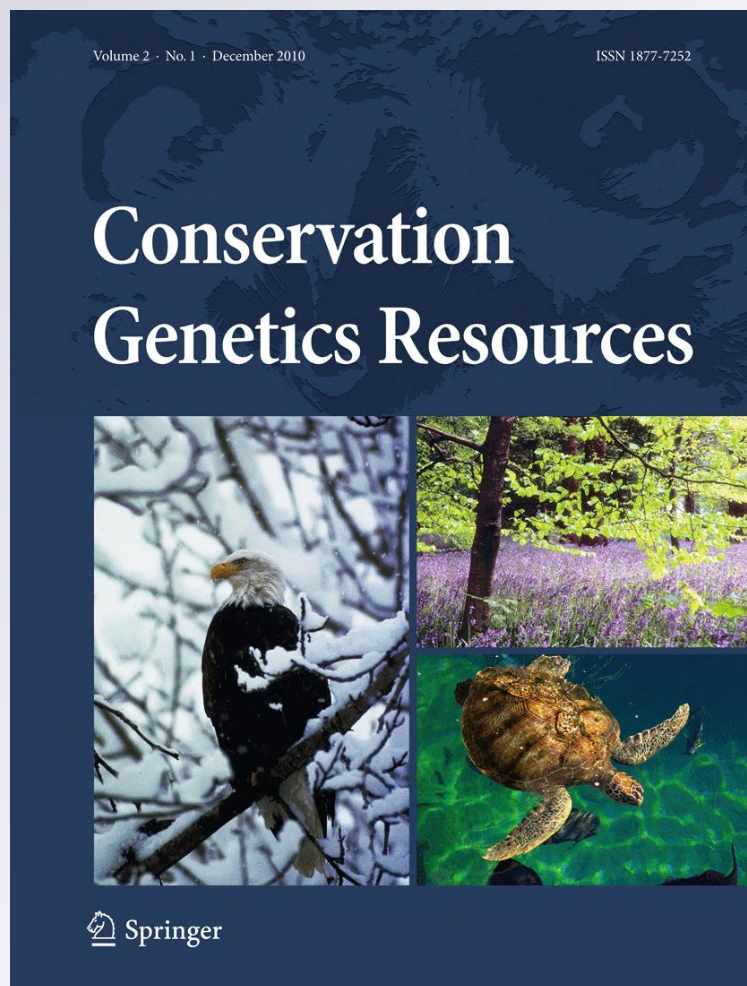
Development and characterization of 17 polymorphic microsatellite loci in the faucet snail, *Bithynia tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae)

Conservation Genetics Resources

ISSN 1877-7252
Volume 2
Number 1

Conservation Genet Resour
(2010) 2:247-250
DOI 10.1007/
s12686-010-9255-9



Springer

Your article is protected by copyright and all rights are held exclusively by Springer Science+Business Media B.V.. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Springer

Author's personal copy

Conservation Genet Resour (2010) 2:247–250
DOI 10.1007/s12686-010-9255-9

TECHNICAL NOTE

Development and characterization of 17 polymorphic microsatellite loci in the faucet snail, *Bithynia tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae)

Justin P. Henningsen · Stacey L. Lance · Kenneth L. Jones · Cris Hagen · Joshua Laurila · Rebecca A. Cole · Kathryn E. Perez

Received: 15 May 2010 / Accepted: 18 May 2010 / Published online: 4 June 2010
© Springer Science+Business Media B.V. 2010

Abstract *Bithynia tentaculata* (Linnaeus, 1758), a snail native to Europe, was introduced into the US Great Lakes in the 1870's and has spread to rivers throughout the Northeastern US and Upper Mississippi River (UMR). Trematode parasites, for which *B. tentaculata* is a host, have also been introduced and are causing widespread waterfowl mortality in the UMR. Waterfowl mortality is caused by ingestion of trematode-infected *B. tentaculata* or insects infected with parasites released from the snails. We isolated and characterized 17 microsatellite loci from the invasive faucet snail, *B. tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae). Loci were screened in 24 individuals of *B. tentaculata*. The number of alleles per locus ranged from 2 to 6, observed heterozygosity ranged from 0.050 to 0.783, and the probability of identity values ranged from 0.10 to 0.91. These new loci provide tools for examining the origin and spread of invasive populations in the US and management activities to prevent waterfowl mortality.

Keywords Microsatellite · PCR primers · SSR · STR · *Bithynia* · Faucet snail

J. P. Henningsen
Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, Amherst, MA 01003, USA

J. P. Henningsen · S. L. Lance · C. Hagen
Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA

K. L. Jones
Georgia Genomics Facility, University of Georgia, Athens, GA 30602, USA

J. Laurila · K. E. Perez (✉)
Biology Department, University of Wisconsin-La Crosse, 1725 State Street, La Crosse, WI 54601, USA
e-mail: perezke@gmail.com

R. A. Cole
United States Geological Survey, BRD National Wildlife Health Center, Madison, WI 53711, USA

Since 2002, large scale (~70,000 birds) die-offs of 15 species of Mississippi river waterfowl have been caused by infection with trematode parasites carried by an invasive aquatic snail, *Bithynia tentaculata* (Wilkins and Otto 2006). This snail was introduced into Lake Michigan from its native range in Europe and Asia in the early 1870s (Mills et al. 1993). Since that time the snail has extended its range into the Great Lakes (except Lake Superior), rivers throughout the northeastern United States, the Wolf River system in Wisconsin and the Upper Mississippi River pools 6–13 (UMR). *B. tentaculata* and its parasites have also been introduced into a number of additional, apparently isolated habitats in the Upper United States including Lake Winnibigoshish and Twin Lakes (MN) and St. Georges Lake, Rattlesnake Reservoir and Smith Lake in Montana. This study was intended to develop microsatellite markers to determine origin of the North American *B. tentaculata* populations and determine colonization routes within U.S. lakes and rivers to facilitate actions to deter further spread of this invasive species.

Total genomic DNA was extracted from several milligrams of *B. tentaculata* foot tissue using CTAB lysis buffer (Saghai-Maroof et al. 1984) and 10 µL Proteinase K (10 mg/ml). After incubation overnight at 37°C, a phenol–chloroform extraction was used to remove cell membranes and mucus, then DNA was precipitated using isopropanol and resuspended in Tris–EDTA with RNase. Enrichment

Author's personal copy

Table 1 Details for 17 polymorphic microsatellite loci developed for *Bithynia tentaculata*

Locus	Primer sequence 5′–>3′	Repeat motif	Size (bp)	*N*	*k*	H_o	H_e	PI
Bite03	F: AGACCTCCCAATGCTTCAGG R: *GCAACGCTCAAGGCAGTTA	$(AGT)_{14}$	188–209	22	5	0.773	0.697	0.14
Bite05	F: ATGGCGCAGCTAGAGTTGTA R: *CGTTTGCAAGGCACAGAGTT	$(ACTC)_{11}$	328–370	21	5	0.476	0.703	0.14
Bite09	F: *CCTGGAGGAATCTGCTGGAA R: TCAAGAGCATTGTATGTACCGC	$(GTT)_8$	206–219	23	6	0.783	0.749	0.10
Bite14	F: *GAATCTCCACCGCTTTGACG R: CGACAAGGCACTTTACCACC	$(GTTT)_6$	400–404	20	2	0.050	0.050	0.91
Bite15	F: *GCACGTTGACCAATCCTACG R: TTGTCGGTCGCAAACATAGT	$(ACAT)_6$	316–320	23	2	0.217	0.485	0.38
Bite16	F: *GCATCACGAGCAGCCTTTA R: CCATCCATGTTAGTGGAGCC	$(GTTT)_6$	296–318	20	4	0.600	0.711	0.14
Bite18	F: *GCTTACTGTCCTTCGGTG R: CCACTAAACAACGTTACCCA	$(ACT)_{10}$	286–308	23	2	0.217	0.485	0.62
Bite19	F: *GCTTCAGCAATGATCAGAAGGG R: CGGCTGTGGAGTACGTTATCA	$(AAC)_{19}$	210–243	23	3	0.565	0.610	0.22
Bite20	F: *GGCTCTTCTTCATCTGGGC R: GGTGGCTGTGTAGGCACTAT	$(ATC)_7$	185–203	24	3	0.250	0.223	0.62
Bite21	F: *GGGACTCAGCCTCGTCAATA R: CCCATAACTCCTGGGATGGT	$(GTTT)_6$	307–311	23	2	0.522	0.499	0.38
Bite22	F: *GGGAGTAGACCAAAGAGTCCA R: ACATGAGATAGGCCTTGCGG	$(AGT)_8$	136–205	24	3	0.208†	0.612	0.23
Bite29	F: *TGCATCGGTGGGTCTGATTA R: GCTAGCCTCGTATTTCCAGC	$(GTTT)_8$	228–240	23	5	0.565	0.639	0.18
Bite38	F: CTTTCAGCACTAACACAGGT R: *CCCTCTTTCATTTCCGAGCG	$(AAGT)_6$	268–316	20	2	0.050	0.219	0.63
Bite40	F: GGCAGCAGCGTTATGTTAGAA R: *GAAGTTGGCTCTGTAAGACCG	$(ATC)_7$	262–281	23	4	0.522	0.566	0.26
Bite41	F: GTCATACTGAAGTTCGCTGCT R: *AAGCCAACCACTGCTAGAT	$(AGT)_{10}$	156–159	24	2	0.125	0.187	0.68
Bite45	F: TCGGCGACGTATGTGAGATT R: *GGACTCAACGGCCTACATC	$(AGT)_7$	186–192	22	3	0.636	0.662	0.19
Bite48	F: TTTCTAGCTGCAATAGCGCC R: *AACCGTAAGGCGAGCAAAC	$(AAAC)_8$	222–228	23	2	0.261	0.287	0.55

The number of individuals genotyped is *N*; size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus

* CAG tag (5′-CAGTCGGGCGTCATCA-3′) label

† Significant deviations from Hardy–Weinberg expectations after Bonferroni corrections

procedures of Glenn and Schable (2005) were followed, with some exceptions. DNA was digested with restriction enzyme *Rsa*I (New England Biolabs), ligated to double-stranded linkers, denatured and hybridized to biotinylated microsatellite oligonucleotide mixes (mix 2 = $(AG)_{12}$ $(TG)_{12}$ $(AAC)_6$ $(AAG)_8$ $(AAT)_{12}$ $(ACT)_{12}$ $(ATC)_8$; mix 3 = $(AAAC)_6$ $(AAAG)_6$ $(AATC)_6$ $(AATG)_6$ $(ACAG)_6$ $(ACCT)_6$ $(ACTC)_6$ $(ACTG)_6$; mix 4 = $(AAAT)_8$ $(AACT)_8$ $(AAGT)_8$ $(ACAT)_8$ $(AGAT)_8$) from IDTDNA, then captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was washed away and remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SimpleX-2 as a primer. There were two primary changes to the Glenn and Schable (2005) protocol. First, a different linker was used (SimpleX-2 Forward 5′-AAAAGCTGCTGGCGAATC and SimpleX-2 Reverse 5′-pGATTCGCCAGCAGC). Second, the enriched libraries were sequenced on a 454 Genome

Author's personal copy

Sequencer FLX System using titanium chemistry following standard Roche 454 library protocols (454 Life Sciences, a Roche company, Branford CT). Sequences were subjected to a 3′ quality trim where only one base in the last 25 bases of the sequence contains a quality score less than 20 or alternatively contains one ambiguous base. CAP3 sequence assembly program (Huang and Madan 1999) was then used to assemble sequences at 98% sequence identity using a minimal overlap of 75 bp. Along with singlets, contigs of two or three sequences were searched for the presence of microsatellite DNA loci using the program MSATCOMMANDER version 0.8.1 (Faircloth 2008) and primers designed with Primer3 (Rozen and Skaletsky 2000). One primer from each pair was modified on the 5′ end with an engineered sequence (CAG tag 5′-CAGTCGGGCGTCA TCA-3′) to enable use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labeled for detection.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals of *B. tentaculata*. PCR amplifications were performed in a 12.5 μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04 μM tag labeled primer, 0.36 μM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units JumpStart Taq DNA Polymerase (Sigma), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures ranging between 60 and 50°C were used for all loci (Table 1). Touchdown cycling parameters consisted of 20 cycles of 96°C for 30 s, highest annealing temperature of 60°C (decreased 0.5°C per cycle) for 30 s, and 72°C for 30 s; and 20 cycles of 96°C for 30 s, 50°C for 30 s, and 72°C for 30 s. PCR products were run on an ABI-3130xl (Applied Biosystems) sequencer and sized with Naurox size standard prepared as described in DeWoody et al. (2004), except that unlabeled primers started with GTTT. Results were analyzed using GeneMapper version 3.7 (Applied Biosystems). Seventeen of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of 17 polymorphic loci in 24 specimens that came from a site in Pool 7 of the Upper Mississippi River (Lake Onalaska). Conditions and characteristics of the 17 loci are given in Table 1. We estimated number of alleles per locus (k), observed and expected heterozygosity (H_o and H_e), and probability of identity (PI) using GenAlEx v6.0 (Peakall and Smouse 2006). Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Bonferroni correction for multiple comparisons 1 locus showed significant deviation from expectations under HWE and no linkage disequilibrium was detected for any pairs of loci out of 136 paired loci comparisons. These microsatellite loci will allow researchers to study the *B. tentaculata* invasion and assist management efforts. Specifically, we plan to use these markers to determine the origins of invasive *B. tentaculata* populations in the United States and examine levels of genetic diversity in invasive populations. This work has the potential to allow us to infer colonization routes and prevent further invasive spread of this species.

Acknowledgments This work was supported by funds from the United States Geological Survey, the River Studies Center at UWL, the University of Wisconsin Institute for Race and Ethnicity Faculty Diversity Research Grant, UWL College of Science and Health Dean's Undergraduate Fellowship Program, UWL Faculty Research Grant, and a National Science Foundation Graduate Research Fellowship to JPH. Tissue collection was assisted by Roger Haro, Greg Sandland, and Ben Walker with funding by the United States Corp of Engineers. Manuscript preparation was partially supported by the Department of Energy under Award Number DE-FC09-07SR22506 to the University of Georgia Research Foundation. *Disclaimer* "This report was prepared as an account of work sponsored by an agency of the United States Government, the Department of Energy. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof."

References

DeWoody AJ, Schupp J, Kenefic LL, Busch J, Murfitt L, Keim P (2004) Universal method for producing ROX-labeled size standards suitable for automated genotyping. BioTechniques 37:348–350

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19:4008

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol 8:92–94

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. Methods Enzym 395:202–222

Huang X, Madan A (1999) CAP3: a DNA sequence assembly program. Genome Res 9:868–877

Mills EL, Leach JH, Carlton JT, Seacor CL (1993) Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. J Great Lakes Res 19:1–54

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molec Ecol Notes 6:288–295

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Res 8:103–106

Author's personal copy

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386 (Source code available at http://fokker.wi.mit.edu/primer3/)

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length variation in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8018–8021

Wilkins KA, Otto MC (2006) Preliminary report: trends in duck breeding populations, 1955–2006. U.S. Fish and Wildlife Service, Division of Migratory Bird Management. 19 pp