ANALYSIS OF THE *CERATITIS CAPITATA* Y CHROMOSOME USING *IN SITU* HYBRIDIZATION TO MITOTIC CHROMOSOMES

U. WILLHOEFT, G. FRANZ
Entomology Unit,
FAO/IAEA Agriculture and Biotechnology Laboratory,
Seibersdorf

Abstract

In *Ceratitis capitata* the Y chromosome is responsible for sex-determination. We used fluorescence *in situ* hybridization (FISH) for cytogenetic analysis of mitotic chromosomes. FISH with the wild-tpye strain EgyptII and two repetitive DNA probes enabled us to differentiate between the short and the long arm of the Y chromosome and gives a much better resolution than C-banding of mitotic chromosomes. We identified the Y-chromosomal breakpoints in Y-autosome translocations using FISH. Even more complex rearrangements i.e. deletions and insertions in some translocation strains were detected by this method. A strategy for mapping the primary sex determination factor in *Ceratitis capitata* by FISH is presented.

1. INTRODUCTION

Genetic Sexing is achieved by linking the wild-type allele of a selectable marker, via a reciprocal translocation, to the Y chromosome. Large scale separation of homozygous females and heterozygous males opens the possibility to release only males in SIT programs. Due to the structure of current sexing strains some disadvantages arise: a) occurrence of unbalanced gametes leads to semi-sterility and b) recombination in heterozygous males, although a rare event, leads to breakdown of the genetic sexing system. It is envisaged that sexing systems developed with molecular approaches will not have these problems. Isolation and germline transformation of a gene suitable for genetic sexing might overcome problems that arise with the use of translocation strains for genetic sexing. Genes involved in sex determination of *Ceratitis capitata* are good candidates for such an approach. This report will summarize a strategy to map the primary sex determination signal in *Ceratitis capitata* in order to facilitate cloning of this factor.

2. MATERIAL AND METHODS

Wild-type strain Egypt II was used to characterize the Y-chromosomal hybridization pattern of two DNA probes. The translocation strains T(Y;3)1-30 [1], T(Y;5)1-61 [2] and T(Y;5)30C [3] were used for mapping the Y-chromosomal breakpoints. As the Y chro-mosome does not polytenize, mitotic chromosome spreads were analyzed. Brain tissue of 3rd instar larvae was incubated for 15 min in 1% Na-citrate, prefixed in methanol:acetic acid (3:1) for 5 min, dissected in a little drop of 60% acetic acid on a slide and fixed on a hot plate. After 2 min in 80% ethanol, the slides were air-dried and frozen at -20°C over night up to one year.

Two probes were used to analyze the Y chromosomes: pY114 is a cloned DNA fragment from *Ceratitis capitata* that contains Y-specific, repetitive DNA [4]. 2H8 is a clone containing ribosomal DNA (rDNA) from *Drosophila hydei* [5]. DNA probes were labelled with DIG-11-UTP according to the instructions of the supplier (Boehringer).

For *in situ* hybridization, slides were baked for 2 h at 80°C and dehydrated in 80% and 100% ethanol. Chromosome spreads were denatured in 25 mM NaOH for 60 sec., washed for 10 sec. in 0.4 x

SSC, 0.1% Tween, and then dehydrated through a series of 70% (precooled at -20°C), 80% and 100% ethanol for 2 min each. The hybridization solution consisted of 20 ng DIG-labelled DNA probe, 50% formamide (deionized) and 35% master mix (1 ml 20 x SSC, 1 ml dextransulfate, 1 ml aqua bidest. and 0.5 ml salmon sperm DNA (10 mg/ml, sheared to 200 - 500 bp). 10 µl hybridization mixture was denatured at 80°C for 8 min, cooled on ice and then applied to the slide, covered with a cover-slip and sealed with rubber cement. Hybridization took place at 37°C in a humid chamber for 12 - 18 h. After two washes in 0,4 x SSC, 0.1% Tween at room temperature, anti-DIG-antibodies (fluorescein-conjugated) were applied according to the instructions of the supplier (Boehringer). Antifade (0.233 g DABCO in 0.9 ml glycerol, 0,1 ml 0.2 M Tris pH 8) containing 0.2 µg/µl propidiumiodide was placed on the slide. Pictures from epifluorescence microscopy were taken with Kodak Ecta GoldII (400 ASA) films.

3. RESULTS AND DISCUSSION

3.1. FISH with wild-type strain EgyptII

Fluorescence *in situ* hybridization using mitotic chromosome spreads of the wild-type strain Egypt II, probed with clone pY114, showed hybridization signals exclusively on the Y chromosome (Fig.1). The long arm of the Y chromosome was labelled except for the region next to the centromere and a gap of approx. 10% of the whole Y chromosome length, near the tip. The centromere and the short arm were not labelled.

In contrast, 2H8 labelled the short arm, the centromere region and approx. 10% of the long arm of the Y chromosome (Fig.1). Besides that, a region at the tip of the short arm of the X chromosome was labelled. This hybridization pattern is in good agreement with published results [6]. In addition, the centromere region of chromosome 4 showed a faint hybridization signal. On the long arm of the Y chromosome the hybridization signals of both probes overlap partially.

In situ hybridization with 2H8 and pY114 proved to increase the resolution compared with C-banding techniques of mitotic chromosomes. Even small parts of the Y chromosome, representing roughly 5 - 10% of the whole Y, can be detected. In addition, probes 2H8 and pY114 allow to distinguish between the long and the short arm of the chromosome. Therefore, *in situ* hybridization was used to identify breakpoints of several Y-autosome translocations.

3.2. Detection of translocation breakpoints

The Y-chromosomal breakpoint of the Y-5 translocation in strain T(Y;5)1-61 was mapped to the middle of the long arm (Fig.2). The Y^5 consists of the short arm, the centromere and a part of the long arm, equivalent to approx. 30 % of the whole Y chromosome, while the 5^Y harbors the rest of the long arm of the Y. Inspecting the chromosomes of four male individuals, two were found to carry only the Y^5, but not the 5^Y chromosome. This karyotype is caused by adjacent-1 segregation and its frequency fits well to the expected 50% unbalanced gametes in this translocation strain.

In strain T(Y;3)1-30 the Y-chromosomal breakpoint was mapped in the neighborhood of the centromere at the long arm (Fig.2). Probe 2H8 labels the Y^5, but not the 5^Y chromosome, indicating that the breakpoint is located approx. 10% of the total length of the Y chromosome distal of the centromere on the long arm. One out of the six individuals that were analyzed proved to have an adjacent-1 karyotype.

The hybridization pattern of T(Y;5)30C showed, besides the Y-5 translocation, a more complex configuration (Fig.2). A considerable part of the long arm of the Y chromosome is deleted in T(Y;5)30C,

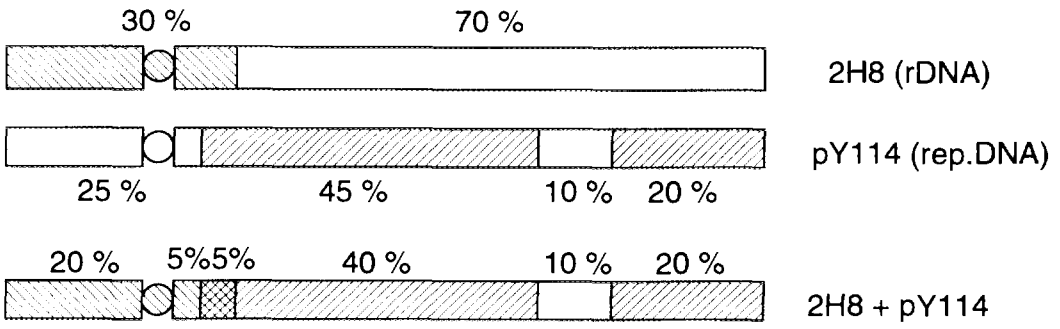

FIG. 1. Y chromosome of the wild-type strain EGYPT II.

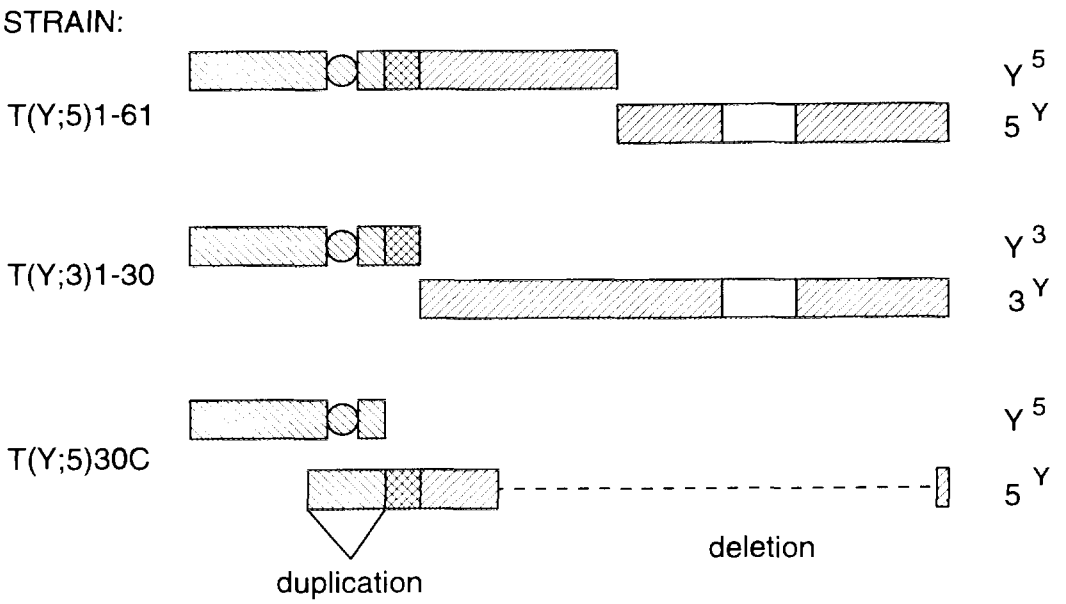

FIG. 2. Y chromosome in translocation strains.

the exact position of the deletion cannot be determined. The Y-chromosomal breakpoint mapped close to the centromere on the long arm of the Y. 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 appeared to be shorter than in the wild-type situation, the region around the "gap" is missing, thus a deletion of half of the long Y chromosome arm has occurred in this strain. Hybridization signals of pY114 and 2H8 overlap, as found in the wild-type karyotype, suggesting that this region of the long arm is not rearranged. As the signal of pY114 labels the tip of the 5^{Y} chromosome, the telomere of this arm should be of Y chromosome origin. Three out of eight individuals analyzed, showed an adjacent-1 karyotype with the Y^{5} and two chromosomes 5.

3.3. A mapping strategy for the sex-determination factor

Mapping of the breakpoints and of deletions in Y-autosome translocation strains opens the possibility to identify those parts of the Y chromosome that are relevant for sex determination. In Y-autosome translocation, the Y chromosome is split into two parts. Adjacent-1 individuals have a part of the translocated autosome in triplicate. Furthermore, the Y-chromosomal segment without centromere is absent. In strains T(Y;5)1-61, T(Y;3)1-30 and T(Y;5)30C 3rd instar larvae with adjacent-1 karyotype were found. In some strains such adjacent individuals develop into adults and they can be distinguished by their phenotype using the appropriate combination of genetic markers. In 1-61 ♂ x *or* ♀, all females are homozygous *or*, while all males are heterozygous and, therefore, wild-type. Adjacent-1 reach the adult stage and have a X,Y^5,5,5 karyotype. The wild-type allele of *or* is located outside of the triplicated chromosome segment [2] and, consequently, adjacent-1 individuals are *or/or*. In this translocation strain adjacent-1 flies are male and can, therefore, be distinguished from the normal, balanced, males. The Y^5 chromosome of strain 1-61 is sufficient for the development of male individuals.

In case of strain T(Y;3)1-30, the Y^3 carries the *dp*$^+$ allele while the free chromosomes 3 carries the mutant allele. This strain generates adjacent-1 females with wild-type phenotype. Therefore, the primary sex determining factor cannot be located on the short arm or on the centromere region of the long arm of the Y chromosome.

In strain 30C, half of the long arm is deleted. As this strain produces viable and fertile males, factors relevant for male sex determination cannot be located in the deleted part of the Y chromosome. Due to the complex rearrangement of the Y chromosome, at least one deletion, one insertion or duplication and one translocation breakpoint, we cannot map the deleted part in detail. *In situ* hybridization with other Y-specific DNA probes might overcome this problem.

Fig.3 summarizes the mapping of the primary sex determining factor on the Y chromosome by analyzing adjacent-1 offspring. Absence of the long arm of the Y chromosome leads to the development of female individuals, presence of this part to the development of male individuals. The primary sex determination factor in *Ceratitis capitata* is therefore a maleness factor.

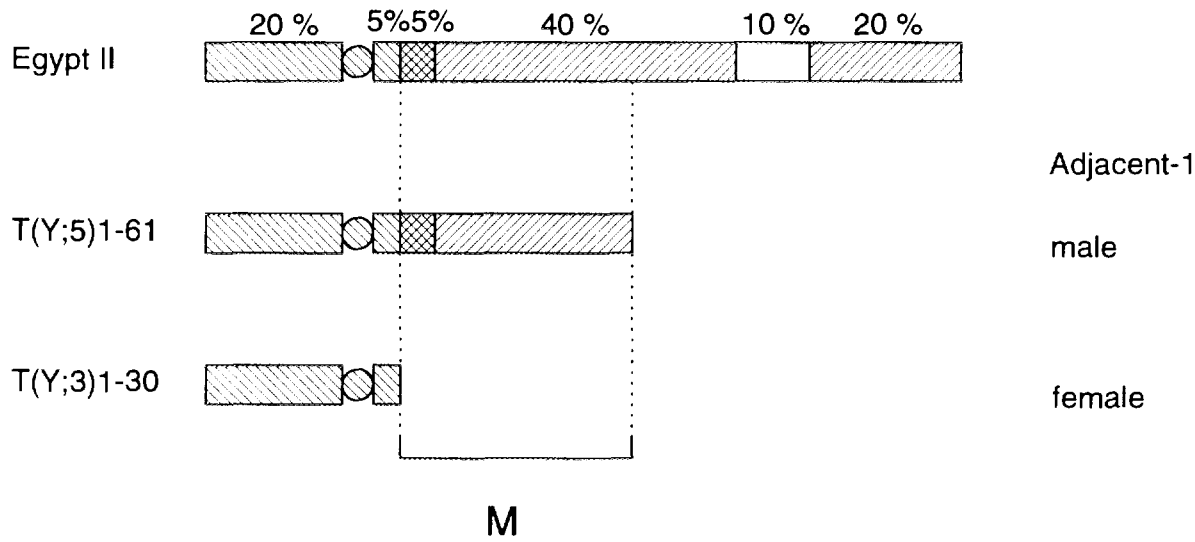

FIG. 3. Deletion mapping of Y factors.

These result is in good agreement with data obtained in studies of sex chromosome aneuploids [7], who found XXX individuals to be females and XXY individuals to be males. The number of X chromosomes is not of importance, while the presence of Y chromosome determines the male sex. A maleness factor responsible for sex determination was found in other Dipterean species like *Megaselia scalaris*, *Musca domestica* and *Culex tritaeniorrhynchus*. A different system of the sex determination is present in *Drosophila melanogaster*, where the X:autosome ratio is responsible for sex determination, while the Y chromosome has no impact.

In theory, a single maleness factor could be sufficient for sex determination. This factor would trigger a cascade of genes that determine somatic and germline development of either female or male characteristics. However, at present we cannot be certain whether one factor or several factors, responsible for sex determination, are located in the mapped part of the Y chromosome of *Ceratitis capitata*.

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