

Studies on mitotic and salivary chromosomes of *Dacus cucurbitae* Coquillett (Diptera: Trypetidae)

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Abstract

Dacus cucurbitae is a serious pest of various types of fleshy fruits and vegetables. The mitotic and salivary chromosomes were reinvestigated using the air-drying and different (C- and H-) banding techniques with a view to rectify the existing controversy regarding the Indian populations of this species. A standard map of its salivary chromosomes was constructed and some important identifiable landmarks were recognized in each arm.

Introduction

In view of the frequent appearance and rapid spreading of resistance to some insecticides, insect vectors and insect pests have been the subject of extensive cytogenetic studies throughout the world. Recently *Dacus cucurbitae*, a serious pest of more than eighty types of fruits and vegetables has been analysed cytogenetically in India (Srivastava & Kaul, 1961; Gopalan, 1972; Gopalan & Dass, 1972; Bhatnagar *et al.*, 1980). Unfortunately the outcome of these studies has been the point of controversy. In the course of the present study the mitotic and salivary chromosomes of Indian populations have been reinvestigated using modern techniques and the data have been compared with that of the earlier ones.

Material and methods

In order to establish cultures of this pest in the laboratory, infested fruits, preferably pumpkin and bitter gourd, were collected from the local markets and transferred into jars containing moist sand. On maturation the larvae left the fruit and penetrated

into the sand for pupation. Several adult flies later emerged from these pupae, which were then housed in large cages. They were mainly fed on yeast hydrolysate and sugar solution. Flies were then induced to lay eggs by placing fresh slices of pumpkin inside the cages, on which the females usually oviposited. The egg-loaded slices were placed in separate covered jars where the larvae were left to grow and develop further. In this way different geographical strains of this species were maintained in the laboratory at $24 \pm 1^\circ\text{C}$.

Mitotic chromosome preparations were made from neuroblast cells of third-instar larvae according to the air-drying technique of Guest and Hsu (1973). Metaphase plates from several different geographic populations were examined. Besides, slides were also processed for C- and H-banding following the techniques of Sumner (1972) and Gatti *et al.* (1976) respectively.

Salivary chromosomes were prepared with the usual squash method. Mature larvae of about similar age were chosen for determining the banding patterns of each arm. Camera-lucida drawings of each arm were made from several nuclei. A composite map was then constructed following the standard scheme of Bridges (1935).

Observations and discussion

Mitotic chromosomes (Fig. 1A—C)

Our analysis, based on a large number of individuals collected from several different geographical areas and also from different host plants has revealed that Indian populations of this species examined so far seem to be homogeneous with respect to their karyotype. The karyotype consists of three pairs of metacentric (including the Xs) and three pairs of submetacentric chromosomes, the Y being dot-like.

This finding is contrary to that of earlier observations. For instance, the karyotype reported by Gopalan (1972) consists of three pairs of metacentric and two pairs of acrocentric autosomes and a slightly heteromorphic acrocentric pair representing the sex chromosomes, while Bhatnagar *et al.* (1980) reported again a different karyotype for this species, with one pair of submetacentric and five pairs of metacentric chromosomes including the X chromosomes, the Y being dot-like in the male (Fig. 2).

Our findings are much closer to those of Bhatnagar *et al.* (1980) than to those of Gopalan (1972). A slight difference in the interpretation of metacentric and submetacentric chromosomes exists which may be due to technical variations, because Bhatnagar *et al.* (1980) used the squash method. However, the possibility that there exist two chromosomal races can not be ruled out. Gopalan (1972) has also used the squash method, but report-

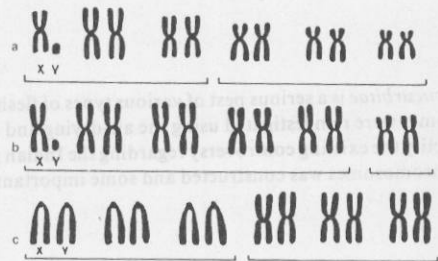
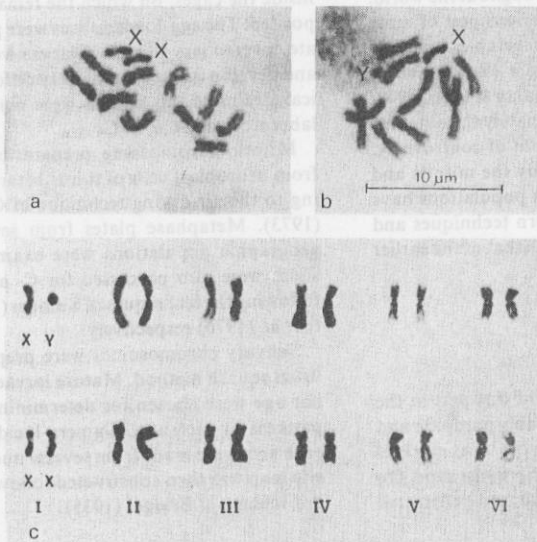


Fig. 2. Schematic representations of karyotypes of *Dacus cucurbitae* reported by different workers: (a) present paper; - (b) Bhatnagar *et al.*, 1980; - (c) Gopalan, 1972.



ed a quite different karyotype for the same species. In fact, such a drastic change in chromosome structure is rather inexplicable by intraspecific chromosomal polymorphism. We, therefore, believe that Gopalan has dealt with a different species, probably a sibling species.

C-banding (with Giemsa staining) (Fig. 3A)

Some slides were processed for C-banding with a view to visualize centromeric positions more precisely in different chromosomes of this species. Four of the five pairs of autosomes show distinct C-band positive bands in their centromeric regions, while the fifth autosome pair shows a larger block of heterochromatin in the vicinity of the centromeric region. The X-chromosome shows a very characteristic pattern, one of its arm being largely C-band positive, while the other arm contains a very small C-band-positive area at its distal end. The centromeric region in this case appears to lie adjacent to the larger block of heterochromatin. The Y chromosome is entirely C-band positive.

H-banding (with Hoechst 33258) (Fig. 3B)

Despite our repeated attempts no definite pattern after treatment with the fluorescent dye Hoechst 33258 could be discerned in this species. In fact, all chromosomes were found to give a uniform dull fluorescence. However, the Y and the C-band posi-

tive areas of the X-chromosome were found to show a slightly brighter fluorescence.

Heterochromatic regions which show bright fluorescence with Hoechst 33258 are generally believed to be relatively rich in A-T base pairs (Hilwig & Gropp, 1972; Raposa & Natarajan, 1974; Comings, 1975; Holmquist, 1975; Gatti *et al.*, 1976; Wheeler & Altenbourg, 1977). During the present study staining with Hoechst 33258 revealed that except the Y and some areas of the X chromosome, all other chromosomes show dull homogeneous fluorescence, suggesting the absence of A-T base pairs in their heterochromatin.

Salivary gland chromosomes (Fig. 4)

Because of the absence of a common chromocenter in this species, in a well spread plate six long chromosomes are observed. The X-chromosome is easily recognized in the male by its thinner and lighter appearance than that of the autosomes. The absence of a chromocenter has made the Y very conspicuous, it appears as a short strand.

These chromosomes are divided into 40 sections and each section is further divided into subsections A, B, C, ... etc. A brief account of some important diagnostic landmarks for each arm is given below:

XL: About 27 μm in length. This arm can be easily recognized by the presence of two closely placed bulb-like structures followed by three dark bands in region 1A-D, a small bulb with several distally placed bands in 3C-D, and section 4A-B with many closely placed bands.

XR: About 9 μm in length. This is the shortest arm, recognizable by its narrow tip, immediately followed by a swelling in region 5C-D and a doublet in region 5B.

II L: About 22 μm in length, recognizable by its narrow tip containing a few thick dark bands, followed immediately by a constriction in region 6A-D.

II R: About 21 μm in length, identifiable by its tapering end, a triplet in 11A and a bulb-like structure with dark bands on either extremity in 10A-B.

III L: About 31 μm in length. Its free end in region 12A is characterized by the presence of light bands followed by a thick dark band. A bulb with two distally placed dark bands, followed by a spindle having several bands in 13A-C and a spindle with a centrally placed diffuse band and a proximal-

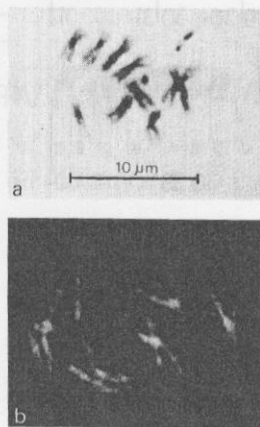


Fig. 3. Chromosome banding in male metaphase plates of *Dacus cucurbitae*: (a) C-banding; - (b) H-banding.



Fig. 4. Map of the salivary gland chromosomes of *Dacus cucurbitae*.

ly placed doublet in 14B-C are also considered of great importance.

III R: About 19 μm in length. Important characters comprise its semiflared free end and two conspicuous bulb like swellings in the region 15B-D.

IV L: About 30 μm in length, identifiable by its characteristically flared free end with several close-

ly packed dark bands in 17A and a large puff with several light diffuse bands in 18A-B.

IV R: About 28 μm in length. Its typically squarish free end containing several light bands distally and two dark bands proximally in region 24B-C, and region 22A-B with several closely placed dark bands are important diagnostic features of this arm.

V L: About 40 μm in length, readily recognized by the presence of two closely placed dark bands near the tip in 25A, two conspicuous swellings in 25D and 26A, a small spindle with dark bands on either extremity in 26C-D and lastly four closely placed bands in 28A.

V R: About 27 μm in length. Diagnostic features include a doublet of thick bands in region 29A and region 31A-D with several sharp bands.

VI L: About 58 μm in length. The free end of this arm is easily marked by the presence of a spindle-like structure with several dark bands distally in 32A-B. Other important characters include a doublet in 33D, a very large bulb with diffuse bands in 34A-B, and a constriction with several dark bands spreading over the region 34E-35A.

VI R: About 23 μm in length. This arm is easily recognized by a considerably large swelling with diffuse bands near the tip in 39D-E and a bulb of moderate size with distally placed thick band in 39B.

Y-Chromosome: About 5 μm in length. It appears as a deeply stained heterochromatic mass with no distinctly visible bands.

Following the results of the present study it is apparent that a detailed comparative analysis of the salivary and mitotic chromosomes in the genus may be of great help in establishing phylogenetic relationships among different species. Also possibly in some instances this will provide further insight into the problem of speciation in this group.

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