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GENETIC VARIATION AMONG POPULATIONS OF RIVER OTTERS IN NORTH AMERICA: CONSIDERATIONS FOR REINTRODUCTION PROJECTS

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Horizontal starch gel electrophoresis was used to assess variability at 23 presumptive gene loci of 732 river otters obtained from fur-trappers in 18 states and three Canadian provinces. States and provinces providing otters were sorted into eight geographic regions for genetic comparisons. Multilocus heterozygosity and polymorphism ranged from 0.018 to 0.032 and 0.044 to 0.087, respectively. One locus, esterase-2, (EST-2) demonstrated a high level of polymorphism throughout all regions. Malate dehydrogenase-1 (MDH-1) was polymorphic throughout the Mississippi drainage but not elsewhere. Heterozygosity, occurrence of rare alleles, and mean number of alleles per locus were associated positively with estimated population sizes. Average heterozygosity and polymorphism values for otters within regions were lower than overall averages reported for mammals but similar to the range of those observed in other mammalian carnivores. Patterns of gene flow suggested by the distribution of polymorphism at the MDH-1 locus do not concur with the current taxonomic classification of river otters. Levels of genetic variation detected in this investigation present a positive outlook for the maintenance of genetic diversity in river otter populations, if sound management principles are applied for reintroductions.

Key words: *Lontra canadensis,* river otter, population genetics, reintroduction

Fossil evidence indicates that ancestors of the North American river otter (*Lontra canadensis*) crossed the Bering land bridge and arrived in North America by the Upper Pliocene (van Zyll de Jong, 1972). Since arriving in North America, otters evolved, expanded their range, and now exploit a wide variety of aquatic habitats throughout most of the continental United States, Canada, and Alaska (Hall, 1981). Hall (1981) recognized seven subspecies of North American river otters. The generic designation for North American river otters has recently been changed from *Lutra* to *Lontra* to reflect morphological differences between New World and Old World otters (Wilson and Reeder, 1993).

Overexploitation, water pollution, and destruction of riparian habitats during the 1800s and early 1900s caused populations of otters to decline throughout most of their historic range and become extirpated in several midwestern states and West Virginia (Nilsson, 1980). Since 1978, wildlife agencies in at least 17 states have implemented reintroduction projects to restore extirpated populations of otters (Ralls, 1990). Otters used in reintroductions were obtained from various areas supporting viable populations. For example, subspecies of otters acquired from geographically distinct areas such as Newfoundland, Wisconsin (both *L. c. canadensis*), and Washington (*L. c. pacifica*) were released in Colorado (Mack, 1985). Similarly, otters from Alaska (*L. c. pacifica* or *L. c. kodiacensis*) and Washington have been translocated to Utah. However, most states used otters from southern Louisiana (*L. c. lataxina*) for reintroduction projects

(Erickson and McCullough, 1987; McDonald, 1989; Ralls, 1990).

The range of the subspecies of otter inhabiting Louisiana encompasses much of the Mississippi River drainage, extending throughout the central and eastern United States and northward through Iowa, Pennsylvania, and into southern New York (Hall, 1981). Although extensive variation in climate and habitat conditions occurs throughout the designated range of *L. c. lataxina,* otters in Louisiana may represent the appropriate subspecies for many of the states conducting reintroduction projects with otter, including Pennsylvania.

In Pennsylvania, populations of river otters declined throughout much of their historic range and became limited to northeastern counties in the state (Eveland, 1978; Rhoads, 1903). In 1982, we initiated the Pennsylvania River Otter Reintroduction Project (PRORP) to restore otters to their historic range (Serfass et al., 1986). During the initial release period (1982–1986), 39 otters were reintroduced to three watersheds in northcentral Pennsylvania (Serfass et al., 1993*a,* 1993*b*). Preliminary plans were to obtain otters by livetrapping in northeastern Pennsylvania and through purchases from a licensed supplier in New York. However, adequate numbers of otters were not obtained from those sources, and 17 otters were obtained from Louisiana and released along Pine Creek in northcentral Pennsylvania during 1983–1984 (Serfass et al., 1986, 1993*a,* 1993*b*). Otters from Louisiana survived and adapted to habitat and climatic conditions at the release area (Serfass et al., 1986, 1993*a*). However, concern arose during initial phases of the project regarding the wisdom of translocating otters from geographically distinct populations without prior knowledge of genetic variation among populations of river otters in North America (Genoways, 1986).

Our objective was to delineate genetic variation among populations of otters in North America and apply the results to selecting sources of otters for reintroductions in Pennsylvania. Because of the otter's dependency on aquatic and riparian habitats for food and shelter, we hypothesized that most gene flow would occur within drainages and, accordingly, that genetic variation among otter populations would be partitioned among major drainage systems. Because the remnant otter population in Pennsylvania has been an important source of otters for PRORP (Serfass et al., 1993*b*), we were also interested in determining if founder effects associated with population declines, such as those experienced by Pennsylvania's otter population, had resulted in loss of genetic variability within the population. This project represents the first attempt to document genetic variability among populations of river otters in North America.

Materials and Methods

During 1987–1989, liver tissues were collected from 732 otters harvested in 18 states and three Canadian provinces (Table 1). Tissue collection was facilitated through cooperative arrangements with furbearer biologists representing wildlife agencies from respective states and provinces. We requested that cooperating biologists coordinate tissue acquisition from trappers, temporary storage of tissues (frozen in conventional freezers), and shipment of samples to the Savannah River Ecology Laboratory for electrophoretic analysis. We obtained an additional 13 samples from otters in Arkansas that died following translocation to Ohio (McDonald, 1989). Samples from Pennsylvania were collected from otters accidentally killed during trapping seasons for legal furbearers. Although not included in statistical comparisons because of tissues degradation, we discuss additional electrophoretic findings for the malate dehydrogenase-1 (MDH-1) locus based on analysis of liver tissue sampled from otters in Ontario and Washington (46 and 12 samples, respectively) and blood from 24 otters from Alaska. Tissue samples were stored individually in 2-ml cryotubes and were shipped to the laboratory frozen in dry ice. Upon arrival at the laboratory, samples were stored from −40 to −70°C until analysis.

Specific capture locations were seldom avail-

TABLE 1.—*Sources of liver tissue from river otters used for electrophoresis.*

Region	State/Province	Samples collected (*n*)
Great Lakes	Michigan	30
	Minnesota	30
	Wisconsin	59
Mid-Atlantic	Delaware	11
	Maryland	21
	New Jersey	20
New England	Connecticut	30
	Massachusetts	83
	New York	30
	Vermont	31
Newfoundland	Newfoundland	39
Northeast	New Brunswick	31
	Nova Scotia	41
	Maine	27
Pennsylvania	Pennsylvania	32
Southcentral	Alabama	20
	Arkansas	13
	Louisiana	120
	Mississippi	23
Southeast	Georgia	26
	South Carolina	15

able for harvested otters used in the study. Consequently, we were unable to define specific local populations and, therefore, decided to merge states and provinces into regional groupings (populations) for genetic comparisons (Table 1). We delineated regions based on geographic proximity of states and provinces and opportunities for interaction among populations of otters through connecting drainage systems. In most cases, regions were defined clearly by barriers to gene flow, such as habitat perturbations caused by human activity and geographic features such as mountain ranges. Otters collected from Pennsylvania were grouped separately from surrounding states to facilitate comparisons to regional populations of otters.

Horizontal starch-gel electrophoresis (Manlove et al., 1975; Selander et al., 1971) was performed on tissue samples to detect genetic variation. After initial screening of 39 enzyme systems, we determined that the following 23 presumptive gene loci had sufficient resolution for inclusion in the study: alkaline phosphatase (ALP), aspartate aminotransferase-1,2 (AAT-1,2), esterase-2 (EST-2), fumerate hydratase (FH), glucose-6-phosphate dehydrogenase (GD), glucosephosphate isomerase (GPI), glutamate dehydrogenase (GTDH), glyceraldehyde dehydrogenase (GAPDH), lactate dehydrogenase-1,2 (LDH-1,2), leucine aminopeptidase-2 (LAP-2), MDH-1, malate dehydrogenase-2 (MDH-2), malic enzyme (ME), mannose-6-phosphate isomerase (MPI), peptidase-1,2 (PEP-1,2), phosphoglucomutase-1 (PGM-1), 6-phosphogluconate dehydrogenase (PGD), purine nucleoside phosphorylase (NP), sorbitol dehydrogenase (SORDH), and xanthine dehydrogenase (XDH).

Polymorphisms were determined through direct side-by-side comparison of protein migrations from the electrophoretic origin. Allozymes were designated alphabetically relative to their migration on the gel. The letter "B" was chosen to represent the most common allele at a locus.

Allele frequencies, mean number of alleles per locus, percentages of polymorphic loci, multilocus heterozygosities (H), deviations from expected Hardy-Weinberg proportions, Wright's (1965) F-statistics, and Rogers' (1972) and Nei's (1978) measures of genetic similarity were calculated using BIOSYS-1 (Swofford and Selander, 1981). Chi-square tests were used to determine if F_{IS} and F_{ST} values differed significantly from zero (Li and Horvitz, 1953; Workman and Niswander, 1970). Analysis of variance (ANOVA) was used to test for among-region differences in heterozygosity and numbers of alleles per locus. Heterozygosities are proportions, and in the present case, exhibited small ranges of values, many of which were zero and were not distributed normally. Consequently, heterozygosity data were arcsine transformed for analysis (Zar, 1984). If an allele was unique and common (frequencies >0.05 in most regions of occurrence) to certain regions, we evaluated distributional and clinal patterns (latitudinal and longitudinal) by comparing the allele's frequency among states comprising those regions.

Spearman's rank correlation (r_s) was used to test if measures of genetic variability were associated significantly ($P \leq 0.05$) with sample size and estimated population size and to evaluate clinal patterns in distributions of unique alleles.

RESULTS

Allozyme phenotypes for 19 enzymes coded by 23 presumptive gene loci were resolved consistently among regions. Tissue

TABLE 2.—*Allele frequencies at 23 loci in river otters sampled from eight regions of North America.*

Enzyme locus	Allele	Great Lakes	Mid-Atlantic	New England	Newfoundland	Northeast	Pennsylvania	Southcentral	Southeast
AAT-1	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-2	A	0.004							
	B	0.996	1.000	0.997	1.000	0.989	1.000	1.000	0.988
	C			0.003		0.011			
ALP	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EST-2	A	0.284	0.250	0.281	0.300	0.351	0.375	0.360	0.485
	B	0.716	0.750	0.719	0.700	0.649	0.625	0.640	0.515
FH	A			0.006					
	B	1.000	1.000	0.994	1.000	1.000	1.000	1.000	1.000
GAPDH	A	0.004				0.006			
	B	0.996	1.000	1.000	1.000	0.994	1.000	1.000	1.000
GD	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI	A			0.009					
	B	1.000	1.000	0.971	1.000	0.995	1.000	1.000	0.962
	C			0.021		0.005			0.038
GTDH	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-2	A		0.011						
	B	0.984	0.989	0.983	1.000	0.971	1.000	1.000	1.000
	C	0.016		0.017		0.029			
LDH-1	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-2	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-1	B	0.861	1.000	1.000	1.000	1.000	1.000	0.844	0.988
	C	0.139						0.156	0.012
MDH-2	A	0.021			0.026				
	B	0.979	1.000	1.000	0.974	1.000	1.000	1.000	1.000
ME	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MPI	B	0.990	1.000	1.000	0.980	1.000	1.000	1.000	1.000
	C	0.010			0.020				
NP	B	1.000	1.000	1.000	1.000	1.000	0.982	1.000	1.000
	C						0.018		
PEP-1	A	0.021							0.012
	B	0.979	1.000	1.000	1.000	1.000	1.000	0.997	0.988
	C							0.003	
PEP-2	A	0.008	0.020	0.009	0.013	0.021		0.017	
	B	0.992	0.980	0.991	0.987	0.979	1.000	0.983	1.000
PGD	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-1	A	0.018							
	B	0.982	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SORDH	A					0.007			
	B	1.000	1.000	1.000	1.000	0.993	1.000	1.000	1.000
XDH	A				0.054			0.004	
	B	1.000	1.000	1.000	0.946	1.000	1.000	0.996	1.000

degradation caused by variability in collection and sample storage conditions prevented inclusion of all individuals for each locus studied (Table 1). Among the 23 loci scored, eight were monomorphic across all regions, 13 were slightly polymorphic in at least one region, and two (MDH-1 and EST-2) were highly polymorphic in some or all regions (Table 2). MDH-1 was polymorphic in Great Lakes and Southcentral regions and slightly polymorphic (most common allele = 0.988) in the Southeast region, but not other regions or in samples from Ontario, Alaska, and Washington. The

TABLE 3.—*Summary of genetic variability for 23 presumptive loci in eight regional populations for the river otter. Tissue degradation caused by variability in collection and storage conditions of samples prevented inclusion of all individuals for each locus studied.*

Region[a]	Sample size/locus[b]	Allele/locus[b]	Polymorphic loci (%) 0.95	0.99	No criterion	Heterozygosity/locus Direct count[b]	Expected Hardy-Weinberg[b]
Great Lakes (7)	102.3 (5.8)	1.43 (0.11)	8.70	26.09	43.84	0.032 (0.017)	0.037 (0.020)
Mid-Atlantic (2)	45.5 (1.9)	1.13 (0.07)	4.35	13.04	13.04	0.018 (0.016)	0.019 (0.016)
New England (5)	145.3 (7.5)	1.30 (0.12)	4.35	13.04	26.09	0.022 (0.017)	0.023 (0.018)
Newfoundland (3)	31.1 (1.8)	1.22 (0.09)	8.70	21.74	21.74	0.028 (0.020)	0.028 (0.019)
Northeast (6)	87.7 (2.7)	1.30 (0.10)	4.35	17.39	30.43	0.023 (0.016)	0.027 (0.020)
Pennsylvania (1)	26.3 (1.2)	1.09 (0.06)	4.35	8.70	8.70	0.020 (0.018)	0.022 (0.021)
Southcentral (7)	132.7 (6.8)	1.22 (0.09)	8.70	13.04	21.74	0.028 (0.019)	0.034 (0.023)
Southeast (4)	34.1 (2.2)	1.22 (0.09)	4.35	21.74	21.74	0.028 (0.021)	0.028 (0.022)

[a] Numbers in parentheses indicate ranking by estimated population sizes for regional populations of otters from smallest (Pennsylvania) to largest (Great Lakes and Southcentral).

[b] Standard errors in parentheses.

MDH-1 polymorphism was expressed by presence of an allele shared among all regions and an allele (C) unique to Great Lakes, Southcentral, and Southeast regions. Frequencies of the C allele ranged from 0.2333 (Minnesota) to 0.0500 (Michigan) in the Great Lakes region, from 0.1829 (Louisiana) to 0.0769 (Arkansas) in the Southcentral region, and from 0.0192 (Georgia) to 0.000 (South Carolina) in the Southeast region. Distribution of the allele among states in those regions was not associated with latitude ($r_s = -0.012$). However, the allele occurred at highest frequencies in states bordering the Mississippi River and declined along a longitudinal gradient from west to east ($r_s = -0.833$). EST-2 was the most variable locus examined and was polymorphic in all regions.

Polymorphism levels among regions ranged from 0.044 to 0.087 (0.95 criterion; Table 3). Percentage of polymorphic loci (0.95 and 0.99 criterion) was not associated significantly with sample size ($r_s = 0.472$ and $r_s = 0.436$, respectively) or estimated population size ($r_s = 0.169$ and $r_s = 0.022$, respectively). Although rare alleles (frequencies <0.01) occurred in all regions (Table 3), occurrence of polymorphisms with no criterion applied was associated significantly with estimated population size ($r_s = 0.824$) but not with sample size ($r_s = 0.602$). Observed heterozygosities ranged from 0.018 (mid-Atlantic) to 0.032 (Great Lakes; overall $H = 0.025 \pm 0.006$ *SE*) and mean numbers of alleles per locus ranged from 1.09 (Pennsylvania) to 1.43 (Great Lakes; $\bar{X} = 1.24 \pm 0.033$) (Table 3), but neither differed significantly among regions ($F = 0.30$, *d.f.* = 7, 176, $P = 0.952$ and $F = 1.43$, *d.f.* = 7, 176, $P = 0.195$, respectively). Observed heterozygosities were significantly associated with estimated population size ($r_s = 0.734$) but not sample size ($r_s = 0.286$). Mean number of alleles was associated significantly with both estimated population size ($r_s = 0.842$) and sample size ($r_s = 0.678$).

Most gene frequencies were in general agreement with Hardy-Weinberg (H-W) expected frequencies. Across all samples, 7.1% (3/42) of polymorphic locus per region combinations (no criterion; Table 2) deviated significantly from H-W equilibrium, and, in each case, deviations were expressed as a deficiency of heterozygotes. Among locus per region combinations deviating from H-W proportions (MDH-1—southcentral, PGM-1 and PEP-2—Great Lakes), only MDH-1—southcentral com-

TABLE 4.—*F-statistics and statistically significant* F_{IS} *and* F_{ST} *values for 15 polymorphic loci (no criterion) in the river otter.*

Locus	F_{IS}	F_{IT}	F_{ST}
AAT-2	−0.010	−0.003	0.007
EST-2	0.078	0.098	0.022**
FH	−0.006	−0.001	0.005
GAPDH	−0.005	−0.001	0.004
GPI	−0.030	−0.008	0.021**
LAP-2	−0.021	−0.008	0.013
MDH-1	0.105	0.202	0.108**
MDH-2	−0.025	−0.006	0.018**
MPI	−0.017	−0.004	0.013*
NP	−0.018	−0.002	0.016**
PEP-1	0.220**	0.230	0.012**
PEP-2	−0.017	−0.011	0.005
PGM-1	0.491**	0.499	0.016*
SORDH	−0.007	−0.001	0.006
XDH	0.438*	0.463	0.044**
Mean	0.079**	0.108	0.031**

* $P < 0.05$; ** $P < 0.01$.

bination ($\chi^2 = 5.00$, *d.f.* = 1, $P = 0.025$) exhibited polymorphism with a common allele frequency of <0.99.

Among loci examined in this investigation, F_{IS} values differed significantly from zero at the PGM-1, XDH, and PEP-1 loci (Table 4). PGM-1, XDH, and PEP-1 were polymorphic in three or less regional populations, and, in each case, the polymorphism was attributed to occurrence of low-frequency alleles (Table 2). F_{IS} measures departure from H-W equilibrium within a subpopulation and may serve as a coefficient of inbreeding. Departures from H-W equilibrium indicated by significant positive F_{IS} values for PGM-1, XDH, and PEP-1 resulted because low-frequency alleles occurred as homozygotes in one of the regional populations for each of the respective loci.

The most commonly reported *F*-statistic, F_{ST}, measures the extent to which populations show spatial heterogeneity in allele frequencies. F_{ST} values differed significantly from zero at nine loci (Table 4). However, with the exception of EST-2 and MDH-1, significant F_{ST} values for other loci resulted primarily as a consequence of large samples and occurrence of low frequency alleles in one or more regional populations (Tables 2 and 4). In addition, Rogers' (1972) and Nei's (1978) measures of genetic similarity indicated only minor differences among regional populations of river otters (Table 5).

DISCUSSION

Comparison to other taxa.—Electrophoretic studies indicate large disparities in genetic variability among organisms from various taxonomic assemblages (Nevo, 1978; Nevo et al., 1984). Based on review of electrophoretic studies from 184 mammalian species, Nevo et al. (1984) calculated an average heterozygosity (*H*) for mammals of 0.041.

Specific factors influencing genetic variability in mammals are poorly understood and have been the focus of considerable

TABLE 5.—*Matrix of Rogers' (1972) genetic similarity (above diagonal) and Nei's (1978) unbiased genetic identity (below diagonal) for comparing genetic similarity of river otters between regions.*

		Region							
	Region	1	2	3	4	5	6	7	8
1	Great Lakes		0.988	0.989	0.987	0.986	0.985	0.981	0.980
2	Mid-Atlantic	0.999		0.996	0.993	0.993	0.992	0.988	0.985
3	New England	0.999	1.000		0.992	0.994	0.993	0.987	0.988
4	Newfoundland	0.999	1.000	1.000		0.991	0.991	0.986	0.984
5	Northeast	0.999	1.000	1.000	1.000		0.995	0.990	0.988
6	Pennsylvania	0.999	1.000	1.000	1.000	1.000		0.991	0.991
7	Southcentral	1.000	0.999	0.999	0.999	0.999	0.999		0.985
8	Southeast	0.998	0.998	0.998	0.999	0.999	1.000	0.999	

discussion (Baccus et al., 1983; Selander and Kaufman, 1973; Wooten and Smith, 1985). Although the literature contains exceptions, in general, levels of genetic variability among mammals tend to be associated inversely with body size, i.e., northern elephant seals (*Mirounga angustirostris*—Bonnell and Selander, 1974); bison (*Bison bison*—McClenaghan et al., 1990). Carnivores such as the cheetah (*Acinoynx jabatus*—O'Brien et al., 1983) and polar bears (*Thalarctos maritimus*—Allendorf et al., 1979) generally display less variability than do herbivores, including manatees (*Trichechus manatus*—McClenaghan and O'Shea, 1988) and white-tailed deer (*Odocoileus virginianus*—Breshears et al., 1987).

The family Mustelidae is comprised of highly mobile carnivores that typically define specialized feeding niches and occur at low population densities (Hall, 1951). Among mustelids, otters are relatively large and are uniquely specialized to exploit semi-aquatic environments (Melquist and Hornocker, 1983; Stephenson, 1977). Consequently, based on models attempting to describe factors controlling genic diversity among animal taxa (Nevo, 1978; Selander and Kaufman, 1973; Wooten and Smith, 1985), otters and other mustelids would be expected to display lower than average levels of heterozygosity and polymorphism. Average heterozygosity (0.025) and polymorphism (0.06) values for otters among the eight regions studied were lower than overall averages reported for mammals but within the range of those observed in other mammalian carnivores, including mustelids (Hartl et al., 1988; Mitton and Raphael, 1990; Nevo et al., 1984; Simonsen, 1982).

Comparison among regions.—Large home ranges and ability of otters to disperse long distances, both overland and within drainages (Melquist and Hornocker, 1983), probably contributed to the overall genetic similarity (Table 5) in our study by facilitating frequent opportunities for transmission of genetic material among adjacent populations of otters. Conversely, anthropogenic factors (i.e., harvest-induced extirpations, water pollution, disruption of riparian habitats) have altered or restricted historic patterns of gene flow and, in addition to natural barriers, may have contributed to significant F_{ST} values among regions, particularly at EST-2 and MDH-1.

Size of regional populations of otters appears to have contributed to levels of genetic diversity observed among regions. Occurrence of rare alleles (polymorphisms with no criterion), heterozygosity, and mean number of alleles per locus were associated positively with estimated population size. Pennsylvania and the mid-Atlantic region probably suffered the most significant population reductions during past periods of unregulated trapping and were judged to support the smallest populations among regions sampled. Low levels of heterozygosity and paucity of rare alleles relative to other regions suggest that bottlenecks associated with population declines may contribute to lower levels of genetic variability detected in those areas.

Evidence of structuring among populations of otters was most evident at MDH-1 (F_{ST} = 0.108). Polymorphism at the MDH-1 locus provides insight into historic patterns of gene flow and relatedness among otter populations. With the exception of a rare occurrence in the Southeast (one heterozygous individual in Georgia), we found no evidence that the allele occurs beyond the Mississippi drainage. Absence of the allele in other regions implies that the Appalachian and Rocky Mountain chains served as barriers to gene flow between populations occurring in the Mississippi drainage and populations to the east and west, respectively.

Extirpation of populations of otters throughout large parts of North America precludes absolute delineation of the historic distribution of the C allele at the MDH-1 locus. Widespread extirpation of populations of otters limited opportunities to collect samples and study distribution of the allele in western portions of the Mississippi

drainage, i.e., Missouri River and associated tributaries. Because the polymorphism at the MDH-1 locus occurs in extant populations of otters throughout the Mississippi drainage, we suspect that the C allele probably occurred in extirpated populations that inhabited upper reaches of the Mississippi drainage (Ohio River and associated tributaries) in western Pennsylvania and southward along tributaries draining western slopes of the Appalachian Mountains.

The C allele appears to have evolved from a mutation that gradually spread throughout the Mississippi River drainage. Because the C allele is distributed throughout a variety of aquatic and climate conditions in the Mississippi drainage, selective forces are unlikely to have limited further spread of the allele to other regions. Consequently, we surmise that overland dispersal by otters eventually would have distributed the allele to populations beyond the Mississippi River drainage. However, such expansion is now impeded by anthropogenic factors.

Taxonomic considerations.—Hall and Kelson (1959) originally recognized 19 subspecies of river otters in North America. However, the taxonomic status of *Lontra canadensis* has been revised subsequently, and taxonomists now recognize seven subspecies (Hall, 1981). Depending on the classification scheme, regions encompassed by our investigation were occupied by either six (*L. c. texensis, L. c. vaga, L. c. canadensis, L. c. lataxina, L. c. interior,* and *L. c. degener*—Hall and Kelson, 1959 or two (*L. c. canadensis* and *L. c. lataxina*—Hall, 1981) subspecies of river otters. Results of our electrophoretic study do not fully correspond with Hall and Kelson's (1959) or Hall's (1981) classifications.

In many regions, Hall and Kelson's (1959) classification partitions subspecies of otters by major drainage patterns and other isolating physiographic barriers. For example, the Appalachian Mountains serve as a geographic boundary to delineate *L. c. interior, L. c texensis,* and *L. c. canadensis* in the Mississippi drainage and *L. c. lataxina* and *L. c. vaga* in the Atlantic Coast drainages. Conversely, Hall's (1981) taxonomic revision expanded the range of *L. c. lataxina* by merging it with *L. c. vaga, L. c. interior,* and *L. c texensis* and by extending the range of *L. c. canadensis* northward. Consequently, Hall's (1981) revision disregards the Appalachian Mountains as a significant isolating barrier to gene flow between coastal and interior drainages.

Distribution of the MDH-1 polymorphism suggests a historic pattern of isolation among populations of otters on opposing sides of the Appalachian Mountains and supports Hall and Kelson's (1959) use of the Appalachians as an important geographic feature defining patterns of gene flow and distribution of subspecies of otters in the eastern United States. Nevertheless, interpretation of the degree of isolation among populations of otters and subsequent delineation of discrete boundaries for use in partitioning subspecies of otters is confounded by the extensive opportunity for gene flow, facilitated by the otter's ability to travel extensive distances within and among drainages (Melquist and Hornocker, 1983).

Pennsylvania serves to illustrate the disparity between electrophoretic results and current taxonomy of otters. Although Pennsylvania is now considered to be occupied by a single subspecies of otter, previous classification indicated the state as a transitional area between *L. c. canadensis* and *L. c. lataxina.* The current classification is not supported by our interpretation of historic patterns of gene flow among populations of otters in the Mississippi River drainage or genetic make-up of the remnant population in northeastern Pennsylvania, based on presence and absence of MDH-1 polymorphism, respectively. However, relatively gentle topography and proximity of headwaters in opposing drainages undoubtedly facilitated overland dispersal and subsequent gene flow among populations in many areas of Pennsylvania. In addition, selective pressures probably contributed lit-

tle to maintenance of genetic differentiation among populations of otters occurring in various drainages, because of similarity in environmental conditions throughout Pennsylvania and the otter's fine-grained exploitation of aquatic and riparian resources (Selander and Kaufman, 1973). Consequently, historic interactions among populations of otters in Pennsylvania probably are described best as a gradient defined by opportunities for overland dispersal by otters occupying adjacent drainages.

Although Hall's (1981) listing of a single subspecies of otter in lower and middle reaches of the Mississippi River drainage is supported by our electrophoretic data, the classification also distinguishes populations of otters in upper reaches of the Mississippi River drainage (Michigan, Wisconsin, and portions of Minnesota) as a separate subspecies from those occupying other regions of the drainage. Based on Hall's (1981) classification, otters from the Great Lakes region represent the same subspecies as otters occupying disparate drainages from Ontario to Atlantic provinces and New England. Failure to detect polymorphism at the MDH-1 locus in otters from Ontario, Newfoundland, Nova Scotia, New Brunswick, and New England states suggests that limited gene flow has occurred among otters from these areas compared with those from the Great Lakes region.

Management Considerations.—In recent years, the value of establishing genetic databases for wildlife species that have undergone extensive population declines has been recognized as a basis for establishing minimum-viable-population criteria and as an important component in design of reintroduction projects (Frankel and Soule, 1981; Haig et al., 1990; Leberg, 1990). Establishing a genetic profile for a species prior to implementing a reintroduction program can be used to determine appropriate sources of founders and in developing strategies to minimize loss of genetic variability in reintroduced populations (Leberg, 1990).

Most states involved with river otter reintroduction projects appear to have selected source populations based on availability (i.e., abundance and opportunity for purchases from trappers). Although otters used in reintroduction projects have been obtained from disparate areas such as Newfoundland, Alaska, and Washington (Mack, 1985; K. P. McDonald, pers. comm.), most were translocated from southern Louisiana (Ralls, 1990). To date, at least 10 states (including northern states such as Ohio, Pennsylvania, and West Virginia) have reintroduced otters from Louisiana.

Although considerable disparities in climatic and habitat conditions existed between Louisiana and most translocation areas, based on current classification, the appropriate subspecies (*L. c. lataxina*) was used. Ironically, if classification of subspecies is applied as the criterion for selecting sources of otters for reintroductions (Ralls, 1990), otters from Louisiana currently would be judged more appropriate for release in Pennsylvania than otters from New York (*L. c. canadensis*). Similarly, using subspecies criteria, otters from Minnesota, Wisconsin, and Michigan (*L. c. canadensis*) would be excluded for use in reintroduction by upper midwestern states (i.e., Iowa, Indiana, and Ohio).

Based on review of the taxonomic literature for the North American river otter (Hall, 1981; Hall and Kelson, 1959; van Zyll de Jong, 1972) and electrophoretic results, we are convinced that use of delineation of subspecies is inappropriate as a sole criterion for selecting sources of otters for restocking. For example, because of extensive connectivity of major drainage systems and occurrence of the unique MDH-1 allele in the Great Lakes region, we find no evidence to suggest that isolating barriers were in place to prevent gene flow between otters occurring in the Great Lakes region and former populations in the upper Midwest. Because of historic opportunities for gene flow and similarities in climate and habitat conditions, the Great Lakes region should be regarded as an appropriate source

of otters for reintroduction in upper midwestern states. Based on similar considerations, PRORP now slects otters exclusively from nearby viable populations (Maryland, New York, and northeastern Pennsylvania), regardless of current subspecies designation.

Reintroduction projects provide potential to restore otters to parts of their historic range (Ralls, 1990). Considering declines suffered by populations of otters in North America (Nilsson, 1980), wildlife managers should be encouraged by levels of genetic variability detected in this investigation and implement strategies to assure that reintroduced populations retain genetic diversity (Leberg, 1990).

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