

Polytene Chromosomes in Salivary Gland of Second Instar Larvae of the Melon Fly, *Bactrocera cucurbitae* (Coquillette) (Diptera: Tephritidae)

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Abstract: The present investigation constitutes a first attempt to study the salivary gland polytene chromosomes of the second instar larvae of the melon fly, *Bactrocera cucurbitae* (Coquillette). A photographic representation of the polytene chromosomes is provided. The identifying tips and banding pattern as well as some important landmarks are described. The whole polytene genome has been mapped by dividing it into 100 sections and the sub sections were lettered. The banding pattern of polytene chromosomes was very clear at this stage and readily workable as cytological material.

Key words: Insect pest control • Sterile Insect Technique • Agricultural pest

INTRODUCTION

The melon fly, *Bactrocera cucurbitae* (Coquillette), a species belonging to the Tephritidae family of Diptera, is one of the major agricultural pests in fruits and vegetable producing countries. They have more than 80 hosts and are major pests of beans, bittermelon, Chinese wax gourd, cucumbers, edible gourds, eggplant, green beans, hyotan, luffa, melons, peppers, pumpkins, squashes, togan, tomatoes, watermelon and zucchini [1]. Originally from the Indo-Malayan region, the fly was introduced into Hawaii from Japan in 1895. It is found in Australia, Burma, Srilanka, Formosa, Guam, Hawaii, India, Bangladesh, Japan, Java, Kenya, Malaya, Mauritius Island, Philippines, Singapore, Southern China and Timor Island [2].

The damage to crops caused by melon flies result from 1) oviposition in fruit and soft tissues of vegetative parts of hosts, 2) feeding by the larvae and 3) decomposition of plant tissue by invading secondary microorganisms. Larval feeding damage in fruits is the most harmful. The annual economic losses are very severe [3]. About 5.5x10⁶ t of vegetables and 2x10⁶ ton of fruit are produced in Bangladesh annually. Usual loss of fruits and vegetables to insects ranges from 15 to 20%. In some cases, it causes a total loss of the crop [4]. Considering the situation the Govt. of Bangladesh has taken eradication plan of fruit fly pests under the National R&D (Research & Development) program.

During the last decades, there has been considerable interest in developing procedures and methods for the biological control of this pest such as the use of natural enemies, mass trapping systems and the sterile insect technique (SIT). Currently, the management and control of this pest is carried out using either cover spray (with organophosphorous insecticides) or bait sprays (with protein hydrolysate and insecticide which attract and kill the adults). However, the heavy reliance on chemical insecticides with the widespread environmental pollution, the presence of pesticide residues in food and water and their possible implications for human welfare and health [5], together with the absence of species-specific mode of action call for more environment-friendly techniques and strategies. The development of environment-friendly genetic methods of control, which have the major advantage of species specificity, can only be realized when there is a thorough understanding and knowledge of the biology, ecology and the genetics of the target pest. The sterile insect technique (SIT) is an area wide strategy for the control of pest insects based on the irradiation and continuous release of a large number of sterile males in the infested area. When sterile males mate with wild fertile females, the eggs produced and/or the subsequent progeny do not develop due to the dominant lethal mutation induced in the parent's reproductive cells. Sterilization with chemosterilant is no longer practiced because of environmental concerns. We need to develop a genetic sexing strain (GSS) of melon fly for the

successful field application of SIT. In such a program, understanding of the genetics of the target species is essential [6], especially cytogenetic analysis is useful to understand the behaviour and genetic stability of a GSS, because GS mechanism is based on the recombination between the autosome breakpoint and the locus [7,8].

The importance of polytene chromosomes as genetic tools is being summarized as follows: (i) In the analysis of chromosome rearrangements for both cytogenetic mapping and construction of specific genotypes [9,10], (ii) the similarity of polytene chromosome maps between closely related species offers a rapid way of inferring their phylogenetic relationships; cytogenetic and genetic approaches have established the chromosomal homologies within same family and could provide the picture of their chromosomal evolution [11, 12], (iii) as diagnostic tools for distinguishing members of a sibling species complex as in the case of the *Anopheles gambiae* species complex [13], (iv) the unique resolution of polytene chromosomes of different developmental stages allows the precise localization of any cloned DNA sequence on the polytene elements by in situ hybridization [14]. This powerful technique provides a versatile tool for linking molecular and genetic information of the target pest.

These attributes have been of great advantage in developing mapping techniques and have contributed to the enrichment of genetic maps in several dipteran species providing novel opportunities for comparative mapping studies [15-17]. For pest species the use of polytene chromosome analysis can make a significant contribution both in terms of understanding population variation and in improving control interventions.

Shahjahan and Yesmin [18] presented the polytene chromosome maps of third instar larvae (6/7 days old) of the melon fly *B. cucurbitae* including mitotic karyotype from neural ganglion. In the present study, characteristics of polytene chromosomes of the same species (three days old) are presented to provide more information as research tools for the development of genetic sexing strain which is a main component of successful SIT application in field.

MATERIALS AND METHODS

Fly Stock: Three-day-old 2nd instar larvae of *B. cucurbitae* originating from a laboratory- reared stock are used for the construction of photographic polytene maps. This population has been maintained in the Fruit fly Laboratory of Radiation Entomology Division at the Institute of Food and Radiation Biology (IFRB), Atomic Energy Research

Establishment (AERE), Savar, Dhaka for more than 23 years. Cultures are maintained at a temperature of 28±2°C with 70-80% relative humidity and 12 h light: 12 h dark cycle. Adult flies are reared on artificial diet (yeast extract: sugar, 1:3) and water in soaked cotton. Eggs are usually collected with sweet gourd and it is served as larval diet.

Salivary Gland Polytene Chromosome Preparation:

Three-day- old second instar larvae were used for the salivary gland polytene chromosome preparations as described by Shahjahan and Yesmin [18]. Larvae were dissected in 45% glacial acetic acid and the glands were fixed in 3N HCl for 3-5 min. They were then transferred to a drop of lactoacetic acid (80% lactic acid-60% acetic acid, 1:2) for about 5 min. The glands were then stained in lactoacetic-orcein. Excess stain was removed by washing the glands two or three times in a drop of lactoacetic acid before squashing. A Nikon (Optiphot-2) phase contrast microscope with relevant photographic attachment was used to examine the polytene chromosome preparations and photographs were taken on Kodak 200 film. Well spread nuclei were used for the construction of polytene maps. Photographs that showed the best morphology for each chromosome region were selected and used for the composite chromosomes presented. The composite maps were scanned at 1200 dpi with Adobe Photoshop 5.5 and printed on a Hewlett Packard 1175 printer.

Chromosome banding patterns are interpreted on the basis of polytene chromosome maps, given by Bedo [19] and Mavragani- Tspidou et al. [20].

RESULTS

Banding Pattern of Polytene Chromosomes at Early Stage of Larval Period: Attempts were made to separate and spread the chromosomes. Weak points in the chromosomes are seen in Sections 2, 19, 63, 76, 81, 91, 97 and 98 (Fig. 1). The total polytene chromosome complement was assigned 100 sections. Although it was not possible to confidently assign chromosome numbers, but the sub sections were lettered, depending on its characteristic features and landmarks (Figs. 1 and 2). The banding pattern was moderately developed but clearly readable in second instar larvae. Dark bands characterize the left end of the chromosome in 4A, 6 and 14A. Sections 9 and 14B each bear one pair faint bands. The expanded regions in sections 3, 16 and 20 are not typical puff; rather section 16 bears a dotted band. Sections 39 and 42 comprise two moderately developed puff regions. There is a poorly developed puff region in section 58.

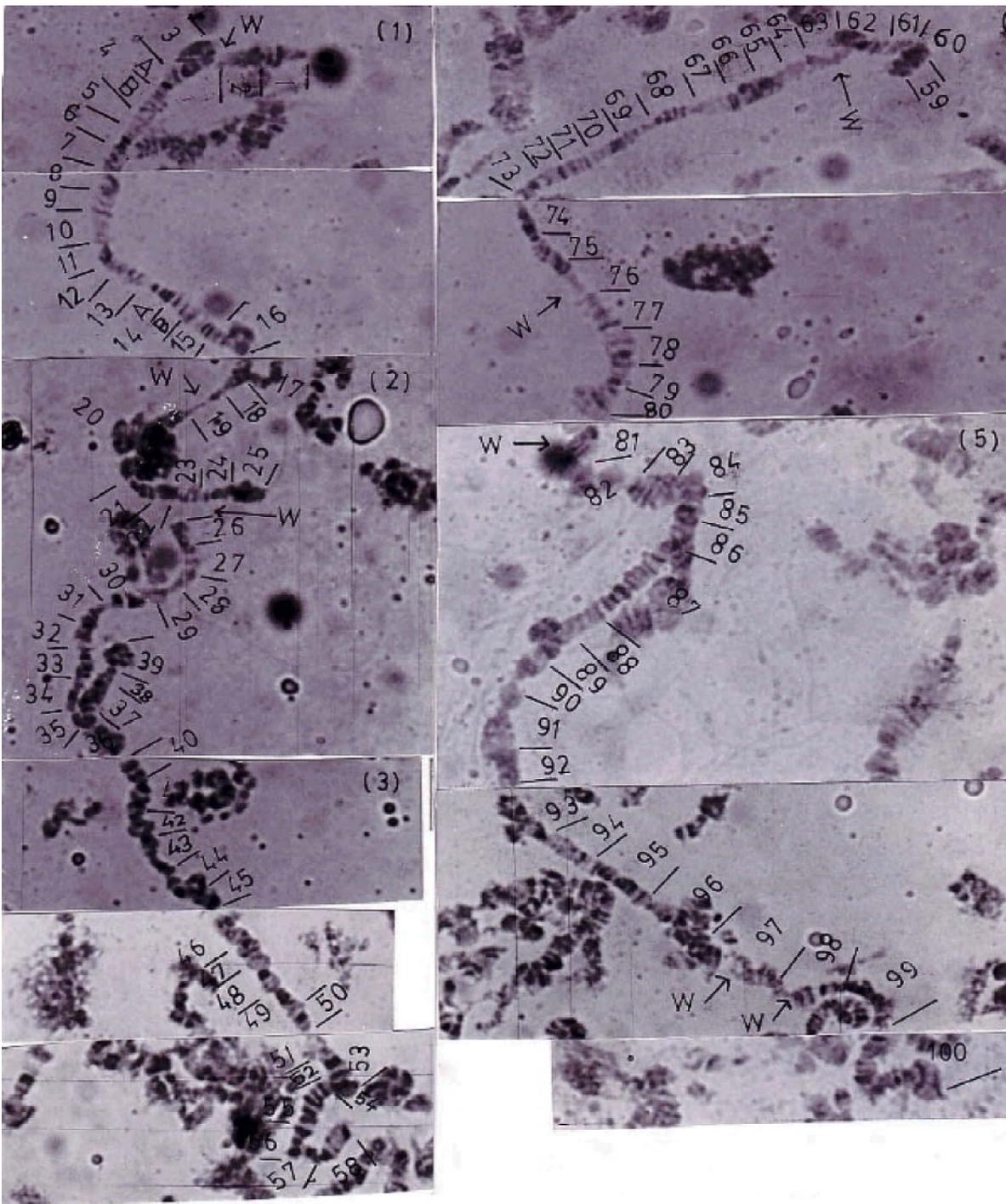


Fig. 1: Salivary gland polytene chromosome maps of second instar larvae of the melon fly, *Bactrocera cucurbitae*, several weak points (W) where chromosome fragmentations are evident, marked by arrows

One pair lightly stained bands occur at 77 and 78. There is a prominent puff in section 80 together with a dark band immediately proximal to it. Section 83 possesses three

clear bands. A well-developed puff is identified in section 100. A schematic diagram of the total genome was provided for better clarification (Fig. 2).

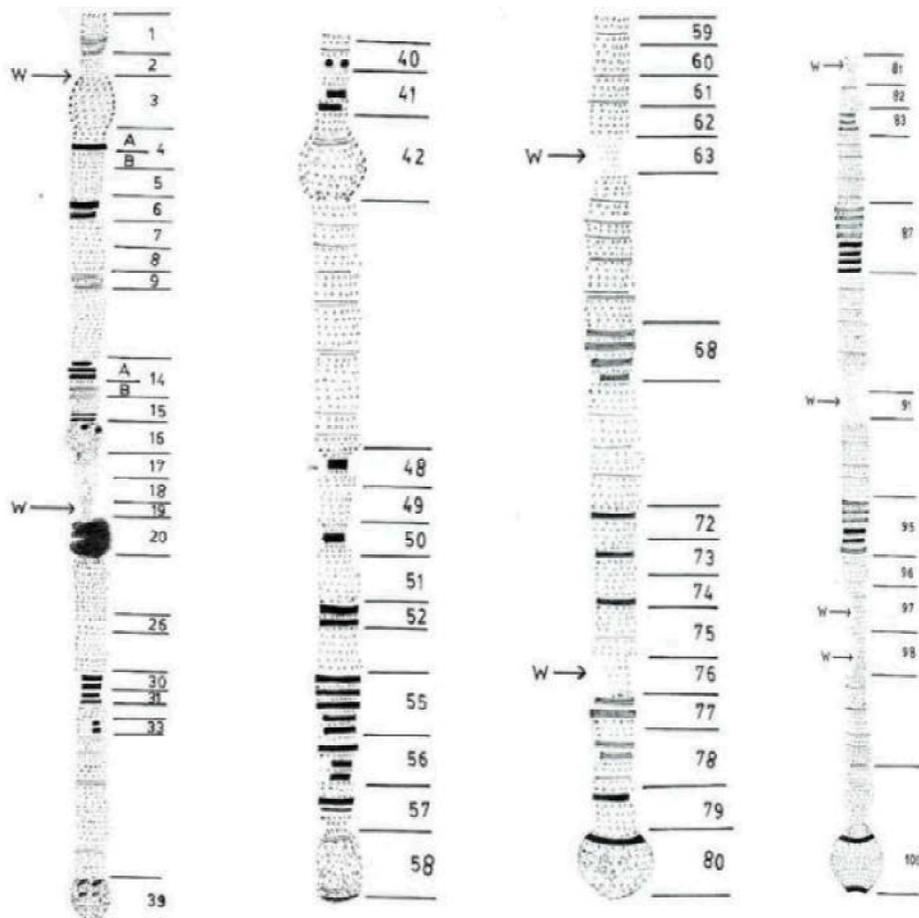


Fig. 2: Diagrammatic representation of salivary gland polytene chromosome maps of second instar larvae of the melon fly, *Bactrocera cucurbitae*, sections 1-100.

Brief accounts of the banding patterns of the salivary gland polytene chromosomes are as follows:

Sections 1-22 (Figs. 1 and 2): Dark bands characterize the left end of the chromosome in 4A, 6 and 14A. At section 9 and 14B, each bears one pair faint bands. On the other hand, 15 comprise one pair moderately dark bands. The expanded regions in sections 3, 16 and 20 are not typical puff, rather section 16 bears a dotted band. The tip (section 1) has faintly stained two bands. Weak points are found in 2 and 19.

Sections 23-42 (Figs. 1 and 2): Deeply stained bands occur in sections 30, 31 and 41. Dotted bands characterize 33 and 40 sections; 39 and 42 comprise two moderately developed puff regions. Breakage occurs at 26.

Sections 43-61 (Figs. 1 and 2): This portion is characterized by a series of dark bands in 52, 55, 56

and 57. Sections 48 and 50 have similar type prominent single band. There is a poorly developed puff region in section 58.

Sections 62 – 81 (Figs. 1 and 2): Lightly stained two pairs of bands are found in section 68. Sections 72, 73, 74 and 79 possess single band. One pair lightly stained bands occurs at 77 and 78. There is a prominent puff in section 80 together with a dark band immediately proximal to it. Weak points are found in 63, 76 and 81 sections.

Sections 82 – 100 (Figs. 1 and 2): Section 83 possesses three clear bands. A series of lightly stained and deeply stained bands are found in the sections 87 and 95. Section 100 appears only as a well– developed puff in the total polytene chromosome complement. Three weak points are found in 91, 97 and 98.

DISCUSSION

A detailed photographic map of third instar larval salivary gland polytene chromosomes of *B. cucurbitae* were presented by Shahjahan and Yesmin [18]. For further advancement, we have tried to present the case as for second instar larvae of the same species. The results of this investigation found *B. cucurbitae* to provide good cytological material in both second and third instar larval salivary gland polytene chromosomes. It is shown that polytene chromosomes, suitable for detailed analysis, can be prepared also from second instar larval stage and are relatively easier than the third instar larvae as a cytological material.

Polytene chromosomes have been an essential tool in genetic analysis since their rediscovery in larval tissues of *Drosophila* and other Diptera [21]. Today polytene chromosome maps are available for about 270 species of Drosophilids and for more than 250 other dipteran insects [22]. Most of the cytogeneticists, working for several important pest species such as *Ceratitis capitata*, *Lucilia cuprina*, *Dacus oleae* and *Bactrocera tryoni*, reported that the salivary gland chromosomes were very difficult to spread because of extensive ectopic pairing [23-25]. In most cases where the chromosomes spread, they were broken and it was difficult to distinguish each chromosome as a separate entity. We also observed such difficulties for *B. cucurbitae*, they are characterized by a large number of weak points that render these chromosomes quite fragile. In this paper, the total polytene chromosome complement of second instar (three days old) larvae was divided into 100 sections. But it was not possible to confidently assign chromosome numbers, because no distinct centromeric position could be ascertained.

Present investigation did not trace any banded sex chromosomes within the polytene nuclei. These chromosomes were represented by a heterochromatic mass like structure in third instar larvae [18]. Here we could not detect such structure. However, further studies are necessary for this characteristic feature.

Insect pests are increasing problem in agriculture and intervention is required to limit losses in crop production. Some insects can be the cause of trade embargoes arising from strict quarantine regulations, or can infest commodities, which require costly and hazardous chemical post-harvest treatments. Different eradication measures are constantly being explored to alleviate the

problem but as yet relatively little research has been conducted on genetically control methods which involve various forms of chromosome analysis (i.e., basic banding pattern, translocation, inversion hybridity) and systems of different isoenzyme activity. An essential prerequisite in these types of work is a thorough understanding of the normal chromosome complement during their life stages. The present research works try to explain a brief outline of these respects.

Application of genetic means for the control of the fly necessitates, in addition to the accumulation of mutations as well as genetic sexing strain, the characterization and recognition of the individual chromosomes of the fly [26]. The unusual size of the salivary gland chromosomes is a product of the type of growth. Salivary and other glands grow by enlargement rather than by duplication of individual cells that can be demonstrated by cell counts and measurements taken at different developmental stages of a larva [21]. Genes have been associated with individual bands. According to Gardner and Snustad [27], the main uses of polytene chromosomes are in locating genes and identifying structural changes in chromosomes.

It is evident that detailed cytogenetic maps would be of significance importance to gain insight into the structure; organization and evolution of the melon fly genome. Cytogenetic analysis of polytene chromosomes is useful to understand the behavior and genetic stability of a genetic sexing strain (GSS) corresponding to wild type strain [7]; serving as a key factor in the field application of SIT [8]. This work has revealed the fact that this species has a readily workable cytological system in larval stages, which may provide interesting results in future. The photographic polytene chromosome maps of the developmental stages may make available a means to pinpoint the precise location of a chromosome breakpoint or a rearrangement. Such chromosome banding techniques may be allowed rapid advancement of our understanding of cytogenetic mechanisms and facilitate chromosome manipulation in this important pest species, *Bactrocera cucurbitae*.

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