TECHNIQUES IN FOREST TREE BREEDING

Yanchuk, A.D.

Senior Scientist and Manager, Forest Genetics Research, British Columbia Ministry of Forests, Victoria, Canada

Keywords: Forest genetics, tree breeding, gene frequencies, gene conservation, breeding populations, superior-tree selection, population improvement, recurrent selection, progeny testing, inbreeding depression, genetic diversity, gene conservation, climate change, risk.

Contents

Summary

Over the last several decades classical plant and animal breeding techniques have been adopted, modified and applied to many important commercial tree species around the world. In contrast with most agricultural breeding approaches, forest tree breeders have had to consider several unique ecological, population and quantitative genetics issues in recurrent selection programs and in deployment strategies with forest trees. Tree breeders have largely been working with wild populations, so factors such as geographic patterns of genetic variation, seed transfer within environmentally similar zones of adaptation, special field and progeny test designs have had to address and meet the special biological features of forest trees. More recently, and part of the future

challenges for tree breeding, are to consider genetic conservation in some forms of a hierarchy as part of applied tree breeding and forest genetics research - which is more appropriately referred to as a system for forest genetic resource management. Now these programs must essentially consider various means and vehicles of *in situ* and *ex situ* conservation, genetic diversity being captured and managed in these conservation populations, as well as genetic diversity of deployment strategies.

1. Introduction

Man has occupied and lived in and near forested lands for thousands of years, and in so doing has had substantial effects on the genetic structure on forests throughout the world. These changes, through harvesting, land clearing, reforestation and afforestation, have, with few exceptions, been undirected in terms of purposeful genetic management objectives. This is quite different to the affects man has had on crops and domesticated animals: there was usually a cognisant awareness to actively manage the genetic resources of his food supply, through selection of seed and breeding stock, and through improving agricultural, horticultural and husbandry techniques.

Forests, in most areas of the world, were viewed in a different manner, as largely self-perpetuating and adequate in their ability to provide wood and other services, so little management was necessary. In rare situations planting of seedlings or trees from local areas was done. Sometimes exotic species were tried, were found to be more desirable than the local species, and were planted in large estates, but little was known about genetic differences among seed sources, and only species differences were viewed as important.

It is clear to most national and international forestry agencies that those days are now over. Almost no forested ecosystems can be considered untouched by man's influence. The idea that man can let forests "evolve" on their own, as they have since they first formed and have re-formed themselves over time, after all types of disturbances, is no longer something that should be considered acceptable to responsible governments, forest managers, conservationists and environmentalists anywhere on the planet. In this regard, great needs and opportunities exist for the development and management of populations of forest trees, as they have been shown to be amongst the most genetically variable organisms on earth.

Relative to genetic improvement in agricultural species, forest tree genetic improvement programs are relatively new endeavors. As the science of genetics developed over the last century, plant breeders took advantage of new discoveries from both theoretical and empirical research. Similarly, many developments in forest genetics and tree improvement were directly applicable, however, many refinements were necessary in order to accommodate the unique biological features of forest trees.

For instance, issues such as reproductive biology, geographic patterns of genetic variation in species, maintaining adaptive genetic variation over very long periods of time, and the maintenance of wild forest reserves are issues that are somewhat unique to forest tree genetic resource management.

2. Genetic Surveys of Natural Populations

As far back as the early and mid-1900s emphasis was placed on wide-range genetic surveys (i.e., provenance testing) using "common garden" experimental principles. The early common garden experiments by ecologists in the 1940s (and earlier) served as the initial models for these investigations in forestry and ecology research. The process was relatively simple in concept. Samples are collected from natural populations of interest (i.e., provenances) then planted in several test environments (usually within the range of the provenance collections) to test performance of the local provenance versus others.

These early studies showed that most tree species had large amounts of genetic variation both within and among populations, but local populations were not always the best. However, forest trees, in general, do show that patterns of adaptation are reasonably well associated with the climate from which they originated. More importantly, there are differences among and within species in the general level of adaptation. Species have also been categorised as generalists, intermediates or specialists in their level of adaptation to climatic and environmental gradients. For instance, species such as western white pine (*Pinus monticola*) are considered generalists, i.e., adaptations are not well correlated to climatic or geographic variables, yet others, such as Douglas-fir, are much more tightly adapted, and movement of seed in this species needs to be restricted.

In the early 1970s genetic surveys of forest trees, with the use of molecular genetic markers, further confirmed large amounts of genetic variation were present, as they had shown in fruit flies, and other organisms. However, these molecular marker studies largely sample neutral genes i.e., genes that vary in the population, but do not have noticeable "phenotypic" effects on individuals. (The term "phenotype" refers to the entire "observed" organism, which is a combination of both the genotype of the organism, environmental effects it has encountered, and the interaction of the two. For instance, genetic variation measured by molecular markers, called isoenzymes (protein variants called allozymes), have been useful for investigating gene flow, mating dynamics within and among stands, heterozygosity (genetic diversity), and various other measures of genetic variation (e.g., effective population size, number of alleles per locus, variation levels among populations and species, and the frequency of rare alleles). It is now clear that both neutral ("random" molecular genetic markers) and non-neutral (adaptive, quantitative genetic variation from common garden studies) were important to study.

The continued development of more complex DNA markers (e.g., Random Amplified Polymorphic DNA (RAPD)), or Simple Sequence Repeat (SSR's or microsatellite markers) are now providing better information on variation at neutral or quasi-neutral gene loci. On the other hand, common garden experiments allow for the complete assessment of the organism (i.e., the phenotype) and this is ultimately what tree breeders must rely on. There are very few ways for breeders to make the progress that is required, without the use of well-designed and executed field experimentation. Observations of the performance of the whole tree, the performance of related groups of trees, and developing an intimate familiarity with all the biological aspects of the species they wish to improve is critical to the success of any kind of genetic improvement in a species.

Whether or not a particular species has had the benefit of some pre-breeding activities i.e., population or provenance studies, it is apparent that geographic genetic variation should be factored into the design of a genetic improvement program. However, many programs have had to start without the benefits of such information. In these situations it is still important to design sampling strategies so that as many populations as possible can be sampled, with several trees per population, to capture and increase the probabilities of having good "provenance effects" in the base population. In the case of exotics, a well developed "land race", if present, may also be an effective base population. These landrace introductions are typically species, which have previously been used for purposes other than timber or pulpwood production, such as windbreaks, shelterbelts or ornamentals. They may have been shown to have some growth and quality advantages over local native species and can therefore be desirable species in "adapted" populations from which to select a base population of trees. Several large industries have been established from such introductions of materials, such as radiata or Montery pine, rubber and coffee, and may have originated from a relatively small collection from wild populations. The point here is that many sources of material may serve as an excellent population with which to make further advances.

3. Improvements Through the Use of Superior-Tree Selection in the Wild

Plants or animals with desirable characteristics have served as breeding stock for hundreds if not thousands of years, without sophisticated genetic theory, or statistical genetic analysis to support selections made by farmers. Genetic testing techniques developed by man over the last several decades have made it possible for much more rapid genetic improvement. However, the underlying reason for genetic gains made throughout the history of agriculture is the same: most traits have genetic variation, and these differences can be transferred to offspring or progeny (i.e., they are heritable, or in a statistical sense, they have a "heritability" (h^2) greater than zero).

Higher heritability traits (e.g., eye color in humans) tend to be those controlled by a few genes with large effect, or several genes not greatly affected by the environmental conditions with which they interact. They usually respond quickly even to mild selection. Low heritability traits (e.g., growth potential in plants and animals) are typically affected by many genes each with a small effect, and can be affected by the environmental conditions to a greater degree. They require more intense selection methods to attain similar levels of gain, compared to higher heritability traits.

Heritability is measured as the ratio of genetic variation (Vg) over that of the total or phenotypic variation (i.e., genetic + environmental variation = phenotypic variation, Vp), observed in the population i.e., h^2 = Vg/Vp. It is intuitive that if environmental variation is minimised our ability to discriminate among better candidate trees is easier. This is the basic idea behind the use of progeny testing techniques i.e., to obtain statistical estimates of Vg and Vp (described below). However, this logic is also relevant to our understanding of why "in the woods" heritabilities are typically low: namely that Vp is very large in wild uneven-aged forests.

For example, if the original selections are made from an unimproved plantation which is nearing harvesting age, this can be one of the most efficient selection approaches from a

non-pedigree population. Gains are certainly lower than they would be with genetic testing, but the costs per unit gain or time may be reasonable. Efficiency of selection in plantations versus wild forests tend to be higher as environmental differences among trees in the plantations (e.g., similar ages, similar cultural treatments to all seedlings) are lower. The least favorable situation tends to be when selection would occur in old-growth forests, where there are typically more variable environmental histories that may have generated uneven regeneration, age class differences, etc.

Overall, most tree improvement programs have focused on improving growth potential, so visual selection or even comparison-tree selection techniques have not been very effective because growth is typically a low heritability trait. However, there are a few other traits that have been successfully improved by phenotypic selection, such as straightness, form characteristics, some disease resistance traits, and wood properties. Again, this is primarily due to the fact that they can have higher heritabilities. So while "in the woods" selections have generally been regarded as not being an efficient means of improvement for some traits, they can be very cost effective if resources are limited and if the trait tends to have a high heritability.

After screening the chosen source, or individuals, the breeder is now in the situation of needing to efficiently establish a program of "recurrent" selection by genetic testing, selection and breeding. Recurrent refers to this continual process of selection, breeding to create a population of new genetic combinations in the offspring, and testing with a new round of selections.

4. Testing Procedures for Genetic Advancement

The first step described above is generally referred to as phenotypic or mass selection (i.e., selection without the use of pedigreed genetic information). However, in forest trees, it is now generally accepted that because the main traits of interest are related to growth potential (and usually of low heritability), and because forest trees have long generations times, selection with the use of genetic information from progeny testing is usually necessary.

Once the breeder has assembled a large enough population from selections in the wild, usually a few to several hundred, decisions are required with respect to the type of progeny testing needed. The first decision is to determine if the testing is for the purpose of further screening of selected trees from the reference (wild) population, or whether it will serve for both purposes of screening and for re-selection for continued breeding and improvement. Second, does the biology of the species allow for vegetative reproduction (i.e., cloning), or is cross-breeding a realistic option and the best means of production of trees for testing and re-selection? This has some influence on the types of testing that should be undertaken, but it is critical to establish the objectives of the program at the outset of the testing scheme.

4.3. Improvements Through the Use of Progeny Testing

As in animal breeding, the past 30 years have seen tree breeders increasingly use techniques that more accurately identify and predict the genetic value or worth of

selected parent trees. Evaluation of a mother tree's true genetic worth can be accomplished by evaluating how its offspring perform relative to offspring from other trees. The use of a tree's offspring, whether they are cuttings (clones) or seedling offspring, is a powerful technique for examining the genetic value of any parent tree. This occurs because offspring share a portion of the mother and father genes, and when planted in sufficient numbers in a proper field experiment, will tend to sample all the micro-environments across the test site. With many offspring trees per parent, planted across the test site(s), some trees will sample better than average sites (i.e., micro-environments), and some worse, but on average the statistical average or mean of the family will reflect the true genetic mean of the family. To carry this point to the extreme, family averages (i.e., the arithmetic mean for a trait measured on all progeny from a parent) from one million offspring would predict the genetic worth or breeding value almost perfectly. This is generally not possible, viable or even necessary and it is common practise to use about 20-30 offspring per family per site, as including more observations per family tends to be inefficient with respect to our interest in being able to compare more families. If multiple test sites are used in the progeny test, it may be desirable to slightly decrease the number of test trees per family per site, but generally it is not desirable to go much less than 20-30 on each site. In more complex test designs, with fewer than a dozen or so offspring per parent on each test site, little loss of experimental trees can be tolerated.

The biology of the species to be tested plays an important part in the actual design and analysis. For instance, in most temperate conifers, the use of open-pollinated seed for testing is an efficient means for screening potential parent trees from the wild populations. However, if such seed is not available, or if insect-pollinated seed are not reliable for insuring that enough individuals from the population are cross fertilizing with the parent tree of interest, controlled pollination can be done with pollen from a few dozen "average" or random trees. This is necessary in order to have the progeny represent the average genetic effect of the population, so that all female (mother) trees are fairly compared. For instance, some of the controlled crossing systems (described below in more detail), such as the factorial-mating scheme, only use a half dozen or so male trees (for pollen). The use of such a small number of male "tester" parents, however, restricts the inferences that a breeder can make about the levels of genetic variation among the female parents being tested. Once again, if ranking the parents is the only criteria or purpose of the test, then a small number of tester pollen parents is adequate. This is typically referred to as polymix testing, especially if one dozen or more random pollen (but unrelated) parents are used in the controlled crossing.

The approach mentioned above has been described as family selection (or sire selection in animal breeding), and has become quite efficient with statistically sophisticated techniques that have been developed over the last two decades. Family selection based on the use of family averages, in well designed progeny tests (see discussion later on test designs) served as the primary technique for most of the genetic advancement in the 1960s and 1970s.

The breeder is also usually interested in determining a genetic worth or score, or more technically correct, a breeding value (BV), for each parent tree. The BV is a measure, or a numerical score, of the additive genetic worth (average genetic effect) of the genes

each female tree contains in relation to the whole population. In the case of an open-pollinated test, the breeder can estimate the BV of each parent by multiplying the family deviations (i.e., family averages minus the grand mean for the test, sometimes referred to as the general combining ability, or GCA) by the family-mean heritability and then multiplying by 2. The family-mean heritability is required to "regress" the environmental noise and lack of precision from the deviations. The multiplier of 2 is required to account for the fact that the average effect of the open-pollinated contribution from the wild is assumed to have a BV of 0. Essentially, then, the family-mean deviations, or GCA's are one-half of what they would be if the parent was crossed with itself, but of course this isn't possible in most cases because of inbreeding depression.

Improved statistical theory and computational capabilities using techniques such as Best Linear Prediction (BLP) and Best Linear Unbiased Prediction (BLUP) have now become the standard statistical approaches for predicting BV's. In forestry, with adequate family sizes mentioned earlier, techniques such as BLP are generally not necessary but should be used if possible as they have been shown to be the optimal method. The reason they are so effective is that they utilise more of the genetic information available to the breeder. This information can be in the form of the performance of relatives (father, grandfather, etc), performance across many environments, and across experiments with age differences. New statistical techniques are being developed, almost yearly, to make prediction of genetic worth more accurate and precise, but these are becoming very specific to the type of breeding problem a breeder is facing and cannot be elaborated upon here.

4.4. Progeny Test Designs

As mentioned above, the objective of progeny testing is to allow the breeder to be more accurate in the ability to evaluate the genetic potential of selected parent trees. The basic principal is again one of reducing or limiting environmental effects. Proper replication and randomisation accomplishes this and underpins all experimental layouts, but several factors need to be considered.

In forest tree genetic testing, early designs tended to follow those in crop breeding but these have been shown not to be the best for forest trees. Historically, large plots containing greater than a dozen trees, with only 2-3 replications per site were used. The thinking behind this was that any genetic differences among genetic entries in the test would be visually striking with large multi-tree plots. Yields could then also be extrapolated to yield an entire plot mean, rather than one based on individual trees. However, these designs tended to bias family means, due to inadequate replication across variable forest environments, i.e., family mean averages or deviations (i.e., GCA's) could be confounded with environmental variations across the test site. Without the use of correct analytical procedures (e.g., BV's or BLP's), and as the estimates become confounded with environmental variations, the results may not be largely different than random numbers. In summary, forest tree field testing environments can be large and quite variable and therefore good field test designs are required.

4.4.1. Field Designs

The types of designs briefly discussed above, i.e., all genetic entries in the study are replicated in each replication or block, with multiple blocks, are referred to as Randomised Complete Block designs (RCB's). In the field, blocks or replications are laid out with the hope of "blocking" out patches of similar micro-sites, but the weakness with the design has typically been that the blocks are too large and still sample across environmental patches. With fewer numbers of trees per family in each block, and therefore more blocks on the test site, the efficiency of the design becomes greater. Carried to the obvious extreme, the use of single tree plots (i.e., only one individual per genetic entry randomly located in each block) is known to be the most efficient RCB designs and are commonly used now in forest tree genetic testing. Row plot designs of 3-5 trees are still sometimes useful tests that may combine other objectives or needs, but their accuracy is somewhat reduced.

For example, in comparing two of these designs, a typical and relatively robust single-tree plot experiment would be one with 25-30 progeny per genetic entry (which means 25-30 blocks or replications). In an experiment with, let's say, four-tree row plots, and 28 trees per genetic entry, the breeder would have to identify seven blocks in the field test. Biases can occur if the seven blocks do not do a good job in stratifying the site into seven homogeneous blocks. It is important to note that if there are not environmental patches or gradients on a test site, then no experimental design is necessary! However, this is usually never the case and in fact forest tree test sites are normally quite patchy, with large gradients, even if it is not visible at the time of establishing the test sites. Recently, many breeding programs are considering new designs, such as incomplete block designs (ICB). ICB designs allow better comparisons among families, as block (replication) sizes are reduced and environmental noise is reduced, although more complex statistical analysis is required.

-
-
-

TO ACCESS ALL THE **21 PAGES** OF THIS CHAPTER,
Visit: http://www.eolss.net/Eolss-sampleAllChapter.aspx

Bibliography

Claussen J., Keck D.D. and W.W. Heisey. (1940). Experimental studies on the nature of species. *Carnegie Inst. Washington Publ.* **520**, 1-452. [One of the original common garden studies examining ecotypic genetic variation in plants.]

Falconer D.S. and Mackay T.F.C. (1995). *Introduction to quantitative genetics*. 4th edition. Longman, Essex. [One of the most well used and well written textbooks on basic population and quantitative genetics.]

Lambeth C.C. (1980). Juvenile-mature correlations in Pinaceae and implications for early selection. *Forest Science* **26**, 571-580. [Examines some of the problems and proposes an approach to make adjustments to predicted genetic gains from early selection in forest trees.]

Magnussen S. and Yanchuk A.D. (1993). Selection age and risk finding the compromise. *Silvae Genetica* **42 (1)**, 25-40. [A more advanced and specific analysis of optimum ages for early selection in coastal Douglas-fir in British Columbia.]

Namkoong G., Kang H.C. and Brouard J.S. (1988). *Tree breeding: principles and strategies*. Springer-Verlag, New York. [One of the most thorough text books on tree breeding theory and strategies suitable for forest geneticists and tree breeders.]

White T.L. and Hodge G.R. (1989). *Predicting breeding values, with applications in forest tree improvement.* Kluwer Academic Publishers, Boston. [An important and well-used textbook by forest tree breeders who are involved with selection and prediction of genetic gains from forest tree breeding experiments.]

Zobel B. and Talbert J. (1984). *Applied forest tree improvement*. John Wiley and Sons Inc., New York. [A basic textbook on the principles and techniques used in forest tree improvement programs.]

Biographical Sketch

Alvin D. Yanchuk is currently a senior scientist and program manager, Forest Genetics Section, Research Branch, of the British Columbia Forest Service, Victoria, British Columbia, Canada. He received his forestry training at the University of Alberta, Edmonton, completing his Ph.D. in 1986. After working for the Alberta Forest Service as the lodgepole pine geneticist, he moved to the B.C. Forest Service genetics and tree breeding program in 1988, as a quantitative geneticist and then as research and program manager for forest genetics research and tree breeding. Most of his current research and applied work in forest genetics has been in the areas of gene conservation, breeding strategies, quantitative genetics, wood property breeding, and research in deployment strategies for genetically selected populations. From June 2000 to January 2001, he worked as a consultant to the Food and Agriculture Organisation, Rome, in the Forest Genetics Resources Program.