

CLONAL FORESTRY, HETEROSIS AND ADVANCED-GENERATION BREEDING

G.A. Tuskan'

Extended Abstract. Clonal forestry has been practiced in *Populus* for hundreds of years; more recently, techniques in vegetative propagation have made it possible to consider clonal forestry for many conifers. Clonal forestry offers several advantages over traditional seedling establishment practices, including planting stock and product uniformity, alternative disease management strategies, and the potential to capture greater amounts of the genetic variation. To date, our breeding strategies have not focused on clonal commercial plantations, even in *Populus*. Most current tree breeding programs rely on methods developed by corn breeders or livestock breeders, where breeding values and progeny tests are used to identify superior parents which are subsequently placed into open-pollinated seed orchards. Progeny are then collected as seeds and distributed to commercial plantations. Alternatively, with clonal planting stock, breeding programs need to develop superior individuals. Traditional advanced-generation breeding designs are based on the primus of population improvement, i.e., advancing the mean of the commercial population over that of the P1 generation. If clonally propagated genotypes are to be deployed operationally then the breeding design needs to be modified to maximize the probability of finding a superior individual. To maximize genetic gains per unit effort breeding strategies should be designed to increase recombination and within-family variance. Statistical theory indicates that means and variances are independent. Even the number of progeny included in present progeny tests are based on optimizing estimates of the mean, not estimates of the variance (30 individuals per family vs. 300-500, respectively). Thus, recurrent selection schemes utilizing factorial or diallel mating designs do not necessarily increase the probability of finding a superior individual.

A second property of clonal forestry that stands apart from seedling propagated forestry is the opportunity to capture heterosis. Heterosis, or hybrid vigor, is defined as the expression of a superior phenotype within the progeny of a single cross relative to either parental genotype (Shull 1911). Heterosis has been demonstrated in everything from chickpeas to chickens. It certainly occurs in inter- and intra-specific crosses and is the rule in most *Populus* breeding programs. In rice and corn, heterosis has been associated with gain yield, disease resistance, plant habit, ripening date, leaf orientation, and many other traits. In *Populus* heterosis has been documented in leaf size, leaf angle, growth rate, water-use efficiency, and branch habit (Bradshaw and Stettler 1995). It is reasonable to expect that heterosis occurs in conifers, particularly if it is the indirect result of mutational load (Franklin 1969, Tuskan and Wiseloge 1985, Strauss 1986). Heterosis occurs in two basic forms--dominance and overdominance. In rice the predominant form of heterosis is dominance; in corn it is overdominance (Stuber *et al.* 1992); in *Populus* both forms have been reported (Bradshaw and Stettler 1995, Li and Wu 1996). Heterosis attributed to dominance is caused by the sum of all dominant alleles at all loci affecting the desirable phenotype. The combination of favorable alleles from alternate parents conveys superiority to the progeny (i.e., AaBbCc . . .). It is this masking of mutational load that creates heterosis (Klekowski 1988). Under dominance heterosis it is possible to create superior, true-breeding genotypes (i.e., AABbCC . . .). Heterosis attributed to overdominance is caused by the

expression of both alleles in the heterozygote. Under overdominance heterosis all alleles manifest themselves and thus have an associated fitness. Classic examples of overdominance include circumstances where a fluctuating environment allows each alternate form of a gene to be favored, e.g., multimeric polypeptides formed from alleles adapted to high and low temperature. In the F₁ generation, the heterozygote appears superior to either parent for either dominance or overdominance heterosis. Thus, the decision on which advanced-generation breeding strategy should be employed will depend upon the form of heterosis expected in the breeding population. If heterosis is attributed to dominance then a simple recurrent mating design will ultimately lead to superior genotypes (Simmonds 1981). Selfing and sib-mating in the breeding population could also be used to accelerate progress toward such genotypes. If heterosis is attributed to overdominance then a reciprocal recurrent mating design works best (Simmonds 1981). Here the clonal planting stock is derived from crosses between the reciprocal populations containing homozygous breeding populations that are used to create production clones with maximum heterozygosity.

Until the extent and form of heterosis is known, maximum genetic gains in a clonal breeding program may not be possible. For example, heterozygosity and thus overdominance heterosis will be lost during repeated simple recurrent selection and mating. Molecular markers can be used to discern the extent and form of heterosis in the breeding population. Genomic mapping and QTL analysis can provide indirect indications of the number and type of heterotic loci present in the mapped population. RAPD, STS, and SSR markers can be used to define the amount of genetic diversity in the breeding populations (Tsaftaris 1995). Experiments can then be designed to associate diversity with hybrid performance. If heterosis increases with increased genetic diversity among crossed parental lines then heterosis is attributed to overdominance. Alternatively, if heterosis increases with decreasing genetic diversity then heterosis is attributed to dominance. Numerous studies involving agricultural species have begun to assess heterosis using this approach (Maroof *et al.* 1997). Finally, molecular markers can be used to characterize gene function. Recent studies in corn indicate that overdominance is due to regulatory genes that convey superiority as a result of heteromeric proteins that interact with both the promoter and enhancer regions of structural genes (Jones 1990, Tsaftaris 1995). Outside of long-term breeding experiments, molecular markers provide the best opportunity to characterize heterosis in forest tree species.

In summary, clonal planting stock offers many advantages to the forest products industry. Advanced-generation breeding strategies should be designed to maximize within-family variance and at the same time allow the capture of heterosis. Certainly there may be a conflict in the choice of breeding strategy based on the trait of interest. It may be that the majority of the traits express heterosis due to overdominance. Alternatively, disease resistance is expressed as the lack of a specific metabolite or infection court then the homozygous recessive genotype may be the most desirable. Nonetheless, as the forest products industry begins to utilize the economic advantages of clonal forestry, breeding strategies will have to be optimized for these commercial plant materials. Here, molecular markers can be used to characterize the nature of heterosis and therefore define the appropriate breeding strategy.

Keywords: Mating designs, molecular markers/diversity, vegetative propagation.

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