## **Optimal Method For Analysis Of Disconnected Diallel Tests**

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### ABSTRACT

The unique feature of diallel mating makes it difficult to analyze with standard statistical programs. A new approach using the SAS PROC MIXED was developed to analyze disconnected diallel tests. The method was validated using computer-simulated data and was compared with other existing special analytical packages. It produced accurate GCA variance component estimates and BLUP of genetic effects as compared with the true values. For a combined analysis of disconnected diallels, three alternative models were evaluated for the treatment of diallel effect, diallel as a fixed effect (Model 1), random effect (Model 2) or no diallel effect (Model 3). Simulated data generated with known parameters were analyzed using BLUP methodology to compare these three alternative models. Results indicated that disconnected diallel design could be efficiently analyzed using Model 3. When a complete model is desired, Model 1 may be preferred to Model 2. Treating diallel as a random effect (Model 2) resulted in a downward bias of variance estimates. Use of a common checklot adjustment was evaluated to connect multiple disconnected diallels tested in separate test series within a breeding region. Results from simulation showed that checklot adjustment was very critical to improve BLUP of genetic values obtained from separate test series analysis. For practical and theoretical considerations, the BLUP analysis using the SAS PROC MIXED method with Model 1, followed by checklot adjustment for GCA and breeding values over multiple test series, would be the best way for analysis of multi-disconnected diallel test series established within a breeding region.

#### Analyzing Diallel Mating using SAS PROC MIXED

Diallel mating is a popular mating design used for crop and tree breeding programs (Griffing 1956; Huber 1993), but its unique feature of a single observation with two levels of the same main effect, general combining ability (GCA), makes it difficult to analyze with standard statistical programs (Johnson and King 1998). A new approach using the SAS PROC MIXED is developed for analyzing genetic data from diallel mating (Xiang 2001). Dummy variables for GCA effects were first constructed with SAS PROC IML, then PROC MIXED procedure was used to estimate variance components and to obtain BLUE (best linear unbiased estimators) of fixed effects and BLUP (best linear unbiased predictors) of random genetic effects (GCA and SCA effects) simultaneously.

The method was validated using computer-simulated data with different levels of data imbalance and produced accurate variance component estimates for all random variables as compared with the true values. The true genetic values (simulated) were strongly correlated with BLUP estimates of GCA, SCA and genetic values (GV) for both balanced and unbalanced data (Fig. 1).



**Figure 1.** Mean correlation of GCA, SCA and full-sib genetic value (GV) estimates with true values for both balanced and imbalanced (75% survival), each with 1000 simulated data sets.

In comparison with other existing analytical packages, the new method produced similar variance estimates as GAREML (Huber 1993) and DIALL (Schaffer and Usanis, 1969) under balanced situations, but superior estimates than DIALL with unbalanced data (Figure 2). The new method also simplifies the difficulty of diallel genetic analysis and provides more flexible options in analysis.



Note: the lines with same 3 symbols (linked) stand for GCA estimates of each parent from 3 methods

Figure 2. Comparison of GCA estimates (tree height in m, as the deviation from the population mean) by SAS PROC MIXED, GAREML and DIALL for 6 parents in each of 2 data sets of different imbalance levels (a, b)

The new method can also be used for predicting individual breeding values with BLUP methodology, applying SAS IML to the outputs provided by PROC MIXED to calculate breeding value for each individual in the progeny test, adjusted for the fixed effects of replicate

and test location. The accurate BLUP prediction, the ability to estimate individual breeding values, and the ease of use would make this new method especially attractive for analyzing tree breeding data.

### **Comparison of Alternative Models For Disconnected Diallel Tests**

Disconnected diallel mating has been used extensively in crop and tree breeding programs, but the critical question remained on how to deal with the diallel effect in the linear model for a combined analysis of disconnected diallels. Simulated data generated with known parameters were analyzed using BLUP to compare three alternative models, which considered diallel as a fixed effect (Model 1), as a random effect (Model 2) or no diallel effect in the model (Model 3).



**Figure 3**. Comparison  $\mathcal{H}$  mean percentage bias of GCA variance estimates for three models over different genetic parameter settings (2 levels of h<sup>2</sup>,  $\gamma$ , r<sub>B</sub>), each with 1000 simulated data sets.

Model 1 and Model 3 produced unbiased GCA variance estimate. Model 2 resulted in downward biased estimate of GCA variance component (Fig. 3) and occasionally produced unrealistically large diallel variance estimate. Model 3 was slightly better than other two models in accuracy of GCA variance estimates, while the difference in accuracy between Model 1 and Model 2 was rather trivial.

The accuracy of BLUP prediction for three models, measured as correlation between true genetic value and prediction, was very close, with the Model 3 slightly better than other two. With a random selection of parents in diallels and random allocation of crosses in field test design, the disconnected diallel mating can be efficiently analyzed using Model 3, where disconnected diallels are treated as a large incomplete diallel.

When a complete model is warranted with nonrandom selection of parents into disconnected diallels or nonrandom allocation of crosses in the field design, Model 1 may be preferred by considering diallel as fixed effect. Model 2 by treating diallel as random effect may

result in downward biased GCA variance estimate, hence underestimate additive genetic variance and heritability.

#### Methods for Combining Multiple Diallel Test Series

Statistical approaches were evaluated for combining multiple disconnected diallel test series in a given region. The best GCA sample variance prediction in the class of linear combination of local variance estimates was derived in this study. This improved local variance estimate can be utilized to adjust both parental GCA and full-sib GV prediction. Another adjustment is checklot adjustment. Its importance is often emphasized for disconnected mating designs and its use has already been in practice of the loblolly pine breeding program of North Carolina State University - Industry Cooperative Tree Improvement Program (NCSU-ICTIP) (Li et al. 1996).

The efficiency of a common checklot adjustment and GCA variance adjustment were investigated using simulated data sets. Results from simulated data showed that analysis of disconnected diallel test series with checklot adjustment is very critical to improve the prediction of genetic values obtained using BLUP analysis. Checklot adjustment increased correlation more than .05 in GCA prediction, and almost .1 in full-sib GV in all parameter settings. Additional adjustment with improved GCA sample variance prediction could improve the correlation slightly beyond checklot adjustment. But this limited improvement is due to the fact that the accuracy of checklot- adjusted prediction alone is already close to the theoretical limit. For practical and other reasons, the checklot adjustment method may be the best way for predicting breeding value for combined multi-disconnected test series for a breeding region.



**Note:** chk-adj: checklot adjustment; best var adj: adjustment using best GCA variance prediction; true var adj: adjustment using true GCA sample variance (theoretical limit)

**Figure 4.** Comparison of adjustment methods applied to GCA prediction from multiple 4-test series (for parameter setting:  $h^2=.2$ ,  $\gamma=.3$ ,  $r_B=.8$ )

## CONCLUSIONS

BLUP analysis of diallel tests can be implemented using the proposed PROC MIXED method. For two-disconnected-diallel design, treating diallel as fixed is preferred for a complete model. Checklot adjustment is important to obtain comparable GCA and BV over multiple test series. For practical and other reasons, BLUP analysis for single diallel test series using the fixed diallel effect model (Model 1) followed with checklot adjustment for GCA and breeding values over multiple test series, is the best way for analysis of multi-disconnected diallel test series established within a breeding region.

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