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MICROSATELLITE LETTERS

# Development of 31 new microsatellite loci for two mole salamanders (*Ambystoma laterale* and *A. jeffersonianum*)

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**Abstract** *Ambystoma* salamanders are amphibians that due to limited dispersal abilities and reliance on wetlands for breeding are susceptible to population declines and local extinctions (Blaustein et al. 2011). Species identification within *Ambystoma* is especially difficult due to the presence of unisexual *Ambystoma* that consist of multiple all-female lineages in which clones can have between two and five nuclear genomes from up to five other *Ambystoma* species (Bogart et al. 2007). The majority of these unisexual *Ambystoma* are composed of nuclear genomes from two species, *A. laterale* (Blue Spotted Salamander) and *A. jeffersonianum* (Jefferson Salamander). We developed species-specific microsatellite markers for these two species as a tool for the identification and investigation of the genetic interactions between sexual and unisexual groups in areas where either sexual species is endangered or of special conservation concern (Ohio, Indiana, and Ontario).

**Keywords** Ambystoma · Microsatellites · Mole salamanders

Microsatellites were isolated as described in Kartzinel et al. (2012). Primers which amplified 144 loci (96 from two separate *A. laterale* libraries, 48 from an *A. jeffersonianum* library) and were initially screened across four individuals from each species. Those that showed amplification and polymorphism within a single species were then assayed in an additional 16 individuals sampled from across the species' geographic range (Supplementary Table S1). PCR reactions for all primers were carried out in 10 µl reactions and consisted of 3.9 µl ddH20, 0.3 µl of mixed forward (CAG or M13) and reverse primer (untagged), 0.3 µl labelled M13R/CAG tag (6-FAM, HEX, or NED), 5 µl BioMix$^{TM}$ Red (BIOLINE), and 0.5 µl of template DNA. Primers were tested using the following temperature profile. First, each reaction was held at 95 °C for 2 min 30 s followed by 20 touchdown cycles (95 °C for 20 s, 60 °C for 20 s with a 0.5 °C decrease per cycle, and 72 °C for 30 s). The reaction was then subject to 15 cycles consisting of 95 °C for 20 s, 50 °C for 20 s, and 72 °C for 30 s. Finally, reactions were held at 72 °C for 10 min. PCR products were analyze on either a Applied Biosystems 3100 or 3730 DNA sequencer using a ROX-labeled internal size standard (GeneScan 500 ROX, Applied Biosystems). Profiles were analyzed using Genemapper software (version 4.1, Applied Biosystems).

We identified 15 species-specific loci in *A. laterale* (11 polymorphic, 4 monomorphic) and 13 species-specific loci in *A. jeffersonianum* (all polymorphic; Table 1). In addition, we characterized four loci that amplify in both species at non-overlapping size ranges. We calculated observed and expected heterozygosities using GenAlEx (Peakall and Smouse 2012; Table 1). There was no strong evidence of linkage disequilibrium (2 of 170 pairwise comparisons). Overall, the loci produced a similar average numbers of alleles (*A. laterale*: 5.29 ± 0.97 SE; *A. jeffersonianum*: 5.47 ± 0.89 SE), and the range of observed and expected heterozygosities varied ($H_o$: 0.053–1.000;

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**Table 1** Description of 31 loci that amplify consistently in *Ambystoma laterale* and *A. jeffersonianum*

| Species | Locus | Primer sequence (5′–3′) | Repeat motif | Size (bp) | N | $N_a$ | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|---|---|
| *Ambystoma laterale* | 1003-626 | F-CGACCACCTGACTAGGACCC<br>R-ACTTGCTTGTTCCCCTGCC | AAAGAG (36) | 150–207 | 27 | 7 | 0.185 | 0.440 |
| | 1400-1610 | F-GGTTGGGGAATTCTATCTATCCC<br>R-GCCTGTTCTGTGTCAGATTTGC | AAAG (44) | 238–357 | 27 | 15 | 0.704 | 0.827 |
| | 1433-688 | F-AGAGCATTGTTCCTCCAGGG<br>R-CAAGGTCGATCTGGTGAGGG | AAAG (76) | 216–250 | 11 | 6 | 0.545 | 0.455 |
| | 1483-1085 | F-ACAATCAGACAACTAAGAGCACTGG<br>R-CCCAGATACCCCTAGGTTTGG | AAAC (40) | 168–176 | 15 | 2 | 1.000 | 0.500 |
| | 1707-1501 | F-TCGATTAATTTCATCAAAATAGCTGC<br>R-TTCTTTACTGTTGCGCCCG | AAAC (24) | 155–159 | 15 | 2 | 0.533 | 0.480 |
| | 1944-51 | F-CAAAGGGGACTATCGGGTAGC<br>R-AACGGTGAAGGGTGACAAGG | ATAC (36) | 206 | 15 | 1 | 0.000 | 0.000 |
| | 540-1773 | F-ATTAAGAGGCCCCTGCTTGG<br>R-ACAGGTGCGTTATGAATGCC | ATATC (35) | 140–195 | 29 | 9 | 0.690 | 0.782 |
| | AmJef13 | F-AAGCCCTTGGTGTCTTATC<br>R-GTTTGCTACCTAACTGCCTGCTAG | AAAC (6) | 270 | 20 | 1 | 0.000 | 0.000 |
| | AmJef44 | F-CTTCAGCCGATCCCTCCC<br>R-GTTTGGTAGTCGGCTGATAGAGTG | AGAT (15) | 194 | 19 | 1 | 0.000 | 0.000 |
| | AmLat13 | F-TTCTTGGGCTTTCTCACAGC<br>R-GTTTGGGTCTGACTGCGCCTTAC[b] | AAAC (6) | 196–200 | 24 | 3 | 0.625 | 0.471 |
| | AmLat24 | F-ACACCTAATGCCCGAGAACC<br>R-GTTTCCTGTGCGCTTACAAATACG | AAAT (8) | 216–235 | 34 | 9 | 0.441 | 0.744 |
| | AmLat26 | F-ACCAGTGAAAGTGCAACAAG<br>R-GTTTAACCTGTAATCTGCAACCTG | AGAT (15), AGAT (18) | 164 | 18 | 1 | 0.000 | 0.000 |
| | AmLat37 | F-TCTTGCAACACTGGGCAC<br>R-GTTTGCGGAACTACTGTGCTGAAC | AATG (12) | 303–327 | 15 | 6 | 0.267 | 0.698 |
| | AmLat38 | F-GACCCTACCCTATAAC<br>R-GTTTGTACAAGCCCGTCTATCTC | AAAC (6) | 245–258 | 34 | 4 | 0.971 | 0.624 |
| | AmLat44 | F-GCTACTTACGGGTCTGGTG<br>R-GTTTGTCAACACCAAATTGCTGCG | AAAC (7) | 199–203 | 19 | 2 | 0.053 | 0.051 |
| | AmJef21[a] | F-GGTGATATGTTCGTTTGTTG<br>R-GTTTGTCTGTTGTCTCCACGCTAC | AAAC (7) | 98–114 | 35 | 3 | 0.743 | 0.490 |
| | AmLat16[a] | F-CGGAACTACAATTCAGGCTCC<br>R-GTTTGGGAAGCTTGCTTACACAGG | ACAT (10) | 312–328 | 20 | 4 | 0.950 | 0.589 |
| | AmLat33[a] | F-TGGACTGTGTAGGAGGCTC<br>R-GTTTAGACACGGAAATTAGCAGCG | AAAG (6) | 440–452 | 15 | 2 | 1.000 | 0.500 |
| | AmLat40[a] | F-CCTCGCATTAGAACTCAGGC<br>R-GTTTGCTGCGGAACTACAAACTG | ACAT (7), AAAT (7) | 359 | 19 | 1 | 0.000 | 0.000 |
| *Ambystoma jeffersonianum* | AmJef01 | F-CCTAGGTTTCACTTGCTTTC<br>R-GTTTCGAACTGGACAATAGCTATG[b] | AAG (9) | 396–408 | 16 | 4 | 0.500 | 0.662 |
| | AmJef09 | F-CTTCCATGCTTGTATCC<br>R-GTTTAGTTGTGATTGGATGCATTC[b] | AAAC (6) | 307–327 | 19 | 5 | 1.000 | 0.632 |
| | AmJef20 | F-GCCATAATTAAATGACTGC<br>R-GTTTGTTATTTCTGTACCGAGTC | AC (9) | 376–426 | 18 | 6 | 0.278 | 0.711 |

**Table 1** continued

| Species | Locus | Primer sequence (5′–3′) | Repeat motif | Size (bp) | N | $N_a$ | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|---|---|
| | AmJef22 | F-CCCTATTAGCACCTTACCAG | AAAC (7) | 356–372 | 52 | 5 | 0.500 | 0.583 |
| | | R-GTTTGGCTACTTACCCATTTATGC | | | | | | |
| | AmJef23 | F-ACCTTTCCTACTGCTCCAC | AAAC (7) | 343–388 | 54 | 10 | 0.685 | 0.704 |
| | | R-GTTTCTGCCTCACGTTAATAGAGG | | | | | | |
| | AmJef25 | F-CTACCCACAACCTTAAGAGC | AAAC (7) | 356–382 | 18 | 4 | 0.278 | 0.250 |
| | | R- GTTTGGCTTAACATCTTGTCAG | | | | | | |
| | AmJef28 | F-CCTTTGATCTTATGGACCTC | AAAC (7) | 354–366 | 18 | 2 | 0.056 | 0.054 |
| | | R-GTTTGGGAGCGTTGTTATGTATTC | | | | | | |
| | AmJef29 | F-TGTGCAAACCATACTACG | AAAC (7) | 244–366 | 52 | 13 | 0.660 | 0.867 |
| | | R-GTTTGTAATCATTCGAGGCATACC | | | | | | |
| | AmJef30 | F-GGAAATAGGCTTCAGAGTTG | AAAC (6) | 356–368 | 18 | 3 | 0.333 | 0.406 |
| | | R-GTTTGTTACCCTTGGGTATTATGC | | | | | | |
| | AmJef32 | F-GGGACTATGAGTTCACGTTTC | AAAC (6) | 355–363 | 18 | 3 | 0.333 | 0.593 |
| | | R-GTTTGCGCTTCTACATGGATAATC | | | | | | |
| | AmJef42 | F-CTTGTTCTCAACCCATTTC | AAAG (16) | 298–354 | 52 | 15 | 0.558 | 0.875 |
| | | R-GTTTAGATAATTGCGCACGTTAC | | | | | | |
| | AmJef46 | F-ACCTCTGCCCTGTAAGATC | AAAC (6) | 302–306 | 18 | 2 | 0.222 | 0.198 |
| | | R-GTTTGTTAAGGGCATTGGTGTG | | | | | | |
| | AmJef21[a] | F-GGTGATATGTTCGTTTGTTG | AAAC (7) | 377–385 | 53 | 3 | 0.415 | 0.412 |
| | | R-GTTTGTCTGTTGTCTCCACGCTAC | | | | | | |
| | AmLat16[a] | F-CGGAACTACAATTCAGGCTCC | ACAT (10) | 307–315 | 13 | 3 | 0.923 | 0.568 |
| | | R-GTTTGGGAAGCTTGCTTACACAGG | | | | | | |
| | AmLat33[a] | F-TGGACTGTGTAGGAGGCTC | AAAG (6) | 441–477 | 16 | 7 | 0.688 | 0.695 |
| | | R-GTTTAGACACGGAAATTAGCAGCG | | | | | | |
| | AmLat40[a] | F-CCTCGCATTAGAACTCAGGC | ACAT (7), AAAT (7) | 284–296 | 17 | 4 | 0.588 | 0.469 |
| | | R-GTTTGCTGCGGAACTACAAACTG | | | | | | |

All other remaining loci were M13 tagged; type is the tagging protocol used for each locus; range is the range of the observed alleles; N is the total number of individuals in which the primer amplifies, $N_a$ is the total number of alleles across all N; $H_e$ and $H_o$ are expected and observed heterozygosities, respectively

[a] Locus that amplifies in both species at different size ranges

[b] Primer that was CAG-tagged

$H_e$: 0.051–0.875). Because of the lack of sufficient numbers of individuals per population, we report no tests of Hardy–Weinberg Equilibrium. However, we acknowledge the importance of detecting null alleles and encourage investigators to test for null alleles if using the loci described here. While the number of alleles per locus is within the lower range of other microsatellite markers, these new loci provide a valuable addition to other microsatellite markers that have been cross-screened with multiple *Ambystoma* species (Peterman et al. 2012). Specifically, the loci that are fixed within species provide a valuable tool for identifying and assessing the population genetics of salamanders with the unisexual *Ambystoma* complex, which mainly consist of genomes from *A. laterale* and *A. jeffersonianum*.

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