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TECHNICAL NOTE

17 novel polymorphic microsatellite markers for the giant water bug, *Abedus herberti* (Belostomatidae)

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Abstract The giant water bug (*Abedus herberti*) is a large flightless insect that is a keystone predator in aridland aquatic habitats. Extended droughts, possibly due to climate change and groundwater pumping, are causing once-perennial aquatic habitats to dry, resulting in serious conservation concern for some populations. *A. herberti* also exhibits exclusive male parental care, which has made it a model organism for studying mating systems evolution. Here we describe 17 novel polymorphic microsatellite loci developed for *A. herberti*. Number of alleles per locus ranged from 2 to 15, and average observed and expected heterozygosities were 0.579 and 0.697, respectively. These loci can successfully resolve both population genetic structure among sites separated by 3–100 km ($F_{ST} = 0.08$–0.21, $P < 0.0001$), and divergent mating strategies within local populations, making them highly useful for conservation genetics studies of this vulnerable species.

Keywords Hemiptera · Stream ecology · Mating systems · Polygyny · Population genetics

The giant water bug *Abedus herberti* (Belostomatidae) is a keystone aquatic invertebrate of conservation concern. This long-lived, flightless predator occupies perennial aquatic habitats in aridlands of Arizona, USA and northern Mexico. Extirpation of populations, possibly due to climate change and water withdrawals by humans, is ongoing and leads to significant ecosystem-level changes (Bogan and Lytle 2011). Its naturally fragmented distribution and conservation status have made *A. herberti* the subject of recent studies on phylogeography (Finn et al. 2007), population stability (Finn et al. 2009), and behavioral adaptations (Lytle et al. 2008). Belostomatids are also interesting because males provide exclusive parental care, and *A. herberti* has become a model system for studying sperm competition (Smith 1979) and mating systems evolution (Smith 1997). Although mitochondrial DNA is useful for resolving broad-scale patterns of population structure (Finn et al. 2007; Daly-Engel et al. 2012), highly polymorphic markers such as microsatellites are needed to understand how genetic diversity varies across complex landscapes in the face of ongoing environmental change (Phillipsen and Lytle in review), and to answer questions at the intrapopulation scale, e.g., assigning parentage and kinship (Daly-Engel et al. 2010). Here, we present 17 polymorphic microsatellite loci that are useful for both purposes.

Tissue samples from two *A. herberti* were used by Genetic Identification Services (GIS; Chatsworth, CA) to construct four enriched microsatellite libraries for CA-, ATG-, CATC- and TAGA-nucleotide repeat motifs using standard cloning protocol (see Murray et al. 2008). GIS used DesignerPCR v.1.03 (Research Genetics, Inc) to design primers for 37 microsatellites that we then optimized in 34–77 unrelated *A. herberti* collected from the Santa Rita Mountains of Arizona. Leg clips from adult bugs were stored in 20 % DMSO-saturated salt buffer (Seutin et al. 1991) and DNA was extracted using Qiagen DNeasy Blood & Tissue kits (Valencia, CA). Loci were electrophoresed for optimal annealing temperature and allelic diversity using a series of 3 % agarose gels run at

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Table 1 Microsatellite loci isolated from *Abedus herberti*

Locus	Primer sequence (5′–3′)	Dye	T_a (°C)	[p] (mM)	Repeat Motif	Size range	N	A	H_o	H_e	*P*
AHA002	F: GCGAGGGTGTCTCACTTTG	NED	52	0.20	di-	195–215	70	6	0.786	0.781	0.174
	R: GCCGATAGAGTCGTTGTCG										
AHA003	F: GGGGGGCTCAAAAAATTA	FAM	52	0.20	di-	190–198	73	5	0.671	0.716	0.842
	R: AGATTGTAGTTGCCCACACA										
AHA03a	F: GTGTGGGCAACTACAATCTG	FAM	52	0.25	di-	106–114	66	5	0.712	0.710	0.760
	R: GCTCCAGGCTTATGCTAATC										
AHA005	F: TTTACTGGTCCCGGATATAGAC	VIC	52	0.15	di-	147–217	71	11	0.775	0.809	0.189
	R: CAAAATGAAGATAGGGGAAGAA										
AHA010	F: GTTCCGCATATAACAAACAATC	NED	52	0.30	di-	149–251	43	10	0.814	0.827	0.228
	R: AATCGACCCCTACAAGTTAATC										
AHA102	F: AGACCCTTCATCCACTACTACC	NED	52	–	di-	257–315	35	10	0.600	0.854	<0.001*
	R: CTACCCCCCTACACTCCAC										
AHA103	F: ACGATTTCGGGTGGAGATAG	NED	52	0.20	di-	197–241	44	15	0.886	0.879	0.135
	R: AAAGCCTCCCTTTGAAGTCC										
AHA104	F: CCTTCATTGCGAATCCTC	FAM	52	–	di-	258–308	41	9	0.220	0.674	<0.001*
	R: TTCCTTCATCCTCAGTAGTGC										
AHA106	F: GCATTATTGGCGTCTGCTA	FAM	52	0.25	di-	258–280	34	4	0.471	0.619	0.148
	R: GCTATCTGTTGCTGTCTCATTC										
AHA111	F: TCGTCGTACAATTCGCAAAC	VIC	52	–	di-	190–204	40	6	0.625	0.661	0.068
	R: CCCCGTGTTCCCACTTAA										
AHA112	F: GGGTTGCGAATGTTATCTC	PET	52	0.15	di-	148–176	72	10	0.806	0.837	0.498
	R: CGGGCTTTTCCTTATGTAG										
AHA114	F: AATAATTTTCTCCCCCCTATCA	PET	52	0.50	di-	251–327	58	15	0.707	0.793	0.239
	R: TTGGCTACTTGACGCATATAGA										
AHB009	F: GCCTTCTGTATCGCCAATAC	VIC	52	0.15	tri-	231–264	75	7	0.360	0.362	0.202
	R: TCAGGTAGAGGAAAACATTGTG										
AHB107	F: GCCCCATTATCCTCATAGTC	VIC	52	0.05	tri-	228–240	77	5	0.701	0.719	0.566
	R: GTGGCTGTGAAAGCGTAAC										
AHC011	F: GAGCCAGCAAGCGTAACA	PET	52	0.30	tet-	243–277	16	5	0.625	0.746	0.621
	R: GCCGCAAGCAGTTCAGTA										
AHD003	F: CTCTCCCCTCTCACTTATTTG	PET	52	–	tet-	100–104	34	2	0.147	0.409	<0.001*
	R: CTCCTGTCGTATTGTTTCT										
AHD006	F: CTCCTGAGGCTCTATTTTATCA	PET	52	–	tet-	100–108	34	3	0.249	0.453	<0.001*
	R: GCCCAAACAGATTTGAATC										

T_a annealing temperature, [*p*] primer concentration in multiplex pools, *Repeat Motif* di-, tri-, and tetra-nucleotide repeats, *N* number of individuals genotyped, *A* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, *P P* value for deviation from HWE, * statistically significant

80v for approximately 2 h. All forward primers were labeled with proprietary dyes obtained from Applied Biosystems (Foster City, California; Table 1) except for AHA003, where the reverse primer was labeled for guanine excess. PCR reactions consisted of 0.1 U Biolase *Taq* DNA polymerase (Bioline; Randolph, Massachusetts), 1x *Taq* buffer, 0.50–0.05 µm of each primer (Table 1), 200 µm each dNTP, and 2.0 mm $MgCl_2$. PCR amplification consisted of an initial denaturation at 95 °C for 4 min, followed by 35 cycles of 1 min at 95 °C, 30 s at 52 °C, and 30 s at 72 °C, followed by a final extension at 72 °C for 20 min. PCR products were resolved with an ABI 3730 automated sequencer and visualized using ABI PRISM GeneMapper Software 3.0 (Applied Biosystems).

Of the 37 loci provided by GIS, 17 (46 %) were successfully genotyped (Table 1). We estimated heterozygosity and tested for deviation from Hardy–Weinberg Equilibrium (HWE) using Genepop 3.4 (Raymond and Rousset 1995). One locus, AHC011, only amplified in 16 individuals, but we included these data in hopes that they will be useful to future investigators. Arlequin 3.11 (Excoffier et al. 2005) indicated that no loci were positive

for linkage disequilibrium after Bonferroni correction. 4 loci were out of HWE because of heterozygote deficiency, which MicroChecker (van Oosterhout et al. 2004) inferred was due to the presence of null alleles.

Data from two studies demonstrate the utility of these loci in identifying fine-scale genetic structure and determining mating system in wild populations of *A. herberti*. A subset of ten loci was used to resolve population genetic structure among 19 collection sites (median sample size = 27) from four Arizona mountain ranges (Phillipsen and Lytle in review). Euclidean distances separating the sites ranged from 3 to 100 km. Loci were amplified in three multiplex PCR sets using Qiagen's Multiplex PCR kits: Set A (AHA010, AHA114, AHB009, AHD006), Set B (AHA103, AHA112, AHA106), and Set C (AHA111, AHA002, AHB107). Exact tests performed in Fstat with 1000 replications (Goudet 1995) showed significant genetic differentiation across loci, with F_{ST} values ranging from 0.08 to 0.21 ($P < 0.0001$; Phillipsen and Lytle in review). A different subset of 12 loci was used to test for heritability in a single male *A. herberti* and 32 of his offspring. Loci were amplified in three different multiplex PCR sets: Set A (AHA005, AHA112, AHA106, AHA103), Set B (AHA114, AHA010, AHA03a, AHB009), and Set C (AHA003, AHA002, AHB107, AHC011). For multiplexing, primers were mixed in three pools at optimal concentration (Table 1) and added to the PCR at 1/5 total reaction volume. Paternal alleles were inherited in expected 50:50 ratios, and four maternal alleles were detected among the brood, indicating a polygynous mating system.

This suite of polymorphic markers is the first developed for a member of the genus *Abedus*, of which the species *A. herberti* has become a model organism in the study of arthropod evolution and aridland conservation ecology. The successful resolution of both broad- and fine-scale population genetic structure and identification of individual mating systems in *A. herberti* demonstrate the utility of these loci for conservation genetics research.

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