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TECHNICAL NOTE

Discovery of 20,000 RAD–SNPs and development of a 52-SNP array for monitoring river otters

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Abstract Many North American river otter (*Lontra canadensis*) populations are threatened or recovering but are difficult to study because they occur at low densities, it is difficult to visually identify individuals, and they inhabit aquatic environments that accelerate degradation of biological samples. Single nucleotide polymorphisms (SNPs) can improve our ability to monitor demographic and genetic parameters of difficult to study species. We used restriction site associated DNA (RAD) sequencing to discover 20,772 SNPs present in Montana, USA, river otter populations, including 14,512 loci that were also variable in at least one other population range-wide. After applying careful filtering criteria meant to minimize ascertainment bias and identify high quality, highly heterozygous (H_o = 0.2–0.50) SNPs, we developed and tested 52 independent SNP qPCR genotyping assays, including 41 that performed well with diluted DNA. The 41 loci provided high power for population assignment tests with only 1 misassignment (1.6 %) between closely neighboring populations. Our SNPs showed high power to differentiate individuals and assign them to population of origin, as well as strong concordance of genotypes from high and diluted concentrations of DNA, and between original RAD and the SNP qPCR array.

Keywords Conservation genomics · RAD · Next generation sequencing · River otter · SNP · Population monitoring · Noninvasive genetic tagging

Like other wide-ranging, elusive species, monitoring river otter populations remains a challenge for management agencies (Melquist et al. 2003). Individual otters are difficult to distinguish visually and are known to travel considerable distances (Melquist and Hornocker 1983; Newton 2012). Radio-tagging studies, which require surgically implanting transmitters, are expensive and logistically difficult, and typically produce small samples sizes. For these and other reasons, studies aimed at estimating abundance, population growth rates, and connectivity have been difficult.

Interest in ensuring long-term population persistence in the face of harvest and habitat loss has led to the development of molecular tools, primarily microsatellite markers, to monitor otter population dynamics (e.g., Mowry et al. 2011). Advancements in discovery and genotyping of single nucleotide polymorphisms (SNPs), with concurrent reduction in costs, present an opportunity to vastly improve our ability to monitor wildlife populations. Relative to

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microsatellites, SNPs are less prone to genotyping errors, easier to transfer and analyze consistently among laboratories, genotyping samples is faster and cheaper, and SNPs can include neutral markers or those linked to regions under selection (Morin et al. 2004; Allendorf et al. 2010; Helyar et al. 2011; Fabbri et al. 2012).

One of the strengths of using genetic methods for monitoring river otter populations is the ease of collecting fecal samples from shared latrine sites along water bodies. Latrines are easy to locate and provide an opportunity to collect fecal material from multiple individuals at one site. Likely because of degradation of DNA due to moisture and ultraviolet light (Vynne et al. 2011; Stetz et al. 2015), these efforts have been largely unsuccessful due to poor genotyping success rates. For example, a study in Montana was able to obtain complete multilocus genotypes for just 6 % of otter scat samples using microsatellites (Newton 2012). As SNPs are considerably shorter than microsatellites, higher genotyping success rates (i.e., lower rates of allelic drop out and false amplifications) are likely, even with poor quality samples such as scat (Morin and McCarthy 2007; Fabbri et al. 2012; Fitak et al. 2015). We therefore set out to develop a SNP array for river otters that would improve our ability to assess and monitor otter populations in Montana and across the species' range.

Although our emphasis was on otter populations in Montana, we used muscle tissue samples from a large geographic area to minimize issues of ascertainment bias and to ensure usefulness range-wide (Fig. 1; Allendorf et al. 2010). Sampling two populations that are somewhat close geographically (i.e., NB and QE) strengthened our test of marker power to assign individuals to population of origin.

DNA was extracted from tissue samples using the Qiagen DNeasy protocol then quantified using the Quant-iT^{TM} PicoGreen$^{®}$ dsDNA assay to ensure DNA concentrations >5 ng/μl, needed for producing restriction-site-associated (RAD) sequencing libraries (Etter et al. 2011). RAD libraries were sequenced on the Illumina HiSeq 2000 platform using 150 base pair paired-end reads.

Following Amish et al. (2012), we selected for informative loci while applying strict data quality and assay design filters. Samples were excluded from downstream analysis if > 50 % of their genotyped loci had <5 reads or >fivefold read count difference between alleles. To facilitate SNP PCR genotyping assay design SNP loci had to be located between 40–70 nucleotides from the end, and we allowed only 1 SNP per RAD locus to avoid RAD loci assembled from paralogs and to avoid physically linked SNPs. We then excluded RAD loci where ≥2 samples had <5 reads or >fivefold read count difference between 2 alleles. We also required that observed heterozygosity (both range-wide and within Montana) be 0.2–0.6, that ≥1 sample from outside Montana was heterozygous at each RAD locus, and that each locus was successfully genotyped in >80 % of Montana samples.

These criteria produced 100 candidate SNPs, from which we selected the 96 with the highest expected heterozygosity for KASP-by-Design Fluidigm Assays (LGC Genomics$^{®}$). We tested these 96 assays on 73 samples from across otter range on a Fluidigm microfluidic SNP-chip. After excluding 7 loci due to high linkage disequilibrium ($p < 0.01$), we identified 52 loci in Hardy–Weinberg proportions, with expected and observed heterozygosity >0.2 for Montana samples, and with ≤1 instance where the initial RAD genotype did not match the SNP-chip genotype (Table S1). Within SNP-chip genotypes, each sample was run at least three times, and there could be ≤1 instance of replicate genotypes not matching. We next required that each of the three possible genotypes from each locus was observed at least once on each of 2 SNP-chips. We set a call rate threshold of 90 % for each SNP-chip (i.e., 90 % of individuals and replicates yielded quality genotypes), and mean genotype confidence had to be >90 % for both chips.

We then identified a subset of 41 loci for use with low quantity DNA samples by testing loci on samples where we reduced the original DNA concentration (≥50 ng/μl) by half (Table S1). We excluded loci where ≥1 instance of normal and low concentration genotypes did not match, and where ≤1 instance of low concentration duplicate genotypes did not match.

We used probability of identity statistics and population assignment tests to examine the SNP panel's power to answer questions of sample identity or population of origin. We used GenAlEx 6.5 (Peakall and Smouse 2006) to calculate probability of identity, and Geneclass 2.0 (Piry et al. 2004; Rannala and Mountain 1997) to test how well individual otters assigned to populations (Paetkau et al. 1995) using our two classes of SNPs. Both sets of loci showed acceptable power to differentiate even closely related individuals within and among populations (Fig. 1). Using 52 loci produced 3 misassignments (5.1 %) compared to just 1 observed in the reduced set of 41 loci (1.6 %). All 3 putatively misassigned samples, however, originated in the closest neighboring population. For example, the 2 individuals from New Brunswick that assigned to Quebec had 46–49 % assignment scores to their native region, suggesting that these may have been related to recent migration events.

The SNPs we report here represent a new tool for monitoring demographic and genetic status and changes in river otter populations across North America. SNPs may be particularly well suited to studying otter populations given the observed increase in genotyping success of fecal

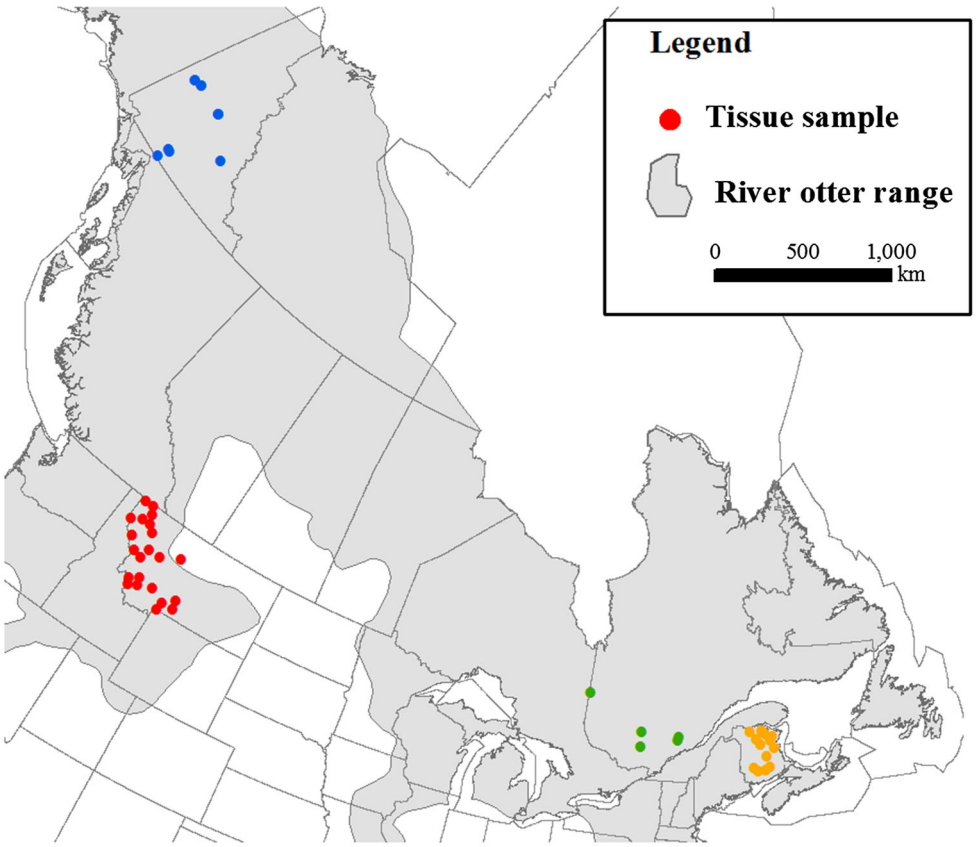

Fig. 1 Locations of tissue samples collected from North American river otter for SNP development; population-specific SNP statistics are reported within the figure

Summary statistics for single nucleotide polymorphism (SNP) loci developed for North American river otter, color coded to match symbols in the above map. See text for SNP selection criteria.

		Normal concentration (52 loci)				Low concentration (41 loci)			
Sample origin	n	H_e	H_o	P_{ID}	$P_{ID\text{-}sibs}$	H_e	H_o	P_{ID}	$P_{ID\text{-}sibs}$
Montana	22	0.451	0.438	1.0E-20	4.3E-11	0.450	0.440	1.9E-16	7.0E-09
Yukon	11	0.297	0.296	1.5E-14	8.1E-08	0.338	0.315	2.6E-12	1.1E-06
Quebec	13	0.317	0.314	1.6E-14	8.1E-08	0.299	0.302	4.5E-11	5.0E-06
New Brunswick	13	0.315	0.247	9.4E-14	2.1E-07	0.279	0.250	8.7E-11	7.0E-06
Total/Average	59	0.345	0.324	3.1E-14	9.3E-08	0.344	0.332	3.4E-11	3.3E-06

samples relative to microsatellite markers in other species (e.g., Campbell and Narum 2009; Fabbri et al. 2012; Fitak et al. 2015). Further, this SNP array may be a powerful tool to explore genetic structure and evolutionary potential of otter populations while taking advantage of noninvasive sampling techniques. Such information is particularly valuable for reintroduction efforts and general questions on river otter ecology. Necessary next steps include optimizing sampling and preservation methods to maximize SNP performance in spraints, and to directly compare performance of SNPs to microsatellites.

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