Applications in Plant Sciences

Applications in Plant Sciences 2016 4(8): 1600032

Primer Note

Development of chloroplast microsatellite markers for the endangered *Maianthemum bicolor* (Asparagaceae s.l.)[1]

Hana Park[2,4], Changkyun Kim[2,4], You-Mi Lee[3], and Joo-Hwan Kim[2,5]

[2]Department of Life Science, Gachon University, Seongnam, Gyeonggi 13120, Republic of Korea; and [3]Korea National Arboretum, Pocheon, Gyeonggi 11186, Republic of Korea

- *Premise of the study:* Ten polymorphic chloroplast microsatellite (cpSSR) markers were developed and characterized in an endemic and endangered herb, *Maianthemum bicolor* (Asparagaceae s.l.), for use in conservation genetics.
- *Methods and Results:* Primer sets flanking each of the 10 cpSSR loci in noncoding regions of the chloroplast genome of *M. bicolor* were designed. These cpSSR markers were tested on a total of 33 adult individuals from three natural populations in South Korea. The number of alleles per locus ranged from two to three. The unbiased haplotype diversity per locus ranged from 0.061 to 0.682. All markers were successfully transferred to the congeneric species *M. japonicum*, *M. bifolium*, and *M. dilatatum* with polymorphisms among the species.
- *Conclusions:* The developed cpSSR markers will be useful in assessing the genetic diversity and population structure of *M. bicolor* and will help to infer its molecular identification, thereby providing a basis for conservation.

Key words: Asparagaceae s.l.; conservation; endangered species; genetic diversity; *Maianthemum bicolor*; microsatellite.

Maianthemum bicolor (Nakai) Cubey (Asparagaceae s.l.) is an endemic perennial herb of South Korea (Oh, 2007). This species is characterized by dioecious flowers and tepal color that is green at the early stage of the flowering period and changes to dark purple as it grows (Yang, 2007). Therefore, it is visually possible to distinguish the species after the flower and fruit appear. Natural populations of *M. bicolor* are facing extinction due to habitat deterioration, low germination rates, and overexploitation by humans due to the species' ornamental value (Chang et al., 2005; Lee et al., 2007). In addition, all local populations of *M. bicolor* growing on the ridges of mountains at altitudes higher than 1300 m above sea level are highly fragmented, and those habitats are isolated with discontinuous distribution (Lee et al., 2007; Oh, 2007). Both in situ and ex situ conservation programs are being undertaken to secure the wild populations (Lee et al., 2007), but neither the extent of genetic diversity nor the population structure of *M. bicolor* has been clearly analyzed. Thus, there is an urgent need for development of useful molecular markers for conservation genetics.

Although nuclear markers are excellent for use in most population genetic analyses (Ding et al., 2008; Kim et al., 2008; Tang et al., 2008; Guichoux et al., 2011), they are not suitable for phylogenetic and phylogeographic studies above species level due to high substitution rates (Powell et al., 1996). Chloroplast simple sequence repeat (cpSSR) markers are mainly distributed throughout noncoding regions, which show higher sequence variation than coding regions (Powell et al., 1995). Moreover, the cpSSR markers developed from a species are frequently applicable to amplify homologous regions across related taxa (Diekmann et al., 2012). Therefore, cpSSR markers can be applied for conservation genetics of endangered plant species and can be used to determine molecular identification and genetic relationships among closely related species (Clark et al., 2000; Huang et al., 2015) as complementary tools of nuclear markers.

Here, we provide 10 cpSSR markers for *M. bicolor* to facilitate conservation and molecular identification. These markers were developed from the complete chloroplast genome sequences of *M. bicolor* and tested on three natural populations. We also examined the transferability of the markers to three congeneric species, *M. japonicum* (A. Gray) LaFrankie, *M. bifolium* (L.) F. W. Schmidt, and *M. dilatatum* (Alph. Wood) A. Nelson & J. F. Macbr.; the former is closely related to *M. bicolor* and the latter two are more distantly related within the genus (Kim and Kim, unpublished).

METHODS AND RESULTS

To determine the complete chloroplast genome sequences of *M. bicolor*, we sampled fresh leaf material from Mt. Deogyu, South Korea. Genomic DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). The chloroplast genome of *M. bicolor* was sequenced using a genome skimming approach on an Illumina MiSeq sequencer (Illumina, San Diego, California, USA) and assembled with Geneious version 7.13 (Biomatters, Auckland, New Zealand). The chloroplast genome of *M. bicolor* is 157,176 bp in length. It displays a typical quadripartite structure, with the large single-copy region (85,698 bp, 54.5% of the total genome) separated from the small single-copy region (18,394 bp, 11.7%) by two inverted repeat regions (26,542 bp, 16.9% each). The GC content is 37.6% (Park et al., unpublished).

The cpSSR regions of *M. bicolor* were searched through the complete chloroplast genome sequence using a tandem repeat search tool in Geneious with the parameters set to ≥7 mononucleotide repeats, ≥4 di- and trinucleotide repeats, and ≥3 tetra-, penta-, and hexanucleotide repeats. A total of 169 repeat motifs were identified in the chloroplast genome, among which the most frequent types

[1]Manuscript received 15 March 2016; revision accepted 13 May 2016.

This work was supported by funding for scientific research (KNA1-2-13, 14-2) from the Korea National Arboretum.

[4]These authors contributed equally to this work.

[5]Author for correspondence: kimjh2009@gachon.ac.kr

doi:10.3732/apps.1600032

TABLE 1. Characteristics of 10 polymorphic chloroplast microsatellite markers developed for *Maianthemum bicolor*.

Locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Position	Allele size range[a] (bp)	GenBank accession no.
MA2	F: GAATTGGGAGAGATGGCTGA	$(AT)_3(A)_{12}$	58	*psbI–trnS_GCU*	326–327	KU845594
	R: TGCTTACTCTCAAACTCTTCGTTT					
MA15	F: TCGATTTATTGAGGCATACTATGAT	$(T)_{10}$	58	*ndhF–rpl32*	265–266	KU845595
	R: TTCCAATTCAAATGATATGACCA					
MA30	F: TTGTTTCGTTCCATTCGATCT	$(T)_{10}$	59	*rpoC1* intron	228–229	KU845596
	R: ATGGTCGATCCCGTAACAGA					
MA32	F: TCACGTTGTCCCAAGTGAAA	$(A)_{11}$	60	*rpoB–trnC-GCA*	325–326	KU845597
	R: ATGATCCGATGGGGTACAAA					
MA33	F: GGGTTCGAATCCCTCTCTCT	$(A)_8$	59	*psbC–trnS-UGA*	189–190	KU845598
	R: TCCATGACTCCCCTTAGCTG					
MA421	F: GGACCCATTCAGGAACAAGA	$(T)_{11}$	60	*clpP* intron	273–274	KU845599
	R: TTTGGATGCATACGGTTCAA					
MA422	F: GAGATAGGATTGGAATCGACACA	$(T)_7$	59	*clpP* intron	200–201	KU845600
	R: CTGAGATATACCATGAGACCAACAA					
MA481	F: GCTTTTATTGCAGCTAAATATCCTTT	$(T)_7$	59	*ndhF–rpl32*	396–397	KU845601
	R: CACGGCAATATGAGCTTATTCA					
MA482	F: AACAAGACATTCTATTACCTAAGAA	$(T)_9$	57	*ndhF–rpl32*	148–150	KU845602
	R: TACCTAACCCATTGCGGAAC					
MA483	F: TTGAGTCGATTTATTGAGGCATAC	$(T)_2(T/G)(T)_7$	59	*ndhF–rpl32*	270–271	KU845603
	R: TTCCAATTCAAATGATATGACCA					

Note: T_a = annealing temperature.
[a]Allele sizes are based on 33 individuals of three populations of *M. bicolor*; see Appendix 1 for population information.

were mononucleotide (124 [73.4%]) and dinucleotide (32 [18.9%]), while tri- (3 [1.8%]), tetra- (8 [4.7%]), and pentanucleotides (2 [1.2%]) were rare. Forty-one loci were randomly selected for screening. We designed 41 primer pairs using Primer3 (Rozen and Skaletsky, 1999) under the following criteria: (1) guanine-cytosine content 30–70%, (2) annealing temperature (T_a) 55–65°C, (3) primer size 18–25 bp in length, and (4) amplicon size 150–500 bp in length. To check the amplification success and variability, two individuals from each of three populations of *M. bicolor* (n = 6; Mt. Deogyu, Mt. Seorak, and Mt. Daeam; Appendix 1) were selected (Appendix 2).

PCR amplification was carried out in a total volume of 25 µL, containing 2.5 µL of 10× PCR buffer (10 mM Tris-HCl [pH 8.3], 1.5 mM $MgCl_2$, and 50 mM KCl), 0.25 mM of each dNTP, 5 µM of each 6-FAM fluorescently labeled forward and unlabeled reverse primer, 1 unit of *Taq* DNA polymerase, and 100 ng of DNA template. Amplification of genomic DNA was performed on a Veriti thermal cycler (Applied Biosystems, Carlsbad, California, USA). Fragment length polymorphism was detected on an ABI 3730 DNA Analyzer with GeneScan 500 ROX Size Standard (Applied Biosystems). Peak data were analyzed using Peak Scanner version 1.0 (Applied Biosystems). The authenticity of the loci amplified in *M. bicolor* populations was confirmed by sequencing the representative PCR products on an ABI 3730 automated sequencer (Applied Biosystems) using the amplification primers. Consensus DNA sequences were assembled for each individual using DNA Baser version 3 (Heracle BioSoft SRL, Piteşti, Romania). Of the 41 primer pairs screened, 10 primer sets showed length variation in cpSSR regions and were developed further (Table 1); 31 were monomorphic and not developed any further (Appendix 2).

Thirty-three adult individuals from three natural populations of *M. bicolor* were used to characterize 10 polymorphic cpSSR loci (Table 2). All polymorphic loci are located in the noncoding regions in which the mutations are usually neutral (Table 1). Polymorphism parameters were calculated with POPGENE version 1.31 (Yeh et al., 1997). The allelic variation (A) in 10 cpSSR loci ranged

TABLE 2. Number of alleles, haplotype diversity, and allele distribution of 10 polymorphic chloroplast microsatellite markers developed for *Maianthemum bicolor*.

			Allele distribution[a]					
			Mt. Deogyu (n = 10)		Mt. Seorak (n = 13)		Mt. Daeam (n = 10)	
Locus	A	H	Repeat motif	n	Repeat motif	n	Repeat motif	n
MA2	2	0.492	$(AT)_3(A)_{12}$	10	$(AT)_3(A)_{11}$*	13	$(TA)_3(A)_{12}$	10
MA15	2	0.436	$(T)_{10}$	10	$(T)_{10}$	13	$(T)_9$*	10
MA30	2	0.511	$(T)_9$	2	$(T)_9$	13	$(T)_9$	10
			$(T)_{10}$*	8				
MA32	2	0.458	$(A)_{11}$	10	$(A)_{10}$	12	$(A)_{10}$	10
					$(A)_{11}$	1		
MA33	2	0.436	$(A)_8$*	10	$(A)_9$	13	$(A)_9$	10
MA421	2	0.515	$(T)_{11}$	8	$(T)_{12}$	13	$(T)_{11}$	9
			$(T)_{12}$	2			$(T)_{12}$	1
MA422	2	0.061	$(T)_7$	9	$(T)_7$	13	$(T)_7$	10
			$(T)_8$*	1				
MA481	2	0.061	$(T)_7$	10	$(T)_7$	12	$(T)_7$	10
					$(T)_8$*	1		
MA482	3	0.682	$(T)_9$*	10	$(T)_7$*	13	$(T)_8$*	10
MA483	2	0.436	$(T)_2(T/G)(T)_7$	10	$(T)_{10}$	13	$T(T/G)(T)_7$*	10
Mean	2.1	0.409	—		—		—	
Total	21	0.775	—		—		—	

Note: A = number of alleles; H = unbiased haplotype diversity; n = number of individuals.
[a]Asterisk denotes specific alleles occurred in one or more individuals of each population.

from two to three, while the unbiased haplotype diversity (*H*) ranged from 0.061 to 0.682, with an average value of 0.409 (Table 2). Ten cpSSR markers amplified a total 21 alleles. Of these, 10 alleles specifically occurred in one or more individuals of each population; four were specific for the Mt. Deogyu population, three for the Mt. Seorak population, and three for the Mt. Daeam population (Table 2). All polymorphic primers developed for *M. bicolor* were able to successfully amplify cpSSR regions in three congeneric species (*M. japonicum*, *M. bifolium*, and *M. dilatatum*) sampled from two populations in South Korea and showed polymorphisms among the species (Appendix 3).

CONCLUSIONS

The cpSSR markers developed here are useful for formulating an in situ conservation strategy of the endangered *M. bicolor* populations by estimating the level of genetic diversity and population structure. The cpSSR markers will also be useful to determine the minimum size of the ex situ core collection that captures the genetic diversity of wild populations of *M. bicolor*. All of the cpSSR markers designed here for amplification of *M. bicolor* are applicable to other congeneric species and are a means for species verification in combination with morphological identification.

LITERATURE CITED

Chang, C.-S., H. S. Lee, T. Y. Park, and H. Kim. 2005. Reconsideration of rare and endangered plant species in Korea based on the IUCN Red List categories. *Journal of Ecology and Environment* 28: 305–320 (in Korean, with English abstract).

Clark, C. M., T. R. Wentworth, and D. M. O'Malley. 2000. Genetic discontinuity revealed by chloroplast microsatellites in eastern North American *Abies* (Pinaceae). *American Journal of Botany* 87: 774–782.

Diekmann, K., T. R. Hodkinson, and S. Barth. 2012. New chloroplast microsatellite markers suitable for assessing genetic diversity of *Lolium perenne* and other related grass species. *Annals of Botany* 110: 1327–1339.

Ding, G., D. Zhang, X. Ding, Q. Zhou, W. Zhang, and X. Li. 2008. Genetic variation and conservation of endangered Chinese endemic herb *Dendrobium officinale* based on SRAP analysis. *Plant Systematics and Evolution* 276: 149–156.

Guichoux, E., L. Lagache, S. Wagner, P. Chaumeil, P. Léger, O. Lepais, C. Lepoittevin, et al. 2011. Current trends in microsatellite genotyping. *Molecular Ecology Resources* 11: 591–611.

Huang, J., X. Yang, C. Zhang, X. Yin, S. Liu, and X. Li. 2015. Development of chloroplast microsatellite markers and analysis of chloroplast diversity in Chinese jujube (*Ziziphus jujuba* Mill.) and wild jujube (*Ziziphus acidojujuba* Mill.). *PLoS ONE* 10: e0134519.

Kim, C., H. R. Na, and H.-K. Choi. 2008. Conservation genetics of endangered *Brasenia schreberi* based on RAPD and AFLP markers. *Journal of Plant Biology* 51: 260–268.

Lee, C. H., C. H. Shin, and K. S. Kim. 2007. Monitoring for population dynamics and conservation of *Smilacina bicolor* Ohwi in Mt. Sobaek and Taebaek. Proceedings of the international scientific conference of The Plant Resources Society of Korea, 131–132, South Korea. Website http://www.dbpia.co.kr/Issue/VOIS00040442 [accessed 6 July 2016] (in Korean).

Oh, B.-U. 2007. *Smilacina* Desf. *In* C.-W. Park [ed.], The genera of vascular plants of Korea, 1312–1313. Academy Publishing Co., Seoul, South Korea.

Powell, W., M. Morgante, R. McDevitt, G. G. Vendramin, and J. A. Rafalski. 1995. Polymorphic simple sequence repeat regions in chloroplast genomes: Applications to the population genetics of pines. *Proceedings of the National Academy of Sciences, USA* 92: 7759–7763.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey, and A. Rafalski. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2: 225–238.

Rozen, S., and H. Skaletsky. 1999. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Tang, S., W. Dai, M. Li, Y. Zhang, Y. Geng, L. Wang, and Y. Zhong. 2008. Genetic diversity of relictual and endangered plant *Abies ziyuanensis* (Pinaceae) revealed by AFLP and SSR markers. *Genetica* 133: 21–30.

Yang, J.-C. 2007. Taxonomy of *Maianthemum* sensu lato (Liliaceae) in Korea. Ms.D. dissertation, University of Chungbuk, Cheongju, South Korea (in Korean, with English abstract).

Yeh, F., R. C. Yang, and T. Boyle. 1997. POPGENE: A user-friendly shareware for population genetic analysis, version 1.31. Molecular and Biotechnology Center, University of Alberta, Edmonton, Canada.

Appendix 1. Collection data for three populations of *Maianthemum bicolor* and three congeneric species.

Species	Location[a]	*N*	Voucher specimens[b]
M. bicolor (Nakai) Cubey	South Korea: Mt. Deogyu, Seolcheon-myeon, Muju-gun, Jeonbuk	10	C.Kim 2015-2
	South Korea: Mt. Seorak, Buk-myeon, Inje-gun, Gangwon	13	C.Kim 2015-42
	South Korea: Mt. Daeam, Buk-myeon, Inje-gun, Gangwon	10	C.Kim 2015-101
M. bifolium (L.) F. W. Schmidt	South Korea: Mt. Deogyu, Seolcheon-myeon, Muju-gun, Jeonbuk	2	C.Kim 2015-69
M. dilatatum (Alph. Wood) A. Nelson & J. F. Macbr.	South Korea: Mt. Seorak, Buk-myeon, Inje-gun, Gangwon	3	C.Kim 2015-28
M. japonicum (A. Gray) LaFrankie	South Korea: Mt. Deogyu, Seolcheon-myeon, Muju-gun, Jeonbuk	1	C.Kim 2015-3
	South Korea: Mt. Seorak, Buk-myeon, Inje-gun, Gangwon	2	C.Kim 2015-35

Note: *N* = number of samples.

[a] GPS coordinates are not included because this is an endangered species in South Korea.

[b] All voucher specimens are deposited at Gachon University herbarium (GCU), Seongnam, South Korea.

Appendix 2. Characteristics of 31 monomorphic chloroplast microsatellite primers designed in *Maianthemum bicolor*.

Locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Position	Allele size (bp)[a]
MA1	F: CCCCGGAAACAGAAAGTCCA R: TCGATCCTTACCAGATCATTCCA	$(A)_{11}$	61	*matK-trnK_UUU*	403
MA8	F: GAGAGGGATTCGAACCCTCG R: CCAGTACTTAACCAGGCCGG	$(AAT)_3$	63	*trnS_GCU-trnG_UCC*	367
MA10	F: TGGCAGTTGAAGGGGAAGTT R: ACGAATCGCACTTTTACCACT	$(AT)_8$	58	*trnS_GCU-trnG_UCC*	440
MA13	F: TCGGTGGAAACCAAATTCTT R: TCTAATGGATAGGACAGAGGT	$(AT)_4$	55	*trnG-UCC-trnR-UCU*	452
MA14	F: TCGGTGGAAACCAAATTCTT R: TCTAATGGATAGGACAGAGGT	$(A)_9$	55	*trnG-UCC-trnR-UCU*	452
MA19	F: TCATAACGTAAACCACCCGCA R: TCCTATACCTGTTATGTTTCTTGGA	$(AT)_5$	59	*atpH-atpI*	344
MA21	F: AACCTTTTCCCGCATCAGGC R: CGAGTACTCTACCGTTGAG	$(A)_{10}$, $(AAT)_3$	61	*matK-trnK_UUU*	393
MA29	F: GACTACTAGAAAAGTTTTGATGCTT R: CATCTATGTAAAAACAGTCAGCCA	$(AAT)_5$	60	*petN-psbM*	416
MA31	F: TCCTAGTTCGGGACTGACGG R: CGGTTAATGGGGACGGACTG	$(A)_9$	63	*trnD_GUC-trnY_GUA*	411
MA34	F: AGGATTCTTCCTATTTGTGG R: TTTTCAAGACCGGAGCTATC	$(AG)_4$	55	*trnS-UGA*	296
MA46	F: TTGCTGACCAACAGTATCTC R: ACGCACTTCACTTACTCACAA	$(A)_{10}$	56	*atpB-rbcL*	385
MA50	F: GGTGGTTCAATTCGATGTTG R: GTTATCTTCGTTTTGAAGTCAGT	$(A)_8$	56	*accD*	430
MA51	F: GAGAAACCCCCGGATCCTTT R: TTTCAGATACGACGGTAAGAAAAA	$(AAT)_3$	61	*psbE-petL*	405
MA53	F: GTCAATAAGGAATCGCCAGGC R: TCAATTCGATCCCCCGATTCA	$(AT)_7$	61	*rpl33-rps18*	377
MA56	F: GTTCGACGTCTCCGAGCTAT R: ACGGACTAACAAGGTCAGCT	$(AAAT)_3$	61	*rpl20-rps12*	449
MA57	F: AGTCGCACATACACCCTAGT R: TAGTTCTTCCGCTTCCAGGA	$(AT)_4$	58	*rps12-clpP*	407
MA61	F: AACGAGTCACACACTAAGCA R: TCCATTCGACACGTGCAATTT	$(A)_9$	56	*rpl16-rps3*	286
MA64	F: TGGTCTACAGGGTTCATAAC R: ATCCAAATCTGCCGAATCAC	$(A)_8$	56	*rpl2intron*	430
MA69	F: TCAACCATTTCATTTTACCA R: CACGATTATATGACCAATTGTAT	$(A)_8$	55	*ycf1-ndhF*	200
MA70	F: ATCATTATTGACGGTCCAAGACC R: CACTTTTATCTAGCTATTTGGTCA	$(A)_9$	61	*ndhF-rpl32*	400
MA73	F: AGTTCCAATTCAAATGATATGACCA R: TCTGATACTTTCTTATTGGATTGGA	$(AAGT)_3$	59	*ndhF-rpl32*	310
MA76	F: CTTCTTAGGTAATAGAATGTCTTGT R: TGACTTCGCTAAAGAGAAAGCT	$(AT)_4$	62	*ndhF-rpl32*	435
MA77	F: TTTGTGCGACAAACAAAAACAAA R: CAACTTGGTCTTGAGTTAGGCG	$(A)_{10}$	56	*rpl32-trnL_UAG*	332
MA86	F: GTTGACGTTTTCCCAGAATT R: CAAATCGTTTCTTTGGCCCA	$(AAT)_4$	55	*rps15-ycf1*	456
MA88	F: TTCAATAGCTTTGCGTCCGC R: AAAATAGCTCGACGCCAGAA	$(AAACG)_3$	58	*trnR_ACG-rrn5*	381
MA89	F: AGGCAAAGGGTCGAGAAACT R: TCGAAAAATGAAATGGCGTGG	$(A)_8$	58	*trnV_GAC-rps12*	389
MA90	F: ACTGGTAGGAGAAGAACCCGA R: TGGACCCTCATGTTGATCCG	$(A)_9$	61	*trnV_GAC-rps12*	372
MA91	F: CGCAACTCAATCATGTATGATGGA R: ATGGTTCCTTACTTCGACAGGGT	$(AAG)_3$	62	*ycf2*	394
MA92	F: CTCAACATGAGGGAGGAGAAAGA R: ATCAACCGTGCTAACCTTGG	$(A)_8$	63	*rps19-psbA*	389
MA115	F: AGAGCAATGCCTATTGTACC R: TAAGGCTTGTTGGAAAACTC	$(A)_{10}$	56	*atpA-atpF*	358
MA132	F: CCTCCTTGAAAGAGAGATGT R: TGGCTTAGTGCGAGATAGTGA	$(C)_8$	56	*trnE_UUC-trnT_GGU*	354

Note: T_a = annealing temperature.

[a]Allele sizes are based on six individuals from three *M. bicolor* populations; see Appendix 1 for population information.

Appendix 3. Comparison of allele distributions of 10 polymorphic cpSSR between *Maianthemum bicolor* and its congeneric species *M. bifolium*, *M. dilatatum*, and *M. japonicum*.

Locus	*M. bicolor*[a] (*n* = 33)	*M. bifolium* (*n* = 2)	*M. dilatatum* (*n* = 3)	*M. japonicum* (*n* = 3)
MA2	$(AT)_3(A)_{12}$, $(AT)_3(A)_{11}$*	$(AT)_7(A)_4$	$(AT)_7(A)_4$	$(AT)_3(A)_8$
MA15	$(T)_9$, $(T)_{10}$	$(T)_{15}$, $(T)_{16}$	$(T)_{14}$	$(T)_9$, $(T)_{10}$
MA30	$(T)_9$*, $(T)_{10}$	$(T)_{11}$	$(T)_{10}$	$(T)_{11}$
MA32	$(A)_{10}$, $(A)_{11}$*	$(A)_9$	$(A)_{10}$	$(A)_9$, $(A)_{10}$
MA33	$(A)_8$*, $(A)_9$	$(A)_{11}$	$(A)_9$	$(A)_{10}$
MA421	$(T)_{11}$, $(T)_{12}$*	$(T)_{10}$, $(T)_{11}$	$(T)_{10}$	$(T)_{10}$
MA422	$(T)_7$, $(T)_8$*	$(T)_7$	$(T)_7$	$(T)_7$
MA481	$(T)_7$, $(T)_8$*	$(T)_6$, $(T)_7$	$(T)_7$	$(T)_7$
MA482	$(T)_7$, $(T)_8$*, $(T)_9$	$(T)_9$	$(T)_9$	$(T)_7$
MA483	$(T)_2(T/G)(T)_7$, $T(T/G)(T)_7$	$(T)_{15}$	$(T)_{14}$	$(T)_9$, $(T)_{10}$

Note: *n* = number of individuals.
[a]Asterisk denotes specific alleles for *M. bicolor*.