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Genetic evaluation of an otter translocation program

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Abstract

The translocation of individuals from one population to another is a common technique in wildlife conservation. However, the outcome of translocation programs is not always properly evaluated and the relative contribution of released individuals to the resident population often remains unknown. We used mitochondrial DNA and autosomal genetic markers to evaluate the success of a translocation program of Eurasian otters (*Lutra lutra*) in Sweden. The program is regarded as successful because of subsequent population growths. Norwegian otters used for the restocking program could be genetically differentiated from Swedish otters. The releases took place at two sites. In an area south of the first site, where 47 otters were released, no genetic contribution of the introduced animals to the population could be observed and the genetic diversity was lower than before the releases. At the second site, the release of seven otters led to a change in genetic composition of the resident population. The results of this study suggest that the growth of the otter population after the restocking may not be as dependent on the releases as initially suspected. The genetic effects of the translocations appear to be restricted to areas in the immediate vicinity of the release sites.

Abbreviations: bp – base pairs; mtDNA – mitochondrial DNA; PCR – polymerase chain reaction; SSCP – single-stranded conformation polymorphism

Introduction

Reintroductions and restocking of populations through translocations of individuals originating from other areas are common tools in conservation (Breitenmoser et al. 2001). Translocations are often the only alternative for the recovery of species and populations that are locally extinct such as Mexican wolves (*Canis lupus*) in southern United States (Parsons 1998), or seriously threatened like the black-footed ferret (*Mustela nigripes*, Dobson and Lyles 2000) and the California condor (*Gymnogyps californianus*, Snyder and Snyder 2000). The degree of success of these management actions varies among groups of species and is particularly low for carnivores (Breitenmoser et al. 2001). Success is typically evaluated by monitoring population growth after the releases and comparing to the initial goals of the program (Breitenmoser et al. 2001). However, most translocation programs lack proper evaluation and the outcome therefore remains unknown (Fischer and Lindenmayer 2000; Breitenmoser et al. 2001).

The degree of success of translocation programs can be indirectly assessed by comparison of the genetic composition of the population before and after the translocation (Ellsworth et al. 1994; Grewe et al. 1994; Leberg et al. 1994; Largiadèr and Scholl 1995; Jones et al. 1996; Nedbal et al. 1997; Rowe et al. 1998; Leberg and Ellsworth 1999). Several studies have reported that for small popula-

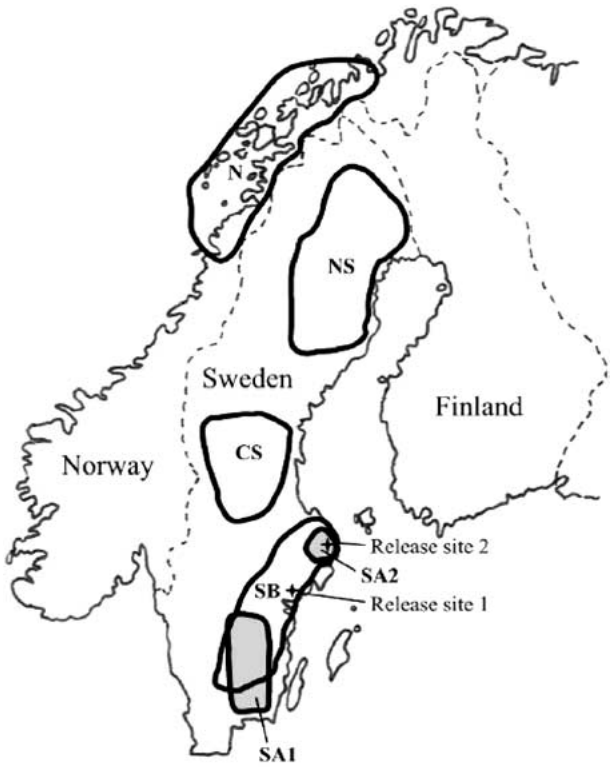

Figure 1. Populations of Eurasian otters included in the study. Populations marked in gray were sampled after the release of otters from Norway (from the population N). The release sites are indicated. Acronyms correspond to those in Table 1.

tions with low genetic diversity, the release of individuals from other areas has led to an increase in the genetic diversity and fitness of the population as well as a demographic expansion (Madsen et al. 1999; Westemeier et al. 1998). This process, known as "genetic rescue" (Ingvarsson 2001), together with the numerical augmentation of the population, are commonly seen as responsible for the population growth following the release programs.

The analysis of the genetic structure of the population after the release can provide valuable information about the relative success of the introduced individuals (Madsen et al. 1999; Ebert et al. 2002; Vilà et al. 2003). If released individuals are contributing to the gene pool of the restocked population and represent a fitness increase, their contribution to new generations should be higher than predicted by demographic considerations alone (Ingvarsson and Whitlock 2000; Ebert et al. 2002). On the other hand, if survival of released individuals is lower than survival of local residents, the contribution of the former should be minor compared to that of the latter. Genetic surveys after the releases could provide an assessment of how much the translocations have contributed to the genetic composition of the local population. In this study, we evaluate the contribution of a restocking program of Eurasian otter (*Lutra lutra*) to a population in Sweden that has subsequently expanded.

The restocking program for otters in Sweden was initiated in the 1980's in attempt to counteract the dramatic decline observed during the last century. Since the decrease was the most severe in the southern parts of the country, a total of 54 otters originating from northern Norway were released into two different areas in the south (47 at one site and 7 at the other) between the years 1987 and 1992 (Figure 1; Sjöåsen 1997; Larsson and Ebenhard 1994). The fate of the 47 otters released at Release site 1 (Figure 1) was monitored by telemetry (Sjöåsen 1996a) and field surveys (Sjöåsen 1996b). The survival of the released otters after one year was 54% and a population growth was observed. The outcome of the program was thus regarded as a success (Sjöåsen 1995; Breitenmoser et al. 2001). During the 1990's, the otter was slowly starting to recover in most parts of Sweden, including in the south (Hammar 1996; Länsstyrelsen Gävle-

Table 1. Distribution of samples within each population

Population (acronym)	Sample size	Sampling period
Norway (N)	20	1988–94
Northern Sweden (NS)	28	1984–99
Central Sweden (CS)	23	1973–99
Southern Sweden before the releases (SB)	15	1966–85
Southern Sweden after the releases, Småland (SA1)	8	1991–98
Southern Sweden after the releases, Uppland (SA2)	20	1993–99

borg 1998; Bisther 2000). Today, otters are scattered through a large part of the suitable habitats in northern Sweden. In the southern half of the country the distribution is less dense, with large areas of suitable habitat remaining unoccupied.

Despite the presumed success of the translocations, it is uncertain to what degree the releases have contributed to the growth of the otter population in southern Sweden. In this study, we used genetic markers to evaluate the effects of the restocking program on the genetic structure of the Swedish otter population. If translocated individuals have successfully contributed to the gene pool of the resident otters, we expect the genetic composition of populations in areas neighboring the release sites to show similarities to that of the Norwegian source population.

Material and methods

Material

Samples from a total of 114 otters were used for the analysis: 20 from northern Norway and 94 from Sweden. The samples were provided by the Environmental Specimen Bank (Contaminant Research Group) and the Department of Vertebrate Zoology, both at the Swedish Museum of Natural History. Most of the animals had been road-killed or accidentally killed in other ways. The Swedish samples dated from between the years 1966 and 1999 (Table 1). The Norwegian samples represented the otters released in the restocking program in the south of Sweden. These otters originally were from the north of Norway, either wild-caught or captive-bred (Sjöåsen 1996a). The sample of the translocated otters consisted of individuals with identification tags that were released and later were found dead.

Samples were grouped into populations based on their geographic origin (Figure 1; Table 1). For the samples in southern Sweden we differentiated the samples dating from before the release of Norwegian otters (SB) from those collected afterwards (SA). We separated the otters from southern Sweden post-dating the releases into two populations (SA1 and SA2), because preliminary genetic analyses indicated they were genetically differentiated and should not be grouped (Arrendal 2000). Only one of these populations (SA2) has been directly subjected to restocking (includes Release site 2, Figure 1), but both of them could be indirectly influenced by dispersing individuals from the other release site. No otters were available for the study from areas closer to the release sites than those from SA1 and SA2. We assume that the releases in the south have not significantly affected the genetic composition of otters in central (CS) and northern Sweden (NS) due to the large distances separating the populations and also due to the fact that the reintroductions were done recently.

Laboratory procedures

Samples of muscle or liver tissue were used in the study and kept frozen at –20 °C prior to the DNA extraction. A small piece (approximately 10–30 μg) of the tissue was sliced with a scalpel and digested in 500 μl of extraction buffer (0.1 M Tris, 0.005 M EDTA-Na_2, 0.2 M NaCl, 0.007 M SDS, adjusted to pH 8.5) with 0.3 mg of proteinase K. The samples were incubated at 37 °C overnight and genomic DNA was extracted following a conventional phenol/chloroform protocol (Sambrook et al. 1989). The extracted DNA was re-suspended in double distilled water and the concentration was measured with a fluorometer (Hoefer DyNA Quant 200).

Three pairs of primers were used to PCR-amplify almost 1000 base pairs (bp) of the mitochondrial DNA (mtDNA) control region excluding a repetitive sequence commonly found in carnivores (Hoelzel et al. 1994): F16355 (5'-CCCAAAGCTGACATTCT AAC-3') and R16812 (5'-CACTAGTCCATCGAGAT GTCCC-3'), F16769 (5'-CATCTGGTTCTTACTTC AGG-3') and R00170 (5'-CCATTGACTGAATAGCA CCTTATGGTT-3'), F00680 (5'-TTAATCAAACCCC CCTTACCCCCCG-3') and R01008 (5'-TAACTGCA GAAGGCTAGGACCAAACCT-3') (K.-P. Koepfli, pers. comm.). Each PCR mixture contained approximately 30 ng of DNA, 1x PCR buffer (Perkin Elmer), 1.5 mM $MgCl_2$, 200 μM dNTP, 30 pmol of each primer and 0.5 units of Ampli Taq polymerase (Perkin Elmer). Thirty-five cycles of amplification were performed in a thermal cycler. Each cycle consisted of denaturation at 94 °C for 60 sec, annealing at 50 °C for 120 sec, and extension at 72 °C for 90 sec, with a final extension at 72 °C for 7 min. The three primer pairs produced fragments of sizes between 260 and 370 bp in length, and were compared by single-stranded conformation polymorphism (SSCP) analyses (Hayashi 1991). The fragments were separated on native 16 cm 12% polyacrylamide gels running at 1 watt for 16–18 h. The different SSCP morphs were visualized using a silver staining technique (Bassam et al. 1991). Representative samples from each SSCP morph and from each population were subsequently sequenced to confirm that they corresponded to the same sequence. Sequencing was conducted using Big Dye^{TM} Terminator Cycle Sequencing chemistry (Perkin Elmer) in an ABI377 semi-automated sequencing instrument (Perkin Elmer) following the manufacturer's protocols.

Six different pairs of microsatellite primers, developed for the Eurasian otter, were used in the study. The markers were Lut717, Lut733, Lut818, Lut832, Lut833 (Dallas and Piertney 1998) and Lut902 (Dallas et al. 1999). PCR was carried out in 10 μl reactions containing ca 50 ng genomic DNA, 1x PCR buffer (Perkin Elmer), 1.5 mM $MgCl_2$, 200 μM dNTP, 2 pmol of each primer and 0.5 units Ampli Taq polymerase (Perkin Elmer). The amplification was performed in a PTC-100 instrument (MJ Research, Inc.). The PCR profiles were different for each one of the markers and are available upon request. The PCR-products were separated on a 6% denaturing polyacrylamide gel and visualized by silver staining (Bassam et al. 1991).

Analyses

Microsatellites were tested for linkage disequilibrium using an exact test based on a Markov chain algorithm as implemented in the program GENEPOP (Raymond and Rousset 1995). The deviation of the allele frequencies from the Hardy-Weinberg equilibrium expectations for each population was tested using the exact test implemented in GENEPOP. The statistical significance of linkage disequilibrium tests across the entire data set and deviations from Hardy-Weinberg equilibrium for each population were assessed using Bonferroni's correction (Rice 1988).

Expected (H_E) and observed (H_O) heterozygosities, as well as average number of alleles per locus for each population were calculated using the program GENETIX (Belkhir 2000). The inbreeding coefficient F_{IS} for each population was also calculated with GENETIX, and the significance of its deviation from zero was tested by a permutation test. Since the sample size differed widely between the populations (Table 1), differences between populations in the genetic diversity (H_E, H_O and average number of alleles per locus) could be due either to real differences between the populations or to sampling bias. One hundred random draws of 8 individuals (sample size for SA1) were studied, and the distribution of the values found for each population was compared to the value observed for SA1. Tests for significance for the differences between other populations were done using Mann-Whitney *U* tests.

The genetic differentiation between the populations under the infinite alleles model was calculated with the θ estimator (Weir and Cockerham 1984) of Wright's (1965) F_{ST}, using the program GENETIX. The significance of the values estimated for this statistic was evaluated using a permutation test. The average number of migrants per generation between populations (Nm) was estimated from θ values using the expression $\theta = 1/(1 + 4Nm)$.

Finally, an assignment test (Paetkau et al. 1995) was used to calculate the likelihood of each individual multi-locus genotype having originated from each of the defined populations. The test was performed using the assignment calculator at http://biodb.biology.ualberta.ca/jbrzusto/alpha/Doh.html. The frequency 1/(2C) was used for alleles missing from one population, where C is the sample size for that population. Since we wanted to know the origin of the individuals from SA1 and SA2, we compared their assignment likelihood to each one of the populations assumed

Table 2. Distribution of mitochondrial DNA control region haplotypes and microsatellite allele *4* at locus Lut832 in the populations. Acronyms correspond to those in Table 1

Population	Haplotype *A* (no. of ind.)	Haplotype *B* (no. of ind.)	Allele *4* (frequency)
N	17	2	0.225
NS	28	0	0.036
CS	23	0	0.022
SB	15	0	0.000
SA1	7	0	0.000
SA2	11	6	0.275

to represent the genetic variability existing in Scandinavia before the releases (SB, CS, NS, or N).

Results

Three fragments of the mtDNA control region were typed by SSCP and/or sequenced in the sampled otters. Two fragments, successfully typed in 82 (primers F16769 and R00170) and 80 (F00680 and R01008) individuals across all populations, did not show any variability. The third fragment, corresponding to control region I, was typed in 109 otters and showed two haplotypes (Table 2). Of these, 101 (93%) had haplotype *A*. Haplotype *B* was only present in two individuals (11% of the sample) from Norway and six (35%) from SA2, one of the post-release populations in southern Sweden. Considering all three control region fragments, the two haplotypes differ by 1 bp (0.1%). Sequencing of two otters from Denmark and two from France revealed that all of them also had haplotype *A* (data not shown).

All microsatellite markers were polymorphic in all populations. Allele frequencies for all loci and populations are given in Appendix I. The only population sample that was not in Hardy-Weinberg equilibrium ($P < 0.05$) was NS, which departed from equilibrium at loci Lut717 and Lut733. The deviation from equilibrium was a result of a heterozygote deficit, which led to a significant positive inbreeding coefficient F_{IS} (0.122; Table 3). One possible explanation for this departure could be some sort of non-random mating, although it is not clear why this would only occur in this area. An alternative and more likely explanation is that the population was not homogenous but internally fragmented (Wahlund effect; Hartl and Clark 1997). In fact, the sample was distributed over an extensive area. In order to simplify analyses, to avoid further reduction of population sample sizes, and because our main interest is on the effects of otter releases in southern Sweden, we retained the northern Sweden (NS) sample as a single population. None of the populations showed significant linkage disequilibrium after Bonferroni correction ($P > 0.05$), indicating that the loci were not linked, although the small sample sizes could be reducing the power of the test.

The average expected heterozygosity was above 0.60 for all populations except for SA1 (H_E = 0.45; Table 3). The largest value was for NS (H_E = 0.75). The average observed heterozygosity shows very similar values (between H_O = 0.64 and 0.72) for all populations except for SA1, for which it was only 0.50. Finally, the average number of alleles per locus gives similar results, suggesting reduced variability for SA1, although this parameter also indicates a reduced variability for SA2.

Some of the measures of variability, for example the expected heterozygosity and the average number of alleles per locus, are strongly affected by the sample size. In our case, the population with the lowest variability corresponds to the one for which only eight samples were obtained (SA1). In order to correct for the large differences in sample size for the populations under study, we randomly sampled 8 individuals 100 times for each one of the larger populations (Table 3). The values observed for the expected heterozygosity and average number of alleles for SA1 were below the minimum observed for the re-samplings for all of the other populations (Table 3). Only two of the 100 re-samplings for SA2 gave lower observed heterozygosity than that observed at SA1. Interestingly, the comparison of the 100 re-samplings suggested that SA2 indeed had lower expected heterozygosity and lower number of alleles per locus than the other populations (Mann-Whitney *U* test, $P < 0.01$ in both cases; Table 3). Consequently, the two southern populations sampled after the releases of otters from Norway (N) showed lower genetic diversity than the other populations.

The degree of differentiation between the populations can be assessed by θ. Although all populations were significantly differentiated (Table 4), the maximum degree of differentiation was between SA1 and SA2, the two southern populations sampled after the releases. SA2 was well differentiated from all populations, but slightly more similar to SB (θ = 0.106). For SA1 the similarity with SB was greater (θ = 0.058). In order to assess the significance of the

Table 3. Sample size (*N*), Nei's (1978) unbiased estimation of the expected heterozygosity (H_E), observed heterozygosity (H_O), average number of alleles per locus (standard deviations in parentheses) and inbreeding coefficient F_{IS}. Figures in bold are mean values from 100 random samplings of 8 individuals (sample size for SA1) and their range. Acronyms correspond to those in Table 1

Pop.	*N*	H_E	(SD)	H_O	(SD)	N. alleles	(SD)	F_{IS}
N	20	0.683	(0.051)	0.715	(0.041)	5.33	(0.82)	–0.048 (ns)
		0.690	**0.619–0.729**	**0.719**	**0.604–0.833**	**4.30**	**3.83–5.00**	
NS	28	0.745	(0.028)	0.656	(0.037)	6.17	(1.72)	0.122**
		0.744	**0.675–0.800**	**0.662**	**0.521–0.833**	**4.87**	**4.00–6.00**	
CS	23	0.695	(0.028)	0.696	(0.039)	5.67	(0.52)	–0.001 (ns)
		0.701	**0.622–0.761**	**0.702**	**0.604–0.812**	**4.39**	**3.67–5.33**	
SB	15	0.694	(0.030)	0.656	(0.050)	4.83	(0.75)	0.057 (ns)
		0.689	**0.632–0.732**	**0.650**	**0.521–0.792**	**4.25**	**3.67–4.67**	
SA2	20	0.613	(0.048)	0.642	(0.044)	3.67	(1.51)	–0.048 (ns)
		0.612	**0.514–0.676**	**0.640**	**0.479–0.833**	**3.24**	**2.67–3.67**	
SA1	8	0.451	(0.091)	0.500	(0.072)	2.50	(0.55)	–0.116 (ns)

**Significantly different from 0 at $P < 0.01$; ns, not significantly different from 0.

Table 4. Inter-population differentiation estimated by θ (Weir and Cockerham 1984), above the diagonal, and number of migrants per generation (*Nm*), below the diagonal. Acronyms correspond to those in Table 1

	N	NS	CS	SB	SA1	SA2
N	—	0.053**	0.082**	0.053**	0.172**	0.134**
NS	4.45	—	0.065**	0.065**	0.177**	0.124**
CS	2.80	3.61	—	0.039**	0.133**	0.126**
SB	4.46	3.60	6.13	—	0.058**	0.106**
SA1	1.20	1.16	1.63	4.09	—	0.221**
SA2	1.62	1.77	1.74	2.11	0.88	—

**Significantly different from 0 at $P < 0.01$.

Table 5. Assignment of individuals from each population (rows) to the populations supposedly not affected by restocking programs (columns). Each individual sample is assigned to the population for which the likelihood of assignment is highest. Acronyms correspond to those in Table 1

	N	NS	CS	SB
N	15 (75%)	2	1	2
NS	2	22 (79%)	2	2
CS	1	1	18 (78%)	3
SB	2	1	4	8 (67%)
SA1	0	0	1	7
SA2	5	3	9	3

difference between these θ values, we subsampled 8 individuals (which is the sample size for SA1) from the SA2 population 100 times and estimated the differentiation between SB and each subsample using θ. The average value was identical to the value estimated for the entire dataset ($\theta = 0.106$) and the lowest 2.5% percentile was 0.058, the same as the value of θ between SB and SA1, suggesting that the difference between these two populations is significantly smaller than between SB and SA2.

Gene flow between the southern populations sampled after the releases (SA1 and SA2) was very low (less than 1 migrant per generation; Table 4) and it was only slightly higher when comparing them to the other populations (between 1 and 2 migrants per generation, except between SA1 and SB). However, gene flow was higher between the other populations (more than 2.5 migrants per generation in all comparisons).

Estimates of gene flow based on θ represent patterns over evolutionary time. An assignment test can be more efficient at portraying recent movements between populations (Paetkau et al. 1995). We studied the assignment of all individual samples to the four populations that represent the genetic variability before the releases: N, NS, CS and SB (Table 5). Between 67% and 79% of the otters from these populations were correctly assigned to the populations from which they were sampled. This indicates that a certain degree of differentiation between populations exists. On the other hand, 7 out of 8 (88%) of the

otters from SA1 were assigned to SB, the population that was living there before the releases. The "misassigned" individual was assigned, instead, to the neighboring population CS. None of the individuals from SA1 seemed similar to the Norwegian otters (N) used for translocation. The individuals from SA2 were assigned to all of the four populations and only 3 of the 20 otters from SA2 (15%) were assigned to SB.

While otters from SA1 do not seem genetically affected by the introgression of DNA from Norwegian otters, the situation is different for the SA2 individuals. Nine alleles that were observed in our Norwegian sample (N) were not observed in SB. Most of them were present in low frequencies in N and, consequently, are not good indicators of the success of the restocking. However, allele *4* at Lut832 represented 22.5% of the alleles at this locus for Norwegian otters (population N, Table 2; Appendix I) whereas it was not present in our sample from SB and accounted for only 3.6 and 2.2% in NS and CS. If this allele was present in SB with the same frequency as observed in N, the probability of missing it in a sample of 15 would be as low as 0.02, which suggests that this allele was not present or was very uncommon in this area before the releases. The allele was not present in SA1, but was frequent (27.5%) in SA2. This result, together with the presence of control region haplotype *B* (Table 2), suggest that while the releases may not have had a genetic impact in SA1, they may have effected the gene pool of SA2.

Discussion

European populations of Eurasian otter are generally characterized by very low genetic diversity, presumably due to founder events during postglacial recolonisation (Effenberger and Suchentrunk 1999; Mucci et al. 1999; Cassens et al. 2000; Pertoldi et al. 2001) or to anthropogenic pressure during the last 2000 years (Pertoldi et al. 2001). Our results are consistent with these hypotheses: only two very similar mtDNA haplotypes have been observed and the variability at microsatellite loci was also low. We observed between 2.5 and 6.2 alleles per microsatellite locus per population (Table 3), similar to values for otters from Scotland (average number of alleles per locus between 2.1 and 5.3 for ten loci; Dallas et al. 1999) and from Denmark (3.1 alleles per locus; Pertoldi et al. 2001).

All studied populations were differentiated from each other (Tables 4 and 5). Subdivision was also indicated by the observed heterogeneity within NS, probably a result of the Wahlund effect (Hartl and Clark 1997). At the same time, in spite of the differentiation, gene flow was quite high between most of the populations (Tables 4 and 5) indicating that exchange between populations may have been higher in the past, when the Scandinavian otter population was denser and the distribution more continuous.

As a result of the population differentiation (at microsatellite loci and, to a lesser extent, mtDNA) it was possible to genetically characterize otters from northern Norway and, hence, detect to what degree the translocation of Norwegian otters to southern Sweden has genetically affected this population. After the release, the otter population in southern Sweden has grown, both in the area around Release site 1 (Figure 1), where the majority of the otters were released (Sjöåsen 1996b), and in areas around SA1 (Bisther 2000) and SA2 (Hammar 1996). Telemetric data have shown that the survivorship of the released otters one year later was 54% (Sjöåsen 1996a). There were also strong indications of two released females reproducing (Sjöåsen 1996a). Consequently, the release of Norwegian otters is regarded as successful (Sjöåsen 1995; Breitenmoser et al. 2001), contributing to the recovery of the population in southern Sweden. However, genetic data are not as supportive. Otters from SA1 were characterized by a low genetic diversity (Table 3). This low variability is expected in a population that has gone through a sharp population decline. The release of 47 otters from Norway at Release site 1 (Figure 1), to the north of SA1, does not seem to have resulted in the arrival of new alleles or an increase of diversity. In fact, the otters in SA1 were most similar to the ones that inhabited the region before the releases (SB, Figure 2; Table 4), with an additional loss of alleles, probably due to random genetic drift (Hartl and Clark 1997).

In SA2, seven Norwegian otters were released (Release site 2; Figure 1) and otters from Release site 1 could also have indirectly contributed to the population if they (or their descendents) dispersed into the area. Our results show that SA2 might have been affected by the restocking. Allele *4* at locus Lut832 was present in a high frequency only in the Norwegian population and in SA2 (Table 2; Appendix I) and additionally, mtDNA haplotype *B* was present only in these two populations (Table 2). Neither the allele nor the haplotype had been observed in the otter population in southern Sweden before the releases (SB). Perhaps as a result of the arrival of Norwegian otters with new

alleles, the allelic diversity in this population has not been as depleted as in SA1 (Table 3). Random genetic drift induced by the small population size, together with the new alleles, have made this population very divergent from all the others. However, even though the genetic composition of SA2 has been affected by the release of otters from northern Norway, it is still most similar to SB (Table 4), the population before the releases, suggesting that the effect of the releases has been limited.

Our results do not imply that the large translocation of otters into Release site 1 had no influence on the local population. Since we were unable to obtain samples from the area where these otters were released, we cannot imply that these have not contributed to the gene pool of the resident otters. However, the absence of an apparent effect of the translocation to otters from an area not very distant (SA1) indicates that the effects may not be far-reaching. Consequently, the results of this study imply that the growth of the otter population observed in southern Sweden during the 1990's (Hammar 1996; Sjöåsen 1996b; Bisther 2000), may not have been as closely related to the restocking program as initially suspected. This conclusion is supported by the fact that population growth has also been observed in areas more distant from the restocking sites (Länsstyrelsen Gävleborg 1998; Bisther 2000). Although half of the group of released otters survived after one year and some reproduced successfully (Sjöåsen 1996a), the genetic effects are restricted to areas directly surrounding the release site, such as SA2, yet even in these regions the releases may not have been able to completely stop the loss of genetic diversity by drift (Table 3). In regions more distant from the release sites, such as SA1, the loss of genetic diversity has been greater and there is no visible arrival of new alleles. Together, these results seem to indicate that the release of otters from northern Norway has had a limited impact on the local population and only at a reduced spatial scale. Similar results have been experienced for translocations of white-tailed deer in southeastern United States (Leberg and Ellsworth 1999). It seems that long distance dispersal of otters has been quite low, perhaps due to the overall low population density in the south of Sweden and subsequent large availability of vacant home ranges.

The evaluation of the success of restocking programs demands *in situ* analyses of the population before and after the implementation of the programs (Leberg and Ellsworth 1999; Fischer and Lindenmayer 2000; Goossens et al. 2002) and not only an assessment of demographic trends. A reliance on demographic data alone may offer an incomplete view of the evolution of a population, leading to misinterpretations of the processes governing the population changes through time. Complementing demographic data with genetic analyses can allow for a more accurate assessment of the success of translocations.

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Appendix I

Allelic frequencies for each locus and population, with sample size in parentheses

	N	NS	CS	SB	SA1	SA2
Lut717	(20)	(28)	(23)	(15)	(8)	(20)
1	0.025	0.071	0.022	0.133	0.000	0.000
2	0.025	0.304	0.022	0.033	0.000	0.000
3	0.700	0.339	0.326	0.633	0.875	0.600
4	0.225	0.054	0.370	0.100	0.000	0.000
5	0.025	0.143	0.174	0.000	0.000	0.400
8	0.000	0.089	0.087	0.100	0.125	0.000
Lut832	(20)	(28)	(23)	(15)	(8)	(20)
1	0.000	0.000	0.022	0.033	0.000	0.025
2	0.175	0.321	0.152	0.200	0.000	0.325
3	0.175	0.321	0.348	0.433	0.500	0.225
4	0.225	0.036	0.022	0.000	0.000	0.275
5	0.175	0.286	0.391	0.267	0.500	0.150
6	0.100	0.036	0.065	0.067	0.000	0.000
7	0.150	0.000	0.000	0.000	0.000	0.000
Lut833	(20)	(28)	(23)	(15)	(8)	(20)
1	0.025	0.000	0.000	0.000	0.000	0.000
2	0.000	0.161	0.130	0.000	0.000	0.025
3	0.400	0.250	0.435	0.400	0.625	0.050
4	0.400	0.250	0.239	0.200	0.313	0.075

5	0.025	0.036	0.065	0.033	0.063	0.350
6	0.150	0.214	0.130	0.267	0.000	0.500
7	0.000	0.089	0.000	0.100	0.000	0.000
Lut733	(20)	(28)	(23)	(15)	(8)	(20)
1	0.000	0.018	0.000	0.000	0.000	0.000
2	0.000	0.089	0.022	0.000	0.000	0.000
3	0.000	0.036	0.000	0.000	0.000	0.000
4	0.050	0.000	0.000	0.000	0.000	0.000
5	0.050	0.000	0.022	0.000	0.000	0.000
6	0.025	0.054	0.000	0.033	0.000	0.000
7	0.350	0.196	0.391	0.300	0.063	0.425
8	0.325	0.411	0.261	0.200	0.000	0.000
9	0.200	0.196	0.304	0.467	0.938	0.575
Lut818	(19)	(26)	(23)	(15)	(8)	(20)
1	0.000	0.000	0.044	0.000	0.000	0.000
2	0.053	0.000	0.087	0.133	0.063	0.000
3	0.290	0.404	0.652	0.400	0.563	0.625
4	0.290	0.462	0.022	0.033	0.000	0.175
5	0.000	0.000	0.130	0.000	0.000	0.000
6	0.000	0.019	0.000	0.000	0.000	0.000
9	0.368	0.115	0.065	0.433	0.375	0.200
Lut902	(20)	(28)	(23)	(15)	(8)	(20)
1	0.075	0.036	0.000	0.000	0.000	0.000
2	0.050	0.071	0.000	0.000	0.000	0.000
3	0.025	0.143	0.000	0.000	0.000	0.000
4	0.000	0.071	0.044	0.100	0.375	0.000
5	0.025	0.089	0.391	0.367	0.375	0.025
6	0.000	0.018	0.044	0.033	0.000	0.350
9	0.325	0.161	0.239	0.133	0.000	0.350
10	0.500	0.393	0.261	0.233	0.250	0.175
11	0.000	0.018	0.022	0.133	0.000	0.100

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