Diallel Crossing Among Doubled Haploids of Cucumber Reveals Significant Reciprocal-cross Differences

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ABSTRACT. Cytoplasmic effects on plant performance have been documented, but are not well understood. Cucumber (*Cucumis sativus*) is a useful plant for studying organellar effects on phenotypes because chloroplasts show maternal transmission and mitochondria paternal transmission. We produced doubled haploids (DH) from divergent cucumber populations, generated reciprocal hybrids in a diallel crossing scheme, measured fresh and dry weights of plants 22–30 days after planting seed, estimated combining abilities and heterosis for early plant growth, and assessed performance differences between reciprocal hybrids with identical nuclear genotypes. Across experiments, general and specific combining abilities and reciprocal effects, as well as their interactions with replicated experiments, were all highly significant ($P < 0.001$). Hybrids consistently out-performed parental lines with average heterosis over midparent values between 14% and 30%. A mitochondrial mutant (MSC3) showed negative effects when used as the male due to paternal transmission of mitochondria, but not as the female parent. Reciprocal hybrids among wild-type DH parents were identified that differed significantly ($P = 0.032$ to 0.001) for dry and fresh weights across experiments, indicating that cucumber breeders should evaluate both directions of crosses when producing hybrid cultivars. Reciprocal hybrids from DH cucumbers offer a unique opportunity to study biological factors contributing to significantly better performances, due to specific nuclear-cytoplasmic combinations and/or parent-of-origin effects in identical nuclear backgrounds.

Cytoplasmic effects on plant performance and fertility are well documented (Kihira, 1982). An example of a useful organellar trait is cytoplasmic male sterility, which is widely used to produce hybrid seed of many crops (Havey, 2004). There are numerous examples of deleterious traits associated with cytoplasm, such as the reduced vigor of maize (*Zea mays*) inbreds possessing the teosinte cytoplasm (Allen, 2005; Edwards et al., 1996) and low-temperature chlorotic phenotypes associated with the ogura cytoplasm of the brassicas [*Brassica* sp. (Bannerot et al., 1977)]. Mitochondrion-associated phenotypes include the chloroplast mutator of *Arabidopsis thaliana* (Abdelnoor et al., 2003; Martínez-Zapater et al., 1992), non-chromosome stripe of maize (Newton and Coe, 1986), and mosaic (MSC) mutants of cucumber (Lilly et al., 2001; Malepszy et al., 1996), all of which show chlorotic

sectors on leaves, reduced vigor, and relatively poor fertility. These mutants are often due to deletions or rearrangements in the mitochondrial DNA that negatively affect gene expression and mitochondrial function (Gu et al., 1993; Hartmann et al., 1994; Hunt and Newton, 1991; Janska et al., 1998; Lauer et al., 1990; Lilly et al., 2001; Marienfeld and Newton, 1994; Newton et al., 1990; Sakamoto et al., 1996).

Whereas the chloroplast DNAs of most plants are similar in size and structure (Raubeson et al., 2007), the mitochondrial DNAs vary greatly in size from about 219 kbp (Palmer and Herbon, 1987) to well over 11 Mbp (Sloan et al., 2012). The larger sizes of plant mitochondrial DNAs are due in part to the accumulation of repetitive motifs (Andre et al., 1992) and DNA transfers from the chloroplast (Cummings et al., 2003). Recombination among repetitive motifs can give rise to structurally rearranged mitochondrial DNAs, which can exist as relatively low-copy subgenomic molecules (sublimons) (Abdelnoor et al., 2003, 2006; Bartoszewski et al., 2004b; Fauron et al., 1995). These sublimons can shift in relative abundance, a process referred to as substoichiometric shifting (Janska et al., 1998; Shedge et al., 2007), to produce mitochondrially associated phenotypes (Abdelnoor et al., 2003; Janska et al., 1998; Kanazawa et al., 1994). The predominance of a specific mitochondrial DNA can be under nuclear control (Abdelnoor et al., 2003; Shedge et al., 2007) to presumably maintain beneficial interactions between the mitochondrial and nuclear genomes.

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Cucumber possesses several characteristics useful for organellar genetics, including differential transmission of the organellar DNAs; a large mitochondrial DNA with repetitive sequences that undergo recombination to produce structurally rearranged molecules; and the existence of the mitochondrially associated, MSC phenotypes. Whereas most plants show maternal transmission of both the chloroplast and mitochondrial DNAs (Birky, 1995; Reboud and Zeyl, 1994), cucumber chloroplasts are maternally and mitochondria paternally transmitted (Havey, 1997; Havey et al., 1998). This unique mode of transmission allows for the separation of chloroplast and mitochondrial effects by reciprocal crossing. The cucumber chloroplast DNA is similar to most plants in size and structure (Kim et al., 2006); however, its mitochondrial DNA is one of the largest among all eukaryotes at 1685 kbp (Alverson et al., 2011; Ward et al., 1981). The mitochondrial DNA of cucumber possesses clusters of repetitive DNAs (Alverson et al., 2011; Bartoszewski et al., 2004a; Lilly and Havey, 2001) that recombine to produce structurally polymorphic molecules among elite cucumber populations (Havey et al., 1998) and are associated with the MSC phenotypes (Bartoszewski et al., 2004b; Lilly et al., 2001).

Because of maternal transmission of the organelles in the vast majority of plants, it is difficult to unequivocally separate chloroplast or mitochondrial effects on phenotypes. Cucumber provides a unique system to identify and characterize chloroplast and mitochondrial effects on plant growth and development by exploiting differential transmission of these organelles. In this study, we produced doubled haploids from divergent cucumber populations and crossed among them to generate a complete diallel mating design. The diallel is a useful tool to estimate the combining abilities of inbreds and heterosis (Griffing, 1956). The general combining ability (GCA) of an inbred is a measure of the average performance of hybrids from crosses with other inbreds. Specific combining ability (SCA) measures the performance of specific hybrid combinations relative to the average performance expected from the inbred parents. Here we report significant differences for plant growth between reciprocal hybrids possessing identical nuclear genotypes, revealing the potential of beneficial organellar effects on plant performance.

Materials and Methods

Doubled haploid plants were produced by culturing immature female flowers (Dirks, 1988) from major market classes of cucumber: GY14 is an elite North American pickling type; 'Marketmore 76' (MM76) and 'Straight 8' (ST8) are North American slicing cucumbers; and TMG1 and 9930 are Asian slicing cucumbers. MSC3 is a mitochondrial mutant selected from the highly inbred (>S₁₈) line B derived from European pickling germplasm (Bartoszewski et al., 2004b). The putative DHs and MSC3 were self-pollinated and progenies grown in the greenhouse. DNA was extracted from pooled leaf tissues of at least 20 plants from each line and from the feral relative of cucumber [*Cucumis sativus* var. *hardwickii* (Csh)], USDA plant introduction 183967. DNAs were genotyped for 190 SSR (SSRs) spread across the cucumber genome (Yang et al., 2012) (Supplemental Table 1). Genetic distances were estimated using the simple matching coefficient and 25 SSRs polymorphic among the DH lines and MSC3 (Supplemental Table 2), and a dendrogram was produced by UPGMA using the program Numerical Taxonomy and Multivariate Analysis System (NTSYS version 2.2; Exeter Software, Setauket, NY).

Five DH lines and MSC3 were grown in the greenhouse and crossed with each other both as the male and female to produce a complete diallel of 30 hybrids. Three plants of each hybrid and parental line (108 plants total) were grown in each of three greenhouses (blocks). The experiment was repeated three times. Growth conditions were optimal for cucumber (16-h days at 28 °C with light intensity of 270 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ at the level of the greenhouse bench; nights at 24 °C). Plants were destructively harvested at 30 d (Expt. 1) and 22 d (Expts. 2 and 3) after planting by cutting stems at the cotyledons, and fresh and dry weights of each plant were measured. The mean weights of the three plants in each block were calculated for hybrids and parents. GCA, SCA, and reciprocal-cross effects were calculated using a SAS (version 9.3; SAS Institute, Cary NC) program based on Griffing's (1956) method 1; fixed effects model 1. Average heterosis was estimated using the program of Burrow and Coors (1994).

Results and Discussion

Putative DH plants were produced by culturing immature female flowers (Dirks, 1988) from each of five cucumber populations (GY14, MM76, ST8, TMG1, and 9930). Because these plants are derived from the female gametophyte, they must possess mitochondrial DNA from the plant producing the female flower. Each putative DH was self-pollinated to produce a DH line, and one DH line from each of the five populations was randomly chosen for genotyping and crossing. MSC3 is a highly inbred mitochondrial mutant of cucumber (Bartoszewski et al., 2004b). We detected no heterozygosity in the five DH

Fig. 1. Genetic relationships among cucumber inbred (MSC3) and doubled haploids from populations Gy14, 'Marketmore 76' (MM76), 'Straight 8' (ST8), 9930, and TMG1 relative to *Cucumis sativus* var. *hardwickii* (Csh). Relationships were estimated using 25 simple sequence repeats (SSRs) and the simple matching coefficient where 1.0 would represent identical genotypes across all SSRs.

Table 1. General combining abilities expressed as deviations from overall mean of zero for fresh and dry weights of progenies from diallel crossing among parental inbred (MSC3) and doubled haploids selected from populations TMG1, 'Marketmore 76' (MM76), 9930, 'Straight 8' (ST8), and Gy14 of cucumber.

Parent	Fresh wt	Dry wt
TMG1	5.21	0.51
MM76	1.81	0.19
9930	1.48	0.39
ST8	-0.72	-0.08
Gy14	-1.88	-0.27
MSC3	-5.90	-0.74

Table 2. Specific combining abilities expressed as deviations from overall mean of zero for fresh and dry weights of progenies from diallel crossing among inbred (MSC3) and doubled haploids selected from populations TMG1, 'Marketmore 76' (MM76), 9930, 'Straight 8' (ST8), and Gy14 of cucumber.

	Fresh wt						Dry wt					
	TMG1	Gy14	ST8	MM76	9930	MSC3	TMG1	Gy14	ST8	MM76	9930	MSC3
TMG1	-6.30	3.44	1.84	-0.32	0.95	0.39	-0.86	0.34	0.22	0.18	0.19	-0.07
Gy14		-7.20	0.09	1.36	1.59	0.72		-0.58	0.14	0.06	-0.04	0.09
ST8			-9.26	0.52	6.20	0.61			-1.22	-0.03	0.81	0.08
MM76				-3.01	1.68	-0.23				-0.49	0.41	-0.13
9930					-11.15	0.74					-1.52	0.15
MSC3						-2.24						-0.14

lines and MSC3 for 190 SSRs (Yang et al., 2012), confirming their highly inbred status. A dendrogram based on 25 SSRs commonly polymorphic among the lines (Supplemental Table 2) revealed two main groups relative to Csh (Fig. 1). The North American cucumbers clustered together (GY14, MM76, and ST8), as did the Asian types (TMG1 and 9930). The mitochondrial mutant MSC3 was placed with the Asian germplasms (Fig. 1). These results are consistent with previous research demonstrating significant genetic divergence between Asian and North American cucumbers (Lv et al., 2012; Staub et al., 1999).

Growth of young plants was measured 22–30 d after planting in greenhouses. Across experiments, overall average fresh and dry weights were 30.4 and 3.0 g, respectively. Fresh and dry weights were significantly correlated at 0.70; coefficients of variation were 15.2% and 19.7%, respectively, and this level of variation may have contributed to the lower correlation between fresh and dry weights. For both traits, genotypes (parents and hybrids), experiments, genotype-by-experiment interactions, and blocks within experiments [block(experiment)] were highly significant ($P < 0.001$). The significance of experiments may have resulted from the weather conditions outside of the greenhouses; cloudy days were typical during Expts. 1 and 2, while clear sunny weather predominated during Expt. 3. Also during Expt. 2, one greenhouse (block) was cooler by an average of 5 °C than the other two greenhouses, which may have contributed to the significant block (experiment) effect.

Across experiments, GCA, SCA, and reciprocal effects, as well as their interactions with experiments, were all highly significant ($P < 0.001$). The DH lines from TMG1, MM76, and 9930 showed positive GCA effects for both fresh and dry weights; while ST8, Gy14, and MSC3 had negative GCA effects for both traits (Table 1). The parental DHs and MSC3 showed negative SCA effects for both fresh and dry weights (Table 2), indicating that hybrids consistently out-performed parental lines. Across experiments, average heterosis over the midparent value was between 14% and 30% (Table 3). Although there are reports of relatively low inbreeding depression and heterosis in cucumber (Cramer and Wehner, 1999; Rubino and Wehner, 1986; Wehner, 1989), the relatively high heterosis in our study supports development of cucumber hybrids, at least with regards to early plant growth. Our study is in agreement with other researchers who reported significant heterosis for various traits in cucumber (Ghaderi and Lower, 1978, 1979; Hormuzdi and More, 1989; Hutchins, 1938; Rubino and Wehner, 1986; Singh et al., 1970).

Reciprocal effects were highly significant ($P < 0.001$) for fresh and dry weights. As expected, the mitochondrial mutant MSC3 showed predominantly negative effects when used as

Table 3. Heterosis observed for fresh and dry weights of progenies across three experiments from diallel crossing among parental doubled-haploid and inbred lines of cucumber.

Expt. no.	Wt	Means			Heterosis ^z
		Parents	Hybrids	Overall	
1	Dry	2.7	3.8	3.7	29.7%
	Fresh	20.1	26.7	25.8	25.6%
2	Dry	1.7	2.1	2.1	19.0%
	Fresh	21.0	24.5	24.0	14.6%
3	Dry	2.5	3.5	3.4	29.4%
	Fresh	35.5	45.7	44.4	23.0%

^zHeterosis reported as percentage of overall mean fresh or dry weight.

a male (due to paternal transmission of mutant mitochondria), but not when used as the female parent (mitochondria transmitted from the wild-type male parent). As a male, MSC3 decreased fresh and dry weights of progenies by 21% and 27% (respectively) relative to experiment-wide means. We detected significant differences between reciprocal hybrids from crosses among wild-type DH lines (Tables 4 and 5). Although the DH from TMG1 showed the highest GCA effects (Table 1), it performed significantly better as a female than male (Table 4). The opposite was true for ST8, which performed better as a male than female (Table 4). As a result, reciprocal hybrids between ST8 and TMG1 were significantly ($P < 0.001$) different for dry and fresh weights across experiments (Table 5), differing by $\approx 30\%$ of the overall experimental means for both traits. Other reciprocal hybrids showing significantly different fresh and dry weights were from crosses of TMG1 by Gy14 or 9930, as well as Gy14 and MM76 for dry weights (Table 5). Therefore, cucumber inbreds showing good GCA and/or SCA should be crossed in both directions to determine if a hybrid produced in one direction outperforms the reciprocal cross (Table 4).

Possible explanations for the significant reciprocal-cross differences include nuclear-cytoplasmic interactions or parent-of-origin effects. The differential transmission of the cucumber organelles (Havey, 1997) allows for the production of reciprocal hybrids with contrasting chloroplast and mitochondrial combinations and identical nuclear genotypes. The significantly better performance of a DH as the male parent could be due to superior mitochondria and/or beneficial mitochondrial interactions with the nucleus. Conversely, better performance as the female parent could indicate better performing or interacting chloroplasts. Well defined heterotic groups exist in maize, and better performing hybrids are produced by crossing inbreds from distinct groups (Hallauer et al., 1988). Our results indicate that different organellar types may exist in cucumber,

Table 4. Reciprocal effects expressed as deviations from overall mean of zero in grams for fresh and dry weights of progenies from diallel crossing among inbred (MSC3) and doubled haploids selected from populations TMG1, ‘Marketmore 76’ (MM76), 9930, ‘Straight 8’ (ST8), and Gy14 of cucumber.

		Female											
		Fresh wt						Dry wt					
		TMG1	Gy14	ST8	MM76	9930	MSC3	TMG1	Gy14	ST8	MM76	9930	MSC3
Male	TMG1	—	−2.37	−4.42	−1.36	−1.93	4.63	—	−0.36	−0.49	−0.21	−0.44	0.60
	Gy14	2.37	—	−0.84	1.50	0.79	3.44	0.36	—	−0.06	0.26	0.18	0.40
	ST8	4.42	0.84	—	0.01	−0.04	4.66	0.49	0.06	—	0.00	0.10	0.49
	MM76	1.36	−1.50	−0.01	—	1.31	1.62	0.21	−0.26	0.00	—	0.33	0.16
	9930	1.93	−0.79	0.04	−1.31	—	−0.14	0.44	−0.18	−0.10	−0.33	—	−0.06
	MSC3	−4.63	−3.44	−4.66	−1.62	0.14	—	−0.60	−0.40	−0.49	−0.16	0.06	—

Table 5. Significance of reciprocal-cross differences for fresh (above diagonal) and dry (below diagonal) weights of progenies from diallel crossing among doubled haploids selected from cucumber populations TMG1, ‘Marketmore 76’ (MM76), 9930, ‘Straight 8’ (ST8), and Gy14.

	TMG1	Gy14	ST8	MM76	9930
TMG1	—	0.032	0.001	NS ^z	0.021
Gy14	0.007	—	NS	NS	NS
ST8	0.005	NS	—	NS	NS
MM76	NS ^z	0.003	NS	—	NS
9930	0.002	NS	NS	NS	—

^zNS = nonsignificant at $P < 0.05$.

and inbreds possessing specific organelles may perform better as the male or female to produce more vigorous hybrids.

In animals, different phenotypes may occur depending on whether an allele is inherited from the mother or father, and these parent-of-origin effects are often due to imprinting in which an allele from one parent is epigenetically modified (Mott et al., 2014). In plants, most parent-of-origin differences are restricted to the endosperm and show maternal expression (reviewed by Köhler et al., 2012). There is an example of parent-of-origin expression in the embryo, the *maternally expressed in embryo 1* gene of maize (Jahnke and Scholten, 2009); however there is no evidence for significant effects on subsequent growth and development of the plant. Nevertheless, the significant reciprocal-cross differences reported in this study could be affected by parent-of-origin effects acting independently of or interacting with the organelles.

The significantly different performances of reciprocal hybrids from DH parents offer a unique opportunity to study biological differences associated with organellar and/or parent-of-origin effects. Transcriptome analyses should reveal important nuclear gene-expression differences associated with contrasting organellar combinations in identical nuclear genotypes. Epigenetic modification of alleles associated with parent-of-origin effects can be determined using tools such as methylation-sensitive enzymes or bisulfite sequencing (Lauria et al., 2014). These studies should provide insights about specific nuclear genes or pathways that interact with the organelles to enhance performance. Expression levels of these genes or pathways could then be assessed as targets for selection in plants showing strict maternal transmission of the organelles, toward the development of superior-performing hybrids.

Literature Cited

Abdelnoor, R.V., A.C. Christensen, S. Mohammed, B. Munoz-Castillo, H. Moriyama, and S.A. Mackenzie. 2006. Mitochondrial genome

dynamics in plants and animals: Convergent gene fusions of a MutS homologue. *J. Mol. Evol.* 63:165–173.

Abdelnoor, R.V., R. Yule, A. Elo, A.C. Christensen, G. Meyer-Gauen, and S.A. Mackenzie. 2003. Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to MutS. *Proc. Natl. Acad. Sci. USA* 100:5968–5973.

Allen, J.O. 2005. Effect of teosinte cytoplasmic genomes on maize phenotypes. *Genetics* 169:863–880.

Alverson, A.J., D.W. Rice, S. Dickinson, K. Barry, and J.D. Palmer. 2011. Origins and recombination of the bacterial-sized multichromosomal mitochondrial genome of cucumber. *Plant Cell* 23:2499–2513.

Andre, C., A. Levy, and V. Walbot. 1992. Small repeated sequences and the structure of plant mitochondrial genomes. *Trends Genet.* 8:128–131.

Bannerot, H., L. Boudlard, and Y. Chupeau. 1977. Unexpected difficulties met with radish cytoplasm in *Brassica oleracea*. *EUCARPIA Cruciferae Nwsl.* 2:16.

Bartoszewski, G., N. Katzir, and M.J. Havey. 2004a. Organization of repetitive DNAs and the genomic regions carrying ribosomal RNA, cob, and atp9 genes in the cucurbit mitochondrial genomes. *Theor. Appl. Genet.* 108:982–992.

Bartoszewski, G., S. Malepszy, and M.J. Havey. 2004b. Mosaic (MSC) cucumbers regenerated from independent cell cultures possess different mitochondrial rearrangements. *Curr. Genet.* 45:45–53.

Birky, C.W. 1995. Uniparental inheritance of mitochondrial and chloroplast genes: Mechanisms and evolution. *Proc. Natl. Acad. Sci. USA* 92:11331–11338.

Burow, M.D. and J.G. Coors. 1994. Diallel: A microcomputer program for the simulation and analysis of diallel crosses. *Agron. J.* 86:154–158.

Cramer, C.S. and T.C. Wehner. 1999. Little heterosis for yield and yield components in hybrids of six cucumber inbreds. *Euphytica* 110:99–108.

Cummings, M.P., J.M. Nugent, R.G. Olmstead, and J.D. Palmer. 2003. Phylogenetic analysis reveals five independent transfers of the chloroplast gene *rbcL* to the mitochondrial genome in angiosperms. *Curr. Genet.* 43:131–138.

Dirks, R. 1988. Method for the production of double-haploid cucumbers. Patent US5492827 A. U.S. Patent Trademark Office, Washington, DC.

Edwards, J.W., J.O. Allen, and J.G. Coors. 1996. Teosinte cytoplasmic genomes. I. Performance of maize inbreds with teosinte cytoplasm. *Crop Sci.* 36:1088–1091.

Fauron, C., M. Casper, Y. Gao, and B. Moore. 1995. The maize mitochondrial genome: Dynamic, yet functional. *Trends Genet.* 6:228–235.

Ghaderi, A. and R.L. Lower. 1978. Heterosis and phenotypic stability of F1 hybrids in cucumber under controlled environment. *J. Amer. Soc. Hort. Sci.* 103:275–278.

Ghaderi, A. and R.L. Lower. 1979. Heterosis and inbreeding depression for yield in populations derived from six crosses of cucumber. *J. Amer. Soc. Hort. Sci.* 104:564–567.

- Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing schemes. *Austral. J. Biol. Sci.* 9:463–493.
- Gu, J., D. Miles, and K.J. Newton. 1993. Analysis of leaf sectors in the NCS6 mitochondrial mutant of maize. *Plant Cell* 5:963–971.
- Hartmann, C., H. Récipon, M.F. Jubier, C. Valon, E. Delcher-Besin, Y. Henry, J. De Buyser, B. Lejeune, and A. Rode. 1994. Mitochondrial DNA variability detected in single wheat regenerant involves a rare recombination event across a short repeat. *Curr. Genet.* 25:456–464.
- Hallauer, A.R., W.A. Russel, and K.R. Lamkey. 1988. Corn breeding, p. 463–564. In: G.F. Sprague and J.W. Dudley (eds.). *Corn and corn improvement*. Amer. Soc. Agron., Madison, WI.
- Havey, M.J. 1997. Predominant paternal transmission of the mitochondrial genome cucumber. *J. Hered.* 88:232–235.
- Havey, M.J. 2004. The use of cytoplasmic male sterility for hybrid seed production, p. 623–634. In: H. Daniel and C. Chase (eds.). *Molecular biology and biotechnology of plant organelles*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Havey, M.J., J.D. McCreight, B. Rhodes, and G. Taurick. 1998. Differential transmission of the *Cucumis* organellar genomes. *Theor. Appl. Genet.* 97:122–128.
- Hormuzdi, S.G. and T.A. More. 1989. Heterosis studies in cucumber (*Cucumis sativus* L.). *Indian J. Hort.* 46:73–79.
- Hunt, M.D. and K.J. Newton. 1991. The NCS3 mutation: Genetic evidence for the expression of ribosomal protein genes in *Zea mays* mitochondria. *EMBO J.* 10:1045–1052.
- Hutchins, A.E. 1938. Some examples of heterosis in the cucumber (*Cucumis sativus* L.). *Proc. Amer. Soc. Hort. Sci.* 36:660–664.
- Jahnke, S. and S. Scholten. 2009. Epigenetic resetting of a gene imprinted in plant embryos. *Curr. Biol.* 19:1677–1681.
- Janska, H., R. Sarria, M. Woloszynka, M. Arrieta-Montiel, and S.A. Mackenzie. 1998. Stoichiometric shifts in the common bean mitochondrial genome leading to male sterility and spontaneous reversion to fertility. *Plant Cell* 10:1163–1180.
- Kanazawa, A., N. Tsutsumi, and A. Hirai. 1994. Reversible changes in the composition of the population of mtDNAs during dedifferentiation and regeneration in tobacco. *Genetics* 138:865–870.
- Kihira, H. 1982. Importance of cytoplasm in plant genetics. *Cytologia (Tokyo)* 47:435–450.
- Kim, J.S., J. Jung, J.A. Lee, H.W. Park, K.H. Oh, W.J. Jeong, D.W. Choi, J. Liu, and K. Cho. 2006. Complete sequence and organization of the cucumber (*Cucumis sativus* L. cv. Baekmibaekdadagi) chloroplast genome. *Plant Cell Rpt.* 25:334–340.
- Köhler, C., P. Wolff, and C. Spillane. 2012. Epigenetic mechanisms underlying genomic imprinting in plants. *Annu. Rev. Plant Biol.* 63:331–352.
- Lauer, M., C. Knudsen, K.J. Newton, S. Gabay-Laughnan, and J.R. Laughnan. 1990. A partially deleted mitochondrial cytochrome oxidase gene in the NCS6 abnormal growth mutant of maize. *New Biol.* 2:179–186.
- Lauria, M., S. Piccinini, R. Pirona, G. Lund, A. Viotti, and M. Morto. 2014. Epigenetic variation, inheritance, and parent-of-origin effects of cytosine methylation in maize (*Zea mays*). *Genetics* 196:653–666.
- Lilly, J.W. and M.J. Havey. 2001. Small, repetitive DNAs contribute significantly to the expanded mitochondrial genome of cucumber. *Genetics* 159:317–328.
- Lilly, J.W., G. Bartoszewski, S. Malepszy, and M.J. Havey. 2001. A major deletion in the cucumber mitochondrial genome sorts with the MSC phenotype. *Curr. Genet.* 40:144–151.
- Lv, J., J. Qi, Q. Shi, D. Shen, S. Zhang, G. Shao, H. Li, Z. Sun, Y. Weng, Y. Shang, X. Gu, X. Li, X. Zhu, J. Zhang, R. van Treuren, W. van Dooijeweert, Z. Zhang, and S. Huang. 2012. Genetic diversity and population structure of cucumber (*Cucumis sativus* L.). *PLoS ONE* 7:e46919.
- Malepszy, S., W. Burza, and M. Śmiech. 1996. Characterization of a cucumber (*Cucumis sativus* L.) somaclonal variant with paternal inheritance. *J. Appl. Genet.* 37:65–78.
- Mariefeld, J.R. and K.J. Newton. 1994. The maize NCS2 abnormal growth mutant has a chimeric nad4-nad7 mitochondrial gene and is associated with reduced complex 1 function. *Genetics* 138:855–863.
- Martínez-Zapater, J.M., P. Gil, J. Capel, and C.R. Somerville. 1992. Mutations at the *Arabidopsis CHM* locus promote rearrangements of the mitochondrial genome. *Plant Cell* 4:889–899.
- Mott, R., W. Yuan, P. Kaisaki, X. Gan, J. Cleak, A. Edwards, A. Baud, and J. Flint. 2014. The architecture of parent-of-origin effects in mice. *Cell* 156:332–342.
- Newton, K.J. and E.H. Coe, Jr. 1986. Mitochondrial DNA changes in abnormal growth (nonchromosomal stripe) mutants of maize. *Proc. Natl. Acad. Sci. USA* 83:7363–7366.
- Newton, K.J., C. Knudsen, S. Gabay-Laughnan, and J.R. Laughnan. 1990. An abnormal growth mutant in maize has a defective mitochondrial cytochrome oxidase gene. *Plant Cell* 2:107–113.
- Palmer, J.D. and L.A. Herbon. 1987. Uncircular structure of the *Brassica hirta* mitochondrial genome. *Curr. Genet.* 11:565–570.
- Raubeson, L.A., R. Peery, T.W. Chumley, C. Dziubek, H.M. Fourcade, J.L. Boore, and R.K. Jansen. 2007. Comparative chloroplast genomics: Analyses including new sequences from the angiosperms *Nuphar advena* and *Ranunculus macranthus*. *BMC Genomics* 8:174.
- Reboud, X. and C. Zeyl. 1994. Organelle inheritance in plants. *Heredity* 72:132–140.
- Rubino, D.B. and T.C. Wehner. 1986. Effect of inbreeding on horticultural performance of lines developed from an open-pollinated pickling cucumber population. *Euphytica* 35:459–464.
- Sakamoto, W., H. Kondo, M. Murata, and F. Motoyoshi. 1996. Altered mitochondrial gene expression in a maternal distorted leaf mutant of *Arabidopsis* induced by chloroplast mutator. *Plant Cell* 8:1377–1390.
- Shedge, V., M. Arrieta-Montiel, A.C. Christensen, and S.A. Mackenzie. 2007. Plant mitochondrial recombination surveillance requires unusual *RecA* and *MutS* homologs. *Plant Cell* 19:1251–1264.
- Singh, J.P., H.S. Gill, and K.S. Ahluwalia. 1970. Studies in hybrid vigour in cucumbers (*Cucumis sativus* L.). *Indian J. Hort.* 27:36–38.
- Sloan, D.B., A.J. Alverson, J.P. Chuckalovcak, M. Wu, D.E. McCauley, J.D. Palmer, and D.R. Taylor. 2012. Rapid evolution of enormous, multichromosomal genomes in flowering plant mitochondria with exceptionally high mutation rates. *PLoS Biol.* 10:e1001241.
- Staub, J.E., F.C. Serquen, T. Horejsi, and J.F. Chen. 1999. Genetic diversity in cucumber (*Cucumis sativus* L.): IV. An evaluation of Chinese germplasm. *Genet. Resources Crop Evol.* 46:297–310.
- Ward, B., R. Anderson, and A. Bendich. 1981. The mitochondrial genome is large and variable in a family of plants (Cucurbitaceae). *Cell* 25:793–803.
- Wehner, T.C. 1989. Breeding for improved yield in cucumber. *Plant Breed. Rev.* 6:323–359.
- Yang, L., D.H. Koo, Y. Li, X. Zhang, F. Luan, M.J. Havey, J. Jiang, and Y. Weng. 2012. Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. *Plant J.* 71:895–906.

Supplemental Table 1. Primer sequences and chromosome locations of simple sequence repeats (SSR) used to establish homozygosity of doubled haploid and inbred lines of cucumber. Primers and locations were previously reported by Yang et al. (2012) and are included here for convenience only.

No.	SSR	Forward	Reverse	Chromosome
1	9A10	GACTAATGCTCCGCTTCAGG	CGACAGGAAATCATCTAGCA	1
2	9C01	GTGCCTCAGTCGGTTTTGTT	GGGAGGGGAAGGATTCAATA	1
3	25C04	TGAATTTCTTTGGTGGATTCAA	ACCTCCCATGGCAATCTACA	2
4	9B07	CAAAATTTGAAGACTAAATTGGCT	TGCTTTGATTTTCATTGCGCA	2
5	9E09	AGCATGATGAGGATCCCTTG	CCGAAGTGCACAAAGTATGG	2
6	9G03	GCTAAGTTATTGTTGACCTCAACTTCT	CGTGTGAAATTCAACCTATTCTCTT	2
7	25B09	GCTCATGCTCGAACCTCTCT	CCAAATTGGCAAACATGAGA	3
8	25D05	TGGATGAATGATGCCACAGT	CAAAAGCCTGTCTTGGTAAAAA	3
9	25D09	CGTGGGTACTTTTCTCTTTATATGTC	TTTTGATTGAATTGTGGTAAGCA	3
10	25G02	GGGTTAGGGTTTGGGAGAGA	AACCTTGGGAGCTATCCACC	3
11	25G07	CAAATGGCGTTTCACCTTCT	GGGTGAGTCCAAAAACAAAGA	3
12	9A03	AGAAGAAACCCAAACCGAAC	AGCCAAATGACTGAGAGGTG	3
13	9B03	ACAGATTCGTTATTTTAAACAATTTTC	CGCGTTGACAAGAGTTTATTG	3
14	25B11	CATGTGTGACGCTTCTTATTTTT	TGGTGCTCCCTTTCTACACA	4
15	25G06	TTTTGCAATCAACCATTTTCC	GCCCATTTCCCAATATATCCC	4
16	21A09	GCAAGAAACAAGTCCAATTC	GTGACAATTCTTATCCCCAA	4
17	25C01	TCACACCTGCATTTTTCATCA	GAGGCGTTCTCAACATACCC	5
18	25F06	TTTTCTTTGATCTTTAGTGCCCA	GGTGAGAGCCCTATGGTGA	5
19	9C04	TCGAGGTAAATCAAAACCGA	ATGTGTCAAACCCACCCATT	5
20	9F01	GGACATGTCAACTCCCCTGT	GCCTCTAGCCTGAAAAGACCA	5
21	25B12	ATACTTCGCCATTGACGCTT	TTACTAGGCGTTGTGGGAGC	6
22	9A02	GCAACTCAGGAAGGAGTTCG	GCTCAGGCTTTGAAACAAGC	6
23	25B06	ACGGTCATCCGAATTCTCAG	CAACAAACGATCCAACATCG	7
24	25C11	ATGAGGGAAGACTTTGGGGT	TTTCGTGAAGAAGGGGAAGA	7
25	25G09	AATTGGACCTTTTGAAGCCC	ATCATCCCATGTGAGGAAGC	7
26	25A10	CAGCTGTTGAGATTCCGAG	GAACAAATGGGGAGAGCAAA	1
27	25C03	TGTGTAAGATTTTATTGGATGCC	CTGAGCTTGATCAATTCCTTCA	1
28	25C05	TTCTCGATTGGTTAGTCGGG	CCATACCTTTGTGTTCCATCAA	1
29	25C10	GCTTGCTGCTAAAGGGGATT	TTCTGTCAAATTTGGTTGTCTG	1
30	25E01	GGCCGTTAACGTTATGTTGC	GTCTCGACGTCAAAGAAGGC	1
31	25E04	TGTTCAAGGTGCTGACCTG	CCACAACAACAAAAGATGTGAA	1
32	25F07	TTGCGCTTTACTTCCTTCCA	CGGCAAAATTCCTCCATTTA	1
33	25F11	TGTTTTCTTTTTCTTTTCTTTTCT	TGGGTAGGTCCATTTTCTCTG	1
34	25G05	AAGGTTGGAAGAGGGTTTGG	TTCTTAGCTTCCGTTTCCA	1
35	25H05	CATGTGAGGAAGGGGTGTTT	GCTTTTTCCAACCAAGCTTTT	1
36	25H08	AAATGATTTCTTCTGCTTTCTTT	GAATGGATCTAGAGATTGTCATCA	1
37	25H09	TTGTGTTCTCTCCACAGTTTGT	AACATGCATAGTCCACACTTTCA	1
38	25H11	CCCACATGAACACAAGCGTA	CAGCTCCTCTCATTCTTCTGG	1
39	25H12	TCGTCGACGCCATAATTCTA	TCATGAGTCGTGCACTTTCAG	1
40	9B01	CAATCAACAAATTCTTGCCA	TGTGAGCAATCCCAAGAACA	1
41	9B02	CTGCCCAAGAAGAAGAGGGT	TGAAACCCCCAAAACAAAAA	1
42	9B11	TGGAAACAAAAAGTTTTGCAC	GCCTCACTTCCAAAAGGACA	1
43	9C02	GCCTGTTTTCGGGCTAAACT	TCCAATAATTTAATTTTGGGCTT	1
44	9C06	CACCCACGTGCTGTAAAAAG	ACCAGTTAACACGTCAATATTTCT	1
45	9C08	TTTGATGACAACAATCTTCATCG	CCTGTGACAACATGGATTCT	1
46	9C11	GGGTCGAGATAAAGCCGTAA	TCGAGCTCGTTGTCGAATAA	1
47	9D09	AGCCATTGAAGAGAGTTTGTG	TTTGTGTTCAACTACACCCATTC	1
48	9E03	TGCCACAGGGATTCTTCTA	TGAAACCCATTTGAGATCTCTTC	1
49	9E07	GGTCTCAAGTTGGGGTTGAA	GAACCTCGTGAAGCAGGGAAG	1
50	9F08	GAAAACATGAAGGCCGTTGT	CCATTTGTCAAGTGCCTTTCA	1
51	9G06	CGAAGGAATGCATGTGAAAG	CCTTTTAACTACCCCAACCA	1
52	9G08	CCCTCTCAACATTTCCACAA	CGAGGAGACTTGATGGGATG	1
53	9H02	CATCCTTTCGACTTTGATGC	CTGCAAAACGTGAAGAGAGCC	1
54	9H07	CACAATCCCACGAAGAACAA	TGCAATTATGGCAAAATCAAAA	1
55	25A06	CGATTGAAATTAGATTTTCCCC	TTTTCGGGAACCAAGTTTCA	2
56	25A08	TTTTTCGCCCCAAGATTACAA	TGTGATCCCAATCAAAGGA	2

Continued next page

Supplemental Table 1. Continued.

No.	SSR	Forward	Reverse	Chromosome
57	25B05	TTATGCAACCATTGGAGAAGG	TGATTTGTTGTCTGTGCCAA	2
58	25B10	ACGTGAGAGACACCCCTCC	TATCCCATGGGTATGGTGTG	2
59	25E02	GAAGGGATGGTAGATGAAGGG	CTTCTCCCTTTCTTGGCTT	2
60	25G01	TGAAACAATCACCATCATCTCA	AAGTGGGAAGCTTTGGAGGT	2
61	25G08	GCCCTTTCCTCTAATTCATTCC	TGAGAAAAAGGACAAAGAAAAAGAA	2
62	25G11	ACTTATTGTAGATACCGTATTTGTCC	TGATGACGAAGATAAAGGGATG	2
63	25H03	AATTCCTGCTAAACCACCCC	GGTTTGTGAAGCCGAAAAA	2
64	9A11	TGCCAGCAAGAAGTTCACAC	CGCATGCTATGAGGAGTCAA	2
65	9B04	GGAGAAATTTGATGGTGTAGCC	TTGCAAATCTCTAATACTTTGCCTT	2
66	9B06	TCACCTTCATGATTTTGCCA	GTCTTCAACGCAACCCAAGT	2
67	9C12	AAGGACAAAGACAACATTAACAAAAA	GGGTTCTACGAAGGAGAGCA	2
68	9D04	TTAAAATTTCCCAACGGAAACC	TGATGGGAGAAAGGTAAACAAGA	2
69	9E02	TTACCTTCCCCACCCCTAACCC	TGACTTTTTGGGGAAACCCCT	2
70	9F06	TGCCTTTACACTCAAATGTGC	CAGGATCCAACCTTCAATGGG	2
71	9G01	GCCAAAAATCCGAGAACAGA	TCCTGGGATTTTGATTTGCT	2
72	9H09	ATCGGTGGTAGTGGTTACGC	GTGGGGTCCAGAGTTGAAAA	2
73	25A01	AAGTGGGAAGGTTTGGAGGT	TTCTTTTTCCCCCTTCATT	3
74	25A02	TACACAGAGGAGGGGTGTCC	TGTGTTGGATCCATTTCTCTG	3
75	25A05	TTGGGTGGTATGGTTATGGAG	ATAACCCCTTTTTCCCCTT	3
76	25B03	GCTGCTTGAATCGGTTCTGT	GAGGAGGTAAATCATGCTCCA	3
77	25B04	GTCTAATCGGACGGCAATGT	GGGAGAGAGAAACCCACCTC	3
78	25B07	AACGGAACAACAAAACTGC	TCAGCAACAGCAATCGTCAT	3
79	25C02	TGATGAGCGATGCAGAGAAT	TGCTTTGGTTTGGTGATTG	3
80	25C08	CAACCCCCAAGCTCTGATAA	ATTGGAGAGTGCTATTGGGG	3
81	25C12	CTTGGAATTCCACGTCACCT	CGTTGAGAAATGCTAATCAATGG	3
82	25D01	CCAAAATTTTAGGATTTCATTGGG	AACGAAAATGCGTTTTTCCA	3
83	25D04	TGCAGCCAAAAGATAGAAAAACA	GGAAAGGGAGTTGTTGGTGA	3
84	25D07	AATGTTGGTGAGTGGGGTTT	AGCCACTTTCTTCCCCCATA	3
85	25D10	TCCCTCCCTCCATTTCTTCT	GAGATGGGATTGGAAGGGTT	3
86	25E03	TGAATACTCCCATTCCTCA	CATGAAGGCAACAAGGAAAAA	3
87	25E05	GAGGAGAGGGTTTGGTGAGA	GGTGGGGAAAGAGCTTTAGG	3
88	25E07	CAGTTCACAACCACATCTTTGAA	CCGAGCTAACAACCCAATCT	3
89	25E11	GGGGTCCAATCCCCTAACTA	TTGTGATAACCCACGAACGA	3
90	25E12	TCCACTTTTGTCTCTCCTCA	CAAAGTAATAGTGTGCTGTGAAAGA	3
91	25F04	GACTTGGTGGTGTCTCGTC	GGATAGAAGCTTCCCTGCGT	3
92	25F05	CATCCCCATCCCTTTCTTTT	ACGTGGAGGGATAAACGTGA	3
93	25F08	TGAAGTGTTGGATGGGTTTG	GACCTTCATTGCTTGGA	3
94	25G03	CCAAAAGTTTAGAGGAAAAAGAAAA	AAGCCATCTTCCACATACTCA	3
95	25G12	TCAAATTAACGAGTGCGAAAGA	GCCACGGGTGACTACAAAT	3
96	25H02	ATGTGACATAGCCGGGAGAG	CGCAAACTATACGGTGCCTT	3
97	9B08	CAAATGGTACGGTTTCGGAC	TGCAAGCCAACCCAATTAT	3
98	9C07	GCAAATATTAAGTATTGAGGACCA	TTTTAATTTGAAACAATCGCAA	3
99	9D01	GCAAGAAACATCTAGGGCCA	CAACATCGGATTATGAGGCA	3
100	9D05	AAGGATGCTGATTGACAGCC	TTTTCATTCCTTCTCGACG	3
101	9D07	TTTGCAGAGGGCAAAGAAAG	TCGGTGGAGGGATTACATAAC	3
102	9E04	TCCGGATCTCTACCAAATGAA	TCAGAGCATAAAACCAGTGTGT	3
103	9E06	TCATCATCATGTCCTTCCCA	TGCTTGTTAATTAGGGAAGATTAAAGA	3
104	9F03	CTCCAAATCCTTGCAATCCAT	TGTTGAGGCAACTGCATAGG	3
105	9F04	GGGATCGTTTTGTACTCCACA	TTAGCAAGGGCTTTGAAAAA	3
106	9F10	TGCTTCACAGAGATTGCGAG	CACCACCTTTGTCTGTCTG	3
107	9F11	CCCTCCCACCTTCACTATCA	ATTGTCAACAAGAAACAGTGTGA	3
108	9G05	GTTGAATTATTGGAGGGATTTTT	CATTGAATTTATTCAACCGTTTGGT	3
109	9H04	TTCTCCCTAAAAAGAACCCTAA	TTTATGGTGGGAGAGATTGA	3
110	25A04	GCAAGGAGTGAACAAACGGT	GTTCTCCACCATAACCCCT	4
111	25A09	TTTTGTGCTGCTGATGAAGC	TTCTCATCGCAGGTGAAGTG	4
112	25C06	CATAAGCCTTTCAAGCTGGG	CTGAGGTCTCTGATGGAGG	4
113	25E06	TCACCCCCACTTTACTCCTC	AGTCAATCAGTCAGCGCCTT	4

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Supplemental Table 1. Continued.

No.	SSR	Forward	Reverse	Chromosome
114	25F09	ACATCGATCATCAAGCCACA	GGCGTTTTGTGAACCTTGT	4
115	25G10	GGATTGGGAAAAGGGATGTT	GGGAAGGTTGAAGGAAAAGC	4
116	9B10	TTGAAGAAACCAAAGCCACC	CAAGCGAGCTCATCTTCTGA	4
117	9C05	ATAGCTGCGTTGTGTTGGTG	TCACAAAGCCGAAGAAAGGT	4
118	9C09	TGCAACCAACAATCACCAGT	TCCCAATTTGATTGTACATCCTT	4
119	9D06	TTTGAAGTCAAATGGTTGAAAT	CCTTGAAAATCTGAACAACAACA	4
120	9D12	GAGCCATCCATCAGAGAGAGA	ACCCACAAGCTTCAGAGGTC	4
121	9E08	GATGAAGAACGTTGGCGTTT	TCCCCTCACATCATTTCATCA	4
122	9E10	GATGTCACCGAACCAAGTGA	CCCACAAAGAAGGTTTCAGA	4
123	9F09	TAGGCCGAGTTGGTGCTAGT	GCTGCATTTGGAATCCTTGT	4
124	9G04	TTATTCCAAGCGTGCTTCCT	AGGGTTTGACGTAGTGACCG	4
125	9G11	GGGGCATTTTGGACTTTACA	CTGAACGGAGACCCAAATGT	4
126	9G12	ACTTACGGCTGGTGGTTAT	GGATTGTCTTTTGGGTTGGTT	4
127	25A07	GGCCTTGGCTTATTGACAAA	TCGATAAGGAGTAAAACTTGCG	5
128	25B08	CAACAATGAGTGGAATGACATTTT	GACTTGGCTTTGGGATTCAA	5
129	25D02	GCCCTATCACTTCTTTGTTTTCA	CAAGAAATGGGGTGGAAGAA	5
130	25D08	CCTCCCTCCATTTTCCACTA	TGAGCCAGTTGGGGTTTTAG	5
131	25E09	AACTCTGGGCAGCTTTTCAA	CTAAATCAGAAAGCCACGGG	5
132	25F02	TGAGATTGGATTGTGGTGTTG	CTTCAATTCCGCAGCACATA	5
133	25F03	TGATTCTCAAAAGGGTCATCG	GATGATCCTCCTCCTCCTCC	5
134	25F12	AGGCTGTGTCTCAACCTATCG	TTTGCATGTTTACAACCATTT	5
135	25G04	GGTGTGATGTTTGAACCTCAAAG	TTATAAGGTTTAATGTGGA AAAACCA	5
136	25H06	TGGAGGGAGTGAGAAGAAAAA	GCTGTACAGATTTGGGGGAA	5
137	19E08	GATTA AAAAATTGGATTTGGTTGTT	CTCAACCAAGTGNCACCTCACA	5
138	9A05	TCCCTTATCTGGCATGAAG	ACCTCCAAAATGAGCTCCAA	5
139	9A08	GCACTACGAGCTGCAAAGG	TTCAGGCTATCTCCCATTCG	5
140	9A12	GGCTAAACTGCTTCCACTGC	CCCCATAGTCCACGTCAAAG	5
141	9B05	CGAAACAAAAGTTTATGATTTTATCG	TTTTTAAACAATCAATACATGTCAAG	5
142	9C03	TATAACCCCGTCTTCTCCCC	GGGAAACCCCTTTGACTCTC	5
143	9C10	CTCAACAAGTTCGGTCCCAT	AATCAAATGTGGGTGGTGGT	5
144	9D03	GGAATATGGAAGGAAAGCCA	ATCCCCAATTCTCCAAAAC	5
145	9D08	TGGGGTTAGGGTTTCAAGTG	CAAGCTCTTCTCAACGGAC	5
146	9E11	CACTTAAACCAGCCGTCCAT	CTCCCCGAAATTTAGGAAT	5
147	9F02	TCATGTCATCAATCACACCTTTC	TTTTTATCTTTTGTCGAGACTTGTTTT	5
148	9F05	TTTCAAATGAACAGAAATCCACA	CTGCTGCAAGGTTGGTTCTT	5
149	9G02	TGGCAAAATATCACATTCTTGAT	TTGTTGTCATCTAATGCAATTTTTC	5
150	9G07	CATGTTGAGTGGAAAAATTGAAA	TTCGTACATTGTCAAATGCG	5
151	9H01	ATGGGAGCTACACGTTGAGG	ATAGGCTTCCATTCCAGGGT	5
152	9H03	ACAAAAGAGAGAGCCCAACA	GTGCAAAACCAACGAAATCAT	5
153	9H05	CCTTTCAATGAATGCCCAAT	GCGCGCTTAATTAAGGAAA	5
154	25A03	TTGGAATCAAACAAAGAAAGAAA	GGCAAAATTTGGGTAGACGA	6
155	25A11	TTCCATCACTTTTACATCCAAGA	TGGTCGATGTAAACCGACTTT	6
156	25A12	AAGATTCAAGGAGGAGGGGAA	AGTTCCACAAGGCACAGGTC	6
157	25B01	TCAGAGAAATGGAGAGGGAAA	CAGGATTTTGTGTTGGGGAA	6
158	25B02	TTGGTTGAAAAAGGAAGGTT	GGGCAGAGGCTTTTCAATA	6
159	25C09	CCCACTCCCACTCTCACTTT	GGGGGTTTTAGGTGAAAAGG	6
160	25D03	TGAAAGTAACACGAACGCCA	GCCGAGTTGCTTCTACCTCT	6
161	25D06	TTTGGCAGAGCGAGGAATAC	AGGAAAGGAAAGGAAAAGGG	6
162	25D12	AAGTGGGCCCTGCTCTAGTT	GGAAGAAAAATTGAAAGCAATG	6
163	25E10	TGCTCACTACCAAGTGACCAAT	TCCACCATACCCCATTTTTC	6
164	25F01	GGCCCAAATAAATGGAGATG	TTTGAAAAGCCGAAAGTAATTC	6
165	25H01	CATAAGTGGGGTTGGGAAA	GTCACATATGGGGTTTGGGA	6
166	25H04	TCAGAGGCAGAGAAAGGAACA	AGCTACCAAGATGCTGCCAT	6
167	25H10	GATGAGATGGAGAGGGTTGG	CACACGAAATAATGTCACTAAAAGTT	6
168	9A04	TGGAGACCTTGTGGAGGAAG	ACAACAGATCCTGCCCAATC	6
169	9A06	TGGGATTGCTAATCCAAAGG	GCTTGAAATCGGCTTGCTAC	6
170	9A07	CAGGAGTGAGAACCTTTGG	AGATTGTGGGTCGTTCTTG	6

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Supplemental Table 1. Continued.

No.	SSR	Forward	Reverse	Chromosome
171	9A09	TCAGACGGGGTTGATTCTC	ACCAGCACAAAGGAAAACCTG	6
172	9B09	GAGTTTCAACAAATGTTTGCTCTT	CTTTTCGCTTTGTGGACCAT	6
173	9D02	ACACCATTTTTCATCGAGATT	GGGATGAGGAGCAAATGGTA	6
174	9D10	CCCACAATTTTAATTGACTCCAA	AATTACCCAATCCAGCCATTC	6
175	9E01	AAACCTTAAGCTCAAAACATTGAA	TGTGCTGGTATTCTTTTCTTGC	6
176	9E05	TATGCCATCAAAACGACACC	GTAGAGATGGGTGCGAAGC	6
177	9E12	TTCTTCTCCATAAAATTTAAACTGCAA	CAAATAAAACAAAACGCAAAAA	6
178	9F07	TATTCTCATCGGAAAACGCC	TTTGCCCTACTACCCCTCCT	6
179	9F12	AGAAAATGGGATTAGAATTATTTAACA	CACCAGCTACGATTTTACGA	6
180	9G09	GGATCATGATTAGCTTCTTTGG	TTGGTGTAAC TCAAATGAGAGAATG	6
181	9H08	CACCTCAACTCCTCCATTCAA	TGGAGGTCATTGAGACTTGCT	6
182	25C07	CTTCTCTCGGCCAGTACAC	TTAATGTCCCACACTTGGCA	7
183	25D11	CCCACCATGCAAAACTCTTT	GTCTTTGCGCACCTCTCACT	7
184	25E08	CTGAGTTGCTTGCTCCTTCC	GGCAGAAACGGAGTTATCCA	7
185	25F10	ATTTGAGTTTGACGCGCTTT	AACAATGAAGGAAAGGTGCC	7
186	25H07	TTTTTGGAATATACGGCGCT	TCCAAAAGATTCCATTTCTG	7
187	9B12	TCATCTAAATATAAAGTTGAATGGTTG	CTCCTCACTCACACTTCGCC	7
188	9D11	CGGGTAGGGTTCTTTGAATG	TTGGAAAGGGAAATGGAAAA	7
189	9G10	AGCTAGCCACGTAACACCGT	CACTCTCAAAATTTAGCCACACA	7
190	9H06	TTTGGGTAGAAATCAAGAAACG	GGGAATCAAACATTCATGGC	7

Supplemental Table 2. Presence (1) versus absence (0) of polymorphic simple sequence repeats (SSR) used for genetic-distance estimates.

SSR	Fragment	Cucumber Accession						
		Csh	ST8	MM76	Gy14	9930	TMG	MSC3
9G03	1	1	0	0	0	0	0	0
9G03	2	0	1	1	0	0	1	1
9G03	3	0	0	0	1	1	0	0
9F01	4	1	0	0	0	0	0	0
9F01	5	0	1	1	1	1	1	0
9F01	6	0	0	0	0	0	0	1
9C04	7	1	0	0	0	0	0	0
9C04	8	0	1	1	1	0	0	0
9C04	9	0	0	0	0	1	1	0
9C04	10	0	0	0	0	0	0	1
9C01	11	0	0	0	0	1	0	0
9C01	12	0	1	0	1	0	0	1
9C01	13	1	0	0	0	0	0	0
9C01	14	0	0	1	0	0	0	0
9B07	15	1	0	0	0	0	0	0
9B07	16	0	0	1	1	1	1	0
9B07	17	0	0	0	0	0	0	1
9B07	18	0	1	0	0	0	0	0
9B03	19	1	0	0	0	0	0	0
9B03	20	0	1	1	0	0	0	0
9B03	21	0	0	0	1	0	0	0
9B03	22	0	0	0	0	1	0	1
9A10	23	0	0	0	1	1	0	0
9A10	24	0	0	0	0	0	1	0
9A10	25	1	0	0	0	0	0	0
9A10	26	0	0	1	0	0	0	0
9A03	27	0	1	1	0	1	1	1
9A03	28	0	0	0	1	0	0	0
9A03	29	1	0	0	0	0	0	0
9A02	30	1	0	0	0	1	1	0
9A02	31	0	0	1	0	0	0	0

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Supplemental Table 2. Continued.

SSR	Fragment	Cucumber Accession						
		Csh	ST8	MM76	Gy14	9930	TMG	MSC3
9A02	32	0	1	0	0	0	0	0
9A02	33	0	0	0	0	0	0	1
9A02	34	0	0	0	1	0	0	0
25G09	35	1	0	0	0	0	0	0
25G09	36	0	0	0	1	1	1	0
25G09	37	0	1	1	0	0	0	0
25G09	38	0	0	0	0	0	0	1
25G07	39	1	0	0	0	0	0	0
25G07	40	0	1	0	1	0	1	1
25G07	41	0	0	1	0	1	0	0
25G06	42	1	0	0	0	0	0	0
25G06	43	0	1	0	1	0	0	0
25G06	44	0	0	1	0	1	0	0
25G06	45	0	0	0	0	0	1	1
25G02	46	1	0	0	0	0	0	0
25G02	47	0	1	0	0	0	0	1
25G02	48	0	0	1	0	1	1	0
25G02	49	0	0	0	1	0	0	0
25F06	50	1	0	0	0	0	0	0
25F06	51	0	1	1	1	1	1	1
25D09	52	1	0	0	1	0	0	0
25D09	53	0	1	1	0	1	1	1
25D05	54	0	1	0	1	0	0	0
25D05	55	1	0	0	0	0	0	0
25D05	56	0	0	1	0	1	1	1
25C11	57	1	0	0	0	0	0	0
25C11	58	0	0	1	1	0	0	0
25C11	59	0	1	0	0	1	1	1
25C04	60	1	0	0	0	0	0	0
25C04	61	0	1	1	0	1	0	1
25C04	62	0	0	0	1	0	1	0
25C01	63	1	0	0	0	0	0	0
25C01	64	0	0	1	0	1	0	1
25C01	65	0	1	0	1	0	0	0
25C01	66	0	0	0	0	0	1	0
25B12	67	1	0	0	0	0	0	0
25B12	68	0	1	1	1	0	0	0
25B12	69	0	0	0	0	1	1	0
25B12	70	0	0	0	0	0	0	1
25B11	71	1	0	0	0	1	1	1
25B11	72	0	1	1	1	0	0	0
25B09	73	0	0	0	0	0	1	0
25B09	74	0	0	1	0	0	0	1
25B09	75	1	0	0	0	0	0	0
25B09	76	0	1	0	1	1	0	0
25B06	77	1	0	0	0	0	0	0
25B06	78	0	1	1	1	0	0	0
25B06	79	0	0	0	0	1	0	0
25B06	80	0	0	0	0	0	1	1
21A09	81	1	0	0	0	0	0	0
21A09	82	0	1	1	1	1	1	1
9E09	83	1	0	0	0	0	1	0
9E09	84	0	1	1	0	1	0	1
9E09	85	0	0	0	1	0	0	0