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Genetic Assessment of Sea Trout Populations within West Sutherland: Report on Microsatellite Analysis

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Executive Summary

From 2006 to 2010, 427 fin clips were taken from brown and sea trout in the West Sutherland area. These samples, which included both river (juveniles) and estuarine (post-smolts, finnock and adults) sites, together with juvenile samples from two rivers outside this area, were screened for microsatellite markers. The genetic analysis was carried out with the aim of providing information on the nature and extent of genetic structuring of brown and sea trout in the area into breeding populations, in order to help inform fisheries management.

The initial genetic analysis supports the view that each river has its own unique breeding population(s), based on significant genetic differences between juvenile populations in different rivers. Analysis of sea trout collected within estuaries indicated the presence of multiple genetic groups in some of these samples, suggesting that the sea trout present in these estuaries originated from a number of different breeding populations, either from the river whose estuary was sampled or from different rivers using the same estuary. Sample from the different estuaries were significantly different from each other.

No genetic differences were found between sea and brown trout samples in the Laxford, which could be due to lack of power because of small sample sizes, the inability of the microsatellite markers used to resolve differences between the two forms, or the possibility that those two life history strategies do belong to a single breeding population.

These early findings, though preliminary, provide some insight into the population structuring within the West Sutherland trout stock. This insight is essential for the design and implementation of effective of management strategies to conserve this species and, in particular, its sea-migrating life from. However, the full extent of structuring remains to be resolved and further, more detailed investigation is needed

to provide a full understanding of this within species biodiversity and determine the most effects approach to its conservation and management.

Introduction

Stocks of the sea-migrating form of brown trout (*Salmo trutta*) are an important part of the fish community in a number of areas of the west coast of Scotland, including West Sutherland. Unfortunately, in many areas, catches have declined dramatically in the recent times (Butler 2002; Butler & Walker 2006). This has led to concerted efforts to try to conserve and enhance stocks. Essential to defining the most effective management strategy is insight into how the species is structured into populations spatially across both broad-and local-scales, among and within rivers.

It may be expected that, as a result of the homing of adults to their natal streams (Elliott, 1994), trout would demonstrate a considerable degree of population structuring, which, in turn, would be expected to lead to the formation and maintenance of discrete breeding units that are more or less reproductively separated. This reproductive isolation provides the basis for the development and maintenance of locally adapted traits and adaptive differentiation within and among river stocks.

Studies of genetic diversity and differentiation among populations of resident brown trout have shown that genetic structuring of brown trout exists across Europe, both among and within rivers (Ferguson, 1989; Hindar et al., 1991). Within West Sutherland, earlier work by Thompson (1995) found that resident brown trout populations from the rivers Polla, Laxford and Loch Assynt in Sutherland were significantly differentiated from each other.

Despite these initial studies, relatively little is known about the genetic diversity and structure of anadromous trout populations, both in general and on the west coast of Scotland in particular. In the Manse system in West Sutherland, it has been suggested that there may be a high degree of straying between rivers (WSFT 2005), resulting in a population structuring where there is a relatively high degree of genetic exchange, often referred to as a meta-population structuring (Hanski, 1991). However, it remains unclear if this straying is common or if it involves non-breeding finnock, which would not have an influence on the genetic structuring of the populations.

To begin to gain insight into the exact nature of population structuring of sea trout stocks, a preliminary study was carried out to examine their genetic structure within the West Sutherland area. The main aim of the study was to define the genetic structure of the samples under investigation. A secondary aim was to place the observed levels of differentiation within West Sutherland into a broader national

context. The samples used in this study had been collected over a number of years and included a mixture of river samples containing juveniles (for which the life history strategy could not be identified), a sample of adult brown and returning sea trout and estuarine samples consisting of post-smolts, finnock and adult sea trout.

The genetic analysis was based on microsatellite markers. Microsatellites are currently the most widely used method for investigating genetic diversity and differentiation within and among populations (Chistiakov et al., 2006). In Atlantic salmon (*Salmo salar*), these markers have been shown in many, though not all, circumstances to elucidate the underlying genetic structure between and within rivers in certain circumstances (Garant et al., 2000; King et al., 2001).

Materials and Methods

Sample sites

Four hundred and twenty-seven fin clips were taken from brown and sea trout caught in four rivers in West Sutherland; the Dionard, Kanaird, Laxford and Polla. In the Dionard, two sets of samples were taken from the estuary (Kyle and Durness) and one set from higher up the river, the Righolter Burn. Further estuary samples included the Kanaird and two sets from the Polla, taken in different years (2006 and 2009). Samples from the Laxford consisted of three sets from the estuary, taken in different years (2006, 2008 and 2009), and four sites further upstream (Boathouse, Loch More, Lone and Maternity Burn) (Fig. 1). Two additional sites were added to put the West Sutherland populations into a broader geographic context. Those samples came from the Ewe (Wester Ross) and Tain (Kyle of Sutherland) (Fig. 1). Sixteen samples were analysed in total, containing a mixture of sites, years and life stages (Table 1).

The Boathouse, Maternity Burn, Righolter Burn, Ewe and Tain samples consisted of juvenile trout, whilst the other, non-estuarine samples (Loch More and Lone) included a mixture of life stages. The Lone sample, used as broodstock, consisted of adult brown and sea trout. Estuarine samples consisted mainly of post-smolts, but also finnock and some adults. Figure 2 shows the box plots of fish fork lengths for each sample and distinct group within each sample (see analysis below).

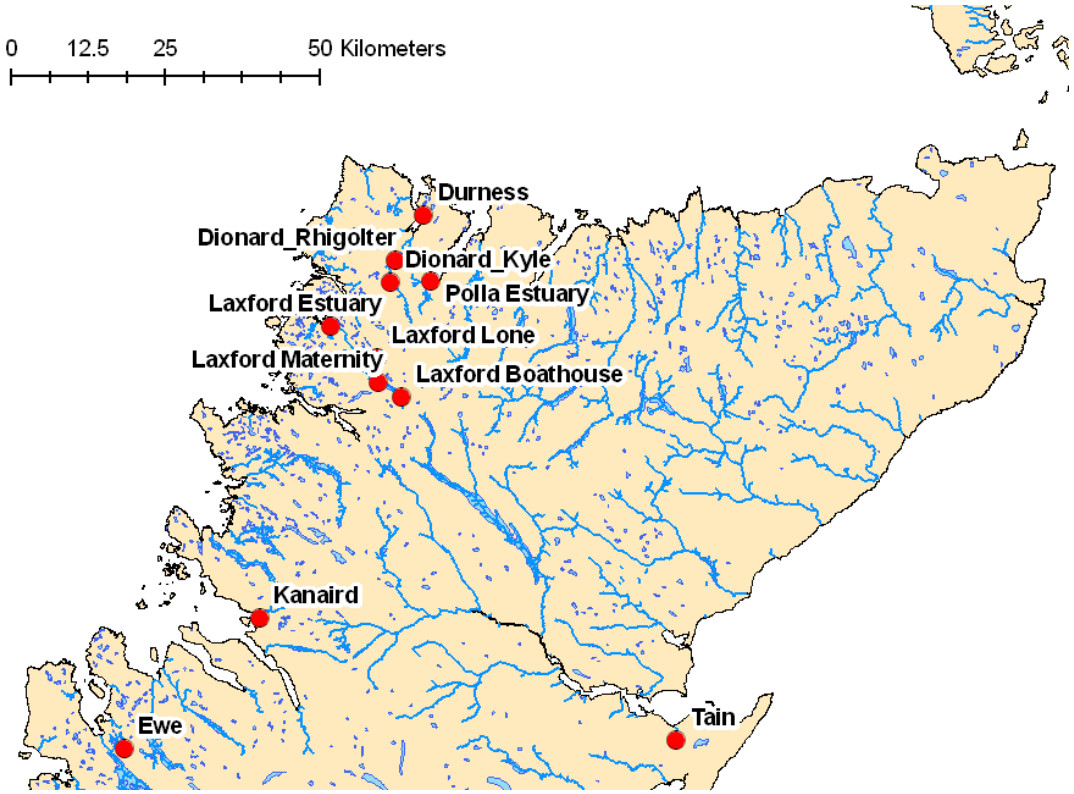

Figure 1: Sample sites included in the analysis.

Table 1:

River, sample, year, code for each sample, along with geographic coordinates (Latitude – Lat, Longitude – Long) and number of individuals (N).

River	Sample	Year	Code	Lat	Long	N
Juvenile samples						
Dionard	Rhigolter Burn	2006	Righolter	58.48	-4.85	24
Ewe	Ewe	2007	Ewe	57.75	-5.54	23
Laxford	Boathouse	2010	Boat	58.28	-4.82	27
	Maternity Burn	2009	LaxMat	58.3	-4.89	16
Tain	Tain Burn	2009	Tain	57.79	-4.03	49
Adult brown vs. sea trout						
Laxford	Lone	2009	Lone 09	58.34	-4.89	32
		2010	Lone 10	58.34	-4.89	24
Estuarine samples (post-smolts, finnock and adults)						
Dionard	Kyle	2009	Dionard	58.54	-4.78	28
	Durness	2008	Durness	58.55	-4.78	24
Kanaird	Estuary	2010	Kanaird	57.95	-5.18	22
Laxford	Estuary	2006	Lax 06	58.38	-5.02	42
		2008	Lax 08	58.38	-5.02	28
		2009	Lax 09	58.38	-5.02	50
Polla	Estuary	2006	Polla 06	58.45	-4.75	20
		2009	Polla 09	58.45	-4.75	27

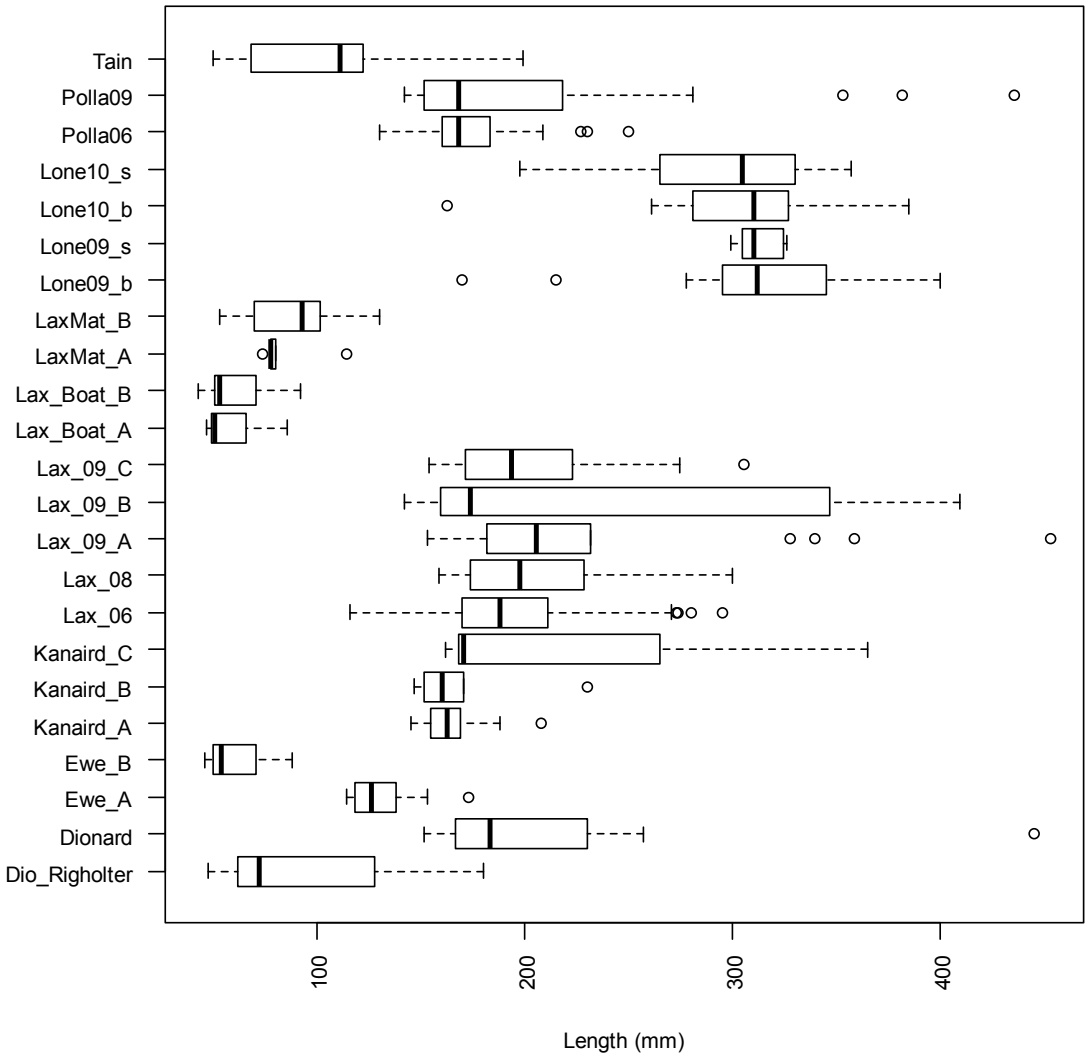

Figure 2: Lengths of trout for the various samples collected for the project. Sample codes can be found in Table 1. Length data from the samples from the Lone show brown (b) and sea (s) trout separately. For each sample, the central boxes represent the sizes within which 50% of the fish fall. The central bold line represents the median value, and the dashed lines extending from the boxes represent the maximum and minimum sizes, excluding outliers, which are identified separately as points.

Genetic analysis

DNA was extracted from fin clips using standard techniques. After DNA extraction, samples were screened for 19 microsatellite markers. The genetic data were quality checked before each sample was examined for the presence of full siblings (brothers and sisters from the same family), as family effects can influence the results. In all samples where full siblings were identified, only one sibling from each family was included in the analysis of variation within and between samples. Samples that

contained more than 15 fish after full siblings were removed were included in the analysis, due to the potential problems associated with smaller sample sizes.

Potential population structure was examined within each sample, to identify whether they could potentially constitute fish from a number of different populations. Furthermore, in the Lone samples, possible differences between the adult brown and sea trout were investigated for each year separately. These results were then taken into account when standard population genetic methods were used to examine genetic diversity and population structuring across West Sutherland. The analysis was first carried out on the samples of juveniles to assess structuring within rivers, based on the assumption that those juveniles originate from a single breeding population. The estuarine samples, which could contain a mixture of fish from different breeding populations, and the adult brown and sea trout from the Lone were then included in the overall analysis to give an idea of their relationship to the juvenile samples. Further details on the methods are given in Appendix 1.

Results

From the 19 microsatellites initially used for the screening, 5 were excluded from the analysis because of technical problems with genetic typing, which meant that the results from these microsatellites could not be relied on. So, the analysis was carried out using the 14 remaining markers, a number that has been shown in other studies to be adequate to begin to gain insight into population structuring (King et al., 2001, 2005).

In some samples, a number of full-siblings were identified. The largest number of full siblings, 3, was found in the Boathouse, whilst none were identified in the Dionard, Durness, Ewe, Kanaird, Laxford 2008, Loch More, Maternity Burn, Polla 2006 and 2009 (Table 2). In the Lone samples, where the two years were analysed together, full siblings were identified across years in three cases.

Table 2

Original sample size, sample size for the analysis, where all but one member of each full sibling family was removed and the largest single family identified. The numbers in brackets relate to the numbers of trout belonging to the different identified clusters within a sample and to adult brown and sea trout, respectively, in the Lone samples.

Sample	Original sample size	Sample size analysis	Largest single family
Juvenile samples			
Righolter	24	23	2
Ewe	23	20 (17/3)	1
Boat	27	24 (8/16)	3
LaxMat	16	16 (5/11)	1
Tain	49	48	2
Adult brown vs. sea trout			
Lone 09	32	30 (25/5)	2
Lone 10	24	19 (10/9)	
Estuarine samples (post-smolts, finnock and adults)			
Dionard	28	28	1
Durness	24	24	1
Kanaird	22	22 (9/6/7)	1
Lax 06	42	37	2
Lax 08	28	23	1
Lax 09	50	49 (18/16/15)	2
Polla 06	20	20	1
Polla 09	27	27	1

Juvenile samples

The within-sample analysis revealed two significantly different genetically distinct groups in the juvenile samples taken from the Ewe, Boathouse and Maternity Burn samples (Table 2). Due to the small resultant group sizes the subsequent genetic analysis was carried out without the Maternity Burn samples or the smaller groups from the Ewe and the Boathouse samples. There were significant differences between all remaining samples (Table A3 in Appendix 2). A spatial plot of the genetic differences between the samples was created to be able to visually assess their genetic relationships (Figure 3). From the plot, it is clear that the sample from the Ewe is the most distinct. The two samples from West Sutherland are closest together, with the sample from the Tain being more distinct.

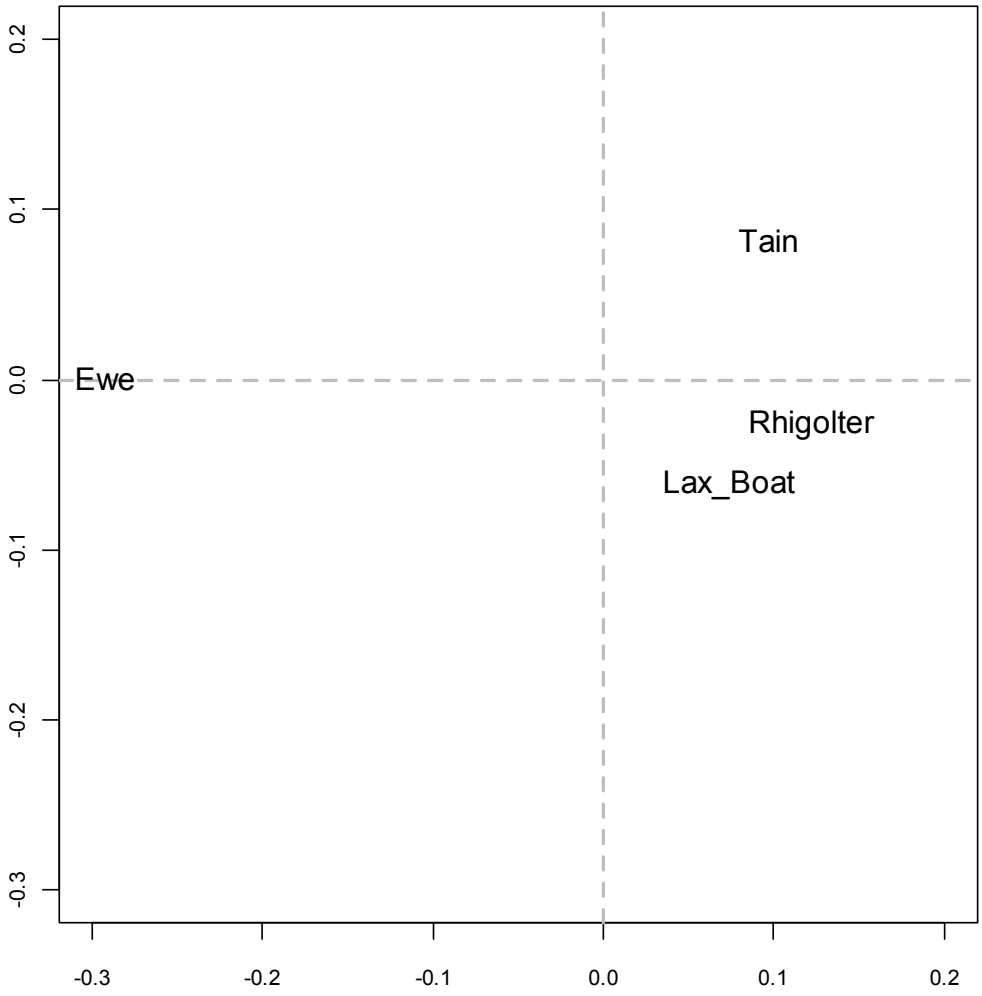

Figure 3: A spatial plot of genetic relationships among the samples of juveniles, based on pairwise estimates of genetic differentiation (see appendix A for details). The closer the samples, the more genetically similar they are.

Adult brown vs. sea trout

Adult brown and sea trout from the Lone were not significantly different when the years 2009 and 2010 were analysed separately; neither were the differences between years. The brown trout from the two year classes were therefore combined for further analysis, as this increased sample sizes. The same was done for the adult sea trout from both years. Similar results were found when the years were combined in that differences between the adult brown and sea trout were not detected (Table A4 in Appendix 2).

Estuarine samples

The within-sample analysis revealed three significantly genetically distinct groups in the Kanaird and Laxford 2009 samples (Table 2). Due to the small resultant group size, subsequent genetic analysis was carried out without the Kanaird samples. In addition to the significant differences within estuaries all of the between estuary comparisons were also significant. The genetic differences between certain Laxford samples (e.g. Lax 06 and Lax 09 C) were found to be greater than some of the those between estuary comparisons (e.g. Lax 09 C is closer to Polla 09 than Lax 09 B; Figure 4).

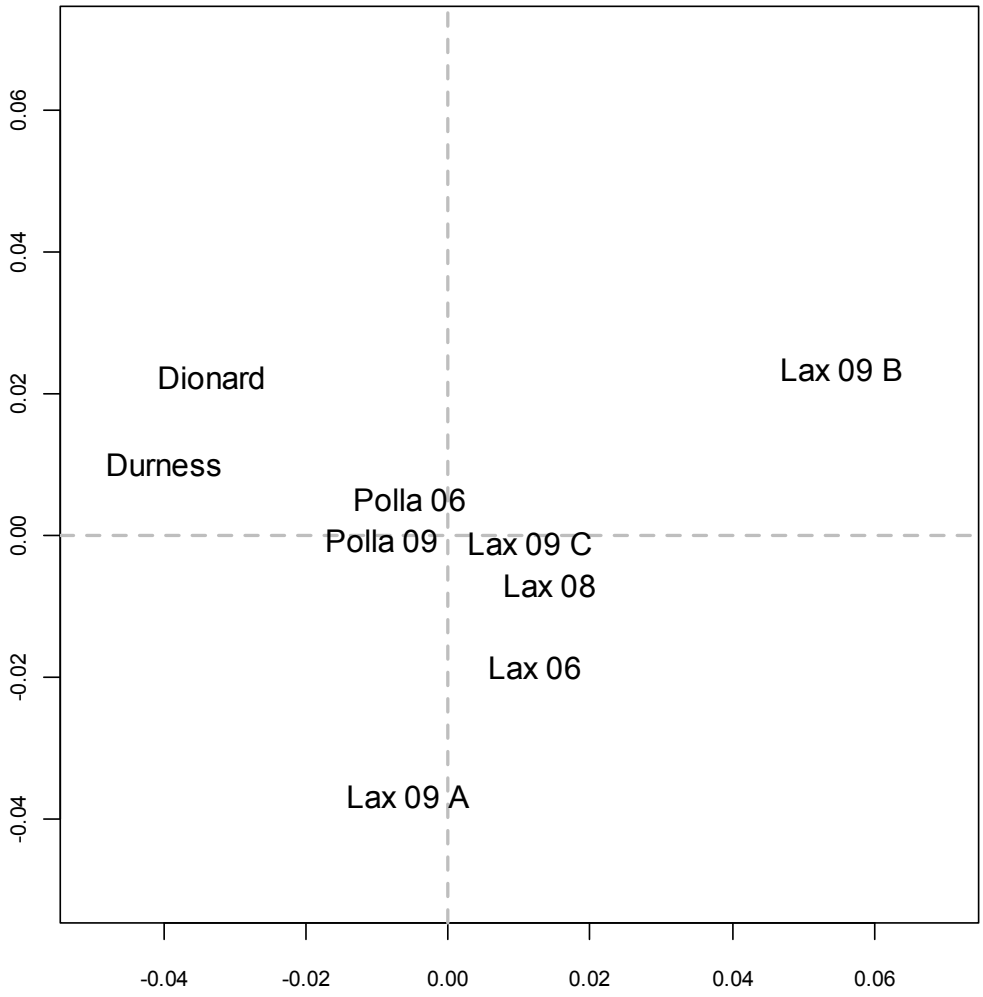

Figure 4: A spatial plot of genetic relationships among estuarine samples, based on pairwise estimates of genetic differentiation (see appendix A for details). The closer the samples, the more genetically similar they are.

Comparison of all samples

A comparison of all samples was undertaken in order to investigate the relationships between the different types of samples in the different catchments studied. Figure 5 clearly shows that the samples of juvenile fish collected in the Ewe and Tain are outliers and they were found to be significantly different from all other samples examined In order to provide better resolution of the West Sutherland samples, these outlier were excluded and the MDS plot redrawn (Figure 6). Within West Sutherland, the samples from the different catchments cluster together (Figure 6), although, as previously noted, some of the within-catchment genetic differences are greater than those found between catchments. There were significant differences between samples taken from the Laxford and Dionard catchments but not the Polla.

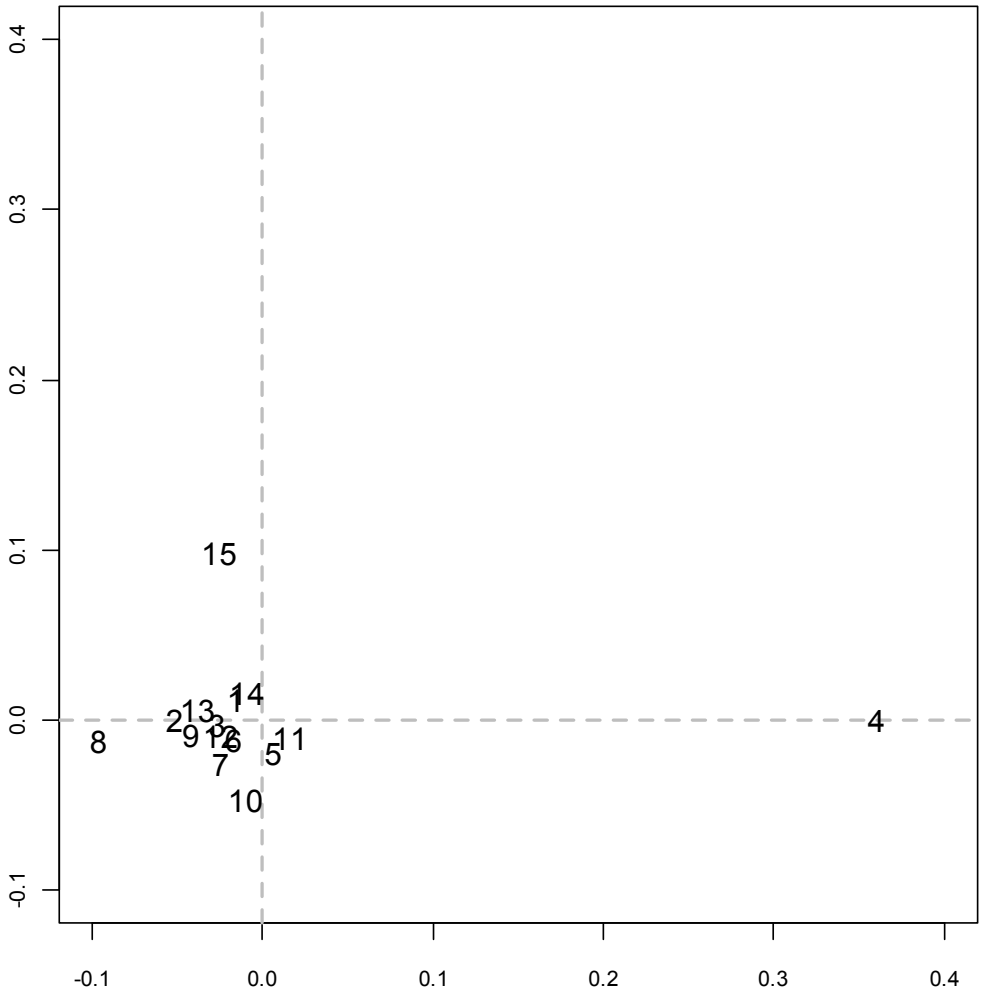

Figure 5: A spatial plot of genetic relationships among all samples (see appendix A for details). Numbers refer to the samples as follows: 1) Dionard, 2) Righolter, 3) Durness, 4) Ewe, 5) Lax 06, 6) Lax 08, 7) Lax 09 cluster A, 8) Lax 09 cluster B, 9) Lax 09 cluster C, 10) Boat, 11) Lone brown trout, 12) Lone sea trout, 13) Polla 06, 14) Polla 09, 15) Tain.

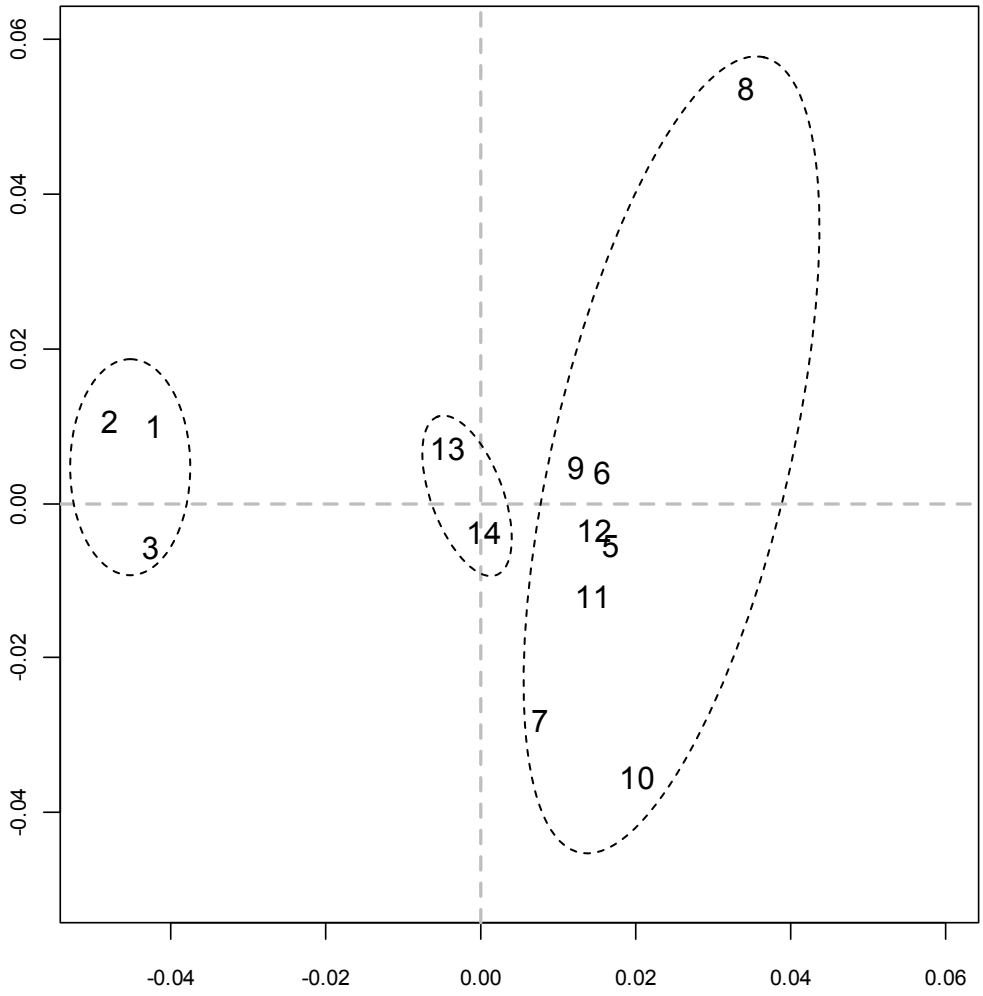

Figure 6: A spatial plot of genetic relationships among all West Sutherland samples (i.e. without the Ewe and Tain). Plot is based on pairwise estimates of genetic differences (see appendix A for details). Circles represent multiple samples within a river. Numbers refer to the samples as follows: 1) Dionard, 2) Righolter, 3) Durness, 5) Lax 06, 6) Lax 08, 7) Lax 09 cluster A, 8) Lax 09 cluster B, 9) Lax 09 cluster C, 10) Boat, 11) Lone brown trout, 12) Lone sea trout, 13) Polla 06, 14) Polla 09

Discussion

The collection of genetic samples taken from the West Sutherland area has been, to an extent, opportunistic rather than strategic, and fish were sampled in a number of locations, over a number of years and from different life stages. The results of the analysis presented can therefore only give preliminary insight into a variety of aspects of population structure of brown and sea trout in West Sutherland, rather than an in-depth analysis. In addition, sample sizes were generally small, which,

when coupled to the presence of more than one genetic group in some samples, led to a number of samples being excluded from the analysis.

There were found to be significant differences between juvenile trout sampled in West Sutherland and the two outlier areas used in the study (Ewe and Tain). McKeown *et al.* (2010) also found geographical structuring of trout populations at the regional level, and noted differences between the Laxford and Ewe. Within West Sutherland, juvenile trout samples from the Laxford and Dionard differed significantly from each other, suggesting they also belong to different breeding populations. Differences between trout populations in the West Sutherland area were also reported by Thompson (1995), who examined the rivers Laxford and Polla.

Within-sample analysis showed that two Laxford samples, Boathouse and Maternity Burn, each consisted of two distinct genetic groups, although, without further investigation, it is unclear what could have produced this result. The results from juvenile sampling do not necessarily apply directly to sea trout, as it is not possible to differentiate between resident and anadromous *Salmo trutta* juveniles taken from areas accessible to sea trout. Samples may therefore be fully resident trout, fully anadromous sea trout or a mixture of both.

In contrast to samples of juvenile trout, those taken in estuaries can be confidently assigned as sea trout. In most cases, samples from the same estuary taken in different years did not differ significantly from each other, which would suggest that the patterns of spatial stock differentiation found may be relatively stable over time. There were significant differences between the estuary samples, suggesting that there is limited gene flow/movement between sea trout in the different catchments studied. As the estuaries examined were reasonably distant (Figure 1), this may not be the case for neighbouring rivers. The existence of multiple genetic groups in two of the estuarine samples, Kanaird and Laxford 2009, suggests the sea trout present in those estuaries originated from a number of breeding populations. It is possible that the river whose estuary was sampled contained multiple breeding populations or that this result was a product of sea trout from different rivers using the same estuary (Middlemas *et al.* 2009; Middlemas *et al.* unpublished data).

No genetic differences were found between adult brown and sea trout caught in the Lone. While this could be due to a lack of power to detect differences, it is also possible that the two life history strategies sampled in this area belong to a single breeding population, in which a number of individuals undergo smoltification and migrate to sea, whilst others do not (Hindar et al., 1991; Thompson, 1995; Walker 2006).

When considered as a whole, the samples from a given catchment were shown to cluster together, although some of the within-catchment genetic differences were greater than those found between catchments. This suggests that, at the geographic

scale examined, trout stocks from different rivers in West Sutherland belong to different breeding populations. It is also possible that there are multiple populations present within each river, although the small sample sizes and fragmented sampling does not provide a convincing test of this hypothesis.

Future work

The results of this study have highlighted some potentially interesting questions that could be addressed using genetic analysis. One of the areas worth exploring is the use of the genetic differences between river stocks and their constituent populations to determine the origin of the trout captured in the estuaries, using the technique of genetic stock identification. This technique has been used in a number of salmon species to assign fish back to population/river/region of origin. However, to be successful, it requires a robust genetic 'baseline' to be established from all the potentially contributing populations or stock groups. This 'baseline' would require a more comprehensive survey of river stocks, as it is not presently available for trout in West Sutherland.

It may be possible to use a different, more recently developed marker, single nucleotide polymorphisms (SNP), instead of microsatellites. Due to the comparatively high number of SNPs available (several thousand candidate SNPs v 14 microsatellites); they are likely to offer a higher degree of resolution to examine trout population structuring. Furthermore, they may provide insight into adaptive differences between brown and sea trout.

However, a more rigorous sampling strategy will be required in order to examine any of these issues in detail. Although the original plan was to use SNPs on the samples used in the study, it is unlikely that this will provide much in the way of further information, as the small sample sizes were more of a limitation to the analysis than the resolution of the microsatellites. Any additional sampling required will be dependent on the exact question that any subsequent work wishes to address.

Acknowledgements

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Appendix 1

Detailed Materials and Methods

Sample preparation and genotyping

Total DNA was extracted from the fin clip using a quick extraction method described by Knox *et al.* (2002). The 19 microsatellites studied were assembled by Queen's University (QUB)/University College Cork/University of Wales Bangor for the EU funded Celtic Sea Trout Project (Table A1). The forward primer of each microsatellite was labeled with a fluorescent dye (FAM, HEX or NED) to allow detection during electrophoresis. Three multiplex PCR reactions were performed in a 10 µl reaction volume containing 5-100 ng DNA, 0.015-0.03 µM of each primer, and 2x Type-it Multiplex PCR Master mix, resulting in a final concentration of 3 mM $MgCl_2$ (Qiagen). The first multiplex (A) contained the 0.05 µM of the primers *SsaD48*, *Ssa85*, *Ssa171*, *Ssa407UOS*, *One102*, *One103*, *CA53293* and *MHC I*. Multiplex B included the microsatellites *SsaD71*, *Ssa416*, *SasaTAP2A* and *CA048828* at 0.05 µM and 0.1 µM of *Cocl_Lav4.* Multiplex C consisted of 0.05 µM of *Ssa197, BG935488*, *CB512797* and *MHC I UTR*, 0.1 µM *Ssa417* and *Ssa289* at 0.15 µM final concentration. PCR cycling conditions consisted of a denaturing phase of 95°C for 5 min, followed by 32 cycles of 95°C for 30 s, 55°C (multiplex C) or 58°C (multiplexes A and B) for 90 s and 72°C for 60 s. The extension time was 30 min at 60°C. Electrophoresis was carried out using MegaBace 500 capillary sequencer and allele size determined using MegaBace Fragment Profiler version 1.0 (GE Healthcare). Quality control of the samples included double checking of all samples for all microsatellites and one in five sample plates were genotyped twice and the results compared.

Table A1

Microsatellite loci screened. Details include name of the microsatellite maker, repeat unit (2-4 base pairs), forward (F-) and reverse (R-) primer sequences and, where possible, reference for the complete microsatellite sequence. GTT sequences added to some of the primer sequences to minimise technical problems with "stuttering".

Microsatellite	Repeat		Primer Sequence	Reference
Ssa85	di	F-	AGG TGG GTC CTC CAA GCT AC	O'Reilly et al., 1996
		R-	GTT TAC CCG CTC CTC ACT TAA TC	
CAO53293	di	F-	TCT CAT GGT GAG CAA CAA ACA	
		R-	GTT TAC TCT GGG GCA TTC ATT CAG	
Ssa171	tetra	F-	GTT TTT ATT ATC CAA AGG GGT CAA AA	O'Reilly et al., 1996
		R-	GAG GTC GCT GGG GTT TAC TAT	
MHC1	di	F-	AGG AAG GTG CTG AAG AGG AAC	
		R-	GTT TCA ATT ACC ACA AGC CCG CTC	
One103	tetra	F-	TGC TAA ATG ACT GAA ATG TTG AGA	Olsen et al., 2000
		R-	GTT TGA GAA TGA ATG GCT GAA TGG A	
Ssa407UOS	tetra	F-	TGT GTA GGC AGG TGT GGA C	Cairney et al., 2000
		R-	GTT TCA CTG CTG TTA CTT TGG TGA TTC	
SsaD48	tetra	F-	GAG CCT GTT CAG AGA AAT GAG	King et al., 2005
		R-	CAG AGG TGT TGA GTC AGA GAA G	
One102	tetra	F-	GGG ATT ATT CTT ACT TTG GCT GTT	Olsen et al., 2000
		R-	GTT TCC TGG TTG GGA ATC ACT GC	
Cocl_Lav_4	di	F-	TGG TGT AAT GGC TTT TCC TG	
		R-	GTT TGG GAG CAA CAT TGG ACT CTC	
SasaTAP2A	di	F-	GTT TGT CCT GAT GTT GGC TCC CAG G	
		R-	GCG GGA CAC CGT CAG GGC AGT	
Ssa416	tetra	F-	TGA CCA ACA ACA AAC GCA CAT	Cairney et al., 2000
		R-	GTT TCC CAC CCA TTA ACA CAA CTA T	
SsaD71	tetra	F-	AAC GTG AAA CAT AAA TCG ATG G	King et al., 2005
		R-	GTT TTT AAG AAT GGG TTG CCT ATG AG	
CAO48828	di	F-	GAG GGC TTC CCA TAC AAC AA	
		R-	GTT TAA GCG GTG AGT TGA CGA GAG	
Ssa197	tetra	F-	GGG TTG AGT AGG GAG GCT TG	O'Reilly et al., 1996
		R-	GTT TTG GCA GGG ATT TGA CAT AAC	
Ssa417	tetra	F-	AGA CAG GTC CAG ACA AGC ACT CA	Cairney et al., 2000
		R-	GTT TAT CAA ATC CAC TGG GGT TAG ACT G	
Ssa289	di	F-	GTT TCT TTA CAA ATA GAC AGA CT	McConnell et al., 1995
		R-	TCA TAC AGT CAC TAT CAT C	
CB512797	di	F-	GGA CGA AGG ACC ACT CCA AT	
		R-	GTT TGG GGG TGC TGA GGA GTA TTT	
BG935488	tetra	F-	GTT TTG ACC CCA CCA AGT TTT TCT	
		R-	AAA CAC AGT AAG CCC ATC TAT TG	
MHC I UTR	di	F-	TGC CCA GAT GAC TTG AGA GAC	
		R-	GTT TCC AAC CTC CTG TGT TGT GTG	

Data analysis

The program MICROCHECKER (Van Oosterhout et al. 2004) was used to screen for genotyping errors and non-amplifying variants (null alleles) in the raw data.

Colony (Wang & Santure, 2009) was run to identify full sibling fish in the sample and calculate an estimated number of breeders (N_b). Family effects can have a large influence on the results of the genetic analysis and, as such, all but one member of each identified full-sib family were removed from the dataset.

The program STRUCTURE 2.3.3 (Pritchard et al., 2000) was used to examine the potential for clustering of individuals into a number of distinct genetic groups within each sample. This analysis was carried out using the admixture model with correlated allele frequencies and four replicates were run with a burn-in phase of 100,000 iterations, followed by a run phase of 300,000. Structure Harvester (Earl, 2001) was then used to examine the most likely number of groups in a sample and the individual assignment probabilities were used to assign each individual to one of the identified groups.

The program GENEPOP 3.1 (Raymond & Rousset, 1995) was used to test if the genotype proportions for each sample were according to Hardy-Weinberg (HW) expectations and to test for linkage disequilibrium (LD).

The genetic diversity of each sample was measured in various ways. Allele frequencies and expected (H_e) and observed (H_o) heterozygosities were calculated using the "Microsatellite Toolkit" (Park, 2001) add-in into Excel, whilst FSTAT 2.9.3 (Goudet, 1995) was used to calculate and the inbreeding coefficient (F_{IS}). HPRare (Kalinowski, 2005) was used to estimate allelic richness (A_R), i.e. the number of alleles per locus corrected by sample size, which was set at 25.

The differentiation between the samples was analysed using two parameters. Pairwise F_{ST}-values, measurements of differences, and associated probabilities were calculated in FSTAT 2.9.3; whilst Nei's pairwise genetic distances (D_A) were estimated using DISPAN (Ota, 1993). Multidimensional scaling, based on the F_{ST} estimates, provided a means of illustrating the relationship between the samples.

Appendix 2

Detailed Results

With the exception of the Ewe, all samples were quite genetically diverse (Table A2). Indeed, the Ewe sample showed very low allelic richness and heterozygosity, though the inbreeding coefficient was also low. The Dionard and Laxford 2008 samples also showed relatively lower diversity, in the sense that the observed heterozygosity (H_o) was a lot lower than the expected heterozygosity (H_e). The estimated inbreeding coefficient was also higher in those two samples, compared to the others (Table A2). With the exception of the Ewe, allelic richness varied from 5.9 in the Tain to 9.3 in the Laxford 2009 cluster C, with an average of 7.2 ± 1.7. Heterozygosity, both expected (H_e) and observed (H_o), was relatively high, with H_e ranging from 0.69 in the Laxford 2009 cluster B sample to 0.79 in the Laxford 2009 cluster C and Polla 2009 samples, and H_o varying between 0.64 (Laxford 2008 and Laxford 2009 cluster B) and 0.75 (Laxford 2009 cluster C). In most cases, the genotype proportions were not in Hardy-Weinberg equilibrium, even after applying a sequential Bonferroni correction. After correcting for multiple tests, significant LD was found only in one case, i.e. between *One171* and *Ssa407* in the adult brown trout sample from the Lone.

Table A2

Measurements of genetic diversity and the results (p-value) of the test for Hardy-Weinberg equilibrium (pHW) for each sample. The measurements of diversity include allelic richness (A_r), expected (H_e) and observed (H_o) heterozygosity and estimates of the inbreeding coefficient (F_{IS}). Significant deviations from HW proportions are in bold. Samples in italics contain juvenile trout.

Sample	A_r	H_e	H_o	F_{IS}	pHW
Dionard	*7.3*	*0.74*	*0.66*	*0.11*	***0.0003***
Righolter	6.9	0.71	0.69	0.04	**0.003**
Durness	6.8	0.73	0.70	0.04	0.198
Ewe	*2.1*	*0.22*	*0.23*	*-0.01*	*0.916*
Lax 06	8.5	0.75	0.69	0.08	**0**
Lax 08	8.5	0.76	0.64	0.16	**< 0.0001**
Lax 09 A	7.1	0.72	0.69	0.04	0.254
Lax 09 B	6.3	0.69	0.64	0.07	0.759
Lax 09 C	9.3	0.79	0.75	0.06	0.269
Boathouse	*7.1*	*0.72*	*0.69*	*0.04*	*0.196*
Lone brown	7.8	0.74	0.69	0.06	**0.004**
Lone sea	8.6	0.75	0.70	0.06	0.103
Polla 06	8.3	0.76	0.71	0.07	0.092
Polla 09	8.0	0.79	0.74	0.05	**0.011**
Tain	*5.9*	*0.71*	*0.71*	*0.01*	*0.217*

The within-sample analysis revealed two genetically distinct groups in the Ewe, Boathouse and Maternity Burn samples and three different groups in the Kanaird and Laxford 2009 samples (Table 1).

The analysis of the juvenile samples, which originated from different rivers, revealed significant differences between all samples (Table A3). The smallest difference (8.5%) was found between the two juvenile samples collected in West Sutherland, i.e. the Laxford Boathouse and the Righolter Burn samples. The largerst difference (41.6%) was seen between the Ewe and Righolter Burn, with an overall average of 25.6% between the samples of juveniles. In general, the average difference between the Ewe and other samples (39.6%) was larger than that found for the Tain (22.2%). Similar results were found for the genetic distance estimates, with the smallest distance found between the West Sutherland samples, whilst the distance with the Ewe were larger than with the Tain (Table A3).

The full analysis of the microsatellite data revealed significant genetic differences between samples taken in different catchments (Table A3). Most sample sites were significantly different from each other, and those that were not were those taken within the same catchment (Table A3). The outgroups, formed by the Ewe and Tain were more different from the West Sutherland samples than those within this region. Indeed, the average estimated differences between the outgroups and the West Sutherland samples was 13.3% in the Tain and 38.8% in the Ewe. Within West Sutherland, the three studied rivers/estuaries were all significantly different from each other. The average difference was largest (6.3%) between the Dionard and the other West Sutherland samples, whilst the average differences between the Polla and the other samples, and between the Laxford and other samples were 4.6% and 5.7%, respectively. In the Laxford, the estuary samples from 2006 and 2008 were not significantly different from the other Laxford samples, with the exception of one of three distinct groups (B) identified in the 2009 estuary sample (Table A3). This latter group was significantly different from all other samples within the Laxford, with and average of 6.7%, similar to differences found between samples from different rivers/estuaries. However, genetic differences between catchments (13.0% ± 13.0%) were, on average, higher than between sites within a catchment (2.5% ± 2.5%) (Table A3). The adult brown trout from the Lone were significantly different from most other samples, which was only the case between the Lone adult sea trout and the Boathouse samples. Furthermore, within the Lone, differences between the adult brown and sea trout were not detected. In the Polla estuary, samples taken in two different years did not show any significant differences. Similar results were found between the Dionard and Durness samples, which were both caught in the Dionard Estuary in two different years. Similar results were found in the genetic distance estimates. The Tain and Ewe samples were more distinct than the samples taken within West Sutherland (Table A3). The smallest values were found within a river/estuary. The Laxford 2009 cluster B sample was more distinct from both the other Laxford estuary samples and the two upstream samples.

Table A3

Pairwise values of F_{ST} (below diagonal) and D_a (above diagonal) for all the sample sites. Significant F_{ST} values are in bold and samples consisting of juveniles are in italics.

	Dionard	*Rhigolter*	Durness	*Ewe*	Lax 06	Lax 08	Lax 09 A	Lax 09 B	Lax 09 C	*Boat*	Lone brown	Lone sea	Polla 06	Polla 09	*Tain*
Dionard	-	0.093	0.090	0.583	0.254	0.256	0.290	0.334	0.271	0.310	0.272	0.296	0.231	0.251	0.327
Rhigolter	0.013	-	0.129	*0.634*	0.272	0.292	0.301	0.350	0.294	*0.317*	0.285	0.331	0.250	0.282	*0.347*
Durness	**0.004**	**0.022**	-	0.595	0.273	0.277	0.286	0.388	0.278	0.311	0.300	0.311	0.254	0.254	0.372
Ewe	**0.377**	***0.416***	**0.390**	-	0.532	0.528	0.538	0.610	0.564	*0.497*	0.507	0.483	0.583	0.566	*0.616*
Lax 06	**0.062**	**0.064**	**0.058**	**0.351**	-	0.094	0.123	0.235	0.129	0.138	0.103	0.135	0.177	0.188	0.338
Lax 08	**0.053**	**0.063**	**0.054**	**0.376**	0	-	0.139	0.227	0.137	0.173	0.121	0.135	0.195	0.197	0.331
Lax 09 A	**0.062**	**0.065**	**0.057**	**0.387**	0.010	0.012	-	0.327	0.201	0.145	0.155	0.187	0.229	0.236	0.367
Lax 09 B	**0.087**	**0.092**	**0.096**	**0.458**	**0.056**	**0.040**	**0.086**	-	0.218	0.329	0.293	0.296	0.293	0.338	0.380
Lax 09 C	**0.050**	**0.061**	**0.049**	**0.403**	0.011	0	**0.028**	**0.041**	-	0.195	0.175	0.191	0.240	0.231	0.318
Boat	**0.077**	***0.085***	**0.065**	***0.373***	0.024	0.027	0.015	**0.090**	0.020	-	0.177	0.208	0.253	0.246	*0.405*
Lone brown	**0.062**	**0.066**	**0.064**	**0.340**	0.007	0.007	**0.020**	**0.071**	**0.022**	**0.036**	-	0.126	0.192	0.182	0.336
Lone sea	**0.056**	**0.067**	**0.063**	**0.386**	0.004	0	0.027	**0.062**	0.014	**0.040**	0	-	0.223	0.196	0.345
Polla 06	**0.038**	**0.057**	**0.046**	**0.399**	**0.030**	**0.023**	**0.046**	**0.065**	**0.027**	**0.057**	**0.037**	**0.026**	-	0.100	0.332
Polla 09	**0.055**	**0.073**	**0.056**	**0.370**	**0.043**	**0.035**	**0.058**	**0.081**	**0.030**	**0.057**	**0.042**	**0.033**	0.001	-	0.301
Tain	**0.105**	***0.120***	**0.118**	***0.398***	**0.118**	**0.107**	**0.124**	**0.126**	**0.103**	***0.147***	**0.111**	**0.108**	**0.096**	**0.087**	-

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