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PRIMER NOTE

Isolation and characterization of 13 polymorphic microsatellite loci from the clam shrimp *Eulimnadia texana* (Crustacea: Spinicaudata)

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Abstract

Thirteen polymorphic microsatellite loci were isolated and characterized from the clam shrimp *Eulimnadia texana*. In analyses of 20–50 individuals from two populations the number of alleles ranged from two to seven with observed heterozygosity ranging between 0.00 and 0.37. The low values for heterozygosity were not unexpected for a group characterized by its unusual androdioecious mating system, in which males compete with self-compatible hermaphrodites for offspring production. These microsatellites are likely to be useful for further evolutionary investigations of this rare mating system in these crustaceans.

Keywords: clam shrimp, *Eulimnadia*, microsatellite, primer

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Androdioecy is extremely rare in both the plant and animal kingdoms (Charlesworth 1984) and has traditionally been viewed as an intermediate evolutionary step between hermaphroditism and dioecy (Lloyd 1975; Charlesworth 1984). The clam shrimp *Eulimnadia texana* exhibits an androdioecious system of reproduction (Sassaman & Weeks 1993). In this system, males coexist with hermaphrodites but unlike some models of androdioecy (Lloyd 1975; 1979), *E. texana* hermaphrodites cannot cross with one another. In response to these unusual organisms, Otto *et al*. (1993) developed a population genetic model to examine the dynamics of *E. texana*'s mating system. The model suggested a number of behavioural and life-history parameters that would allow for stable coexistence of both males and hermaphrodites. Crucial to evaluating the model is the estimation of a number of model parameters from field populations, including male mating success. For this purpose we have isolated and characterized a number of microsatellite loci in *E. texana*.

Genomic DNA from entire clam shrimps collected from Portal, Arizona were extracted using the DNeasy Plant kit (Qiagen) and a microsatellite library was developed using the methods below and by following the protocols outlined using a TOPO TA cloning kit (Version K) from Invitrogen.

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DNA was digested using a *Sau*3 AI digestion, and the digested DNA was purified using Clontech Chroma Spin columns. The fractionated genomic DNA was then ligated to *Sau*3 AI linkers, and excess *Sau*3 AI linker was removed using the Chroma Spin columns. The linker-ligated fragments were amplified by polymerase chain reaction (PCR) using a Dynazyme TAQ kit to form the whole genome PCR library. The library was then denatured and hybridized to biotinylated probe [5′-$(CA)_{15}$TATAAGATA-Biotin]. The resulting probe–target fragments were captured using Vectrex Afidin D, and nonbound DNA was removed with TBST to enrich the library for DNA containing CA repeats. The captured fragments were eluted by resuspending the Vectrex matrix in water and heating at 65 °C for 15 min. The suspension was then centrifuged at 14 000 ***g*** for 30 s, and the resulting supernatant was collected. The $(CA)_n$-enriched library was again amplified by PCR, and an aliquot of the amplification was diluted 100 : 1, 500 : 1 and 2500 : 1. Successful CA enrichment was verified using a dot-blot technique on these dilutions.

The enriched library was randomly cloned into *Escherichia coli* colonies using at TOPA TA cloning kit following procedures outlined in Version K of this product (Invitrogen, 2000). The One Shot® Chemical Transformation procedures were implemented to transform the bacteria. The transformed colonies were grown on standard LB bacterial

Table 1 Characterization of 13 microsatellite loci in the clam shrimp *Eulimnadia texana* (Crustacea: Spinicaudata)

Locus	Primer sequence (5′–3′)	Repeat motif	T_a (°C)	Clone size (bp)*	N_A	H_O	GenBank no.
CS3	CTTCTCCTGACCCGGCCTGAA GGTGTGTCTCACACTGACTGCT	$(GTA)_6$ …$(AGT)_6$ … $(GGA)_6$ … $(TAG)_7$ … $(TAG)_7$	58–46	643	5	0.37	AY515020
CS4	GATCATAAACTCGACTGTCA GGACCGGGTACTTTCTACGTG	$(AG)_{15}$	48	321	5	0.16	AY515021
CS5	GTCTAAACCCCGTCAGTGCCGA CTAACTTGCGTCCTTGGCGCT	$(CT)_6$	44	496	3	0.00	AY515022
CS7	GAAGCTTGGGATCACGGGTA AGCTAGACTCGGTTCATGGT	$(TAC)_4$ … $(CTA)_4$	56–42	395	7	0.27	AY515023
CS8	GACCACGTGGTACGTATCT GTACGGCGTTATCAGAAC	$(GA)_{14}$	44	318	6	0.25	AY515024
CS10	TAGCCATGATTCTCATCGCA GGTATAGTCACGCAAGATGA	$(AC)_6$	44	325	4	0.03	AY515025
CS11	GAGGCTGACATGAATGAACT AGTGAACTACTGCATGACT	$(GT)_{10}$	48	512	4	0.06	AY515026
CS12	GGTCGAACTACAGGCTTGA GAGATGAAGCAGACGCCAT	$(GTT)_4$	56–42	429	2	0.00	AY515027
CS15	CAGCAGGGGTGATTCAAACG CCCCCTCTATGAAAACACG	$(CA)_7$	48	189	5	0.32	AY515028
CS16	GAGTGAGTCAGTATCAGCTGA GATCACTCTGTCTGTTTGTCCGAGT	$(CAGA)_9$ … $(GACA)_5$ … $(GACA)_4$ … $(GACA)_5$	58–46	584	2	0.25	AY515029
CS17	GATCAACCAGGTAGCTTGCC ATCGTCTAAACCCCGTCAGT	$(AG)_6$ … $(CTT)_5$	58–46	502	2	0.04	AY515030
CS19	GCCCGATTCTTACGTTTTCTC AGATGGTCAACAGAGGGGTC	$(CA)_7$	52	178	3	0.21	AY515031
CS20	GGAAACACTGATCGTCGCAATGGGCA TAAGATTAGGAGGAATAGTAGCAG	$(ACT)_{13}$ … $(TAC)_8$	56–42	390	6	0.17	AY515032

*From original sequenced clone.

T_a, annealing temperature. Ranges refer to touchdown protocol in which the higher temperature (left) is used in the first two cycles followed by decreases in annealing temperature of 2 °C every two cycles followed by 20 cycles at the lower (right) temperature. N_A, number of alleles; H_O, observed heterozygosity.

plates. Positive clones were identified as being white or light blue, and these colonies were randomly screened for CA repeats by directly sequencing their inserts. Positive clones were sequenced producing 26 products with putative microsatellite sequences. Primers were developed for 23 of these clones with 19 resulting in positive amplification products.

Initially, 20 individuals of *E. texana* from a New Mexico population SWP were amplified in 10-μL PCR reactions containing about 25 ng genomic DNA, 10 μM of each primer and 0.2 U *Taq* (Panvera, LA PCR 2.1) in the manufacturer's GC buffer (#11). PCR amplification was confirmed using 2–4% agarose gels and polymorphism was assessed using 6% acrylamide gels with silver staining (Promega) or by 6.5% acrylamide gels on a LiCor 4200S DNA analyser. Of the 19 loci, 13 were shown to be polymorphic with two to four alleles. For several loci (CS3, CS5, CS15, CS16, CS17 and CS19), 20 or more additional individuals from a second population (WAL) from New Mexico were examined, from which several additional alleles were identified.

The number of alleles, product sizes, primer annealing temperatures and observed heterozygosity for each of the 13 polymorphic loci are presented in Table 1. Observed heterozygosity ranged from 0.00 to 0.37. Lower heterozygosity and polymorphism can be expected from members of these populations because selfing rates are quite high (Weeks *et al*. 1999; Weeks & Zucker 1999). Nonetheless, the polymorphic microsatellite loci reported here will probably be valuable to future studies on inbreeding, gene flow and other population genetic aspects of these organisms.

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