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TECHNICAL NOTE

Characterization of novel microsatellite loci for *Hetaerina americana* damselflies, and cross-amplification in other species

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Abstract *Hetaerina* damselflies are distributed throughout the neotropics. We developed eleven microsatellite loci for the damselfly *Hetaerina americana*. Microsatellites were tested for polymorphism on a panel of 24 individuals. The number of alleles ranged from 2 to 6, observed heterozygosity from 0.080 to 0.701, and the fixation index from −0.266 to 1.000. Cross-amplification was tested in 7 different species in the genus *Hetaerina* from the United States and Mexico. These microsatellite loci will be useful for studies of population structure and gene flow in *H. americana*.

Keywords Microsatellite · Damselfly · *Hetaerina americana*

Hetaerina is a neotropical damselfly genus with 37 species (Garrison 1990). *H. americana* is among the most broadly distributed representative of the genus, ranging from Canada to northern Central Americana and is among the most highly studied with regards to its behavioral ecology and immunocompetence (e.g. Grether 1996; Contreras-Garduño et al. 2006). Although *Hetaerina americana* is widespread in North America, several populations are vulnerable (NatureServe 2012). Furthermore, we aim to use these microsatellite loci to characterize the population genetics of several species in the genus, including those that have imperiled national or regional statuses (e.g. *H. titia*, NatureServe 2012).

Here we describe the isolation and characterization of 11 novel microsatellite loci from *H. americana* as a population genetics characterization resource. Individuals were obtained from riparian sites near Castroville, Texas, United States (29.343°N 98.882°W); Arroyo de Piedra, Veracruz, Mexico (19.456°N 96.482°W); and Armeria, Colima, Mexico (18.961°N 103.965°W). Insects were captured with aerial nets, stored in 95 % ethanol, and then refrigerated at −80 °C until analysis. DNA was extracted from wing muscle using the Qiagen QIAamp DNA Mini Kit using the manufacturer's protocol. 500 ng of DNA was prepared for whole genome shotgun sequencing on the Roche Genome Sequencer FLX instrument using the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Sciences, Indianapolis, USA) following the manufacturer's protocol. The library was quantified for DNA fragment size distribution and concentration (Agilent 2100 Bioanalyzer) and then processed with the GS FLX emulsion polymerase chain reaction (PCR) and sequencing kits. Sequencing was performed using 1/16th of a picotiterplate and yielded 24,421 sequences.

The sequences were screened for potential microsatellite loci by MSATCOMMANDER (Faircloth 2008) under the default settings. Of the 24,421 sequences, 1,063 contained putative microsatellite loci. Primers for trinucleotide (minimum repeat number = 5) and tetranucleotide (minimum repeat number = 4) were designed by PRIMER3 software (Rozen and Skaletsky 2000) embedded in MSATCOMMANDER using default settings. In total, 22 tetranucleotide primer pairs and 2 trinucleotide primer pairs were used for amplification trials. The 11 loci that amplified cleanly and were polymorphic in a panel of 8

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individuals were used for characterization across a sample set of 24 individuals from 3 different sampling sites.

Fragments were amplified by means of the M13-hybrid primer process. In this process, a 16-bp fragment is added to the 5′ end of the forward primer for binding of the dye-labeled M13-hybrid primer (Boutin-Ganach et al. 2001; Schuelke 2000). Primer mixes were prepared as follows: 2 µl reverse primer (100 µM); 4 µl forward primer (2.5 µM); 4 µl 6FAM dye-labeled M13-hybrid primer (2.5 µM); 90 µl RNAse/DNAse-free water. Amplification was carried out in 10 µl reactions containing 5 µl Qiagen Multiplex Mastermix, 0.5 µl BSA (10 mg/ml), 1 µl primer mix, 2 µl RNAse/DNAse-free water, and 1.5 µl template DNA (30–100 ng in total). The following cycling conditions were used: an initial step of 95 °C for 15 min; 25 cycles of: 30 s at 94 °C, 90 s at 59 °C, 60 s at 72 °C; 20 cycles of: 30 s at 94 °C, 90 s at 53 °C, 60 s at 72 °C; and 30 min at 60 °C. PCR products were run on an ABI 3730XL capillary sequencer, and allele sizes were scored manually using GS 500-LIZ size standard in GeneMapper v3.7 genotyping software (ABI).

Tests for deviations from Hardy–Weinberg equilibrium (HWE) were carried out in GENALEX v6.4 (Peakall and Smouse 2006) and tests for linkage disequilibrium (LD) were performed in GENEPOP 4.0 (Rousset 2008) for each of the 11 loci and 3 populations (24 individuals), with Bonferroni correction applied for multiple comparisons. Tests for linkage disequilibrium (LD) were performed in Genepop 4.0 (Rousset 2008). After Bonferroni correction for multiple comparisons, one locus (H11) showed significant departure from Hardy–Weinberg equilibrium in the Arroyo de Piedra population ($p < 0.004$). LD was suggested for loci H18 and H22 in Arroyo de Piedra population ($p = 0.012$), loci H18 and H8 in the Arroyo de Piedra population ($p = 0.013$), and loci H1 and H15 in the Armeria population ($p = 0.021$). The number of alleles for the sample set of all 24 samples ranged from 2 to 6 (Table 1). Calculations of observed (H_O) and expected heterozygosity (H_E) and fixation indices (F_{IS}) were carried out in GENALEX v6.4 and results are shown in Table 1. Observed and expected heterozygosities ranged from 0.000 to 0.458 and from 0.080 to 0.701, respectively. Finally, F_{IS} ranged between −0.266 and 1.000.

Cross-amplification of primers in other species was evaluated in 7 different *Hetaerina* species. DNA was extracted wing muscle using the Qiagen QIAampDNA Mini Kit according to the manufacturer's protocol for tissue. Most loci amplified well (Table 2), however, because only a single individual per species was available, polymorphism could not be tested. The microsatellites developed here should prove a useful resource in support of studies of population structure and gene flow in *H. americana* and other *Hetaerina* species.

Table 1 Characterization of 12 microsatellite loci for *Hetaerina americana*

Locus	Forward primer 5′–3′	Reverse primer 5′–3′	Motif	Genbank accession #	Size (bp)	A	H_O	H_E	F_{IS}
H1	CCTATATTGTGTCTTATACCTGTTTCC	GGCCAGTTCATTTCCCTGC	TTTA	JX515535	272–305	6	0.458	0.701	−0.266
H10	CCGCACCTATGTTTGTCCC	GTGAAGGTCTTTCTTCACGTTG	ATCT	JX515536	126–210	2	0.000	0.080	1.000
H11	CGCCACTCGATGGACTACG	TGTGACGGGAGGAAGTGTC	TCCC	JX515537	116–377	7	0.208	0.588	0.328
H15	GTGGTGCTACCGAGTTTCC	ACTTCTGGCTTAGGTGCGG	GGAT	JX515538	282–297	4	0.208	0.665	0.111
H17	TCACGGCTCACCTCTGAAC	GGGAAGATGGGTACATGGAG	GCAT	JX515539	130–337	5	0.292	0.563	0.300
H18	TCGTCGTTAAATGTTATCGTAGAGC	CACAAATTCAGGTATATCGTAATGC	GAAT	JX515540	243–266	5	0.333	0.600	−0.094
H22	CACTGTGTCGATTAGGTGGTC	ATGTATGTCCGCCCTTTGC	CAT	JX515541	124–236	4	0.250	0.608	0.143
H3	TCCATGAGGTCCAATGGCG	GTTCTCCTTCCAACAGCGG	GGAG	JX515542	199–329	3	0.042	0.494	0.628
H4	CTTGCCAACTAGTGTGACGG	GGAGGGAGTGGTTAGTACTTCG	AATG	JX515543	174–177	2	0.000	0.469	1.000
H7	GCTGGGTGCTGGAAGGAG	AAGTCACACGGATTTGCCC	GGGA	JX515544	238–255	4	0.083	0.296	0.670
H8	GAGCAAGACGGAGTCGGG	ACCCTTCTTCTTGGCGAGG	GGGA	JX515545	189–196	3	0.333	0.588	0.147

Size includes 16 bp M13F(−20) tag, A number of alleles, *HO* observed heterozygosity, *HE* expected heterozygosity, *FIS* fixation indices

Table 2 Cross-amplification of developed microsatellite loci in members of the genus *Hetaerina*

Species	H1	H10	H11	H15	H17	H18	H22	H3	H4	H7	H8
H. capitalis	+	+	+	−	+	+	+	+	+	+	+
H. cruentata	−	+	+	+	+	−	−	+	+	+	+
H. occisa	+	+	+	+	+	+	+	+	+	+	−
H. pilula	+	+	+	+	+	−	+	+	−	+	+
H. sempronia	+	+	+	+	+	+	−	+	+	+	+
H. titia	+	+	+	+	−	−	+	+	+	+	+
H. vulnerata	+	−	+	+	+	+	+	−	+	+	+

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References

Boutin-Ganach I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improved the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechnic 31:24–28

Contreras-Garduño J, Canales-Lazcano J, Cordoba-Aguilar A (2006) Wing pigmentation, immune ability and fat reserves in males of the rubyspot damselfly, *Hetaerina americana*. J Ethol 24:165–173

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94

Garrison RW (1990) A synopsis of the genus *Hetaerina* with descriptions of 4 new species (Odonata, Calopterygidae). Trans Am Entomol Soc 116:175–259

Grether GF (1996) Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. Evolution 50:1949–1957

NatureServe (2012) NatureServe Explorer: an online encyclopedia of life. www.natureserve.org/explorer. Accessed Aug 2012

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Res 8:103–106

Rozen S, Skaletsky HJ (2000) PRIMER3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234