Genetic Diversity among *Cucumis metuliferus* Populations Revealed by Cucumber Microsatellites

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Abstract. *Cucumis metuliferus* E. Mey. ex Naud (African horned cucumber, horned melon) is endemic to Africa and is a relative of cultivated cucumber (*C. sativus* L.) and melon (*C. melo* L.). In the present study, genetic variation among the USDA *C. metuliferus* collection was evaluated with microsatellite [simple sequence repeat (SSR)] markers derived from *C. sativus* genomic DNA sequences. Of 564 cucumber SSRs tested, 51.8% were able to produce polymerase chain reaction amplicons in *C. metuliferus* suggesting a high degree of DNA sequence homology between the two species. Forty-two cross-species SSRs were used to assess genetic variation among 36 *C. metuliferus* accessions. Genetic diversity among these accessions was relatively low. Of the 42 SSRs, 12 were monomorphic, and each marker, on average, was able to detect 3.3 alleles among the 36 accessions. Neighbor-joining clustering analysis revealed a positive relationship between genetic divergence and geographic distances among these accessions. Genetic distance of *C. metuliferus* to melon is smaller than that of *C. metuliferus* to cucumber.

The African horned cucumber or horned melon, *Cucumis metuliferus* ($2n = 2x = 24$), is a member of the Cucurbitaceae family, which includes several economically important vegetable crops (cucurbits) like cucumber, melon, squash (*Cucurbita* spp.), and watermelon (*Citrullus lanatus* Matsum. & Nakai). This monoecious, climbing, annual vine species occurs naturally throughout sub-Saharan regions of Africa and is consumed by some local populations as a vegetable. *C. metuliferus* fruit is believed to contain antioxidant compounds (Mothlanka, 2008; Wannang et al., 2007). More recently, as a result of the characteristic appealing orange-reddish color and long shelf life of its fruits, *C. metuliferus* is being promoted as a specialty horticultural fruit and has commercial production in several countries, including New Zealand, Israel, Australia, and the United States (National Research Council, 2008; Wilkins-Eller, 2004).

Cucurbit breeders have a strong interest in *C. metuliferus* because it is a reservoir of potentially useful genes for cucurbit crop improvement. For example, cucumber is highly susceptible to Southern root-knot nematode, *Meloidogyne incognita* (Kofoid

& White) Chitwood, whereas high-level resistance has been identified in several accessions of *C. metuliferus* (Fassuliotis, 1967, 1979; Nugent and Dukes, 1997; Walters et al., 1993, 2006). In addition, *C. metuliferus* also has been shown to possess heat tolerance, the ability to germinate under high saline conditions (Benzioni et al., 1991) as well as resistance to whitefly, *Papaya ringspot virus*, and *Watermelon mosaic virus* (National Research Council, 2008; Provvidenti and Gonsalves, 1982; Provvidenti and Robinson, 1974). However, attempts to produce viable interspecific hybrids between cucumber or melon with *C. metuliferus* have been unsuccessful (Deakin et al., 1971; Fassuliotis, 1977, 1979; Kho et al., 1980; Kroon et al., 1979; Norton and Barker, 1980; Walters and Wehner, 2002). Recent progress in plant genomics and biotechnological research, particularly in the field of comparative genomics, is offering new opportunities to use these untapped genetic resources. To design proper strategies of introducing useful genes into elite cultivated germplasm, it is necessary to understand the genetic diversity and phylogenetic relationships among wild and cultivated *Cucumis* species. The objective of this study was to assess genetic variation among *C. metuliferus* accessions in the USDA collection.

Materials and Methods

Plant materials and simple sequence repeat markers. Thirty-six *C. metuliferus* accessions, six cucumber, melon, watermelon, and squash (*Cucurbita pepo*) cultivars or breeding lines, were used. Detailed information on all germplasm used is summa-

rized in Table 1. Of the 36 *C. metuliferus* accessions, 35 (CM01 to CM35) were from the U.S. National Plant Germplasm System (NPGS) at North Central Region Plant Introduction Station in Ames, IA, and one (CM36) was from a commercial source. The remaining six genotypes included two cucumber lines, 'Gy14' and 'Lemon', two melon lines, 'Top Mark' and 'Q3-2-2', the watermelon cultivar New Hampshire Midget, and one yellow summer crookneck squash line (Table 1). The DNA sample of 'New Hampshire Midget' was provided by Amnon Levi at the USDA-ARS in Charleston, SC.

All simple sequence repeat (SSR) markers were developed from the whole genome sequence of a Chinese cucumber inbred line, '9930' (Huang et al., 2009; Ren et al., 2009) and the primer pairs were synthesized commercially. Initially, 552 genomic SSRs were tested for their ability to amplify CM15 genomic DNA. Among those markers that produced amplicons in CM15, the first 42 were chosen for evaluation of genetic diversity among *C. metuliferus* accessions. Cucumber primer sequence information, SSR motifs, and chromosome locations of the 42 SSR markers are listed in Table 2.

Morphological variation, genomic DNA isolation, and polymerase chain reaction amplification. All plant material was grown in a greenhouse on the University of Wisconsin–Madison campus. Each accession was hand-pollinated to produce seeds for the next generation. Morphological variations in fruits such as fruit skin color, fruit shape, and fruit flesh color among the 36 accessions were recorded.

Genomic DNA was extracted from unexpanded young leaves by the CTAB method (Murray and Thompson, 1980). Each amplification reaction contained 25 ng template DNA, 0.5 μ M each of two primers, and 1 $\times$ polymerase chain reaction (PCR) master mix (Fermentas, Glen Burnie, MD) in a total volume of 10 μ L. A touchdown PCR program (Weng et al., 2005) was used for all primer sets. The majority of tested SSR primer pairs amplified a single band from each sample, and the variation in the size of the PCR products allowed the patterns to be resolved in 3% agarose gels stained with ethidium bromide. Markers detecting null alleles in PCR were repeated at least once to rule out the possibility of PCR failure.

Data analysis. The discriminatory power of each marker was assessed with Polymorphic Information Content (PIC). PIC was calculated as $PIC = 1 - \sum(p_i^2)$, in which p_i is the frequency of the i th allele detected in all 36 *C. metuliferus* accessions (Anderson et al., 1992).

PCR products were scored in binary format with the presence of a band being scored as 1 and its absence scored as 0, thus generating a binary matrix, which was used in data analysis with the software package PHYLIP 3.66 (Felsenstein, 1989). Specifically, 1000 bootstrap trials were performed on the data using SEQBOOT after which RESTDIST was used to construct pairwise

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Table 1. Plant materials used in this study.

No.	Species	Accession	Abbreviation	Origin	Location ²	Altitude (m)	Seed source	Notes ³
1	<i>C. metuliferus</i>	Ames 19146	CM01	South Africa	Unknown	Unknown	NPGS	
2	<i>C. metuliferus</i>	Ames 23535	CM02	South Africa	24.7 S, 28.2 E	0	NPGS	
3	<i>C. metuliferus</i>	PI 202681	CM03	South Africa	Unknown	Unknown	NPGS	
4	<i>C. metuliferus</i>	PI 292190	CM04	South Africa	Unknown	Unknown	NPGS	Resistant to PRSV
5	<i>C. metuliferus</i>	PI 422520	CM05	Former USSR	Unknown	Unknown	NPGS	
6	<i>C. metuliferus</i>	PI 441995	CM06	Netherlands	Unknown	Unknown	NPGS	
7	<i>C. metuliferus</i>	PI 482435	CM07	Zimbabwe	17.5 S, 31.3 E	1300	NPGS	
8	<i>C. metuliferus</i>	PI 482436	CM08	Zimbabwe	19.5 S, 31.4 E	1150	NPGS	
9	<i>C. metuliferus</i>	PI 482438	CM09	Zimbabwe	18.8 S, 29.1 E	1000	NPGS	
10	<i>C. metuliferus</i>	PI 482439	CM10	Zimbabwe	18.8 S, 31.0 E	1490	NPGS	
11	<i>C. metuliferus</i>	PI 482440	CM11	Zimbabwe	18.3 S, 28.7 E	1100	NPGS	
12	<i>C. metuliferus</i>	PI 482441	CM12	Zimbabwe	19.2 S, 29.9 E	1300	NPGS	
13	<i>C. metuliferus</i>	PI 482442	CM13	Zimbabwe	19.7 S, 30.2 E	1250	NPGS	
14	<i>C. metuliferus</i>	PI 482443	CM14	Zimbabwe	19.7 S, 30.3 E	1200	NPGS	Resistant to RKN
15	<i>C. metuliferus</i>	PI 482444	CM15	Zimbabwe	17.8 S, 32.6 E	1100	NPGS	
16	<i>C. metuliferus</i>	PI 482446	CM16	Zimbabwe	17.9 S, 32.8 E	900	NPGS	
17	<i>C. metuliferus</i>	PI 482448	CM17	Zimbabwe	17.6 S, 29.5 E	850	NPGS	
18	<i>C. metuliferus</i>	PI 482449	CM18	Zimbabwe	17.7 S, 30.1 E	1100	NPGS	
19	<i>C. metuliferus</i>	PI 482450	CM19	Zimbabwe	16.9 S, 29.5 E	1200	NPGS	
20	<i>C. metuliferus</i>	PI 482451	CM20	Zimbabwe	16.8 S, 29.4 E	1180	NPGS	
21	<i>C. metuliferus</i>	PI 482452	CM21	Zimbabwe	16.8 S, 29.4 E	1100	NPGS	Resistant to RKN
22	<i>C. metuliferus</i>	PI 482453	CM22	Zimbabwe	16.4 S, 29.6 E	1200	NPGS	
23	<i>C. metuliferus</i>	PI 482455	CM23	Zimbabwe	16.4 S, 29.6 E	1200	NPGS	Mature white fruit
24	<i>C. metuliferus</i>	PI 482456	CM24	Zimbabwe	16.4 S, 29.6 E	1200	NPGS	Round fruit
25	<i>C. metuliferus</i>	PI 482458	CM25	Zimbabwe	16.6 S, 32.2 E	1000	NPGS	
26	<i>C. metuliferus</i>	PI 482459	CM26	Zimbabwe	16.6 S, 31.8 E	980	NPGS	
27	<i>C. metuliferus</i>	PI 482460	CM27	Zimbabwe	16.6 S, 31.8 E	980	NPGS	
28	<i>C. metuliferus</i>	PI 482461	CM28	Zimbabwe	16.6 S, 31.7 E	960	NPGS	
29	<i>C. metuliferus</i>	PI 482462	CM29	Zimbabwe	16.8 S, 30.8 E	1340	NPGS	
30	<i>C. metuliferus</i>	PI 500380	CM30	Zimbabwe	17.7 S, 25.9 E	1084	NPGS	
31	<i>C. metuliferus</i>	PI 500424	CM31	Zambia	13.1 S, 28.2 E	Unknown	NPGS	
32	<i>C. metuliferus</i>	PI 504556	CM32	Netherlands	Unknown	Unknown	NPGS	
33	<i>C. metuliferus</i>	PI 505598	CM33	Zambia	13.3 S, 23.1 E	960	NPGS	
34	<i>C. metuliferus</i>	PI 526242	CM34	Zambia	18.9 S, 27.8 E	1,000	NPGS	
35	<i>C. metuliferus</i>	PI 527568	CM35	Burundi	3.81 S, 30.3 E	1210	NPGS	
36	<i>C. metuliferus</i>	Jelly melon	CM36	Unknown	Cultivated	Cultivated	Reimerseeds.com	
37	<i>C. sativus</i>	Gy14	GY14	USA	Cultivated	Cultivated	This study	Cucumber, outgroup
38	<i>C. sativus</i>	NSL5714	LEMON	USA	Landrace	Cultivated	NPGS	Cucumber, outgroup
39	<i>C. melo</i>	TopMark	MELON	USA	Cultivated	Cultivated	This study	Melon, outgroup
40	<i>C. melo</i>	Q 3-2-2	MELON	USA	Cultivated	Cultivated	This study	Melon, outgroup
41	<i>Citrullus lanatus</i>	New Hampshire Midget		USA	Cultivated	Cultivated	Amnon Levi	Watermelon, outgroup
42	<i>Cucurbita pepo</i>	yellow summer crookneck squash		USA	Cultivated	Cultivated	This study	Outgroup

²Geographic ordinate information was from USDA Germplasm Resource Information Network web site (<http://www.ars-grin.gov/>). S = south (altitude); E = east (latitude).

³PRSV = papaya ringspot virus (Provvidenti and Gonsalves, 1982); RKN = root-knot nematode (Walters et al., 1993).

distance matrices (based on Nei and Li, 1979), then subjected to neighbor-joining (NJ) cluster analysis (Saitou and Nei, 1987) from which a consensus tree was generated with CONSENSE. Non-*C. metuliferus* species were used as outgroups in tree construction.

The binary data matrix was also used to calculate Jaccard's similarity coefficients (Jaccard, 1901) to estimate the genetic diversity among the *C. metuliferus* accessions and between these and the outgroup members. Genetic distance estimates were calculated as the complement of Jaccard's similarity coefficient (Spooner et al., 1996).

Results

Morphological variation among *C. metuliferus* accessions. Under greenhouse conditions, all *C. metuliferus* plants were monoecious with staminate flowers typically appearing several days before pistillate flowers, but variation in flowering time (days

from germination to anthesis) among these lines was observed. Most female flowers and fruits were concentrated at the base of the vines. The immature fruits were dark green with mottled green spots. Ripened fruits were typically and characteristically ellipsoid in shape and bright reddish orange in color with large, sharp spines. All mature fruits had green flesh color, but variation in fruit shape and color was observed. CM23 (PI 482455) had white immature fruit, which turned pale yellow when ripe. CM24 (PI 482456) had round mature fruits.

Cross-species transferability of cucumber genomic simple sequence repeats. Initially, 552 cucumber genomic SSRs were examined for their ability to amplify genomic DNA of CM15. A total of 284 primer pairs amplified PCR products in CM15 yielding a 51.8% success rate of amplification. The first 42 of the 284 SSRs were further tested in an additional 41 cucurbit lines described in Table 1. As expected, all SSRs had successful

amplification in 36 *C. metuliferus* accessions as well as 'Gy14' and 'Lemon' cucumbers. Cross-species transferability of the 42 cucumber SSRs across melon, watermelon, and squash was 83.3% (35 of 42), 45.0% (19 of 42), and 50.0% (21 of 42), respectively. The PCR amplification details for each marker are shown in Table 2.

A total of 209 putative alleles was revealed by the 42 SSRs generated from 41 DNA templates. Each of the 42 primer pairs amplified a single band in 'Gy14' or 'Lemon'. In 'Top Mark' melon, seven markers failed to amplify; two markers, SSR01115 and SSR04534, amplified two and three bands, respectively; and the remaining 33 each amplified one band (Table 2). In *C. metuliferus*, 33 of 42 SSRs each amplified a single band; nine amplified two or three bands in each DNA template, of which SSR01115 and SSR04534 had two to three bands in all accessions, whereas the remaining seven markers (SSR00004, SSR00931, SSR01178,

Table 2. Primer sequence information, chromosome location, PIC, and number of PCR bands in four cucurbit crop species of 42 SSRs used in this study.

No.	Markers	Cucumber chromosome	No. of alleles	PIC	Cucumber	No. of bands in PCR			Forward primer	Reverse primer	SSR motif ^a
						Melon	Watermelon	Squash			
1	SSR00004	6	9	0.760	1	1	1	0	ttcattgcaaaagcacacaca	tgaaaaaggaggacaaagga	(AT)15
2	SSR00129	1	1	0.000	1	1	0	0	acgtcaacactcctcatcgtc	tcatacaatggcgactcaca	(TC)13
3	SSR00170	5	1	0.000	1	1	0	0	ttgcaatttgtgcaggagata	aggattatggcccaaaagg	(AG)14
4	SSR00193	6	2	0.054	1	1	0	0	gccaatccaatggaacaagt	ttgtaaaccaaaaccttacccc	(AT)12
5	SSR00219 ^z	Unknown	3	0.480	1	1	2	2	gtgaatgagagccgttgat	gagacgcatcattactctgc	(GAA)10
6	SSR00398	5	3	0.199	1	0	0	0	attcaaaccccggttaaccc	agtgaatatggcggaactg	(TCT)10
7	SSR00507	2	2	0.054	1	1	0	0	caccgagaacgaaagaagga	tctaaggtgggagggaacc	(GAAGCA)6
8	SSR00647	6	3	0.426	1	1	1	1	cgattttccagcattgatgt	tagttttgggcttcgatgg	(GAA)10
9	SSR00710 ^z	Unknown	3	0.517	1	1	1	0	gagaaccatctcaaaagatgaa	ataaatgggttcggtctcc	(AT)13
10	SSR00931 ^y	7	7	0.538	1	1	0	0	ggacatccccattgaatttg	acgtggggtgcagctaataa	(TA)21
11	SSR01012	6	4	0.443	1	1	1	1	tcacaaaatcgcgacctaaa	gtgagccgttgattctcgt	(ATA)9
12	SSR01115	1	12	0.820	1	2	1	1	attccaatcccaaaaagggt	ctcctctccaatgagcaag	(TACA)15
13	SSR01178 ^z	Unknown	6	0.648	1	1	0	1	aattgacttttggccctcag	tgctctcagtgaaaaatgctc	(AG)14
14	SSR01179	6	1	0.000	1	1	1	1	accaaaaatgccaaccttc	cgtccctcacaccttttctc	(AG)13
15	SSR01191	6	4	0.465	1	0	1	0	ttcttcaaatgccctcaaa	aaagatgggtgggagcaaa	(TG)15
16	SSR01253	2	2	0.054	1	0	0	0	cgtctggatttgttgtaa	aatgtcgggagtgatgcacat	(ATC)8
17	SSR01254 ^z	Unknown	3	0.494	1	1	2	1	atcttcggcaaaaagcaaac	atactacccccaccgtcact	(GA)16
18	SSR01379	1	6	0.729	1	1	0	1	tgttactctggcgaatgctt	tcagaaatcaggagctggaga	(TC)18
19	SSR01552	4	3	0.484	1	1	0	0	cacgacttgccattaacctt	cccaaaccttccctcaaat	(TCT)8
20	SSR01566 ^z	Unknown	6	0.588	1	0	0	1	tggaaaagctcagttgtttga	tgagtgcgaaccaactaca	(AG)14
21	SSR01903	6	4	0.452	1	1	0	0	cattgtctctgatgggaaag	accctttctcagcggtcatt	(AT)12
22	SSR02309 ^y	6	1	0.000	1	1	1	1	tgaatgcctctgcaatgac	tcattgactagacgccagc	(TA)14
23	SSR02355 ^z	Unknown	3	0.487	1	0	0	0	tggaaaaagagagcaataaagga	ccctaaactcccgagagaatg	(TA)20
24	SSR02384	6	5	0.680	1	1	1	1	aaaaatccgacaaatcgtgc	ggcctcaatgttgccttttgc	(AT)14
25	SSR03514	5	4	0.451	1	1	1	1	taggggtccccctccctcata	gggtaccacaaaagcaagtga	(TC)14
26	SSR03610	2	1	0.000	1	1	0	0	gggggaatacgtgaaagagg	ggcctcaatgtcaaaagcgg	(CTTT)7
27	SSR04534	4	6	0.682	1	3	1	1	gggttcaaacctccctcaact	gggggttggtgagacagttat	(TA)27
28	SSR04570 ^y	3	5	0.638	1	1	0	1	aaaggttgccaaagggaat	tcgcgtaaagaacagaggc	(TGA)8
29	SSR04649	4	2	0.389	1	1	0	0	ttgaaattgatgacatccca	acatggaggagacagggcac	(GAA)9
30	SSR04704	7	1	0.000	1	1	0	1	cattcccttttgcgaacctca	aggccgatttgaaagaaat	(TC)15
31	SSR04870	2	1	0.000	1	1	0	0	tacatgccccccttgaaagaag	ttcttgaggagattgcagaaa	(CT)20
32	SSR04905	4	1	0.000	1	1	1	2	gccctagggtctgcttctt	ttctacaactggcccaaccc	(TCT)8
33	SSR06184	5	4	0.302	1	1	2	0	actaaccaaaccaacgtccg	caggcttaattgttaagaaacagaa	(TCATC)6
34	SSR07100	5	2	0.153	1	1	1	1	cacaccattacggttatggg	catttggttcagaaagggga	(CT)16
35	SSR07220	3	3	0.461	1	0	0	0	ccattgtctcttcttcgaaaa	catctgttggtggagcattg	(ATA)12
36	SSR07248	6	7	0.697	1	1	0	0	cgattggaaaatcgcgcac	cgaatcgcttcagttctt	(AAG)9
37	SSR07627 ^y	4	1	0.000	1	1	1	1	atgggattgaacaacaagca	aaatggaaggtcacgtggag	(AGA)8
38	SSR10335	6	3	0.370	1	1	1	1	aaatggaacccaacaatca	tagattaatttgggcagccg	(CAG)13
39	SSR10697	3	2	0.375	1	0	0	0	ttcttgggggaacagaaatgc	tgccacttgaggatgtgtcaa	(AAG)14
40	SSR11043	4	1	0.000	1	1	0	0	aggtagaacaacacggcaat	tcgcactactctttaccga	(GAA)16
41	SSR11343	6	1	0.000	1	1	0	1	gtggggttgcttttgataa	caatgggtgcttgccttcaa	(AT)19
42	SSR11742	7	1	0.000	1	1	1	1	gctatcccaaggatgatga	agcttggcttctgcttttga	(AG)30

^aPrimer sequence information for these markers was not listed in Ren et al. (2009).

^yChromosome location information for these markers was from Weng et al. (unpublished data).

^zRepeat numbers of motifs shown were in the Chinese cucumber inbred line 9930 (Ren et al., 2009).

PIC = Polymorphic Information Content; PCR = polymerase chain reaction; SSR = simple sequence repeat.

SSR01552, SSR03514, SSR04570, and SSR06184) amplified more than one band in only some accessions, which may suggest heterozygosity at these SSR loci.

The 42 SSRs detected 140 putative alleles among 36 *C. metuliferus* accessions with an average of 3.33 alleles per SSR locus (Table 2). Twelve of the 42 markers were not informative among the 36 accessions. The PIC values of these markers ranged from 0 to 0.820 with the average of 0.331 across 42 markers (Table 2).

Genetic diversity of *C. metuliferus* accessions. Bootstrapping and NJ cluster analysis resulted in a consensus tree as shown in Figure 1. Not all branch points in the consensus tree were strongly supported. Of the 1000 bootstraps, those supported by more than 50% probability were mostly inner nodes. Nevertheless, it is evident that most of the 36 *C. metuliferus* accessions were grouped according to their geographic origins. Six groups (A through F in Fig. 1) could be recognized in the dendrogram. Accessions

that were collected from nearby locations were clustered in the same group (Table 1).

Several accessions, including CM31 (PI 500424), CM 33 (PI 505598), CM34 (PI 526242), and CM35 (PI 527268), were collected from relatively isolated locations (Table 1). CM31 and CM33 were from Zambia and consistent with this, they were clustered in one group. However, CM34 and CM35 and a commercial line, CM36, were placed in the same Group E, although they were geographically distant from each other.

For CM05, CM06, and CM32, their geographic coordinate information was not available in the NPGS database. Clustering analysis placed CM05 and CM06 in Group A with four accessions from South Africa, and CM32 was in the same group with CM31 and CM33 (Fig. 1).

The Jaccard's similarity coefficient-based genetic distances among tested materials were calculated. The genetic distance matrix between 11 selected *C. metuliferus* accessions and two outgroup members ('Gy14'

and 'Top Mark') is listed in Table 3, which agreed very well with the consensus tree (Fig. 1). From Table 3, it is evident that the genetic distance from *C. metuliferus* to *C. melo* (cv. Top Mark) was smaller than that to cucumber (cv. Gy14). Although the genetic distance between melon and cucumber was 0.933, that between *C. metuliferus* and melon was 0.897 and between *C. metuliferus* and cucumber was 0.954 (average across 11 accessions in Table 3) suggesting *C. metuliferus* is more similar to *C. melo* than to *C. sativus*.

Discussion

In this study, the genetic variation of USDA *C. metuliferus* collections was evaluated using molecular markers. Data obtained here suggested that genetic diversity among the examined *C. metuliferus* populations was relatively low. The 42 SSRs were able to detect on average 3.3 alleles across 36 *C. metuliferus* accessions, and 12 of them were monomorphic. Considering that *C. metuliferus*

Fig. 1. Dendrogram of 36 *C. metuliferus* accessions based on 42 cucumber (*C. sativus*) genomic simple sequence repeat markers. Accession numbers were shown to the immediate right of the dendrogram branches. Bootstrap sampling of alleles was carried out for 1000 repetitions. Only percentages of those supporting nodes with more than 50% probability were shown. Accessions delimited by vertical bars and capital letters are member of cluster from the same geographic region.

Table 3. Jaccard's similarity coefficient-based genetic distance matrix among 11 selected *C. metuliferus* accessions, 'Gy14' cucumber and 'Top Mark' melon.

	CM04	CM08	CM14	CM21	CM24	CM26	CM27	CM31	CM32	CM33	M36	Gy14
CM04	—											
CM08	0.531	—										
CM14	0.431	0.450	—									
CM21	0.548	0.563	0.492	—								
CM24	0.508	0.475	0.421	0.352	—							
CM26	0.407	0.426	0.450	0.540	0.424	—						
CM27	0.516	0.554	0.532	0.525	0.458	0.259	—					
CM31	0.585	0.597	0.508	0.548	0.483	0.508	0.414	—				
CM32	0.642	0.652	0.636	0.609	0.571	0.500	0.431	0.431	—			
CM33	0.652	0.621	0.706	0.682	0.646	0.556	0.492	0.492	0.456	—		
CM36	0.667	0.620	0.603	0.676	0.623	0.537	0.523	0.567	0.623	0.632	—	
Gy14	0.977	0.941	0.976	0.988	0.964	0.966	0.965	0.927	0.939	0.910	0.943	—
Top Mark	0.895	0.897	0.893	0.949	0.908	0.897	0.880	0.880	0.908	0.859	0.900	0.933

is indigenous to Africa, which was thought to be the center of *Cucumis* evolution (Kirkbride, 1993), this low level of diversity was unexpected. The markers used were selected based on their transferability to *C. metuliferus*. It may be argued that this selection process might bias toward those evolutionarily conserved DNA sequences and underestimate genetic variation because conserved sequences tend to be less polymorphic across taxa (e.g., Sourdille et al., 2001; Yu et al., 2004; Zhang et al., 2005). If this is true, low polymorphism should also be expected in

cucumber and melon for these markers, which was not the case. Each of the 42 markers amplified a single DNA fragment in the two cucumber lines, 'Gy14' and 'Lemon'. The polymorphism between them was 33.3% (14 of 42), which was much higher than the overall polymorphism reported among cucumber lines (7% to 12%) using other marker types (Dijkhuizen et al., 1996; Staub et al., 2002). Meanwhile, the polymorphism rate of 30 SSRs between 'Top Mark' and 'Q3-2-2' melons was 20% (six of 30), which was comparable to that reported

by Cuevas et al. (2008, 2009). Therefore, the marker selection process used in this study did not seem to contribute to the observed low genetic diversity among the *C. metuliferus* accessions.

One possible explanation for the low diversity is the sample size examined in this study. *C. metuliferus* is endemic to the vast areas from South Africa to tropical Africa (National Research Council, 2008; Wilkins-Eller, 2004). However, only 36 *C. metuliferus* accessions are currently available in NPGS. Thirty-three of the 35 accessions used in this

study came from only four countries, 24 of which were collected in a relatively concentrated area in Zimbabwe (Table 1). It is possible that the genetic diversity of the whole *C. metuliferus* population might not be well represented by the accessions examined. Nevertheless, it seems that low genetic diversity is common among cucurbits as compared with other crop species. For example, in genetic diversity studies with rice, *Oryza sativa* L. (Ni et al., 2002), cotton, *Gossypium hirsutum* L. (Lacape et al., 2007), or soybean, *Glycine max* (L.) Merr. (Kuroda et al., 2009), the average number of alleles per marker locus revealed by different marker types (mainly SSRs) was generally over 5.0, which was even higher among their respective wild relatives. This number was far fewer (less than 3.5) in cucurbit crops like cucumber (Staub et al., 2002; Watcharawongpaiboon and Chunwongs, 2008), melon (Lpez-Sese et al., 2003; Luan et al., 2008; Nakata et al., 2005; Tanaka et al., 2007), watermelon (Levi et al., 2001), and *C. metuliferus* (this study). Allard (1960) explained that because cucurbit plants are large and require a lot of space to grow, a few plants are likely to satisfy the wants of any one grower. Cucurbits may consequently have existed in small colonies both in nature and under cultivation, and this restriction in population size may have produced inbreeding (and reduced heterozygosity) despite the monoecious floral mechanism favoring outcrossing. It is not known if the low genetic variation represents a bottleneck during the evolution of *C. metuliferus*.

The primary motivation for the present study was to evaluate genetic diversity among the *C. metuliferus* accessions, thus providing some guidance for exploring this gene pool for cucumber and melon improvement. Early attempts of wide hybridization between *C. metuliferus* and melon or cucumber were unsuccessful (Deakin et al., 1971; Fassuliotis, 1977, 1979; Walters and Wehner, 2002). An alternative strategy may be through comparative genetic mapping of target genes such as nematode resistance in *C. metuliferus* using cross-species molecular markers. The transferability of molecular markers developed in cucumber is usually more than 50% across other *Cucumis* species (Ren et al., 2009; this study). A large amount of molecular markers can be developed from whole genome sequences of cucumber or melon (Gonzalez et al., 2009; Huang et al., 2009), which can be used for molecular mapping of interested genes or quantitative trait loci (for example, nematode resistance genes) in *C. metuliferus*. The target genes could then be cloned from *C. metuliferus* and transferred into cultivated cucurbit crops through biotechnological approaches.

Despite the generally low genetic diversity among the 36 *C. metuliferus* accessions, the polymorphism level between specific accessions was adequate for genetic mapping. For example, among the 42 SSR markers, the polymorphism level between CM33 (PI 505598) with CM04 (PI292120,

resistant to papaya ringspot virus) and CM14 (PI 482443, resistant to root-knot nematode) (Walters et al., 1993) was 45.4% and 50.0%, respectively. Considering the availability of large numbers of molecular markers from cucumber or melon, this level of polymorphism makes it relatively straightforward to construct a linkage map in *C. metuliferus* to locate target resistance loci.

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