

Decision for round #1 : Revision needed

Decision on preprint "Does the seed fall far from the tree?..."

We have received three reviews on your paper. The reviewers were overall very positive, pointing out a well-motivated and well conducted study. They give suggestions for clarifications, improvements and further developments that I invite you to consider in a revised version.

- Thank you for the positive and constructive feedback on our manuscript.

I have just a few additional comment and suggestions for clarification.

On family relationships: You use two pairwise metrics, the GRM relationship coefficient, and Loiselle et al.'s 1999 kinship coefficient, to assess pairwise relatedness and its decay with distance in two sites in a Scots pine forest. Following reviewer 3, it would be interesting to see if both statistics pick up the same relationship degrees between individuals. For this, it would be useful to clarify what are the expected values and assignment ranges of each of these metrics for different degrees of relatedness. Currently, you report degrees of relatedness using the relationship coefficient but cite Manichaikul et al. 2010 who give expectations and ranges for the kinship coefficient, which can be confusing for the reader.

- Thank you for the comment, we have now clarified that pairwise relatedness estimates are double the value of pairwise kinship estimates presented in Manichaikul et al. 2010. In addition, we added a comparison between the pairwise relatedness estimates (GRM) and pairwise kinship estimates (Loiselle) as a supplementary Figure S3. There we also show that the estimates behave as expected despite, e.g., being estimated with a different set of SNPs, and follow the expected 2:1 relatedness:kinship ratio.

Also, we classified the pairwise relationship based on the kinship estimates and added supplementary Table S2 to compare the classification done using relatedness or kinship estimates. They do have a few differences, but the main pattern of family relationship classification is very similar.

On the spatial extent of the observed SGS patterns: You report some differences between sites, such as relatedness in the first distance class and distance up to which individuals are more related to each other "compared to individuals in other distance classes". It would be useful to investigate to what extent these (admittedly small) differences are biological properties, or a product of the design of distance classes and sampling range, e.g., using the same definition of classes and restricting the analysis of decay of relatedness to the same distance.

- We sampled the two areas as two "replicates" of the same population, but the sampled areas are not identical in shape or size, which, e.g., can cause differences in the results. We do not expect the study sites to differ in biological properties as such and the idea of the sampling is not to try to find biological differences, so we would like to keep the full range of distances of the larger study site in the manuscript. Looking at the decay of relatedness with distance (Figure 3 and the new Figure S2), the two samples look very similar. However, to make the comparison between sites easier, we have added a plot of GRM against spatial distance where we use the same distance classes for both study sites and restrict the examination to maximum distance of Mäkrä (Figure S2). We also added a Mantel correlogram plot where Ranta-Halola and Mäkrä are divided into equally long distance classes (Figure S4). The correlation between relatedness and distance is significant in longer distances in Ranta-Halola than Mäkrä, but there are more pairwise comparisons in

Ranta-Halola and the shapes of the sampled areas differ. There are age differences in the sampled areas, and we have added age information to the manuscript.

On the significance of SGS: It would be interesting to assess significance of the decay of kinship with distance using permutations for blog, so as to obtain an assessment of significance of SGS based on the decay of kinship in a single test as recommended by Vekemans and Hardy 2004; this significance test can be used to assess significance of Sp.

- As suggested, we have done 1000 permutations of individual locations in SPAGeDi to estimate the significance of Sp. The p-value was below 0.001 for the Sp estimates of both sampling sites.

Carefully revise the English language please, there are some problems with missing articles and prepositions.

- We have carefully checked and corrected the language throughout the manuscript.

L.65 Unnecessarily

- As suggested, we have changed the word.

L.72-76 Dispersal distances are not necessarily the same in within pops (closed canopy) and for colonization (open landscapes, especially in a boreal context), perhaps to link with the discussion of dispersal distances.

- This is a good point! We have modified the sentence accordingly: "Dispersal information can be used, for example, in predicting species' potential for adaptation (Kuparinen et al. 2010; Kremer et al. 2012; Barton 1979; Slatkin 1973), bearing in mind that dispersal rates e.g. in open landscapes of colonization stage may be different than those estimated here ."

L.115 What means "no minor homozygote"? Please explain more clearly the order of application of the filters; L.144-150 match the filtering mentioned briefly in L.115-116.

- As suggested, we have now clarified the terminology and changed the order in which we present the SNP filtering in the methods.

L.196 Spagedi doesn't consider distance classes for blog, the regression is done over all pairwise distances between individuals.

- Thank you for the comment. In fact, our estimation only uses blog over the whole distance. SPAGeDi uses only the mean kinship based on the first distance class to estimate neighborhood size, but blog for the whole distance. Here is a quote from the Spagedi manual: "Starting from a global regression slope, the procedure consists in estimating Nb as $Nb = -(1-F(1))/blog$, where $F(1)$ is the kinship coefficient between individuals for the first distance class (assumed to correspond to pairs of neighbours), and σ is estimated as $\sigma = [Nb/(2 \pi.k.D)]^{1/2}$." However, we did notice that there was a small mistake in the formula ($Nb = -(1/F(1))/blog$ instead of $Nb = -(1-F(1))/blog$), so thank you for drawing our attention to the formula!

L.199-200, please make sure subscript letters for individuals and loci are easy to distinguish and defined in the text. Currently it's not the case.

- We have modified the formulas so that the subscript letters are now easier to read. We also added more definitions to the formula of Loiselle's kinship estimate, all other formulas should already have all definitions in place.

L254: What was the value of the GRM in Mäkrä in the shortest distance class, 0.004? Comparing with Fig 3, 0.0004 looks like a typo? The class with the shortest distance was not defined in the same way, making it difficult to compare GRM values.

- Thank you for pointing this out, we have corrected the mean value of GRM in the shortest distance class for Mäkrä to 0.004.

L.256, spell “at a similar rate”, L. 258 “a negative correlation”, L. 260 “decay of relatedness with spatial distance”

- As suggested, we have made the corrections to the text.

L.275 Do you mean “similar” instead of “comparable” (=possible to compare)?

- Yes, we mean “similar” and have changed the word in the text.

L.345 both values are virtually the same

- We agree on the comment, so we changed the sentence to show the highest values of each study to make the point clearer.

L.347 not spatial structure, but spatial genetic structure. Consider using FSGS or SGS, for (fine-scale) spatial genetic structure throughout the manuscript; L.350 not population structure, but FSGS; L.356, not the spatial structure but the FSGS; verify the correct usage throughout manuscript

- We have gone through the text and corrected the text in all places we found a discrepancy in the wording of spatial genetic structure. We decided not to use an abbreviation for the term.

by Myriam Heuertz, 20 Sep 2023 16:43

Review by Joachim Mergeay, 25 Aug 2023 14:44

Does the seed fall far from the tree? Weak fine-scale structure in a continuous Scots pine population

This ms provides a detailed genomic insight into the population genetic structure of a conifer, and attempts to calculate the spatial extent of gene flow (pollen and seed dispersal combined), and parameters related to genetic neighborhood size.

Overall, it is a sound piece of work showing that even with wind dispersal of pollen the dispersal distance is very limited. This results in clear local genetic structure (at the level of kinship and isolation by distance), even though at the level of inbreeding coefficients the effects are very weak, yet deviating clearly from random mating.

This genetic structure and the presence of rather small neighborhoods (in terms of spatial extent) has consequences for how we calculate effective sizes in trees, even those with wind-dispersed pollen. Many methods assume that a sample used to calculate N_e or N_S was taken from a randomly mating population (e.g., LD-based methods), and this paper shows that non-random mating (as a result IBD) is pervasive, even when the estimated genetic structure (F-statistics) is very weak.

A technical comment: I needed both the pdf and the html version: the pdf missed figures, the html missed formulas (embedded figures not shown). That was a bit of a nuisance.

- Thank you for the constructive comments on our manuscript and we apologize for the inconvenience of the missing figures/formulas.

I do have questions about parts of the methodology, especially the calculation of neighborhood size and the gene dispersal distance.

Let me start with confusion about the abbreviations used:

A. N_b is used here to denote Neighborhood Size, but N_b it is generally also used in the literature for the effective number of breeders (the effective number of parents of a single cohort in populations with overlapping generations). Nunney et al. (2016) suggest to use N_n instead of N_b for neighborhood size, to avoid this confusion. Neel et al. (2013) use N_S , Wright's Neighborhood Size. I'm using N_S in my comments.

- Thank you for the comment, but we decided to keep the original abbreviation N_b because it is used in the software SPAGeDi and in the literature referring to that software. Quick Google Scholar search with the term ' N_b "neighborhood size"' finds about 3000 results from a literature and ' N_b "effective number of breeders"' finds about 700. Thus, we concluded that the term N_b for neighborhood size is widely used.

B. You calculate N_S on the basis of spatial kinship decay (line 193), and from that you deduce the gene dispersal distance σ , using the effective density D_e . You could actually calculate N_S also genetically by means of a LD-based method. Neel et al. (2013) showed that in spatially structured populations (like this one), $LDNe$ will estimate N_S when the sample originates from an area smaller than or equal to the spatial extent of the neighborhood size (a circle with radius 2 times σ). Since you have an idea how large σ is (even though you are using N_S to estimate σ), you can resample independent batches of genotypes and calculate the average N_S across replicates. This would be a welcome independent estimate of N_S .

- This was an interesting idea and it prompted us to test the $LDNe$ method as implicated in NeEstimator v2.1 (via an R package $RLDNe$) to get an independent estimate of the neighborhood size. However, due to the large variation in the results depending on the data used, we decided not to include the results in the manuscript.

More precisely, we tested the method for separate generations (20 year cohorts) and sampled a circle of $2\pi\sigma^2$. Then we estimated the N_e (N_b) using 500-5000 randomly chosen SNPs with MAF 0.05-0.10. The point estimates of N_e varied between -3000 and over 2000, also, often at least the upper Jackknife confidence interval was infinite. In addition, the N_e estimate from the same individuals changed considerably depending on how many loci were used in the estimate. Also estimates based on a different sampling of individuals (with approximately the same number of individuals and SNPs) could have a tenfold difference.

C. I'm not convinced that the effective density D_e is a good metric to use here, and I refer to lines 205 (methods) and 290 (results). why would the mean distance between parents and

offspring (=effective dispersal distance) change as a function of the N_e/N_c ratio? You sampled individuals from the N_c and observed the realized (=effective) dispersal from that same N_c , not from a theoretical equivalent N_e . Isn't the "effective" part already accounted for when you estimated spatial kinship decay?

- We use effective population density (D_e) to take into account the variance in reproductive output of individuals in time and space (e.g., Vekemans & Hardy: New insights from fine-scale spatial genetic structure analyses in plant populations, Molecular Ecology 2004). More specifically, it is stated in Vekemans & Hardy (2004), that in $4\pi D\sigma^2$ (that equals N_b), D is the effective density. They also state that: "D is the product of the census density and the N_e/N ratio".

D. Mark that you already wrote that the median distance of the closest GRM class was 51 and 59 m, which represents dispersal rather over 2 than 1 generations (since over 1 generation GRM would be around 0.5, not 0.25). Hence these distances are 1.27 times the median (or average? unclear wording in the ms) distance across a single generation (see calculations below). As a result, the expected sigma value would be closer to 40 and 46 m.

- We have answered the comment about estimating sigma directly from the data in the point considering Figure 5. Here is the same answer:

What comes to the suggestion of estimating sigma directly from the data, we do not believe that our sampling scheme is suitable for that. We have sampled only two regions with individuals in approximately 20 meter distances and aimed for trees of similar size (i.e., age, although still with some variation in age), which does not capture many close relatives and is not dense enough to directly estimate the dispersal distance. Taken together, our data and sampling are better suited for estimating sigma from regression of kinship on spatial distance.

We went through the text describing the GRM estimates in the family relationship classes and found only "median" mentioned. We have used the average when describing the relatedness within distance classes, which are depicted in Figure 3, whereas median is used while describing the family relationship classes in Figure 5.

E. All of this is under the assumption that the tree (census) density is 2000 /ha, but that would be easy to actually count and extrapolate, no? Maybe even using geospatial data?

- We tested two different ratios of effective to census densities (0.25 and 0.5), which gives quite a large range of potential population densities. We do not have current estimates of density in our study region, but we think that the estimate should be from the time when our adult study trees germinated. The histories of the sample sites include seed tree cuttings, but the age structure shows that, especially in Ranta-Halola, there is a large number of trees that are older and younger than the cutting. However, we do not have historical estimates of the population densities of the study populations either, so we decided to use a more general mean density estimate from the Finnish Scots pine forests. Note that not all individuals in the study areas were sampled in our study so we cannot infer the density just from our geospatial data. We have clarified the density issue in the Material and methods and Discussion parts of the manuscript.

Now, there seem to be a few very relevant things to be done with these data and results:

1. You can independently estimate N_e for the sampled area from sibship assessments (Colony), and use that to calculate N_e/N_c given that you can know N_c pretty accurately. From that result, you can check if the sigma you calculated is correct and if you should use "effective" density instead of actual density. (Mark that this sibship method is much less

sensitive to spatial structure than LD-based methods, assuming that you sampled the entire area under investigation with equal intensity).

- Thank you for the idea, but, unfortunately, we do not have any siblings in the data, the closest relatives are second degree relatives. We have answered the question about using the effective population density above in C.

2. You can independently estimate sigma (directly from the kinship data and the spatial coordinates). See below comment pertaining to fig 5.

- We have answered this point along the comments on Figure 5.

3. Linkage disequilibrium Ne calculations are very sensitive to the assumption of spatial genetic structure, and in continuous spatially structured populations like this one, Ne estimates will yield NS when samples are taken within a circle of radius 2σ . Since you can resample the >400 genotypes in batches only containing individuals within the spatial extent of a Neighborhood, you can estimate NS independently of De. Once you know NS and Nc (within an area of $2\pi\sigma^2$), you can calculate how many neighborhoods there are (and extrapolate $N_e = NS \times (\text{number of neighborhoods})$). Or if you know the spatial extent of a Neighborhood (through the Sigma value), you can now reliably calculate the Ne/Nc ratio (actually, the NS/Nc ratio within a neighborhood, which should be the same as the overall Ne/Nc, as this is just a function of the variance in reproductive success). You can also extrapolate the Ne of the entire population: if the area occupied by the population is X, the total number of neighborhoods equals $X/(4\pi\sigma^2)$, and the Ne should be $(X NS)/(4\pi\sigma^2)$

- We have answered the first part of this comment and explained the LD estimation of Ne/neighborhood size in point B above. In addition, there is a recent paper about estimating contemporary Ne in a continuous (Maritime) pine population and why it is not very feasible (Santos-del-Blanco et al. 2022 Biological Conservation). The same problems apply to Scots pine. In addition, estimating Ne is out of the scope of this paper.

Minor Comments:

the authors talk about “a continuous populations”, but that requires some clarification. The Märkä population is situated on an island in a freshwater lake, for example. Although it may experience pollen flow with trees on adjacent islands or on the , it is not what I understand under the term “continuous”.

- Scots pine has a continuous cover across most of Finland, and especially in southern Finland. See, e.g., this map:

<https://koivu.luomus.fi/kasviatlas/maps.php?taxon=107143&size=0&year=2015>

The rather uniform environment, e.g. low altitudinal differences (but a steep latitudinal climatic gradient), suggests very little differentiation across populations (except for some latitudinal clines). The populations are all rather young, established since the last glaciation. Because of the gene flow, there is little isolation. All of this has resulted in very low differentiation between populations. Märkä island is adjacent to the mainland and surrounded by other islands and it also represents a very typical Finnish landscape. Therefore it is reasonable to treat it as a representative sample from a Scots pine continuous population. Similarity between the patterns in Märkä (island) and Ranta-Halola (mainland) support the conclusion that being an island does not play a significant role in its SGS patterns. We have added some clarification to the methods.

Line 29: 3210 or 3120? Differs with results section.

- Thank you for spotting this mistake out, the correct number is 3210 and we have checked that it is now consistently written in the manuscript.

Line 107: the coordinates of the Mäkrä site are situated in the lake adjacent to the Mäkrä island.

- The reason for this is that the coordinates were given with less detail to improve readability, the exact points of the sampled trees are given with more decimal places in the supplementary material. To avoid confusion, we have now added seconds to the coordinates of the study sites in the main text.

Line 153: this needs better explanation. Phi-ST is generally derived from Nucleotide diversity, and adds weight to alleles that are more different from each other. How was this done in practice if you don't know the phase of haplotypes?

- Phi-ST is estimated using AMOVA in which the molecular variance is originally estimated from distance matrix derived from human mtDNA restriction site polymorphism (Excoffier 1992), but one can equally well use any genetic distances between individuals. Here, we used Nei's D distances estimated from SNP data. However, we decided to remove this estimate from the manuscript, since it didn't add considerably to the results.

Line 173 - 187: Mantel tests are notoriously weak (and have a high type 2 error), and there are far better ways of addressing the question of spatial genetic structure (as noted in the MS). See Legendre & Fortin 2010 and Legendre et al. 2015. Legendre, P., and M.-J. Fortin. 2010. Why not merely use a spatial autocorrelation analysis from Moran's I? It may not make a big difference here though.

- We have used both the traditional Mantel test over the whole study distance, which has the mentioned weaknesses, and Mantel correlogram analysis, which overcomes the problem of handling the whole distance as a one homogeneous sample. In addition, we actually had statistically "significant" results using Mantel tests, both for the whole distance in Ranta-Halola and Mantel correlograms for both Mäkrä and Ranta-halola for the first distance classes.

Figure 5: could you provide sample sizes (numbers of pairwise family relationships) for the different categories? You calculated sigma indirectly by estimating NS, assuming D and assuming a certain N_e/N_c to infer D_e , but can't you calculate Sigma, or even estimate a dispersal kernel function, directly from the data? Sigma is the average dispersal distance between offspring and parents, or between two offspring of the same parent. However, you have no first degree relations in your data: the smallest kinship coefficients are around 0.25 instead of 0.5. (However, half-sib relations are also just 0.25). Using information on age of trees (if you have estimates for those, e.g. from trunk diameter) you could infer which kinship combinations are parent-offspring (0.5, but absent from dataset), avuncular (0.25) and which are half-sibs (0.25). Using that information, you can estimate dispersal across two generations. Gene dispersal over 1 generation is sigma. The distance over 2 generations should (according to my deductions) be $\text{Sigma} * \text{Pi}/4$. (average dispersal across 1 generation from the parent will give a circle with radius sigma. Dispersal from the second generation starts on the circumference of this first circle, and a second circle is drawn. Part of the dispersal is back in the direction of the grandparent. The average distance of all points on this second circle to the origin of the first circle gives you the average distance across 2 generations. The calculation is nicely explained here <https://mindyourdecisions.com/blog/2018/10/18/whats-the-average-distance-of-two-points-on-a-circle/>). So across 2 generations you expect the gene dispersal distance to be $1.27 * \text{Sigma}$.

- As suggested, we have added the numbers of pairwise family relationships to the figure legend of Figure 5. What comes to the suggestion of estimating sigma directly from the data, we do not believe that our sampling scheme is suitable for that. We have sampled only two regions with individuals in approximately 20 meter distances and aimed for trees of similar size (i.e., age, although still with some variation in age), which does not capture many close relatives and is not dense enough to directly estimate the dispersal distance. Taken together, our data and sampling are better suited for estimating sigma from regression of kinship on spatial distance.

Line 328: This has important implications for estimates of N_e , and how samples should be taken when estimating N_e . In effect, this spatial structure allows one to only estimate N_S reliably, and then to extrapolate N_S to the total area of occupation to get N_e for that population.

- We agree, but the estimation of total population N_e is outside the scope of the manuscript.

Line 360: Fig. 5 shows the median, not the average dispersal distance? Still, if I'm correct to deduce that this is across 2 generations instead of 1, the average dispersal distance is $54\text{m}/1.27=42\text{ m}$

- Figure 5 shows the distribution of distance classes of different family relationships and yes the figure legend says: "Boxplots show the median (central vertical line), the lower and upper quantiles (boxes), and up to 1.5 interquartile range (whiskers) distances." For estimating the average dispersal distance based on this information, please see the detailed response above.

Line 391: Not a minor comment: IMHO, this statement very wrongly assumes that each parent produces on average just 2 offspring. Instead, each parent produces many thousands of seeds and seedlings across its lifetime, and selection acts on those seedlings, weeding out less fit genotypes. Most seedlings never reach adulthood, whereas the theoretical model assumes all reach adulthood but less fit genotypes have lower reproductive output. There is already very strong selection since orders of magnitude more offspring are produced than the number that can survive to adulthood. So I strongly question the assumption that adaptation on a very fine local scale would require extraordinary selection pressures or steep ecological gradients. Because there are so many targets of selection (=very high genotypic diversity) and because ecological space is so limited (only 1 seed per tree is expected to reach adulthood, whereas hundreds of thousands are produced across its lifetime), the response to even weak selection must be strong.

- Thank you for your comment. We prefer to keep this part of the manuscript to provide a theoretical perspective on what this level of gene flow implies on the strength of selection and capacity for very fine-scale adaptation. We agree that the number of offspring generated offers many opportunities for selection to act and acknowledge the fact that in species with large N_e , selection can indeed be very effective. However, our point is not merely on the strength/effectiveness of selection as such, but also on the spatial scale that species can track the environmental differences and adapt to them via allele frequency changes. Dispersal distances play an important part in defining that scale – together with strength of selection. We have now rephrased this part of discussion to be clearer in our intended message.

dLine 397: That is just a neutral consequence of being related, no? You share large chromosomal chunks of DNA due to a shared ancestry, hence you share alleles, including rare ones.

- That is correct, here we wanted to point out what consequences that sharing of especially rare (and potentially harmful) alleles can have.

I always sign my reports.

Joachim Mergeay, Research Institute for Nature and Forest, Belgium

Review by Jean-Baptiste Ledoux, 22 Jul 2023 22:45

In this manuscript, entitled “Does the seed fall far from the tree? - weak fine scale genetic structure in a continuous Scots pine population” Niskanen and collaborators characterize the spatial genetic structure among Scots pine individuals in two naturally regenerated sites separated by 20 km and located in a continuous South-Eastern Finnish Forest (l. 23-25). The Authors genotyped 469 adult trees from different age (33-145 years) using a custom-made Affymetrix SNP array including 407 540 markers resulting in 157 325 polymorphic SNPs (l.111-115). All the trees were georeferenced using a portable GPS locator allowing for estimation of pairwise geographical distances (l.123-130).

From this dataset, the Authors implemented different filtering steps function of the analyses (e.g. excluding related individuals, considering different MAF; details l. 133-138; 140-150; l.225-230). They conducted usual population genetics analyses to characterize population genetic structure between the two populations (FST, PCA, FST, ϕ ST), spatial genetic structure among individuals (l.157-187), estimation of related demographic parameters (Nb, sigma, Sp, l.188-223) and characterize the spread of rare alleles (l. 224-235).

Regarding the analyses of spatial structure at the individual scale:

1) they regressed the pairwise genetic distances (estimated as relatedness coefficient; GRM l.158-163) on the geographic distances and test the correlation using a Mantel test and Mantel correlogram dividing their samples in pre-defined geographic distance classes (l. 176-187).

2) They estimated Nb, sigma and Sp following Hardy & Vekemans (2004) by using a second estimator of pairwise genetic distances, the kinship estimator of Loiselle et al. (1995). Briefly, Nb, the neighborhood size, was estimated based on the regression of kinship vs. natural log of the genetic distance. Sigma, the effective dispersal, was estimated using different effective population density value and an iteration procedure while Sp, which quantifies the strength of the genetic structure among individuals, was estimated as the inverse of the regression slope divided by 1-FN (FN= mean kinship in the first distance class).

Regarding the analyses of the spread of rare alleles, the Authors focused on SNP with MAF < 0.01 and tested the regression between the GRM and the geographic distance among individuals using Mantel correlogram. They fitted the proportion of rare alleles on relatedness using local regression with sample site as fixed predictor (l. 233-235).

The Authors demonstrated a very weak genetic structure between the two sites (l.238-247). Overall, mean pairwise relatedness was low (l.253-255) but the Mantel correlogram evidenced a slight decrease of genetic relatedness with pairwise geographic distances. Interestingly, this decrease was observed at similar rate among the two sampling sites (l. 255-259). Yet, the Mantel test on the whole dataset (i.e. not considering distance classes)

was significant only in one of the two populations (l.260-262). The resulting S_p were low, confirming the low spatial structure (l.262-266).

Regarding the GRM, the Authors identified 24 closely related pairs coming from the same sampling site. Decomposing the GRM in family relationships, the Authors showed the spatial aggregation of closely related individuals to be similar (same distance) in the two sites.

The estimator of dispersal (Sigma) is low.

Finally, the Authors confirmed the aggregation of related individuals also when considering rare alleles, with more related individuals sharing higher proportion of rare alleles.

The Authors discussed the spatial genetic structure among individuals in the light of non-random mating patterns previously reported (l. 324-337), highlighting their study as the first to demonstrate such structure in a large continuous population of Scott Pines.

They discussed the biological processes explaining potentially the weakness of the spatial structure focusing on long distance pollination, continuous distribution range and high population density (l. 338-343) and contrasted their results with what has been previously reported in fragmented populations of the same and other species (l.341-357).

The Authors involved the short dispersal (sigma value) estimated in their study in existing literature on wind and animal pollinated species and mentioned the limitations of the model used (i.e. Gaussian distribution of dispersal distances vs. leptokurtic dispersal kernel in wind pollinated species) (l. 368-373). Based on estimated sigma values, they inferred the strength of selection (selection coefficient) needed for their population to locally adapt. Owing to the strong coefficient of selection, they considered the likelihood for local adaptation to be low (l.374-392).

They concluded the study discussing the implications of fine scale spatial structure for the conservation and management of Scott Pine populations (l. 393-419).

Overall, this is an interesting and well written paper addressing an important issue (spatial genetic structure) in a keystone species with high economic value. The motivation of the study is well explained as well as the particular interest of the two populations (two areas within a dense continuous populations). The main objectives of the study are well exposed and references are recent and relevant in the context of the study.

The methods are relatively well explained and appropriated (but see below). The results are clear while, in my opinion, the discussion may be a bit improved.

Below I list some comments that, hopefully, will help the Authors to improve the current version of the manuscript.

- Thank you for the positive and fruitful comments on our manuscript.

#: I would expect some more details regarding demographic data. In the present form the Authors only mentioned they sampled "469 adult (33–145 years) Scots pines" l. 108. If possible, I would like to know about 1) the respective age of the two populations and 2) the size (age?) structure and 3) the density of each population. The Scott pine is the kind of species in which such data should be relatively easily estimated. In this line, I was wondering whether or not the Authors tested the evolution of the spatial genetic structure among individuals accounting for these demographic data. For instance, are the differences between the two populations in term of significance of the Mantel test and S_p (l. 260-266) potentially explained by different demographics or recolonization histories? This is probably

something to discuss in the paper, particularly considering that the two populations were naturally regenerated.

- As suggested, we have added the age distribution of the sampling sites to the supplementary data (Figure S1) and more details of the sampling sites to the Material and Methods section. Density estimates from the time periods that the study trees germinated are not available. We have given a more detailed answer about the relevant density estimate below. The sites were naturally regenerated after seed tree cuttings. There is very low genetic differentiation and high similarity in terms of spatial genetic structure. Some small differences in the results may be caused by, e.g., the differences in the sampled area and possibly the age structure. We have added a paragraph about the small differences between the samples to the discussion.

The Authors also based their estimates of census density on literature (l.207-212). Does it mean it was not possible to estimate these parameters for each of the two populations directly from the field (see above?)

- We repeat here the answer we gave to the first reviewer about using geospatial data to estimate the census density of the study sites:

We do not have current estimates of density in our study region, but we think that the estimate should be from the time when our adult study trees germinated. The histories of the sample sites include seed tree cuttings, but the age structure shows that, especially in Ranta-Halola, there is a large number of trees that are older and younger than the cutting. However, we do not have historical estimates of the population densities of the study populations either, so we decided to use a more general mean density estimate from the Finnish Scots pine forests. Note that not all individuals were sampled in our study so we cannot infer the density just from our geospatial data. We have clarified the density matter in the Material and methods and Discussion parts of the manuscript.

#: Following Rousset 2007, the regression between genetic and geographic distances in a 2D model (as it is the case in the present study) should be done considering the natural log of the geographic distance. Is there a reason to explain why the Authors used the geographic distance per se?

- While estimating neighborhood size and dispersal distance, we performed the regression analyses between relatedness and $\ln(\text{spatial distance})$ as explained in the methods section "Neighborhood size and dispersal distance" (copied from the manuscript here: "where F_N is the mean kinship (Loiselle et al. 1995) of the first distance class (0–50 m in Mäkrä and 0–60 m in Ranta-Halola) and blog is the regression slope of kinship on the natural logarithm of spatial distance over all distance classes (Rousset 2000; Hardy & Vekemans 2002)").

#: l.189-190: The definition of the neighborhood size should be rephrased according to Vekemans & Hardy 2004 (MolEcol see p.922-923) and Rousset 2008 (https://kimura.univ-montp2.fr/~rousset/hsg029_proofs_corrected.pdf; see p.14). The approximation of neighborhood size as panmictic population is not recommended.

- We have now rephrased the definition of the neighborhood size.

#: Still on the neighborhood size, it is estimated in the paper from the formulae $N_b = -(1 - F_n) / \text{blog}$. The Authors then reported two different estimates of N_b for each population considering different effective density values (l.285-287). It is not clear to me how these two N_b values were involved in the computation of N_b . To my understanding N_b is estimated from the slope of the linear model as supported by the formulae above (see also all the work by Rousset). Could the Authors clarify this issue?

- N_b is first estimated using the given formula. Then, using the first estimate of N_b as a starting value, while simultaneously estimating σ , an iterative process starts where effective density is used as shown in this formula:

$$\sigma_g = [N_b / (4\pi D_e)]^{1/2}$$

We have clarified this in the Material and Methods of the manuscript.

#: Regarding the family relationships:

1) how did the Authors define the interval for each of the considered degree? In my understanding, these intervals are also impacted by the reference allele frequencies.

- Thank you for the comment. It is true that the reference allele frequencies and the relatedness estimation method affect the exact boundaries of the family relationship classes. However, as shown by Jiang et al. (BMC Bioinformatics 23:525, 2022), the difference between the kinship (we used relatedness, but Jiang et al. used kinship in their work) estimate using a similar genomic relationship method we used and the value expected in theory is very small, when kinship is estimated from a population sample. For example, for a pair of full siblings the bias from true value was -0.00953 and standard deviation 0.002864. Given the large range of values that each family relationship class already spans, we do not see this as a problem for drawing conclusions about the spatial proximity of the distribution of distances of differently related individuals. In addition, we have added a supplementary Table S2, where we compare the classification based on relatedness and kinship, and show that they agree very well.

Another potential issue with the estimation of relatedness using GRM is the inflation of relatedness estimates when close relatives are included in the reference allele set. This we avoided by only including the unrelated individuals ($GRM < 0.0625$) when estimating the reference allele set.

2) The Authors used two different estimators of genetic distance among individuals: GRM based on relatedness and Loiselle's kinship. Do the two estimators gave the same levels of family relationship among pairs of related individuals (e.g. all the 2nd degree family relationships are found with GRM and Loiselle?)

- We have added family relationship classes using Loiselle's kinship and compared these categories with the ones estimated using relatedness (GRM) in Table S2. The estimates and family relationship classifications were very similar with both estimators. See also our answer to Myriam Heuerz above (the second answer).

3) It is very interesting to see that the size of the populations does not impact the mean distance among highly related individuals (Figure 5). I encourage the Authors and to emphasize this result and to improve its discussion. Is this something commonly reported in SGS studies? What is the (biologic, life strategy) hypothesis of the Authors to explain this result?

- Due to the extremely low genetic differentiation between the sampling sites (and in Scots pine in general), we handle the study sites as one population and refer to them as separate sampling sites with differing ranges of distances. Due to this reason, we are hesitant to go any deeper in the discussion of the potential effects of population size in relatedness patterns.

#: The discussion is likely the part that requires more rephrasing. I believe the discussion of most of the results can be improved. The same is true for the implications of the results.

1) The reasoning of the Authors is sometimes a bit hard to follow, particularly when it comes to the contrast between the restricted dispersal sigma (50m) and the weak genetic structure. Depending on the part of the discussion, it appears that there is no gene flow (e.g. l. 330, 361) or that there is high gene flow through pollen dispersal (l. 350-353). Beyond clarification, one way to improve the issue could be to better link the part of the discussion in which the Authors compared the S_p values among species (l. 346-350) with the part of the discussion in which they compared the sigma obtained in different tree species (l. 360-368). Adding the range of geographic distances at which F_{ST} are significant in other species may also be interesting. What about the estimation of D_e ? Is it realistic to consider values of D_e that will result in much higher sigma?

- We have added discussion about the effect of considering smaller values of D_e and how likely those are in our study sites. We also added that the estimated sigmas can be thought as minimum estimates, since we do not consider the leptokurtic dispersal kernel of pollen and the effective density is not known (but can also be smaller than what we used).

There is already discussion considering the small sigma and small S_p , and why they are actually not conflicting in a continuous population with constant gene flow, so this part we changed only little. One has to remember that the estimate of sigma depicts the mean dispersal, so there are gametes that disperse shorter distances and some that disperse longer distances. We added more information about pollen dispersal and considering the breeding contribution of surrounding forests in different places of the discussion, so we think that we have clarified the subject.

2) The last two paragraphs from l. 402-419 are a bit too general. They introduced interesting considerations for management regarding for instance inbreeding and inbreeding depression but the Authors keep this very general perspective without linking these considerations with their own study.

- Thank you for the comment, we made some additions to the text, but we decided to keep the mentioned paragraphs of the discussion. These parts of the discussion are related to the results and their potential applications, so we find them relevant.

3) I found the paragraph on selection (l. 374-392) potentially interesting. Could the Authors be more precise regarding the known agents of selection in the Scott Pine? They computed the coefficient of selection considering a characteristic length (L) = 100m. Based on this value and their estimate of sigma they estimated that the coefficient of selection is high (at least following Authors reasoning because this value is not involved in existing published values; see below) and they mentioned that their landscape is homogenous, which limits the likelihood for strong selection. My concern here is that the reasoning may be a bit biased. Maybe the Authors should consider a characteristic length related to their homogenous landscape instead of arbitrarily choosing 100m? Or if 100m is meaningful, they should likely explain why? It would be nice also to put the value of the coefficient of selection in a broader context with example supporting the fact that 0.29 is a high value supporting strong selection.

- Thank you for your comment. We have rephrased this part of discussion to be clearer. In the original version 100 m was chosen as an arbitrary example. We now present an example in a different form. We give some examples of known adaptations to climate that occur at the between populations scale (populations from different latitudinal origins). At that spatial level, adaptation can undoubtedly be seen. We also highlight the fact that there is no evidence for fine-scale adaptations (e.g., to different types of soils) in this species.

l. 386: do we really talk about the "size" of the coefficient selection? Maybe value would be better?

- We have rewritten that section of the discussion, so this comment is no longer relevant.

#: Long distance events and their implications in the observed patterns are mentioned on l. 338 and 372. In this context, I was wondering why the Authors did not try to identify these long distance events. One way would be to check for migrants (using for instance assignment tests) between the two populations? Those migrants may be proof of the long-distance events and the deviation from the Gaussian dispersal.

- Thank you for the comment, but any assignment program would require some genetic differentiation between the populations which the individuals are tried to be assigned to. In our case, we do not have separate populations, but we have sampled the same population and have two sampling sites which show almost no differentiation in PC analysis (Figure 2) or FST analysis ($F_{ST} = 0.0005$). In addition, all pairwise relationships in the classes 1st-4rd degree (i.e., higher than “unrelated”; Figure 5, Table S2), were between individuals within a sampling site, which does not point to very recent or clear dispersal events within our sample. Given these results, we do not see assignment as a useful analysis to try to find long-distance dispersal events. Note that the two sampling sites and the surrounding regions contain a vast number of non-genotyped individuals.

Minor issues:

The sentences l.111-112 and 113-115 are confusing (at least to me, sorry for that!). What is the number of genotyped SNPs? Is this number 407504 or 157325? What are the markers mentioned in l. 111-112?

- We have now clarified the text on SNP numbers and filtering of SNPs for the analyses.

From the legend (l.301-302), it seems that figures 5a and b are missing. The red line mentioned in the Figure 5 legend is not visible on the graph.

- We apologize for this mistake, we had forgotten to update the figure to the BiorXiv version of the manuscript! Now the parts a) and b) are added to Figure 5.

l.105: the provided link does not work.

- Thank you for pointing that out, we have updated the link.

l.334-335: What about the expected values of GRM and Loiselle kinship in case of pairs of individuals linked by selfing?

- The expected kinship between a selfed individual and its parent is 1 for relatedness (GRM) and 0.5 for kinship (Loiselle). We did not find relatedness values that would be this high and added a sentence about this in the discussion.

L.232: is “constructed” correct?

- We changed “constructed” to “obtained” in the text.

l.332-334: it would be nice to have estimation of selfing and survival rates.

- We have added the percentages of selfing and mortality related to selfing to the discussion. Selfing rate in mature seeds is 5%–10% and the average mortality in selfed seeds is 75%–85%.

I.353-357: not sure how to link this part on southern/northern range limits / adaptation with the rest of the paragraph. This is an interesting point, yet it deserves some more details.

- Thank you for the comment, we have elaborated this part to make it more connected to the rest of the paragraph.

I.395: what is a “lethal equivalent”?

- Lethal equivalent is a measure of genetic load describing the number of recessive deleterious alleles with equivalent impact as a homozygote to a single lethal allele. In other words: how many times an individual would die if all its genome would be homozygous.

Review by Roberta Loh

Does the seed fall far from the tree? - weak fine scale genetic structure in a continuous Scots pine population

The present study describes the fine scale spatial genetic structure of *Pinus sylvestris* in two collection sites from the same population in south-eastern Finland. The study was well conducted and has a clear methodology appropriate to the question.

The text is clear and well written and presents a relevant and interesting discussion regarding the importance of knowledge on SGS for practical forest management issues.

Minor suggestions were made throughout the text.

Title:

The title says that the study is conducted on a continuous Scots pine population. However, the images of the landscape in Punkaharju area show several patches of land separated by water. Also, the two sampling sites seem to be located in different sides of a series of ridges. In each site, the area is continuous, but considering a bigger picture, it seems to be an area that is naturally subdivided and trees are located in different population patches separated by water.

This is genuine curiosity: could this be considered a continuous population?

I mean, genetically, the study demonstrates that it is a single population. But is that the intention in the title? Or the term “continuous” in the title is referring to the fact that both study sites are in the same area? Because it looks like a very discontinuous landscape, although the study showed that the physical discontinuity of the land does not promote a strong genetic structure.

- Of course this is a matter of scale, but from an evolutionary and genetic point of view, the populations and the species main distribution area are continuous. Scots pine has a continuous cover across most of Finland, and especially in southern Finland. See, e.g., this map: <https://koivu.luomus.fi/kasviatlas/maps.php?taxon=107143&size=0&year=2015>

Similar to any other species, continuity is lost when the resolution is reduced enough. In our opinion, it would be more dishonest to claim that there would be subpopulations separated by bodies of water, when in reality the patches are genetically well connected and almost impossible to separate from each other. Mäkrä island is adjacent to the mainland and

surrounded by other islands and it also represents a very typical Finnish landscape. Therefore it is reasonable to treat it as a representative sample from a Scots pine continuous population. Similarity between the patterns in Mäkrä (island) and Ranta-Halola (mainland) support the conclusion that being an island does not play a significant role in its SGS patterns. No species of land plants of course has a completely continuous population but it is hard to imagine a land plant species that would be closer to being continuous than Scots pine in the boreal region. Scots pine is the most common tree species in Finland, and during natural regeneration of forests in Mäkrä and Ranta-Halola, the proportion of Scots pine in southeastern Finland was very high (results from the First Finnish National Forest Inventory analysed e.g., in Aakala et al. Landsc Ecol (2023) 38:2417–2431).

Abstract

Line 26: While we detected some pairwise relatedness at short distances, the relatedness decreased with increasing distance, as expected.

R: I believe that this sentence would be better written by stating that the relatedness was restricted to a very short distance, and not that it has simply decreased with increasing distance, because it is expected indeed. But I think that the authors wanted to state that relatedness was weak and restricted to a few meters.

- Thank you for the suggestion. We changed the sentence a little, but we do think that the observation of decreasing relatedness with distance is important to state, since it is the core of spatial genetic structure.

Line 32: Detailed empirical estimates of dispersal are necessary both in studying post-glacial colonization and predicting the response of forest trees to climate change.

R: I think this last sentence could be relocated to the beginning of the abstract. It is an important remark to give context to the relevance of the study but it's not a conclusion of this study.

- Thank you for the comment, but we prefer to keep the last sentence as it is.

R: No keywords?

- We did not include keywords, as they were not required in the formatting guidelines. The main keywords are included in the title.

Introduction

The introduction is well-written and provides the necessary context for understanding the issue being addressed.

Line 79: As a major source of timber, paper and pulp, Scots pine holds also high economic value.

R: Add reference.

- We have added reference to the Natural Resources Institute of Finland page about the wood consumption in Finland 2021:

<https://www.luke.fi/en/statistics/wood-consumption/forest-industries-wood-consumption-2021>

Material and Methods

Statistical analyses are well described and carefully conducted. The chosen estimators are suitable to describe SGS and the marker has sufficient resolution to differentiate genotypes at the individual level.

Regarding spatial distribution of genetic variation, I find it interesting to use the term “gene flow” to describe the exchange of alleles between groups with estimators such as F_{st} and Φ_{ST} and the term “gene movement” to describe the intragroup (intrapopulation) spatial genetic structure.

- We were not sure whether the reviewer is suggesting that we should use the term “gene movement” in our manuscript. Thus we preferred to keep using “gene flow”.

Line 108: We sampled 469 adult (33–145 years) Scots pines approximately at 20 m distance, with shortest within sampling site distance of 10 and 14 m and longest 464 and 1164 m in Makra and Ranta-Halola, respectively.

R: How many samples were collected on each site?

- We have now added the numbers of samples collected from each site to the “Samples and genotypes” chapter (113 from Mäkrä and 356 from Ranta-Halola).

Line 121: Figure 1. Maps of the sampling sites. Sampled trees are indicated as red dots.

R: Include source of the images in the figure legend. Include more details of the location of the study area.

Suggestion:

Maps of the sampling sites in Punkaharju intensive study site located in south-eastern Finland. Sampled trees are indicated as red dots.

- Thank you for the suggestion, we have changed the figure legend as suggested and added information about the R-package used to draw the maps.

Line 169: “first cousins once removed”

R: what do you mean by “First cousins once removed”?

- “First cousins once removed” is a way to describe a family relationship where the compared pair are a first cousin and a first cousin’s offspring. We think “First cousins once removed” is the most explicit way to explain the relationship.

Results

Line 248: Figure 2 – PCA analysis.

R: The percentage of total variance explained by PC1 And PC2 seems very low. From 0.35% to 0.42%.

- That is correct and quite often the case with large SNP datasets and small genetic differences. See for example Kastally & Niskanen et al. 2022 (The Plant Journal, 109: 1337–1350).

Line 292: Figure 4 – Mantel test results

R: Authors chose to present Mantel test results on relatedness versus distance by plotting r^2 values of Mantel test versus each distance class. I find the graphic a bit confusing and hard to interpret. I understand this was done because the test was performed separately for each distance class. But wouldn’t it be possible to plot the relatedness values (GRM) versus distance and show the significant r^2 values within the plot, next to the data point?

- Thank you for the comment. We have already plotted relatedness versus spatial distance in Figure 3. The Mantel correlogram plot (Figure 4) is quite widely used, so we think it is more convenient to keep it as it is.

Lines 254 – 258: Parameters in Ranta appear to be twice as large as in Makra. What could be the reason for this difference? The larger area of the collection site in Ranta? Different population densities?

- We have now made comparisons between Mäkrä and Ranta-Halola using Mäkrä's distance classes in both sampling sites (Figure S2). One can see from the figure that the decay of relatedness with distance is very similar in both places, except for the first distance class. The number of comparisons differs quite a lot (Table S1), and in addition, the sampling areas differ in shape, so these are potential reasons for the small differences. But it is also possible that Mäkrä has more half-siblings (or other 2nd degree relatives) due to the younger sampling and age structure. We have added a paragraph about the possible causes of these small differences in the discussion.

Line 300: Figure 5. The relation between family relationship classes and spatial distance. The pairwise distances of the second degree related individuals in a) Makra and b) Ranta-Halola are shown as red lines. The distribution of all pairwise distances of individuals in different family relationship classes are shown in c) for Makra (turquoise) and Ranta-Halola (orange). Boxplots show the median (central vertical line), the lower and upper quantiles (boxes), and up to 1.5 interquartile range (whiskers) distances. The family relationships are classified based on pairwise GRM: second degree between 0.177–0.354 (e.g., half-sibling), third degree between 0.088–0.177 (e.g., first cousin), fourth degree between 0.044–0.088 (e.g., first cousin once removed), and unrelated below 0.044.
R: Is there supposed to be another part in this figure? I couldn't find any red lines.

- We apologize for this mistake, we had forgotten to update the figure to the BiorXiv version of the manuscript! Now the parts a) and b) are added to Figure 5.

Discussion

The discussion is well constructed and refers to many studies with pines and other tree species, providing a suitable overview for contextualizing the results. Authors refer to both classic studies on the spatial genetic structure of plants and more recent works in the field. The work is relevant and brings an interesting discussion regarding the effect of dispersion on adaptive potential, which is not usually addressed in an objective way in studies describing the spatial genetic structure of plants.

Line 335: Fine scale genetic structure has previously been found in smaller fragmented Scots pine populations (Robledo-Arnuncio & Gil 2005; Sofletea et al. 2020) but here we show this pattern within a large continuous population for the first time.

R: Could this weak SGS be the result of the discontinuous landscape with vegetation patches separated by series of meandering waterways that create unsuitable habitats for seed germination? Therefore, either pollen or seeds can only thrive if they fall close to the parent plant or if they cross larger distances to land on a different ridge with proper conditions to succeed in finding another plant to pollinate or a place to germinate.

- This is an interesting idea, but here we show that the pattern of decreasing relatedness with increasing distance does not reach very far - and the pattern is lost already within a sampling site. And within a sampling site there are no water bodies or other larger obstacles to explain this. Also see our responses to the "continuity" issue above.