Theor Appl Genet (1990) 80:177–182

Cytogenetic analysis of three genetic sexing strains of *Ceratitits capitata*

Ph. Kerremans [1], **K. Bourtzis** [2] **and A. Zacharopoulou** [2,*]

[1] Entomology Unit, Joint FAO/IAEA Program, IAEA Laboratories, A-2444 Seibersdorf, Austria
[2] Department of Biology, Division of Genetics, Cell and Developmental Biology, University of Patras, Patras, Greece

Received February 15, 1990; Accepted February 23, 1990
Communicated by H. F. Linskens

Summary. Polytene chromosomes of three genetic sexing strains of *Ceratitis capitata* were analyzed. The genetic sexing mechanism is based on a pupal color dimorphism (white – brown) and is the result of a reciprocal translocation between the Y chromosome and the autosome bearing the *w* locus (white pupal case). The analyzed polytene chromosomes were derived from two different pupal tissues, the orbital bristle and fat body cells. The Y chromosome is visible in both tissues, while the autosomes present a different banding pattern. Based on these features, the autosome breakpoints in the three Y; autosome translocations were mapped, and the homology of the translocated autosome in both tissues was established. In addition, the location of the break-points was compared to the stability of these three strains.

Key words: Genetic sexing – Polytene chromosome – *Ceratitis capitata*

Introduction

The advantages of a genetic sexing (GS) system for *Ceratitis capitata* for improving the efficiency of the sterile insect technique (SIT) have been extensively pointed out (Lachance 1979; Rossler 1979; Robinson and Van Heemert 1982).

In a such a program, understanding the genetics of the target species is essential. In recent years knowledge of the genetics of *C. capitata* has rapidly increased and a considerable number of mutants have been obtained (Medfly Genetics Information Circular 1987). Moreover, polytene chromosomes of *C. capitata* are now available, thereby providing material for cytogenetic analysis. Polytene maps have been made from pupal orbital trichogen cells (Bedo 1986, 1987) and larval salivary glands (Zacharopoulou 1987, 1990). Chromosome maps of both tissues are necessary because autosomes in each tissue have quite different banding patterns (Bedo and Zacharopoulou 1988).

Cytogenetic analysis is useful to understand the behavior and genetic stability of a GS strain. One of the problems in the existing strains is the breakdown of the GS mechanism, probably due to the recombination between the autosome breakpoint and the locus on which this mechanism is based.

Although the recombination occurs infrequently in males of *C. capitata,* it increases in the presence of chromosomal rearrangements (Rossler 1982). The occurrence of breakdown of the GS mechanism is a major concern when these strains are mass reared and released for population control by the SIT. Replacing a contaminated colony in which the sexing mechanism has broken down by a new "clean" population in the factory might interrupt the program and increase the costs of rearing, if several "clean" back-up colonies are maintained ready for replacement.

In the present study, polytene chromosomes from orbital trichogen and fat body cells of three GS strains based on puparial color were cytogenetically analyzed and mapped to determine the autosomal breakpoints. Based on this map, the homology of the translocated autosome between the two tissues was established. Since fat body polytene chromosomes have the same banding pattern as salivary gland (SG) chromosomes, we could correlate the translocated autosome between the polytene chromosomes of the trichogen cells (TC) and salivary glands.

* To whom correspondence should be addressed

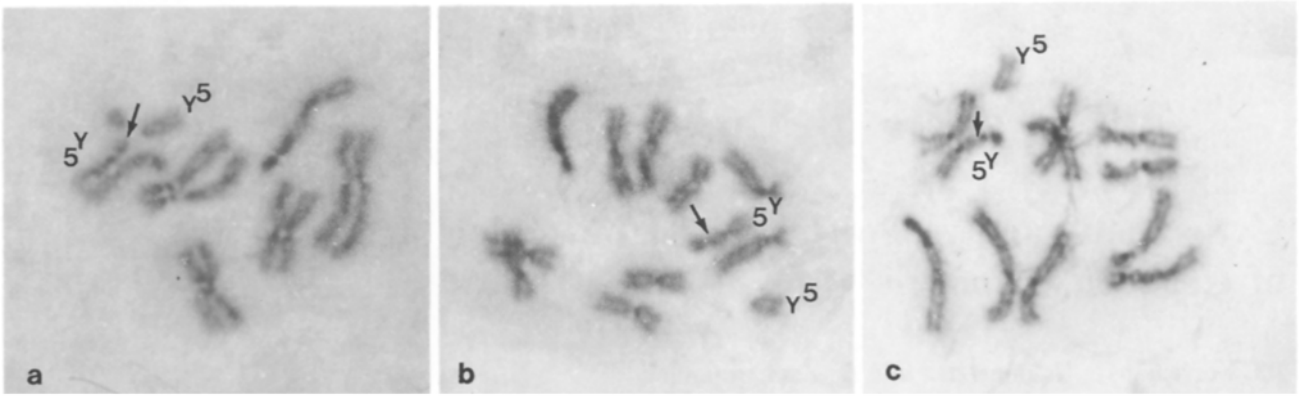

Fig. 1 a–c. Neuroblast mitotic metaphases from the three genetic sexing strains **a** wp23, **b** wp30c, **c** wp101 showing the reciprocal translocation between the chromosomes 5 and Y. *Arrows* indicate the autosome breakpoints

Moreover, we tried to correlate the different stabilities of these strains to their autosomal breakpoints; hypothesis have been provided to explain these differences.

Materials and methods

Strains

With all three of the genetic sexing strains used, the separation mechanism is based on the puparial color. The male puparia are brown whereas female puparia are white. The strains are designated *wp23, wp101* (Robinson and Van Heemert 1982), and *wp30C* (Bush-Petersen and Southern 1987).

Cytology

Mitotic preparations. Mitotic neuroblast metaphases were examined using the same technique as previously reported (Zacharopoulou 1987).

Polytene chromosome preparations. Polytene chromosome preparations from pupal orbital trichogen cells were made as described by Bedo (1987); for pupal fat body cells, the same method was used with a minor modification of the squash. The orbital trichogen cells were gently squashed as more pressure was applied to the fat body cells, since these are very condensed and not easily spread out. The orbital trichogen polytene chromosomes were interpreted on the basis of the standard chromosome maps for the trichogen chromosomes (Bedo 1987), while the salivary gland chromosome maps (Zacharopoulou 1990) were used for the fat body cells.

Results and discussion

Mitotic chromosome analysis of the three strains revealed that there is a reciprocal translocation between the Y chromosome and chromosome 5, as it was arbitrarily designated in previous work (Zacharopoulou 1990). Figure 1 shows that the Y breakpoint is on the long arm close to the centromere. The autosome breakpoint is also on its long arm, near the centromere. From the same figure it is also clear that both breakpoints are similar in all strains.

Detailed mapping of the autosome breakpoints was made by analysis of the polytene chromosomes. The Y breakpoint could not be determined since the sex chromosomes do not polytenize in *Ceratitis capitata* (Bedo 1987; Zacharopoulou 1987).

The Y chromosome is represented in pupal trichogen cells as a spherical dark body and a nucleolus (Bedo 1987). A similar structure can also observed in pupal fat body cells but not in larval fat body cells and salivary glands (Zacharopoulou 1987; Bedo and Zacharopoulou 1988). Whereas the sex chromosome structures of larval and pupal fat body cells are different, the autosomes have a banding pattern similar to the salivary gland polytene chromosomes. This observation is important because it could be used to identify homologous chromosomes from tissues where they cannot otherwise be recognized.

The structure of a Y; autosome translocation in polytene chromosomes depends mainly on the Y chromosome breakpoint. Bedo (1987) suggested that the Y nucleolar organizer is located at the secondary constriction of the short arm of the Y chromosome. If this chromosomal region is present in the karyotype, the nucleolus or the spherical body may be attached to the autosome breakpoint. This depends mainly on the chromosome pairing. Figure 2 shows that in the orbital trichogen polytene chromosomes of the three strains, the nucleolus was attached to the autosome for strains *wp30C* (Fig. 2a) and *wp101* (Fig. 2b), while the spherical body was connected to the autosome in strain *wp23* (Fig. 2c). It must be emphasized that in the first two strains the nucleolus was always attached to the autosome in all preparations examined. In contrast, in *wp23* the spherical body or nucleolus was connected to the autosome. We could not explain this observation. Perhaps small differences in the Y breakpoints between the strains or artifacts could have

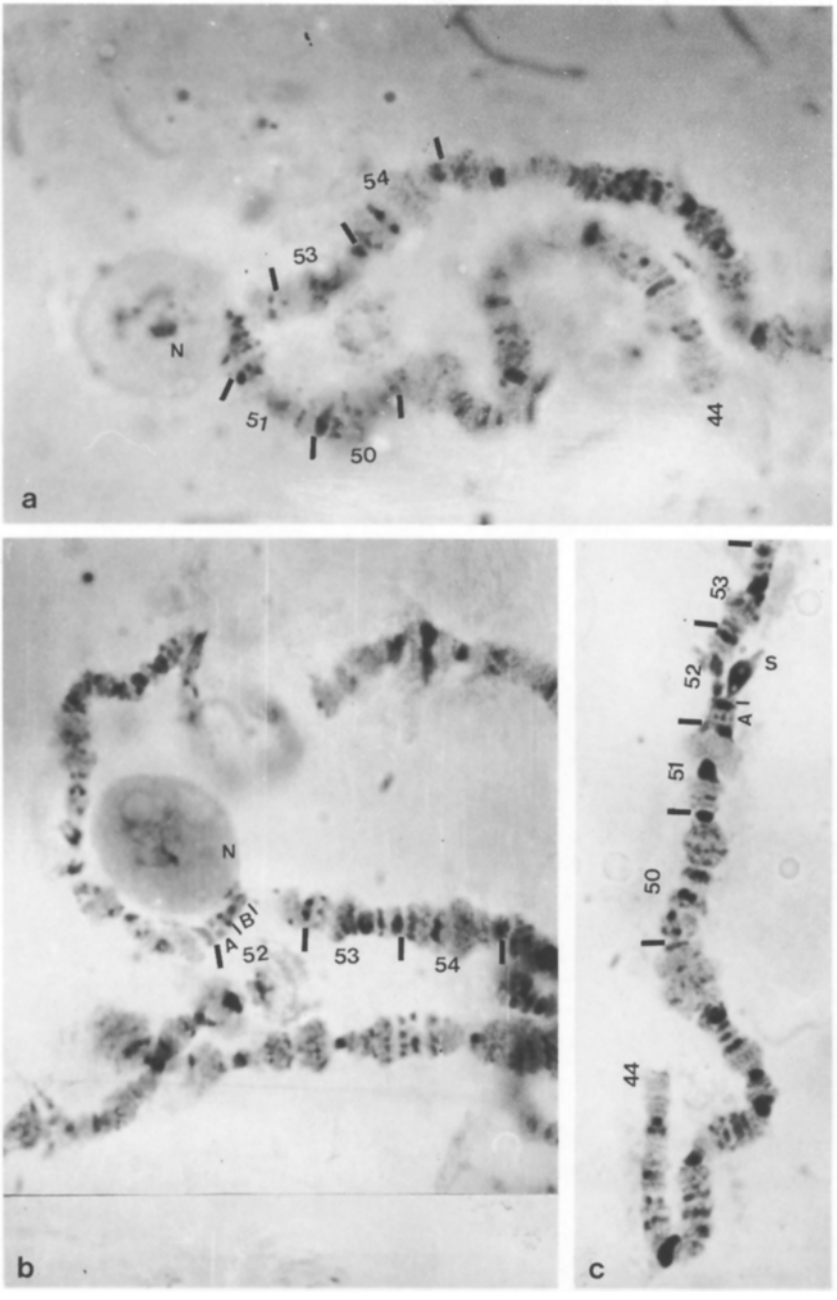

Fig. 2a–c. Orbital bristle polytene chromosomes in the strains **a** wp101, **b** wp30c, **c** wp23. Note that the nucleolus (N) is attached in the 52B region in **a** and **b**, while the spherical body(s) is attached in the region 52A in the strain wp23

affected the structure of these Y; autosome translocations.

Analysis of the pupal fat body polytene chromosomes also identified the autosome involved in the translocation. Although the quality of the chromosomes was poor, the autosome breakpoint could be recognized. Figure 3 shows that the nucleolus is now attached to the same autosome in all strains and in about the same region. This polytene element can easily be identified as the salivary gland polytene chromosome 61–80 (Fig. 3b), which was correlated to the arbitrarily designated mitotic chromosome 5 (Zacharopoulou 1990).

Using the above data we mapped the autosomal breakpoints in all three strains (Table 1). The breakpoints are identical for the *wp30c* and *wp101*, but there is a small difference for the *wp23*. This agrees well with the mitotic karyotype, where the autosome breakpoint appears approximately in the same region in each strain. By using the autosome breakpoints, we identified the homologous polytene element from orbital trichogen cell salivary glands, which have different banding patterns.

The observation that the three breakpoints are all located very close together seems to reflect "a hot spot" of this region for radiation-induced chromosome aberra-

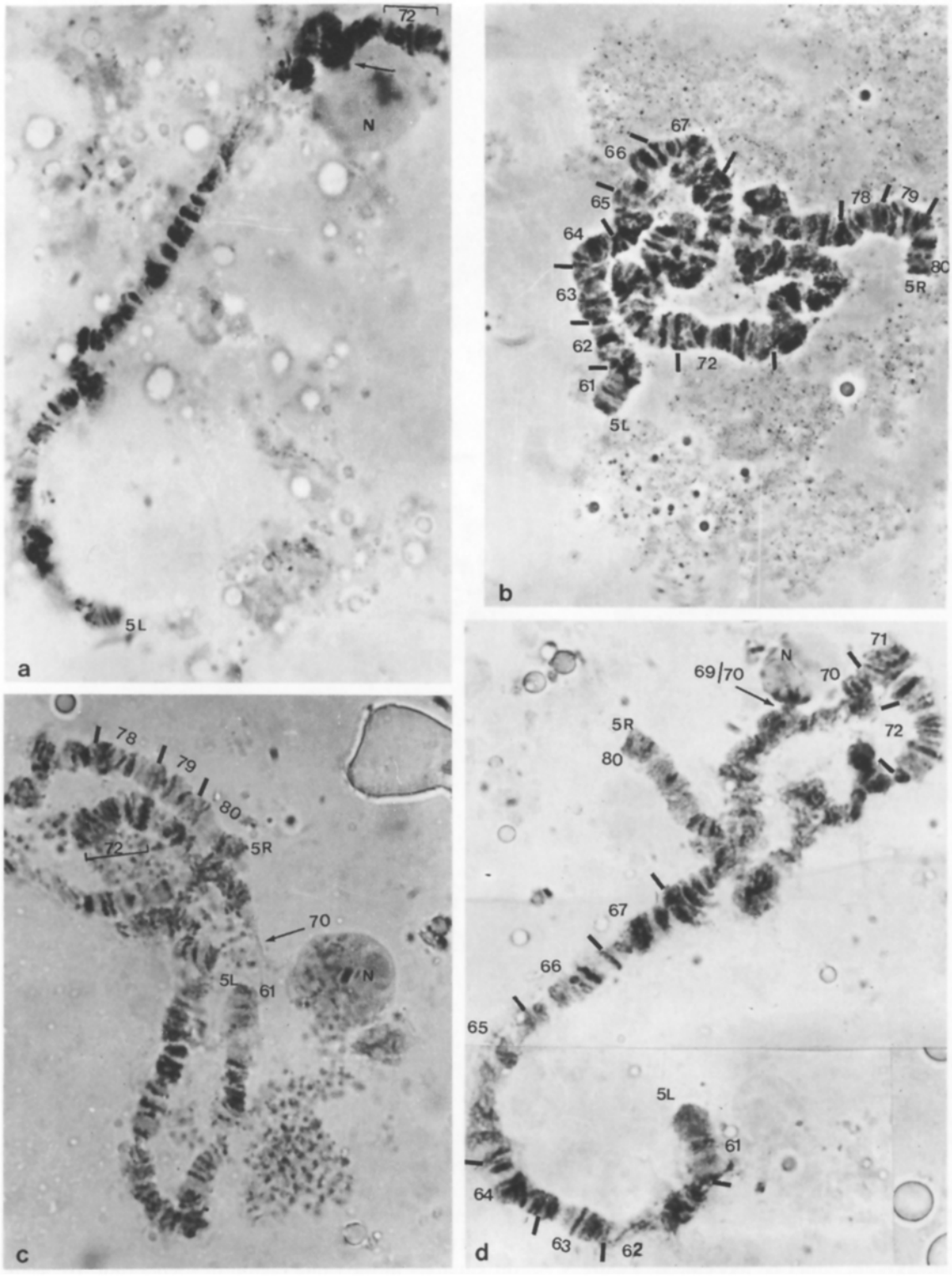

Fig. 3a–d. Polytene chromosomes from pupal fat body cells in the strains **a** wp101, **c** wp30c, **d** wp23. The nucleolus is attached in all strains. *Arrows* indicate the autosome breakpoints. **b** Salivary gland polytene chromosome 5 (sections 61–80). The similarity of banding patterns between salivary gland and fat body element is indicated

Table 1. Autosomal translocations breakpoints in *Ceratitis capitata*

Strain	Chromosomal breakpoints	
	TC	SG
wp23	52A	69/70
wp30C	52B	70
wp101	52B	70

tions. A similar but larger radio-sensitive region was found in *Anopheles stephenci* (Robinson et al. 1986).

The three GS strains were checked for a period of one year for true breeding, i.e., the brown male puparia and white female puparia. Aberrant types, i.e., brown female puparia and white male puparia, occurred at a frequency ranging from 0.2% to 0.8%, with the highest frequency in *wp23* and the lowest in *wp30c*. This frequency was stable if the aberrant types were removed from the colony in each generation. When they were not removed, their frequency increased; the highest frequency (almost 50%, within one year) occurred in *wp23*.

Similar observations were reported from mass rearing experiments. Bush-Petersen and Kafu (1989) showed that *wp30C* was more stable than *wp101*. However, McInnis et al. (1990) observed a such instability in the strain *wp23* that is higher as compared to that reported for the *wp101* strains. Thus, we can conclude that *wp30c* is very stable, *wp101* is intermediate, and *wp23* is the most unstable.

The occurrence of these aberrant types was not due to external contamination but was the result of some internal processes in these strains. Genetic recombination in the males between the *w* locus and the translocation breakpoint could be a plausible explanation for the phenomenon. Rossler (1982) showed that male genetic recombination occurred in some other Y; autosome translocations in *Ceratitis capitata,* but was absent or very low in the normal strains. The same phenomenon has also been observed in strains of other *Diptera* species that contain Y; autosome translocation (Lester et al. 1979; Whitten 1979).

In some of these cases male recombination was observed, not only on the autosomes involved in the translocation, but also in other autosomes. Whitten (1979) assumed that the loss of suppression of male crossing-over might be the result of a position effect in certain Y; autosome translocation. If male crossing-over is responsible for the recombinants, then recombination must vary between strains. Since the breakpoint are all close together, we assume that the distance between the breakpoints and the *w* locus is not the only factor affecting the difference in recombination frequency. Perhaps the location of the breakpoint itself or the genetic background of these strains also has an affect. Experiments to introduce a different genetic background into an unstable strain of the medfly showed that the stability could be improved dramatically (McInnis et al. 1990). Studies with various *Drosophila* strains revealed that genetic recombination occurred in males (Hiraizumi 1971) and that transposable elements are responsible (Finnegan and Fawcett 1986; Louis and Yannopoulos 1988). Transposable elements have not been found yet in *Ceratitis capitata.*

Moreover, we cannot exclude the possibility of the emergence of aneuploid individuals that may result in aberrant phenotypes. We noticed that several larvae of the *wp23* strain had a trisomic karyotype for the translocated autosome (Zacharopoulou 1990), but it is not known if these larvae would have been viable as adults.

The effect of genetic background and recombination on strain stability has important consequences for the selection of suitable GS strains for mass rearing. If recombination were the only factor affecting stability, inversions would be needed to decrease the frequency of recombinants. Isolation of inversions is, however, a long process. In contrast, the introduction of another genetic background could be accomplished much faster.

Acknowledgements. We thank A. Robinson and E. Bush-Petersen for providing us with the *Ceratitis* strains. R. Gingrich is thanked for his comments of the manuscript. This work forms part of a joint FAO/IAEA research program for the development of genetic sexing mechanisms for the Mediterranean fruit fly, *Ceratitis capitata.*

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