Demographics and landscape features determine intra-river population structure in Atlantic salmon (*Salmo salar* L.): the case of the River Moy in Ireland.

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Abstract

Contemporary genetic structure of Atlantic salmon (*Salmo salar* L.) in the River Moy in Ireland is shown here to be strongly related to landscape features and population demographics, with populations being defined largely by their degree of physical isolation and their size. Samples of juvenile salmon were collected from the 17 major spawning areas on the river Moy and from one spawning area in each of five smaller nearby rivers. No temporal allele frequency differences were observed within locations for 12 microsatellite loci, whereas nearly all spatial samples differed significantly suggesting that each was a separate population. Bayesian clustering and landscape genetic analyses suggest that these populations can be combined hierarchically into five genetically-informative larger groupings. Lakes were found to be the single most important determinant of the observed population structure. Spawning area size was also an important factor. The salmon population of the closest nearby river resembled genetically the largest Moy population grouping. In addition we showed that anthropogenic influences on spawning habitats, in this case arterial drainage, can affect relationships between populations. Our results show that Atlantic salmon biodiversity can be largely defined by geography and thus knowledge of landscape features (for example, as characterised within Geographical Information Systems) has the potential, to predict population structure in other rivers without an intensive genetic survey, or at least to help direct sampling. This approach of combining genetics and geography, for sampling and in subsequent statistical analyses, has wider application to the investigation of population structure in other freshwater/anadromous fish species and possibly in marine fish and other organisms.

Introduction

For many terrestrial species landscape features and habitat heterogeneity are critical determinants of the spatial pattern of genetic variation and population demographics (e.g. Opdam 1991; Sork *et al.* 1999; Manel *et al.* 2003). Both can present barriers to gene flow and limit carrying capacity and hence, population size within discrete habitats. For marine fish, physical barriers to dispersal are less apparent, although such mechanisms have been identified in some species. For example, oceanography and bathymetry have been identified as isolating mechanisms in European flounder (Hemmer-Hansen *et al*. 2007), Atlantic herring (Bekkevold *et al*. 2005) and Atlantic cod (Ruzzante *et al*. 1999). In freshwater, the landscape genetics approach has been applied primarily on salmonid fishes (e.g. Rieman & Dunham 2000) and, for example, in cutthroat trout populations (Neville *et al*. 2006), migratory life history, stream connectivity and carrying capacity of individual habitats have been identified as being important determinants of genetic patterns. There is considerable evidence for the structuring of Atlantic salmon (*Salmo salar* L.) into distinct reproductively isolated populations at a range of geographic levels (e.g. King *et al.* 2001; Verspoor *et al.* 2005; Dillane *et al*. 2007), although it is only recently that the potential of habitat features, e.g. spawning habitats, to influence population structuring has been investigated (Garant *et al*. 2000, Primmer *et al*. 2006, Dionne *et al*. 2008). Atlantic salmon are obligate river gravel spawners requiring specific substrate conditions for the successful retention and incubation of their eggs (Gibson 1993). Spawning habitats occur in identifiable zones or reaches, as a function of geo-fluvial processes unique to each individual river system and related to sediment production and transfer (Davey & Lapointe 2004). Depending on the pattern of, and the distance between these habitats, groups of breeding fish will be separated from each other to varying degrees. These groups of fish form the basis of putative populations, which may be substantially reproductively-isolated from other spawning groups. Natal homing maintains population structuring arising as a consequence of this habitat heterogeneity. The role of spawning habitats in maintaining and promoting salmon population structuring within rivers, in terms of their distribution, frequency and isolation, has been identified previously by Primmer *et al*. (2006) and by Vaha *et al*. (2007). However their importance, through their possible influence on population size, has not been studied previously and would represent a valuable additional insight into our understanding of how spawning habitats might affect population structure.

The study of landscape genetics attempts to quantify the effects of geographical composition, configuration and matrix quality on gene flow and spatial genetic variation (Storfer *et al*. 2007), and provides a useful way of determining the relative influences of landscape on gene flow, genetic discontinuities and population structure. This approach has been successful in elucidating habitat factors which influence population structure in a number of species in various ecosystems (e.g. Bockelmann *et al.* 2003; Coulon *et al.* 2004; Petren *et al.* 2005). Sampling design is central to the determination of genetic population structure within a landscape genetics framework (Storfer *et al.* 2007) and Manel *et al.* (2003) suggest that the most useful approach is to sample individuals over the entire study area (using either systematic or random sampling designs). The subsequent use of spatial statistics to determine genetically significant population structuring can help to ascertain the likely boundaries where populations begin and end. Given that the biology of Atlantic salmon is well known, particularly the importance of specific habitats to successful reproduction, it seems reasonable that knowledge of the distribution and dimensions of spawning areas be incorporated into sampling programme design.

The river Moy in north western Ireland represents a useful case study of population dynamics in Atlantic salmon. Among the largest catchment in Ireland (2000km^2), the system is divided into two sub-catchments by a series of large lakes. It has remained unaffected by artificial stock enhancement or farm escapes, both of which have the potential to influence the genetic make-up of

wild populations (McGinnity *et al*. 2003). As stated earlier, spawning habitats are discontinuous, occurring in zones or reaches unique to each individual catchment (Davey & Lapointe 2004), and in the Moy, there are many such spawning areas distributed throughout making it easier to observe the consequences of natural evolutionary processes and responses to landscape features in the genetic structure of salmon populations. The present study represents in part a development of work previously undertaken on Atlantic salmon, e.g. Garant *et al.* (2000), Primmer *et al.* (2006), Dionne *et al*. (2008), by assessing the effect of the size of spawning habitats on genetic structure within a river. Small spawning areas (assuming spawning area size to be a reasonable surrogate of the number of spawners or, at least, of carrying capacity), especially if they are substantially isolated from other spawning areas, may be particularly important in this regard, since the populations that use them are susceptible to high levels of random genetic drift and predisposed to local extinctions. A study of genetic variation in neighbouring rivers provides an opportunity to explore inter-river factors that influence population structuring, in addition to those factors that promote intra-river structuring. The sampling design and analysis used here are spatially informed, rather than relying on opportunistically collected samples from familiar or easily accessible areas, and advances in technology both in the collection of genetic and geographical data and in the integration and analysis of these data, are utilised to delineate genetically significant population boundaries (for comprehensive reviews of the subject see Manel *et al.* (2003) and Storfer *et al.* (2007)). Thus the specific aims of the present study are to; 1) elucidate salmon population structure in the river Moy and nearby rivers using sampling of specific spawning sites, 2) determine how salmon populations are distributed relative to landscape features, and 3) assess the role of population demographics (potential for genetic drift) in promoting and maintaining genetic structure in Atlantic salmon, as a model for other fish species.

Materials & Methods

Study area and sampling

The Moy river catchment has 177km of main river channel draining an area of approximately 2,000km^2 (Figure 1). The system comprises two 6th order sub-catchment basins of approximately equal size. The eastern sub-catchment consists of main river channel and tributaries while the western sub-catchment consists of two large interconnected lakes into each of which flows a single main tributary, the Deel into Lough Conn (57km^2), and the Clydagh/Manulla into Lough Cullin (8km^2). Most recent estimates of pre-fisheries abundance of Atlantic salmon in the Moy are in the region of 100,000 adult fish, although returning stocks have been estimated in the mid 1970s to be as high as 270,000 (unpublished reports of the Standing Scientific Committee of the Irish National Salmon Commission). Approximately 60% of returning fish were harvested in commercial and recreational fisheries until 2006. Most commercial fisheries ceased in 2007, but angling catches accounting annually for an average 7,913 salmon (Anon 2001-2005) continue. An arterial drainage programme, initiated in 1960, has had major physical impact on the geomorphological structure of the river bed in large parts of the eastern main river system, resulting in fragmentation of spawning habitats.

Extensive field surveys undertaken during the winter of 2002/2003 identified the distribution of suitable spawning habitats throughout the system (Figure 1) on the basis of the presence of redds, and sightings of spawning fish at the time of survey. Hard copy map information was integrated into a geographical information system (GIS) (ArcView 3.2) held by the Central Fisheries Board, Co. Dublin (see Appendix 1 for geographical variables associated with each site sampled) and 17 discrete and fragmented spawning areas were selected, from which juvenile salmon were sampled

(collected by back pack electro-fishing during the summer months of 2003 and 2004). Three of these sampling sites were in the Deel (Glendavolagh, lower and upper Shanvolahan), which flows into Lough Conn, and three were from the tributaries flowing into Lough Cullin (Clydagh, upper and lower Manulla). The remaining 11 Moy sampling sites were in the eastern sub-catchment. Additional samples were obtained from spawning areas identified in four neighbouring river systems: the Brusna (2 locations), catchment area 95 km^2; Cloonaghmore (1), catchment area 130 km^2; Easky (1), catchment area 101 km^2; Ballysadare (1), catchment area 646 km^2. Samples were taken over an area of 0.5-1.5km to eliminate the potential effects of sampling families. In order to assess short-term temporal stability between cohorts, 24-48 0+ and 1+ parr were collected from each location (the only exception was the upper Shanvolahan site, where no 0+ specimens were caught). Samples of fin or muscle tissue were stored in 99% ethanol.

Molecular analysis

DNA was released from specimens by taking a small piece of muscle tissue (approximately $2mm^3$) and boiling at 99°C for one hour in 100µl 10% chelex™ resin solution. Individuals were screened for variation at 12 microsatellite loci: *Ssa*197, *Ssa*171, *Ssa*202, *Ssa*85 (O'Reilly *et al.* 1996); *Ssa*170 (EMBL accession number: AF525205); *Sssp*2201, *Sssp*2215, *Sssp*2216, *Sssp*2210, *Sssp*G7 (Paterson *et al.* 2004); and *SSOSL*85 and *SSOSL*417 (Slettan *et al.* 1995). Amplifications were carried out in 10µl volumes, including 1µl of chelex extracted DNA, 0.25mM dNTPs, 0.5U *Taq* DNA Polymerase (Promega™), 2µl of 5x buffer (Promega™) supplemented with 0.5mM $MgCl_2$ and 1µM each of forward (3'-end-labelled with IRD800 or IRD700 (MWG BIOTECH™)) and reverse primers. Reactions were carried out on a Hybaid™ thermocycler and consisted of an initial denaturation step of 3 min at 95°C, followed by 30 cycles of denaturation at 95°C for 30s, annealing at 56°C for 30s and extension at 72°C for 30s. Alleles were resolved on 6% denaturing polyacrylamide gels using a LiCOR4200™ automated DNA sequencer. Allele sizes and genotypes were determined using a combination of a molecular weight marker (LiCOR™) and allele cocktail standards to ensure consistent scoring of genotypes.

Statistical analysis

Genetic data from 1606 salmon (from 17 sites within the Moy catchment and five from neighbouring systems) were analysed. Genotypic data was checked for inconsistencies and errors using Micro-Checker software v 2.2.3 (van Oosterhout *et al*. 2004). Population descriptive statistics (e.g. observed and expected heterozygosity and allelic richness) were calculated using FSTAT 2.9.3 (Goudet 2001). GENEPOP 3.0 (Raymond & Rousset 1995) was used to test samples from all locations and all loci for conformance with Hardy-Weinberg expectations and gametic disequilibrium. The modified false discovery method (Narum 2006) was used to correct for multiple tests. All loci were checked for evidence of natural selective pressure using the LOSITAN selection detection workbench, which uses the F_{ST} outlier approach as described in Beaumont & Nichols (1996). Pairwise and overall F_{ST} values were calculated using F-STAT 1.2 (Goudet 1995). Significance values for each locus were determined by bootstrapping over loci and permutation over samples, and significance values for all loci were calculated by jack-knifing over loci.

STRUCTURE (Pritchard *et al*. 2000) analysis was used to estimate the distribution and number of major population groups. This was carried out using an admixture model (where each individual is deemed to have drawn some fraction of its genome from each of the populations under consideration) with correlated allele frequencies (which assumes that frequencies in the different

populations are likely to be similar due to migration or shared ancestry). Burn-in and MCMC (Markov chain Monte Carlo) lengths of 10,000 each were used (a number considered to be sufficient to achieve reliable results (Evanno *et al.* 2005)). Twenty runs were carried out in order to quantify the amount of variation of the likelihood for each K (putative number of populations; in this case K values of one to 20 were simulated) and the mean value of the log likelihood ($L(K)$) of the data was calculated. The most likely value of K was identified using the maximal value of $L(K)$ returned by STRUCTURE (e.g. Zeisset and Beebee 2001). A bar plot showing each individual as a line segment partitioned in K coloured components (representing the individual's estimated membership coefficients in the K clusters) was created from the STRUCTURE output using the program DISTRUCT (Rosenberg, 2002). Isolating mechanisms influencing genetic differentiation among populations were identified using the software BARRIER 2.2 (Manni *et al.* 2004), which implements a method based on computational geometry and a Monmonier's maximum-difference algorithm to identify possible barriers to gene flow. In the first instance, the number of barriers among samples was identified on the basis of geomorphological features, which are assumed to impede migration and gene flow between putative populations. This assessment was compared with those estimated using BARRIER 2.2. The robustness of the computed barriers was tested following the approach described in the BARRIER manual, which is based upon the analysis of a 100 re-sampled bootstrapped matrices of the pairwise F_{ST} data. Genetic distances (from Nei's D_A (1983)) were calculated using POPULATIONS (http://www.cnrs-gif.fr/pge) and a neighbour joining dendrogram was constructed from these using TREEVIEW (http://taxonomy.zoology.gla.ac.uk/rod/rod.html).

Effective population sizes were estimated using the linkage disequilibrium method of Bartley *et al.* (1992) in NeEstimator (Peel *et al.* 2004) (other software options and methods were investigated and all gave similar results). Geographical data on a number of spawning area specific variables within the Moy were acquired from the field data collected in the project and integrated into the GIS. Key variables included the distance between spawning areas, the size of spawning areas, and the height of a spawning area above sea level (Appendix 1). Data on variables related to discharge at each of the identified spawning areas were also estimated from the GIS, including the size of the contributing catchment, stream order (Shreve 1969), and winter discharge levels as measured by the National Hydrological Gauging Station network. The relationships between genetic differentiation and geographic/demographic factors were assessed using GESTE 1 (Foll & Gaggiotti 2006) which relates F_{ST} values for each population to geographic/demographic parameters using a generalised linear model. This program only allows for analysis of two factors at a time, but allows the assessment of interactions between variables other than F_{ST}. Specifically, we examined the relationships between spawning area size, N_e, and F_{ST}, and the relationship between distance from the sea, N_e and F_{ST}. In addition, these factors were examined at two levels; the entire Moy catchment, and the eastern tributaries (excluding the Cloonacool) only. GENALEX 6 (Peakall & Smouse 2006) was used to perform a Mantel test to investigate the linear relationship between genetic differentiation in the Moy, as determined by F_{ST}, and geographic distance (in river km between sites). Spatial autocorrelation was applied to investigate the spatial genetic structure within the Moy at the individual level using GENALEX 6, and following methods proposed by Smouse & Peakall (1999) allowing for the analysis of spatial genetic structure for multi-allelic and multi-locus data sets.

Evidence for genetic population bottleneck-effects was sought using some of the methods suggested by Ramstad *et al.* (2004). Firstly, evidence of increased heterozygosity relative to that expected at mutation-drift equilibrium was assessed using the program BOTTLENECK 1.2.02 (Cornuet & Luikart 1996) assuming a two-phase model (TPM) of mutation, and the significance of heterozygosity excess over all loci was assessed with a Wilcoxon sign-rank test. The second approach was to test for a mode shift away from a normal L-shaped distribution of allele

frequencies (Luikart *et al*. 1998), also using BOTTLENECK. Reductions in population size detected using both of these methods could be associated with recent demographic declines. The third approach was to measure M-ratio, which is the mean ratio of number of alleles to range in allele size (Garza & Williamson 2001) and this was done using the program AGARst v. 3.3 (Harley 2002). Finally, reductions in the proportions of rare alleles, which can also signify historical demographic declines, were noted.

Results

No statistically significant differences in genetic composition were found between year-classes at any of the locations sampled, which indicates temporal stability of allele frequencies. Thus, cohorts were combined and all further analyses were undertaken on composite samples. The loci used showed no evidence of pair-wise linkage disequilibrium, nor was there evidence that any were under the influence of natural selection within the confines of the present study. Micro-Checker did not reveal any problems with null alleles or consistent genotyping errors. Composite samples were found to be in Hardy-Weinberg equilibrium in all but six of the 22 locations (Table 1). Where deviations occurred they were not consistent across loci (data not shown). Summary descriptive statistics of levels of variability at each location are given in Table 1. Most spatial samples were significantly differentiated from one another, with the exceptions of those from the spawning areas located in the upper and lower Shanvolahan (western Moy) and the upper and lower Brusna river (outside Moy) (Table 2). F_{ST} among all samples was 0.024 ($p<0.001$) (with per locus F_{ST} ranging from 0.021 to 0.029) and within the Moy only, was 0.020 ($p<0.001$). Pairwise population F_{ST} ranged from 0.002 to 0.057 (Table 2).

Results from STRUCTURE, suggested that a *K* value of nine best described population groupings, since the estimated log probability of the data (L(*K*)) peaked at this value, and variance between runs of successive values of *K* increased substantially thereafter (Figure 2a). A bar plot (Figure 2b) indicated that these clusters were probably associated with the Deel, Clydagh, Manulla, eastern Moy, Cloonacool, and with the nearby smaller rivers the Brusna, Cloonaghmore, Easkey and Ballysadare. These groupings are consistent with the physical geography of the Moy catchment and surrounding area (lakes in the west, extensive tributaries joined by the main river stem in the east, separate catchments), and are supported by the results of the BARRIER analysis (Figure 3). All seven barriers (a to g) identified by the Monmonier's algorithm were highly supported by bootstrap analysis. The most important barriers identified by that algorithm, in order, were the lakes (north/south (a) and east/west (b)), the marine environment (c & d), isolation within the Lough Cullin tributaries, probably due to genetic drift (e & f), and the top of the Moy system (g), the latter possibly being due to removal of spawning habitats because of arterial drainage. A neighbour joining dendrogram from Nei's D_A (Figure 4) indicated patterns of spatial variation associated with barriers identified in the previous analysis, i.e. an east/west division; high levels of differentiation within the western catchment; lower levels of differentiation within the eastern Moy catchment where tributary populations were characterised by low bootstrap values, and separation of neighbouring catchments from the Moy (with the exception of the Brusna, which groups closely with the eastern Moy).

Results of the GESTE 1 analysis (Foll & Gaggiotti 2006) used here to help identify the environmental factors that are responsible for the observed spatial structuring of genetic diversity show the importance of spawning area size in determining the level of genetic differentiation among samples within the eastern Moy (Table 3). The model containing spawning area size only (model 3) had the highest posterior probability of all those calculated in this part of the river system. Here spawning area size was negatively correlated with Fst (Pearson correlation: $r = -0.44$, n=36,

$p<0.05$), which indicates that as spawning area size decreases the level of genetic differentiation between populations increases. GESTE 1 analysis suggests N_e as the most important factor when data from both eastern and western parts of the river system are combined. Estimates of N_e in the Moy catchment were lower by an order of magnitude (4,613) than might have been predicted (see Table 1); given that the escapement of adult salmon to the Moy is estimated in the region of 40,000. Isolation by distance analysis (Mantel test) shows a positive relationship between geographic distance and genetic differentiation (F_{ST}) considered at the scale of the entire river Moy ($r^2=0.2652$, $p<0.001$) and within the eastern basin of the river ($r^2=0.1303$, $p<0.013$). Spatial autocorrelation (Figure 5) suggests a patch size, below which there is an absence of assortative mating, of approximately 29km.

There was limited evidence for contemporary population size reductions (bottlenecks). However, heterozygote excess, indicative of recent declines occurred in three tributaries of the Moy (Table 1) two of which are characterised by limited spawning habitat (Lower Shanvolahan and Eighnagh). Mode shifts were not evident in any of the sampling sites. In the case of a test for historical population reductions, M-ratios ranged from 0.65 (Upper Manulla) to 0.85 (Owengarve). Lower values are associated, with low proportions of rare alleles, suggestive of historical demographic declines.

Discussion

Seventeen spawning areas within 13 tributaries of the Moy were identified *a priori* for sampling and analysis in this study. Significant population structuring was detected between all tributaries, and in some cases within tributaries, and is reflective of the discontinuous distribution of spawning habitats (Davey & Lapointe 2004). Temporal stability of allele frequencies was observed at all locations sampled, suggesting that fry and parr within sampling sites represent the same populations and that movement of older juveniles is largely consistent with the dispersal of younger fish within nursery areas associated with the spawning zones sampled. This suggests that the collection of juveniles is the appropriate strategy for elucidating population structure in Atlantic salmon. Six sites showed deviations from Hardy-Weinberg expectations, but these deviations never occurred at more than one or two of the 12 loci examined, and were not consistent across loci. Given that the populations showing deviations were not characterised by any losses of variability (which could suggest a potential family effect) they were included in the analysis and were considered to be representative of contemporary populations in the area. The level of F_{ST} observed in the present study was comparable to within river levels observed among salmon populations in Canadian (Garant *et al*. 2000), Russian (Primmer *et al*. 2006) and other European rivers (Vähä *et al*. 2007). The observed levels of differentiation were consistent across loci (data not shown) and although variation at *Ssa*202 is believed to be weakly sex linked (Gilbey *et al*. 2004), and may be under the influence of natural selection (de Eyto *et al*. 2007), there was no evidence from this study that patterns of variation differed from those at any of the other loci used.

Our results suggest that salmon in the Moy can be broadly divided into five population groupings using the Bayesian clustering approach of Pritchard *et al*. (2000) and that these groupings are consistent with potential obstacles to gene flow suggested by BARRIER analysis. It would seem that the single most important of these physical impediments in the Moy river system are the large lakes which effectively divide the catchment into distinct three areas; north-west, south-west and east. These lakes possibly limit gene flow among the catchments, in a way that might be similar to the kind of processes described by Dionne *et al*. (2008) in their 'difficulty of upstream migration' index. Lake migration behaviour in smolts has been previously suggested by Aarestrup & Koed

(2003) to be adaptive, and it could be reasonable to similarly assume that the spawning migration of adult fish through lakes would also have an adaptive basis, although we are unaware of studies on this phenomenon. While this kind of pattern has previously been demonstrated in Pacific salmon species (e.g. sockeye salmon, Ramstad *et al.* (2004)), this is the first study to show that lakes, by limiting within river migration, can be a significant landscape feature in the shaping of genetic population structure in Atlantic salmon. Interestingly, the levels of differentiation observed between the fish in the Moy and some of its neighbouring rivers (separated from each other by the sea) are substantially less than the amount of differentiation found between fish in the Moy's eastern and western catchments, which are separated from each other by the presence of the freshwater lakes. Individual rivers have previously been considered (and observed) to represent population units (e.g. King *et al.* 2001; Dillane *et al.* 2007) and studies have generally shown that differentiation within rivers is smaller than between, implying that the sea distance between river mouths is critical as a discriminator of populations. Our findings suggest that this may not always be the case. One explanation might be that gene flow between populations of ocean migrating Atlantic salmon occurring in the Moy and adjacent Brusna river, could be as great if not greater than within the Moy.

Population size, either estimated in terms of spawning area size or based on genetic data N_e, was found to be an important variable in explaining relationships between populations in the Moy; differentiation among small populations being significantly greater than among large ones. Here, random genetic drift accentuated by small population numbers, might be expected to be the principal cause. These results suggest that the size of spawning areas (which should be finite with respect to the number of spawning fish that can be potentially accommodated) might be a useful approximation of population size at carrying capacity. This relationship is likely to apply to salmon populations generally and could prove informative in elucidating observed population structure in other species, where habitats necessary for reproduction are specialised and defined in space. GESTE analysis undertaken at the level of the entire catchment identifies N_e as being an important determinant of population structure in the Moy. It is unclear why this effect is not still apparent when the western Moy samples are excluded from the analysis. It has been suggested by Foll and Gaggiotti (2006), that the analytical power of GESTE can be limited by the number of populations included in the analysis; an analysis of the eastern Moy (11 distinct populations) being substantially poorer than analysis of the populations in the Moy as a whole (17 populations). Foll and Gaggiotti (2006) warn that GESTE has limited power with <10 populations. N_e estimates were approximately an order of magnitude lower than local estimates of census population size. Estimates of population size using genetic data have previously been demonstrated to provide very small values of N_e compared with census population size (Vucetich *et al.* 1997).

It is apparent from the results presented here that random genetic drift in small isolated populations, usually constrained by the availability of spawning habitat, can promote substantial genetic differentiation. However, there are a number of populations in the Moy where it is evident that drift is likely to have been amplified by reductions in population size due to either historical or contemporary bottle neck events, the signature of which remain still in the genetic make-up of the current population. Low M-ratio values and proportions of rare alleles, indicative of historical reductions in population size, were observed in the present study in a few cases. Similarly to Schöfl & Schlötterer (2006), we did not determine whether M-values were lower than expectations in an equilibrium situation (given that we could not be sure of the mutation model most closely associated with the microsatellite markers used here). M values below 0.7 have been associated with declines in population size or with island/founder effects, while values of M above 0.8 are usually seen in demographically stable populations (Garza & Williamson, 2001). The latter authors showed that the value of M is dependant on a number of factors, most notably the number of loci screened and number of individuals sampled. Here, taking both the sample size and the observed

values of M into account, it seems reasonable to infer that some populations (e.g. the Glendavolagh and Clydagh tributaries, as well as the neighbouring Easkey and Ballysadare rivers) exhibit signs of historical losses (or founder effects), and these are mostly populations that are highly differentiated genetically from neighbouring population groupings. The Ballysadare is an interesting case in this regard. Records show the installation of a series of fish passes and the founding of a salmon population from twelve fish, transplanted directly into the river from the Moy in mid 1800s (Wilkins, 1989).

Straight forward isolation by distance (IBD) analyses can provide a useful insight into the genetic structure of salmon populations particularly, in linear fluvial systems and where spawning habitats are distributed continuously throughout a system. In the Moy such simple analysis of IBD is confounded by the geomorphological complexity of the river system, especially the location and size of the lakes, and by the fragmented nature of areas available for reproduction. Spatial autocorrelation analysis is an important refinement of classical IBD, providing higher resolution information, informative IBD signals over smaller scales, and the delineation of areas or 'patches' that are ecologically relevant. The typical patch size detected in the Moy study (29km) is similar to the patch size (34km) detected by Primmer *et al*. (2006) in the Varzuga river on the Kola Peninsula using this approach. This distance corresponds with the typical distances between tributaries in the eastern Moy catchment (Table 2), which tend to group together both with STRUCTURE and genetic distance analyses. While patch size will reflect to a large degree the heterogeneity of spawning areas, the consistency between patch size in our study and that of Primmer *et al.* (2006) suggests that some of the biological characteristics inherent in salmon, e.g. mobility and dispersal of juveniles, homing fidelity, promote genetic isolation-by-distance and play an important role in genetically separating populations within large river systems.

Salmon within tributaries in the eastern Moy constitute the largest observed grouping of populations in the present study. The populations within this group are potentially larger (Table 1) and associated spawning areas are generally closer together than in other parts of the river system. There may therefore be increased opportunities for gene flow between them. Small numbers of successfully spawning migrants can be enough to prevent detectable genetic differentiation at neutral loci (Grant & Waples 2000). While spawning habitats in the eastern Moy are now fragmented into tributaries, there were previously more continuous spawning opportunities throughout the main channel linking these tributaries. Drawings from a pre-drainage engineering survey of the Moy (map record held by the Office of Public Works, Ballina, Co. Mayo, Ireland) show that the main channel of the Moy (Figure 1) historically consisted of a single and very extensive area of suitable spawning habitat, distributed almost continuously over the entire main stem of the river and linking many of what are now seemingly discrete areas of tributary spawning habitat of varying sizes. Gravels from this large area of spawning habitat were excavated as part of an extensive arterial drainage of the river system undertaken in the 1960s. As a consequence of this activity, there are now few spawning opportunities in the main channel of the river and this could account for the genetic differentiation between fish sampled in spawning areas at Cloonacool in the upper reaches of the system and the other populations that from the eastern Moy grouping (Figure 1).

Salmon from small neighbouring rivers, discharging directly into the sea, namely the Brusna, Cloonaghmore and Easkey, group closely with the eastern Moy populations. This may be the result of gene flow from the large eastern Moy salmon production area (estimated to produce 75-80% of the total fish production of the catchment; also supported by N_e estimates (Table 1)) to these smaller neighbouring rivers, and would be consistent with the mainland-island metapopulation concept discussed in Hanski & Simberloff (1997), and also with results of a study undertaken by Hindar *et*

al. (2004) of rivers flowing into Hardangerfjorden in Norway. On the other hand, Palstra *et al* (2007) caution against the assumption that directionality of gene flow is from large to small populations and suggest that while large populations can serve as 'sources' over contemporary time scales, the reverse may be the case on evolutionary time scales.

The combination of geo-spatial information derived here from the GIS platform with molecular genetics suggests that the geographical information on the spatial positioning or patterning of spawning areas offers the opportunity to detect population groupings of salmon in other rivers. Using this knowledge it should be possible to predict the occurrence and extent of genetic population structuring in this species and to design appropriate sampling strategies for other rivers. Furthermore, geo-spatial modelling (Davey & Lapointe,2004) and the application of advances in remote sensing such as high resolution aerial digital photography and satellite imaging make the collection of highly accurate salmon habitat information at large regional scales practicable, superseding the labour intense field approach used in this study for identifying and mapping of spawning habitats. The principles of the approach illustrated here could also be applied to other freshwater species, particularly salmonid species, where habitat preferences, reproductive strategies and life histories are well known.

In conservation biology and resource management, the landscape genetics approach has the potential to identify evolutionary significant units, management units or conservation units (e.g. Youngson *et al.* 2003) allowing insights into the ecological and geographical processes that promote population structuring. This ability to identify biological organisation at the below species level, could be very important with respect to determining impact of climate change, selective resource exploitation, introgression of cultured strains with wild populations and disease impacts (McGinnity *et al*. 2003; de Eyto *et al*. 2007). There exists an increasing demand from management authorities for genetic stock identification (GSI). Critical to the successful application of GSI is an ability to identify distinct population or management units, and predict where these may occur. The local population is the basic unit of production and evolution, and therefore should be the preferred unit of management. As has been observed here, salmon populations within river systems can be numerous, small in size, and structure may be influenced by a number of geographic and demographic factors. The combined analysis of genetics and landscape features illustrated here offers the best opportunity for effective future management of this and other similar species.

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727

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741 Figure 1. The Moy, Brusna, Cloonaghmore, Easkey and Ballysadare catchments. Sampling areas
742 are shown in yellow. Adjacent spawning zones (only within the Moy) are shown in black. The
743 green area represents historical spawning areas which existed prior to the drainage work carried out
744 in the 1960s. (1 Glendavolagh; 2 Lower Shanvolahan; 3 Upper Shanvolahan ; 4 Clydagh; 5 Lower
745 Manulla; 6 Upper Manulla; 7 Pollagh; 8 Glore; 9 Trimoge; 10 Killeen; 11 Upper Spaddagh; 12
746 Lower Spaddagh; 13 Sonnagh; 14 Eighnagh; 15 Owengarve; 16 Lower Cloonacool; 17 Upper
747 Cloonacool; 18 Lower Brusna; 19 Upper Brusna; 20 Cloonaghmore; 21 Easkey; 22 Ballysadare)
748
749 Figure 2a. Mean L(k) (± SD) over 20 runs for each k value in STRUCTURE analysis
750
751 Figure 2b. Bar plot of a STRUCTURE k=9 simulation. Each bar constitutes an individual fish, and
752 the y-axis measures the proportion of each individual attributable to each cluster, which can be
753 estimated from the colour composition of bars
754
755 Figure 3. Output from BARRIER analysis showing where barriers (a-g) to gene flow occur within
756 the study area. The blue lines represent the Voronoï tessellation of the population samples (in red)
757 according to their geographical locations, and corresponding Delaunay triangulation are shown by
758 green lines. See Manni *et al*. 2004 for further detail.
759
760 Figure 4. Neighbour joining phylogram from Nei's D_A, with bootstrap values
761
762 Figure 5. Correlogram showing genetic correlation as a function of geographic distance over all
763 Moy samples (Intercept 27.12). r is the genetic correlation, U and L dotted lines indicate the 95%
764 confidence interval about the null hypothesis of no genetic structure and error bars about r indicate
765 95% confidence interval determined by bootstrapping
766

Table 1. Levels of variability, diagnostic parameters for bottlenecks, and estimated population sizes for each sampling location. **H_E** – gene diversity, **H-W p-value** – probability of compliance with Hardy-Weinberg expectations, **N_A total** - total number of alleles observed, **N_A mean**– average number of alleles across loci, **A_R** – allelic richness. **H_O excess p-value** – probability of no heterozygote excess assessed with Wilcoxon sign rank test, **M-Ratio**, **Proportion of rare alleles** – proportion of the total number of alleles observed at each location which occur at a frequency of less than 0.1. **N_e** – effective population size with associated 95% confidence intervals using the linkage disequilibrium method in NeEstimator, **Spawning area** – Size of available spawning area (m^2). Significant p-values for a global probability value of 0.05 are given in bold (adjusted p-value is 0.013, after correction for multiple tests using the modified false discovery method of Narum (2006))

		n	H_E	H-W (p-value)	N_A total	N_A mean	A_R	H_O excess (p-value)	M-Ratio	Proportion of rare alleles (%)	N_e (95% CI)	Spawning area (m^2)
1. Glendavolagh	*West*	96	0.87	0.169	166	13.8	10.4	0.026	0.71	76.5	192 (162-234)	31414
2. Lower Shanvolahan		48	0.88	**0.002**	168	14.0	11.5	**0.001**	0.73	75.6	246 (173-416)	14697
3. Upper Shanvolahan		24	0.87	0.651	150	12.5	11.8	0.259	0.67	69.3	125 (83-242)	n/a
4. Clydagh		96	0.87	**0.000**	187	15.6	11.4	0.055	0.77	80.7	220 (186-267)	22767
5. Lower Manulla		48	0.86	**0.005**	160	13.3	10.7	0.575	0.69	72.5	79 (68-93)	45552
6. Upper Manulla		48	0.85	0.336	142	11.8	9.9	0.311	0.65	70.4	58 (51-67)	27712
7. Pollagh	*East*	93	0.88	0.090	201	16.8	12.1	0.055	0.82	82.1	259 (216-322)	66420
8. Glore		111	0.90	0.065	211	17.6	12.5	0.021	0.83	83.4	489 (377-690)	55891
9. Trimoge		95	0.89	0.076	208	17.3	12.5	**0.000**	0.83	79.3	456 (345-667)	70183
10. Killeen		96	0.89	**0.001**	212	17.7	12.7	0.032	0.82	81.6	168 (148-193)	51375
11. Upper Spaddagh		48	0.89	**0.001**	184	15.3	12.1	0.190	0.75	76.1	105 (89-127)	30865
12. Lower Spaddagh		48	0.89	**0.001**	188	15.7	12.5	0.102	0.78	79.8	160 (127-213)	30865
13. Sonnagh		96	0.89	0.067	206	17.2	12.5	0.017	0.84	81.6	375 (294-510)	29260
14. Eighnagh		96	0.89	0.018	199	16.6	12.2	**0.005**	0.79	80.4	370 (282-529)	15304
15. Owengarve		95	0.89	0.014	218	18.2	12.9	0.039	0.85	83.9	1042 (620-3074)	59928
16. Lower Cloonacool		48	0.88	0.225	180	15.0	12.1	0.102	0.77	80.6	211 (158-314)	99424
17. Upper Cloonacool		48	0.86	0.042	167	13.9	11.4	0.311	0.75	77.8	58 (52-65)	n/a

773

18. Lower Brusna	*Other rivers*	48	0.89	0.022	184	15.3	12.4		0.065	0.75	79.3		376 (240-835)	n/a
19. Upper Brusna		48	0.89	0.577	184	15.3	12.2		0.133	0.77	77.7		169 (132-229)	n/a
20. Cloonaghmore		89	0.87	0.372	190	15.8	11.1		0.515	0.79	77.4		394 (294-588)	n/a
21. Easkey		96	0.88	0.018	196	16.3	11.8		0.076	0.77	77.0		276 (227-351)	n/a
22. Ballysadare		91	0.85	0.018	163	13.6	9.9		0.455	0.68	74.8		159 (136-190)	n/a

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Table 2. Matrix of pairwise F_{ST} estimates (below diagonal) and pairwise geographic distances between sampling sites within the Moy (above diagonal). All sampling sites are significantly differentiated from one another ($p<0.05$, corrected to 0.014 using false discovery method), except those given in bold. Samples are numbered as in Table 1.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
1		17.2	20.9	67.5	69.7	82.8	74.5	78.5	85.0	78.5	82.9	79.0	94.1	105.2	116.6	107.6	111.5	-	-	-	-	-
2	0.009		1.7	52.7	54.9	68.0	64.7	63.7	70.3	61.8	68.1	64.2	79.3	90.4	101.8	92.8	96.7	-	-	-	-	-
3	0.010	**0.004**		54.5	56.7	69.7	66.4	65.5	72.0	63.5	69.8	65.8	81.1	92.1	103.5	94.6	98.4	-	-	-	-	-
4	0.042	0.027	0.030		33.2	46.3	43.0	42.1	48.6	40.1	46.4	42.5	57.7	68.7	80.1	71.2	75.0	-	-	-	-	-
5	0.037	0.024	0.034	0.039		41.8	38.6	37.6	44.1	35.6	41.9	38.1	53.2	64.3	75.7	68.7	72.6	-	-	-	-	-
6	0.039	0.037	0.047	0.047	0.022		51.6	50.7	57.2	48.7	55.0	51.1	66.3	77.3	88.7	79.8	83.6	-	-	-	-	-
7	0.025	0.017	0.019	0.027	0.026	0.037		19.8	30.8	22.4	28.7	24.8	39.9	51.0	62.4	53.4	57.3	-	-	-	-	-
8	0.022	0.013	0.017	0.027	0.026	0.034	0.008		29.9	21.4	27.7	23.8	38.9	50.0	61.4	52.4	56.3	-	-	-	-	-
9	0.021	0.016	0.017	0.028	0.029	0.033	0.011	0.007		23.4	29.7	25.8	40.9	52.0	63.4	54.4	58.3	-	-	-	-	-
10	0.027	0.019	0.020	0.019	0.024	0.031	0.011	0.007	0.008		14.7	10.8	25.9	37.0	48.4	37.2	41.1	-	-	-	-	-
11	0.027	0.021	0.020	0.029	0.030	0.034	0.016	0.011	0.012	0.008		3.9	25.1	36.2	47.6	38.6	42.5	-	-	-	-	-
12	0.026	0.016	0.020	0.026	0.023	0.029	0.010	0.009	0.010	0.006	0.007		23.7	34.8	46.2	34.8	38.6	-	-	-	-	-
13	0.024	0.018	0.020	0.024	0.025	0.035	0.007	0.007	0.010	0.007	0.011	0.006		18.9	30.2	21.3	25.1	-	-	-	-	-
14	0.027	0.020	0.022	0.030	0.031	0.039	0.017	0.015	0.013	0.013	0.008	0.012	0.012		30.4	20.5	24.4	-	-	-	-	-
15	0.027	0.018	0.021	0.024	0.028	0.037	0.011	0.013	0.012	0.008	0.011	0.008	0.009	0.012		32.8	36.6	-	-	-	-	-
16	0.034	0.027	0.022	0.024	0.033	0.039	0.012	0.017	0.015	0.014	0.021	0.016	0.016	0.020	0.013		3.9	-	-	-	-	-
17	0.047	0.038	0.032	0.036	0.047	0.048	0.022	0.030	0.026	0.026	0.030	0.025	0.029	0.030	0.024	0.006			-	-	-	-
18	0.025	0.019	0.016	0.027	0.032	0.037	0.013	0.013	0.009	0.011	0.009	0.007	0.011	0.012	0.010	0.018	0.024		-	-	-	-
19	0.031	0.026	0.018	0.029	0.035	0.044	0.015	0.018	0.014	0.016	0.014	0.014	0.013	0.016	0.013	0.017	0.021	**0.002**		-	-	-
20	0.051	0.044	0.036	0.036	0.053	0.051	0.027	0.029	0.027	0.023	0.029	0.030	0.027	0.033	0.026	0.024	0.033	0.024	0.026		-	-
21	0.035	0.034	0.023	0.037	0.044	0.043	0.022	0.021	0.019	0.016	0.018	0.020	0.021	0.021	0.017	0.020	0.032	0.019	0.019	0.025		-
22	0.046	0.044	0.037	0.047	0.057	0.057	0.039	0.039	0.035	0.032	0.041	0.037	0.037	0.041	0.037	0.037	0.049	0.036	0.037	0.051	0.037	

Table 3. Posterior probabilities of nine possible models associated with (a) spawning area size and N_e and (b) distance from the sea and N_e within the entire Moy catchment and within the eastern Moy tributaries only. The analysis was undertaken to both include and exclude the possible effect of the lakes on this analysis. These probabilities illustrate the degree of association between geographic/demographic factors and genetic differentiation. Models with the highest posterior probabilities include the factors most strongly associated with the observed patterns in genetic differentiation.

(a)		**Probability**	
Model	**Factors**	**Entire Moy**	**Eastern Moy only**
1	Constant	0.46	0.81
2	Spawning area size	0	0
3	Constant & Spawning area size	0.17	0.11
4	N_e	0	0
5	Constant & N_e	0.34	0.08
6	Spawning area size & N_e	0	0
7	Constant, Spawning area size & N_e	0.03	0
8	Spawning area size, N_e & interaction	0	0
9	All	0	0
(b)			
1	Constant	0.38	0.83
2	Distance from sea	0	0
3	Constant & Distance from sea	0.03	0.07
4	N_e	0	0
5	Constant & N_e	0.54	0.08
6	Distance from sea & N_e	0	0
7	Constant, Distance from sea & N_e	0.05	0.01
8	Distance from sea, N_e & interaction	0	0
9	All	0	0.01

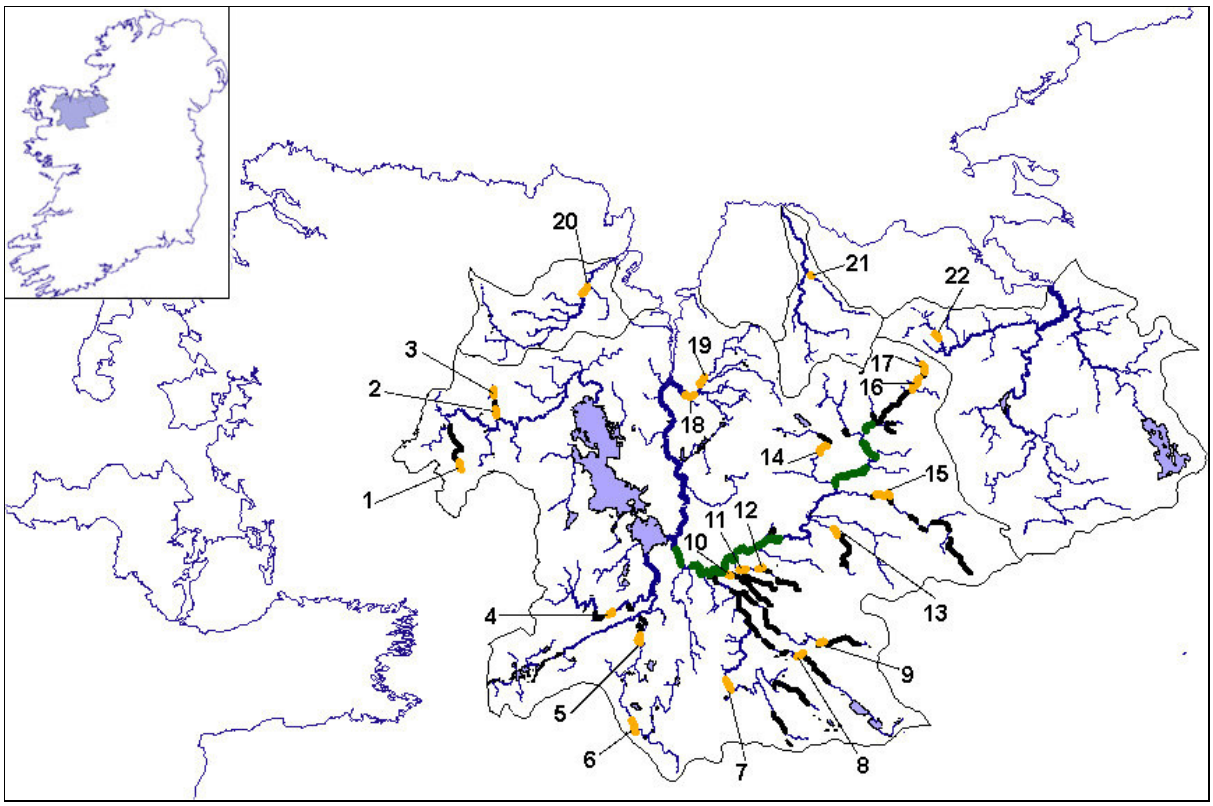
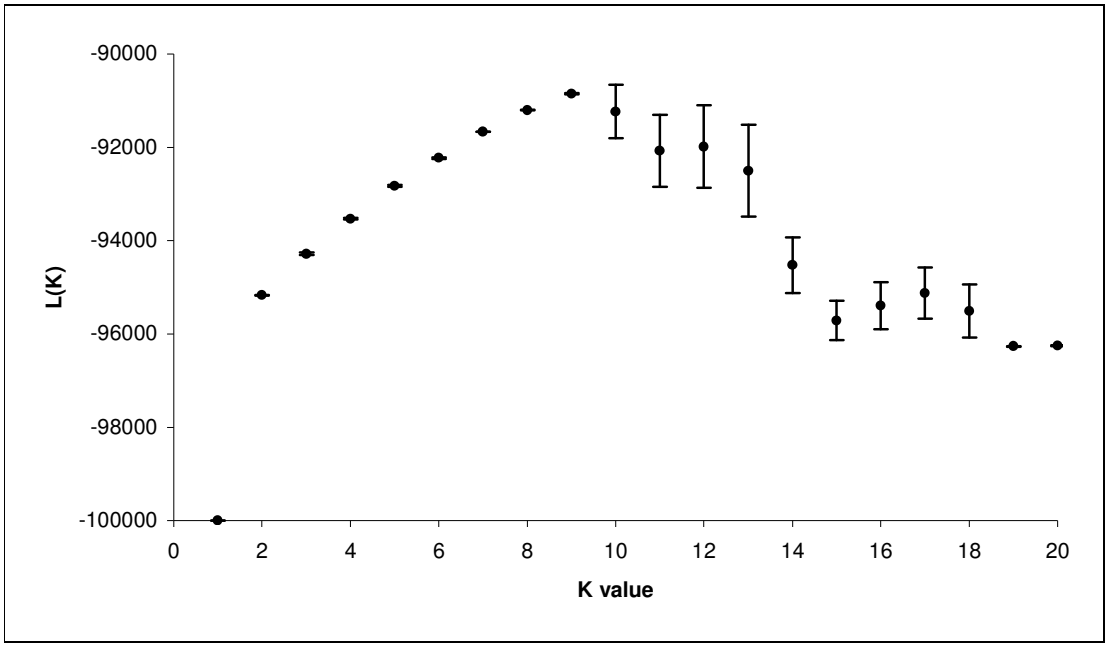


Figure 1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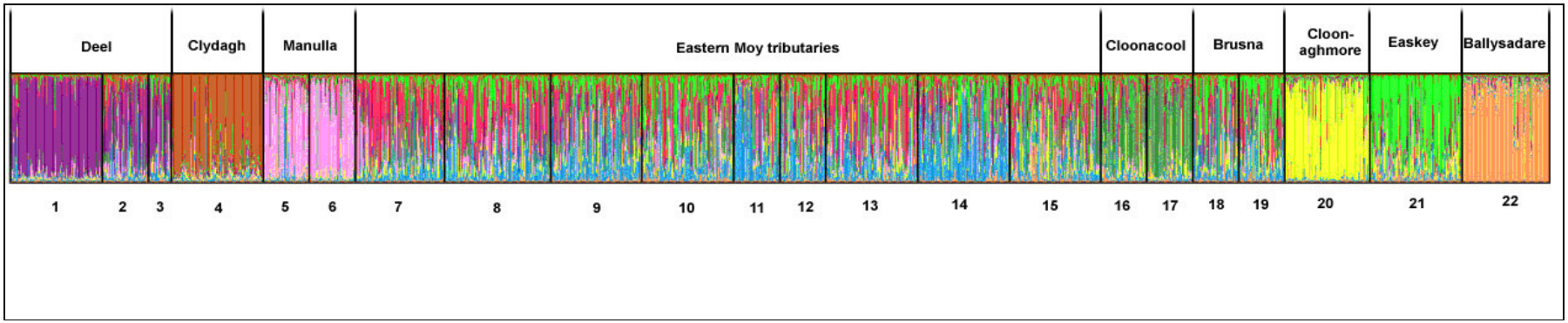
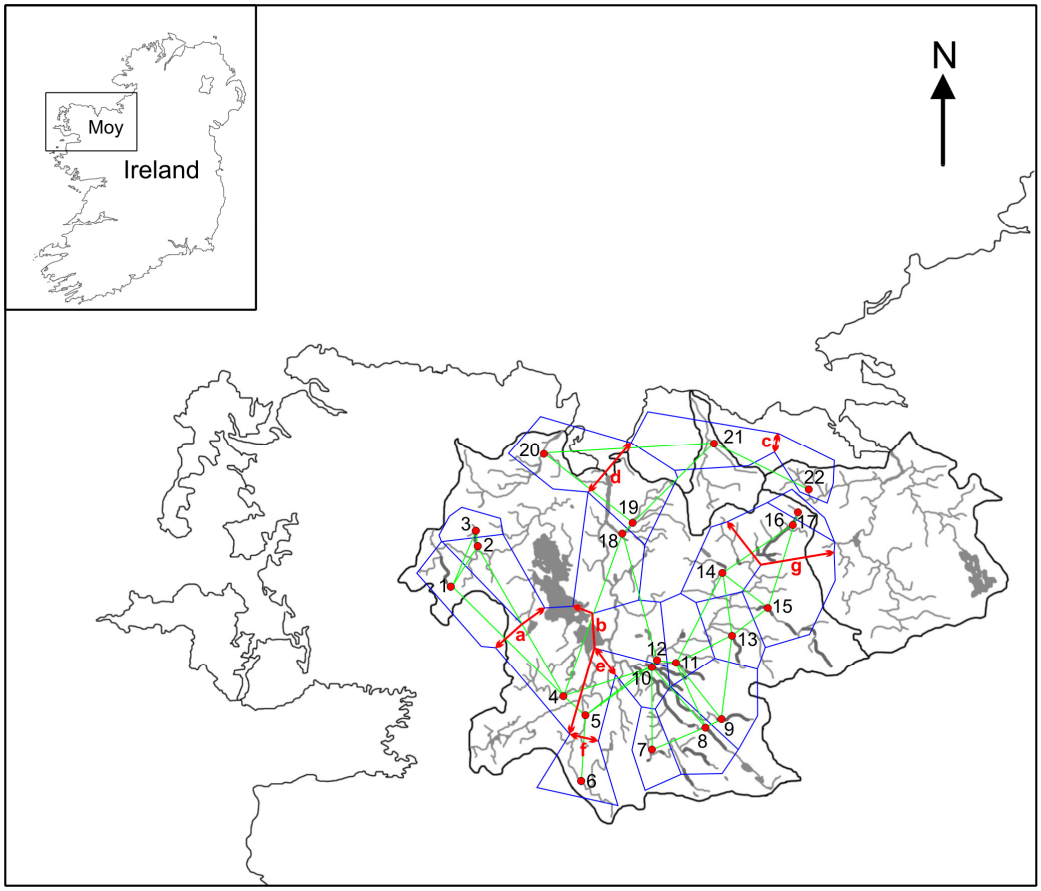
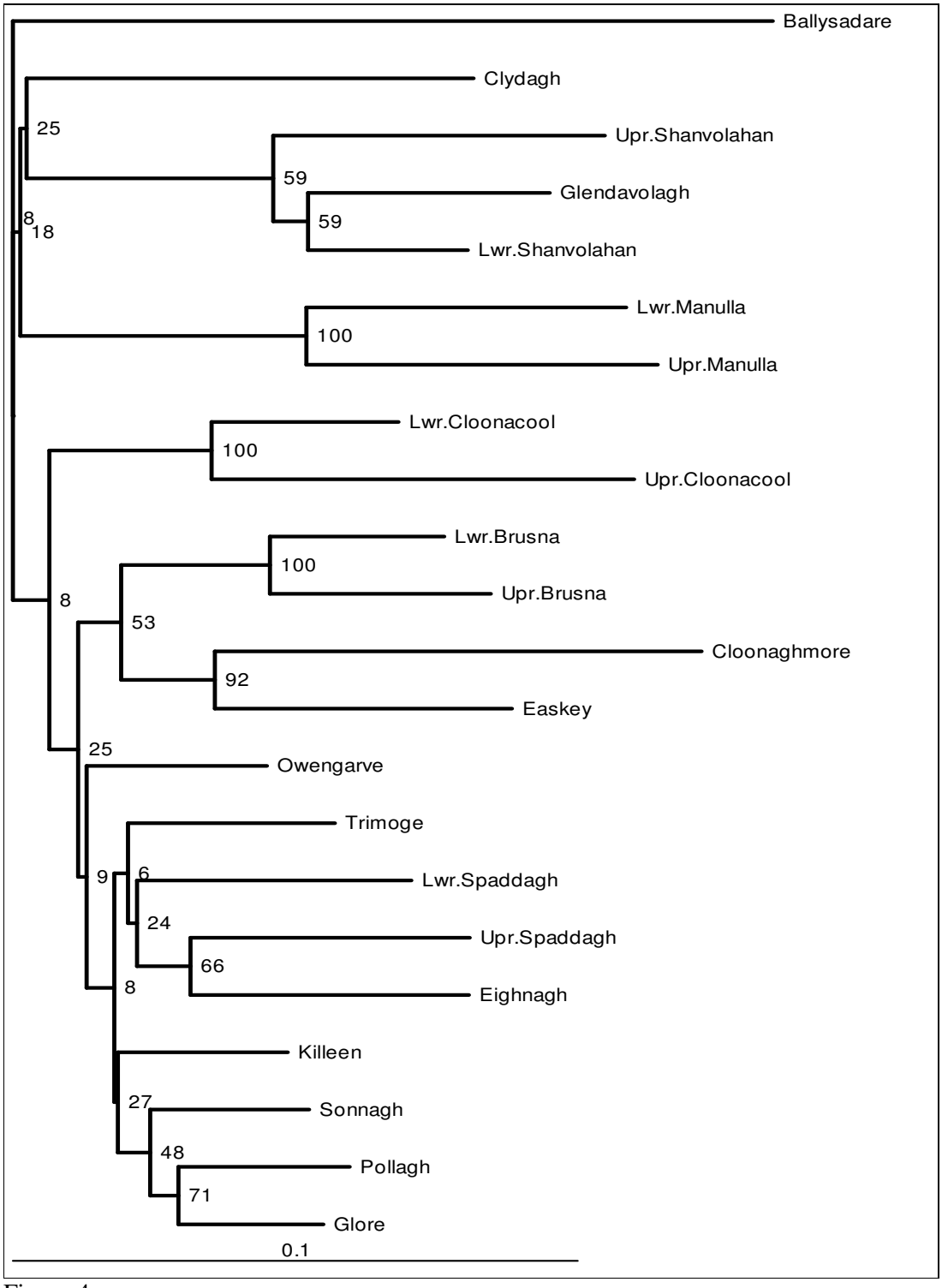
-92000
-94000
-96000
-98000
-100000
L(K)
0
2
4
6
8
10
12
14
16
18
20
K value
Figure 2a.


Deel
Clydagh
Manulla
Eastern Moy tributaries
Cloonacool
Brusna
Cloon-
aghmore
Easkey
Ballysadare
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Figure 2b


Moy
Ireland
N
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
a
b
c
d
e
f
g
Figure 3


Ballysadare
Clydagh
25
Upr.Shanvolahan
59
Glendavolagh
8
18
59
Lwr.Shanvolahan
Lwr.Manulla
100
Upr.Manulla
Lwr.Cloonacool
100
Upr.Cloonacool
Lwr.Brusna
100
Upr.Brusna
8
53
Cloonaghmore
92
Easkey
25
Owengarve
Trimoge
9
6
Lwr.Spaddagh
24
Upr.Spaddagh
66
8
Eighnagh
Killeen
27
Sonnagh
48
Pollagh
71
Glore
0.1
Figure 4.

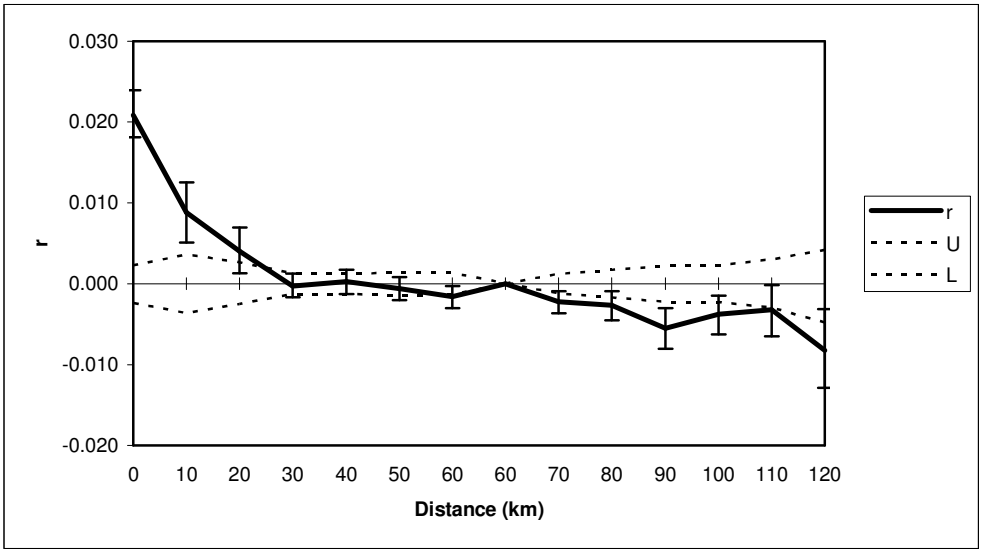

0.030
0.020
0.010
0.000
-0.010
-0.020
r
0
10
20
30
40
50
60
60
70
80
90
100
110
120
Distance (km)
r
U
L

Figure 5

Appendix 1. Geographic variables associated with each site sampled within the Moy (derived from GIS platform for comparisons with genetic data)

Population	**Spawning area size (m^2)**	**Altitude (m)**	**Discharge (cumecs)**	**Stream order (Shreve)**	**Contributing catchment area (m^2)**
1. Glendavolagh	31414	84.1	0.422	5	92204
2. Lwr Shanvolahan	14697	50.0	0.813	31	275116
3. Upr Shanvolahan	n/a	56.6	0.813	15	121268
4. Clydagh	22767	36.7	1.667	60	444516
5. Lwr Manulla	45552	19.9	4.564	108	164318
6. Upr Manulla	27712	27.1	4.564	55	1045132
7. Pollagh	66420	51.9	2.786	57	1157036
8. Glore	55891	66.1	1.603	20	596416
9. Trimoge	70183	69.5	1.517	18	605592
10. Killeen	51375	28.4	0.611	11	239376
11. Upr Spaddagh	30865	36.5	0.377	8	147244
12. Lwr Spaddagh	30865	54.4	0.377	5	118604
13. Sonnagh	29260	54.9	0.938	5	26436
14. Eighnagh	15304	58.3	0.659	20	169868
15. Owengarve	59928	75.2	3.052	19	442688
16. Lwr Cloonacool	99424	67.5	2.438	24	148272
17. Upr Cloonacool	n/a	202.9	0.231	11	7716

Author information box

The population genetics group based in University College Cork specialise in the application of molecular genetics in fisheries, aquaculture and conservation biology. At present they are particularly interested in how geographic features relate to population structure, methods of genetic stock identification in mixed fisheries, and in the genetic consequences of interactions between wild and reared strains of aquatic species. Dr. Elvira de Eyto is a research scientist with Ireland's Marine Institute whose research interests are in ecology, population biology and genetics of Atlantic salmon. Dr. Ellen Kenchington is a research scientist with Fisheries and Oceans, Canada. Her research interests include the landscape genetics of aquatic organisms. Dr. Paulo Prodohl is a Reader in population genetics and evolutionary biology at Queen's University Belfast. Most of his research activity is in the area of population genetics of aquatic organisms.