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Deciphering Ecological Barriers to North American River Otter (*Lontra canadensis*) Gene Flow in the Louisiana Landscape

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Abstract

For North American river otters (*Lontra canadensis*) in Louisiana, statewide distribution, availability of aquatic habitats, and the absence of physical barriers to dispersal might suggest that they exist as a large, panmictic population. However, the wide variety of habitat types in this region, and the dynamic nature of these habitats over time, could potentially structure river otter populations in accordance with cryptic landscape features. Recently developed landscape genetic models offer a spatially explicit approach that could be useful in identifying potential barriers to the movement of river otters through the dynamic aquatic landscape of Louisiana. We used georeferenced multilocus microsatellite genotypes in spatially implicit (STRUCTURE) and spatially explicit (GENELAND) models to characterize patterns of landscape genetic structure. All models identified 3 subpopulations of river otters in Louisiana, corresponding to Inland, Atchafalaya River, and Mississippi River regions. Variation in breeding seasonality, brought about by variation in prey abundance between inland and coastal populations, may have contributed to genetic differentiation among populations. It is also possible that the genetic discontinuities we observed indicate a correlation between otter distribution and access to freshwater. Regardless of the mechanism, it is likely that any genetic differentiation among subpopulations is exacerbated by relatively poor dispersal.

The North American river otter (*Lontra canadensis*) historically was found in freshwater riparian and brackish coastal habitats throughout most of North America (Hall 1981; Larivière and Walton 1998; Melquist et al. 2003); however, the species became locally extinct in many areas by the mid-1800s and early 1900s, primarily as a consequence of human-related activities such as overharvest, pollution, and urbanization (Armstrong 1972; Nilsson 1980; Melquist and Dronkert 1987). Reintroduction programs, initiated in the late 1970s, were extremely successful in restoring extirpated otter populations to their historic range (Ralls 1990; Melquist et al. 2003). The vast majority of these reintroduction programs used river otters from Louisiana as founding stock (Ralls 1990; Raesly 2001).

In Louisiana, North American river otters are common throughout the state, with higher abundances occurring along the coast (Ensminger and Linscombe 1980; Chabreck et al. 1985; Shirley et al. 1988). Their importance as a furbearer species in Louisiana has led to intensive annual harvest of more than 3300 otters per year (1998–2003 average; Scognamillo 2005). Beyond basic distribution and harvest information, however, knowledge about the ecology, natural history, population dynamics, and genetic structure of river otters in this region is limited or nonexistent (Lowery 1974; Holcombe 1980; Chabreck et al. 1982, 1985; Edwards 1983; Scognamillo 2005). This lack of data is surprising, given the extent to which Louisiana river otters have been transplanted throughout North America.

The Louisiana landscape appears to be ideal for promoting river otter movements. Prominent physical barriers known to limit gene flow of otters at a regional scale (e.g., mountain ranges, Serfass et al. 1998) are absent.

In addition, the complex network of aquatic habitats throughout Louisiana predicts that river otters might be moving freely throughout the region as one large, panmictic population. However, the manner in which animals use a landscape is determined both by their habitat requirements and social structure; thus, populations may exhibit spatial genetic structure even in the absence of physical barriers (Chesser 1991; Blundell et al. 2002). Given the wide diversity of aquatic and terrestrial habitats available to otters in Louisiana, and the dynamic nature of these landscapes over time, we instead might expect otter populations to be spatially structured in accordance with cryptic landscape features and/or biological attributes such as social interactions among individual animals and dispersal capabilities.

Our purpose in this research was to utilize genetic information from harvested river otters to investigate competing hypotheses regarding the spatial genetic structure and population connectivity of river otter populations in Louisiana. To achieve this goal, we utilized spatially implicit and spatially explicit models to investigate patterns of landscape genetic structure in North American river otters in Louisiana. From a biological perspective, characterization of genetic structure as it relates to underlying features of the landscape will provide some insight into the ecology of the species and help to identify potential barriers to the movement of river otters through the dynamic aquatic landscapes of Louisiana. Likewise, reintroduction programs are likely to continue using Louisiana populations as source stock, and a more comprehensive picture of the distribution of genetic diversity within the region will serve to guide selection of founders to minimize loss of genetic diversity.

Methods

Sample Collection

We collected tissue samples from 111 North American river otters harvested by professional trappers. Although trapping is permitted throughout the state, samples were made available from 3 areas, 2 of them in coastal Louisiana (mouth of the Mississippi River and Atchafalaya River) and 1 in inland habitats in the area of the intersection between the Mississippi River and the Red River. Carcasses were frozen on harvest, and tissue samples remained frozen until DNA extraction. For each sample, trappers identified their harvest location on a map, and the geographic coordinates of these catch locations were manually entered into an ArcGIS layer (ESRI, v9).

Laboratory Methods

We extracted DNA from tissue samples using either a potassium acetate protocol (Sambrook and Russell 2001) or a modified ammonium acetate protocol (modified from the PUREGENE kit; Gentra Systems, Minneapolis, MN). We assessed the quantity and quality of extracted DNA via electrophoresis through an agarose gel stained with ethidium bromide and diluted each sample to approximately 10 ng/μl in Tris-low-EDTA buffer (10 mM Tris–HCl, 0.1 mM ethylenediaminetetraacetic acid).

We amplified 12 microsatellite loci for each sample, using the primers and reaction conditions outlined in Beheler et al. (2004, 2005; RIO 02, 06, 07, 08, 11, 12, 13, 14, 15, 16, 17, and 19). We combined amplified loci into 3 gel sets (Set A: RIO 02, 11, 15, 16; Set B: RIO 07, 12, 13, 19; and Set C: RIO 06, 08, 14, 17) based on locus-specific allele sizes and fluorescent label color (6-FAM, HEX, or NED). We added combined polymerase chain reaction products to ROX 400HD internal lane standard (Applied Biosystems, Foster City, CA) and electrophoresed them through a 5% polyacrylamide gel (Long Ranger Singel Packs; Cambrex, East Rutherford, NJ) on an ABI 377 DNA sequencer. Allele sizes were determined for each locus using GeneScan 3.1 and Genotyper 2.5 software (Applied Biosystems).

To maximize quality within our microsatellite dataset, we developed a known set of alleles for each locus, representing the full range of allele sizes. We combined these into gel sets as above, and ran this allelic standard every 12 lanes to minimize genotyping errors due to electrophoretic variability both within and among gels. Additionally, we re-electrophoresed and/or reamplified all genotypes with low signal intensity (<100 as determined by Genotyper 2.5 software).

Genetic Analyses on the Total Population

We used the software CONVERT (version 1.2; Glaubitz 2004) to facilitate input file preparation for all software used for microsatellite data analysis. We tested for a deficiency of heterozygotes relative to Hardy–Weinberg expectations for each locus and globally using a score test (Rousset and Raymond 1995) and a Markov chain method for estimating *P* values (Guo and Thompson 1992; parameter values of dememorization = 10 000, number of batches = 500, number of iterations per batch = 5000) in GENEPOP software (version 3.4; Raymond and Rousset 1995). The number of batches and the number of iterations per batch were increased from default values to minimize standard errors for the *P*-value estimates (standard error < 0.005). We quantified the level of deviation from Hardy–Weinberg equilibrium by estimating F_{IT} for each locus and globally, using the method of Weir and Cockerham (1984) as implemented in SPAGEDI software (version 1.2; Hardy and Vekemans 2002) and tested for significance using a permutation test (20 000 permutations of genotypes among individuals). Due to the large number of comparisons involved in locus-specific tests for Hardy–Weinberg disequilibrium and locus-specific tests for deviation of F_{IT} estimates from zero, we used a false discovery rate (FDR) method (Benjamini and Yekutieli 2001) to obtain an experiment-wide alpha level (α_{EW}) prior to assessing significance. FDR methods offer an increase in power and a more stringent control over type II error than the more widely used Bonferroni correction (Moran 2003; Narum 2006). We chose to use the Benjamini and Yekutieli (2001)

modification of the original FDR control method (Benjamini and Hochberg 1995), as it allows for dependence among tests.

Inferring Subpopulations

To infer the number of subpopulations in our sample set and to assign individual samples to these groups, we employed 2 Bayesian clustering techniques. First, we used the popular STRUCTURE software (version 2.1; Pritchard et al. 2000; Falush et al. 2003) because the variety of modeling options available make it well suited to the detection of various patterns of population genetic structure (Evanno et al. 2005; Latch et al. 2006). Second, we used GENELAND software (version 0.3; Guillot et al. 2005b), which has been developed to optimize the delineation of subpopulations by incorporating spatial coordinates for each sample into the model. In the STRUCTURE approach, all clustering solutions are equally likely (i.e., the prior for the clustering is uniform), and spatial coordinates only are considered after data processing as a method of visualizing subpopulation membership. Although this makes sense in some situations where geographical barriers do not mimic genetic partitions, it often is true that genetically differentiated populations exist in geographically distinct areas. GENELAND was designed to incorporate spatial coordinates at an earlier stage of model development in order to better define geographical boundaries among populations and allows for uncertainty in spatial coordinates by introducing an additive noise parameter to the coordinates (Guillot et al. 2005a).

In STRUCTURE, we performed 5 runs at each value of the fixed parameter K (the number of subpopulations), from $K = 1$ to $K = 10$. Each run consisted of 100 000 replicates of the MCMC after a burn-in of 30 000 replicates. We used the admixture model and allowed the allele frequencies to be correlated among subpopulations. All other parameters were set to default values (Pritchard and Wen 2003). This configuration, using the admixture model and correlated allele frequencies, is thought to provide the best resolution in the case of potentially subtle population structure (Falush et al. 2003). The highest average likelihood was used as a point estimate of K. We did observe the phenomenon that once the true K was reached, likelihoods for larger Ks plateaued and the variance among runs increased (Pritchard and Wen 2003). Thus, we also used a ΔK measure that has been proposed to alleviate this problem and provide a more robust estimate of K (Evanno et al. 2005). Once we had inferred the most likely number of subpopulations, we ran 5 longer runs each (100 000 burnin and 500 000 replicates) at the inferred K, $K_{(\text{inferred})} + 1$, and $K_{(\text{inferred})} - 1$ to verify chain convergence and consistency among runs. To assign individuals to subpopulations, we performed a final run (100 000 burn-in and 500 000 replicates) at the inferred K. Values of q, the proportion of an individual's sampled genome characteristic of each subpopulation, were used to assign individuals to subpopulations. Individuals were unambiguously assigned to a single subpopulation when q values were greater than 0.70. When q values fell within the range $0.25 < q < 0.70$, assignments were made to multiple subpopulations.

To infer the number of subpopulations (K) in GENELAND, we first varied the number of subpopulations from 1 to 10, using a matrix of genotypes, spatial coordinates for each individual, and 100 000 stored MCMC iterations (500 000 iterations, thinning = 5). Allele frequencies were drawn from independent Dirichlet distributions (Pritchard et al. 2000) as this model has been shown to perform better than the alternative model (F-model; Guillot et al. 2005a). We set the maximum rate of the Poisson process to 100, a value close to the number of individuals in our dataset as suggested by Guillot et al. (2005a). This parameter controls the number of polygons in the geographical area under study, and the value used herein corresponds to strongly fragmented partitions and weak dependence on the spatial organization of populations. In the spatially explicit GENELAND model, subpopulations are assumed to be spatially organized through the Poisson–Voronoi tessellation (Dupanloup et al. 2002); we set the maximum number of nuclei within this tessellation to 300 (3 × maximum rate as suggested by Guillot et al. 2005a).

We ran the GENELAND MCMC 5 times with the level of uncertainty attached to our spatial coordinates set to 10 km, a liberal estimate of the dispersal ability of individual otters in the Gulf coast (Foy 1984). We also executed GENELAND runs with additive noise parameters of 0 and 100 km, to account for the possibility that inaccurate coordinates were provided. Because trappers are not restricted in where they may trap otters, we did not anticipate errors in spatial coordinates; however, whether errors in the recorded spatial coordinates of individuals exist or not, accounting for uncertainty in the positioning of individuals has been demonstrated to substantially improve precision in the detection of borders and allows individuals with the same spatial coordinates to belong to different populations (Guillot et al. 2005a). Additionally, we performed 5 runs of the model without spatial coordinates (spatially implicit model) for comparative purposes. We used the mode of the distribution of K as a point estimate of K. The assignment of individuals to subpopulations was performed in a separate run as suggested by Guillot et al. (2005a). For these runs, K was set to the inferred number of subpopulations and all other parameters were similar to those runs with variable K. Ten runs with fixed K were performed for the spatially explicit model (uncertainty = 10 000 m), and 10 runs with fixed K were performed for the spatially implicit model. For each of the 20 runs with fixed K, the posterior probability of subpopulation membership was computed for each pixel of the spatial domain (100 × 100 pixels), using a burn-in of 100 iterations. Individuals with a posterior probability of population membership of greater than 0.70 were unambiguously assigned to the modal subpopulation. Individuals were assigned to multiple subpopulations if the posterior probability of population membership was greater than 0.25 in more than 1 subpopulation.

Table 1. Estimates of genetic diversity in the total sample and in the 3 identified subpopulations. Sample size (n), observed (H_O) and expected (H_E) heterozygosities, the average number of alleles per locus (A), number (A_U) and average frequency of unique alleles, allelic richness (A_R), and deviation from random mating (F_{IS}) are provided. P values for F_{IS} estimates are given in parentheses

	n	H_O	H_E	A	A_U	A_R	F_{IS}
Total sample	111	0.590	0.651	6.75	N/A	6.12	0.0942 (0.0000)[a]
Inland	47	0.603	0.630	5.92	8 (0.043)	5.66	0.0437 (0.0500)
Atchafalaya River	32	0.539	0.622	5.50	3 (0.109)	5.50	0.1350 (0.0000)
Mississippi River	32	0.625	0.648	5.25	2 (0.070)	5.25	0.0366 (0.1250)

[a] The quantity estimated is for the total population (F_{IT}).

Bayesian clustering algorithms such as STRUCTURE and GENELAND can infer strict barriers when in fact the zone of contact may be more diffuse (Worley et al. 2004; Evanno et al. 2005; Frantz et al. 2006). To clarify the nature of these zones of contact, partial and multiple Mantel tests can be used to partition the effects of contemporary microevolutionary processes such as isolation by distance and long-term historical divergence associated with a possible historical barrier to gene flow (Telles and Diniz-Filho 2005). Thus, for each pair of inferred subpopulations, we generated 3 matrices for analysis: 1) a matrix of Rousset's genetic distance measure between individuals a (Rousset 2000; as calculated in SPAGEDI); 2) a matrix of pairwise Euclidean distances between individuals; and (3) a binary matrix expressing long-term historical divergence, created by allocating a value of zero to pairs of individuals located on the same side of a putative barrier, and a value of 1 to pairs located on different sides of the barrier (i.e., pairwise model matrix; see Manly 1997; Sokal et al. 1997; Telles and Diniz-Filho 2005). We performed both simple and partial Mantel tests using program ZT (Bonnet and Van de Peer 2002; with MantelTester GUI frontend) to calculate Pearson's correlation coefficient to measure correlation between the matrices. Significance was assessed using 10^6 randomizations. We also performed multiple Mantel regression (Legendre et al. 1994; Legendre and Legendre 1998) using Permute! (version 3.4; Casgrain 2001) to partition contemporary and historical effects on genetic divergence among subpopulations, according to the method of Telles and Diniz-Filho (2005).

Population Genetic Analyses of the Inferred Subpopulations

For each of the inferred subpopulations (based on spatially explicit GENELAND results), we tested for a deficiency of heterozygotes and quantified deviations from Hardy–Weinberg equilibrium by estimating F_{IS} as described previously. We controlled for multiple testing using the FDR control method as described previously.

We estimated levels of genetic diversity within each of the inferred subpopulations by calculating observed (H_O) and expected (H_E) heterozygosities, the number of alleles per locus (A), and the number (A_U) and frequency of unique alleles using GDA software (version 1.1; Lewis and Zaykin 2001). We adjusted the average number of alleles per locus for variation in subpopulation sample size (A_R) with rarefaction in FSTAT software (version 2.9.3; Goudet 1995), to alleviate the sensitivity of allelic diversity estimates to sample size (Hurlbert 1971; Petit et al. 1998).

We estimated levels of genetic differentiation among the inferred subpopulations by calculating F_{ST} according to the method of Weir and Cockerham (1984) in SPAGEDI software. We tested for significance of the observed F_{ST} values by comparing them to those obtained by 20 000 permutations of individuals among populations.

Results

Genetic Analyses on the Total Population

We obtained complete multilocus genotypes for all 111 river otters sampled. Within the total sample, the number of alleles per locus ranged from 3 (RIO14, RIO17) to 15 (RIO13), with an average of 6.75. Global tests on the total sample indicated a significant deviation from Hardy–Weinberg equilibrium ($P < 0.0001$), due to disequilibrium at 3 loci ($\alpha_{EW} = 0.0161$; $P_{RIO08} = 0.0001$; $P_{RIO11} = 0.0154$; $P_{RIO19} = 0.0020$). We found a significant deficiency of heterozygotes globally ($F_{IT} = 0.0942$, $P < 0.0001$; Table 1) and in 3 loci ($\alpha_{EW} = 0.0161$; $P_{RIO06} = 0.0134$; $P_{RIO08} = 0.0141$; $P_{RIO15} = 0.0037$).

Inferring Subpopulations

The STRUCTURE analysis indicated that the most likely number of subpopulations in the total sample was 3. The mean estimated logarithm of probability of the data (ln $\Pr(X|K)$) was maximum for $K = 3$ (Figure 1a), and the ΔK measure yielded a strong peak at $K = 3$ (Figure 1b). The 15 longer runs (5 each at $K = 2$, 3, and 4) gave results consistent with those of the comparable shorter runs (data not shown). Individual assignments are summarized in Figure 2a. Most individuals ($n = 99$) were unambiguously assigned to 1 of the 3 subpopulations ($\bar{q}$=0.903), weakly divided into geographic regions, hereafter referred to as: Inland ($n = 37$), Atchafalaya River ($n = 30$), and Mississippi River ($n = 32$). Twelve individuals were assigned to 2 subpopulations, but no individuals were assigned to all 3 subpopulations.

The GENELAND analysis to infer the number of subpopulations in the total dataset corroborated our

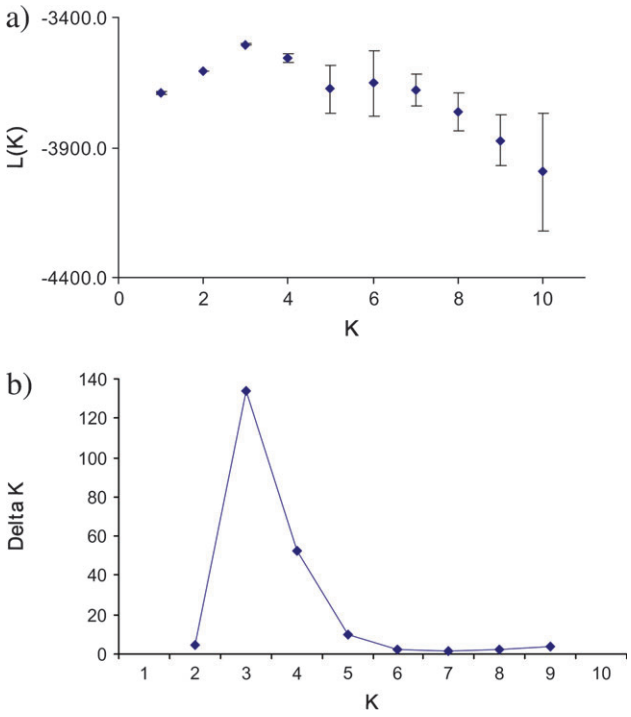

Figure 1. Plot of (a) mean likelihood values (averaged across runs) and (b) estimate of ΔK for each possible value of K using data obtained from STRUCTURE.

STRUCTURE analysis, indicating that the most likely value of K was 3. For all runs to infer K using spatial data (5 runs with 10 000 m uncertainty, 1 run with 0 m uncertainty, and 1 run with 100 000 m uncertainty), the mode of the distribution of K was 3. For the 5 runs performed without spatial coordinates, the mode of the distribution of K was either 4 or 5; however, in all runs there were only 3 subpopulations to which all individuals were assigned. Inferred subpopulations beyond 3 were 'ghost' subpopulations with no individuals assigned to them. This phenomenon has been noted previously (particularly when subpopulations are weakly differentiated) and it is recommended that such subpopulations be ignored (Guillot et al. 2005a, 2005b).

Individual assignments performed in GENELAND with K fixed to 3 were consistent across each of the 10 runs with and without spatial data (Figure 2b,c). In the spatially explicit model, GENELAND consistently identified 3 well-defined, geographically distinct subpopulations that generally corresponded to those identified in STRUCTURE: Inland (n = 47), Atchafalaya River (n = 28), and Mississippi River (n = 32; Figure 2b). Only 4 individuals were ambiguously assigned; 3 individuals were assigned to both the Inland and Atchafalaya River subpopulations and 1 individual within the spatially defined Atchafalaya region was genetically assigned to the Mississippi River subpopulation (Figure 2b). In the spatially implicit model, the 3 subpopulations were geographically less well defined; however, a general pattern separating Inland (n = 37), Atchafalaya River (n = 40), and Mississippi River (n = 29) subpopulations was observed (Figure 2c). Five individuals were assigned to 2 subpopulations.

The overall correlation between the physical and genetic distance matrices was 0.13 ($P < 0.00001$), indicating that the genetic similarity between individual otters decreased as the physical distance between them increased. However, this could be due to the genetic similarity of otters within subpopulations, which also happen to be spatially proximate. To specifically address isolation by distance across our inferred boundaries, we considered each pair of inferred subpopulations separately. Partial Mantel tests revealed the presence of a barrier to gene flow other than geographic distance for the Inland–Atchafalaya River subpopulations (r = 0.191, $P < 0.000001$) but not for the Inland–Mississippi River ($r = -0.032$, P = 0.061) or Atchafalaya River–Mississippi River (r = 0.029, P = 0.117). Similar results were obtained using a Mantel test based on a multiple regression design (Table 2). For the Inland–Atchafalaya River subpopulations, the R_T^2 of the full model (by combining geographic distances and the binary model matrix as predictors of genetic distances) was 0.049 ($P < 0.0001$). Using the equations provided in Telles and Diniz-Filho (2005), we determined that 8% of the variation in genetic distances that can be explained by geographic processes can be attributed to relationships within groups, due to geographically structured gene flow counteracting genetic drift, as in a contemporary isolation by distance model, whereas 73% can be attributed to long-term historical divergence alone. Although overlap between these 2 processes prevents us from entirely partitioning population divergence between historical and contemporary processes, the relative magnitude of the effects suggests that the Inland and Atchafalaya River subpopulations are genetically structured in accordance with a historical barrier to gene flow, rather than contemporary isolation by distance (Table 2). We used the same analyses and reasoning to identify the opposite pattern in the Atchafalaya River–Mississippi River comparison; that spatial patterns in genetic distances across these pairs of subpopulations are better explained by contemporary isolation by distance relationships among individuals than by historical divergence (Table 2). Although the Inland–Mississippi River subpopulations also showed an overall pattern consistent with isolation by distance as the primary effect, a large overlap term made straightforward interpretation more difficult for this comparison (Table 2).

Population Genetic Analyses of the Inferred Subpopulations

Despite the overall pattern of isolation by distance, we observed relatively consistent patterns of genetic clustering across the 3 models (STRUCTURE, spatially implicit GENELAND, and spatially explicit GENELAND). However, subpopulation boundaries were more clearly delineated in the spatially explicit model than in either of the spatially implicit models. Thus, we decided to use the individual

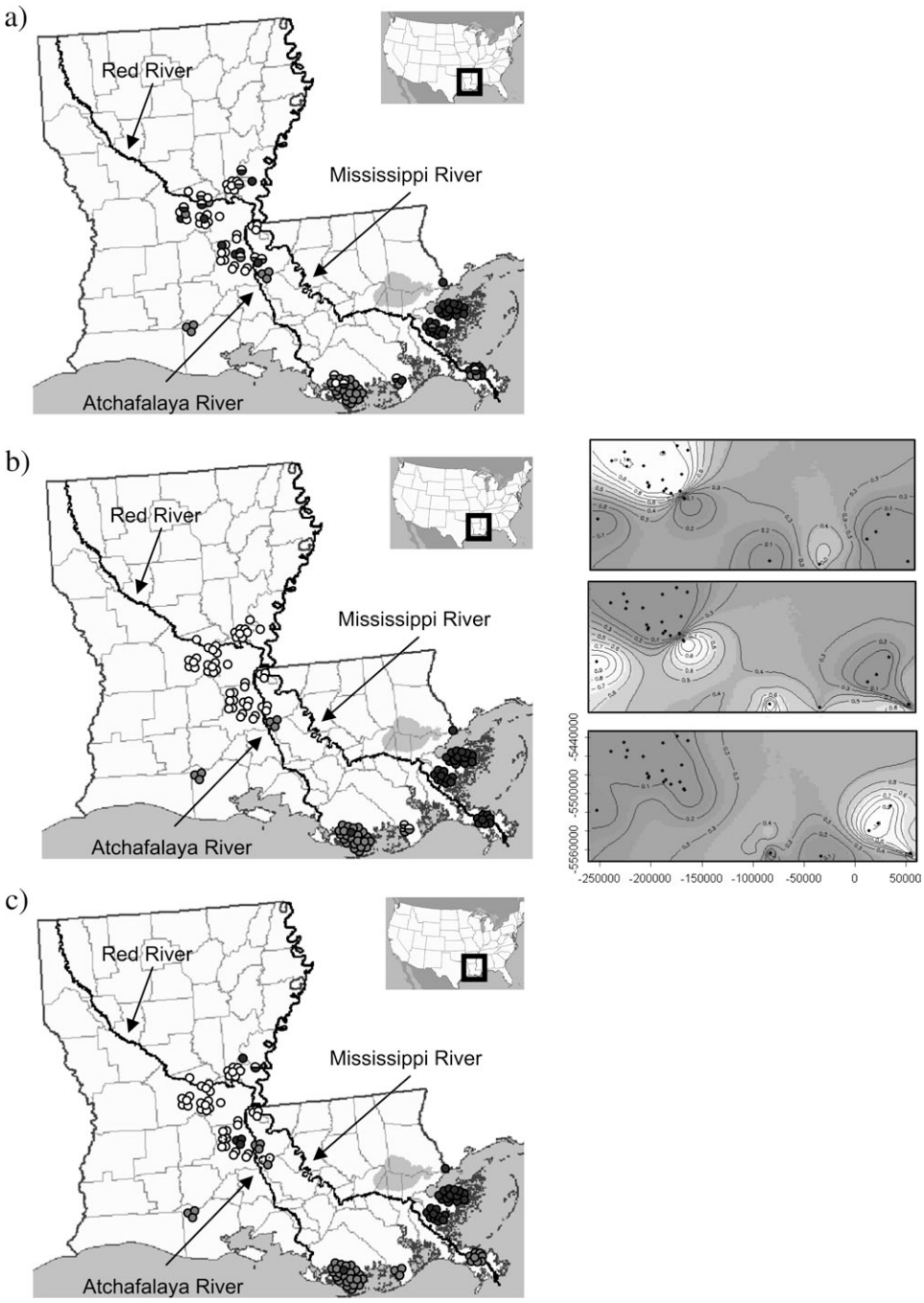

Figure 2. Individual assignments for (a) STRUCTURE, (b) spatially explicit GENELAND, and (c) spatially implicit GENELAND. Circles indicate unique samples, and multiple samples with identical spatial coordinates are clustered. Samples are grouped into 1 of 3 color-coded groups (white, black, gray). Ambiguous assignments are coded using half-circles (or dotted circles) to indicate the 2 (or 3) subpopulations to which the individual was assigned. Tessellations provided for the spatially explicit GENELAND analysis indicate the probability that a sample belongs to a particular group and range from low (dark color) to high (light color).

assignments generated by the spatially explicit GENELAND model to define subpopulation membership for subsequent genetic analyses.

The Atchafalaya subpopulation exhibited a significant deviation from Hardy–Weinberg equilibrium ($\alpha_{EW} = 0.0273$, $P < 0.0001$) due to disequilibrium at 2 loci ($\alpha_{EW} = 0.0161$, $P_{RIO08} = 0.0080$, $P_{RIO13} = 0.0134$). Conversely, we observed no deviation from Hardy–Weinberg equilibrium in the Inland or Mississippi subpopulations ($P_{Inland} = 0.0369$, $P_{Mississippi} = 0.0742$). We found a significant deficiency

Table 2. Results of multiple Mantel regressions using long-term historical divergence between subpopulations and IBD as predictors of genetic divergence between subpopulation pairs of North American river otters in Louisiana. The variation in genetic distance explained by both geographic effects (a + b + c) is equal to the R_T^2 of a multiple Mantel test using both effects as predictors ($1 - R_T^2$ = unexplained variation (d)). The R_I^2 of the regression using IBD alone (geographic distance matrix) gives (a + b), and the R_H^2 of the regression using history alone (binary model matrix) gives (b + c). The overlap between historical and contemporary predictors is equal to (a + b) + (b + c) – (a + b + c). a, b, and c are given as proportion of the total variation and as the percentage of explained variation. Equations follow Telles and Diniz-Filho (2005)

	Inland–Atchafalaya River	Inland–Mississippi River	Atchafalaya River–Mississippi River
IBD only (a)	0.0041 (8%)	0.0062 (21%)	0.0447 (50%)
Overlap (b)	0.0093 (19%)	0.0232 (76%)	0.0433 (49%)
Historical only (c)	0.0360 (73%)	0.0010 (3%)	0.0007 (1%)
Unexplained (d)	0.9506	0.9696	0.9113

IBD, Isolation by distance

of heterozygotes only in the Atchafalaya subpopulation ($F_{IS} = 0.1350$, $P < 0.0001$; Table 1). This deficiency was evident in almost all loci, so it may indicate additional cryptic subdivision within the Atchafalaya River subpopulation. We did run the Atchafalaya subpopulation through STRUCTURE to detect any additional subdivision; however, we did not detect any subdivision, which could be due to a lack of power at this scale.

Similar levels of genetic diversity were observed within each of the 3 subpopulations (Table 1). Observed heterozygosities ranged from 0.539 in the Atchafalaya River subpopulation to 0.625 in the Mississippi River subpopulation. The Inland subpopulation contained slightly more alleles per locus on average than did the Atchafalaya and Mississippi River subpopulations, a pattern that was retained when we adjusted for variation in subpopulation sample sizes (Table 1). This pattern reflected the large number of unique alleles in the Inland subpopulation ($n = 8$) relative to the Atchafalaya River ($n = 3$) and Mississippi River ($n = 2$) subpopulations. Most of the unique alleles detected were at low frequency, and while it is possible that many could be the result of small sample sizes, 1 allele (allele 214 at the RIO08 locus in the Atchafalaya River subpopulation) was observed at a frequency of 21%, more than twice as frequent as any other unique allele.

Genetic differentiation among populations was small but highly significant ($F_{ST} = 0.0429$; $P < 0.0001$). Pairwise F_{ST} estimates among subpopulations all were significant (Inland–Atchafalaya river = 0.0370, $P < 0.0001$; Inland–Mississippi River = 0.0421, $P < 0.0001$; and Atchafalaya River–Mississippi River = 0.0524, $P < 0.0001$).

Discussion

Our data do not support the hypothesis that the North American river otter population in Louisiana exists as a single, panmictic population. When we considered the entire sample of otters from Louisiana, we detected a significant deviation from random mating, indicating that otters were not distributed in a random fashion and were instead associated in genetically defined subpopulations. All Bayesian clustering methods (STRUCTURE, spatially implicit GENELAND, and spatially explicit GENELAND) divided river otters in Louisiana into 3 subpopulations. However, each method differed slightly in the exact location of subpopulation boundaries and in the assignment of individual river otters to subpopulations.

Spatially implicit models often assigned individual otters to multiple subpopulations. Ambiguous assignments of otters in the spatially implicit models likely resulted from a combination of similar allele frequency distributions among subpopulations (caused by recent isolation or contemporary gene flow) and a corresponding lack of power to differentiate among subpopulations in these models (Latch et al. 2006). As allele frequency distributions between 2 populations increase in similarity, it has been demonstrated that spatially implicit models are increasingly unable to correctly identify the number of subpopulations in a dataset or to unambiguously assign individuals to populations (Evanno et al. 2005; Latch et al. 2006). The levels of genetic differentiation we observed among the inferred subpopulations approached the level at which spatially implicit models like STRUCTURE are still able to correctly identify the number of subpopulations but may have a relatively high rate of ambiguous or incorrect assignments of individuals to these subpopulations (Latch et al. 2006).

By including geographic information into the spatially explicit GENELAND model, genetic discontinuities in the dataset were clearly identified as barriers to gene flow between subpopulations. However, inferred boundaries between subpopulations should be interpreted with caution. Locations where river otters were sampled were somewhat geographically disjunct, and it is possible that this resulted in inefficient inference of genetic discontinuities (Manel et al. 2003). To help alleviate this potential problem, we employed conservative priors, including a high lambda value (indicating weak spatial organization) and a liberal additive noise to the spatial coordinates, resulting in a relatively weak dependence on spatial data. Even so, genetic discontinuities may still be open to interpretation, particularly when boundaries are inferred along a continuum of genetic types, as in situations where populations are connected by contemporary processes such as isolation by distance.

Further investigation of the inferred boundaries between subpopulations revealed that both contemporary and

historical processes influence the genetic structure of river otters across Louisiana. At the Inland–Atchafalaya River boundary, results from both partial Mantel and multiple regression analyses suggest that long-term historical divergence may be a more powerful explanation for genetic divergence than contemporary processes such as isolation by distance. At the Atchafalaya River–Mississippi River boundary, the inferred genetic discontinuity could not be unequivocally associated with a long-term barrier to gene flow. Isolation by distance, due to a contemporary balance between local genetic drift and geographically mediated gene flow, was the primary force shaping patterns of genetic divergence between the Atchafalaya River and Mississippi River subpopulations. At the Inland–Mississippi River boundary, partial Mantel tests suggested isolation by distance, but the large overlap term in the multiple regression analysis makes interpretation less obvious. Nevertheless, contemporary gene flow seems to be playing a large role in genetic structure between the Inland and Mississippi River subpopulations.

Within the inferred subpopulations, conformation of the Inland and Mississippi River subpopulations to Hardy–Weinberg expectations suggests that these subpopulations have been appropriately defined. While it is possible that the deviation from random mating observed in the Atchafayala River subpopulation indicates the presence of additional cryptic subdivision within this sample, the diffuse sampling effort in this subpopulation may have prohibited us from detecting it. Levels of genetic diversity within the Louisiana otter subpopulations are relatively high and similar to those found in other populations of otters across North America (Fike 2005). One interesting observation is the high frequency unique allele found throughout the Atchafalaya River subpopulation. Although we cannot unambiguously identify the origin of this unique allele, it is likely that isolation and subsequent genetic drift are responsible for the presence of this allele at high frequency in the Atchafalaya River subpopulation. Alternatively, perhaps otters in this region are connected to unsampled populations to the west that contain this allele at high frequency.

No obvious physical barriers exist to restrict gene flow between the coastal and inland populations of river otters in Louisiana, yet these 2 groups remain distinct. Our data indicate a historical boundary to gene flow between the Inland and Atchafalaya River subpopulations, yet no obvious landscape-level barriers are present. The Inland population also is diverged from the Mississippi River subpopulation, although the large amount of overlap variance does not allow for a definitive conclusion regarding the scale of this divergence (historical vs. contemporary). Genetic differentiation between coastal and inland populations of North American river otters in the absence of physical barriers has been documented in Alaska and was attributed to sex-biased dispersal (Blundell et al. 2002). Although available data do not allow us to evaluate this hypothesis, it is feasible that similar dispersal patterns could be shaping the genetic structure in Louisiana.

Coastal and inland habitats in Louisiana differ rather dramatically, and river otters may be utilizing their respective habitats in fundamentally different ways. Social organization of North American river otters (both in terms of degree of sociality and group composition) varies widely both among and within habitats (Melquist and Hornocker 1983; Erickson and McCullough 1987; Reid et al. 1994; Blundell et al. 2002), and although data are absent regarding the social organization of river otters in Louisiana in either coastal or inland habitats, it is reasonable to hypothesize that river otter social organization may differ between these aquatic landscapes.

In Louisiana, coastal and inland otter populations have different diet composition; and although both rely on fish as their main prey, blue crab (which peaks in late fall) is the second most important item found in the diet of coastal otters, whereas crayfish (which peaks in early winter) is the second most important for inland otters (Chabreck et al. 1982; Mouton E, Louisiana Department of Wildlife and Fisheries, personal communication). Thus, it is possible that prey availability could be contributing to a difference in breeding seasonality between coastal otter populations and upland otter populations. Such shifts have been observed for the European otter (Rui Beja 1996) and the North American river otter in Montana (Crait et al. 2006).

Regardless of the mechanism, it is likely that the isolated distribution of coastal otters may be exacerbated by relatively poor dispersal, which has been documented for otters along the Gulf coast (Foy 1984). Contrary to what has been observed in Idaho (32km; Melquist and Hornocker 1983) and Alaska (more than 60 km; Blundell et al. 2002), a study of river otters inhabiting coastal marshes in East Texas suggests that otters in the Gulf coast may disperse no more than a few kilometers (7 km; Foy 1984). Thus, it can be hypothesized that the isolation by distance pattern of gene flow observed between the Atchafalaya and Mississippi populations could at least in part be the result of isolation caused by the dependency of coastal otters on sources of freshwater combined with poor dispersal among isolated subpopulations.

It is clear that North American river otters do not utilize Louisiana's landscape uniformly and that contrasting dynamics in social organization and habitat utilization of others between coastal and inland aquatic habitats could be playing an important role in structuring otter populations in Louisiana. This has significant implications for conservation and management of the species within Louisiana, where we can now use this information to set management regulations to retain the distinct genetic diversity present in each subpopulation. On a larger scale, the implications of these data are particularly relevant because nearly all extant populations of North American river otter have been established using at least some otters from Louisiana. We do not know whether, and to what extent, coastal or inland subpopulations have been used as founding populations in the past, and certainly this could significantly affect the viability of reestablished populations throughout North America. Our findings can help guide selection of founding

stock for future reintroduction efforts to maximize chance of persistence in reintroduced populations and to minimize loss of unique genetic types within Louisiana.

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References

Armstrong DM. 1972. Distribution of mammals in Colorado. University of Kansas Museum of Natural History Monograph No. 3:415.

Beheler AS, Fike JA, Dharmarajan G, Rhodes OE, Serfass TS. 2005. Ten new polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and their utility in related mustelids. Mol Ecol Notes. 5:602–604.

Beheler AS, Fike JA, Murfitt LM, Rhodes OE, Serfass TS. 2004. Development of polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and amplification in related Mustelids. Mol Ecol Notes. 4:56–58.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B. 57:289–300.

Benjamini Y, Yekutieli D. 2001. The control of the false discovery rate in multiple testing under dependency. Ann Stat. 29:1165–1188.

Blundell GM, Ben-David M, Groves P, Bowyer RT, Geffen E. 2002. Characteristics of sex-biased dispersal and gene flow in coastal river otters: implications for natural recolonization of extirpated populations. Mol Ecol. 11:289–303.

Bonnet E, Van de Peer Y. 2002. zt: a software tool for simple and partial Mantel tests. J Stat Soft. 7:1–12.

Casgrain P. 2004. Permute! Multiple regression over distance, ultrametric, and additive matrices with permutation test. Computer program and documentation. Available from URL http://www.bio.umontreal.ca/Casgrain/en/labo/permute

Chabreck R, Edwards T, Linscombe G. 1985. Factors affecting the distribution and harvest of river otters in Louisiana. Proc Ann Conf Southeast Assoc Fish Wildl. 39:520–527.

Chabreck R, Holcombe J, Linscombe G, Kinler N. 1982. Winter food of river otters from saline and fresh environments in Louisiana. Proc Ann Conf Southeast Assoc Fish Wildl. 36:373–383.

Chesser RK. 1991. Influence of gene flow and breeding tactics on gene diversity within populations. Genetics. 129:573–583.

Crait JR, Blundell GM, Herreman JK, Ben-David M. 2006. Late seasonal breeding of river otters in Yellowstone National Park. Am Midl Nat. 156:189–192.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. Mol Ecol. 11:2571–2581.

Edwards T. 1983. River otter abundance, distribution and habitat use in Louisiana [thesis]. [Baton Rouge (LA)]: Louisiana State University.

Ensminger A, Linscombe G. 1980. The fur animals, the alligator, and the fur industry in Louisiana. Louisiana Dept Wildl Fish Bull. 109.

Erickson DW, McCullough CR. 1987. Fates of translocated river otters in Missouri. Wildl Soc Bull. 15:511–517.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 14:2611–2620.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 164:1567–1587.

Fike JA. 2005. Genetic structure of river otter populations in eastern North America and an assessment of genotyping error rates associated with alternative sources of DNA [thesis]. [Frostburg (MD)]: Frostburg State University.

Foy MK. 1984. Seasonal movement, home range. habitat use of river otter in Southern Texas [thesis]. [College Station (TX)]: Texas A&M University.

Frantz AC, Tigel Pourtois J, Heurtz M, Schley L, Flamand MC, Krier A, Bertouille S, Chaumont F, Burke T. 2006. Genetic structure and assignment tests demonstrate illegal translocation of red deer (*Cervus elaphus*) into a continuous population. Mol Ecol. 15:3191–3203.

Glaubitz JC. 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol Ecol Notes. 4:309–310.

Goudet J. 1995. FSTAT version 1.2: a computer program to calculate F-statistics. J Hered. 86:485–486.

Guillot G, Estoup A, Mortier F, Cosson J-F. 2005a. A spatial statistical model for landscape genetics. Genetics. 170:1261–1280.

Guillot G, Mortier F, Estoup A. 2005b. Geneland: a computer package for landscape genetics. Mol Ecol Notes. 5:708–711.

Guo SW, Thompson EA. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics. 48:361–372.

Hall ER. 1981. The mammals of North America. New York (NY): John Wiley and Sons.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. Mol Ecol Notes. 2:618–620.

Holcombe J. 1980. Winter food habits of the river otter in southern Louisiana [thesis]. [Baton Rouge (LA)]: Louisiana State University.

Hurlbert SH. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology. 52:577–586.

Latch EK, Dharmarajan D, Glaubitz JC, Rhodes OE. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conserv Genet. 7:295–302.

Legendre P, Lapointe F-J, Casgrain P. 1994. Modeling brain evolution from behavior: a permutational regression approach. Evolution. 48:1487–1499.

Legendre P, Legendre L. 1998. Numerical ecology. Amsterdam (the Netherlands): Elsevier.

Lewis PO, Zaykin D. 2001. Genetic Data Analysis: computer program for the analysis of allelic data. Version 1.1. Available from: URL http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php

Larivière S, Walton LR. 1998. Lontra canadensis. Mammalian Species. 587:1–8.

Lowery G. 1974. The mammals of Louisiana and its adjacent waters. Baton Rouge (LA): Louisiana State University Press.

Manel S, Schwartz MK, Luikart G, Taberlet P. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol. 18:189–197.

Manly BJF. 1997. Randomization, bootstrap and Monte Carlo methods in biology. London: Chapman and Hall.

Melquist WE, Dronkert AE. 1987. River otter. In: Novak M, Baker JA, Obbard ME, Malloch B, editors. Wild furbearer management and conservation in North America. Ontario: Ontario Ministry of Natural Resources. p. 627–641.

Melquist WE, Hornocker MG. 1983. Ecology of river otter in west central Idaho. Wildl Monog. 83.

Moran MD. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos. 100:403–404.

Melquist WE, Polechla PJ, Toweill D. 2003. River otter (*Lontra canadensis*). In: Feldhamer GA, Thompson BC, Chapman JA, editors. Wild Mammals of North America: biology, management, and conservation. Baltimore (MD): Johns Hopkins University Press. p. 708–734.

Narum S. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. Conserv Genet. 7:783–87.

Nilsson G. 1980. River otter research workshop. Gainesville (FL): Florida State Museum.

Petit RJ, El Mousadik A, Pons O. 1998. Identifying populations for conservation on the basis of genetic markers. Conserv Biol. 12:844–855.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155:945–959.

Pritchard JK, Wen W. 2003. Documentation for STRUCTURE software: Version 2. Available from: URL http://pritch.bsd.uchicago.edu

Ralls K. 1990. Reintroductions. In: Foster-Turley P, Macdonald S, Manson C, editors. Otters: an action plan for their conservation. Brookfield (IL): Kelvyn Press. p. 20–21.

Raesly EJ. 2001. Progress and status of river otter reintroduction projects in the United States. Wildl Soc Bull. 29:856–862.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population-genetics software for exact tests and ecumenicism. J Hered. 86:248–249.

Reid DG, Code TE, Reid ACH, Herrero SM. 1994. Spacing, movements, and habitat selection of the river otter in boreal Alberta. Can J Zool. 72:1314–1324.

Rousset F. 2000. Genetic differentiation between individuals. J Evol Biol. 13:58–62.

Rousset F, Raymond M. 1995. Testing heterozygote excess and deficiency. Genetics. 140:1413–1419.

Rui Beja P. 1996. Seasonal breeding and food resources of otters, *Lutra lutra* (Carnivora, Mustelidae), in south-west Portugal: a comparison between coastal and inland habitats. Mammalia. 60:27–34.

Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual. 3rd edn. New York: Cold Spring Harbor Laboratory Press.

Scognamillo DG. 2005. Temporal and spatial harvest patterns of river otter in Louisiana and its potential use as a bioindicator species of water quality [dissertation]. [Baton Rouge (LA)]: Louisiana State University.

Serfass TL, Brooks RP, Novak JM, Johns PE, Rhodes OE. 1998. Genetic variation among populations of river otters in North America: considerations for reintroduction projects. J Mammal. 79: 736–746.

Shirley MG, Linscombe G, Kinler NW, Knaus RM, Wright VL. 1988. Population estimates of river otters in a Louisiana coastal marshland. J Wildl Manag. 52:512–515.

Sokal RR, Oden NL, Walker J, Waddle DM. 1997. Using distance matrices to choose between competing theories and an application to the origin of modern humans. J Hum Evol. 32:501–522.

Telles MPC, Diniz-Filho JAF. 2005. Multiple Mantel tests and isolation by distance, taking into account long-term historical divergence. Gen Mol Res. 4:742–748.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for analysis of population structure. Evolution. 38:1358–1370.

Worley K, Strobeck C, Arthur S, Carey J, Schwantje H, Veitch A, Coltman DW. 2004. Population genetic structure of North American thinhorn sheep *Ovis dalli*. Mol Ecol. 13:2545–2556.

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