DEVELOPMENTS IN CYTOLOGICAL MAPPING OF *Ceratitis capitata* BY IN SITU HYBRIDIZATION

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Abstract

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Cloned DNA sequences were hybridized to salivary gland polytene chromosomes of the medfly, *Ceratitis capitata*, establishing molecular markers for 55 sites on all (ten) autosome arms. Fourteen of the markers correspond to characterized medfly transcription units, while the function of the remaining clones is unknown. Five additional markers were identified as repetitive elements that hybridized to a large number of autosomal sites and also to the granular network that represents the X chromosome. Some of the clones were also hybridized to polytene chromosomes from the orbital bristle cells of *C. capitata* to align the two types of polytene chromosomes, which differ significantly in their banding pattern.

1. INTRODUCTION

The correlation in the Mediterranean fruit fly, *Ceratitis capitata*, of polytene chromosomes [1, 2] to the mitotic chromosomes and to the genetic linkage groups [2] opened up the possibility of localizing genes by cytological analysis of chromosome rearrangements [3] and by in situ hybridization [4]. The latter technique provides a powerful tool for linking molecular and genetic studies, and supplements the classical approaches to establishing chromosome homologies among related species.

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Here, we summarize the current results of in situ hybridization studies *C. capitata.* In addition to the salivary gland chromosomes, the male orbital bris cells (trichogen cells) were used for the first time. This study permits a detai comparison of the two chromosome maps in order to resolve the banding pa differences between the polytene chromosomes from the two tissues [5].

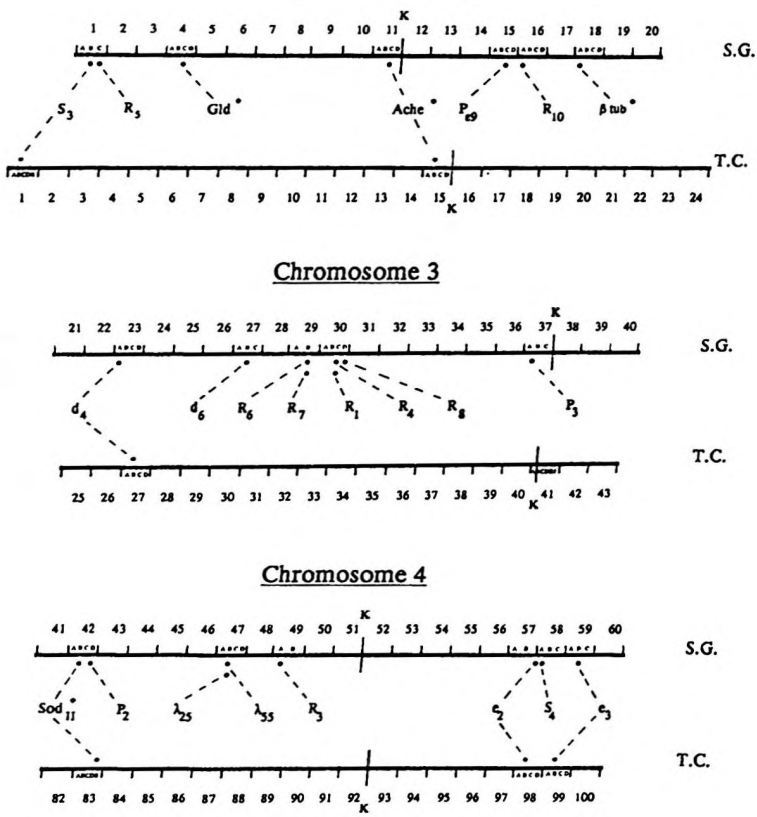

FIG. 1. The DNA cloned sequences and their cytological sites on salivary gland (S.G.) and trichogen cell (T.C.) polytene chromosomes. Asterisks indicate cloned medfly genes.

2. MATERIALS AND METHODS

Larvae and pupae of the Benakeion mass rearing strain were used for chromosome preparations. Squash preparations of salivary gland chromosomes were made from larvae five to six days old following the method of Zacharopoulou et al. [3]. In addition, squash preparations of trichogen cell chromosomes were obtained from male orbital cells according to the technique reported by Bedo [1], but omitting the staining step.

The cloned DNA sequences were isolated from genomic and/or cDNA libraries using in several cases *Drosophila* sequences as probes. Other clones represent random cDNA clones, clones obtained through amplification by the polymerase chain reaction, or clones originating from random amplified polymorphic DNA (RAPD) analysis. DNA labelled using biotin was used in a standard hybridization procedure [3]. The resulting hybridization signals were mapped on the basis of published maps for salivary gland chromosomes [2] and trichogen cell chromosomes [1].

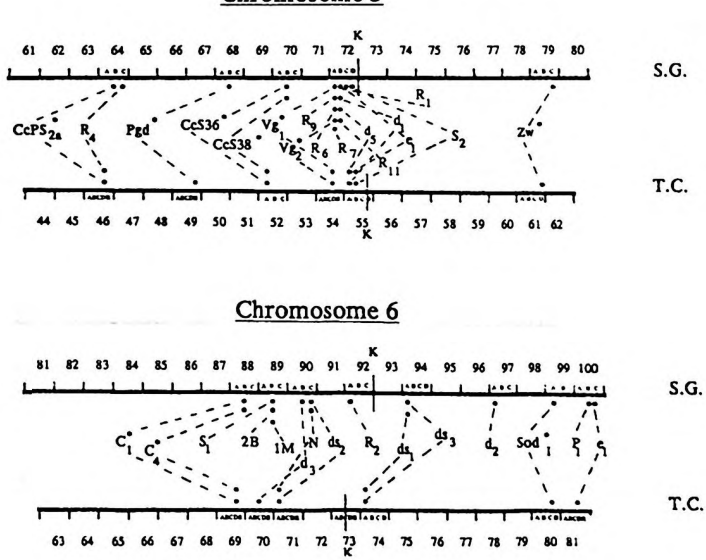

FIG. 1. (cont.)

3. RESULTS AND DISCUSSION

Polytene chromosomes in the medfly are difficult biological material. They often break and show extensive ectopic pairing, especially in salivary gland nuclei. Furthermore, there are only two orbital bristle cells in each male, a fact that adds to the above difficulties. In addition, resolution is lost during the in situ hybridization procedure. Nevertheless, our results clearly show that chromosomes from both tissues can be used for in situ hybridization. Figure 1 lists the clones used and the chromosomal sites to which they hybridize on both types of polytene chromosomes; in total this provides 55 molecular markers for all autosome arms.

Although we used almost all the clones for in situ hybridization in trichogen cells, only a small number could be mapped. This is, in addition to the difficulties mentioned above, due to possible differences in chromosome organization (e.g. degree of polytenization) between the two tissues. A comparison between the two sites in all different chromosomes favours the idea that differential activity of chromosome loci in different stages of development could be one reason for banding pattern differences [6, 7]. However, we cannot exclude other reasons, especially those concerning the structural organization of the chromosomes [8].

Among the molecular markers already mapped, 14 correspond to characterized genes of *C. capitata*. An interesting observation concerns the chromosomal localization of those genes that are homologous to sex linked *Drosophila* genes. All these genes not only mapped to medfly chromosome 5 but also showed a gene order similar to their homologues in *Drosophila* (Fig. 2). This is clear molecular evidence that the two chromosomes are homologous, and further supports the concept that distantly related Diptera species essentially maintain their chromosome identity [9, 10].

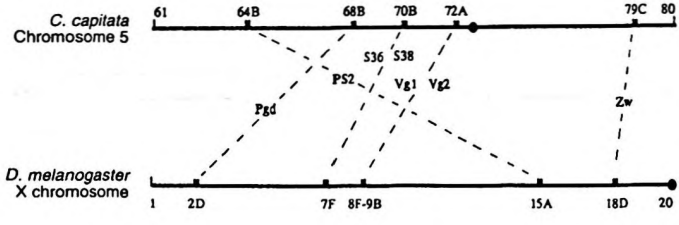

FIG. 2. Cytological sites of several genes mapped on chromosome 5 of C. capitata *compared with homologous sex linked genes of* D. melanogaster.

The function of all other markers is unknown, although some of them were isolated by using *Drosophila* genes as probes. Nevertheless, a number of them could be very useful in genetic mapping experiments to correlate genetic and cytological maps. Some of these clones, symbolized as 'R' in Fig. 1, are polymorphic in different medfly populations (restriction fragment length polymorphisms and RAPDs).

Finally, six of the DNA clones tested showed a hybridization pattern characteristic for repetitive sequences. They hybridized to a large number of chromosomal sites in all autosomes and to the heterochromatic network that represents the X chromosome [6, 7]. It would be interesting to examine whether such elements may be transposable in different medfly strains.

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