NOTES

Genetics of cold hardiness in a cloned full-sib family of coastal Douglas-fir[1]

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Abstract: Variation in cold-hardiness traits, and their extent of genetic control and interrelationships, were investigated among individuals (clones) within a single large full-sib family of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) from Oregon. Cold injury to needle, stem, and bud tissues was evaluated in fall 1996 and spring 1997 following artificial freeze testing of detached shoots collected from 4-year-old ramets (rooted cuttings). Variation among clones in cold-injury scores was significant ($p < 0.01$) for all shoot tissues in both fall and spring and averaged about three times the magnitude previously observed among open-pollinated families of this species. Thus, improving cold hardiness by within-family selection appears to hold much promise. Striking similarities in relative magnitudes of heritability estimates and genetic correlations in the full-sib family, compared with breeding populations, support the following hypotheses about the quantitative genetics of cold hardiness in this species: (*i*) heritability of cold hardiness (both broad-and-narrow-sense) is stronger in the spring than in the fall; (*ii*) cold hardiness of different shoot tissues in the same season is controlled by many of the same genes; and (*iii*) genetic control of fall cold hardiness is largely independent of cold hardiness in the spring.

Résumé : La variation entre individus pour les caractères associés à la tolérance au froid, ainsi que le degré de contrôle génétique et d'inter-relations, ont été étudiés sur des clones appartenant à une seule grande fratrie de douglas (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) côtier originaire de l'Oregon. Les dommages au froid observés sur les aiguilles, sur la tige et sur les tissus des bourgeons ont été évalués à l'automne de 1996 et au printemps de 1997 à la suite d'un test de gel artificiel sur des pousses excisées recueillies sur des ramets âgés de 4 ans (boutures enracinées). La variation observée dans les dommages au froid parmi les clones était significative ($p < 0{,}01$) pour tous les tissus des pousses à l'automne et au printemps et était en moyenne trois fois plus élevée que celle observée parmi les familles de cette espèce issues de la pollinisation libre. Ainsi, l'amélioration de la tolérance au froid par une sélection à l'intérieur d'une famille apparaît prometteuse. Les fortes similitudes observées dans le niveau relatif des estimés d'héritabilité et de corrélations génétiques entre la fratrie et les populations qui interagissent supportent les hypothèses suivantes quant à la génétique quantitative de la tolérance au froid de cette espèce : (*i*) l'héritabilité de la tolérance au froid (au sens large et au sens strict) est plus forte au printemps qu'en automne; (*ii*) la tolérance au froid de différents tissus des pousses est contrôlée, pour une même saison, par plusieurs des mêmes gènes; et (*iii*) le contrôle génétique de la tolérance au froid à l'automne est largement indépendant du même contrôle au printemps.

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Introduction

Much has been learned in recent years about genetic variation and control of cold hardiness in coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) based on artificial freeze testing of shoot samples from sapling-age trees in progeny tests (Aitken and Adams 1996, 1997; Aitken et al. 1995). Considerable variation among families within populations for

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Table 1. Comparison of genetic (clonal) variation within a single full-sib family with variation among open-pollinated families within breeding populations of coastal Douglas-fir, for fall and spring cold injury (% damage) to three shoot tissues.

		Within single full-sib family[a]				Within breeding populations[b]			
Season	Tissue	Mean	Range	CV_c (%)[c]	H^2[d]	Mean	Range	CV_f (%)[e]	h^2[f]
Fall	Needle	75	30–95	87	0.21	52	25–82	17	0.34
	Stem	82	25–95	85	0.14	24	10–49	27	0.21
	Bud	62	10–95	137	0.16	20	5–42	37	0.14
Spring	Needle	71	19–95	107	0.45	45	32–62	16	0.49
	Stem	65	43–90	72	0.27	48	14–86	42	0.77
	Bud	63	41–90	78	0.20	36	22–73	56	0.56

[a]Based on 186 clones (mean of 3.5 rooted cuttings per clone) of progeny in the fall and a subset of 171 of these clones (mean of 3.3 rooted cuttings per clone) in the spring.
[b]Based on two western Oregon breeding zones (40 families sampled per zone).
[c]Coefficient of variation in clonal means, ($CV_c = (\sigma_c^2)^{0.5}/X_c$), where σ_c^2 is the estimated clonal variance component and X_c is the mean over all clones.
[d]Estimated individual-tree broad-sense heritability.
[e]Coefficient of variation in family means, ($CV_f = (\sigma_f^2)^{0.5}/X_f$), where σ_f^2 is the estimated family variance component and X_f is the mean over all families.
[f]Estimated individual-tree narrow-sense heritability.

shoot cold injury in both spring and fall indicates there is much room for improving cold hardiness in conventional breeding programs. This is especially true for spring cold hardiness, as indicated by the strong narrow-sense heritability estimates for this trait (Aitken and Adams 1997).

In this note we describe genetic variation for cold-hardiness traits within a single full-sib family of Douglas-fir with parents originating from western Oregon. This three-generation pedigree was created to genetically map and estimate magnitudes of effects of quantitative trait loci (QTLs) controlling adaptive traits including bud phenology, growth rhythm, and cold hardiness (Jermstad et al. 1998). Because individuals were vegetatively replicated by rooting cuttings, it is possible to quantify genetic variation within this family and compare it with levels of variation reported previously for cold-hardiness traits at the population level. We were also interested in the degree to which the genetic control of cold-hardiness traits and genetic relationships among traits in this family compare with those observed in breeding populations.

Material and methods

Clonal materials and freeze testing

The second filial generation (F_2) was constructed by crossing two individuals in the first filial generation (F_1s); and the F_1s were first created by crossing two pairs of unrelated parents from western Oregon, with the parents in each pair having contrasting timing of bud burst (early versus late). The original F_2 family had 298 progeny, of which 48 were used for DNA isolation from needles to construct a preliminary linkage map (Jermstad et al. 1998). Cuttings from seedlings of 192 of the remaining 250 progeny were successfully rooted (Ritchie 1990) in the spring of 1993 and transplanted to a bare-root nursery for one season. The rooted cuttings were then outplanted in the spring of 1995 at two test sites, one in western Washington, and the other in Oregon. At planting, each clone was represented by a row plot of three ramets in each of four randomized complete blocks on each site.

All sampling for cold-hardiness testing was from the Washington test site. Four lateral-shoot tips (5 cm long) were harvested from each sampled ramet, 4 years after rooting, in the fall of 1996 (October 7) and in spring of 1997 (April 8) prior to bud burst. Not all 192 clones had sufficient numbers of ramets for sampling. In the fall, one or two ramets from each of 186 clones were sampled in two of the four blocks (mean number of ramets per clone from both blocks = 3.5). In the spring, one or two ramets from each of 171 clones included in the fall collection were sampled in each of the remaining two blocks (mean number of ramets per clone from both blocks = 3.3). Different blocks were utilized in each season to minimize damage to the trees.

Table 2. Comparison of estimated genetic correlations in a single cloned full-sib family with measurements from two breeding populations of coastal Douglas-fir for cold-injury scores between different tissues in the fall (above diagonal) and spring (below diagonal), and between the same tissue in the two seasons (on the diagonal).

	Within single full-sib family[a]			Within breeding populations[b]		
	Needle	Stem	Bud	Needle	Stem	Bud
Needle	0.26	0.75	0.60	—[c]	0.78	0.85
Stem	0.47	–0.43	0.72	0.89	–0.25	0.92
Bud	0.69	0.84	–0.40	0.88	0.98	—[c]

[a]Broad-sense correlations (r_g).
[b]Narrow-sense (additive) correlations (r_A).
[c]Estimates were not available.

Details on freeze-testing procedures are presented elsewhere (Aitken and Adams 1996, 1997; Aitken et al. 1995), so we give only a brief outline here. On each date, shoot samples from each ramet were subjected to four freezing temperatures chosen from preliminary tests to inflict a range of injury scores, from high to low (–9 to –15°C in fall, –12 to –18°C in spring). Samples were put into a programmable freezer (at –2°C) which was slowly reduced in temperature until the test temperature was reached. After 1 h, the samples were removed, stored overnight at 2°C, and then kept in the dark at room temperature for 7 days to allow cold-injury symptoms to develop. Needle, stem (phloem and cambium), and bud tissues were visually assessed by two individuals and scored independently into 10 damage classes based on the proportion of tissue showing injury symptoms (browning).

Statistical analysis

An initial analysis showed that the clonal component of injury to all tissues was significant ($p < 0.05$) only for the two intermediate freezing temperatures on each sampling date (–11 and –13°C in fall; –14 and –16°C in spring) as damage levels were too high at the lower temperature and too low at the higher temperature to allow for the detection of genetic differences. Thus, all further analyses were conducted using the mean injury score for each ramet at the two intermediate temperatures for each season. Analyses first employed the general linear models procedure of the SAS statistical software package (SAS Institute Inc. 1993) to test the significances of clone differences (type III sums of squares). Components of variance in the model were then estimated using the restricted maximum likelihood (REML) method of the SAS VARCOMP procedure. To quantify the magnitudes of genetic variation within the full-sib family we calculated clonal coefficients of variation (CV_c; Table 1). To evaluate the genetic control of each trait, we estimated their broad-sense heritabilities:

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{cb}^2 + \sigma_e^2}$$

where σ_c^2 is the estimated variance component due to clone differences, σ_{cb}^2 is the variance due to clone-by-block interaction, and σ_e^2 is the variance due to differences between ramets within clonal-row plots. We also estimated broad-sense genetic correlations (r_g) between cold-injury scores for different tissues in the same season and between scores for the same tissue in different seasons (Burdon 1977).

To compare genetic parameters estimated from data in our single full-sib family to population-level estimates, we used mean results from two western Oregon breeding populations reported previously (Aitken and Adams 1996, 1997). In this investigation, shoots were harvested from 7-year-old individuals of 40 open-pollinated families sampled from two progeny tests within each population and subjected to freeze-testing procedures similar to those used in this study. We quantified genetic variation in these materials by calculating mean family coefficients of variation (CV_f; Table 1). Genetic control was quantified by mean estimated narrow-sense heritabilities (h^2) and genetic relationships between traits by mean estimates of narrow-sense (additive) genetic correlations (r_A). Using the same families and populations, O'Neill (1999) found strong narrow-sense genetic correlations ($r_A \geq 0.79$) between cold injury scores of 7- and 2-year-old trees. Because of this result, we assume that our comparison of the genetics of cold hardiness in 4-year-old rooted cuttings with that of 7-year-old saplings is not influenced by the age differences between the two sets of materials.

Results and discussion

Variation among clones in cold-injury score was significant ($p < 0.01$) and extensive for all tissues in both fall and spring, with the clonal coefficient of variation averaging 94% (Table 1). This considerable variation among clones is nearly three times the average coefficient of family variance observed for the 40 open-pollinated families in two breeding zones (mean CV = 32.5%) and suggests there is much promise for improving cold hardiness by within family selection. Assuming that genetic variance among open-pollinated families equals one third of the additive variance (V_A) in a breeding population (Campbell 1979) and genetic variance within full-sib families equals $V_A/2$ plus three quarters of dominance variance (V_D) (Falconer and Mackay 1996), genetic variance within an average full-sib family derived by random pairing of parents is expected to be at least 1.5 times (i.e., $(1/2V_A + 3/4V_D)/(1/3V_A) = 3/2V_A + 9/4(V_D/V_A)$) the variance among open-pollinated family means. The large coefficient of variation within this one family relative to that observed among open-pollinated families within breeding zones may, at least, partially be due to the fact that the number of clones was more than four times greater than the number of families sampled in the breeding zones. Also, estimates for full-sib families were obtained from a single test site; thus, additive genetic variance components were increased in the absence of genotype-by-environment interaction. In addition, recall that the grandparents of our full-sib family were chosen to maximize segregation of bud-burst timing in the F_2 generation. Because stem hardiness and bud phenology are closely associated (Campbell and Sorensen 1973; Campbell and Sugano 1975, 1979; Aitken and Adams 1997), especially in spring, within family variation in cold injury is likely accentuated by the selection of grandparents with extreme phenotypes in this pedigree.

The absolute magnitudes of broad-sense and narrow-sense heritabilities in Table 1 are not comparable, because they are based on different materials and experimental designs. Nevertheless, relative magnitudes of heritabilities in the two seasons were the same in the two sets of materials. That is, heritability estimates for cold-injury score were always greater in the spring than in the fall for the same tissue. In addition, the two sets of materials had similar patterns of genetic correlations between cold-injury traits (Table 2). Estimates of both broad-sense and narrow-sense genetic correlations in cold injury were strong and positive between tissues in both seasons but were weak or negative between the same tissue in different seasons. Corresponding estimated correlations between clone means and between family means for cold injury (data not shown) were of the same sign but weaker than the genetic correlations (T.S. Anekonda, unpublished data). In all cases, cold-injury correlations between different tissues in the same season based on clone or family means were significant ($p < 0.001$) but were not significant ($p > 0.05$) between the same tissue in different seasons. Thus, genetic correlations within the full-sib family support earlier conclusions about the inheritance of cold hardiness in coastal Douglas-fir (Aitken and Adams 1996, 1997). That is, the cold hardiness of different shoot tissues within the same season are controlled by many of the same genes, while genetic control of cold hardiness in the spring is stronger and largely independent of genetic control of cold hardiness in the fall. Results of the QTL mapping study involving our full-sib family will not only provide evidence supporting or refuting the above hypotheses (e.g., to what extent are QTLs for spring and fall cold hardiness the same) but will aid our understanding of the inheritance of cold-hardiness traits (e.g., how many genes influence each trait and how are they distributed in the genome).

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