Journal of Heredity 2013:104(2):192–201
doi:10.1093/jhered/ess094
Advance Access publication December 5, 2012

Genetic Diversity of the Eurasian Otter (*Lutra lutra*) Population in Israel

TALI MAGORY COHEN, TAMAR NARKISS, AMIT DOLEV, YOSSI BEN-ARI, NOGA KRONFELD-SCHOR, AMICHAI GUTER, DAVID SALTZ, AND GILA KAHILA BAR-GAL

From the Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel (Cohen, Narkiss, and Bar-Gal); Israel Mammal Research Centre, Society for the Protection of Nature in Israel, Tel-Aviv 66183, Israel (Dolev and Guter); Department of Zoology, Tel Aviv University, Tel-Aviv 69978, Israel (Ben-Ari and Kronfeld-Schor); and Mitrani Department for Desert Ecology, Jacob Blaustein Institute for Desert Research, Ben Gurion University of the Negev, Israel (Saltz).

Address correspondence to Gila Kahila Bar-Gal at the address above, or e-mail: bargal@agri.huji.ac.il.

Abstract

The Israeli population of Eurasian otter (*Lutra lutra*) marks the Palearctic southern boundary of the species' distribution in the Levant. During the 20th century, the otter population in Israel experienced a dramatic decline due to anthropogenic habitat alterations. Currently, the otter population in Israel is estimated at about 100 individuals and defined as "Critically Endangered". The aim of this research was to characterize the Israeli otter population in order to determine its genetic diversity and fragmentation state for conservation purposes. Monitoring spraint sites during 2000–2011 along active and historic otter distribution regions indicate both stable and unstable otter subpopulations, mainly along the Jordan River. Four otter subpopulations, representing 57 individuals, were characterized by 12 microsatellites, previously used to characterize the European otter populations. The genetic results indicated three subpopulations correlating with three geographical regions: the Hula Valley, Sea of Galilee, and the Harod Valley. A moderate genetic diversity ($F_{\mathrm{st}} = 0.087–0.123$) was found among the subpopulations, suggesting sporadic interactions between individuals from distinct geographical locations along the Jordan Rift Valley. The Israeli otter population was found to be very small, demographically remote and genetically distinct, harboring unique alleles absent from the studied European populations. Therefore, immediate conservation actions are recommended to prevent the deterioration of the isolated, unique, and critically endangered otter population in Israel.

Key words: *conservation genetics, Israeli otter population, microsatellites, spraint*

The distribution of the Eurasian otter (*Lutra lutra*) is mostly Palearctic, with Israel marking the southern boundary of its range in the Levant (Mason and Macdonald 1986; Ruiz-Olmo et al. 2008). Its habitat is composed of wetlands such as rivers with clean running water and thick brush on their banks, as well as water reservoirs adjacent to these streams, and fishponds (Kruuk 2006). Israel is located in a semi-arid region, where wetlands are found only along the drainage basin of streams in the northern Jordan Rift Valley, dictating available habitats for the otter. Until the mid-20th century, otters were abundant in Israel in all coastal rivers extending from the Lebanese border to the Tel-Aviv region, as well as along the Jordan River Basin from its source to the Dead Sea, including the Hula Lake and the shores of the Sea of Galilee. The Harod and Yisrael Valleys served as a corridor between the Jordan River catchments and the coastal plain populations (Dolev et al. 2006).

During the last century, rapid human population growth, agriculture, and urbanization caused fragmentation and reduction in natural habitats. These changes led to a decrease in the population size of many wildlife species, including the Eurasian otter. Since the 1960s, the otter population has undergone a dramatic decline due to elimination of wetland habitats, illegal hunting, water pollution, drainage of water sources (Mendelson and Yom-Tov 1999), and road kills (Guter et al. 2005). The last evidence of otter activity in the coastal plains was recorded at the end of the 1980s, and the otter has been considered to be extinct in this region ever since (Shalmon 1994). The eastern distribution of the otter population in Israel surrounds the main natural water reservoirs: the Sea of Galilee and the Jordan River. These water bodies support the largest otter population in Israel and serve as an ecological corridor by connecting the upper Galilee, the Golan Heights, and the Beit-She'an Valley through the natural water system (Guter 2004; Dolev et al. 2006). However, recent development activities, including replacement of the open trenches with closed pipes, have damaged the routes of migration and reduced available food (Guter and Dolev 2009).

A similar process was reported in Europe, where otter populations have suffered a major decline in range and numbers since the 1950s, and the species is now considered endangered (Macdonald and Mason 1994; Mason and Macdonald 2004). Several factors promoted this decline, including water pollution, loss of habitat, poaching, and road kills. For example, during the 1960s, otter populations in Denmark, France, and Britain vanished from multiple regions, leaving small remote areas with diminutive remnant populations (Chanin and Jefferies 1978; Lode 1993; Pertoldi et al. 2001). An awareness of this situation by scientists and governments in Europe has encouraged research and enhanced knowledge relative to the ecology and genetics of the surviving otter populations (Dallas et al. 2002; Pertoldi et al. 2001; Hajkova et al. 2007; Lanszki et al. 2008; Mucci 2008). This increased information has supported conservation actions, including reintroductions of otters to the wild (Randi et al. 2003; Mucci et al. 2010).

In 1986, Macdonald and Mason (1986) estimated the otter population in Israel at about 100 individuals, and there has been no new population size estimation since then (Dolev and Perevolotsky 2004; Guter 2004). Therefore, currently the Eurasian otter is listed in Israel as "Critically Endangered" (Dolev and Perevolotsky 2004) and in the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species as "Near Threatened" (Vié et al. 2009). The fragmentation of the otter population in Israel and the decrease of migration events between the subpopulations can increase inbreeding, loss of genetic diversity, and susceptibility to disease (Templeton et al. 1990; Young et al. 1996; Hanski 1998; Bohonak 1999; Brook et al. 2002; Crooks 2002). Reduction of genetic variability can result in the fixation of negative alleles and loss of fitness, such as survival, reproductive output, and growth rates, along with impaired ability to adapt to long-term changes in the environment (Crooks 2002). Overall, this process can have profound negative effects on the short- and long-term viability of the population (Lacy 1997; Sherwin and Moritz 2000; Coulon et al. 2004). Therefore, understanding the genetic structure of this threatened population is crucial for its conservation (Maudet et al. 2002; Eggert et al. 2004; Proctor et al. 2005; Aspi et al. 2006). Moreover, a recent study conducted in Italy determined that the Israeli–Eurasian otter population is genetically different from European populations (Mucci 2008). Thus, there is a critical need to determine the distribution, population size, and the genetic structure of the otter population in Israel before recommending any management strategies.

The goal of this research was to characterize the genetic profile of the Israeli–Eurasian otter population in order to determine its genetic diversity and state of fragmentation for conservation purposes. The genetic analysis demonstrates relationships among individuals, the degree of inbreeding, and the influence of habitat fragmentation on the migration of individuals and possible gene flow or genetic drift.

Materials and Methods

Materials for the Genetic Study

Tissue samples were collected from otter carcasses, mainly from road kills that were found by the Nature and Parks Authority rangers during 2000–2011. The tissue samples consisted of liver, muscle, and skin collected during autopsy at the National Collections of Natural History at Tel Aviv University, Israel, and excised ears from the Wildlife Tissue Collection of the National Natural History Collections at the Hebrew University of Jerusalem. All samples were frozen (−20 °C). For each carcass, detailed information was recorded, including location, probable cause of death, sex, and age (Supplementary Table S1). Fresh spraints found during the surveys were collected, kept in 70% ethanol, and refrigerated for genetic analysis (see Supplementary Material online). Multiple attempts were made to extract DNA from the spraints. Due to low success rates in amplifying DNA from spraints, these samples were not included in this study. Overall, 57 specimens were studied, representing four subpopulations based on the geographic location of collection: (1) the Hula Valley, (2) the Harod and Yisrael Valleys, (3) the Sea of Galilee Basin and the Jordan Valley, and (4) the Golan Heights (Supplementary Table S1, Supplementary Figure S1a).

DNA Amplification and Genotyping

DNA was extracted using guanidine thiocyanate (GuHCL) and silica-based purification methods (Boom et al. 1990; Hoss and Paabo 1993). Twelve microsatellite markers (LUT701, LUT832, LUT715 LUT717, LUT733, LUT818, 040T05, LUT435, LUT453, LUT457, LUT615, and LUT833) known to be highly variable among European otter populations (Dallas and Piertney 1998; Huang et al. 2005) were amplified via polymerase chain reaction (PCR). PCR was carried out in 25-µl volumes (5–7 µl of DNA extract, 10× PCR buffer, 0.2 mM of dNTPs, 3 mM of $MgCl_2$, 0.4 µM of each primer, and 0.5 units/reaction of Taq polymerase). A touchdown PCR program was performed with an initial denaturation at 94 °C for 10 min followed by a total of 40 cycles of 15 s at 94 °C, 30-s annealing for 2 cycles each at 60, 58, 56, 54, 52 °C, and 26 cycles at 48 °C, and 45-s elongation at 72 °C, with a final extension time of 10 min at 72 °C. All samples were examined by gel electrophoresis to determine positive/negative amplification. Multiple negative extraction and amplification controls were included in each PCR to detect contamination. For all amplifications performed, no contamination was detected. The samples amplified successfully were diluted in to 1:100 ratio and genotyped at the Center of Genomic Technologies of the Hebrew University of Jerusalem on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems).

Genotyping Analysis

Microsatellite alleles and initial statistical computations were examined and determined using the ABI software Genotyper and ABI PRISM GeneMapper (Chatterji and Pachter 2006). Further analysis included a microsatellite quality check by MicroChecker® (Van Oosterhout et al. 2004) to detect abnormal allele frequencies, null alleles, and allele drop out.

Population genetic analysis was performed with Arlequin (Excoffier et al. 2005) and GenAlEx 6.5b2 (Peakall and Smouse 2001). Clustering analysis using principal component analysis (PCA) was calculated on the basis of a pairwise, individual-by-individual ($N \times N$) genetic distance matrix via GenAlEx (Peakall and Smouse 2001). Population structure and hybridization was detected using the software STRUCTURE 2.4.4® (Pritchard et al. 2000) using a Bayesian clustering method with a burn-in period of 200 000 followed by 200 000 Markov chain Monte Carlo (MCMC) repeats and considering microsatellite frequencies correlated among populations. STRUCTURE was run under the admixture model, with correlated allele frequencies from $K = 2$ to $K = 10$ to explore clustering. The location prioritization (LOC PRIOR) model was employed in order to use sampling locations as prior information to assist the clustering of datasets of a relatively small number of samples. Each run was repeated three times to avoid deviations among the different runs. In order to identify the optimal value of K, we used the Structure Harvester (Earl and vonHoldt 2012), which detects the number of groups that best fits the dataset based on the ΔK recommended by Evanno et al. (2005).

Calculation of the inter-population genetic variance was performed via Arlequin 3.01 software (Excoffier et al. 2005). This included estimation of allele frequencies for each locus relevant to each population and calculation of expected (H_e) versus observed (H_o) heterozygosity values. Additionally, an analysis of variances (AMOVA) test was performed to examine the variable distribution within and between the Israeli populations and determining the fixation index (F_{st}) values (Excoffier et al. 2005).

Results

Genetic Characterization of Otter Population

A total of 57 Eurasian otter carcass samples from four different geographic regions in Israel (Hula Valley, Sea of Galilee, Golan Heights, and Harod Valley) were genetically analyzed using 12 microsatellite markers. This sample collection comprises a large sampling of the estimated otter population in Israel, as the population was previously estimated at about 100 individuals (Macdonald and Mason 1986). Unfortunately, the DNA quantity of 14 samples was limited; therefore, they were genotyped with only seven microsatellite markers. As a result of the limited sample size, we repeated the genetic analysis on the full set of 57 samples with 7 loci and on a subset of 43 samples with 12 loci. In most of the analyses, the results of the two sets were similar, indicating that the sample size or the number of loci studied had no significant effect on the results.

Sample distribution included 31 specimens (54.4%) found at the Hula Valley and the upper Jordan Valley, 11 specimens (19.3%) from the Harod and Yisrael Valleys, 11 specimens (19.3%) from the Sea of Galilee Basin and the Jordan Valley, and 4 specimens (7%) from the Golan Heights (Supplementary Table S1). Significant differences in sample size from the different habitats were due to unequal distribution of the otter, with the majority of the population observed in the Hula Valley. The majority (~84%) of the otter samples originated from road kills ($n = 28$, 49%), and an additional 20 specimens were found on road banks (35%). Other causes of death included poaching (two specimens, 3.5%), entrapment in bird and fish nets (four specimens, 7%), electrocution by an electric fence (one specimen, 1.75%), and unknown causes (Supplementary Table S1). Sex was determined for most carcasses based on visual inspection, identifying 20 males (35.1%), 26 females (45.6%), and 11 of undetermined sex (19.3%) (Supplementary Table S1).

All 12 microsatellite markers, originally used to genotype European otter populations, successfully amplified the DNA of the Israeli otter samples. All loci were polymorphic, but the levels of polymorphism were low, with a total of two to five alleles per locus. The mean number of alleles per locus was 2.6 (Table 1). The allele frequency of each of the seven loci (LUT701, LUT832, LUT715, LUT717, LUT733, LUT818, 040T05) was similar in the two sets (set 1 and set 2) regardless of the sample size (Supplementary Table S2). Significant variation in the allele frequency was detected among the subpopulations for all microsatellite loci. For example, the frequency of allele 167 in locus LUT733 was 0.35 in the Hula Valley, 0.84 in the Harod Valley, 0.11 in the Sea of Galilee, and absent in the Golan Heights (Supplementary Table S2).

Private alleles were detected in the Hula Valley subpopulation (LUT701, allele 200; LUT733, allele 179; 040T05, allele180; LUT457, allele 188) among 14 individuals and in two samples from the Golan Heights (LUT715, allele 171; LUT733, allele 171; LUT453, allele 131) (Supplementary Table S2). Null alleles and allele dropouts were not suggested in any of the 12 microsatellite markers.

Genetic variability as estimated by observed and expected heterozygosity within the region-based subpopulations was moderate, suggesting a genetically diverse population where outbreeding and dispersal may take place (Table 1). Fixation index values were close to zero, indicating random mating (Table 1). Fixation values in the Hula Valley subpopulation were higher than in the other subpopulations and ranged from 0.080 to 0.142, raising the possibility that selective breeding may occur in the subpopulations. It is possible that these values are the result of the small sample size studied and do not reflect the status of the subpopulations. In addition, no statistically significant deviations from Hardy–Weinberg were found when calculated for each locus separately. One locus (LUT818) was found to be monomorphic in the Harod Valley and Golan Heights subpopulations (Supplementary Table S2).

Table 1 Genetic diversity of the Israeli otter subpopulations as estimated by 12 microsatellites

	Set 1 (12 loci)							Set 2 (7 loci)						
Population	N	N_a	H_e	Ho	F	Significance	Assign (%)	N	N_a	H_e	H_o	F	Significance	Assign (%)
Hula Valley	24	3.083	0.499	0.474	0.080	$P < 0.05$	87.5	31	2.857	0.449	0.384	0.142	$P < 0.05$	83.87
Harod Valley	6	1.917	0.346	0.514	−0.398	n.s.	100	11	2.286	0.360	0.364	−0.027	n.s.	72.73
Sea of Galilee and Golan Heights	13	2.917	0.454	0.459	0.009	$P < 0.05$	76.92	15	2.714	0.472	0.465	0.006	$P < 0.05$	73.33
Total	43	2.639	0.433	0.482	0.094		79	57	2.619	0.427	0.404	0.040		86

N, number of specimens studied; N_a, number of observed alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; *F*, fixation index; significance of the *F* probability value (*P*-value: n.s. [not significant]; $P < 0.05$); Assign%, currently assigned to source. Set 1 = 43 specimens genotyped with 12 microsatellite markers; set 2 = 57 specimens genotyped with 7 microsatellite markers.

Genotyping results, using the microsatellite loci, assigned the sampled specimens into one of three clusters. STRUCTURE analysis, for both 7 and 12 microsatellite datasets, yielded a maximum probability of three clusters (Figure 1), supporting the subdivision of the samples into three subpopulations according to their geographical regions: (1) the Hula Valley, (2) the Harod Valley, and (3) the Sea of Galilee and the Golan Heights, with the exception of two samples from the Yisrael Valley. One sample was assigned to the Harod Valley (E2) and the other to the Sea of Galilee and the Golan Heights (E1) (Figure 1). Four additional samples did not cluster with their original region of origin: three samples from Hula Valley (A8, A12, and A13) clustered with the Harod Valley and one sample from the Sea of Galilee clustered with Hula Valley (C11) (Figure 1).

In the PCA, patterns of both sets (7 and 12 microsatellites) included clustering of three major groups, Hula Valley, Harod Valley, and the Sea of Galilee, with the Golan Heights. The analysis of both sets clustered the Harod Valley samples in close proximity to each other. Most of the Hula samples were also clustered in close proximity, but some were closer to samples of the Sea of Galilee and the Golan Heights. The same trend was detected for samples from the Sea of Galilee and the Golan Heights as well (Figure 2a,b). Population PCA analysis showed that the three subpopulations clustered separately from each other (Figure 2c,d). These findings were repeated in both datasets (Figure 2).

A summary of the population assignment test indicated that in set 1 (43 samples, 12 markers), 86% of the samples were assigned to the subpopulation that was identified based on their geographical origin. In set 2 (57 samples and 7 markers), 79% of the samples were assigned to their original subpopulation (Table 1). Three samples from the Hula Valley (A3, A8, A12) were assigned to the Sea of Galilee, and two samples of the Sea of Galilee (C9, C11) were assigned to the Hula Valley. All of these samples are adjacent to the upper Jordan River region, indicating a route of dispersal among these subpopulations. In the Harod Valley, two samples were assigned to the Sea of Galilee (B1, B3) and one to the Hula Valley (B6). In addition, one sample from the Hula Valley (A36) and two from the Sea of Galilee (C2, C12) were assigned to the Harod Valley, suggesting a dispersal route through the lower Jordan River. The assignments of different samples to other subpopulations can indicate possible gene flow between the subpopulations along the Jordan River and the Sea of Galilee, connected by a suitable wetland habitat. The assignment test results support the PCA and the STRUCTURE analysis.

Clustering analysis, combined with biological and geographical data, divided the samples into three subpopulations, corresponding with their place of origin. Based on these results, the estimated genetic diversity between the subpopulations (F_{st} and D_{est}) was calculated (Table 2). Both indices showed significant values ranging between 0.087 and 0.192 ($P \leq 0.001$, computed after 1000 bootstraps over loci), generating similar results for both datasets (Table 2). Overall, D_{est} values were higher than F_{st} values, yet both indices reflected a genetic diversity that supported three distinct subpopulations. The Israeli otter population F_{st} values correspond with diversity values previously found among subpopulations across Europe (Mucci et al. 2010).

To evaluate the genetic diversity of the Israeli otter population relative to other populations that have been surveyed with microsatellites, we compared our results with published results of European otter populations (Pertoldi et al. 2001; Dallas et al. 2002; Arrendal et al. 2004; Kalza et al. 2006; Hajkova et al. 2007; Janssens et al. 2008; Lanszki et al. 2008). Among the Israeli population, most loci showed a low number of alleles (<5 alleles per locus) compared to the European populations, which had as many as nine different alleles per locus (Table 3) (Pertoldi et al. 2001; Janssens et al. 2008; Mucci 2008). The average number of alleles per locus in the Israeli population was 2.6, lower than that found in France, although sample sizes were similar (Table 3) (Pertoldi et al. 2001; Janssens et al. 2008). As the allele number may be influenced by the sample size studied, we compared the overall observed heterozygosity to evaluate the genetic diversity of the Israel population and found it to be similar to observed heterozygosity in some European populations (Mucci et al. 2010; Table 3). We, therefore, believe that the small sample size studied in this research (equivalent to about a half of the estimated Israeli otter population based on previous estimates) represents the real genetic diversity of the otter population in Israel.

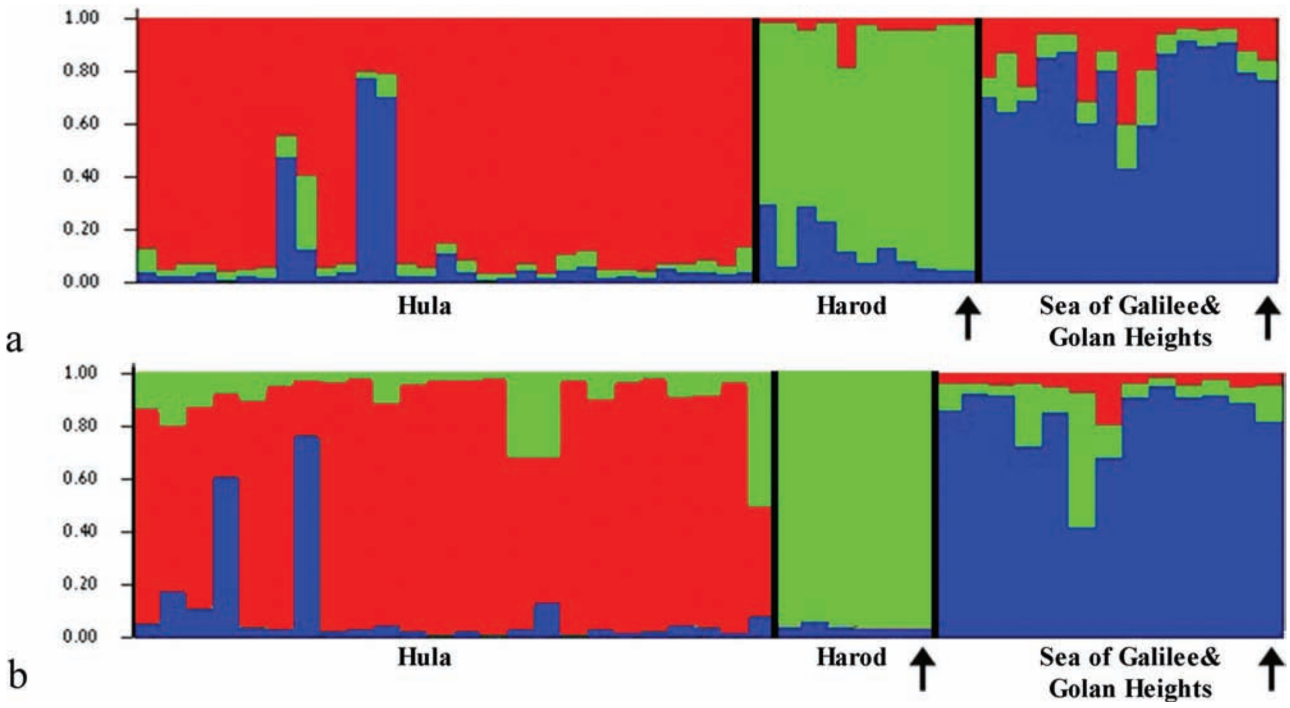

Figure 1. Partitions of otter specimens from Israel into three subpopulations. Structure-based populations resolved otter samples into three groups according to the percent representation of resolved populations (Q-value) similarity, presuming three subpopulations ($K = 3$). The Y-axis represents Q-value within each individual (listed on X-axis, not shown in figure). The left arrow represents sample E1 (Yisrael Valley) and the right arrow represents sample E2 (Yisrael Valley).

Discussion

The genetic examination of otter specimens collected from various geographical locations in Israel revealed moderate genetic variability among individuals, suggesting recent or ancient gene flow among individuals from various locations. The high percentage (~88.3%) of samples that were assigned to their subpopulation of geographic origin indicates that there are differences among the subpopulations (Table 1). The samples assigned to more than one subpopulation were mainly from the Sea of Galilee and from the Hula Valley. The connection between these subpopulations is through the Jordan Rift Valley, which may represent a possible dispersal route (Figure 3). Moreover, the clustering of the Golan Heights specimens with samples from the Sea of Galilee indicates a close connection between the regions. The prevalence of otters in the Golan Heights may designate a possible subpopulation of the Sea of Galilee or Hula Valley with occasional connections along “winter streams” from the Golan Heights to the Jordan Valley. A connection between the Golan Heights otter population and otter populations in Syria is not reasonable because there are no wetland corridors that can be used for migration. In the dry climate of the Mediterranean region, wetland corridors are crucial for otter dispersal, as they can only cross a limited distance of terrestrial terrain (Kruuk 2006). A reduction in heterozygosity, which may be caused by subpopulation structure, resulting in the different allele frequencies among subpopulations could be caused by the “Wahlund effect” (Hartl and Clarck 1997). The underlying cause of this population subdivision could be geographic barriers to gene flow, suggesting that otters may be differentiated at a lower geographical scale similar to the scenario shown for the France and German otter populations in Europe (Randi et al. 2003). Furthermore, it is possible that the differentiation of the Hula Valley and Harod Valley subpopulations is due to isolation by distance owing to limited dispersal rate and reduced gene flow (Dallas et al. 2002). Moreover, specimens found in the Golan Heights periodically over the years suggest that otters inhabiting this area are exposed to random immigrations and local extinctions repeatedly.

The specimens from the Hula Valley were characterized by four private unique alleles and two additional alleles that were present in high frequencies compared to the other subpopulations (Supplementary Table S2). The presence of certain alleles only among individuals from the Hula Valley suggests a low rate of migration events as well as inbreeding and lack of gene flow with the other subpopulations. Although otters can disperse to the Sea of Galilee through the Jordan Valley, this migration was not very common. This supposed inferred barrier cannot be explained by geographical features because none are present in the region. On the contrary, the Hula Valley provides adequate conditions for migration of otters, offering a large area of wetland habitat, fishponds, natural water reservoirs, and sites with thick vegetation. Active spraints sites have been

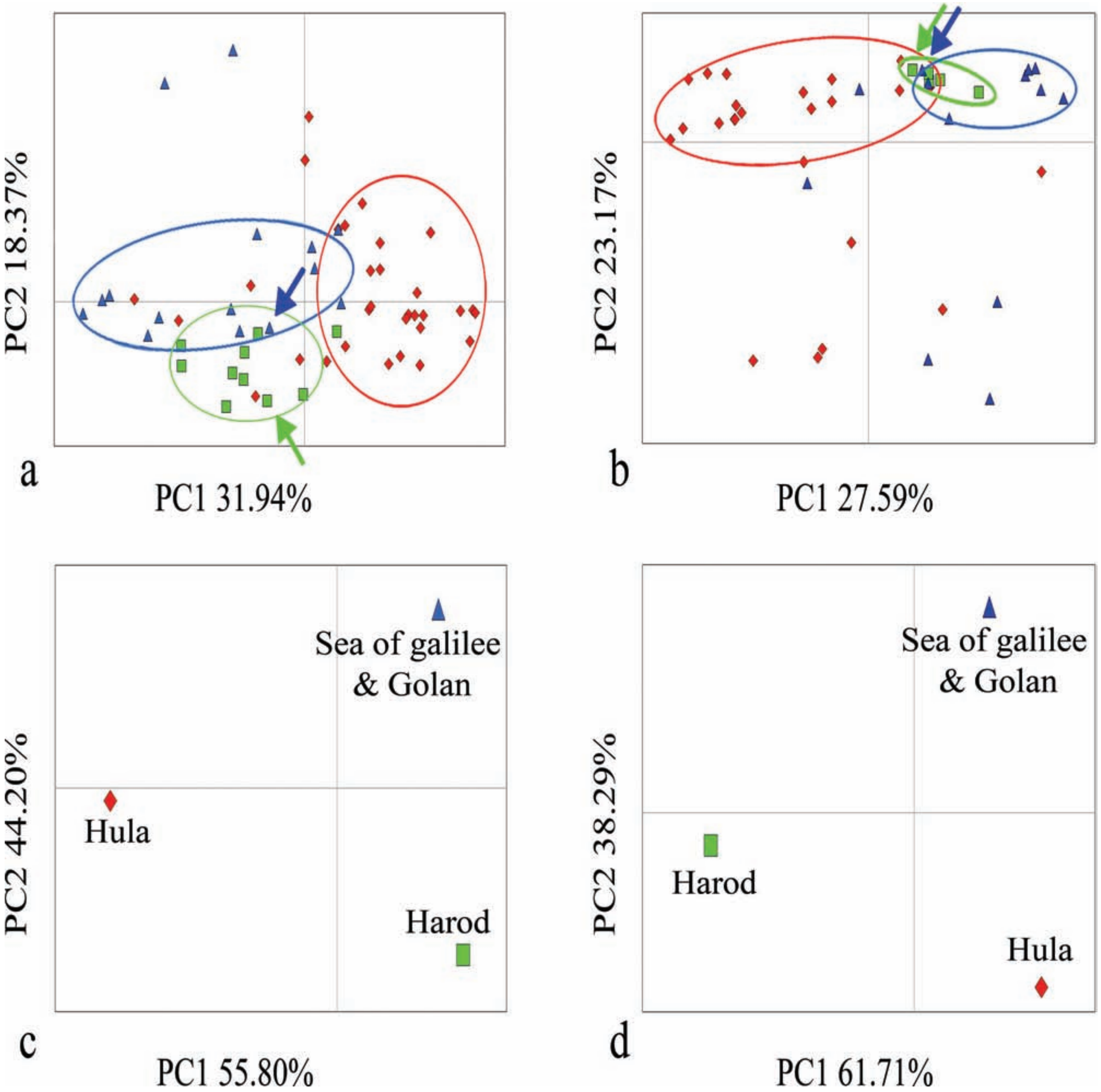

Figure 2. PCA of 12 and 7 microsatellite loci in the otter population in Israel. (**a**) PCA analysis of otter individuals (57 specimens genotyped with 7 microsatellite markers); (**b**) PCA analysis of otter individuals (43 specimens genotyped with 12 microsatellite markers); (**c**) PCA analysis of otter subpopulations (57 specimens genotyped with 7 microsatellite markers); (**d**) PCA analysis of otter subpopulations (43 specimens genotyped with 12 microsatellite markers). Geographic regions are marked by shades and shapes as follows: Hula Valley, diamond; Sea of Galilee and the Golan Heights, triangle; Harod Valley, square. In addition, major groups of samples of the same geographic origin were circled according to the original shade of the group. The blue arrow represents sample E1 (Yisrael Valley) and the green arrow represents sample E2 (Yisrael Valley).

documented since 2000 (Supplementary Figure S1), further supporting a dynamic population in the region. Therefore, we hypothesize that ecological factors, such as abundance of food and presence of wetland habitats, decrease dispersal rate. These environmental conditions, offering an optimal habitat for otters, especially the abundance of different prey species (native versus cultivated fish), may decrease dispersal events, resulting in semi-fragmentation of the Hula Valley otter subpopulations and increasing the regional genetic differences within the Hula Valley. A similar situation was found in France, where despite the high mobility of otters, their distribution was limited by

Table 2 F_{st} and D_{est} values between Israeli otter subpopulations

	Sample sites	Hula	Harod Valley	Sea of Galilee, Golan Heights
Set 1	Hula	—	0.175	0.173
	Harod Valley	0.123	—	0.136
	Sea of Galilee, Golan Heights	0.099	0.113	—
Set 2	Hula	—	0.161	0.192
	Harod Valley	0.118	—	0.107
	Sea of Galilee, Golan Heights	0.111	0.087	—

Above the diagonal: D_{est} values; below the diagonal: pairwise F_{st} values. All values were found to be significant ($P < 0.001$). Set 1 = 43 specimens genotyped with 12 microsatellite markers; set 2 = 57 specimens genotyped with 7 microsatellite markers.

Table 3 Number of alleles found in seven microsatellite loci in the Israeli and European Eurasian otter populations

Country locus	Israel (43)	France (117)	Hungary (17)	Sweden (114)	Germany (52–58)	Great Britain (618)	Denmark (125)	Czech Republic (132)	Slovakia (65)
LUT701	3	5	4	—	—	5	3	5	5
LUT832	3	3	4	7	—	7	4	4	4
LUT715	4	3	4	—	4	5	—	3	3
LUT717	2	4	9	8	5	7	2	6	6
LUT733	4	3	4	9	5	6	5	4	4
LUT818	3	4	—	9	-	6	4	6	5
LUT833	2	5	—	5	6	7	—	6	6
Mean number of alleles	3	3.86	5	7.6	5	6.14	3.6	4.86	4.71

(X) = number of individuals sampled.

watershed limits (Janssens et al. 2006). Moreover, although the otter is opportunistic, exploiting the most abundant prey available (Erlinge 1969; Taastrom and Jacobsen 1999; Guter 2004; Sales-Luís et al. 2009), in southwest England and Spain, otters were found to prefer native fish in their diet, although nonnative fish were of relative prominence in the area (Blanco-Garrido et al. 2008; Miranda et al. 2008).

Genetic microsatellite markers used to characterize the European otter populations (Dallas and Piertney 1998; Huang et al. 2005; Mucci 2008) indicate that the Israeli–Eurasian otter population is genetically unique and differs from European otter populations. Overall, the European otter populations were found to be more variable than the Israeli population, representing different alleles with different frequencies. The low genetic variability found in the Israeli otter subpopulations studied cannot be explained only by the small sample size, as shown in a comprehensive study of European otter populations (Mucci et al. 2010). Therefore, other factors, such as distance between subpopulations (Dallas et al. 2002; Mucci et al. 2010), genetic drift, inbreeding events (Larson et al. 2002; Frankham 2005), or relatively recent demographic expansion from a small population, can be the cause of the low genetic diversity (Rogers and Harpending 1992; Harpending et al. 1998). The genetic differences between the European and the Israeli otter population may indicate the isolation of the Israeli population and limited or absent gene flow between the European and Israeli populations. This finding correlates with the broad study of the European otter populations that indicated that otters in Europe are currently subdivided into a mosaic of subpopulations generated by both historical (fragmentation) and current (limited dispersal) factors (Mucci et al. 2010). Conserving the otter population in Israel should be a priority as it is genetically distinct from the studied European otter populations, demographically remote, marks the southern boundary of the species' distribution in the Levant, and its population size is very small. Moreover, the otter is considered the top predator of wetland habitats in Israel and serves as a flagship species for biodiversity conservation of these habitats.

The surveys of otter spraints sites conducted in Israel during 2000–2011 support the genetic relationship found among the otter subpopulations (see Supplementary Material online) and suggest a stable population activity along the northeastern areas (Hula Valley, Sea of Galilee, and Jordan Rift Valley). In addition, groups of individuals that cannot be sustained were found to inhabit certain areas, such as the Golan Heights and the coastal plains. Spraints found occasionally since 2006 on the banks of the few streams along the northern part of the Israeli coastal area (Guter et al. 2006), and two otter road kills found in 2007 and 2008 along the coast and in the Yisrael Valley, indicate that these areas are periodically occupied by opportunistic otters, but this activity is only temporary (Guter and Dolev 2009). These findings suggest migration of otters into suitable unoccupied habitats along the coast, although the possibility of false absence must be taken into consideration. Based on our observations, we can consider two migration options: the first is a migration route from the Harod Valley to the Yisrael Valley (east to

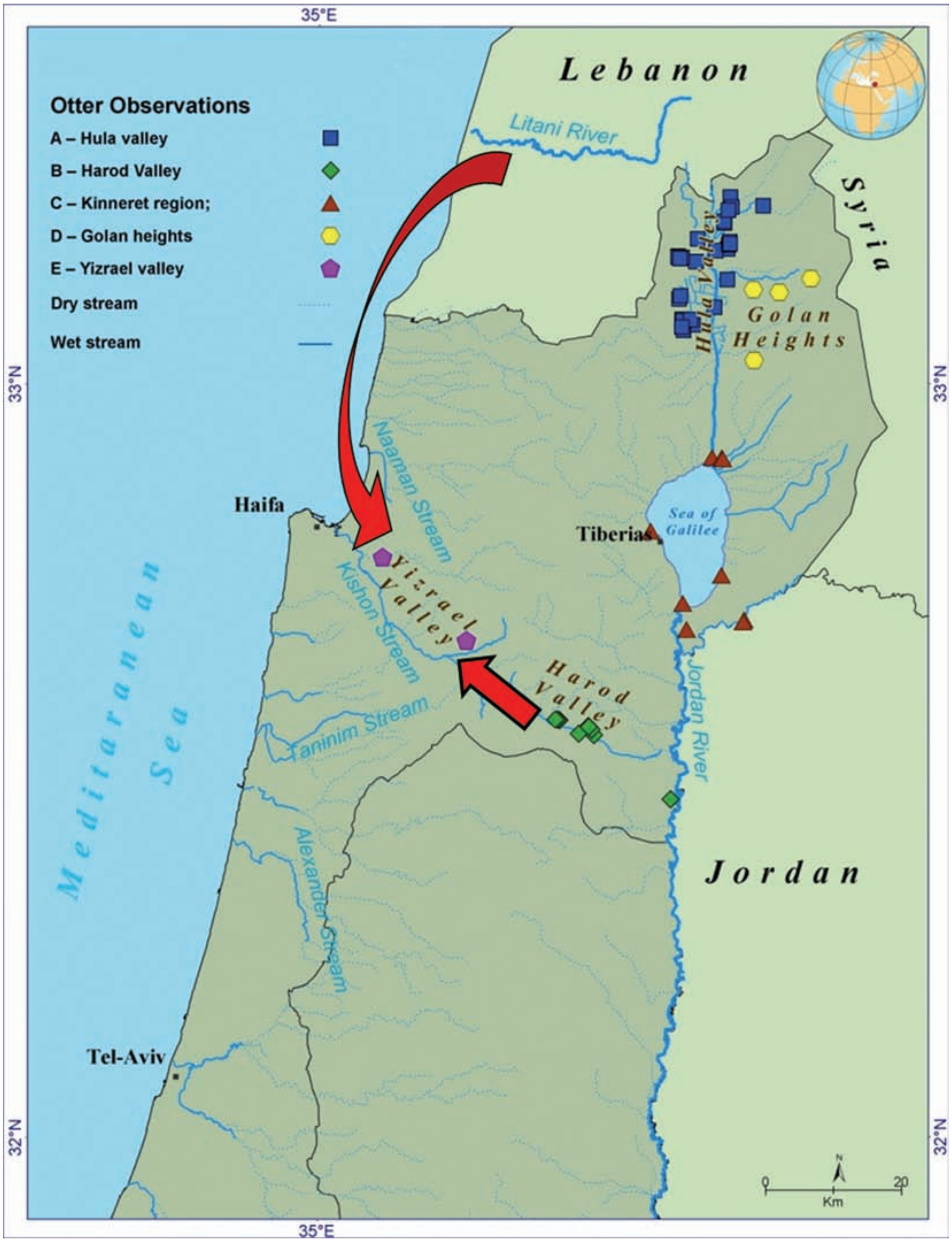

Figure 3. Geographical location of tissuse studied and possible dispersal routes.

west) as exemplified by sample E2 from Kfar Baruch, which clustered with and was assigned to the Harod Valley subpopulation (Figure 3); the second possibility is a migration route along the Mediterranean Sea, presumably from the Lebanese otter population along the coastal area, perhaps from the Litani River (Guter et al. 2006). The Lebanese otters, located at southeast Lebanon, may also interact with the otters in the Hula Valley that may disperse via the Jordan Valley to the Sea of Galilee. This may explain the similarity between the genetic profiles of samples as shown in the various clustering analyses and assignment tests (E1, Yisrael Valley) with samples from the Sea of Galilee (Figure 3). However, genetic or ecological information on the Lebanese otter population is absent, and a single dispersal event cannot account for continuous dispersal between the Israeli and Lebanese otter populations. Regardless of the dispersal route, the presence of otters in the Yisrael Valley is important for the connection of the coastal plain population with the eastern wetland habitats along the

Jordan Valley. Movement of individuals between the northeastern and the west coastal plains may serve as a successful way for otter populations to recover in vacant habitats along the Mediterranean Sea (Janssens et al. 2006). It is important to note that the limited number of specimens and spraints found in the Yisrael Valley during 2006–2010 (Supplementary Figure S1b), and the absence of positive observations in 2011, cannot be explained by a lack of wetland habitats. Therefore, factors preventing otters from establishing a subpopulation in this geographical area are currently unclear.

To summarize, otters are present in wetland habitats of Israel, exhibiting stable activity along the northeastern regions and previously populated (2006–2010) the northern coastal plains of the Mediterranean Sea. We believe that other environmental changes caused by anthropogenic activities (food availability, frequency of favored prey species, and changes in water quantity and quality), which were not studied in this research, also pose a threat to the otter population in Israel, as a decrease in active spraints sites has been documented in the past few years (Supplementary Figure S1). Considering that the otter population in Israel is critically endangered and given the fact that natural colonization is not possible provides compelling argument for consideration of reintroduction programs as a viable alternative. We recommend the release of offsprings of captive Israeli otters in order to maintain the distinctive Israeli otter population. Moreover, the genetic characteristics of the otter subpopulations can be used as guideline in future noninvasive projects, based on otter spraints, to monitor the dynamics of the population.

Supplementary Material

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

Funding

This work was partially supported by a grant from the Israel Mammal Research Centre, Society for the Protection of Nature in Israel.

Acknowledgments

We thank the rangers and volunteers of the Nature and Parks Authority for their help with collecting otter carcasses; the National Collections of Natural History at Tel Aviv University, Israel, for the autopsy work; and G. Nizri and R. Goldblat (GIS division, SPNI) for preparing the maps. We also thank Dr Marilyn Menotti-Raymond for her critical reading and comments.

References

Arrendal J, Walker CW, Sundqvist AK, Hellborg L, Vila C. 2004. Genetic evaluation of an otter translocation program. Conserv Genet. 5:79–88.

Aspi J, Roininen E, Ruokonen M, Kojola I, Vilà C. 2006. Genetic diversity, population structure, effective population size and demographic history of the Finnish wolf population. Mol Ecol. 15:1561–1576.

Bandelt HJ, Forster P, Sykes BC, Richards MB. 1995. Mitochondrial portraits of human populations using median networks. Genetics. 141:743–753.

Blanco-Garrido F, Prenda J, Narvaez M. 2008. Eurasian otter (*Lutra lutra*) diet and prey selection in Mediterranean streams invaded by centrarchid fishes. Biol Invasions. 10:641–648.

Bohonak AJ. 1999. Dispersal, gene flow, and population structure. Q Rev Biol. 74:21–45.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 28:495–503.

Brook BW, Tonkyn DW, Q'Grady JJ, Frankham R. 2002. Contribution of inbreeding to extinction risk in threatened species. Conserv Ecol. 6:16.

Chanin PRF, Jefferies DJ. 1978. The decline of the otter Lutra lutra L. in Britain: an analysis of hunting records and discussion of causes. Biol J Linn Soc. 10:305–328.

Chatterji S, Pachter L. 2006. Reference based annotation with GeneMapper. Genome Biol. 7:R29.

Coulon A, Cosson JF, Angibault JM, Cargnelutti B, Galan M, Morellet N, Petit E, Aulagnier S, Hewison AJ. 2004. Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. Mol Ecol. 13:2841–2850.

Crooks KR. 2002. Relative sensitivities of mammalian carnivores to habitat fragmentation. Conserv Biol. 16:488–502.

Dallas JF, Piertney SB. 1998. Microsatellite primers for the Eurasian otter. Mol Ecol. 7:1248–1251.

Dallas JF, Marshall F, Piertney SB, Bacon PJ, Racey PA. 2002. Spatially restricted gene flow and reduced microsatellite polymorphism in the Eurasian otter Lutra lutra in Britain. Conserv Genet. 3:15–29.

Dolev A, Guter A, Saltz D, Kronfeld-Schor N. 2006. Israel's otter (*Lutra lutra*) population, status and future conservation. In: Conroy JWH, Yoxon G, Gutleb AC, Ruiz-Olmo J, editors. The return of the Otter in Europe—where and how? 2003 June 30– July 5, Sabhal Mor Ostaig, Isle of Skye. Broadford: International Otter Survival Fund. p. 1–7.

Dolev A, Kronfeld-Schor N, Kahila G, Ben-Ari Y, Shahal R, Guter A. 2011. Long term monitoring of the endangered Eurasian otter population in Israel: implication for conservation. Hystrix, It J Mamm. (n.s.) Supp. 72.

Dolev A, Perevolotsky A, editors. 2004. The red book of vertebrates in Israel. Jerusalem (Israel): Israel Nature and National Parks Protection Authority and Society for Protection of Nature in Israel Press.

Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genetics Resour. 4:359–361.

Eggert LS, Mundy NI, Woodruff DS. 2004. Population structure of loggerhead shrikes in the California Channel Islands. Mol Ecol. 13:2121–2133.

Erlinge S. 1969. Food habits of the otter Lutra lutra L. and the mink Mustela vison Schreber in a trout water in southern Sweden. Oikos. 20:1–7.

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.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 1:47–50.

Frankham R. 2005. Genetics and extinction. Biol Conserv. 126:131–140.

Guter A. 2004. The Eurasian otter (*Lutra lutra*) in Israel: ecology and conservation. Tel Aviv (Israel): Tel-Aviv University. [in Hebrew, English summary].

Guter A, Dolev A. 2009. Annually Israel otter survey–2009. Tel-Aviv: Israel Mammals Research Center Society for the Protection of Nature in Israel. [in Hebrew].

Guter A, Dolev A, Saltz D, Kronfeld-Schor N. 2005. Temporal and spatial influences on road mortality in otters: conservation implications. Israel Zool. 51:199–207.

Guter A, Dolev A, Saltz D, Kronfeld-Schor N. 2006. Do otters occasionaly visit Israel's coastal plain? IUCN Otter Specialist Group Bulletin. 23:12–14.

Hajkova P, Pertoldi C, Zemanova B, Roche K, Hajek B, Bryja J, Zima J. 2007. Genetic structure and evidence for recent population decline in Eurasian otter populations in the Czech and Slovak Republics: implications for conservation. J Zool. 272:1–9.

Hanski I. 1998. Metapopulation dynamics. Nature. 396:41–49.

Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherry ST. 1998. Genetic traces of ancient demography. Proc Natl Acad Sci USA. 95:1961–1967.

Hartl DL, Clark AG. 1997. Principles of population genetics. 3rd ed. Sunderland (MA): Sinauer Associates.

Höss M, Pääbo S. 1993. DNA extraction from Pleistocene bones by a silica-based purification method. Nucleic Acids Res. 21:3913–3914.

Huang CC, Hsu YC, Lee LL, Li SH. 2005. Isolation and characterization of tetramicrosatellite DNA markers in the Eurasian otter (*Lutra lutra*). Mol Ecol Notes. 5:314–316.

Janssens X, Defourny P, Kermabon J, Baret PV. 2006. The recovery of the otter in the Cevennes (France): a GIS-based model. Hystrix, It J Mamm. 17:5–14.

Janssens X, Fontaine MC, Michaux JR, Libois R, De Kermabon J, Defourny P, Baret PV. 2008. Genetic pattern of the recent recovery of European otters in southern France. Ecography. 31:176–186.

Kalza B, Jewgenowa K, Fickel J. 2006. Structure of an otter (*Lutra lutra*) population in Germany–results of DNA and hormone analyses from faecal samples. Mamm Biol. 71:321–335.

Kruuk H. 2006. Otters: ecology, behavior and conservation. Oxford: Oxford University Press.

Lacy RC. 1997. Importance of genetic variation to the viability of mammalian populations. J Mammal. 78:320–335.

Lanszki J, Hidas A, Szentes K, Revay T, Lehoczkzy I, Weiss S. 2008. Relative spraint density and genetic structure of otter (*Lutra lutra*) along the Darva river in Hungary. Mamm Biol. 73:40–47.

Larson S, Jameson R, Etnier M, Fleming M, Bentzen P. 2002. Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. Mol Ecol. 11:1899–1903.

Lode T. 1993. The decline of otter (*Lutra lutra*) populations in the region of the pays De Loire, Western France. Biol Conserv. 65:9–13.

Macdonald SM, Mason CF. 1994. Status and conservation needs of the otter (*Lutra lutra*) in the western Palearctic. Strasbourg (France): Council of Europe Press.

Macdonald SM, Mason CF, Shalmon B. 1986. A survey for otters in Israel. Oryx. 20:233– 236.

Mason CF, Macdonald SM. 1986. Otters—ecology and conservation. Cambridge (UK): Cambridge University Press.

Mason CF, Macdonald SM. 2004. Growth in otter (*Lutra lutra*) populations in the UK as shown by long-term monitoring. Ambio. 33:148–152.

Maudetr C, Miller C, Bassano B, Breitenmoser-Würsten C, Gauthier D, Obexer-Ruff G, Michallet J, Taberlet P, Luikart G. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [Capra ibex(ibex)]. Mol Ecol. 11:421–436.

Mendelson H, Yom-Tov Y. 1999. Fauna Palaestina-Mammalia of Israel. Jerusalem (Israel): Keter Press.

Miranda R, Copp GH, Williams J, Beyer K, Gozlan RE. 2008. Do Eurasian otters Lutra lutra (L.) in the Somerset Levels prey preferentially on non-native fish species? Fundam Appl Limnol. 172:339–347.

Mucci N. 2008. Assessiing the paterns of genetic diversity in otter (*Lutra lutra*) populations in Europe. Bologna (Italy): Università di Bologna.

Mucci N, Arrendal J, Ansorge H, Bailey M, Bodner M, Delibes M, Ferrando A, Fournier P, Fournier C, Godoy JA. 2010. Genetic diversity and landscape genetic structure of otter (*Lutra lutra*) populations in Europe. Conserv Genet. 11:583–599.

Peakall R, Smouse PE. 2006. genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 6:288–295.

Pertoldi C, Hansen MM, Loeschcke V, Madsen AB, Jacobsen L, Baagoe H. 2001. Genetic consequences of population decline in the European otter (*Lutra lutra*): an assessment of microsatellite DNA variation in Danish otters from 1883 to 1993. Proc Biol Sci. 268:1775–1781.

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

Proctor MF, McLellan BN, Strobeck C, Barclay RM. 2005. Genetic analysis reveals demographic fragmentation of grizzly bears yielding vulnerably small populations. Proc Biol Sci. 272:2409–2416.

Randi E, Davoli F, Pierpaoli M, Pertoldi C, Madsen AB, Loeschcke V. 2003. Genetic structure in otter (*Lutra lutra*) populations in Europe: implications for conservation. Anim Conserv. 6:93–100.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol. 9:552–569.

Ruiz-Olmo J, Loy A, Cianfrani C, Yoxon P, Yoxon G, de Silva PK, Roos A, Bisther M, Hajkova P, Zemanova B. 2008. Lutra lutra. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. [cited 2012 November 9]. Available from www.iucnredlist.org.

Sales-Luís T, Freitas D, Santos-Reis M. 2009. Key landscape factors for Eurasian otter Lutra lutra visiting rates and fish loss in estuarine fish farms. Eur J Wildlife Res. 55:345–355.

Shalmon B. 1994. Otter survey in Israel. Internal report for the Society for the Protection of Nature in Israel (S.P.N.I.), Tel-Aviv, p. 4. [in Hebrew].

Sherwin WB, Moritz C. 2000. Managing and monitoring genetic erosion. In: Young AJ, Clarke G, editors. Demography genetics viability of fragmented populations. New York: Cambridge University Press.

Taastrom HM, Jacobsen L. 1999. The diet of otters (*Lutra lutra* L.) in Danish freshwater habitats: comparisons of prey fish populations. J Zool. 248:1–13.

Templeton AR, Shaw K, Routman E, Davis SK. 1990. The genetic consequences of habitat fragmentation. Ann Mo Bot Gard. 77:13–27.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 4:535–538.

Vié JC, Hilton-Taylor C, Stuart SN, editors. 2009. Wildlife in a changing world–an analysis of the 2008 IUCN Red List of Threatened Species. Gland (Switzerland): IUCN.

Young A, Boyle T, Brown T. 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol (Amst). 11:413–418.

**Received March 21, 2012; Revised October 8, 2012;
Accepted October 16, 2012**

Corresponding Editor: William Murphy