Impact of Population Expansion on Genetic Diversity and Structure of River Otters (*Lontra canadensis*) in Central North America

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Abstract

Populations of North American river otters (*Lontra canadensis*) declined throughout large portions of the continent during the early 1900s due to habitat degradation and unregulated trapping. River otters had been extirpated in North Dakota (ND), but the Red River Valley has since been recolonized, with potential source populations including the neighboring states of Minnesota or South Dakota, or the Canadian province of Manitoba (MB). We genotyped 9 microsatellite loci in 121 samples to determine the source population of river otters in the Red River Valley of ND, as well as to assess population structure and diversity of river otters in central North America. Overall, genetic diversity was high, with an average observed heterozygosity of 0.58. Genetic differentiation was low ($F_{ST} < 0.05$) between river otters in ND and those of Minnesota, suggesting that eastern ND was recolonized by river otters from Minnesota. River otters from MB were genetically distinct from all other sampled populations. Low genetic differentiation ($F_{ST} = 0.044$) between South Dakota and Louisiana (LA) suggested that reintroductions using LA stock were successful. The genetic distinctiveness of river otters from different geographic regions should be considered when deciding on source populations for future translocations.

Key words: conservation genetics, Louisiana, Manitoba, microsatellites, Minnesota, North Dakota, South Dakota

Many members of the family Mustelidae have experienced severe declines and regional extirpation, with recovery following human-mediated reintroductions or natural expansion of remnant populations (Williams and Scribner 2010; Hapeman et al. 2011; Hobbs et al. 2011). Historically, North American river otters, *Lontra canadensis*, occurred in most major drainages of the continental United States and Canada (Hall 1981; Toweill and Tabor 1982; Melquist et al. 2003). By the early 1900s, unregulated trapping, riparian habitat disturbances, and water pollution had resulted in the extirpation of river otters throughout much of their original range (Nilsson 1980; Melquist and Dronkert 1987; Lariviere and Walton 1998; Raesly 2001; Melquist et al. 2003; Stevens et al. 2011). Particularly, severe declines occurred in central North America and other regions dominated by riverine systems, which support smaller river otter populations than areas with greater abundance and variety of aquatic habitats (e.g., the Great Lakes region and coastal marshes along the Gulf of Mexico) (Melquist and Dronkert 1987; Melquist et al. 2003).

Implementation of laws to improve water quality (e.g., the Clean Water Act of 1972) and gradual advances in furbearer management practices (e.g., trapping limits) have contributed to the recovery of river otter populations across much of their historic range (Raesly 2001; Melquist et al. 2003). Along with natural expansion of remnant populations, reintroduction programs undertaken by 22 states and 1 Canadian province have contributed substantially to the widespread restoration of river otter populations (Raesly 2001; Melquist et al. 2003; Spinola et al. 2008). River otters now occupy waterways in at least some portion of all states in which they historically occurred. Fourteen of the states undertaking reintroduction projects translocated river otters from the coastal marshes of southern Louisiana (LA), the region supporting the highest density of river otters in North America (Raesly 2001; Melquist et al. 2003). The genetic consequences of translocations from one
geographic region to another are often not considered prior to movement of individuals (Williams et al. 2000; Williams et al. 2002; Latch et al. 2008). For example, the widespread use of river otters from LA for reintroductions may have considerable influence on the genetic composition of remnant populations through introgression as expansion occurs, and this would likely alter allele frequencies in the recipient regions to more closely resemble the otter population of the Gulf Coast (Serfass et al. 1998; Fike et al. 2004). Consequently, a potential long-term outcome of these reintroduction projects may be the reduction in genetic differences among river otter populations across central North America.

In North Dakota (ND), river otters were relatively common in most river drainages prior to the 1890s, but became extirpated through overhunting (Bailey 1927; Stearns and Serfass 2011). However, beginning in the early 2000s, river otters have been recolonizing portions of the Red River of the North (hereafter referred to as the Red River) Drainage in eastern ND (Stearns and Serfass 2011; Triska et al. 2011). River otters in ND are classified as a species of conservation concern and trapping is prohibited (Hagan et al. 2005; North Dakota Game and Fish Department 2012). With the exception of a study of river otter food habits by Stearns and Serfass (2011), virtually nothing is known about the general ecology or genetic diversity and structure of the newly established river otter population in ND.

Considering drainage patterns in the region, potential source(s) for the river otter population in ND may include Manitoba (MB), Minnesota, and/or South Dakota. The Red River flows north, forming the border between ND and Minnesota before entering the Canadian province of MB. The river otter population of MB is secure, and an annual recreational trapping season is open throughout much of the province (Manitoba Department of Conservation and Water Stewardship 2012). However, although river otters occupy the Red River drainage in MB, the population density is low (Stenson 1986), and this area is, therefore, closed to fur trapping.

In Minnesota, river otters historically occurred throughout the state. Nonetheless, the population experienced substantive declines and regional extirpation, particularly in the south. Between 1979 and 1982, 24 river otters, collected from a large remnant population in northeastern Minnesota (MN-NE), were reintroduced at 2 sites in southern Minnesota (Raesly 2001). The demographics of the river otter populations in southern Minnesota are not well characterized, but an increase in incidental trappings, reported sightings, and motor vehicle fatalities suggest that the population is growing (Gorman et al. 2008). Additionally, the remnant MN-NE population has been naturally expanding, and is suspected as a likely source of the river otter population now present in the Red River of western Minnesota (Erb 2011). Statewide trapping of river otters was initiated in Minnesota in 2010, including counties bordering the Red River (Erb 2011).

In South Dakota, river otters had been extirpated by the late 1800s and are currently listed as a state-threatened species and legally protected from trapping (Kiesow and Dieter 2005). In 1998 and 1999, the Flandreau Santee Sioux Native American Tribe released 34 river otters translocated from LA onto tribal lands along the Big Sioux River in Moody County in east-central South Dakota (Figure 1) (Kiesow and Dieter

![Figure 1. Map of river otter sampling locations in North America. Shading indicates approximate regions from which river otter samples were obtained: MB, MN-NE, MN-RR, ND, SD-NE, SD-RE, and LA. The dotted line between LA and MN-NE represents habitat connectivity in the Mississippi River drainage historically before the species underwent decline. Closed arrows indicate likely paths of natural population expansion and the open arrow indicates the translocation of individuals. The dashed line shows the approximate northernmost extent of the Mississippi River drainage basin.](https://academic.oup.com/jhered/article-abstract/105/1/39/857574/10539)
Success of the reintroduction effort has not been formally evaluated, but numerous sightings in the release area indicate that a population has become established (Kiesow 2003). Furthermore, in recent years, there has been an increase in the number of verified reports of river otters in northeastern South Dakota (SD-NE). The origin of river otters in the northeast is most likely from expansion of the reintroduced population but also could be from individuals dispersing from adjacent states or a combination of these potential sources (Kiesow and Dieter 2003).

Here, we examined the genetic diversity and structure of river otter populations in central North America. Our primary objectives were to: 1) determine the origin(s) of the river otter population now occupying the Red River drainage in eastern ND (focal study area), 2) compare and contrast the genetic structure and diversity of the river otter populations in MB, Minnesota, ND, and South Dakota, and 3) evaluate the current influence and the potential long-term influence of translocating river otters from LA on the genetic composition of populations in South Dakota and surrounding regions.

Materials and Methods

Samples

Samples were collected from 121 river otters taken by legal trapping, found dead, or accidently killed in LA, MB, Minnesota, ND, and South Dakota (Figure 1). Dried pelt samples from MB were obtained from river otters taken by legal trapping and provided to Manitoba’s Conservation District and Water Stewardship Wildlife Branch (n = 28); no samples were obtained from Trapping Area Zone 1. Minnesota samples (n = 39) were collected from 2 regions: 15 tissue samples from otters taken by legal trapping in Polk or Clearwater Counties in the Red River Drainage of Minnesota (Minnesota Red River Valley [MN-RR]) and provided to Minnesota’s Department of Natural Resources and 24 DNA isolates of individuals originating from the large remnant population in MN-NE. DNA isolates from the MN-NE individuals have not been used in any previous studies to our knowledge. Tissue samples from river otter carcasses incidentally collected (i.e., found dead, hit by vehicles, or accidently trapped) and recovered by the North Dakota Department of Game and Fish from the Red River drainage in eastern ND (n = 21) were acquired. Additional tissue samples were obtained from river otters incidentally recovered by the South Dakota Department of Game, Fish and Parks in the reintroduction region in the Big Sioux River (South Dakota reintroduction region [SD-RE]) (n = 16) and 3 northeastern counties in the Minnesota River drainage (SD-NE) (n = 5). DNA isolates, used in a previous study by Latch et al. (2008), from tissue of LA river otters (n = 12) taken by legal trapping from both inland and coastal parishes were obtained.

Microsatellite Amplification and Genotyping

Genomic DNA was isolated from all tissue samples using the Qiagen DNeasy Blood and Tissue Kit (Valencia, CA) following the manufacturer’s protocol for isolation from soft tissue. DNA from dried pelt samples (e.g., hair and skin tissue) was isolated using the Bio Rad InstaGene Matrix (Hercules, CA) chelex-based protocol. Nine polymorphic microsatellite loci described by Beheler et al. (2004) (RIO01, 02, 04, 06, 08) and Beheler et al. (2005) (RIO13, 17, 18, 19) were amplified by polymerase chain reaction (PCR) with fluorescently labeled forward primers and genotyped for analysis (Supplementary Table S1 online). PCR amplification for the microsatellite loci was performed in 10 µL reactions following protocols previously described (Beheler et al. 2004; Beheler et al. 2005); each round of PCR completed included a negative amplification control. Genotyping and visualization of amplified products relied on capillary electrophoresis on an ABI 310 or an ABI 3730Xl Genetic Analyzer. The genotype of each sample at each microsatellite locus was determined by comparison of amplicon length to an internal size standard analyzed by GeneMapper (Applied Biosystems, Carlsbad, CA) software.

Genetic Analyses

 Genetic variation at the 9 microsatellite loci was determined for each individual locus and for composite genotypes of individuals within each of the 7 sampled river otter populations. Microsatellite variability was assessed using the following parameters calculated by FSTAT, v. 2.9.3.2 (Goudet 1995), GENEPOP, v.4.0 (Raymond and Rousset 1995), POPGENE, v.1.32 (Yeh and Boyle 1997), or ARLEQUIN, v.3.11 (Excoffier et al. 2005): allelic frequencies, number of alleles per locus in each individual population, total number of alleles at each locus, private alleles per population, expected heterozygosity, and observed heterozygosity. Standard deviation values for expected and observed heterozygosity were calculated in the Excel Microsatellite Toolkit (Park 2001). Linkage disequilibrium between pairs of loci using a log-likelihood ratio statistic was calculated with FSTAT. Exact tests (Guo and Thompson 1992) were performed in GENEPOP to determine whether each microsatellite locus within each population was in Hardy–Weinberg equilibrium, and if there were loci with heterozygote excess or deficits. Significance of Hardy–Weinberg equilibrium and linkage disequilibrium was determined after using a Bonferroni correction on an initial significance level of α = 0.05 (Rice 1989).

To assess genetic variation, FIS and FST values (Wright 1951) were estimated using FSTAT and GENEPOP, respectively. FIS values, estimating the reduction of heterozygosity due to nonrandom mating, were calculated for all microsatellite loci across all river otter populations and for each population. FST values were assessed to determine pairwise population differentiation with corrected P values calculated in FSTAT, in addition to Nei’s unbiased genetic distance (D) corrected for small sample size (Nei 1978), which was calculated in POPGENE. Microsatellite allele frequency distributions were analyzed and compared for the 7 river otter populations.
STRUCTURE, 2.3.3 (Pritchard et al. 2000; Falush et al. 2003; Falush et al. 2007; Hubisz et al. 2009) was used to apply a model-based clustering algorithm to infer population structure and admixture. All sample data were run for values of $K = 1–8$ for a total of 20 iterations to ensure an accurate estimate of $K$ could be obtained despite variation in likelihood values among runs. Each run used 500 000 Markov chain Monte Carlo generations following a burn-in of 50 000 steps. STRUCTURE was run for each of 4 models combining assumptions on genetic ancestry of individuals and genetic relatedness among populations: admixture-correlated, admixture-independent, no admixture-correlated, and no admixture-independent. Models of admixture (or no admixture) allow (or disallow) for an individual to originate from more than 1 genetic cluster, and models of correlation allow for the association of allele frequencies across very closely related populations. All other parameters were set to the default values. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to evaluate the most likely number of population clusters ($K$) in the data by $2 \text{ ad hoc}$ methods: calculating $\Delta K$ values (Evanno et al. 2005) and identifying the $K$ value that maximizes the log probability of data, $\ln P(D)$ (Pritchard et al. 2000). STRUCTURE was also run using $K = 1–3$ for the following population groupings: SD-RE with LA and ND with both Minnesota groups combined. A multivariate representation of the analyzed individuals was completed through to Factorial Correspondence Analysis in GENETIX, v. 4.02.2, using the “3D-sur populations” options to assess the overall relationship across individuals in the populations (Belkhir et al. 2004).

**Results**

Complete multilocus genotypes were obtained for 116 of the river otters sampled; the other 5 samples were successfully genotyped at 8 of the 9 microsatellite loci (RIO04, $n = 120$; RIO13, $n = 118$; RIO18, $n = 120$). All 9 microsatellite loci were polymorphic; the observed number of alleles per locus ranged from 5 (RIO17) to 14 (RIO13); an overall mean of 6.1 alleles per locus per population was observed. Overall mean observed heterozygosity was 0.58 and ranged from a low of 0.51 in the SD-NE population to a high of 0.64 in the MB population (Table 1). $F_{IS}$ values calculated for all microsatellite loci indicated that the ND population had the lowest $F_{IS}$ value ($F_{IS} = 0.103$), whereas SD-NE had the highest $F_{IS}$ ($F_{IS} = 0.258$) (Table 1). The average $F_{IS}$ value for each locus was lowest for RIO08 ($F_{IS} = -0.051$) and highest for RIO01 ($F_{IS} = 0.344$) (Supplementary Table S2 online). Tests for linkage disequilibrium found no significant linkage between loci ($P < 0.001$, after correction). Two-tailed tests for departure from Hardy–Weinberg equilibrium indicated significant deviation after the Bonferroni adjustment for multiple comparisons was applied ($P < 0.0004$). Significant departures from Hardy–Weinberg equilibrium occurred at 2 loci in the MN-NE population (RIO01 and RIO04) and 1 locus in Red River Valley Minnesota population (RIO01). Heterozygote deficiencies occurred at 1 locus in each of the following populations: SD-RE (RIO17), MN-NE (RIO01), MN-RR (RIO06), and MB (RIO13). Multilocus tests failed to detect significant heterozygote excess.

Measures of genetic differentiation found moderate values between many of the sampled river otter populations. $F_{ST}$ ($P < 0.002$) and Nei’s $D$ found similar patterns of genetic differentiation between population pairs (Table 2). Low, nonsignificant levels of genetic differentiation were found between river otter populations in ND and MN-RR ($F_{ST} = 0.0294$, $P = 0.0045$; $D = 0.0943$). Similar low values occurred between MN-RR and MN-NE ($F_{ST} = 0.0250$, $P = 0.01$; $D = 0.0978$), and slightly higher, significant values of differentiation were found between ND and MN-NE populations ($F_{ST} = 0.0360$, $P < 0.002$; $D = 0.1152$). SD-RE and LA populations had nonsignificant genetic differentiation based on $F_{ST}$ but moderate differentiation according to Nei’s D ($F_{ST} = 0.0439$, $P = 0.005$; $D = 0.2029$); however, SD-NE and LA show moderate to great differentiation for both measures ($F_{ST} = 0.1143$, $P < 0.002$; $D = 0.5168$). Genetic differentiation between the 2 South Dakota populations was low when estimated by $F_{ST}$ ($F_{ST} = 0.0470$, $P = 0.035$), but moderate when small population size is corrected for by Nei’s $D$ ($D = 0.2268$). $F_{ST}$ values for LA indicated negligible, yet significant levels of genetic differentiation ($F_{ST} < 0.05$) when compared to most other populations. Pairwise comparisons

<table>
<thead>
<tr>
<th>Location</th>
<th>Region</th>
<th>Code</th>
<th>Sample type</th>
<th>Collection years</th>
<th>Collection method</th>
<th>$N$</th>
<th>$A$</th>
<th>$H_D$ (SD)</th>
<th>$H_E$ (SD)</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manitoba</td>
<td></td>
<td>MB</td>
<td>Dried pelt</td>
<td>2006–2009</td>
<td>Trapping$^a$</td>
<td>28</td>
<td>8.4</td>
<td>0.64 (0.03)</td>
<td>0.76 (0.04)</td>
<td>0.151</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Northeast</td>
<td>MN-NE</td>
<td>DNA</td>
<td>Unknown</td>
<td>Trapping$^a$</td>
<td>24</td>
<td>6.6</td>
<td>0.60 (0.03)</td>
<td>0.74 (0.03)</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>Red River</td>
<td>MN-RR</td>
<td>Tissue</td>
<td>Mid-2000s</td>
<td>Trapping$^a$</td>
<td>15</td>
<td>5.7</td>
<td>0.53 (0.04)</td>
<td>0.65 (0.05)</td>
<td>0.199</td>
</tr>
<tr>
<td>North Dakota</td>
<td></td>
<td>ND</td>
<td>Tissue</td>
<td>2004–2008</td>
<td>Opportunistic</td>
<td>21</td>
<td>6.0</td>
<td>0.59 (0.03)</td>
<td>0.65 (0.05)</td>
<td>0.103</td>
</tr>
<tr>
<td>South Dakota</td>
<td></td>
<td>SD-NE</td>
<td>Tissue</td>
<td>2003–2008</td>
<td>Opportunistic</td>
<td>5</td>
<td>3.7</td>
<td>0.51 (0.07)</td>
<td>0.67 (0.04)</td>
<td>0.258</td>
</tr>
<tr>
<td></td>
<td>Reintroduction</td>
<td>SD-RE</td>
<td>Tissue</td>
<td>2003–2008</td>
<td>Opportunistic</td>
<td>16</td>
<td>5.7</td>
<td>0.57 (0.04)</td>
<td>0.72 (0.04)</td>
<td>0.211</td>
</tr>
<tr>
<td>Louisiana</td>
<td></td>
<td>LA</td>
<td>DNA</td>
<td>2003–2004</td>
<td>Trapping$^a$</td>
<td>12</td>
<td>6.2</td>
<td>0.62 (0.05)</td>
<td>0.74 (0.04)</td>
<td>0.157</td>
</tr>
</tbody>
</table>

$A$ is the mean number of alleles per locus; $H_D$ is observed heterozygosity (SD); $H_E$ is the mean expected heterozygosity (SD); $F_{IS}$ is the estimate of reduction in heterozygosity due to nonrandom mating; $N$ is the number of samples; SD, standard deviation.

$^a$ Samples were obtained from individuals taken by legal trapping.
between MB and all other river otter populations suggested moderate to great levels of genetic differentiation.

Broad and continuous microsatellite allele distributions were observed in the sampled river otter populations (Supplementary Figure S1 online). Similar allele size distributions and frequency patterns occurred between the Minnesota and ND populations (e.g., RIO02; RIO17; RIO19) with allele sizes of the ND otters generally falling within the combined range observed in the Minnesota populations. Similarly, allele sizes of the SD-RE samples fell within the range observed in the LA river otters for all alleles except RIO02. MB generally exhibited the broadest range within the range observed in the LA river otters for all alleles. Eighteen private alleles were observed among the populations: 2 in the ND population, 2 in each of the Minnesota populations, and 12 in the MB population.

Genetic partitioning across river otter populations was examined using STRUCTURE. Ad hoc methods to determine the number of partitions provided support for a varying number of clusters with a minimum of 2 and a maximum of 5 using the admixture, correlated model (Figure 2). Information regarding the geography of the study area must also be taken into consideration when assessing the potential number of genetic clusters identified by STRUCTURE (Pritchard et al. 2000). Using this guideline, evident, and important differences in clustering patterns could be identified when the value of K was raised from 2 to 3, yet the biogeographically relevant information that could be derived was minimal when the value of K was set higher than K = 3 (Figure 2). At K ≥ 3, the genetic distinctiveness of the MB population was evident, as was clustering of river otters in the SD-RE with those of LA, while the Minnesota and ND populations clustered together (Figure 2); these partitions were also supported by analysis using GENETIX (see below). The clustering patterns among river otter populations remained consistent across all model parameters in STRUCTURE, indicating robust genetic relationships among the populations (Supplementary Figure S2 online). The river otters from SD-NE appeared to be of mixed ancestry, and there was a high degree of genetic differentiation between these river otters and those from other populations. This may indicate the movement of river otters from multiple, perhaps unsampled, populations into SD-NE, or may be an artifact of the small sample size for this region. Given the biology and history of river otters in the study region, we expected to find moderate levels of mixed ancestry throughout the dataset due to historical and potentially current gene flow. Clustering patterns were not different when STRUCTURE analysis included only a subset of the sampled populations (data not shown).

A factor correspondence analysis conducted using the software GENETIX also found genetic similarity between river otters in ND and Minnesota (Figure 3). Likewise, the river otter populations in South Dakota and LA were genetically similar, whereas the population of MB was distinctive. Most of the variation in the dataset was accounted for by Axes 1 and 2, 40.57% and 22.52%, respectively; a small amount of variation occurred along Axis 3, 12.28% (which did not distinguish among populations; data not shown). In a 2D plot, ND and Minnesota river otters formed a distinct group, whereas South Dakota river otters grouped with some of the individuals from LA (Figure 3). In both STRUCTURE and GENETIX analyses, MB river otters were genetically distinct from other populations (Figure 3).

### Discussion

Recovery of river otters across much of their range through widespread reintroduction efforts and natural expansion of remnant populations has affected patterns of genetic structuring. The ND river otter population has a genetic composition similar to that of the Minnesota population (Table 2; Supplementary Figure S1 online), and river otters in the 2 states clustered together genetically (Figures 2 and 3), suggesting that Minnesota was the source population for river otters recolonizing ND (Figure 1). A decrease in observed heterozygosity between the core MN-NE population ($H_O = 0.60$) and the population in Minnesota’s Red River Valley ($H_O = 0.53$) (Table 1) is typical of range expansion, where levels of genetic variation decrease along the path of expansion (Excoffier and Ray 2008; Latch et al. 2009). The low $F_{ST}$ and Nei’s $D$ values (Table 2) between the ND and Minnesota populations were indicative of minimal differentiation or high gene flow between the populations, which are connected by abundant aquatic habitat in the Red River drainage.

Across river otter populations sampled for our study, the genetic diversity detected was high. Despite a history of population declines followed by multiple recolonization events, the average observed heterozygosity remained high.

### Table 2. Matrix of $F_{ST}$ (above diagonal) and Nei’s $D$ (below diagonal) values between river otter populations

<table>
<thead>
<tr>
<th></th>
<th>MB</th>
<th>MN-NE</th>
<th>MN-RR</th>
<th>ND</th>
<th>SD-NE</th>
<th>SD-RE</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>—</td>
<td>0.1023</td>
<td>0.1252</td>
<td>0.1524</td>
<td>0.0807</td>
<td>0.9977</td>
<td>0.0926</td>
</tr>
<tr>
<td>MN-NE</td>
<td>0.4656</td>
<td>—</td>
<td>0.0250</td>
<td>0.0360</td>
<td>0.0827</td>
<td>0.0785</td>
<td>0.0231</td>
</tr>
<tr>
<td>MN-RR</td>
<td>0.4664</td>
<td>0.0978</td>
<td>—</td>
<td>0.0295</td>
<td>0.1278</td>
<td>0.1219</td>
<td>0.0437</td>
</tr>
<tr>
<td>ND</td>
<td>0.5925</td>
<td>0.1152</td>
<td>0.0943</td>
<td>—</td>
<td>0.1299</td>
<td>0.1137</td>
<td>0.0464</td>
</tr>
<tr>
<td>SD-NE</td>
<td>0.3648</td>
<td>0.3712</td>
<td>0.4434</td>
<td>0.4235</td>
<td>—</td>
<td>0.0470</td>
<td>0.1147</td>
</tr>
<tr>
<td>SD-RE</td>
<td>0.4271</td>
<td>0.3188</td>
<td>0.4149</td>
<td>0.3626</td>
<td>0.2368</td>
<td>—</td>
<td>0.0444</td>
</tr>
<tr>
<td>LA</td>
<td>0.4264</td>
<td>0.1335</td>
<td>0.1613</td>
<td>0.1538</td>
<td>0.5168</td>
<td>0.2029</td>
<td>—</td>
</tr>
</tbody>
</table>

$F_{ST}$ values that are significant at $P < 0.002$, after correction for multiple comparisons, are in indicated in bold.
Similarly, high levels of diversity have been found in several other mustelid species that have undergone population declines with subsequent increase (Williams and Scribner 2010; Pickles et al. 2012; Zigouris et al. 2012); by contrast, low levels of diversity in other mustelid species may reflect a history of extirpation and reintroduction (Larson et al. 2002; Hapeman et al. 2011).

In our study, the highest levels of heterozygosity occurred in the 3 large, remnant populations of LA ($H_O = 0.62$), MB ($H_O = 0.64$), and MN-NE ($H_O = 0.60$) (Table 1). High genetic diversity is likely attributable to the long-term persistence of relatively large river otter populations in these regions, notably in LA where high genetic diversity has been reported by a previous study (Latch et al. 2008). The other populations from which we sampled would likely have undergone founder effects during recolonization, reducing genetic diversity and altering allele frequencies as the population expanded. Deviations from Hardy–Weinberg equilibrium in the Minnesota populations and high $F_{IS}$ across sampled populations indicates a deficiency of heterozygotes

**Figure 2.** Genetic partitioning across river otter populations. (A) STRUCTURE analysis using 9 microsatellite loci genotyped in river otters from MB, MN-NE, MN-RR, ND, SD-NE, SD-RE, and LA. Software settings assumed admixture between populations and correlated allele frequencies, for $K = 2$–5. At $K = 3$, the populations approximately subdivided into clusters geographically: MB (red); MN-NE/MN-RR/ND (blue); and SD-RE/LA (green). At $K = 5$, a cluster (yellow) includes both the SD-NE river otters along with a few individuals from SD-RE. (B) The most likely number of clusters suggested by ad hoc methods are circled in red on the graphs: $\Delta K$ (Evanno et al. 2005) supports $K = 2$, whereas $\ln P(D)$ (Pritchard et al. 2000) supports $K = 5$. 

($H_O = 0.58$) (Supplementary Table S2 online).
and potential subpopulation structuring, a pattern observed in other mustelid species (Mucci et al. 2010; Hapeman et al. 2011).

The limited genetic differentiation (Table 2) and moderate overlap in clusters (Figure 3) between LA and the persistent population in MN-NE could be a remnant of the formerly continuous range of river otters across the Mississippi River drainage, enabling gene flow throughout the region until disruption of the species range (Serfass et al. 1998). The ability of river otters to disperse long distances (Melquist and Hornocker 1983; Blundell et al. 2002) and maintain large home ranges (Melquist and Hornocker 1983) coupled with the lack of barriers to movement within the Mississippi River drainage would have enabled unobstructed gene flow among contiguous populations across the drainage; this may also be supported by a previous report of an allele unique to river otter populations in the Mississippi River drainage (Serfass et al. 1998).

The river otters of MB were distinctive from all other populations, exhibiting high genetic differentiation (Table 2), distinct clustering (Figures 2 and 3), and unique allele frequency patterns with a high number of private alleles (Supplementary Figure S1 online). These suggested that MB river otters had been genetically isolated from those in surrounding drainages, perhaps exacerbated by the historical extirpation and present-day low abundance of river otters in MB’s portion of the Red River (Stenson 1986).

Source populations for river otter reintroductions generally have been selected based on the ease by which individuals could be obtained (e.g., LA) with little attention paid to genetic considerations (Serfass et al. 1998; Raesly 2001). South Dakota river otters from the reintroduction region appear to be genetically distinct from those of ND and Minnesota, likely due to the release of individuals from LA. Heterozygosity of a source population is expected to be high in comparison to newly colonized populations, as seen between LA’s native river otters ($H_O = 0.62$) and South Dakota’s reintroduction region population ($H_O = 0.57$) (Table 1) (Ibrahim et al. 1996; Latch et al. 2009). We detected low, nonsignificant genetic differentiation ($F_{ST} = 0.044$) between LA and South Dakota’s reintroduction region; however, the modest levels of genetic distance (Nei’s $D = 0.203$) and variation in allele frequency patterns between the locations may reflect shifts in allele frequencies and genetic drift that occur in populations founded by reintroductions (Williams et al. 2002; Mock et al. 2004; Latch and Rhodes 2005). Additionally, 3 genetically distinctive river otter subpopulations exist within LA (Latch et al. 2008), and collection information on the subpopulation from which translocated individuals were obtained is unavailable. Between-population comparisons together with a positive $F_{IS}$ value in South Dakota’s reintroduction
region ($F_{RS} = 0.211$) suggest potential recent admixture between the reintroduced individuals and otters moving from unsampled areas into the region. Overall, the newly founded population of South Dakota is similar to that of LA (Figures 2 and 3), as would be expected following a successful translocation (Williams et al. 2000; Latch and Rhodes 2005).

Available population genetic information should be considered by wildlife management agencies during development and implementation of plans designed to ensure the retention of unique or distinctive regional genetic patterns. Careful selection of a source population when translocating individuals is important for the preservation of genetically distinctive populations (Leberg 1990; Allendorf and Luikart 2007). As observed in other species for which reintroductions have been successful, the movement of individuals between geographic regions can alter population structure and result in the retention of genetic signatures from the source population in the reintroduced population (Latch and Rhodes 2005; Ferrando et al. 2008; Williams and Scribner 2010; Hapeman et al. 2011). River otter reintroduction programs have often relied on the translocation of LA individuals, yet our current results indicate that regional populations persist that are genetically distinctive from the LA otter population. This regional distinctiveness should be considered when deciding which source population to use during future reintroductions of river otters. Additionally, the long-term monitoring of regions where LA river otters have been released will be important for gaining a better understanding of how the genetic composition of expanding remnant populations will be altered as introgression occurs.

Supplementary Material
Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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