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OLIVER BRENDEL*[1], LINDA HANDLEY* and HOWARD GRIFFITHS**
* Scottish Crop Research Institute (SCRI), Invergowrie, Dundee DD2 5DA, Scotland, UK
** Department of Plant Sciences, University of Cambridge, Downing Street Cambridge CB2 3EA, England, UK
[1]Corresponding authors present address: UMR INRA-UHP Ecologie et Ecophysiologie Forestière, INRA Centre de Nancy, 54280 Champenoux, France; Tel.: +33.383.39.40.41 ; Fax : +33.383.39.40.69 ; e-mail: brendel@nancy.inra.fr
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Summary

New data suggest that variations for $\delta^{13}C$ and ring width among natural populations of Scottish Scots pine (*Pinus sylvestris* L.) may be due to the persistence of palaeotypes, of various post–glacial, migratory origins. We assessed differences in wood cellulose carbon isotope composition ($\delta^{13}C$) and ring width between Scottish Scots pine populations grown in a clone bank or in natural populations. Ring width and $\delta^{13}C$ were significantly different among natural populations. Potential water deficit positively correlated with $\delta^{13}C$ or ring width in the natural populations. $\delta^{13}C$ and ring width of clone bank trees did not correlate with any climate variables at their sites-of-origin. The absence of correlation suggests little adaptation to climate for these two traits. Ring width showed site–times–population interaction for the two types of sites (natural populations and clone bank), but $\delta^{13}C$ did not. These results suggest, that there may be insufficient climate variation within Scotland to cause significant genetic differences in $\delta^{13}C$ or ring width at the population level.

Words 164

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Introduction

Postglacial history suggests two, and possibly three, sources for modern Scottish Scots Pine (*Pinus sylvestris* L.) populations. Following Pleistocene glaciations (1,800,000 to 10,000 before present [BP]; Steven and Carlisle 1959), Scots pine migrated within Britain to Scotland from southern England (before 9000 BP, Bennett 1984) and from south-western Ireland (before 8,500 BP, Birks 1989). The pines migrating from England probably had a different, non–British origin than did migrants from Ireland. Between 8,500 BP and 8,000 BP a third group of pines emerged in Northwest Scotland, in the West Wester Ross area around Loch Maree and north of the Cairngorm Mountains (Bennett 1984). It is unclear whether this population arrived *via* one of the British routes or *via* longer distance spread of seeds or originated from a Scottish pre-glacial refugium (Bennett 1984, Birks 1989).

Through the founder effects of these palaeo-migrations, different "palaeotypes" established in Scotland. A palaeotype is defined here as a range of genotypes characteristic of a given population and resulting from a unique post-glacial, migratory origin. Strong morphological variations (Steven and Carlisle 1959) and monoterpene and isoenzyme analyses (Forrest 1980, Kinloch et al. 1986) suggested considerable genetic variability for Scottish Scots pine. In particular, the West Wester Ross Scots pines are markedly different from other populations in Scotland (Forrest 1982).

Scots pine was established in Britain as a dominant tree before 4000 BP (Bennett 1984). Changed climate and anthropogenic influences have reduced Scots pine woodlands to about 80 large stands, totalling less than 11,000 ha, plus smaller remnant populations of varying sizes, which are scattered throughout Scotland (British

Forestry Commission, pers. comm.). Conservation policies for the remnant populations are based on the classification of Scots pine populations into seven Biochemical Zones. The Zones were erected on the basis of monoterpene and isoenzyme contents (Figure 1; based on Forrest 1980, Forrest 1982 and Kinloch et al. 1986). Monoterpenes and isoenzymes are thought to be neutral markers indicating genetic differences among plants. We deemed it useful to refine this classification by adding new traits to the demographic analysis.

We studied the two functional traits, carbon isotope composition ($\delta^{13}C$) and ring width. These traits reflect genetic variation and are sensitive to environmental conditions. At the leaf level, the $\delta^{13}C$ of plant material is determined by the ratio of stomatal conductance to the CO_2 assimilation rate (Farquhar et al. 1982). Therefore, the main environmental factors affecting within-species variations for $\delta^{13}C$ are those which relate to plant water availability and atmospheric water demand, e.g. soil moisture, air humidity and temperature (Francey and Farquhar et al. 1982, Yakir et al. 1994) and irradiation (Brendel 2001). Consequently, among–population differences of $\delta^{13}C$ can result from: 1) acclimation to environmental conditions at the time of carbon fixation giving rise to phenotypic plasticity, 2) adaptation to local climate and environmental conditions yielding ecotypes and 3) historical differences in genetic background, assumed here to represent palaeotypes. When plants have a common environmental history, the remaining variation of plant $\delta^{13}C$ reflects largely genetic differences. The genetic basis of variation for $\delta^{13}C$ has been demonstrated in a number of woody plant species (e.g. Meinzer et al.1990, Meinzer et al.1992, Zhang et al. 1993, Zhang and Marshall 1994, Zhang and Cregg 1996, Fan et al. 1999, Pennington et al. 1999). Therefore, in a combined approach of natural and clone bank populations

it should be possible to partition palaeotype from ecotype and/or phenotypic plasticity. Our specific objectives were 1) To investigate differences in $\delta^{13}C$ and ring width among natural populations with respect to climate, 2) To investigate adaptation to environmental conditions (ecotypes) by comparing the $\delta^{13}C$ of clone bank material with climate conditions at the sites-of-origin, 3) To explore the geographical groupings of populations on the basis of $\delta^{13}C$ and ring width data and 4) To investigate the extent of genetic variation *versus* phenotypic plasticity for $\delta^{13}C$ and ring width for Scottish Scots pine populations by comparing natural populations with clone bank ones.

Material and Methods

Sites

The clone bank is located at the Newton Nursery of the Forestry Commission Research Division, Elgin, Scotland, UK (3°20'W 57°39'N; Figure 1). The weather conditions are relatively warm in summer (1400 day-°C) as well as in winter (35 day-°C frost) compared with the conditions of the other sites sampled (Table 1). Shoots from mother trees at the sites-of-origin were grafted onto root stocks in 1957, and the in1997 sampled adult trees were 40 years old. All rootstocks originated from one English provenance. We are conscious that using grafted material adds variation introduced by rootstock variability. However it was shown in a rootstock-times-scion crossing experiment for growth traits of *Pinus taeda* L. (Jayawickrama *et al.* 1997), that rootstock accounted for only a minor part of the observed variation, whereas the major part was attributable to scion variation. Originally several ramets of the same clone were planted. Due to regular thinning, the adult trees are now regularly spaced (about 200 stems/ha).

In the clone bank, trunk cores were taken from 89 trees from 16 sites-of-origin, which were derived from all Biochemical Zones in Scotland (Figure 1: based on Forrest 1980, Forrest 1982 and Kinloch et al. 1986) except the 'North' zone. The number of trees sampled for this study per site-of-origin (2 to 10) was proportional to the population size at the respective site–of–origin, to yield a good coverage of Scotland without giving disproportionate emphasis to smaller populations. Because the British National Grid map reference (Ordnance Survey Maps, Landranger Series, 1988) of each mother tree was known, the trees in the clone bank can be related to their original environments (Figure 1), however it was not possible to locate the

mother-tree itself. The parent populations covered an area from Loch Maree in the west (NG 898; 5°22'W) to Crathes in the East (NO 743; 3°00'W) and Blackmount in the south (NN 331; 56°30'N) to Amat in the North (NC 889; 57°45'N). This covers an area of roughly 160 km north-south by 180 km east-west. Using climate maps (Birse and Dry 1970, Birse and Robertson 1970), site-of-origin environmental variables (Table 1) were determined for the British National Grid reference of each mother tree. Altitude in [m] above mean sea level was taken for each mother tree from Ordnance Survey maps, Landranger Series, Scale 1:50.000 (1988).

Trunk cores from 150 trees were taken from five natural Scots pine populations in Scotland (Figure 1; Table 1): 25 trees from Glenmore (GM; Eastcentral Biochemical zone), Glen Einig (GE; North), Ben Eighe (BE; Northwest) and Loch Clair (LC; Northwest), and 50 trees from Rannoch (RA; Southcentral). All Scots pines sampled were trees from areas classified by the Forestry Commission as native Scots pine. Only trees from the oldest age classes were sampled. The available environmental variables were significantly different for most sites (Table 1).

Although we were unable to relocate the mother trees for the clone bank genotypes, four of the five wild populations could be compared with representatives of these same populations in the clone bank (Ben Eighe = Loch Maree ; Loch Claire = Coulin; Rannoch; Glenmore = Rothiemurchus). The same climate maps as for the clone bank site-of-origins were used for environmental data.

Sampling and analyses

Main stems of the clone bank and natural population trees were sampled with a 5-mm diameter incremental ring borer at breast-height, and the resulting samples

were analysed for ring width and $\delta^{13}C$ of extracted cellulose. To account for anticipated within-ring circumferential variation of $\delta^{13}C$ (e.g. Mazany et al. 1980, Stuiver et al. 1984, Nguyen-Queyrens et al. 1998), samples from three cores taken from each tree (one each from the south, northeast and northwest compass points) were combined after ring width measurements prior to processing for $\delta^{13}C$ analysis.

Ring width was measured for all sampled cores using an analytical imaging system (MILLIPORE, Bio Image System) calibrated for ring width measurements (Brendel 1998) with an estimated resolution of 0.1 mm. For $\delta^{13}C$ analysis, the sampled wood was ground to a fine powder in a freezer mill (Glen Creston model 6700), and a 50 mg (or less) sub-sample of the wood powder was used to isolate cellulose using an acetic acid / nitric acid method (Brendel et al. 2000). $\delta^{13}C$ measurements were done on main stem cellulose extracts to avoid the short-term integration properties of needle $\delta^{13}C$ (Brendel 2001). The carbon isotope composition of the extracted cellulose was measured with a precision of ±0.15‰ standard deviations using a Europa Scientific Tracermass CF-IRMS (continuous-flow isotope ratio mass spectrometer; Handley et al. 1993).

For samples from the clone bank and the natural populations, the width of each growth ring was averaged for three cores. Due to strong growth at the clone bank, it was possible to analyse two periods for $\delta^{13}C$, representing annual growth from 1991 to 1994 (4 years) and from 1995 to 1996 (2 years). The samples taken from the natural populations did not yield enough material for $\delta^{13}C$ analysis to measure two periods separately. Therefore $\delta^{13}C$ was measured for the whole period from 1991 to 1996 and mean ring widths (MRW) were calculated for this period. To compare the clone bank samples with those from the natural populations, the ring-width-weighted mean of

$\delta^{13}C$ of the Period 1995 to 1996 ($\delta^{13}C_1$) and $\delta^{13}C$ of the Period 1991 to 1994 ($\delta^{13}C_2$) were calculated to yield an overall MRW $\delta^{13}C$ for the six rings.

The two periods analysed for the clone bank were used to test the temporal consistency of $\delta^{13}C$ and ring width classifications. To use functional traits to distinguish populations where historical differences are suspected, it is necessary that these functional traits yield a consistent classification of trees or populations over time. Linear Correlation analyses (Sokal and Rohlf 1995) were used to compare $\delta^{13}C$ and MRW for the two sampled periods. The $\delta^{13}C$ of Period 2 and Period 1 for each tree were highly correlated ($R^2 = 0.86$; $P < 0.005$; p1 = -0.84 + 0.98 * p2), as were the MRWs of the two Periods ($R^2 = 0.82$; $p<0.005$; p1 = 0.10 + 1.11 * p2) and the correlations for both variables had slopes close to one. This suggests a high temporal consistency for the two measured traits in terms of the consistency of differences in growth and $\delta^{13}C$ among individual trees in the clone bank and an effective smoothing of annual environmental variation by the pooling of rings. Similar temporal consistencies were found for the $\delta^{13}C$ between juvenile and adult pools of rings in a *Pinus pinaster* (Ait.) field trial (Nguyen-Queyrens et al. 1998) and for between-needle-generation within a *Picea mariana* (Mill.) half diallel experiment (Johnsen et al. 1999)

Statistical Analysis

Statistical analyses were performed using either the STATISTICA 6 (StatSoft France. 94700 Maisons-Alfort, France) or SAS Ver 8.01 (SAS Institute Inc., Cary, NC, USA) software packages. Correlations between factors were estimated using an analysis of variance (ANOVA) linear regression model. The groupings of populations

were tested using ANOVA linear model. The Tukey's Studentized Range (HSD) Test was used for among group comparisons. Significance levels, if not otherwise mentioned, were $P < 0.05$. Where necessary, linear correlation analyses were programmed after Sokal and Rohlf (1995) using STATISTICA 6 Basic programming language.

Results

Natural populations

MRW and $\delta^{13}C$ were significantly (ANOVA: $P < 0.0001$; Table 2) different among natural populations. Tukey's HSD comparison of means revealed two groups for MRW: a) Glen Einig, Ben Eighe, Loch Claire and Glenmore and b) Rannoch (Table 3). The trees at Rannoch had significantly wider rings than all other populations. For population means of $\delta^{13}C$, post-hoc tests (Tukey) revealed two distinctly different groups (Table 3): a) Loch Claire and Ben Eighe (West Wester Ross group) with significantly more negative $\delta^{13}C$ values and b) Glen Einig, Glenmore and Rannoch with significantly less negative $\delta^{13}C$ values.

Within-population variations for MRW and $\delta^{13}C$ (Table 4) were unrelated to size of sampled area, number of trees sampled, sampling density or each other. There were also no relationships between $\delta^{13}C$ and MRW, neither for each population nor for all trees together ($P > 0.05$; $R^2 < 0.2$; data not shown).

There were no significant ($P > 0.05$) linear regressions between the climate variables, temperature or frost (from Birse and Dry 1970 and Birse and Robertson 1970) and $\delta^{13}C$ or MRW of each tree. Altitude did not correlate significantly with $\delta^{13}C$, however it correlated significantly with MRW ($P < 0.05$; $R^2 = 0.10$). For potential water deficit, the natural populations fell into two well-defined classes (0 mm and 0-to-25 mm potential water deficit). Therefore, ANOVA was a more appropriate statistical method than was linear regression analysis. A third class for potential water deficit would be the excess of precipitation during summer months over potential evapotranspiration by more than 500mm (shown in figure 2). However,

as this class was only descriptive in the climate maps (Birse and Dry 1970 and Birse and Robertson 1970; see Table 1), trees from this environment were classed for the ANOVA consistent with the climate maps as 0.0 mm potential water deficit. ANOVAs for $\delta^{13}C$ and MRW data with potential water deficit as a classed factor were highly significant ($P < 0.0005$). The coefficient of determination for MRW was moderate ($R^2 = 0.24$) and for $\delta^{13}C$ rather low ($R^2 = 0.09$). For the class with higher potential water deficit, the trees had significantly (Tukey-Kramer; $P < 0.05$) less negative $\delta^{13}C$ values and an increased MRW. When trees from the environments with summer excess of precipitation over potential evapotranspiration were separated, they tended to less negative $\delta^{13}C$ values and lower growth compared with trees having higher potential water deficits, consistent with the ANOVA results (Figure 2).

Clone bank

When the clone bank trees were grouped by sites-of-origin, the large variation among individual trees within the clone bank (Table 4) resolved into a range of population means (1.12‰ for $\delta^{13}C$ and 2.97 mm for MRW). There was no correlation between the number of trees sampled for each population and the respective within-population standard deviation of $\delta^{13}C$ or MRW. There were also no relationships between $\delta^{13}C$ and MRW of the clone bank trees, neither for the two periods separately nor for the entire six years (all $R^2 < 0.07$; data not shown). Grouping the trees by sites-of-origin was significant for MRW (ANOVA; $P < 0.05$) and not significant for $\delta^{13}C$ (Table 2).

Yet, on the basis of two or three palaeotypes much larger groupings than those represented by separate populations would be expected. K-means clustering was used as a means to investigate clustering of populations by $\delta^{13}C$ or MRW *versus* the geographic distribution of the populations. The K-means clustering for three clusters was highly significant for $\delta^{13}C$ and MRW (ANOVA; $P < 0.0005$, Table 4). K-means clustering was also highly significant (ANOVA; $P < 0.0005$) for both traits when only populations that were represented by more than three trees ($N > 3$ in Table 4) in the clone bank were included in the statistical analyses. For MRW, cluster one consisted of the populations in a central east-west corridor, cluster two was mainly in the northwest and consisted of the populations at Maree, Coulin, Achnashellach and Ballochbuie in the east. Cluster three was divided into a south part with Blackmount and Rannoch and a north part with Affric, Strathfarrar, Amat and Grant. For $\delta^{13}C$, cluster one corresponded to a central east-west corridor including Garry, Rothiemurchus, Ballochbuie, Rannoch and Tanar, cluster 2 corresponded to populations in the north-west (Maree, Coulin, Achnashellach, Strathfarrar, Affric) and one population in the east (Crathes) and cluster 3 was divided into a north part (Guisachan, Amat, Grant) and a south part (Blackmount). The extent of the three clusters was different for MRW and $\delta^{13}C$, however the general pattern was similar: a north-west cluster with the smallest MRW and average $\delta^{13}C$ values, a central east-west corridor with average growth and the least negative $\delta^{13}C$ values and a cluster that is split into a north and south part with the highest MRW and the most negative $\delta^{13}C$ values (Table 4, Figure 4). Some populations were consistently part of these general clusters: in the central corridor (cluster one) Garry, Rothiemurchus and Tanar, in the north-east (cluster two) Maree, Coulin and Achnashellach, and in cluster three, in the

north Amat and Grant and in the cluster three south Blackmount. The combination of the clusters results in cluster one with the least negative $\delta^{13}C$ values and average MRW, in cluster two with medium $\delta^{13}C$ values and the least MRW and in cluster three with the most negative $\delta^{13}C$ and the highest MRW.

The grouping into Biochemical Zones was not significant (ANOVA; $P > 0.05$) for $\delta^{13}C$. For MRW the grouping into Biochemical Zones was significant at $P = 0.04$, however, a post-hoc test (LSD planned comparisons) showed that this significance was chiefly caused by the strong influence of the NW group (West Wester Ross), comprising Maree and Coulin (Figure 1). Without this group an ANOVA for MRW and Biochemical Zones was not significant ($P > 0.05$).

For sites-of-origin of the clone bank trees, an analysis of variance showed the environmental variables to be significantly different among populations. Linear regressions between singletree data of $\delta^{13}C$ or growth and the available environmental variables (Table 1), including altitude, were not significant at a 5% level ($P = 0.05$).

Comparison of natural populations and clone bank populations

Rankings for most-to-least negative means of $\delta^{13}C$ of the comparable populations between clone bank and natural populations were for the clone bank trees: LC > BE > GM > RA (Table 2) and for trees of the natural populations: BE > LC > RA > GM (Table 2). The linear regression between the population means of $\delta^{13}C$ for the natural populations and for the clone bank is not significant ($P = 0.19$), however the means are close to the bisector (Figure 3a). Population means for MRW from natural and clone bank populations were highly correlated ($R^2 = 0.93$; $P_{slope} < 0.05$; correlation analysis after Sokal and Rohlf (1995); Figure 3b) and MRW ranked

populations in the same order in the clone bank and in the natural populations (Figure 3b). Because four populations were available in both their natural settings and the clone bank, it was possible to test the following model for population-times-environment interaction:

$$\delta^{13}C \text{ (or MRW)} = \mu + \text{population}_i + \text{site}_j + \text{pop*site}_{ij}\ \varepsilon,$$

where $population_i$ are the four comparable populations, $site_j$ is either natural environment or clone bank and $pop*site_{ij}$ is the population times site interaction. The model was highly significant for $\delta^{13}C$ and MRW ($P < 0.0001$; table 5). For $\delta^{13}C$ the population-times-site interaction was not significant ($P > 0.05$) and Tukeys HSD test for differences among populations revealed Loch Clair and Ben Eighe to have significantly more negative $\delta^{13}C$ values than Glenmore and Rannoch (Table 3). For MRW there was a significant population-times-site interaction and the populations were ranked from smallest to widest rings: Loch Claire, Ben Eighe, Glenmore and Rannoch, with only Loch Claire and Rannoch significantly different (Table 3).

Discussion

Differences for $\delta^{13}C$ and MRW found in the natural populations and in relation to climatic variables

Wild-site populations were found to be significantly different for $\delta^{13}C$ and MRW. The two populations from West Wester Ross had significantly more negative $\delta^{13}C$ values than the other three populations at Glenmore, Rannoch and Glen Einig. These three populations had nearly identical means for $\delta^{13}C$ (Table 3), despite significant differences in temperature, potential water deficit and accumulated frost (Table 1). This suggests that the difference in population mean-$\delta^{13}C$ found for the natural populations could not be explained by environmental differences alone. No significantly different MRW's were observed for the West Wester Ross populations. Only the Rannoch population had significantly wider rings than other populations.

The $\delta^{13}C$ and MRW data of the trees from the natural populations co-varied with the potential water deficit of the locations. Trees at locations with higher potential water deficit tended toward less negative $\delta^{13}C$ values and larger MRW. Commonly, for drier conditions a reduction in growth would be expected, compared with less dry conditions (Robertson et al. 1989). However, Grace and Norton (1990) found mainly negative correlation coefficients between ring-width and precipitation for Scottish Scots pine populations at different altitudes for late summer, and also positive correlations in early spring. They interpreted the late summer negative relationship as rainy summers reducing growth, which might also be the cause for the positive relationship between potential water deficit and MRW found in our study. It is, however, also possible that the unusual Scottish climate conditions might cause the positive relationship. Potential water deficit varied from excess precipitation (negative

potential water deficit) to little potential water deficit. Where precipitation is higher than evapotranspiration there is a tendency for create conditions waterlogging and root hypoxia. Such conditions reduce stomatal conductance and assimilation rates (Pezeshki 1993, Wagner and Dreyer 1997, Gravatt and Kirby 1998), hence the production of assimilates and tree growth (Terazawa and Kikuzawa 1994, Wagner and Dreyer 1997). Environments having an excess of water might also be characterized by frequent cloud cover and reduced light irradiation. The effect of light-limited assimilation rate could also reduce growth, consistent with Figure 2.

The observed, positive relationship between potential water deficit and $\delta^{13}C$ is in agreement with the model of carbon isotope discrimination published by, e.g. Farquhar, Ehleringer & Hubick (1989), where a reduction in stomatal conductance due to drier conditions should result in less negative $\delta^{13}C$ values. This was confirmed in several studies (Dupouey *et al.* 1993, Yakir et al 1994, Livingston and Spittlehouse 1996). Moreover, Topa and Cheeseman (1992) suggested that flooding decreases assimilation primarily *via* a reduction in photosynthetic capacity with an indirect subsequent down-regulation of stomatal conductance. This should increase intercellular CO_2 concentration and therefore lead to more negative $\delta^{13}C$ values (Guy and Wample, 1984), in the same direction as the Farquhar model and consistent with Figure 2. Therefore, the results suggest that the variation of $\delta^{13}C$ and MRW related to potential water deficit observed in the natural populations might be due to hypoxic soil conditions rather than to drought. However, despite the co-variation between potential water deficit and $\delta^{13}C$, three natural populations (Glen Einig, Rannoch and Glenmore) with significantly different populations means for potential water deficit (Table 1) had the same population mean-$\delta^{13}C$ (Table 3).

Differences among populations within the clone bank, geographical grouping and relation to climate at the sites-of-origin

Regrouping of populations by K-means clustering resulted in a similar general geographical pattern for $\delta^{13}C$ and MRW. Differences between $\delta^{13}C$ and MRW in the attribution of populations to the different clusters were mainly found for populations close to the borders between clusters. Within border-populations, genotypes from both sides might influence the functional traits. Therefore, especially in a spatially restricted situation such as the Scottish peninsula, a distinct distribution of genotypes might not to be expected, but rather centres of difference with less sharply defined borders.

The variation of functional traits of trees grown in a common environment can be due to microenvironmental variation within the plantation or to genotypic differences among trees. It is likely that the microenvironmental variation within the clone bank lowers the statistical power to detect differences among populations. Therefore the significant effects found might be underestimated. However, the clustering should be due to genetic differences among trees.

When foliar (needle) $\delta^{13}C$ of coniferous clone bank trees has been related to the climates associated with their seed sources (vapour pressure deficit: Zhang and Marshall 1995; precipitation and evapotranspiration: Zhang and Cregg 1996; altitude: Zhang et al.1993, Zhang and Marshall 1994), the results suggested that responses of conifers to climate are specific to taxon and/or climatic situation. MRW and $\delta^{13}C$ of the trees in the clone bank did not reflect the climate variables of their sites-of-origin. The variation of climate conditions within Scotland might not be large enough to

result in an effective selection pressure, resulting in characteristic ecotypes. Therefore either genetic differences among populations are not large enough to be detected with this experimental design, possibly masked by microenvironmental variation within the clone bank, and/or adaptation of trees to local climates in Scotland occurs within phenotypic plasticity.

Comparison between natural populations and clone bank

The same ranking among populations was found for MRW in the natural populations and in the clone bank. Growth, however, was much stronger and differences were larger in the clone bank than in the natural populations. This is also reflected by the population-site-interaction found in the model including the data from both experimental settings. This suggests that the climate conditions at the natural population sites had a generally restricting influence on growth, which did not change the ranking by growth found in the clone bank. For $\delta^{13}C$, a similar clustering was found for the natural populations and for the clone bank. The data points were close to the bisector and the model did not include a significant-population-times site interaction. This suggests that either the differences in environmental conditions at the natural populations were too small to have a detectable influence in the $\delta^{13}C$ values, or the clone bank was situated in an environmental condition that would result in similar $\delta^{13}C$ values as at the natural population sites.

The two significantly different groups found for $\delta^{13}C$ at the natural population sites, and using the model combining natural and clone bank populations, corresponded well to the cluster found for the clone bank alone: Ben Eighe (Loch

Maree) and Coulin (Loch Claire) are in cluster two whereas Glenmore (Rothiemurchus) and Rannoch are in cluster one. For MRW only Loch Clair and Rannoch were significantly different in the natural populations and for the model.

Conclusions

From the natural populations data alone no clear distinction can be made between palaeotypes or ecotypes, because palaeotypes might coincide with differences in climate. However, results from the clone bank, and the comparison between clone bank and natural populations, suggest that an ecotypic differentiation of Scots pines within Scotland may not be strong. However, significant among-population or among-cluster differences were found, which were not entirely explicable by acclimation or adaptation to local environments. Palaeotypes, resulting from different post-glacial migration routes, might provide an explanation for the geographic distribution of functional traits. The West Wester Ross populations, characterized as markedly different (Forrest 1982) were distinguished clearly within the $\delta^{13}C$ natural populations dataset, although not so clearly in the model output for MRW, and they were not separated by the clone bank data set for $\delta^{13}C$ and MRW. A clone bank experiment with seedlings from selected populations based on the k-means cluster found in the present study could clarify the situation.

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Tables

Table 1 Location of experimental sites and environmental variables (from Birse and Dry 1970, Birse and Robertson 1970): accumulated temperature as integrated excess of temperature over 5.6 ºC in [day-ºC]; potential water deficit as excess of potential evaporation over precipitation in [mm]; accumulated frost as integrated deficiency of temperature under 0 ºC in [day-ºC]), altitude (in [m]). Potential water deficit is the sum of the months were a potential water deficit exists. Areas with an excess of precipitation over potential evapotranspiration during summer months are indicated with "0+". The values for the natural populations are means of the environmental data for the individual trees, not significantly different sites (Tukey, $P < 0.05$) are coded with the same lower case letters. For the sites-of-origin of the clone bank trees the range (minimum to maximum) is given for each variable.

	Longitude W	Latitude N	Altitude mean (range)	Tempe-rature	Water deficit	Frost
Glen Einig	4°45'	57°57'	60 d (40)	1226 a	0.0 c	80.0 c
Ben Eighe	5°22'	57°38'	70 d (180)	1146 ab	0^+ c	76.3 c
Loch Clair	5°22'	57°33'	169 c (200)	1051 bc	0^+ c	80.0 c
Rannoch	4°21'	56°40'	282 b (160)	963 cd	12.3 a	170.0 b
Glen-more	3°44	57°09'	376 a (200)	863 d	10.2 b	194.6 a
Clone-bank	3°20'	57°39'	30	1400	75	35
sites-of-origin	5°22' to 3°00'	56°30'to 57°45'	30 to 700 (0 to 400)	413 to 1238	0^+ to 37.5	70 to 350

Table 2: ANOVA results, testing among-population differences for natural populations and clone bank sites-of-origin for a) $\delta^{13}C$ and b) MRW, showing degrees of freedom (*DF*), sum of squares (*SS*), F test (*F*) and level of significance (*P*).

Site	Trait	Source	*DF*	*SS*	*F*	*P*
Natural populations	$\delta^{13}C$	Model	4	15.18	8.18	<0.0001
		Error	140	64.95		
		Total	144	80.13		
	MRW	Model	4	17.55	12.66	<0.0001
		Error	140	48.53		
		Total	144	66.07		
Clone bank	$\delta^{13}C$	Model	15	10.35	1.17	0.32
		Error	73	43.21		
		Total	88	53.56		
	MRW	Model	15	46.80	2.30	0.0097
		Error	73	98.96		
		Total	88	145.76		

Table 3 Within-population means of MRW and $\delta^{13}C$ for natural populations and comparative clone bank trees, including standard deviations and number of trees sampled in brackets. Also shown are the least square means resulting from the ANOVA model including natural populations and clone bank data. Lowercase letters indicate populations that are not significantly different (ANOVA, $P < 0.05$, Tukey-Kremer; for clone bank calculated using only the four comparative populations).

	$\delta^{13}C_{nat.pop.}$	$\delta^{13}C_{clone\ bank}$	$\delta^{13}C_{model}$	$MRW_{nat.pop.}$	$MRW_{clone\ bank}$	MRW_{model}
LC	-26.7±0.6 (25) a	-26.0±0.1 (2) a	-26.4 a	0.7±0.4 (25) a	1.1±0.3 (2) a	0.9 ab
BE	-26.7±0.6 (25) a	-25.8±0.5 (6) a	-26.3 a	1.0±0.6 (25) a	2.3±0.9 (6) a	1.6 abc
GM	-26.0±0.8 (25) b	-25.7±0.9 (5) a	-25.7 b	1.1±0.5 (25) a	3.2±1.2 (5) a	2.13 bcd
RA	-26.0±0.8 (50) b	-25.5±0.8 (7) a	-25.7 b	1.5±0.7 (50) b	3.8±1.5 (7) a	2.68 cd
GE	-26.0±0.6 (25) b	----	----	0.7±0.4 (25) a	----	----

LC: Loch Clair; BE: Ben Eighe; GM: Glenmore; RA: Rannoch, GE: Glen Einig

Table 4: Means and standard deviations (SD) for the clone bank populations, including results of the K-means cluster analysis and the biochemical zones (BZ). The trees in the clone bank yielded for $\delta^{13}C$ a range of 3.7‰ and for MRW a range of 6.0mm. Means for the cluster for $\delta^{13}C$ and MRW were: -25.5‰ / 3.0 mm, -25.9‰ / 1.9 mm and –26.3‰ / 3.8 mm, for cluster one, two and three, respectively. Populations that belong to the same cluster of $\delta^{13}C$ and MRW are in normal, populations that belong to different clusters for $\delta^{13}C$ and MRW are in italics.

	N	$\delta^{13}C$±SD	MRW±SD	Cluster $\delta^{13}C$	Cluster MRW	BZ
Garry	6	-25.2±1.0	2.6±0.7	1	1	SW
Tanar	9	-25.5±0.9	2.9±1.6	1	1	NE
Rothiemurchus	5	-25.7±0.9	3.2±1.2	1	1	EC
Ballochbuie	*3*	*-26.6±0.5*	*2.1±0.5*	*1*	*2*	*NE*
Rannoch	*7*	*-25.5±0.8*	*3.8±1.4*	*1*	*3*	*SC*
Crathes	*3*	*-26.1±0.3*	*3.1±1.7*	*2*	*1*	*NE*
Maree	6	-25.8±0.5	2.3±0.9	2	2	NW
Achnashellach	6	-25.8±0.4	2.0±0.4	2	2	NC
Coulin	2	-26.0±0.1	1.1±0.3	2	2	NW
Affric	*9*	*-25.8±1.0*	*3.9±0.7*	*2*	*3*	*NC*
Strathfarrar	*7*	*-26.0±1.2*	*3.6±0.9*	*2*	*3*	*NC*
Guisachan	*3*	*-26.1±0.6*	*3.2±1.8*	*3*	*1*	*SW*
Mar	*3*	*-26.2±0.9*	*2.9±0.7*	*3*	*1*	*EC*
Grant	10	-26.2±0.3	3.7±1.4	3	3	EC
Blackmount	7	-26.4±0.5	4.1±1.2	3	3	SW
Amat	3	-26.5±0.7	3.7±1.1	3	3	NC

Table 5. ANOVA model for the four comparable populations (*population*) from natural environments and clone bank (*site*) for $\delta^{13}C$ and MRW. The table is showing degrees of freedom (*DF*), sum of squares (*SS*), F test (*F*) and level of significance (*P*). To test the statistical significance of each factor Type III Sum of squares were used. There was no significant *populations–times–site* interaction for $\delta^{13}C$.

	Source	*DF*	*SS*	*F*	*P*	R^2
$\delta^{13}C$	Model	7	20.15	5.87	<0.0001	0.226
	Error	133	65.23			
	Total	140	85.38			
	Population	3	13.30	9.12	<0.0001	
	Site	1	5.78	11.89	0.0008	
MRW	Model	7	81.09	22.69	<0.0001	0.544
	Error	133	67.90			
	Total	140	149.00			
	Population	3	22.68	14.81	<0.0001	
	Site	1	32.28	63.24	<0.0001	
	Popxsite	3	6.34	4.14	0.008	

Figure Titles

Figure 1. Biochemical Zones and locations of natural populations (larger filled circles and bold-italics labelling), original sites of clone bank trees (smaller symbols and smaller labelling) and the clone bank (star sign); Biochemical Zones (North [N], Northwest [NW], Northcentral [NC], Northeast [NE], Eastcentral [EC], Southwest [SW] and Southcentral [SC]) modified from a map as used by the Forestry Commission Scotland (based on Forrest 1980, Forrest 1982 and Kinloch 1986); graph origin (0 km N / 0 km E) corresponds to 56°6'N / 6°50'W, upper right corner (200 km N / 300 km E) corresponds to 57°59'N / 2°0'W.

Figure2. Means of individual tree data from the natural populations for $\delta^{13}C$ and MRW for three classes of potential water deficit : a) precipitation exceeds evaporation by 500mm per year ('excess'), b) no potential water deficit with precipitation equal to or exceeding evaporation ('no pwd') and c) potential water deficits from 0 to 25mm per year ('pwd 0-25mm'). Error bars are standard deviations.

Figure 3. Comparison of clone bank *versus* natural population means for a) $\delta^{13}C$ and b) MRW. Error bars are standard deviations. Broken line is 1:1; GM: Glenmore. RA: Rannoch, LC: Loch Clair, BE: Ben Eighe. For MRW the significant linear correlation is shown.

Figure 4. Clone bank: K-means clustering for a) $\delta^{13}C$ and b) MRW population means; individual trees plotted at their sites-of-origin in Scotland. Circled numbers refer to k-means cluster as listed in Table 4, lines separate populations that belong to different cluster; graph origin as in Figure 1.

Figures

Figure 1.

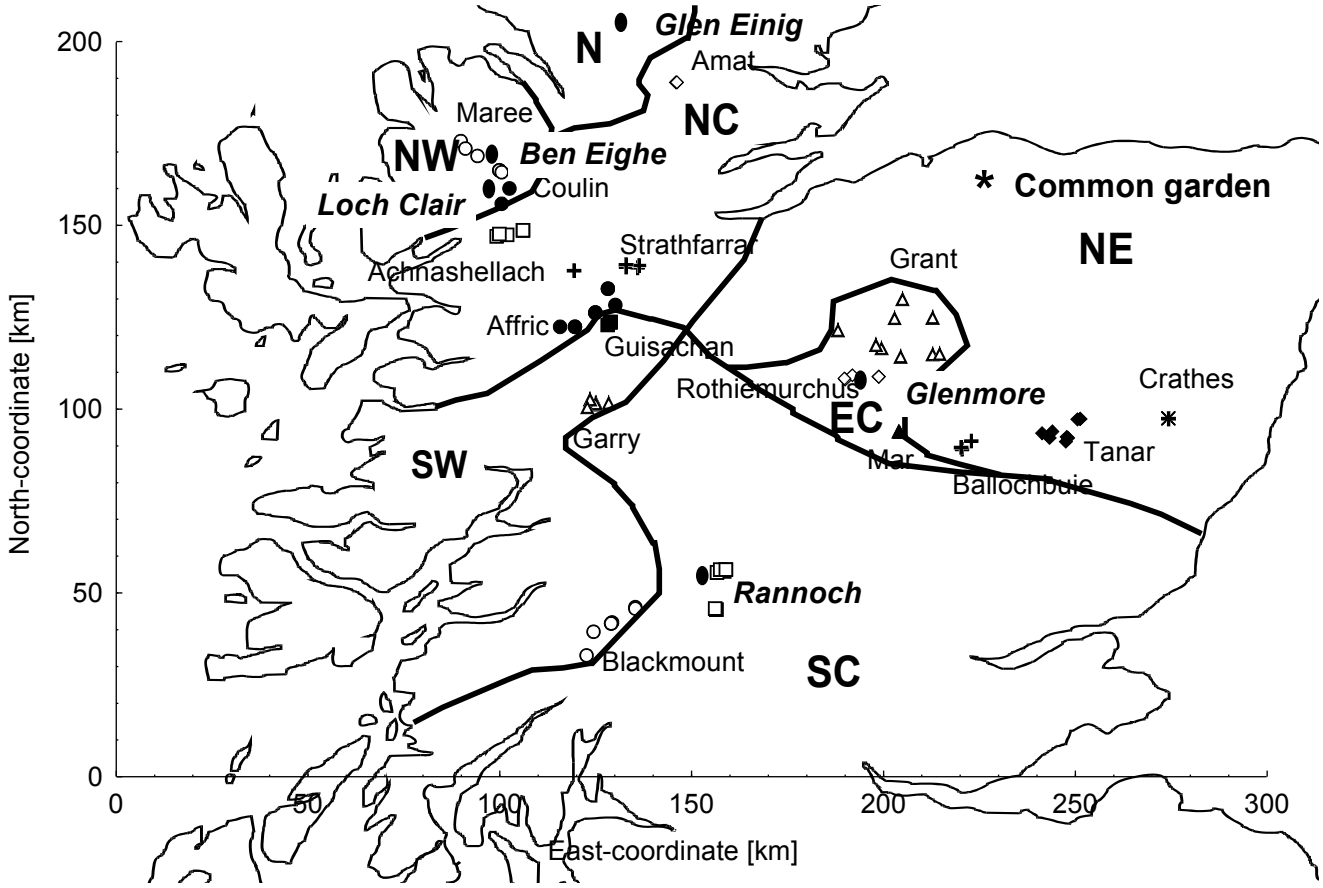


Figure 2

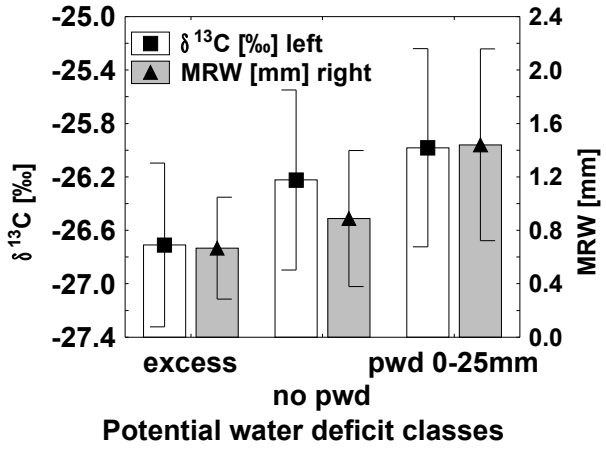
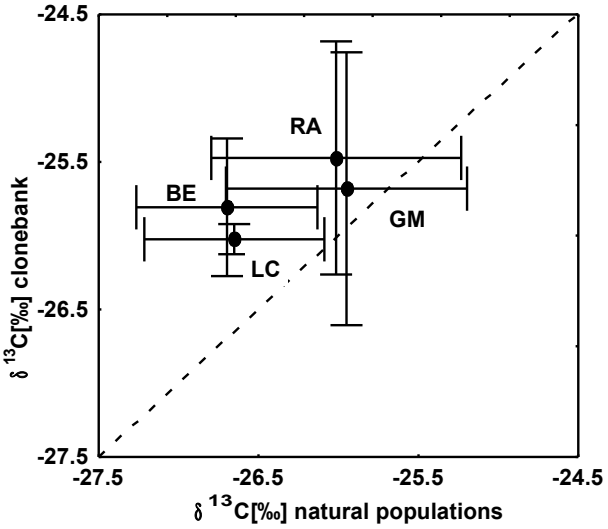


Figure 3.

a)



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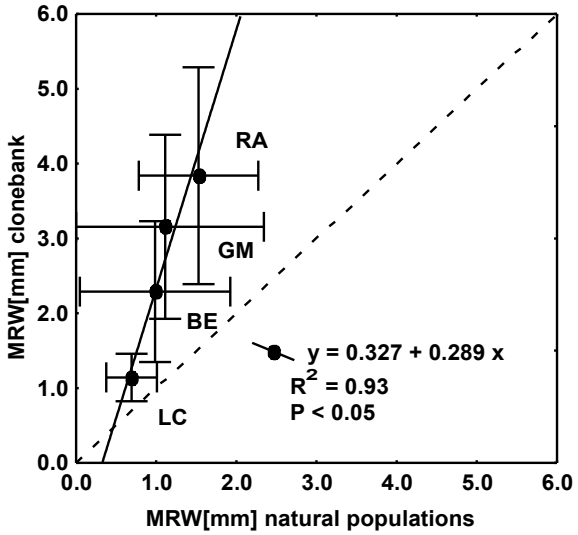

Figure 4.