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# *Y* enriched and *Y* specific DNA sequences from the genome of the Mediterranean fruit fly, *Ceratitis capitata*

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**Abstract.** DNA sequences that are enriched or specific to the genome of the male medfly, *Ceratitis capitata,* have been isolated using a differential hybridization approach. Twelve phage clones from a genomic library have been identified that consistently display more intense hybridization with a genomic DNA probe from males as opposed to one from females. Southern DNA blot analysis reveals that these recombinant clones contain at least one *Eco*RI fragment that is either specific to the male genome, or more highly represented in it, as compared with the female genome. These *Eco*RI fragments, when used as probes, all generate a similar pattern of intense multiple bands in genomic DNA of males. This suggests the presence of repetitive sequences that are at least partially homologous in these regions of the genome that are specific to or enriched in males. In situ hybridization to mitotic chromosomes confirms a *Y* chromosomal origin for the male specific repetitive sequences. Data on the genomic organization, representation and evolutionary conservation of these sequences that are specific to or enriched in males are presented. Studies of the genomic organization and representation of flanking sequences that are not male specific are presented as well.

## Introduction

The genetic structure and function of the *Y* chromosome varies greatly over taxa. While it is functionally nonessential in a variety of insect species such as those in the genus *Protenor,* in most *Drosophila* species the *Y* chromosome is at least essential for male fertility (White 1973). In mammals, genes that are critical for the process of sex determination are *Y* linked (Cooke 1990). However, even in the case of *Drosophila,* where the *Y* is indispensable only in terms of fertility, there are other genetic functions that can be assigned to this chromosome. These include ribosomal DNAs, collochores or meiotic pairing sites and elements such as a suppressor of the *X* linked *Stellate* gene (Ashburner 1989), and ABO, which exerts an influence over the expression of the autosomal gene *abnormal oocyte* (Verma 1988).

From a structural point of view, both cytological and molecular analyses have revealed that in the dipteran species examined the *Y* chromosome is heterochromatic, largely consisting of repetitive DNAs that are specific or enriched in this chromosome (Hennig 1990). Repetitive sequences that are specific to the *Y* chromosome are somewhat rare, although those that have been found are present in relatively high copy numbers of 200–2,000 per genome. In *Drosophila* these *Y* specific sequences are interspersed with another class of more moderately repetitive elements referred to as *Y* associated sequences; these usually number less than 50 copies per genome. The *Y* associated sequences may be enriched on the *Y* chromosome, but they are not limited to it as are the *Y* specific sequences (Hennig 1990). From the *Y* associated class, sequences that have been classified as transposable elements have been recovered including *micropia* (Lankenau et al. 1988), and the Type II ribosomal DNA insertion sequence (Jakubczak et al. 1990). Features such as these, in conjunction with the hemizygous nature of this chromosome, render the *Y* as a fast evolving chromosome in both dipteran and mammalian genomes, making it useful for studies of evolutionary relationships within and between closely related species (Hennig 1985; Eicher et al. 1989).

Although much of our understanding of the structure and function of the dipteran *Y* chromosome stems from work with *Drosophila* species, studies of this chromosome in another dipteran *Ceratitis capitata* (commonly referred to as the Mediterranean fruit fly) have already demonstrated that the picture is not yet complete. Studies of naturally occurring or laboratory induced sex chromosome aneuploids in the medfly suggest that at least a portion of the *Y* chromosome is necessary for male viability and sex determination. In addition, a considerable amount of evidence suggests that a *Drosophila*

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type of "balance" mechanism of sex determination does not operate in this species (Lifschitz and Cladera 1989).

Structurally, Bedo (1989) has clearly documented important differences in the cytology of the medfly *Y* and *X* chromosomes, again as compared with *Drosophila*. In documenting the pattern of banding in medfly mitotic and meiotic chromosomes, Bedo has shown that it is the *X* chromosome of *C. capitata* that is rich in fluorescent bands, in contrast to the case in *Drosophila* where it is the *Y* that has many bands. Fluorescent banding of this type is thought to indicate regions of repetitive DNA in heterochromatin.

Functionally, evidence has been obtained suggesting the presence of a male sex determinant on the *Y* chromosome in the medlfy (Lifschitz and Cladera 1989). In sex chromosome aneuploids of this species, viable and fertile females are detected with up to four *X* chromosomes, whereas *XXY* flies are fertile males. In *Drosophila melanogaster,* all these genotypes would be phenotypically manifested as females (Baker 1989). In addition, for the medfly, studies of three translocation lines involving the relocation of segments of the *Y* to an autosome have enabled the localization of the putative male sex determinant, at least at the cytological level (Lifschitz and Cladera 1989; Zapater and Robinson 1986). The results suggest a location near the centromere for this factor on the long arm of the *Y* chromosome.

As a first step in the characterization of the medfly *Y* chromosome in our laboratory, the isolation of male specific DNA sequences by a differential hybridization approach has been completed. This method entailed the annealing of labeled female and male specific genomic DNAs to duplicate representatives of a medfly genomic library. Recombinant phage that exhibited a preferential hybridization to the DNA probe from males were selected for further characterization. This approach resulted in the identification of what appear to be *Y* linked sequences. Plasmid subclones derived from each of the phage were analyzed with regard to genome organization and representation in both sexes, restriction enzyme map and evolutionary conservation within different natural isolates of the medfly.

## Materials and methods

*Fly strains.* The medfly strain designated as the laboratory strain is the USDA Hi-Lab line kept in continuous laboratory culture for over 30 years. The medfly strains Mauna Loa (M. Loa), Kula and ISL 6 were obtained from Don McInnis of the USDA facility in Manoa, Hawaii and maintained in our laboratory. The Mauna Loa strain was collected from Mauna Loa, Hawaii over 2 years ago whereas the ISL 6 line was initiated in laboratory culture less than 1 year ago from the same area. The Kula medfly strain was derived from flies eclosing from fruits collected at Kula, Maui approximately 1 year ago.

*DNAs.* A medfly actin sequence contained within the plasmid pmed85 that is identical to the medfly actin clone pmed21 (Haymer et al. 1990) was used as a control. All other plasmid clones used were generated in the course of this study from the shotgun subcloning of phage DNA EcoRI fragments into either pUC19 or pUC9.

*DNA isolations.* Plasmid DNAs were isolated by the alkaline lysis method (Birnboim and Doly 1979). Phage DNAs were purified from lysates of intact phage obtained from either confluent plates (Maniatis et al. 1982) or liquid cultures (Miller 1987) according to the following method (I. Felger, personal communication). Phage lysate (5 ml) was treated with 25 µl each of DNAse I (stock 10 mg/ml) and RNAse A (stock 10 mg/ml) at 37° C for 1 h. One milliliter of lysis buffer (0.5 M Tris, pH 8.5, 0.25 mM EDTA, 2.5% SDS) prewarmed to 70° C was added with vortexing. After incubation at 70° C for 35 min and subsequent cooling, cellular debris was precipitated with 1.25 ml 8 M potassium acetate on ice for 30 min and pelleted by centrifugation at 10,000 rpm in a Sorvall SS-34 rotor at 4° C for 20 min. The recovered supernatant was mixed with 4 ml isopropanol, incubated for 10 min at room temperature and centrifuged at 10,000 rpm for 10 min at 20° C. The phage pellet was dissolved in 900 µl 3 M sodium acetate, pH 6.0, transferred to a microfuge tube and reprecipitated with 600 µl isopropanol. The phage was repelleted by centrifuging at room temperature for 10 min at 10,000 rpm, resuspended in 500 µl 0.3 M sodium acetate, pH 6.0 and subjected to two rounds of phenol extraction. DNA was precipitated with a 10 min incubation at room temperature in the presence of 0.6 vol. isopropanol. After another centrifugation, the pellet was washed with 500 µl 70% ethanol, air dried and resuspended in 25 µl 1 × TE, pH 8.0 with 1 µl RNAse A added.

Genomic DNA was isolated by the following method. Sex specific DNA was prepared from males or females that were collected within 8 h after eclosure from the pupal stage to prevent the possibility of any matings and/or sperm transfer. Genomic DNA was isolated from the laboratory strain of medfly according to the method of Herrmann and Frischauf (1987). All other genomic DNAs were prepared using a rapid DNA isolation protocol (Rick Lifton, personal communication). Ten etherized flies of one sex (~100 mg tissue) were placed into a chilled 15 ml Dounce homogenizer containing 2.5 ml of grind buffer (0.2 M sucrose, 0.05 M EDTA, 0.001 M Tris, pH 9.0, 0.5% SDS) and homogenized briefly on ice. The homogenate was gently filtered through sterile polyfil into a 14 ml polypropylene tube to which proteinase K was then added to a final concentration of 200 µg/ml and heated at 65° C for 1 h. After the addition of 375 µl 8 M potassium acetate, the solution was placed at −20° C overnight. Cell debris was then pelleted by centrifuging the mixture for 15 min at 12,100 *g* at 4° C in a Sorvall SS-34 rotor. DNA was precipitated from the supernatant with 2 vol. 95% ethanol at 22° C, pelleted by centrifugation and resuspended in 250 µl 1 × TE. After one phenol extraction, and one extraction with 24:1 chlorophorm:isoamyl alcohol, 0.1 vol. 3 M sodium acetate, pH 5.2 and 2.5 vol. ice cold 95% ethanol were added to precipitate the nucleic acid at −20° C. Pelleted DNA was recovered by spinning tubes in an Eppendorf centrifuge for 15 min at 10,000 rpm at room temperature. The resuspended DNA in 200 µl 1 × TE was treated with RNAse A.

*Other molecular biology manipulations.* Genomic library manipulations, Southern DNA blots and nucleic acid hybridizations were carried out following protocols from Maniatis et al. (1982). Hybridization stringency for most experiments was 50% formamide at 42° C with three washes in 2 × SSC at room temperature, one each in 1 × SSC at 42° C and 50° C and in 0.2 × SSC at 50° and 55° C in the presence of 0.1% SDS. (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate.) For lower stringency, DNAs were reannealed in 42% formamide with a final wash in 1 × SSC at 45°–50° C. Synthesis of radioactive probes was completed using the random priming method (Feinberg and Vogelstein 1983) with the kit supplied by Beohringer Mannheim Biochemicals. Prior to radiolabeling, genomic DNAs were cleaved with *Bam*HI and *Hin*dIII to minimize the reannealing of large DNA molecules in solution (Wahl et al. 1987). The plasmid subclone inserts used as probes were eluted by "genecleaning" (Bio 101) before labeling. Restriction enzymes were obtained from Boehringer Mannheim Biochemicals and digests were conducted according to the manufacturer's recommen-

dations. The genomic library was constructed by ligating genomic DNA partially digested with *Mbo*I into the *Bam*HI site of EMBL4.

*In situ hybridizations.* Neural ganglia were dissected from third instar larvae and squashed according to the procedure of Ashburner (1989). Mitotic chromosomes were denatured by immersing slides in 70% formamide at 70° C for 2 min. Denatured chromosomes were then hybridized with a biotin labeled probe. Color detection was accomplished using 3-3′-diaminobenzidine as described by Ashburner (1989).

## Results

### *Selection and characterization of λYmed phage*

In order to isolate sequences localized to the *Y* chromosome of the medfly, replicate plaque lifts of a genomic library in λEMBL4 were differentially screened with labeled genomic DNA of males and females (see Materials and methods). Of 3,400 phage from the medfly genomic library that were differentially screened in this way, 12 clones that appeared consistently to hybridize more intensely with the DNA probe from males as compared with the that from females were identified and plaque purified. In all 12 cases, hybridization was observed to some extent on the filters probed with the DNA of females but only to a fraction of that observed for the probe from males. These were designated as λYmed phage to indicate enriched in males, and therefore potentially *Y* chromosomal in origin, sequences from the medfly.

To characterize these phage clones further, DNA was isolated and digested with *Eco*RI. The digestion mix was split between two wells on separate sides of the same gel. After Southern blotting, each half of the filter was probed with genomic DNA of either males or females using conditions identical to those described for the initial library screening. The filter hybridized to the probe from males was exposed overnight with one intensifying screen in comparison with a 5 day exposure with two screens for the filter probed with DNA of females (data not shown). The results of this experiment reveal several points. First, non-identical stretches of genomic DNA are carried by the different phage. However, similar size *Eco*RI fragments between certain phage can be seen suggesting the possibility that overlapping sections of the genome have been cloned. Second, at least one *Eco*RI fragment in each phage isolate produces a stronger hybridization signal when probed with DNA of males as opposed to DNA of females. Finally, the pattern of hybridization with the two probes can help predict whether any of the phage insert sequences are *Y* specific, or non *Y* specific but enriched on the *Y*. Accordingly, phage designated as λYmed-1, 6, 8, 9, 10, 12 and 13 appear to carry DNA sequences that are *Y* chromosomal in origin. Phage such as λYmed-3, 4, 5, 7 and 11 contain *Eco*RI fragments that appear to be well represented in both sexes in addition to those that are male biased and appear to be enriched on the *Y*. The medfly DNA segments within these particular phage may contain repetitive elements that are enriched in copy number on the *Y* but present on other chromosomes as well.

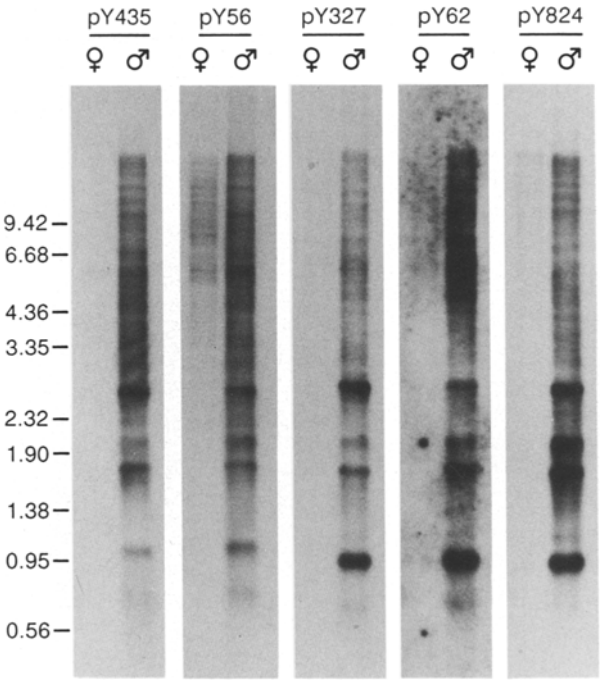


**Fig. 1.** Genomic DNA of males and females digested with *Eco*RI and probed with individual *Eco*RI fragments identified as male biased in the initial library screen. Size markers (kb) are from a *Hin*dIII + *Eco*RI digest of lambda DNA

### *Genome representation of selected EcoRI fragments or subclones*

Those *Eco*RI fragments within the phage that displayed the most disparate intensity differences between the sexes were selected for further study. To facilitate this, fragments were subcloned into the plasmid vector pUC9 (pUC19 for λYmed-1). Plasmid subclones that contained an insert correlated in size to the original phage *Eco*RI fragments of interest were checked by Southern blotting and differential hybridization to confirm the presence of a genomic sequence represented in a manner consistent with the results discussed above. The first number of the numerical designations of these plasmid subclones corresponds to the phage of origin. The rest of the designation refers to the size of the subclone.

The insert fragments from these subclones were then used to probe adjacent lanes of equal amounts of *Eco*RI digested genomic DNA of females and males in order to compare the organization and representation of the insert sequences between the two sexes. At least one subcloned *Eco*RI fragment was checked from each phage (except λYmed-11).

Figure 1 shows the pattern of hybridization seen in genomic DNA of males vs females for several fragments from different phage. For all of these fragments (except subclone pY56) hybridization to sequences present only in genomic DNA of males is evident, although after an even more extended exposure, some weak bands can be detected in the lanes containing gneomic DNA of females for these cases. Within the DNA of males, four predominant bands of hybridization of 2.97, 2.17, 1.87

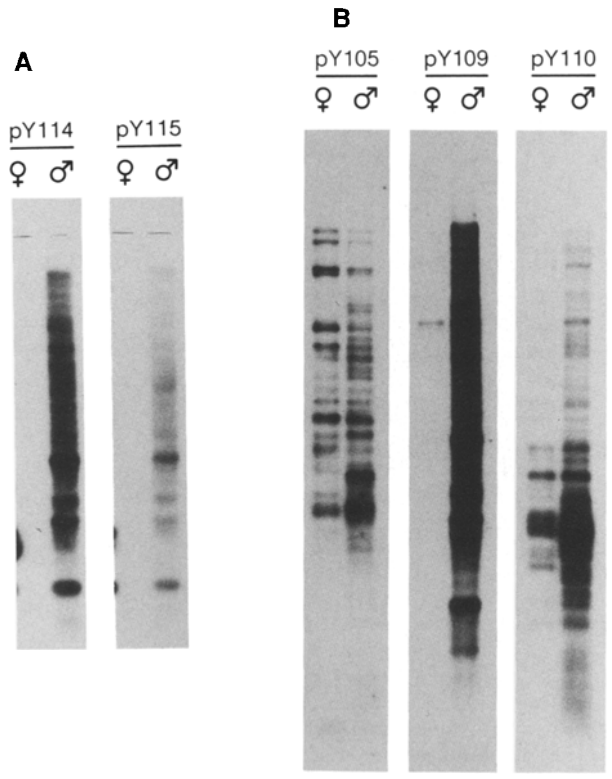


**Fig. 2A, B.** Genomic DNA of males and females digested with *Eco*RI and probed with selected *Eco*RI fragments all taken from λYmed-1. *Panel* **A** shows two fragments for which no signal was detectable in DNA of females even after a 5 day exposure. *Panel* **B** shows examples of several male biased sequences also present in the same phage

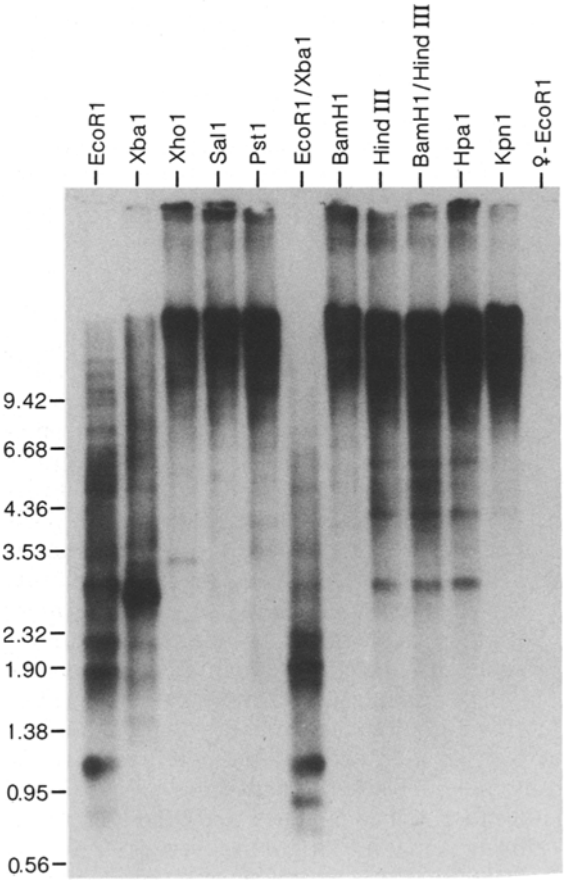


**Fig. 3.** Genomic organization of male specific repetitive sequences. Various digests of genomic DNA of males probes with subclone pY115, a male specific fragment are shown. One lane containing genomic DNA of females is included as a control

and 1.1 kb in size are identified by all of these fragments. This pattern of hybridization was observed for a variety of similarly identified male specific fragments, irrespective of which parental phage the fragment was derived from (not shown).

Although heterogeneous in length, the ability of these fragments to detect a similar pattern of bands in the genome of medfly males suggests that these DNAs may contain a repetitive or homologous element that is enriched in this genome. To investigate their homology at a gross level, one subclone from each phage was selected for restriction enzyme mapping. Most of the enzymes checked, such as *Bam*HI, *Hpa*I, *Eco*RV, *Bgl*II, *Xho*I, *Pst*I, *Sal*I and *Cla*I, were found to cut infrequently. The notable exception was the presence of *Xba*I sites in 12/14 plasmid inserts. Also *Hin*dIII sites were found in 8/14 subclones. No single map is identical to another although some similarities exist.

*Detailed analysis of the phage λYmed-1*

Two classes of sequences were identified from the phage λYmed1 (Fig. 2). For the insert sequences from plasmid subclones pY114 and pY115 shown in panel A, no signal is detected in the sample of genomic DNA of females even after extended exposure to X-ray film. Panel B shows that this phage also contains sequences not unique to the *Y* (especially pY105 and pY110), and sequences such as pY109 that appear to be enriched on the *Y*. These sequences are identified by a weak band of hybridization in the lane containing DNA of females, but this is much less intense than the hybridization in the lane containing DNA of males. In addition pY109 identified the same prominent four band pattern seen in the male specific fragments such as pY114 and pY115 described from Fig. 1. The sublcones pY105 and pY110 each clearly identify sequences present in genomic DNA of both males and females, although both roduce some notable restriction fragment length polymorphisms (RFLPs) between the sex specific DNAs. In addition, none of these fragments produces the predominant four band pattern seen previously in the male specific or male biased cloned fragments.

*Genomic organization of male specific sequences in λYmed-1*

To investigate further how the λYmed-1 sequence is organized within the medfly genome, genomic DNA of males was digested with each of nine enzymes as well

as double digests with *Eco*RI + *Xba*I and *Bam*HI + *Hin*dIII, electrophoresed, blotted and probed with pY115. A lane loaded with *Eco*RI digested genomic DNA of females was also included to confirm the male specificity of this subclone. The resulting autoradiogram is shown in Fig. 3.

Within the *Eco*RI digested genomic DNA of males is seen the pattern of bands described earlier. In the lane containing *Xba*I digested DNA, a significant amount of the hybridization is found in sequences that are approximately 2.80 kb in size. When cleaved with *Eco*RI and *Xba*I together, the homologous sequences appear to be fractionated into five major fragments estimated at 2.25, 1.88, 1.72, 1.10 and 0.89 kb in length. Most of the higher molecular weight bands observed in the single digests with *Eco*RI and *Xba*I are also affected by the double digest reaction. For other restriction enzymes, the regions in the male medfly genome that contain DNA squences homologous to the probe appear to be largely devoid of recognition sites. Exceptions are the restriction enzymes *Hin*dIII and *Hpa*I, which identify three similarly sized fragments containing homologous sequences, although the majority of the signal is contained within high molecular weight bands.

*Internal structure of a male biased sequence*

From studies of sequences enriched in or specific to males by other groups, the increased level of hybridization to the genomic DNA of males was attributed to a relatively short element contained within certain sequences. In order to investigate the possibility of a short element within the repetitive DNA sequences isolated here, one of the plasmid subclones that generated a signal enriched in males was selected for further characterization. Subclone pY9.6 was chosen for this experiment because of the presence of several internal *Xba*I sites. After digestion with *Eco*RI and *Xba*I and elution, the four resulting fragments were hybridized individually to adjacent lanes of EcoRI digested DNA of male and female medflies (Fig. 4). The restriction map for the insert of subclone pY9.6 is included for reference.

Each of the fragments appears to detect the four major bands in the lane containing DNA of males. Most notable, however, is the level of male specificity of each fragment. The 2.45 kb *Eco*RI-*Xba*I subfragment, which is relatively rich in recognition sites for restriction enzymes, is the only fragment that identifies sequences present in DNA of females under these conditions. The other fragments produce the typical male specific hybridization pattern seen previously. With much longer exposures here again, some weak bands can be detected in the lanes containing DNA of females. At least for the smallest fragment, this was most likely due to some cross contamination that occurred during the elution. However, clearly at the level where the repetitive sequences are identified, the 2.45 kb *Xba*I-*Eco*RI fragment appears to define a junction between a male specific sequence and a sequence common to the genomes of both sexes.

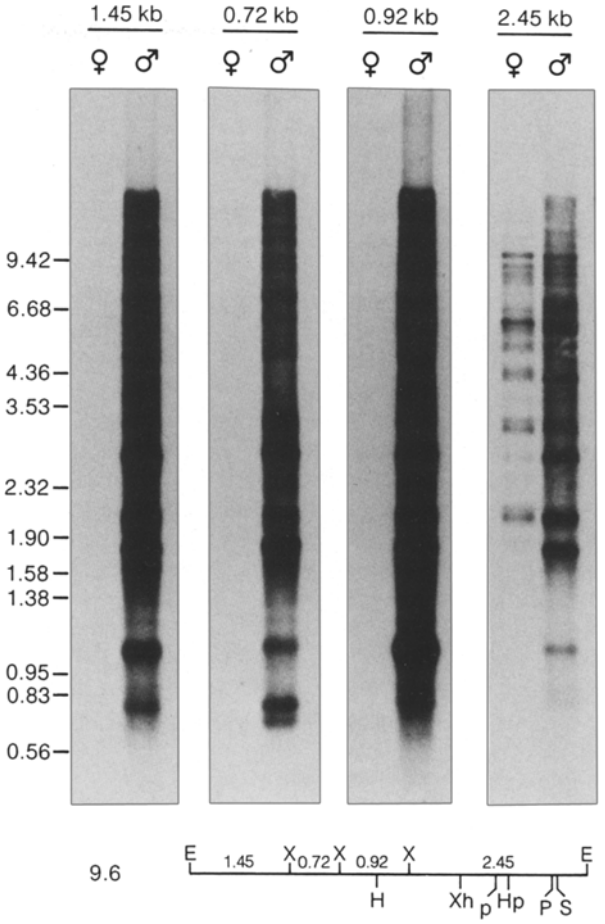


**Fig. 4.** Internal structure of a male biased sequence. Subclone pY9.6 was subdivided into four fragments defined by *Eco*RI-*Xba*I sites (restriction map shown below). Each fragment was used individually to probe genomic DNA of males vs females. Restriction enzyme sites: *E*, EcoRI; *H*, HindIII; *Hp*, HpaI; *P*, PstI; *S*, SalI; *X*, XbaI; *Xh*, XhoI

*In situ hybridization*

Several of the subcloned fragments isolated in this study were used as probes for hybridization in situ to mitotic chromosomes of the medfly. Figure 5A shows an example of the hybridization pattern produced by the pY9.6 subclone described above. In this figure it can be seen that the *Y* chromosome is heavily labeled. In addition, portions of at least two autosomes appear to be labeled as well. This probe does not appear to hybridize with the *X* chromosome. In Fig. 5B, the male specific element in pY114 is used as a probe. In this figure the *Y* appears to be labeled to the same extent as with pY9.6, but in this case clearly the autosomes are not labeled. Although the *X* chromosome is not clearly visible in this cell, the fact that pY9.6 did not hybridize to the *X* suggests that pY114, containing common male specific sequences, would not either.

*Evolutionary conservation of the element*

Previous studies have revealed that *Y* chromosome DNA sequences can be associated with transposable elements,

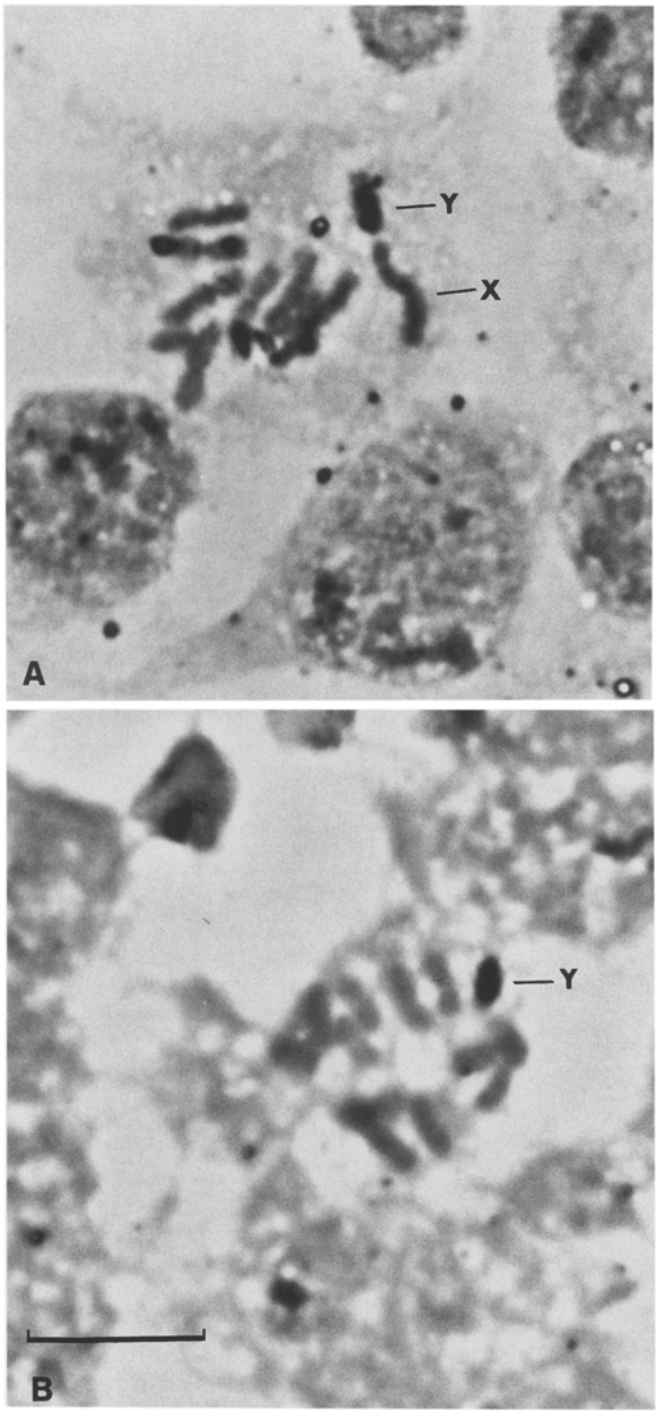


**Fig. 5A, B.** In situ hybridization of cloned sequences to mitotic chromosomes. **A** Probe is subclone pY9.6. Label is found over extensive regions of the *Y* chromosome and smaller regions of two autosomes. *X* chromosome is not labeled. **B** Probe is subclone pY114. Labeling is evident only on the *Y* chromosome. Bar represents 10 μm

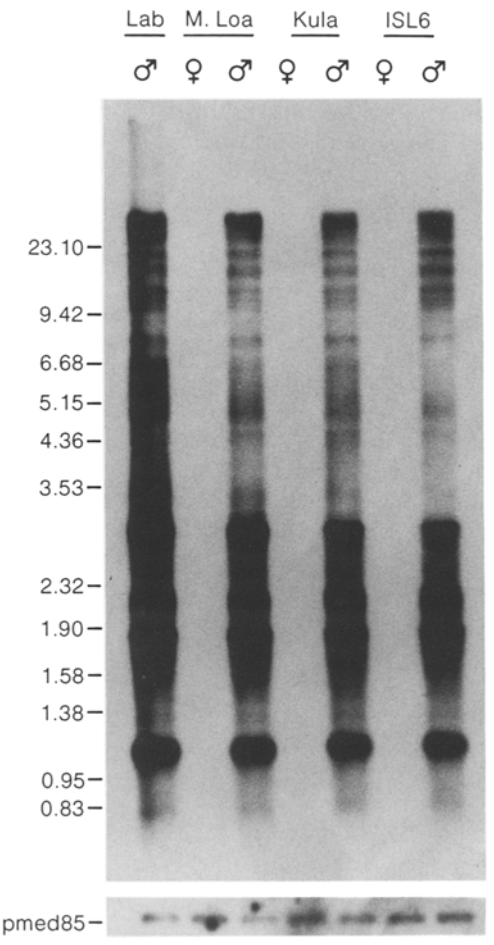


**Fig. 6.** Evolutionary conservation of male specific repetitive sequences in DNA from various medfly strains (see text for strain list). As a control, the same blot was stripped and reprobed with pmed85, a subclone containing a medfly actin gene (Haymer et al. 1990)

based on the observation of RFLPs of homologous sequences between geographically isolated strains of the same species or different subgenera. Also, *Y* specific sequences have been noted to be conserved only within closely related species, often being consistent in genome location based on banding pattern data (Vogt et al. 1986). To examine these ideas with sequences isolated in this study, sex specific DNA was isolated from additional geographical strains of medfly from the Hawaiian islands. These DNAs, after cleaving with *Eco*RI, were probed with the male specific 1.60 kb *Eco*RI fragment derived from λYmed-1.

Figure 6 shows the results of this experiment conducted under lower stringency conditions (see Materials and methods) for the laboratory, Mauna Loa and Kula strains of medfly. Signal is observed only within the samples from males of the medfly strains and there are no discernible RFLPs between the strains. When this blot was stripped and reprobed at a reduced stringency with the same DNA, no difference in hybridization was observed with the exception of a faint high molecular weight band seen in the samples from the female medfly strains. A control, the medfly actin gene pmed85 (Haymer et al. 1990), shows that approximately equal amounts of DNA were loaded for the medfly strains between the sexes. A similar probing of genomic DNA from *D. melanogaster* and *Dacus dorsalis* (a related Tephritid species) produced no detectable hybridization.

## Discussion

In this paper, phage clones from a medfly genomic library that carry repeat sequences that are enriched in the genome of the male medfly, and presumably *Y* linked, are described. Three lines of evidence suggest that these phage contain sequences preferentially localized to the *Y* chromosome. First, differential hybridization was consistently observed for these clones between the DNA probe specific for males and females in the initial screening of the library, subsequent plaque purifications and the Southern blot analysis of the digested DNAs. In the absence of evidence for sex specific changes in chromosome number or endopolyploidy during development, these sequences are attributable to a location on the *Y* chromosome in the genome of the Mediterranean fruit fly.

Second, Southern transfers of adjacent lanes of DNA of females and males probed with individual *Eco*RI fragments derived from the phage clones suggest that these phage contain repetitive elements that originate from the *Y* chromosome. For these blots, *Eco*RI fragments were selected from each phage on the basis of extreme hybridization signal differences between the sex specific probes in the initial series of hybridizations. Irrespective of phage origin, these plasmid subclone probes detected more bands in DNA of males than in that of females. Between the different plasmid subclone probes, the banding pattern of genomic DNA of males was also similar. Four predominant male specific bands migrating at 2.97, 2.17, 1.87 and 1.10 kb were apparent as well as several higher molecular weight bands. For most of the fragments, hybridization to the genomic DNA of the female was observed to varying extents. However, for two plasmid subclones from the same phage, pY114 and pY115 from λYmed-1, hybridization was restricted to the DNA of males even after extended exposure of the blot. This strongly supports a *Y* chromosomal origin for these sequences.

Finally, the results of the in situ hybridization are consistent with a *Y* chromosomal origin of these sequences. Subclones pY114 and pY9.6, which contain male specific repetitive sequences, both hybridize strongly to extensive areas of the *Y* chromosome. In addition, only pY9.6, which also contains a non-sex specific region (Fig. 4) hybridizes as well to portions of the autosomes.

The detection of similar bands in the genomic DNA of males with various subclones implies that these DNA elements are related in sequence. Restriction enzyme mapping of the subclones, which enables a comparison of sequence resemblance at a gross level, revealed that no two subclones were identical in their restriction enzyme profile. These findings, however, do not exclude the possibility that each of these fragments contain a conserved repeat unit on the order of a few to several hundred base pairs in length, such as those characterized from the *Drosophila Y* chromosome (Vogt and Hennig 1986a; Wlaschek et al. 1988). Such short homologous regions may not be detected by a restriction enzyme that recognizes a 6 bp sequence and therefore cleaves the DNA less frequently.

Studies of the genomic organization of these sequences enriched in males reveal that these regions appear to have a paucity of recognition sites for certain restriction enzymes including *Xho*I, *Sal*I, *Pst*I, *Bam*HI and *Kpn*I. However, the areas of the genome that contain a male specific sequence have a relatively frequent number of recognition sites for the enzymes *Eco*RI and *Xba*I, as evidenced by the detection of a few intense bands at lower molecular weight range.

Another question pertaining to repetitive sequences is whether they are organized primarily in a dispersed or tandemly repeated fashion. The four prevalent fragments consistently generated and identified in the DNA of males by the *Y* specific elements, together with the higher molecular weight fragments seen, suggest that the repeat element could be both tandemly organized and dispersed on the *Y* chromosome of the medfly. For the *Y* associated sequences, the type of signal generated in the genomic DNA of the female infers that these elements might be dispersed in the genome outside of the *Y* chromosome, as one might expect for a transposable element. Additional evidence for a dispersed arrangement of the element is the *Eco*RI restriction profiles of the 11 phage from which these sequences are derived. These phage appear to contain different configurations of *Eco*RI generated bands, indicating that these phage contain copies of the element flanked by different regions of the genome. However, a portion of the bands in four phage clones, λYmed 6, 7, 8 and 13, appear to comigrate, potentially because they represent overlapping or common regions of the genome.

These repetitive sequences vary in the pattern of hybridization to genomic DNA of females. In many instances, faint high molecular bands are observed. These bands could be due to sequences flanking a *Y* specific element that are located on another chromosome in addition to being localized on the *Y*. The male bias seen may actually be due to the presence of a short repeat unit(s) contained within these subclones. This hypothesis was investigated by analyzing the sex specificity of regions of the subclone 9.6. The largest fragment (2.45 kb) appears to be the only portion of this subclone to produce clear hybridization in the genome of females. This fragment is also endowed with a variety of restriction enzyme sites, in contrast to the reduced number of sites in adjoining fragments that are male specific. This pattern of hybridization, taken together with the restriction site profile of this subclone, suggests that this region of the genome consists of *Y* chromosomal sequences adjoining sequences that are distributed on other chromosomes. Again, the results of the in situ hybridization are consistent with this interpretation.

The genomic insert DNA in λYmed-1 may well represent the best case for a *Y* chromosomal origin of sequences. First, two plasmid subclones from this phage exhibit the clearest male specific hybridization (Fig. 2). The remaining *Eco*RI fragments derived from this phage reflect *Y* chromosomal characteristics as well. These fragments consistently display sex specific RFLPs suggesting that over a ~13.0 kb contiguous stretch of DNA, sequences enriched in or specific to the genome

of males are present. These types of sequences that are variable in their sex specificity resemble those of phage clones containing *Y* chromosomal DNA isolated from *Drosophila hydei*, where both *Y* specific and *Y* associated sequences have been found (Vogt and Hennig 1986a, b). The genomic representation of the male specific sequences also appears to be invariable between the three different medfly strains examined. This level of conservation mimics that found for other *Y* specific sequences from other species (Vogt et al. 1986). However, these three strains may not have been separated sufficiently in temporal and spatial terms to detect differences. Further studies of DNAs from other strains of medfly obtained from Israel, Egypt, Austria and Guatemala currently underway in our laboratory may be a truer test of the conservation, and subsequent *Y* specific character, of the elements described here.

In conclusion, we report here the isolation and preliminary characterization of homologous repetitive elements that are enriched in males or male specific and *Y* linked, in the genome of the medfly, *C. capitata*. We have confirmed by in situ hybridization that subclones sharing male specific repetitive regions hybridize in common to extensive areas of the *Y* chromosome. Another confirmation may come from the analysis of organizational differences of these sequences in strains with rearrangements of the *Y* chromosome such as translocations. The proposed homology between the subclones derived from different phage may be elucidated through sequencing of those regions in which the repetitive element can be localized. Genetic function potential can be deduced from RNA blotting analysis if a messenger RNA can be detected. An investigation of the *Y* enriched sequences between different strains can be conducted to detect variation that could result from transposable elements. Finally, these sequences, if both tandemly organized and dispersed on the *Y* chromosome as proposed, could be used to initiate the physical mapping of the medfly *Y* chromosome leading to the eventual isolation of a *Y* linked sex determinant.

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