–3 and 4–5 respectively. Both species contain asymmetric karyotypes with 2n = 12 chromosomes. Karyotypes of these species exhibit variation in size and shape of sex chromosomes and autosomes attributable to the amount and distribution of c-heterochromatin at their centromeric and telomeric regions (Fig. 5). Morphologically *B. zonata* consists of three submetacentric (2, 5 and 6) and two metacentric (3, 4) autosome pairs while *Z. tau* karyotype compose of four metacentric (2, 3, 4, and 6) and one (5) submetacentric autosome pairs. The X chromosome of both species is metacentric and the Y chromosome is found dot-shaped (Figs 1, 3, 4, *m-o*).

Table 1. **Distribution of C-band in mitotic metaphase chromosomes of *Bactrocera zonata* and *Zeugodacus tau***

NO. C	*Bactrocera zonata*								*Zeugodacus tau*							
	NO. MN.Ex.	PC	C	I	L	S	ST	LT	NO. MN.Ex.	PC	C	I	L	S	ST	LT
C2	30	–	+	–	–	–	–	–	30	+	+	–	–	–	–	–
C3	30	+	+ +	–	–	–	–	–	30	+	+ +	–	–	–	–	–
C4	30	+ + +	+ +	–	–	–	–	–	30	+	+ + +	–	–	–	–	–
C5	30	–	+ + +	–	–	–	–	–	30	+ +	+ + +	–	–	–	–	–
C6	30	–	+ + +	–	–	–	–	–	30	–	+ + +	–	–	–	–	–
X	30	+/+ +/+ +/+	+/+ +/+	–	–	–	+	–	30	+/+ +/+	+/+ /+ +/+	–	+++	–	–	–
X	30	–	+/– +/– +/–	–	–	–	+	–	30	+/–+/–+/–	+/– +/–	–	+++	–	+	–
Y	30	+ + +	+ +	–	–	–	–	–	30	+ + +	+ +	–	–	–	–	–

*NO. C. = chromosome number; NO. MN. Ex. = number of metaphase nucleus examined; PC = pericentric region; C = centromere region; I = interstitial region; L = long arm; S = short arm; ST = telomere region of short arm; LT = telomere region of long arm; + = faint band; ++ = deep band; +++ = highly deep band; – = no band; +/– = heterozygous sex chromosome; +/+ = homozygous sex chromosome

C-banding pattern of mitotic metaphase chromosomes of *Bactrocera zonata*:

C-bands are preferentially localised in the pericentric region in the autosome set (chromosome pairs 2–6). Large pericentric C-band with 18.33% heterochromatin block is observed in the female X chromosome. In male, the X chromosome is decorated with a large heterochromatin block (17.11%) in the centromere region (Table 2). A faint telomeric C^+ band in the telomere region of the short arm is located in the X chromosome in both sexes (Figs. 1–3 and 6, *a*). The telomeric band consists of 17.45% heterochromatin accumulation in females and 17.03% in male (Table 2). The dot-like Y chromosome is highly heterochromatic and in most cases full of c-heterochromatin (Figs 1, 3 and 5, *a*). The details heterochromatin distribution in the autosomes of *B. zonata* are as follows:

Chromosome 2:
It is the longest chromosome among the autosome set (Table 2) and figured out with submetacentric appearance (Fig. 5, *a*). It has one C-band at the centromeric region (Table 1). This chromosome is composed of the minimal amount of heterochromatin block (9.65% in female and 10.99% in male) (Table 2) among the karyotype set, as the heterochromatin block sometimes appears invisible in Giemsa stain.

Chromosome 3:
It is the second longest chromosome of *B. zonata* metaphase karyotype (Table 2) and appears with metacentric appearance (Fig. 5, *a*). This chromosome is decorated with one centromeric C^+ band in the pericentric region (Table 1). It is composed of comparatively a larger heterochromatin block (11.88% in female and 12.61% in male) (Table 2).

Chromosome 4:
Chromosome 4 is metacentric (Fig. 5, *a*). It is characterised by a large pericentric C^+ band in the centromere region (Table 1) and heterochromatin block (12.99% in female and 12.74% in male) is almost similar with chromosome 3 (Table 2).

Chromosome 5:
This chromosome has a submetacentric appearance with medium size in length (Fig. 5, *a*). It is composed of a prominent C^+ band in the centromere region (Table 1). Hetero-

Table 2. **Size and volume of constitutive heterochromatin blocks in mitotic metaphase chromosomes of *Bactrocera zonata* and *Zeugodacus tau Female***

Chromosome length and region- statistical description (15 female and 15 male metaphase plates)										
Chromosome No.	Female					Male				
	R L (μm)	CH L (μm)	CH%	CH range (μm)	T %	C L (μm)	CH L (μm)	CH%	CH range (μm)	T%
	M ± S.E.	M ± S.E.	M ± S.E.	–	M ± S.E.	M ± S.E.	M ± S.E.	M ± S.E.	–	M ± S.E.
Bactrocera zonata										
2	7.87 ± 0.36	0.74 ± 0.04	9.65 ± 0.64	4.55–13.38	–	8.52 ± 0.39	0.90 ± 0.06	10.99 ± 1.07	3.82–17.49	–
3	7.45 ± 0.36	0.87 ± 0.04	11.88 ± 0.64	8.49–16.02	–	7.62 ± 0.23	0.96 ± 0.20	12.61 ± 0.45	8.19–16.72	–
4	6.36 ± 0.31	0.82 ± 0.05	12.99 ± 0.58	9.15–17.08	–	6.48 ± 0.23	0.82 ± 0.06	12.74 ± 0.72	9.48–17.61	–
5	5 .57 ± 0.24	0.80 ± 0.05	14.24 ± 0.45	11.76–-18.19	–	5.99 ± 0.21	0.93 ± 0.06	15.43 ± 0.52	12.37–19.04	–
6	5.24 ± 0.22	0.86 ± 0.05	16.41 ± 0.77	12.85–21.23	–	5.53 ± 0.18	0.96 ± 0.06	17.30 ± 0.79	12.61–24.85	–
X	3.09 ± 0.12	0.56 ± 0.02	18.33 ± 0.58	15.18–23.25	–	3.50 ± 0.13	0.60 ± 0.33	17.11 ± 0.94	13.67–26.93	–
T in X	0.53 ± 0.02	–	–	12.12–23.99	17.45 ± 0.81	0.59 ± 0.03	–	–	11.56–21.21	17.03 ± 0.66
Zeugodacus tau										
2	8.43 ± 0.42	0.86 ± 0.05	10.39 ± 0.52	6.06–14.15	–	8.15 ± 0.45	0.82 ± 0.06	10.19 ± 0.62	6.18–14.72	–
3	7.56 ± 0.35	0.79 ± 0.05	10.51 ± 0.47	7.49–14.23	–	7.22 ± 0.41	0.70 ± 0.04	10.07 ± 0.40	6.63–16.39	–
4	7.18 ± 0.32	0.76 ± 0.04	10.65 ± 0.43	7.81–13.26	–	7.06 ± 0.40	0.87 ± 0.06	12.36 ± 0.66	8.89–18.42	–
5	6.79 ± 0.28	0.78 ± 0.05	11.55 ± 0.62	7.92–17.18	–	6.37 ± 0.33	0.67 ± 0.05	10.70 ± 0.59	7.00–14.48	–
6	6.49 ± 0.28	0.79 ± 0.06	12.28 ± 0.77	8.51–18.58	–	6.03 ± 0.32	0.85 ± 0.05	14.17 ± 0.74	–	–
X	5.48 ± 0.23	0.75 ± 0.04	67.23 ± 0.61	63.87–74.23	–	5.55 ± 0.31	0.79 ± 0.06	63.59 ± 0.86	55.98–68.72	–
T in X	0.77 ± 0.05	–	–	9.63–17.68	13.97 ± 0.63	0.87 ± 0.07			10.26–19.66	15.54 ± 0.68

*RL = relative length; C= centromere; L = length; H = heterochromatin; T = telomeric band; X = female sex chromosome; Y chromosomes were not consider in the measurement.

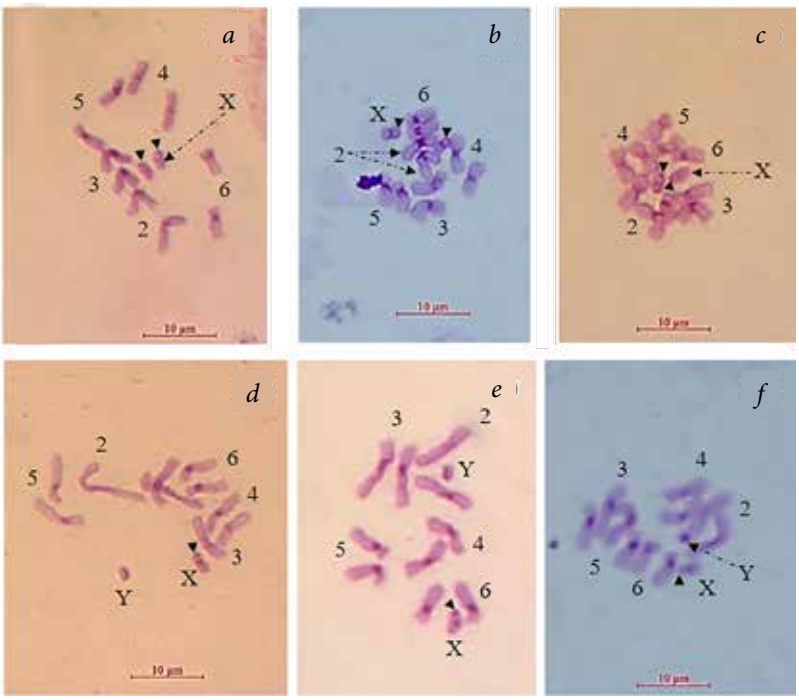

Fig. 1. C-banding patterns of mitotic metaphase karyotypes of *Bactrocera zonata*: *a–c* female karyotypes: *d–f* male karyotypes. Arrow heads indicate the telomeric band in X chromosome

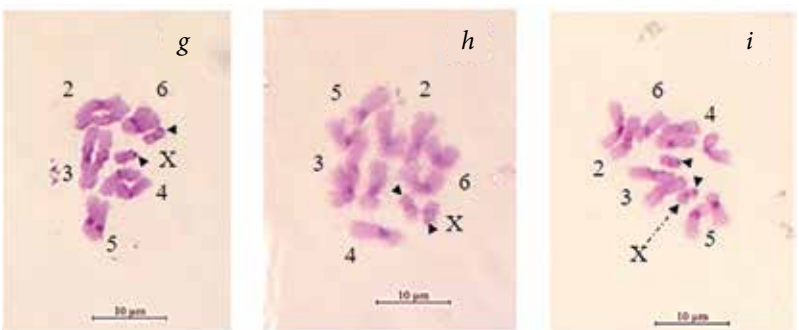

Fig. 2. C-banding patterns of female metaphase karyotypes of *Bactrocera zonata* (*g–i*). Arrow heads indicate the faint C^+ band in the telomere region in X chromosome

chromatic accumulation of this chromosome is 14.24% and 15.43% of female and male respectively (Table 2).

Chromosome 6:

This is the smallest chromosome (Table 2) in the autosome set and displayed with submetacentric appearance (Fig. 5, *a*). It is decorated with the largest heterochromatin block (16.41% in female and 17.30% in male) in the centromere region of the metaphase genome (Table 2).

C-banding pattern of mitotic metaphase chromosomes of *Zeugodacus tau*:

C-bands are located in the pericentric region in the autosome set (chromosome pairs 2–6). The C-banding pattern reveals that the prominent C-banded block (67% in female and 63% in male) covers the whole long arm of X chromosome in both sexes (Table 2) with a telomeric C^+ band in the short arm of male only (Fig. 5, *b*). Long arm of the X chromosome is totally heterochromatic,

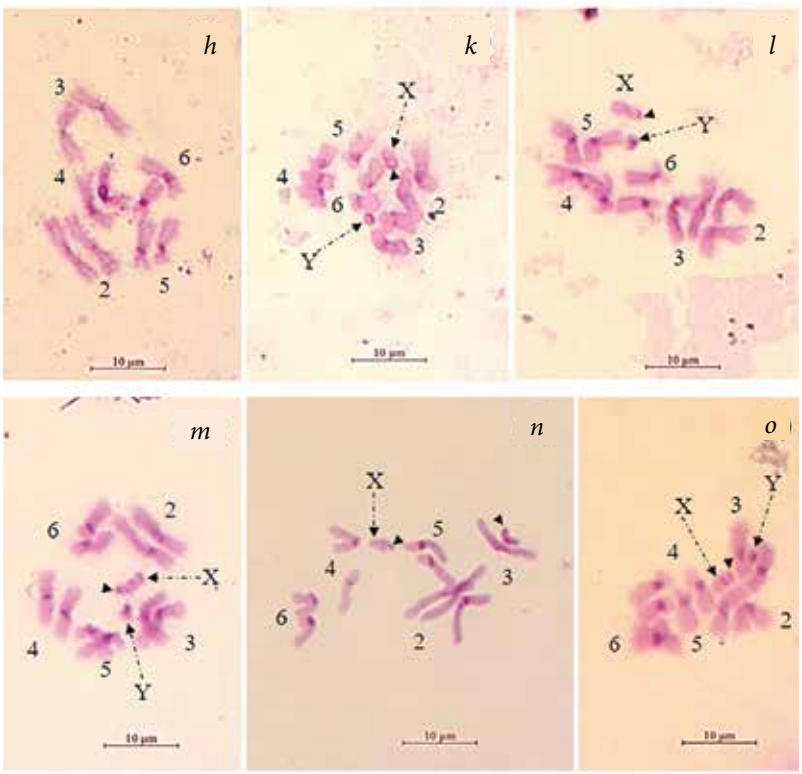

Fig. 3. C-banding patterns of mitotic metaphase chromosomes of *Bactrocera zonata* (*j–o*). Arrow heads indicate the faint C⁺ band in the telomere region in X chromosome

while the opposite arm is entirely euchromatic (Fig. 4). The dot shape Y chromosome is highly heterochromatic and almost full of c-heterochromatin (Figs 4 and 5, *b*). The distinct morphological characters and C-banding patterns for each chromosome (autosomes) of *Z. tau* are described as follows:

Chromosome 2:
Chromosome 2 is the longest chromosome (Table 2) among the karyotype set. It appears with metacentric morphology (Fig. 5, *b*). There is a faint C+ band found in the pericentric region (Table 1) of this chromosome. It contains a minimal amount (10.39% in female and 10.19% in male) of heterochromatin distribution (Table 2).

Chromosome 3:
This chromosome consists of one thin heterochromatin block comparatively darker than chromosome 2 (Table 1). It is metacentric (Fig. 5, *b*) chromosome and fashioned with pericentric C-band. Female and male contain 10.51% and 10.07% C-heterochromatin blocks of this chromosome, respectively (Table 2).

Chromosome 4:
Alike the chromosome 2 and 3, this chromosome is characterised with a dark C⁺ band in the centromere region (Table 1). Its metacentric morphology with pericentric C-band makes it easily visible in the karyotype set (Fig. 5, *b*). Chromosomes of females composed of 11.55% and male with 12.36% of C-heterochromatin (Table 2).

Chromosome 5:
It is the most conspicuous chromosome pair because of its submetacentric morphology among the karyotype genome of *Z. tau* (Fig. 5, *b*). A dark heterochromatin block is found in the pericentric region (Table 1). These features enable them to be distinguished from other chromosomes in

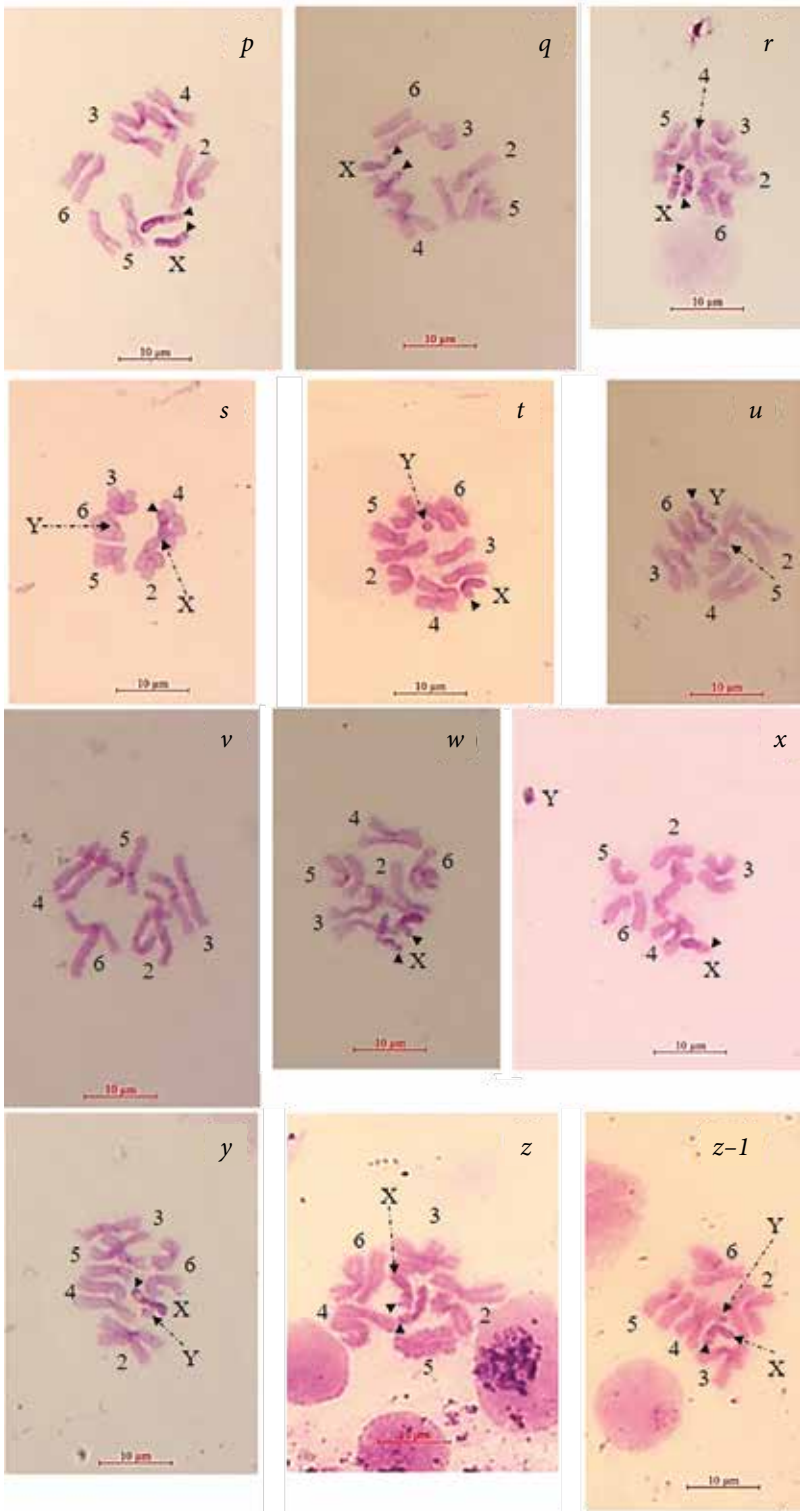

Fig. 4. C-banding patterns of mitotic metaphase karyotypes of *Zeugodacus tau* (*p–z–1*). *p–r*, *w*, *z* female karyotypes; *s–u*, *x*, *y*, z*–1* — male karyotypes. Arrow heads indicate the telomeric band in X chromosome

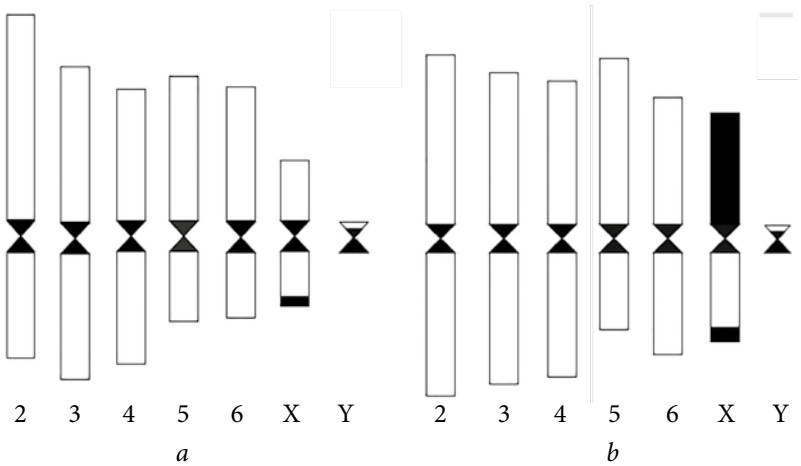

Fig. 5. Diagrammatic representation of haploid ideograms of mitotic metaphase karyotypes of *Bactrocera zonata* (*a*) and *Zeugodacus tau* (*b*). Heterochromatin portions are depicted in black

the karyotype complement. Female and male constitute 11.55% and 10.70% C-heterochromatin blocks in this chromosome, respectively (Table 2).

Chromosome 5:
It is the smallest chromosome in the karyotype set characterised with metacentric morphology (Fig. 5, *b*). A prominent heterochromatin block found in the centromere region (Table 1) of this chromosome is similar with chromosome 2 and 3. Maximum amount of C-heterochromatin accumulation (female consists of 12.28% and male with 14.17%) found in this chromosome (Table 2).

Discussion

Cytological evidence suggests that heterochromatin accumulation in some cases is involved in species differentiation of the dipteran insects. Heterochromatin variation in natural populations becomes a common phenomenon in higher organisms (John, 1981). Since the discovery of heterochromatin, c-heterochromatin apparently is observed in higher organisms (both plants and animals) particularly in the dipteran insects. C-heterochromatin is often found in the pericentric region of mitotic chromosomes, as blocks of dark staining (Giemsa) flanking the centromeres. It consists of highly repetitive DNA or satellite DNA stationed in tandem in the eukaryotic genome that might extend 60% of the metaphase period of the sex chromosome (X chromosome) (Table 2, X chromosome in *Z. tau*) and near about 50% of certain autosomes (Bonaccorsi & Lohe, 1991). Modern cytogenetic evidences reveal that C-heterochromatin of *Drosophila melanogaster* chromosomes contain 30 active gene that work as suppressor of forked gene at the proximal region of X chromosome (Gatti & Pimpinelli, 1992) and Y chromosome contains certain active gene specially role as fertility factors. Thus, the presence of heterochromatin in the eukaryotic genome indicates its significant role in the controlling activities and evolutionary linked to the genome (Irick, 1994; Zuckerkandi & Hennig, 1995).

The study of tephritid fruit fly genetics and cytogenetics has conferred considerable attention in recent years (Kounatidis et al., 2008; Stratikopoulos et al., 2009;

Drosopoulou et al., 2011; Tsoumani et al., 2011; Zacharopoulou et al., 2011 a, b) after conforming them as the most destructive agricultural pests in the world (White & Elson-Harris, 1992). The analysis of karyotype showed that both *B. zonata* and *Z. tau* have 2n = 12 chromosomes (Figs 1 and 4) (Yesmin et al., 2019, 2020). It is consistent with the model number of chromosome pairs in most Tephritidae species. The metaphase complement is composed of six pairs of chromosomes: five pairs of sub-metacentric or metacentric autosomes and one pair of sex chromosomes. In female mitotic elements, XX chromosomes present a homologous pair, while in male X and Y chromosomes are separated from each other (Figs 1 and 4). X chromosomes in both species (*B. zonata and Z. tau*) are metacentric and smallest in the karyotype set (Table 2). It contains a dark heterochromatic block in the pericentric region (Table 1). The Y chromosome is square-circle (semi-circle) dot-shaped with highly heterochromatic as shown by the C-banding technique (Figs 1, 3 and 4, *s–u*). These are consistent with most of the tephritid species analysed so far *Bactrocera cucurbitae* (Singh & Gupta, 1984 — as "*Dacus cucurbitae*"); *Ceratitis capitata* (Bedo, 1986; Zacharopoulou, 1987); *Bactrocera oleae* (Mavragani-Tsipidou et al., 1992 — as "*Dacus oleae*"); *Bactrocera* species namely *B. dorsalis*, *Zeugodacus tau* (as "*B. tau*"), *Zeugodacus cucurbitae* (as "*B. cucurbitae*") and *B. correcta* (Hunwattanakul & Baimai, 1994); *B. dorsalis* complex (Baimai et al., 1995, 1996); *Anastrepha* species (Selivon & Perondini, 1997); *B. tryoni* (Zhao et al., 1998); *Z. tau* (Baimai et al., 2000 — as "*B. tau*"); *B. cucurbitae* (Shahjahan & Yesmin, 2002); *A. suspensa* (Cevallos & Nation, 2004); *Anastrepha* fruit fly (Selivon et al., 2005); *A. ludens* (Garcia-Martinez et al., 2009); *Rhagoletis completa* (Drosopoulou et al., 2010); *R. cingulata* (Drosopoulou et al., 2011); *B. dorsalis* (Zacharopoulou et al., 2011 a); *B. cucurbitae* (Zacharopoulou et al., 2011 b) and most *Rhagoletis* species, i. e., *R. pomonella, R. berberidis, R. nova, R. conversa, R. brincidi, R. cerasi* and *R. completa* (Procunier & Smith, 1993; Frias, 2002; Kounatidis et al., 2008; Drosopoulou et al., 2010). In every cases, the authors concluded that all were diploid species with the base chromosome number of 2n = 12.

Sex chromosomes in most tephritid fruit flies are easily identified with their highly heteromorphic appearance as described by Zacharopoulou (1987), Mavragani-Tsipidou et al. (1992), Hunwattanakul and Baimai (1994), Zhao et al. (1998), Baimai et al. (2000) and Mavragani-Tsipidou (2002). The findings of Cevallos and Nation (2004), Selivon et al. (2005), Garcia-Martinez et al. (2009) and Zacharopoulou et al. (2011a, 2011b) also support the present results. Heterochromatin block is accumulated to the centromere region of all autosomes as well as the X chromosome of *B. zonata* and *Z. tau*. Mavragani-Tsipidou et al. (1992) reported that the Y chromosome of *D. oleae* attained with a dark dot-shaped appearance while the X chromosome was found considerably larger in size with densely stained in most parts. Their finding indicated that sex chromosomes (X and Y) of *D. oleae* are somewhat similar with those of Z. *tau*. Moreover, molecular analysis of *B. oleae* Y chromosomes attained a high accumulation of repetitive DNA sequences (Gabrieli et al., 2011) that were constructed by C-heterochromatin (Dimitri et al., 2009). Zhao et al. (Zhao et al., 1998) introduced the X chromosome of *B. tryoni* was larger and highly heterochromatic as densely stained by Giemsa. These are consistent with the present results in case of *Z. tau*'s sex chromosome. Shahjahan and Yesmin (2002) presented

the ideogram of the mitotic karyotypes of *B. cucurbitae*, where they showed the heterochromatin distribution in Sex chromosomes. The X chromosome was almost heterochromatic and the dot like Y chromosome was totally heterochromatic. It also supported the present findings.

Hunwattanakul and Baimai (1994) reported mitotic karyotypes of four species of fruit flies (*Bactrocera*) in Thailand. They found five metacentric (chromosome 2, 3, 4, 6) including X and one submetacentric (chromosome 5) chromosome in *B. tau* karyotype set. Moreover, H-banding showed brighter fluorescence in centromeric regions of all autosomes. Fluorescence reflecting in the pericentric region of X chromosome with a large block of heterochromatin. It is completely similar with the present findings of *Z. tau*. Baimai et al. (2000) published a report on cytological evidence of *B. tau* complex in Thailand where they explained seven karyotype structures under three groups based on the amount and distribution of heterochromatin in sex chromosomes and autosomes. They distinguished species 'A' karyotypes as model species of *B. tau* and compared it to other six species (B–G) karyotypes. All species of the B. tau complex exhibited 2n = 12 mitotic karyotypes compared with other species groups under the genus *Bactrocera* as described earlier by Hunwattanakul and Baimai (1994) and Baimai et al. (1999). They showed that metaphase karyotypes consisted of one heteromorphic sex chromosome (XX in female and XY in male) and five autosome pairs containing different amounts and distribution of C-heterochromatin. Female sex chromosomes (XX) were varied in size and the amount of pericentric heterochromatin. The Y chromosome in all cases was dot-shaped. The autosome pairs on the other hand, were characterised by conspicuous heterochromatin blocks mostly in their pericentric region. Those cytological evidences are somewhat similar with the present findings of *Z. tau* karyotypes, containing different amount of heterochromatin block in their pericentric region of the autosomes, though female sex (one X) chromosome has decorated with a faint telomeric C^+ band in their short arm and Y chromosome appears as square-circle (semi-circle) heterochromatic dot-shaped (Fig. 4).

Similar (*Z. tau*) banding pattern (telomeric C^+ band) of X chromosome was described by Selivon et al. (2005) in *Anastrepha* species. They reported that *A. zenildae*, *A. grandis* and *A. leptozona* have a C^+ band in their telomere region of the X chromosome. X chromosome with a large heterochromatin part also found in other insect species i. e. Drosophila (*D. kikkawai* complex and *D. montium* subgroup), *Anopheles* (*A. dirus* complex and *A. maculates* group), *Bactrocera* (*B. dorsalis* complex and *Zeugodacus* group) by Baimai (1998). These are similar to the present finding of *B. zonata* and *Z. tau*. Bhatnagar et al. (1980) reported the karyotypes of three species of *Dacus* (*Bactrocera*) fruit flies (*D. cucurbitae*, *D. zonatas* and *D. diversus*). They found five pairs of long euchromatic autosomes and one pair of smaller sex chromosomes in these three species. Females were homomorphic (XX) and males were of heteromorphic (XY) sex. In the case of *D. zonatas*, they found three pairs of metacentric (pairs II, III and IV) and two pairs of submetacentric (pairs V and VI) autosomes. There is an inconsistency of autosome pair II, where we find it is of a submetacentric appearance. The X chromosomes in both findings are metacentric and Y chromosomes are dot-shaped. For C-banding,

Bhatnagar et al. (1980) shown that the autosomes were decorated with centromeric heterochromatic blocks (C-bands) where autosome pairs II, V and VI with small C-bands, autosomes pair IV possessed larger C-band and autosome pair III had two C-bands. There are some inconsistencies comparing the present findings. We find comparatively smaller in (pairs 2, 3 and 4) and larger pericentric/centromeric C-band in autosomes (pairs 5 and 6) (Fig. 5, *a*, Table 2). No intercalary or telomeric band is found in the present study. In the X chromosome, they found only centromeric C-band where we find centromeric C-band with a telomeric band in the short arm of X chromosome. Y chromosomes in both cases are highly heterochromatic (Fig. 5, *a*) following the consistency of the present findings.

The cytogenetic evidences of *B. zonata* and *Z. tau* available so far suggest that heterochromatin distribution in mitotic metaphase karyotype plays an important role in karyotypic evolutions, phylogenetic affinity and consequently the evolutionary divergence of sibling/closely related species of these dipteran pests. Therefore, detailed information of heterochromatin accumulation, especially at the molecular level and its evolutionary significance remains intriguing and challenging.

Conclusions

Present study accomplishes the karyotypes of *Bactrocera zonata* and *Zeugodacus tau* in terms of their diploid chromosome numbers (2n = 12) and C-heterochromatin distribution (pericentromeric/centromeric) in autosomes and sex chromosomes. These cytological approaches constitute new tools for chromosomal evidence in taxonomic and evolutionary issues of these tephritid flies. It facilitates the knowledge to markup the boundary of the cryptic and/or sibling species based on the genomic characteristics of these economically important fruit flies.

Declarations

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Conflicts of interests. The authors have no conflict of interests.

Consent: This is hereby ensuring that all authors read and approved to submit the manuscript.

Availability of data and material. Experiments were conducted at the Fruit Fly Lab of Cytology and Biocontrol Research (CBR), Radiation Entomology and Acarology Division (READ), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka, Bangladesh. Therefore, all data and materials were accumulated from our own experiments and are available, if required.

Code availability. Carl Zeiss Zen Blue Software packages were used for snapshot and image processing. Statistical analyses were done with IBM SPSS Version 24 software. Image alignments were done with Microsoft Office PowerPoint 2013 software package.

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REFERENCES

Ata, A. M. 2005. Constitutive heterochromatin diversification of two allium species cultivated in Egypt. *Minia Journal of Agricultural Research and Development*, **25** (4), 663–676.

Baimai, V. 1998. Heterochromatin accumulation and karyotypic evolution in some Dipteran insects. *Zoological Studies*, **37** (2), 75–88.

Baimai, V., Phinchongsakuldit, J. & Sumrandee, C. 2000. Cytological evidence for a complex of species within the taxon *Bactrocera tau* (Diptera: Tephritidae) in Thailand. *Biological Journal of the Linnean Society*, **69**, 399–409. doi: 10.1111/j.1095-8312.2000.tb01213.x

Baimai, V., Phinchongsakuldit, J. & Trnachartvanit, W. 1999. Metaphase karyotypes of fruit flies of Thailand (IV): Evidence for six new species of the *Bactrocera dorsalis* complex. *Cytologia*, **64** (4), 371–377. doi: 10.1508/cytologia.64.371

Baimai, V., Trinachartvanit, W., Tigvattananont, S. & Grote, P. J. 1996. Metaphase karyotypes of fruit flies of Thailand. II. Five species of four subgenera of *Bactrocera*. *Journal of the Science Society of Thailand*, **22**, 97–104.

Baimai, V., Trinachartvanit, W., Tigvattananont, S., Grote, P. J., Poramarcom, R. & Kijchalao, U. 1995. Metaphase karyotypes of fruit flies of Thailand. I. Five sibling species of the *Bactrocera dorsalis* complex. *Genome*, **38** (5), 1015–1022. https://doi.org/10.1139/g95-134

Bauchan, G. R. & Hossain, M. A. 1999. Constitutive heterochromatin DNA polymorphism in diploid *Medicago sativa* spp. Falcate. *Genome*, **42** (5), 930–935. doi: 10.1139/g99-038

Bedo, D. G. 1986. Polytene and mitotic chromosome analysis in *Ceratitis capitata* (Diptera: Tephritidae). *Canadian Journal of Genetics and Cytology*, **28** (2), 180–188. https://doi.org/10.1139/g86-025

Bhatnagar, S., Kaul, D. & Caturvedi, R. 1980. Chromosomal studies in three species of the genus *Dacus* (Trypetidae: Diptera). *Genetica*, **54**, 11–15. https://doi.org/10.1007/BF00122402

Bonaccorsi, S. & Lohe, A. 1991. Fine mapping of satellite DNA sequences along the Y chromosome of *Drosophila melanogaster*: relationships between the satellite sequences and fertility factors. *Genetics*, **129** (1), 177–189. PMID: 1936957

Buonomo, S. B. C. 2010. Heterochromatin DNA replication and Rif1. *Experimental Cell Research,* **316,**(12), 1907–1913. https://doi.org/10.1016/j.yexcr.2010.03.015

Cáceres, C., Segura, D.F., Teresa Vera, M., Wornoayporn, V., Cladera, J. L., Teal, P., Sapountzis, P., Bourtzis, K., Zacharopoulou, A. & Robinson, A. S. 2009. Incipient speciation revealed in *Anastrepha fraterculus* (Diptera: Tephritidae) by studies on mating compatibility, sex pheromones, hybridization, and cytology. *Biological Journal of the Linnean Society*, **97** (1), 152–165. https://doi.org/10.1111/j.1095-8312.2008.01193.x

Cevallos, V. E. & Nation, J. L. 2004. Chromosomes of the Caribbean fruit fly (Diptera: Tephritidae). *Florida Entomologist*, **87** (3), 361–364. https://doi.org/10.1653/0015-4040(2004)087[0361:COTCFF]2.0.CO;2

Dimitri, P., Caizzi, R., Giordano, E., Accardo, M. C., Lattanzi, G. & Baimonti, G. 2009. Constitutive heterochromatin: a surprising variety of expressed sequences. *Chromosoma*, **118** (4), 419–435. https://doi.org/10.1007/s00412-009-0211-y

Drosopoulou, E., Augustinos, A. A., Nakou, I., Koeppler, K., Kounatidis, I., Vogt, H., Papadopoulos, N. T., Bourtzis, K. & Mavragani-Tsipidou, P. 2011. Genetic and cytogenetic analysis of the American cherry fruit fly, *Rhagoletis cingulata* (Diptera: Tephritidae). *Genetica*, **139** (11–12), 1449–1464. doi: 10.1007/s10709-012-9644-y

Drosopoulou, E., Koeppler, K., Kounatidis, I., Nakou, I., Papadopoulos, N. T. & Mavragani-Tsipidou, P. 2010. Genetic and cytogenetic analysis of the walnut-husk fly (Diptera: Tephritidae). *Annals of the Entomological Society of America*, **103** (6), 1003–1011. https://doi.org/10.1603/AN10059

Elder, J. F. & Turner, B. J. 1995. Concerted evolution of repetitive DNA sequences in eukaryotes. *The Quarterly Review of Biology*, **70** (3), 297–320. doi: 10.1086/419073.

Falistocco, E., Falcinelli, M. & Veronesi, F. 1995. Karyotype and C-banding pattern of mitotic chromosomes in alfalfa, *Medicago sativa* L. *Plant Breed*, **114** (5), 451–453. doi: 10.1111/j.1439-0523.1995.tb00831.x

Frias, D. 2002. Importance of larval morphology and heterochromatic variation in the identification and evolution of sibling species in the genus *Rhagoletis* (Diptera: Tephritidae) in Chile. *Proceedings of the 6th International Fruit Fly Symposium*. Stellenbosch, Irene, South Africa, Isteg Scientific Publications, 267–276.

Gabrieli, P., Gomulski, L. M., Bonomi, A., Siciliano, P., Scolari, F., Franz, G., Jessup, A., Malacrida, A. R. & Gasperi, G. 2011. Interchromosomal duplications on the *Bactrocera oleae* Y chromosome imply a distinct evolutionary origin of the sex chromosomes compared to *Drosophila*. *PlosOne*, **6** (3), e17747. doi: 10.1371/journal.pone.0017747

Garcia-Martinez, V., Hernandez-Ortiz, E., Zepeda-Cisneros, C. S., Robinson, A. S., Zacharopoulou, A. & Franz, G. 2009. Mitotic and Polytene analysis in the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae). *Genome*, **52** (1), 20–30. doi: 10.1139/G08-099

Gatti, M. & Pimpinelli, S. 1992. Functional elements in *Drosophila melanogaster* heterochromatin. *Annual Review of Genetics*, **26**, 239–275. https://doi.org/10.1146/annurev.ge.26.120192.001323

Giardini, M. C., Milla, F. H., Lanzavecchia, S., Nieves, M. & Cladera, J. L. 2015. Sex chromosomes in mitotic and polytene tissues of *Anastrepha fraterculus* (Diptera, Tephritidae) from Argentina: a review. *Zookeys*, **540**, 83–94. https://doi.org/10.3897/zookeys.540.6058

Heitz, E. 1928. Das heterochromatin der moose. *Jahrbucher für Wissenschaftliche Botanik*, **69**, 762–818.

Hernández-Ortiz, V., Bartolucci, A. F., Morales-Valles, P., Frias, D. & Selivon, D. 2012. Cryptic species of the *Anastrepha fraterculus* complex (Diptera: Tephritidae): a multivariate approach for the recognition of South American morphotypes. *Annals of the Entomological Society of America*, **105** (2), 305–318. https://doi.org/10.1603/AN11123

Hunwattanakul, N. & Baimai, V. 1994. Mitotic karyotypes of four species of fruit flies (*Bactrocera*) in Thailand. *Kasetsart Journal (Natural Science)*, **28** (1), 142–148. ISSN: 0075-5192

Irick, H. 1994. A new function for heterochromatin. *Chromosoma*, **103** (1), 1–3. https://doi.org/10.1007/BF00364720.

John, B. 1981. *Heterochromatin variation in natural populations, Chromosome Today*. 7. George Allen & Unwin, 128–137.

Kounatidis, I., Papadopoulos, N., Bourtzis, K. & Mavragani-Tsipidou, P. 2008. Genetic and cytogenetic analysis of the fruit fly *R hagoletis cerasi* (Diptera: Tephritidae). *Genome*, **51**(7): 479–491. https://doi.org/10.1139/G08-032

Kuvangkadilok, C., Boonkemtong, C. & Phayuhasena, S. 1998. C-banding in polytene chromosomes of six *Simulium* species (Diptera: Simuliidae) from Doi inthanon national park, Northern Thailand. *Journal of the Science Society of Thailand*, **24**, 245–250.

Levan, A., Fredga, K. & Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. *Heriditas*, **52** (2), 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Liu, X., & Ji, Q. 2024. Review of *Zeugodacus tau* (Walker) (Diptera: Tephritidae): Blogical characteristics and control strategy. *CABI Agric Biosci* **5**, 90. https://doi.org/10.1186/s43170-024-00284-5

Lohe, A. R., Hilliker, A. J. & Roberts, P. A. 1993. Mapping simple repeated DNA sequences in heterochromatin of *Drosophila melanogaster*. *Genetics*, **134** (4), 1149–1174. https://doi.org/10.1016/0168-9525(93)90135-5

Mavragani-Tsipidou, P. 2002. Genetic and cytogenetic analysis of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae). *Genetica*, **116** (1), 45–57. doi: 10.1023/a:1020907624816

Mavragani-Tsipidou, P., Karamanlidou, G., Zacharopoulou, A., Koliais, S. & Kastritsis, C. 1992. Mitotic and polytene chromosome analysis in *Dacus oleae* (Diptera: Tephritidae). *Genome*, **35** (3), 373–378. https://doi.org/10.1139/g92-056

Pardue, M. L. & Hennig, W. 1990. Heterochromatin: Junk or collectors item? *Chromosoma*, **100** (1), 3–7. https://doi.org/10.1007/BF00337597

Procunier, W. S. & Smith, J. J. 1993. Localization of ribosomal DNA in *Rhagoletis pomonella* (Diptera, Tephritidae) by in situ hybridization. *Insect Molecular Biology*, **2** (3), 163–174. https://doi.org/10.1111/j.1365-2583.1993.tb00136.x

Ranganath, H. A. & Hagele, K. 1982. The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. n. albomicana*. I. Distribution and differentiation of heterochromatin. *Chromosoma*, **85** (1), 83–92. https://doi.org/10.1007/BF00344595

Schutze, M. K. et al. 2015. Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioral and chemoecological data. *Systematic Entomology,* **40** (2), 456–471. https://doi.org/10.1111/syen.12113.

Selivon, D. & Perondini, A. L. P. 1997. Evaluation of techniques for C and ASG banding of the mitotic chromosomes of *Anastrepha* species (Diptera: Tephritidae). *Brazilian Journal of Genetics*, **20** (4), 651–653. http://dx.doi.org/10.1590/S0100-84551997000400015

Selivon, D., Perondini, A. L. P., Rocha, L. S. 2005. Karyotype characterization of *Anastrepha* fruit flies (Diptera: Tephritidae). *Neotropical Entomology*, **34** (2), 273–279. https://doi.org/10.1590/S1519-566X2005000200015

Shahjahan, R. M. & Yesmin, F. 2002. Polytene chromosome maps of the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). *Genome*, **45** (6), 1167–1174. doi: 10.1139/g02-081

Singh, O. P. & Gupta, J. P. 1984. Studies on mitotic and salivary chromosomes of *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). *Genetica*, **62** (3), 217–221. https://doi.org/10.1007/BF00056439

Stratikopoulos, E. E., Augustinos, A. A., Pavlopoulos, I., Economou, K. P. H., Mintzas, A., Mathiopoulos, K. D. & Zacharopoulou, A. 2009. Isolation and characterization of microsatellite markers from the Mediterranean fruit fly, *Ceratitis capitata*: cross-species amplification in other Tephritidae species reveals a varying degree of motif conservation. *Molecular Genetics and Genomics*, **282** (3), 283–306. doi: 10.1007/s00438-009-0465-3

Sullivan, B. A. 2013. Centromeres. *In*: Lennarz, W. J., & Lane, M. D., eds. *Encyclopedia of Biological Chemistry.* Elsevier Inc., Academic Press, 446–450. https://doi.org/10.1016/B978-0-12-378630-2.00471-0

Sumner, A. T. 1990. *Chromosome Banding*, Unwin Hyman Ltd., London, UK.

Tsoumani, K. T., Augustinos, A. A., Kakani, E. G., Drosopoulou, E., Mavragani-Tsipidou, P. & Mathiopoulos, K. D. 2011. Isolation, annotation and applications of expressed sequence tags from the olive fly, *Bactrocera oleae*. *Molecular Genetics and Genomics*, **285** (1), 33–45. doi: 10.1007/s00438-010-0583-y

Tuna, M., Vogel, K. P., Arumuganathan, K. & Gill, K. 2004. C-banding analyses of *Bromus inermis* genomes. *Crop Science,* **44** (1), 31–37. doi: 10.2135/cropsci2004.3100

White, I. M. & Elson-Harris, M. M. 1992. *Fruit flies of economic significance: their identification and bionomics*. CAB international, Wallingford, Oxon, UK. https://doi.org/10.1093/ee/22.6.1408

Wichman, H. A., Payne, C. T., Ryder, O. A., Hamilton, M. J., Maltbie, M. & Baker, R. J. 1990. Genomic distribution of heterochromatic sequences in Equids: implications to rapid chromosomal evolution. *Journal of Heredity*, **82** (5), 369–377. doi: 10.1093/oxfordjournals.jhered.a111106

Yasuhara, J. C. & Wakimoto, B. T. 2008. Molecular landscape of modified histones in *Drosophila* heterochromatic Genes and euchromatic-heterochromatin transition zones. *PLOS Genetics*, **4** (1), e16. https://doi.org/10.1371/journal.pgen.0040016

Yesmin, F. 2013. *Chromosomes of Bactrocera papayae Drew and Hancock and Bactrocera carambolae Drew and Hancock (Diptera: Tephritidae): Mitotic metaphase karyotype and polytene genome*. PhD thesis, UKM, Malaysia.

Yesmin, F., Uddin, M. N., Rahman, G. M. S. & Hasanuzzaman, M. 2019. Identification of larval salivary gland polytene chromosomes of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Journal of Biological Control*, **33** (3), 295–302. doi: 10.18311/jbc/2019/22718

Yesmin, F., Haymer, D., Uddin, M. N. & Hasanuzzaman, M. 2025. Cytological characterisation of autosomes and sex chromosomes in tephritid fruit flies *Bactrocera zonata* and *Zeugodacus tau* (Diptera, Tephritidae). *Zoodiversity*, **59** (1), 21–34. https://doi.org/10.15407/zoo2025.01.021

Zacharopoulou, A. 1987. Cytogenetic analysis of mitotic and salivary gland chromosomes in the Medfly *Ceratitis capitata*. *Genome*, **29** (1), 67–71. https://doi.org/10.1139/g87-011

Zacharopoulou, A., Augustinos, A. A., Sayed, W., Robinson, A. S. & Franz, G. 2011 a. Mitotic and polytene chromosomes analysis of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Genetica*, **139** (1), 79–90. https://doi.org/10.1007/s10709-010-9495-3

Zacharopoulou, A., Sayed, W. A. A., Augustinos, A. A., Yesmin, F., Robinson, A. S. & Franz, G. 2011 b. Analysis of Mitotic and polytene chromosomes and photographic polytene chromosome maps in *Bactrocera cucurbitae* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, **104** (2), 306–318. https://doi.org/10.1603/AN10113

Zhao, J. T., Frommer, M., Sved, J. A. & Zacharopoulou, A. 1998. Mitotic and polytene chromosome analysis in the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae). *Genome*, **41** (4), 510–526. https://doi.org/10.1139/g98-053

Zingore, K. M., Sithole, G., Abdel-Rahman, E. M., Mohamed, S. A., Ekesi, S., Tanga, C. M. et al. 2020. Global risk of invasion by *Bactrocera zonata*: Implications on horticultural crop production under changing climatic conditions. *PLoS ONE*, **15** (12), e0243047. https://doi.org/10.1371/journal.pone.0243047

Zuckerkandi, E. & Hennig, W. 1995. Tracking heterochromatin. *Chromosoma*, **104** (2), 75–83. doi.org/10.1007/BF00347689

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