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TECHNICAL NOTE

Development of 10 microsatellite loci in the wolf spider *Arctosa sancterosae* (Araneae: Lycosidae)

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Abstract Ten novel microsatellite loci were isolated from *Arctosa sancterosae*, a white dune dwelling species of wolf spider. Diversity was assessed in 273 individuals sampled from 11 populations along the Northern coast of the Gulf of Mexico. These new genetic markers will be useful for the description and conservation of these limited populations.

Keywords Arachnids · Coastal dune ecosystem · Microsatellites · Enriched library

The white beach spider, *Arctosa sancterosae*, is a burrowing wolf spider endemic to the dune ecosystem of the northern coast of the Gulf of Mexico (NGC). Species endemic to this ecosystem are ideal for examining the effects of disturbance (e.g. hurricanes, habitat fragmentation/degradation) on population persistence. It is widely recognized that the primary threat to these populations is habitat fragmentation, but with the reduced gene flow associated with anthropogenic habitat modification and a predicted increase in the intensity of tropical storms (Goldenberg et al. 2001), the outlook for this dynamic, fragile system is bleak. *Arctosa sancterosae* shares this habitat with several species of endangered beach mouse for which small population size has limited the inference of population structure and dynamics. Our hope is to develop *A. sancterosae* as a complimentary, sister system to aid in conservation planning for the entire dune ecosystem that is itself in an advanced stage of degradation (Martínez and Psuty 2004).

We developed 10 novel microsatellite loci using the enrichment protocol of Glenn and Schable 2005. Whole genomic DNA was extracted from the legs of *A. sancterosae* using the DNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions. DNA concentration was determined using a spectrophotometer and genomic DNA was then digested with the restriction enzymes *Rsa*I and *Xmn*I to yield fragments between 300 and 1,000 bp long. To the ends of these fragments we then ligated SuperSNX24 linkers (F; GTTTAAGG CCTAGCTAGCAGAATC, R; GATTCT GCTAGCTAGGCCTTAAACAAAA) and a polymerase chain reaction was performed to ensure ligation was successful. Genomic fragments were enriched using a probe mix containing four biotinylated oligonucleotides (AAT_{10}, $AAAT_7$, AAC_6 and $AGAT_8$) and separated with streptavidin magnetic beads. This mixture was washed with a 2× SSC, 0.1% SDS solution twice and a 1× SSC, 0.1% SDS solution four times. A magnetic particle collector was used between washes to capture the magnetic beads. After the last wash, fragments were removed from the probes by denaturing at 95°C for 5 min and precipitating with 95% ethanol and 3 M sodium acetate. These fragments were then air-dried and resuspended in 25 µL of TLE. To increase the quantity of these recovered enriched DNA we amplified the enriched pool by PCR using the SuperSNX24-F primer. These amplified fragments were then transformed and cloned using a TOPO TA Cloning Kit (45-0641). Blue-white selection revealed 288 clones that were then screened for inserts suitable (large enough) for microsatellite development by PCR using M13 forward and reverse primers (Glenn and Schable 2005). After amplification, PCR products

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containing inserts of suitable size were cleaned using Exo-SAP-IT® (USB) per the manufacturer's protocol. Sequencing reactions used the Big Dye Terminator v. 3.5 (Applied Biosystems) chemistry and were cleaned using G-50 Sephadex in a MultiScreen PCR Filter Plates (Millipore). Sequences were generated using an ABI 3130 sequence analyzer. These were edited using Geneious (Drummond et al. 2009) and checked for short tandem repeats (STR) using TROLL (Tandem Repeat Occurrence Locator; Castelo et al. 2002). Primers were then designed for STR containing loci using PRIMER3 (Rozen and Skaletsky 1996) in Geneious (Drummond et al. 2009).

These loci were evaluated for primer performance, variation among individuals, and populations were assayed for allelic frequencies, yielding ten polymorphic loci that were scored for 273 individuals. Fluorescence-labeled fragments were visualized on an ABI 3130 and allele sizes were determined through comparison with a known size standard (GeneScan-500 Rox) using GeneMapper version 3.7. Polymorphism was screened on 273 individuals from 11 populations. The number of alleles ranged from 3 to 11. Expected heterozygosity was computed among and within populations using Levene 1949 in the software package PopGen (Yeh et al. 1999). The loci were tested and each was found to be segregating independently (Table 1). Tests for departure from HWE showed populations exhibiting evidence of inbreeding for a number of loci (Table 2).

Table 1 Characterization and level of variability at 10 microsatellite loci in 11 populations of *Arctosa sancterosae*

Marker name	Primer sequence	Motif	No. of alleles	Fragment size	Total size range	H_O	H_E[a]
AS6	F: AATCGCAGGGTCAATAATGC R: GGCCTATTTGTTCAGCCATC	TA	11	419	416–442	0.6114	0.6288
AS7	F: TTCATGGCTGTTTGCCACTA R: TTGTCCTGCACTTGCATAATT	TA	4	308	310–324	0.1152	0.1377
AS8	F: TGATTTCGCGAATCCCTAGT R: TGAAAGCCGTTTAATCCTTACA	TAGA	3	281	282–286	0.1042	0.1275
AS9	F: CCATTGAAACTGGGACATTCT R: GATCCGGTGGAACGAAAGTA	TA	3	398	420–428	0.1262	0.1552
AS10	F: TTTACGCGCAATCGTGTTTA R: ATGCCCTGGAACTAATGCAG	AAC	3	325	324–330	0.3241	0.3592
AS11	F: CATAAATTCGTAATTATTTTTGATGC R: AACAACTTTCAAAAGGCATGTG	TAT	3	150	168–174	0.2136	0.2206
AS13	F: CAGCGTTTCCCACACCATA R: TCTACCCTGCCACGTGATATT	GAG	4	297	291–303	0.2968	0.3105
AS15	F: TCAAGTTTGACGTCCAGTCG R: ACCCCTCATGACAGGTAAGG	TA	3	291	308–312	0.1206	0.1368
AS16	F: AGCAGAAAAATTCAAACGTGA R: CAAGTCGCCAAAAGGGATTA	TG	5	269	270–290	0.6044	0.6176

[a] Expected heterozygosity was computed using the method of Levene (1949)

Table 2 Within population heterozygosity levels across loci and Fis estimates

Population	$\bar{X}$ H_O	$\bar{X}$ H_E[a]	Fis
Dauphin Island Sea Lab, Dauphin Island, AL	0.140	0.149	0.060
Ft. Morgan, Bon Secor, AL	0.143	0.150	0.043
Pine Beach, Bon Secor, AL	0.112	0.121	0.074
Perdido Key Area, Gulf Islands National Seashore, FL	0.206	0.216	0.047
Ft. Pickens, Pensacola Beach, FL	0.172	0.187	0.084
Gulf Islands National Seashore, Navarre Beach, FL	0.183	0.203	0.098
Destin Point, Public Beach, FL	0.213	0.244	0.124
Henderson State Park, Destin, FL	0.277	0.306	0.094
Public Beach Acess, Mexico Beach, FL	0.347	0.405	0.145
Public Beach Acess, St. Joe, FL	0.341	0.423	0.194
St. George Island, FL	0.354	0.444	0.203

[a] Expected heterozygosity was computed using the method of Levene (1949)

The isolation and characterization of these 10 loci will enable us to determine heterozygosity levels in each population and examine historical levels of gene flow between what are now disjunct populations of *A. sancterosae* along the NGC. In conjunction with loci previously developed for wolf spiders, we hope to explore in fine detail the dynamics of these populations and their responses to both anthropogenic (development) and natural (hurricanes) disturbance. The identification of source and sink populations and tests for genetic bottlenecks will allow us to measure relative health of these habitat fragments and provide a new faunal model for the study of these highly endangered ecosystems.

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