Breeding Methods in Tree Improvement

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The two previous papers have presented a very thorough account of current information on heritabilities for wood and morphological characteristics. A primary purpose for determining heritability figures is to better orient breeding methods. How, then, can this information be used in the current tree improvement programs and how will it affect silviculture in the long run?

Ten years ago the descriptive words "phenotype" and "genotype," commonly used in genetics, had very little meaning to management foresters. These terms are relatively common today and are employed freely in discussions of tree improvement programs. They exemplify the new set of terms used in advancing techniques in genetics and breeding procedures. It will be necessary to define and develop some other new terms as they are used throughout this paper.

Heritability, a term so freely used in previous papers, is defined in the tree improvement glossary (Snyder 1959) as a "measure of the relative degree to which a character (or characteristic) is influenced by heredity as compared to environment." Variation among trees can be expressed by formula as follows:

Total Variation = Hereditary Variation + Environmental Variation

A properly designed heritability experiment can separate variation due to heredity from the variation due to environment. Heritability is usually expressed as a decimal or percent figure. For example, if a broad sense heritability of 0.7 or 70 percent is found for a characteristic such as specific gravity, it means that 70 percent of the total variation in specific gravity is due to heredity and 30 percent is due to environmental influences.

What elements are included in the heritability portion of the estimate and what do they mean? The term "broad sense heritability," or the total hereditary variation, refers to a numerical estimate including three values: namely, additive variance, dominance variance, and variation due to epistasis. Additive variance is due to the average or additive effect of a gene or genes. Each gene contributes a small addition to the overall effect. Dominance variance is due to the interaction of alleles. Epistatic variance is due to the interaction between non-alleles. The term "narrow sense heritability" refers to ratio of additive to phenotypic variation.

Broad sense heritability is determined by dividing total genetic variance (V_G) by phenotypic variance (V_p) and is expressed mathematically by the equation $h^2_{\text{broad sense}} = V_G/V_p$. Narrow sense heritability is obtained by dividing additive variance (V_A) by phenotypic variance (V_p) and can be expressed by the formula $h^2_{\text{narrow sense}} = V_a/V_p$

If the narrow sense heritability figure is high, then the mass selection method of breeding will be most productive. On the other hand, if the dominance or epistatic variation is very high, the greatest gain can be accomplished by an intraspecific hybridization approach.

Figures on inheritance, represented by heritability values, are essential to the development of any tree breeding program. If, for example, specific gravity were controlled to a considerable extent by environment, the field forester would be the one who could most easily increase or decrease the specific gravity of stands by silvicultural manipulations. The tree breeder would be able to add very little by genetic or breeding techniques. If, on the other hand, as Mr. McElwee has shown, specific gravity is controlled to a considerable extent by heredity, the silviculturist can do less to affect it, and management techniques must be quite drastic to bring about major changes. However, the tree breeder can accomplish a great deal.

Initiation of a Tree Improvement Program

Assuming that a tree improvement program is about to be initiated, the ideal procedure is to make a thorough study of the species to be improved. Detailed information concerning variation between and within individuals must be gathered and a complete study made of this species, with an assessment of causes of variation over the entire range. Heritability of important characteristics and the mode of inheritance must be determined. Controlled crosses with related species should be made and their progenies studied. Armed with this information the tree breeder could then devise a breeding scheme and proceed with improving the species.

Since one rarely approaches ideal conditions, chances are that a tree breeder will find himself with the situation that confronted forest geneticists approximately 10 years ago, when the first southern pine tree improvement programs were initiated. Little of the necessary information was available as a base for an efficient breeding design. There was not time to make the required surveys and conduct experiments that would provide knowledge needed to choose among the several immediate avenues of approach available. These had already been explored, developed, and exploited in various crop improvement activities with other plant species. The problem in forestry in the Southeast was to determine which breeding method would result in the greatest improvement in the shortest time so as to provide improved seed for the very large and expanding planting program then underway.

Methods to Produce "New Creations"

Polyploidy.—The "go for broke" approach was characterized by polyploidy adherents. In this breeding system, the fundamental structure of the cell is manipulated to change its genetic constitution. Each cell in every species has two sets of a fixed number of heredity units or chromosomes. Pine species have 12; aspen, 19; human beings, 23. Normally this number is characteristic to the species, but occasionally something occurs that upsets the normal condition and then the resulting progeny have an abnormal number of chromosomes. There are certain plants which are improved by such a change in chromosome number; some persons have suggested that this might be true for forest trees.

Probably the most intensive search for polyploid trees has been made in the aspen improvement program, where individuals were located that were extremely vigorous and had exceptionally large leaves. A microscopic examination of cells from these selections demonstrated that they had 3 sets of 19 chromosomes instead of the normal 2 sets. Since these trees were so very vigorous, it was hoped that such triploids might be especially desirable.

Geneticists artificially recreated triploid individuals with colchicine, a chemical that interrupts the natural processes of cell division (Inst. Paper Chem. 1955). When a seed is placed in a solution of colchicine at the time of germination, a few individuals with twice the normal number of chromosomes develop. An aspen seed with 2 sets of 19 chromosomes can be forced to develop twice this number. This individual is partly fertile and, when crossed to a normal aspen, develops seed which grow into very vigorous seedlings with 3 sets of 19 chromosomes. These triploids are normally sterile or have very low fertility so could not be reproduced by seed. However, it might be possible to produce triploids in sufficient number by establishing seed orchards of alternating rows of normal diploid and tetraploid individuals. If so, triploid individuals could be used in practical forest management if desired.

Although examples of polyploidy in pines have been reported, (Hyun 1954; Mergen 1958, 1959) in every case the seedlings are malformed and grow poorly, with undesirable form; thus polyploid seedlings in the pines are of little value. Polyploidy as a breeding method in pines must be relegated to the laboratory, at least for the foreseeable future.

Induced chromosomal *changes.—Another* means for producing artificial genetic variation is to subject seed or plants to X-ray (Snyder et al. 1961) or other radiation (Beers 1962). This causes actual physical disturbances in one or several chromosomes. These can be large changes involving whole sections of chromosomes or minute "gene changes" which are reflected in changes in the developing seedlings; such seedlings with artificially induced changes or mutations have up to now been very difficult to keep alive, and this technique can also be considered in the laboratory stage as a practical breeding tool.

Interspecific hybridization.—Crossing two species of pine is another method that sometimes produces seedlings with spectacular characteristics. The resulting progeny are compared to each parent to determine what improvement, if any, has been gained. This phase of forest genetics has been extensively pursued at the Institute of Forest Genetics at Placerville, Calif. Control crossing techniques were developed here in the early 1930's, and the major effort of this station has been directed into the methodology and usefulness of interspecific hybridization. Such crosses between species have produced some remarkable hybrids (Callaham 1957), but for one reason or another very few of them have been of practical use. Two of the hybrids, the Jeffrey x Coulter and the knobcone X Monterey, have been quite successful. Jeffrey x Coulter hybrids are now being planted in California forest plantations and the Jeffrey x Coulter hybrid backcrossed to Jeffrey is also being planted in the Jeffrey pine range where it is resistant to the pine reproduction weevil (Righter 1960).

The most impressive practical use of the hybridization technique has been developed in Korea (Hyun 1961) where in 1 year over 1 million control-pollinated seedlings of a pitch pine x loblolly pine hybrid were produced. This hybrid has much of the growth habit of loblolly pine and some of the frost resistance of the pitch pine parent. The labor cost of such hand-produced hybrids would be prohibitive in this country, but a few have been produced to be used on an experimental basis. Hybridization, as a completely practical method, cannot be used until some inexpensive system of control pollination is developed.

Some research has been done to develop practical means of mass producing hybrids. Wakeley and Campbell (personal communication) have tested a method of applying slash pine pollen to unbagged longleaf strobili, but with indifferent success. Brown and Greene (1961) and Hyun (1961) are working with chemicals that will cause male sterility.

If Wakeley's or some other simple method can be developed so that successful hybrids are produced consistently it would be relatively simple to make a mass collection of pollen and dust a large number of trees in the seed orchard inexpensively. On the other hand, if male sterility can be induced, orchards could be so designed that the species to be crossed would be planted in alternating rows.

The pollen of the seed parents would be rendered sterile so that all of the seed would be hybrid. These techniques are still in the experimental stages but may hold some promise.

Improving Existing Species

Intraspecific hybridization.—The technique of making controlled crosses between members of the same species may be used to combine desirable characteristics from different individuals. It always must be remembered that some selection is implied regardless of the breeding scheme used. However, the difficulty of making selections varies considerably with the type of breeding method in use, and the size of the population that must be examined. As the size of the population available for selection and the number of characteristics being considered increases, selection difficulties are compounded.

The problem of selecting usable breeding stock is minimized with the intraspecific hybridization method of plant improvement. Usually in this system individuals with one particular outstanding trait are selected. The original selections are crossed in an attempt to combine the outstanding features of each into one hybrid. The system is time-consuming, though, especially with species that have considerable time lapse between seed germination and the development of reproductive organs. After the original crosses are made, progeny must be grown and new selections made which contain the desirable characteristics. These are then cross bred to fix the characteristics in a high proportion of the progeny and to establish a seed source. In order to prevent inbreeding depression, several selections must be made and carried on concurrently. The seed source is eventually developed from these selections.

Mass selection.—This is a system of breeding for improvement that promises slow steady gains. Normally, the increase one can expect is limited by the genetic capability of the most outstanding individual in the population. With a large amount of natural variation of the additive type, large increases can be expected; conversely, with little natural variation, there can be **little** improvement by this method.

As the number of characteristics to be improved increases, selection difficulty increases. In order to make the greatest possible gain a high intensity of selection must be practiced—and suitable individuals are difficult to locate. On the other hand, once the selections are made, seed production problems are simplified as a continuous supply of large quantities of seed can be produced from vegetatively propagated ramets of the original selections.

Practical Tree Improvement

With these methods available, the pioneer southern pine tree breeders took a long, hard look at their species. Little or no information was available from previous data to guide them. A few preliminary surveys indicated that variation was present and seed source important. Interspecific hybridization had not been particularly promising. The problem at hand was to develop a genetically improved source of seed as soon as possible. The decision was: mass selection.

The data now available testify to the validity of this choice for most characteristics. It must be remembered that many of the experiments from which these data have been drawn were designed for other purposes and that much is from relatively young material, but the significance cannot be denied. Heritability values made from measurements of immature material can be expected to increase as the plantings come closer to maturity, at least for some species. Let us now, as the politicians say, examine the record.

Barber and McElwee have quoted heritabilities for many characteristics and indicated the amount of improvement that can be expected based on these figures. They have urged the immediate pursuit of an aggressive tree improvement program based on the mass selection method of breeding, or some variation thereof.

A brief review of some of the heritability figures quoted will emphasize the soundness of the recommendation.

Some of the oldest plantations which can be used for estimating heritabilities were established by personnel of the Southeastern Forest Experiment Station at Lake City, Fla. Planting consisting of grafted clones, open pollinated progeny, and control pollinated progenies are available for estimating the genetic improvement possible. Squillace and Bengtson (1961) have reported heritability figures for several characteristics from these 10- to 14-year-old plantations. Narrow sense heritabilities of 56 percent for specific gravity were obtained with control pollinated progeny while the broad sense or total heritability was estimated to be 73 percent from clonal data. Fairly high heritabilities for diameter growth (25 to 58 percent), stem volume (18 to 35 percent), crown width (24 to 48 percent), and bark thickness (33 to 67 percent) were obtained. A fairly low heritability for height growth of 5 to 10 percent was obtained. A tentative hypothesis advanced by Squillace indicates that this is probably due to the wide spacing (20 by 20 feet) of the plantation and the attendant lack of competition. This is somewhat substantiated by Barber's[1] figure of 27 to 37 percent for material planted at a spacing of 10 by 10 feet, van Buijtenen's figure of 20 percent for material planted at an 8 by 8 foot spacing (personal communication), and by recent results obtained by Stonecypher (personal communication) from seedlings planted also at an 8 by 8 foot spacing. It is interesting to note that the broad sense heritability of oleoresin yield is estimated at 90 percent while the narrow sense estimates vary from 45 to 90 percent depending on the methods used.

[1] Barber, John Clark. An evaluation of the slash pine progeny tests of the Ida Cason Callaway Foundation (Pinus elliottii Engelm.). Ph.D. Diss., Univ. Minn. 206 pp., illus. 1961.

van Buijtenen (1962) estimated broad sense heritability of 64 percent and 84 percent from 5-year-old grafts for specific gravity, and narrow sense values of 37 and 49 percent for 2-year-old control pollinated material. He also reported diameter heritability of 20 percent for 6-year-old loblolly pine progenies. He estimated that an improvement of 10 percent for each of these characteristics may be expective from one cycle of selection and that an increase of 25 percent in total wood production for the several factors combined would not be out of reason (personal communication).

Stonecypher (personal communication) found a low narrow sense heritability for first year height growth, which increased with second year measurements. He noted that narrow sense specific gravity heritability estimates for 1- and 2-year-old progenies approach the actual values given by van Buijtenen and that they remain constant for the 2 years.

Selection severity can be varied from slight to intense, with selection of low to medium intensity. Forestry practices can be guided so that any landowner can achieve a moderate amount of genetic improvement in his forest stands. Some gain will be achieved, if, in a seed tree cutting, selection of seed trees from which cones will be collected is made as the first step in harvesting. Improvement here is controlled by the nature and number of trees in the stand being cut. There is no doubt that a minimum amount of improvement can be expected when the selections represent only 10 to 20 percent of the stand, which is normally the case. As soon as the area is sufficiently stocked, the seed trees are harvested during a year of heavy cone production. Such areas are designated as seed collection areas. Careful planning is necessary as harvesting of the seed trees must be scheduled to coincide with the optimum period for cone collection. Cost of cone collection from trees which have been cut is relatively inexpensive, especially when large crops are present.

The next highest level of selection severity is represented by the seed production area, where better than average stands are selected, carefully rogued of undesirable individuals, and managed for a continuing supply of genetically improved seed. Since mature trees must be climbed for cone collection it would seem at first that the expense would be prohibitive, but collection costs as low as $3 to $5 per bushel have been attained as compared to an average cost of $2.50 per bushel when cones are purchased on the open market (Goddard 1958; Cole 1962). According to Easley (1963), seedlings from one seed production area had a height advantage of 24 percent at the age of 8 years on sandy soil as compared to nursery run seed; this same seed production area produced seedlings with a height advantage of 7 percent on heavy clay soil. He concludes that the collection of seed from a local source of selected parent stock is advantageous.

The most severe selection that can be commercially applied is through seed orchards. Individual elite selections are made only after the examination of thousands of acres of forest land. These selections are rigidly graded in comparison to a number of surrounding dominant specimens, and included in the orchard only after they indicate a maximum amount of advantage. The selections are vegetatively propagated and planted in a central orchard location, or a seed orchard of seedlings from the selected parents is established. The orchard is designed to insure a minimum amount of inbreeding. Here the trees are cultivated as intensely as in fruit orchards and will serve as a source of high quality seed for the future.

Based upon the figures quoted today and if we take into consideration improvement due to increased vigor, finer limbs, straighter boles, and less disease, it seems probable that the yield increase of 10 percent suggested by Barber can be obtained with ease and the suggested figure of 25 percent advanced by van Buijtenen is within reach. Assuming no change in pulpwood stumpage values from the figure advanced by Perry and Wang (1958) and figuring a 20 percent increase in yield due to tree improvement efforts, we can realize a gross increased profit of some $600 per pound of seed at a 25-year rotation, or an extra $2.10 per acre per year. This profit, they say, justifies the expenditure of $181.27 per pound of seed, allowing 5-percent interest on the invested money.

Perry's figures are based on yield alone, and no attempt was made to include other advantages which cannot be represented easily by monetary values—for example, the morphological characteristics of bole straightness. It is difficult to determine how much more solid wood content would be delivered per cord by minimizing the amount of crook, spiral, and sweep. It is difficult to learn how much more cellulose is in each cord and how much less cooking liquor will be needed at the mill. One can only estimate the increase in usable fiber contained in the straight pulpwood stick. Add to these figures a decrease in knot wood volume, a concomitant decrease in compression wood associated with knots, and an increase in the number of seedlings growing to maturity by virtue of increased disease resistance. Consider also the pulp increase reported earlier by McElwee due to increases in specific gravity and the probable improvement in paper sheet formation due to having wood with more uniform fibers. All these advantages, nebulous as they may be, are to be gained as a result of the activities now taking place. Estimates of possible improvements made several years ago covered the entire scale from a minimum 1, 2, and 5 percent made by the conservative members of this group to a maximum 100 percent by the most optimistic. Actual values today indicate an intermediate expected increase of 15 to 25 percent based on yield, plus the additive increment due to quality. Perry's figures, which seemed so unobtainable 5 years ago, are becoming more realistic as we gain additional knowledge of the inheritance patterns in the species we are using.