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Among population differentiation at nuclear genes in native Scots pine (*Pinus sylvestris* L.) in Scotland

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Abstract

In the Scottish Highlands, Scots pine is at the north-western extreme of its wide natural distribution. Here, the remaining native populations are patchily distributed in highly variable environments, from the more continental, drier eastern Highlands to the milder, wetter Atlantic Ocean coast. As these pinewoods are the remnants of a naturally established forest, they form a valuable system for analysis of genetic and adaptive variation in heterogeneous environments. Using samples from across the Scottish population, we analysed data from nuclear and mitochondrial genes to assess patterns of within and between population genetic variation. Within population diversity levels were high, and significant genetic differentiation among pairs of Scottish populations at relatively small spatial scales was present at several nuclear loci. At these loci, no differentiation had been found among continental populations, even those separated by large geographic distances. Overall, no clear clustering of Scottish samples was found in population structure analysis suggesting that geographically distant populations with high intra-population nucleotide diversity are not strongly isolated or diverged from each other. Scottish populations lacked a mitotype that is widespread in eastern and north-eastern Europe, indicating that pines from that area may not have participated in the most recent colonisation of the British Isles.

Introduction

The extent of genetic differentiation between populations depends on several factors including demographic history related to range shifts and population size changes, natural selection due to local adaptation and the level of gene flow (Savolainen et al., 2007). The

homogenizing effects of gene flow on genetic diversity are known for highly outcrossing wind pollinated species. For instance low genetic differentiation at neutral markers has been documented for many forest tree species across geographical ranges that can span thousands of kilometres (Karhu et al., 1996). However, less is known about patterns of genetic differentiation within and between populations at the species margin in environments that are spatially heterogeneous at a relatively fine scale.

Scots pine (*Pinus sylvestris* L.) is the most widely distributed conifer species in the world, covering a huge range of environments across Eurasia (Critchfield and Little, 1966). In Scotland, the species is at the north-western extreme of its distribution. According to palynological data, it reached Britain by about 10,500 years ago (Huntley and Birks, 1983) and Scotland around 8,000 years ago (Bennett, 1995; Birks, 1989), though fossil remains indicate a presence in northern Scotland at low abundance from at least 9,600 years ago (Froyd, 2005; Froyd & Bennett, 2006). The possibility that the postglacial colonisation of the Highlands involved migrants from different geographical sources has been suggested by significant differences between contemporary western populations and those in the rest of Scotland at allozyme and monoterpene (3-carene) loci (Forrest, 1980, 1982; Kinloch *et al.*, 1986) and unique restriction fragment length polymorphisms in the mitochondrial (*mt*DNA, Sinclair *et al.* 1999) and chloroplast (cpDNA) genomes (Provan *et al.*, 1998). Previous work using candidate nuclear genes has indicated similar levels of diversity in Scottish populations as in mainland European populations, and patterns of allelic frequency incompatible with a simple colonisation and expansion model (Wachowiak *et al.*, 2010). However, the origin of the colonists of the British Isles remains unresolved.

Scots pine's current range in Scotland is only a fraction of what it used to be. Following postglacial colonisation, Scots pine rapidly expanded its range, reaching its maximum extent by ~5,000 years ago (Ennos et al., 1997). Due to competition from deciduous trees, climate change and human activity native pinewoods now remain only in the Highlands of Scotland, physically separated by at least 500 km from the nearest mainland populations in continental Europe (Ennos et al., 1997). Currently, around 84 fragments of Scots pine woodland are recognized as native, with total area of about 18,000 ha (Jones, 1999; Mason et al., 2004). Despite occupying a relatively small geographic area, the patchily distributed Scottish Scots pine populations occur in very heterogeneous environments due to differences in average temperature and precipitation (Salmela et al., 2010) and the complex topography of the

Highlands. For example, the westernmost pinewoods experience annual rainfall close to 3000 mm while eastern populations experience about 740 mm, and the length of the growing season (number of days with average temperature above +5°C) varies from about 300 days on the west coast to 100 in the highest-altitude eastern pinewoods. Thus, at both regional and local scales in the Scottish Highlands, there is high environmental heterogeneity and a high potential for divergent selection and the development of local adaptation.

Empirical studies and simulations suggest that spatial heterogeneity in the environment, and hence in the pattern of natural selection, can lead to local adaptation and genetic differentiation between populations (González-Martinez et al., 2006; Howe et al., 2003; Savolainen et al., 2007; Wegmann et al., 2006). Patchily distributed populations with low average densities may be particularly affected by such spatial variability compared to higher-density populations. However, genetic differentiation may result not only from diversifying selection, which may affect patterns of nucleotide variation at adaptively important loci, but also from demographic processes related to population size changes, genetic drift or surfing (Excoffier et al., 2009). Given their relatively recent colonisation history and the spatial heterogeneity of the environments in which they live, the native Scottish Scots pine fragments seem likely to have experienced the interacting effects of both demographic changes and spatially variable environmental selection. They therefore comprise a valuable system for resolving the effects of these processes on variation at the molecular scale.

In this study, nucleotide diversity at nuclear and mitochondrial loci was used to assess genetic variation within and among Scots pine populations from different environments across Scotland. In a previous study, we analysed nucleotide diversity at a regional scale in Scotland and compared it to that in continental European populations (Wachowiak et al. 2010). Here, we increased within-population sampling to focus on genetic differentiation among populations at a fine spatial scale. Considering the environmental differences between sites, their spatial separation and recent recolonization history, we aimed to test to what extent Scottish populations are differentiated at nuclear and mitochondrial loci.

Material and Methods

Sampling

Seeds of *P. sylvestris* were collected from twelve natural populations of Scots pine, covering the full spatial extent of the Scottish distribution (Figure 1). The populations occupy climatically variable areas across a broadly east-west climatic gradient within Scotland ranging from the eastern Highlands to the Atlantic coast. The environmental gradient combined differences in length of growing season (108-279 days), annual rainfall (785-2905 mm) and average mean temperature in winter (-2.01 to 3.38°C) (Table 1). Environmental variables for the sites were derived from UK Met Office data, which is collated at a 5x5 km grid scale and includes interpolated values, particularly in the Highlands (Perry and Hollis, 2005). Cones were collected from ten mature trees from each population in recognised old-growth Scots pine forest; at these sites trees were typically over 150 years old and often much older (Steven and Carlisle, 1959). Trees were separated by at least 50 m to minimise sampling of closely related individuals.

Nuclear and mitochondrial loci, PCR amplification and haplotype analysis

Genomic DNA was extracted from megagametophytes (a maternally derived haploid tissue surrounding the embryo) of germinated seeds from ten trees per population from each location (Table 1) following the protocol provided with the DNeasy Plant Mini Kit (Qiagen). Nuclear DNA polymorphism was determined for twelve genes including early response to dehydration 3 protein (*erd3*), abscissic acid, water dehydrative stress and ripening induced gene family members 1 and 3 (*lp3-1, lp3-3*), Caffeoyl CoA *O*-methyltransferase (*ccoaomt*) (Eveno et al., 2008); ABI3-interacting protein 2 (*a3ip2*) and chalcone synthase (*chcs*) (Pyhäjärvi et al., 2007); abscissic acid responsive protein (*abaR)* and dehydrin gene family members including *dhn2*, *dhn3*, *dhn7*, *dhn9* analysed in Scots pine by Wachowiak *et al.* (2009) and *dhy*2PP described in *P. pinaster* by Eveno *et al.* (2008). Nomenclature of dehydrins corresponds to the notation of gene family members described in Scots pine (Wachowiak et al., 2009). Variation in the mitochondrial genome was determined for the *nad*1 intron B/C and *nad*7 intron 1 (Jaramillo-Correa et al., 2004; Soranzo et al., 2000). Both nuclear (*n*DNA) and mitochondrial (*mt*DNA) markers were used as they are useful in tracking variation in species migration routes due to different mode of inheritance (biparental vs. maternal) and dispersal (pollen vs. seeds) (Neale and Sederoff 1989). PCR (polymerase chain reaction) amplification was performed with PTC-200 (MJ Research) and carried out in a total volume of 25µl and 0.25U *Taq* DNA polymerase with the respective 1x PCR buffer (BioLabs) following standard amplification conditions as described for each gene in original papers. DNA was amplified from haploid megagametophyte which allowed determination of

the nuclear gene haplotypes (alleles) by direct sequencing. PCR fragments were purified using QIAquick^{TM}PCR Purification Kit (Qiagen). About 20 ng of PCR product was used as a template in 10 µl sequencing reactions with the Big Dye Terminator DNA Sequencing Kit (Applied Biosystems) performed by the GenePool sequencing service, University of Edinburgh. For each population, about 6 thousand nucleotides base pairs (bp) of *n*DNA were aligned across genes excluding the sequence of PCR primer sites. To amplify a polymorphic 31 bp insertion/deletion (indel) in *nad1 mt*DNA region, diagnostic primers *nad1*H-I were used (Soranzo et al., 2000). To score the size differences in the *mt*DNA *nad*7 intron 1 caused by two single indels of 5 and 32 bp found in continental populations of Scots pine (Naydenov et al., 2007; Pyhäjärvi et al., 2008), the samples were digested with 0.5 U of *Dra*II restriction enzyme. The PCR products (~5µl) of both *mt*DNA polymorphic regions were electrophoretically separated on 2% agarose gel and scored for indel variation. Three samples from each population were also sequence-characterised to check for presence of other polymorphisms or potential fragment length homoplasies and compared to each other and the nucleotide sequences available in GeneBank (NCBI). CodonCode Aligner software was used for editing and assembling of the sequence data and all sequence polymorphisms were visually rechecked from chromatograms. Scots pine DNA sequences for each nuclear locus reported in this paper are deposited in the EMBL sequence database under accession numbers HQ108916 – HQ110050.

Nucleotide and haplotype polymorphisms

Descriptive statistics for DNA polymorphism at nuclear loci were computed with DnaSP (Librado and Rozas, 2009) to look at the difference in the amount of nucleotide and haplotype diversity and the allelic frequency distribution between populations. Nucleotide diversity was measured as the average number of nucleotide differences per site (π) between two sequences (Lukens and Doebley, 2001). Multilocus estimates of the population mutation parameter theta (θ_W, equal to $4N_e\mu$, where N_e is the effective population size and μ is the mutation rate per nucleotide site per generation) (Watterson, 1975) was computed based on the number of segregating sites and the length of each locus using Markov Chain Monte Carlo

(MCMC) simulation under a Bayesian model (Pyhäjärvi et al., 2007). The average number of pairwise differences and the number of shared and exclusive polymorphic sites and their distribution for each nuclear locus between populations (excluding indels) were determined

using SITES 1.1. software (Hey and Wakeley, 1997). The number of haplotypes (unique alleles) and haplotype diversity at each locus were calculated in DnaSP. The recombination rates per site for the 12 loci were obtained using composite-likelihood methods (LDhat, McVean et al., 2002). Estimates of the amount of nucleotide diversity and correlation between polymorphic sites were conducted for all individuals from each population separately and also for all individuals combined to obtain species-wide estimates compiled from all populations.

Departures from the standard neutral model at *n*DNA loci

Neutrality tests were applied to each locus to check for departures from a neutral model of evolution. Deviations from the frequency distribution spectrum of polymorphic sites at individual populations were assessed by Tajima's *D* (Tajima, 1989) and Fay and Wu's *H* (Fay and Wu, 2000). The outgroup species *P. pinaster* (subgenus *Pinus*) was used in two heterogeneity tests, the McDonald-Kreitman (MK) test (Thornton, 2005) and the Hudson-Kreitman-Aguadé (HKA) test (Jiggins et al., 2008). The orthologous *P. pinaster* sequences were obtained previously from different studies (Eveno et al., 2008; Pyhäjärvi et al., 2007; Wachowiak et al., 2009). The significance of multilocus estimates of the Tajima's *D* and HKA tests statistic were evaluated by comparison to a distribution generated by 1000 coalescent simulations using the HKA program (http://lifesci.rutgers.edu/~heylab). The MK test was conducted in DnaSP.

Population structure and environmental associations

The allelic frequency distribution spectrum was assessed for each locus and population and also at the multilocus level. To measure differentiation among populations Wright's fixation index (Weir and Cockerham, 1984), F_{ST}, was calculated for each locus and tested for significance by 1000 permutations as implemented in Arlequin 3.0 (Excoffier et al. 2005). The hierarchical distribution of genetic variation within and among populations based on all polymorphic sites detected was estimated using an analysis of molecular variance (AMOVA). Differentiation between the populations was measured as a weighted average over all polymorphic sites and tested for significance in Arlequin 3.0. Population structure from the haplotypic data was tested by S_{nn} statistics (Hudson, 2000) and its significance evaluated by 1000 permutations of the samples for every pairwise comparison between populations as implemented in DnaSP. S_{nn} measures the average proportion of nearest-neighbor haplotypes that are present in the same locality and it is expected to be near one for strong population

differentiation, while an estimate of 0.5 would indicate that two groups are part of the same panmictic population.

To check for signatures of population structure we applied cluster analysis using the admixture model implemented in STRUCTURE 2.3.3 (Pritchard et al., 2000) and genetic mixture analysis of linked haploid sequences data as implemented in BAPS software (Corander and Tang, 2007). We chose to use both methods since they make different assumptions, for example on linkage. To estimate the number of clusters in the data in the STRUCTURE analysis, K of 1 to 12 was explored and ten independent runs were conducted for each K. The burnin was set to at least 100 000 and the run length to at least 1 000 000. The dataset included polymorphic sites from all nuclear genes and individuals were represented by a single allele. Linked sites, as determined by significant Fisher's exact test after Bonferroni correction, were excluded (data not shown). In BAPS, the MLST-format as a separate fasta file was used for each locus and ten independent runs were conducted for each K (1-12) to estimate the number of clusters for all samples and also for groups of individuals from different populations.

To check for association between single nucleotide polymorphic sites (SNPs)/haplotype frequencies at individual loci and environmental variables that reflect between population differences in selective gradients related to precipitation and temperature at the home sites we used the spatial analysis software MatSAM (Joost et al., 2008). Two likelihood ratio tests (G and Wald tests) were applied to test the null hypothesis of no association between the genetic and environmental variables (at the 5% level).

Results

Nucleotide variation

All of the nuclear loci were polymorphic and there was a substantial difference in the amount of nucleotide and haplotype variation at the individual loci. The least polymorphic locus was *ccoaomt* (π_{total} = 0.00191) and most polymorphic was *lp3-3* (π_{total} = 0.03562) (Table 2). Average total nucleotide diversity was π_{total} =0.0098 and the average nonsynonymous and silent divergence were 0.0045 and 0.0150, respectively (Table 3). At individual locations the lowest (0.0080) and highest (0.0124) diversity were observed for Coille Coire Chuilc and Meggernie, respectively (Table 3, Supplementary Table S1). Multilocus estimates of

Watterson's theta for all populations combined was θ_{sil}=0.0111 (with 95% credibility intervals of 0.0091-0.0134) and in pairwise comparisons between populations the values were similar between the least differentiated population, Shieldaig (θ_{sil}=0.0088 (0.0060-0.0129)) and the most differentiated population, Meggernie (θ_{sil}=0.0136 (0.0098-0.0189)) (Table 3). The average pairwise differentiation was about 1% and it was very similar between all pairs of populations (0.008-0.011, Supplementary Table S2). The lowest numbers of shared polymorphisms (~65%) as compared to other populations were found at Shieldaig, Glen Tanar and Black Wood of Rannoch (Supplementary Table S3). The average number of haplotypes per gene was 4.2 and the average haplotype diversity was high (H_d =0.74±0.13) and similar across populations with the highest values in Meggernie (N=5, H_d =0.81±0.11) (Table 3). The average recombination rate per site for Scottish populations was ρ=0.0101. The values varied between population with highest values observed for western Glen Affric (ρ=0.0511) and the lowest for Glen Tanar (ρ=0.0004) (Table 3). The high recombination rate found for *dhn2* was largely responsible for the high average ρ at Glen Affric. At *mt*DNA loci, all populations were fixed for the 31bp indel at the *nad1* intron B/C and the 5bp indel at the *nad7* intron 1, cosmopolitan mtDNA variants abundant in Western Europe and also present in eastern Russia and China (Naydenov et al., 2007; Pyhäjärvi et al., 2008).

Neutrality tests

Significant positive Tajima's *D* was found at Beinn Eighe (*D*=0.887, P<0.01) and a tendency towards an excess of common over low frequency variants was found in most individual populations (Table 3). The exception was Rothiemurchus with slightly negative values of *D* (Table 3). At individual loci, significant excess of intermediate frequency mutations (P<0.05) was found at *dhn3* (*D*=2.205), *a3iP* (*D*=2.160) and *ccoaomt* (*D*=2.195) in Shieldaig, Beinn Eighe and Glen Derry, respectively. An excess of rare variants was found at *dhn3* (*D*=-1.783) in Ballochbuie and *ccoaomt* (*D*=-1.741) in Glen Einig (Supplementary Table S1). For pooled samples across populations, a significantly negative value of Tajima's *D* was found at *erd3* (Table 2). Overall, an excess of high-frequency derived variants (indicated by negative mean values of Fay and Wu's *H*) was found in all Scottish populations (*H*= -0.405) and at individual loci (Table 2), however the pattern was heterogeneous across individual populations with *H* values ranging from -1.421 in Glen Einig to 0.435 in Meggernie (Table 3).

An excess of nonsynonymous sites as compared to synonymous sites was found at *abaR* (Ka/Ks=1.77), *dhn7* (1.09) and *erd* (9.45). A significant reduction of polymorphism (π_{total}) relative to divergence (K) was found at *dhn3* and *lp33* in the multilocus HKA test. The two loci showed deviations from neutral expectations in most populations except Shieldaig, Glen Tanar and Meggernie. No deviations from standard neutral expectations were found at any locus with the MK test.

Population structure

When all polymorphic sites at nuclear loci were analysed jointly, significant genetic differentiation was detected between Shieldaig, and both Glen Einig and Ballochbuie (P=<0.05) in the AMOVA analysis, however most of the genetic variation was found within populations (Supplementary Table S4). In general, pairwise population differentiation was locally variable, with no consistent pattern suggesting isolation by distance. This extended to variation among loci, in that significant pairwise differentiation among populations was detectable in some comparisons, for some loci, but consistent patterns were rare.

Overall, nine out of twelve loci showed significant differentiation for frequency spectra in pairwise comparisons between certain populations (Supplementary Table S5). Based on the number of differences between haplotypes, significant differentiation in at least one pairwise comparison was found for all loci except *erd* (Supplementary Table S6). The most differentiated were Glen Tanar and Glen Affric which showed significant differentiation for at least one locus with all other populations except Shieldaig. Similarly Glen Loy was differentiated from all other populations except Glen Derry (Supplementary Table S6).

High haplotype structure was found at *abaR* for Glen Tanar and at *chcs* for Glen Loy as compared to other populations (Supplementary Tables S6). The locus *a3ip* at Glen Affric was completely fixed for the most common haplotype at this locus in Scottish populations of the species (Supplementary Table S1). No polymorphism was found at *ccoaomt* at Beinn Eighe and Rothiemurchus populations and *dhn7* at Beinn Eighe and Coille Coire Chuilc. Both loci were fixed in these populations for the most common haplotypes found in Scottish populations. Reduced polymorphism at *dhn3* relative to other populations was found for Black Wood of Rannoch and Glen Tanar, at *dhn9* for Glen Einig and Black Wood of Rannoch, and at *lp33* at Shieldaig.

The clustering analysis in STRUCTURE and BAPS suggests the presence of four genetic clusters (K = 4) (Figure 2). Some evidence of admixture was found in 9 samples in total (Figure 2). Overall however, individuals representing different clusters were mixed between populations and there was no correspondence to geographical regions, which indicates a lack of real population structure. Similarly, no among-population structure was detected by the clustering analysis in BAPS software when geographical information was used as a prior. In this case, despite the inclusion of all twelve populations, a single cluster was most likely.

At all loci, the frequency spectra showed no association with environmental variables across populations as indicated by likelihood ratio tests with the exception of *dhn7* locus that showed significant associations at haplotype level (Wald test). The frequency of the main haplotype at this locus was significantly associated with latitude, whilst the frequency of the second most frequent haplotype was significantly associated with altitude and mean February temperature.

Discussion

In this study, levels of genetic diversity were analysed in a series of native pinewoods across Scotland. The data indicated high within population genetic diversity not compatible with a simple recolonization model. We found striking among population heterogeneity at individual nuclear gene loci, which was in marked contrast to what has been observed over much larger geographical scales among populations from the continental range of the species. Scottish populations showed no evidence of population structure and were missing a common mitochondrial haplotype present in the continental part of the species distribution. Together, these findings indicated that geographically distant Scottish populations were not strongly diverged or isolated from each other and suggest that they may have experienced a quite different recolonization history from continental European populations.

High genetic diversity at nuclear loci

High levels of nucleotide diversity were present within populations, comparable to levels observed for regionally-pooled samples in previous work (Wachowiak et al. 2010). Considering the recent decline of Scots pine in Scotland, population contraction appears to have left no molecular signature, either in the amount of nucleotide diversity or the intragenic recombination rates. Both are similar to those previously reported for populations from the

continuous continental parts of the species range (Wachowiak et al., 2009). High nucleotide diversity suggests that reduction of the Scottish Scots pine populations has been too recent to have had an effect on diversity level and that there has been consistent high gene flow between populations (Nielsen and Wakeley, 2001). High levels of diversity in Scottish populations were also observed in previous studies using monoterpenes (Forrest, 1980, 1982), allozymes (Kinloch et al., 1986; Prus-Glowacki et al., 2012) and chloroplast DNA (Provan et al., 1998). Considering the significant variation observed for quantitative phenotypic traits, the Scottish pinewoods do not fit expectations that increasing environmental heterogeneity – allied to local adaptation – leads to reduced genetic diversity within populations (Excoffier et al., 2009; Wegmann et al., 2006). However, the expectation of a decrease of genetic diversity with distance from refugia assumes limited recent and past gene flow between subpopulations (Excoffier, 2004; Ray et al., 2003). As gene flow rates in wind pollinated pines may be efficient even at large distances, this assumption is unlikely to hold. In addition, the life history characteristics of trees such as longevity, multiple age and size classes, overlapping generations and late reproduction buffer against the decrease of genetic variation due to population contractions (Austerlitz et al., 2000). High genetic diversity within populations together with high heritable phenotypic variation observed at several quantitative traits (Perks and McKay, 1997)) suggests that Scottish populations have a high potential to produce a diverse adaptive responses to environmental variation in the complex landscape of the Highlands.

Significant among-population differentiation at individual loci

The most striking result was the relatively high and significant among-population differentiation at nuclear gene loci; a completely distinct pattern to that seen among populations in the continuous ranges of the species, where differentiation was negligible even over large distances. Out of 12 loci analysed, 11 showed some evidence of population differentiation among at least one pair of Scottish populations. Six of these loci (*dhn2, dhn7, dhn9, abaR a3ip2* and *chcs*) showed no significant differentiation between Scandinavian and Central European populations (Pyhäjärvi et al., 2007; Wachowiak et al., 2009), indicating the remarkably high differentiation levels among Scottish pinewoods on a much smaller geographic scale.

Reflecting the fact that the majority of genetic diversity was found within populations and the idiosyncratic nature of pairwise among-population differentiation, clustering analyses showed

no evidence for large scale population structure using the dataset as a whole. This result indicated that the populations were not strongly isolated or diverged from each other, possibly due to their common origins and at least historically, effective gene flow between them. However, more intensive within-population sampling (for example, to target different age groups within populations), and a larger number of nuclear markers would be required to resolve population substructure. Similarly, there was no clear correspondence between patterns of nucleotide variation and gross environmental gradients, although at one locus (*dhn7*), haplotype variation was significantly correlated with latitude, altitude and mean winter temperature. It is clear that, if natural selection has acted in Scottish populations as suggested by previous studies of quantitative traits (Salmela et al., 2011), the mode of action is different to that observed in continental populations, where clinal patterns of adaptive variation are present in Scots pine and other species (Ingvarsson et al., 2008). However, considering the lack of evidence for departures from neutrality or for selection across loci in this or previous studies, at this stage it is equally likely that demographic factors are responsible for the observed patterns of nucleotide variation and higher resolution studies are needed.

Admixture in Scottish populations?

In populations Glen Tanar, Glen Loy and Glen Affric, three loci - *abaR*, *chcs* and *erd* - showed significant differentiation between these and most other populations. If these populations were originally established under a scenario of range expansion during early colonisation, such patterns of nucleotide diversity could arise from genetic surfing, in which standing genetic variation may increase in frequency and be propagated, reaching very high frequencies and even fixation far from their place of origin (Klopfstein et al., 2006). However, the overall picture for Scottish populations does not fit simple expectations for recent population expansion, i.e. an excess of rare alleles and low frequency mutations, and reduced nucleotide diversity relative to putative refugial populations. On the contrary, allelic frequency spectra within most populations were shifted towards intermediate frequency variants and within population diversity levels were as high, or higher, than in continental populations. An alternative explanation would be that the pattern of among-population differentiation in Scottish populations is the result of admixture between colonists from different refugial populations. In this scenario, despite the effectiveness of gene flow and recombination in this species, its longevity (trees may live for several hundred years), overlapping generations and relatively recent colonization history (~10,000 years) have

prevented complete homogenisation of gene pools across populations originating from different refugial origins. Signs of admixture were present in patterns of nucleotide diversity and the detailed allelic frequency spectra at nuclear loci (Wachowiak et al., 2010). Interestingly, our *mt*DNA data showed that Scottish populations lacked the 5bp indel at nad7 intron 1, which is widespread in eastern and north-eastern Europe, suggesting that pines from that area may not have participated in most recent colonisation of British Isles. Previous findings of private organelle variants in Scotland (Provan et al., 1998; Sinclair et al., 1999) provide some evidence of unique diversity and hint that these populations may have experienced a quite different recolonisation history from continental European populations. In the latter, evidence for admixture of diverged *mt*DNA lineages was found in northern Fennoscandia (Pyhäjärvi et al., 2008). However, the low level of *mt*DNA divergence observed in conifers in general and the low resolution of current markers makes it difficult to provide more evidence for geographic structuring and/or admixture in Scottish populations. Again, it is clear that more nuclear and *mt*DNA markers are needed for Scots pine in order to reconstruct with adequate precision its postglacial recolonisation routes. Considering the very low rate of *mt*DNA sequence evolution, comparative analyses of whole *mt*DNA genomes between samples from geographically distant locations may be needed to successfully identify such polymorphic regions.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Supplementary information.

Available online in the NERC Open Research Archive (http://nora.nerc.ac.uk/id/eprint/20477).

Supplementary Table S1. Summary statistics of nucleotide and haplotype variation, neutrality tests and recombination rate estimates at the loci studied in the Scots pine populations in Scotland. Population names and locations are given in Table 1.

Supplementary Table S2. Average pairwise differentiation in comparisons between populations for the combined dataset of 12 loci.

Supplementary Table S3.

Average percentage of shared polymorphisms in pairwise comparisons between populations for the combined dataset of 12 loci.

Supplementary Table S4. AMOVA results for all SNP_S combined and all populations studied.

Supplementary Table S5.

Significant values of *Fst* statistics for corresponding loci (marked in superscript) in pairwise comparisons between populations ($P<0.05$)

Supplementary Table S6.

Significant values of S_{nn} for pairwise comparisons between populations.

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598 **Tables and Figures**

599 **Table 1.** Geographic coordinates and environmental data (mean average estimate for 1961–1990) of the 12 sampled *P. sylvestris* populations
600 from Scotland.

Nr	Population (acronym) Name (acronym)	Seed zone	Latitude	Longitude	Average altitude	Length of the growing season (days)	Annual precipitation (mm)	February mean temp. (C^0)
1.	Beinn Eighe (BE)	North West	57.63	5.35	63	279	2411	3.38
2.	Glen Affric (GA)	North Central	57.27	4.92	256	204	1686	0.62
3.	Glen Einig (GE)	North	57.95	4.76	55	237	1463	1.85
4.	Shieldaig (SD)	North West	57.51	5.64	81	267	2385	2.99
5.	Ballochbuie (BB)	North East	56.99	3.30	475	108	1343	-2.01
6.	Glen Derry (GD)	East Central	57.03	3.58	462	160	1056	-0.84
7.	Glen Tanar (GT)	North East	57.05	2.86	334	231	785	1.82
8.	Rothiemurchus (RM)	East Central	57.15	3.77	318	216	1042	1.03
9.	Black Wood of Rannoch (BW)	South Central	56.67	4.32	275	252	1160	1.77
10.	Coille Coire Chuilc (CCC)	South Central	56.41	4.71	257	223	2905	1.39
11.	Glen Loy (GL)	South West	56.91	5.13	170	187	2156	0.31
12.	Meggernie (MG)	South Central	56.58	4.35	306	219	1497	0.81

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Table 2. Nucleotide and Haplotype variation at 12 nuclear gene in Scottish populations of Scots pine

					Nucleotide diversity						Haplotype diversity	
Gene	*n*	L	I (L)	S	π_{total}	π_{nonsyn}	π_{silent}	ρ^a	D^b	H^c	N	H_d (SD)
dhn2	71	610	8 (39)	18 (3)	0.00879	0.00326	0.01154	0.02404	1.039	-0.520	21	0.852 (0.033)
dhn3	98	339	1 (6)	28 (8)	0.01603	0.01079	0.02367	0	-0.164	3.227	14	0.839 (0.017)
dhn7	83	329	2 (8)	11 (5)	0.00455	0.00406	0.0052	0	-0.905	-5.446	9	0.642 (0.043)
dhn9	80	733	2 (11)	36 (6)	0.0103	0.00899	0.01295	0.00773	0.072	-4.403	17	0.834 (0.026)
dhn2PP	117	428	1 (8)	20 (5)	0.00964	0.00129	0.02276	0.05218	0.082	1.357	27	0.918 (0.13)
abaR	116	442	4 (22)	11 (1)	0.00508	0.00522	0.00493	0.03677	0.082	-1.278	12	0.848 (0.017)
a3iP2	110	885	2 (23)	16 (4)	0.00327	0.00055	0.00376	0.00339	-0.198	-3.634	14	0.61 (0.046)
ccoaomt	119	523	1 (4)	5 (1)	0.00191	0	0.00318	0.00765	0.129	-0.642	4	0.264 (0.048)
chcs	84	306	1 (1)	14 (6)	0.00676	0	0.0081	0.00545	-0.738	-2.022	10	0.753 (0.043)
erd3	118	583	0	16 (11)	0.00167	0.00035	0.003	0.00686	-1.847*	-3.484	14	0.673 (0.027)
lp3-1	72	373	1 (8)	22 (6)	0.01387	0.00425	0.01674	0.09383	0.352	0.659	38	0.968 (0.009)
lp3-3	67	463	3 (153)	32 (1)	0.03562	0.01616	0.06439	0.00216	1.926	2.674	23	0.912 (0.018)
Mean	94.6	501.2	2.2 (23.6)	19.1 (4.8)	0.0098	0.0046	0.0150	0.0200	-0.014	-1.959	16.9	0.759 (0.038)

n total sample size; L – length of gene fragment including indels; I – number of indels (length); S – number of polymorphic sites (singleton); π – nucleotide diversity (Nei 1987); [a] - recombination rate; [b] Tajima's *D* test (Tajima 1989), [c] Fay and Wu *H* test (Fay and Wu 2000); N – number of haplotypes (number of unique haplotypes at the locus), H_d – haplotype diversity (standard deviation); [*]P<0.05;

Table 3. Summary statistics of nucleotide and haplotype variation and frequency distribution spectra across 12 nuclear genes in Scottish populations of Scots pine.

					Nucleotide diversity								Haplotype diversity	
Population	***n***	**L**	**I (L)**	**SNPs**	π_{total}	π_{nonsyn}	π_{silent}	θ^a	**C.I. (95%)[b]**	ρ^c	D^d	H^e	**N**	H_d **(SD)**
1. Beinn Eighe	7.6	498.0	25 (252)	111(20)	0.0106	0.0046	0.0170	0.0106	0.0075-0.0152	0.0154	0.887*	0.043	3.75	0.67 (0.11)
2. Glen Affric	8.3	498.8	26 (260)	106(49)	0.0093	0.0042	0.0147	0.0102	0.0072-0.0145	0.0511	0. 153	0.163	4.25	0.71 (0.10)
3. Glen Einig	7.7	496.4	27 (252)	101(54)	0.0096	0.0045	0.0151	0.0102	0.0071-0.0145	0.0045	0.110	-1.421	4.00	0.72 (0.14)
4. Shieldaig	6.8	500.0	23 (195)	92(41)	0.0089	0.0051	0.0124	0.0088	0.0060-0.0129	0.0028	0.286	-0.307	3.33	0.73 (0.19)
5. Ballochbuie	6.8	495.8	25 (251)	98(62)	0.0112	0.0052	0.0180	0.0102	0.0071-0.0145	0.0034	0.076	0.015	3.92	0.75 (0.16)
6. Glen Derry	7.1	496.8	26 (260)	124(64)	0.0098	0.0046	0.0151	0.0119	0.0084-0.0168	0.0055	0.032	-0.816	4.50	0.79 (0.13)
7. Glen Tanar	8.0	497.0	26 (265)	98(35)	0.0085	0.0038	0.0131	0.0098	0.0068-0.0139	0.0004	0.113	-0.423	3.83	0.72 (0.14)
8.Rothiemurchus	7.8	497.4	26 (265)	121(70)	0.0092	0.0047	0.0135	0.0109	0.0077-0.0154	0.0010	-0.131	-1.015	4.33	0.75 (0.11)
9. Black Wood of Rannoch	7.9	498.0	26 (256)	98(32)	0.0091	0.0034	0.0140	0.0106	0.0075-0.0149	0.0131	0.228	-0.196	4.42	0.73 (0.13)
10. Coille Coire Chuilc	7.8	501.2	24 (248)	117(58)	0.0080	0.0036	0.0124	0.0105	0.0074-0.0148	0.0087	0.082	0.007	4.58	0.72 (0.11)
11. Glen Loy	8.0	497.3	25 (257)	126(49)	0.0105	0.0054	0.0155	0.0117	0.0083-0.0165	0.0084	0.116	-1.342	4.58	0.79 (0.13)
12. Meggernie	7.9	498.6	26 (263)	157(53)	0.0124	0.0060	0.0187	0.0136	0.0098-0.0189	0.0071	0.294	0.435	5.00	0.81 (0.11)
Total/Mean	**7.7**	**497.9**	**25 (252)**	**112(49)**	**0.0098**	**0.0046**	**0.0150**	**0.0111**	**0.0091-0.0134**	**0.0101**	**0.174**	**-0.405**	**4.21**	**0.74(0.13)**

n- average number of sequences analysed per locus; L – average length of the sequences in base pairs excluding indels; I – number of idels (total length in bp); SNPs- number of polymorphic sites detected (singletons in parenthesis); π – nucleotide diversity (Nei 1987); [a] median for silent sites; [b] 95% credibility intervals for θ; [c] ρ – average recombination rate estimates for a set of 8 loci including *a3iP*, *abaR*, *ccoam*, *dhn2*, *dhn3*, *dhn7*, *dhn9*, *erd*; [d] *D* test (Tajima 1989); [e] *H* test (Fay and Wu 2000); N – number of haplotypes; H_d – haplotype diversity (standard deviation); *P<0.01

Fig. 1
Geographic location of the sampled Scots pine populations in Scotland.

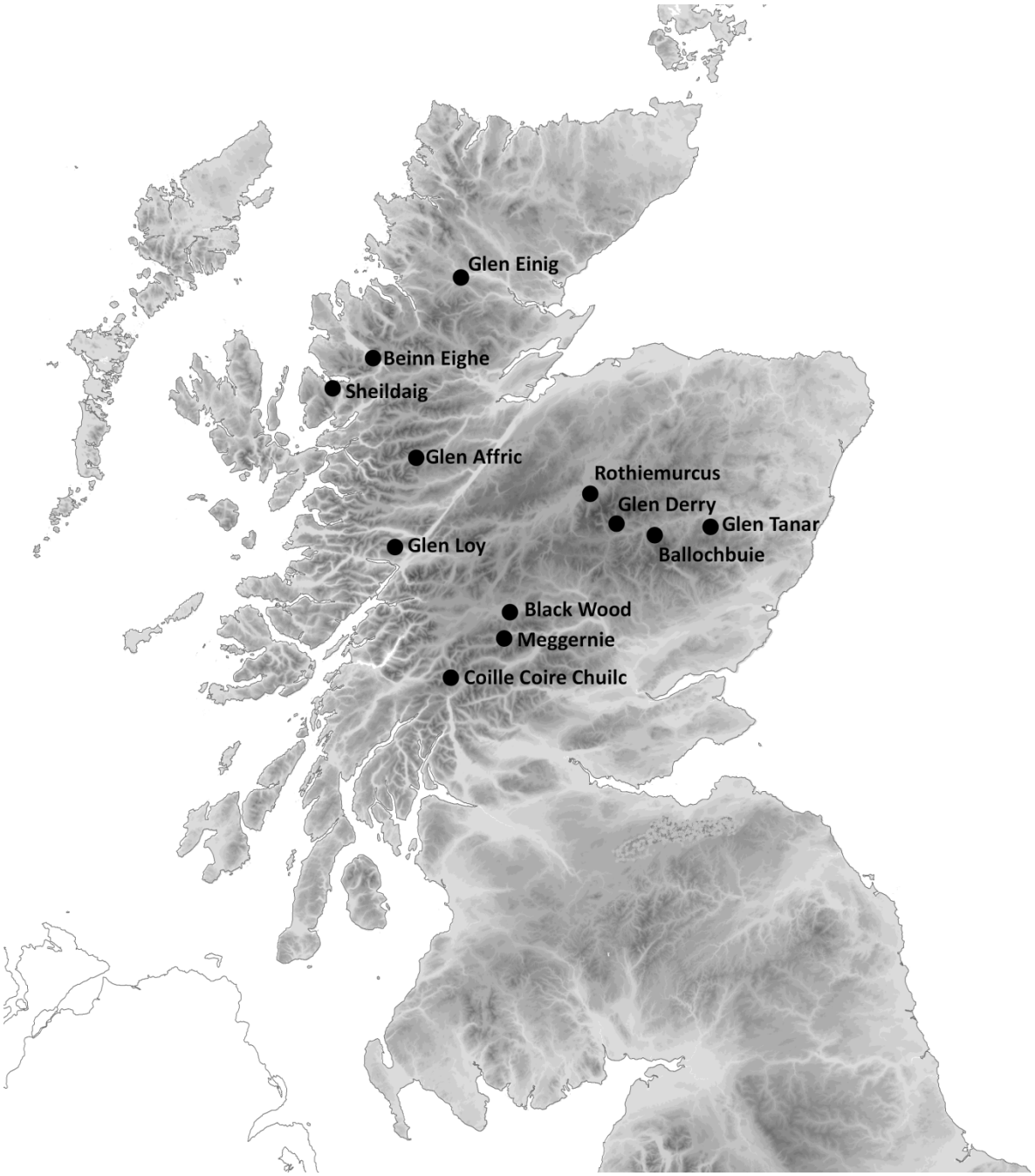

Figure 2.

Bar plots describing the result of the BAPS clustering analysis in Scottish Scots pine *P. sylvestris* with 4 assumed genetic clusters (K). Samples (not delineated) are arranged for each cluster and corresponding population following the population number (below the chart) as in Table 1. The greyscale represent the estimated membership in the inferred genetic clusters. Some evidence on admixture was found at nine individuals in total including populations 4 (SD, 1 individual), 5 (BB, 2), 6 (GD, 1), 7 (GT, 2), 8 (RM, 1), 10 (CCC, 1) and 11 (GL, 1).

Supplementary Material for online publication only

Supplementary Table S1. Summary statistics of nucleotide and haplotype variation, neutrality tests and recombination rate estimates at the loci studied in the Scots pine populations in Scotland. Population names and locations are given in Table 1.

					Nucleotide diversity							
					Total		**Nonsynonymous**				**Haplotype diversity**	
Locus	Pop.	n	L	I (L)	S	π	S	π	D^a	H^b	N	H_d (SD)
dhn2	BE	8	591.5	7 (29)	12	0.0099	1	0.0030	1.220	-1.429	5(3)	0.857(0.108)
	GA	5	596.6	7 (29)	11	0.0093	1	0.0032	0.164	-0.3	5(1)	1(0.126)
	GE	7	588.9	9 (29)	11	0.0067	1	0.0015	-0.705	-1.571	3(2)	0.524(0.209)
	SD	6	585.5	7 (38)	12	0.0090	3	0.0064	-0.141	0.267	4(3)	0.867(0.129)
	BB	6	594.5	6 (19)	8	0.0079	1	0.0032	1.957	0.533	4(2)	0.8(0.172)
	GD	5	588.5	7 (29)	11	0.0090	1	0.0021	-0.109	-3.7	3(1)	0.7(0.218)
	GT	4	587.2	6 (28)	10	0.0086	1	0.0027	-0.834	-0.667	3(1)	0.833(0.222)
	RM	6	588.8	7 (29)	10	0.0071	1	0.0028	-0.338	0.267	3(1)	0.6(0.215)
	BW	6	591.9	7 (20)	7	0.0053	1	0.0028	0.128	0.267	3(1)	0.6(0.215)
	CCC	7	598.4	7 (20)	13	0.0097	1	0.0030	0.421	-2.714	6(2)	0.952(0.096)
	GL	5	590.3	6 (28)	10	0.0083	2	0.0053	0.000	-1.3	4(1)	0.9(0.161)
	MG	6	594.7	6 (28)	15	0.0115	1	0.0028	0.091	0.267	6(3)	1(0.096)
dhn3	BE	9	333.2	1 (6)	16	0.0197	4	0.0130	0.551	4.111	4(1)	0.833(0.08)
	GA	7	333.0	1 (6)	17	0.0152	4	0.0096	-1.518	3.095	4(1)	0.810(0.130)
	GE	9	333.2	1 (6)	19	0.0205	7	0.0170	-0.110	3.667	5(0)	0.861(0.087)
	SD	6	334.2	1 (6)	14	0.0250	3	0.0170	2.205*	-0.533	3(0)	0.733(0.155)
	BB	10	333.0	1 (6)	18	0.0118	5	0.0077	-1.783*	1.422	6(0)	0.867(0.085)
	GD	8	333.2	1 (6)	21	0.0168	3	0.0106	-1.606	2.929	5(1)	0.786(0.151)
	GT	7	333.0	1 (6)	4	0.0040	1	0.0015	-0.876	-0.667	4(0)	0.810(0.130)
	RM	9	333.0	1 (6)	16	0.0124	4	0.0074	-1.462	2.472	5(0)	0.861(0.087)
	BW	9	333.0	1 (6)	3	0.0033	0	0.0000	0.025	-0.528	4(1)	0.806(0.089)
	CCC	8	333.0	1 (6)	16	0.0130	3	0.0071	-1.540	3.214	4(1)	0.750(0.139)
	GL	7	333.3	1 (6)	17	0.0226	6	0.0179	0.469	4.714	5(1)	0.905(0.103)
	MG	9	333.8	2 (7)	17	0.0253	5	0.0183	1.661	1.528	4(1)	0.750(0.112)
dhn7	BE	7	329.0	0 (0)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	GA	7	327.6	1 (2)	3	0.0044	2	0.0047	0.755	-1.143	3(0)	0.714(0.127)
	GE	8	328.5	1 (2)	4	0.0049	3	0.0057	0.182	-3.357	4(1)	0.750(0.139)
	SD	4	329.0	0 (0)	2	0.0041	1	0.0036	1.893	0	2(0)	0.667(0.204)
	BB	9	327.8	1 (2)	3	0.0041	2	0.0043	0.794	-1.833	3(0)	0.639(0.126)

	GD	4	327.3	1 (2)	4	0.0061	1	0.0027	-0.78	1.333	3(1)	0.833(0.222)
	GT	8	326.2	2 (8)	6	0.0067	2	0.0045	-0.345	-0.857	4(1)	0.786(0.113)
	RM	8	328.5	1 (2)	3	0.0042	2	0.0045	0.712	-1.286	4(0)	0.786(0.113)
	BW	10	326.5	2 (8)	6	0.0059	3	0.0051	-0.453	-1.333	5(2)	0.756(0.130)
	CCC	7	329.0	0 (0)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	GL	4	328.0	1 (2)	3	0.0056	2	0.0064	1.09	-1	3(0)	0.833(0.222)
	MG	7	326.7	2 (8)	6	0.0068	3	0.0062	-0.536	-1.952	5(0)	0.905(0.103)
dhn9	BE	6	724.4	2 (11)	25	0.0178	6	0.0136	1.103	-5.333	4(0)	0.8(0.172)
	GA	7	729.4	1 (5)	7	0.0048	5	0.0053	1.208	1.095	4(0)	0.857(0.102)
	GE	6	729.0	1 (5)	3	0.0024	2	0.0024	1.648	-0.533	3(1)	0.733(0.155)
	SD	5	728.0	1 (5)	15	0.0115	9	0.0104	1.219	-0.6	3(0)	0.7(0.218)
	BB	6	730.0	1 (5)	7	0.0038	5	0.0043	-0.631	-1.6	4(1)	0.8(0.172)
	GD	6	728.0	1 (5)	14	0.0071	9	0.0072	-1	-6.667	3(1)	0.733(0.155)
	GT	10	728.0	1 (5)	16	0.0101	9	0.0089	1.373	-1.867	5(2)	0.8(0.1)
	RM	7	728.7	2 (11)	27	0.0120	7	0.0102	-1.214	-15.81	5(0)	0.905(0.103)
	BW	6	730.0	1 (5)	3	0.0022	2	0.0022	1.124	0	2(0)	0.533(0.172)
	CCC	7	728.7	1 (5)	17	0.0099	12	0.0101	0.24	-4.095	5(1)	0.857(0.137)
	GL	8	726.7	2 (11)	29	0.0139	8	0.0118	-0.561	-12.571	6(1)	0.893(0.111)
	MG	6	724.4	2 (11)	30	0.0207	10	0.0156	1.103	0.267	6(4)	1(0.096)
dhn2PP	BE	10	591.5	1 (8)	12	0.0106	1	0.0014	0.666	0.711	8(1)	0.844(0.08)
	GA	10	596.6	1 (8)	11	0.0106	2	0.0016	0.216	0.444	8(1)	0.933(0.077)
	GE	10	588.9	0 (0)	11	0.0103	0	0.0000	0.615	1.067	8(1)	0.933(0.077)
	SD	9	585.5	1 (8)	12	0.0111	1	0.0009	0.719	1.583	5(0)	0.833(0.098)
	BB	10	594.5	1 (8)	8	0.0107	1	0.0014	0.717	1.689	8(0)	0.956(0.059)
	GD	10	588.5	1 (8)	11	0.0070	0	0.0000	0.195	-0.978	9(2)	0.978(0.054)
	GT	10	587.2	1 (8)	10	0.0103	1	0.0008	0.487	1.333	6(1)	0.889(0.075)
	RM	9	588.8	1 (8)	10	0.0111	1	0.0015	0.719	2.333	7(1)	0.944(0.070)
	BW	10	591.9	1 (8)	7	0.0114	3	0.0023	-0.456	1.778	7(2)	0.911(0.077)
	CCC	10	598.4	1 (8)	13	0.0088	2	0.0016	0.221	-0.356	7(0)	0.867(0.107)
	GL	9	590.3	0 (0)	10	0.0103	3	0.0033	-0.71	1.778	8(3)	0.972(0.064)
	MG	10	594.7	0 (0)	15	0.0086	1	0.0008	1.314	1.156	7(0)	0.911(0.077)
abaR	BE	9	421.1	4 (22)	5	0.0052	2	0.0042	0.753	-0.667	5(0)	0.861(0.087)
	GA	9	420.8	4 (22)	6	0.0052	3	0.0055	-0.081	-2.111	5(0)	0.833(0.098)
	GE	9	421.2	4 (22)	4	0.0040	2	0.0037	0.538	0.833	5(1)	0.861(0.087)
	SD	10	421.3	3 (21)	5	0.0043	3	0.0043	0.074	-1.333	5(2)	0.822(0.097)
	BB	9	420.8	4 (22)	3	0.0040	2	0.0053	1.948	0.333	3(0)	0.667(0.105)
	GD	10	421.0	4 (22)	4	0.0039	2	0.0042	0.626	-0.995	5(1)	0.8(0.1)
	GT	10	421.4	4 (22)	6	0.0046	3	0.0045	-0.409	-3.022	4(0)	0.711(0.117)
	RM	10	420.7	4 (22)	4	0.0039	2	0.0051	0.566	0.356	5(1)	0.822(0.097)

	BW	10	421.1	3 (21)	6	0.0057	3	0.0061	0.501	0.711	6(1)	0.844(0.103)
	CCC	10	421.4	3 (21)	8	0.0064	3	0.0056	-0.212	0.533	8(4)	0.956(0.059)
	GL	10	421.2	4 (22)	5	0.0042	3	0.0041	-0.027	-2.044	5(4)	0.756(0.130)
	MG	10	421.1	3 (21)	6	0.0054	3	0.0060	0.328	0.49	5(0)	0.8(0.1)
a3ip2	BE	9	862.0	2 (23)	5	0.0032	0	0.0000	2.16*	0.194	2(0)	0.556(0.09)
	GA	10	862.0	2 (23)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	GE	8	862.0	2 (23)	9	0.0044	0	0.0000	0.394	-3.857	4(1)	0.786(0.113)
	SD	10	862.0	2 (23)	6	0.0016	0	0.0000	-1.493	-0.8	4(2)	0.533(0.180)
	BB	8	862.0	2 (23)	5	0.0027	0	0.0000	1.008	0.5	3(0)	0.607(0.164)
	GD	10	862.0	2 (23)	11	0.0037	1	0.0031	-0.793	-4.089	4(0)	0.644(0.152)
	GT	9	863.8	2 (23)	10	0.0051	0	0.0000	0.898	-0.083	5(1)	0.806(0.120)
	RM	9	862.0	2 (23)	9	0.0029	0	0.0000	-1.128	-2.204	4(1)	0.694(0.147)
	BW	10	862.5	2 (23)	10	0.0044	0	0.0000	0.277	-2.222	4(1)	0.644(0.152)
	CCC	9	862.6	2 (23)	12	0.0054	1	0.0034	0.27	-1.278	5(2)	0.833(0.098)
	GL	8	862.0	2 (23)	6	0.0020	0	0.0000	-1.28	0	3(0)	0.464(0.2)
	MG	10	862.0	2 (23)	9	0.0034	0	0.0000	-0.311	-3.822	5(0)	0.756(0.130)
ccoaomt	BE	10	519.0	1 (4)	0	0.0000	0	0.0000	-	-	1(1)	0(0)
	GA	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
	GE	10	519.0	1 (4)	5	0.0019	0	0.0000	-1.741*	-0.889	3(1)	0.378(0.181)
	SD	9	519.0	1 (4)	4	0.0017	0	0.0000	-1.61	-0.972	2(0)	0.222(0.166)
	BB	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
	GD	10	519.0	1 (4)	4	0.0043	0	0.0000	2.195*	0	2(0)	0.556(0.075)
	GT	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
	RM	10	519.0	1 (4)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	BW	10	519.0	1 (4)	4	0.0027	0	0.0000	0.022	-0.267	2(0)	0.356(0.159)
	CCC	10	519.0	1 (4)	4	0.0027	0	0.0000	0.022	-0.267	2(0)	0.356(0.159)
	GL	10	519.0	1 (4)	4	0.0024	0	0.0000	-0.4	-0.8	3(1)	0.378(0.181)
	MG	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
chcs	BE	6	305.1	1 (1)	4	0.0066	0	0.0000	0.768	-0.8	5(0)	0.933(0.122)
	GA	9	305.4	1 (1)	4	0.0046	0	0.0000	-0.229	-1.472	4(0)	0.694(0.147)
	GE	7	305.5	1 (1)	2	0.0031	0	0.0000	0.687	-0.429	2(0)	0.476(0.171)
	SD	8	305.2	1 (1)	7	0.0084	0	0.0000	-0.226	0	4(0)	0.750(0.139)
	BB	6	305.7	1 (1)	4	0.0050	0	0.0000	-0.676	-0.533	3(0)	0.733(0.155)
	GD	7	305.3	1 (1)	5	0.0072	0	0.0000	0.363	0.381	5(0)	0.857(0.137)
	GT	8	305.5	1 (1)	4	0.0048	0	0.0000	-0.222	-1.357	4(0)	0.643(0.184)
	RM	6	306.0	0	2	0.0028	0	0.0000	-0.05	-1.067	3(1)	0.733(0.155)
	BW	6	305.2	1 (1)	10	0.0138	0	0.0000	-0.246	0	3(1)	0.733(0.155)
	CCC	7	305.5	1 (1)	2	0.0031	0	0.0000	0.687	-0.429	4(0)	0.714(0.181)
	GL	8	305.0	1 (1)	6	0.0077	0	0.0000	0.087	-0.429	3(0)	0.679(0.122)

	MG	6	305.2	1 (1)	6	0.0096	0	0.0000	0.666	0.267	3(0)	0.733(0.155)
erd3	BE	9	583.0	0	2	0.0011	0	0.0000	-0.583	0.472	3(0)	0.417(0.191)
	GA	10	583.0	0	4	0.0020	2	0.0014	-0.702	0.533	4(1)	0.733(0.101)
	GE	10	583.0	0	4	0.0019	0	0.0000	-0.762	-1.511	4(1)	0.711(0.117)
	SD	10	583.0	0	2	0.0013	0	0.0000	0.222	-2.321	3(1)	0.644(0.101)
	BB	10	583.0	0	7	0.0029	2	0.0014	-1.269	-0.978	5(2)	0.800(0.1)
	GD	10	583.0	0	4	0.0020	1	0.0007	-0.702	0.492	4(2)	0.733(0.101)
	GT	9	583.0	0	1	0.0009	0	0.0000	0.986	0.178	2(0)	0.5(0.128)
	RM	10	583.0	0	3	0.0019	0	0.0000	0.096	0.178	5(0)	0.756(0.130)
	BW	10	583.0	0	3	0.0016	1	0.0007	-0.431	0.597	4(1)	0.733(0.101)
	CCC	10	583.0	0	1	0.0010	0	0.0000	1.464	0	2(0)	0.556(0.075)
	GL	10	583.0	0	3	0.0021	0	0.0000	0.473	0.178	4(0)	0.800(0.076)
	MG	10	583.0	0	3	0.0019	0	0.0000	0.096	0.178	4(1)	0.778(0.091)
lp3-1	BE	3	373.0	0	5	0.0089	0	0.0000	-	-1.333	3(3)	1(0.272)
	GA	8	367.9	1 (8)	13	0.0141	1	0.0030	0.13	1.143	6(3)	0.929(0.084)
	GE	5	365.8	1 (8)	9	0.0126	1	0.0049	0.461	-0.8	5(0)	1(0.126)
	SD	3	373.0	0	7	0.0125	1	0.0081	-	1.333	3(2)	1(0.272)
	BB	5	369.8	1 (8)	11	0.0153	1	0.0073	0.436	1.7	4(1)	0.9(0.161)
	GD	6	365.5	1 (8)	9	0.0121	1	0.0073	0.693	1.6	4(2)	0.867(0.129)
	GT	5	365.8	1 (8)	8	0.0110	1	0.0049	0.294	0	4(2)	0.9(0.161)
	RM	7	366.1	1 (8)	12	0.0128	1	0.0058	-0.257	-1	6(2)	0.952(0.096)
	BW	9	369.7	1 (8)	13	0.0152	1	0.0027	0.77	0.861	7(4)	0.944(0.07)
	CCC	8	367.9	1 (8)	10	0.0114	1	0.0030	0.367	2	6(5)	0.893(0.111)
	GL	7	366.1	1 (8)	7	0.0099	0	0.0000	1.381	-0.095	6(2)	0.952(0.096)
	MG	6	370.3	1 (8)	15	0.0185	1	0.0065	0.154	3.467	5(2)	0.933(0.122)
lp3-3	BE	5	342.8	6 (152)	25	0.0437	7	0.0203	1.342	4.5	4(1)	0.9(0.161)
	GA	8	343.6	6 (152)	26	0.0402	6	0.0157	1.56	1.571	5(1)	0.857(0.108)
	GE	3	332.0	6 (152)	20	0.0429	5	0.0183	-	-9.667	2(0)	0.667(0.314)
	SD	2	374.0	6 (89)	6	0.0160	2	0.0110	-	-	2(0)	1(0.5)
	BB	2	310.0	6 (153)	20	0.0645	5	0.0275	-	-	2(2)	1(0.5)
	GD	7	341.0	6 (152)	26	0.0387	7	0.0167	1.301	-0.095	7(3)	1(0.076)
	GT	5	344.0	6 (152)	19	0.0341	6	0.0181	1.671	3	3(1)	0.8(0.164)
	RM	5	344.0	6 (152)	25	0.0399	7	0.0187	0.915	4.6	4(1)	0.9(0.161)
	BW	8	342.6	6 (152)	26	0.0381	7	0.0188	1.476	-2.214	6(2)	0.929(0.084)
	CCC	6	366.7	6 (152)	21	0.0247	5	0.0090	-1.042	3.467	5(2)	0.933(0.122)
	GL	6	341.7	6 (152)	26	0.0369	6	0.0165	0.869	-4.533	5(0)	0.933(0.122)
	MG	10	347.9	6 (152)	31	0.0360	8	0.0154	0.63	4.444	8(3)	0.956(0.059)
Average	BE	91	5975.6	2.1 (21.0)	111	0.0106	21	0.0046	0.887*	0.043	3.75	0.67 (0.11)
/Total	GA	100	5984.9	2.2 (21.7)	106	0.0093	26	0.0042	0. 153	0.163	4.25	0.71 (0.10)

GE	92	5957.0	2.3 (21.0)	101	0.0096	21	0.0045	0.110	-1.421	4.00	0.72 (0.14)
SD	82	5999.7	1.9 (16.3)	92	0.0089	23	0.0051	0.286	-0.307	3.33	0.73 (0.19)
BB	91	5950.1	2.1 (20.9)	98	0.0112	24	0.0052	0.076	0.015	3.92	0.75 (0.16)
GD	93	5962.3	2.2 (21.7)	124	0.0098	26	0.0046	0.032	-0.816	4.50	0.79 (0.13)
GT	95	5964.1	2.2 (22.1)	98	0.0085	24	0.0038	0.113	-0.423	3.83	0.72 (0.14)
RM	96	5968.6	2.2 (22.1)	121	0.0092	25	0.0047	-0.131	-1.015	4.33	0.75 (0.11)
BW	104	5976.4	2.2 (21.3)	98	0.0091	21	0.0034	0.228	-0.196	4.42	0.73 (0.13)
CCC	99	6013.6	2.0 (20.7)	117	0.0080	28	0.0036	0.082	0.007	4.58	0.72 (0.11)
GL	92	5966.6	2.1 (21.4)	126	0.0105	30	0.0054	0.116	-1.342	4.58	0.79 (0.13)
MG	100	5982.8	2.2 (21.9)	157	0.0124	32	0.0060	0.294	0.435	5.00	0.81 (0.11)

n – haploid sample size; L – average length of the sequences in base pairs excluding indels; I (L) – number of indels (total length); S – number of polymorphic sites (singleton); π – nucleotide diversity (Nei 1987); [a] Tajima's *D* test (Tajima 1989), [b] Fay and Wu *H* test (Fay and Wu 2000); [c] - least-squares estimate of recombination parameter (standard error), [d] average values excluding *lp3-3* locis; [e] multilocus least-squares estimate of recombination parameter at the loci excluding *lp3-3* (standard error); N – number of haplotypes (number of unique haplotypes at the locus), H_d – haplotype diversity (standard deviation); "-" not estimated due to low number of informative sites or samples; * significance relative to expectations based on coalescent simulations with recombination (see material and methods for details), $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Supplementary Table S2. Average pairwise differentiation in comparisons between populations for the combined dataset of 12 loci.

	BE	**GA**	**GE**	**SD**	**BB**	**GD**	**GT**	**RM**	**BW**	**CCC**	**GL**	**MG**
Beinn Eighe	***											
Glen Affric	0.0094	***										
Glen Einig	0.0096	0.0086	***									
Shieldaig	0.0109	0.0099	0.0095	***								
Ballochbuie	0.0097	0.0089	0.0088	0.0107	***							
Glen Derry	0.0098	0.0086	0.0089	0.0099	0.0091	***						
Glen Tanar	0.0097	0.0088	0.0087	0.0105	0.0090	0.0087	***					
Rothiemurchus	0.0097	0.0086	0.0086	0.0104	0.0090	0.0092	0.0082	***				
Black Wood of Rannoch	0.0098	0.0088	0.0086	0.0103	0.0086	0.0089	0.0085	0.0088	***			
Coille Coire Chuilc	0.0086	0.0084	0.0090	0.0113	0.0088	0.0090	0.0080	0.0084	0.0090	***		
Glen Loy	0.0103	0.0096	0.0089	0.0097	0.0100	0.0098	0.0094	0.0097	0.0094	0.0098	***	
Meggernie	0.0110	0.0107	0.0110	0.0112	0.0110	0.0107	0.0106	0.0105	0.0110	0.0101	0.0110	***
Average	**0.0099**	**0.0091**	**0.0091**	**0.0104**	**0.0094**	**0.0093**	**0.0091**	**0.0092**	**0.0092**	**0.0091**	**0.0098**	**0.0108**

Supplementary Table S3.
Average percentage of shared polymorphisms in pairwise comparisons between populations for the combined dataset of 12 loci.

	BE	GA	GE	SD	BB	GD	GT	RM	BW	CCC	GL	MG
Beinn Eighe	***											
Glen Affric	70.7	***										
Glen Einig	73.7	77.7	***									
Shieldaig	64.0	60.9	62.8	***								
Ballochbuie	69.5	79.2	80.6	64.6	***							
Glen Derry	74.9	69.6	76.1	70.8	73.1	***						
Glen Tanar	63.1	63.1	67.0	59.6	66.7	75.3	***					
Rothiemurchus	82.1	73.5	76.4	65.4	74.2	77.3	66.4	***				
Black Wood of Rannoch	59.2	65.7	69.6	52.3	70.2	68.5	71.6	65.2	***			
Coille Coire Chuilc	77.1	73.6	76.6	68.3	74.4	81.9	71.1	76.1	64.2	***		
Glen Loy	83.1	71.2	74.9	70.6	73.7	75.1	65.2	82.6	60.6	75.5	***	
Meggernie	73.4	68.8	70.4	63.0	71.0	72.5	65.6	80.1	64.5	78.8	78.1	***
Average	71.9	70.4	73.3	63.9	72.5	74.1	66.8	74.5	64.7	74.3	73.7	71.5

Supplementary Table S4. AMOVA results for all SNP_S combined and all populations studied.

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	11	157.392	0.06546 (Va)	0.48
Within populations	108	1474.600	13.65370 (Vb)	99.52
Total	119	1631.992	13.71917	
Fixation Index	0.0048			

Supplementary Table S5.

Significant values of *Fst* statistics for corresponding loci (marked in superscript) in pairwise comparisons between populations (P<0.05)

Population	BE	GA	GE	SD	BB	GD	GT	RM	BW	CCC	GL
Beinn Eighe (BE)											
Glen Affric (GA)	0.222 ^{dhn9}										
Glen Einig (GE)		0.258 ^{a3ip}									
Shieldaig (SD)		0.319 ^{dhn9}	0.141 ^{a3ip} 0.133 ^{abaR} 0.235 ^{dhn2} 0.141 ^{All}								
Ballochuie (BB)	0.236 ^{dhn9}			0.231 ^{dhn3} 0.136 ^{All}							
Glen Derry (GD)	0.370 ^{ccoam} 0.579 ^{dhn7}										
Glen Tanar (GT)	0.149 ^{abaR}	0.282 ^{a3ip} 0.186 ^{abaR}	0.312 ^{abaR}	0.235 ^{a3ip} 0.391 ^{dhn3}	0.245 ^{abaR} 0.206 ^{dhn9}	0.186 ^{abaR}					
Rothiemurchus (RM)	0.356 ^{dhn7}	0.066 ^{a3ip}	0.183 ^{dhn2PP}	0.116 ^{abaR}		0.444 ^{ccoam}	0.340 ^{abaR}				
Black Wood of Rannoch (BW)	0.143 ^{dhn3} 0.271 ^{dhn9}		0.076 ^{abaR}	0.260 ^{dhn2} 0.437 ^{dhn3} 0.396 ^{dhn9}		0.300 ^{dhn2}	0.249 ^{abaR}				
Coille Coire Chuilc (CCC)		0.199 ^{a3ip}		0.134 ^{a3ip}		0.579 ^{dhn7}		0.356 ^{dhn7}			
Glen Loy (GL)		0.440 ^{chcs}	0.459 ^{chcs}	0.248 ^{chcs}	0.404 ^{chcs}	0.255 ^{chcs}	0.212 ^{abaR} 0.414 ^{chcs}	0.618 ^{chcs}	0.217 ^{chcs}	0.459 ^{chcs}	
Meggernie (MG)	0.254 ^{dhn7}	0.106 ^{a3ip} 0.221 ^{dhn9}	0.206 ^{dhn9}		0.159 ^{dhn3} 0.231 ^{dhn9}		0.279 ^{abaR}	0.255 ^{dhn2PP}	0.254 ^{dhn7} 0.234 ^{dhn9}		

Supplementary Table S6.

Significant values of S_{nn} for pairwise comparisons between populations.

Population	BE	GA	GE	SD	BB	GD	GT	RM	BW	CCC	GL
Beinn Eighe(BE)											
Glen Affric (GA)	0.624^{a3ip*}										
Glen Einig (GE)		0.683^{a3ip**}									
Shieldaig (SD)			0.683^{dhn2pp*}								
Ballochuie (BB)	0.857^{dhn2**}	0.863^{dhn2*}		0.833^{dhn2*}							
Glen Derry (GD)	0.642^{ccoam*} 0.764^{dhn3*} 0.818^{dhn7*}	0.537^{a3ip*}		0.916^{lp31*}							
Glen Tanar (GT)	0.698^{abaR*} 0.642^{dhn7*} 0.712^{dhn9*}	0.676^{a3ip*} 0.788^{lp33*}	0.692^{abaR*}		0.707^{abaR*}	0.640^{abaR*} 0.705^{dhn9*}					
Rothiemurchus (RM)	0.652^{dhn7*}	0.624^{a3ip*}	0.711^{dhn2pp*}			0.642^{ccoam*} 0.736^{dhn2pp*}	0.730^{abaR*}				
Black Wood of Rannoch (BW)	0.660^{dhn2*} 0.833^{dhn3*}	0.600^{a3ip*}	0.740^{dhn3*}	0.805^{dhn2*}		0.753^{dhn9*}	0.759^{abaR**}				
Coille Coire Chuilc (CCC)		0.696^{a3ip**} 0.667^{dhn7*}				0.818^{dhn7*}	0.642^{dhn7*}	0.652^{dhn7*} 0.720^{lp31*}	0.708^{dhn9*} 0.691^{lp31*}		
Glen Loy (GL)	0.745^{2pp*} 0.802^{dhn3*}	0.835^{chs*} 0.750^{lp31*}	0.933^{chs***}	0.688^{chs*}	0.857^{chs**}		0.825^{chs**} 0.854^{lp31*}	0.928^{chs***} 0.842^{dhn2pp*}	0.661^{abaR*} 0.910^{chs**} 0.755^{lp31*}	0.889^{chs***} 0.755^{lp31*}	
Meggernie (MG)	0.705^{dhn7*} 0.833^{lp31*}	0.580^{a3ip*}		0.679^{2pp*}	0.747^{abaR**}		0.688^{abaR*} 0.725^{dhn2pp*}	0.811^{dhn2pp**}	0.696^{abaR*} 0.667^{dhn3*}	0.704^{dhn7*} 0.785^{lp31*}	0.785^{chs*} 0.807^{lp31*}

*, 0.01<P<0.05; **, 0.001<P<0.01; ***, P<0.001

Appendices, <u>for online publication only</u>

Appendix 1. Summary statistics of nucleotide and haplotype variation, neutrality tests and recombination rate estimates at the loci studied in the Scots pine populations in Scotland. Population names and locations are given in Table 1.

					Nucleotide diversity							
					Total		**Nonsynonymous**				**Haplotype diversity**	
Locus	Pop.	n	L	I (L)	S	π	S	π	D^a	H^b	N	H_d (SD)
dhn2	BE	8	591.5	7 (29)	12	0.0099	1	0.0030	1.220	-1.429	5(3)	0.857(0.108)
	GA	5	596.6	7 (29)	11	0.0093	1	0.0032	0.164	-0.3	5(1)	1(0.126)
	GE	7	588.9	9 (29)	11	0.0067	1	0.0015	-0.705	-1.571	3(2)	0.524(0.209)
	SD	6	585.5	7 (38)	12	0.0090	3	0.0064	-0.141	0.267	4(3)	0.867(0.129)
	BB	6	594.5	6 (19)	8	0.0079	1	0.0032	1.957	0.533	4(2)	0.8(0.172)
	GD	5	588.5	7 (29)	11	0.0090	1	0.0021	-0.109	-3.7	3(1)	0.7(0.218)
	GT	4	587.2	6 (28)	10	0.0086	1	0.0027	-0.834	-0.667	3(1)	0.833(0.222)
	RM	6	588.8	7 (29)	10	0.0071	1	0.0028	-0.338	0.267	3(1)	0.6(0.215)
	BW	6	591.9	7 (20)	7	0.0053	1	0.0028	0.128	0.267	3(1)	0.6(0.215)
	CCC	7	598.4	7 (20)	13	0.0097	1	0.0030	0.421	-2.714	6(2)	0.952(0.096)
	GL	5	590.3	6 (28)	10	0.0083	2	0.0053	0.000	-1.3	4(1)	0.9(0.161)
	MG	6	594.7	6 (28)	15	0.0115	1	0.0028	0.091	0.267	6(3)	1(0.096)
dhn3	BE	9	333.2	1 (6)	16	0.0197	4	0.0130	0.551	4.111	4(1)	0.833(0.08)
	GA	7	333.0	1 (6)	17	0.0152	4	0.0096	-1.518	3.095	4(1)	0.810(0.130)
	GE	9	333.2	1 (6)	19	0.0205	7	0.0170	-0.110	3.667	5(0)	0.861(0.087)
	SD	6	334.2	1 (6)	14	0.0250	3	0.0170	2.205*	-0.533	3(0)	0.733(0.155)
	BB	10	333.0	1 (6)	18	0.0118	5	0.0077	-1.783*	1.422	6(0)	0.867(0.085)
	GD	8	333.2	1 (6)	21	0.0168	3	0.0106	-1.606	2.929	5(1)	0.786(0.151)
	GT	7	333.0	1 (6)	4	0.0040	1	0.0015	-0.876	-0.667	4(0)	0.810(0.130)
	RM	9	333.0	1 (6)	16	0.0124	4	0.0074	-1.462	2.472	5(0)	0.861(0.087)
	BW	9	333.0	1 (6)	3	0.0033	0	0.0000	0.025	-0.528	4(1)	0.806(0.089)
	CCC	8	333.0	1 (6)	16	0.0130	3	0.0071	-1.540	3.214	4(1)	0.750(0.139)
	GL	7	333.3	1 (6)	17	0.0226	6	0.0179	0.469	4.714	5(1)	0.905(0.103)
	MG	9	333.8	2 (7)	17	0.0253	5	0.0183	1.661	1.528	4(1)	0.750(0.112)
dhn7	BE	7	329.0	0 (0)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	GA	7	327.6	1 (2)	3	0.0044	2	0.0047	0.755	-1.143	3(0)	0.714(0.127)
	GE	8	328.5	1 (2)	4	0.0049	3	0.0057	0.182	-3.357	4(1)	0.750(0.139)
	SD	4	329.0	0 (0)	2	0.0041	1	0.0036	1.893	0	2(0)	0.667(0.204)
	BB	9	327.8	1 (2)	3	0.0041	2	0.0043	0.794	-1.833	3(0)	0.639(0.126)
	GD	4	327.3	1 (2)	4	0.0061	1	0.0027	-0.78	1.333	3(1)	0.833(0.222)
	GT	8	326.2	2 (8)	6	0.0067	2	0.0045	-0.345	-0.857	4(1)	0.786(0.113)

	RM	8	328.5	1 (2)	3	0.0042	2	0.0045	0.712	-1.286	4(0)	0.786(0.113)
	BW	10	326.5	2 (8)	6	0.0059	3	0.0051	-0.453	-1.333	5(2)	0.756(0.130)
	CCC	7	329.0	0 (0)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	GL	4	328.0	1 (2)	3	0.0056	2	0.0064	1.09	-1	3(0)	0.833(0.222)
	MG	7	326.7	2 (8)	6	0.0068	3	0.0062	-0.536	-1.952	5(0)	0.905(0.103)
dhn9	BE	6	724.4	2 (11)	25	0.0178	6	0.0136	1.103	-5.333	4(0)	0.8(0.172)
	GA	7	729.4	1 (5)	7	0.0048	5	0.0053	1.208	1.095	4(0)	0.857(0.102)
	GE	6	729.0	1 (5)	3	0.0024	2	0.0024	1.648	-0.533	3(1)	0.733(0.155)
	SD	5	728.0	1 (5)	15	0.0115	9	0.0104	1.219	-0.6	3(0)	0.7(0.218)
	BB	6	730.0	1 (5)	7	0.0038	5	0.0043	-0.631	-1.6	4(1)	0.8(0.172)
	GD	6	728.0	1 (5)	14	0.0071	9	0.0072	-1	-6.667	3(1)	0.733(0.155)
	GT	10	728.0	1 (5)	16	0.0101	9	0.0089	1.373	-1.867	5(2)	0.8(0.1)
	RM	7	728.7	2 (11)	27	0.0120	7	0.0102	-1.214	-15.81	5(0)	0.905(0.103)
	BW	6	730.0	1 (5)	3	0.0022	2	0.0022	1.124	0	2(0)	0.533(0.172)
	CCC	7	728.7	1 (5)	17	0.0099	12	0.0101	0.24	-4.095	5(1)	0.857(0.137)
	GL	8	726.7	2 (11)	29	0.0139	8	0.0118	-0.561	-12.571	6(1)	0.893(0.111)
	MG	6	724.4	2 (11)	30	0.0207	10	0.0156	1.103	0.267	6(4)	1(0.096)
dhn2PP	BE	10	591.5	1 (8)	12	0.0106	1	0.0014	0.666	0.711	8(1)	0.844(0.08)
	GA	10	596.6	1 (8)	11	0.0106	2	0.0016	0.216	0.444	8(1)	0.933(0.077)
	GE	10	588.9	0 (0)	11	0.0103	0	0.0000	0.615	1.067	8(1)	0.933(0.077)
	SD	9	585.5	1 (8)	12	0.0111	1	0.0009	0.719	1.583	5(0)	0.833(0.098)
	BB	10	594.5	1 (8)	8	0.0107	1	0.0014	0.717	1.689	8(0)	0.956(0.059)
	GD	10	588.5	1 (8)	11	0.0070	0	0.0000	0.195	-0.978	9(2)	0.978(0.054)
	GT	10	587.2	1 (8)	10	0.0103	1	0.0008	0.487	1.333	6(1)	0.889(0.075)
	RM	9	588.8	1 (8)	10	0.0111	1	0.0015	0.719	2.333	7(1)	0.944(0.070)
	BW	10	591.9	1 (8)	7	0.0114	3	0.0023	-0.456	1.778	7(2)	0.911(0.077)
	CCC	10	598.4	1 (8)	13	0.0088	2	0.0016	0.221	-0.356	7(0)	0.867(0.107)
	GL	9	590.3	0 (0)	10	0.0103	3	0.0033	-0.71	1.778	8(3)	0.972(0.064)
	MG	10	594.7	0 (0)	15	0.0086	1	0.0008	1.314	1.156	7(0)	0.911(0.077)
abaR	BE	9	421.1	4 (22)	5	0.0052	2	0.0042	0.753	-0.667	5(0)	0.861(0.087)
	GA	9	420.8	4 (22)	6	0.0052	3	0.0055	-0.081	-2.111	5(0)	0.833(0.098)
	GE	9	421.2	4 (22)	4	0.0040	2	0.0037	0.538	0.833	5(1)	0.861(0.087)
	SD	10	421.3	3 (21)	5	0.0043	3	0.0043	0.074	-1.333	5(2)	0.822(0.097)
	BB	9	420.8	4 (22)	3	0.0040	2	0.0053	1.948	0.333	3(0)	0.667(0.105)
	GD	10	421.0	4 (22)	4	0.0039	2	0.0042	0.626	-0.995	5(1)	0.8(0.1)
	GT	10	421.4	4 (22)	6	0.0046	3	0.0045	-0.409	-3.022	4(0)	0.711(0.117)
	RM	10	420.7	4 (22)	4	0.0039	2	0.0051	0.566	0.356	5(1)	0.822(0.097)
	BW	10	421.1	3 (21)	6	0.0057	3	0.0061	0.501	0.711	6(1)	0.844(0.103)
	CCC	10	421.4	3 (21)	8	0.0064	3	0.0056	-0.212	0.533	8(4)	0.956(0.059)

	GL	10	421.2	4 (22)	5	0.0042	3	0.0041	-0.027	-2.044	5(4)	0.756(0.130)
	MG	10	421.1	3 (21)	6	0.0054	3	0.0060	0.328	0.49	5(0)	0.8(0.1)
a3ip2	BE	9	862.0	2 (23)	5	0.0032	0	0.0000	2.16*	0.194	2(0)	0.556(0.09)
	GA	10	862.0	2 (23)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	GE	8	862.0	2 (23)	9	0.0044	0	0.0000	0.394	-3.857	4(1)	0.786(0.113)
	SD	10	862.0	2 (23)	6	0.0016	0	0.0000	-1.493	-0.8	4(2)	0.533(0.180)
	BB	8	862.0	2 (23)	5	0.0027	0	0.0000	1.008	0.5	3(0)	0.607(0.164)
	GD	10	862.0	2 (23)	11	0.0037	1	0.0031	-0.793	-4.089	4(0)	0.644(0.152)
	GT	9	863.8	2 (23)	10	0.0051	0	0.0000	0.898	-0.083	5(1)	0.806(0.120)
	RM	9	862.0	2 (23)	9	0.0029	0	0.0000	-1.128	-2.204	4(1)	0.694(0.147)
	BW	10	862.5	2 (23)	10	0.0044	0	0.0000	0.277	-2.222	4(1)	0.644(0.152)
	CCC	9	862.6	2 (23)	12	0.0054	1	0.0034	0.27	-1.278	5(2)	0.833(0.098)
	GL	8	862.0	2 (23)	6	0.0020	0	0.0000	-1.28	0	3(0)	0.464(0.2)
	MG	10	862.0	2 (23)	9	0.0034	0	0.0000	-0.311	-3.822	5(0)	0.756(0.130)
ccoaomt	BE	10	519.0	1 (4)	0	0.0000	0	0.0000	-	-	1(1)	0(0)
	GA	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
	GE	10	519.0	1 (4)	5	0.0019	0	0.0000	-1.741*	-0.889	3(1)	0.378(0.181)
	SD	9	519.0	1 (4)	4	0.0017	0	0.0000	-1.61	-0.972	2(0)	0.222(0.166)
	BB	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
	GD	10	519.0	1 (4)	4	0.0043	0	0.0000	2.195*	0	2(0)	0.556(0.075)
	GT	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
	RM	10	519.0	1 (4)	0	0.0000	0	0.0000	-	-	1(0)	0(0)
	BW	10	519.0	1 (4)	4	0.0027	0	0.0000	0.022	-0.267	2(0)	0.356(0.159)
	CCC	10	519.0	1 (4)	4	0.0027	0	0.0000	0.022	-0.267	2(0)	0.356(0.159)
	GL	10	519.0	1 (4)	4	0.0024	0	0.0000	-0.4	-0.8	3(1)	0.378(0.181)
	MG	10	519.0	1 (4)	4	0.0015	0	0.0000	-1.667	-1.067	2(0)	0.2(0.154)
chcs	BE	6	305.1	1 (1)	4	0.0066	0	0.0000	0.768	-0.8	5(0)	0.933(0.122)
	GA	9	305.4	1 (1)	4	0.0046	0	0.0000	-0.229	-1.472	4(0)	0.694(0.147)
	GE	7	305.5	1 (1)	2	0.0031	0	0.0000	0.687	-0.429	2(0)	0.476(0.171)
	SD	8	305.2	1 (1)	7	0.0084	0	0.0000	-0.226	0	4(0)	0.750(0.139)
	BB	6	305.7	1 (1)	4	0.0050	0	0.0000	-0.676	-0.533	3(0)	0.733(0.155)
	GD	7	305.3	1 (1)	5	0.0072	0	0.0000	0.363	0.381	5(0)	0.857(0.137)
	GT	8	305.5	1 (1)	4	0.0048	0	0.0000	-0.222	-1.357	4(0)	0.643(0.184)
	RM	6	306.0	0	2	0.0028	0	0.0000	-0.05	-1.067	3(1)	0.733(0.155)
	BW	6	305.2	1 (1)	10	0.0138	0	0.0000	-0.246	0	3(1)	0.733(0.155)
	CCC	7	305.5	1 (1)	2	0.0031	0	0.0000	0.687	-0.429	4(0)	0.714(0.181)
	GL	8	305.0	1 (1)	6	0.0077	0	0.0000	0.087	-0.429	3(0)	0.679(0.122)
	MG	6	305.2	1 (1)	6	0.0096	0	0.0000	0.666	0.267	3(0)	0.733(0.155)
erd3	BE	9	583.0	0	2	0.0011	0	0.0000	-0.583	0.472	3(0)	0.417(0.191)

	GA	10	583.0	0	4	0.0020	2	0.0014	-0.702	0.533	4(1)	0.733(0.101)
	GE	10	583.0	0	4	0.0019	0	0.0000	-0.762	-1.511	4(1)	0.711(0.117)
	SD	10	583.0	0	2	0.0013	0	0.0000	0.222	-2.321	3(1)	0.644(0.101)
	BB	10	583.0	0	7	0.0029	2	0.0014	-1.269	-0.978	5(2)	0.800(0.1)
	GD	10	583.0	0	4	0.0020	1	0.0007	-0.702	0.492	4(2)	0.733(0.101)
	GT	9	583.0	0	1	0.0009	0	0.0000	0.986	0.178	2(0)	0.5(0.128)
	RM	10	583.0	0	3	0.0019	0	0.0000	0.096	0.178	5(0)	0.756(0.130)
	BW	10	583.0	0	3	0.0016	1	0.0007	-0.431	0.597	4(1)	0.733(0.101)
	CCC	10	583.0	0	1	0.0010	0	0.0000	1.464	0	2(0)	0.556(0.075)
	GL	10	583.0	0	3	0.0021	0	0.0000	0.473	0.178	4(0)	0.800(0.076)
	MG	10	583.0	0	3	0.0019	0	0.0000	0.096	0.178	4(1)	0.778(0.091)
lp3-1	BE	3	373.0	0	5	0.0089	0	0.0000	-	-1.333	3(3)	1(0.272)
	GA	8	367.9	1 (8)	13	0.0141	1	0.0030	0.13	1.143	6(3)	0.929(0.084)
	GE	5	365.8	1 (8)	9	0.0126	1	0.0049	0.461	-0.8	5(0)	1(0.126)
	SD	3	373.0	0	7	0.0125	1	0.0081	-	1.333	3(2)	1(0.272)
	BB	5	369.8	1 (8)	11	0.0153	1	0.0073	0.436	1.7	4(1)	0.9(0.161)
	GD	6	365.5	1 (8)	9	0.0121	1	0.0073	0.693	1.6	4(2)	0.867(0.129)
	GT	5	365.8	1 (8)	8	0.0110	1	0.0049	0.294	0	4(2)	0.9(0.161)
	RM	7	366.1	1 (8)	12	0.0128	1	0.0058	-0.257	-1	6(2)	0.952(0.096)
	BW	9	369.7	1 (8)	13	0.0152	1	0.0027	0.77	0.861	7(4)	0.944(0.07)
	CCC	8	367.9	1 (8)	10	0.0114	1	0.0030	0.367	2	6(5)	0.893(0.111)
	GL	7	366.1	1 (8)	7	0.0099	0	0.0000	1.381	-0.095	6(2)	0.952(0.096)
	MG	6	370.3	1 (8)	15	0.0185	1	0.0065	0.154	3.467	5(2)	0.933(0.122)
lp3-3	BE	5	342.8	6 (152)	25	0.0437	7	0.0203	1.342	4.5	4(1)	0.9(0.161)
	GA	8	343.6	6 (152)	26	0.0402	6	0.0157	1.56	1.571	5(1)	0.857(0.108)
	GE	3	332.0	6 (152)	20	0.0429	5	0.0183	-	-9.667	2(0)	0.667(0.314)
	SD	2	374.0	6 (89)	6	0.0160	2	0.0110	-	-	2(0)	1(0.5)
	BB	2	310.0	6 (153)	20	0.0645	5	0.0275	-	-	2(2)	1(0.5)
	GD	7	341.0	6 (152)	26	0.0387	7	0.0167	1.301	-0.095	7(3)	1(0.076)
	GT	5	344.0	6 (152)	19	0.0341	6	0.0181	1.671	3	3(1)	0.8(0.164)
	RM	5	344.0	6 (152)	25	0.0399	7	0.0187	0.915	4.6	4(1)	0.9(0.161)
	BW	8	342.6	6 (152)	26	0.0381	7	0.0188	1.476	-2.214	6(2)	0.929(0.084)
	CCC	6	366.7	6 (152)	21	0.0247	5	0.0090	-1.042	3.467	5(2)	0.933(0.122)
	GL	6	341.7	6 (152)	26	0.0369	6	0.0165	0.869	-4.533	5(0)	0.933(0.122)
	MG	10	347.9	6 (152)	31	0.0360	8	0.0154	0.63	4.444	8(3)	0.956(0.059)
Average /Total	BE	91	5975.6	2.1 (21.0)	111	0.0106	21	0.0046	0.887*	0.043	3.75	0.67 (0.11)
	GA	100	5984.9	2.2 (21.7)	106	0.0093	26	0.0042	0. 153	0.163	4.25	0.71 (0.10)
	GE	92	5957.0	2.3 (21.0)	101	0.0096	21	0.0045	0.110	-1.421	4.00	0.72 (0.14)
	SD	82	5999.7	1.9 (16.3)	92	0.0089	23	0.0051	0.286	-0.307	3.33	0.73 (0.19)

BB	91	5950.1	2.1 (20.9)	98	0.0112	24	0.0052	0.076	0.015	3.92	0.75 (0.16)
GD	93	5962.3	2.2 (21.7)	124	0.0098	26	0.0046	0.032	-0.816	4.50	0.79 (0.13)
GT	95	5964.1	2.2 (22.1)	98	0.0085	24	0.0038	0.113	-0.423	3.83	0.72 (0.14)
RM	96	5968.6	2.2 (22.1)	121	0.0092	25	0.0047	-0.131	-1.015	4.33	0.75 (0.11)
BW	104	5976.4	2.2 (21.3)	98	0.0091	21	0.0034	0.228	-0.196	4.42	0.73 (0.13)
CCC	99	6013.6	2.0 (20.7)	117	0.0080	28	0.0036	0.082	0.007	4.58	0.72 (0.11)
GL	92	5966.6	2.1 (21.4)	126	0.0105	30	0.0054	0.116	-1.342	4.58	0.79 (0.13)
MG	100	5982.8	2.2 (21.9)	157	0.0124	32	0.0060	0.294	0.435	5.00	0.81 (0.11)

n – haploid sample size; L – average length of the sequences in base pairs excluding indels; I (L) – number of indels (total length); S – number of polymorphic sites (singleton); π – nucleotide diversity (Nei 1987); [a] Tajima's *D* test (Tajima 1989), [b] Fay and Wu *H* test (Fay and Wu 2000); [c] - least-squares estimate of recombination parameter (standard error), [d] average values excluding *lp3-3* locus; [e] multilocus least-squares estimate of recombination parameter at the loci excluding *lp3-3* (standard error); N – number of haplotypes (number of unique haplotypes at the locus), H_d – haplotype diversity (standard deviation); "-" not estimated due to low number of informative sites or samples; [*] significance relative to expectations based on coalescent simulations with recombination (see material and methods for details), [*]P<0.05; [**] P<0.01; [***] P<0.001.

Appendix 2. Average pairwise differentiation in comparisons between populations for the combined dataset of 12 loci.

	BE	**GA**	**GE**	**SD**	**BB**	**GD**	**GT**	**RM**	**BW**	**CCC**	**GL**	**MG**
Beinn Eighe	***											
Glen Affric	0.0094	***										
Glen Einig	0.0096	0.0086	***									
Shieldaig	0.0109	0.0099	0.0095	***								
Ballochbuie	0.0097	0.0089	0.0088	0.0107	***							
Glen Derry	0.0098	0.0086	0.0089	0.0099	0.0091	***						
Glen Tanar	0.0097	0.0088	0.0087	0.0105	0.0090	0.0087	***					
Rothiemurchus	0.0097	0.0086	0.0086	0.0104	0.0090	0.0092	0.0082	***				
Black Wood of Rannoch	0.0098	0.0088	0.0086	0.0103	0.0086	0.0089	0.0085	0.0088	***			
Coille Coire Chuilc	0.0086	0.0084	0.0090	0.0113	0.0088	0.0090	0.0080	0.0084	0.0090	***		
Glen Loy	0.0103	0.0096	0.0089	0.0097	0.0100	0.0098	0.0094	0.0097	0.0094	0.0098	***	
Meggernie	0.0110	0.0107	0.0110	0.0112	0.0110	0.0107	0.0106	0.0105	0.0110	0.0101	0.0110	***
Average	**0.0099**	**0.0091**	**0.0091**	**0.0104**	**0.0094**	**0.0093**	**0.0091**	**0.0092**	**0.0092**	**0.0091**	**0.0098**	**0.0108**

Appendix 3.

Average percentage of shared polymorphisms in pairwise comparisons between populations for the combined dataset of 12 loci.

	BE	GA	GE	SD	BB	GD	GT	RM	BW	CCC	GL	MG
Beinn Eighe	***											
Glen Affric	70.7	***										
Glen Einig	73.7	77.7	***									
Shieldaig	64.0	60.9	62.8	***								
Ballochbuie	69.5	79.2	80.6	64.6	***							
Glen Derry	74.9	69.6	76.1	70.8	73.1	***						
Glen Tanar	63.1	63.1	67.0	59.6	66.7	75.3	***					
Rothiemurchus	82.1	73.5	76.4	65.4	74.2	77.3	66.4	***				
Black Wood of Rannoch	59.2	65.7	69.6	52.3	70.2	68.5	71.6	65.2	***			
Coille Coire Chuilc	77.1	73.6	76.6	68.3	74.4	81.9	71.1	76.1	64.2	***		
Glen Loy	83.1	71.2	74.9	70.6	73.7	75.1	65.2	82.6	60.6	75.5	***	
Meggernie	73.4	68.8	70.4	63.0	71.0	72.5	65.6	80.1	64.5	78.8	78.1	***
Average	71.9	70.4	73.3	63.9	72.5	74.1	66.8	74.5	64.7	74.3	73.7	71.5

Appendix 4. AMOVA results for all SNPs combined and all populations studied.

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	11	157.392	0.06546 (Va)	0.48
Within populations	108	1474.600	13.65370 (Vb)	99.52
Total	119	1631.992	13.71917	
Fixation Index	0.0048			

Appendix 5.
Significant values of F_{ST} statistics for corresponding loci (marked in superscript) in pairwise comparisons between populations (P<0.05)

Population	BE	GA	GE	SD	BB	GD	GT	RM	BW	CCC	GL
Beinn Eighe (BE)											
Glen Affric (GA)	0.222 ^{dhn9}										
Glen Einig (GE)		0.258 ^{a3ip}									
Shieldaig (SD)		0.319 ^{dhn9}	0.141 ^{a3ip}								

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			0.133 abaR 0.235 dhn2 0.141 All								
Ballochuie (BB)	0.236 dhn9			0.231 dhn3 0.136 All							
Glen Derry (GD)	0.370 ccoam 0.579 dhn7										
Glen Tanar (GT)	0.149 abaR	0.282 a3ip 0.186 abaR	0.312 abaR	0.235 a3ip 0.391 dhn3	0.245 abaR 0.206 dhn9	0.186 abaR					
Rothiemurchus (RM)	0.356 dhn7	0.066 a3ip	0.183 dhn2PP	0.116 abaR		0.444 ccoam	0.340 abaR				
Black Wood of Rannoch (BW)	0.143 dhn3 0.271 dhn9		0.076 abaR	0.260 dhn2 0.437 dhn3 0.396 dhn9		0.300 dhn2	0.249 abaR				
Coille Coire Chuilc (CCC)		0.199 a3ip		0.134 a3ip		0.579 dhn7		0.356 dhn7			
Glen Loy (GL)		0.440 chcs	0.459 chcs	0.248 chcs	0.404 chcs	0.255 chcs	0.212 abaR 0.414 chcs	0.618 chcs	0.217 chcs	0.459 chcs	
Meggernie (MG)	0.254 dhn7	0.106 a3ip 0.221 dhn9	0.206 dhn9		0.159 dhn3 0.231 dhn9		0.279 abaR	0.255 dhn2PP	0.254 dhn7 0.234 dhn9		

Appendix 6.
Significant values of S_{nn} for pairwise comparisons between populations.

Population	**BE**	**GA**	**GE**	**SD**	**BB**	**GD**	**GT**	**RM**	**BW**	**CCC**	**GL**
Beinn Eighe(BE)											
Glen Affric (GA)	0.624 a3ip*										
Glen Einig (GE)		0.683 a3ip**									
Shieldaig (SD)			0.683 dhn2pp*								
Ballochuie (BB)	0.857 dhn2**	0.863 dhn2*		0.833 dhn2*							
Glen Derry (GD)	0.642 ccoam* 0.764 dhn3*	0.537 a3ip*		0.916 lp31*							

	0.818^{dhn7*}											
Glen Tanar (GT)	0.698 ^{abaR*} 0.642^{dhn7*} 0.712^{dhn9*}	0.676 ^{a3ip*} 0.788^{lp33*}	0.692^{abaR*}		0.707^{abaR*}	0.640 ^{abaR*} 0.705 ^{dhn9*}						
Rothiemurchus (RM)	0.652^{dhn7*}	0.624 ^{a3ip*}	0.711^{dhn2pp*}			0.642^{ccoam*} 0.736^{dhn2pp*}	0.730^{abaR*}					
Black Wood of Rannoch (BW)	0.660^{dhn2*} 0.833^{dhn3*}	0.600 ^{a3ip*}	0.740^{dhn3*}	0.805^{dhn2*}		0.753 ^{dhn9*}	0.759^{abaR**}					
Coille Coire Chuilc (CCC)		0.696^{a3ip**} 0.667^{dhn7*}				0.818^{dhn7*}	0.642^{dhn7*}	0.652^{dhn7*} 0.720^{lp31*}	0.708^{dhn9*} 0.691^{lp31*}			
Glen Loy (GL)	0.745^{2pp*} 0.802^{dhn3*}	0.835^{chs*} 0.750^{lp31*}	0.933^{chs***}	0.688^{chs*}	0.857^{chs**}		0.825^{chs**} 0.854^{lp31*}	0.928^{chs***} 0.842^{dhn2pp*}	0.661 ^{abaR*} 0.910^{chs**} 0.755^{lp31*}	0.889^{chs***} 0.755^{lp31*}		
Meggernie (MG)	0.705^{dhn7*} 0.833^{lp31*}	0.580 ^{a3ip*}		0.679^{2pp*}	0.747^{abaR**}		0.688^{abaR*} 0.725^{dhn2pp*}	0.811^{dhn2pp**}	0.696 ^{abaR*} 0.667^{dhn3*}	0.704^{dhn7*} 0.785^{lp31*}	0.785^{chs*} 0.807^{lp31*}	

*, 0.01<P<0.05; **, 0.001<P<0.01; ***, P<0.001