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PERMANENT GENETIC RESOURCES

Polymorphic tetranucleotide microsatellites for Cope's giant salamander (*Dicamptodon copei*) and Pacific giant salamander (*Dicamptodon tenebrosus*)

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Abstract

We present primers and amplification conditions for 15 microsatellite loci developed for the Cope's giant salamander (*Dicamptodon copei*), 14 of which are tetranucleotide repeats. Cross-species amplification revealed 10 of these loci to also be polymorphic in the Pacific giant salamander (*Dicamptodon tenebrosus*). Several loci produced nonoverlapping allelic ranges between the two species and may be useful in species identification. These polymorphic microsatellite loci are potentially useful for future studies of population genetics in dicamptodontid salamanders.

Keywords: cross-species amplification, Dicamptodontidae, molecular markers, Pacific Northwest, population genetics

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The Cope's giant salamander (*Dicamptodon copei*) and the Pacific giant salamander (*Dicamptodon tenebrosus*) are endemic to the Pacific Northwest of the USA. Both species are stream breeding, but only *D. tenebrosus* regularly metamorphoses into a terrestrial adult. Because of its resemblance to larval *D. tenebrosus*, *D. copei* was not recognized as a species until a detailed morphological analysis elevated it to species status (Nussbaum 1970, 1976). Recently, a genetic study was initiated in order to assess comparative levels of population structure for sympatric populations of the two species (Steele *et al*. submitted) and necessitated the development of microsatellite markers for population-level genetic studies. Despite several microsatellite loci available for *D. tenebrosus*, most of which have been found to cross-amplify in *D. copei* (Curtis & Taylor 2000), there remained a need for additional, highly polymorphic loci to address specific questions of population genetics in these species. We describe highly polymorphic tetranucleotide microsatellite markers for *D. copei* and *D. tenebrosus* that can be used in conjunction with previously developed microsatellite markers to facilitate future studies of population genetics of these species.

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Total genomic DNA from one *D. copei* was extracted from a tail clip using a standard proteinase-K digestion and phenol–chloroform extraction protocol (Sambrook *et al*. 1989). An enriched library was made by ecogenics GmbH from size-selected genomic DNA ligated into *Sau*LA/*Sau*LB-linker (Armour *et al*. 1994) and enriched by magnetic bead selection with biotin-labelled $(ACAG)_7$ and $(GATA)_7$ oligonucleotide repeats (Gautschi *et al*. 2000a, b). Of 566 recombinant colonies screened, 82 gave a positive signal after hybridization. Plasmids from 65 positive clones were sequenced and primers designed for 25 microsatellite inserts. Of these, 21 were tested for polymorphism. Primer design was carried out using PRIMER 3 software (Rozen & Skaletsky 2000).

To assay variation among individuals, polymerase chain reaction (PCR) amplification of each primer pair was conducted in a 15.0-μL total volume on an iCycler (Bio-Rad, Inc) or an ABI 2720 (Applied Biosystems) thermocycler. PCRs consisted of: 1× PCR buffer, 1.5 mM $MgCl_2$, 10–20 ng genomic DNA, 0.2 μM each of a labelled forward and unlabelled reverse primer, 150 μM dNTPs and 0.5 U *Taq* DNA polymerase (Applied Biosystems). The amplification profile included: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53–60 °C (Table 1) for 30 s and elongation at 72 °C for 30 s, and a final extension at 72 °C for 10 min. PCR

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Table 1 Locus name, forward (F) primer with fluorescent dye label and reverse (R) primer sequences, repeat unit, annealing temperature (T_a) in °C, and GenBank Accession number for microsatellite loci

Locus	Primer sequence (5′–3′)	Repeat unit	T_a	GenBank ID
D04	F: (PET)GAAACTATTTTATCAAAAGCATGC R: TCTAAATATGTGTATGGGTGTATAAG	$(TATC)_{18}(TGTC)_{14}$	53	EU257639
D05	F: (NED)ATCCGACCTACCACGAGATG R: GGCAATTATCATAGCCAGCAG	$(GATA)_{16}(GA)_9$	60	EU257640
D06	F: (PET)GACAAATGGATAGCTGCATAGC R: GCCTTCTGAATTGGGTGAAG	$(GATA)_2GACA(GATA)_3GACA(GATA)_9$	56	EU257641
D07	F: (NED)TGGTGACCTGGGAACACTAAG R: CATGTTGCACAGCATCGTC	$(GATA)_{24}$	60	EU257642
D08	F: (NED)TGTGCACGGACTACACTTTAGG R: CCAAGATGCCTCTTTTGGTG	$(TATC)_{16}$	53	EU257643
D13	F: (VIC)CAGGGCAATATGACCTAGTCG R: TGGGGGTAACCTGCAACAG	$(CTAT)_{11}$	53	EU257644
D14	F: (6-FAM)TGCTTCTGAGCAATTATTGTGG R: AGATTGGTGTGTAGGTGGTTG	$(CTAT)_{17}$	53	EU257645
D15	F: (6-FAM)GTGTGTCTGAAGTGGCAAGG R: AGCCCACTGATTCTACGAGAG	$(CTAT)_{13}CTGT(CTAT)_5CTAC(CTAT)_2$	56	EU257646
D17	F: (PET)TCCCCAGTAGGCCTTGTGAC R: GCTCTTGTCCGGTCTTACTTG	$(CTAT)_7CTAA(CTAT)_{12}$	60	EU257647
D18	F: (6-FAM)AAGGCTGGAAGGTTTTATGC R: TGCTAACCGCTCAGATTCAC	$(CTAT)_{15}$	53	EU257648
D20	F: (VIC)TCAGTGTGGAGGGTGAGTTG R: GGCGCAGGTCATAAAAGG	$(GATA)_{18}$	60	EU257649
D22	F: (PET)ATAGACAGGTAGGTAGGGGTAGC R: CCTATCTACATCTATCAATCCCTACG	$(GATA)_3TGTA(GATA)_9$	53	EU257650
D23	F: (VIC)GGTTGGTCTTACCTCCAAGG R: CATTACCCCTACCATTCGTG	$(GATA)_{12}$	56	EU257651
D24	F: (6-FAM)CAACATAATACTGATGGTGTTTGC R: AGAATAAATGGCCGTTTTGG	$(CTAT)_{24}$	60	EU257652
D25	F: (NED)TGGGCAATGTGACTTTATGTC R: CTGTTATCCCGACTAAAGAATTTC	$(GATA)_{10}$	53	EU257653

products were multiplexed on an ABI 3730 automated sequencer (Applied Biosystems) with a LIZ 500 size standard run concurrently with each sample. The software GENEMAPPER version 3.7 (Applied Biosystems) was used to score alleles. The presence of null alleles was tested using MICRO-CHECKER (van Oosterhout *et al*. 2004). Levels of observed and expected heterozygosities were determined and tests for departure from Hardy–Weinberg equilibrium and linkage disequilibrium were performed using GENEPOP (Raymond & Rousset 1995).

All 15 loci were polymorphic in *D. copei*, while 10 of the 11 cross-amplifying loci were polymorphic in *D. tenebrosus* (Table 2). No loci were identified as having null alleles in *D. copei*. Loci D04 and D05 in *D. tenebrosus* contained an excess of homozygotes ($P < 0.05$) which may indicate null alleles. The number of homozygous genotypes at these loci (Table 2) was adjusted to reflect the estimated 'real' numbers of homozygotes (van Oosterhout *et al*. 2004). Two loci in *D. copei* (D07 and D13) and two loci in *D. tenebrosus* (D04 and D18) were significantly out of Hardy–Weinberg equilibrium. No loci were in linkage disequilibrium in either species after correcting for multiple comparisons. Three loci (D07, D14 and D18) had different allelic ranges that

Table 2 Number of detected alleles (A), size range of PCR products in base pairs and expected (H_E) and observed (H_O) heterozygosities in two species of giant salamander, asterisks indicate loci out of Hardy–Weinberg equilibrium. Polymorphism data are based on screening 20 individuals of each species from a single population. Four loci did not amplify in *Dicamptodon tenebrosus*

	Dicamptodon copei				*Dicamptodon tenebrosus*			
Locus	A	Range	H_E	H_O	A	Range	H_E	H_O
D04	11	110–166	0.9	0.9	6	124–184	0.44*	0.2*
D05	11	143–179	0.87	0.95	6	151–175	0.52	0.4
D06	7	197–221	0.83	0.95	3	185–201	0.47	0.45
D07	8	192–236	0.85*	0.75*	4	271–315	0.5	0.65
D08	6	107–127	0.74	0.75	—	—	—	—
D13	12	108–164	0.84*	0.7*	9	115–183	0.84	0.75
D14	9	91–131	0.84	0.85	7	149–173	0.74	0.75
D15	9	197–233	0.86	0.85	—	—	—	—
D17	8	156–192	0.82	0.75	4	184–196	0.64	0.65
D18	6	148–168	0.79	0.7	7	214–242	0.81*	0.8*
D20	14	166–226	0.93	0.95	—	—	—	—
D22	7	95–119	0.83	0.95	—	—	—	—
D23	5	100–144	0.78	0.85	1	105	—	—
D24	4	120–132	0.69	0.65	2	128–132	0.05	0.05
D25	6	81–105	0.7	0.7	4	97–109	0.19	0.2

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could be used for species identification purposes. These loci are potentially useful for future studies of population genetics of dicamptodontid salamanders.

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