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ORIGINAL PAPER

Genetic mapping of paternal sorting of mitochondria in cucumber

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Abstract Mitochondria are organelles that have their own DNA; serve as the powerhouses of eukaryotic cells; play important roles in stress responses, programmed cell death, and ageing; and in the vast majority of eukaryotes, are maternally transmitted. Strict maternal transmission of mitochondria makes it difficult to select for better-performing mitochondria, or against deleterious mutations in the mitochondrial DNA. Cucumber is a useful plant for organellar genetics because its mitochondria are paternally transmitted and it possesses one of the largest mitochondrial genomes among all eukaryotes. Recombination among repetitive motifs in the cucumber mitochondrial DNA produces rearrangements associated with strongly mosaic (MSC) phenotypes. We previously reported nuclear control of sorting among paternally transmitted mitochondrial DNAs. The goal of this project was to map paternal sorting of mitochondria as a step towards its eventual cloning. We crossed single plants from plant introduction (PI) 401734 and *Cucumis sativus* var. *hardwickii* and produced an F_2 family. A total of 425 F_2 plants were genotyped for molecular markers and testcrossed as the female with MSC16. Testcross families were scored for frequencies of wild-type versus MSC progenies. Discrete segregations for percent wild-type progenies were not observed and paternal sorting of mitochondria was therefore analyzed as a quantitative trait. A major quantitative trait locus (QTL; LOD >23) was mapped between two simple sequence repeats encompassing a 459-kb region on chromosome 3. Nuclear genes previously shown to affect the prevalence of mitochondrial DNAs (*MSH1*, *OSB1*, and *RECA* homologs) were not located near this major QTL on chromosome 3. Sequencing of this region from PI 401734, together with improved annotation of the cucumber genome, should result in the eventual cloning of paternal sorting of mitochondria and provide insights about nuclear control of organellar-DNA sorting.

Names are necessary to report factually on available data; however, the US Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Introduction

Plants possess DNA in the nucleus, chloroplast, and mitochondrion. For the vast majority of plants, the organellar (chloroplast and mitochondrial) DNAs are maternally transmitted; although biparental (Smith 1989; Reboud and Zeyl 1994; Zhang and Sodmergen 2003; Weihe et al. 2009) and paternal (Neale et al. 1989; Neale and Sederoff 1989; Fauré et al. 1994; Havey 1997; Havey et al. 1998; Chat et al. 1999; McCauley et al. 2005) transmissions of organellar DNAs are known. The plant mitochondrial DNA is generally much larger (367 and 570 kb for *Arabidopsis* and maize, respectively) than those of most animals or fungi (~17 kb for humans and ~15 kb for yeast) (Gillham 1994;

Unseld et al. 1997). Plant mitochondrial DNAs can undergo intramolecular recombination to produce different gene orders among relatively closely related individuals (Fauron et al. 1995), as well as chimeric reading frames associated with cytoplasmic male sterilities (Fujii and Toriyama 2008) and variegated phenotypes (Martinez-Zapater et al. 1992; Newton 1995; Bartoszewski et al. 2004). Nuclear genes, such as *MSH1*, *RECA*, and *OSB1* (Abdelnoor et al. 2003; Zaegel et al. 2006; Shedge et al. 2007; Arrieta-Montiel et al. 2009); affect the generation and prevalence of specific mitochondrial-DNA variants (sublimons).

Cucumber (*Cucumis sativus* L.) represents a unique model plant for organellar genetics because its three genomes show different modes of transmission maternal for chloroplast, paternal for mitochondrial, and biparental for nuclear genes (Havey et al. 1998), and the plant has a very large mitochondrial genome at ~1.7 Mb (Ward et al. 1981; Alverson et al. 2011). This large mitochondrial genome is due in part to the accumulation of short repetitive sequences (Lilly and Havey 2001). Recombination among these repetitive DNAs can produce mosaic (MSC) plants, which have deformed leaves and sectors of white and green tissues (Malepszy et al. 1996; Bartoszewski et al. 2004). MSC is a valuable tool for studying mitochondrial transmission because the phenotype can be easily scored at the seedling stage (Malepszy et al. 1996; Lilly et al. 2001). We previously identified a nuclear locus, *Psm* for paternal sorting of mitochondria, which preferentially sorts for rare wild-type mitochondrial DNAs transmitted paternally from MSC plants (Havey et al. 2004; Al-Faifi et al. 2008). The goal of this research was to fine-map paternal sorting of mitochondria as a step towards its eventual cloning in order to understand nuclear control of mitochondrial sorting.

Materials and methods

Mapping population

A single cucumber plant (3-A) from USDA Plant Introduction 401734 (Css) was selected because it produced a relatively high frequency (79%) of wild-type progenies when crossed as the female with MSC16. This plant was crossed as the female with a single plant of *C. sativus* var. *hardwickii* (Csh), a feral relative of cucumber, which produced a high frequency (>90%) of MSC progenies in crosses with MSC16. A single F_1 plant (C10106D) was propagated by stem cuttings and propagules were self-pollinated to produce an F_2 family (C10115). A total of 447 F_2 plants were grown in the greenhouse, self-pollinated, and testcrossed with MSC16 as the male parent. We attempted to produce more than one self and testcross family when multiple female flowers were present on individual F_2 plants. Of these 447 F_2 plants, 425 were genotyped for molecular markers and used to create the genetic map.

Segregation analyses

Black spines on cucumber fruit are conditioned by a dominant allele at the *B* locus (Pierce and Wehner 1990). Css has white and Csh has black spines and fruits from F_2 plants were visually scored for spine color. For paternal sorting of mitochondria, 50–60 seeds per testcross family were sown in vermiculite and placed on a warm bench (30°C) in the greenhouse for approximately 3 weeks. MSC plants possess chlorotic spots on cotyledons and leaves and grow slower relative to wild-type plants (Lilly et al. 2001). The numbers of wild-type and MSC testcross progenies were counted and overall germination calculated. To assess any effects of seed dormancy on germination and to determine the reproducibility of phenotypic scores, three different plantings of testcross families were completed. The first planting was done from spring 2008 to the spring of 2009, the second was done during summer and fall of 2009, and the third during spring and summer 2010. Seeds sown in the second planting were preferentially from the same fruit as the first planting. When there was too little remnant seed, the seeds were planted from independent testcrosses of MSC16 to the same F_2 plant. In the third planting, independent testcrosses to the same F_2 plant were evaluated.

Genotyping of molecular markers

Genomic DNA was extracted from the two parental plants and F_2 progenies as described by (Krysan 2004) with modifications. Fresh leaf tissue from young plants was placed into a 96-well plate and a 1-mm diameter stainless steel ball was added to each well. 200 μL of TE (9.5 mM EDTA and 50 mM Tris pH 9) were added; the plate was sealed and placed on a Geno Grinder 2000 shaker (Glen-Mills, Clifton, NJ, USA) for 2 min at 450 strokes/min. The 96-well plate was then centrifuged for 5 min at $2{,}000 \times g$. Serial dilutions of the supernatant were tested by PCR (1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each primer, 0.5 unit of Taq polymerase) to identify the best amount for amplifications. Primers used for DNA testing were AEST25 F (GCAAAGACCAATCTTAATGT) and R (TGGGACATCATCGTTTCTGA) with PCR conditions of 3 min at 95°C; followed by 35 cycles of 94°C for 25 s, 55°C for 35 s, and 72°C for 20 s; and a final extension of 3 min at 72°C.

Melon (*Cucumis melo* L.) markers MC60 and A_23-C03 were previously shown to be linked to paternal sorting of mitochondria (Al-Faifi et al. 2008). Primers (Table 1) were designed for these genes and used to produce amplicons from DNA of the parental plants. At least 25 amplicons of

each marker were sequenced in both directions as previously described (Al-Faifi et al. 2008). Sequences were edited and aligned using Sequencher v.4.6 (GeneCodes, Ann Arbor, MI, USA) to identify single nucleotide polymorphisms (SNPs). Melt-curve assays (Mader et al. 2008) were also used to genotype F_2 progenies. Primers were designed using LightTyper (Roche, Indianapolis, IN, USA) to produce amplicons of approximately 200 bp with melting temperatures of 60–65°C (Table 1). SimpleProbes were synthesized by TIB MOLBIO (Jackson, NJ, USA) and labeled with fluorescein. The best PCR conditions for SimpleProbes were a primer ratio of 1:5 (forward:reverse) and 0.4 μM of the labeled probe. Melting analysis was performed directly after PCR. SimpleProbes from marker MC60 were used to genotype the F_2 population based on the melt curve of the first negative derivative of fluorescence with respect to temperature ($-dF/dT$). The iCycler iQ™ real-time PCR detection system (Bio-Rad, Hercules, CA, USA) was also used for DNA-melting analyses, using parameters of 10 s at 95°C, 2 min at 35°C and a temperature melting range from 35 to 75°C with 1°C increments per cycle and a hold time of 5 s. Differential melting temperatures of alleles were used to assign a genotype to each plant.

Primers for 960 simple sequence repeats (SSR) of cucumber (Ren et al. 2009) were used in bulk segregant analysis (Michelmore et al. 1991) to identify polymorphisms associated with paternal sorting of mitochondria. Two pools of DNA were used, one containing DNAs from 15 F_2 plants which produced >90% MSC testcross progenies and the second with seven DNAs of F_2 plants which produced >90% wild-type testcross progenies. Equal amounts of each DNA were pooled. 10 μL PCR reactions were performed (1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each primer, 0.5 unit of Taq polymerase, and 20 ng of bulked DNA) using the same cycling program as described above. Amplicons were resolved through 3% (w/v) Super Fine Resolution (Amresco, Solon, OH, USA) agarose gels for 4–7 h, at constant voltage of 70. Markers polymorphic between the two bulks were evaluated using DNA from 425 F_2 progenies and the two parental DNAs. Genotyping of polymorphic SSRs was performed using polyacrylamide gels prepared with 39 mL of 40% acrylamide stock, 130 mL of 1× TBE, 89 mL of ddH_20, 1.82 mL of 10% APS, and 208 μL of TEMED. 5–10 μL of PCR reactions were mixed with 2.5 μL of loading dye (0.25% bromophenol blue and 40% (w/v) sucrose dissolved in water) and 1.25 μL of a 1:100 dilution of 10,000× GelRed nucleic acid Gel Stain (Biotium, Hayward, CA, USA). Gels were run at 300 volts for 1.5 h. Additional primer pairs (SSR_Psm5, SSR_Psm7, and SSR_Psm8 in Table 1) were designed for microsatellites between SSR06011 and SSR15124 based on the genomic sequence from cucumber line 9930 (Huang et al. 2009) using Tandem Repeat Occurrence Locator program (Castelo et al. 2002) or the SSR identification tool from the Cucurbit Genomic Database (http://www.icugi.org/).

Fragment analysis by capillary electrophoresis and melt-curve analyses were also used to genotype the F_2 family.

Table 1 Primer sequences used for genotyping of molecular markers

Primer	Genotyping method[a]	Primer sequence (5′–3′)
MC60-F	Sequencing	GGAGCAAGAAGCTCAACACA
MC60-R	Sequencing	TGCAATCTGCAAAATCCTGA
MC60-F	Melt curve	TGCTTCAGGAGCATGTT
MC60-R	Melt curve	CTGCATATCGTGCCCAA
A_23-C03-F	Sequencing	CTGGAACACCACCAGTTCCT
A_23-C03-R	Sequencing	GTCAGGTAGCCCAAGACAGG
A_23-C03-F	Melt curve	GCTTTAGCACTTCCACAA
A_23-C03-R	Melt curve	TGAAGGGGCACTTTACG
MSH1-F	Sequencing	TAGGAGGCTGAATGGTTGCT
MSH1-R	Sequencing	TCCCTCACTCTCCCATGTTT
SSR_Psm5-F	Fragment	CACGACGTTGTAAAACGACTG AAGAGAAGACCCCACTGA
SSR_Psm5-R	Fragment	GTTTCACCCAAATAACATGAAG GAGA
SSR_Psm7-F	Fragment	CACGACGTTGTAAAACGACTCC TACCCATCTCCATGTCA
SSR_Psm7-R	Fragment	GTTTCCAAGAATCTTACTTTCTT TGTCAATC
SSR_Psm8-F	Fragment	CACGACGTTGTAAAACGACTGG ACAAAATCATGAAGTCATTC
SSR_Psm8-R	Fragment	GTGCTTTATAGCGTAGAAAAACATT

[a] Fragment = Capillary resolution of fragment size differences

Fluorescently labeled primers (FAM or HEX) were used for PCR amplifications to label fragments that were subsequently mixed with Geneflo 625 DNA ladder labeled with ROX (CHIMERx, Milwaukee, WI, USA) as the size standard. DNA fragments and standards are denatured with Hi-Di™ Formamide (Applied Biosystems (ABI), Foster City, CA, USA) prior to loading on the ABI 3700 at the University of Wisconsin Biotechnology Center. Peaks were visualized using the Genescan software package from ABI. For melt-curves, intercalating dyes such as SybrGreen (Promega, Madison, WI, USA) or EvaGreen (Biotium, Hayward, CA, USA) were added to the master mix and the iCycler real-time detection system was used to perform the analyses.

Linkage and QTL analyses

Goodness-of-fits to the expected segregations (1:2:1 for SSRs or SNPs or 3:1 for spine color) were calculated using Microsoft (Redmond, WA, USA) Excel 2007 and detection of linkages in centiMorgans (cM) using the Kosambi mapping function were completed using Map manager QTXb20 (Manly and Olson 1999; Manly et al. 2001). Orders and recombinations among SSRs were compared to the genetic map of Ren et al. (2009).

Due to the semi-continuous distribution of the percentage of wild-type and MSC progenies, composite interval mapping (Zeng 1993, 1994; Jiang and Zeng 1995) was completed using QTL Cartographer v2.5 (Wang et al. 2010) with a walking speed of 0.5 cM and window size from 1 to 10 cM. A maximum of five marker loci were selected as cofactors by stepwise forward regression to reduce background effects. Simple interval mapping was also completed using R/qtl for binary, two-part, normal, and non-parametric data (Broman and Sen 2009). A QTL was declared significant when its LOD score was higher than the LOD threshold calculated using 1,000 permutations for and experimental-wise (type I) error rate of $P = 0.05$ (Churchill and Doerge 1994).

Evaluation of candidate genes

MSH1, *RECA*, and *OSB1* are nuclear genes that influence recombination and prevalence among mitochondrial DNAs (Martinez-Zapater et al. 1992; Abdelnoor et al. 2003; Zaegel et al. 2006; Shedge et al. 2007; Arrieta-Montiel et al. 2009). Putative homologs to these genes in the cucumber genome were identified using translated BLAST searches and their locations compared to the major QTL controlling paternal sorting of mitochondria. Primers were designed for MSH1 (Table 1), SNPs identified by sequencing amplicons as previously described, and segregations determined.

Results

Segregation of genetic markers

Cucumber has a narrow genetic background (Dijkhuizen et al. 1996; Staub et al. 2005) and a previous study revealed that the genomic region associated with paternal sorting of mitochondria was highly monomorphic in a cross between two cultivated cucumbers (PI 401734 and 'Straight 8'), with an average of one SNP every 25 kb of genomic sequence (Al-Faifi et al. 2008). Therefore, we developed a segregating family from more genetically diverse parents (Css and Csh). Spine color on fruits from F_2 plants yielded 228 black (*B-*) and 77 white-spined (*bb*), fitting the expected 3:1 ratio ($P = 0.921$) (Table 2).

Al-Faifi et al. (2008) previously reported that paternal sorting of mitochondria showed linkage to an RFLP revealed by CsP483 and melon markers MC60 and A_23-C03. The sequence of genomic clone CsP483 (Genbank accession CC144388) showed significant similarity to sequences on chromosome 3 of cucumber (Huang et al. 2009). Bulk segregant analysis revealed two polymorphic SSRs (SSR06791 and SSR00733) also on chromosome 3

Table 2 Goodness-of-fit to observed segregations for morphological and molecular markers

Marker	Maternal	Observed		Expected	Prob.[a]
		Heterozygous	Paternal		
B	77	0	228	3:1	0.921
SNP_MSH1	12	23	21	1:2:1	0.096
SSR21012	46	187	51	1:2:1	0.000*
SSR17264	68	165	81	1:2:1	0.388
SNP_MC60	85	141	73	1:2:1	0.381
SSR01647	19	45	29	1:2:1	0.325
A_23-C03	31	91	59	1:2:1	0.013*
SSR13163	18	63	28	1:2:1	0.106
SSR02856	52	110	61	1:2:1	0.682
SSR13949	72	172	102	1:2:1	0.074
SSR11397	15	32	35	1:2:1	0.001*
SSR11594	80	202	97	1:2:1	0.205
SSR16238	67	166	81	1:2:1	0.320
SSR21456	85	205	100	1:2:1	0.336
SSR20338	81	177	85	1:2:1	0.800
SSR06011	82	192	93	1:2:1	0.485
SSR_Psm5	106	198	92	1:2:1	0.610
SSR_Psm7	101	171	82	1:2:1	0.294
SSR_Psm8	44	106	40	1:2:1	0.257
SSR15124	93	190	85	1:2:1	0.691
SSR23177	108	201	64	1:2:1	0.002*
SSR00733	97	212	100	1:2:1	0.743
SSR06791	67	144	64	1:2:1	0.712

[a] Asterisk indicates poor fit to expected ratio

(Ren et al. 2009). These two SSRs, together with 17 additional SSRs on chromosome 3 and melon markers MC60 and A_23-C03, were genotyped using DNAs from 425 F_2 progenies. Three (21012, 11397, and 23177) out of the 19 SSRs and melon marker A_23-C03 did not fit the expected 1:2:1 segregation for codominant loci; all other molecular markers fit expected segregations (Table 2).

Linkages among the SSR markers agreed closely with those previously reported by Ren et al. (2009), except for the position of two markers at the end of the linkage group (Fig. 1). We observed higher rates of recombination in our F_2 population than those reported by Ren et al. (2009); genetic linkages among markers in our map spanned 88 cM as compared to 35 cM in the Ren et al. (2009) map (Fig. 1). This discrepancy could be due to the relatively few (77) recombinant inbred lines used by Ren et al. (2009), as compared to 425 F_2 progenies used for our genetic map. Melon markers MC60 and A_23-C03, previously shown to be linked to paternal sorting of mitochondria in a different cross (Al-Faifi et al. 2008), were linked to these SSR markers on chromosome 3 of cucumber (Fig. 1).

Mapping of paternal sorting of mitochondria

Although we previously reported that paternal sorting of mitochondria was controlled by a single locus (Havey et al. 2004; Al-Faifi et al. 2008), non-discrete segregations were observed in the Css by Csh cross used in this study (Fig. 2). This discrepancy could be due to environmental conditions when testcrosses were made, seed dormancy from Csh (Lower and Edwards 1986), or poor germination of MSC

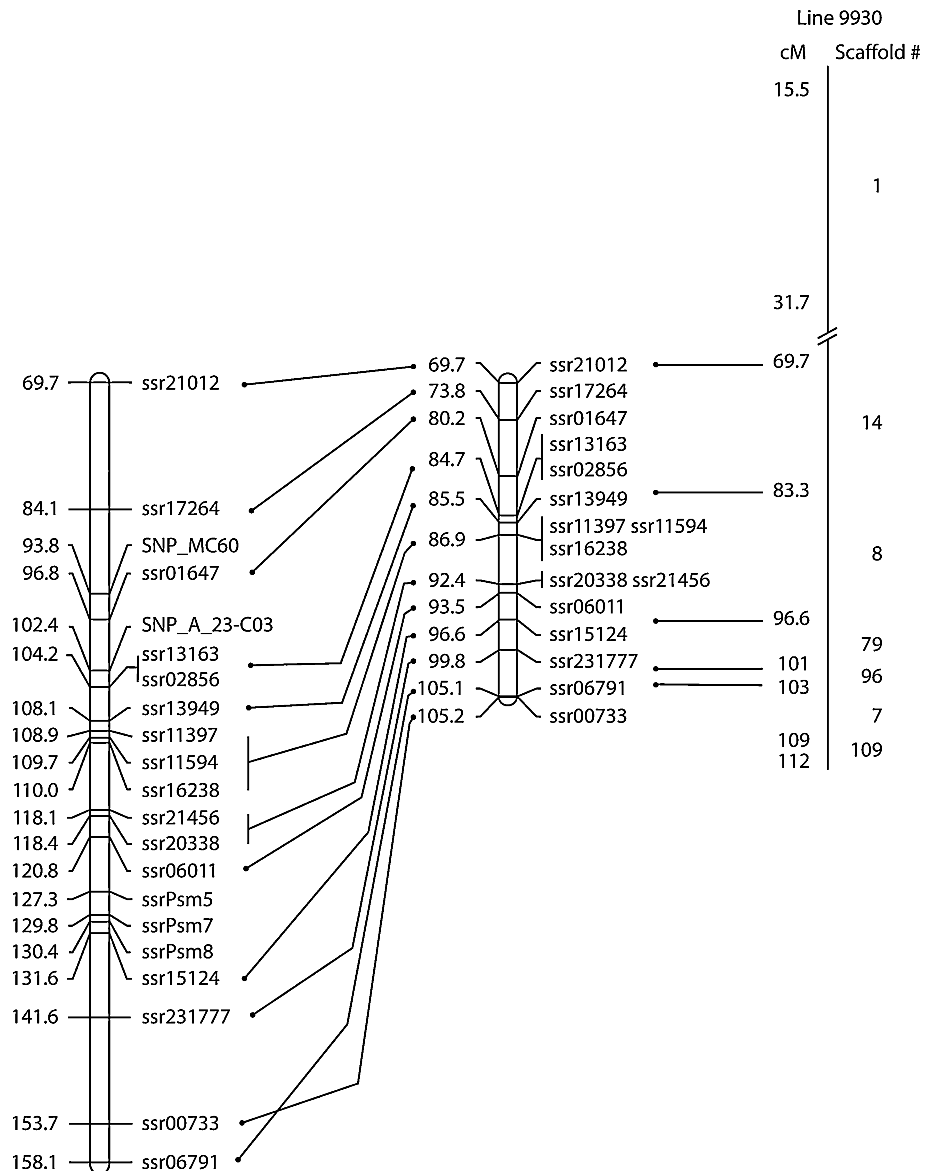

Fig. 1 Linkages and genetic distances in centiMorgans (cM) among markers on chromosome 3 from two segregating families of cucumber. The map on the *left* is from the 425 F_2 progenies from Plant Introduction 401734 × *Cucumis sativus* var. *hardwickii* (this study); the map on the *right* is from 77 recombinant *inbred lines* from the cross of GY14 × *Cucumis sativus* var. *hardwickii* (Ren et al. 2009). Positions of markers segregating in both families are shown by *lines*. Scaffolds from the genomic sequence from cucumber line 9930 (Huang et al. 2009) are shown on the *right*

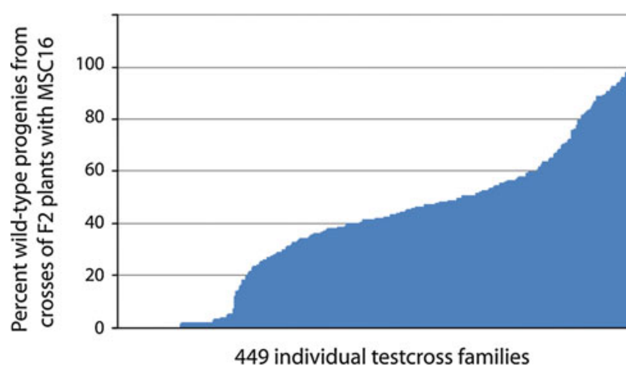

Fig. 2 Percentage of wild-type progenies (y-axis) in families (x-axis) from F_2 female plants testcrossed with MSC16 as the male

progenies. To assess potential environmental effects on the proportion of MSC to wild-type testcross progenies, seed from independent testcrosses to the same F_2 plant were evaluated. Out of 447 F_2 plants with successful testcrosses with MSC16 as the male (Supplemental Table 1), 340 had more than one fruit derived from independent testcrosses on different days. Only three (IY, O, and X) plants showed noticeably different numbers of wild-type versus MSC progenies from independent testcrosses (Supplemental Table 1). For e.g., from four independent testcrosses to F_2 plant IY, two families had 3 or 4% wild-type progenies, while the other two families had 26 or 52% wild-type progenies (Supplemental Table 1). These few differences among replicated testcross families could be due to an environmental effect on the ability of pollen to grow and successfully reach the ovule on the day the testcross was made. To assess the potential effect of seed dormancy, we planted testcross seed from the same fruit across 3 years and observed that germination significantly ($P < 0.001$) increased over time. Nevertheless, the relative proportions of MSC and wild-type progenies were consistent across time (Supplemental Table 1). Pairwise t tests revealed significant differences ($P < 0.001$) between the mean germination rate of testcross families that were predominantly MSC (mean of 68%) compared to that from families with approximately equal numbers of wild-type and MSC progenies (mean of 76%) or largely wild-type progenies (mean of 83%). An overall reduction in numbers of MSC seedlings relative to wild-type supports a deleterious effect of MSC on progenies, which could increase the proportion of wild-type testcross progenies.

Because of its continuous distribution, we analyzed paternal sorting of mitochondria as a quantitative trait using the mean proportion of wild-type testcross progenies across all plantings of seed from the same fruit as well as seed from independent testcrosses. Interval mapping using QTL cartographer, as well as different quantitative models (binary, two-part, normal, and non-parametric) in R/qtl, revealed a highly significant QTL (LOD >23) between SSRs 21456 and 06011 on chromosome 3 (Fig. 3). This major QTL was placed approximately 25 cM from melon marker MC60, closely agreeing with the 17 cM between MC60 and *Psm* previously reported by Al-Faifi et al. (2008). The genetic distance between SSRs 21456 and 06011 was 2.7 cM and both SSRs have been assigned to sequence scaffold 00008 of cucumber line 9930 (Huang et al. 2009).

Evaluation of candidate genes

Nuclear genes, such as *MSH1*, *RECA*, and *OSB1* affect recombination and prevalence among plant mitochondrial DNAs (Abdelnoor et al. 2003; Zaegel et al. 2006; Shedge et al. 2007; Arrieta-Montiel et al. 2009). These genes could also control the predominance of specific mitochondrial DNAs in the developing embryo. Putative cucumber homologs of *MSH1*, *REC1A*, *REC2A*, *REC3A*, and *OSB1* were identified by translated searches and assigned to scaffolds 000001, 000366, 000031, 000040, and 000109, respectively, of cucumber 9930 (Huang et al. 2009). Of these, scaffolds 000001 (*MSH1*) and 000109 (*OSB1*) have been assigned to chromosome 3 of cucumber; however,

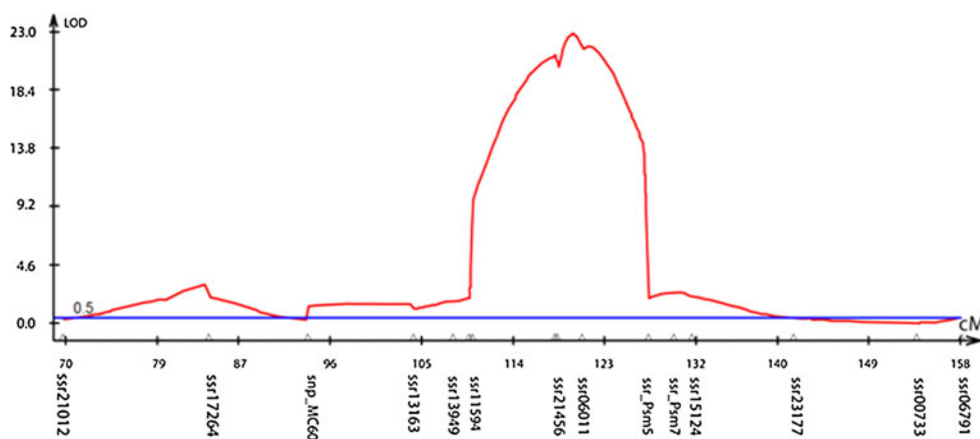

Fig. 3 LOD values (y-axis) from QTL cartographer for percent wild-type progenies from crosses of MSC16 as the male to F_2 progenies. Linkages of molecular markers are shown on x-axis. *Horizontal line* shows LOD threshold from permutation analysis

scaffold 000001 is approximately 60 cM and scaffold 000109 is located about 20 cM from scaffold 00008 where the major QTL for paternal sorting of mitochondria was placed. Single nucleotide polymorphisms in *MSH1* (Table 2) confirmed that this gene segregated independently from the genetic region carrying the major QTL controlling paternal sorting of mitochondria, agreeing with Al-Faifi et al. (2008).

Discussion

Although we previously reported that paternal sorting of mitochondria was controlled by the single locus *Psm* (Havey et al. 2004; Al-Faifi et al. 2008), non-discrete segregations were observed in this study (Fig. 2). This discrepancy could be due to a combination of reduced viability of MSC progenies, seed dormancy, or environmental effects during fertilization and embryo development. Nevertheless, our results reveal that paternal sorting of mitochondria is controlled by a major QTL (LOD >23) on chromosome 3. This major QTL showed linkage to marker MC60 in agreement with the previous segregation analysis of Al-Faifi et al. (2008) using a different cross. Therefore, we are confident that the major QTL mapped using the Css by Csh cross is the same as the *Psm* locus described by Havey et al. (2004) and Al-Faifi et al. (2008).

Nuclear genes, such as *MSH1*, *REC1A*, *REC2A*, *REC3A*, and *OSB1*, affect recombination among and the prevalence of specific mitochondrial DNAs (Arrieta-Montiel et al. 2009). The plant MutS homolog 1 (*MSH1*) gene is targeted to the organelles and is involved in mismatch repair (Xu et al. 2011). In *Arabidopsis*, mutations in *MSH1* are associated with accumulation of rearranged mitochondrial DNAs and variegated green tissues (Martinez-Zapater et al. 1992). Mutations in the RECA genes result in illegitimate recombination among mitochondrial DNAs (Odahara et al. 2009). OSBs are plant-specific single-stranded DNA-binding proteins targeted to organelles that control stoichiometries of different mitochondrial DNAs after recombination events (Zaegel et al. 2006; Maréchal and Brisson 2010). Importantly, the region on chromosome 3 that controls paternal sorting of mitochondria does not carry cucumber homologs of the *MSH1*, *RECA*, and *OSB1* genes, indicating that paternal sorting of mitochondria in cucumber may provide new and unique insights about nuclear control of mitochondrial sorting.

The 2.7-cM region between SSRs 21456 and 06011 corresponds to 459 kb based on the genomic sequence of cucumber GY14 on Phytozome v 5.0 (http://www.phytozome.net/). Database searches revealed at least 69 putative genes supported by cDNAs in this genomic region (Supplemental Table 2). Our research will now focus on sequencing through this genomic region in PI 401734 to identify and evaluate candidate genes, in order to better understand nuclear control of mitochondrial-DNA sorting.

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