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MICROSATELLITE LETTERS

Isolation and characterization of polymorphic microsatellite loci in the endangered San Diego fairy shrimp (*Branchinecta sandiegonensis*)

Joelle M. Andrews · Andrew J. Bohonak · Marie A. Simovich

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Abstract A set of ten polymorphic microsatellite loci were isolated and characterized for the endangered San Diego fairy shrimp (*Branchinecta sandiegonensis*). These loci were amplified on a set of 24 fairy shrimp collected from vernal pools located throughout San Diego County. The loci selected are highly variable across a wide sampling range (3–29 alleles per locus, 3–20 heterozygotes observed). After directly testing for Mendelian inheritance through family screens, eight markers did not show evidence of null alleles. This novel set of microsatellite markers will be useful in future genetic studies to assess intraspecific diversity, population connectivity and mating patterns.

Keywords Vernal pools · Fairy shrimp · Anostraca · *Branchinecta* · Microsatellites · Population genetics

The San Diego fairy shrimp (*Branchinecta sandiegonensis*) inhabits a narrow, discontinuous range of vernal pool habitat on coastal chaparral-covered mesas and inland foothills of southern California, and is the most commonly found fairy shrimp species in this range (Eriksen and Belk 1999). Loss of vernal pool habitat in San Diego due to human disturbance has been extensive, with only small remnants of most vernal pool landscapes remaining (Bauder and McMillan 1998). As a result, *Branchinecta sandiegonensis* was listed as an endangered species (U.S. Fish and Wildlife Service [USFWS] 1997) among other sensitive species unique to these habitats. Here, we report the isolation and characterization of polymorphic *B. sandiegonensis* microsatellite loci that will be useful in future population genetic studies.

Genomic DNA from 10 adult *B. sandiegonensis* individuals, each from a different population, was extracted using the DNeasy Blood and Tissue kit (Qiagen), and sent to Genetic Identification Services (GIS; Chatsworth CA, USA) to develop eight enriched microsatellite libraries with CA-, GA-, AAC-, CAG-, AAG-, ATG-, TACA-, and TAGA- repeats. Following digestion of genomic DNA with *HindIII* restriction enzyme, fragments 350–700 bp long were subjected to magnetic bead capture using biotinylated capture molecules. Enriched fragments were ligated into pUC19 plasmids and electroporated into *E. coli*. Colonies containing the insert were isolated and plasmid DNA was amplified via PCR. Resulting PCR products were sequenced using Amersham's DYEnamic™ ET Terminator Cycle Sequencing Kit, followed by electrophoresis on an Applied Biosystems Model 377 DNA Sequencer in order to detect the presence of microsatellites. The eight enrichment libraries yielded a total of 98 cloned sequences containing microsatellites, and 66 of these sequences have suitable flanking regions. Primers were designed and tested by GIS against fifteen individuals and genomic library DNA. From these 66 sequences, 20 loci were found in preliminary screens to be polymorphic. All polymorphic loci underwent additional quality control screens to ensure that they were suitable for population genetic analysis. Additional primer sets for each locus were designed using

J. M. Andrews (✉) · A. J. Bohonak
Department of Biology, San Diego State University, 5500 Campanile Dr., San Diego, CA 92182-4614, USA
e-mail: jswerdna23@aol.com

A. J. Bohonak
e-mail: abohonak@mail.sdsu.edu

M. A. Simovich
Department of Biology, University of San Diego, 5998 Alcalá Park, San Diego, CA 92110, USA
e-mail: simo@sandiego.edu

Table 1 Variation in polymorphic loci for *Branchinecta sandiegonensis* in a screen of 24 individuals from 14 pools

Locus	Repeat motif[a]	Primer sequence (5′–3′)	Multiplex Mix[b]	Expected product size[a]	N_a	Actual size range	N_H	N_0	Null alleles?
BsB8	$(CT)_{30}$	F: GTGTCTCACTGCCGGTTGTTTTCATT	3	180–190	21	138–283	13	0	No
		R: GGCAAGTTCCTACTTTCATCCTTTTACCGA							
BsB201	$(CT)_{10}$, TT insert	F: CAAACCCAACCCATCGTGACATCCTAC	2	398	7	386–399	12	1	No
		R: CTGGCACCACTTACAATCGGCTATCATC							
BsE1	$(AAG)_{14}$, AG and A insert	F: AGAGAGAAGAAGTCGCAGAGGGTCAGTG	3	153	9	133–161	9	2	No
		R: GCCACGACACAAGGTTTCCGACAT							
BsH106	$(ATCT)_8$	F: GTCGGCGCTACGAGTTGGATCAATAAAA	2	141	8	112–145	11	0	No
		R: TGGAGTCGAAACGAACGTCTGTCCA							
BsH120	$(TAGA)_{23}$	F: CGGACGGTTTGGGTCTCCTTTTATCTTT	4	365	12	261–400	10	3	No
		R: TCGGGAGGATCTAATGTAAGCGGTA							
BsH101	$(TCTA)_5$	F: ACAGGACGGACGAGGCATCAAAATC	1	317	3	310–318	3	3	No
		R: AAAGACGAAAACCGCCAAAAAGCATCT							
BsF128	$(TGA)_7$	F: TCCGTTCTATGATTAAGTGTGG	3	230	3	217–229	8	2	No
		R: GCACTCCATCTAGGAAGAAGA							
BsB208	$(GA)_{28}$, AA insert	F: ACGAAAATTTTTGGAAGGTAGCCTAACTGG	3	277	29	303–495	20	2	No
		R: CAGCAGCACCAGACAGAAACTCAAACTG							
BsH123	$(TAGA)_9$	F: GATGCCACCAGCCATTAAC	5	230	10	198–234	13	0	Yes
		R: TTTCGGGCTTTCAGTGTTG							
BsH128	$(TAGA)_7$	F: CAAACCATGAGAACCAACCTCCCATTTC	6	260	7	242–261	4	4	Yes
		R: ACTAGCAAAACCGGGACAGAACGACAGA							

N_a number of alleles, N_H number of heterozygotes, N_0 number of failed samples

[a] Determined from cloned sequences

[b] Multiplex reactions are as follows: mix 1 (95 °C for 15 min, 30 cycles of {94 °C for 30 s, 69 °C for 3 min, 72 °C for 1 min with 16 % ramp}, 60 °C for 30 min), mix 2 (95 °C for 15 min, 30 cycles of {94 °C for 30 s, 69 °C for 3 min, 72 °C for 1 min with 16 % ramp}, 60 °C for 30 min), mix 3 (95 °C for 15 min, 40 cycles of {94 °C for 30 s, 61 °C for 3 min, 72 °C for 1 min with 16 % ramp}, 60 °C for 30 min), mix 4 (95 °C for 15 min, 40 cycles of {94 °C for 30 s, 63 °C for 3 min, 72 °C for 2 min with 16 % ramp}, 60 °C for 30 min), mix 5 (95 °C for 15 min, 30 cycles of {94 °C for 30 s, 56 °C for 3 min, 72 °C for 1 min with 16 % ramp}, 60 °C for 30 min), mix 6 (95 °C for 15 min, 30 cycles of {94 °C for 30 s, 61 °C for 3 min, 72 °C for 1 min with 16 % ramp}, 60 °C for 30 min)

Primer3 (http://frodo.wi.mit.edu/primer3/) and were tested against additional samples.

We genotyped families of known *B. sandiegonensis* crosses to directly test for Mendelian inheritance and null alleles. Families were obtained from mating experiments using adult fairy shrimp collected from pools in the Maddox complex located in Mira Mesa, CA. DNA was extracted from cyst offspring following protocol by Steele et al. (2009). Problematic loci with unexpected products, null alleles or non-Mendelian inheritance were either discarded or the flanking regions were sequenced to redesign primers. At least three polymorphic families were screened per locus, with the exception of BsH106 due to its low variation in these pools (only two families turned out to be polymorphic). We chose 10 loci for further screening, of which 2 show possible evidence of null alleles (Table 1).

We screened these genes in 24 adult *B. sandiegonensis* individuals from 14 pools throughout the San Diego region (Table 1). PCR protocols were optimized for single and multiplex PCRs using a multiplex PCR kit (Qiagen). After each primer pair was optimized across an annealing temperature gradient, several multiplex reactions were tested. PCR reactions were carried out in 10.5 μL volumes using the following recipe: 5 μL of 2× Master Mix; 1 μL of 10× primer mix, resulting in a final concentration of 0.2–0.4 μM of each primer; 1.5 μL of 1:10 diluted template DNA; and 3 μL of sterile PCR-grade water. Reactions were run in on a Mastercycler© epgradient (Eppendorf) thermocycler according to the following protocol: 95 °C for 15 min, 30–40 cycles of (94 °C for 30 s, annealing temperature for 3 min, 72 °C for 1–2 min), 60 °C for 30 min. To enable genotyping, forward primers were 5′ fluorescently labeled with 6-FAM, NED, VIC, and PET dyes. All DNA samples were genotyped on an ABI 3730xl DNA Analyzer (Applied Biosystems). Raw allele sizes were scored using Genemapper 4.0 with LIZ500 size standard. Raw fragment sizes were visualized and binned to integer allele codes using the R package MsatAllele (Alberto 2009). Any scoring errors were checked and re-scored manually. Variation for these ten loci was high in the individuals we screened (Table 1).

We were able to select reliable, informative, highly variable *B. sandiegonensis* microsatellite markers from quality screens that have the potential to further enhance understanding of population genetic structure and connectivity.

These markers may also be useful in other *Branchinecta* species, including *B. lindahli*, and the detection of *B. sandiegonensis* × *B. lindahli* hybrids. Information on the historic distribution and genetic integrity of *B. sandiegonensis* is necessary to improve conservation and management strategies of this federally endangered species.

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