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Original Investigation

Genetic analysis of Eurasian otters (*Lutra lutra*) reveals high admixture in Finland and pronounced differentiation in Sweden

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ABSTRACT

A number of mammal species in Europe, including the Eurasian otter (*Lutra lutra*), have experienced a decline in population size in the 20th century due to persecution, environmental pollution and ongoing habitat fragmentation. This has often led to a substantial loss of genetic diversity which may threaten population viability. While otters have been studied in some detail genetically, the northern part of the Fennoscandian range has not been covered well so far. By explicitly focussing on the genetics of otter populations from northern Sweden and Finland we aimed at closing that gap. To infer their genetic structure and diversity, we analysed sequences of the mitochondrial control region and 12 nuclear microsatellite markers in 197 Eurasian otters from Sweden and Finland. Variability of the mitochondrial control region was low overall but still revealed previously undetected haplotypes unique to the Finnish otter population. Expected heterozygosities in Fennoscandia were within the range previously reported.

Bayesian cluster analysis of our microsatellite data revealed genetic structuring of the Swedish otter populations. In contrast, we observed a high degree of admixture among the Finnish populations that we also found at the geographic border of the two countries (Lapland).

Admixed ancestry in Finnish otters suggests that gene flow from the Swedish to Central European populations is potentially facilitated via the Finnish otter populations connecting the Swedish animals with otter populations in mainland Europe.

Introduction

One key feature to ensure population viability is to maintain genetic variability within populations, this is most efficiently done by ensuring migration, which will lead to genetic exchange between populations and thus contribute to their variability (e.g. Frankham et al. 2004; Allendorf and Luikart 2007). In turn, habitat fragmentation due to spatially disconnected suitable territories or migration barriers (e.g. motorways) can have detrimental effects on genetic variability. In the worst case, the concomitant high levels of genetic drift and local inbreeding can result in genetic depletion and inbreeding depression (Zachos et al. 2007; Johnson et al. 2010). It is therefore necessary to evaluate both the genetic variability and the degree of differentiation and connectivity among populations to determine the current status and future potential viability of a species.

A number of mammalian carnivore species across Europe (e.g. *Mustela lutreola*: Maran et al. 2011; *Vormela peregusna*: Tikhonov et al. 2008; *Lynx pardinus* and *Lynx lynx*: Von Arx and Breitenmoser-Wursten 2008; Schmidt et al. 2011; *Canis lupus*: Randi 2011; *Ursus arctos*: Swenson et al. 2011) have suffered from a decline in population size. Among them is the Eurasian otter (*Lutra lutra*), a species widely distributed across the Palaearctic and reaching also the Asian Tropics. It is particularly vulnerable to anthropogenic influences such as water pollution (Olsson and Sandegren 1991) but also to other human activities, for example fishing with fyke or drift nets (Ruiz-Olmo et al. 2008). As a result, local extinctions or severe declines in population size have been documented until the 1980s (Randi et al. 2003; Elmeros et al. 2006; Stanton et al.

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2009). Fortunately, very recently numbers have increased again due to successful conservation measurements such as improved habitat quality or captive breeding programmes (Ferrando et al. 2008; Stanton et al. 2009; Koelewijn et al. 2010; Honnen et al. 2011; Roos et al. 2012). A decade ago, the species status was evaluated as "Vulnerable" in the IUCN Red List of Threatened Species, but since a re-evaluation in 2004 the otter has been classified as "Near Threatened" (Ruiz-Olmo et al., 2008). Although there is no imminent extinction risk, conservation of the species is still an issue, and regional restocking programmes have been carried out to help the species recolonise parts of their former distribution range, e.g. in the Netherlands (Koelewijn et al. 2010), Spain (Ferrando et al. 2008) and England (Stanton et al. 2009), but also in Sweden where 47 otters from Northern Norway and seven captive-bred otters were released in two regions of central Sweden (Uppsala län, seven individuals; Södermanlands län, 47 individuals) between 1987 and 1992 (Sjöåsen, 1996). Within its distribution range, the Eurasian otter is a well-studied flagship species (e.g. Cassens et al. 2000; Pertoldi et al. 2001; Mucci et al., 2004, 2010; Kalz et al. 2006; Lanszki et al. 2010). The most detailed genetic study to date covered large parts of Europe also considering the Fennoscandian populations (Mucci et al., 2010). Yet, particularly the northern parts of Finland and Sweden lacked comprehensive sampling for genetic analyses in that study.

Lapland is a cultural region that covers the Northern parts of Sweden and Finland and is the connecting area between Scandinavia and the European mainland. It is mostly located north of the Arctic Circle with short summers, while winters are long and dark (restricting the growing and breeding seasons) with continuous ice cover of water bodies. This causes seasonally scarce food supply, especially for species relying on aquatic prey (Sulkava et al. 2007). A number of generalist species are not able to expand their distribution range into this area (e.g. Wild boar and Red deer). The White-tailed Sea Eagle (*Haliaeetus albicilla*), another predator largely dependent on aquatic prey, cannot sustain large population sizes in this environment (Cederberg et al. 2003). In this bird species, a pronounced genetic differentiation among Finnish populations has been found (Ponnikas et al. 2013). The distribution of the Eurasian otter stretches into this area. Considering that Lapland connects Scandinavia with the European mainland via Russia and that the harsh environment may keep population sizes small – Central Finland: 52 individuals (study range: 1650 km^2, Sulkava et al. 2007); Finnish Lapland: 450–500 individuals (study range: 95,000 km^2, Sulkava and Sulkava, 2009) – one would expect low genetic variability and a pronounced population differentiation. Furthermore, ongoing habitat fragmentation due to road construction and forest management could strongly influence the dispersal of otters and thereby hinder gene flow resulting in decreased variability and increased differentiation, thus reducing the viability of the species in Northern Europe. Contrary to these expectations Mucci et al. (2010) only found three large genetic clusters in Fennoscandia: (i) south-west Norway; (ii) north and central Norway/central and southern Sweden; and (iii) northern Sweden and Finland. However, particularly the last grouping was not represented well in their study, with only few samples from northern Sweden and none at all from northern Finland.

In the present study, we therefore analysed 197 Eurasian otters sampled throughout Sweden and Finland with an explicit focus on the Lapland region (northern Sweden and northern Finland). Samples were analysed by means of sequencing a fragment of the mitochondrial control region and genotyping the specimens at 12 nuclear microsatellite loci. In particular, we wanted to test whether Swedish and Finnish otters exhibit as little substructuring as indicated by previous results based on limited geographic coverage (Mucci et al., 2010) or whether the genetic pattern in otters from this northern part of the distribution range is more complex than previously thought.

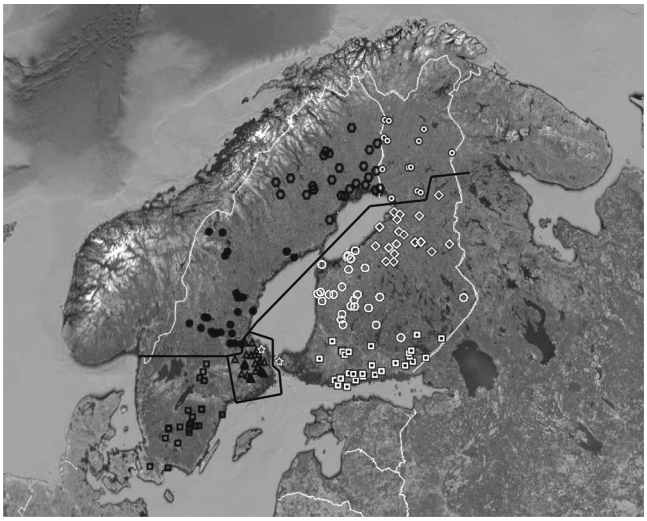

Fig. 1. Map of sampling locations. Black open squares (SSW, $N = 29$), open triangles (CESW, $N = 30$), dots (CWSW, $N = 28$) open hexagons (NSW, $N = 22$) represent individuals sampled In Sweden. White open squares (SF, $N = 25$), donuts (CF, $N = 32$), diamonds (NF, $N = 18$), dots (LF, $N = 13$) denote Finnish individuals. The two stars represent the island specimen from Gräsö and Åland. Black lines denote clusters detected by the Structure programme ($K = 4$).

Material and methods

Sampling

Otters found dead as by-catches in fishing gear or on roads were collected (2001 to 2010 in Sweden, $n = 107$; 1983 to 2009 in Finland, $n = 90$) and frozen. Two samples from islands in the Baltic Sea between Sweden and Finland (Gräsö and Åland) were also included to infer their origin. Sample locations are given in Fig. 1. An approximately 1×1 cm piece of muscle tissue was dissected and preserved in 70% ethanol for genetic analyses. DNA was extracted with the Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) according to the manufacturer's protocol.

MtDNA analysis

The mitochondrial control region was amplified using the primer pair DLH (5′-CCTGAAGTAAGAACCAGATG-3′; Tiedemann et al., 1996) and ProL (5′-CACCACCAACACCCAAGCT-3′; Kocher et al., 1989) and following PCR conditions described in Cassens et al. (2000), with a reduced annealing temperature of 53 °C. The PCR products were sequenced on an automated sequencer (3730xl DNA Analyzer, Applied Biosystems, Carlsbad, CA, USA). Electropherograms were corrected visually and aligned using BioEdit version 7.8.9.0 (Hall, 1999). The data set was collapsed into haplotypes with the FaBOX package (Villesen, 2007; http://www.birc.au.dk/fabox/). Amplification and sequencing were repeated in cases of ambiguous results.

We obtained a fragment of 345 bp for 181 individuals. To integrate the detected haplotypes into the documented diversity of the control region of the Eurasian otter, 13 haplotype sequences (Lut1–Lut13, accession no.: AJ006174–78, EU294255, EU294258, FJ971618–22, HQ113947) were downloaded from GenBank. Sequences were aligned and then cut to equal length (255 bp) which allowed us to also include two additional individuals from the Fennoscandian data set (total number of individuals then 183). A median-joining network, as implemented in the Network

programme (Bandelt et al. 1999), was calculated with this data set. To obtain a general overview, a haplotype frequency of one was used for the sequences from GenBank, whereas the remaining frequencies are reported as they were found in our original data set. Haplotype and nucleotide diversities were calculated using DnaSP 5.1 software (Librado and Rozas, 2009). Since mtDNA variation was very low (see Results), we did not perform any further analyses on our sequence data.

Microsatellite analysis

Genotyping of the specimens was done at 12 nuclear microsatellite loci (Lut435, Lut457, Lut604, Lut615, Lut701, Lut715, Lut717, Lut733, Lut782, Lut818, Lut832 and Lut833, Dallas and Piertney 1998) using the following PCR conditions: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation (94 °C, 1:30 min), annealing (54–58 °C, 1:15 min) and elongation (72 °C, 1:30 min), and terminal elongation at 72 °C for 10 min. Genotyping was performed with an automated sequencer (MegaBACE™1000, GE-Healthcare Bio-Sciences AB, Uppsala, Sweden), and fragment length was determined with the software GENETIC PROFILER 2.0 (Amersham Biosciences, GE-Healthcare, Uppsala, Sweden). The microsatellite data were tested for genotyping errors (stutter bands, null alleles, large-allele dropout) using the Micro-Checker software (van Oosterhout et al., 2004). A test of linkage disequilibrium between each pair of loci was performed with GenePop (Raymond and Rousset 1995). Expected and observed heterozygosities as well as deviations from Hardy–Weinberg equilibrium were calculated using Arlequin version 3.11 (Excoffier et al. 2005). Allelic richness, a measure of allelic diversity corrected for different sample sizes and calculated with a rarefaction approach, was determined with FStat version 2.9.3.2. (Goudet 1995).

Preliminary analyses suggested a further population substructuring in the microsatellite data set. Therefore we divided, in a first approach, the Swedish and Finnish otter populations into subgroups according to their geographic origin (see Fig. 1). We calculated pairwise F_{ST}-values (based on allele frequencies) and R_{ST} values (based on differences in allele length) with the Arlequin software for these groups.

To determine the number of genetic subpopulations, we ran analyses with the STRUCTURE software (Pritchard et al. 2000) for different parameter sets. This Bayesian approach groups individuals based on their genotypes into K clusters under the assumption that these clusters meet the conditions of Hardy–Weinberg and linkage equilibria. Furthermore, we allowed for admixture and correlated allele frequencies (Falush et al. 2003). In a first step, no prior information on the individuals' origin was given. We tested our data for $K = 1$ to $K = 10$ with ten iterations per K. The burn-in period was set at 100,000 steps in the Markov Chain Monte-Carlo procedure followed by 500,000 replications. We then repeated the analysis with the same parameters but specified a potential source population. Last we used the locprior model implemented in the STRUCTURE software. While the 'model with prior population information' embedded in STRUCTURE assumes that the probability for an individual's assignment to a cluster may vary among populations, it still relies on highly informative (near exact) location information. The locprior model is placing more weight on clustering outcomes correlated with location information and is thus more suitable for data with few individuals and loci or not enough divergence (Hubisz et al. 2009). For each of these runs, the most probable number of K was also evaluated with the ad-hoc statistic ΔK (Evanno et al. 2005) calculated as,

$$\Delta K = \frac{\left(\left|mL(K+1) - 2mL(K) + mL(K-1)\right|\right)}{sdL(K)}$$

where $L(K)$ is the natural logarithm of the probability that K is the correct number of clusters, m is the mean and SD is the standard deviation of the replicate runs for the same K value. An Analysis of Molecular Variance (AMOVA), based on F-statistics implemented in the Arlequin software, was done with a data set of groups based on their geographic origin (eight populations) and compared to the K groups inferred with the STRUCTURE program.

To test for a pattern of isolation by distance (IBD) we performed Mantel tests on matrices of genetic and geographic distances based on the STRUCTURE results (see below). We followed two approaches: (i) among-group IBD was tested for with IBDWS (Jensen et al. 2005; http://ibdws.sdsu.edu/~ibdws/); (ii) IBD among individuals within groups was calculated with GenAlEx (Peakall and Smouse, 2012; http://biology.anu.edu.au/GenAlEx/Welcome.html).

For among-group IBD, we first calculated the arithmetic mean of the coordinates of all individuals within each group, thus accounting for uneven distribution of samples within the geographic ranges of the groups. The resulting four locations were used to measure the minimum overland Euclidean distances between each pair using Google Earth. We then used linearised F_{ST}-values calculated as $F_{ST}/(1 - F_{ST})$ and logarithmic geographic distance matrices for the Mantel test implemented in IBDWS. Individual-based IBD calculations within groups were carried out following the recommendations of the GenAlEx manual as Mantel tests on matrices of linearised genetic vs. logarithmic geographic distances.

As a last approach assignment tests were conducted using the GeneClass2 program (version 2.0). The tests were run as follows: according to the original question the individuals from (i) Lapland (LF) and the island individuals from Gräsö and Åland were assigned, and the two stocked populations (ii) Central Eastern (CESW) and (iii) Southern Sweden (SSW) were assigned to a data set comprising all other groups. We did this by means of two different approaches: (i) allele-frequency based (assignment computation following Paetkau et al. 1995, probability criterion: Paetkau et al. 2004) and (ii) Bayesian (assignment: Rannala and Mountain 1997, probability criterion: Paetkau et al. 2004).

Results

MtDNA

We obtained an alignment of a total of 181 (183) D-loop sequences of the mitochondrial genome for 345 bp (255 bp) from specimens collected across Finland, $n = 81$, and Sweden, $n = 100$ (102).

Although fewer samples were examined for Finland, haplotype (HD) and nucleotide diversities (π) were higher in Finnish than in Swedish otters (Table 1).

We found six haplotypes in our dataset. Haplotype Lut1 (96 individuals from Sweden, 70 individuals from Finland) was the most common (91%) (Fig. 2). Haplotypes Lut3 (five individuals) and Lut13 (one individual) were exclusive to Sweden, while Lut4 (one individual), Lut14 (nine individuals) and Lut15 (one individual) were only detected in Finland. Haplotypes Lut14 and Lut15 are described here for the first time (GenBank: KC823048 and KC823049), whereas all other haplotypes have been found before (Cassens et al. 2000 (Lut1-Lut5), Stanton et al. 2009 (Lut6 and Lut7), Finnegan and Néill 2010 (Lut8–Lut12), Honnen et al. 2011 (Lut13)). All haplotypes found in the present study differed from the ancestral Lut1 by only a single mutational step producing a star-like phylogeny in the median-joining network (Fig. 2).

Table 1
Sample sizes and variability parameters of Fennoscandian otter populations. *N*, sample size for microsatellite and, in parentheses, mtDNA analyses; H_O and H_E, observed and expected heterozygosities; *A*, number of alleles; A_R, allelic richness (based on 28 individuals); A_{HT}, number of haplotypes found; HD, haplotype diversity; π, nucleotide diversity. (Group 1: NSW = Northern Sweden, CWSW = Central Western Sweden, LF = Lapland (Finland); Group 2: CESW = Central Eastern Sweden; Group 3: SSW = Southern Sweden; Group 4: NF = Northern Finland, CF = Central Finland and SF = Southern Finland, [number of individuals per group]).

	Sweden												Finland			
N	109 (102)												88 (81)			
A_{HT}	3												4			
HD	0.115												0.224			
Π [%]	0.033												0.06			
	Group 1				Group 2				Group 3				Group 4			
Locus	*A*	A_R	H_O	H_E	*A*	A_R	H_O	H_E	*A*	A_R	H_O	H_E	*A*	A_R	H_O	H_E
Lut435	10	8.88	0.84	0.85	5	4.93	0.60	0.66	6	5.93	0.21	0.29	9	7.67	0.73	0.76
Lut457	9	8.07	0.70	0.70	10	9.86	0.87	0.83	8	7.86	0.52	0.64	9	8.05	0.64	0.77
Lut604	7	6.33	0.68	0.66	5	4.93	0.80	0.68	5	4.93	0.21	0.20	7	6.35	0.57	0.68
Lut615	10	9.85	0.84	0.88	9	8.87	0.83	0.84	9	8.93	0.55	0.82	9	8.30	0.83	0.84
Lut701	6	5.78	0.68	0.76	5	5.00	0.73	0.75	6	5.93	0.69	0.65	7	6.15	0.65	0.69
Lut715	11	9.01	0.71	0.79	3	3.00	0.57	0.58	6	6.00	0.68	0.77	7	5.34	0.44	0.53
Lut717	8	6.99	0.68	0.81	4	3.97	0.48	0.57	4	3.97	0.21	0.28	6	5.23	0.64	0.63
Lut733	10	8.29	0.67	0.78	3	3.00	0.70	0.58	3	3.00	0.31	0.28	6	4.89	0.64	0.67
Lut782	6	5.40	0.47	0.67	3	3.00	0.70	0.59	4	4.00	0.32	0.54	7	5.89	0.63	0.70
Lut818	7	5.75	0.65	0.68	3	3.00	0.50	0.54	5	5.00	0.62	0.58	6	5.13	0.61	0.68
Lut832	8	6.60	0.76	0.75	6	5.93	0.73	0.76	5	5.00	0.62	0.62	5	4.98	0.72	0.68
Lut833	8	7.18	0.77	0.80	4	4.00	0.70	0.61	4	4.00	0.55	0.65	5	3.75	0.67	0.62
Total	100				60				65				83			
Mean	8.33	7.34	0.70	0.76	5.00	4.96	0.68	0.66	5.42	5.38	0.46	0.53	6.92	5.98	0.65	0.69

Microsatellites

One-hundred-and-ninety-seven individuals were genotyped at 12 nuclear microsatellite loci. The Micro-Checker programme detected possible stutter bands at three out of twelve loci (Lut717, Lut733, and Lut782). Careful re-inspection of the electropherograms confirmed their correct scoring. Large-allele dropout was not detected, but potential null-alleles were found at nine out of 12 loci when all individuals were pooled. Since our data set covered a large geographic range and we did not encounter any problems during the PCR, we assumed that the null allele signal was either an artefact due to genetic substructuring or, if null alleles are indeed present, they are so at a frequency that is not likely to distort our results. Linkage disequilibrium was not detected between any pair of loci, and all loci were included in subsequent analyses. Hardy–Weinberg conditions were not met in the overall data set, indicating genetic substructuring (Wahlund effect).

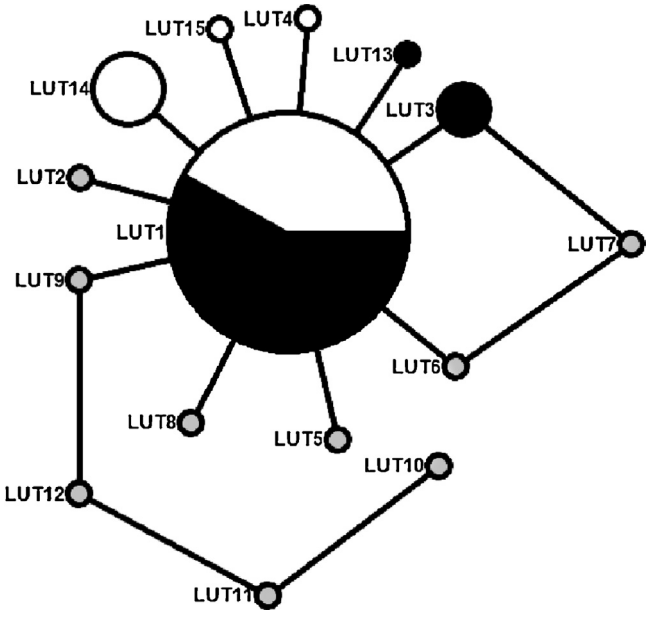

Fig. 2. Median-joining network of the Fennoscandian dataset. Circle size corresponds to haplotype frequency (present data set only); lines between haplotypes denote one mutational step. To give an overview of European haplotype diversity, we added sequences from GenBank to the data set (frequency = 1). Sweden = black, Finland = white, GenBank = grey; Lut1-Lut13 denote the names of the haplotypes as published in GenBank (Accession no. (Lut1–Lut13, accession no.: AJ006174–78, EU294255, EU294258, FJ971618–22, HQ113947), Lut14 and Lut15 are newly discovered haplotypes (GenBank: Lut14 KC823048 and Lut15 KC823049).

When separated into geographic groups (see Fig. 1), mean observed and expected heterozygosities were highest in the Northern part of Sweden and Finland (NSW and NF, respectively) and lowest in Southern Sweden (SSW). An increase of allele numbers and allelic richness from South to North was visible (Supplementary Table S1). The groups resulting from the STRUCTURE analysis (see below) roughly followed this pattern with group 1 (NSW, CWSW, LF) displaying the highest values for mean observed and expected heterozygosities, allele number and allelic richness (Table 1). A southward decline was visible in groups 2 (CESW) and 3 (SSW) (Table 1). In the large group 4 (NF, CF, SF), comprising only individuals from Finland, expected and observed heterozygosities were comparable to group 2, but the number of alleles and allelic richness were higher (Table 1).

In the pairwise comparisons based on geographic groups all Swedish groups were significantly different from each other, whereas the Finnish populations were not except for the northernmost (Lapland LF) (Table S2). This result is also reflected by R_{ST} values except that the significant difference between LF and NSW disappears (Table S2).

The STRUCTURE software yielded $K = 4$ clusters to be the most likely scenario for a sub-structuring of the Finnish and Swedish otter populations (Fig. 3). This was confirmed by calculating the ad-hoc statistic ΔK. The clusters consisted of (i) a group comprising the Northern (NSW) and Central Western Swedish (CWSW) otters together with otters from Finnish Lapland (LF) (group 1); (ii) Central Eastern Swedish otters (CESW, group 2); (iii) Southern Swedish otters (SSW, group 3); and (iv) the remaining Finnish otters (NF, CF, SF, group 4) some of which, however, displayed admixed ancestry (Fig. 3). The fixation index F_{ST} was 0.075 ($p < 0.01$; $R_{ST} = 0.082$, $p < 0.01$), indicating that 7.5% (8.2%, respectively) of the overall genetic diversity was due to differentiation among the four groups detected by STRUCTURE as opposed to diversity within

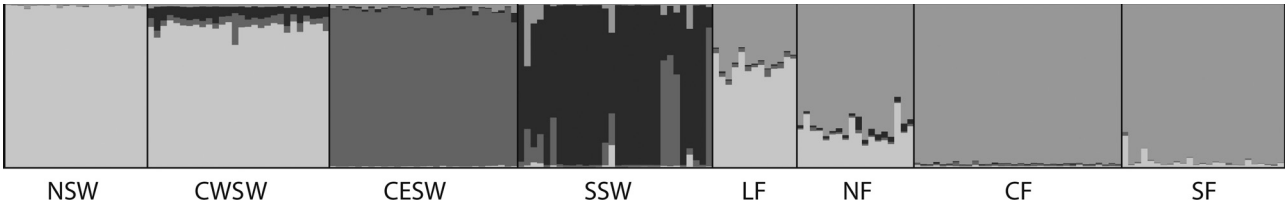

Fig. 3. (A) Bar plots showing the results including prior information on the individuals' geographic origin. (B) Bar plots based on the results of the STRUCTURE software with prior information on the individual's origin and the locprior function. Each individual is represented by a vertical bar which is subdivided according to the percentage of assignment to the $K = 4$ different genetic clusters.

Table 2
Pair wise comparisons of F_{ST} (No. of different alleles, below diagonal) and R_{ST} (sum of squared size difference, above diagonal) values based on groups supported by STRUCTURE analysis. Group 1 (NSW = Northern Sweden, CWSW = Central Western Sweden, LF = Finnish Lapland), group 2 (CESW = Central Eastern Sweden), group 3 (SSW = Southern Sweden), group 4 (NF = Northern Finland, CF = Central Finland, SF = Southern Finland) significant differentiation after Bonferroni correction is indicated in bold.

	Group 1	Group 2	Group 3	Group 4
Group 1	–	**0.09664**	**0.09358**	**0.06011**
Group 2	**0.08765**	–	**0.16724**	**0.13971**
Group 3	**0.10777**	**0.16696**	–	**0.12869**
Group 4	**0.05569**	**0.09807**	**0.11675**	–

them. Pairwise F_{ST} values between the four groups were all statistically significant (Table 2).

Fixation indices for six groups (without the previously stocked Swedish populations) were $F_{ST} = 0.056$ and $R_{ST} = 0.061$ and for all eight sampled populations $F_{ST} = 0.090$ and $R_{ST} = 0.098$, (for all AMOVAS $p < 0.0001$). There was no IBD pattern among the four STRUCTURE groups ($p = 0.701$, $R^2 = 0.178$). Among individuals within these groups, however, IBD was weak but statistically significant in all but group 2 (Central Eastern Sweden, Table 3).

The probability to be assigned to one of the eight populations for the Lapland samples was overall low. Only few samples displayed a probability of more than 50% of belonging to a particular population (with both approaches used), most notably to NSW followed by NF (Table S3). One individual was not assigned to any of the populations (F89, 0% assignment probability to any of the groups tested with both approaches), suggesting that this individual is a migrant (or a descendant of migrants), possibly from Northern Norway. The Gräsö individual was assigned to the closest mainland population (CESW) but only with a very low probability of 4% (Table S3). The individual sampled from Åland showed highest probability to belong to one of the Finnish populations. Since the populations CESW and SSW were subject to re-stocking in the past, assignment tests were undertaken to assess whether the stocking has left a genetic signal in these populations. The majority of the individuals comprising the CESW group were not assigned to any of the other groups (Table S4). However, two individuals were found to be assigned to NF (frequency-based: 57%, Bayesian: 47%) and LF (frequency-based: 65%, Bayesian: 71%), respectively (Table S4). Tests carried out on the SSW otters revealed high probabilities for the majority of individuals to be assigned to the CWSW population. In contrast, no individual was assigned to CESW. Interestingly, two individuals from Småland (SSW) were assigned with high probabilities to all Finnish populations using both assignment criteria (Table S5).

Table 3
Isolation by distance for individuals within the four STRUCTURE groups. Significant values are bold. For geographic groups, see Table 2 (Rxy is the correlation coefficient for the geographic and genetic data matrices, significant values in bold).

		n	Rxy	P (rxy-rand ≥ rxy-data)	R^2
Group 1	LF NSW CWSW	61	**0.196**	0.001	0.038
Group 2	CESW	30	−0.035	0.319	0.001
Group 3	SSW	22	**0.395**	0.001	0.156
Group 4	NF CF SF	75	**0.127**	0.001	0.016

Discussion

In line with our expectations and contrary to Mucci et al. (2010) we found further substructuring among the Swedish and Finnish otters, indicating that the large genetic cluster found by Mucci et al. (2010) covering parts of Sweden and the whole Finnish population was an artefact due to incomplete sampling, particularly in Lapland. In accordance with Mucci et al. (2010) and earlier findings, our mtDNA data revealed low variability and no phylogeographic structuring. Nevertheless, we found two previously undescribed haplotypes in the Finnish otter population (one of them in no less than nine individuals), further evidence of the insufficient coverage of this population in previous studies. These two haplotypes fit into the known star-like network pattern which suggests a single glacial refugium from which Europe was recolonised in the Holocene (Sommer and Benecke 2004; Mucci et al., 2010). Variability in the nuclear genome was much higher and increased from South to North. Testing for substructuring of the data set revealed a clear differentiation of the Swedish otter populations from each other and from the Finnish ones. No structuring was found among the Finnish populations south of Lapland, indicating a high degree of admixture throughout most of Finland which suggests that migration between geographically distant groups is occurring frequently.

Population numbers in Sweden declined dramatically in the past leaving behind isolated subpopulations with relatively few breeding individuals (Roos et al. 2001). In Finland, the decline was not as pronounced and mainly locally restricted to coastal regions and archipelagos (Stjernberg and Hagner-Wahlsten 1994), which probably explains the lower extent of differentiation. The Åland islands, an extensive archipelago consisting of a main island surrounded by thousands of islands and islets off the southwestern coast of Finland, suffered complete extinction but have recently been recolonised (T. Stjernberg, unpublished data). They are located roughly halfway between the two countries' coastlines, yielding two possible migration ways for otters: (i) from Sweden via the winter ice cover (~25 km distance) or (ii) from Finland via "island-hopping" in the archipelago. Our data set contained two island individuals, one from the Western part of Åland and one from Gräsö (Uppland, Sweden). Our results clearly indicate that Gräsö has been recolonised from the Swedish mainland but the Åland individual tells a somewhat more ambiguous story. Its genetic make-up shows high genetic contributions from all over Finland and, to some degree, even from the northernmost Swedish population. Although it is possible for an otter to cover large distances (e.g. , p. 60), it is much more likely that Åland has been recolonised from the Finnish

side. This is also reflected by the large portion of admixed Finnish ancestry in this individual's genome.

The genetic diversities of the present study (as measured by heterozygosity) ranged from 0.46–0.72 (H_O) to 0.53–0.76 (H_E), respectively (Table 1 and Table S1). This is within the range previously reported for European otter populations: Mucci et al. (2010), based on a set of 11 microsatellites (nine of which were also included in our panel of 12 loci), reported values between 0.35 and 0.69 for H_O and between 0.37 and 0.71 for H_E from otter populations in 17 European countries. The highest values in their study were found in Sweden and Finland, the lowest for Italy, Denmark and England. Very low diversity for the isolated and bottlenecked Danish otters was also reported by Honnen et al. (2011).

The increase in genetic variability in the nuclear genome from South to North in our study area reflects the geographic characteristics of Finland and Sweden. The two countries are in large parts separated by the Baltic Sea; therefore, admixture between the populations of these two countries can only occur in the far North, i.e. in Lapland. Here, a connection of the Norwegian and Finnish and of the Swedish with the Russian populations is possible, which allows mixing of their respective gene pools.

Within Sweden, the populations displayed significant genetic differentiation which reflected the geographic regions sampled. Gene flow among these populations is limited, although some individuals with mixed ancestry were found. This structuring is also shown by the F_{ST} and R_{ST} values calculated for the groups based on geographical origin (Table S2). All Swedish geographic groups differed significantly from each other and from the Finnish. The aforementioned decline in numbers of the Swedish otter population is the likely cause for this pattern of differentiation. Additionally, there was a restocking programme in Sweden in the late 1980s and early 1990s (Sjöåsen 1996; Arrendal et al. 2004). The releases took place in two locations: in Central Sweden (seven otters in Uppsala län, CESW) and in Södermanlands län (47 otters, Southern Sweden). A genetic contribution to the local population could only be found in Upland which is possibly an effect of sampling too far away from the release site in southern Sweden (Sjöåsen 1996; Arrendal et al. 2004). We have found that the Southern Swedish population, although a distinct Structure cluster, shows diverse microsatellite ancestry, which is reflected by the possible assignment to a variety of other groups, most notably the geographically closest one (CWSW). In this area, a large number of individuals have been released (wild-caught and captive-bred, cf Sjöåsen, 1996; Arrendal et al. 2004), which is the likely cause for the ambiguous assignment, especially for the two individuals (SM5315 and SM5027, Table S5) seemingly belonging to the Finnish populations. A completely different picture was obtained for the Central Eastern Swedish population. This is clearly a distinct entity with only two individuals (Up5293 and 9UP5028, Table S4) showing some probability of belonging to Finnish populations, suggesting gene flow from other populations in addition to possible influence of the seven stocked individuals.

Within the Finnish data set, the picture is more ambiguous. There was no sign of differentiation between either Lapland and Northern Finland nor between Northern and Central Finland, but all other comparisons among Finnish geographic groups were significant. Despite the fact that forestry and rivers (as transportation ways) are important for the country's economy, habitat fragmentation and shipping traffic did not preclude gene flow. This is further underlined by the fact that Finnish otters did not show a strong structuring into, or differentiation between, geographically defined subpopulations, although geographic distances covered by our dataset were comparable to those in Sweden. Accordingly, we conclude that dispersal capacity and migration propensity in *Lutra lutra* are comparatively higher in Finnish than in Swedish populations.

Especially in Northern Finland admixture is more pronounced. The comparably harsh and long winters might be one key reason for this. Sulkava and Sulkava (2009) reported that otters have to move relatively long distances between ice-free feeding grounds. Consequently, the probability of encounters of genetically different individuals during mating season is enhanced and thus facilitates admixture, creating a bridge between the Northern Swedish and the Finnish populations. Similar migration routes existed in other parts of Europe and have recently been shown to have been re-established in the wake of population recovery and range expansion. Previous work has shown, for instance, that the genetically deprived Danish population has been reconnected, via recent recolonisation of Germany's northernmost federal state Schleswig-Holstein, to the large East-German otter population (Honnen et al. 2011). This has created a new route for genetic exchange between these populations.

With regard to the Europe-wide study of Mucci et al. (2010) we were able to complement the picture on genetic structuring in Fennoscandian otter populations. While we were able to show an enhanced migration propensity in Northern Finnish otter populations, our data also reveal significant differentiation between Finnish Lapland and Northern Swedish otters. Further research is needed which closely monitors the populations in question and identifies migration barriers to implement meaningful management measures. Our results have implications for the viability of the population in Finland as a whole and for adjacent populations. The admixed ancestry prevalent in the Finnish otters suggests that strong gene flow is maintained by high migration activity. High dispersal rates within this population may result in these animals functioning as connecting links for populations of neighbouring countries which underlines their importance in the species' overall management.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mambio.2014.09.005.

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