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Comparative Phylogeography of Salmonids in the British Isles: Implications for the Management of Biodiversity

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Abstract

Intraspecific biodiversity underpins species' character and abundance. An understanding of its nature and distribution is needed by managers to develop effective programmes for conserving local fish stocks. Prior to 18,000 yrs bp, much of northern Europe was glaciated. The fish fauna of the British Isles, much of which was glaciated at the time, is dominated by salmonids and has largely evolved since this time. A comparative study was made of the distribution of mitochondrial DNA variation in Atlantic salmon, brown trout and Arctic charr based on restriction enzyme analysis of the D-loop, cytochrome b, and the ND1 gene regions of the mitochondrial genome. The study found all three species to display significant spatial diversity, both within and among river systems. Arctic charr populations and nonanadromous populations of brown trout showed the greatest spatial divergence, including major regional differences, as well as the lowest within population diversity. Significant divergence was also found for Atlantic salmon and anadromous brown trout, even between tributaries of the same river but with only limited regional differentiation. The implications of the work for understanding of the post-glacial evolution of biodiversity in salmonids in Europe, and the implications of the results for species management, are considered.

Introduction

Biodiversity, the fundamental focus of conservation as regards fishes and other groups of organisms, is fundamentally genetic diversity (Avise, 1994). It exists not only at the species level but, as is becoming increasingly clear, is universally present within species and a major determinant of local species character and recruitment success. Not surprisingly, the conservation of intraspecific diversity is now recognized as an important objective in fisheries management. However, its achievement is often difficult as understanding of the nature and distribution of intraspecific biodiversity in most species remains limited or is lacking for most species. Providing this understanding represents a major challenge given the numbers of species, their often wide geographical ranges, the complexity of their genomes, and the finite research resources available, and is in many contexts unlikely to be attainable. However, where detailed information is lacking, meaningful action can still be taken if there is an understanding of the historical development of intraspecific biodiversity

and its distribution in different groups of fish species and in different regional settings. This can be used to predict where important biodiversity is likely to be found.

The development of this understanding lies within the purview of the newly emerging field of phylogeography. This field of study is "…concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species." (Avise, 2000). Its focus is on the genetic relatedness of lineages and the historical origins of their contemporary spatial distributions, and represents a sub-discipline of biogeography. A major tool in phylogeographic studies is the analysis of the distribution of mitochondrial DNA (mtDNA) variation. Variation in this part of the species genome can serve as a marker of overall genetic diversity and provide insight into population structuring and divergence, and its historical development. These insights are needed to develop effective overall programmes for conserving intraspecific biodiversity.

The mtDNA genome is maternally inherited, has high rate of mutation compared to coding regions of the nuclear DNA and, in most species, is inherited without recombination. Thus the mtDNA character of a fish provides information on maternal ancestry and lineages, and the distribution of these lineages can provide insight into population relatedness and serves as a marker of biodiversity. When considered in the context of historical geography, the findings allow inferences to be drawn regarding the general historical processes responsible for contemporary biodiversity. Where populations can be shown to have distinct historical origins, it is likely that they will also have diverged at nuclear genes, creating population differences which in many cases are likely to be of adaptive relevance (Avise, 2000). Thus the distribution of mtDNA provides valuable insights which can be used to guide the development of more effective conservation programmes.

Much of the fish fauna of northern and western European land mass has been established since the retreat of the Pleistocene glaciers starting c.18,000 yrs bp from populations in non-glaciated regions. Recent work on a number of nearctic and palearctic fish species suggest events during the colonisation of these deglaciated regions are a major determinant of the nature and distribution of contemporary diversity among populations (e.g. Bernatchez and Wilson, 1998; Verspoor et al., 2005). Considerable insight now exists on a trans-range basis for a number of species (e.g. Bernatchez, 2001; Brunner et al. 2001). However, detailed understanding of species phylogeography of fish species on a regional level is essential for the development of conservation programmes. Some studies have been carried out (e.g. Verspoor et al. 2002) but detailed work is still required for most species in most regions.

A preliminary qualitative overview of observations of regional mtDNA variation in three closely related species of salmonids - the Atlantic salmon *Salmo salar*, the brown trout *S. trutta*, and the Arctic charr *Salvelinus alpinus* in the British Isles is presented below. Basic levels and patterns of variation observed in the three species are compared and a few of the main insights these give into the phylogeography of the species, and of relevance to species conservation, are discussed. The merits of the comparative approach, highlighted by others (e.g. Moritz and Faith, 1998), and the implications of the insights gained from the study are considered.

Materials and Methods

An analysis was made of samples of salmon, brown trout and Arctic charr from 26, 44 and 30 locations, respectively, in the British Isles, both within and among river systems. Most of the samples are from Scotland. Sample sizes ranged from X to Y. Genetic screening for mtDNA variation was carried out on total genomic DNA extracted from fin clips from fish collected both destructively and non-destructively. Sequence variation was characterized in three regions of the mtDNA genome – the D-loop, the cytochrome B gene and the ND1 gene, by polymerase chain reaction amplification of the selected regions followed by restriction endonuclease enzyme digestion using standard methods (e.g. Verspoor et al., 1999; Knox et al., 2002) and a set of specific PCR primers (Verspoor et al., unpublished). Preliminary analysis of a subset of the samples had identified restriction enzymes which detected sequence variation.

The analysis presented includes the construction of lineage networks showing the most parsimonious assessment of their likely evolutionary relatedness. Based on this relatedness, the higher order, older lineages containing the individual lineages are identified following the nested approach of Templeton et al. (1992). The distribution of lineage diversity within samples and the basic geographical distributions of lineages among samples has been plotted and compared for the three species.

Results

Seven restriction enzymes resolved variation in the mtDNA regions screened defining18 genetic lineages in the Atlantic salmon. These were identified by a seven letter name e.g. AAABCDE (Figure 1a). In each of the brown trout and the Arctic char, six informative enzymes were found. These defined 18 and 20 different lineages, respectively, for these two species, with each given a distinguishing six letter name e.g. BABABB (Figure 1b, c). The genetic relatedness of these lineages and the relative proportion of samples in which they were detected are shown in Figures 1a,b,c. In the three species, the greatest divergence was found among Atlantic salmon lineages with the most differentiated lineages separated by nine genetic steps; in brown trout and Arctic charr, the greatest divergence seen among lineages were eight and six genetic steps, respectively.

The most parsimonious relatedness network for the salmon lineages shows all lineages to be related to at least one other lineage by a single genetic difference (Figure 1a). Nesting analysis puts the 18 lineages into six first order, higher lineages (1-1 to 1-6) which in turn cluster into three higher order lineages (2-1, 2-2 and 2-3). The two most common lineages are found in the central part of the network, as expected for older lineages. In general, lineages in the centre of the network are the most common and those at the tips of the network are found in only one or a few samples.

Nesting analysis shows the 18 brown trout lineages as part of five first order higher lineages (Figure 1b) which are in turn linked into two second order lineage groups. One of these, 3-1 is large and contains 14 lineages and the other is small and contains only two. As with the

Atlantic salmon, there are two central lineages with a wide distribution among samples and in general central lineages are more widespread than peripheral lineages. However, the main second order cluster of lineages, 3-1, shows a more complex radiation of lineages than seen in the higher order lineage clusters defined for the Atlantic salmon; compared to Atlantic salmon, a higher proportion of lineages in the main cluster are two steps removed from the central lineages. At the same time, in contrast to the Atlantic salmon, the genetic link between the higher order lineage groups is ambiguous, with three equally parsimonious potential links, each involving a single intermediate lineage which was not detected.

The network resolved for the 22 Arctic charr lineages contains seven first order higher lineage groups (Figure 1c). These in turn form three higher second-order clusters. one of the lineage groups (1-1) as seen in brown trout, is disconnected from the remainder of the network by a missing intermediate and its linkage to rest of the network is ambiguous. However, as with the other species, the most widespread lineages are those at the central part of the lineage, again these most probably being the oldest.

The samples from all three species, both within and among river systems show significant heterogeneity in the frequencies of lineages (analysis not presented). However, the pattern of heterogeneity for each species is markedly different for each species even though the number of lineages detected for each was similar,. All Atlantic salmon samples contained at least two lineages, with more than 50% having four or more (Figure 2a). In contrast, 65% of Arctic charr samples had only a single lineage and only one sample (6.5%) had more than three lineages. Samples of brown trout were intermediate. Over 60% had two or more lineages, and only 7% had more than four. Within the brown trout (Figure 2b), where both anadromous and resident populations were sampled, 85% of resident fish samples had 1 or 2 lineages while in sea trout samples 32% had 3 or more.

The geographic distribution of lineages among samples also differs among the species. In the Atlantic salmon, no particular geographic pattern was apparent with all lineages appearing across the study area (Figure 3). In contrast, in brown trout (Figure 4), a number of variant types appear to be regionally restricted and no lineages were found in all samples. However, the two lineages at the centre of the main second order lineage cluster are present in most samples. Marked regional distributions appear are found in relation to resident populations and for lineages AAAAAB, AAABAD and BBABAA. Differentiation in Arctic charr is even more marked. The two main lineages (AAABAB and AAABBB), which lie at the centre of the lineage network (Figure 1c), are less common in samples than are the main trout lineages (Figure 4 and 5). At the same time, regionally restricted lineage distributions are also found for charr. There is an almost exclusive association of the first order lineage group 1-7 with the eastern river systems of Scotland, this lineage group only being detected in one sample from a western Scottish catchment. Other lineage groups which are highly restricted in their distributions are 1-1, 1-2 and 1-6, as well as lineage BBBBAB and BBBBAB which are each restricted to a single sampling location.

Comparison of the geographical distributions of lineages in the species, the most striking feature revealed is that the samples with the greatest lineage diversity in trout and charr are from the same relatively restricted region, encompassing lakes in and around the adjacent

Rannoch Moor. Two of the trout samples with the greatest number of lineages (4 and 5) are from resident loch populations in this area. This stands out as most resident samples have only one or two lineages and only one sea trout sample had the same number or more lineages. This region also has the most diverse char sample, with 7 lineages, from Loch Rannoch; all other char samples examined have three or less and most only one or two. Coincidentally, trout samples in this general area, including those just to the south in the vicinity of Loch Awe, are the only ones with lineages in small, disconnected lineage cluster 1-5/2-2 (BBABAA and BAABAA - see Figure 1b). In Arctic charr, the Loch Rannoch sample from the same area is the only sample to have lineages from the small disconnected higher order lineage group 1-1 comprised of AAAACA, AAABCA, BAABCA (Figure 1c). At the same time, the Rannoch sample contains the unique BBBABB lineage, one of the most distantly related lineages from 1-1. Thus the most divergent charr lineages, are both unique and associated with the same location.

Discussion

The results indicate overall levels of genetic variability in the three species in the study region to be more or less equivalent. The higher number of lineages found in Arctic charr can be accounted for by the geographic distribution of charr samples being wider. The analysis included nine charr samples from Ireland and 2 from Wales, a third of the samples analysed, while only a single sample from each of the two *Salmo* species derived from outside of Scotland. Three lineages observed in charr were found only in Irish samples. In contrast, in both salmon and charr, the lineages present in the single Irish samples were present in the Scottish samples. Though the sampling intensity for brown trout in Scotland was much greater, as lineages in trout and charr appear to be regionally restricted, the greater regional coverage could still account for the greater number of charr lineages found compared to trout. In contrast, both the regional coverage and the intensity of sampling of Atlantic salmon were lower than charr and brown trout, respectively, suggesting that all other factors equal, fewer lineages might have been expected to be observed for this salmonid. However, that they do not may reflect the effect of the differing biologies of the species on historical patterns of gene flow; the fact that highly significant genetic differentiation occurs among tributaries within rivers (e.g. Verspoor et al. 2005) and little straying appears to occur even among tributaries with rivers (e.g. River Dee, Aberdeenshire, A. Youngson, pers. com.). The markedly lower level of differentiation seen among salmon samples suggests that historical gene flow has been higher among salmon populations than among those of other species. As such, it is likely that genetic drift in salmon populations has been much less pronounced. This view is supported by the fact that the overall level of diversity in resident trout samples is approximately 35% lower than in sea trout samples.

The equivalent overall levels of diversity in the three species is somewhat surprising given the general perception that brown trout are more genetically diverse than salmon, and that Arctic charr are relatively invariant. However, the present study represents one of the first to undertake a more rigorous single region comparison; previous studies have been based largely on limited studies and primarily on the analysis of protein loci (e.g. Gyllensten,

1985). It suggests that the general impression of species differences in overall diversity are the result of the samples being compared in the past being derived from a limited region or from different regions for each species. The differences would be expected given the major differences in the distribution of diversity within and among samples seen here.

The observed distribution of diversity within and among samples is consistent with results from studies of protein loci. Protein studies show a higher proportion of diversity to occur within locations in Atlantic salmon than in resident brown trout or Arctic charr (Gyllensten, 1985). Based on a broad survey of species, Gyllensten (1985) suggested that this was a reflection of their life history differences. The differences in the distributions of variation observed in the present study between salmon and charr, as well as between anadromous and resident trout, support this view. This would also account for the difference in the level of diversity reported between salmon and trout by Gyllensten (1985).

The observed regional patterns of differentiation at protein loci (e.g. Verspoor et al. 2005), and of mtDNA lineages (e.g. Verspoor et al., 2002; present study), point to the importance of factors operating early on in the development of populations in determining the pattern of distribution of diversity seen among contemporary populations (e.g. Bernatchez and Wilson 1998; Verspoor et al., 2005). Only more a few components of observed diversity (e.g. *MEP-2** enzyme locus, Verspoor et al., 2005) is there evidence for contemporary regionally varying selective forces being important. Most probably in the region being considered, as well as much of the ranges of the three salmonid species studied, this means that events associated with colonisation of regions as they emerged from under Plesiocene ice sheets since the last glacial maximum, as well as the distribution of refugial populations, are likely to be particularly critical. Much of the British Isles, as well as laarge parts of northern Europe, were covered in ice (Boulten et al. 2001; Bowen et al. 2002; Figure 6).

Understanding of the influence of these historical factors, the remit of phylogeographic study, can help to understand the nature and distribution of this diversity, and its biological significance. Comparative phylogeographic studies can be of particular value by providing insight into the factors which have been important to species general and to understand how species differ. The comparative analysis of the distribution of genetic diversity in the three salmonid species in the British Isles presented here is only preliminary. However, it already provides valuable insight into the nature of genetic diversity in these species.

The first important insight is that the three species in the study region have similar overall levels of genetic diversity. However, and of particular importance to managers, the species show major differences in how their diversity is distributed within and among populations. In line with previous work (e.g. Gyllensten, 1985), it shows that life history patterns shown by populations are a major determinant, both within and among and within the species (see also Verspoor et al. 2005 for evidence of the same life history difference at protein loci in Atlantic salmon). This means that the loss of resident populations is likely to represents a greater loss of overall diversity then does that of an anadromous population. Whether this will also be the case with regard to adaptive variation in the nuclear genome, which is the main management concern, needs to be investigated. At least to some degree it may well do so if there is a general association of lineage divergence with adaptive divergence as

suggested by Avise (2000). At the same time, this is not to say that the potential loss of an anadromous population is not a serious concern. Studies such as those by McGinnity et al. (2005) and Donaghy and Verspoor (1997) on salmon show that major adaptive difference exist between anadromous populations which indicate that populations, once lost, are not likely to be easily re-established by restocking from other sources; this may explain the poor success rate associated with stock restoration programmes based on the use of non-native fish.

The present analysis also suggests that there are biodiversity "hotspots". The most diverse and evolutionarily divergent populations in Scotland for both brown trout and Arctic char occur in the same region around Rannoch Moor. One also appears to occur in the Loch Melvin region in Ireland. Here the greatest diversity so far observed in the brown trout in the study region has previously been reported. This coincides with the observation in the present study of a unique Arctic charr lineage whose distribution appears restricted to this part of Ireland. The comparative approach taken is particularly useful in identifying such "hotspots", both by providing evidence of their occurrence but also, by increasing understanding of how they arise, by allowing locations where further hotspots might be expected to be identified and investigated. Such hotspots are worthy of particular protection in conservation initiatives as they represent a disproportionate amount of the overall salmonid diversity in the region examined.

The present analysis has not explored in detail the historical processes which may be responsible for the observed distribution of diversity in the species studied. This requires a careful and rigorous assessment of the lineage distributions and their associations with historical environmental conditions, something currently underway. However, one aspect of the observed distribution of diversity suggests the importance of differential migration from multiple glacial refuges. The charr of Loch Rannoch belong to two unique and highly divergent lineages. These will have evolved from post-glacial colonists belonging to the closely related lineages found elsewhere in the study area (Figure 1c), and that these derived lineages have, with time, through a process of genetic drift and, potentially at least, selection become fixed in the charr in the loch.. The higher level lineage group of which the Rannoch BBBABB is apart is strongly associated with North Sea drainages in the eastern part of the study area and the main clade in the Baltic Sea (unpublished data). On the otherhand, the higher level lineage group to which the other unique lineages belong (i.e. AAAACA, AAABCA, BAABCA) is strongly associated with Atlantic drainages in the study area. This suggests that the study area was colonised by fish from two distinct groups of colonists, a western one characterised by the central lineages with the higher 2-1 and 2-2 lineage groups, and an eastern one characterised by the central lineate within the higher 2-3 lineage group.

It is not clear whether the unique lineages in Loch Rannoch represent diversity within a single genetic population or whether the diverse lineages are associated with different genetic populations. The loch contains a morphologically distinct zooplankton feeder inhabiting the pelagic zone as well as two distinctive but similar benthic forms. Both of the latter appear to feed on bottom invertebrates but one becomes piscivorous when older (refs). The association of the different genetic lineages found with these morphs seems likely as

there is genetic evidence that the loch has more than one distinct genetic population (Wilson et al. 2004) and this question is currently being investigated. Certainly, mtDNA lineages are associated with ecologically different and reproductively isolated morphological types in Loch Melvin in Ireland (Ferguson 2004). Studies of both charr and brown trout suggest that the occurrence of sympatric populations of charr and trout may be widespread (e.g. Wilson et al. 2000) in the study area. Atlantic salmon stocks in many larger rivers appear to be composed of multiple genetic populations (e.g. Verspoor et al. 2005) but these are believed to be largely parapatric (i.e. occurring in adjacent locations rather than in the same location). However, sympatric populations of resident and anadromous Atlantic salmon are known to occur in other parts of the species range (e.g. Verspoor and Cole, 2005).

The same east-west disjunction of the distribution of lineages is not seen in the study area in relation to the two *Salmo* species. However, work outside the study area shows lineage group 2-3 in salmon to characterize most salmon found in the Baltic Sea. If so, it may be that at least some lineages in this group may have been associated with a more easterly Pleistocene refuge and Clades 2-1 and 2-2, with a more western refuge. However, if so, it would appear that their contributions to diversity in salmon populations in all parts of the study area. In contrast, in brown trout, there are two higher level clades but one is rare and associated with the Loch Rannoch area while the other is widespread. These may or may not reflect the presence of different eastern and western refuges. However, the pattern of distribution of these lineage groups suggests that they are associated with two distinct refugial groups in the western part of the study region, or further south along the Atlantic seaboard. Potentially informative mitochondrial studies have been carried out outside the study area but the equivalence of the lineages observed with those detected in the present study remains to be established.

The qualitative evaluation of the data presented already gives some interesting insights into biodiversity in the species studied. This shows that comparative phylogeographic studies have considerable potential to provide valuable insights into fish biodiversity which can aid in developing more effective management initiatives for its conservation. However, the findings remain preliminary and a more detailed analysis, including rigorous statistical testing of observed differentiation, must still be carried out. Benefits from this approach could be enhanced considerably by the development of integrated genetic data bases for species which allowed broader based and more detailed phylogeographic analyses to be carried out. The additional insights which can be gained from taking a broader approach is well illustrated by recent integrations of protein data in Atlantic salmon (e.g. Verspoor et al, 2005). Despite the benefits gained from this latter work, much more would have been gained if research groups had early on developed common methodology and established the equivalence or not of the variation resolved in different studies. Collaborations to achieve these aims should be encouraged. One such international collaboration currently being established on an informal basis is **SALMAN** – Atlantic **Sal**mon **M**icrosatellite **A**nalysis **N**etwork (contact corresponding author for details). Its objective is to link researchers working on genetic variation at microsatellite loci in Atlantic salmon, to develop common methods and link data bases. Similar initiatives need to be developed with respect to

mtDNA variation for which a considerable amount of information already exists in the literature for many species, including Atlantic salmon and brown trout.

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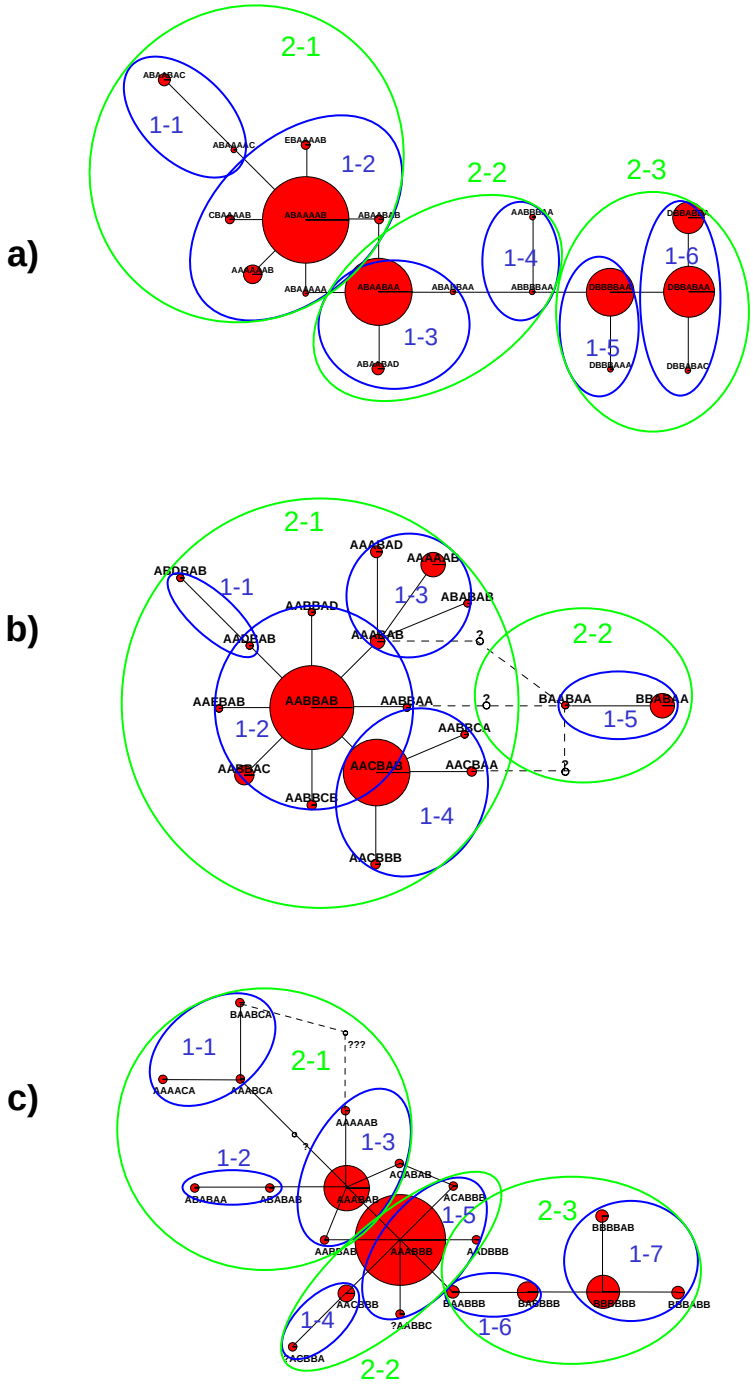

Figure 1 Parsimony networks showing the relatedness of the maternal mtDNA lineages resolved in a) Atlantic salmon, b) brown trout, and c) Arctic charr. The size of the node in the network reflects the relative proportion of the samples screened for each species in which the particular lineage was found. The smallest circles in each case represent a single

sample; the small empty circles with “?” next to them indicate intermediate lineages which are expected but have not been observed.

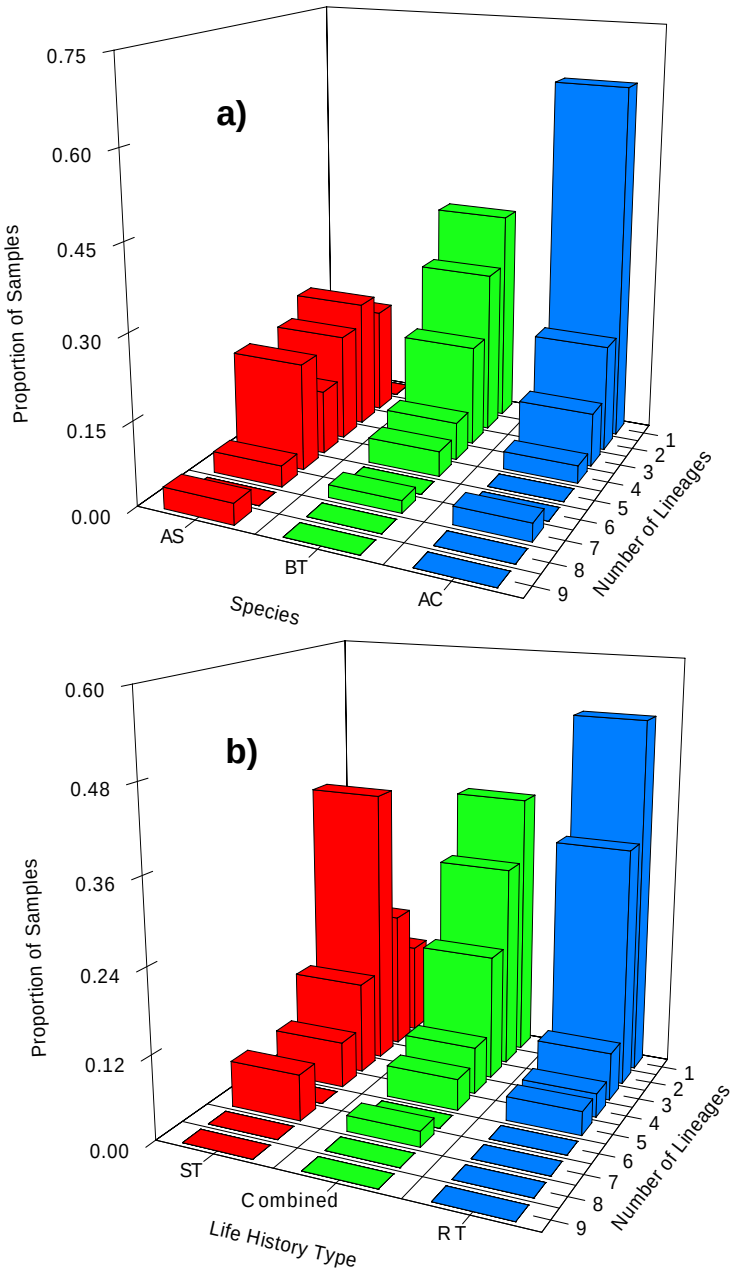

Figure 2 The number of lineages observed in samples a) comparing Atlantic salmon, brown trout and Arctic charr, and b) comparing resident and anadromous samples of brown trout.

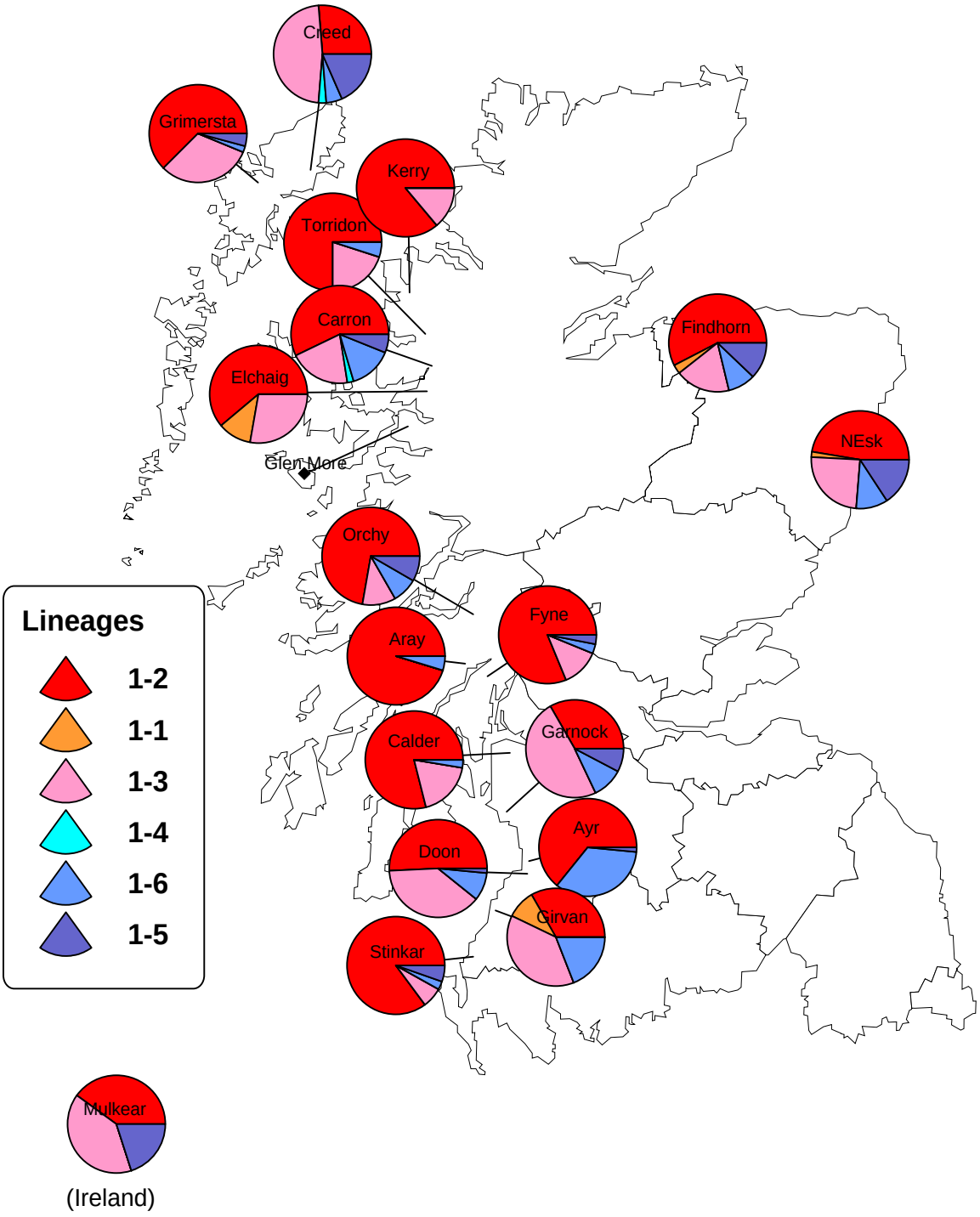

Figure 3 The distribution of lineage types among locations in the study region for samples of Atlantic salmon. The lineage types are as defined in Figure 1a.

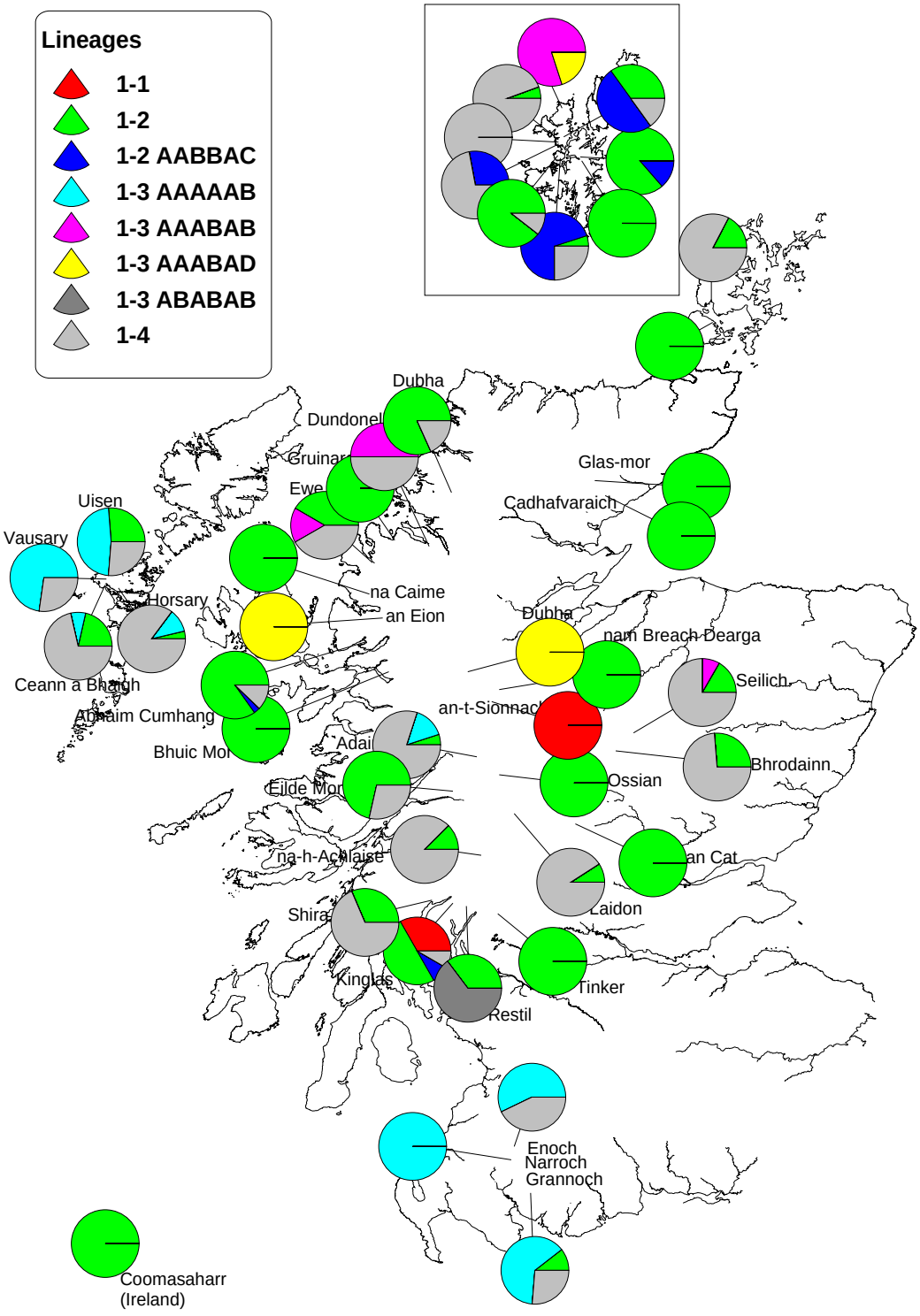

Figure 4 The distribution of lineage types among locations in the study region for samples of brown trout. The lineage types are as defined in Figure 1b.

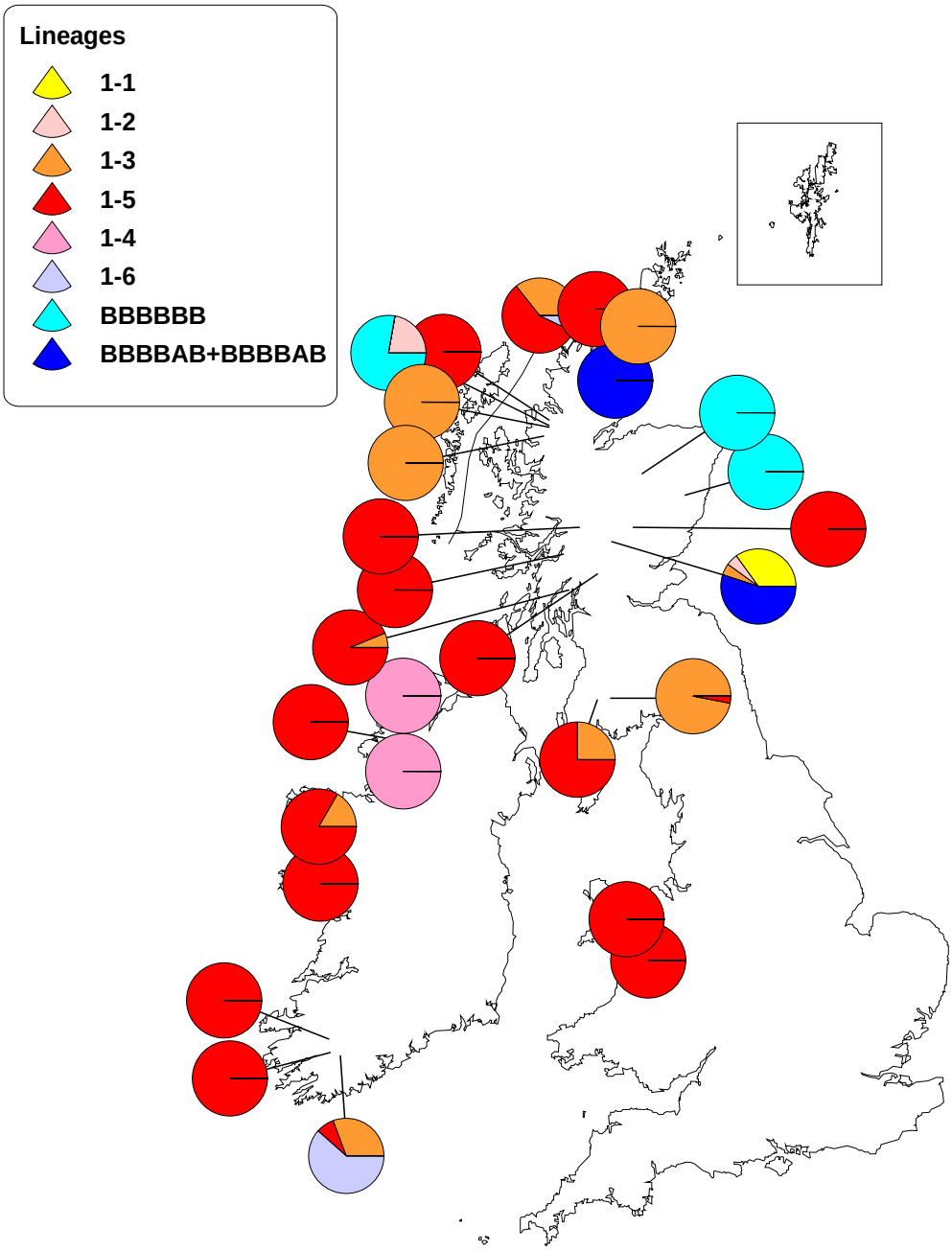

Figure 5 The distribution of lineage types among locations in the study region for samples of Arctic charr. The lineage types are as defined in Figure 1c.

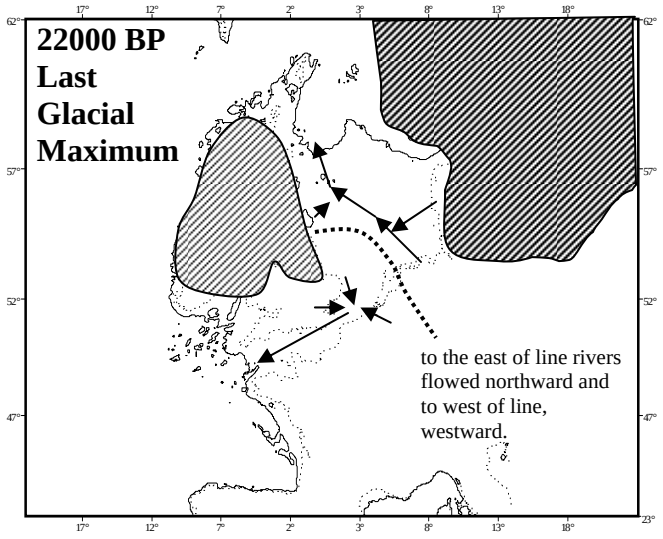

Figure 6 Approximate distribution of ice at the time of the last glacial maximum around 22,000 years bp. Sea levels were much lower and the regional landmass much different. Particularly noteworthy is the major division of the regional watersheds around the British Isles. What is now the southeastern and central North Sea area and linked to the modern Elbe and Wesser Rivers drained northward between the two icesheets into the Norwegian Sea while the Rhine, Thames and what is now southwestern North Sea region flowed to the west into the Atlantic, providing a geographical basis for two distinct refugial areas for anadromous salmonid fishes such as Atlantic salmon, brown trout and Arctic charr.