Contrasting genetic structure of the Eurasian otter (Lutra lutra) across a latitudinal divide

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The Eurasian otter (Lutra lutra) is recovering from well-documented population declines that occurred during the 20th century. Little is known about the genetic impact of these declines in northern Britain and the current understanding of otter genetic structure in Britain is incomplete. This study reexamines genetic structure in Scotland, one of the otter’s major strongholds in the United Kingdom, and combines data with a published microsatellite data set from the remainder of the United Kingdom to produce the 1st comprehensive assessment of genetic structure across the entirety of mainland Britain. We show that there is a remarkable contrast in genetic structure of otters in northern Britain compared to the south. Population fragmentation and high levels of genetic structure were typical of southern Britain, whereas in the north we observed a virtually panmictic population. These results imply very different demographic histories of otters in these 2 regions. Our findings also suggest a more favorable environment for the Eurasian otter in recent times in the north of the United Kingdom, possibly linked to human population density and anthropogenic habitat impacts. This study therefore provides a complete description of population genetics of the Eurasian otter in the United Kingdom, and allows inferences to be made regarding the relative importance of landscape characteristics on the recovery of otter populations.

Key words: declines, fragmentation, microsatellite, population

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DOI: 10.1644/13-MAMM-A-201

The Eurasian otter (Lutra lutra) is known to have undergone substantial demographic declines throughout Europe during the last half of the 20th century (Jefferies 1989). The most significant cause of this decline is usually attributed to water contamination by organochlorine pesticides and polychlorinated biphenyls (Mason 1995; Murk et al. 1998). The magnitude of decline varied between otter populations in Britain such that by the mid-1980s, otters were absent from most of England, but a relatively large population remained in the southwest. They were absent from southern Wales and parts of northern Wales but not from central Wales. There was evidence of a decline in the southern and east-central lowlands of Scotland, but the population remained large in the highlands and islands (MacDonald 1983). In recent years, otter recovery has been documented in several parts of western Eurasia and especially in the United Kingdom (Green and Green 1997; Strachan 2006). The population genetics of Eurasian otters have been investigated previously in Scotland (Dallas et al. 1999); Scotland, mid-Wales, and southwestern England (Dallas et al. 2002); and in England and Wales (excluding northern England and Scotland—Hobbs et al. 2011). The genetic consequences of the decline and structure of contemporary populations have yet to be described jointly throughout the whole of the United Kingdom, making direct comparisons between regions problematic. This information is important, because declines in natural populations may cause population

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isolation and increased Allee effects that include localized inbreeding leading to reduced population viability (Mukai and Yamaguchi 1974; Frankham 1995; Keller and Waller 2002; Nielsen et al. 2012). In addition, it is important to identify admixture (gene flow among previously isolated populations) because, contrary to expectation, admixture sometimes proves detrimental to fitness if populations previously had high degrees of local adaptation (e.g., Edmunds 2007).

Northern Britain presents a heterogeneous environment, containing a high percentage of built-up land in southern Scotland and a high percentage of arable and horticultural land in the southeast and eastern part of northern Scotland. The uplands are under much less intensive agriculture (Countryside Information System 2007). Agricultural land use is associated with many pesticides linked to the decline of otters. Scotland contains the highest mountains in Britain, expected to be difficult environments for otters to penetrate, but also a substantial coastline and inland water system that otters use extensively. Heterogeneity of landscape factors such as these can influence the way in which populations are genetically structured (Manel et al. 2003). The southern part of Britain has already been shown to contain considerable levels of genetic subdivision among otter populations (Stanton et al. 2009; Hobbs et al. 2011). The current genetic subdivision in England corresponds to isolation due to the known demographic history of otter declines. Wales and Scotland both appear to contain contiguous otter populations (Jones and Jones 2004; Strachan 2006), but considerable genetic substructuring has been revealed in Wales (Hobbs et al. 2011). The Scottish otter population therefore also may be highly fragmented, despite maintaining high numbers. The study of Hobbs et al. (2011) also investigated the effect of an otter reintroduction program that occurred over the past 10–20 years in East Anglia and North Yorkshire, and showed that otter dispersal between regional populations has been limited. This suggests that the genetic effect of any reintroduced individuals is likely to have remained limited to those regional populations.

The response of otters to landscape or anthropogenic factors in northern Britain may not be the same as that in the south, in part because otter behavior shows considerable geographic variation. In the western and northern parts of Scotland, otters are widely found in sea lochs and coastal habitats, whereas in much of England and Wales, otters are more typically found in freshwater lakes and rivers (Kruuk 2006). Also, prey resources are different in the north than in the south, with the biomass of key prey species such as Atlantic salmon (Salmo salar) and the European eel (Anguilla anguilla) being considerably higher in Scotland than in England (Malcolm et al. 2010).

The otter populations of Britain therefore provide a means for assessing how different ecological pressures influence genetic structure in this species. This study aimed to analyze otter genetic structure in Scotland using Bayesian clustering methods, and to compare the genetic structure to previous studies elsewhere in the United Kingdom (Hobbs et al. 2011), thereby providing a complete description of genetic structure of the Eurasian otter throughout Britain. We predicted that populations in mainland northern Britain would be highly genetically differentiated, in common with those in southern Britain, due to a combination of physical barriers (i.e., the highlands, islands, and the Great Glen), anthropogenic features (i.e., the urban belt running between Glasgow and Edinburgh), and historic pesticide use in southern Scotland (Jeffries and Hanson 2000). The present study also aimed to investigate potential causes of any genetic structure identified in Scotland. This was done by investigating the relationship between geographic distance and genetic distance of otter samples in Scotland, using an “isolation-by-cost-distance” landscape genetics approach that accounts for putative landscape features that may positively or negatively influence movement in this species (Manel et al. 2003).

**Materials and Methods**

Otter tissue samples were collected from mainland Scotland, from recent roadkills. DNA was extracted from muscle of 103 otters using the Qiagen tissue extraction kit (Qiagen GMBH, Hilden, Germany) following the manufacturer’s instructions (Qiagen 2006). Polymerase chain reaction amplifications were in a 6.5-μl reaction, with 3.5-μl Multiplex Mix (Qiagen), 4 μg of bovine serum albumin (New England Biolabs, Ipswich, Massachusetts), 0.3 pmol of each primer, and approximately 50 ng of DNA. The following cycling conditions were used: 95°C for 15 min; 30 cycles of 94°C for 30 s, 58°C for 90 s, and 72°C for 60 s; and a final extension of 60°C for 30 min. Samples were genotyped using 15 microsatellite markers: Lut 435, 457, 604, 615, 701, 715, 717, 733, 782, 818, 832, 833, and 902 (Dallas and Piertney 1998; Dallas et al. 2002), and 04OT05 and 04OT22 (Huang et al. 2005), using Genemapper version 4.0 (AppliedBiosystems 2006), with > 99% success rate. A published data set of 454 genotypes using the same markers from Welsh and English roadkilled otters also was incorporated (Hobbs et al. 2011). To cross-calibrate the 2 data sets, 20 samples from the study of Hobbs et al. (2011) were regenotyped and compared to the genotypes of this study. The genotypes were identical, except for 2 loci, where there was a 1-base-pair difference between data sets for all alleles, which were subsequently adjusted.

**Bayesian clustering.**—In order to describe the genetic structure of otters in Britain, Bayesian clustering was carried out with all 557 individuals. The approaches implemented in the software GENELAND (Guillot et al. 2005) and STRUCTURE (Pritchard et al. 2000) were used, in each case with 500,000 Markov chain Monte Carlo iterations, a burn-in of 50,000, independent allele frequencies (lambda set to 1.0), an admixture model (alpha inferred, with initial alpha set to 1.0), and K set at 1–10. The STRUCTURE analysis also was repeated, using the same settings, except with correlated allele frequencies. The number of genetic clusters was determined using the method of Evanno et al. (2005). A spatial model was used for GENELAND but not for STRUCTURE. Genetic clusters identified by GENELAND will hereafter be referred to as “groups.” Bayesian clustering was then repeated once on
each of the genetic groups identified by GENELAND, but with $K$ fixed at 2. This was carried out to designate hierarchical levels of population clustering in our samples for use in other analyses. Each of these additionally bifurcated genetic clusters will hereafter be referred to as “subpopulations.” This designation of hierarchical groups was only carried out using GENELAND to ensure that subpopulation designations remained consistent for subsequent analyses.

Progressive partitioning.—Progressive partitioning (Hobbs et al. 2011) was then carried out on all 557 samples to identify the strongest genetic partitions in mainland Britain. Progressive partitioning uses Bayesian clustering to examine the distribution of genotypes at $K = 2$ clusters successively to a higher level of structuring than is identified using traditional Bayesian clustering methodologies. This was carried out in the programs GENELAND and STRUCTURE with correlated allele frequencies in both cases. The other parameters were the same as for the GENELAND and STRUCTURE analysis described above.

Genetic variation and partitioning.—Pairwise $F$-statistics (genetic differentiation between a given sample grouping, relative to a 2nd, more-inclusive sample grouping—Wright 1951) was estimated between different combinations of the following sets of hierarchical grouping: all individuals (i.e., the total [T]), the groups (G) identified by GENELAND, the subpopulations (S) identified by the bifurcation of these genetic groups, and individuals (I). $F_{IS}$ (individuals relative to subpopulations) was estimated for each of the subpopulations separately. $F$-statistics were calculated between each of the hierarchical levels of genetic structure separately for the northern (Scotland and northern England) and southern (Wales, eastern England, and southwestern England) populations as follows: $F_{GT}$ (groups relative to total), $F_{ST}$ (subpopulations relative to total), $F_{IS}$ (individuals relative to subpopulations), and $F_{IT}$ (individuals relative to total). All $F$-statistics were calculated in Arlequin version 3.5 (Excoffier and Lischer 2010). Two analyses of molecular variance (AMOVAs) were carried out on all samples, using Arlequin version 3.5 (Excoffier and Lischer 2010), once each for the northern and southern populations. Percentage of variation was estimated among groups, between subpopulations within groups, among individuals within subpopulations, and within individuals.

An isolation-by-cost-distance analytical approach was carried out, using Mantel tests to investigate the correlation between genetic and cost-distance, over a number of cost grids generated for Scotland. This analysis used 63 samples identified as the Scottish group by GENELAND. Seven sets of cost grids were created based on the landscape features shown in Supporting Information S1 (DOI: 10.1644/13-MAMM-A-201.S1). Data for the cost grids was obtained from the Countryside Information System (2007) and from the Environmental Research Institute (Malcolm et al. 2010). Cost grids investigated the following features: altitude, urbanized areas, arable and horticultural land, main salmon rivers, main roads, coastal areas, and special areas of conservation. These cost grids are shown in Supporting Information S1, with a description of each one.

Cost grids were created as follows: The feature in question (dark shade in Supporting Information S1) was assigned a range of cost multipliers to movement using ARCGIS version 8.3 (ESRI 2003). These multipliers ranged from 0.01 to 100 multiples of the cost associated with $1 \times$ the prevalence of the barrier or facilitator estimated for each cell (light shade in Supporting Information S1). A cost value of $< 1$ implies facilitation of gene flow, whereas $> 1$ implies restriction of gene flow, with a value of 1 implying simple isolation by Euclidean distance (assuming open sea to be a complete barrier to gene flow). Seven cost grids were created for each of the 7 features (total of 49 grids). Open sea was given a default cost of 1,000× background (except for the “coastal” grid, where it varied between a cost of 0.01 and 100 to investigate sea as a feature itself). Data matrices were then created from cost distances between all individuals, for all of the cost grids, using the program PATHMATRIX (Ray 2005). Correlation coefficients were calculated for each of these matrices against a matrix of pairwise genetic distances for all 65 samples (calculated in GenAleX—Peakall and Smouse 2012), using R (R Development Core Team 2011). For each of the landscape features, the geographic distance matrix for the cost grid with the highest correlation coefficient was used in a partial Mantel test against genetic distance (Peakall and Smouse 2012), with Euclidean distance (cost value equal to 1) partialed out.

**RESULTS**

Bayesian clustering.—GENELAND analysis identified 5 clusters with the highest likelihood for British otter samples (Fig. 1). These were located in southwestern England, Wales, eastern England, northern England, and mainland Scotland. STRUCTURE identified only 2 clusters when using independent allele frequencies, but also identified 5 when using correlated allele frequencies (Supporting Information S2, DOI: 10.1644/13-MAMM-A-201.S2). With $K = 5$, clustering results for both GENELAND and STRUCTURE were similar, except STRUCTURE split the Welsh group into 2 clusters and kept the northern England and Scottish group as a single cluster, whereas GENELAND did the opposite. Each of the 5 GENELAND clusters was successfully split into $K = 2$ using GENELAND (Fig. 1). Notably, the Scottish mainland group was split into 2 subpopulations on either side of the Great Glen. Each of the subpopulations is hereafter referred to by the group name with a suffix of either A or B (shown in Fig. 1).

Progressive partitioning.—Progressive partitioning assumes that clusters with the strongest genetic differentiation will separate first, with weaker partitions being detected subsequently (Hobbs et al. 2011). A separate analysis was therefore carried out using progressive partitioning on the full data set ($n = 557$), to identify the relative strength of genetic structuring in Britain. Using both GENELAND and STRUCTURE, the 1st partition split the Welsh and southwestern England cluster from the rest of Britain. The
2nd partition split southwestern England from the Welsh cluster, and eastern England from northern England and Scotland.

*Genetic variation and partitioning.*—Pairwise $F_{GT}$-values (Table 1) were high between most groups, with 40% above 0.2 and 90% above 0.1. The exception to this was between Scotland and northern England ($F_{GT} = 0.054$), which was 49% smaller than the next lowest $F_{GT}$-value (northern England versus eastern England; $F_{GT} = 0.104$). Mean pairwise $F_{GT}$-values between groups ranged from 0.226 for southwestern England to 0.135 for Scotland. Between-subpopulation pairwise $F_{ST}$-values (Table 2) ranged between 0.342 (southwestern England [B] and Wales [A]) and 0.022 (Scotland [A] and Scotland [B]). $F_{ST}$-values between
subpopulations that were not in the same group ranged between 0.342 (southwestern England [B] and Wales [A]) and 0.063 (northern England [A] and Scotland [B]). All $F_{ST}$-values were significantly different from 0 ($P < 0.001$). Overall $F_{ST}$-values were relative to total) values were low for the northern groups (Scotland and northern England) at 0.032, and high for the southern groups (Wales, eastern England, and southwestern England) at 0.217. Overall $F_{IT}$ was 0.066 for the northern subpopulations and 0.030 for the southern subpopulations. $F_{IT}$ was 0.132 for the northern subpopulations and 0.288 for the southern subpopulations. Wales (A), Wales (B), northern England (A), and Scotland (A) had $F_{IS}$-values significantly greater than 0 ($P < 0.05$; 0.032, 0.045, 0.045, and 0.061, respectively) and Scotland (B) had a highly significant ($P < 0.001$) $F_{IS}$-value (0.134).

Analysis of molecular variance was applied to the data set for comparison with $F$-statistics. For the northern subpopulations, the great majority of genetic variation was partitioned within subpopulations (93.9%), 86.8% of which was found within individuals, and 6.1% among individuals. Relatively little was explained among subpopulations within groups (4.0%) and among groups (3.2%). In contrast, for the southern subpopulations, a smaller proportion of the genetic variation was explained within subpopulations (73.4%, of which only 2.2% was explained among individuals within subpopulations), with much more explained among groups (21.8%); 4.9% was explained among subpopulations within groups (Tables 3 and 4). The AMOVA results therefore corroborate what is shown by the $F$-statistics.

The highest correlation coefficient for each of the landscape features investigated in the isolation-by-cost-distance analyses are given in Table 5, and shown graphically in Supporting Information S3 (DOI: 10.1644/13-MAMM-A-201.S3) All of the highest correlation coefficients were significant based on the Mantel tests of genetic distance against cost distance. The highest correlation coefficient of all the comparisons was for the special areas of conservation cost grid ($r = 0.174$). However, partial Mantel tests did not indicate a significant correlation between genetic distance and cost distance when Euclidean distance was partialed out.

**DISCUSSION**

The most striking result of our study is the lack of genetic structure among subpopulations of the Eurasian otter in northern Britain, in stark contrast to the high genetic structure in the south. This is shown by the contrast in the results of Bayesian clustering, $F$-statistic values, and AMOVA between the north and the south. This finding is somewhat unexpected, given the heterogeneous landscape that otters in Scotland occupy, and the history of pesticide use in northern Britain. Also striking is the presence of significant $F_{IS}$-values, and that the subpopulations that have these significant values are the ones that are in the regions with the least genetic structure. Dallas et al. (1999) showed that multiple island and mainland otter populations in Scotland exhibited departure from mutation–drift equilibrium. These departures from mutation–drift equilibrium were found in apparently large continuous otter populations, also true of the subpopulations with significant $F_{IS}$-values in the present study. Dallas et al. (1999) attributed these departures from mutation–drift equilibrium to population bottlenecks, but also acknowledged the possibility of demographic processes, such as local fluctuations in population size, harem mating, or extinction–recolonization events. This may also be the case in the subpopulations with significant $F_{IS}$-values in this study (Wales [A] and northern

**TABLE 1.**—Pairwise $F_{GT}$-values (calculated in ARLEQUIN) of Eurasian otter (*Lutra lutra*) genetic clusters (subpopulations) identified by GENELAND analysis, using samples from throughout mainland Britain. These subpopulations refer to the following locations in Fig. 1: eastern England subpopulations A and B (E_Eng_A and B), Welsh subpopulations A and B (Wales_A and B), southwestern England subpopulations A and B (SW_Eng_A and B), Scottish subpopulations A and B (Scot_A and B), northern England subpopulations A and B (N_Eng_A and B). All $F_{GT}$-values are significant at $P < 0.001$.

<table>
<thead>
<tr>
<th></th>
<th>Eastern England</th>
<th>Wales</th>
<th>Southwestern England</th>
<th>Scotland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wales_A</td>
<td>0.224</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Southwestern England</td>
<td>0.228</td>
<td>0.278</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Scotland_A</td>
<td>0.112</td>
<td>0.170</td>
<td>0.202</td>
<td>—</td>
</tr>
<tr>
<td>Northern England</td>
<td>0.104</td>
<td>0.187</td>
<td>0.197</td>
<td>0.054</td>
</tr>
</tbody>
</table>

**TABLE 2.**—Pairwise $F_{ST}$-values (calculated in ARLEQUIN) of Eurasian otter (*Lutra lutra*) genetic clusters (subpopulations) identified by GENELAND analysis, using samples from throughout mainland Britain. These subpopulations refer to the following locations in Fig. 1: eastern England subpopulations A and B (E_Eng_A and B), Welsh subpopulations A and B (Wales_A and B), southwestern England subpopulations A and B (SW_Eng_A and B), Scottish subpopulations A and B (Scot_A and B), northern England subpopulations A and B (N_Eng_A and B). All $F_{ST}$-values are significant at $P < 0.001$.

<table>
<thead>
<tr>
<th></th>
<th>E_Eng_A</th>
<th>E_Eng_B</th>
<th>Wales_A</th>
<th>Wales_B</th>
<th>SW_Eng_A</th>
<th>SW_Eng_B</th>
<th>Scot_A</th>
<th>Scot_B</th>
<th>N_Eng_A</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_Eng_B</td>
<td>0.073</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Wales_A</td>
<td>0.261</td>
<td>0.265</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Wales_B</td>
<td>0.224</td>
<td>0.226</td>
<td>0.045</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SW_Eng_A</td>
<td>0.267</td>
<td>0.247</td>
<td>0.298</td>
<td>0.288</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SW_Eng_B</td>
<td>0.278</td>
<td>0.260</td>
<td>0.342</td>
<td>0.320</td>
<td>0.145</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Scotland_A</td>
<td>0.151</td>
<td>0.125</td>
<td>0.202</td>
<td>0.181</td>
<td>0.241</td>
<td>0.245</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Scotland_B</td>
<td>0.134</td>
<td>0.106</td>
<td>0.189</td>
<td>0.163</td>
<td>0.218</td>
<td>0.209</td>
<td>0.022</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N_Eng_A</td>
<td>0.145</td>
<td>0.116</td>
<td>0.225</td>
<td>0.195</td>
<td>0.220</td>
<td>0.252</td>
<td>0.085</td>
<td>0.063</td>
<td>—</td>
</tr>
<tr>
<td>N_Eng_B</td>
<td>0.118</td>
<td>0.105</td>
<td>0.193</td>
<td>0.166</td>
<td>0.219</td>
<td>0.234</td>
<td>0.066</td>
<td>0.065</td>
<td>0.062</td>
</tr>
</tbody>
</table>
England [A] [Supporting Information S4, DOI: 10.1644/13-MAMM-A-201.S4]).

Ooters in the north sustained 20th century population declines as in the rest of Britain (Crawford 2010). In fact, populations in Scotland sustained heavy losses during this period. Two areas in Britain particularly suffered from attack by the wheat bulb fly (Leptohylemyia coarctata—Gough et al. 1961), with one of these being southeastern Scotland, and the other being eastern England. It was these areas that were treated most heavily with the insecticides dieldrin and aldrin. In addition, because of the presence of the wheat bulb fly, use of these pesticides continued in these areas until 1975, more than 10 years after a voluntary ban was agreed upon throughout the rest of Britain. Mean dieldrin residues and lethal casualties from dieldrin poisoning were higher in wildlife from these 2 areas (Jefferies and Hanson 2000). These population declines during the 20th century have been hypothesized as the cause for the population genetic structure observed in otters in southern Britain (Hobbs et al. 2011). This led us to hypothesize that otter populations in northern Britain would be highly genetically structured, similar to those in the south. However, the northern otter populations are, genetically, relatively continuous in comparison to the southern populations. The STRUCTURE analysis with correlated allele frequencies (Supporting Information S2 and S5, DOI: 10.1644/13-MAMM-A-201.S5) estimates $K = 5$ genetic clusters and shows the north (northern England and Scotland) effectively forming a single genetic cluster, whereas the south (Wales, eastern England, and southwestern England) is composed of the remaining 4 clusters. This is despite the fact that, in this study, sample coverage by area for the north (87,070 km$^2$) was more than 10% greater than that of the south (77,410 km$^2$) [Supporting Information S6, DOI: 10.1644/13-MAMM-A-201.S6]). The contrast in the genetic structure is corroborated by the results of the progressive partitioning analysis (Hobbs et al. 2011), which initially split the Welsh and southwestern England clusters from the rest of Britain, and then split the Welsh cluster from the southwestern England cluster, and the eastern England cluster from the 2 northern clusters. This methodology splits the most differentiated first (Hobbs et al. 2011). This result therefore suggests that the 3 adjacent genetic clusters in southern Britain are all more genetically differentiated from each other than any part of the entirety of northern mainland Britain. Hobbs et al. (2011) also identified the presence of discrete regional populations and evidence for substructuring within Wales. However, because that study did not include samples from Scotland, it was unable to describe population structure throughout the entirety of mainland Britain.

**GENELAND and STRUCTURE both identified 5 genetic clusters in mainland Britain. However, GENELAND grouped Wales into a single cluster and northern England and Scotland separately, whereas STRUCTURE results were the opposite. The spatial model in GENELAND assumes that the spatial domain of each population can be approximated by the union of a few polygonal domains (Guillot et al. 2005). This leads to a greater emphasis on spatial information using GENELAND than the nonspatial version of STRUCTURE that was used in this study. This may have led to GENELAND overestimating the genetic differentiation within northern Britain, and underestimating it in Wales, due to the greater maximum distance between the samples in northern Britain. Another potentially confounding factor when analyzing population genetic structure is the otter reintroduction program that...**

**Table 3.—Results of AMOVA for northern subpopulations of Eurasian otter (Lutra lutra), using samples from mainland Britain. Group designations for the AMOVA were group 1 = (subpopulation 1 = Scotland mainland A; subpopulation 2 = Scotland mainland B); group 2 = (subpopulation 1 = northern England A; subpopulation 2 = northern England B).**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>Fixation indexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups</td>
<td>1</td>
<td>56.2</td>
<td>0.176</td>
<td>3.15</td>
<td>$F_{ST}$: 0.032</td>
</tr>
<tr>
<td>Among subpopulations within groups</td>
<td>2</td>
<td>43.4</td>
<td>0.221</td>
<td>3.96</td>
<td>$F_{ST}$: 0.041*</td>
</tr>
<tr>
<td>Among individuals within subpopulations</td>
<td>167</td>
<td>922</td>
<td>0.341</td>
<td>6.11</td>
<td>$F_{ST}$: 0.066*</td>
</tr>
<tr>
<td>Within individuals</td>
<td>171</td>
<td>828</td>
<td>4.842</td>
<td>86.8</td>
<td>$F_{ST}$: 0.132*</td>
</tr>
<tr>
<td>Total</td>
<td>341</td>
<td>1,850</td>
<td>5.580</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.001.$

**Table 4.—Results of AMOVA for southern subpopulations of Eurasian otter (Lutra lutra), using samples from mainland Britain. Group designations for the AMOVA were group 1 = (subpopulation 1 = Wales A; subpopulation 2 = Wales B); group 2 = (subpopulation 1 = eastern England A; subpopulation 2 = eastern England B); group 3 = (subpopulation 1 = southwestern England A; subpopulation 2 = southwestern England B).**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>Fixation indexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups</td>
<td>2</td>
<td>495</td>
<td>1.17</td>
<td>21.75</td>
<td>$F_{ST}$: 0.217</td>
</tr>
<tr>
<td>Among subpopulations within groups</td>
<td>3</td>
<td>105</td>
<td>0.262</td>
<td>4.86</td>
<td>$F_{ST}$: 0.062*</td>
</tr>
<tr>
<td>Among individuals within subpopulations</td>
<td>380</td>
<td>1,550</td>
<td>0.121</td>
<td>2.23</td>
<td>$F_{ST}$: 0.030**</td>
</tr>
<tr>
<td>Within individuals</td>
<td>386</td>
<td>1,481</td>
<td>3.84</td>
<td>71.16</td>
<td>$F_{ST}$: 0.288*</td>
</tr>
<tr>
<td>Total</td>
<td>771</td>
<td>3,631</td>
<td>5.39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.001.$

** $P < 0.05.$
TABLE 5.—Isolation-by-cost-distance analysis for Eurasian otter (Lutra lutra) in Britain. Highest correlation coefficient (between cost distance [estimated using PATHMATRIX] and genetic distance [estimated using GenAlEx]) for each landscape feature investigated, and its corresponding cost value. P-values for each of these landscape features also are given, once Euclidean distance (no cost value assigned to the feature in question, i.e., cost value = 1) has been partialed out. NA = not applicable.

<table>
<thead>
<tr>
<th>Landscape feature</th>
<th>Maximum correlation coefficient</th>
<th>Cost value</th>
<th>P-value (partial Mantel test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation</td>
<td>0.1691*</td>
<td>× 0.1</td>
<td>0.185</td>
</tr>
<tr>
<td>Built-up land</td>
<td>0.1640*</td>
<td>× 1</td>
<td>NA</td>
</tr>
<tr>
<td>Arable and horticultural land</td>
<td>0.1643*</td>
<td>× 2</td>
<td>0.390</td>
</tr>
<tr>
<td>Main salmon rivers</td>
<td>0.1656*</td>
<td>× 2</td>
<td>0.421</td>
</tr>
<tr>
<td>Coastal</td>
<td>0.1676*</td>
<td>× 100</td>
<td>0.137</td>
</tr>
<tr>
<td>Main roads</td>
<td>0.1657*</td>
<td>× 1</td>
<td>NA</td>
</tr>
<tr>
<td>Special areas of conservation</td>
<td>0.1739*</td>
<td>× 0.1</td>
<td>0.096</td>
</tr>
</tbody>
</table>

*p < 0.01.

The genetic consequences of these reintroductions are discussed in detail in Hobbs et al. (2011). In short, these reintroductions have clearly influenced the genetic composition of otters in Britain; however, this effect has been found to be quite localized. As demonstrated in the present study, high $F_{ST}$ and $F_{GT}$ values typify southern British sites, indicating little gene flow between them. The 2 most genetically differentiated groups in the present study were southwestern England and Wales ($F_{GT} = 0.28$), neither of which have experienced reintroductions to our knowledge. This therefore strongly implies that the reintroductions are not the main contributory factor of the genetic structure present in southern Britain, except in a few known, localized cases.

The genetic structure results in the present study expand on the findings of Dallas et al. (2002), who showed high levels of genetic subdivision within regions in southwestern England, and between southwestern England and Wales. The present study puts this finding of high differentiation into context. Southwestern England had the highest mean pairwise $F_{GT}$-value, whereas Scotland had the lowest. Scotland and northern England had the lowest intragroup $F_{GT}$-value. This again highlights the difference between otters in the south and north of the United Kingdom.

Among groups (Table 1), a low $F_{GT}$-value was estimated between northern England and Scotland (0.054) and among subpopulations (Table 2), a low $F_{ST}$-value was estimated between Scotland A and Scotland B (0.022) and between Wales A and Wales B (0.045). The low $F_{GT}$-values between northern England and Scotland, and low $F_{GT}$-values between Scotland A and Scotland B, are further evidence of low levels of genetic structure in northern Britain. The low among-subpopulation $F_{ST}$-value for Wales is perhaps surprising because this region has previously been shown to contain considerable amounts of substructuring (Hobbs et al. 2011). This, however, simply emphasizes the extent of substructuring in the other British regions. The among-group variation shown in the south ($F_{GT} = 0.217$) is high compared to most other studies of microsatellite variation in medium–large carnivores, even though the spatial distribution of samples here is relatively small (Paetkau et al. 1999; Schwartz et al. 2002; Rueness et al. 2003; McRae et al. 2005).

The significant $F_{IS}$-estimates indicate localized inbreeding in the Welsh subpopulations, the Scottish subpopulations, and 1 of the northern England subpopulations. Significant $F_{IS}$-values are indicative of inbreeding, which can occur in fragmented populations due to restricted population size and subsequent genetic drift (Wright 1922; Crow and Kimura 1970). Counterintuitively, the 2 groups where both subpopulations had significant $F_{IS}$-values (Scotland and Wales) also were the 2 groups with the lowest within-group $F_{ST}$-values. The 2 groups where neither subpopulation showed significant $F_{IS}$-values (southernwestern England and eastern England) were the 2 groups with the highest within-group $F_{ST}$, and northern England had 1 subpopulation with a significant $F_{IS}$-value and showed an intermediate within-group $F_{ST}$. Significant $F_{IS}$-values also were observed by Hobbs et al. (2011), and were attributed to a Wahlund effect in those cases. This might be expected in most of the groupings in Britain, due to the presence of substantial genetic structure. However, the Scotland B population had a notable lack of genetic structure (based on a low $F_{ST}$-value with its neighboring population). Inbreeding depression can, and does, occur regularly in wild populations (Packer 1979; Coulson et al. 1999; Rowe et al. 1999). However, as discussed above, the otter populations in northern Britain are not genetically fragmented, implying high levels of connectivity, and gene flow. The significant inbreeding coefficients detected may therefore be evidence of local population isolation or bottlenecks in Scotland, as discussed by Dallas et al. (1999). Many mechanisms exist that enable animals to avoid inbreeding (Keller and Waller 2002) but there is no evidence of any detrimental effects of inbreeding in the British otter population currently. Because of the improving conservation situation for otters in Britain, these significant inbreeding coefficients seem unlikely to be an indication of a major problem.

The AMOVA (Tables 3 and 4) emphasizes the contrast between northern and southern Britain, with only 3.2% of the variation in the north explained among groups, but 21.8% in the south. This north–south divide must reflect highly contrasting demographic histories for each half of Britain. The high $F_{IS}$-values are reflected in the percentage of variation among individuals within subpopulations, which are nearly 3 times higher for the northern subpopulations than the those in the south (6.11 versus 2.23). The contrasting nature of the genetic differentiation of otters in northern compared to southern Britain is unexpected considering the presence of physical barriers, anthropogenic features, and historic pesticide use in Scotland (Jefferies and Hanson 2000). These factors might all be expected to lead to a highly fragmented otter population in northern Britain, which is clearly not the case. Hobbs et al. (2011) and Dallas et al. (2002) both discussed the fragmented nature of the otter population in Britain, but neither...
the dichotomy between the north and south. Hobbs et al. (2011) conclude that there is little evidence of population expansion from strongholds including northern Britain. This study shows that although strongholds in southern Britain have remained relatively genetically isolated, northern Britain appears to currently have little or no population genetic fragmentation. Demographic declines and expansions could potentially be investigated using mitochondrial DNA analysis, providing an alternative perspective of genetic structure of this species. Mitochondrial genetic diversity of this species has, however, been shown to be low outside of Wales (Stanton et al. 2009) and Ireland (Finnegan and Néill 2010) in the United Kingdom, limiting the power of this analysis and we therefore do not include it here.

The genetic partition that splits the Scottish otter population occurs along the length of the Great Glen. This has been shown to be a barrier to gene flow in red deer (Cervus elaphus—Perez-Espona et al. 2008). Perez-Espona et al. (2008) estimated a low average pairwise $F_{ST}$ between their samples of 0.019. A significant $F_{ST}$-value between otter populations either side of the Great Glen of 0.022 shows that otters are susceptible to population fragmentation by landscape features. However, this was the lowest $F_{ST}$-value between any pairwise otter population in this study, highlighting that other factors appear to be of much greater importance in determining gene flow in this species.

The present study identifies a unique and unusual genetic structure in this British carnivore. The findings may reflect a more favorable environment for the Eurasian otter in northern Britain than the south. A preliminary investigation of the interaction between genetic and geographic distance in Scottish otters was carried out in the present study using Mantel and partial Mantel tests. The highest correlation coefficient was found for facilitation of gene flow by the special areas of conservation landscape feature (Table 5; Supporting Information S3). This may again be evidence of a favorable environment for otters in the north. However, although this analysis identified multiple significant correlations between genetic distance and cost distance, none of the correlations were significant once Euclidean distance was partialled out. This is likely due to the uneven spatial distribution of the samples used from Scotland. These landscape features (Table 5) provide a useful reference for future investigation however. Scotland is known to have highly productive rivers, especially in terms of food that is likely to be favorable for otters, e.g., large fish such as salmon and trout (International Council for the Exploration of the Sea 2009; Malcolm et al. 2010). The different patterns of genetic structure observed also may reflect variation in the ecology of otters in the north compared to the south. In the western and northern parts of Scotland, otters are widely found in sea lochs and coastal habitats (Kruuk 2006). This affinity to the coast may facilitate movement in this northern part of their range in Britain. The results imply that high-quality habitat may be very effective at mitigating the effects of population fragmentation in this iconic British carnivore. These findings advance current knowledge of the relative importance of landscape features on fragmentation in the Eurasian otter. This information may be of principal use outside the United Kingdom, where conservation efforts have been less successful. Future studies on population genetics of otters would benefit from continuing the preliminary landscape genetic analysis carried out in the present study, possibly at a finer spatial scale and where sampling is more evenly spaced. The special areas of conservation in Scotland should be focused on to further elucidate if these features are indeed of particular importance in facilitating gene flow in this species.

Acknowledgments

Many thanks to the Cardiff University Otter Project for sample collection in England and Wales. Sample collection in Scotland was coordinated by R. Maclachlan, University of Glasgow. We are grateful for tissue samples supplied by Loch Lomond and Trossachs National Park Ranger Service, International Otter Survival Fund (Isle of Skye), and Glasgow Museums. We thank the 2 anonymous reviewers for their comments on the manuscript. Funding was provided by the British Ecological Society (grant SEPG 1133/1404) and the Glasgow Natural History Society (Blodwen Lloyd Binns Bequest).

Supporting Information

Supporting Information S1.—Cost grids investigated in the isolation-by-distance analysis. Figs. A–G are each of the 7 landscape features investigated. Data for Figs. A–D and F were from the Countryside Information System (2007), and data for Fig. G were from Malcolm et al. (2010). The feature being investigated (shaded): A) altitude [highest 50%], B) built-up land, C) arable and horticultural land, D) main salmon rivers, E) coastal, F) main roads, and G) special areas of conservation for each of the cost grids was assigned 7 values (0.01, 0.1, 0.5, 1, 2, 10, or 100), corresponding to 49 grids in total using ARCGIS version 8.3. These values are interpreted by PATHMATRIX (Ray 2005) as a “cost to movement.” The values were chosen to account for both facilitation and restriction of gene flow across several orders of magnitude, a method that has been used in the literature previously (Perez-Espona et al. 2008). PATHMATRIX uses these cost maps to create a pairwise matrix of “cost distances,” which can be used as a measure of geographic distance for a Mantel test (Supporting Information S3). Found at DOI: 10.1644/13-MAMM-A-201.S1

Supporting Information S2.—Plot to identify the most likely number of genetic clusters (for both correlated and independent allele frequencies), using the program STRUCTURE version 2.3.4 (Pritchard et al. 2000) and the method of Evanno et al. (2005). The highest value of delta $K$ indicates the most likely number of clusters. This was carried out for a) correlated allele frequencies and b) independent allele frequencies. Found at DOI: 10.1644/13-MAMM-A-201.S2

Supporting Information S3.—Correlation coefficients (calculated in R—R Development Core Team 2011) versus cost to movement for the landscape features from Supporting Information S1. The highest r value is indicated by a dotted line. Found at DOI: 10.1644/13-MAMM-A-201.S3

Supporting Information S4.—The $F_{ST}$-values for all subpopulations. Stars indicate $F_{ST}$-values that are significantly different from zero. These subpopulations refer to the following locations: eastern England subpopulations A and B, E, Eng A and B; Welsh subpopulations A and B, Wales A and B; southwestern England subpopulations A and B;


Submitted 18 August 2013. Accepted 3 March 2014.

Associate Editor was Bradley J. Swanson.