Genetic Analysis of Early Field Growth of Loblolly Pine Clones and Seedlings from the Same Full-Sib Families

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The Forest Biology Research Cooperative recently established a series of loblolly pine clonal trials known as CCLONES (Comparing Clonal Lines on Experimental Sites). There are three primary levels of genetic structure in this study (parental, full-sib family, clone) that strengthen the power of CCLONES for examining genetic mechanisms and interactions with cultural treatments and locations. A fourth level of genetic structure can be added by considering the provenance of the parents. This report includes some preliminary results from the genetic analyses of 2nd year growth traits that were recently measured at the CCLONES loblolly pine trials. The specific objectives of this report are 1) to determine heritability estimates for various growth traits for loblolly pine clones and seedlings, 2) to compare the genetic correlations between parents and families when grown as cuttings *versus* seedlings, and 3) to determine the *genotype x environment* interaction by looking at the genetic correlations for parents, families, and clones for paired trials.

MATERIALS AND METHODS

The parental population consisted of twenty first-generation and ten second-generation selections from a larger population that is part of the Loblolly Pine Lower Gulf Elite Population. In addition two slow-growing parents were included. These selections represent the Atlantic Coastal Plain (ACP), Florida (FL), and Lower Gulf (LG) provenances of loblolly pine. These thirty-two elite loblolly pine parents were mated in a partial diallel design and created 70 full-sib families from which a total of 2,000 vegetatively propagated clones were generated. Rooted cuttings from approximately 1,000 of these clones from 61 full-sib families and seedlings from the same full-sib families were established at seven field sites across the southeastern United States utilizing a resolvable incomplete block design (Tests A-G).

Each growth variable (2nd year height, height increment, and crown width) was analyzed for cuttings and seedlings simultaneously with a bivariate analysis in ASREML. Narrow-sense heritability ($\hat{h}^2$) was estimated using the corresponding variance components. Type B genetic correlations for general combining ability ($r_{B_{propGCA}}$) and specific combining ability ($r_{B_{propSCA}}$) between cuttings and seedlings were estimated in order to compare parental and family performance between propagule types. In order to quantify the extent of *genotype x* environment interaction, type B genetic correlations across pairs of trials were estimated for the clonal data.

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RESULTS AND DISCUSSION

There are three provenances represented in this population. Therefore, genetic groups were added to the pedigree file representing the three provenances (ACP, FL, and LG). By doing this we do not assume that all of the parents come from one population, but rather a separate provenance mean was estimated for each of the three genetic groups. The Florida source of loblolly pine ranked highest, followed by Atlantic Coastal Plain and then Lower Gulf provenances for all growth variables measured at all of the tests with one exception. At the northern most test (Test G) in Virginia, the Atlantic Coastal Plain ranked higher than the Florida source for 2nd year height, but the Lower Gulf source still performed the poorest.

Narrow-sense heritability was estimated for all measured variables by site for each propagule type and was always greater for the clones *versus* seedlings. For example, estimates of $\hat{h}^2$ for 2nd year height of rooted cuttings ranged from 0.14 to 0.26, while for seedlings, $\hat{h}^2$ estimates ranged from ~ 0 to 0.2. Similar trends were observed for the other growth traits measured. Non-additive genetic variance was detected at all of the sites for 2nd year height. Heritability estimates for crown width and height increment of rooted cuttings were more weakly controlled by additive effects than 2nd year height, ranging from about 0.07 to 0.24 for both traits.

At the time of planting, seedlings were generally taller than rooted cuttings, and this trend has continued through year two. However, height increments were very similar between both propagule types. Height increment ranged from 1.1 to 2.1m for cuttings growing in high intensity culture, while for seedlings, height increment ranged from 1.1 to 1.9m. Generally, seedlings also had a wider crown than rooted cuttings.

In order to compare cuttings to seedlings, type B genetic correlations between propagule types were calculated for additive and dominance effects. The genetic correlation between propagule types for additive effects, for example, gives us an indication of whether parental ranks are dependent upon whether their progeny are grown as cuttings or seedlings, while type B genetic correlations for dominance effects measure the correspondence of dominance across propagule types. For all growth traits measured the type B genetic correlation between propagule types for additive effects was strongly high. $r_{B_{propGCA}}$ ranged from 0.71 to 0.99 for all of the traits. This tells us that the parents ranked the same regardless of propagule type.

The type B genetic correlations between propagule types for dominance effects were more variable. A low $r_{B_{propSCA}}$ indicates that full-sib families deviated differently around their half-sib family mean depending on whether they were grown as cuttings or seedlings. The most probable reason for inconsistency between dominance effects is a sampling problem. Within a test there were about 32 seedlings per full-sib family, while only 15 clones per full-sib family. Therefore, full-sib means may have been located with more error for cuttings than seedlings. This could also be extended to half-sib families. However, correlations of full-sib family values (GCA parent1 + GCA parent2 + SCA) between propagule types for 2nd year height were moderate to high, ranging from 0.68 to 0.95, indicating that full-sib family performance was independent of propagule type.

The extent of *genotype x environment* interaction was investigated by analyzing data across pairs of trials. The type B correlations were highly variable indicating the presence of GxE on some sites while absent from others. The best type B genetic correlations appear to be between Tests B, C, and D, all of which contained rooted cuttings from the Spring 2002 setting. For example, there were some slight changing of ranks of parents at tests B and D, however, the overall genetic correlation was high ($r_{B_{gxeGCA}} = 0.87$) indicating little GxE. Not only did full-sib families deviate similarly around their parental means ($r_{B_{gxeSCA}} = 0.82$) at Tests B and D, but the full-sib families also ranked similarly, indicating little GxE at the full-sib family level. Clonal ranking was also consistent at Tests B and D indicating that a good ranking clone for age two height at Test B also ranked high at Test D, and this was the case at for all paired analyses involving trials originating from the Spring 2002 setting

Extensive *genotype x environment* interaction was observed between some sites. For example, the worst type B genetic correlations were between Test A and Tests B, C, D, E, or F. Rooted cuttings for Test A originated from the Winter 2002 setting, while those from B, C, D, and E originated from the Spring 2002 setting, and rooted cuttings planted in Test F came from the Summer 2002 setting. Perhaps there was a propagation effect that has carried over to the field. A propagation effect of season was observed for rooting as well. The lowest type B genetic correlations for rooting were observed between the winter setting and either the spring or summer settings, while the highest correlations were observed between the two spring settings. Another indication of a propagation (C effect) effect for field growth comes from the genetic correlations involving Test G (cuttings also originating from the Winter 2002 setting). There was a stronger relationship between the clonal rankings at Test A and G, than between the clonal rankings at these sites and any of the other trials.

CONCLUSION

All of the growth traits analyzed, 2nd year height, height increment and crown width, were heritable as evidenced by the heritabilities for these traits. However, higher heritability estimates were obtained with clones compared to seedlings for all traits measured. Genetic correlations of additive effects between propagule types indicated that parental rankings were stable regardless of whether their progeny were grown as rooted cuttings or seedlings. There appears to be a propagation effect of the season the cuttings were set that has carried over to field growth. This was evidenced by the type B genetic correlations from paired site analyses.

Accounting for Spatial Variability in Clonal Forestry Trials

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Experimental sites in forestry tend to have high environmental variability, which is usually expressed in the form of patches, gradients or both, together with considerable random microsite noise (Costa e Silva *et al.* 2001). With environmental heterogeneity, the estimates obtained for the analysis of a particular dataset will tend to be highly variable. With an adequate experimental design or by using an appropriate statistical spatial analysis, it is possible to account for part of this heterogeneity and produce considerable improvements in heritabilities and therefore in the precision of genetic value predictions. The goal of this study is to quantify relative efficiencies of using classical and non-classical statistical techniques to account for spatial variability in clonal trials, and also to decide which technique is the most parsimonious and yet performs well across a broad set of environmental conditions.

MATERIALS AND METHODS

The study was based on simulations of single site clonal trials of 2,048 ramets planted in single tree plots on a rectangular grid of 64 rows and 32 columns with 8 ramets for each of the 256 clones. Two factors were considered in simulating the environment over which the different experiments were located: a gradient generated with a polynomial function depending on the x and y coordinates of the grid, and patches that were modeled by incorporating a covariance structure based in a first-order separable autoregressive process or AR1 $\otimes$ AR1 with nugget (microsite error) (Gilmour *et al.* 1997). The three surface patterns simulated included: only patches (PATCH), only gradient (GRAD), and a combination of both (ALL). More details of the simulation process can be found in Gezan *et al.* (2005).

Several sets of spatial analysis techniques were studied and compared with classical approaches. All models and datasets were analyzed using ASREML (Gilmour *et al.* 2002), and estimated variance components were summarized and empirical correlations (CORR) between the true and predicted clonal values were calculated by surface pattern and statistical model.

The first group of statistical analysis assumed that the errors were independent and identically distributed (ID), and included: classical analysis, simple global trend functions and nearest neighbor (NN) techniques. For the classical analyses the designs considered were: completely randomized (CR), randomized complete block with 8 replicates (RCB), an incomplete block designs with 32 blocks (IB 32), and a row-column design (R-C). The global trend was modeled with 3 different continuous functions of the x and y coordinates: a) Linear, b) Reduced polynomial, and 3) Full polynomial. The NN techniques used linear covariates in the linear model to correct for similar microenvironments which were calculated by averaging residuals of neighbor plots. Here, a number of different variants of Papadakis (PAP) (Atkinson 1969) were implemented.

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The second group of spatial techniques was based in modeling patches by incorporating a separable autoregressive error structure that was fitted with two variants: a) without nugget (AR1⊗AR1), and b) with nugget effect (AR1⊗AR1+η). These variants were fitted alone, in combination with design effects (RCB, IB 32 and RC), or with different functions of the global trend (Linear, Reduced and Full polynomial).

RESULTS AND CONCLUSIONS

The use of classical design with independent errors, as is done in many studies using IB 32 and R-C helped to successfully control for trend and patches, but it was not optimal (Figure 1a). The R-C design had average CORR values of 0.89, 0.88 and 0.87 for ALL, GRAD and PATCH surface pattern which were only 0.01 lower than the maximum, and it was the best of the models with independent errors for ALL and PATCH surface patterns, but deficient in GRAD surfaces.

Incorporating an error structure different than independent errors produced an important increase in the average CORR values, with larger improvements in PATCH surfaces (Figure 1a and 1b). Also, differences between classical experimental designs were almost non-existent when the error structure was modeled together with the design effects. As expected, the best results were found in the AR1⊗AR1+η error structure with average CORR values as high as 0.90 with the Full-polynomial model, and yielded average variance components close to their parametric values. Finally, failing to model the error structure correctly (as with AR1⊗AR1) produced bias in some variance component parameters (data not shown).

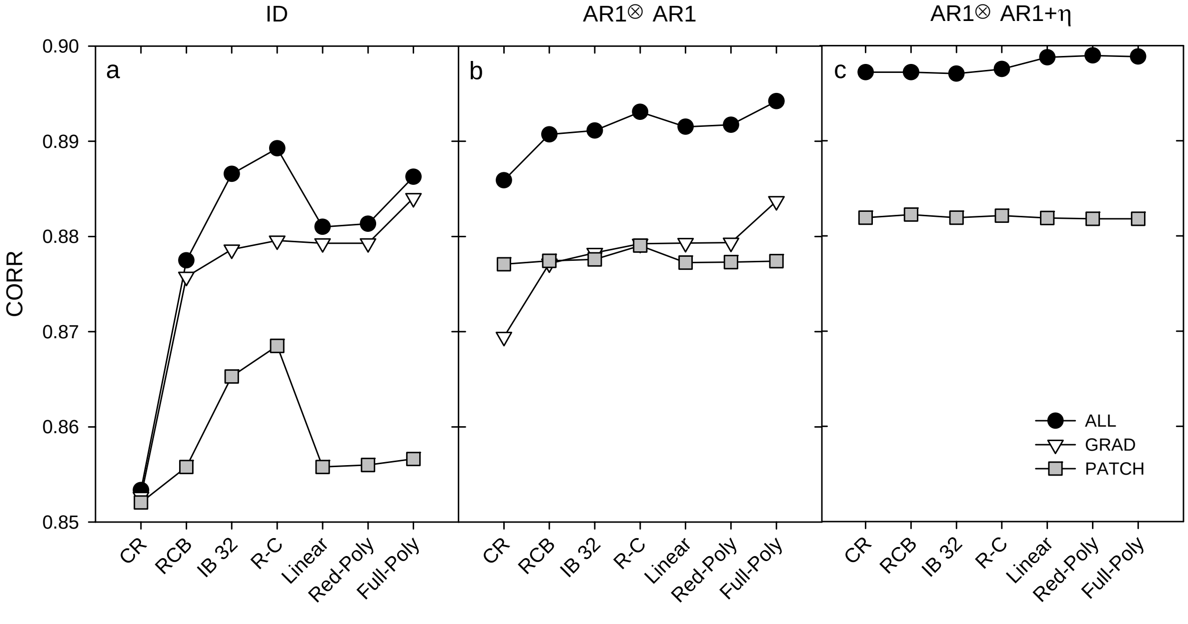

Figure 2. Average correlations between true and predicted treatment effects (CORR) in 3 different surface patterns for classical experimental design analyses and polynomial models fitted for the following error structures: a) independent errors (ID), b) autoregresive without nugget (AR1⊗AR1) and, c) autoregresive with nugget (AR1⊗AR1+η).

Promising results were obtained with the Papadakis methods, particularly for surfaces with patches, but also in surfaces with gradients (Figure 2). These methods were almost as good as models that modeling the error structure, particularly those variants that considered more plots and/or covariates. PAP-11 was the best followed by PAP-6. The use of the latter is recommended because of its simplicity (only 4 covariates).

In summary, if simple analyses are preferred, R-C and IB designs should be used. For further improvements, it is recommended to use some form of PAP methods, but several covariates must be tried and tested. Finally, spatial analysis incorporating error structures are promising, and they should be used whenever possible; but the computational requirements and some uncertainty about the correct procedures and model testing limit its practical use.

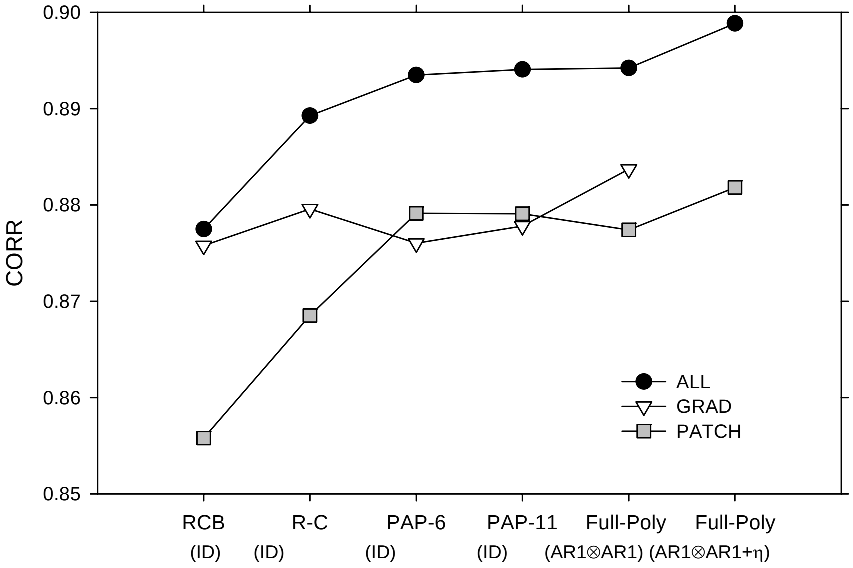

Figure 4. Average correlations between true and predicted treatment effects (CORR) in 3 different surface patterns for selected methods: randomized complete block (RCB), row-column (R-C), and Papadakis (PAP).

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