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Title

Ecology and management history drive spatial genetic structure in Scots pine

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Abstract

Forest management practices that remove trees from stands can promote substantial changes in the distribution of genetic diversity within and among populations at multiple spatial scales. In small and isolated populations, elevated inbreeding levels might reduce fitness of subsequent generations and threaten forest resilience in the long term. Comparing fine-scale spatial genetic structure (SGS) between life stages (e.g. adult and juvenile cohorts) can identify when populations have undergone disturbance, even in species with long generation times. Here, we studied the effects of historical and contemporary forest management, characterized by intense felling and natural regeneration respectively, on genetic diversity and fine-scale SGS in adult and juvenile cohorts. We examined fragmented Scots pine (*Pinus sylvestris* L.) stands in the Scottish Highlands, and compared them with a remote, unmanaged stand. A total of 777 trees were genotyped using 12 nuclear microsatellite markers. No difference was identified in allelic richness or gene diversity among stands or life stages, suggesting that historical and contemporary management have not impacted levels of genetic variation. However, management appears to have changed the spatial distribution of genetic variation. Adult genotypes from managed stands were more spatially structured than in the unmanaged stand, a difference mediated by contrasts in tree density, degree of fragmentation of stands at the time of establishment and rate of gap creation. Surprisingly, juveniles were less spatially structured than adults in the managed stands, suggesting an historical erosion of the structure of the adult cohort but contemporary recovery to natural dynamics, and indicating a high capacity of the species to recover after disturbance. Here we showed that using the spatial component of genetic diversity can help to detect both historical and contemporary effects of disturbance in tree populations. Evaluation of successional change is important to adequately detect early responses of tree populations to forest management practices. Overall, our study suggests that combining sustainable management with forest conservation practices that ensure larger effective population sizes is key to successfully maintaining genetic diversity in Scots pine.

1. Introduction

A prolonged history of forest exploitation based on the harvesting of trees has resulted in widespread modification of Europe's forests, impacting genetic diversity within and among populations (FAO, 2014). Currently, over 70% of European forests (representing some 15% of European forest area) are subject to a management plan or its equivalent (Forest Europe, 2015) but, despite a substantial shift toward sustainable practices over the past 25 years (FAO, 2015), the consequences of historical management practices such as extensive felling on the distribution of genetic diversity in tree species remain largely uncertain. Genetic diversity plays an essential role in underpinning forest resilience by facilitating evolutionary processes, and it is key in forest responses to disturbances, such as habitat loss, fragmentation or pathogen attack (Schaberg et al., 2008; Cavers and Cottrell, 2014). Consequently, understanding how historical and contemporary forest management have shaped patterns of genetic diversity allows evaluation of the potential resilience of European forests and informs the development of adaptive management plans.

The impact that tree removal can have on population genetics has been addressed through exploration of levels of neutral genetic variation, revealing changes in gene frequencies (Schaberg et al., 2008) and loss of alleles (Adams et al., 1998; Rajora et al., 2000; Kettle et al., 2007; Ortego et al., 2010), yet many studies have failed to detect significant effects (Bradshaw, 2004; García-Gil et al., 2015; Rajora and Pluhar, 2003; Schaberg et al., 2008; Young et al., 1996). Some authors attribute the lack of effect to the long generation time in trees, because changes in genetic diversity after disturbance may take many generations (Lowe et al., 2005). However, changes in tree distribution and age structures can alter the spatial organisation of genetic variation, even when overall levels of variation are maintained, allowing us to explore the genetic legacy of forest management (Piotti et al., 2013; Sjölund and Jump, 2015).

In naturally regenerated tree populations, genotypes are not distributed randomly. Typically, individuals become less genetically similar as the distance between them increases (Jump and Peñuelas, 2007; Paffetti et al., 2012; Vekemans and Hardy, 2004), causing a phenomenon known

as spatial genetic structure (SGS). Restricted dispersal results in offspring being more likely to establish close to the mother tree (Jump et al., 2012; Pandey et al., 2012). Consequently, the pollen and seed dispersal mechanism will strongly influence the extent and magnitude of SGS within a species. For example, plants with animal dispersed pollen usually show greater SGS than those with wind dispersed pollen (Vekemans and Hardy 2004). Furthermore, individual density is usually inversely correlated with SGS. For example, the extent of SGS in low density populations of *Acer pseudoplatanus* is nine times greater than in high density populations (Vekemans and Hardy 2004).

The ecological determinants of SGS (such as recruitment frequency, seed and pollen dispersal distance, and individual density) are commonly modified by forest management practices that remove trees. Consequent changes in SGS may alter local mating patterns and the distribution of genetic diversity in subsequent generations (Smouse and Peakall, 1999). Furthermore, different forest management practices, such as felling, coppicing or thinning, will differentially impact selection of individuals and seedling establishment potentially leading to a broad range of genetic impacts (Cottrell et al., 2003; Paffetti et al., 2012; Piotti et al., 2013; Sjölund and Jump, 2015). Distinguishing the effects of forest management on SGS is, therefore, a challenging task.

SGS of plant populations is dynamic and can change across life stages. In individuals that reproduce sexually, seedlings might be affected by compensatory mortality and competitive thinning, post dispersal, thereby altering spatial distribution patterns with age (Ng et al., 2004). Most studies have found greater SGS in early regeneration stages than in mature individuals (González-Martínez et al., 2002; Hardesty et al., 2005; Ng et al., 2004; Soto et al., 2007; Troupin et al., 2006). The successional component of SGS (e.g. comparing SGS between adult and juvenile cohorts) has mainly been studied in order to understand the natural development of SGS (Berens et al., 2014; González-Martínez et al., 2002; Jones and Hubbell, 2006). Such changes in SGS have rarely been used to assess the influence of forest management practices (but see Jones et al., 2006; Leclerc et al., 2015; Troupin et al., 2006).

This study focuses on the remaining fragmented Scots pine (*Pinus sylvestris* L.) forests of the Scottish Highlands (known as Caledonian pine forests), which are believed to be the only native pine forests in the UK. These fragmented remnants represent a valuable system in which to study the impacts of historical forest management practices because numerous records of management history exist. To understand the effects of historical and contemporary forest management practices, we investigated genetic diversity and fine-scale SGS in adult and juvenile cohorts in two native managed pine forests and compared these with a remote, unmanaged stand. We selected two life stages that were established in distinct periods with contrasting forest management systems: (1) adult trees that established during 19th Century, characterised by high browsing pressure by deer and after a period of intense felling (hereafter historical management); and (2) juveniles that established during the last two decades, characterised by conservation policies promoting natural regeneration (hereafter contemporary management). Specifically we sought to determine: 1) did historical management practice impact genetic diversity and SGS – comparing mature managed and unmanaged stands? and 2) how has contemporary management practice affected diversity and SGS – comparing adults and juveniles from managed stands? We hypothesised that in the absence of effects of historical management, mature managed stands would display similar values of genetic diversity and SGS as those in an unmanaged stand, while in the absence of effects of contemporary management, stronger SGS would be found in the juvenile stages, and similar values of genetic diversity will be evident in both juvenile and adult cohorts.

2. Material and methods

2.1. Study species

Scots pine is a wind-pollinated outcrossing conifer and is the most widely distributed pine species in the world, with a range that spans Eurasia, from the Arctic circle in Norway in the north to the south of Spain and south of Turkey and from the west coast of Scotland to the far east of Russia (Carlisle and Brown, 1968). Populations from southern Europe, Scotland and Asia Minor

generally represent isolated occurrences. In Scotland this species occurs at the western limit of its global distribution and constitutes the iconic species of the Caledonian pine forest. Scots pine is typically a pioneer species (together with birch and aspen) that readily regenerates after natural or human disturbances, if competition and grazing pressure are low (Mátyás et al., 2004). It grows well on most soils, nevertheless, due to shade and competition intolerance, it is often restricted to poor soils (Steven and Carlisle, 1959). It is a monoecious species, and female flowering can start at the age of 15 to 30 years, in open to closed stands respectively (Mátyás et al., 2004). Pollen movement is predominantly over tens of metres within a stand (Robledo-Arnuncio et al., 2004b), but it may reach 100 km (Robledo-Arnuncio, 2011). Seeds are primarily wind and gravity dispersed, and typically travel up to 100 metres (McVean, 1963).

2.2. Study sites and history of forest management

From a peak distribution around 6,000 years ago, Scots pine in Scotland has been in decline for millennia, with a major retreat 4,000 years ago, initially attributed to a climate shift to wetter conditions (Bennett, 1984), although human and grazing pressures may have also played a significant role (Tipping et al., 2008). The exploitation and reduction in Scots pine extent has been particularly intense from the 18th Century onwards (Hobbs, 2009), mainly characterized by felling and selective logging to provide construction timber (Smout, 2003). The general decrease in forest extent, together with poor natural regeneration in the Caledonian pine forest (due to extensive browsing pressure by deer and sheep), kept this forest at low tree density for many years (McVean, 1963) and has strongly suppressed regeneration during the last 200 years (Steven and Carlisle, 1959). During the last few decades, however, forest management has moved to protect and expand the remaining Caledonian pine forest (Forestry Commission, 2016).

We selected two study sites in Scotland, Abernethy (57°20’N, 3°61’W) and Glen Affric (57°15’N, 5°00’W). Nowadays, these sites constitute some of the largest ancient pine forest in Scotland covering areas of 2452 ha and 1532 ha, respectively (Mason et al., 2004). In each site, an old open native stand was selected, where trees are expected to have been established through

natural regeneration of native provenance. Hereafter these stands will be referred to as managed stands. The fire regime in the UK is largely human driven (Davies et al., 2008), but tree mortality through large fires is uncommon in Scotland. In addition, wind-blow and snow can cause some casualties through the year, and fungi and insects will be minor effects. However, intense forest disturbance in recent centuries can be attributed mainly to forest management practices.

The study site in Abernethy is located at 370 m a.s.l., with south westerly prevailing winds and densities of 160 stems ha^{-1}. Stand composition is formed by Scots pine, with presence of *Juniperus communis*. The understory is predominantly *Calluna vulgaris, Vaccinium myrtillus* and small patches of *V. vitis-idaea*. Historical exploitation at Abernethy has taken place over millennia and high felling and browsing pressure during the 18th Century are reflected in the progressive contraction of the extent of Abernethy forest in historical maps from 1750 until 1830 (Smout et al., 2005, Summers et al. 2008). By 1858, the forest was represented only by scattered trees in the study site and followed by enclosure of the forest as deer forest occurred in 1869 (O'Sullivan, 1973). In the 1980s the area was designated a National Natural Reserve. Seasonal grazing by sheep was stopped in 1990 and deer fences were removed (Beaumont et al., 1995). Since then, culling of deer has kept the population stable and compatible with forest regeneration. By 1992 the percentage of seedlings with evidence of browsing had fallen from 72% to 43% with an increase of 20% in the number of established seedlings and saplings (Beaumont et al., 1995).

The study site in Glen Affric is located at 260 m a.s.l., south west of Loch Affric, where the prevailing winds are south westerly, and stand density is 103 stems ha^{-1}. Stand composition is dominated by Scots pine and the vegetation layer is predominantly *C. vulgaris* with small patches of *V. vitis-idaea* and *V. myrtillus*. Evidence from pollen records from west Glen Affric, where our stand is located, show a sustained low tree cover around these sites for several thousand years as a result of prolonged human impact, with the recent expansion of the forest when the present tree cohort developed around 1880 (Shaw, 2006). Historical documents report felling of trees during the 18th and 19th Centuries (Smout et al., 2005) with the decline evident in pollen records.

Following a period of intensive sheep and deer grazing in the late 20th Century a major effort was made to protect and restore the remaining native pine forest (Bain, 2013). Glen Affric was initially declared as a Caledonian Forest Reserve in 1961 by the Forestry Commission (Bain, 2013) and later, in 1984, a National Natural Reserve.

To compare our heavily managed stands with an unmanaged case, and since unmanaged stands do not exist in Scotland, pre-existing samples from a boreal site in Western Siberia were used (60°54'N, 68°42'E). These samples were taken from within a continuous population with extensive areas of natural forest, with a stand density of 470 stems ha^{-1}. These forests have never been altered by humans, but are subject to regular natural disturbance by fire on roughly 50 year timescales. In these boreal forests, competition forces Scots pine to forest edges and onto poor quality sites such as sandy soils or bogs, and it is outcompeted on better soils by *Pinus sibirica*, *Larix sibirica* and *Populus tremula*. As a result even mature individuals may be small. Hereafter this stand will be referred to as the unmanaged stand.

In Scots pine, genetic variation is partitioned predominantly within rather than among populations, and levels of within-population genetic diversity across the range of Scots pine are similarly high (Wachowiak et al., 2014, 2011). Previous studies of diversity across the range of this species have shown that genetic differentiation among even distant populations of Scots pine is low (Naydenov et al., 2007; Provan et al., 1998; Prus-Glowacki and Stephan, 1994; Wang et al., 1991) but see (Forrest, 1982; Prus-Glowacki et al., 2012). Some authors attribute this homogeneity to common ancestry, as well as extensive gene flow (Chybicki et al., 2008) and lack of major physical barriers (Naydenov et al., 2007). As absolute genetic diversity levels in the managed and unmanaged stands are of similar magnitude, and the physical capacity for gene movement should be similar in each, we can assume that the primary driver of genetic structure will have been the presence or absence of significant human intervention. Therefore, this comparison can be informative regarding the processes that are likely responsible for the observed spatial pattern of genetic diversity at fine scales.

225

226 2.3. Sample collection, life stages and stand structure

227 We selected stands of 200 m × 200 m in Abernethy and Glen Affric, respectively. Sampling
228 strategy was designed to account for short distance classes in order to detect fine-scale SGS,
229 choosing individuals randomly and assuring sufficient numbers of pairwise comparisons in each
230 distance class, as recommended by Cavers et al. (2005). We sampled needles from two life stages,
231 juveniles and adults. Sample size per stand in each life stage varied from 131 to 181 (Table 1). All
232 individuals were mapped using a GARMIN 62s handheld GPS and diameter was measured at
233 breast height (d.b.h.). The d.b.h. was used as a proxy of age, defining juveniles as individuals with
234 d.b.h. below 10 cm. Existing data from trunk cores from nearby adult pines in Abernethy
235 (Summers et al., 2008) were used to calibrate the relationship between d.b.h. and age.

236

237 The unmanaged study site was sampled in three sub-stands of 50 x 50 m along a linear transect of
238 300 m, which were combined to give a single stand sample for subsequent analysis. All sampled
239 individuals were mapped, measured for d.b.h. and tree height classified as short (<2m) or tall
240 (>2m). Juveniles were defined as short individuals with d.b.h. below 10 cm. Sample size in each
241 life stage varied from 57 to 73 (Table 1). Thirty random trunk sections from adult pines were taken
242 from the unmanaged site to calibrate the d.b.h.-age relationship. We evaluated the relationship
243 between d.b.h. and tree age, and whether this relationship varied among sites using a linear model
244 in R 3.0.1 (R Core Team, 2013). We chose d.b.h. as the response variable and tree age and site
245 (Abernethy and unmanaged) were the predictor variables. The interaction between the predictor
246 variables was tested and compared with a model without interactions by using the Akaike
247 Information Criterion.

248

249 2.4. Microsatellite analyses

250 Total genomic DNA was extracted from 50 mg silica gel dried needles using QIAGEN DNeasy
251 96 Plant Kit (QIAGEN Ltd. Crawley, UK) following the manufacturer's protocol. All individuals
252 were genotyped at twelve nuclear microsatellite markers (SSR): psyl2, psyl16, psyl17, psyl36,

253 psyl42, psyl44, psyl57 (Sebastiani et al., 2011), SPAC7.14, SPAC12.5 (Soranzo et al., 1998),
254 PtTX4001, PtTX4011 (Auckland et al., 2002) and SsrPt_ctg4698 (Chagné et al., 2004), combined
255 in two multiplexes of six loci each. Multiplex 1 consisted of primers psyl2, psyl17, psyl42, psyl44,
256 PtTX4001 and PtTX4011 at concentrations of 3 µl, 2 µl, 2 µl, 2 µl, 3 µl and 2 µl respectively.
257 Multiplex 2 consisted of primers psyl16, psyl36, psyl57, SPAC7.14, SPAC12.5 and
258 SsrPt_ctg4698 at concentrations of 2 µl each. Reactions were carried out in a final volume of 10
259 µl with 1X of QIAGEN Type-it Multiplex PCR Master Mix, 1 µM of each multiplex and 25 ng
260 of template DNA. Annealing temperature for both multiplexes was 56°C. Polymerase chain
261 reactions (PCR) were performed in Veriti™ Thermal cycler (Applied Biosystems, Bleiswijk,
262 Netherlands), with the following programme: 1 cycle at 95°C for 4 min followed by 35 cycles at
263 95°C for 45 s, 56°C for 45 s, 72°C for 45 s, and a final step at 72°C for 5 min. PCR products were
264 analysed by DNA Sequencing and Services, Dundee, UK, using an Applied Biosystems 3730
265 DNA Sequencer with reference to a LIZ 500 size standard. Fragment analysis results were scored
266 using GENEMARKER V.2.6.0. (SoftGenetics, State College, PA, USA). FLEXIBIN (Amos et
267 al., 2007) was used to check discrete classes of raw allele sizes and MICRO-CHECKER (Van
268 Oosterhout et al., 2004) to check genotyping errors and null allele frequencies. Several markers
269 showed evidence of null alleles at very low frequencies (maximum frequency of 0.04, data not
270 shown), which is far below to the threshold at which null alleles can result in a significant
271 underestimate of expected heterozygosity, estimated as 0.2 (Belletti et al., 2012; Chapuis and
272 Estoup, 2007). Therefore, all markers were kept for further analysis.
273
274 2.5. Genetic diversity and spatial genetic structure analysis
275 Genetic diversity estimators within stands and life stages were estimated using FSTAT 2.9.3.2
276 (Goudet, 1995): mean number of alleles per locus (A), rarefied allelic richness (A_R) (rarefied to 57
277 individuals for each stand and life stage), expected heterozygosity (H_E) and inbreeding coefficient
278 (F_{IS}). We conducted ANOVAs to test for differences in A, A_R, and H_E between stands and life
279 stages in R 3.0.1 (R Core Team 2013). We calculated F_{ST} among stands and life stages in
280 ARLEQUIN v3.5 (Excoffier and Lischer, 2010), and the differentiation index D (Jost, 2008)

implemented in the R package DEMEtics (Gerlach et al., 2010). In both cases, significance values were determined for a 5% nominal level after Bonferonni correction. F_{ST} estimates differences in allele frequencies among stands, whereas differentiation index *D* measures the fraction of allelic variation among them.

We implemented fine scale SGS analyses in SPAGeDi 1.4b (Hardy and Vekemans, 2002). In order to test for significance in genetic relatedness, the Kinship coefficient of Loiselle et al. (1995) (F_{ij}) was estimated as $F_{ij}=(Q_{ij}-Q_m)/(1-Q_m)$, where Q_{ij} is the probability of identity in state for random gene copies from two individuals *i* and *j*, and Q_m is the average probability of identity by state for gene copies coming from random individuals from the sample. A regression between the Kinship coefficient F_{ij} and the logarithm of pairwise geographic distances of individuals was computed (b_F). Standard errors of the regression slope were computed using a jackknife procedure over loci. The significance of the slope of the regression was tested using 10,000 permutations of locations among individuals. To visualize the SGS, we plotted average pairwise estimates of genetic relatedness as a function of distance to generate spatial autocorrelograms. Analyses were conducted for each stand and life stage separately across the full distance range. SGS_{MAX} was also calculated for each stand and life stage, which is the greatest distance at which the Kinship coefficient of a given distance class *F(d)* is significant at $p<0.05$ (Jump et al., 2012). We also calculated the *Sp* statistic, as suggested by Vekemans and Hardy (2004), to allow comparability among stands and life stages with other studies. The *Sp* statistic was determined as $-b_F/(1 - F_1)$, where b_F is the regression slope of kinship coefficient estimate (*F*) on distance classes and F_1 is the kinship coefficient for adjacent individuals in the first distance interval.

Because the number of pairs within each distance class should ideally exceed 50 pairs of individuals, we set the distance intervals of at least 10 metres (Cavers et al., 2005; Jump and Peñuelas, 2007). Overall, we established 10 distance classes for the managed stands (0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100), and 8 distances classes in the unmanaged stand (0-10, 10-20, 20-30, 30-60, 60-70, 70-80, 80-90, 90-100). Distance classes

between 30 and 60 metres were combined in the unmanaged stand to ensure sufficient numbers of pairs in the class. We also tested other distance class options and longer final distances up to 200 metres, and found they revealed similar and no more informative results. In addition, in the unmanaged stand, analysis of each sub-stand was also conducted separately, and the same results were obtained.

3. Results

3.1. Stand structure

Tree diameter distribution for managed stands was bimodal, with the highest frequencies for juvenile individuals at diameters between 0 and 10 cm (Fig. 1). A gap of adult individuals with diameter classes between 10 to 30 cm and 10 to 25 cm occurred in Abernethy and Glen Affric, respectively (Fig. 1). Contrastingly, tree diameter distribution in unmanaged stand was more skewed towards smaller diameters. There was no gap in the distribution in this case, instead there was a gradual decrease in the numbers of individuals with increasing diameter class (Fig. 1).

We found that d.b.h. was dependent on age and site (F=29.85, R^2=0.31), showing strong differences among age (t=3.81, p<0.001), and among sites (t=-6.03, p<0.001). However, we did not find significant interactions between age and study site (Fig. 2). The relationship between d.b.h. and age suggested that differences in age profiles in the two sites were smaller than differences in tree size (e.g. trees with different d.b.h. could have a similar age).

3.2. Genetic diversity

Genetic diversity parameters did not significantly differ between managed and unmanaged stands (Table 1). Among the twelve nuclear loci analysed, the number of alleles (A) in the managed stands ranged from 3 to 31 and 4 to 29 per locus for Abernethy and Glen Affric respectively for both life stages combined (multilocus average of 9.92 for each site). A ranged from 3 to 31 in the unmanaged stand, with a multilocus average of 9.83 again for both life stages combined. For rarefied allele richness (A_R) in the managed stands, multilocus estimates obtained mean values of

8.99 and 8.83 for Abernethy and Glen Affric respectively and 8.95 for the unmanaged stand both life stages combined, based on a minimum number of 126 individuals. Expected heterozygosity levels (H_E) showed multilocus estimates of 0.58 in Abernethy and 0.56 in Glen Affric, and similar values of 0.58 for the unmanaged stand for both life stages combined (See Table 1 for genetic diversity estimators on each site and life stage and Appendix A, Table A1, for detailed information of each microsatellite). Neither A, A_R or H_E significantly differed between managed vs. unmanaged stands (all p-values > 0.05). However, some differences appeared in the inbreeding coefficient (F_{IS}) which was significant and higher for both managed stands, indicating significant departure from Hardy–Weinberg equilibrium, whereas it was not significant for the unmanaged stand (Table 1). F_{ST} values indicated low but significant differences among the two managed stands (F_{ST}=0.004, p<0.001), and higher differences when comparing them with the unmanaged stand (F_{ST}=0.03 and F_{ST}=0.04, p<0.001, for Abernethy vs. unmanaged and Glen Affric vs. unmanaged respectively), indicating that despite remarkably similar overall levels of genetic diversity, their genetic composition differs to some extent.

When comparing life stages within stands, neither A, A_R or H_E significantly differed (all p-values > 0.05). F_{ST} values indicated no significant differences among life stages in Abernethy and the unmanaged stand, however low but significant F_{ST} occurred among life stages in Glen Affric. In agreement, differentiation index D showed the same pattern, although values were consistently larger (See Appendix A, Table A2).

3.3. Spatial genetic structure

We found significant SGS in all managed stands for both life stages which extended up to 40 metres further than the unmanaged stand (Table 1 and Fig. 3). The kinship coefficient for the first distance class $F_{(1)}$ and the Sp statistic also reflected the relationship between the extent of SGS and historical management, which was larger for managed than for unmanaged stands (Table 1).

When comparing SGS among life stages within stands, both SGS_{MAX} and $F_{(1)}$ were larger for adult than for juvenile stages in the managed stands (e.g. SGS_{MAX} extended up to 20 metres further in adults than juveniles) (Table 1 and Fig. 3). In contrast, SGS was larger for juveniles than for adults for the unmanaged stand, with significant SGS only at distances of less than 10 metres in the juvenile stage (Table 1 and Fig. 3). In the managed site of Glen Affric, we found that at 50-80 m trees were less genetically similar than expected compared with a random distribution of genotypes (see significant negative values of Kinship coefficient at distances between 50 and 80 metres in Glen Affric in Fig. 2). The minimum number of pairwise comparisons per distance class in the managed stands for each life stage was 106 individuals, whereas it was 63 individuals in the unmanaged stand. The *Sp* values did not reflect the same relationship between the extent of SGS with contemporary management as SGS_{MAX} and $F_{(1)}$ did. Thus, of the managed stands, *Sp* value was not significantly different between adults and juveniles in Abernethy, whereas it increased from adults to juveniles in Glen Affric (Table 1).

4. Discussion

We found two main results: 1) although overall levels of genetic diversity were strikingly similar, more extensive spatial structuring of genetic diversity was found in the mature managed stands when compared with the unmanaged one; 2) in contrast to expectations, a reduced extent of spatial genetic structure was found in the early stages of regeneration of the managed stands compared with the adult cohorts, again despite no difference in overall levels of genetic diversity between life stages. These patterns suggest that both historical and contemporary management can significantly alter spatial genetic structure of Scots pine. Here, we combine ecological information with historical data on the stands to better understand the mechanisms that are likely responsible for these differences in spatial genetic structure.

4.1. Impact of historical forest management practices

Notable differences in size profiles appeared between managed and unmanaged stands, (e.g. mean d.b.h. generally bigger in managed stands (Fig. 1)). However, the d.b.h.-age relationship was

different among managed and unmanaged stands (Fig. 2), linked to the growth-retarding effect of the bog habitat of the latter. Hence, the contrast in age profiles –a more important comparison for SGS analysis– was much smaller than for size profiles (e.g. small trees from the unmanaged stand often had similar ages to much larger trees from the managed one). The age profile of the stands was strongly reflective of their distinct histories, with large, old trees present in the managed sites plus a pulse of recent regeneration, whilst a much wider range of ages was present in the unmanaged one, with fewer very old trees. The structure in the unmanaged site is likely to reflect the natural fire disturbance dynamics to which it is exposed. These dynamics are likely in turn to affect genetic structure, with a higher turnover in the unmanaged stand –shown by the diverse, but generally young age profile– indicating a higher potential for gene dispersal and therefore erosion of spatial structure.

Genetic diversity of both mature managed sites, as indicated by allelic richness and expected heterozygosity, did not differ significantly from the unmanaged stand but instead was remarkably similar (e.g. H_E: 0.57-0.59 vs. H_E: 0.58, respectively). Although average diversity levels were lower than those reported in mainland European populations of Scots pine using nuclear SSR (H_E: 0.62-0.85) (Scalfi et al. 2009; Naydenov et al. 2011; Nowakowska et al. 2014; García-Gil et al. 2015) differences might be explained by the number of markers used and their specific levels of polymorphism. Thus, for example, selecting two of the three markers used by Scalfi et al. (2009), SPAC 7.41 and SPAC 12.5, the mean value of genetic diversity in our study (0.9) would be higher than previously reported. Also, the markers with the lowest values of diversity in our study, psy144 and psy12, had very similar low values in mainland European populations (Sebastiani et al., 2011) (see Appendix A, Table A1). Previous studies in Scottish populations of Scots pine have also reported relatively high levels of genetic variation using other molecular markers (Forrest, 1982, 1980; Kinloch et al., 1986; Provan et al., 1998; Sinclair et al., 1998; Wachowiak et al., 2013, 2011).

High levels of genetic variation at the population level suggests that effective population size has been sufficiently high to restrict effects of genetic drift despite intensive management and geographical isolation of populations. Scots pine is a wind-pollinated tree with wind-dispersed seed, and achieves high levels of gene flow by: (1) long seed wings, up to four times as long as the seed (Steven and Carlisle, 1959), (2) low seed mass (Castro, 1999) (here 2.9 to 12.64 mg), on average smaller than other pine species (9.1 to 233 mg) (Vander Wall, 2003), and (3) extensive pollen flow, from 17-22 m (Robledo-Arnuncio et al., 2004b) and up to 100 km in small fragments (Robledo-Arnuncio, 2011) (similar to other wind-pollinated tree species). Therefore, it appears that gene flow has been sufficient to prevent erosion of genetic diversity. F_{IS} values, an indirect measure of inbreeding, were not high in the managed sites although they were significantly higher than in the unmanaged site (0.05-0.06 vs. 0.01 respectively), suggesting that although gene flow has prevented loss of genetic diversity at the population level, fine scale alterations to gene flow might have taken place. Drastic reduction of population sizes can induce higher rates of selfing and mating between relatives (Robledo-Arnuncio et al., 2004a). The small size of the population at the time of establishment of the current adult cohorts, as indicated by historical data (Shaw, 2006; Summers et al., 2008)**,** might explain this pattern.

Consistent differences in SGS were found in the mature managed stands which showed greater extent and magnitude of structure, as indicated by SGS_{MAX} up to 40 metres and higher $F_{(1)}$, compared with the unmanaged one. The extent of SGS of the mature managed stands was also larger than the values reported for Scots pine (Chybicki et al., 2008) and other *Pinus* species, which typically had values below 15 metres (De-Lucas et al., 2009; González-Martínez et al., 2002; Jones et al., 2006; Marquardt and Epperson, 2004; Parker et al., 2001; Troupin et al., 2006; Williams et al., 2007). It should be noted, however, that SGS estimates in Parker et al. 2001 and Jones et al. 2006 were based on allozyme markers, and the need for caution when comparing SGS extent with different molecular markers has been previously highlighted (Jump and Peñuelas, 2007).

447 Values of SGS extent more comparable to those in our managed stands were observed in
448 fragmented populations of *Pinus pinaster* (~ 20 metres) (De-Lucas et al., 2009). The high values
449 of SGS_{MAX} in the managed stands are likely a consequence of the drastic reductions in the number
450 of seed and pollen donors, which are two of the main drivers of SGS (e.g. due to felling practices).
451 The larger extent of SGS observed in Glen Affric may arise from local differences in historical
452 management, with a prolonged limited tree cover due to human activities (Shaw, 2006), which is
453 also reflected in the lower density of the site. The very short spatial scale of genetic structure in
454 the mature unmanaged stand was remarkably similar to that in undisturbed continuous populations
455 of *P. pinaster* which displayed either weak or no relatedness, with maximum values of SGS_{MAX} of
456 10 metres (De-Lucas et al. 2009). As these populations have contrasting local contexts, the
457 unmanaged stand being part of the extensive core Eurasian population whereas the undisturbed
458 population of *P. pinaster* being a distributional edge population, the similarity in SGS values
459 observed seems likely to be due to their common unmanaged state. Therefore, it seems clear that
460 tree density, degree of fragmentation of stands at the time of establishment and rate of gap creation
461 play a major role in the formation of SGS in populations. *Sp* values for the mature managed stands
462 (0.0045 and 0.0098) were remarkably higher than for the non-managed stand (-0.0006). Similarly,
463 De-Lucas et al. (2009) found differences in the *Sp* values between fragmented and continuous
464 populations of *P. pinaster*, although they were generally higher than the values reported in this
465 study.
466
467 4.2. Impact of contemporary forest management practices
468 In the managed stands, there were no differences among life stages in the levels of allelic richness
469 or gene diversity, suggesting contemporary management has not impacted genetic variation.
470 However, we found higher relatedness – as higher SGS intensity and extent – in adults than in
471 juveniles, with a greater discrepancy in the Glen Affric site. In contrast, the unmanaged site had
472 stronger relatedness in the juvenile stage than in adults, as is usually found in natural tree
473 populations. Natural populations often show greater SGS in the early stages of regeneration, due
474 to the higher spatial aggregation of trees (Rozas et al., 2009; Szwagrzyk and Czerwczak, 1993).

This pattern has been reported in other species of *Pinus* (González-Martínez et al., 2002), in *Quercus* (Hampe et al., 2010), tropical trees (Hardesty et al., 2005; Ng et al., 2004) and other plant species (Yamagishi et al., 2007). Nevertheless, a few studies have found greater SGS in adult life stages, such as in *Jacaranda copaia* (Jones and Hubbell, 2006), where it was attributed to very low recruitment and high mortality rates, or in the tropical tree *Dicorynia guianensis*, linked to overlapping of generations in the adult cohort (Latouche-Hallé et al. 2003). A subsequent study of the latter species found stronger SGS in saplings (Leclerc et al., 2015), suggesting that earlier observations were probably specific to the particular study cohort. Stronger SGS in adults than in late juveniles was also found for *Prunus africana* and it was attributed to a reduction in gene flow due to past logging (Berens et al., 2014). In our study, the most probable explanation seems to be the influence of changes in contemporary management. In the managed populations of Scots pine investigated here, high felling pressure at the time of establishment of the adult cohort, together with high browsing pressure, has suppressed regeneration for decades, which is also reflected in the absence of individuals estimated between 25 and 100 years old (Fig. 2). In the last 25 years, there has been a deliberate policy to encourage regeneration in the pine forest (Mason et al., 2004), with a consequent increase in forest density. This increment in forest density appears to have maintained diversity levels, increased gene flow and produced a more randomized distribution of genotypes in the new generation.

The observed reduction in juvenile *SGS* shows an erosion of the structure present in the adult cohort and contemporary recovery to natural dynamics, reflecting the high capacity of the species to recover after disturbance. Overall, *Sp* was higher in Glen Affric than in Abernethy, as for *SGS*. Although the spatial extent of *SGS* was higher in adults at Glen Affric, *Sp* was slightly higher in the juvenile stage. This means more distant pairs of juveniles were less related than they would be by chance (juveniles showed a lack of relatedness among individuals at 50-80 m separation). The biological cause of this trend is not clear but, together with F_{ST} values that showed a small but significant difference among juveniles and adults, it may indicate introgression from populations not present in our sample.

503

504 4.3. Conclusions

505 In this study we investigated how historical and contemporary forest management have shaped
506 patterns of genetic diversity and spatial distribution of genotypes of Scots pine. We provide
507 evidence to show that although overall levels of genetic diversity in historically managed
508 populations can be similar to unmanaged populations and as high as continental populations,
509 spatial genetic structure can be considerably altered. Our results suggest that intense management
510 practices that remove trees from the stand, such as felling, could alter fine-scale patterns of gene
511 flow and increase genetic relatedness of individuals at fine scales with implications for inbreeding
512 levels and, potentially, long-term adaptability. As a consequence, the extent of family clusters can
513 be modified, as for instance in our study which increased up to 40 metres in managed sites. From
514 a practical point of view, to ensure a broad sample of genetic variability, guidelines for seed
515 collection should aim for minimum sampling distances between mother trees of at least 40m.

516

517 The reduction of SGS observed in juveniles following contemporary management to promote
518 regeneration, indicates a high capacity of the species to recover after intense forest management.
519 Here, we suggest that combining sustainable management with forest conservation practices that
520 ensure larger effective population sizes is key to successfully maintaining genetic diversity in
521 Scots pine. This capacity of the early stages of regeneration to capture gene flow might have
522 implications for forest resilience and will be particularly important in the context of climate
523 change (Alfaro et al., 2014; Fady et al., 2015; Hoffmann and Sgrò, 2011; Millar et al., 2007) under
524 which selection pressures are expected to change.

525

526 Here we showed how investigating the spatial component of genetic diversity alongside tree
527 demographic structure can help to detect both historical and contemporary effects of disturbances
528 in tree populations. The effects of forest management were not reflected in overall levels of
529 genetic diversity, but they were manifested in SGS, as has been seen in previous studies (Paffetti
530 et al. 2012; Leclerc et al. 2015; Sjölund and Jump 2015). Therefore, incorporating a spatial

component when evaluating the effects of forest management practices is highly recommended. The evaluation of successional change is also essential to properly assess genetic dynamics within populations and to adequately detect early responses to forest management practices.

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Tables

Table 1: Summary of multilocus genetic diversity and SGS estimators for each study site and life stage.

Figures

Fig. 1: Tree diameter (d.b.h.) distribution in the three study sites: Abernethy (ABE), Glen Affric (GLA) and the unmanaged site (UNM). Juvenile stem diameter was measured at 10 cm height. Data are presented in intervals of 5 cm.

Fig. 2: Relationship between d.b.h. and age for the managed site of Abernethy (ABE) and the unmanaged site (UNM). Lines of best fit are represented by solid lines and 95% CI by dashed lines. Dots represent observed values.

Fig. 3: Spatial autocorrelograms for each study site: Abernethy (ABE), Glen Affric (GLA) and the unmanaged site (UNM); and life stage (adult and juvenile) based on the kinship coefficient F_{ij}, estimated from 12 microsatellite loci, and consecutive 10 m distance classes (note that for the unmanaged stand distance classes were combined between 30 to 60 metres). Shaded areas represent 95% confident intervals obtained from 10,000 permutations of genotypes among locations. Black bars around mean F_{ij} values represent standard errors derived through jackknifing over loci.

Appendix A. Supplementary material

Table A1: Genetic diversity estimators for each locus, study site and life stage.

Table A2: Pairwise F_{ST} values (below diagonal) and differentiation index D (Jost, 2008) (above diagonal) among study sites and life stages.

Table 1: Summary of multilocus genetic diversity and SGS estimators for each study site and life stage.

Study site	Life stage		Genetic diversity estimators				Spatial genetic structure estimators			
		N	*A*	A_R	H_E	F_{IS}	$F_{(1)}$	SGS_{MAX} (m)	b_F ± SE	*Sp* ± SE
Abernethy	Adult	181	9.50	7.11	0.587	0.052**	0.0291***	20	-0.0044 ± 0.0006***	0.0045 ± 0.0028
	Juvenile	170	9.25	6.72	0.583	0.080**	0.0183***	18	-0.0028 ± 0.0009**	0.0029 ± 0.0023
Glen Affric	Adult	165	8.92	6.79	0.568	0.063**	0.0298***	40	-0.0097 ± 0.0023***	0.0098 ± 0.0010
	Juvenile	131	9.25	6.74	0.561	0.049**	0.0156***	20	-0.0118 ± 0.0027***	0.0119 ± 0.0006
Unmanaged	Adult	57	7.58	6.51	0.576	0.012	-0.0033	0	0.0006 ± 0.0005	-0.0006 ± 0.0005
	Juvenile	73	8.17	6.94	0.582	0.021	0.0067	5	-0.0017 ± 0.0010*	0.0018 ± 0.0011

N, sample size; *A*, mean number of alleles per locus; A_R, rarefied allelic richness; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. $F_{(1)}$, Kinship coefficient for first distance class (0-10m); SGS_{MAX}, greatest distance at which the Kinship coefficient of a given distance class *F(d)* is significant at $p<0.05$; b_F ± SE, regression slope of the Kinship coefficient *Fij* computed among all individuals against geographical distances ± standard error; *Sp* ± SE, *Sp* statistic ± standard error. Significant *P*-values are indicated as $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. *P*-values for F_{IS} are obtained after 10,000 permutations of gene copies within individuals of each stand.

Fig. 1: Tree diameter (d.b.h.) distribution in the three study sites: Abernethy (ABE), Glen Affric (GLA) and the unmanaged site (UNM). Juvenile stem diameter was measured at 10 cm height. Data are presented in intervals of 5 cm.

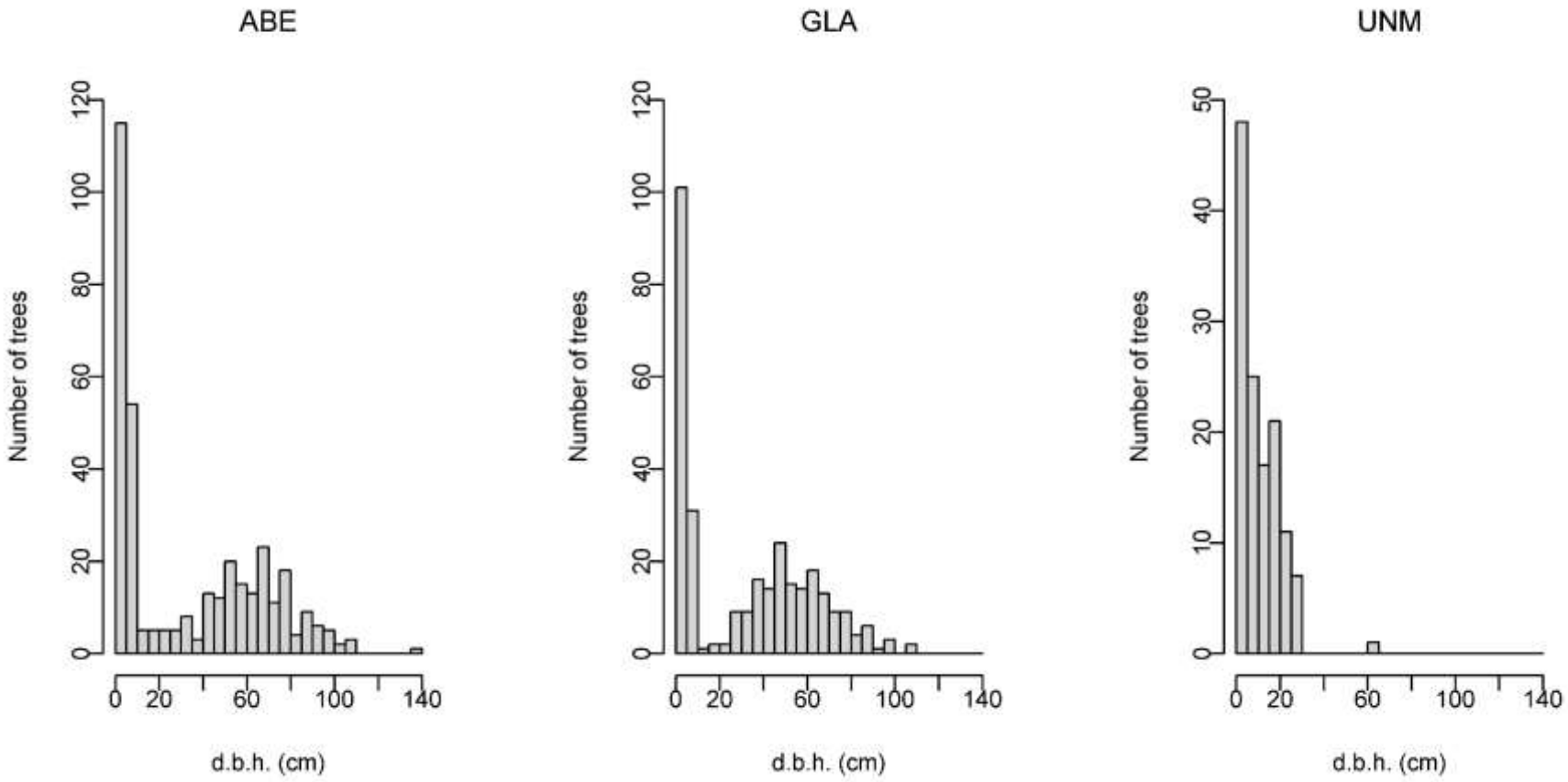


Fig. 2: Relationship between d.b.h. and age for the managed site of Abernethy (ABE) and the unmanaged site (UNM). Lines of best fit are represented by solid lines and 95% CI by dashed lines. Dots represent observed values.

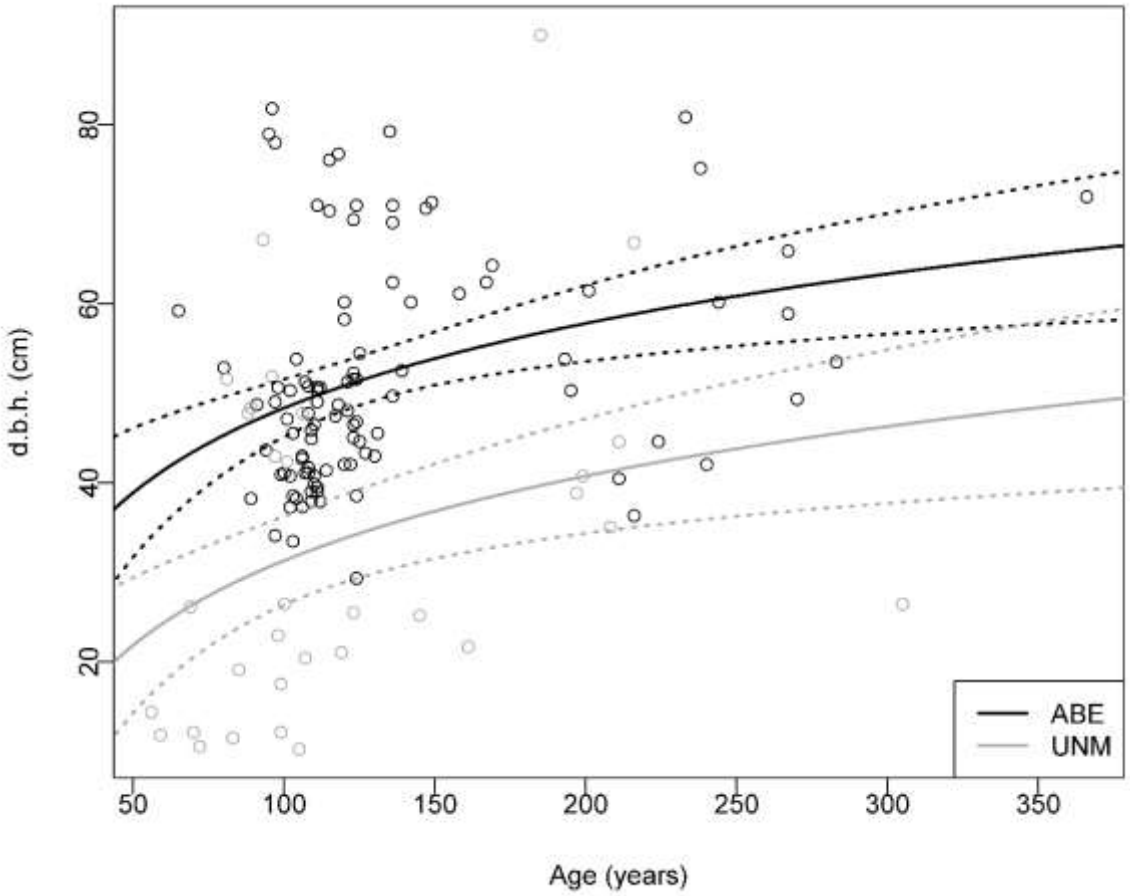
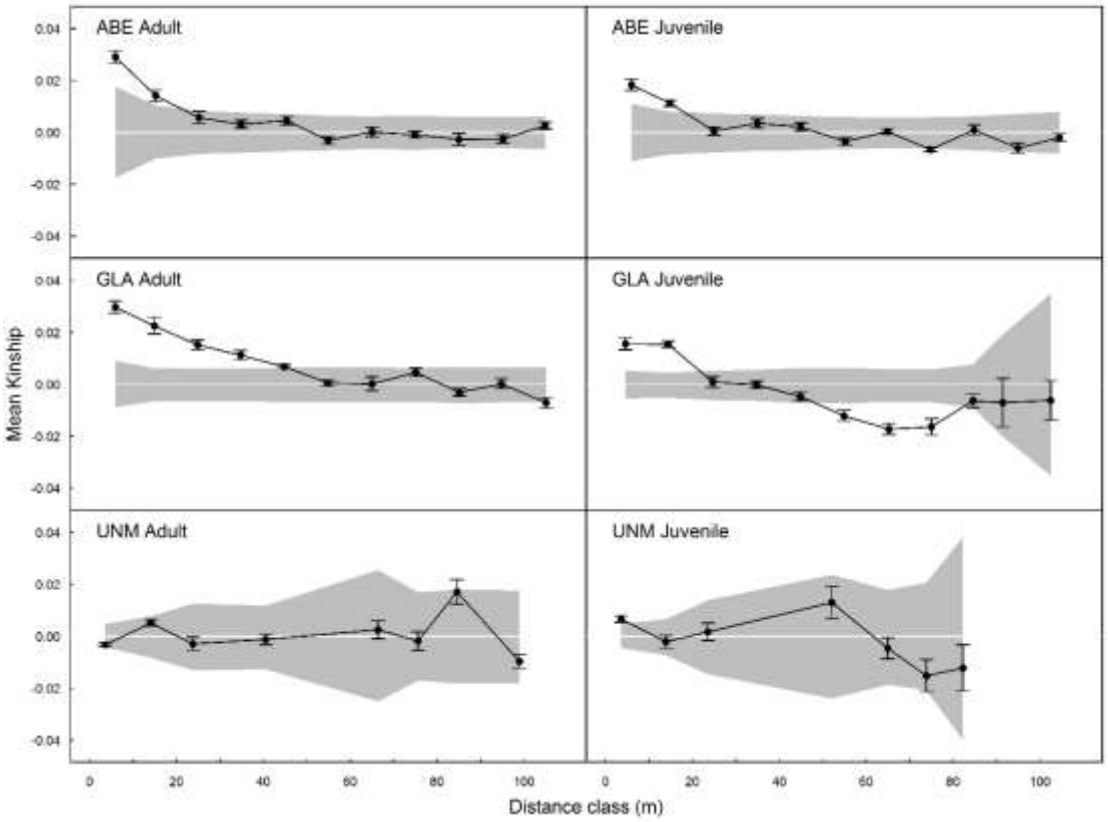


Fig. 3: Spatial autocorrelograms for each study site: Abernethy (ABE), Glen Affric (GLA) and the unmanaged site (UNM); and life stage (adult and juvenile) based on the kinship coefficient F_{ij}, estimated from 12 microsatellite loci, and consecutive 10 m distance classes (note that for the unmanaged stand distance classes were combined between 30 to 60 metres). Shaded areas represent 95% confident intervals obtained from 10,000 permutations of genotypes among locations. Black bars around mean F_{ij} values represent standard errors derived through jackknifing over loci.



Appendix A. Supplementary material

Table A1: Genetic diversity estimators for each locus, study site and life stage.

Locus	Life stage	Abernethy					Glen Affric					Unmanaged				
		N	*A*	A_R	H_E	F_{IS}	*N*	*A*	A_R	H_E	F_{IS}	*N*	*A*	A_R	H_E	F_{IS}
PtTX4001	Adult	181	11	9.28	0.8306	-0.028	165	9	7.59	0.7783	0.03	57	7	6.22	0.5951	-0.002
	Juvenile	170	12	9.32	0.8430	-0.06	131	11	8.79	0.8074	0.054	73	9	6.22	0.5073	0.028
PtTX4011	Adult	181	7	4.61	0.5920	0.099*	165	7	5.12	0.5423	0.213***	57	6	5.66	0.6717	0.204*
	Juvenile	170	6	4.73	0.6144	0.22***	131	6	5.05	0.6094	0.097	73	5	4.96	0.6922	0.3*
psy144	Adult	181	5	3.08	0.1166	-0.042	165	5	3.12	0.1380	-0.054	57	2	1.88	0.0517	-0.018
	Juvenile	170	5	2.88	0.0804	-0.024	131	5	3.2	0.1581	-0.067	73	3	2.39	0.1293	-0.06
psy117	Adult	181	8	6.32	0.7820	0.054	165	10	6.97	0.7907	-0.004	57	8	7.03	0.8224	-0.065
	Juvenile	170	8	5.98	0.7600	0.133**	131	8	6.56	0.7580	0.016	73	7	6.8	0.8247	-0.025
psy142	Adult	181	5	4.15	0.6466	0	165	6	5.22	0.6669	0.019	57	4	3.51	0.6479	-0.084
	Juvenile	170	6	4.34	0.6632	0.104*	131	6	5.07	0.6551	0.01	73	5	4.32	0.6411	-0.155*
psy12	Adult	181	3	2.17	0.3193	0.163*	165	3	2.18	0.2727	-0.096	57	2	2	0.3354	0.059
	Juvenile	170	3	2.17	0.3539	0.087	131	3	2.23	0.2386	0.393***	73	2	2	0.2314	-0.017
psy116	Adult	181	7	5.95	0.7862	-0.03	165	6	5.5	0.7736	0.011	57	6	5.5	0.7399	-0.092
	Juvenile	170	8	5.95	0.7720	0.063	131	7	5.42	0.7512	-0.024	73	6	5.87	0.7598	-0.01
psy157	Adult	181	5	4.23	0.3652	0.002	165	6	4.52	0.3483	-0.009	57	4	3.99	0.3892	-0.128
	Juvenile	170	5	4.19	0.3517	0.064	131	5	4.05	0.2984	-0.024	73	5	4.39	0.5168	-0.087
CTG4698	Adult	181	8	6.24	0.6044	0.019	165	8	5.17	0.5635	-0.043	57	5	5	0.6500	0.049
	Juvenile	170	6	5.34	0.6124	-0.034	131	6	5.27	0.5721	-0.068	73	5	4.64	0.6065	-0.016
SPAC7.14	Adult	181	29	19.08	0.9174	0.194***	165	26	18.6	0.9150	0.236***	57	22	17.95	0.9023	0.09*
	Juvenile	170	28	17.13	0.9093	0.179***	131	28	17.83	0.9072	0.21***	73	28	22.47	0.9513	0.097**

SPAC12.5	Adult	181	21	16.15	0.8989	-0.007	165	17	14.62	0.9058	0.098***	57	22	16.58	0.8475	0.048
	Juvenile	170	19	15.33	0.8956	0.054*	131	22	14.58	0.8814	0.005	73	19	15.85	0.8629	0.032
psy136	Adult	181	5	4.06	0.1877	0.438***	165	4	2.82	0.1166	0.216***	57	3	2.76	0.2607	-0.01
	Juvenile	170	5	3.23	0.1451	0.108	131	4	2.82	0.0897	-0.029	73	4	3.35	0.2578	-0.01

N, sample size; *A*, mean number of alleles per locus; A_R, rarefied allelic richness; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. Significant *P*-values are indicated as *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. *P*-values for F_{IS} are obtained after 10,000 permutations of gene copies within individuals of each stand.

Table A2: Pairwise F_{ST} values (below diagonal) and differentiation index *D* (Jost, 2008) (above diagonal) among study sites and life stages.

	ABE Adults	ABE Juveniles	GLA Adults	GLA Juveniles	UNM Adults	UNM Juveniles
ABE Adults	-	-0.00134	0.01367***	0.01694***	0.09089***	0.08407***
ABE Juveniles	-0.00085	-	0.01925***	0.01836***	0.09777***	0.09615***
GLA Adults	0.00531***	0.00504***	-	0.01223**	0.08486***	0.08469***
GLA Juveniles	0.00794***	0.00712***	0.00514***	-	0.09852***	0.09642***
UNM Adults	0.04973***	0.05174***	0.04434***	0.05228***	-	0.00843
UNM Juveniles	0.04923***	0.05132***	0.04586***	0.05382***	-0.00254	-

ABE refers to Abernethy, GLA refers to Glen Affric, UNM refers to the unmanaged site. Significant *P*-values are indicated as *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.