1. male flowering early and female cone late type: fp/mp =< 1.5. the following clones belong to this group: 7434, 7430, 7416, 16, 7407, 33, 07 and 13.

2. female and male flowering coincidence: 1.5 < fp/mp < 5.0. the following clones belong to this group: 7408, 7405 and 25.

3. female flowering early and male late type: fp/mp >= 5.0 the following clones belong to this group: 7420, 43, 04, 7316, 7431, 7444, 431 and 7415.

Discussion

When the seed orchard of Chinese fir at Chongyang was established, there was little knowledge about the flowering characteristics, so the seed production was low and the self-fertilization could not be avoided. Knowledge of flowering characteristics is very useful in the improvement or establishment a new seed orchard (e.g. the clone selection and the number of clones, the ratio of mainly female clones to mainly male clones, the ratio of mainly female trees to pollen trees, the number of clones). We need to have as many mainly female clones as possible together with enough mainly male clones to produce enough pollen for dispersal and good fertilization depending on the seed orchard size, the main wind in pollen dispersal season, and the topography of this seed orchard. The reasonable ratio of pollen trees to female can be estimated (ZHANG ZHUO-WEN et al., 1990, 2001; CHEN XIAOYANG et al., 1991 and 1996). This can be improved by using clones which female cones and male cones do not flower coincidence at the same time to avoid self-fertilization and produce high genetic quality seeds. Only in this way can we be able to control or avoid self-fertilization in seed orchard.

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Improving Precision of Breeding Values by Removing Spatially Autocorrelated Variation in Forestry Field Experiments

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Summary

Adjustment for micro-environmental heterogeneity in inadequately blocked field experiments is desirable to improve estimates of genetic parameters and to maximize genetic gains from selection. In three forestry field trials of red alder (*Alnus rubra* Bong.) we removed spatially autocorrelated variation with kriging and evaluated the effect on estimates of treatment means and heritabilitiy compared to standard analysis. Kriging removed block effects and reduced the family × block interaction in all traits. The variation due to interactions was recovered in simple family or provenance variance components, which increased by up to 40 % in some traits. Heritability estimates and expected gains from selection increased accordingly, while the standard error of the estimate for family and provenance means decreased. The improvement was largest in experiments where blocking was clearly inadequate to capture site variation, when block size was large, and for traits that could be influenced by variation in soil properties. Bud break and leaf abscission, which are presumed to be independent from variation in soil, were spatially independent. Heritabilities estimated from an experiment with incomplete block design with nine trees per block could only be minimally improved. We recommend that variograms should routinely be constructed in the analysis of forestry field trials to test if residuals from standard models are spatially autocorrelated. If they are, kriging is proposed as a useful supplement to ANOVA in tree breeding experiments and other forestry field trials.

Key words: Kriging, heritability, tree breeding, red alder.

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Introduction

Data collected from forestry field experiments often varies within replicated blocks due to environmental gradients such as soil fertility, moisture, or previous land use. Careful layout of blocks and intensive site preparation can reduce this residual error in field experiments, but at the time of evaluation inadequate blocking is frequently discovered nonetheless. One of the most effective means to ameliorate effects of site variation has been the use of incomplete block designs with small blocks. Alpha designs, a class of generalized lattice designs, have become popular for managing environmental gradients in forestry experiments (CORRELL and CELLIER, 1987; KLEIN, 1989; WILLIAMS and MATHESON, 1994; JOHN, 1995). Nonetheless, complete block designs are still being used, and the design of many progeny trials in long-term forestry experiments pose a serious impediment to precise estimates of genetic parameters and breeding values. ERICSSON (1997) suggested that incomplete blocks should be imposed on a trial at the time of evaluation regardless how the experiment was originally designed, and that the optimal block size be determined as the one that yields the highest heritability. However, since heritabilities or other genetic parameters are estimated with an error, choosing the experimental design by trial and error may result in overestimation of heritabilities.

An alternative approach is to model site variation as a smooth surface, which is appealing since blocks with distinct boundaries are conceptually not ideal to model continuous site variation. Numerous methods have been proposed to model spatial variation, such as trend surface analysis (THOMSON and EL-KASSABY, 1988), least squares smoothing (GREEN et al., 1985; CLARKE et al., 1994, 1996), separable ARIMA process (CULLIS and CLEESON, 1991), nearest neighbor methods (WILKINSON et al., 1983; STROUP and MULITZE, 1991) or kriging (BRESLER et al., 1981; SAMBRA et al., 1989; LOPEZ and ARRUE, 1995). Several authors compared these and other methods for use in agricultural field trials (SAMBRA et al., 1990; BROWNIE et al., 1993; STROUP et al., 1994; CLARKE and BAKER, 1996). Although rarely used, kriging proved to be suitable to remove micro-environmental heterogeneity in agricultural experiments, while some other methods have been shown to increase the error of treatment estimates occasionally. Moreover, there is a wealth of literature in soil science showing that soil properties such as soil water, nutrient, silt, and clay content are spatially dependent and can best be modeled with kriging (e.g., BURGESS and WEBSTER, 1980a, 1980b; BURROUGH, 1993). Since variation in growth of trees is often caused by variation in soil properties, it appears sensible to apply the same methodology to spatial variation in forestry field experiments.

The objective of this study was to investigate whether the kriging method is suitable to recover family × block interactions that are due to environmental within-block variation in forestry field trials, and whether the precision of breeding values could be increased. Further, we provide estimates of genetic parameters in red alder populations for the purpose of genetic resources management. Such information provides a measure of genetic variability in traits, and is necessary to predict gains from selection in a tree improvement program.

Materials and Methods

Field experiments

We evaluated two trials with a split-plot design for genetic testing (experiment 1 and 2) and one trial with an incomplete block design (experiment 3). The traits measured were three-year height and diameter, the date of leaf abscission, and the date of bud break as described in HAMANN et al. (1998). The two standard trials were split-plot designs with up to 55 provenances from British Columbia. The trials had three replications, provenances located in main-plots, and five families per provenance located in sub-plots. They were analyzed according to the following model:

$$Y_{ijkl} = \mu + B_i + P_j + P \times B_{ji} + F(P)_{k(j)} + F(P) \times B_{k(ij)} + \varepsilon_{l(ijk)}$$

where Y is the measurement of seedling l in block i from provenance j and family k; μ is the overall mean; B, P, and $F(P)$ are the effects of block, provenance, and family within provenance, respectively; $P \times B$ is the interaction effects of block with provenance and also the main-plot error; $F(P) \times B$ is the interaction effects of block with family within provenance; and ε is the experimental error.

The third trial with advanced experimental design was an incomplete block design with 4 provenances from Alaska and 20 families per provenance. We used a lattice square design with 10 replications, each subdivided into nine incomplete blocks with nine trees. Since nine mutually orthogonal latin squares are known (GRAHAM et al., 1995), a layout could be derived, where each family shared an incomplete block once and only once with every other family. Although this design is for 81 treatments and had a slight imbalance due to one missing treatment in each replication, the statistical efficiency calculated from the incidence matrix according to WILLIAMS and MATHESON (1994) was 10 % better than what could be achieved with computer generated alpha-designs (CSIRO/BIOSS, 1994). The following linear model was used for analysis:

$$Y_{ijkl} = \mu + B_i + I(B)_{j(i)} + P_k + P \times I_{ik} + P \times I(B)_{jk(i)} + F(P)_{l(k)} + F(P) \times B_{il(k)} + F(P) \times I(B)_{jk(i)} + \varepsilon_{m(ijkl)}$$

where $I(B)$ are incomplete blocks within complete blocks. The other symbols are as above. Variance components, assuming a random model, were estimated using the restricted maximum likelihood method of PROC MIXED (SAS INSTITUTE, 1999) in all experiments.

Parameter estimates

From the variance components, σ^2, individual heritabilities, h^2, were estimated as follows:

$$h^2 = 4\ \sigma^2_{F(P)} / (\sigma^2_{F(P)} + \sigma^2_{B \times F(P)} + \sigma^2_{I(B) \times F(P)} + \sigma^2_E)$$

This heritability would be used to estimate gains from within provenance selection. Selection in a breeding program for British Columbia would likely be across provenances. We therefore also calculated heritabilities for individual selection across all provenances in B. C. as an upper bound according to the formula:

$$h^2_{BC} = 4\ \sigma^2_{F(P)} + \sigma^2_P / (\sigma^2_{F(P)} + \sigma^2_P + \sigma^2_{B \times F(P)} + \sigma^2_{I(B) \times F(P)} + \sigma^2_{B \times P} + \sigma^2_{I(B) \times P} + \sigma^2_E)$$

This value should be an overestimate because the assumption of random mating is likely to be violated, and because a tree breeding program for red alder in British Columbia would likely comprise several breeding populations for different environments. Heritabilities of traits for selection within such populations will be between the two values h^2 and h^2_{BC}. Standard errors of heritabilities, $SE(h^2)$, were estimated according to the delta-method (LYNCH and WALSH, 1998, Appendix 1) based on the matrix of variances and covariances associated with estimates of variance components generated using the restricted maximum likelihood method of PROC MIXED (SAS INSTITUTE, 1999).

Spatial analysis

Environmental heterogeneity was detected as spatial dependence of residual errors after partitioning of variance components according to the previous models. Maps of residuals from
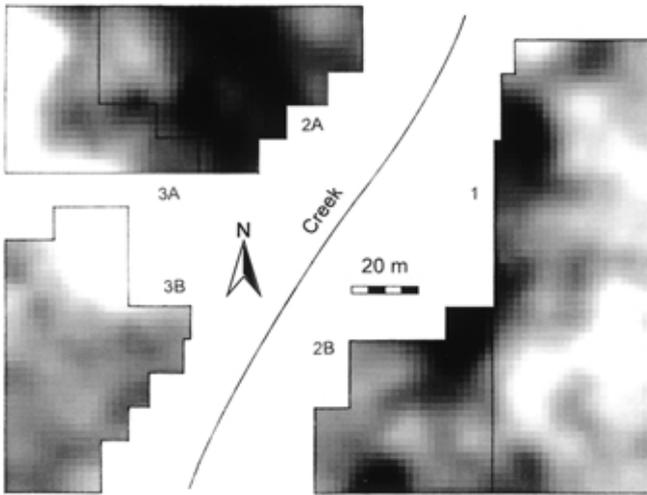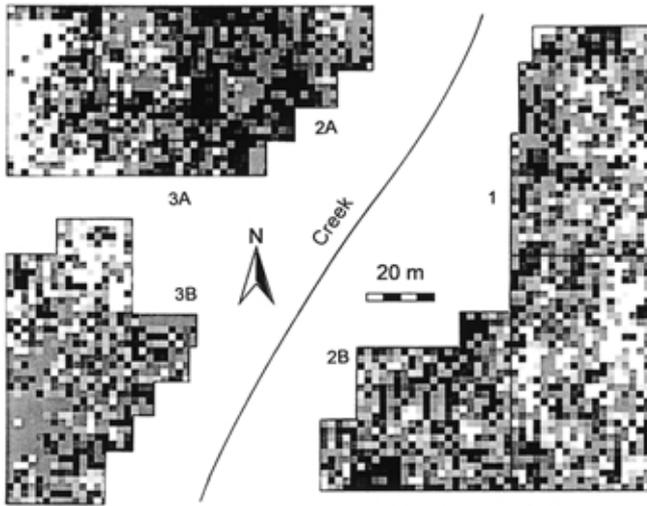
Figure 1. – Residual error variation after analysis of variance (top) and variation due to autocorrelation in residual values (bottom). Light shades indicate negative values, dark shades represent positive values (residuals range from –38 to 35 cm). The numbers refer to (discontinuous) blocks.

Figure 2. – Semiovariograms for height (top) and bud break (bottom) for experiment #1.

the above models without the block effects were generated, and this variation was partitioned into spatially autocorrelated variation and random error with the kriging method. Kriging is an optimal interpolation method that yields smooth varying surfaces of best linear unbiased predictions (BLUP) of values on a spatial grid. Kriging is a two step process involving the construction of a variogram that provides information for optimal interpolation weights, and the interpolation itself. Variograms are functions that are fitted on a plot of the squared differences among all pairs of sample points against their Euclidean distances. Usually, a so-called "spherical model" is fitted that starts at a minimal value at zero distance (indicating the highest spatial dependence), increases linearly at first and then gradually approaches a maximum value (indicating no spatial dependence beyond a certain distance among sample points). The interpolation is then performed with the equation:

$$z(x_0) = \Sigma(\lambda_i \cdot z(x_i))$$

where $z(x_0)$ is the value to be predicted at location x_0, $z(x_i)$ is the value of a variable sampled at location x_i, and λ_i is the interpolation weight. This weight depends on the distance between xi and x_0 and is derived by complex calculations based on the variogram function so that the estimate of $z(x_0)$ is unbiased and
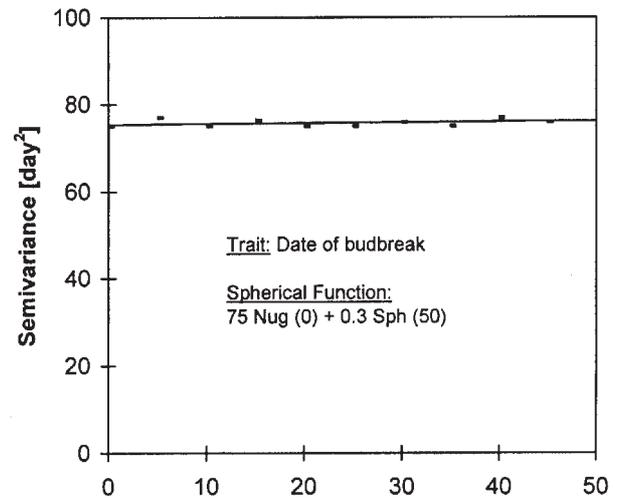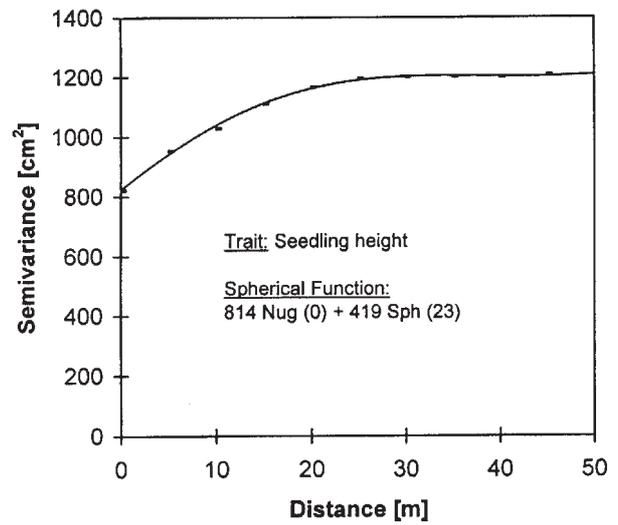
that the estimation variance for $z(x_0)$ is less than for any other linear combination of the observed values (BURROUGH and MCDONNELL, 1998)[1]. The program GSTAT[2] by PEBESMA and WESSELING (1998) was used to fit variogram models and to generate maps of predicted values and variances. Subsequently, we compared results from standard analysis, and the same analysis after original data had been corrected by subtracting the amount that was determined to be due to spatial autocorrelation. The GIS software ARCVIEW was used to generate maps of residuals and predicted values.

Results and Discussion

Spatial dependence of residuals

Plots of the residual error variation after analysis of variance revealed non-random patterns in height and diameter that were not accounted for by blocking. Spatial patterns of residuals are illustrated for experiment 1 (*Figure 1, top*), where

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[1] Refer to Chapter 6 of BURROUGH and MCDONNELL (1998) for a thorough introduction to geostatistical methodology.

[2] Freeware available at http://www.gstat.org

Table 1. – Variance components and individual heritabilities using the normal model (N), and adjustment for spatially autocorrelated variation (A).

Trait	Model	Variance components									h^2	(Sterr)	h^2_{BC}	(Sterr)
		B	P	P*B	I(B)	P*I	F(P)	F*B	F*I	E				
Experiment 1 (Split-Plot Design)														
Height	N	61	151	280			168	63		1604	0.37	(0.05)	0.56	(0.05)
Height	A	1	191	0			181	27		1563	0.41	(0.05)	0.76	(0.05)
Diameter	N	1.3	0.6	1.0			2.7	0.8		61.2	0.17	(0.04)	0.20	(0.04)
Diameter	A	0.6	1.0	0.0			2.9	0.3		60.9	0.18	(0.04)	0.24	(0.04)
Leaf absc.	N	2.6	21.3	2.7			8.1	1.4		50.4	0.54	(0.06)	1.41	(0.06)
Leaf absc.	A	0.2	21.9	0.0			8.3	0.8		49.7	0.56	(0.06)	1.50	(0.06)
Bud break	N	0.2	4.6	1.6			3.8	1.5		38.4	0.35	(0.05)	0.67	(0.05)
Bud break	A	0.1	4.9	0.0			4.0	0.9		38.4	0.37	(0.05)	0.74	(0.05)
Experiment 2 (Split-Plot Design)														
Height	N	124	187	214			231	92		2740	0.30	(0.09)	0.48	(0.09)
Height	A	5	265	18			260	15		2811	0.34	(0.09)	0.62	(0.09)
Experiment 3 (Incomplete Block Design)														
Height	N	5.4	2.6	1.1	0.2	0.0	7.9	12.1	0.2	35.3	0.57	(0.11)	0.70	(0.11)
Height	A	0.0	2.6	0.5	0.0	0.0	8.1	10.9	0.0	35.2	0.59	(0.11)	0.75	(0.11)

Note: B, block; I(B), incomplete block within block; P, provenance; F(P), family within provenance; E, experimental error; h^2, heritability; h^2_{BC}, heritability for selection across provenances in British Columbia; Sterr, standard error.

dark shades indicate larger values than those predicted by ANOVA and light shades represent values below expectations. Residuals are spatially dependent up to a distance of approximately 25 m according to the semivariogram in the upper part of *Figure 2*, where the squared difference among any two observations is plotted as a function of their distance. The squared difference or semivariance does not increase beyond 25 m indicating spatial independence, and it has a value of approximately 800 cm² at zero distance, which is the residual variance that cannot be explained by spatial autocorrelations. The variogram function was then used to model site variation with kriging (*Figure 1, bottom*). Strong spatial dependence in height growth was also observed in experiment 2 with a patch size of approximately 20 m. Spatial dependence in experiment 3 could be observed up to approximately 40 m only when analyzed as a randomized complete block design. The patch size of micro-site variation is useful information to determine optimal block size for alpha-designs laid out on comparable sites (FU *et al.,* 1999). In contrast to growth traits, residuals for phenological traits were spatially independent, illustrated for bud break in the lower part of *Figure 2*. The likely explanation is that these traits are less influenced by site variation and only respond to variables such as temperature and day length.

When constructing variograms several problems could be encountered. A stable variogram cannot be obtained when the site has a global environmental gradient, because the squared difference of two observations will linearly increase with distance instead of leveling off. In this case, the global gradient must be modeled separately, e. g. by including a regression function in the general linear model. A similar problem can arise if blocks are laid out with large distances between them, and large differences among blocks. Then, separate variograms should be constructed for each block. Finally, this methodology should not be used if site variation is discrete, for example if a previous road through the trial caused soil compaction. Stable variogram functions can only be obtained if the site variation is continuos and the gradients across patches are more or less homogeneous.

Another problem may arise when experimental units are multi-tree plots, and a portion of the spatial dependence observed in the variogram is due to individuals within one plot receiving the same treatment or being the same genotype. This effect would be apparent in the variogram as a sudden drop in semivariance at the distance equivalent to the plot size. One solution could be to ignore the small distance values when developing the variogram function. This is possible because fitting a variogram function is an interactive process in most software packages. Alternatively, the plot mean of a multi-tree plot should be used for kriging.

Variance components and heritabilities

After predicted residuals had been subtracted from the original data, the analysis was repeated and the changes in variance components are shown in *Table 1*. Block effects approach zero as expected, and also interactions of provenance and family effects are eliminated or substantially reduced. Simple effects due to families and provenances increase accordingly, while the residual error variation stays approximately the same. Particularly in experiments 1 and 2, variance components due to provenances are substantially increased for growth traits (between 20 and 40 %). This is reflected in an increase of heritability for selection across provenances (h^2_{BC}). The explanation for the limited effect on heritability for within provenance selection (h^2) lies in the experimental design. The split-plot design with families in subplots ensures a better estimate of family within provenance effects, because they are physically close and therefore less affected by site variation. Changes in heritability, as well as provenance and family variance components, for the phenology traits were relatively minor. These results suggest that there would be little harm in subtracting values predicted by kriging, even when there is no spatial dependence in the experimental data. In experiment 3 the incomplete block design accounted for most of the environmental variation and changes in heritability were equally small.

Estimated individual heritabilities for growth traits were moderate with values between 0.30 and 0.59. The difference between h^2 and the upper bound for heritabilities that can be expected in breeding populations for British Columbia (h_{BC}^2) was quite narrow, and heritabilities in the order of 0.40 for can

Table 2. – Rank changes among provenance means for height in experiment #1 (left normal model, and right adjusted for spatially autocorrelated variation).

Rank	Prov	Mean	Stderr		Prov	Mean	Stderr
1	27	386.9	(5.2)		59	387.5	(4.9)
2	59	386.8	(6.6)		27	378.1	(6.5)
3	19	376.6	(4.5)		19	377.0	(3.8)
4	32	376.2	(5.1)		32	372.7	(3.8)
5	51	371.1	(4.4)		51	372.6	(4.0)
6	41	371.0	(6.8)		35	368.4	(4.3)
7	35	368.7	(4.5)		8	364.7	(4.1)
8	8	367.3	(4.3)		12	364.3	(3.8)
9	13	363.8	(5.9)		41	364.3	(6.1)
10	12	363.3	(4.2)		34	361.0	(3.8)
11	33	361.4	(5.6)		15	359.5	(4.6)
12	34	360.6	(3.9)		13	358.9	(5.3)
13	15	358.8	(5.7)		33	358.6	(4.9)
14	43	358.7	(5.3)		22	355.6	(3.5)
15	6	354.8	(4.0)		50	354.2	(3.9)
16	46	354.3	(5.6)		43	353.9	(5.1)
17	52	352.9	(4.0)		6	352.5	(3.6)
18	50	352.2	(4.2)		46	351.9	(4.1)
19	11	349.7	(4.2)		52	351.8	(3.8)
20	54	349.2	(4.9)		58	350.4	(5.0)
21	22	348.4	(3.9)		11	349.0	(4.1)
22	30	348.1	(3.5)		47	348.2	(3.7)
23	14	347.5	(4.0)		30	346.7	(3.3)
24	58	345.8	(5.0)		54	343.9	(4.4)
25	47	344.0	(4.4)		14	342.0	(3.9)
26	20	342.9	(4.6)		20	340.9	(4.2)
27	68	341.5	(4.0)		9	338.7	(4.5)
28	45	339.8	(5.7)		45	338.0	(4.3)
29	48	336.7	(4.9)		68	337.8	(3.8)
30	9	335.2	(5.6)		48	337.4	(5.4)
31	26	333.3	(5.4)		64	333.6	(5.3)
32	63	333.3	(4.1)		26	333.0	(5.2)
33	64	331.5	(5.6)		56	332.9	(4.9)
34	56	330.0	(4.9)		42	331.0	(3.9)
35	42	328.8	(4.5)		28	329.9	(4.5)
36	28	328.3	(5.8)		63	329.4	(3.9)
37	62	327.1	(5.1)		37	327.5	(4.2)
38	37	325.2	(4.3)		17	326.6	(3.8)
39	17	321.2	(4.6)		62	324.0	(4.9)
Average		350.6	(4.9)			350.3	(4.4)

Note: *) indicates a significant rank change among a pair of provenance at p<0.05 according to Azzalini and Cox (1984).

be expected for growth traits in practical tree improvement programs. It should be noted that h_{BC}^2 estimates for leaf abscission exceeding one reflect restricted gene flow and strong genetic differentiation among provenances for this trait. The results in this study differ from those of Ager and Stettler (1994), who reported large genetic variation between populations but very limited within-population genetic variance, which suggested that there is little opportunity for selection within populations. Substantial within-population variance observed in this study is an important finding and shows that genetic improvement through selection and breeding is possible.

Precision of treatment means

Heritability is equivalent to the correlation between the observed phenotype and the true breeding value, which is unknown. An increase in heritability therefore implies that the precision of the estimate of the true breeding values has increased. The effect can indirectly be confirmed by investigating the standard error of treatment estimates by ANOVA. After correction for autocorrelated variation, precision of estimates of family means were 0 to 5 % better, and precision of provenance means were improved by 7 to 12 %. The rank order of family and provenance means also changed sometimes, which is relevant when selecting genotypes for tree breeding. The effect is illustrated in *Table 2* for experiment 1, where the standard error of provenance means decreased by 10 % and six significant rank changes were observed when ordering 39 provenances according to their performance in height growth. Although these field trials were specialized genetic experiments, there is no reason why this methodology would not prove to be beneficial for other kinds of forestry field trials where measured traits are influenced by micro-site variation.

Conclusions

Kriging removed block effects and reduced the family × block interaction in all traits. The variation due to interactions was recovered in simple family or provenance variance components, which increased by up to 40 % in some traits. The improvement was largest in experiments where blocking was clearly inadequate to capture site variation, when block size was large, and for traits that could be influenced by variation in soil properties. Changes in provenance and family variance components for phenology traits were relatively minor, which suggests that there would be little harm in subtracting values predicted by kriging, even when there is no spatial dependence in the field measurements. We recommend that variograms should routinely be constructed in the analysis of forestry field trials with a standard design to test if residuals of the models are spatially autocorrelated. If they are, kriging is proposed as a useful supplement to ANOVA in tree breeding experiments and other forestry field trials.

Acknowledgements

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Genetic Variation and Structure of *Rhododendron brachycarpum* D. Don, a Rare and Endangered Tree Species in Korea

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Summary

Rare and endangered plant species are commonly hypothesized to have little genetic variation because of inbreeding, genetic drifts, genetic bottlenecks and/or other factors. We investigated genetic variation in *Rhododendron brachycarpum* D. Don, a rare and endangered evergreen tree species in Korea, by examining allozyme variation at 13 loci in 200 individuals distributed among 6 populations. As expected, low level of genetic diversity was observed ($A = 1.3$, $P = 29.5\%$, $H_e = 0.075$). While a single allele was the most common in almost all the polymorphic loci, two allele frequencies were completely or nearly intermediate at the locus *Pgi-2* in all populations, suggesting a selection effect on the *Pgi-2*. The Uleung Island population (*R. brachycarpum* var. *roseum*) had a unique genetic structure and was most distinctive from the inland populations.

Key words: *Rhododendron brachycarpum*, rare and endangered species, genetic variation, allozyme, selection.

Introduction

The genus *Rhododendron* is a large taxonomic group, including evergreen and deciduous woody species and is primarily distributed in the Northern Hemisphere. In Korea, up to 12 different rhododendron species have been recorded (LEE, 1989). *Rhododendron brachycarpum* D. Don is an insect-pollinated, alpine evergreen tree species, native to central and southern Korea, the Kuril Islands, and mountainous northern and central parts of Japan. The species can reach a maximum height of about 4m, withstand wind and dry mineral soil better than any other rhododendrons occurring in Korea, and can live in temperature under –30 °C ~ –40 °C. In Korea, *R. brachycarpum* has been traditionally used as a medicinal plant and has been designated as a rare and endangered plant.

Rhododendrons have been the subject of extensive genetic studies in recent years (KREBS, 1996; ESCARVAGE et al., 1998; NAITO et al., 1999; DE RIEK et al., 1999; PORNON and ESCARAVAGE, 1999; PORNON et al., 2000; MILNE and ABBOT, 2000; NG and CORLETT, 2000; KAMEYAMA et al., 2001), but there have been no published studies of population genetics in *R. brachycarpum*. The objectives of this study were: (1) to estimate levels of genetic diversity in six native populations of *R. brachycarpum* in Korea employing allozyme markers; (2) to measure the distribution of genetic variation within and among populations; and (3) to assess the implications for conservation of this species in Korea.

Materials and Methods

Plant materials

From the late June to the mid-July of 2001, foliage tissues were collected from six natural stands located throughout the native range of *R. brachycarpum* (*Fig. 1*) in Korea. Within each stand, 31–36 trees were selected for foliage collection with a minimum distance of 30 m between trees in order to decrease the risk of relatedness. In several stands such as HONGCHEON and JIRI, however, the scarcity of suitable trees dictated sampling in close proximity (within 30 m) until the goal of 30 trees was reached. Foliages collected were placed in ice chests, and transported to the laboratory within 48 h, where they were stored at 4 °C.

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