

Report

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**DO SEED ZONES CONSERVE
ADAPTIVE VARIATION?
TESTING THE ADAPTIVE
SIGNIFICANCE OF SEED ZONES IN
SCOTS PINE**

**Nine month progress report for
Scottish Forestry Trust
June 2008**

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Objectives of the project:

The project will use a combination of molecular markers and growth experiments to assess the effectiveness of designated seed zones in Scots pine for the conservation of adaptive variation, by answering the following questions:

1. Do designated seed zones accurately reflect patterns of neutral and adaptive variation in Scots pine?
2. Is local seed better adapted to the local environment, both now and in the future, than seed from further afield?

Progress from September 2007 to June 2008**1. Seed collection from populations of Scots pine across the native range in Scotland**

Between January and March 2007 seed collections of *Pinus sylvestris* were made by Dr. Glenn Iason and colleagues from the Macaulay Institute from 21 native pine populations located in all seven of the designated native pine zones (Appendix I). Within each population four maternal trees were sampled for cones to yield at least 50 viable seeds per maternal tree. Maternal trees were chosen to cover the full elevational range within the relevant pinewood.

2. Establishment of seedling provenance/progeny trial

Approximately 100 seeds were extracted from cones collected from each maternal tree, germinated and potted up in late spring 2007. The resulting plants were used to establish a provenance/progeny seedling trial located in a cool glasshouse at CEH Edinburgh in summer 2007. The trial comprises 84 families, each of 40 offspring, derived from the 21 populations of native pine from which seed was originally collected. The trial is arranged as a randomised block design with 40 blocks each containing one individual from each of the 84 families (a total of 3360 seedlings).

3. Measurement of adaptive character variation in provenance/progeny trial

Preliminary investigation into the patterns of adaptive genetic variation in *P. sylvestris* has concentrated on the character of date of bud flushing. Seedlings were scored twice a week from 25/3/08 to 9/5/08 to determine the date at which the terminal bud flushed. Table 1 shows a simple ANOVA of the results which indicates that time of bud flush shows significant variation both among populations and among families within populations.

| Source of Variation | d.f. | Mean Square | Significance |
|---------------------|------|-------------|--------------|
| Blocks | 39 | 171 | P<0.001 |
| Population | 20 | 1103 | P<0.05 |
| Family(Population) | 63 | 608 | P<0.01 |
| Within Family | 3120 | 50 | |

Table 1. Analysis of variation for date of bud flushing in a seedling provenance/progeny trial containing 21 populations of *P. sylvestris* from the native range within Scotland and grown in a cool glasshouse near Edinburgh.

4. Estimation of heritability of time of bud flush variation

The results of the ANOVA of bud flush date were used to estimate the heritability of variation for this character. If we assume that the families of seeds collected were composed of a mixture of full and half sibs, the heritability of variation h^2 is estimated as 0.66, suggesting very strong genetic control of variation in bud burst date. However it should be noted that heritability assessed by this method may be an overestimate if there are significant maternal effects on bud burst date. Further estimates of bud flushing date will be made over future years to determine whether a reduction in the estimate of h^2 is found, a result that would be expected if there were significant maternal effects.

5. Estimation of the extent of population differentiation for time of bud flush

Where variation among populations and among families within populations can both be measured, a quantitative estimate of genetic differentiation among populations can be calculated (Qst). Assuming, as above, that families are a mixture of full and half sibs, a Qst value of 0.03 is calculated. This indicates that only 3% of the variation for date of bud flush is found among populations, a similar level of differentiation to that found for neutral genetic markers (Fst). This value may be an underestimate if maternal effects are large.

6. Geographic and Ecological determinants of population differentiation for bud flush date

Simple regression analysis indicates significant relationships between bud flush date of a family, and latitude and elevation of origin of that family (Table 2). Earlier flushing in the glasshouse is shown by populations from further north and at higher elevation. However, only a small amount of the variation ($R^2 = 6-10\%$) is accounted for by either of these factors (Table 2). When latitude, elevation and longitude are included in a multiple regression, only elevation accounts for a significant amount of variation in bud flush date. Initial analysis of possible ecological factors that may explain variation in bud flush date indicate later flushing for populations with higher accumulated temperature values ($R^2 = 8.9\%$), and greater moisture deficits ($R^2 = 10.5\%$) (Table 2).

| Source of Variation | d.f | M. S. Latitude | M.S. Longitude | M.S. Elevation | M.S. Moisture deficit | M.S. Accumulated temperature |
|---------------------|-----|----------------|----------------|----------------|-----------------------|------------------------------|
| Regression | 1 | 112** | 63 | 191** | 192** | 166** |
| Error | 82 | 19 | 20 | 18 | 18 | 18 |
| R^2 | | 5.6 | 2.6 | 10.4 | 10.5 | 8.9 |

Table 2. Results of ANOVA for regression of date of bud flush on both geographic and ecological variables at site of origin of *P. sylvestris* family in Scotland. R^2 indicates amount of variation accounted for by regression. * $P < 0.05$; ** $P < 0.01$.

7. Development of methods for scoring Molecular Marker variation

Seven microsatellite primers (SPAC_11.6, SPAG_7.14, SPAC_11.5, SPAC_11.4, SPAG_3.7, SPAC_12.5 (Soranzo *et al.* (1998)) and PtTX4001 (González-Martínez *et al.* (2004)) have been tested for use in estimating both population differentiation and outcrossing rate in the *P. sylvestris* populations collected for the provenance/progeny trial. Large scale scoring of these genetic markers will be conducted in the autumn of 2008.

8. Identification of Candidate Genes linked to local adaptation

To facilitate the identification of candidate genes and ensure delivery of the this part of the project, additional funding has been secured from the EC-funded Network of Excellence EVOLTREE, to bring Dr Witold Wachowiak from the Institute of Dendrology of the Polish Academy of Sciences to Edinburgh for 6 months to apply his experience with analysis of Scots Pine genes to our collection from the Scottish remnant populations. Following his Ph.D. (studying hybridisation between *P. sylvestris* and *P. mugo*), Dr. Wachowiak has worked for three years at the University of Oulu, Finland identifying variations in genes linked to cold tolerance in Scots Pine and has extensive experience of the procedures for location, characterisation and screening of DNA for candidate genes. During the visit to Edinburgh, he will use his own knowledge and an additional database of 'candidate' genes, made available through the EVOLTREE network, to analyse variation in populations across Scottish environmental gradients. He will identify variable regions and establish the screening protocols, to ensure subsequent analysis of the full experimental trial proceeds quickly. Dr. Wachowiak will start work in August 2008.

Conclusions

The large scale seedling provenance/progeny trial needed to deliver information on adaptive genetic differentiation across the natural range of Scots pine in Scotland has been successfully established. Data on a relevant adaptive character has been gathered and analysed. This work will be extended to include further characters in the next year. Preliminary work required for scoring molecular marker variation has been completed. EC funding has been secured for developing methods for screening variation in candidate adaptive genes. This will provide the opportunity to study candidate genes and incorporate the information into the current project.

References

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- Soranzo N, Provan J, Powell, W. (1998) Characterization of microsatellite loci in *Pinus sylvestris* L. *Molecular Ecology* (1998) 7, 1260-1261.

Appendix I. Location of maternal families of *P. sylvestris* sampled for the provenance/progeny trial, together with information on ecological variables at the site.

| Population | Core pinewood area (ha) | Pinewood zone | Family | Altitude (m) | Latitude | Longitude (W) | Accumulated temperature | Moisture deficit |
|-----------------------|-------------------------|---------------|---------|--------------|----------|---------------|-------------------------|------------------|
| Abernethy | 2452 | East Central | AB1832 | 365 | 57.2 | 3.6 | 873 | 47 |
| | | | AB1834 | 363 | 57.2 | 3.6 | 876 | 48 |
| | | | AB1837 | 311 | 57.2 | 3.6 | 939 | 61 |
| | | | AB1839 | 327 | 57.2 | 3.6 | 919 | 57 |
| Allt Cull | 13 | North East | AC1802 | 512 | 57.0 | 3.4 | 699 | 13 |
| | | | AC1803 | 493 | 57.0 | 3.4 | 723 | 18 |
| | | | AC1806 | 464 | 57.0 | 3.4 | 759 | 26 |
| | | | AC1808 | 435 | 57.0 | 3.4 | 795 | 33 |
| Amat | 181 | North Central | AM41 | 39 | 57.9 | 4.6 | 1202 | 116 |
| | | | AM44 | 201 | 57.9 | 4.6 | 1019 | 74 |
| | | | AM45 | 153 | 57.9 | 4.6 | 1073 | 86 |
| | | | AM50 | 156 | 57.9 | 4.6 | 1070 | 85 |
| Ballochuie | 775 | North East | BB74 | 500 | 57.0 | 3.3 | 717 | 17 |
| | | | BB75 | 524 | 57.0 | 3.3 | 687 | 11 |
| | | | BB80 | 456 | 57.0 | 3.3 | 772 | 29 |
| | | | BB97 | 421 | 57.0 | 3.3 | 816 | 38 |
| Beinn Eighe | 182 | North West | BE21 | 59 | 57.6 | 5.4 | 1210 | 103 |
| | | | BE23 | 91 | 57.6 | 5.4 | 1173 | 94 |
| | | | BE26 | 83 | 57.6 | 5.3 | 1183 | 97 |
| | | | BE30 | 17 | 57.6 | 5.4 | 1259 | 113 |
| Black Wood of Rannoch | 1011 | South Central | BW1822 | 307 | 56.7 | 4.3 | 990 | 57 |
| | | | BW1825 | 257 | 56.7 | 4.3 | 1054 | 69 |
| | | | BW1828 | 250 | 56.7 | 4.3 | 1063 | 71 |
| | | | BW1830 | 285 | 56.7 | 4.3 | 1082 | 62 |
| Coille Coire Chuilc | 67 | South Central | CCC1801 | 298 | 56.4 | 4.7 | 1024 | 56 |
| | | | CCC1806 | 222 | 56.4 | 4.7 | 1125 | 75 |
| | | | CCC1807 | 237 | 56.4 | 4.7 | 1105 | 71 |
| | | | CCC1809 | 269 | 56.4 | 4.7 | 1062 | 63 |
| Cona Glen | 189 | South West | CG1862 | 89 | 56.8 | 5.3 | 1265 | 100 |
| | | | CG1864 | 154 | 56.8 | 5.3 | 1182 | 83 |
| | | | CG1867 | 180 | 56.8 | 5.3 | 1149 | 76 |
| | | | CG1868 | 169 | 56.8 | 5.3 | 1163 | 79 |
| Crannach | 70 | South West | CR1881 | 258 | 56.6 | 4.7 | 1063 | 65 |
| | | | CR1885 | 307 | 56.6 | 4.7 | 999 | 53 |
| | | | CR1888 | 338 | 56.6 | 4.7 | 959 | 45 |
| | | | CR1889 | 280 | 56.6 | 4.7 | 1034 | 60 |
| Glen Affric | 1532 | North Central | GA1892 | 205 | 57.3 | 5.0 | 1073 | 71 |
| | | | GA1893 | 274 | 57.3 | 5.0 | 990 | 53 |
| | | | GA1897 | 274 | 57.3 | 5.0 | 989 | 53 |
| | | | GA1900 | 270 | 57.3 | 5.0 | 994 | 54 |
| Glen Cannich | 301 | North Central | GC85 | 348 | 57.3 | 4.9 | 892 | 36 |
| | | | GC88 | 361 | 57.3 | 4.9 | 876 | 32 |
| | | | GC89 | 182 | 57.4 | 4.9 | 1091 | 78 |
| | | | GC90 | 189 | 57.4 | 4.9 | 1082 | 77 |

Appendix I ctd..

| | | | | | | | | |
|---------------|------|---------------|--------|-----|------|-----|------|-----|
| Glen Derry | 235 | East Central | GD94 | 493 | 57.0 | 3.6 | 726 | 16 |
| | | | GD95 | 492 | 57.0 | 3.6 | 727 | 16 |
| | | | GD98 | 437 | 57.0 | 3.6 | 795 | 30 |
| | | | GD99 | 426 | 57.0 | 3.6 | 809 | 33 |
| Glen Einig | 27 | North | GE33 | 59 | 58.0 | 4.7 | 1171 | 108 |
| | | | GE36 | 69 | 58.0 | 4.7 | 1160 | 106 |
| | | | GE37 | 45 | 58.0 | 4.7 | 1187 | 112 |
| | | | GE38 | 46 | 58.0 | 4.8 | 1186 | 111 |
| Glen Loy | 74 | South West | GL1868 | 155 | 56.9 | 5.1 | 1168 | 85 |
| | | | GL1872 | 161 | 56.9 | 5.1 | 1160 | 83 |
| | | | GL1876 | 197 | 56.9 | 5.1 | 1114 | 74 |
| | | | GL1877 | 170 | 56.9 | 5.1 | 1149 | 81 |
| Glen Tanar | 1564 | North East | GT1851 | 289 | 57.0 | 2.9 | 975 | 76 |
| | | | GT1856 | 422 | 57.0 | 2.9 | 810 | 42 |
| | | | GT1858 | 345 | 57.0 | 2.9 | 906 | 62 |
| | | | GT1860 | 330 | 57.0 | 2.9 | 924 | 66 |
| Loch Clair | 126 | North West | LC3 | 166 | 57.6 | 5.4 | 1070 | 62 |
| | | | LC5 | 153 | 57.6 | 5.4 | 1108 | 79 |
| | | | LC7 | 107 | 57.6 | 5.4 | 1162 | 91 |
| | | | LC8 | 102 | 57.6 | 5.4 | 1168 | 92 |
| Meggernie | 277 | South Central | MG1813 | 286 | 56.6 | 4.4 | 1025 | 62 |
| | | | MG1815 | 264 | 56.6 | 4.4 | 1053 | 68 |
| | | | MG1816 | 287 | 56.6 | 4.4 | 1023 | 62 |
| | | | MG1820 | 385 | 56.6 | 4.3 | 895 | 37 |
| Rhidorroch | 103 | North | RD61 | 175 | 57.9 | 5.0 | 1049 | 76 |
| | | | RD63 | 220 | 57.9 | 5.0 | 998 | 64 |
| | | | RD64 | 193 | 57.9 | 5.0 | 1028 | 71 |
| | | | RD70 | 138 | 57.9 | 5.0 | 1090 | 85 |
| Rothiemurchus | 1744 | East Central | RM1841 | 306 | 57.1 | 3.8 | 951 | 61 |
| | | | RM1845 | 325 | 57.1 | 3.8 | 928 | 56 |
| | | | RM1846 | 329 | 57.1 | 3.8 | 923 | 55 |
| | | | RM1848 | 311 | 57.1 | 3.8 | 944 | 60 |
| Shieldaig | 103 | North West | SD11 | 91 | 57.5 | 5.6 | 1182 | 92 |
| | | | SD13 | 44 | 57.5 | 5.6 | 1243 | 104 |
| | | | SD14 | 56 | 57.5 | 5.6 | 1229 | 101 |
| | | | SD16 | 132 | 57.5 | 5.6 | 1197 | 68 |
| Strath Oykel | 14 | North | SO51 | 38 | 58.0 | 4.6 | 1192 | 115 |
| | | | SO55 | 152 | 58.0 | 4.6 | 1064 | 86 |
| | | | SO56 | 158 | 58.0 | 4.6 | 1058 | 84 |
| | | | SO60 | 63 | 58.0 | 4.6 | 1164 | 109 |

