

PRIMER NOTE

Genetic variation in two populations of the rough-skinned newt (*Taricha granulosa*) assessed using novel tetranucleotide microsatellite loci

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Abstract

Seven novel tetranucleotide microsatellite loci were identified from a partial genomic DNA library, enriched for GATA-motif microsatellites, from the rough-skinned newt (*Taricha granulosa*). All loci were polymorphic, and one displayed a high frequency null allele. A related species, *T. rivularis*, displays strong site fidelity and detectable population structure over small spatial scales, so we assessed genetic variation in two samples of *T. granulosa* separated by 16 km. Distributions of allele frequencies differ significantly between our two sites, but small values of F_{ST} and Rho_{ST} suggest that the populations are linked by a large amount of gene flow.

Keywords: homing, microsatellites, newts, parentage, population structure, site fidelity

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Newts of the genus *Taricha* are evolutionarily and ecologically interesting for a variety of reasons, including homing behaviour (Twitty *et al.* 1964), site fidelity (Twitty 1966), predator–prey coevolution (Brodie & Brodie 1999), and sexual selection (Janzen & Brodie 1989). Polymorphic microsatellite loci should contribute to continued progress in these and other areas of newt research. Here we report the characterization of novel *Taricha granulosa* microsatellite loci. Our primary use of these markers has been in the assessment of parentage, and as a by-product of these studies we have generated genotypic data that allow a preliminary assessment of small-scale population structure in two Oregon populations of this species.

Adult *T. granulosa* were collected from two sites, one near the Benton County Fair Grounds (44°33'56" N, 123°19'17" W) in Corvallis, Oregon, and the other in the E. E. Wilson Wildlife Area (44°41'18" N, 123°12'29" W), approximately 16 km to the north of the Fair Grounds. We collected newts either by picking up migrating individuals during rainy nights or by submerging plastic minnow traps in bodies of water in which newts resided. A segment of tail 2–5 mm in length was cut from each newt and preserved at –80 °C. Genomic DNA was extracted from each tissue

sample by a standard proteinase K, phenol–chloroform procedure (Sambrook *et al.* 1989).

Genomic DNA from a single *T. granulosa* specimen was used to construct a partial genomic library enriched for GATA-motif microsatellite sequences. The enrichment protocol was a modification of the biotinylated oligonucleotide procedure described by Kijas *et al.* (1994). In our version of the procedure, genomic DNA was digested in two separate reactions with *RsaI* and *HaeIII*, and the 400–700 bp fragments were isolated by gel electrophoresis. These blunt-ended fragments were ligated into *EcoRV* digested, dephosphorylated pBluescript phagemid (Stratagene). We performed asymmetric polymerase chain reaction (PCR) with this ligation as the template by using a 10-fold excess of one primer, resulting in a final concentration of 50 µM of the T3 primer (5'-ATTAACCCTCACTAAAGGGA-3') and 5 µM of the T7 primer (5'-TAATACGACTCACTATAGGG-3'). A biotinylated (GATA)₆ oligonucleotide was affixed to 50 µL streptavidin-coated magnetic beads (Promega) following the manufacturer's recommendations. Hybridization of our asymmetric PCR product to the bead-bound oligonucleotide and subsequent washes followed the procedures outlined in Kijas *et al.* (1994), except that our wash solutions contained the T3 and T7 primers instead of the USP and RSP primers.

Enriched DNA was released from the oligonucleotide-bead complex by heating a final high stringency wash to

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Table 1 Key information for seven novel *Taricha granulosa* microsatellite loci. Shown for each locus are the primer sequences, the fluorescent dye used, the microsatellite sequence that was detected in the original positive clone, and the absolute size of the PCR product expected from the clone in base pairs (a value that differs slightly from the allele size reported by the ABI377). Also shown, for each of the two focal populations are the number of adults assayed, the number of alleles per locus, and heterozygosities. The final two columns show the F_{ST} and Rho_{ST} measures of population structure for these two populations

Locus	GenBank accession no.	Primer sequence (5'–3')	Fluor. label	Cloned repeat	Size of cloned allele	Benton County Fair Grounds			E. E. Wilson			F_{ST}	Rho_{ST}
						n	no. of alleles	H_O/H_E	n	no. of alleles	H_O/H_E		
<i>Tgr01</i>	AF388949	GGACGGGACATCTGCTGA GTGTGCGAGTTGTGGATTTC	6-FAM	(TATC) ₁₃	228	121	12	0.90/0.87	134	12	0.83/0.88	0.010	–0.003
<i>Tgr02</i>	AF388950	CCATATTACACTGTTACCTGTTA AGTGGGGGTTGTCTTTCT	HEX	(GATA) ₁₃	195	87	18	0.90/0.91	134	18	0.93/0.91	0.003	–0.003
<i>Tgr04</i>	AF388951	AAACGGCACCAAAGCTATT AATGAACCCAAACTATAACTACC	TET	(GATA) ₁₂	225	121	10	0.54/0.79	134	9	0.63/0.84	–	–
<i>Tgr06</i>	AF388952	AAAACCAGCAGCATCAGTCC AGAGTGAGCCAAGTGACAATCC	HEX	(AGAT) ₁₄	158	121	8	0.80/0.83	134	9	0.81/0.80	0.003	–0.002
<i>Tgr10</i>	AF388953	TGTTGCCCTCTACCAATCC GACCAGATACCCACAGTTGTC	TET	(TAGA) ₁₄	183	121	16	0.93/0.91	134	15	0.89/0.92	0.003	0.005
<i>Tgr13</i>	AF388954	CAACTTGGGAAAGCTGTAAC ATGCCACTTCTAACAACGGT	6-FAM	(TAGA) ₇	140	21	3	0.57/0.61	–	–	–	–	–
<i>Tgr14</i>	AF388955	CCAGCAAATCAGGTCAGAA TTTGAAGGGCAAGTTGGTAAT	6-FAM	(GATA) ₃₁ *	279	121	26	0.94/0.95	134	28	0.92/0.95	0.004	–0.004

*This microsatellite departed from perfection in two places, yielding an overall sequence of (GATA)₁₂GATTA(GATA)₂GAAA(GATA)₁₇.

90 °C for 10 min and retaining the supernatant. The enriched DNA was then purified using a PCR purification spin column (Qiagen) and was used as template for PCR with the T3 and T7 primers. The double-stranded product of this PCR was then spin-column purified, digested with *Bam*HI and *Hind*III, and ligated into *Bam*HI, *Hind*III digested, dephosphorylated pBluescript phagemid. The ligation was transformed into competent *Escherichia coli*, and the resulting library was screened using a (GATA)₄ oligonucleotide end-labelled with ³²P. Inserts of positive clones were sequenced and PCR primers were designed to amplify the microsatellites of those with adequate flanking sequences. Seven functional pairs of primers were obtained (Table 1).

For the assay of microsatellite genotypes from newt genomic DNA, each PCR was carried out in a 20- μ L reaction volume, containing *Taq* buffer (50 mM KCl, 10 mM Tris-HCl, pH 9.0, and 0.1% Triton X-100), an optimal MgCl₂ concentration, 0.15 μ M each primer, 0.2 mM of each dNTP, and 0.5 units *Taq* polymerase. Thermal cycling was preceded by a denaturation period of 2 min at 95 °C and followed by a 2 min extension period at 72 °C. Each PCR run comprised 35 cycles, each of which consisted of 1 min at 92 °C, 1 min at the optimal annealing temperature, and

2 min at 72 °C. For loci *Tgr01*, *Tgr02*, *Tgr04*, and *Tgr06*, 1.5 mM MgCl₂ and an annealing temperature of 56 °C were used. *Tgr10*, *Tgr14* and *Tgr13* were annealed at 52 °C, with MgCl₂ concentrations of 1.75 mM, 1.75 mM and 2.0 mM, respectively. One primer from each pair was synthesized with an attached fluorescent dye (Table 1) capable of being detected by an Applied Biosystems (ABI) sequencer. Fragments were separated and visualized by the Central Services Laboratory at Oregon State University on an ABI 377 sequencer with appropriate fragment-analysis software. Alleles were sized relative to the GeneScan-500 internal standard. Consistent with expectations for tetranucleotide loci, all alleles appeared to differ from one another by multiples of 4 bp, and their reported sizes were rounded to reflect this observation (e.g. at *Tgr14*, 241.21 was rounded to 241, whereas 308.17 was rounded to 309; Fig. 1). Analyses of Hardy–Weinberg equilibrium, genotypic disequilibrium, F_{ST} , and Rho_{ST} were conducted using GENEPOP, version 3.1b (Raymond & Rousset 1995).

All microsatellite loci identified from our *T. granulosa* library were polymorphic (Table 1). Highly significant departures from Hardy–Weinberg equilibrium (Table 1) and unexpected segregation patterns in progeny arrays

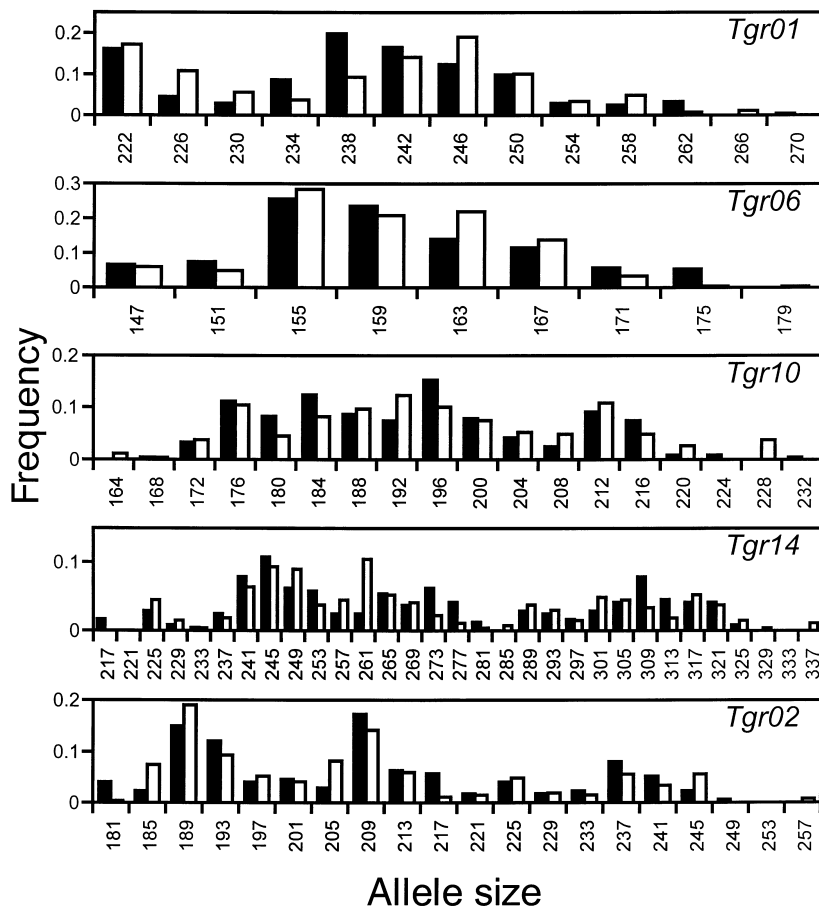


Fig. 1 Allele frequency histograms for the Benton County Fair Grounds (solid bars) and E. E. Wilson (open bars) populations of *Taricha granulosa*. Allele names represent the sizes of PCR fragments as assessed by an ABI 377 sequencer. For each locus, the distribution of allele frequencies differs significantly between populations ($P < 0.001$).

(data not shown) clearly demonstrated that a nonamplifying (null) allele was segregating at *Tgr04* in both populations. Only three alleles were observed at *Tgr13*, rendering it almost useless for parentage analysis (our primary use of these markers). For these reasons, *Tgr04* and *Tgr13* were not included in our analysis of population structure.

Aside from those seen at *Tgr04*, the only significant departures from Hardy–Weinberg expectations were observed at *Tgr10* in the Fair Grounds population ($P = 0.02$) and *Tgr01* in the E. E. Wilson population ($P = 0.01$). We found no evidence that any pair of loci was in genotypic disequilibrium in either population. The distributions of allele frequencies in the two populations (Fig. 1) differed significantly at every locus (all P -values < 0.001). However, the average F_{ST} between these populations for our five microsatellite loci was merely 0.005 (Table 1) and the average Rho_{ST} was -0.002 (Table 1).

Thus, even though our samples clearly are not drawn from a single panmictic population, we failed to detect appreciable genetic structure over the small geographical scale under consideration. This result contrasts with the genetic results from a similar, allozyme-based study of *T. rivularis* (Hedgecock 1978). *T. rivularis* displayed small but detectable population structure over a similar geographical scale. For 12 samples collected from a transect no more than 26 km in length, Hedgecock (1978) reported significant heterogeneity in allele frequencies and an F_{ST} of 0.062, a value 12 times the size of ours. To the extent that we can trust comparisons between genetic studies utilizing markers with very different levels of polymorphism (Hedrick 1999), our results suggest that *T. granulosa* may exhibit less site fidelity than *T. rivularis*. Further research using these microsatellite loci should shed additional light on this and other interesting topics in newt ecology and evolution.

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References

- Brodie ED III & Brodie ED Jr (1999) Costs of exploiting poisonous prey: Evolutionary trade-offs in a predator-prey arms race. *Evolution*, **53**, 626–631.
- Hedgecock D (1978) Population subdivision and genetic divergence in the red-bellied newt, *Taricha rivularis*. *Evolution*, **32**, 271–286.
- Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.
- Janzen FJ & Brodie ED III (1989) Tall tails and sexy males: Sexual behavior of rough-skinned newts (*Taricha granulosa*) in a natural breeding pond. *Copeia*, **1989**, 1068–1071.
- Kijas JMH, Fowler JCS, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *Biotechniques*, **16**, 657–662.
- Raymond M & Rousset F (1995) GENEPOP: population genetics software for exact tests and ecumenicism. Vers. 1.2. *Journal of Heredity*, **86**, 248–249.
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Twitty VC (1966) *Of Scientists and Salamanders*. W. H. Freeman, San Francisco, California.
- Twitty VC, Grant D, Anderson O (1964) Long distance homing in the newt *Taricha rivularis*. *Proceedings of the National Academy of Sciences of the USA*, **51**, 51–58.