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Genetic Variation for Needle Traits in Scots Pine (*Pinus sylvestris* L.)

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Abstract

The remnants of the Caledonian Native Pinewood are distributed across a relatively narrow geographic area in the Scottish Highlands, yet inhabit a steep environmental gradient in terms of rainfall, temperature, and altitude. Previous work based on common garden trials has demonstrated that native pine populations (*Pinus sylvestris* (L.)) exhibit differentiation in terms of growth, phenology, and frost resistance. However, despite their important role in plant fitness, no such information is available on leaf traits, which have shown both plastic and adaptive genetic responses to environmental variation in other species. We analysed a subset of 11 needle characters in 192 saplings grown in a population-progeny common garden trial based on seedlots from eight native pinewoods. Narrow-sense heritability (h^2) was estimated for each trait, and found to be particularly high (1.30 ± 0.33) for resin canal density. The majority of the phenotypic variation found was within populations, although interpopulation differentiation was detected for needle length (ΔAICc = 2.55). Resin canal density was positively correlated with longitude ($\beta = 0.45$, ΔAICc = 4.23), whereas stomatal row density was negatively correlated ($\beta = -0.12$, ΔAICc = 2.55). These trends may reflect adaptation for differences in moisture availability and altitude between east and western populations in Scotland.

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Introduction

Once widespread across the Scottish Highlands, Scots pine (*Pinus sylvestris* (L.)) populations have been in decline in the region for the past 5000 years. The fragmented remnants that endure are estimated to cover only one percent of the area that was once occupied (McVean and Ratcliffe, 1962). Despite this dramatic contraction, native Scots pine populations have retained levels of molecular genetic diversity comparable to those observed in continental European populations (Wachowiak et al., 2011, 2013). Moreover, they continue to survive under a wide variety of environmental conditions within this limited geographic area. Thus the westernmost pinewoods occur at sea level and receive more than double the annual precipitation of their easternmost counterparts (~3000 vs 800 mm), which are located at an elevation of 500 metres in the Cairngorm Mountains.

In conifers, needles are the key organs responsible for photosynthesis, carbon assimilation, and exchange of gas and water with the atmosphere. Needles may vary in overall dimensions within and between individual trees, as well as in details of key anatomical characteristics important in adaptation. Such features include stomatal density, which influences rates of gas exchange and water loss, and the abundance of resin canals, which provides the needle with defence against both herbivores and pathogens (Christiansen et al., 1987; Schroeder, 1990). Numerous studies have been conducted on variation in needle dimensions and anatomical characters of Scots pine and other conifers growing *in situ* (Bobowicz and Korczyk, 1994; Urbaniak et al., 2003; Androsiuk and Urbaniak, 2006). These have demonstrated very significant differences between and within populations. Relating morphological to environmental variation, reductions in needle length have been reported for wild-growing *P. sylvestris* (James et al., 1994) and *P. roxburghii* (Tiwari et al., 2013) with increasing altitude. However, common garden trials are required in order to disentangle environmentally dependent, or plastic, variation from genetic.

Local adaptation can be described in terms of a balance between gene flow and selection: specifically, it occurs only if differential selective pressures are of sufficient strength to overcome the homogenising effects of gene flow (Kawecki and Ebert, 2004). Scots pine is wind-pollinated and a highly outcrossing tree species (Muona and Harju, 1989; Robledo-Arnuncio et al., 2004), and in Scotland genetic structure has been shown to be extremely low for nuclear and chloroplast markers which are subject to pollen-mediated gene flow (Provan et al., 1998, Wachowiak et al., 2011, 2013). Despite this, common garden studies have demonstrated that these populations show adaptive genetic differentiation associated with this environmental variation for physiological characters such as growth rate, phenology, photochemical capacity, and response to cold temperature (Perks and Mckay, 1997; Perks and Ennos, 1999; Salmela et al., 2011, 2013). In contrast, little information has been gathered on the extent of genetically determined variation in anatomical characters that may be related to adaptation of these populations to the diverse environments present within Scotland.

In this study we assess the potential for differential adaptation of leaf morphological characters among populations of a wind-pollinated tree species subject to extensive gene flow. Within a common garden we grow collections of open pollinated families sampled from populations located across the Scottish rainfall and temperature gradient. The design allows us to estimate the extent of genetic variation present both within and among populations, and the heritability of traits across populations. This provides an indication of the ability of the population to respond to selection on the character concerned. Lower stomatal densities have been reported for plants growing in drier environments; for example among Canadian hardwood species (Carpenter and Smith, 1975), and in *Pinus* decreases in length have been observed with increasing altitude in the wild (James et al., 1994; Tiwari et al., 2013). We hypothesise that genetic differences among populations may reflect the differences in climate between east and west: specifically that the significantly drier, high altitude populations in the east may exhibit shorter needles with a lower density of stomatal rows. It has been suggested that poor survival of Scots pine transplanted into western sites may be in part due to the greater prevalence of fungal pathogens within the damper oceanic climate (Mason et al., 2004). We therefore also anticipate that resin canal density may be greater for western populations than others in the Scottish distribution.

Methods

Study Populations

The trial consisted of material grown from seed collected from eight native populations from sites across the Scottish Highlands, which experience different climates (Figure 1, Table 1). Mean annual temperatures ranged from 6.44 ^{o}C at Ballochbuie to 8.53 ^{o}C at Beinn Eighe and mean annual rainfall was lowest at Glen Tanar (850 mm) and highest at Coille Coire Chuilc (3091 mm). Within each population seed was collected in March 2007 from four open-pollinated mother trees, sampled across the altitudinal range of the site. A substantial degree of collinearity exists among environmental predictors for native sites included in the study (Table 2). Both mean temperature and precipitation decrease with longitude, while altitude increases from sea level on the west coast toward the Cairngorm Mountains in the east.

Experimental Design

A common garden trial was established outside at the Centre for Ecology and Hydrology, located ~10 km south of Edinburgh, Scotland, UK. The trial site receives similar levels of precipitation to the easternmost populations included in the trial, but is at lower altitude and latitude. The material described in this paper is a subsample of that described by Salmela et al., 2013, with populations chosen to provide coverage across the range of the Scottish distribution. Seed was sown in summer 2007; shortly after germination seedlings were transferred to 11 cm diameter × 9.6 cm pots, randomised, and maintained in the glasshouse under natural lighting conditions. In spring 2008, seedlings were re-potted into 11 × 11 × 12 cm pots, randomised a second time, and relocated to outdoor benches where plants were watered only during periods of unusually low precipitation to prevent droughting. In spring 2011 plants were re-potted once more to 13 × 13 × 13 cm pots, and maintained at a spacing of approximately one pot width.

In total, the trial consisted of 192 individuals, which comprised six blocks of one member from each half-sib family from each population (6 x 4 x 8) with randomisation within blocks.

Sampling Protocol

Needles were harvested for analysis during summer 2012, when saplings were approximately five years old. In order to ensure that only fully expanded tissues were measured, all needles were taken from previous-year whorl branches. In Scots pine a fascicle consists of a pair of similarly sized needles. Five fascicles were removed from each individual and placed immediately onto damp tissue and stored in Petri dishes for no longer than two hours prior to dissection or mass recording. One needle from each pair was used for destructive measurements, and the other was weighed intact. In total, 960 needle pairs were sampled (5 pairs × 192 individuals): a small number of measurements were unsuccessful, reducing the total number of observations to 933.

Anatomical Measurements

Needles subject to dissection (the first of each pair in a fascicle) were first scanned using a flatbed scanner to provide images for length estimation, and then viewed under a stereo microscope. In pines, stomata are arranged into longitudinal rows: the numbers of stomatal rows on the adaxial (upper) and abaxial (lower) surfaces of the needle were counted, after which the needle was sectioned by hand using a sharp razor blade to provide a transverse section (TS) (Figure 2). Internal and external characters may vary throughout the length of a needle, and therefore in all cases both stomatal row counts and cross sections were obtained from the approximate centre of each. Each TS was stained with 0.05 % Aniline Blue, and digitally photographed via a camera mounted on a *Leica DM2500* light microscope (×10 objective). All measurements on captured images were made via *MacBiophotonics ImageJ* (Abramoff et al., 2004). Fresh mass was obtained from the second needle in each pair, and dry mass was recorded after needles had been held for a minimum of three days in a desiccating oven at ~60 °C.

In summary, the following traits were recorded from intact needles: length (mm), number of stomatal rows (adaxial and abaxial), fresh mass and dry mass (mg); and the remaining from transverse cross-sections: TS area (mm^2), vascular bundle VB area (mm^2), width (mm), depth (mm), resin canal density (expressed as the number of resin canals per mm^2 of TS area), and stomatal row density (the total number of stomatal rows on adaxial and abaxial surfaces divided by the perimeter of a transverse cross-section in mm).

Estimation of SLA, LDMC

Specific leaf area (SLA) is defined as the ratio of leaf surface area to dry mass. For the purposes of estimating area, needles were envisaged as open-ended semi-cylinders, and SLA (mm^2mg^{-1}) was approximated by the following:-

$$SLA = \frac{\pi rl + 2rl}{m} \qquad 1$$

where *r* (mm) is the radius (i.e. depth) of a needle, *l* (mm) is length, and *m* (mg) is dry mass. Leaf dry matter content (LDMC) was expressed as the ratio of leaf dry mass to fresh mass, and was logit transformed prior to mixed-model analyses.

Statistical Analysis

All analyses were carried out in *R* (http://www.R-project.org/). Mixed models were fitted by *maximum likelihood* (ML) using the package ‘lme4’ (Bates et al., 2013). Comparisons between models were made on the basis of their *corrected Akaike Information Criterion* (AICc) scores using the package ‘MuMIn’ (Barton, 2013); the resulting best models were then fitted via *restricted* *maximum likelihood* (REML) to obtain parameter estimates. ΔAICc scores are reported in terms of the AICc score of the null model relative to the best model; following (Burnham and Anderson, 2004), additional parameters which provide an improvement in AICc of < 2 were not considered to provide adequate improvement over the simpler model. Figures were produced using 'ggplot2' (Wickham, 2009).

Phenotypic correlations between family trait means were commonly high (> 0.5), particularly those which describe the dimensions of a needle cross-section, where correlation coefficients were > 0.9 (Table 3). The following traits were therefore chosen to be representative of the data: TS area, SLA, length, resin canal density, stomatal row density, and LDMC.

Estimation of Pooled Genetic Components

Genetic variance components were estimated after pooling across populations by first fitting a model to each trait in which Population, Family within Population, Individual within family (five observations were made per individual), and Block were incorporated as random effects with random intercepts:-

$$Trait = \mu + Population + Family + Individual + Block + \varepsilon \tag{2}$$

Pooled narrow-sense heritabilities (h^2) were estimated as follows using data from all populations:-

$$h^2 = \frac{V_A}{V_P} = \frac{4V_{fam}}{V_{fam} + V_{ind} + V_{block} + V_{res}} \tag{3}$$

where V_A is additive genetic variance, V_P is phenotypic variance, V_{fam}, V_{ind}, and V_{blk} are the among family within population, among individual within family, and among block components, and V_{res} is the residual variance. *Pinus sylvestris* is highly outcrossing, and with the exception of stands at extremely low population density, the proportion of full-sibs within maternal progenies has been shown to be almost zero (Robledo-Arnuncio et al., 2004). Within families siblings were therefore assumed to be half-sibs, and V_A was estimated as four times V_{fam}. The interpopulation component, V_{pop}, was excluded from the estimate of V_P. Very large sample sizes are required to provide accurate estimates of h^2, however, with smaller samples it is still possible to detect significant heritabilities when estimates are large. Standard errors for the heritability values were estimated according to (Visscher, 1998) as:-

$$\mathrm{SE}_{h^2} = 4\sqrt{\frac{2(1-\frac{h^2}{4})^2[1+(s-1)\frac{h^2}{4}]^2}{s(s-1)(f-1)}} \tag{4}$$

where *s* is the number of offspring per family, and *f* is the number of families.

The genetic coefficient of variation CV_A (Houle, 1992) is a measure of additive genetic variability normalised by the trait mean, and was estimated pooling across populations by the following:-

$$\mathrm{CV}_A = \frac{\sqrt{V_A}}{\mu_{Trait}} \times 100 \tag{5}$$

where μ_{Trait} is the mean of the given trait. Note that CV_A was not estimated for logit transformed LDMC, as CV estimates are not appropriate for variables which contain both positive and negative values.

As five observations were collected for each plant, estimates of repeatability were produced for each of the traits analysed as the proportion of total variance accounted for by differences between individuals (Sokal and Rohlf, 1995; Nakagawa and Schielzeth, 2010).

Interpopulation Differentiation

Interpopulation differences were evaluated by comparison of model pairs by AICc for each trait. A model with the same form as equation 2, but with Population as a fixed rather than random effect, was compared to a null model which did not include a Population effect.

Environmental Covariation

To investigate the explanatory power of environmental parameters, trait values were modelled against different combinations of covariates (altitude was excluded due to the high degree of correlation with both longitude and temperature). The meteorological data were obtained from a UK Met Office set of monthly data spanning the years 1961 – 1990 (Perry and Hollis, 2005), at 5 km grid square resolution. A global model was first specified for each of the traits examined:-

$$Trait = \mu + Latitude + Longitude + Temperature + Rainfall + Population \\ + Family + Individual + Block + \varepsilon \qquad 6$$

whereby environmental parameters were fixed effects, and the remaining were random. Model subsets were derived from the global model to describe all possible combinations of fixed effects, with a restriction that each model could contain a maximum of two covariates, and the best models were determined by AICc.

Results

Pooled Genetic Components

Narrow-sense heritability (h^2) estimates ranged from 0.15 to 1.30 (Table 4) across traits, although SEs were large. h^2 estimates were significant for TS Area, length, and resin canal density in the respect that 95 % CIs for these traits (one-tailed) did not contain zero. SLA and LDMC were subject to large block variances: when the block component was excluded from Vp, h^2 estimates for these traits rose to 0.43 (± 0.25) and 0.48 (± 0.26), respectively. CV_A ranged between 6.91 – 25.17: the lowest value was for functional leaf trait SLA, and the highest was for resin canal density.

Repeatability estimates ranged from 0.31 to 0.70 (Table 4). Resin canal and stomatal row density exhibited the lowest repeatabilities (0.31 and 0.36), and needle length the highest. As with h^2, repeatability was substantially greater for SLA and LDMC with block excluded from the total variance at 0.49 and 0.74, respectively.

Interpopulation Differentiation

Generally, little variation could be explained by interpopulation differences, and only for needle length was there adequate support for the inclusion of population as a fixed effect (Table 5). Family variances were typically estimated to be greater than population variances with the exception of needle length, and the family component for resin canal density was particularly large (21.73%, Table 4). For all traits the major proportion of the variance was attributable to differences between individuals within families (31.19 – 69.75%). The proportion of total variance attributable to between-block differences (Table 4) was generally low (typically < 3%), with the notable exceptions of SLA and LDMC (17.95 and 37.27%). A complete table of population means and standard errors is available in the supplementary.

Needle length ranged from 14.11 – 54.91 mm, with an overall mean of 30.95 and a standard error of ± 1.75 mm; the longest needles on average were from Rothiemurchus at 37.42 (± 1.68) mm, followed by Glen Affric at 34.82 (± 2.96) mm, and the shortest were from Beinn Eighe at 29.66 (± 1.16) mm (Figure 3). The Rothiemurchus population exhibited the greatest deviance from the mean needle length, and evidence of a population effect was lost when it was excluded from the analysis. Mass (not included in analyses above) was highly correlated with length (Table 3), and individual needle dry masses ranged from 1.53 – 25.03 mg, the heaviest being around ×16 that of the lightest; average dry mass was 7.98 (± 0.41) mg.

Some evidence was found for a population effect for stomatal row density, however it was very weak (ΔAICc = 0.31). Individuals originating from the westernmost and easternmost populations in the study (Beinn Eighe and Glen Tanar) exhibited the greatest and lowest stomatal row density at 4.65 (± 0.07) and 4.21 (± 0.15) rows per mm, respectively.

There was no evidence for a fixed effect of population for resin canal density, which ranged from 3.0 – 12.7 per mm^2, and averaged at 3.78 (± 0.26). Similarly, no evidence was found for interpopulation differentiation with regard to the functional trait SLA, which averaged 14.18 (±0.43) mm^{2}mg^{-1}, nor for LDMC which was around ~46 % on average.

Environmental Covariation

Evidence was found for a positive relationship between resin canal density and longitude (β_{long} = 0.45 (± 0.18), ΔAICc = 4.23, Figure 4a), indicating that on average eastern populations possessed a larger number of canals per cross-sectional area than western. In contrast, average stomatal row density was found to decrease with longitude (β_{long} = -0.115 (± 0.05), ΔAICc = 2.55, Figure 4b). Model testing tables for these traits are available in the supplementary.

Discussion

In this study, genetic variation for needle traits in native Scots pine grown under common garden conditions in Scotland was assessed within and among populations and in relation to environmental variation at sites of origin. The total sample size was constrained in part by the time-consuming nature of the measurements, but nevertheless population differences and environmental trends were identified. Among populations we found evidence of genetic differences for needle length and the number of stomatal rows. Both stomatal row numbers and resin canal density exhibited a relationship with longitude, the former being in the opposite direction expected. Additionally, significant heritable variation was detected for several traits, indicating that they should be responsive to selection.

In Scots pine, needle length and stomatal row number have been reported to show sizeable variation between and within sites *in situ* (Bobowicz and Korczyk, 1994; Urbaniak et al., 2003; Androsiuk and Urbaniak, 2006), and therefore make good candidate traits for studies of local adaptation in a common garden environment. In our trial needle lengths were found to vary in relation to sites of origin, and this effect was due primarily to the larger, heavier needles possessed by individuals from Rothiemurchus which lies within the Cairngorm Mountain range. Previous studies have identified negative relationships between needle length and altitude *in situ*, for *P. sylvestris* growing in Scotland, and *P. roxburghii* in the Indian Himalayas (James et al. 1994, Tiwari et al., 2013). We did not find evidence of this effect at the population level, as Rothiemurchus is among the sites with the greatest altitude and yet exhibited the largest needles: high altitude populations may therefore not possess a genetic predisposition to shorter needle sizes. An obvious caveat is that different phenotypes may be produced in the common garden than on native sites, and so it would be of benefit to determine whether the same pattern is observed in the needle lengths of wild trees. Steven and Carlisle (1959) described variation in needle length among Scottish pinewoods measured at native sites which could not be explained in relation to any specific topocline. Similarly, as we were unable to explain this variation satisfactorily in terms of the environmental parameters considered, the drivers remain unclear.

Control of plant gas exchange and water loss is regulated primarily via stomatal pores: in Scots pine, the stomata are arranged in longitudinal rows on both the flat upper (adaxial) and curved lower (abaxial) needle surfaces. We measured the density of stomatal rows as a proxy for the stomatal density typically reported for broadleaf species; it is worth noting that as the number of rows present varies throughout the length of the needle, so too does the stomatal density (our observations were collected consistently from the needle midpoint). A relationship was identified between stomatal row density and longitude, decreasing on average from west to east. The density of stomata on leaf surfaces *in situ* has been attributed to differences in moisture availability (Carpenter and Smith, 1975; Hogan et al., 1994; Brewer and Nuñez, 2007), and Hultine and Marshall (2000) have previously reported a decrease in stomatal density in needles of Scots pine with increasing altitude. Conversely, Tiwari et al., 2013 reported that stomatal density increased with altitude, however, across their study site rainfall and humidity also increased substantially with altitude. As with the other traits measured, within-population variation for stomatal row density was large, and the predictive power of the model was very limited. Contrary to our expectation, resin canal density was observed to increase with longitude, being marginally lower in the west where pathogen prevalence is thought to be higher. Though not well understood, pine resin may play a role in water regulation (Farrell et al., 1991), and a higher canal density may be desirable in areas of lower moisture availability if water loss can be reduced. Despite inclusion of meteorological data, longitude was preferred as a covariate over precipitation or temperature. The climate data used in the study was data was at 5×5 km resolution, and interpolated as monitoring stations do not exist in all grid squares. In Scotland, longitude is broadly representative of a number of axes of variation including climate and soil type. It seems plausible that the phenotypic trends observed correspond to decreases in moisture availability in conjunction with increasing altitude; however, longitude is the more powerful predictor either because of the greater accuracy afforded in its measurement, or potentially closely related to relevant environmental variation than site means of temperature and rainfall.

Although longitudinal trends were observed for stomatal row density and resin canal density, population differentiation was not sufficiently strong in either case to provide support for inclusion of a fixed population effect. An earlier study conducted on the same trial material identified interpopulation differences in photochemical capacity during a particularly harsh winter, whereby the largest reduction in capacity was observed for the low altitude western population Benn Eighe (Salmela et al., 2011). During the same study, a relationship was observed between spring needle flush and site altitude, although variation was greater within than between populations. Our own result was similar in that trends could be detected, even when interpopulation effects could not. This once again emphasises the large variability present within populations, as the majority of trait variance was accounted for between families, and individuals within families. Interfamily variance was particularly high for resin canal density, producing a large and significant h^2. Narrow-sense heritability represents the proportion of phenotypic variance attributable to genetic differences between individuals, and the potential for a given trait to respond to selection. A large h^2 may be indicative of fluctuating selection for fewer or greater numbers of resin canals over time (Bell, 2010), or of variable selection across small spatial scales; however, our trial size did not permit h^2 to be estimated with a high degree of accuracy.

As the extent of within-plant variation was unknown, five needle pairs were collected per individual. The repeatability of within-plant measurements varied among traits: needle length was highly repeatable (i.e. needles from the same individual tended to be highly similar), whereas resin canal and stomatal row density we substantially lower. Low repeatability may be a consequence of genuine variability for these traits, particularly as resin canal and stomatal row number vary throughout the length a needle; it is also possible that these traits are subject to a higher degree of measurement error. For the purpose of future studies, it is worth noting that the number of observations required to characterise individual variation is dependent on the trait in question.

SLA and LDMC are leaf functional traits, which are increasingly used to generalise the `resource-use' strategies of plant species based on the premise that plant species exhibit a common suite of adaptations to environmental stresses (Grime, 1977; Chapin, 1991; Reich et al., 2003). SLA values have been observed to decrease with rainfall and soil nutrient gradients *in situ* for a variety of species, being predominantly lower when availability to either becomes limited (Givnish, 1987; Cunningham et al., 1999; Warren et al., 2005). LDMC reflects leaf tissue density, and higher values indicate a larger proportion of structural compounds. Neither trait was differentiated among populations, nor showed covariation with the environmental parameters considered. They did, however, exhibit very large block variation in contrast to the morphometric traits. SLA has been reported to depend on canopy position and light levels in both broadleaf and conifer species (Lewandowska and Jarvis, 1977; Bond-Lamberty et al., 2002; Lombardini et al., 2009), and the block effect may have been caused by partial shading of the trial from a nearby tree. The exclusion of block variance from total phenotypic variance (Vp), resulted in substantially greater estimates of h^2. Because of the sensitivity of these traits to environmental variation, researchers may wish to exercise additional caution drawing inferences from these traits when measured in the field.

We may have anticipated that populations from the Cairngorm Mountains would exhibit similar phenotypes, as they occupy seemingly comparable environments, and should more readily share genes on account of their geographic proximity. However, the results suggest that this is an unwarranted assumption, as needles of Rothiemurchus origin were differentiated in size from those of the other Cairngorm sites (Ballochbuie, and Glen Tanar, located ~33 and 56km away respectively), which were more similar to the remainder of the distribution. Rothiemurchus has the lowest elevation of the Cairngorm sites, and it is possible that the selective pressures related to altitude increase more rapidly approaching the tree line. Given the short distances between these sites, this result implies that local adaptation is possible over small spatial scales in Scots pine, despite its capacity for long-distance gene flow. As our study was conducted at a single common garden, the phenotypes observed may differ from those produced on native sites. It would be of interest to determine whether the same phenotypic patterns can be observed in the wild. Additionally, as the evidence suggested that neighbouring populations exhibited genetic differences with respect to morphology, future work should entail evaluation of the scope for differential adaptation at the local scale.

Conclusion

The Caledonian Pine Forest remnants represent a valuable study system for local adaptation, as they are remote from the main species distribution, and occupy a compact geographical area which exhibits marked environmental heterogeneity. In this study we examined morphological variation between saplings from native Scots pine stands grown under common garden conditions in order to evaluate evidence for local adaptation. The main findings were that populations differed in needle length, and that stomatal density and resin canal density exhibit a relationship with longitude in a manner that suggests adaptation to moisture availability and/or altitude at sites of origin. Variation within populations was high, and between-family variance was particularly high for resin canal density, resulting in a large h^2 estimate.

Functional leaf traits SLA, and LDMC, which are often used to characterise plant resource-use strategies (e.g. Reich et al., 1992; Wilson et al., 1999), were found to be highly sensitive to microenvironmental variation, and care should therefore be taken when drawing inferences from measurements of these traits, particularly when measured outwith a controlled environment.

Despite the relatively small geographic area and the highly outcrossing nature of Scots pine, our results demonstrate that genetic differentiation has occurred for needle traits in Scotland, and that this is possible over short distances. As previously little to no spatial genetic structure has been identified among native pinewoods (Provan et al., 1998, Wachowiak et al., 2011, 2013), it is plausible that the differences and trends observed have an adaptive basis, and it would be of interest to investigate whether the same patterns can be observed *in situ*.

Conflict of interest statement

None declared.

Data Archiving Statement

The needle morphological data will be deposited in the Environmental Information Data Centre (EIDC) upon acceptance.

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Table and Figure captions

Figure 1 Map of native populations sampled for use in the study, including site of common garden trial. Refer to table 1 for population key.

Figure 2 Schematics of a) an intact pine needle (stomatal rows are present on both sides), and b) a transverse cross-section. *Pinus sylvestris* needles are typically curved to some extent, and are often twisted; they possess a minimum of two resin canals, but commonly have more distributed around the needle perimeter. Scale bars based upon average values.

Figure 3 Barplots of needle length by population. Error bars represent ± 1 SE, and were estimated by modelling each population separately using REML. Refer to Table 1 for population key.

Figure 4 Positive and negative relationships between a) resin canal density ($\beta = 0.45$ (± 0.18), $\Delta AICc = 2.55$) and b) stomatal row density ($\beta = -0.12$ (± 0.05), $\Delta AICc = 2.55$) with longitude. Coefficients were estimated via REML; error bars represent ± 1 SE, and were estimated for each population individually. Refer to table 1 for population key.

Table 1 Coordinates of trial populations, the range of altitudes at which the mother trees were sampled, alongside mean annual temperature and rainfall taken from Met Office estimates for each site.

Table 2 Pearson’s correlation coefficients between site averaged values (n = 8) for longitude, latitude, altitude (m.a.s.l.), mean monthly temperature (°C), and mean monthly rainfall (mm).

Table 3 Pearson's correlation coefficients among needle traits recorded (n = 933). TS Area, Width, and Depth are parameters derived for transverse cross-sections, and VB Area represents the area of the vascular bundle within a transverse cross-section.

Table 4 Narrow-sense heritabilities (h^2) with their associated standard errors, the coefficient of genetic variation (CV_A), the proportions of variance attributable to population, family within population, individual within family, and block effects, and repeatability (individual variance as a proportion of the total). CV_A was not estimated for logit transformed LDMC, as negative and positive values were present following logit transformation.

Table 5 Comparisons of models with and without population as a fixed effect by AICc. ΔAICc values represent the difference in AICc between the null model (i.e. no population effect) and the best (a value of zero indicates that the null model was the best), and Akaike Weight represents the probability of that model being the best of the two considered. Inclusion of population as a fixed effect necessitated an additional 7 degrees of freedom.

Table 1

Population	Latitude	Longitude	Altitudinal Range (m.a.s.l.)	Mean Annual Temp (°C)	Mean Annual Rainfall (mm)
Ballochbuie (BB)	56.99	-3.30	421-524	6.44	958
Beinn Eighe (BE)	57.63	-5.35	17-91	8.53	2202
Black Wood (BW)	56.67	-4.32	250-307	7.06	1159
Coille Coire Chuilc (CCC)	56.41	-4.71	222-298	6.46	3091
Glen Affric (GA)	57.27	−4.92	205–274	7.02	1837
Glen Loy (GL)	56.91	−5.13	136–197	7.96	1824
Glen Tanar (GT)	57.05	−2.86	293–422	7.40	850
Rothiemurchus (RM)	57.15	−3.77	306–329	6.82	811
Common Garden	55.86	−3.21	190	7.08	932

Table 2

	Latitude	Altitude	Temperature	Rainfall
Longitude	-0.16	0.86	-0.50	-0.74
Latitude		-0.35	0.62	-0.21
Altitude			-0.81	-0.59
Temperature				0.14

Table 3

	VB Area	Width	Depth	SLA	Length	Canal Density	S Row Density	Fresh Mass	Dry Mass	LDMC
TS Area	0.91	0.94	0.91	-0.56	0.54	-0.14	0.18	0.78	0.78	0.09
VB Area		0.85	0.83	-0.54	0.56	-0.09	0.23	0.76	0.76	0.06
Width			0.78	-0.61	0.58	-0.18	0.19	0.77	0.77	0.08
Depth				-0.48	0.44	-0.13	0.20	0.68	0.70	0.15
SLA					-0.41	0.06	-0.33	-0.62	-0.72	-0.58
Length						-0.07	0.18	0.90	0.85	-0.13
Canal Density							0.02	-0.07	-0.06	0.02
S Row Density								0.26	0.27	0.07
Fresh Mass									0.97	-0.03
Dry Mass										0.19

Table 4

Trait	h^2 (SE)	CV_A	Var Pop (%)	Var Fam (%)	Var Ind (%)	Var Block (%)	Repeatability
TS Area	0.68 (0.29)	11.38	1.08	7.42	55.90	1.79	0.56
SLA	0.30 (0.24)	6.91	1.27	4.39	39.96	17.95	0.40
Length	0.88 (0.30)	11.10	6.28	5.26	69.75	2.99	0.70
Canal Density	1.30 (0.33)	25.17	2.01	21.73	31.19	0.00	0.31
Stom Row Dens	0.22 (0.22)	10.23	4.99	5.13	37.90	1.84	0.36
logit LDMC	0.15 (0.21)	-	0.00	1.95	46.66	37.27	0.47

Table 5

Trait	Intercept (SE)	Population as Fixed Effect	df	ΔAICc	Akaike Weight
TS Area (mm^2)	0.62 (0.01)	-	5	0.00	0.90
SLA (mm^{2}mg^{-1})	14.18 (0.43)	-	5	0.00	0.86
Length (mm)	30.95 (1.75)	+	12	2.55	0.78
Canal Density (per mm^2)	3.78 (0.26)	-	5	0.00	0.89
Stom Row Dens (per mm)	4.39 (0.11)	+	12	0.31	0.54
logit LDMC	−0.14 (0.04)	-	5	0.00	0.99

Figure 1


70°
60°
50°
40°
-10°
0°
10°
20°
Beinn Eighe
Glen Affric
Rothiemurchus
Glen Tanar
Glen Loy
Ballochbuie
Black Wood
Coille Coire Chuilc
Common Garden

Figure 2

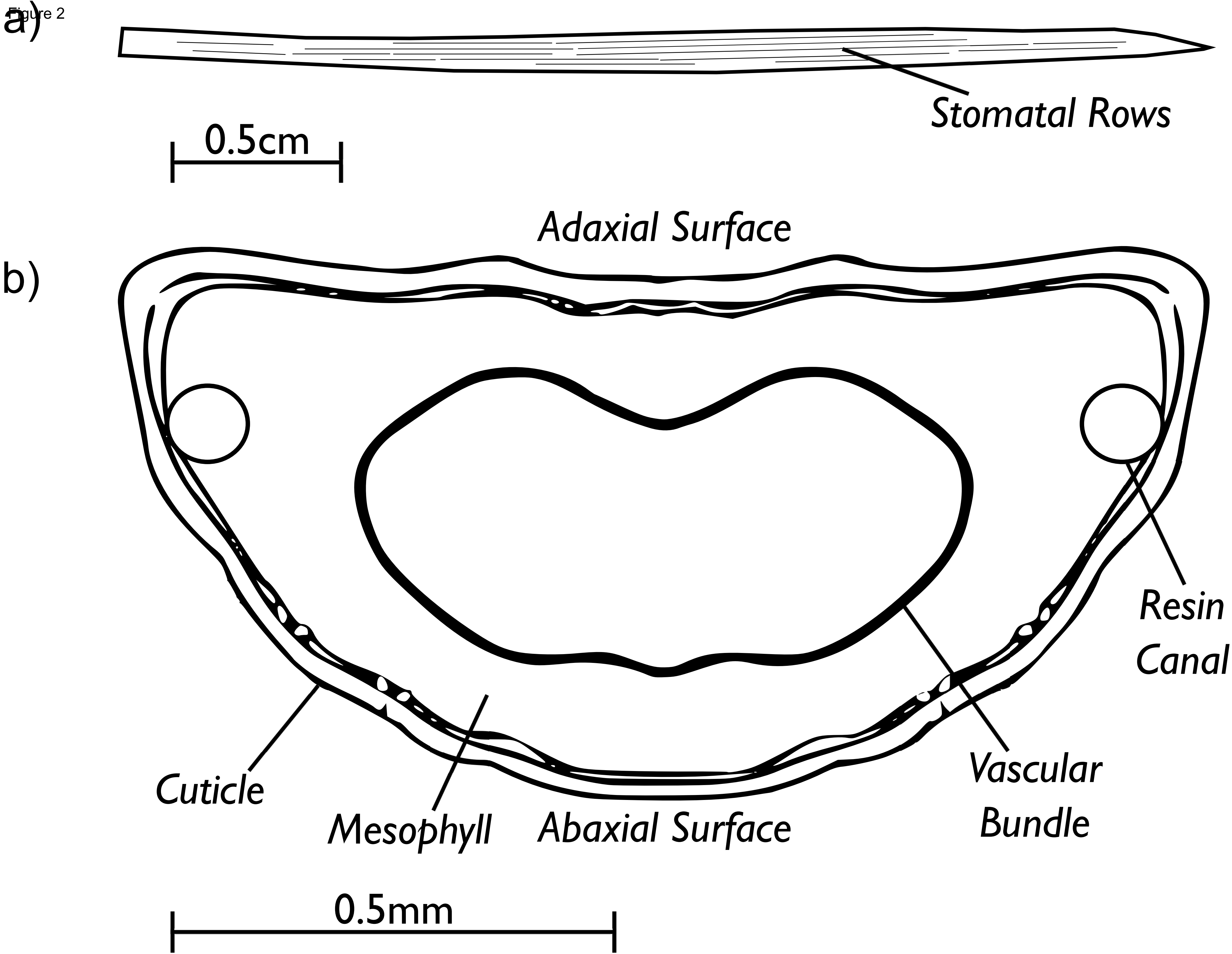

a)
Stomatal Rows
0.5cm
Adaxial Surface
b)
Resin Canal
Vascular Bundle
Cuticle
Mesophyll
Abaxial Surface
0.5mm

Figure 3

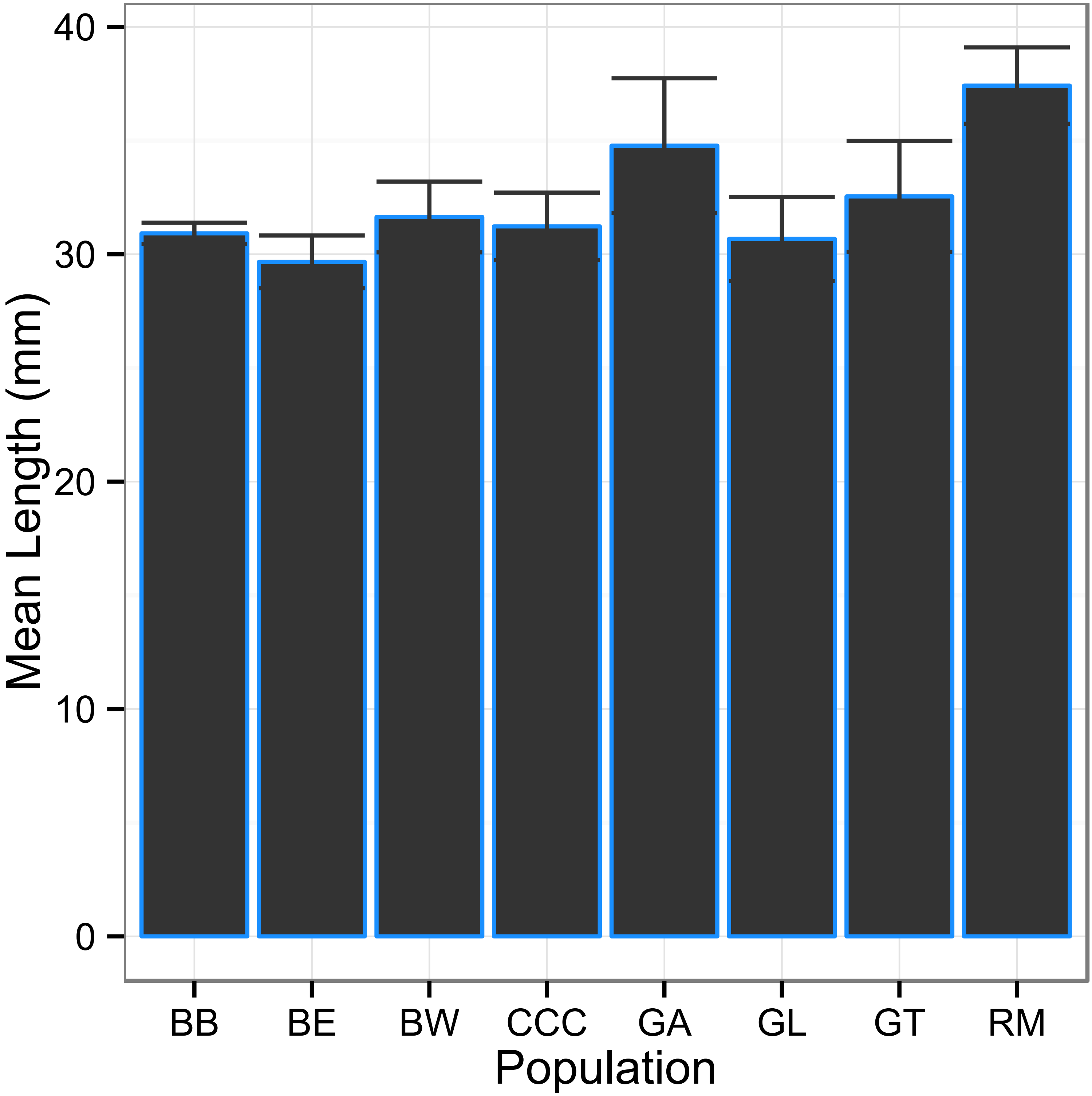
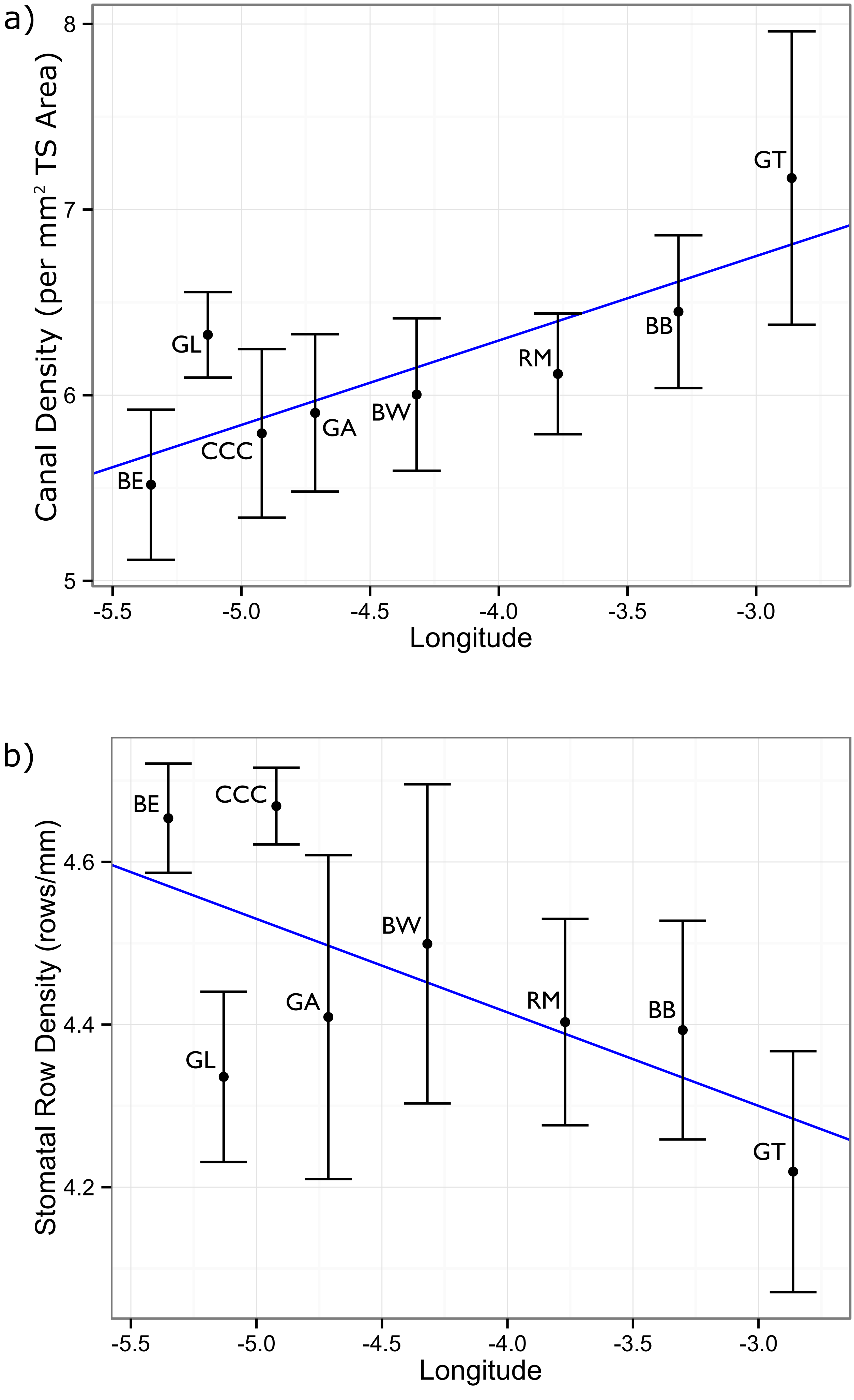


Figure 4



a)
Canal Density (per mm^2 TS Area)
8
7
6
5
GT
BB
RM
BW
GA
CCC
GL
BE
-5.5
-5.0
-4.5
-4.0
-3.5
-3.0
Longitude
b)
Stomatal Row Density (rows/mm)
4.6
4.4
4.2
BE
CCC
BW
GA
GL
RM
BB
GT
-5.5
-5.0
-4.5
-4.0
-3.5
-3.0
Longitude