

PRIMER NOTE

New microsatellite primers for *Daphnia galeata mendotae*

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In order to study genetic changes in populations of *Daphnia galeata mendotae*, I characterized seven polymorphic microsatellite loci. Primers to amplify these loci were tested on individual eggs from the resting egg bank of Onondaga Lake, NY. Levels of polymorphism and cross-amplification in *D. g. galeata* indicate that they will be useful markers for ecological genetic studies on both adults and diapausing eggs of these species.

Keywords: *Daphnia*; egg bank; microsatellites

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Daphnia is a genus of freshwater crustacean common throughout temperate and arctic regions. It is one of the most abundant zooplankton species in many lakes and serves as an important biotic control on algae and food source for fish (Brooks & Dodson 1965). Sexual *Daphnia* females produce diapausing eggs that can remain viable in lake sediments for decades. Researchers have begun exploiting the record of eggs buried in sediments to examine phenotypic and genetic changes occurring over known intervals (Hairston *et al.* 1999, 2001; Kerfoot *et al.* 1999; Cousyn *et al.* 2001; Reid *et al.* 2002). Because of the extremely small size of individual eggs (actually early stage embryos), extensive genetic analyses on resting egg banks are limited to polymerase chain reaction (PCR)-based methods. PCR amplification of DNA has been successful on individual eggs as old as 2000 years (Limburg & Weider 2002). Here I describe the development of seven microsatellite loci for *Daphnia galeata mendotae*, a species common in northern North America.

DNA for constructing the genomic library was isolated from multiple individuals of an isoclonal line of a single female from Oneida Lake, NY using a DNeasy Tissue Kit (Qiagen). A library enriched for di- and trinucleotide repeats was created using a modified version of the procedure of Hamilton *et al.* (1999). DNA was digested using one of *HaeIII*, *RsaI*, or *AluI* or a combination of *NheI*, *HhaI*, and *AluI* (New England Biolabs). Digested DNA was first ligated to blunt-ended SNX linker sequences containing known restriction enzyme sites (Hamilton *et al.* 1999) then

enriched for di- and tri-nucleotide 30 bp repeats using streptavidin-magnetic capture (Hamilton *et al.* 1999). Repeat-enriched DNA fragments were ligated into the *XbaI* site of plasmid vector pUC19 (New England Biolabs) and DH5- α *E. coli* cells (Gibco, BRL) were transformed with these plasmids. Positive transformants were detected using dot-blot hybridization (Sambrook & Russell 2001) or colony lifts onto nylon membranes (Micron Separation, Inc.) and probed with a Phototope Star chemiluminescent kit (New England Biolabs). Clones containing microsatellite repeats were sequenced using BigDye Terminator Cycle Sequencing chemistry and an ABI377 Automated Sequencer (Applied Biosystems).

Primer pairs were designed for 11 sequences containing microsatellite repeats. Three loci showed nonspecific amplification and eight loci yielded consistent product. Forward primers of seven of these loci were labelled with 5' fluorescent tags [6-FAM, HEX (Integrated DNA Technologies) or NED (Applied Biosystems)] for genotyping. DNA from individual *D. galeata mendotae* eggs from the sediments of Onondaga Lake, Syracuse, NY was tested for polymorphism. Individuals came from three time horizons (depths) in the sediments, with a maximum age of about 20 years. An individual egg was removed from its ephippial case, rinsed in sterile water, and placed in 15–40 μ L 5% chelex solution. Extractions were heated at 60 °C for 3 h to overnight, boiled at 99 °C for 10 min, centrifuged for 2 min, and stored at –20 °C until used. PCR reactions were performed in 96-well plates or 0.2 μ L thin-walled strip tubes using a PCR Express or OMNE thermocycler (Thermo Hybaid). PCR amplifications were performed in 10 μ L reactions containing 10 ng DNA template, 20 mM

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Table 1 Characteristics of microsatellite loci for *Daphnia galeata mendotae*. T_a , optimized annealing temperature; n , number of individuals genotyped; H_O , heterozygosity observed; H_E , heterozygosity expected

Locus	Primer Sequence (5'–3')	Repeat	T_a (°C)	Size Range (bp)	No. Alleles	n	H_O	H_E	GenBank Accession	<i>D. galeata galeata</i> Amplification
Dgm105	F: ATGTGAGCGCGGAGCATTT R: GTCCAGCCGGCCATTTTCAGTT	(CAG) ₈ AG	58	188–197	3	103	0.58	0.56	AY542269	+
Dgm106	F: ACCACCACCTCCTCCGCCACAT R: TTCGTGATTTTCCTCACCCATTTTC	(CAA) ₈ CCTAA	58	130–145	5	103	0.66	0.67	AY542270	+
Dgm107	F: CCTTTGGCATCGTTTCTTAATTCCT R: CTGCGCAACCTCCAGTCCT	(TGC) ₇	58	120–128	4	38	0.42	0.47	AY542271	+
Dgm109	F: CCAGCTGTTGACCACCTG R: TGC GCGAGGATTTCCAACAC	(ACC) ₇ AC	58	258–266	6	102	0.57	0.66	AY542272	+
Dgm111	F: ATCCAGCACTGCGGTCAATTG R: TTATCGCGTGCAGCTATFACATTC	(CGT) ₆	58	173–210	4	75	0.15	0.64	AY542273	+
Dgm112	F: GGAAATAGGCTAGATGCTGTGT R: TTATTGATCTTCCGGCTGACTTTTA	(TGC) ₆ TGG	58	121–130	3	39	0.49	0.54	AY542274	+
Dgm113	F: TGCCACGAATCGTCTATAATGGTG R: AGCCACATGTAGGCACAAGTCA	(GCT) ₇	58	135–155	5	94	0.61	0.74	AY542279	+

Tris-HCl (pH 8.4), 50 mM KCl, 2 mM MgCl₂, 0.25 mM dNTPs, 0.5 µM each forward and reverse primers, 0.5 units Taq DNA polymerase (Gibco Life Technologies), and for some individuals 0.11 units TaqStart antibody (Clontech). Thermocycler conditions were 94 °C for two minutes, 35 cycles of 94 °C, 58 °C, 72 °C for one minute each, followed by a final extension at 72 °C for 30 min. PCR products were diluted, electrophoresed on an ABI377 Automated Sequencer with a ROX500 size standard, and analysed using GENESCAN version 3.1 and GENOTYPER version 2.1 (PE Biosystems).

All seven loci showed polymorphism (Table 1). Allele numbers ranged from three to six (average = 4.3). Observed heterozygosity ranged from 0.15 to 0.66 (average = 0.50), expected heterozygosity ranged from 0.47 to 0.74 (average = 0.61). Six of the seven loci showed no significant deviation from Hardy–Weinberg equilibrium, while one locus (Dgm111) did show deviation.

In two cases the reverse primers given here are degenerate and differ from the sequence of the clone used to identify the locus at the 5' end by one (Dgm107) or three (Dgm113) base pairs. However, amplification of these loci using the primer sequences given in this paper produces consistent, reproducible allele sizes when separate PCR reactions are performed on the same individual. Correct sequences are on file with GenBank.

All primer pairs have also been tested on 12–16 samples of *D. galeata galeata* from Lake Constance, Germany. All loci are polymorphic with allele sizes similar to those found in the North American *D. galeata mendotae* samples. High levels of polymorphism and cross-amplification indicate that these microsatellite loci will be useful for studies of population structure in these species. The identification

of neutral, nuclear markers amplified through PCR will permit genetic analyses of both adult and resting egg stages of *D. galeata*.

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