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ORIGINAL PAPER

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The nematode-resistance gene, *Mi-1*, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato

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Abstract The gene *Mi-1* confers effective resistance in tomato (*Lycopersicon esculentum*) against root-knot nematodes and some isolates of potato aphid. This locus was introgressed from *L. peruvianum* into the corresponding region on chromosome 6 in tomato. In nematode-resistant tomato, *Mi-1* and six homologs are grouped into two clusters separated by 300 kb. Analysis of BAC clones revealed that the *Mi-1* locus from susceptible tomato carried the same number and distribution of *Mi-1* homologs, as did the resistant locus. Molecular markers flanking the resistant and susceptible loci were in the same relative orientation, but markers between the two clusters were in an inverse orientation. The simplest explanation for these observations is that there is an inversion between the two clusters of homologs when comparing the *Mi-1* loci from *L. esculentum* and *L. peruvianum*. Such an inversion may explain previous observations of severe recombination suppression in the region. Two *Mi-1* homologs identified from the BAC library derived from susceptible tomato are not linked to the chromosome 6 locus, but map to chromosome 5 in regions known to contain resistance gene loci in other solanaceous species.

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Introduction

Plant disease resistance (R) genes are single, mainly dominant genes that confer resistance against specific pathogens that carry the corresponding avirulence gene (reviewed in Dangl and Jones 2001). The largest group of R genes that have been so far characterized encodes a class of proteins characterized by the presence of a domain with a nucleotide-binding site (NBS) and a C-terminal region with leucine-rich repeats (LRR) (reviewed in Dangl and Jones 2001). These NBS-LRR R-genes are distributed across a wide range of plant taxa and are commonly found as clustered gene families (Hulbert et al 2001; Cannon et al. 2002; Bakker et al. 2003). In tomato, members of this family are involved in resistance against nematodes (*Mi-1* and *Hero*, Milligan et al. 1998; Ernst et al. 2002), insects (*Mi-1*, Rossi et al. 1998), viruses (*Sw-5*, Brommonschenkel et al. 2000), bacteria (*Prf*, Salmeron et al. 1996), and fungi (*I2*, Ori et al. 1997; Simons et al. 1998).

Many of the described R-gene loci are made up of clusters consisting of several homologs or paralogs (Michelmore and Meyers 1998), which in some instances are also functional R-genes with other resistance specificities (Bendahmane et al. 1999; van der Vossen et al. 2000). The clustering of R-gene loci may facilitate gene conversion events through mispaired recombination between genes from different loci that can potentially lead to the development of novel R-gene families and/or resistance specificities. Generation of new R-gene families and specificities is crucial in the struggle by the plant host to overcome new pathogen types that might arise. Clearly, the study of the structure and function of R-genes and R-gene loci is important for mobilizing various resistance sources effectively and for engineering new strategies and R-genes for disease resistance in agriculture.

The R-gene, *Mi-1*, which originates from the wild tomato species *Lycopersicon peruvianum*, confers effective resistance in cultivated tomato (*L. esculentum*) against several species of root-knot nematode (*Meloidog-*

yne spp.) (Milligan et al. 1998), potato aphids (*Macrosyphum euphorbiae*) (Rossi et al. 1998), and white flies (Nombela et al. 2003). The *Mi-1* gene belongs to the NBS-LRR class of R-genes that contains a putative coiled-coil domain preceding the NBS (Williamson et al. 2000). The *Mi-1* locus maps to the short arm of chromosome 6 in tomato and is contained within a large, introgressed chromosomal segment from the corresponding region in *L. peruvianum* (Ho et al. 1992). The physical and genetic structure surrounding the *Mi-1* locus has been well characterized using linked genetic markers (Messeguer et al. 1991; Ho et al. 1992; van Wordragen et al. 1994; Kaloshian et al. 1998). The amount of introgressed DNA varies among cultivars and lines, with the line Motelle generally thought to have the smallest introgressed region (Messeguer et al. 1991; Ho et al. 1992). Analyses of yeast artificial chromosome (YAC) clones spanning the 650 kb introgressed segment containing the *Mi-1* locus in Motelle identified the presence of two separate clusters of *Mi-1* homologs ~300 kb apart (Vos et al. 1998). In this paper, we refer to the cluster consisting of the functional *Mi-1* gene and two homologs as "cluster 1p" (Milligan et al. 1998); "p" indicates its *L. peruvianum* origin (Fig. 1A). We designate the second cluster of four *Mi-1* homologs at this locus as "cluster 2p". The three homologs in cluster 1p have previously been designated *Mi-1.1*, *Mi-1.2*, and *Mi-1.3*. Of these, only *Mi-1.2* confers nematode and aphid resistance in transgenic tomato. *Mi-1.1* is transcribed and appears to be a functional gene, whereas *Mi-1.3* does not appear to be an intact gene. The DNA sequence of the 60-kb region of the genome carrying the three homologs in cluster 1p has been previously determined (Milligan et al. 1998).

Severe recombination suppression has been observed in the *Mi-1* region in crosses between root-knot nematode-resistant and -susceptible *L. esculentum* lines (Messeguer et al. 1991; Ho et al. 1992; Ganal and Tanksley 1996: Kaloshian et al. 1998). From more than 21,000 F_2 produced from crosses between resistant tomato carrying the introgressed *Mi-1* locus from *L. peruvianum* and a susceptible tomato, five recombinants were found at the *Mi-1* locus (Kaloshian et al. 1998). Three recombination points were found to reside between cluster 1p and the tightly linked centromere-proximal marker C93.1, whereas the remaining recombination points were located telomeric to cluster 2p (Fig. 1A). No recombination event was identified in the ~300-kb region between the two *Mi-1* homolog clusters. These data localize the recombination suppression to the region spanning the two *Mi* homolog clusters. This repression of recombination has been attributed to the fact that the *Mi-1* locus is of alien origin in *L. esculentum*, and that it is located in close proximity to the heterochromatic region surrounding the centromere of chromosome 6 (Kaloshian et al. 1998; Zhong et al. 1999).

In this paper, we study the genetic structure of the *Mi-1* locus. We present a comparative analysis of the locus from susceptible tomato (*Le Mi-1* locus) and the corresponding locus from resistant tomato (*Lp Mi-1* locus), which is derived from *L. peruvianum.* Several RFLP markers from regions of the *Lp Mi-1* locus were developed and used to investigate the genetic structure of the *Le Mi-1* locus. Analysis of the distribution and organization of *Mi-1* homologs in susceptible tomato is also described.

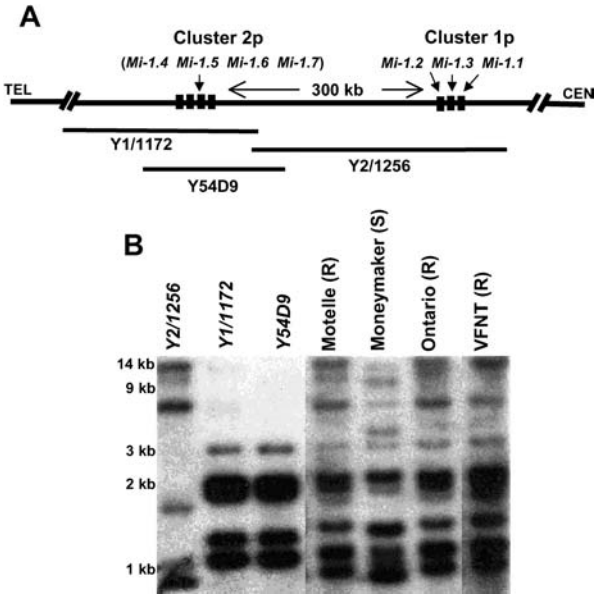

Fig. 1A, B The *Mi-1* locus from *Lycopersicon peruvianum* consists of two clusters of *Mi-1* homologs. **A** Genetic and physical map of the *Mi-1* locus derived from a contig of yeast artificial chromosome (YAC) clones, Y1/1172, Y2/1256, and Y54D9, which span the *Mi-1* locus on chromosome 6 from root-knot nematode-resistant tomato. Diagram is not drawn to scale. *Black boxes* represent *Mi-1* homologs present in the locus. **B** Hybridization pattern of DNA from YAC clones spanning the *Lp Mi-1* locus and from tomato lines Motelle, Moneymaker, Ontario, and VFNT. *R* Root-knot nematode-resistant tomato lines containing the introgressed segment derived from *L. peruvianum* that carries the *Mi-1* locus, *S* root-knot nematode-susceptible tomato line. All DNA samples were digested with *Eco*RV and probed with SM101, a DNA fragment containing part of the 5′-terminal region and the NBS domain of the *Mi-1* gene

Materials and methods

Genetic material and nucleic acid extraction

Three tomato (*L. esculentum*) cultivars (VFNT, Motelle, and Ontario) that contain the *Lp Mi-1* locus introgressed from *L. peruvianum* accession PI 128657, and two root-knot nematode susceptible cultivars of tomato (Heinz 1706, Moneymaker) with the *Le Mi-1* locus were used in this study. Seeds of *L. esculentum* cv. Heinz 1706 were kindly supplied by M.A. Budiman (Clemson, S.C., USA). Tomato DNA extraction was based on a scaled-up version of the protocol described in Dellaporta et al. (1983).

Three tomato YAC clones that span the *Lp Mi-1* locus were used. The ~210-kb YAC clone, Y54D9, derived from root-knot nematode-resistant tomato cv. VFNT (Bonnema et al. 1996) was a gift from P. Zabel (Wageningen, The Netherlands). The ~570-kb YAC clone, Y1/1172, and the ~540-kb YAC clone, Y2/1256, derived from root-knot nematode-resistant tomato (cv. E22) containing the introgressed *Mi-1* locus (Vos et al. 1998) were provided

by P. Vos (Keygene, Wageningen, The Netherlands). DNA from yeast carrying these YAC clones was extracted according to Hoffman and Winston (1987).

A BAC library derived from *L. esculentum* cv. Heinz 1706 was obtained from the Clemson University Genomics Institute [(CUGI) Clemson, S.C., USA] and used to isolate large insert clones containing *Mi-1* homologs. Two filters out of a set of seven filters that represented 15 genome equivalents were screened with the *Mi*-NBS probe (a PCR-amplified DNA fragment spanning the NBS domain of the *Mi-1* R-gene) that had been labeled with αP^{32}-dCTP. Hybridizing tomato BAC clones from the library screen were obtained from CUGI.

Large insert plasmid DNA from the BAC clones was extracted using the CONCERTHigh Purity Midiprep System (Life Technologies/Invitrogen, San Diego, Calif., USA).

Mapping with introgression lines

To map the positions of the isolated BAC clone inserts in the tomato genome, end fragments from selected BAC clones were isolated by TAIL-PCR (Liu and Huang 1998). These BAC-end clones were used as probes in RFLP analyses of introgression lines of *L. pennellii* (LA 716) in *L. esculentum* (M82) as described in Eshed and Zamir (1994). These lines were kindly made available through seeds provided by the C.M. Rick Tomato Genetic Resource Center (University of California, Davis, Calif., USA). DNA was extracted from 50 introgression lines and digested with either *Eco*RI or *Hin*dIII restriction enzyme for RFLP analysis.

RFLP analysis and molecular markers

Genomic DNA from tomato plants (6 μg) or DNA from YAC or BAC clones (1–2 μg) was digested with the indicated restriction enzymes in 30 μl-reaction volumes at the recommended temperature for 3–4 h. Digested fragments were separated overnight on a 1% TAE agarose gel and blotted onto Nytran supercharged nylon membranes (Schleicher and Schuell, Keene, N.H.) using 20× SSC transfer buffer. DNA was UV cross-linked to the membrane as described in Kaloshian et al. (1998). DNA probes were prepared by PCR amplification or restriction-enzyme digestion of appropriate plasmid clones followed by gel extraction and purification using the CONCERT Rapid Gel Extraction System (Life Technologies/Invitrogen). The DNA probes were labeled with αP^{32}-dCTP using a Multiprime labeling kit (Amersham, N.J., USA). Conditions for DNA hybridization and filter membrane washing were according to Seah et al. (1998).

The molecular markers 54D9L and 54D9R were derived from the left and right borders of YAC clone Y54D9 according to the method of Schmidt et al. (1992). The RFLP markers C264.2, C32.5, C32.1, C11.24, C12.27, and C93.1 were derived from cosmid subclones as described in Kaloshian et al. (1998).

Results and discussion

Physical organization and distribution of *Mi-1* homologs in susceptible and resistant tomato

A DNA blot of three YAC clones spanning the *Lp Mi-1* locus was probed with SM101, a DNA fragment containing part of the N-terminal region and the NBS domain of *Mi-1.2* (Milligan et al. 1998). The hybridization pattern (Fig. 1B) verified that the *Lp Mi-1* locus consists of two clusters of *Mi-1* homologs as described in Vos et al. (1998). YACs Y1/1172 and Y54D9, which harbor cluster 2p, contain at least four homologs, which we designate *Mi-1.4*, *1.5*, *1.6*, and *1.7*, at the telomeric end of the introgressed *L. peruvianum* segment in Motelle (Fig. 1A). The Y2/1256 YAC clone with cluster 1p carries the functional *Mi-1* gene (*Mi-1.2*) and two homologs (*Mi-1.1* and *1.3*) and is centromeric to cluster 2p (Fig. 1A; Milligan et al. 1998; Vos et al. 1998). The four distinct homologs from cluster 2p have been localized to a 60-kb BAC clone derived from Y54D9 and partially sequence characterized; however the orientation and order of these four homologs have not been determined (Seah, Rossi, Yaghoobi and Williamson, unpublished). Cluster 1p has been localized to a 52-kb BAC clone that has been previously sequenced (Milligan et al. 1998). All of the strongly hybridizing bands in the resistant cultivars (Motelle, Ontario, and VFNT) are accounted for on these three YAC clones, indicating that the *Mi-1* homologs most similar to the functional resistance gene are clustered in the introgressed region on chromosome 6 (Fig. 1B).

The level of DNA polymorphism at the *Le Mi-1* locus is low among root-knot nematode-susceptible tomato plants. We have observed similar RFLP banding patterns for Moneymaker (Fig. 1B), Del Oro, and Heinz 1706 (not shown), using the *Mi*-NBS sequence probe. Hence, the *Le Mi-1* locus from the susceptible tomato cultivar Heinz 1706, from which a BAC library resource is available, should be representative of the structure of the *Le Mi-1* locus and is suitable for comparison with the resistant *Lp Mi-1* locus.

By screening a BAC library of Heinz 1706 with the *Mi*-NBS probe, we identified 18 BAC clones (average size of 120 kb) that contain sequences homologous to *Mi-1.2*. RFLP analysis of these BAC clones indicated that there are at least nine different *Mi-1* homologs present in tomato (Fig. 2A). Sequence analysis confirmed that there were nine distinct homologs (Seah, unpublished). The hybridizing bands of the BAC clones accounted for all strongly hybridizing bands on a digest using genomic DNA of Heinz 1706, indicating that most, if not all, *Mi-1* homologs from susceptible tomato are contained within these isolated BAC clones (Fig. 2A).

Based on their hybridization patterns, the 18 BAC clones from *L. esculentum* (Ble1–Ble18) were grouped into four non-overlapping contigs that place the nine *Mi-1* homologs (*Mi-1a* through *Mi-1i*) into four clusters, clusters 1e–4e (Fig. 2B). By RFLP analysis with the *Mi*-NBS probe, the seven *Mi-1* homologs in cluster 1e and cluster 2e of susceptible tomato could be mapped to the short arm of chromosome 6, the region corresponding to the *Lp Mi-1* locus in resistant tomato (not shown). To determine the genomic location of the other two clusters, BAC-end fragments from Ble4 and Ble14 were cloned and used as probes on DNA blots of *L. pennellii*/*L. esculentum* introgression lines (Eshed and Zamir 1994). BAC clones Ble4 (cluster 3e) and Ble14 (cluster 4e) mapped to two different regions of chromosome 5 in tomato (Fig. 3). Interestingly, both of these regions carry multiple resistance genes in other solanaceous species (summarized in Grube et al. 2000). *Gpa*, *R1*, *Rx2*, *nb*,

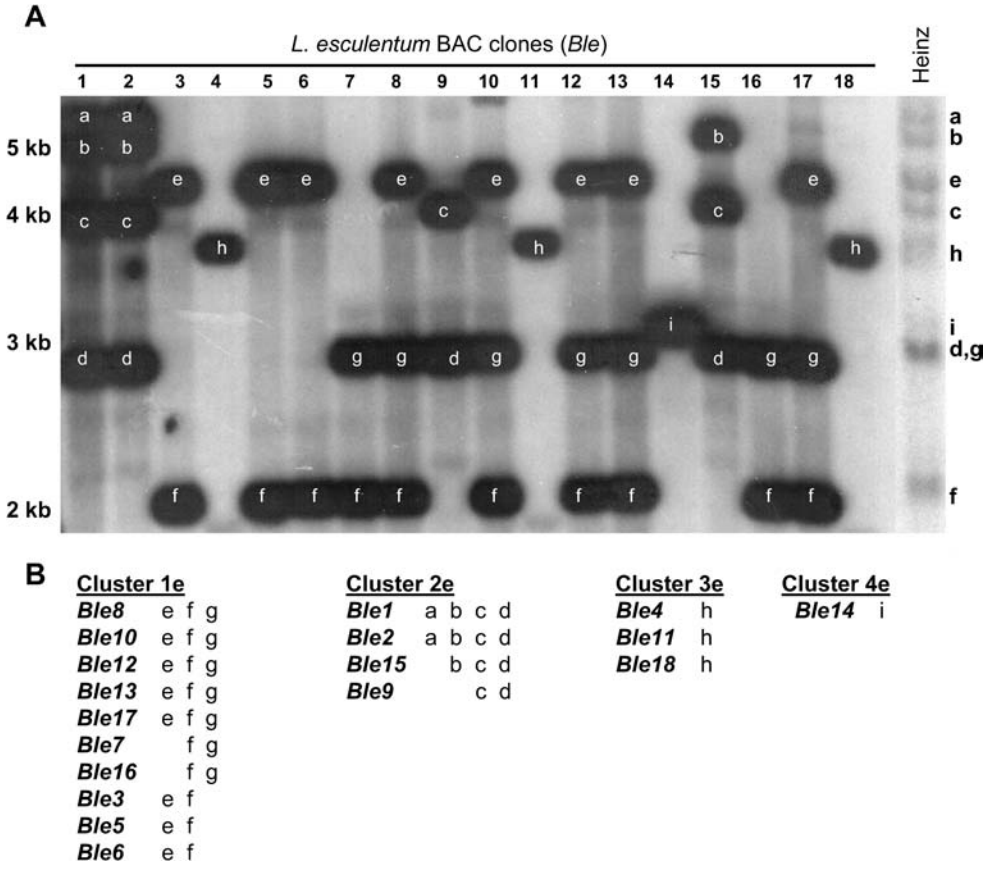

Fig. 2A, B Clusters of *Mi-1* homologs in the root-knot nematode-susceptible tomato cultivar Heinz 1706. **A** Hybridization pattern of DNA from BAC clones (Ble1–18) and Heinz 1706 digested with *Hin*dIII and probed with the *Mi*-NBS fragment. Distinct *Mi-1* homologs are labeled *a–i*. **B** The homologs from Heinz 1706 can be grouped into four non-overlapping BAC contigs or clusters

Grp1, and *phyt3* resistance loci in *Solanum* spp. are linked with the marker TG432, which resides between markers TG441 and CD41 (Fig. 3) in the region where we have positioned *Mi-1h* in tomato (De Jong et al. 1997). The *Pto*, *Fen*, and *Prf* loci (Grube et al. 2000), which are linked to marker TG619 in *L. pimpinellifolium*, are located in the same chromosomal region as *Mi-1i* (Fig. 3; Tanksley et al. 1992).

Evidence for an inversion at the *Mi-1* locus

Six molecular markers previously localized to the *Lp Mi-1* locus (Kaloshian et al. 1998) and the two YAC end probes 54D9R and 54D9L were hybridized to a set of nylon filters containing DNA from YACs spanning the *Lp Mi-1* locus and BACs from the *Le Mi-1* locus. Genomic DNA from tomato cultivars VFNT, Heinz 1706, and Moneymaker was included on the blots to assess the copy number of the marker in the genomes of resistant and susceptible plants and to allow comparison of sizes of hybridizing fragments with those from the YAC and BAC clones.

Marker C93.1 is present as a single copy at both the *Le Mi-1* and *Lp Mi-1* loci in tomato (Kaloshian et al. 1998; Fig. 4A). Recombinant analysis from previous studies indicated that the polymorphic marker C93.1 is allelic between resistant and susceptible tomato, and that the hybridizing fragment maps just centromeric to cluster 1p (Kaloshian et al. 1998; Milligan et al. 1998). The hybridization of C93.1 to *L. esculentum* BAC clones Ble7, -12, -13, -16, and -17 (Fig. 4A) indicates that cluster 1e is orthologous to cluster 1p. The absence of the C93.1 marker sequence on Ble3 and Ble5 indicates that this marker is closer to *Mi-1g* than it is to *Mi-1e*. By aligning the C93.1 marker in the resistant and susceptible loci, the order of the homologs in cluster 1e from telomeric to centromeric direction is *Mi-1e*, *Mi-1f*, and *Mi-1g* (Fig. 5D).

The YAC clone Y54D9 carries all four homologs present in cluster 2p. By probing DNA blots of YAC clones Y1/1172 and Y2/1256 with 54D9R and 54D9L, we were able to determine that 54D9R is centromeric to cluster 2p (Figs. 4, 5). Surprisingly, hybridization with the *L. esculentum* BACs indicated that 54D9R was associated with cluster 1e (present on Ble3, -5, -10, and -12) on the opposite side of the cluster relative to C93.1 (Figs. 4B, 5).

Although 54D9L is present in several copies in the tomato genome, there is only one copy within the *Lp Mi-1* locus (Fig. 4C). 54D9L did not hybridize to Y2/1256 on DNA blots of the YAC clones that span the *Lp Mi-1* locus, indicating that this marker is telomeric to cluster 2p (Fig. 5A). There is a *Hin*dIII restriction-enzyme cleavage site within the 54D9L marker sequence, resulting in two hybridizing bands with the Y1/1172 and Y54D9 clones. Since 54D9L is a YAC-end marker, it was not unexpected

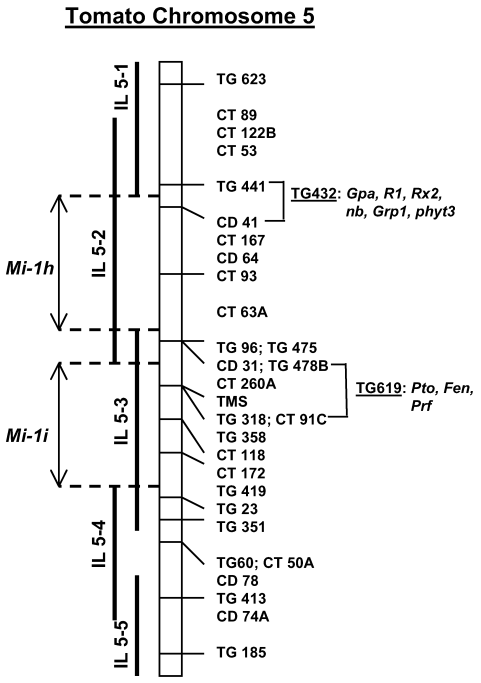

Fig 3 Positions of *Mi-1h* and *Mi-1i* on chromosome 5 of tomato. DNA blots of *L. esculentum* /*L. pennellii* introgression lines probed with BAC-end fragments derived from clones Ble4 and Ble14, which carry *Mi-1h* and *Mi-1i*, respectively. The *black bars IL5-1* thru *IL5-5* represent the introgressed regions of *L. pennellii* segments in five lines spanning chromosome 5 (Eshed and Zamir 1994). *Double-headed arrows* indicate the regions to which *Mi-1h* and *Mi-1i* are localized based on the introgression map. Deduced positions of the markers TG432 or TG619 with linked R-gene loci are indicated

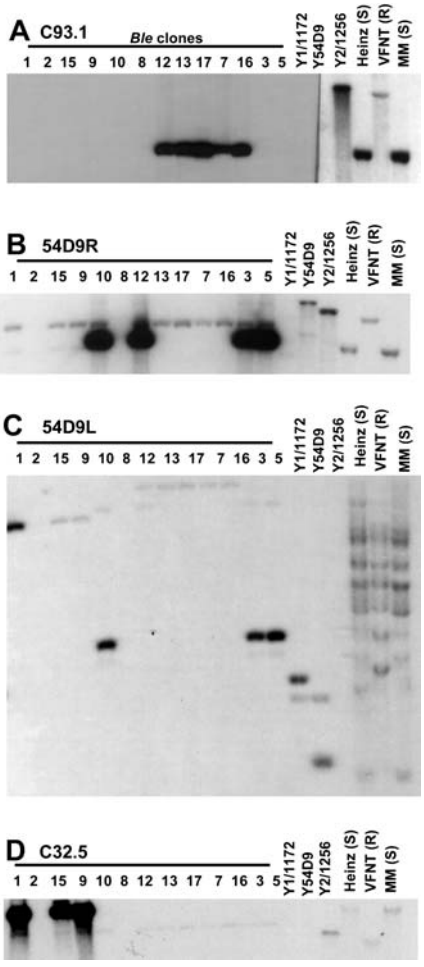

Fig. 4A–D RFLP analysis of molecular markers of *Lp Mi-1* and *Le Mi-1* loci. DNA from 13 Ble clones (Fig. 2), three YAC clones (Y1/1172, Y54D9, and Y2/1256) that span the *Lp Mi-1* locus and tomato genomic DNA from Heinz 1706, VFNT, and Moneymaker (*MM*) was digested with *Eco*RV (**A**), *Hin*dIII (**B**, **C**) or *Eco*RI (**D**). DNA blots were probed with (**A**) C93.1, (**B**) 54D9R, (**C**) 54D9L, or (**D**) C32.5. *R* Root-knot nematode-resistant tomato containing the *Lp Mi-1* locus, *S* root-knot nematode-susceptible tomato lines with the *Le Mi-1* locus

that the band hybridizing to Y54D9 is smaller than the corresponding band in Y1/1172. Analysis of the BAC clones from susceptible tomato shows the presence of at least two copies of the 54D9L sequence at the *Le Mi* locus. One of the copies, 54D9La, hybridizes only to the BAC clone Ble1 that is at one end of cluster 2e. The second copy, 54D9Lb, is present on BAC clones Ble3, -5, and -10 indicating it is just telomeric to cluster 1e (Figs. 4C, 5D). Since cluster 2e, carrying the homologs *Mi-1a*, *Mi-1b*, *Mi-1c*, and *Mi-1d*, maps to the short arm of chromosome 6 and telomeric to cluster 1e, it is likely to be orthologous to cluster 2p. Marker 54D9La is associated with cluster 2e (hybridizing to Ble1, Figs. 4D, 5D) in a position consistent with being allelic to 54D9L in *L. peruvianum*, thus giving the order of the *Mi-1* homologs in cluster 2e from the telomeric to centromeric direction as *Mi-1a*, *Mi-1b*, *Mi-1c* and *Mi-1d*.

Marker C32.5 is a single-copy marker positioned about 20 kb telomeric to *Mi-1.2* (Fig. 4D; Kaloshian et al. 1998). If the relative positions of the molecular markers at the *Le* and *Lp Mi-1* loci were conserved, one would expect marker C32.5 to be associated with cluster 1e. However, DNA blots indicate that C32.5 is associated with cluster 2e (present on Ble1, -9, and -15) (Figs. 4D, 5). The molecular markers C32.1 and C11.24 are known from DNA sequence analysis to be 0.9 kb apart and located 8.5 kb telomeric to *Mi-1.2* in cluster 1p**.** These probes show the same hybridization pattern on all DNA blots probed and can thus be considered a single marker (data not shown). A second copy of the C32.1/11.24 sequence (C32.1/11.24b) was found in this study to be associated with cluster 2p. However, we were not able to position this marker relative to cluster 2p since the YAC clones Y1/1172 and Y54D9 span the entire cluster. At the *Le Mi-1* locus, however, there was only one copy of C32.1/11.24, and it was localized to Ble1, -2, -15, and -9, positioning it between *Mi-1b* and C32.5 (Table 1; Fig. 5).

C12.27 hybridized to a single band at the *Lp Mi-1* locus and was shown by genetic and physical mapping to be located between C93.1 and *Mi-1.2* (Kaloshian et al. 1998). However, hybridization to the Ble BACs indicates that there are three copies of the C12.27 sequence at the

Table 1 RFLP patterns at the *Mi-1* loci of *Lycopersicon esculentum* and *L. peruvianum*

Cluster	Clone	Molecular markers[a]								
		54D9L	54D9R	Rex-1	C264.2	C32.5	C32.1	C11.24	C12.27	C93.1
2p	Y1/1172	p	-	-	-	-	p-b	p-b	-	-
	Y54D9	p	p	-	-	-	p-b	p-b	-	-
1p	Y2/1256	-	p	p	p	p	p-a	p-a	p	p
2e	Ble1	e-a	-	-	-	e	e	e	e-b	-
	Ble2	-	-	-	-	-	e	e	e-b	-
	Ble15	-	-	-	-	e	e	e	e-b	-
	Ble9	-	-	-	-	e	e	e	e-b	-
1e	Ble10	e-b	e	e-a	-	-	-	-	e-c	-
	Ble8	-	-	-	-	-	-	-	-	-
	Ble12	-	e	-	-	-	-	-	e-a	e
	Ble13	-	-	-	-	-	-	-	e-a	e
	Ble17	-	-	-	-	-	-	-	e-a	e
	Ble7	-	-	-	-	-	-	-	e-a	e
	Ble16	-	-	-	-	-	-	-	e-a	e
	Ble3	e-b	e	e-a, e-b	-	-	-	-	e-c	-
	Ble5	e-b	e	e	-	-	-	-	e-c	-

[a] *p* markers of *L. peruvianum* origin, *e* markers of *L. esculentum* origin, letters following *e* or *p* distinguish different bands that hybridize with a particular probe, - no hybridization

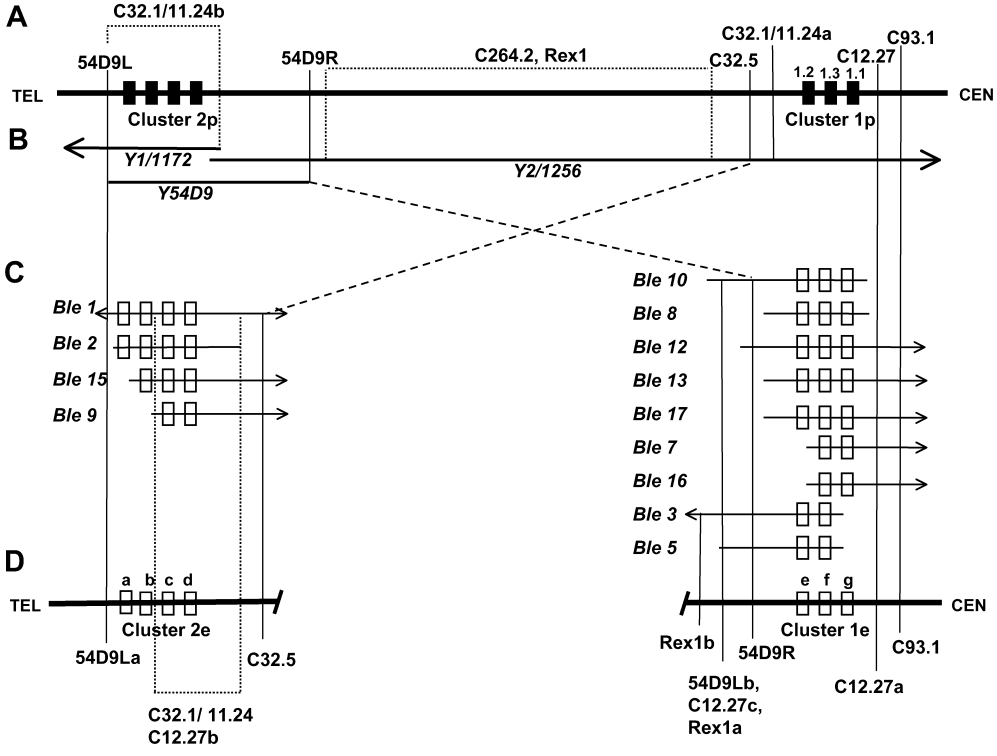

Fig. 5A–D Comparative physical maps of *Le* and *Lp Mi-1* loci. **A** *Lp Mi-1* locus with centromeric and telomeric directions indicated. *Black boxes* indicate the distribution of *Mi-1* homologs present in cluster 1p and cluster 2p at the *Lp Mi-1* locus. **B** YAC clones spanning the *Lp Mi-1* locus on chromosome 6. Only part of YACs Y1/1172 and Y2/1256 are diagrammed as indicated by the *arrowhead* on the *left* or *right end* of the YACs. **C** Contigs of BAC clones derived from *L. esculentum* cultivar Heinz 1706 that map to the *Le Mi-1* locus. *Open boxes* represent *Mi-1* homologs present in the BACs. BAC ends with *arrows* indicate ends of the BACs that have not been delimited by molecular markers used in this study. **D** Genetic structure of the *Le Mi-1* locus. Map is not drawn to scale

Le Mi-1 locus with similar hybridization intensities (not shown). One copy, C12.27a, hybridized to Ble12, -13, -17, -7, and -16, placing it on the right flank of cluster 1e and probably allelic to the C12.27 marker in the *Lp Mi-1* locus. A second copy, C12.27b, hybridized to all four BAC clones associated with cluster 2e, and a third copy, C12.27c hybridized to Ble3, -5, and -10 placing it on the left flank of cluster 1e.

Rex-1 is a PCR-based marker that maps to the *Mi-1* locus and is used diagnostically to differentiate root-knot nematode-resistant and -susceptible tomato (Williamson et al. 1994). When the amplified fragment produced with these primers was used to probe a DNA blot, it hybridized to a single band at the *Lp Mi-1* locus (not shown). In susceptible tomato however, the Rex-1 probe hybridized to two bands on Ble3 (Rex-1a and Rex-1b) and to a single band co-migrating with Rex-1a on Ble5 and Ble10, placing Rex-1a centromeric to Rex-1b in the contig (Table 1; Fig. 5). This hybridization pattern locates Rex-1b telomeric to 54D9R in cluster 1e.

The marker C264.2 had previously been shown to map to the *Le Mi-1* locus, but this probe did not hybridize to

any of the Ble clones analyzed, suggesting that clusters 1e and 2e are separated by a gap containing this sequence. The physical distance between these two clusters in susceptible tomato on chromosome 6 has not been determined.

The simplest explanation for the above results is that there is an inversion in the chromosomal region between markers 54D9L and C93.1 when *L. esculentum*- and *L. peruvianum*-derived regions are compared. Such an inversion would account for the observed exchange of C32.5 from the left flank of the cluster 1 in *L. peruvianum* to the right flank of the cluster 2 in susceptible *L. esculentum*. Conversely, an inversion would explain the observation that 54D9R is associated with cluster 1e.

Evolution of the *Mi-1* locus

If *L. peruvianum* is the ancestral progenitor of the *Lycopersicon* genera (Miller and Tanksley 1990; Baudry et al. 2001), the similarity in structure and organization of the *Le* and *Lp Mi-1* loci (Fig. 5) suggests that a genetic duplication event to produce the two clusters of *Mi-1* homologs occurred within *L. peruvianum* or a progenitor and was carried over into *L. esculentum*. It is interesting that the copy number of homologs at each of the two clusters is conserved between *L. esculentum* and *L. peruvianum*, suggesting that intrachromosomal recombination or unequal crossing over may not have played a role during the time since these two species diverged. We suggest that the inversion between the two clusters occurred after the divergence of *L. esculentum* and the *L. peruvianum* accession that was the source of nematode resistance. However, it is also possible that an inversion occurred in the *L. peruvianum* region during the process of introgression.

We have not been able to determine whether the endpoints of the recombination event are within or between the two clusters of homologs. If the clusters were arranged in an inverted orientation, a simple intrachromosomal recombination event between them could result in an inversion of the intervening structure. However, we have not yet been able to determine the orientations of the *Mi-1* homologs in cluster 2e or 2p. The differences in copy number and positions of other markers in the region suggest that the evolution leading to the current configuration of these two loci has involved additional events. For example, C32.1/11.24 is associated with both cluster 1p and 2p at the *Lp Mi-1* locus, but is only in cluster 2e at the *Le Mi-1* locus. Conversely, C12.27 is present in both clusters for the *Le Mi-1* locus, but only near cluster 1p at the *Lp Mi-1* locus (Fig. 5). Fluorescence in situ hybridization to extended DNA fibers has been previously used in the study of cluster 1p (Zhong et al. 1999), and this technique may be able to provide additional information on the organization of the other clusters and the structure of the inversion.

Recombination suppression at the *Lp Mi-1*/*Le Mi-1* locus

The apparent chromosomal inversion event between the two *Mi-1* homolog clusters in *L. esculentum* or *L. peruvianum* may account for the suppression of recombination around the *Mi-1* locus in crosses between resistant and susceptible accessions of *L. esculentum* (Kaloshian et al. 1998). Recombination suppression has also been observed at the tomato *Tm2a* R-gene locus, which is also derived from *L. peruvianum* (Ganal and Tanksley 1996). Suppression of recombination hinders positional cloning of a gene as this strategy depends on the generation of recombinants around the desired locus. Furthermore, suppressed recombination increases the potential for linkage drag that reduces the efficiency of backcrossing in plant breeding programs.

Recombination is also suppressed, but to a lesser extent, in the *Mi-1* region in progeny of crosses of the *Mi-1*-donor accession of *L. peruvianum* with a susceptible accession of *L. peruvianum* (Kaloshian et al. 1998). While several recombinants were observed between the C11.24 and C93.1 markers flanking cluster 1, recombination was suppressed telomeric to the C11.24 marker (Kaloshian et al. 1998). Recombination also appears to be lower in this region in an intraspecific cross of *L. peruvianum* segregating for *Mi-9*, a heat-stable, nematode-resistance gene that maps to the same region of chromosome 6 as *Mi-1* (Ammiraju et al. 2003). In contrast to cultivated tomato, *L. peruvianum* is a self-incompatible species with high genetic variability within the species (Baudry et al. 2001). Whether any accessions of *L. peruvianum* contain the inversion that is found between the two clusters of homologs in nematode susceptible *L. esculentum* has not been determined. Further comparison of the structure of the *Mi-1* locus in various accessions of *L. peruvianum* is likely to be a rich source of information on the genetic mechanisms associated with the evolution of the *Mi-1* locus.

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