New microsatellite markers for population genetic studies of fishers

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Abstract

We have developed new microsatellite markers from fishers in the Kings River population in the southern Sierra Nevada. Using ear tissue samples, we screened 50 new microsatellite markers in fishers from this region. Of these, 44 loci (88%) amplified, and 6 (12%) were polymorphic. Four of the polymorphic loci have 4 base pair (bp) repeats, one has a 2 bp motif, and one has a compound repeat. The low level of polymorphism among screened loci in our samples corroborates other studies that have found reduced genetic diversity in Sierra Nevada fisher populations. These new genetic markers will be useful for a variety of population genetic studies of fishers, such as population monitoring using genetic tagging.

Microsatellites are regions of DNA comprised of repeats of the same, short sequence. These markers have a high degree of variability, making them useful in population genetic studies.

Example microsatellite sequence:

...GACAGAAAGAAAGAA...AGAAGGAC...

The number of repeats can vary, generating different genotypes based on the size of the allele (in base pairs).

	MP0059		MP0144	
Fisher 1	166	166	214	214
Fisher 2	166	170	210	214

To develop the loci, we used ear tissue samples collected from live-caught fishers in the Kings River Project Area, Sierra National Forest, CA. The ear tissue was removed during implantation of ear tags for a demographic study.

Live trap

Recently tagged fisher waking up from anethesia.

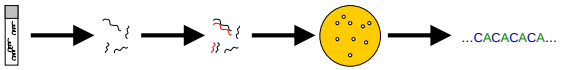
Objectives

Develop microsatellites for fishers in the Southern Sierra Nevada that can be used to:

- identify individuals from hair samples
- determine male reproductive success
- estimate effective population size
- measure dispersal among subpopulations
- detect past genetic bottlenecks in the population

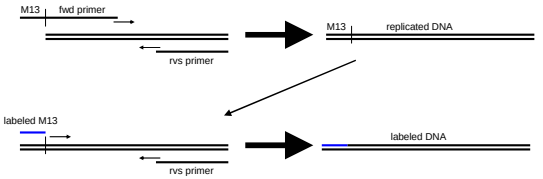
Microsatellite development methods

Step 1: Isolate microsatellites by cloning



1) Restriction digest whole DNA
2) Probe fragments with a known repeat sequence
3) Insert probed fragments into bacteria with Invitrogen TOPO TA Cloning® Kit
4) Sequence bacterial clones

Step 2: Screen primers for polymorphism using M13 label



The fwd primer has an M13 sequence "tag" that it incorporates into the replicated DNA in the first step. This DNA is used as template in a second step that uses a universal, fluorescently labeled primer to label the product that will be screened.

The Conservation Genetics Laboratory at UC Berkeley.

Overview of microsatellite development progress

Step	Number	% from prev step	% from beginning
Clones amplified	288	100	100
Clones sequenced	179	62	62
Useable μsat loci	104	58	36
Primers ordered	50	48	17
Loci that amplified	44	88	15
Polymorphic loci	**6**	**14**	**2**

Conclusions

- To date, we have found 6 loci that are polymorphic in fishers from the Southern Sierra Nevada
- Of the loci that amplified, only 14% were polymorphic
- This low level of polymorphism reinforces previous findings of low genetic diversity in this population
- Future work:
  - Screen monomorphic loci with samples from other populations from across North America
  - Determine the number of loci needed to meet the study objectives and sequence more clones if needed

Summary data for new loci

Locus	Repeat	Samples screened	Size range	Alleles	H_E	H_O*	PI**
MP0059	CA_{19}	40	166-170	2	0.46	0.27	0.39
MP0144	$(AGAA)_{16}$	35	206-218	4	0.34	0.19	0.44
MP0175	$(CTTT)_{11}(CCTT)_3$	41	174-182	3	0.51	0.50	0.35
MP0197	$(TTTC)_{10}TCTC(TTTC)_2$	36	232-240	3	0.54	0.40	0.28
MP0200	$(GAAA)_{16}$	31	173-177	2	0.21	0.13	0.63
MP0247	$(GAAA)_3(GA)_8(GAA)_{11}$	5	139-155	3	0.62	0.06	0.21

*No locus out of Hardy-Weinberg equilibrium ($p<0.05$ HW exact test)
**Overall PI across loci: 2.2×10^{-3}

Acknowledgments

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