

POLYTENE CHROMOSOME MAP OF THREE *DROSOPHILA* SPECIES BASED ON BANDING PATTERN

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ABSTRACT: In this study, polytene chromosome maps of three *Drosophila* species namely *D. ananassae*, *D. bipectinata*, and *D. melanogaster* belonging to the melanogaster group were constructed based on their banding patterns obtained through analysis of images generated by aceto-orcein staining of salivary gland chromosomes. The nuclei of the salivary gland cells in each of the three *Drosophila* species contained six chromosome arms (X, 2L, 2R, 3L, 3R, and 4). The chromosomal inversion number and banding patterns of six chromosome arms were slightly changed among *D. melanogaster*, *D. ananassae* and *D. bipectinata*. Banding patterns were similar in *D. melanogaster* and *D. ananassae*. However, *D. bipectinata* polytene chromosome banding pattern were slightly different from that of *D. melanogaster* and *D. ananassae*. Chromosome number 4 was detected as the smallest among the *Drosophila* chromosomes. The species-specific polytene chromosome banding patterns can be valuable tools for chromosomal aberration detection. Thus, the results might provide a background to study their evolutionary history, genetic diversity, and phylogenetic relationships.

Key words: Polytene chromosome, Banding pattern, Chromosome map, Chromosomal inversion.

INTRODUCTION

Polytene chromosomes are gigantic chromosomes commonly known as salivary gland chromosomes, mostly found in dipteran insects. Polytene chromosome maps are used to study genetic diversity in species and to analyze the overall cytological arrangement of transcription and replication (Zhimulev *et al.* 2004). Dobzhansky and Sturtevant (1938) and Dobzhansky and Epling (1944) conducted groundbreaking research on *Drosophila pseudoobscura* and related species, utilizing polytene chromosomal banding patterns to create phylogenies across many species groups and subgroups.

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The significant chromosomal structure changes brought about by chromosomal inversions in natural populations exhibit sharp frequency variations that frequently correlate to temporal and spatial climatic trends, indicating that they may be used to track the effects of global warming (Rezende *et al.* 2010). According to Hoffman and Rieseberg (2008), genetic diversity in the genes linked to the inversions may be a major factor in population divergence and speciation. Chromosome inversion polymorphisms in *Drosophila* species have been the subject of extensive global research (Dobzhansky and Sturtevant, 1938). Lemeunier and Ashburner (1976) showed the phylogenetic relationships between six *melanogaster* species subgroup species based upon polytene chromosome banding. The building of phylogenetic links was made possible by cytological techniques, which revealed polymorphism in natural populations of *D. pseudoobscura* (Dobzhansky 1937). Moreover, the distinct dark and thin banding patterns have been used to construct chromosome map and in taxonomic identification (Alanen 1981). Polytene chromosome preparations from three *Drosophila* species, *D. melanogaster*, *D. ananassae* and *D. bipectinata* were analyzed to construct chromosome maps for each species. Then, the chromosome maps were compared to find out the relationship of these species in terms of polytene chromosome banding patterns.

MATERIAL AND METHODS

Three species of *Drosophila* were collected from three different geographical locations of Bangladesh such as, *D. ananassae* from Satchari (Sylhet), *D. melanogaster* from Hiron point (Sundarban), and *D. bipectinata* from Dohar (Dhaka).

Orcein was used in the form of a 1% solution in 45% acetic acid. This solution was prepared by pouring 55 mL boiling glacial acetic acid over 1gm orcein powder. The solution was cooled, 45 mL of distilled water was added, and filtered. This solution was unstable, so it needed to be prepared fresh before use. The staining procedure was like the aceto-carmin method (Cai *et al.* 2010). For salivary gland dissections forceps were used to remove 8-10 actively crawling larvae from the sides of the rearing vials. The larvae were placed on a drop of saline solution on a microscope slide. Salivary glands were dissected and separated under a stereomicroscope. A segment of the salivary gland was placed on a fresh slide. Then a drop of 45% acetic acid fixative was added to it. Then the glands were transferred immediately to the drop of aceto-orcein stain on the slide. A clean cover slip was placed onto the surface of the glands and forceps were used to gently tap the surface of the coverslip in a circular pattern to facilitate chromosomal spreading (Smith. 1947).

Polytene chromosomes were seen through a light microscope at 10x or above magnifications. At 40x magnification, the band and interband became visualized. Well-spread chromosomes were examined and photographed using a Bio-blue microscope (BB1152-PL, Euromex Bio-blue) with 100x magnification. Best photographs showing the clean morphology were used for the construction of composite chromosomes using Adobe Photoshop cascading style sheets CSS and Picasa photo editor. The best pictures were selected to construct a chromosome map. Each arm of a polytene chromosome was separated and cut into a linear form by a photo editor. Each chromosome arm was labeled with its band number. The same arm of polytene chromosome was compared with other *Drosophila* polytene chromosome arms.

RESULTS AND DISCUSSION

Polytene chromosome preparations from three *Drosophila* species *viz.*, *D. melanogaster* (Fig 1. A-C), *D. ananassae* (Fig 1 D - F), and *D. bipectinata* (Fig.1 G-I) were analyzed to find the banding pattern. In our study, six chromosome arms (X, 2L, 2R, 3L, 3R and 4) were detected in polytene chromosomes of all three studied *Drosophila* species. Bands were counted in each polytene chromosome arm of three *Drosophila* species. In *D. melanogaster*, 21, 51, 29, 37, and 72 bands were detected in X, 2L, 2R, 3L, and 3R chromosomes respectively (Fig.2 and Table 1). Among them, the X chromosome was the shortest and the 3R chromosome was the longest. Band 1 is related to the centromere, and asynapsis was found between 2-8 bands in the 2R chromosome. Chromosome inversion was found between 6-7,19-20,31-33 bands in the 3L arm (Fig.2) and 35-36 and 59-60,65-66 bands in the 3R arm (Fig.2). In *D. ananassae*, 20, 50, 39, 60, and 51 bands were detected in X, 2L, 2R, 3L, and 3R chromosomes respectively (Fig.3 and Table 1). Among them, the X chromosome was the shortest and the 3L chromosome was the longest. Band numbers 24, 25, 32, 40, 45, and 46 indicated weak points in the 2L chromosome arm, and 27 and 36 indicated the dotted band in the 2R chromosome arm of *D. ananassae* (Fig. 3 and Table 1).

In *D. bipectinata*, 21, 40, 43, 43, and 41 bands were detected in X, 2L, 2R, 3L, and 3R chromosomes respectively (Fig.4 and Table 1). Among them, the X chromosome was the shortest, and the 3R chromosome was the longest. Band numbers 16-18 indicated inversion in the 2L chromosome arm, and asynapsis was found between 2 and 3 bands in the 2R chromosome arms of *D. bipectinata* (Fig. 4). *D. melanogaster*, *D. ananassae*, and *D. bipectinata* contained 21, 20 and 21 bands in the X arm, 51, 50 and 40 in the 2L arm, 29, 39 and 41 in the 2R, 37, 60, 43 in the 3L arm and 72, 51, 41 in 3R arm of polytene chromosomes respectively (Table 1). Dark stain band regions of X, 2L, 2R, 3L, 3R chromosome

Table 1. Polytene chromosome bands of three *Drosophila* species

Spe	Chromosome arm	Thin band region	Dark band region	Number of bands in each arm	Total number of bands in each species
<i>D. melanogaster</i>	X	4, 17, 18	1* , 6 , 7 , 10, 13 , 16, 20 , 21	21	210
	2L	10, 11, 20, 29, 37, 38	1, 2, 6 , 7 , 24 , 25 , 30, 32, 33 , 34 , 35, 40, 48 , 51	51	
	2R	1, 4-8, 12, 24, 28	2 , 11 , 13, 14 , 21 , 22 , 25 , 26 , 27 , 29	29	
	3L	12-15, 28-31, 34-37	1 , 2 , 4 , 5 , 8 , 11, 20, 22, 23, 24 , 33 , 35	37	
	3R	2-7, 14-17, 23-26, 39-42, 54-59, 66-71	1, 9 , 10, 11 , 13 , 18, 21 , 22, 20, 22, 30, 38, 43, 50, 53, 60 , 62 , 63 , 69, 72	72	
<i>D. ananassae</i>	X	2, 3, 5, 6, 10, 11, 12, 15, 16, 17	1 , 7 , 8 , 9, 20	20	220
	2L	7-9, 11-23, 30, 33-35, 42, 43, 45	2 , 3 , 4 , 5, 6, 10, 28 , 29, 38 , 39 , 41, 44, 48	50	
	2R	2-5, 8-14, 30-35, 37	1 , 6 , 7 , 10, 15 , 18, 19 , 20, 23 , 24, 28, 38, 39	39	
	3L	6-9, 11, 17, 18, 19, 20, 22, 23, 24, 25, 30, 33-38, 53-55, 57, 58	1 , 2 , 3, 4, 5 , 10, 12, 14, 15, 16, 21, 26, 29, 31, 32, 34, 39, 44, 46, 49, 52, 59, 60	60	
	3R	7, 11, 20, 22, 23, 37, 45, 49, 50	1, 2 , 3, 4, 8, 9 , 10 , 12 , 12, 14 , 17 , 18, 21, 27, 29-33	51	
<i>D. bipectinata</i>	X	1, 8, 9, 12	2 , 3, 4, 5, 6 , 10, 11 , 13, 14, 15, 16 , 17 , 19, 20, 21	21	188
	2L	9, 10, 12, 15, 18, 21-25, 27, 28, 31-34, 36, 37, 40	1 , 2 , 5, 7, 13 , 17 , 19, 26, 29, 30 , 35, 39	40	
	2R	1, 2, 6, 14, 19-21, 23, 24, 25, 26, 27-30, 36	1, 4, 5 , 10 , 15, 16 , 17, 22, 31, 32, 35, 38, 39, 43	43	
	3L	2, 3, 4, 7, 11, 26, 31, 36, 38, 40	1 , 5 , 10 , 23 , 32 , 34, 37 , 39, 42, 43	43	
	3R	2, 5, 6, 7, 14, 18, 26, 31, 32, 41	1 , 3, 4, 9, 12 , 13 , 15, 16, 17, 19, 20, 22, 23, 27, 30 , 35 , 37	41	

*Highly dark bands are in bold numbers.

arm of *D. melanogaster*, *D. ananassae*, and *D. bipectinata* are presented in Table 1. Based on band number, the second and third chromosomes of *D. bipectinata* were shorter than those of *D. melanogaster* and *D. ananassae*. The largest chromosome arm was (3R) in *D. melanogaster* and 3L chromosome in *D. ananassae*. However, the third arm of *D. ananassae* contained more total band than that of *D. melanogaster*. Band numbers varied among the species. The highest and lowest band numbers were detected in *D. melanogaster* and *D. bipectinata* respectively (Table.1). Chromosome arm 3L contained more bands

than 3R chromosome arm in *D. melanogaster* (Fig.2). But, 3R chromosome arm contained more bands than 3L chromosome arm in *D. bipectinata* and *D. ananassae* (Fig.3 and Fig.4). Total band number found in third polytene chromosome arm of three *Drosophila* species, *D. melanogaster*, *D. ananassae* and *D. bipectinata* was 109, 111 and 84, respectively (Table 1).

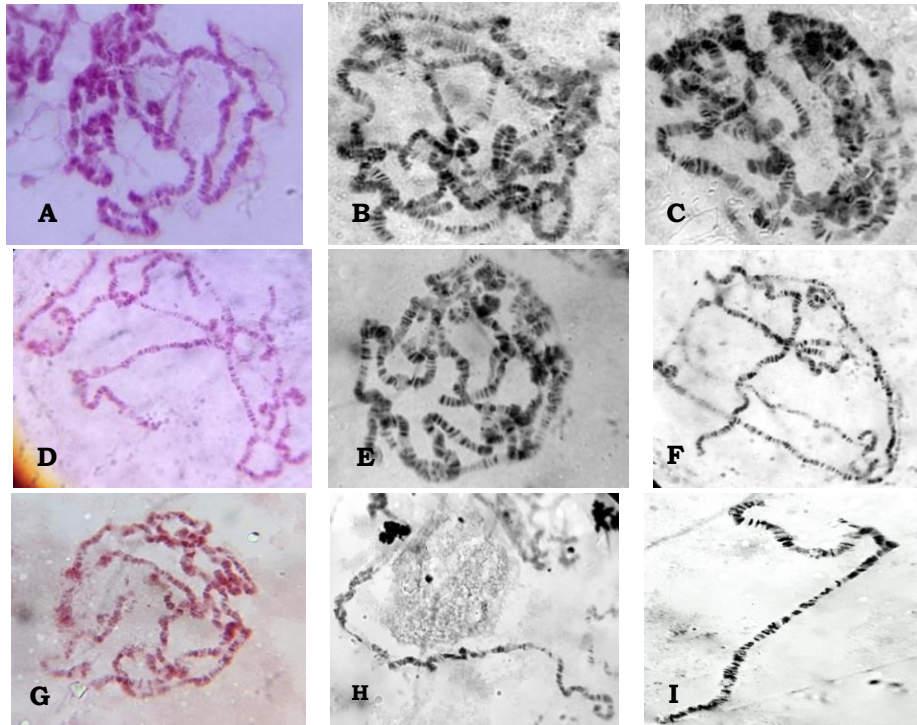


Fig. 1. Polytene chromosomes of three *Drosophila* species. Selected images of polytene chromosome preparations of *D. melanogaster*, *D. ananassae* and *D. bipectinata* are presented in A-C, D-F, and G-I, respectively. Orcein stained chromosomes observed at 1000 \times .

It could be detected that X, 2L and 3R chromosome arms in *D. melanogaster* contained more bands than that of *D. ananassae* and *D. bipectinata* chromosome (Fig.2, Fig.3, Fig.4 and Table 1). 2R and 3L chromosome arms of *D. ananassae* contained more bands than that of others (Fig.3 and Table 1). The band-interband, dark and thin band arrangement patterns showed some difference in every chromosome map (Table 1). Polytene chromosomes, composed of thousands of DNA strands, play crucial roles in various tissue functions as in the salivary gland (Kuzin *et al.*, 2002). Researchers have extensively explored the structural, functional, and genetic aspects of polytene chromosomes (Zhimulev, 1999; Zhimulev and Belyaeva, 1974; Semeshin, 2004), as well as the heterochromatin within them in *D. melanogaster* (Koltzoff, 1934;

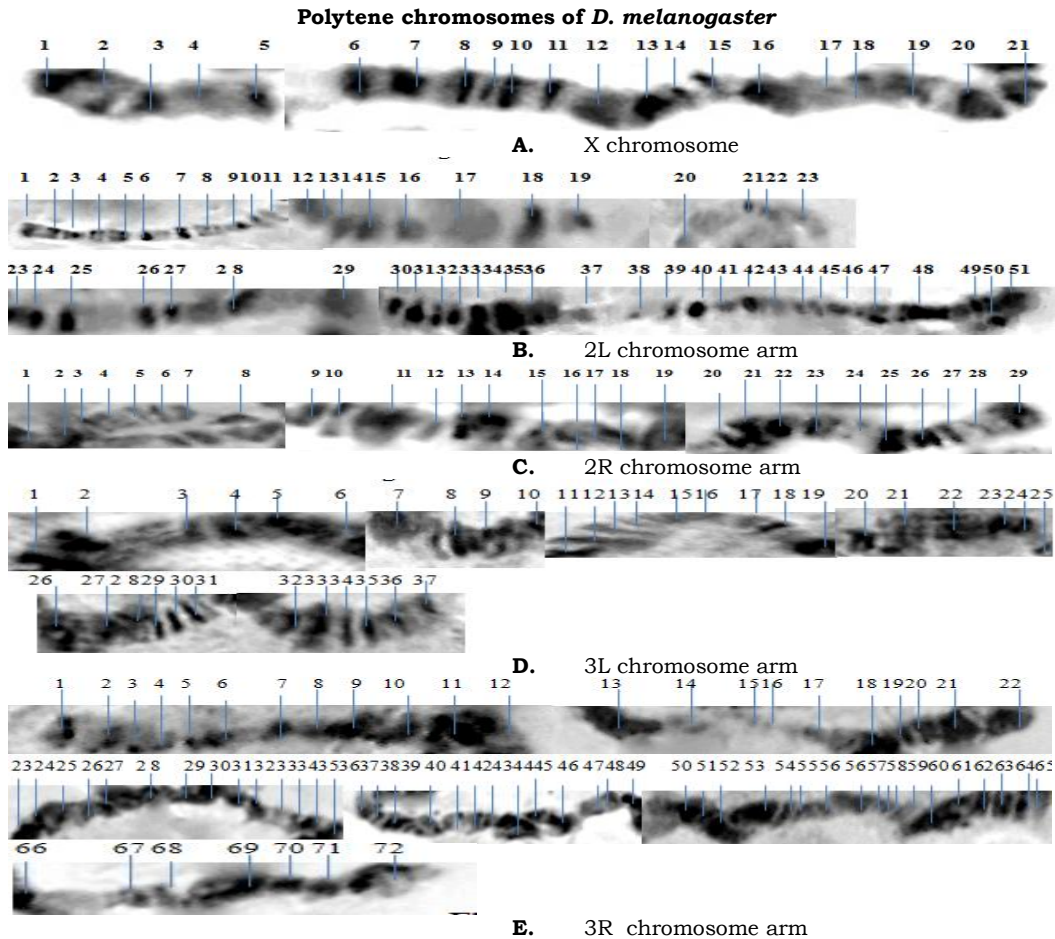
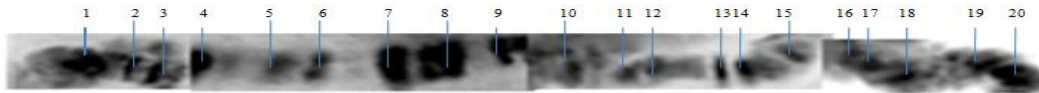


Fig. 2. Polytene chromosome of *D. melanogaster*. A, B, C, D, and E present X, 2L, 2R, 3L, and 3R polytene chromosome arms, respectively. Orcein stained chromosome arms observed at 1000 \times (increased from original magnification).

Belyaeva *et al.*, 2008). In this study, the numbers of thin and thick bands on each chromosome arm of three *Drosophila* species—*D. melanogaster*, *D. ananassae*, and *D. bipectinata* are detailed (Fig. 2-4 and Table 1). *Drosophila* polytene chromosomes exhibit over 5000 distinct bands when study using different methods (Zykova *et al.*). Our investigation specifically quantifies total band counts in the polytene chromosomes of these three species as follows: 210 for *D. melanogaster*, 220 for *D. ananassae*, and 188 for *D. bipectinata* (Fig. 2-4 and Table 1). Early studies by Bridges (1935, 1938, 1939, 1941, 1942) provided foundational chromosome maps of *Drosophila melanogaster*, detailing individual chromosome arms and their features. Notably, Bridges (1935) described chromosomes 4 and 3R as the shortest and longest chromosomes, respectively, aligning with findings from our present study.

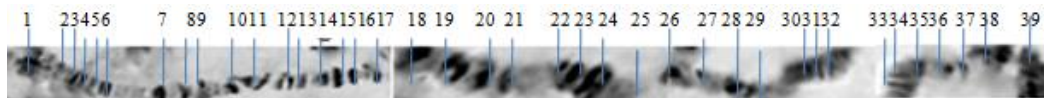
Polytene chromosome of *D. ananassae*



F. X chromosome



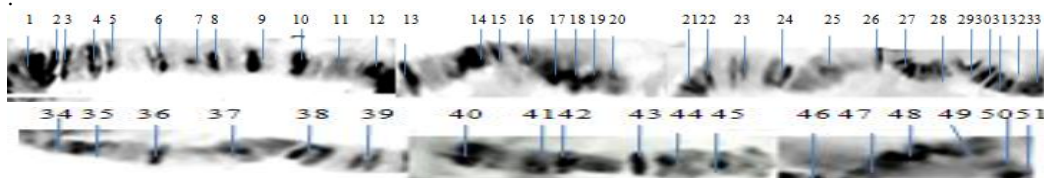
G. 2L chromosome



H. 2R chromosome



I. 3L chromosome



J. 3R chromosome

Fig.3. Polytene chromosome of *D. ananassae*. F, G, H, I, and J present X, 2L, 2R, 3L, and 3R polytene chromosome arms, respectively. Orcein stained chromosome arms observed at 1000× (increased from original magnification).

The banding pattern of polytene chromosomes showed a similarity between *D. melanogaster* and *D. ananassae*. However, there were slight differences in the banding pattern of *D. bipectinata* compared to *D. melanogaster* and *D. ananassae*. These findings suggest a closer genetic relationship between *D. melanogaster* and *D. ananassae* than between either of these species and *D. bipectinata*.

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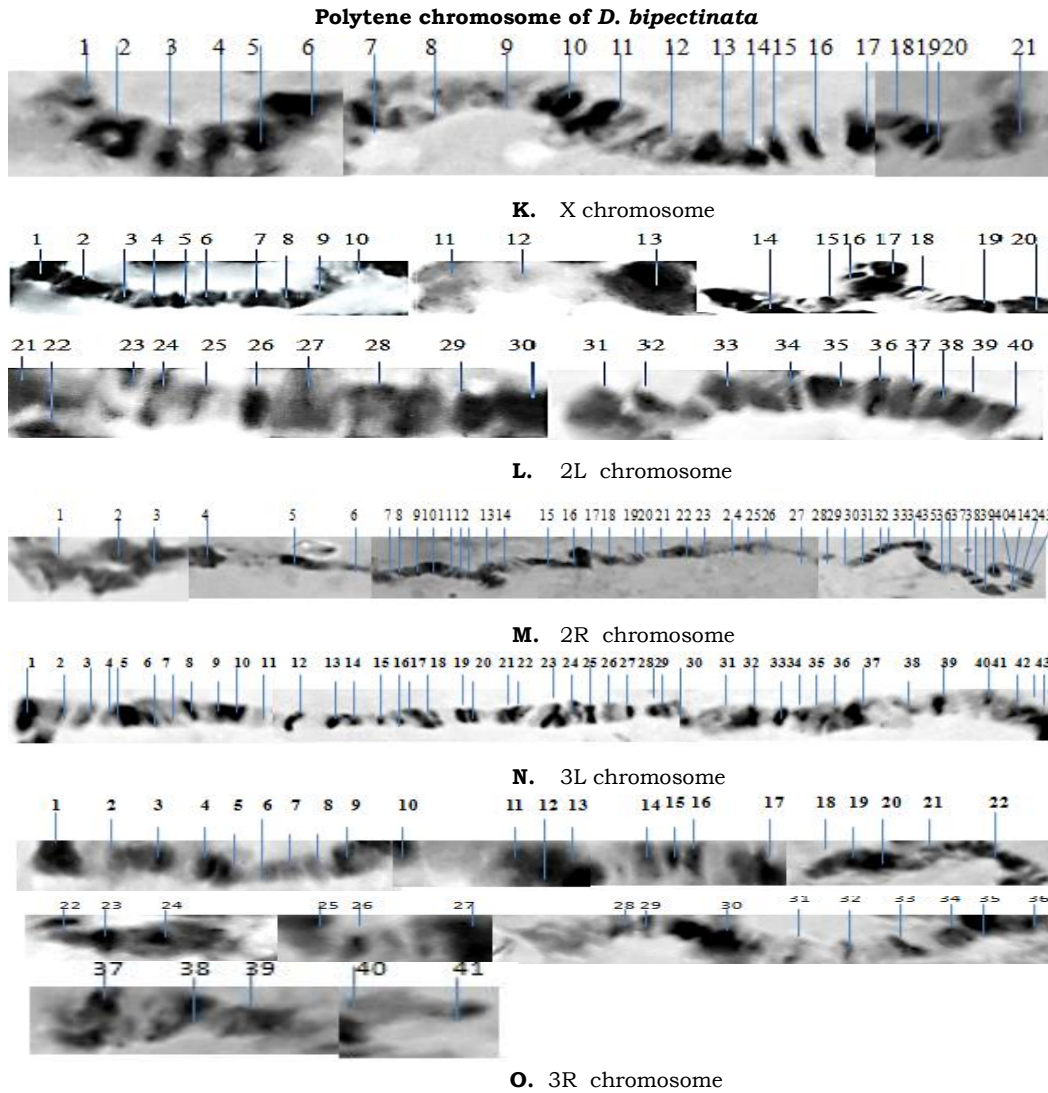


Fig. 4. Polytene chromosome of *D. bipectinata*. K, L, M, N, and O present X, 2L, 2R, 3L, and 3R polytene chromosome arms, respectively. Orcein stained chromosome arms observed at 1000 \times (increased from original magnification).

CONCLUSION

Banding patterns and band interband numbers of six chromosome arms of polytene chromosomes were slightly changed among *D. melanogaster*, *D.*

ananassae, and *D. bipectinata*. The polytene chromosomal inversion number, dark and thin band number, and chromosome arm differences are also slightly variable among these *Drosophila* species. However, other experimental procedures using fluorescence staining and high-resolution imaging could be instrumental in revealing actual positions and number of bands and interbands. Still, this study could be used as a background in future studies of Bangladeshi *Drosophila* species.

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