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INBREEDING DEPRESSION AND VARIANCE STRUCTURES FOR HEIGHT AND ADAPTATION IN SELF- AND OUTCROSS *THUJA PLICATA* FAMILIES IN VARYING ENVIRONMENTS

J. H. Russell [1], **R. D. Burdon** [2] **& A. D. Yanchuk** [3]

[1] Cowichan Lake Research Station, Research Branch, British Columbia Ministry of Forests, P.O. Box 335, Mesachie Lake, B.C., Canada V0R 2N0 (corresp.author)
[2] New Zealand Forest Research Institute, Private Bag 3020, Rotorua, New Zealand
[3] Research Branch, British Columbia Ministry of Forests, P.O. Box 9519, Station Provincial Government, Victoria, B.C., Canada V8W 1L4

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ABSTRACT

Field trials of self- and outcross families from 24 parent trees of western redcedar, a species that has a high rate of natural self-pollination, were established on three sites: a low-elevation nursery site, Cowichan Lake Research Station (CLRS), at close spacing (24 parents); a fertile low-elevation site, Jordan River Low (JRL), (16 parents); and a nearby high elevation site, Jordan River High (JRH), (12 parents). Heights and survival were recorded to age 9 except at CLRS (to age 7 only). Growth was fastest at JRL (mean year-9 height 4.6 m) followed by CLRS (2.1 m at age 7), and slowest at JRH (1.2 m at age 9). Mortality remained very low (<3 %) throughout. Inbreeding depression (ID) was almost nil for seed production and growth in the nursery, however it became appreciable with time at both JRL and CLRS, reaching about 10 % at mean height *ca* 2.5 m, but with little subsequent increase. ID at JRH, however, was very slight (<3 %). Genetic correlations for seed-parent performance between mating types were generally high (r_g >0.7, and not declining with time), indicating that self-family performance predicts parental breeding values well, but there was some statistically significant seed-parent × mating-type interactions reflecting r_g<1. There was evidence of appreciable genetic variation in height for recurrent selection with individual narrow-sense heritabilities of *ca* 0.3 and additive genetic coefficients of variation of over 10%. In general, the results fit the expectation that an inbreeding species will have little if any genetic load resulting from strongly deleterious recessive genes affecting viability and general fitness, but significant genetic load of mildly suboptimal effects and not necessarily strongly recessive. The implications are discussed in relation to the putative Quaternary history of the species and the likely evolutionary stability of mating systems.

Key words: Western redcedar, inbreeding depression, self-pollination, field trials

INTRODUCTION

Inbreeding depression (ID) for a given type of mating among relatives varies widely among plant species in its severity and its timing (HUSBAND & SCHEMSKE 1996). ID is usually severe in coniferous species, beginning at early life stages (HUSBAND & SCHEMSKE 1996; WILLIAMS & SAVOLAINEN 1996), and is usually the result of the expression of lethal (or at least strongly deleterious) recessive mutations at one or more loci causing embryo abortion, a lack of filled seed, reduced germination, early seedling mortality or poor initial growth. Such recessives, while rare at individual loci, may involve a very large number of different loci within the population. ID in growth and adaptation also occurs in later stages of the life cycle of trees and this is most likely caused by homozygous genes with small, mildly recessive effects (LANDE & SCHEMSKE 1985; FU & RITLAND 1994), with the degree and timing depending upon species (HUSBAND & SCHEMSKE 1996; WILLIAMS & SAVOLAINEN 1996).

Western redcedar (*Thuja plicata* D. Don) is considered a notable exception among conifers. Past selfing studies in western redcedar showed minimal ID in initial life-cycle stages from zygotic development to germination (OWENS *et al.* 1990), and minimal ID in subsequent survival and early growth traits in a benign greenhouse environment (CHERRY 1995). This apparent lack of ID seemed to fit well with early biochemical studies which showed little to no genetic variation and/or heterozygosity (VON RUDLOFF & LAPP 1979; COPES 1981; VON RUDLOFF *et al.* 1988; YEH 1988; EL-KASSABY *et al.* 1994; GLAUBITZ *et al. 2000*) and a high level of selfing (*t* [effective rate of outcrossing] = 0.40) in an experimental population of unrelated parents (EL-KASSABY *et al.* 1994) and ($t = 0.7$) in natural populations (O'CONNELL *et al.* 2001). A lack of field trials designed to measure quantitative genetic traits prior to 1995, coupled with the above studies, resulted in the conclusion that western redcedar has minimal, if any, genetic variability (COPES 1981; YEH 1988; EL-KASSABY *et al.* 1994) which was attributed to a severe bottleneck during the last glaciation (CRITCHFIELD 1984; YEH 1988). This led EL-KASSABY (1999) to conclude that western redcedar depends on phenotypic plasticity for evolutionary survival rather than on genetic variability.

Recent studies, however, have indicated significant variation among populations in microsatellite loci (O´CONNELL 2003) and in needle monoterpenes among families and individuals (VOURC'H *et al.* 2002). In addition, quantitative studies have shown significant additive genetic variation in early growth and cold-hardiness (REHFELDT 1994; CHERRY 1995), however, these trials used open-pollinated progenies which have inherent biases in estimating genetic parameters due to variable selfing rates (EL-KASSABY *et al.* 1994) and levels of ID among individual parents (this study).

In the current study, the effects of selfing, as compared to outcrossing using common parents on seedling height and adaptability, were studied for western redcedar after outplanting on three diverse field sites. Estimation of ID for an individual pedigree is subject to various biases which stem largely from the typical differences between outcrosses and inbreds in family coefficients of relationship. This can bias comparisons among pedigrees in ID, and tests for both the general presence of ID and individual cases of ID. Three approaches were employed to address these potential biases: adjustment of self and outcross family effects for differential coefficients of relationship, estimation of genetic correlations between self- and outcross performance, and use of analyses of covariance to adjust for putative maternal effects. Adjustments to traditional measures of ID and associated variances that had been developed previously (BURDON & RUSSELL 1999, 2001) were used in data analyses presented here.

The amount and timing of ID were interpreted with respect to genetic variability and the wider evolutionary implications are discussed.

MATERIAL AND METHODS

Material and crossing plan

Forty-four parent trees selected from wild populations within the Maritime Seed Planning Zone on Vancouver Island, British Columbia were used in the study. No selected trees were within 5 km of each other. Scions from the selected trees were grafted and established in the clonebanks at Cowichan Lake Research Station on Vancouver Island (CLRS; Latitude 48° 50' N, Longitude 124° 08' W, elevation 200 m). Twenty-four of the parent trees were used as seed parents and were both selfed and outcrossed. The other 20 trees were used as pollen parents, with 17 of the female trees being crossed with a common, unrelated 10-male polymix, and the other seven with another unrelated 10-male polymix. Differences between polymix lots were non-significant ($p > 0.45$) in all exploratory data analyses, so this effect was ignored in subsequent analyses. The resulting seed was container-sown and grown in a greenhouse for the first year.

Seedlings were outplanted at the beginning of the second growing season at three sites in coastal British Columbia (B.C.): (1) CLRS; (2) Jordan River Low (JRL; Latitude 48° 25´ N, Longitude 124° 02´ W, elevation 100 m), and; (3) Jordan River High (JRH; Latitude 48° 31´ N, Longitude 124° 07´ W, elevation 1000 m). These sites were chosen to represent diverse environmental conditions: CLRS was planted at close spacing, to hasten competition due to crown closure; JRL is a low-elevation, nutrient-rich site, and the trees were expected to exhibit near-optimum growth; and JRH is at the upper elevational limit of the coastal B.C. distribution of western redcedar, and the trees were expected to be exposed to cold-related damage.

Each site was planted with 24 seedlings per mating type (self or outcross) per seed parent. The seedlings at the CLRS nurserybed site were planted at a close spacing of 0.25 m x 0.25 m in 4-tree square plots with six fully randomized complete blocks. The two sites at Jordan River (JRL and JRH) were planted at 2 m x 2 m spacing with 4-tree row plots in each of six fully randomized complete blocks. The CLRS site had all 24 seed-parents represented while JRL had 16, and JRH, 12. The CLRS site was systematically thinned to 12 seedlings per mating-type per seed-parent (*i.e.*, reduced to two diagonal trees per plot) after the fourth growing season, and was terminated after the seventh season because of competition due to the close spacing.

Initial heights in the field were recorded at all sites upon planting (first-year nursery heights), and then heights were recorded annually up to age-7 at CLRS and age-9 at JRL from seed. Four-, five-, and nine-year heights were measured at the JRH site. Mortality was recorded at age-7 at CLRS and at age-9 at JRL and JRH. Cold-damage to leaders was recorded at age-9 at JRH.

Statistical Analyses

Inbreeding depression overall, at any one site, was expressed as a percentage

$$ID = (1 - I / O) \times 100 \qquad [1]$$

where I and O are the means for inbred and outcross material respectively for all height measurements, for survival at age-7 at CLRS and age-9 at JRL and JRH, and for cold-damage at age-9 at JRH. No further statistical analyses were performed for JRH data.

Analysis of variance (ANOVA) was performed using Proc GLM, Type III SS (SAS Inst. Ver. 8.1) to test for mating-type differences for each height measurement at CLRS and JRL, using the model outlined in Table 1. The test for mating-type effects is stringent, being based on treating seed-parent × mating-type interaction as a random effect. However, the seed-parent × mating-type interaction sums of squares were adjusted to a common basis of additive genetic coefficient of relationship for self- and outcross progeny as described in BURDON & RUSSELL (2001).

Tests for individual cases of ID were made using Eq. 26 of BURDON & RUSSELL (1999), using an experimentwise α-level. These tests were also made adjusting for age-1 height using analysis of covariance (ANCOVA). The focus was on possible 'negative' ID or 'outbreeding depression'.

Effects were tested and variance components were estimated for all heights at CLRS and JRL, using PROC GLM Type III SS (SAS Inst. Ver. 8.1) separately by mating-type at each site, according to the ANOVA model presented in Table 2. In addition, all heights for age-2 onwards were adjusted for potential maternal effects using age-1 height as a covariate in ANCOVA; this allowed for both tests of significance and adjusted estimates of variance components free from the effects of any ID expressed in the first year's growth. Further ANCOVAs were carried out, adjusting

Table 1. Form of analysis of variance for data from both mating types at a site.

Source of variance	Degrees of freedom	Expected mean square
Mating type (T)	1	$\sigma_w^2 + n\sigma_{tpr}^2 + nr\sigma_{tp}^2 + npr\Phi_t$
Parents (P)	p–1	$\sigma_w^2 + n\sigma_{tpr}^2 + \quad 2n\sigma_p^2$
Replicates (R)	r–1	$\sigma_w^2 + n\sigma_{tpr}^2 + 2n\sigma_{pr}^2 + 2p\Phi_r$
T × P	p–1	$\sigma_w^2 + n\sigma_{tpr}^2 + nr\sigma_{tp}^2$
T × R	r–1	$\sigma_w^2 + n\sigma_{tpr}^2 + np\sigma_{tr}^2$
P × R	(p–1) (r–1)	$\sigma_w^2 + n\sigma_{tpr}^2 + 2n\sigma_{pr}^2$
T × P × R	(p–1) (r–1)	$\sigma_w^2 + n\sigma_{tpr}^2$
Within-plot	2 p r (n–1)	σ_w^2

where σ_w^2 denotes the random-effect within-plot variance, etc. and Φ_t and Φ_r denote the fixed-effect 'variances' due to mating type and replicates, respectively.

Table 2. Form of analysis of variance for a single mating type within a site.

Source of variance	Degrees of freedom	Expected mean square
Parents (P)	p–1	$\sigma_w^2 + n\sigma_{fr}^2 + nr\sigma_f^2$
Replicates (R)	r–1	$\sigma_w^2 + n\sigma_{fr}^2 + \Phi_r$
P × R	(p–1) (r–1)	$\sigma_w^2 + n\sigma_{fr}^2$
Within-plot	p r (n–1)	σ_w^2

see footnotes of Table 1 for explanation.

later heights for covariance on all subsequent ages for testing and estimating treatment effects, and for age-4 heights in testing all other effects and estimating variance components for subsequent heights.

Estimated variance components for selfs and outcrosses respectively were compared with the theoretical expectations (assuming no maternal effects or epistasis) of (1) the variances among self-families being four times those among outcross families (cf BURDON & RUSSELL 1999, Eqs. 11 and 13), and (2) ratios of between 0.6 and 1.0 for variance within self-families relative to within outcross families (*i.e.*, within-plot variances for the respective types of families) (cf *op. cit.*, Eqs. 14 and 15).

Family-mean repeatabilities were estimated by site for self- and polycross families respectively, the most convenient formula being (F – 1)/F where F is the F-ratio for the family effect in ANOVA. Genetic correlations (r_g) were then estimated between self and outcross family means within sites (BURDON & RUSSELL 1999, Eq. 27) and between sites according to BURDON (1977). Repeatabilities and genetic correlations were estimated for unadjusted heights, as well as age-2 and later heights adjusted for covariance on age-1 and age-4 heights, for both CLRS and JRL. As a cross-check, genetic correlations were also estimated according to the relationship (from Table 1):

$$r_g = \sigma_f^2/(\sigma_f^2+\sigma_{ft}^2) \qquad [2]$$

where, σ_f^2 = among-family variance component and σ_{ft}^2 = family*mating-type interaction variance component. Individual-tree narrow-sense heritability (h^2), the ratio of additive genetic to phenotypic variance (σ_A^2/σ_P^2), was also estimated from polycross family data (Table 2), using the relationship (BURDON & RUSSELL 1999):

$$h_{wo}^2 = 4\sigma_f^2/(\sigma_f^2+\sigma_{fr}^2+\sigma_w^2) \qquad [3]$$

where, σ_{fr}^2 = family * replication variance component.

Alternative estimates, assuming r_g (above) = 1, were obtained from self-family data according to:

$$h_{ws}^2 = \sigma_f^2/(\sigma_f^2+\sigma_{fr}^2+\sigma_w^2) \qquad [4]$$

Using σ_{tpr}^2 (Table 1) in place of σ_{fr}^2 (Table 2) in Eqs. 3 and 4 did not substantially affect the heritabilty or genetic correlation estimates.

Additive genetic and phenotypic coefficients of variation were estimated by dividing σ_A and σ_P respectively by the population mean. All of the above genetic parameters were estimated with and without adjustment on age-1 heights using ANCOVA.

RESULTS

Nursery observations and growth

There was no detectable ID in the self progenies for germination or nursery survival (data not presented), and no morphological aberrations. There was also very little indication of any ID at early stages of growth in a greenhouse environment (Tables 3, age-1 height). Survival was close to 100 % for both self- and outcross seedlings. Population ID (reflected in mating-type effects) for height at the end of the first year was non-significant (p = 0.50 and 0.12 at CLRS and JRL,

Table 3. Mean growth and adaptational values for self- and outcross progeny at successive ages at three field sites.

Trait	Age (years)	CLRS		Jordan R. Low		Jordan R. High	
		self	out	self	out	self	out
Height (m)	1	0.282	0.287	0.293	0.303	0.303	0.30
	2	0.512	0.532	0.606	0.639		
	3	0.765	0.827	1.12	1.2		
	4	1.1	1.18	1.55	1.68	0.681	0.7
	5	1.43	1.54	2.03	2.25	0.75	0.764
	6	1.71	1.86	2.56	2.89		
	7	1.98	2.22	3.19	3.6		
	8			3.75	4.24		
	9			4.36	4.86	1.15	1.18
Survival (%)	7	97.5	98.6				
	9			97.4	100.	98.3	99.3
Cold-damage (%)	9					46.2	46.9

Table 4. Results of ANOVA tests (p-value) for seed-parent x mating-type interaction for heights at successive ages at two field sites, with and without adjustment for covariance on year-1 height.

Site	Test [1]	Age 1	2	3	4	5	6	7	8	9
JR Low	T*P	.00627 **	.00927 ns	.0874 ns	.192 ns	.514 **	1.05 **	1.80 **	2.51**	3.09 **
	T*Padj	.000846 ***	.00707 ns	.0824 ns	.157 ns	.431 **	.866 *	1.33 *	1.71 ns	2.31 *
	T*Pcovariance		.00827 ns	.0892 ns	.198 ns	.568 **	1.13 **	1.93 **	2.65 **	3.14 **
	T*Padj/covariance		.0290 ns	.0519 ns	.120 ns	.430 *	0.843 *	1.31 *	1.66 ns	2.23 *
CLRS	T*P	.0145 ***	.0272 ***	.0663 ***	.108 **	.111 *	.164 ns	.255 ns		
	T*Padj	.0166 ***	.0309 ***	.0754 ***	.130 **	.136 *	.245 ns	.319 ns		
	T*Pcovariance		.0144 **	.0449 ***	.0677 *	.0712 ns	.0997 ns	.181 ns		
	T*Padj/covariance		.0202 **	.0568 ***	.0694 *	.0686 ns	.117 ns	.169 ns		

[1] T = mating type; P = Parents; adj. = interaction adjusted for common basis of additive genetic coefficient of relationship (see text).

[2] n.s. = non significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

respectively) at 1.5 % averaged over all 24 seed-parents. There was significant ($p < 0.01$) mating-type × seed-parent interaction for the samples that went to both the CLRS and JRL site.

Outplanting performance

Mortality was very low (<3 %) at all three sites, with no significant difference between self- and outcross trees (Table 3) and thus no significant ID. Overall, the trees were healthy at both the JRL and CLRS sites, but cold-damage was severe at the JRH site, affecting form and growth. However, there was no significant difference among self- and outcross trees for cold damage (Table 3). Height growth was roughly linear with respect to age at all sites (Table 3), with age-9 heights averaging 4.6 m, and 1.2 m at JRL and JRH respectively and age-7 height 2.1 m at CLRS.

Population ID for height was evident in statistically significant ($p < 0.05$) mating-type effects. It increased generally over time at all sites, but was more prevalent at JRL and CLRS, and low at JRH (Table 3). When plotted against mean height at each site for each measurement year, ID at CLRS and JRL was very similar, reaching around 10 % at about 2 m mean height (Fig. 1). However, there was little subsequent increase in ID at JRL, and a slight drop from age 8 to age 9 which evidently reflected the deaths of just a very few trees. Adjustment for covariance on age-1 (nursery) height did not affect these results. However, adjustment of later heights for covariance on age-6 heights reduced estimated ID to essentially zero at JRL as did adjustment for age-3 and age-4 heights at CLRS. ID subsequently increased after age five at CLRS.

Seed-parent effects across the two mating-types were large and statistically significant ($p < 0.01$) for all heights at both sites. However, mating-type × seed-parent interaction effects (duly adjusted for different coefficients of relationship between the mating-types) were significant up to age 5 at CLRS but non-significant for age-6 and age-7 heights (Table 4). The interaction of mating-type × seed-parent at JRL was not significant between ages 2 and 4, but became significant at age 5 onwards, except for age 8 (Table 4). Adjustment for covariance on age-1 height had no affect on the interaction at JRL, but resulted in a non-significant interaction one year earlier at age 5 at CLRS.

There was no indication of outbreeding depression (OD) at JRL site but there was one seed-parent that exhibited significant OD (experimentwise $p < 0.003$) for just the first two years at CLRS. Adjustment using ANCOVA on age-1 height also did not materially

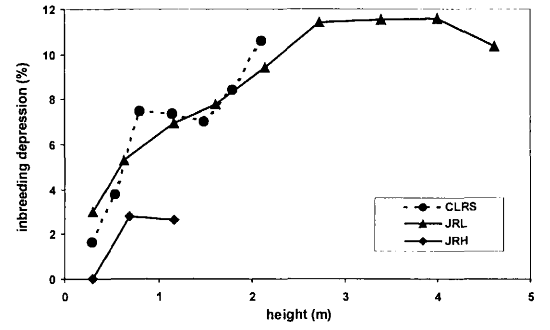

Figure 1. Inbreeding depression for height versus mean heights at three field sites.

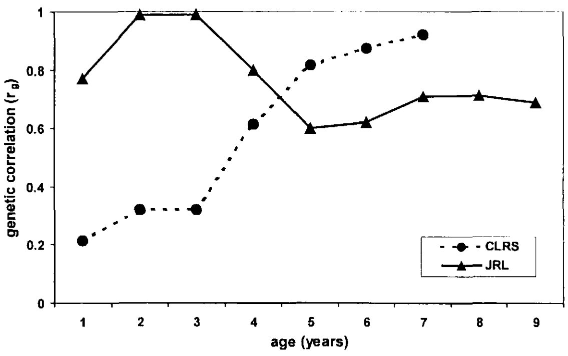

Figure 2. Genetic correlations between mating types for height versus age at two field sites.

change these results.

Looking at mating-type × seed-parent interaction from the approach of genetic correlation estimates, self- and outcross families usually showed statistically significant correlations, indicating only modest interaction. At JRL they increased from age 1 to age 2, decreased from ages 2–5, and then gradually increased to around 0.7 at age 9 (Fig. 2). At CLRS they gradually increased from age 1 (0.2) to age 2 (0.3) and then rapidly increased to 0.9 at age 7 (Fig. 2). Two estimates exceeding the theoretical upper bound of +1 are readily attributable to random estimation error. Phenotypic correlations (details not shown) were necessarily lower, but followed much the same trends, and set the statistical significance of the genetic correlations. Use of Eq. 2, as the cross-check, did not materially change the results (details not shown). Adjusting later heights for covariance on age-1 heights had minimal effect on the genetic correlation estimates. Estimates of between-site genetic correlations are variable; however, they point overall to little rank-change interaction among sites, being positive and for the most part significant, irrespective of the mating-type or the combined mating types (Table 5).

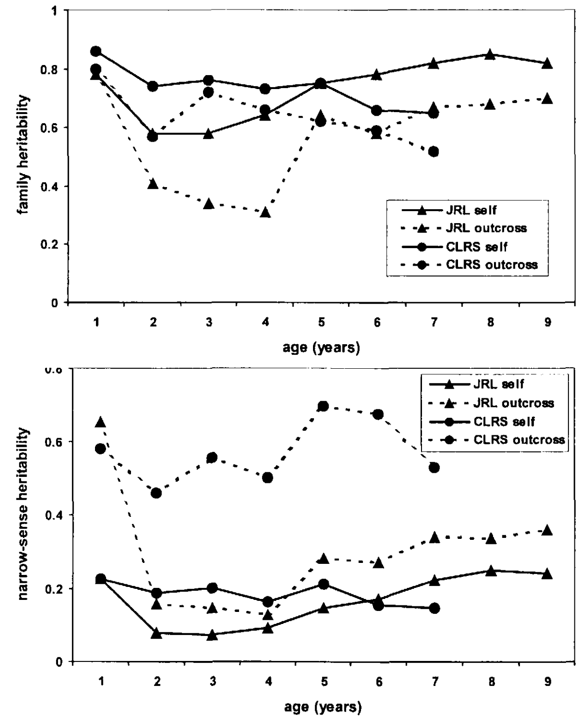

Figure 3. (a) Family-mean repeatabilities for self- and outcross progeny heights versus age at two field sites; (b) individual narrow-sense heritability estimates for self- and outcross progeny heights in relation to age at two field sites.

Family-mean repeatabilities for heights in both selfs and outcrosses at JRL initially decreased, but gradually increased after the third year for self-families and after the fourth year for outcross families (Fig. 3a). Repeatabilities of self-family means were 17 to 100 % larger than those for outcrosses, except at outplanting (age 1) when they were essentially equal. At CLRS family-mean repeatabilities gradually declined over time with self-family repeatabilities 6 to 30% greater than for the outcrosses after age 1.

Estimates of individual narrow-sense heritability (Fig. 3b) showed fluctuations that were similar to those for family-mean repeatabilities except that they were more marked. At JRL they drastically decreased from age 1 to age 2 but gradually increased over time, and self estimates became, on average, much closer to outcross estimates. At CLRS, the individual narrow-sense heritabilities tended to be markedly lower from the self-families than from the outcrosses. Covariance adjustments on age-1 height tended to slightly reduce all heritability estimates across all ages (details not shown).

Estimated coefficients of phenotypic variation for height were consistently greater for self families as compared to outcrosses at both sites (Fig. 4a), whereas

Table 5. **Genetic correlation estimates within and between mating-type for heights at different ages at two field sites.**

		Jordan River Low							
		S1	P1	S2	P2	S4	P4	S7	P7
CLRS	S1	1.00***	0.51 ns						
	P1	0.53 ns	0.99 ***						
	S2			0.87 *	0.64 ns				
	P2			0.96 *	1.2 **				
	S4					0.9 **	0.81 ns		
	P4					0.42 ns	0.89 ns		
	S7							0.74 *	0.5 ns
	P7							1.08 **	1.08 **

[1] Sx, Px = self- and outcross families, respectively, at age – x.
[2] n.s. = non significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

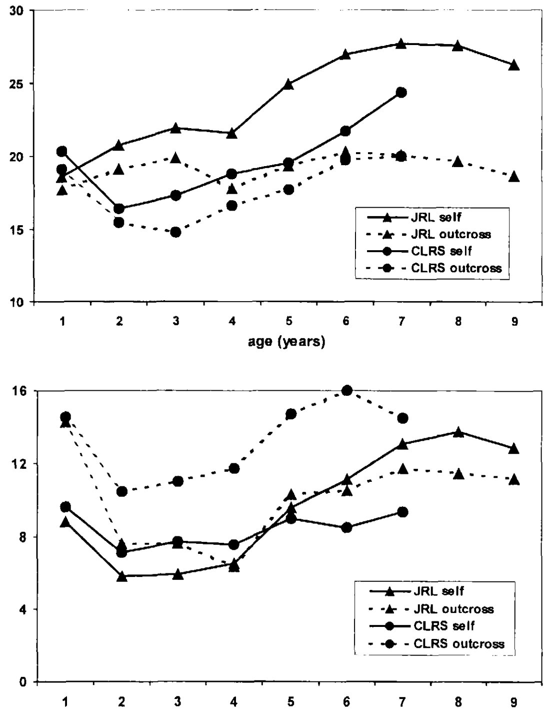

Figure 4. a) Phenotypic coefficients of variation for western redcedar self and outcross progeny heights versus age at two field sites; b) additive coefficients of variation for western redcedar self and outcross progeny heights versus age at two field sites.

self-family estimates of additive coefficients were less than outcross values at CLRS but similar or greater at JRL (Fig. 4b). All estimates of coefficients of variation tended to increase over time after an initial decrease from age 1 to age 2. Adjustments to heights using ANCOVA on age-1 values tended to decrease all estimated coefficients although there seemed to be a greater effect on outcross estimates (details not shown).

Ratios of estimated among-parent variance components for self-families relative to those for outcross families at JRL approached 4 as the trees aged (Table 6), and the corresponding ratios for estimated within-plot error variances remained at or near 1. The corresponding ratios of estimated among-family variances were lower at CLRS, fluctuating between 1.5 and 2, which paralleled the pattern for heritability estimates from the self-families which were markedly lower than those from the polycross families there. However, after the covariance adjustment for age-1 heights was used, the ratio increased considerably, to around 2.5 (Table 6). For within-plot variance estimates these self- to outcross ratios gradually increased from 1 to 1.3 at age 7 at both sites.

DISCUSSION

Nursery growth and observations

As has been reported previously (OWENS *et al.* 1990; CHERRY 1995), there was no detectable ID in the self progenies for very early life-history traits, which represents one of the few known exceptions to the norm for tree species (HUSBAND & SCHEMSKE 1996; WILLIAMS & SAVOLAINEN 1996). In this study, there were no evident differences in germination or nursery survival, and no visible aberrations indicative of the expression of markedly deleterious alleles as are

Table 6. Ratios of self- to outcross among-progeny and within-plot variances for heights at different ages at two field sites with and without covariance adjustment as ht-1.

	Famila variance ratios				Error variance ratios			
	Jordan R Low		CLRS		Jordan R Low		CLRS	
	no covar	covar	no covar	covar	no covar	covar	no covar	covar
1	1.42	-	1.70	-	0.96	-	0.99	-
2	2.10	3.10	1.70	0.90	1.01	1.00	1.07	1.18
3	2.10	8.49	1.69	1.39	1.10	1.06	1.19	1.22
4	3.60	12.27	1.45	2.28	1.30	1.27	1.07	1.23
5	2.81	4.60	1.28	1.89	1.13	1.12	1.12	1.32
6	3.51	4.63	0.93	1.46	1.19	1.12	1.18	1.38
7	3.92	4.78	1.32	1.92	1.18	1.12	1.30	1.57
8	4.55	5.21			1.17	1.14		
9	4.29	4.72			1.18	1.18		

typically seen in other conifer species. Also, there was very little indication of any ID at early stages of growth in a greenhouse environment.

Post-nursery ID

It is clear that ID, which became marked at JRL and CLRS, arose almost entirely in the post-nursery phase. Percent ID became much greater in this phase, and ANCOVA results supported this interpretation by showing differences in ID after adjusting for covariance on first-year and subsequent years' heights. The onset of significant ID for western redcedar after the nursery phase suggests that ID for height growth was expressed more strongly in the less benign field environments. Examples of ID being weakly expressed in near-optimal nursery environments have been reported in conifers (ERIKSSON & LINDGREN 1975; LINDGREN 1975) and other plant species (see HUSBAND & SCHEMSKE 1996). However, ID did not appear to become more severe after the first few years in the field, and this accords with some results reviewed by WILLIAMS & SAVOLAINEN (1996). At JRL and CLRS the pattern may have been influenced by early canopy closure. At JRL, the leveling- off of ID after age 6, corresponding roughly to an average tree height of three metres, could have been the result of canopy closure affecting environmental influence on ID. On the other hand, the slight decrease in ID between ages 3 and 5 at CLRS coincided with severe competition due to tight spacing, slowing growth of all trees. ID subsequently increased at CLRS after 50 % of the trees were systematically thinned, and was still on a linear trajectory at the termination of the trial when average tree height was over 2 metres. Examples of ID reduction due to size-specific mortality resulting from competition after crown closure has been reported elsewhere (HARDNER & POTTS 1997; KOELEWIJN *et al.* 1999; SORENSEN 1999). Unlike the tree species in the above studies, western redcedar is a late successional species and competition-induced mortality may have less of an impact on ID as stand development progresses over time.

The lack of evident ID at JRH is noteworthy, given the intuitive expectation (cf ERIKSSON & LINDGREN 1975; LINDGREN 1975) that ID would be more strongly expressed in a harsh environment than in a benign one. Certainly this trial, which was severely affected by exposure to cold, failed to give any precise information. It may have been that height was not the best growth variable to study for ID, since it would seem that any height advantage had been negated by repeated cold-damage. However, the high survival and similar cold-hardiness of both self- and outcross individuals raises some interesting questions on the effect of ID on adaptability. These results are partly supported by CHERRY (1995), who was unable to find significant differences in seedling cold-hardiness between self- and outcross families of western redcedar. In addition, there were minimal physiological differences in the ability of western redcedar self progenies to withstand drought as compared to outcross (N. LIVINGSTON & RUSSELL, unpubl. data). It would seem that, although exposure to a less benign environment results in reduced height growth for self trees, measurable physiological functions do not appear to be affected.

The majority of conifer species exhibit substantial, yet variable, ID for growth in young trees (see reviews in HUSBAND & SCHEMSKE 1996; WILLIAMS & SAVOLAINEN 1996). Results from these studies are not directly comparable to results presented here, since the effects of deleterious recessive alleles and associated mortality are generally not taken into account. Poor survival of self progenies is common for most conifers thus the greater ID in response to selfing reported in other studies would be conservative given the associated mortality. Certainly most tree species exhibit very high population selfing ID as compared to western redcedar at a similar age (*eg.* HARDNER & POTTS 1997; KOELEWIJN *et al.* 1999; SORENSEN 1999).

It is interesting to note that in coastal Douglas-fir, which is often a close associate of western redcedar but a member of the Pinaceae, population ID for seedling height upon selfing increased from 5 % at outplanting (after considerable mortality and nursery culling) to 30% at age 5 when planted on a site similar to CLRS (J. H. WOODS pers. comm.).

Results from a pollination study with western redcedar (OWENS *et al.* 1990) indicated that selfing may be less detrimental to seed production than other conifers, and EL KASSABY *et al.* (1994) concluded that this species may experience significant outbreeding depression rather than a lack of inbreeding depression. This study, however, using a rigorous statistical criterion (cf BURDON & RUSSELL 1999, Eqs. 4 and 5) clearly shows that although there were very occasional statistically significant cases of early outbreeding depression, these were not persistent over time and inbreeding depression became more marked.

Comparative ID among seed-parents

Considerable variation among individual seed-parents for height growth ID upon selfing, as was seen in this study, has been reported in other tree species (*e.g.* WILCOX 1983; SNIEZKO & ZOBEL 1988; TAJIMI 1990; MORRICA *et al.* 1994). While proper account was often not taken of the differential additive coefficients of relationship between self- and outcross families, this individual variation observed in other studies was almost certainly valid. For example, in the Douglas-fir trial mentioned above, GCA-adjusted individual selfed ID varied from 17.2 % to 38.0 % at age 5 (J. RUSSELL unpubl. data).

In our study the estimated genetic correlations between self- and outcross performance were generally imperfect, which also reflects some seed-parent × mating-type interaction. Nevertheless, while imperfect, these correlations were strong and positive, and certainly significantly above 0.5, a number which has been suggested as an appropriate minimum value in considering the use of self-families for GCA estimations (WILLIAMS & SAVOLAINEN 1996). In stark contrast, correlations between self- and outcross family means reported in the literature for growth have tended to be low to nonexistent (see WILLIAMS & SAVOLAINEN 1996). An exception was a study with sugi (*Cryptomeria japonica* D. Don) which had a phenotypic correlation of 0.8 for height at 4 years despite 36 % ID, however, the correlation was reduced to 0.53 by age 15 (TAJIMA 1990).

A recent study by KUMAR (2003) indicated near-zero genetic correlation between selfed and outcrossed families of *Pinus radiata* D. Don for stem diameter (ages 9 and 26), but strong positive correlations for wood density and for straightness- and branch-habit scores. Diameter in *P. radiata* appeared to be more sensitive than height to maladaptation to a site (BURDON 1971), suggesting that it may be more subject to ID than height and show lower self-outcross genetic correlations than height.

Additive genetic variation among seed parents

Evidence of significant additive genetic variation in fitness traits, as reported in this study, is not unique, despite depauparate genetic variation in neutral biochemical and DNA markers. In fact, there is both theoretical and empirical evidence that functional additive genetic variation can recover more rapidly after a severe bottleneck than neutral genetic marker variation (see review in LYNCH 1996). This could be attributed to both a greater genomic mutation rate for quantitative traits and the conversion of nonadditive genetic variation to additive following a population bottleneck.

This is the first published report of significant additive genetic variation in western redcedar using material that is not biased by differential amounts of selfing and ID (as is common with open-pollinated material). The level of genetic variation certainly offers scope for achieving genetic gains in growth rate. In fact, the estimates of heritability and additive genetic coefficients of variation for height are high compared with those for Monterey pine (*Pinus radiata* D. Don) at comparable stages of development (BURDON 1992; BURDON *et al.* 1992), this latter species having responded very well to recurrent selection. In addition to significant variation among polycross progenies, strong positive genetic correlations between mating-types and between-site genetic correlations strengthens the argument for significant genetic variation in western redcedar for height growth.

The strong positive genetic correlations indicate that in this species, self-family performance can be a good guide to parental breeding values. However, the higher repeatabilities of self-family means would need to effectively off-set imperfect correlations. On the other hand, there are logistical advantages with respect to length of breeding for selfing as opposed to an outcrossing mating scheme, as well as potential increases in effective selection intensity (WILLIAMS & SAVOLAINEN 1996). In comparison, the use of open-pollinated progeny for evaluating parents will be complicated by variation among seed-parents in percentage selfing (EL-KASSABY *et al.* 1994), in combination with differential susceptibility to ID.

Actual versus theoretical variances

There were marked departures at CLRS as compared to JRL below the theoretical ratio of 4:1, assuming an additive genetic model, for estimated self- to polycross family variance. However, the ratio approached 3:1 for age-4 heights, after adjusting for covariance on nursery heights, indicating that possibly with time and fully efficient adjustment for maternal effects, near-theoretical variance structures may be realized. If there was severe and highly variable ID among seed parents one might have expected departures from theoretical ratios in the opposite direction.

Within-family self- to outcross variance ratios were close to unity, or slightly higher; thus indicating that variation within self-families was higher than expected, on the basis of an additive genetic model, relative to the polycross families. This is typical of other gymnosperm studies (*e.g.* SNIEZKO & ZOBEL 1988), but unlike in this study, the increased variation is usually attributed to the expression of strongly deleterious mutations. In fact, ROBERTSON (1952) identified elevated variation within self-families as an expected consequence of low-frequency recessive alleles constituting genetic load, although in this case the evident absence of markedly deleterious recessive alleles (namely a notable lack of runts or chlorophyll-defectives, etc.) would seem to argue against a major role of dominance.

Another possible factor contributing to elevated variance within self-families is lower developmental homeostasis. FALCONER (1989) illustrated the increase in expression of environmental variance within inbred lines of various organisms and hypothesized that under directional dominance, inbred individuals may have less homeostasis. There is growing evidence that western redcedar has a generalist adaptive strategy (REHFELDT 1994; CHERRY 1995) and displays phenotypic plasticity in growth and adaptive traits (EL-KASSABY 1999; RUSSELL unpubl. data). It is conceivable that self progeny have less developmental homeostasis when outplanted into natural environments. However, this would not seem to be the case at JRH, where both self- and outcross trees exhibited similar responses to a harsh environment.

Broader interpretation in relation to breeding system

Western redcedar, along with other *Thuja* spp., have some of the highest measured selfing rates of any conifer species (PERRY & KNOWLES 1990; XIE *et al.* 1991; EL-KASSABY *et al.* 1994; LAMY *et al.* 1999; O'CONNELL *et al.* 2001; O'CONNELL 2003). There is no indication in this study of any recessive lethals or sublethals in any traits observed from reproduction through to growth and survival. There was a small but increasing ID for seedling growth, but not for survival or adaptability, during the first few years in the field. There is also substantial variability among populations and genotypes for selfing rate (EL-KASSABY *et al.* 1994; O'CONNELL *et al.* 2001). Although we observed some variability among seed- parents for ID for growth after a year in the field, estimated genetic correlations between self- and outcross families, although imperfect (reflecting the differential levels of ID), were strongly positive and generally very significant statistically. Evidence of significant additive genetic variation in growth traits was found in this study, as well as in other studies (REHFELDT 1994; CHERRY 1995; RUSSELL unpubl. data), despite a lack of detectable isozyme and RFLP variation (COPES 1981; VON RUDLOFF *et al.* 1988; YEH 1988; EL-KASSABY *et al.* 1994; GLAUBITZ *et al.* 2000). Recently, O'CONNELL (2003) has shown substantial among-population variation in microsatellites for western redcedar, and variation among families and individuals for needle monoterpene levels correlated with deer-browse intensity has been reported by VOURC'H *et al.* (2002).

It would seem that western redcedar has either purged all or most of its recessive and strongly deleterious mutations (at least those affecting early life history fitness), or maintains a lower equilibrium frequency of lethals through partial selfing with selection as opposed to random mating. There is paleontological evidence that western redcedar went through some recent bottleneck(s) during the last glaciation and possibly through repeated bottlenecks during the Pleistocene Epoch (CRITCHFIELD 1984; O'CONNELL 2003). Western redcedar maintains low levels of inbreeding depression that are expressed after reproduction and early growth, and substantial additive genetic variation in growth traits. If the background level of inbreeding depression is low, selfing rates of a few percent are theoretically very effective in purging recessive lethal or sublethal mutations from a population by increasing the frequency of rare lethal genotypes in selfing progeny (LANDE & SCHEMSKE 1985; CHARLESWORTH & CHARLESWORTH 1987). On the other hand, given the apparent high mutation rate of western redcedar

(O'CONNELL 2003), the absence of early ID does not necessarily translate to an absence of a substantial mutation load if deleterious recessives have become fixed within populations (LYNCH 1996).

In contrast to the situation for early-acting deleterious recessives, theoretical analysis (LANDE & SCHEMSKE 1985; HEDRICK 1994) has indicated that the genetic load component of ID comprising nearly additive, mildly deleterious lethals, as well as genetic variation in quantitative traits, is difficult to purge by inbreeding, since some of it can become fixed by random processes in the absence of periodic outcrossing. It would seem that the observable amounts of polygenic mutations in conifers appear to be capable of maintaining much of the heritable variation in quantitative traits even in predominantly selfing populations (HEDRICK 1994). In any event, western redcedar evidently possesses additive genetic variation in growth traits, despite high levels of selfing and the early results using biochemical markers.

Gymnosperms are known to have high genomic mutation rates per generation which can favour the evolution of outcrossing (MORGAN 2001). This may or may not reflect somatic mutations accumulating during mitosis prior to meiosis – in this connection, conifers are generally much more sensitive than angiosperms to short-wavelength ionising radiation (OLEKSYN 1991). As well, conifers that reproduce asexually, as western redcedar often does, could well accumulate more mutations per sexual Generation. O'CONNELL (2003) estimated a mutation rate of 6.3×10^{-4} per locus per generation based on a single somatic mutation using microsatellites. Western redcedar evidently maintains an effectively low load at mutation-selection equilibrium, with effective purging in nature of deleterious mutations which do not show up even in a benign experimental environment. This significant per-generation mutation rate could account for the high genetic diversity reported for microsatellite markers that are evidently neutral (O'CONNELL 2003), while the lack of polymorphisms for isozymes would accord with the proposition (STRAUSS & LIBBY 1987) that low-frequency isozyme alleles do carry a slight fitness cost even in the heterozygous state.

Archegonial polyembryony may play an important role here. An apparently high number of archegonia per ovule (average of 10–12; OWENS & MOLDER 1980), which is typical of Cupressaceae, is likely to have a paradoxical role: it would seem conducive to purging the load, yet at the same time it would allow for high self-fertility despite a considerable embryo lethal load. On the other hand, others have found little evidence of a significant reduction in the genetic load due to polyembryony (HEDRICK *et al.* 1999; O'CONNELL (2003). It may be that, while significant load remains, repulsion linkages between deleterious recessives at different loci reduce the potential of archegonial polyembryony to assist the process of purging. There still remains the question of why archegonial poyembryony should persist so strongly in conifers if it is not playing a positive role in the genetic system.

Although juvenile offspring of three *Thuja* species have resulted from prevalent selfing, the adult parental populations tend to present a different picture. In three populations of *T. occidentalis* and four of *T. orientalis*, the parental trees were either in Hardy-Weinberg equilibrium or had an excess of heterozygotes (PERRY & KNOWLES 1990; XIE *et al.* 1991). As well, YEH (1988) showed that the parental populations of western redcedar were in Hardy-Weinberg (H-W) equilibrium using F-statistics inferred from haploid tissue. The trees selected for the YEH (1988) study were mature dominant or codominants stems in extensive mixed coniferous stands. Thus there was a greater potential for outcrossing in these populations as opposed to isolated, sparse, open-grown stands of immature to mature redcedar that are more typical of peripheral and riparian populations. More recently, O'CONNELL (2003) obtained a positive inbreeding coefficient of $F = 0.085$ (cf $F = 0$ effectively assumed in our Eq. 3), indicative of inbred seedlings surviving to sexual maturity. However, this still translates into an ID rate of 0.55 with respect to survival to sexual maturity.

If parental populations are at or near H-W equilibrium, then most selfed seedlings do not survive to the reproductive stage. It is conceivable that western redcedar harbours deleterious recessive mutations that are expressed in later life stages affecting survival and reproductive capacity. If this is the case, then cumulative ID in western redcedar should approach 1 despite the lack of early recessive lethals. This would be more typical of other conifer species (KOELEWIJN *et al.* 1999, SORENSEN 1999) except in the timing of ID. LANDE *et al.* (1994) explained the apparent contradiction in gymnopsperms, which have high ID despite intermediate primary selfing rates, as either high mutations to recessive lethals or selective interference among loci with deleterious recessive mutations under partial selfing. Purging of recessive lethals does not occur until a threshold selfing rate is met or exceeded. O'CONNELL (2003) estimated a high mutation rate for western redcedar which has been proposed for conifers by KLEKOWSKI (1988) due to the lack of a separate germ line. As well, mitotic (*i.e.*, somatic) mutations can accumulate at a greater per-generation rate since conifers, such as western redcedar, have a long life span and can propagate vegetatively. Recent studies with other conifer species, however, indicate that there is not as high a mutation rate as anticipated (DVORNYK *et al.* 2002, CLOUTIER *et al.* 2003).

However, it seems conceivable that a 10% reduction in growth of self trees five or more years after germination – even though we did not find any intensification of ID at JRL in the later stages of our study – could translate to decreased fitness relative to outcross progeny in a pure or mixed coniferous stand in which density-dependent selection can operate. It may be noted that stem diameter growth, which is far more sensitive to competition than height growth, is much the more important component of individual tree size in closed stands and, as such is likely to dominate variation in reproductive capacity. On the other hand, in an open-grown population where tree-to-tree competition is less, the small inbreeding depression in growth may allow self trees, especially in the absence of outcrossed pollen due to isolation, to reach reproductive maturity. This, however, may also apply in large measure to predominantly outcrossing species. It is interesting to note the results of SORENSEN (2001) which indicated that in the outbreeding *Pinus contorta* a peripheral population showed signs of some purging of deleterous recessives, with less severe inbreeding depression compared with two non-peripheral populations. Although the selfs of western redcedar may survive to reproductive maturity and thus result in potential purging of its genetic load, there is as yet no information on the viability of seed after repeated generations of selfing.

Evolutionary stability of outcrossing

The evolutionary stability of outcrossing, despite the strong theoretical selective advantage of genes that tend to increase the rate of selfing, is attributable to the high inbreeding depression maintained by recessive lethals and sublethal mutations in large outcrossing populations (FISHER 1941). Genetic models have shown a variety of results from species evolving to complete outcrossing or selfing, depending upon the degree of ID (*e.g.* LANDE & SCHEMSKE 1985; HOLSINGER 1988), to stable mixed mating (*e.g.* DAMGAARD *et al.* 1992; CHEPTOU & MATHIAS 2001). In addition, models that include essential information on plant ecology and reproductive biology that can influence the evolution of mating systems, can result in stable intermediate selfing rates (*e.g.* HOLSINGER 1991). PERRY & KNOWLES (1990) found a higher selfing rate in a population of scattered eastern white cedar that had less availability of pollen from outside sources. More recently, O'CONNELL (2003) showed a positive relationship between outcrossing rate and tree height, but no difference between ecologically-divergent populations. Pollen availability was cited as the factor contributing to these trends.

Whether the mixed mating system is a transitory condition evolving towards nearly-complete selfing or is evolutionarily stable is conjectural. However, the importance of inbreeding and associated ID in determining population genetic structure in western redcedar, cannot be discounted. The use of open-pollinated families and populations, with its associated variation in individual selfing rates and ID, is likely to incur major bias in studying quantitative traits in western redcedar. On the other hand, the ability of this species to self freely in the absence of deleterious recessives and minimal effect of late-acting mildly deleterious alleles, as well as to successfully propagate vegetatively, makes western redcedar an excellent candidate for pursuing alternative tree breeding strategies. It is also an ideal tree species to investigate the genetic basis of ID using methods such as those developed by FU & RITLAND (1994).

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