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PRIMER NOTE

Characterization of 17 microsatellite loci in the Japanese land snail genera *Mandarina*, *Ainohelix*, and *Euhadra* (Mollusca, Gastropoda, Pulmonata)

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Abstract

Land snails of the genera *Mandarina*, *Euhadra* and *Ainohelix* are useful for understanding the ecology and evolution of speciation and adaptation, so we have developed 17 microsatellite loci for these species. As in other land snails, most of the loci are highly polymorphic compound repeats, with a great size range between alleles. The loci should be useful in understanding gene-flow, genetic structure and speciation in these species.

Keywords: *Mandarina*, Japan, land snail, microsatellites

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Japan is characterized by a high degree of endemism in the land snail fauna. The genus *Mandarina* (Bradybaenidae) is found only on the oceanic Bonin Islands (Ogasawara), where it has undergone an extensive adaptive radiation into approximately 14 species (Chiba 1999). In contrast, 22 *Euhadra* (Bradybaenidae) species are distributed throughout the four main Japanese Islands and the neighbouring Korean island of Jeju. Both genera are recognized by differences in their genitalia, shell shape and banding patterns, and *Euhadra* also has a variable body symmetry. Finally, *Ainohelix editha* (Bradybaenidae) is a polytypic species, restricted to the northern Japanese island of Hokkaido. *A. editha* is characterized by an exceptional degree of intraspecific shell variation. In particular, *A. editha* in two quite separate locations are striking because they are extremely flat and have a sharp keel, whereas at adjacent sites the shells are globular or depressed-globular (Teshima *et al*. 2003). We have now developed microsatellite primers for these snails, concentrating on *Mandarina* and *Ainohelix*, with the continuing aim of studying the relationships between populations of land snails.

DNA was extracted from foot tissue using the CTAB method of Doyle & Doyle (1987). DNA was then enriched for GT dinucleotide repeats following a protocol modified by Travis Glenn from Armour *et al*. (1994), available at www.uga.edu/srel/DNA_Lab/protocols.htm. Portions of the ligation were transformed into *E. coli* and screened for inserts using M13 forward and reverse primers and colony polymerase chain reaction (PCR). The PCR products of 300–800 base pairs (bp) were sequenced using BigDye (PE Applied Biosystems) chemistry and an ABI Prism® 310 Genetic Analyser. Sequences from both strands were edited in BIOEDIT (available at www.mbio.ncsu.edu/BioEdit/bioedit.html). Primers for PCR were developed using PRIMER 3.0 (www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Four loci were isolated following this method, one for *E. quaesita* and three for *M. ponderosa*.

As an alternative strategy, 10 μg genomic DNA from *M. ponderosa* and *A. editha* was sent to Ecogenics GmbH (www.ecogenics.ch) for commercial enrichment and isolation of GT-repeat containing clones. The purified clone DNA was then sequenced and primers designed, as described above. The remaining 13 loci, nine for *M. ponderosa* and four for *A. editha* were isolated using this method.

For PCR, 50 ng DNA was used as template. With the exception of one locus (*Mpo*3), 5 pmol of each primer were provided with 0.1 mM of each dNTP, 0.35 U Takara rTaq™ (Takara Biomedicals, Japan) and 1 μL of supplied 10 X PCR buffer (10 mM Tris-HCL, pH 8.3; 50 mM KCl; 1.5 mm $MgCl_2$). All PCR amplifications were carried out in a 10 μL final volume on an MJ Research PTC-200 thermal cycler. With the exception of *Mpo*3, the standard PCR conditions were 95 °C for 1 min, followed by 34 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s, and a final extension of 72 °C for

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30 min. *Mpo*3 was amplified using 0.35 U Qiagen Hot Star Taq™ and buffer (1.5 mM $MgCl_2$ final concentration; remaining ingredients not detailed). PCR conditions were 95 °C for 15 min, followed by 9 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 25 s, and 19 cycles of 89 °C for 15 s, 55 °C for 15 s, and 72 °C for 25 s, with a final extension of 72 °C for 30 min.

Fragment size analysis was performed on the ABI Prism® 310 Genetic Analyser, DATA COLLECTION Software v1.2.2 and GENESCAN® Analysis Software v3.1.2 (Applied Biosystems). Forward primers were labelled with either NED, HEX or 6-FAM, and the PCR products electrophoresed with the ROX-500 size standard.

Details of the loci, primer sequences, and numbers of alleles in the three species are shown in Table 1, along with the product size and observed and expected heterozygosities. One locus (*Mpo*11) had a deficit of heterozygotes (Table 1), which may be due to null alleles. Details of the GenBank accession number and clone sequence repeat are shown in Table 2. In general, the isolated microsatellites are highly polymorphic, compound repeats, as has been reported in other land snails (Davison 1999). Some of the loci cross-amplify between genera (Table 1), and may work in other bradybaenids. These microsatellite DNA markers may be useful in assessing population genetic structure, gene flow and speciation in these species.

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Table 1 Characterization of microsatellite loci in Japanese bradybaenid land snails

Species	Locus	Primers (5′–3′)	Clone size (bp)	Allele size range (bp)	No. alleles/ samples	H_O	H_E
E. quaesita	*Equ*1*	CAGTAGACAATTCAGCAGCTTTGG CGGCGACTTGCCTACCCGAG	144	129–132	2/11	0.080	0.080
M. ponderosa	*Mpo*1	CGAGGGCTCTCAGATACCAG CTTAAGTAAGCGCGATTCACG	272	250–286	19/62	0.871	0.912
	*Mpo*2	TTCACAGTGACAGCATTGGTC AATATTACCTACTTTCAAAACCACTTG	201	178–216	19/62	0.823	0.896
	*Mpo*3	CAATACAATTCGCACTCCCTGG TCAGTCATCGACTATGCCCTGG	144	127–177	17/62	0.919	0.916
	*Mpo*4	CACTGTGATATCCACTCTGTGC AGCTGGGATTGATTGCTACC	292	243–321	20/62	0.936	0.919
	*Mpo*5	TACTCTTGCGAAAGCCGAAC TCCACTGAGGGTTTGTTATATTTG	403	226–>500	50/62	0.984	0.968
	*Mpo*6	TTCTTCCTTCTAACATTGGGACC CAGCTAAACTCTCAGAACGGCAC	135	107–157	20/62	0.980	0.924
	*Mpo*7	AAACCGTAGCACATGGAGAAC AGCCTGTCATTACTGCTGAAC	219	191–276	24/62	1	0.938
	*Mpo*8	TCGACTGCTGCATTCTAAGG GACATTTCCGGAGTGATGTG	282	260–346	24/62	0.903	0.918
	*Mpo*9	TGTGGCTTAACGGATTCAGG CCCCCTTTGGCCCTTAAC	231	169–258	26/62	0.984	0.940
	*Mpo*10†	CCACGTACTTCCGTGCTTAC GGCATAGTCTCAGACATAATGAAATAC	177	177	0/62		
	*Mpo*11	CACTCACGAACCCATTCTCTC CATCCAGCAATAGTCTTTGTCC	178	164–186	11/62	0.758‡	0.812
	*Mpo*12	CATGTTGAATCACGTTGATGG CATGTCAACAGACACTGTACCAC	175	127–>500	17/62	0.951	0.899
A. editha	*Aed*1	GGCCCGTACGTTGTTTTATC TGCACAAACCCCCTAGTACC	275	198–483	28/18	0.889	0.958
	*Aed*2	CACATACTCACTCGAACAAACG TGTTCGAGGTATGTGGGTATGA	237	242–324	31/35	0.943	0.949
	*Aed*3	GCCTTTATTACGGCGAGTTC GGTGAGGCAAGCAATAGGAG	192	297–335	17/16	0.938	0.916
	*Aed*4	GTGCAGGGGTCTAGCACTAAG TAGCTTGCTCAGGACATCTG	217	375–399	3/17	0.356	0.476

*also polymorphic in *M. ponderosa*.
†polymorphic in *M. mandarina*.
‡heterozygote deficit using exact test in GENEPOP.

Table 2 Repeat motifs of the microsatellite clones and GenBank accession numbers

Locus	Repeat motif	GenBank
*Equ*1	$(CAA)_8$	AY453826
*Mpo*1	$(CA)_5N(CA)_3N_6(CA)_3(CG)_2(CA)_2N_2(CA)_8(CG)_4(CA)_9$	AY453827
*Mpo*2	$(GT)_7(CTGT)_2CT(GT)_2N_2(GT)_3N_2(GT)_4N_4(GT)_4N_2(GTGC)_6(GC)_2(GTGC)_4(GT)_8$	AY453828
*Mpo*3	$(GT)_{28}$	AY453829
*Mpo*4	$(CG)_6(CA)_5N_2(CA)_{34}$	AY453830
*Mpo*5	$(CA)_{26}N_4(CG)_{10}N_{14}(GA)_3N_2(GA)_2(TAGA)_{24}N_4(TAGA)_{30}(GA)_9$	AY453831
*Mpo*6	$(CT)_{22}(GT)_{24}$	AY453832
*Mpo*7	$(GA)_{27}N_9(GA)_6N_2(GA)_{24}$	AY453833
*Mpo*8	$(CT)_{13}(CA)_{17}N_{14}(CA)_9(CG)_{11}$	AY174175
*Mpo*9	$(CA)_{26}N_2(CA)_3N_5(GC)_4N_4(CA)_{20}$	AY174176
*Mpo*10	$(ST)_7GC(ST)_4$	AY174177
*Mpo*11	$(CT)_4N_2(CT)_5N_2(CT)_5N_2(CT)_8(CA)_2N_2(CA)_5$	AY174178
*Mpo*12	$(GT)_3N_2(GT)_{13}N_2(GT)_4(GA)_6N_8(GT)_{13}N_6(GT)_6$	AY453834
*Aed*1	$(CT)_3N(CT)_4N_2(CT)_3N_2(CT)_4N_2(CT)_5N_2(CT)_4N_4(CT)_5N_8(CT)_3N_2(CT)_3N_8(CT)_4N(CT)_3$ $N_6(CT)_3N_6(CT)_{25}$	AY453836
*Aed*2	$(CT)_5N_2(CT)_8N_2(CT)_{27}N_2(CT)_3N_6(CT)_3N_6(CT)_4N_{10}(CAGA)_4(CA)_{13}$	AY453837
*Aed*3	$(CT)_3N_2(CT)_2N_3(CT)_4N_2(CT)_3$	AY453838
*Aed*4	$(CT)_{36}$	AY453839

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