Identification of the Sex-Determining Region of the *Ceratitis capitata Y* Chromosome by Deletion Mapping

Ute Willhoeft and Gerald Franz

FAO/IAEA Agriculture and Biotechnology Laboratory, Entomology Unit, A-2444 Seibersdorf, Austria

Manuscript received January 22, 1996
Accepted for publication July 5, 1996

ABSTRACT

In the medfly *Ceratitis capitata,* the *Y* chromosome is responsible for determining the male sex. We have mapped the region containing the relevant factor through the analysis of *Y*-autosome translocations using fluorescence *in situ* hybridization with two different probes. One probe, the clone pY114, contains repetitive, *Y*-specific DNA sequences from *C. capitata,* while the second clone, pDh2-H8, consists of ribosomal DNA sequences from *Drosophila hydei.* Clone pY114 labeled most of the long arm and pDh2-H8 hybridizes to the short arm and the centromeric region of the long arm. In 12 of the analyzed 19 *Y*-autosome translocation strains, adjacent-1 segregation products survive to the late pupal or even adult stage and can, therefore, be sexed. This was correlated with the length of the *Y* fragment still present in these aberrant individuals and allowed us to map the male-determining factor to a region of the long arm representing ~15% of the entire *Y* chromosome. No additional factors, affecting for example fertility, were detected outside the male-determining region.

THE Mediterranean fruit fly, *Ceratitis capitata* (Wied.), is one of the major insect pests in agriculture. The knowledge of the genetics of medfly is increasing rapidly, as there is an interest in its application to develop control technologies for this pest. Many mutant markers for the five autosomal linkage groups are available (ROESSLER and KOLTIN 1976; ROESSLER and ROSENTHAL 1992) together with two polytene maps, one from salivary glands (ZACHAROPOULOU 1987) and one from orbital bristle trichogen cells (BEDO 1987). Recently, several molecular markers were mapped to the polytene chromosomes using *in situ* hybridization (ZACHAROPOULOU *et al.* 1992).

The *XY* chromosome pair of *C. capitata* is heteromorphic and heterochromatic (BEDO and WEBB 1989), and despite extensive mutagenesis only a single mutant has been found to be *X*-linked (for a review see ROESSLER *et al.* 1994). The sex chromosomes do not polytenize, the *X* chromosome forms a granular network and the *Y* chromosome appears as a heterochromatic sphere in some polytene tissues (BEDO and WEBB 1989; ZACHAROPOULOU *et al.* 1990). The ribosomal DNA (rDNA) loci are located on the *XY* pair (BEDO and WEBB 1989). Recently, several *Y*-specific and *Y*-enriched sequences, which proved to be repetitive, were described by ANLEITNER and HAYMER (1992). Aneuploid $XX22^{Y}$ individuals from a male-linked translocation strain were males and it was therefore suggested that the *Y* chromosome is male-determining (ZAPATER and ROBINSON 1986).

Many *Y*-autosome translocation strains have been isolated in our laboratory to link selectable markers to the *Y* chromosome and produce strains for large-scale separation of females and males (KERREMANS *et al.* 1992; FRANZ and KERREMANS 1994; FRANZ *et al.* 1994; KERREMANS and FRANZ 1994) in Sterile Insect Technique programmes (MCINNIS *et al.* 1994; HENDRICHS *et al.* 1995). The translocation strains have been analyzed genetically and cytogenetically using C-banding of mitotic chromosomes and polytene chromosome analysis to map the autosomal breakpoints.

Several of the translocation strains produce aneuploid individuals that survive until adulthood. These individuals, descending from adjacent-1 segregation in the male meiosis, contain a duplication of a part of the autosome involved in the translocation and they were used to map autosomal, recessive mutations on chromosome 5 (KERREMANS and FRANZ 1994). Such adjacent-1 individuals also carry *Y*-chromosome deletions, where the length of the deletion depends on the position of the breakpoint on the *Y* chromosome. However, it was impossible to determine the exact position of the breakpoint in the *Y* chromosome in C-banded, mitotic chromosome spreads.

Here, we report the mapping of the *Y*-chromosome breakpoints of 19 *Y*-autosome translocations by fluorescence *in situ* hybridizations (FISH) using repetitive DNA probes. Adjacent-1 adults were viable in 12 of these strains and they were used to map the male-determining region on the *Y* chromosome. Furthermore, these aneuploid individuals were analyzed with respect to the presence or absence of male fertility factors on the *Y* chromosome.

MATERIALS AND METHODS

Strains: Egypt II (Eg II) is a wild-type strain that has been reared in the laboratory since 1983. Genetic and cytogenetic

Corresponding author: Gerald Franz, Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria. E-mail: franz@rial1.iaea.or.at

analysis of the translocation strains *T(Y;4;5)1-49, T(Y;5)1-59, T(Y;5)1-61, T(Y;3;5)2-5, T(Y;5)2-22, T(Y;5)2-40, T(Y;2;5)2-42, T(Y;2;4;5)2-54* and *T(Y;2;5)2-82* was described by FRANZ *et al.* (1994). Data on translocation strains *T(Y;5)3-86, T(Y;5)3-129, T(Y;3;5;6)3-136, T(Y;3;5)3-139, T(Y;5)3-179, T(Y;5)3-192, T(Y;5)3-245* and *T(Y;5)3-263* were published by KERREMANS and FRANZ (1995), while the description of *T(Y;3)1-30* and *T(Y;5)30C* can be found in KERREMANS *et al.* (1992) and KERREMANS *et al.* (1990), respectively. The *T(Y;5)* translocation males carry the wild-type alleles of the recessive markers *white pupae* (*wp*) (ROESSLER 1979) and *white eye* (*we*) (ROESSLER and ROSENTHAL 1992) linked to the *Y* chromosome, while the females are homozygous for the mutant alleles; *we* was mapped to the left arm and *wp* to the right arm of autosome 5 (KERREMANS and FRANZ 1994). In *T(Y;3)1-30,* the marker *dark pupae* (*dp*) (ROESSLER and KOLTIN 1976) is used.

Rearing conditions and fertility tests: Larvae were grown in a carrot-yeast medium and adults fed on a sugar-yeast medium. Pupation occurred in fine sand. For testing fertility, single adjacent-1 males were mated with two wild-type females in small cages (10 cm length, 5 cm diameter equipped with an egging net) kept under 12 hr light-dark cycle. The presence of mating couples (which, on average, last 2 hr) was monitored in regular intervals for ≥8 days. Egg hatch of all eggs, sampled during this period, was assessed 4 days after egg deposition. In addition, any offspring that reached the pupal or adult stage were recorded. After 8 days, the males were removed from the cages, dissected and analyzed by phase contrast microscopy at 160× magnification to determine whether motile sperm were present.

DNA probes and labeling: Clone pY114 contains a genomic, repetitive DNA fragment from the *Y* chromosome of *C. capitata;* Southern hybridization experiments demonstrated that this fragment does not hybridize to genomic DNA from females (ANLEITNER and HAYMER 1992). The rDNA probe, pDh2-H8, is a cloned fragment from *Drosophila hydei* containing a 28S ribosomal DNA (rDNA) coding region interrupted by an intervening sequence (RENKAWITZ-POHL *et al.* 1980). pmed21 is a genomic clone from *C. capitata* containing a part of an actin gene (HAYMER *et al.* 1990).

Plasmid DNA was isolated from liquid cultures with the alkaline lysis method according to BIRNBOIM and DOLY (1979). For FISH and Southern hybridizations, inserts of the clones were gel purified according to WEICHENHAN (1991) and labeled with digoxigenin-11-dUTP using a random primed DNA labeling kit (Boehringer).

Southern analysis: Genomic DNA of single individuals was isolated as described by BLIN and STAFFORD (1976) and used for Southern blotting following standard procedures (SAMBROOK *et al.* 1989). Southern hybridization of DIG-labeled probes and detection by Lumigen PPD was done according to the supplier's instructions (Boehringer). For the analysis of adjacent-1 adults, we used the bodies of those individuals from which we obtained chromosome preparations for karyotype analysis.

Chromosome spreads: Brain tissue from third instar larvae and testes or ovaries from adults were used for chromosome spreads. After dissection of the tissue in Ringer's solution (0.9% NaCl, 0.02% CaCl, 0.02% KCl, 0.01% $NaHCO_3$ pH 6.9), it was pretreated in 1% citric acid for 10–15 min, prefixed in methanol:acetic acid (3:1) for 1 min and transferred to a small drop of 60% acetic acid on a slide. The tissue was disrupted with forceps and heat-fixed. Chromosome preparations were preselected for male karyotypes by the identification of the sex chromosomes using phase contrast microscopy, then dehydrated in 70% ethanol, air dried and stored at −20° for one night or up to 10 months until further use.

Fluorescence *in situ* hybridization: Slides were baked at 80° for 2 hr followed by dehydration in an ethanol series and air dried. For fluorescence *in situ* hybridizations using the rDNA probe pDh2-H8, an RNase digest was performed at 37° for 30 min in 25 μl of RNase A (50 μg/ml), slides were washed in 0.4× SSC, 0.1% Tween and dehydrated through an ethanol series. Chromosomes were denatured in 0.05 M NaOH for 60 sec, transferred to 0.4× SSC, 0.1% Tween for 10 sec and dehydrated as described above. For each slide, 10 ng (in 1.5 μl double distilled water) of DIG-labeled probe DNA was mixed with 3.5 μl master mix and 5 μl deionized formamide. The master mix consisted of 100 μl dextran sulfate (50% in double distilled water, autoclaved), 100 μl double distilled water, 100 μl 20× SSC and 50 μl salmon sperm DNA (10 μg/μl, sheared to 500 bp length). The hybridization mixture was heated to 80° for 8 min, immediately cooled on ice and applied to the slide with the denatured chromosomes. The slide was covered by a 22 × 22 mm coverslip and sealed with rubber cement. Hybridization was performed in a humid chamber at 37° for 14–17 hr. Slides were washed twice for 5 min in 0.4× SSC, 0.1% Tween at room temperature. Hybridization signals were detected with anti-digoxigenin-fluorescein Fab-fragments according to the instructions of the supplier (Boehringer). Chromosomes were counter stained with propidium iodide in antifade (0.233 g 1,4-diazobicyclo(2,2,2)octane in 1 ml Tris pH 8 and 9 ml glycerol). Photographs were taken with a 400 ASA Ektachrome film using a Leitz Dialux Epifluorescence microscope with a 63× or 100× lens. At least three male individuals of each strain were analyzed using probes pY114 and pDh2-H8.

RESULTS

FISH banding patterns of the *Y* chromosome: FISH with clone pY114 using mitotic chromosome spreads of the wild-type strain Egypt II showed hybridization signals located exclusively on the *Y* chromosome (Figure 1A). The whole of the long arm of the *Y* chromosome was labeled with the exception of the region close to the centromere and a gap near the tip representing ~10% of the whole *Y* chromosome length. The centromere and the short arm were not labeled.

In contrast, pDh2-H8 labeled the short arm, the centromere region and ~10% of the long arm of the *Y* chromosome (example in Figure 1B). On the long arm of the *Y* chromosome the hybridization signals of both probes partially overlapped. The rDNA clone also hybridized to the tip of the short arm of the *X* chromosome. The overall hybridization pattern as well as the relative signal intensities of the *X* and the *Y* chromosome are in good agreement with published results (BEDO and WEBB 1989) with the exception that we also obtained signals on the long arm of the *Y* chromosome.

FISH with clones pDh2-H8 and pY114 increased the resolution as compared to standard C-banding, especially for the short arm of the *Y* that stains only weakly with C-banding. Even small parts of the *Y* chromosome, representing roughly 5%, can be detected (see, for example, Figure 1E, Y^5 chromosome labeled by probe pY114).

Detection of *Y* chromosome breakpoints in *Y*-autosome translocations: Chromosome spreads from third instar larval brain tissue were selected under a phase contrast microscope for male karyotypes. For the detection of the *Y*-chromosome breakpoints in 19 *Y*-autosome translocations we used at least three preparations for

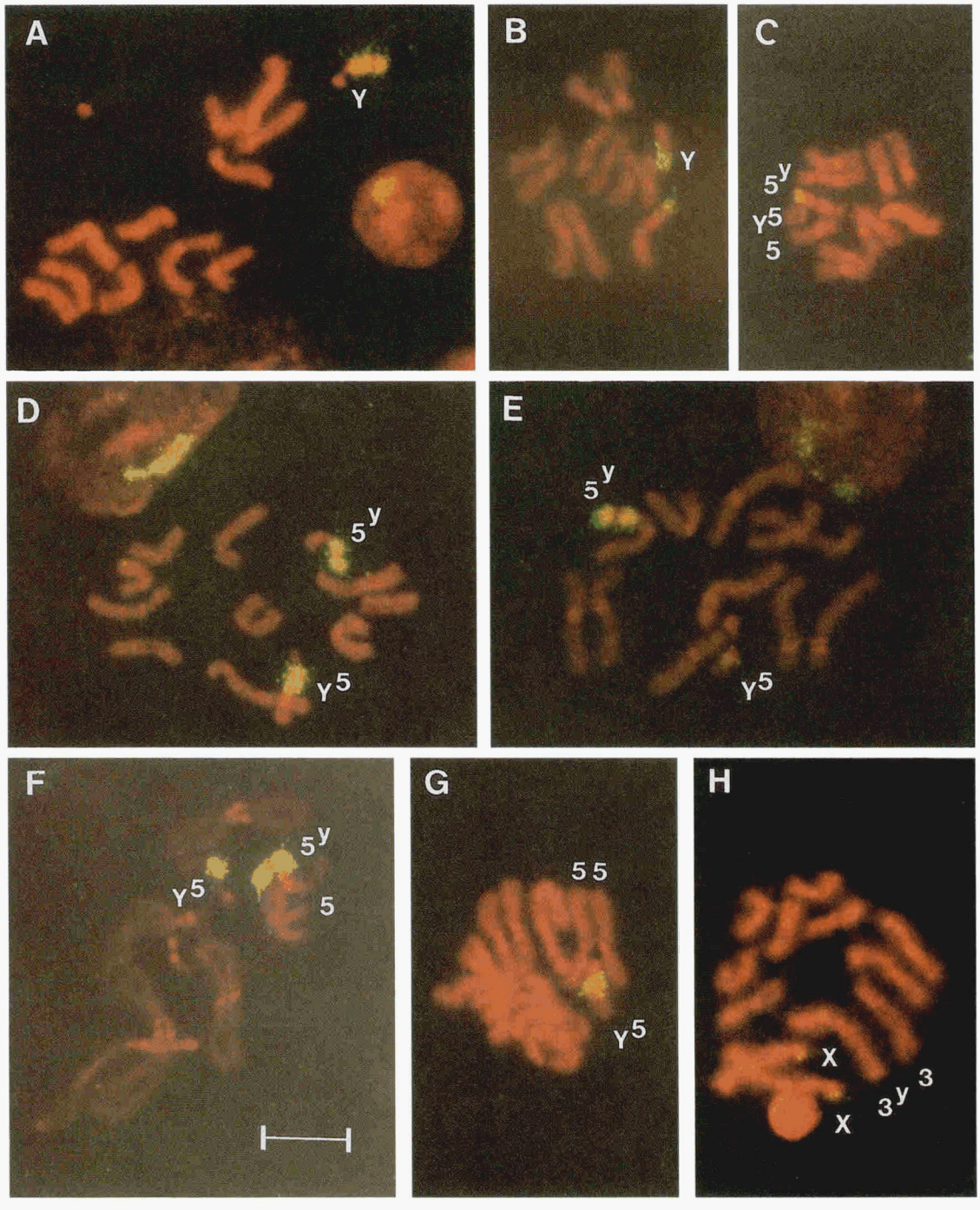

FIGURE 1.—FISH on mitotic chromosomes of wild-type and translocation strains. (A and B) Wild-type strain Egypt II, (C) *T(Y;5)30C,* (D) *T(Y;5)2-40,* (E) *T(Y;5)3-129,* (F) *T(Y;5)3-263,* (G) *T(Y;5)3-263* adjacent-1, (H) *T(Y;3;5)2-5* adjacent-1. (A and C–G) Probed with clone pY114, (B and H) probed with clone pDh2-H8. (A–F) Mitotic chromosomes of third instar larva brain tissue, (G and H) mitotic chromosomes from testes of male adjacent-1 adults. Bar, 10 μm.

probe pY114 and pDh2-H8 and documented three chromosome spreads per slide (examples in Figure 1, D–F, schematic drawings in Figure 2, B and C). In 12 translocation strains a single *Y*-chromosome breakpoint was detected, although in some of these, namely *T(Y;4;5)1-49, T(Y;2;4;5)2-54* and *T(Y;3;5)2-5,* more than one autosome is involved in the translocation. The *Y*-chromosome translocation breakpoints in the different strains are distributed randomly over the whole *Y* chromosome.

Seven *Y*-autosome translocations showed complex rearrangements (Figure 2). In strain *T(Y;5)3-129* a *Y*-chromosome deletion was found, as the signal on 5^{Y} was clearly smaller than expected (Figure 1E). In strains *T(Y;2;5)2-42* and *T(Y;2;5)2-82,* two *Y* chromosome breakpoints split the *Y* chromosome into three fragments. The hybridization pattern of *T(Y;5)30C* showed that the breakpoint is close to the centromere on the long arm of the *Y*. In addition, a considerable part of

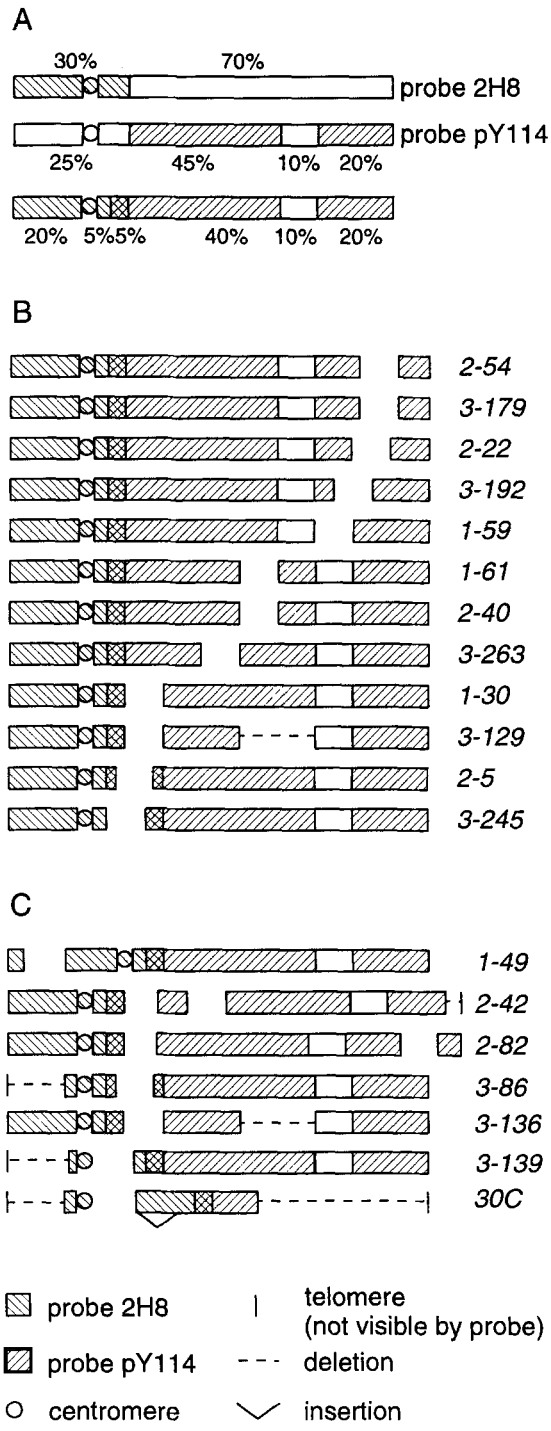

FIGURE 2.—FISH banding pattern and map of *Y*-chromosomal breakpoints in *Y*-autosome translocation strains. (A) Labeling (given in percentage of the whole *Y* chromosome) of probes pDh2-H8 (ribosomal DNA) and pY114 (*Y*-specific, repetitive DNA) on the *Y* chromosome of wild-type strain Egypt II. (B) Map of *Y*-chromosome breakpoints in translocation strains that produce adjacent-1 offspring that were used for the deletion mapping of *Y*-chromosome factors. (C) Map of breakpoints in strains that do not produce adjacent-1 offspring. The location of deletions and insertions is arbitrary.

the long arm of the *Y* chromosome is rearranged (Figure 1C). The rDNA signal on the 5^Y chromosome appeared to be twice as long as the one found in the wild-type karyotype. This is caused either by a duplication of this region or by an insertion of an rDNA containing segment of unknown origin. The pY114 signal was significantly shorter than in the wild type and no gap is visible. Thus a deletion of more than half of the long *Y*-chromosome arm has occurred. Hybridization signals of pY114 and pDh2-H8 overlap, as found with the wild-type *Y* chromosome, suggesting that this region of the long arm is not rearranged. The pY114 probe labeled the tip of the long arm of chromosome 5^Y indicating that the telomere of this arm should be of *Y*-chromosome origin. Another deletion was observed in the short arm of the *Y* chromosome, as the segment labeled with probe pDh2-H8 is shorter than the corresponding region in a wild-type strain. The same is true for strain *T(Y;3;5)3-139* (Figure 2C). In *T(Y;3;5;6)3-136* a segment, somewhere in the middle of the long arm, is deleted (Figure 2C), but the exact location of the deletion cannot be determined. These results show that even large deletions of the long or the short *Y*-chromosome arm do not cause sterility in these strains, indicating that large regions of the *Y* chromosome do not contain genes important for sex determination or male fertility.

Deletion mapping of the *Maleness* factor on the *Y* chromosome: Combining the genetic data with the position of the *Y*-chromosome breakpoints enabled those parts of the *Y* chromosome to be identified that are relevant for male determination. Twelve of the strains, listed in Figure 2B, produce unbalanced offspring due to adjacent-1 segregation in male meiosis.

Figure 3 shows the general structure of these two types of unbalanced karyotypes. One ($X/X/A^Y/A$) carries an autosomal deletion and dies usually during early stages of development. The reciprocal segregation product has a duplication for a part of the autosome and a deletion for a part of the *Y* chromosome ($X/Y^A/A/A$, example in Figure 1G). In all strains listed in Figure 4, some of these adjacent-1 individuals develop into adults and they can be distinguished from the progeny with balanced karyotypes by employing the appropriate combination of genetic markers, *i.e.*, markers covered by the duplication in case of female adjacent-1 individuals and markers not covered by the duplication in case of male adjacent-1 individuals.

In each case, four aberrant individuals were analyzed using chromosome preparations from testis or ovary tissue to verify that they were indeed adjacent-1 individuals, lacking the A^Y chromosome, but still carrying the Y^A chromosome. In strains *T(Y;5)2-22* and *T(Y;3)1-30* one out of four individuals was found to carry the complete translocation. These two aberrant individuals probably arose by recombination in the male parent heterozygous for the respective markers.

In eight translocation strains the adjacent-1 individuals are males (Table 1). In some strains, *e.g.*, *T(Y;5)2-22*, a relatively high number of adjacent-1 offspring develop into adults, while in other strains, *e.g.*, *T(Y;5)2-40*, only a small percentage of adjacent-1 individuals survive until

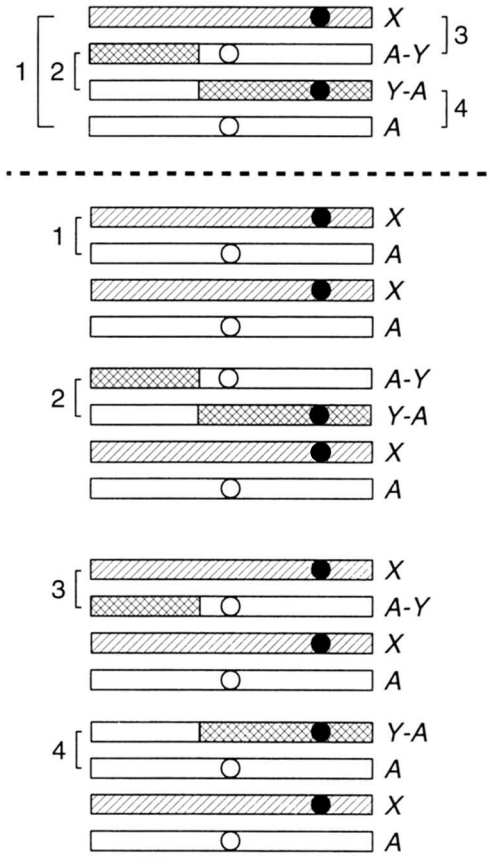

FIGURE 3.—Segregation of *Y*-autosome translocations. Two types of segregation are possible (top), alternate (1 and 2) and adjacent-1 (3 and 4). In the following generation (bottom), four different karyotypes are produced where one autosome and one *X* chromosome is derived from the mother. Alternate segregation (1 and 2) leads to balanced offspring. Adjacent-1 segregation results in unbalanced offspring; (3) two *X* chromosomes, one *Y* fragment and a deletion for part of the autosome; these individuals usually die during early embryogenesis, and (4) one *X* chromosome, one *Y* fragment and a duplication for part of the autosome; a certain percentage of these individuals survives until the adult stage.

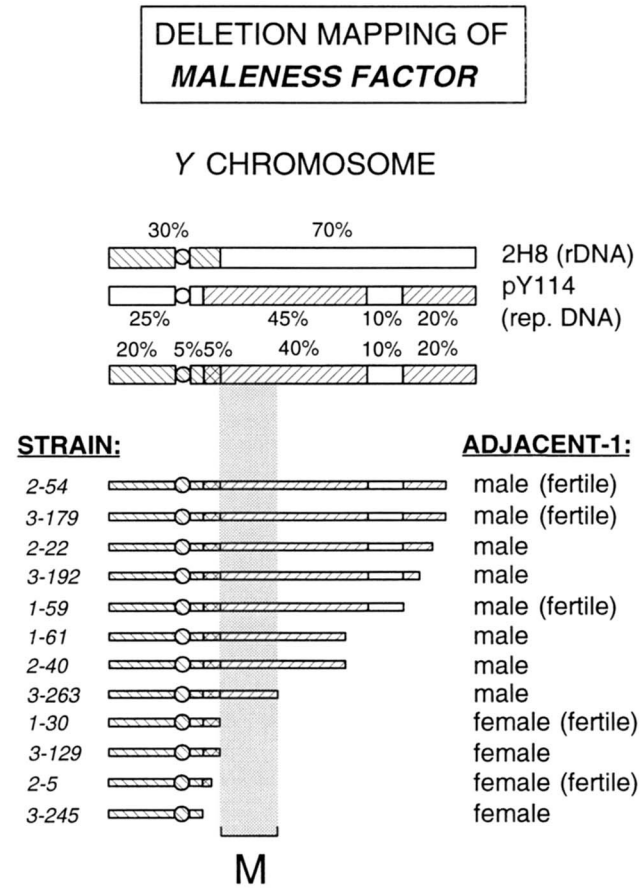

FIGURE 4.—Deletion mapping of the *Maleness* factor. Karyotype analysis of adjacent-1 adults served for the identification of the *Y* segment present in each strain. Sex and fertility of the individuals is indicated on the right. For labeling pattern of the *Y* chromosome see legend of Figure 2. M, male-determining region.

adulthood. In addition, in all strains a certain percentage of these adjacent-1 males did not completely emerge and died during eclosion (Table 1). Both, the overall recovery of adjacent-1 adults as well as the percentage of half-emerged flies correlates well with the length of the duplication of chromosome *5*. We assume that the general fitness of adjacent-1 males is decreased by the increased length of the duplicated part of chromosome *5*.

The remaining four strains produce female adjacent-1 offspring. In *T(Y;5)3-245* no marker is available to identify adjacent-1 flies as the autosomal breakpoint is distal to the only morphological marker on this arm of chromosome *5* (*i.e., wp*). However, a considerable proportion of the females died during eclosion indicating the presence of individuals with unbalanced karyotypes. For this strain only these half emerged flies were used for FISH and Southern analysis. Both methods confirmed the predicted adjacent-1 karyotype.

By combining the results from all 12 strains the *Maleness* factor could be mapped (Figure 4). In strain *T(Y;5)3-263* ~55% of the long *Y*-chromosome arm is deleted (Figure 1G) and as these individuals were found to be males, the *Maleness* factor cannot be located in this region. In strains *T(Y;3)1-30* and *T(Y;5)3-129* most of the long arm (~70% of the whole *Y*) is deleted and the adjacent-1 individuals were females. Therefore, the *Maleness* factor cannot be located in the region of the short arm of the *Y* and in the region of the long arm next to the centromere. The *Maleness* factor must be located in the region of the long arm between the breakpoints of *T(Y;5)3-263* and *T(Y;5)1-30* that represents ~15% of the whole *Y* chromosome (Figure 4).

In strain *T(Y;3;5)2-5*, we analyzed not only aberrant females but also 10 aberrant males that occurred as a consequence of the complexity of this translocation. Nine of these males showed a small deletion of chromosome *3* with a expected karyotype of *X* $3^{Y}3$ *55*, but one male was *XX* $3^{Y}3$ *55* (Figure 1H). Thus, the presence of the *Y* segment containing the *Maleness* factor causes male development irrespective of the number of *X* chromosomes present.

TABLE 1

Karyotype, frequency and fertility of adjacent-1 males in *Y*-autosome translocation strains

Strains	Karyotype		Frequency of aberrant males			Fertility of aberrant males			
	Chromosome 5 duplication (%)	*Y* chromosome deletion (%)	No. of flies analyzed	Aberrant males (%)	Aberrant males half emerged (%)	No. of males tested	No. of males observed mating	Mean egg hatch (%)	Offspring produced
	5L								
T(Y;2;4;5)2-54	10	8	16519	17.2	11.8	10	6	56	+
T(Y;5)1-59	33	20	23007	18.6	37	10	7	17	+
T(Y;5)3-192	50	15	23827	10.9	81.9	10	1	0	−
T(Y;5)2-40	54	35	19835	6.9	90	10	2	0	−
	Cen.								
T(Y;5)3-263	31	55	32630	9.2	87.6	10	0	0	−
T(Y;5)1-61	28	35	27943	17.9	81	12	0	0	−
T(Y;5)2-22	25	12	34697	22	75	12	0	0	−
T(Y;5)3-179	25	8	39980	16.8	79.7	10	1	3	−
	5R								

Length of duplication of chromosome *5* (chromosome *5* duplication, in %) and *Y*-chromosomal deletion (*Y* chromosome deletion, in %) in adjacent-1 males; frequency of aberrant males and fraction of half-emerged aberrant males (% of total aberrant individuals, note for a comparison: 0.8% occur in the wild-type strain Egypt II); and fertility of adjacent-1 males tested for 10–12 single males with respect to observed mating couples, egg hatch and offspring. Percentage of egg hatch is calculated only from families that showed egg hatch. For a comparison, balanced *T(Y;5)* translocation males produce 65–70% egg hatch (FRANZ *et al.* 1994). The translocation strains are listed in the order of size and region of autosome *5* duplications. 5L, left arm of chromosome *5*, *Cen.*, centromere; 5R, right arm of chromosome *5*.

Adjacent-1 individuals were also subject to Southern analysis using pY114 as a probe. DNA was isolated from the fly material that remained after removing ovaries or testes for the karyotype analysis. Figure 5A shows that the number and intensity of the resulting bands is reduced significantly when at least half of the *Y* chromosome is deleted. Only few and relatively weak hybridization signals are observed in individuals carrying large deletions, *e.g.*, in adjacent-1 individuals from strain *T(Y;5)3-245*, thereby corroborating the results from FISH. As a comparison, the results obtained with the respective balanced genotypes are shown in Figure 5B.

Test for fertility of adjacent-1 males: To determine whether the *Y* chromosome of the medfly carries male fertility factors as is the case in Drosophila, single male crosses were set up with 10 or 12 adjacent-1 males from each strain. The cages were checked at regular intervals for 8 days for the presence of mating couples. At least one mating was observed for seven *T(Y;5)1-59* adjacent-1 males, six in the case of *T(Y;2;4;5)2-54*, two in the case of *T(Y;5)2-40* and one in the case of *T(Y;5)3-179* and *T(Y;5)3-192*. The males of the other strains were never observed mating. All eggs produced were collected, egg hatch was determined and any offspring was noted (Table 1).

At the end of the test, testes were dissected. In all adjacent-1 males motile sperm was detected and no obvious defect in sperm morphology was observed. The same was true for those individuals that were used in the karyotype analysis, including the adjacent-1 males produced by translocation *T(Y;3;5)2-5* where the short arm and the proximal part of the long arm are deleted. Consequently, if there are male fertility factors on the medfly *Y* chromosome, they have to be located in the segment identified as carrying the *Maleness* factor.

Females mated with all adjacent-1 males showed a reduced fertility, although with varying degree. However, this seems to be caused primarily by the inability of the males to mate successfully. These males are generally less fit and have a shorter life span than the balanced males. Reduced viability and fertility are correlated with the length of the duplication of the autosome rather than as a consequence of the *Y* chromosome deletion.

Aberrant females of strains *T(Y;3)1-30, T(Y;3)3-129, T(Y;3;5)2-5* and *T(Y;5)3-245* that carry only one *X* chromosome and a short part of the *Y* chromosome showed no obvious change in ovary morphology or egg production. Aberrant females of strain *T(Y;3;5)2-5* were fertility tested and three out of 10 individuals produced offspring. Strain *T(Y;3)1-30* was shown previously to produce fertile adjacent-1 females (KERREMANS *et al.* 1992). These results lead to the conclusions that (1) one *X* chromosome is sufficient for female development and fertility and (2) no *Y*-specific factor, compromising the development of fertile females, is located on the short arm and on a small region of the long arm next to the centromere of the *Y* chromosome.

DISCUSSION

Mapping *Y* chromosome rearrangements: FISH increased the mapping resolution as compared to the C-

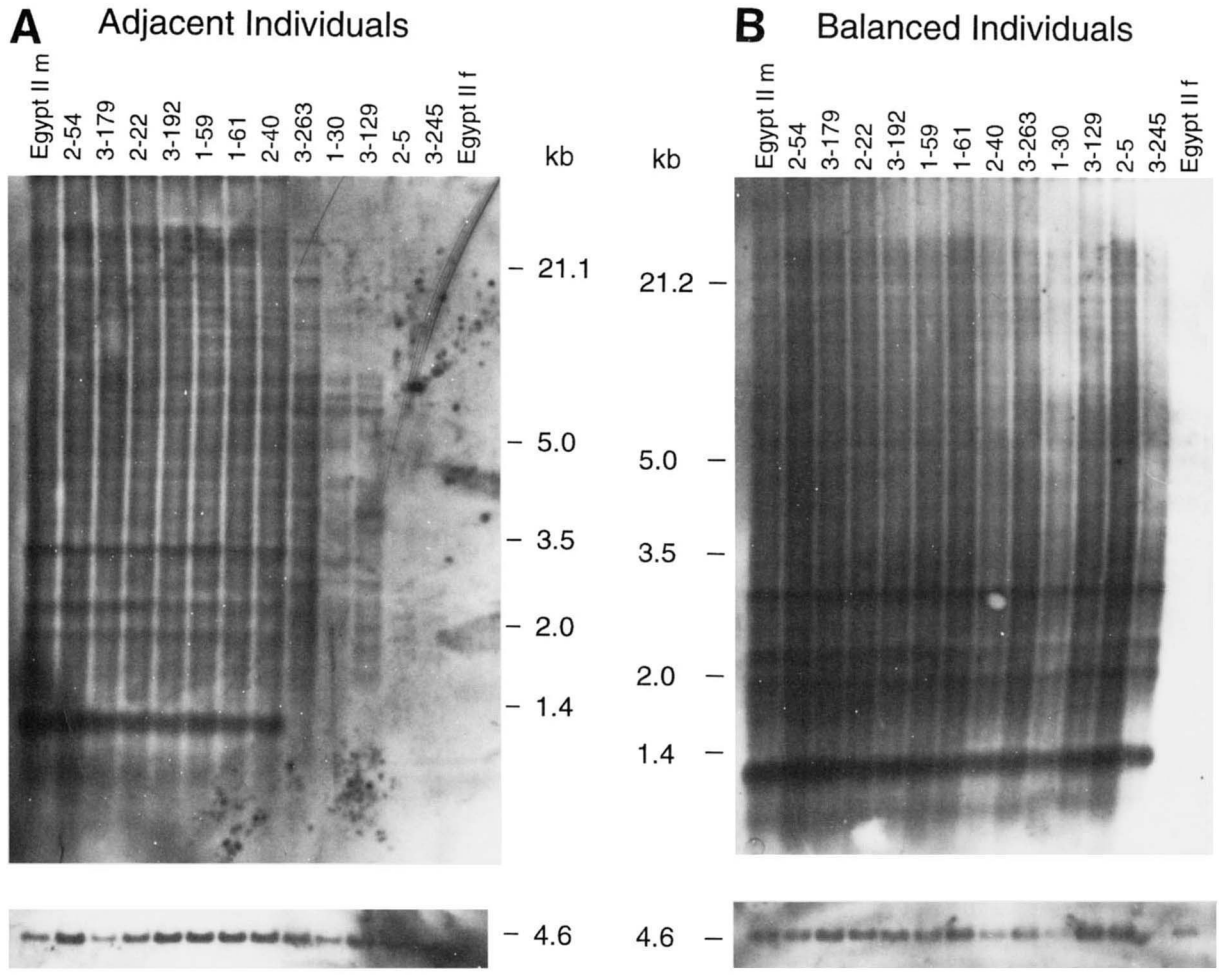

FIGURE 5.—Southern analysis of *Eco*RI-digested genomic DNAs of single individuals from *Y*-autosome translocation strains. (A) Adjacent-1 individuals, (B) balanced individuals. (Top) Hybridization with DNA probe pY114. (Bottom) Rehybridization with DNA probe pmed21.

banding technique. Even the translocation of small parts of the *Y* can be detected by this method. Insertions and deletions are also detectable with repetitive probes, as demonstrated in five strains (Figure 2), but such rearrangements have to be relatively large for a secure detection. As we used a whole series of *Y*-chromosome deletions, we minimized the chance of overlooking small rearrangements that affect the *Y*-chromosome region of interest.

The *Maleness* factor of *C. capitata*: Two studies have shown that sex determination in *C. capitata* is different from that found in *D. melanogaster*. LIFSCHITZ and CLADERA (1989) found *XXX* females and *XXY* males in a wild population and concluded that the *Y* chromosome determines the male sex. ZAPATER and ROBINSON (1986) predicted the presence of male determinants in the long arm of the *Y* chromosome based on the analysis of aneuploid offspring generated by a *T(Y;2)* translocation.

Our results confirm (1) that the long arm of the *Y* chromosome is sufficient to determine maleness and (2) that this chromosome arm determines maleness despite the presence of two *X* chromosomes. Consequently, the sex-determination mechanism in *C. capitata* differs from the system found in *D. melanogaster*. In the medfly, a dominant *Maleness* factor exists while in Drosophila the *X* chromosome:autosome ratio is responsible for sex determination. In *D. melanogaster* several *X*-chromosomal "numerator" genes and autosomal "denominator" elements control the key gene *Sex-lethal* in early embryonic development that then initiates a cascade of genes for the development of the embryo into either a male or female individual (for a review see CLINE 1993). Our results demonstrate clearly that the very first step of sex determination is different in *C. capitata*.

Deletion mapping enabled the location of the factor(s) responsible for male sex determination to be more precisely defined. Using a series of *Y*-chromosome deletions we mapped the *Maleness* factor to a region representing ~15% of the whole *Y* chromosome. This region is located in the first third of the long arm next to the centromere. Whether sex determination is caused by a single *Maleness* factor or by several factors located in this region cannot be determined from our data. However in theory, a single, dominant *Maleness*

factor should be sufficient. The positional information obtained in our experiments could be an essential first step for the cloning of the factor(s). Based on this information, we have isolated the respective region of the *Y* chromosome via microdissection (U. WILLHOEFT, J. MUELLER-NAVIA and G. FRANZ, unpublished data).

A male-determining region present on the *Y* chromosome has also been reported in several other Dipteran species. *Lucilia cuprina* shows, as medfly, a heterogametic and heterochromatic *XY* pair and the male-determining region was mapped, using adjacent segregation products, to the centromeric portion of the *Y* chromosome (BEDO and FOSTER 1985). In contrast, a distal location of the male-determining region was found in *Chironomus thummi* (KRAEMER and SCHMIDT 1993) and in *Megaselia scalaris* (WILLHOEFT and TRAUT 1995). In both species the mitotic *XY* chromosomes cannot be distinguished cytologically, although differences can be found by RFLP studies. A location near to the centromere might be favored in species possessing heteromorphic sex chromosomes and *Y* chromosomes containing a high proportion of repetitive DNA.

Are fertility factors present on the *C. capitata Y* chromosome?: We have detected *Y*-autosome translocations where ≤50% of the *Y* chromosome is deleted. Furthermore, in the most extreme adjacent-1 karyotypes the short arm and the proximal part of the long arm (*e.g., T(Y;3;5)2-5*) or the distal half of the long arm (*e.g., T(Y;5)3-263*) are deleted. In all these cases we observed motile sperm with, apparently, normal morphology. If *Y*-chromosomal fertility factors are at all present in *C. capitata,* they can only be located in the same region of the *Y* chromosome in which we mapped the *Maleness* factor. This situation is very similar to that found in *L. cuprina* (BEDO and FOSTER 1985), but quite different from *D. melanogaster* and *D. hydei* where several factors essential for spermiogenesis are distributed over the entire *Y* chromosome (for reviews see LIFSCHYTZ 1987; HENNIG *et al.* 1989; HACKSTEIN and HOCHSTENBACH 1995).

The deletion mapping of the medfly *Y* chromosome demonstrated that 85% of the *Y* chromosome contain no material required for sex determination and testes development. This could lead to the occurrence of *Y* chromosome length polymorphism in wild populations. However, no such polymorphism in three mutant strains and six wild-type strains of different origin has been found (U. WILLHOEFT and G. FRANZ, unpublished results). The only exception reported so far is a *Y* chromosome, detected in a wild population from Argentina, that lacks approximately half of the long arm (LIFSCHITZ and CLADERA 1989). *Y* chromosome polymorphism does not seem to be frequently present in medfly, suggesting that *Y* chromosome size is essential, although not for sex determination and spermiogenesis.

We are grateful to Dr. D. HAYMER for the gift of probe pY114 and pmed21 and to Dr. R. RENKAWITZ-POHL for the gift of probe pDh2-H8. We thank H. GENSTHALER, O. IBANTSCHITZ, E. SCHORN and M. TAHER for excellent technical assistance in breeding the strains. M. ASHBURNER and A. ROBINSON are thanked for critical reading of the manuscript. U.W. was supported by a grant of the German Government.

LITERATURE CITED

ANLEITNER, J. E., and D. S. HAYMER, 1992 *Y* enriched and *Y* specific DNA sequences from the genome of the Mediterranean fruit fly, *Ceratitis capitata.* Chromosoma **101:** 271–278.

BEDO, D. G., 1987 Polytene chromosome mapping in *Ceratitis capitata* (Diptera : Tephritidae). Genome **29:** 598–611.

BEDO, D. G., and G. G. FOSTER, 1985 Cytogenetic mapping of the male-determining region of *Lucilia cuprina* (Diptera : Calliphoridae). Chromosoma **92:** 344–350.

BEDO, D. G., and G. C. WEBB, 1989 Conservation of nucleolar structure in polytene tissues of *Ceratitis capitata* (Diptera : Tephritidae). Chromosoma **98:** 443–449.

BIRNBOIM, H. C., and J. DOLY, 1979 A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:** 1513–1523.

BLIN, N., and D. W. STAFFORD, 1976 A general method for the isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res. **3:** 2303–2308.

CLINE, T. W., 1993 The *Drosophila* sex determination signal: how do flies count to two? Trends Genet. **9:** 385–390.

FRANZ, G., and P. KERREMANS, 1994 Requirements and strategies for the development of genetic sex separation systems with special reference to the Mediterranean fruit fly, *Ceratitis capitata,* pp. 113–122 in *Fruit Flies and the Sterile Insect Technique,* edited by C. A. CALKINS, W. KLASSEN and P. LIEDO. CRC Press, Boca Raton, FL.

FRANZ, G., E. GENCHEVA and P. KERREMANS, 1994 Improved stability of genetic sex-separation strains for the Mediterranean fruit fly, *Ceratitis capitata.* Genome **37:** 72–82.

HACKSTEIN, J. H. P., and R. HOCHSTENBACH, 1995 The elusive fertility genes of *Drosophila:* the ultimate haven for selfish genetic elements. Trends Genet. **11:** 195–200.

HAYMER, D. S., J. E. ANLEITNER, M. HE, S. THANAPHUM, S. H. SAUL *et al.,* 1990 Actin genes in the Mediterranean fruit fly, *Ceratitis capitata.* Genetics **125:** 155–160.

HENDRICHS, J., G. FRANZ and P. RENDON, 1995 Increased effectiveness and applicability of the sterile insect technique through male-only releases for control of Mediterranean fruit flies during fruiting seasons. J. Appl. Entomol. **119:** 371–377.

HENNIG, W., R. C. BRAND, J. HACKSTEIN, R. HOCHSTENBACH, H. KREMER *et al.,* 1989 *Y* chromosomal fertility genes of *Drosophila:* a new type of eukaryotic genes. Genome **31:** 561–571.

KERREMANS, P., and G. FRANZ, 1994 Cytogenetic analysis of chromosome 5 from the Mediterranean fruit fly, *Ceratitis capitata.* Chromosoma **103:** 142–146.

KERREMANS, P., and G. FRANZ, 1995 Isolation and cytogenetic analyses of genetic sexing strains for the medfly, *Ceratitis capitata.* Theor. Appl. Genet. **91:** 255–261.

KERREMANS, P., K. BOURTZIS and A. ZACHAROPOULOU, 1990 Cytogenetic analysis of three genetic sexing strains of *Ceratitis capitata.* Theor. Appl. Genet. **80:** 177–182.

KERREMANS, P., E. GENCHEVA and G. FRANZ, 1992 Genetic and cytogenetic analysis of *Y*-autosome translocations in the Mediterranean fruit fly, *Ceratitis capitata.* Genome **35:** 264–272.

KRAEMER, C., and E. R. SCHMIDT, 1993 The sex determining region of *Chironomus thummi* is associated with highly repetitive DNA and transposable elements. Chromosoma **102:** 553–562.

LIFSCHITZ, E., and J. L. CLADERA, 1989 Cytogenetics and sex determination, pp. 3–11 in *Fruit Flies Their Biology, Natural Enemies and Control, volume 3B,* edited by A. S. ROBINSON and G. HOOPER. Elsevier, Amsterdam.

LIFSCHYTZ, E., 1987 The developmental program of spermiogenesis in *Drosophila:* a genetic analysis. Int. Rev. Cytol. **109:** 211–257.

MCINNIS, D. O., S. Y. T. TAM, C. GRACE and D. MIYASHITA, 1994 Population suppression and sterility rates induced by variable sex ratio, sterile insect releases of *Ceratitis capitata* (Diptera: Tephritidae) in Hawaii. Ann. Entomol. Soc. Am. **87:** 231–240.

RENKAWITZ-POHL, R., K. H. GLAETZER and W. KUNZ, 1980 Characterization of cloned ribosomal DNA from *Drosophila hydei.* Nucleic Acids Res. **8:** 4593- 4611.

ROESSLER, Y., 1979 The genetics of the Mediterranean fruit fly: a "white pupae" mutant. Ann. Entomol. Soc. Am. **72:** 583–585.

ROESSLER, Y., and Y. KOLTIN, 1976 The genetics of the Mediterranean fruit fly, *Ceratitis capitata:* three morphological mutations. Ann. Entomol. Soc. Am. **69:** 604–608.

ROESSLER, Y., and H. ROSENTHAL, 1992 Genetics of the Mediterranean fruit fly (Diptera: Tephritidae): morphological mutants on chromosome five. Ann. Entomol. Soc. Am. **85:** 525–531.

ROESSLER, Y., A. MALACRIDA and M. C. ZAPATER, 1994 Mutants, chromosomes, and genetic maps in the Mediterranean fruit fly, pp. 97–112 in *Fruit Flies and the Sterile Insect Technique,* edited by C. A. CALKINS, W. KLASSEN and P. LIEDO. CRC Press, Boca Raton, FL.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning. A Laboratory Manual,* Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

WEICHENHAN, D., 1991 Fast recovery of DNA from agarose gels by centrifugation through blotting paper. Trends Genet. **7:** 109.

WILLHOEFT, U., and W. TRAUT, 1995 The sex-determining region of the *Megaselia scalaris* (Diptera) *Y* chromosome. Chromosome Res. **3:** 59–65.

ZACHAROPOULOU, A., 1987 Cytogenetic analysis of mitotic and salivary gland chromosomes in the medfly *Ceratitis capitata.* Genome **29:** 67–71.

ZACHAROPOULOU, A., K. BOURTZIS and P. KERREMANS, 1990 A comparison of polytene chromosomes in salivary glands and orbital bristle trichogen cells in *Ceratitis capitata.* Genome **34:** 215–219.

ZACHAROPOULOU, A., M. FRISARDI, C. SAVAKIS, A. S. ROBINSON, P. TOLIAS *et al.,* 1992 The genome of the Mediterranean fruitfly *Ceratitis capitata:* localization of molecular markers by *in situ* hybridization to salivary gland polytene chromosomes. Chromosoma **101:** 448–455.

ZAPATER, M., and A. S. ROBINSON, 1986 Sex chromosome aneuploidy in a male-linked translocation in *Ceratitis capitata.* Can. J. Genet. Cytol. **28:** 161–167.

Communicating editor: L. PARTRIDGE