An integrated genetic and cytogenetic map for the Mediterranean fruit fly, *Ceratitis capitata*, based on microsatellite and morphological markers

Elias E. Stratikopoulos · Antonios A. Augustinos · Yannis G. Petalas ·
Michael N. Vrahatis · Anastasios Mintzas · Konstantinos D. Mathiopoulos ·
Antigone Zacharopoulou

Abstract A genetic map based on microsatellite poly-morphisms and visible mutations of the Mediterranean fruit fly (medfly), *Ceratitis capitata* is presented. Genotyping was performed on single flies from several backcross families. The map is composed of 67 microsatellites and 16 visible markers distributed over four linkage groups. Fluorescence in situ hybridization of selected microsatellite markers on salivary gland polytene chromosomes allowed the alignment of these groups to the second, fourth, fifth and sixth chromosome. None of the markers tested showed segregation either with the X or the third chromosome. However, this map constitutes a substantial starting point for a detailed genetic map of *C. capitata*. The construction of an integrated map covering the whole genome should greatly facilitate genetic studies and future genome sequence projects of the species.

Introduction

The Mediterranean fruit fly, *Ceratitis capitata*, is a species of great economic importance since it is a major pest of fruit crops in many parts of the world, including tropical and sub-tropical areas (Harris 1989; Fletcher 1989). In the last two decades, significant genetic information has been accumulated for the species and several genetic tools are now available. The karyotype consists of five pairs of autosomes and a XX/XY pair of sex chromosomes. The map of morphological and biochemical variants includes ∼50 loci comprising five linkage groups, which correspond to the five autosomes (Saul and Roessler 1984; Malacrida et al. 1990; Roessler and Rosenthal 1992). Till now, only one X-linked locus, the ceratotoxin gene family, has been reported (Rosetto et al. 2000). In medfly, recombination is restricted to the females (Roessler and Rosenthal 1992); however a very low recombination in males has been observed in some strains occurring mainly in pre-meiotic clusters (Franz 2002).

In addition, polytene chromosome maps have been constructed for two different types of banding patterns found in medfly (Bedo 1987; Zacharopoulou 1990) and these maps have been correlated with mitotic chromosomes and the genetic linkage groups (Zacharopoulou 1990). Both X and Y chromosomes are heterochromatic and do not form polytene elements. The Y chromosome carries the maleness factor and is sex determining (Willhoeft and Franz 1996) while the linkage group corresponding to the *Drosophila* X chromosome has been assigned by in situ hybridization to the fifth medfly autosome (Zacharopoulou et al. 1992;

E. E. Stratikopoulos · A. A. Augustinos · A. Mintzas ·
K. D. Mathiopoulos · A. Zacharopoulou (✉)
Department of Biology, University of Patras, Patras 26500,
Greece
e-mail: zacharop@upatras.gr

Y. G. Petalas · M. N. Vrahatis
Computational Intelligence Laboratory, Department
of Mathematics, University of Patras Artificial Intelligence
Research Center, University of Patras, Patras, Greece

Present Address:
E. E. Stratikopoulos
Department of Genetics and Development, Columbia University,
New York, NY 10032, USA

Present Address:
K. D. Mathiopoulos
Department of Biochemistry and Biotechnology, University
of Thessaly, Thessaly, Greece

Gariou-Paralexiou et al. 2002). In situ hybridization of several characterized medfly genes (Zacharopoulou et al. 1992; Kriticou 1997) allowed unambiguous inferences for chromosome homologies between medfly and *Drosophila* to be made (Gariou-Paralexiou et al. 2002). Furthermore, polytene chromosomes have been a key factor in the analysis of genetic sexing strains (for a review see Robinson et al. 1999; Robinson 2002) and the construction of the first balancer chromosome for the species (Gourzi et al. 2000).

Despite these advances, medfly genetics is still very limited and the identified linkage groups, with the exception of that representing the fifth chromosome (Kerremans and Franz 1994; Zwiebel et al. 1995; Niyazi et al. 2005), have not yet been integrated to their respective cytogenetic maps.

Medfly belongs to the Tephritidae family, which includes many serious agricultural pests. Advances in medfly genetics/genomics will have a great impact not only in basic and applied research within the species but also in comparative studies, establishing *C. capitata* as a genetic model for the whole family. In genetic model species, high-resolution genetic mapping is an important step towards the discovery and characterization of genes and gene pathways affecting important or useful traits. Furthermore, the integration of genetic and cytogenetic maps constitutes a key factor in defining the basic genome organization and provides physical anchor points for future genome sequencing efforts.

The present study is our first attempt towards the construction of a genetic linkage map using molecular markers. A total of 73 microsatellites and 16 visible markers were used for the genetic linkage analysis. In addition, we localized 23 of these microsatellite markers by in situ hybridization to salivary gland polytene chromosomes. Thus, an integrated genetic and cytogenetic map corresponding to four out of the five medfly autosomes is now available. This map provides a starting point that lays the foundation for a detailed genetic mapping in the species. The construction of an integrated map covering the whole genome should greatly facilitate molecular genetic studies that will help to develop/improve genetic sexing systems for the medfly and future genome sequence projects of the species.

Material and methods

Fly stocks

Two wild-type strains, *Benakeion*, which is maintained in mass culture in our laboratory for more than 27 years and *Madeira* that is maintained at FAO/IAEA laboratories at Seibersdorf, Austria.

Multiple marker strains for the four out of the five autosomes: In particular, strains *bb lp Wb be* and *bb lp be*, which carry the morphological mutations *bb* (*blond body*) and *lp* (*long pupae*) (Cladera, personal communication; Cladera 1997), *Wb* (*Wide Bar*) (Roessler and Rosenthal 1990) and *be* (*brown eye*), a spontaneous mutation isolated in our lab, all mapped on the second chromosome. Strain *ew dp*, which carries the recessive morphological markers *ew* (*eroded wings*) and *dp* (*dark pupae*) on the third autosome (Roessler and Rosenthal 1990). Strain *CyBar*, which is homozygous for the dominant *Cy* (*Curly wings*) mutation (Gubb et al. 1998) and heterozygous for the dominant *Bar* (*Bar eye*) mutation, which is homozygous lethal (Roessler and Rosenthal 1990), both mapped on the fourth chromosome. Strains $w^2 y^2 wp Sr^2$ and $w^2 y^2 wp$ (Gubb et al. 1998; Gourzi et al. 2000) which carry the recessive mutations w^2 (*white eye*), y^2 (*yellow body*), *wp* (*white pupae*) and the dominant Sr^2 (*Sergeant*) mutation, which is homozygous lethal (Gourzi et al. 2000; Niyazi et al. 2005) all mapped on the fifth chromosome. No visible markers for the sixth chromosome were available.

Genetic crosses

In a pilot experiment, selected primer pairs were tested for variability on several wild type and multiple marker strains in order to determine the most suitable lines for constructing a microsatellite based map. The high variability of microsatellites allowed us to set up informative crosses that could be scored for segregation of the available markers.

Crosses were set up involving each of the four multiple marker strains and the *Benakeion* strain and virgin F_1 females were individually backcrossed to a single male from the same marker strain. In the F_2 a total of 3,425 progeny derived from 30 families representing the four multiple strains were used to map the morphological markers while only five of them were used for mapping both visible mutations and microsatellite markers (Table 2). In addition, 20 F_1 virgin females originating from crosses between "*Benakeion*" and "*Madeira*" were individually backcrossed to a single male of one of the parental strains. Two of these families, containing 57 and 118 progeny respectively, were only used for microsatellite mapping (Table 2).

Microsatellite markers

Plasmid Bluescript SK+ (Stratagene) and pUC18 microsatellite containing clones were identified from either several size selected genomic libraries using an equal mix

of ^{32}P end-labeled d(TG)$_{15}$ and d(TC)$_{15}$ oligonucleotides as probes or microsatellite-enriched libraries of total genomic DNA from the wild-type strain *Benakeion* (E. E. Stratikopoulos et al. unpublished data). DNA sequence for each clone was determined and a pair of primers flanking the microsatellite sequence was designed. In order to test the efficacy of the PCR with a particular primer pair, reactions were performed in 10 μl that contained ∼10 ng of DNA, 2.5 mM MgCl$_2$, 1 × reaction buffer [Promega: 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100], 0.2 U Taq polymerase (Promega), 0.25 mM of each dNTP and 0.5 μM of each primer using genomic DNA from the *Benakeion* strain as template. Amplifications were performed on a PTC-100 thermocycler (MJ Research Inc) for 30 cycles of 1 min at 95°C, 1 min at 55°C and 1 min at 72°C. PCR products were analyzed in agarose gels.

Genotyping and linkage analysis

Genomic DNA from single flies was prepared following the procedure described by Ashburner (1989a). PCR was performed as above with the only difference that 0.1 μM of the forward primer was end-labeled with [γ^{32}P]-ATP, using T4 polynucleotide kinase (MBI, Fermentas). PCR products were denatured at 80°C for 10 min, chilled on ice and 2.5 μl were loaded into a preheated 5% polyacrylamide gel containing 8 M urea. The genotype of each fly was identified after autoradiography. Three members of our group scored each marker independently and conflicting data were re-examined. The phenotypes for all visible mutations were scored at the adult stage.

Segregation of markers in F$_2$ progeny of each family was tested for goodness-of-fit to the expected Mendelian (1:1) segregation ratio. A few markers that showed significant deviation ($P < 0.05$ and/or $P < 0.01$) were included in linkage groups only if their presence did not disturb the order established without them.

Grouping of markers was performed in each family separately with JoinMap 3.0 (Van Ooijen and Voorrips 2001) with a LOD score of 3.0. LOD was then increased from 3 to 5 by increments of 1 to test the robustness of the linkage groups in the genotype data set. Once groups were established, a recombination linkage map was produced with a LOD score of ≥3.0 for each group. The identified groups and the order of markers in each group were checked with MapMaker 3.0 (Lander et al. 1987) and Carthagene (Schiex and Gaspin 1997). Both software packages use a multipoint maximum likelihood criterion for map construction while JoinMap uses the two-point likelihood. It has been suggested (Laurent et al. 1998) that packages, using the multipoint maximum likelihood usually achieve better performance than those using the two- point likelihood criterion. Markers that could

not be included in the maps are listed as belonging to the respective group. Finally, data from each family were combined in order to calculate the composite map by applying the "*Combine Groups for Map Integration*" function of Join-Map. The linkage LOD score was set at ≥3.0 with Kosambi's (1944) mapping function, which assumes positive interference in crossing over events. Furthermore, available recombination data from previously established crosses involving six visible markers mapped on fifth chromosome were incorporated into the composite maps. These included the *elbowed bristles* (*el*), the *ruby eye* (*ru*), the *orange eye* (*or*) and *the rough eye* (*ro*), characterized by Roessler and Rorenthal, (1992) and the *reduced bristles* (*rb*) and the *rumpled wings* (*rmp*), which have been isolated in our lab following an EMS mutagenesis screen (Rapti 2000).

Polytene chromosomes in situ hybridization

Microsatellite containing clones were labeled using the DIG-Nick Translation Mix (Roche Diagnostics GmbH, following the instructions of the manufacturer). For some markers, their PCR products were used for labeling. Detection of hybridization signals was performed using the Fluorescent Antibody Enhancer Set for DIG Detection (Roche Diagnostics GmbH). Polytene chromosomes squashes were prepared from larval salivary glands as described in Zacharopoulou et al. (1992). The salivary gland polytene maps of Zacharopoulou, (1990) and their revised version of Gariou-Papalexiou et al. (2002) were used for mapping the hybridization signals.

Results

Genetic markers

Screening either genomic or microsatellite-enriched libraries identified a total of 122 microsatellite markers. Sixty-six of these markers were found to be informative in at least one of the families analyzed. In addition, five primer pairs, named *Ccmic 7, 8, 13, 14,* and *15* (Bonizzoni et al. 2000) and two primer pairs designed for a closely related species, *Bactrocera oleae*, named *Boms61* (Augustinos et al. 2005) and *Boms64* (A. Augustinos et al. unpublished data), were also used (Table 1). Markers were tested for a 1:1 segregation ratio using χ^2 goodness-of-fit tests and most of the loci segregated in a 1:1 ratio. Three markers, *Medflymic 23, 34,* and *46,* showed a significant deviation from the expected Mendelian ratio of 1:1 at $P < 0.05$ but only in one of the families analysed. Since their presence did not give rise to conflicts in the order established without them, these markers were not excluded

Table 1 Description of isolated microsatellite loci used in linkage analysis

Marker	Cytological localization	5′ primer (5′→3′)	Repeats	3′ primer (5′→3′)	Allele size	GenBank acces. No.
Medftymic5	X	ATACTAAACCGCCTCCGAATG	$(TC)_{15}$	GAGCGGAATTAAGAGCAAATGAG	204	329382
Medftymic9	X	TAAGTACTGGCGTTCACGCATC	$(TA)_7$	ATGCGTTGCACGTTAGAAAAAG	170	329390
Medftymic18	4L (50B)	TATTGAGCAGGCTGTTGATAAC	$A_{11}(GA)_9$	TCGCACTTCTTATCAACCC	137	329399
Medftymic23	2R (13A)	TAAAGGGCTGATGCTGGAGAC	$(TG)_{13}$	CGGAGAAATTCAAAACACGAAC	200	329404
Medftymic24	m.s	ACTGCCTTGGCGATAGAAATTC	$(TTGG)_4$	CCGGCATTACTCAGTGAAGC	183	329405
Medftymic25	X	AGGCAAAGACAAGAAATTCG	$(TA)_9$	GCAGCTTGAGGTGTAGTTTAAC	284	329406
Medftymic27	X	TCTAAAACATCATTTGGCGG	$(TG)_4TA(TG)_6$	GTGGAGAAAATGATGAACGC	189	329408
Medftymic28	–	TGAAAACAACGAACCCTCTACC	$(TG)_{13}$	TGAGAAGGCAATGACACCAAC	201	329409
Medftymic29	m.s	TTCACACAACGCCAGCAG	$(TG)_{14}$	CATTCTCTCTGGTCTCGGTTTG	221	329410
Medftymic30	–	TACTGGACAACGGGTTAACAGC	$(TG)_{12}$	TTTTATGTTCAACGCTGCGAC	135	329411
Medftymic31	X	TCATGAGATCAGTCGACCTACC	$(TG)_{11}$	AGTTCATGTCAAGTTTAGGCAG	118	329412
Medftymic32	–	TCTGTTTATGCGCTCTTTGTTG	$(TG)_{27}$	CGAGCATACAATAAACACAGGC	167	329413
Medftymic33	m.s	TCTACATATCTGTGTCGCTTC	$(TG)_7$	CGAAAGGACATTTAAAGGCTCG	131	329414
Medftymic34	–	TATGATACGCCAGCACCTTCAG	$(TG)_{11}$	AAATTGCCGTCAGATTTCCG	121	329415
Medftymic36	X	TTGTGAAATGAGCGGAAAGC	$(TG)_7$	TTCTACTTTGTACACAAGCCAC	114	329417
Medftymic37	X	AGGAAAACCCCACCTAACACATC	$(TG)_{12}$	TAAATTAAGCGACGACGCGAC	228	329418
Medftymic38	X	AATCACCACTATCATCACAC	$(TG)_5CG(TG)_{10}$	ACACTAATGCAGTGGCGAG	134	329419
Medftymic40	6L (84A)	AGACAAACGGACAGACAAAAAG	$(TG)_8$	TAATGTTCGTCGTTAGGTTCGC	177	329421
Medftymic42	f.s	CTCCACCGCTCTTTTATATATC	$(TG)_{12}$	ATTCATCTTCGTTTTCGGCTTC	136	329423
Medftymic43	f.s	TTTTCGAACGGCTGCATC	$(TG)_{31}$	TTAGAGGCAAGCCACCAGG	214	329424
Medftymic44	6R (98B)	TATGCCCTCTATCATGCAAATG	$(TG)_{13}$	ACAATGAGGCAACAAAGTGG	171	329425
Medftymic46	X	TTCATATTCATTCGCTGCACTC	$(TG)_6AG(TG)_4$	AACACGTTCATTTATGCCGTTC	206	329427
Medftymic47	6R (99)	TGGGTTTCACTTGGAGGCG	$(TG)_4TT(TG)_6$	CGGGGCATATCTTTCAATTC	126	329428
Medftymic48	5L (72B)	CCTCTGATGCCCCAATAAAC	$(TG)_4AA(TG)_6$	TTGTGGCTTAACAACGAATC	197	329429
Medftymic49	f.s	CGTGTGAGGAGATGGTCAG	$(TG)_3CG(TG)_5$	GAATTCAAGCGCCACAAAG	154	329430
Medftymic53	5R (75)	TATGCTTACATATGGACTCG	$(TG)_{14}$	CGGTATAGAAACCGTTAGTCAG	189	329434
Medftymic54	X	TGCGTGTATGTGTAAGATTG	$A_{11}GA_8$	TGCTTTTGACGTGCATTAACAC	163	329435
Medftymic55	6R (98)	TTTTAGGAGCATGGTAAAGGC	A_{10}	CAATACTTTGATGTTTTGTTAC	102	329436
Medftymic56	2L (4C)	ATTCATACTCGCATATCCGGTG	$(TG)_{11}$	CGGCAGTCATTTACATAAATGC	164	329437
Medftymic60	4L (49D)	CAGGCAGTTCAGCAGTTCAG	$(TG)_5TCTC(TG)_8$	GCTTACCACCTATCTCTTGAC	201	329441
Medftymic61	m.s	CCAATCACACTCAAACACTTTC	$(TG)_3TA(TG)_{14}$	GAAGTCTTCATCAAATTGCAGG	186	329442
Medftymic62	6L(91B)	GCAAACAAACAACAGACACAAG	$(TG)_{12}$	GCCCTAATGTGTTCAGTCCATC	241	329443
Medftymic63	6R (100A)	GGAGAGAATGCCACAAAATAAC	$(TG)_{14}$	TGCCACAAAACGATGTAACTG	197	329444
Medftymic64	m.s	CTGTCACCATACCGATGCC	$(TG)_7$	AATCCTATTCGCCTTCATACCG	241	329445
Medftymic65	X	CCCCCAAAGTCTAAGCGAAACC	$(TG)_8$	CGCCTTTTGGCATATCAATAAG	175	329446
Medftymic66	4L (43A)	ATGACGACATGTGCAAACTGA	$(TG)_{14}$	CCCACCATTTTGGTGATAACAC	161	329447

Table 1 continued

Marker	Cytological localization	5' primer (5'→3')	Repeats	3' primer (5'→3')	Allele size	GenBank acces. No.
Medflymic67	f.s	AAAATCCCCTTGATGCCTG	$(TG)_8$	ACATAAGCGTGTACCTTGTGCC	166	329448
Medflymic71	m.s	TAAATACATGCGGCGTCAATG	$(TC)_{15}$	GCTCGTTTGTTCGATTGTTCAG	204	329452
Medflymic74	4L (44C)	TCAAAGAAACAAAGAGGCGTG	$(TG)_{11}$	TAAGCAGCAGACACAAGTGTTC	194	329455
Medflymic78	4R (59C)	ATTTGCCCGTCATTCAAACAAC	$(TG)_{10}$	ATTTATACACCCAGTCATGCCC	157	329459
Medflymic82	X	GTTTTCGCCGTTACTTTTTCG	$(TG)_5$	ACAACAACAAAAACAGCGGAG	206	329463
Medflymic84	6R (100A)	CGCGAAGAAAGAAGAGAAGTTGTC	$(TG)_7GC(TG)_7$	GCCGACAGCGACTAACCTAAC	198	329465
Medflymic87	f.s	ATGACAACCGTGTCAGGAGTAG	$(TG)_5TT(TG)_4$	CTAGGATTTGGTGAAGGCGAAGG	170	329468
Medflymic89	X	TGGGACCACAATTCACCAC	$(TG)_{11}$	CCCACACTTTGAGTTGATGAAC	165	329470
Medflymic90	4L (51B)	TCGGGAACAACAACAAATCTGTG	$(ACG)_9$	ATCATCAGCCGCCATCGTC	111	329471
Medflymic91	X	AATTGCTCGGCTTATCCTTACG	$(TG)_7AG(TG)_4$	CATGAGCACTCAGAAAGCCTAC	194	329472
Medflymic92	2L (9A)	AAATGACACAAAACCGTAACCC	$(TC)_{11}$	GCAACCGTTTACTGCTCAATG	142	329473
Medflymic96	X	GCCGCAACTATTTCTACACCC	$(TG)_{11}$	ATGCTGCTGTTACTGTTCCTTC	203	329477
Medflymic97	2R (14A)	GCTTGAATCCCTTTGGAAAAC	$(TC)_9$	AGACGGAAAATTTGGTGCAAAG	196	329478
Medflymic99	f.s	CCGCCGTTAAAACTGAAATC	$(TG)_5TC(TG)_9$	AAGAAGGGCGGAAAAAATTC	230	329480
Medflymic104	X	TAACCGTCGGCTGTGAGCTTC	$(TG)_7$	TCCACGGCAATTTGTCTTC	209	329485
Medflymic105	X	TTATCTCTTCCCTTGCCTTAC	$(TA)_5$	AGAATCAACCAACCAACTTTGC	192	329486
Medflymic107	2L (1A)	TGGAGGCTCGTAGAAGCAAAG	$(TG)_5TA(TG)_5$	TAACGATTATGCTCAAGGCAAG	161	329488
Medflymic108	X	GACGCCGAGGACATATTTAA	$(TG)_{11}$	CACCAAATTGCCGAATTCATAAC	124	329489
Medflymic109	2L (3D)	GATTGAATGCCCGGTAAATG	$(TA)_3(TG)_3$	TCAACTGTTTGCCACAATC	197	329490
Medflymic112	f.s	CTAACTACGACGACACCAAAGC	$(CAA)_9$	CGCTGCTTCCACTAACCTCTC	136	329493
Medflymic113	2L (6B)	TATCTTTCCTAGCACAATGGCG	$(TG)_9$	AAACAATGTGAGCAAACAGCAG	149	329494
Medflymic114	X	AAAGTGATAAAAGCCCCATGAG	$(TG)_6TCTC(TG)_9$	GCAGGCAGTTCAGCAGTTCAG	159	329495
Medflymic115	2L (7A)	TAAGGCGCATTTGGGAGTAAAAC	$(TG)_{12}$	GCAATATGCTCTTTCCCCAC	166	329496
Medflymic116	5L (61C)	TATGTGTTGCAAACTCATTGCC	$(TG)_9$	TTGCCGCATTCACACATAC	186	329497
Medflymic122	2L(2B)	CAACAATTTACGCCTTTTTTCGG	$(TG)_2AG(TG)_8$	CACAGCACACAAAAATTGCATC	124	329503
Medflymic123	f.s	TCTACAAGCAATGACCAAC	G_8	GACTAAAACCAAACTGCC	174	329504
Medflymic126	f.s	TGTAATGAAGCGAAAGAAGTGG	$(TG)_{18}$	GCAGTCTTGCTGCTCTAAAACC	230	329507
Medflymic146	–	TCACAACGAGCGTAAAAAGG	$(TCGG)_3$	CGACACCAACGGATAGAC	220	NA
Medflymic156	–	GTACTTGGTAACAAAGTGAGG	T_9	CCTTTTGCGAGTTACAAC	160	NA
Medflymic160	–	ATTGTTAGGTGATGGTGTAG	$(GTT)_5$	GGCAGAGCACTGAAGTTAATAC	177	NA
Boms64	–	TGCTAGGCTGAACATTCG	$(CA)_2C(CA)_4 N_{12}(CA)_4C(CA)_2$	TGTTTTGCTGTTTCCAGG	129	NA

X, no hybridization signal; m.s, multiple hybridization signals; f.s, 2–5 hybridization signals; –, non tested; NA, not available

from further linkage analysis. In addition to microsatellite markers, 16 visible markers were also used in this study.

Linkage analysis and map construction

A total of 175 F_2 progeny and backcross parents from two families (3 and 9) were genotyped for several sets of 72 out of the totally 73 microsatellites analyzed (Table 2). None of these markers showed linkage to the X chromosome.

Furthermore, several microsatellites with a unique position on polytene chromosomes (see below) were tested on F_1 parents of single pair backcrosses between the *Benakeion* and the multiple visible marker strains for each of the four autosomes (second, third, fourth and fifth) respectively. Seven of the above microsatellites were informative in specific backcrosses representing the second, fourth and fifth autosome respectively. None of the microsatellites mapped on the third chromosome was found polymorphic in the families analyzed (Data not shown). A total of 733 F_2 progeny, derived from five single pair backcrosses were genotyped for both the visible mutations and various sets of seven microsatellite loci (Table 2). An example of this procedure is shown in Fig. 1.

Genotype data for each family were analyzed separately to sort the markers into groups, using the JoinMap 3.0 computer program. In this analysis, 73 microsatellite loci were included as well as 16 morphological markers, the latter distributed amongst three autosomes (4 for the second, 2 for the fourth and 10 for the fifth chromosome). The MapMaker and the Carthagene programs were also used in order to test the robustness of the resulting linkage groups and maps. Both software packages produced the same results concerning either the grouping or the order of markers in each group. Data from all analysed families were pooled and analysed by JoinMap at LOD 3.0 to construct the composite map. The composite map contains 67 microsatellites and 16 visible

markers organized into nine autosomal individual linkage maps. Five of the remaining microsatellites, although showed linkage (at LOD ≥ 3.0) to markers belonging to specific groups, could not be included into the final linkage map at LOD 3.0. Only one marker, *Medflymic 27*, did not show linkage to any of the analyzed loci. Using the cytological localization of selected microsatellite and visible markers on polytene chromosomes, the nine individual maps were aligned to the second, fourth, fifth and sixth chromosome. The final map is presented in Fig. 3. Characteristics of each linkage map and its alignment to the respective chromosome are given below.

Second Chromosome

The linkage group corresponding to the second chromosome encompasses 18 microsatellite markers and four morphological markers. These markers fall into three distinct groups. Although each of them was assigned to the same chromosome using the nine cytologically mapped loci, *Medflymic 107*, *122*, *109*, *56*, *113*, *115*, *92*, *23* and *97*, as anchor points, these groups were freely recombined from each other. Moreover, three additional microsatellites, *Boms61*, *Medflymic126* and *156* show linkage to the group consisting of the loci *Medflymic92*, *71*, *42*, *23*, and *97*.

Fourth chromosome

Two groups of loci were aligned to the fourth autosome. One group consists of two markers and is freely recombined with the markers belonging to the second group. A total of 13 microsatellite and two visible markers are included in this linkage group. The cytological position of the loci used to assign the two groups to the fourth chromosome is indicated (Fig. 3).

Table 2 Informative loci analysed in each family

Family	No. of progeny	Polymorphic markers
3	57	*9*, *23*, *24*, *25*, *27*, *28*, *29*, *30*, *32*, *33*, *34*, *36*, *37*, *38*, *40*, *42*, *43*, *44*, *46*, *49*, *54*, *55*, *56*, *62*, *63*, *65*, *67*, *71*, *74*, *78*, *82*, *84*, *87*, *89*, *90*, *91*, *92*, *96*, *97*, *104*, *105*, *108*, *109*, *112*, *113*, *115*, *116*, *122*, *123*, *146*, *156*, *160*, *Boms64*, *Ccmic7*, *Ccmic8*, *Ccmic13*, *Ccmic14*, *Ccmic15*
9	118	*5*, *23*, *27*, *29*, *30*, *31*, *34*, *40*, *42*, *46*, *47*, *48*, *49*, *53*, *56*, *60*, *61*, *63*, *64*, *65*, *66*, *67*, *71*, *74*, *82*, *84*, *89*, *90*, *92*, *96*, *97*, *99*, *105*, *107*, *114*, *116*, *126*, *160*, *Ccmic7*, *Boms61*
2.1	100	*56* , *107*, *bb*, *lp*, *Wb*, *be*
4.9	120	*18*, *Cy*, *Bar*
4.15	97	*74*, *Cy*, *Bar*
4.16	117	*60*, *78*, *Cy*, *Bar*
5.13	82	*116*, w^2 , y^2, *wp*, Sr^2

Markers analyzed in more than one family are underlined. The prefix *Medflymic* has been omitted from the respective microsatellite markers
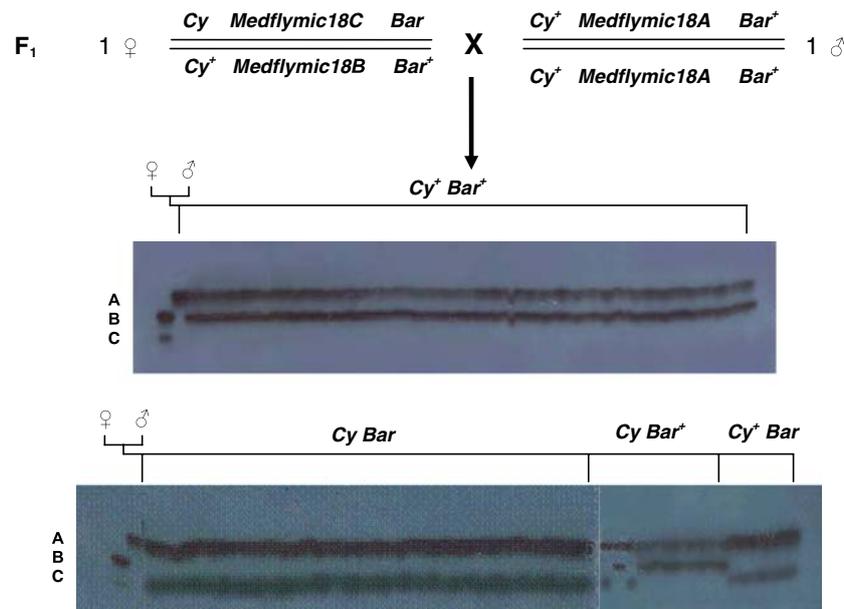
Fig. 1 Genetic mapping of morphological and microsatellite markers. A *Cy Bar* heterozygous F_1 female was backcrossed to a male exhibited the wild type phenotype. The genotypes for each individual, the two parents (♀, ♂) and the 120 progeny at microsatellite locus *Medflymic18* were scored by the indicated PCR typing pattern. The female parent carried the *B* and *C* alleles, while the male parent was homozygous for the *A* allele of the microsatellite marker. It can be deduced from the relative frequencies that in the F_1 female parent, the *Medflymic18C* allele is associated to the *Cy* and *Bar* alleles, while the *Medflymic18B* allele is linked to the wild type alleles of both morphological loci. The relative frequencies of the recombinant progeny clearly indicate a strong linkage of the microsatellite marker with the two morphological markers and especially with the *Bar* locus

Fig. 2 Fluorescence in situ hybridization of microsatellite clones to salivary gland polytene chromosomes. Panels **A–C** show the hybridization signals of the clones *Medflymic109*, *Medflymic18* and *Medflymic55* at 2L(3D), 4L(50B), and 6R(98) respectively. Panel **D** shows the multiple hybridization signals of the clone *Medflymic29*
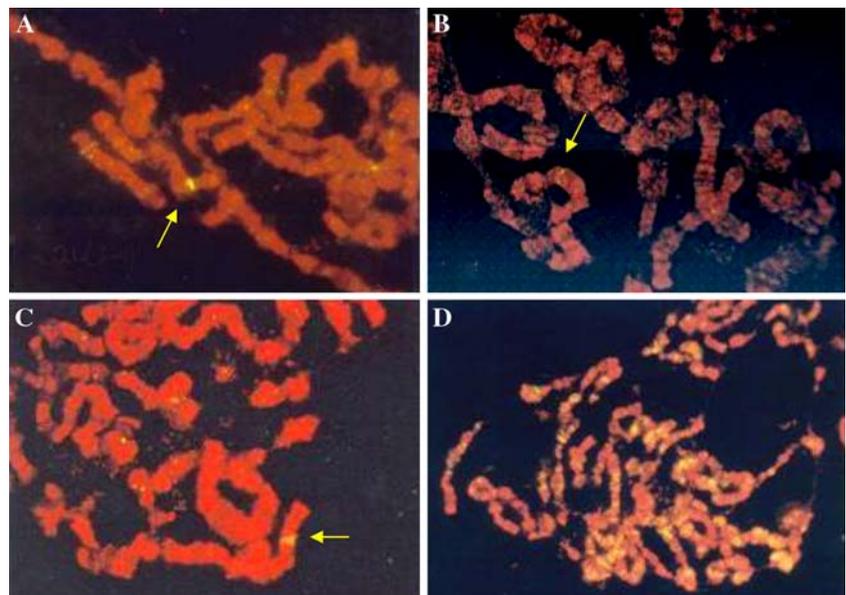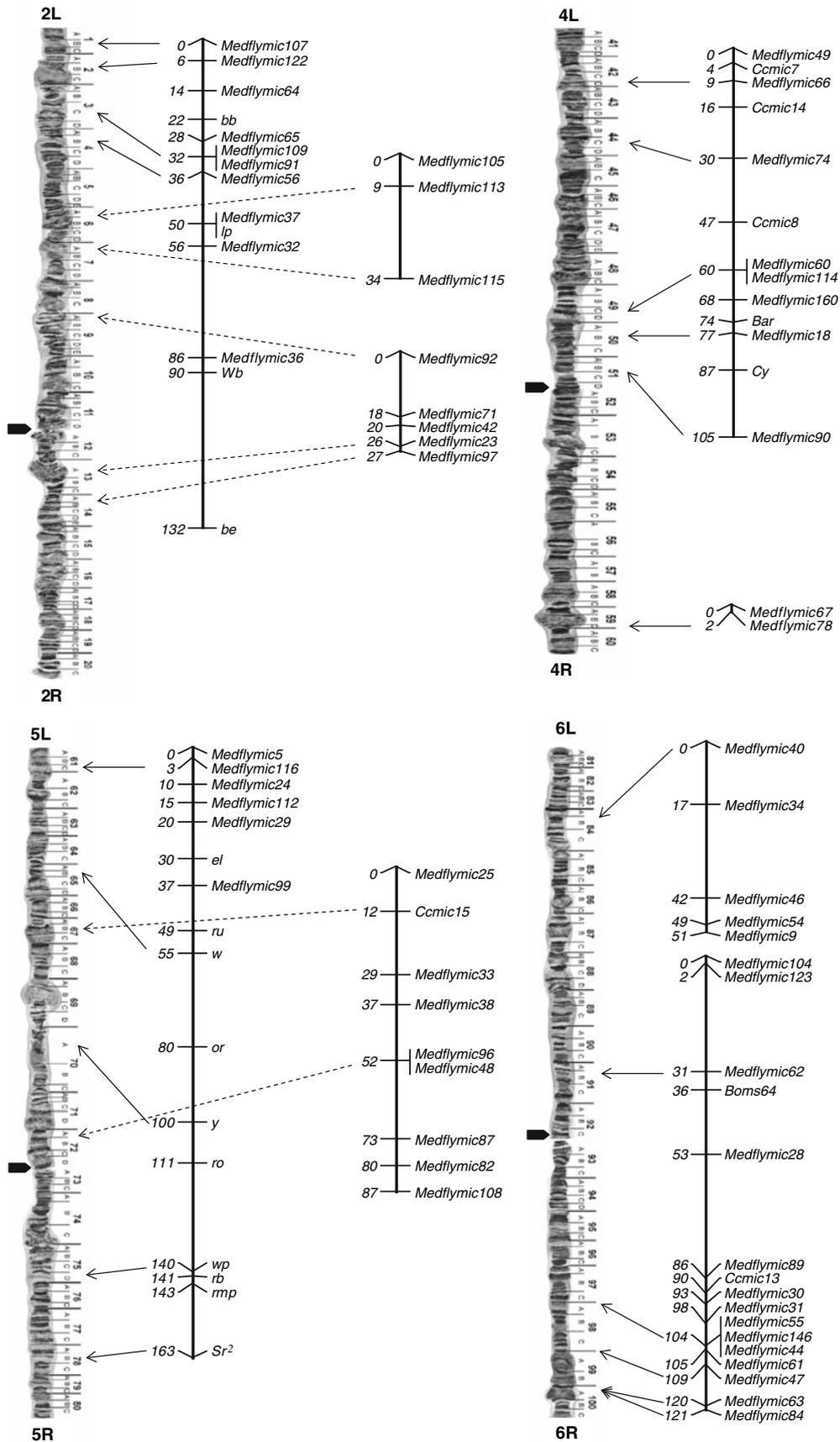
Fifth chromosome

Linkage map representing the fifth chromosome is composed of two distinct parts, which have been aligned to it using as anchor points the cytological localizations of microsatellites and genes mapped on the same chromosome (Fig. 3). Fifteen microsatellites and 10 morphological markers are included in this linkage group.

Two additional microsatellite markers, *Medflymic43* and *Medflymic53*, are included to this linkage group.

Sixth chromosome

Linkage map representing the sixth chromosome encompasses 21 microsatellite markers. No morphological

2L

0 — Medflymic107
6 — Medflymic122
14 — Medflymic64
22 — bb
28 — Medflymic65
32 — Medflymic109
Medflymic91
36 — Medflymic56
50 — Medflymic37
lp
56 — Medflymic32
86 — Medflymic36
90 — Wb
132 — be

0 — Medflymic105
9 — Medflymic113
34 — Medflymic115

0 — Medflymic92
18 — Medflymic71
20 — Medflymic42
26 — Medflymic23
27 — Medflymic97

2R

4L

0 — Medflymic49
4 — Ccmic7
9 — Medflymic66
16 — Ccmic14
30 — Medflymic74
47 — Ccmic8
60 — Medflymic60
Medflymic114
68 — Medflymic160
74 — Bar
77 — Medflymic18
87 — Cy
105 — Medflymic90

0 — Medflymic67
2 — Medflymic78

4R

5L

0 — Medflymic5
3 — Medflymic116
10 — Medflymic24
15 — Medflymic112
20 — Medflymic29
30 — el
37 — Medflymic99
49 — ru
55 — w
80 — or
100 — y
111 — ro
140 — wp
141 — rb
143 — rmp
163 — Sr^2

0 — Medflymic25
12 — Ccmic15
29 — Medflymic33
37 — Medflymic38
52 — Medflymic96
Medflymic48
73 — Medflymic87
80 — Medflymic82
87 — Medflymic108

5R

6L

0 — Medflymic40
17 — Medflymic34
42 — Medflymic46
49 — Medflymic54
51 — Medflymic9

0 — Medflymic104
2 — Medflymic123
31 — Medflymic62
36 — Boms64
53 — Medflymic28
86 — Medflymic89
90 — Ccmic13
93 — Medflymic30
98 — Medflymic31
Medflymic55
104 — Medflymic146
Medflymic44
105 — Medflymic61
109 — Medflymic47
120 — Medflymic63
121 — Medflymic84

6R

◄ **Fig. 3** Genetic map of *C. capitata*. Microsatellites and visible marker loci are shown on the right. Genetic distances in centimorgans are shown on the left. Thin arrows link the genetic loci to their appropriate location on polytene chromosomes. Thick arrows show the centromere position of each polytene chromosome

markers are available for this linkage group. This linkage group consists of two groups of markers and their alignment to the sixth chromosome was achieved using the cytological position of several markers as anchor points (Fig. 3).

In situ hybridization

The linkage groups were anchored to the polytene chromosomes by in situ hybridization using either the microsatellite bearing clones or the PCR product of selective markers, as probes (Fig. 2, Table 1). Some of the tested markers showed multiple hybridization signals or did not show any signal at all. As it is evident from Fig. 3, the unique cytological localization of mapped loci is consistent with their genetic order.

Discussion

We present the first integrated genetic and cytogenetic map of the Mediterranean fruit fly, *Ceratitis capitata*, consisting of microsatellites and visible markers. Four autosomal linkage groups have been defined which were assigned by in situ hybridization of selected microsatellite markers to the salivary gland polytene chromosomes. The map includes 67 microsatellite and 16 visible markers. Five of the remaining analyzed microsatellite markers (*Medflymic43, 53, 126, 156 and Boms61*), although they have been assigned to specific groups, they could not be included into the final genetic linkage map. This may be attributed to the size of the mapping families and/or the low presence of informative meioses. Only one marker, *Medflymic 27*, did not show linkage to any of the markers analyzed.

The recombination frequency does not correlate closely with physical distance as judged by polytene chromosome length (Fig 3). It is worthwhile noticing that the genetic distances of loci, *Medflymic60, 18* and *90* on the 4L chromosome arm, are surprising large as compared to their cytological positions. Similarly, markers in the middle of the 2L arm either present large genetic distances as compared to their physical distances or they recombine freely to each other. However, the recombination frequency is comparable to their respective physical distance for markers mapped close to the chromosome ends (e.g. 2L and 6R arm, respectively). This probably reflects the

distribution of crossing–over events along the medfly chromosomes. Similar observations have been reported for *A. gambiae* (Zheng et al. 1996). It is well known that crossing-over is reduced in pericentromeric areas of *D. melanogaster*, while it is generally maximal per unit length of polytene chromosome near the middle of each chromosome arm (Ashburner 1989b).

The map presented here is the statistically best estimate from the described data sets. The availability of polytene chromosomes allowed the alignment of the identified groups to their respective chromosomes. Furthermore, the overall consistency between the recombinational and cytogenetic order of the markers is a strong indication of the robustness of this map. Cytological mapping of more of our microsatellite markers could further strengthen their established order. Significant improvement of the in situ hybridization signal, at least in some cases as it is clearly indicated from our experiments, can be achieved if a PCR product, representing the least repetitive sequence of a microsatellite clone, is used as probe. However, this was attempted only in a low scale in this study as in most cases the whole microsatellite clone was used as probe. The multiple hybridization signals could be attributed either to the long simple repeat arrays or to the sequence of their unique regions that can be a part of a long repeat dispersed in the genome. The non-detection of hybridization possibly reflects the under- or not polytenization of the respective chromosomal regions. It is obvious that this map is far from its completion and does not cover a significant portion of the genome. The third autosome and the X chromosome are not included in this map. In addition, we found nine groups representing parts of the four autosomes, the second, fourth, fifth and sixth chromosomes. The independent assortment of markers belonging to the same linkage group is more likely due to large genetic distances and/or the lack of markers in the vicinity of the gaps. Because of these reasons as well as the non-random distribution of crossing-over along the chromosomes it is difficult to give a total recombination length of the medfly genetic map.

A similar situation has been observed in a number of studies including *D. mojanensis* (Staten et al. 2004) and *A. funestus* (Wondji et al. 2005) in which markers of the same chromosome were recombined freely from each other. Additional linkage groups to the chromosome number have been observed in Hymenoptera as the *A. mellifera* (Hunt and Page 1995), the *B. hebetor* (Antolin et al. 1996), *T. brassicae* (Laurent et al. 1998) and the *A. rosae* (Nishimori et al. 2000), indicating the existence of gaps in the presented maps.

Many factors can affect marker coverage and genome map density, such as number and distribution of markers on the genome, crossing-over distribution on the genome, the degree of polymorphism as well as the genome size and

mapping population size (Liu 1998). In the present study a total of 73 microsatellite and 16 visible markers were analyzed. The linkage data revealed a non-random distribution of the microsatellite loci throughout the medfly genome. In particular, their distribution was neither analogous to the size of the chromosome nor uniform throughout each chromosome. Several microsatellite loci tend to accumulate to specific chromosomal locations, such as the distal regions of chromosome arms (2L, 5L and 6R). Significant nonrandom distribution of microsatellite markers has been noted in human, mouse and rat linkage maps (Dib et al. 1996; Dietrich et al. 1996; Jacob et al. 1995). A clustering of microsatellite markers on ends of linkage groups has been observed in zebrafish (Shimoda et al. 1999), an observation that was attributed to the accumulation of CA/GT microsatellites on these chromosome regions. The majority of identified microsatellite markers in medfly during this study as well as in Bonizzoni et al. (2000) are CA_n/GT_n dinucleotides. Linkage analysis of additional microsatellite markers could clarify whether an analogous situation holds for *C. capitata*. Given the present results we would suggest that the non-random distribution of the microsatellite loci and the number of loci analyzed in respect to the genome size are the main reasons for the incomplete genome coverage. However, additional reasons probably exist related to the structure and/or organization of the medfly genome. Interestingly, no X-linked markers were observed. We would assume that the structural organization of the X chromosome, which is mainly heterochromatic, as well as parts of the autosomes with a similar structure, could affect the cloning procedures resulting in their under-representation in the constructed genomic libraries (International Human Genome Sequencing Consortium 2004).

Despite the non-completion of the current map, the present work constitutes a significant advance in the genetics of *C. capitata*. It provides a large set of molecular markers that could act as sequence tagged sites (STS) in genome mapping studies of the species.

Furthermore, the level of polymorphism of microsatellites observed amongst laboratory strains and several natural populations (our unpublished results) indicates that these markers can prove a useful tool for field studies of population structure and dynamics in the species (Bonizzoni et al. 2000, 2001). These markers can also be scored in other Tephritidae species, as current experiments in our lab clearly show. This can greatly facilitate the development of molecular markers in species with limited genetic information but of a great economic importance providing thus a mean for comparative mapping.

The integrated genetic and cytogenetic map presented in this study provides a starting point for a complete genetic mapping in medfly. This could greatly facilitate molecular genetic studies that will help to develop/ improve genetic

sexing systems for the medfly and provide a basis for future genomic research in the species.

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