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 FST and RhoST 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’-ATTACCCTCACTAAAGGGA-3’) and 5 μM of the T7 primer (5’-TAATACGACTCACTATAGGG-3’). A biotinylated (GATA) 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
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.

<table>
<thead>
<tr>
<th>Locus</th>
<th>GenBank</th>
<th>Primer sequence (5'-3')</th>
<th>Fluor. label</th>
<th>Cloned repeat</th>
<th>Size of cloned allele</th>
<th>Benton County Fair Grounds</th>
<th>E. E. Wilson</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>accession no.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>no. of alleles</td>
<td>$H_{O}$/$H_{E}$</td>
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<tr>
<td>Tgr01</td>
<td>AF388949</td>
<td>GGACGGGACATCTGCTGA</td>
<td>6-FAM</td>
<td>(TATC)$_{13}$</td>
<td>228</td>
<td>121</td>
<td>12</td>
<td>0.90/0.87</td>
</tr>
<tr>
<td>Tgr02</td>
<td>AF388950</td>
<td>GCTGCGATGGGAGATTTCC</td>
<td>HEX</td>
<td>(GATA)$_{13}$</td>
<td>195</td>
<td>87</td>
<td>18</td>
<td>0.90/0.91</td>
</tr>
<tr>
<td>Tgr04</td>
<td>AF388951</td>
<td>AATACATTACCTACTACATTGTA</td>
<td>TET</td>
<td>(GATA)$_{12}$</td>
<td>225</td>
<td>121</td>
<td>10</td>
<td>0.54/0.79</td>
</tr>
<tr>
<td>Tgr06</td>
<td>AF388952</td>
<td>AACTACGGCAAGATGAATTC</td>
<td>HEX</td>
<td>(AGAT)$_{14}$</td>
<td>158</td>
<td>121</td>
<td>8</td>
<td>0.80/0.83</td>
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<tr>
<td>Tgr10</td>
<td>AF388953</td>
<td>TGTCCCTCTACACAATCC</td>
<td>TET</td>
<td>(TACA)$_{14}$</td>
<td>183</td>
<td>121</td>
<td>16</td>
<td>0.93/0.91</td>
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<td>Tgr13</td>
<td>AF388954</td>
<td>CAATTGAAAGAATGAACTCT</td>
<td>6-FAM</td>
<td>(TACA)$_{14}$</td>
<td>140</td>
<td>21</td>
<td>3</td>
<td>0.57/0.61</td>
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<tr>
<td>Tgr14</td>
<td>AF388955</td>
<td>CCAAGGCAATCAGGGAGA</td>
<td>6-FAM</td>
<td>(GATA)$_{2}^*$</td>
<td>279</td>
<td>121</td>
<td>26</td>
<td>0.94/0.95</td>
</tr>
</tbody>
</table>

*This microsatellite departed from perfection in two places, yielding an overall sequence of (GATA)$_{12}$GATTA(GATA)$_{12}$GAAA(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 BamHI and HindIII, and ligated into BamHI, HindIII 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-μL 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 μM 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, Fₛₜ and Rₛₜ 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...
(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 $R_{HI}$ 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