Non–invasive sampling of endangered neotropical river otters reveals high levels of dispersion in the Lacantun River System of Chiapas, Mexico

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Abstract

Non–invasive sampling of endangered neotropical river otters reveals high levels of dispersion in the Lacantun River System of Chiapas, Mexico.— Patterns of genetic dispersion, levels of population genetic structure, and movement of the neotropical river otter (*Lontra longicaudis*) were investigated by screening eight polymorphic microsatellites from DNA extracted from fecal samples, collected in a hydrologic system of the Lacandon rainforest in Chiapas, Mexico. A total of 34 unique genotypes were detected from our surveys along six different rivers, and the effect of landscape genetic structure was studied. We recovered 16 of the 34 individuals in multiple rivers at multiple times. We found high levels of dispersion and low levels of genetic differentiation among otters from the six surveyed rivers ($P > 0.05$), except for the pairwise comparison among the Lacantún and José rivers ($P < 0.05$). We recommend that conservation management plans for the species consider the entire Lacantún River System and its tributaries as a single management unit to ensure the maintenance of current levels of population genetic diversity, because the population analyzed seems to follow a source–sink dynamic mainly determined by the existence of the major river.

Key words: Assignment test, Chiapas, Lacandon rainforest, Low genetic structure, Microsatellites, Neotropical river otter.

Resumen

El muestreo no invasivo de las nutrias neotropicales, una especie amenazada, revela altos niveles de dispersión en el sistema fluvial del Lacantún, en Chiapas, México.— Se investigaron patrones de dispersión genética, niveles de estructura poblacional genética, y desplazamientos de las nutrias neotropicales (*Lontra longicaudis*) mediante el screening de ocho microsatélites polimórficos tomados del DNA de muestras fecales, que fueron recogidas en el sistema hidrológico del río Lacantún de la Selva Lacandona, Chiapas, México. Se detectó un total de 34 genotipos únicos a lo largo de seis ríos, estudiándose el efecto de la estructura genética según el paisaje. Se identificaron 16 de los 34 individuos estudiados en varios de los ríos y en múltiples ocasiones. Encontramos altos niveles de dispersión y bajos niveles de diferenciación genética entre las nutrias de los seis ríos estudiados ($P > 0{,}05$), con excepción de las comparaciones por pares entre los ríos José y Lacantún ($P < 0{,}05$). Se recomienda que en los planes de gestión para esta especie se considere el sistema hidrológico del río Lacantún como una sola entidad, para así mantener los niveles de diversidad genética encontrados, dado que parece que la población analizada sigue una dinámica de fuente-sumidero determinada por el pricipal río de la zona.

Palabras clave: Test de asignación, Chiapas, Selva Lacandona, Estructura genética baja, Microsatélites, Nutria neotropical.

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Introduction

The neotropical river otter (*Lontra longicaudis*) is a semi–aquatic carnivore that has been protected by Mexican law since 1994 (NOM, 1994) and is also considered an endangered species according to CITES Appendix I (Larivière 1999). Its current distribution includes Central America, South America, and the island of Trinidad in the Caribbean. It is found in different riverine habitats, including deciduous and evergreen forests, but it is thought to prefer clear rivers and streams. In Mexico, populations of the neotropical river otter currently exist in larger rivers and their tributaries along the northern states of Sinaloa and Jalisco to the southern states of Chiapas, Veracruz, Oaxaca, and Tabasco, including the Yucatan Peninsula. he Lacandon forest is located in the southeast part of the Mexican state of Chiapas and occupies an area of ca. 957, 240 ha (fig. 1). The protected area has an intricate hydrologic system composed of permanent rivers that are well interconnected by smaller tributaries that become desiccated during the dry season. In the Lacantún River System, neotropical river otters subsist in moderate densities, in part because the Lacandon forest has been protected by the Mexican government and has been minimally impacted by anthropogenic activities. The most obvious way of dispersal for otters is the use of water currents to increase their speed and mobility. They also inhabit transformed rivers where the original vegetation has been cleared by anthropogenic activities.

Molecular markers have proven to be an important tool for investigating the patterns of gene flow and levels of genetic structure of otters in different parts of the world. Microsatellite genotype variation in North American river otters (*Lontra canadensis)* revealed that ecological barriers prevented dispersal, and this resulted in the genetic differentiation of three inland and coastal subpopulations in Louisiana (Latch et al., 2008). Another microsatellite study revealed high levels of gene flow among seven different populations of river otters, where gender differences were fundamental to the understanding of genetic diversity and recolonization in the area (Blundell et al., 2002; Reid et al., 1994). In European otters (*Lutra lutra*), microsatellite markers revealed that steep and dry areas prevented proper dispersal in 10 river basins and two genetically differentiated otter populations were described for the area (Janssens et al., 2008). In addition, mitochondrial DNA (mtDNA) data helped discover two well–defined subpopulations of the Southern river otter (*Lontra provocax*) in the southern part of Argentina (Centrón et al., 2008). On a broader scale, allozyme data revealed that the North American river otters (*L. canadensis*) were sorted in eight geographic regions and that gene flow was more restricted (Serfass et al., 1998).

River otters are elusive and difficult to capture. Therefore, in this study, we used non–invasive sampling because it has demonstrated to be an efficient method to conduct genetics studies of wild animals without having to capture them, or even observe them (Dallas et al., 2003). In addition, because the same individual can be recaptured multiple times in different areas non–invasive sampling can be very useful to more directly study individual movement and dispersion. We extracted DNA from non–invasively collected fecal samples and used eight polymorphic microsatellite loci to study movement patterns and levels of genetic structure among populations of the Neotropical river otter (*L. longicaudis*) inhabiting the Lacantún River System and its tributaries in the Lacandon forest, Chiapas, Mexico. Our goal was to characterize the levels of genetic diversity from animals sampled in six major rivers to assess movement of individuals and dispersion in an environment without intense anthropogenic activities and low levels of habitat fragmentation. We hypothesized that the complex network of rivers and tributaries throughout the study area would allow neotropical river otters to move freely through the region as one large, panmictic population, especially because during the dry season, tributaries are desiccated and force animals to move back to the larger permanent rivers. To assess our hypothesis we used microsatellites specifically designed for river otters, because they have demonstrated to have sufficient power to examine fine scale genetic structure and tracking dispersion patterns of river otters among the different tributaries (Latch et al., 2008).

Material and methods

Biological sample

We sampled an area that encompassed most of the known current distributional range of the neotropical river otter (*Lontra longicaudis*) in the southern part of the Lacandon Rainforest Reserve, in the southern state of Chiapas, Mexico. The maximum linear distance between the most distant rivers is 34 km and our geographic scale is limited to the Lacandon Rainforest Reserve extension (957,240 ha). Our sampling was designed to obtain fecal samples from as many individuals as possible in an effort to accomplish an exhaustive sampling of the population and to assess individual movement patterns and dispersion in an environment with low levels of habitat fragmentation. We surveyed eight transects along the six rivers, walking a maximum distance of 10 km from the main Lacantún River, searching for otter latrines with fresh fecal material. Transects were surveyed during two field seasons in the early morning (dry and cold seasons of 2006 and 2007) to avoid the negative effect of the weather on the feces (Hájková et al., 2006). Fresh fecal samples were placed in Falcon tubes with silica gel and transported to the laboratory for analysis. In total, we collected 623 fecal samples along the Lacantún River (LcR, n = 154), Colorado River (CR, n = 65), José River (JR, n = 85), Lagartos River (LgR, n = 97), Miranda River (MR, n = 129), and Tzendales River (TR, n = 93) (fig. 1). We did not identify fecal samples using a genetic marker because visual identification of feces from neotropical river otter from latrines and by the type of food ingested (fish and crustaceans) is an established

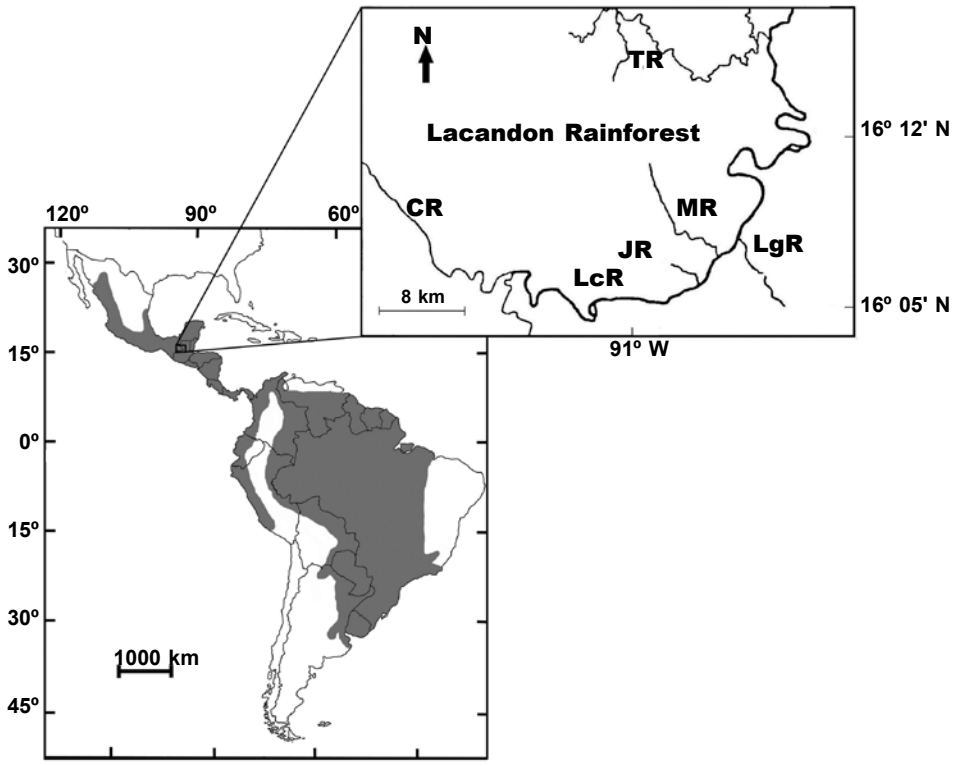

Fig. 1. Rivers sampled in the Lacandon Rainforest, Chiapas, Mexico. Location of the rivers is shown as follows: Miranda (MR), José (JR), Lacantún (LcR), Tzendales (TR), Lagartos (LgR) and Colorado (CR). We included the historical distribution map of the species, and merged the collecting site that is restricted to the extension of the Lacandon Rainforest Reserve.

Fig. 1. Ríos muestreados en la Selva Lacandona, Chiapas, México. La localización de los ríos se indica de la forma siguiente: Miranda (MR), José (JR), Lacantún (LcR), Tzendales (TR), Lagartos (LgR) y Colorado (CR). Se ha incluido el mapa de la distribución histórica de la especie y destacado el lugar de recolección que está dentro de la Reserva de la Selva Lacandona.

and very reliable method for documentation of river otters in the field (Medina–Vogel & Gonzalez–Lagos, 2008). DNA was also extracted from blood and fecal samples from five captive *L. longicaudis* housed at two different zoological parks. This DNA was used as positive controls and to validate the accuracy of our fecal DNA genotypes.

Laboratory procedures

Whole genomic DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit and from blood using the QIAamp Tissue and Blood Kit (QIAGEN) in a separate room from PCR products (Eggert et al., 2005). Quality of DNA was assessed by electrophoresis on 1% agarose gels in combination with molecular weight standards. We optimized DNA amplification following the standard protocol suggested by Lampa et al. (2008). In total, eight polymorphic microsatellite loci designed for the sister species *L. canadensis* (Beheler et al., 2004, 2005) were optimized and amplified for this species. PCR conditions consisted of an initial denaturation at 95ºC for 2', followed by 35 cycles at 94ºC for 30s, respective annealing temperature for 30s, 72ºC for 30s, then 72ºC for 10'. We incorporated variations for the PCR conditions (Protocol 1, 2, and 3) as suggested by Beheler et al., 2004 and Beheler et al., 2005. All reactions were performed in a Perkin Elmer 9700 Thermocycler. Amplifications of microsatellite loci were carried out in a 15 µL volume containing 30 ng of DNA, 0.3 mM of dNTP´s, 0.5 µM of each primer, 1x *Taq* buffer (2.0 µM of $MgCl_2$, 10 mM of Tris–HCl, 50 mM of KCl), 2.5x of BSA, and 1.0 U of Flexi*Taq* polymerase (PROMEGA). Each fecal sample was genotyped four independent times to validate our data and to overcome problems associated with low quality DNA obtained from non–invasive samples, and to be consistent with the multi–tube approach (Taberlet et al., 1996). We sexed samples by using the amplification of the zinc finger gene region on agarose gels, following the protocol proposed by Ortega et al. (2004). PCR products were verified by electrophoresis on 1% agarose gels stained with ethidium bromide. Fragment

analysis was performed on an ABI Prism 3100 Genetic Analyzer. Computer–generated results were analysed using the GeneScan (version 2.1) software, and final allele–sizing was done using the ABI Genotyper package (version 2.1).

Genetic analysis

We estimated the proportion of polymorphic loci and the average number of alleles per locus using GDA software (Lewis & Zaykin, 2001), and the observed and the expected heterozygosity using POPGENE (Yeh & Boyle, 1997) by the algorithm proposed by Levene (1949). We analysed differences in allele frequency distribution between the different sampled sites for each locus individually and across all loci, and also tested departures from Hardy–Weinberg equilibrium and linkage disequilibrium using GENEPOP version 4.0 (Rousset, 2008). Due to the variation in sample sizes between collecting localities, inbreeding coefficients (*Fis*) were calculated using the algorithm suggested by Weir & Cockerham (1984).

Proportions of allelic dropout and false allele detection associated with the non–invasive sampling were estimated by comparing the genotypes of DNA extracts from fecal samples of captive otters with genotypes of blood DNA extracted from the same otters. The proportion of allelic dropout was estimated as the proportional number of all–PCRs in which a fragment observed in the DNA extracted from blood was undetected in PCRs derived from fecal DNA. The proportion of the false allele detection was estimated as the proportional number of all PCRs in which a fragment observed in the DNA extracted from feces was not present in PCRs derived from DNA extracted from blood (Dallas et al., 2002, 2003). Using the proportion of allele dropout and false allele, we used the program GEMINI (Valière et al., 2002) to obtain the expected proportions of incorrect genotypes estimated for the entire population. We used the same software to determine the ability of our eight microsatellites to distinguish between individuals, the probability of identity (P_{ID}) (*i.e.* the probability of different individuals sharing an identical genotype at random; Mills et al., 2000; Waits et al., 2001) and the P_{ID} between siblings using the 5 samples from captive otters caught in the same area, but under the custody of a zoological park.

We calculated population substructure and heterozygote deficits using FSTAT version 2.9.3 (Goudet, 1995). Because genotypes from all six sampling localities were in Hardy–Weinberg equilibrium, we calculated pairwise F_{st} for all sampled sites and tested the significance of gene diversity across different sites using 1,000 permutations with the same program; *P*–values were obtained by multiple comparisons after Bonferroni corrections. We used *Fst* as an estimator of genetic variation between populations because it is more sensitive to test the hypothesis of panmixia.

We also investigated population genetic structure using an analysis of molecular variance (AMOVA), as implemented by ARLEQUIN (version 3.01, Excoffier et al., 2005). The variance components are used to calculate differences among populations and between individuals indifferent rivers. To assess inter–population dispersal, we used assignment tests in the GENECLASS 2 software (Piry et al., 2004), by using the algorithm of Paetkau et al. (1995). These assignment tests use individual genotypes and population allele frequencies to calculate potential migrants and to allocate them to their original populations (Cornuet et al., 1999). In addition, we obtained a graph with the GENETIX v. 4.05 software (Belkhir et al., 1996–2004), and we performed a Factorial Correspondence Analysis (FCA) to visualize the distribution of sampled individuals according to their sample site.

In order to understand how genetic variation was distributed within the study area, we first used Bayesian assignment techniques to test for population structure using the program STRUCTURE (version 2.2, Pritchard et al., 2000). This method identifies clusters of genetically similar individuals from multilocus genotypes without prior knowledge of their origin and genetic relationships. We ran a series of pilot runs to estimate Pr(X|*K*), where X represents the data for *K* between 1 (the expected value if all individuals belong to the same cluster) and 6 (the maximum number of sampling localities in different rivers). In our final runs to determine the most likely *K*, we assumed that populations had correlated allele frequencies, inferred alpha from the data, and used a burn–in and Markov chain Monte Carlo (MCMC) of 200,000 followed by 1,000,000. Longer burn–in or MCMC did not change the results.

We also conducted estimates of population size by using the mark–recapture data of neotropical river otter genotypes presented in table 1 and the CAPWIRE software (Miller et al., 2005). This program has been recently developed to maximize the use of DNA–based mark–recapture data and performs well for smaller populations (N < 100) with substantial capture heterogeneity. The program has two models to estimate population size, both based on presence or absence of captured heterogeneity. We assumed demographic resolution on the basis of the relatively short sampling time, while geographical resolution was maximized by collecting the scats in the closest rivers. In order to remove pseudo–replicates, scats collected from the same latrine on the same day that originated from the same individual were considered as a single observation.

Results

During our scat collection surveys of the six rivers we recovered a total of 623 presumed river otter fecal samples. We attempted to isolate DNA from all 623 samples and obtained complete microsatellite and sex data for 154 fecal samples. While our amplification success was low (24.4%), these 154 samples were successfully amplified for all eight microsatellite loci designed by Beheler et al. (2004, 2005) and for a zinc finger sexing marker (Ortega et al., 2004). Furthermore, they met the scoring requirements of our strict protocol applying the multipletube approach. DNA extracted from neotropical river otter feces is

Table 1. We obtained a total of 154 genotypes for 8 microsatellite loci from the collected fecal samples. We identified 34 unique genotypes and 16 of these were recaptured many times and in many rivers (denoted with an *). This table shows the number of repeated feces collected with each of the 34 unique genotypes, the location, sex of the individual, and the field season ($^{+}$ 2006 *and* $^{-}$ 2007): #G. Genotype. (For abbreviations, see figure 1.)

*Tabla 1. Se obtuvo un total de 154 genotipos para los 8 loci de microsatélites a partir de las muestras fecales recogidas. Se identificaron 34 genotipos únicos, 16 de los cuales fueron recapturados en múltiples ocasiones y en varios ríos (seguidos de un *). Esta tabla muestra el número de heces recogidas repetidamente, con cada uno de los 34 genotipos únicos, la localización, el sexo del individuo y la estación del año ($^{+}$ 2006 and $^{-}$ 2007).* #G. Genotipos. *(Para las abreviaturas ver figura 1.)*

#G	LcR	MR	JR	CR	LgR	TR
1♀	3^{+}	0	0	0	0	0
2♀*	2^{-}	0	0	1^{-}	0	1^{+}
3♀*	4^{+}	0	0	2^{+}	0	1^{+}
4♂	5^{-}	0	0	0	0	0
5♀	0	2^{+}	0	0	0	0
6♀	0	3^{-}	0	0	0	0
7♂	0	2^{+}	0	0	0	0
8♂	0	0	0	5^{-}	0	0
9♀*	0	0	6^{+}	0	0	1^{-}
10♂	0	0	0	0	4^{-}	0
11♂	0	2^{+}	0	0	0	
12♀*	1^{-}	0	0	1^{-}	0	1^{+}
13♀*	3^{-}	0	0	2^{+}	0	0
14♂*	0	2^{-}	2^{+}	0	0	2^{-}
15♀*	2^{+}	0^{+}	2^{-}	0	3^{-}	1
16♀*	0	1^{-}	1^{-}	0	3^{-}	1^{-}
17♂	0	0	0	1^{+}	0	0
18♀*	2^{+}	0	0	2^{-}	1^{+}	0
19♀*	1^{+}	1^{-}	0	1^{+}	0	0
20♂	5	0	0	0	0	0
21♂	0	0	5^{+}	0		0
22♂*	2^{+}	3^{+}	0	0	2^{-}	0
23♂	0	0	0	2^{-}	0	0
24♀	0	0	0	0	5^{-}	0
25♀	2^{-}	0	0	0	0	0
26♀	0	0	0	5^{-}	0	0
27♂	0	0	0	0	0	3^{-}
28♂	6^{+}	0	0	0	0	0
29♀*	3^{+}	2–	0	2^{-}	0	0
30♀	0	0	4^{+}	0	0	0
31♀*	3^{-}	0	2^{-}	0	0	1^{-}
32♂*	0	0	1^{+}	0	1^{+}	1^{-}
33♀*	0	2	2^{-}	0	2^{-}	0
34♂*	3^{+}	0	2^{+}	0	0	2^{-}

notoriously difficult to work with and several factors have been reported to affect PCR amplifications of microsatellite loci in otters (Hajkova et al., 2006). However, results from the comparison of our four replicates per loci suggested that we had low levels of genotyping error for these samples. In addition, PCR error values due to allelic dropout averaged 0.023 for the eight microsatellites tested on feces and blood samples of five captive individuals, and 0.065 for the field samples. For false alleles, we obtained an average value of 0.019 in the control samples, and 0.034 in the field samples. With eight microsatellites in a tissue sample set of 5 otters, we estimated that the probability of random match between unrelated individuals for all multilocus genotypes was 1.3–2.8 10^{-6} (P_{ID} unbiased), and the probability of a random match between siblings for all multilocus genotypes was 4.2–5.5 10^{-3} (P_{ID} sibs). Thus, the overall P_{ID} was low, suggesting our selected microsatellite were adequate to differentiate between individual otters, including relatives.

Samples with complete microsatellite data came from along the LcR (n = 47), CR (n = 24), JR (n = 27), LgR (n = 21), MR (n = 20), and TR (n = 15) and represented all of the six rivers from our original surveys (fig. 1). From these 154 fecal samples, we identified 34 unique genotypes, of which 16 were found multiple times in feces from the same river and 18 were also found in multiple rivers (table 1). Among these unique genotypes, sex ratio did not differ from unit. With data for six rivers pooled, fecal samples represented 19 females and 15 males (two tailed binomial test, P = 0.72; table 1). Both sexes were found in the six distinct rivers but in a different spread pattern. Eleven males were collected only once in one river, and 4 individuals were detected at least in 2 different rivers in the area. CR was the river where we found fewest males (3), and it was the site that

Table 2. Expected (H_E) and observed (H_O) heterozygosities and number of alleles per locus (A) for every river sampled. Hardy–Weinberg Equilibrium (HWE) and inbreeding coefficient (F_{IS}) were calculated according to Weir & Cockerham (1984). Significant P values after Bonferroni corrections are denoted in bold. (For abbreviations, see figure 1.)

Tabla 2. Heterocigosidad esperada (H_E) y observada (H_O) y número de alelos por locus (A) para cada río muestreado. Se calcularon el equilibrio de Hardy–Weinberg (HWE) y el coeficiente de consanguinidad (F_{IS}) según Weir & Cockerham (1984). En negrita se consignan del valores P significativos tras las correcciones de Bonferroni. (Para las abreviaturas ver figura 1.)

		HWE			LcR	MR	JR	CR	LgR	TR
Locus	A	*P*–value	F_{IS}		(n = 47)	(n = 20)	(n = 27)	(n = 24)	(n = 21)	(n = 15)
RIO02	5	**0.01**	0.131	H_E	0.743	0.702	0.796	0.678	0.738	0.706
				H_O	0.643	0.71	0.437	0.5	0.667	0.667
RIO04	10	0.083	0.122	H_E	0.878	0.813	0.814	0.857	0.77	0.811
				H_O	0.857	0.684	0.75	0.75	0.6	0.833
RIO07	6	0.16	0.241	H_E	0.778	0.815	0.79	0.893	0.761	0.841
				H_O	0.571	0.632	0.812	0.25	0.467	0.667
RIO08	4	0.07	0.294	H_E	0.606	0.701	0.619	0.75	0.689	0.627
				H_O	0.071	0.5	0.5	0.75	0.533	0.583
RIO10	3	0.064	–0.047	H_E	0.593	0.412	0.458	0.679	0.515	0.598
				H_O	0.428	0.474	0.437	1	0.6	0.583
RIO11	4	**0.03**	0.139	H_E	0.518	0.685	0.704	0.536	0.687	0.746
				H_O	0.571	0.579	0.5	0.25	0.733	0.583
RIO13	3	0.16	0.049	H_E	0.5	0.547	0.627	0.571	0.653	0.648
				H_O	0.571	0.579	0.5	1	0.467	0.583
RIO16	3	0.089	0.167	H_E	0.14	0.313	0.377	0.25	0.439	0.42
				H_O	0.143	0.263	0.312	0.25	0.467	0.167
ALL	38			H_E	0.59 ± 0.2	0.62 ± 0.2	0.65 ± 0.2	0.65 ± 0.2	0.66 ± 0.1	0.67 ± 0.2
				H_O	0.48 ± 0.3	0.55 ± 0.1	0.53 ± 0.2	0.59 ± 0.3	0.57 ± 0.1	0.58 ± 0.1

did not share genotypes with other rivers. Females appeared to move more among rivers than males because we found 12 female genotypes many times in different rivers: 2 female genotypes were detected in 4 rivers, 8 were recorded in 3 distinct rivers, and 2 were present in 2 rivers. LcR had 11 out of 19 of the recorded genotypes, while Lag contained the fewest female genotypes (4). Also, 7 genotypes found in females in CR were also found in other rivers in the area, which was different from the male pattern.

We recorded 38 different alleles with an average of 4.75 ± 2.3 alleles per locus in all of the populations (table 2). The most polymorphic locus was RIO 04 with 10 alleles, and the least polymorphic were RIO 10, RIO 13, and RIO 16, each with 3 alleles. All but three loci were under HWE. The three loci that deviated from HWE showed a heterozygote deficit; none of the loci showed linkage disequilibrium. We did not observe differences in the *Fis* values among the different collection sites. We used the multilocus *F*–statistic because it has been shown that it is not affected by sample size (Pearson coefficient $r = 0.29$, $P > 0.05$). Expected heterozygosity values ranged from a minimum 0.59 in LcR to a maximum 0.67 in TR. Observed heterozygosity ranged from 0.48 in LcR to 0.59 in CR (table 2). The results of the AMOVA analysis showed that the greatest source of genetic variation was found within rivers and not between different rivers (table 3). Results from the assignment test in GENECLASS 2 did not show genetic differences between rivers, and many of the samples were not assigned to their original sample site. The sites LgR and CR showed values equal to zero for the assignment test, while MR presented the highest assignment value (36.84). This test revealed a dispersal pattern among individuals from the different rivers, in a well–connected area, suggesting that the level of dispersion among sites is high. The FCA

test showed a clear lack of genetic structure in the entire area (fig. 2).

F–statistics were not significantly different from $P < 0.05$, and two loci (RIO 07 and RIO 08) showed the largest fixation indices when compared among the six rivers (table 4). The *Fst* pairwise comparisons among rivers ranged from 0.0 to 0.018, with no genetic differentiation in any comparisons except for the LcR and JR comparison ($P < 0.05$). LcR and JR are neighbouring localities and there are no apparent barriers that can prevent dispersal, but JR is a small, seasonally dry river that is unsuitable for the neotropical river otter during the dry season. Overall, our results suggest that there are no biogeographic or ecologic barriers to dispersal because there appears to be continuous flow and *Fst* values did not differ significantly from zero between rivers.

The STRUCTURE analysis showed $\Delta K_{MAX} = 2.176$, with a high score at $K = 1$, meaning one potential genetic unit in the Lacandon area. The cluster for all individuals in the structure diagram revealed a great deal of admixture (fig. 3). The highest posterior probability was Ln Pr(X|*K*) = –2,107.1 for the suggested 20 runs for $K = 1$ (Pritchard et al., 2000). Our results showed similarities among individuals; these results show a high degree of admixture among the different rivers at 90% confidence intervals of the individual's posterior probabilities.

Table 3. Analysis of molecular variance (AMOVA) for all samples of *L. longicaudis:* Sv. Source of variation; Sq. Sum of squares; Vc. Variance components; Pv. Percentage variation; Ap. Among populations; Wp. Within populations.

Tabla 3. Análisis de varianza molecular (AMOVA) para todas las muestras de L. lingicaudis*: Sv. Fuente de variación; Sq. Suma de cuadrados; Vc. Componentes de la variancia; Pv. Variación de porcentages; Ap. Entre poblaciones; Wp. En una misma población.*

Sv	Sq	Vc	Pv
Ap	515.24	1.58	2.80%
Wp	10,584.34	55.13	97.20%
Total	11,099.58	56.71	

Estimates of population size using CAPWIRE yielded a total of 154 individuals in the area; we reported 34 distinct genotypes, that is, 22% of the

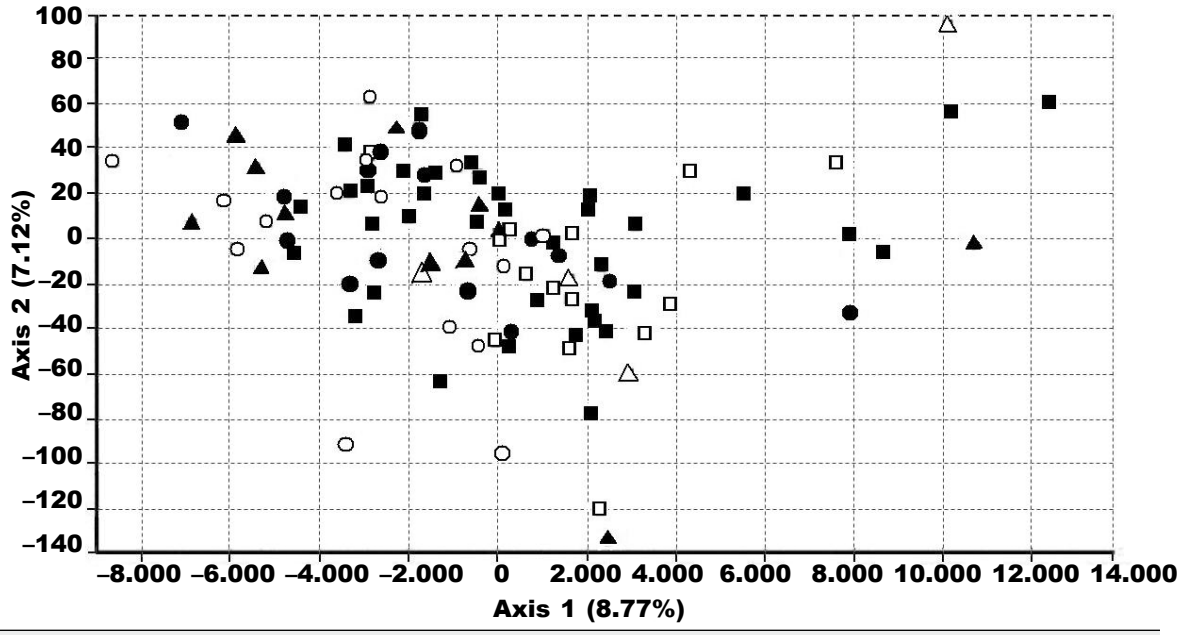

Fig. 2. Correspondence factorial analysis (CFA) for the samples of the neotropical otter in the six rivers of the area: ∗ Miranda River (MR); □ Lacantún River (LcR); ○ José River; ◆ Colorado River; ● Lagartos River; △ Tzendales River.

Fig. 2. Análisis de correspondencia factorial (CFA) para las muestras de la nutria neotropical en los seis ríos de la zona. Los símbolos del gráfico son: ∗ Río Miranda (MR); □ Río Lacantún (LcR); ○ Río José (JR); ◆ Río Colorado (CR); ● Río Lagartos (LgR); △ Río Tzendales (TR).

Table 4. Pairwise comparisons between the different rivers of the Lacandon forest. Values of F_{ST} are shown under the diagonal. In bold, the significant P–values ($P < 0.05$). (For abbreviations, see figure 1.)

Tabla 4. Comparaciones por pares entre los distintos ríos de la Selva Lacandona. Los valores de F_{ST} se encuentran por debajo de la diagonal. En negrita, los valores de P significativos (P < 0,05). (Para las abreviaturas ver figura 1.)

	MR	LcR	JR	CR	LgR	TR
MR	–	–	–	–	–	–
LcR	0.0182	–	–	–	–	–
JR	0.0172	**0.017**	–	–	–	–
CR	–0.0013	–0 .0197	0.0149	–	–	–
LgR	0.0011	0.0192	–0.0091	–0.0059	–	–
TZ	–0.0034	0.0185	–0.0182	0.0026	–0.0135	–

estimated population. The total population size of otters was subtracted from data obtained on the capture/recapture genotypes presented in table 1. According to simulation results using CAPWIRE, conducting surveys during the two dry seasons allowed us to obtain DNA samples from 22% of the population (95% CI). With regards to the length of the sampling period needed to estimate the population size, CAPWIRE estimated a period of 45 days (CI: 27–50) which was less than our scat collecting efforts.

Discussion

Non–invasive surveys conducted in the rivers of the Lacandon rainforest revealed that otters were present in all six major rivers. We detected a total of 34 neotropical river otters over two dry seasons (19 females and 15 males) and determined that 16 of these unique genotypes were recaptured multiple times and in multiple rivers, and during both dry seasons. These results are direct evidence that individuals move freely between different rivers and suggest that there are no barriers for movement among the Lacandon River System (table 1). Furthermore, our estimates of dispersion levels between rivers suggest that they are high enough to prevent genetic differentiation. The pattern of low genetic structure is also strongly supported by the high resolution provided by microsatellite markers in these types of studies (Selkoe & Toonen 2006; Weber et al., 2009). In particular, Latch et al. (2008) found that the North American river otter (*L. canadensis*) in Louisiana is affected by a variety of landscape habitats that have structured the river otter populations and have prohibited an adequate level of gene flow, but the scale of that study is several times larger than the area surveyed in this study.

In comparison, neotropical river otters in the Lacandon rivers showed moderate levels of genetic diversity, but also showed a lack of genetic differentiation among the surveyed sites. This pattern of panmixia could be explained by a recent colonization event into the area. However, given the moderate levels of allelic diversity, a recent invasion of the species seems doubtful. We did not find genetic differentiation among populations of the neotropical river otter. Our results reflected a well–interconnected network of rivers in the Lacandon forest, where individuals show high rates of dispersal. Male North American river otters tend to disperse in close populations (approx. 16 km), whereas females disperse more broadly (approx. 60 km, Blundell et al., 2002). We found that our non–invasive surveys provided direct evidence that both male and female otters move between rivers, athough females appear to move larger distances. More tracking inferences are needed, however, to confirm our results. The hydrological system in the Lacandon forest is composed of a series of permanent rivers, seasonal fishponds, channels, and marshes that provide a continuous flow of water in the area (Inda–Díaz et al., 2009). The lack of variation in *Fis* values among different localities also supports our findings of a lack of population structure. The home range for the neotropical river otter during the dry season ranges between 2 to 4 km, and can be larger during the rainy season (7 to 9 km; Gallo–Reynoso, 1989). The average distance between rivers that we surveyed was 7 km and therefore, it was not surprising that we recovered the same individuals in adjacent river systems. In well–structured populations, assignments are correctly allocated to their collecting site. Our results do not support the idea that this species is highly territorial and they are concordant with a species that has low levels of genetic structure and disperses over considerable distances (Lorenzen et al., 2008; Paetkau et al., 1995). In addition, these otters can swim a distance of 5 km every day and they may have overlapping home ranges with several individuals in the area (Gallo–Reynoso, 1989).

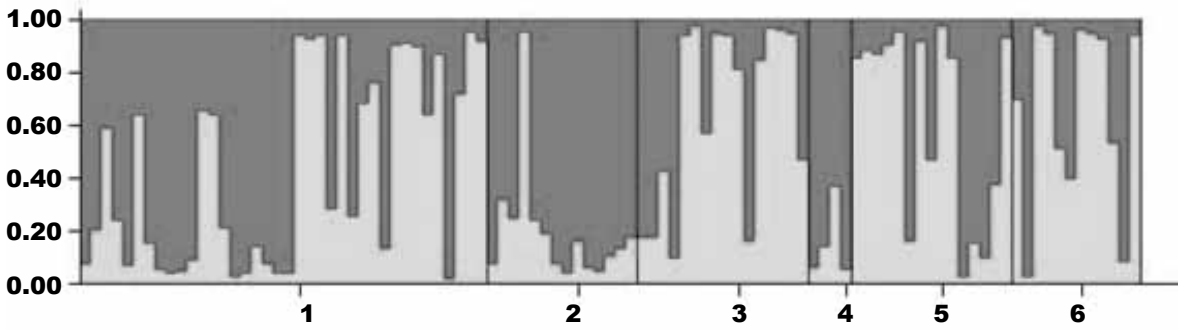

Fig. 3. Bayesian analysis of the nuclear genetic structure of river otter populations among the six sampled rivers in the Lacandon Reserve. Data are based on eight microsatellite loci with 90% probability intervals of probabilities to belong to one of each group.

Fig. 3. Análisis bayesiano de la estructura genética nuclear de las poblaciones de nutrias de los seis ríos muestreados en la Reserva de Lacandon. Los datos se basan en ocho locus microsatétile con un 90% de intérvalos de probabilidad del 90% pertenecientes a cada uno de los grupos.

Neotropical river otters are a relatively abundant species in the Lacandon forest. Because of the inhospitality of the tropical rainforest anthropogenic activities are not currently well–developed. The area has remained isolated for long periods of time, colonized only by native Lacandon tribes with low population densities. In 2000, this same area was surveyed for a dietary study based on visual observations of animals, feces and footprints. It was determined that neotropical river otters should be widely and continuously distributed along the rivers of the Lacandon forest because they are apparently connected by a complex network of tributaries without obvious subdivisions (Soler, 2004). The major geographic division in the area is found in the LcR, because it is complex and continuous and separates two major reserves: the Lacandon forest reserve in Mexico and the Peten reserve in Guatemala. For example, the distance between the CR and the TR is approximately 75 km, but both were associated with high levels of genetic diversity, and no genetic differentiation was found between them. This is consistent with current dispersion. JR is located half way between CR and TR and it showed the same levels of uniformity as the other rivers. However, JR is a seasonal river and neotropical river otters were not frequently found there. Physical barriers to the dispersal of neotropical river otters were not obvious and dispersion among the different individuals in the area appears constant.

The overall genetic diversity of the species in the area can be considered adequate to maintain a viable population, and even the smallest and most seasonal river (JR) contributed to the genetic diversity of the species. Wildlife managers should therefore focus on maintaining connectivity between the different rivers to avoid fragmentation of this large population into genetically distinct subpopulations.

To date, most studies have been conducted in the European otter, *L. lutra* (Dallas et al., 2003; Janssens et al., 2008) and the North American river otter, *L. canadensis* (Centrón et al., 2008; Latch et al., 2008), and very few studies have been conducted on populations of the neotropical river otter (*L. longicaudis)*. Information concerning population dynamics of the species is scarce, and even less is known about the genetic structure of their populations. Only two studies related to the genetic structure of the neotropical river otter have been conducted previously in Brazil, but both were carried out in larger areas than our present study (Trinca et al., 2007; Weber et al., 2009). The first study found no significant mtDNA genetic differentiation between populations in the southern and south–eastern regions of Brazil and the latter study, using microsatellite markers, found significant levels of genetic differentiation in populations in the southernmost part of Brazil. This is not surprising since microsatellite markers have been widely used successfully to reveal a finer scale genetic structure in areas where mtDNA or allozymes have failed to detect it (Zhang & Hewitt, 2003). Genetic studies at different spatial scales are important because levels of variation at a large scale may help to understand how ecological and evolutionary processes act on the adaptive capacity of the species while those at a finer scale may help to elucidate patterns of kinship and social organization.

Our study shows no detectable genetic differentiation in this species among the major rivers. The geographic position of the LcR suggests that it could be managed as the main connector for this river network, and can be used as a suitable dispersal corridor for neotropical river otters in the area. As we documented that all localities have similar levels of heterozygosity, this population does not currently require translocation efforts to improve genetic diversity. However, should future efforts be required, the

genetic affinities to otters from neighbouring rivers in the Peten and Calakmul reserves in Guatemala should first be investigated before they can be considered as a potential source.

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