

regimes on the structure and diversity of populations. However, the most appropriate measure of genetic diversity is problematic. Diversity reflected in neutral genetic markers is often poorly correlated with the functional diversity that is often crucial to site adaptation and to response to artificial selection, such that common-garden field trials are often needed to relate functional diversity to marker diversity as well as elucidating genecology and within-species taxonomy. Common-garden experiments, however, while they can reveal much, will often need to be complemented by ecophysiological studies. Such studies can identify the specific environmental factors that cause stress, and the ways in which stress arises. As such, they can be powerful tools for applying results of the common-garden experiments beyond the particular environments where they are located. Of interest are indications that major functional diversity can be governed by promoter regions rather than coding regions of genes.

The genetic component of conservation management is likely to depend on the ecological validity of management regimes. Misguided exclusion of fire has often proved very counterproductive. Questions must now arise concerning the appropriateness of trying to impose uneven-aged and essentially continuous canopy structures on each and every species.

See also: Genetics and Genetic Resources: Genetic Systems of Forest Trees; Molecular Biology of Forest Trees; Population, Conservation and Ecological Genetics. **Tree Breeding, Practices:** Breeding for Disease and Insect Resistance; Genetics and Improvement of Wood Properties. **Tree Breeding, Principles:** A Historical Overview of Forest Tree Improvement; Breeding Theory and Genetic Testing; Economic Returns from Tree Breeding. **Tree Physiology:** Stress.

Further Reading

- Burdon RD (2003) Genetically modified forest trees. *International Forestry Review* 5(1): 58–64.
- Dutkowski GW, Costa e Silva J, Gilmour AR, and Lopez GA (2002) Spatial analysis methods for forest trials. *Canadian Journal of Forest Research* 32: 2201–2214.
- Kibblewhite RP and Shelbourne CJA (1997) Genetic selection of designer trees for different paper and pulp grades. In: *Transactions of the 11th Fundamental Research Symposium Fundamentals of Papermaking Materials*. Cambridge, UK, pp. 439–472.
- Lambeth C, Lee B-C, O'Malley D, and Wheeler N (2001) Polymix breeding with parental analysis of progeny: an alternative to full-sib breeding and testing. *Theoretical and Applied Genetics* 103: 930–943.
- Lambeth C, Lee B-C, O'Malley D, and Wheeler N (2001) Polymix breeding with parental analysis of progeny: an

alternative to full-sib breeding and testing. *Theoretical and Applied Genetics* 103: 930–943.

- Mátyás C (ed.) (1999) *Forest Genetics and Sustainability*. Dordrecht The Netherlands: Kluwer Academic.
- Muller-Starck G and Schubert R (2001) *Genetic Response of Forests to Changing Environmental Conditions*. Dordrecht, The Netherlands: Kluwer Academic.
- Plomion C (2001) Wood formation in trees. *Plant Physiology* 127: 1513–1523.
- Skårppa T and Johnsen Ø (1999) Patterns of adaptive variation in forest tree species: the reproductive environment as an evolutionary force in *Picea abies*. In: Mátyás C (ed.) *Forest Genetics and Sustainability*. Dordrecht, The Netherlands: Kluwer pp. 49–58.
- Strauss SH (2003) Genomics, genetic engineering, and domestication of crops. *Science* 300: 61–62.
- Strauss SH and Bradshaw HD (eds) (2004) *The Bioengineered Forest: Challenges for Science and Society*. Washington, DC: RFF Press.
- Stroup WW (2002) Power analysis based on spatial effects mixed models: a tool for comparing design and analysis strategies in the presence of spatial variability. *Journal of Agricultural, Biological and Environmental Statistics* 7: 491–511.
- Unifying perspectives of evolution, conservation and breeding (2001) Proceedings (eight papers), Symposium in Honour of Dr Gene Namkoong. *Canadian Journal of Forest Research* 31: 561–632.
- Walter C and Carson MJ (eds) (2004) *Plantation Forest Biotechnology for the 21st Century*. Trivandrum, Kerala, India: Research Signpost.
- White TL, Neale DB, and Adams WT (2003) *Forest Genetics*. Wallingford, UK: CAB International.
- Yanchuk AD (2001) The role and implications of biotechnological tools in forestry. *Unasylva* 204(52): 53–61.

Conifer Breeding Principles and Processes

A D Yanchuk, British Columbia Ministry of Forests, Victoria, BC, Canada

© 2004, Elsevier Ltd. All Rights Reserved.

Introduction

Conifers or gymnosperms occupy many important forest ecosystems and are some of the oldest known species on the planet. Of the thousands of conifer species known to exist on earth, probably fewer than 100 have received even preliminary genetic studies, and only a dozen or so are under any kind of intensive tree improvement and breeding efforts. Some of these species, such as Monterey pine (*Pinus radiata*), have provided some of the most successful

commercial forest tree plantations in the world. According to the Food and Agriculture Organization, of the approximately 187 million ha of forest tree plantations around the world, 31% are coniferous and of these approximately 20% are pine species.

Relative to genetic improvement in agricultural species, forest tree genetic improvement programs are very new. However, many changes to classical breeding approaches used in domesticated plants and animal species have had to be made in order to accommodate the unique biological features of forest trees. The reproductive biology, geographic patterns of genetic variation in the species, preservation of adaptive genetic variation, and the maintenance of wild forest reserves are issues that are unique to forest tree genetic resource management.

Genetic Surveys and Selection in Natural or Naturalized Populations

As far back as the early and mid twentieth century emphasis was placed on range-wide genetic surveys (i.e., provenance testing) using 'common garden' experiments, first established by research ecologists. Samples (seed) were collected from natural populations of interest (i.e., provenances) then raised and planted together in several environments to test performance of the local provenance versus nonlocal.

These studies showed that most conifers have large amounts of genetic variation, both within and among populations, and local populations are generally slightly suboptimal (e.g., adaptational lag can be present, among other factors). 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 this type of adaptation. Conifers can be classified 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 with climatic or geographic variables, yet others, such as Douglas-fir (*Pseudotsuga menziesii*), are much more tightly adapted; hence, movement of seed in this species needs to be restricted. A few species, such as some of the *Thuja* species, have genetic variation substantially lower than most other conifers, and at the extreme, *Pinus resinosa* shows almost no genetic variation.

Sampling strategies for breeding programs have sometimes been designed to take advantage of natural levels of variation among populations. In some cases, usually where exotic species are used, 'provenance effects' may be available to use, or a

well-developed 'landrace,' if present, may also be an effective base population. Several large industries have been established from such introductions of materials, as with Monterey pine in New Zealand, Douglas-fir in France, Sitka spruce (*Picea sitchensis*) in the United Kingdom, and *Eucalyptus* spp. in many tropical and semitropical countries.

Genetic Testing

Genetic testing techniques developed over the last several decades have made it possible for much more rapid genetic improvement. However, the underlying reason for genetic gains is the same: most traits have genetic variation, and these differences can be transferred to offspring or progeny (i.e., they are heritable). Overall, most tree improvement programs have focused on improving growth potential, so selection on individual phenotypes has generally not been very effective because growth is typically a lower-heritability trait. However, a few other traits have been successfully improved by phenotypic selection, such as straightness, form characteristics, some disease resistance traits, and wood properties.

Field Test Designs

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. The use of a mother tree's offspring, whether vegetative (clonal) or seedling offspring, is a powerful technique for examining the genetic value of any parent tree. With many offspring trees per parent, planted across several test sites, some trees will sample better than average (i.e., microenvironments), and some worse, but on average the statistical average or mean of the family will reflect closely the true genetic mean of the family. It is a widespread practice 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.

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 randomized complete block designs (RCBs). In the field, blocks or replications are laid out with the hope of 'blocking' out patches of similar microsites, but the weakness with the design has typically been that the blocks are too large and fail to partition off much of the environmental variation that occurs within recognizable 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.

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 replicates). It is important to note that if there were no environmental patches or gradients on a test site (which is usually never the case), no test site designs would be necessary. Forest tree test sites are normally quite patchy, with large gradients, even if this is not visible at the time of establishing the test sites. Of late, many breeding programs are considering new designs, such as incomplete block designs (ICB).

One significant problem with progeny testing to identify superior parent trees is that when competitive effects among trees take place, it is effectively no longer useful as a genetic trial to compare all genotypes. Slower-growing families will continually be outcompeted and among-family growth differences inflated. Hence, predicting genetic gain from older progeny tests is biased, as the trees are not in a true plantation situation. In recent decades, many genetic-gain trials in many programs have been established to help verify estimates from replicated progeny trials to volume yields on a per-hectare basis. Many institutions working with fast-growing species, such as Douglas-fir and radiata pine, now have very good measures of yield per hectare, with commercially well-known clones or families.

The Problem of Early Selection

In forestry, it is rare (except with some fast-growing hybrid poplars or *Eucalyptus* spp.) for selection ages to approach the final harvest age. This is particularly true for most conifer tree species bred for timber production, where rotation ages can be 25–80 years, depending upon the species and site productivity. In this situation, if the genes that affect the expression of a trait at an early age change their expression, then errors in selection accuracy and prediction in gain will be made. Family selection ages in forest tree breeding have usually been shown to be optimal (based on growth data) at ages 6–12 years. Tree breeders, then, face the issue of making adjustments to the predictions of genetic gain at rotation, to accommodate the juvenile to mature-age genetic correlation being imperfect. It will take several more decades for forest geneticists to understand the magnitudes of age–age genetic correlations in forest trees, so this is an inherent risk in tree breeding – little else can be done at this stage.

Genotype × Environment Interactions

It is also known that a particular genotype may perform differently, relative to others, in different environments. Thus genetic differences can be

expressed differently in different environments. This is usually referred to as genotype × environment interaction ($G \times E$) and has been a very important issue for geneticists to consider for many decades. Most conifer breeding programs use several test sites to look for stability in performance. For the most part, conifer genotypes exhibit reasonable performance across many variable sites, except in extreme conditions (e.g., such as low-elevation selections being tested at high elevations). Therefore, it is important that in initial testing schemes, multiple-site field-testing be used in order to establish the magnitude of $G \times E$ in the population and identify specific families of interest.

It is generally accepted now that any satisfactory method of analysis of $G \times E$ must allow the breeder the ability to differentiate between true changes in rank among genotypes in different environments, and that of statistical artifact (e.g., scale effects caused by different means and variances among different sites). The quality of the genetic tests, measured by heritability, is one of the factors that must be considered, along with the similarity in averages and variances.

Advanced-Generation Breeding and Testing

If it proves to be economically and biologically feasible to carry on with a genetic improvement program, the best parents identified through the first round of progeny testing (P_1 selection) need to be mated together. With recurrent selection continuing on this basis, the breeders are essentially creating new allelic combinations from which to change the frequency of genes for the trait of interest by selection. The most advanced conifer programs in the world, e.g., *Pinus taeda*, *P. radiata*, *Pseudotsuga menziesii*, and a few others, are at the filial third (F_3) or filial fourth (F_4) generations.

Traits of Economic Interest

Besides the improvements in growth performance, wood density has been studied and has been found to have higher heritabilities than growth rate, typically twice that of growth rate. However, there are often lower coefficients of phenotypic variation so gains are not large in most wood quality traits particularly when growth rate is also selected. As well, there are typically adverse genetic associations between these two traits. Many other traits are also important in conifer breeding and genetics, such as pest and disease resistance, tree form as it would affect log and timber quality, and many other wood properties. Whether these are being directly selected for

economic reasons or not, conifer breeders have investigated these traits to establish the genetic and phenotypic relationships that could affect selection on other main traits (i.e., typically growth).

Mating Designs

There are several ways in which conifer breeders have been making controlled crosses (i.e., mating designs) for advanced-generation breeding. Complete matings of all possible desirable crosses is an ideal, but generally not practical and not economically or biologically necessary. Balanced mating designs are preferred in that estimates of general combining ability (GCA) and specific combining ability (SCA) are unbiased, but this has generally been shown not to be overly important. Making as many crosses as possible in the shortest period of time (as long as each parent is crossed a few times) tends to be a more cost-effective approach, as genetic gain per unit of time is generally the most important measure of long-term achievement in forest tree breeding.

A mating design that has been used often is the factorial design. This is where a tree can serve exclusively as a male or a female in some small mating group. This is one distinct advantage of the factorial design; e.g., if the breeder chooses a breeding group of eight unrelated trees, and mates them in the factorial design, four parents only need to provide pollen, and the other four only need to provide female cones for isolation and breeding. The total number of crosses in this situation is therefore 16. In the most commonly used mating design, the diallel, trees are used both as males or females. Complete diallel mating designs are generally not necessary, and the partial half-diallel scheme is usually employed by most tree breeding programs. However, after three to five crosses per tree it has been shown that there is little added benefit for the purposes of family and within-family selection.

Although there has been substantial debate about which approaches are best for advanced-generation field tests and selections (i.e., 'forward' selections), the most effective method will likely be determined by local operational and practical matters more than by theoretical ones. For instance, large homogenous single-family blocks (e.g., 100 full-sibling trees in each block with no replication) have been established, which allow the breeder a much better visual comparison of all cohorts in that family. With homogeneous test environments, this method can be very effective. On the other hand, breeders are usually still interested in applying selection at the family level, before selecting a few individual trees within families. In such cases, the designs, such as

those discussed above (i.e., RCB designs), need to be used. Selecting individual trees for the next generation from single-tree plot or incomplete block designs is still possible with these designs (relative to the use of RCBs, or large single-family plots). However, it is likely that more reliance must be put on the family level of selection and therefore the use of more statistical assumptions and methodologies.

Breeding Population Structure

It has been shown that high short-term gains are possible with a relatively small breeding population, without losing much genetic variability (sometimes it may actually increase) even over several generations. If, for example, an aggressive program is considered appropriate, an elite breeding population of about 30 parents could be crossed together for the second generation population. In this situation, however, many of the lower-frequency genes, which may not have strong effects on the trait of interest, will be lost by genetic sampling effects. But including dozens more, or even a hundred, will only slightly reduce the chance of these losses. Moreover, these low-frequency genes do not have much affect on the genetic variation under selection. So other approaches should be developed to accommodate these kinds of genes (i.e., gene conservation programs). Most conifer breeding programs around the world now work with relatively small population sizes in the F_2 and F_3 generations, but maintain larger genetic reserves by other means.

Once this issue of population size has been resolved, it would be important to consider issues of relatedness that will be built up over time in the breeding population. This is inevitable in any breeding program, so tree breeders have to design methods to minimize the effects of accumulated inbreeding as best as possible. Subdividing the breeding population into groups that remain separate from intercrossing, sometimes referred to as sublines, allows the breeder to make matings among trees within sublines over generations but never crossing trees among sublines. This approach is now common in most tree breeding programs, as it allows the breeder the option of making selections among unrelated groups, which will be important for commercial seed production, usually in seed orchards.

Inbreeding Depression

As discussed above, it is inevitable that there will be relatedness among future-generation selections. Inbreeding is generally undesirable, because when related individuals are intermated, the deleterious

genes (typically present even in highly selected parents) will become homozygous at frequencies higher than expected by chance. If too few individuals are selected, or selection is not strong enough to eliminate these homozygous individuals, some deleterious alleles can become fixed in the selected population and overall performance or fitness will be lowered in the population.

Most tree species simply grow too slowly and are too difficult to work with to develop inbred lines, as has been done in some crop species. In forest tree seed orchards, or production populations of trees, as mentioned earlier, a breeder can take one or a small number of trees from each breeding group (subline) and inbreeding is effectively zero and production is maximized.

In the breeding population, mating decisions that minimize crosses among close relatives over time, and generate a large number of offspring per generation, will reduce the risks and hazards of inbreeding depression. With other experimental organisms (e.g., mice, maize) adequate population sizes with strong selection, has, over time, offset these effects and made genetic improvement a long-term possibility.

Genetic Diversity and Risk in Forestry

There are essentially two types of risks that foresters and forest genetic resource managers need to consider:

1. Is there a threat from a currently known pest that may be able to overcome current levels of natural resistance in the species and inflict unacceptable losses in planted forests?

There are several ways in which this can be addressed, but all will depend upon a good understanding of the genetic basis of the resistance. For instance, several types of resistance mechanisms could be deployed in mixtures to make it difficult for the insect or pest to mutate to overcome all resistance genes at once. Another approach could be to deploy some susceptible genotypes along with resistant genotypes that rely on a single resistance gene or mechanism. The hope is that there is less pressure on the insect to evolve virulence to the resistant gene, while allowing for a commercially viable crop.

2. The geneticist must consider that there may be some future risk of a currently unknown threat (e.g., the introduction of an exotic pest). This is likely the case in which some natural resistance, controlled by genetic variations present in the population, is lost by mismanagement of genetic

diversity in the deployment population. Responsible genetic management policy needs to be founded on this basic premise.

Several research approaches have been considered with respect to this problem. As a general rule it has been shown that a very large number of genotypes, say much greater than 30–40, do not add any additional levels of ‘genetic safety’ over what natural forest regeneration might be able to provide. In fact, a relatively safe range can be between 10 and 30 unrelated genotypes. This is due to the following: genes at low frequency in the original wild population, which may be able to provide some resistance or increase fitness of the tree, will not be at frequencies high enough to protect even these natural stands. In other words, there would not be enough surviving and unrelated individuals in the wild stand with the resistant gene to serve as parents to regenerate the stand.

In summary, the deployment of genetic variation across the landscape for timber production must balance several factors, but a linear increase in some measure of diversity does not, in itself, provide a linear decrease in risk.

Gene Conservation

Basic measures in most tree breeding and conservation programs are to outline, document, and establish where and how certain classes of genes and genetic variation are best maintained over time and space, in case they are needed. Gene conservation should be viewed as a type of genetic ‘insurance,’ but the breeding populations, if present, should maintain most of the variation needed for the short and medium term future.

The loss of additive genetic variation in a population can be approximated by the formula $1/(2N_e)$, where N_e is the effective population size, so a sample of 30 trees from an infinitely large population will contain over 98% of the original genetic variation in the population. However, what will not be present in relatively small breeding populations are most of the genes that were at relatively low frequency in the original population. Conservation of these genes, either singly or in some complex of frequencies, is why gene conservation programs are needed and have to be designed to achieve over time. Breeding populations, clone banks, provenance trials, progeny tests, can all be linked, along with *in situ* populations (protected areas, such as parks, or managed forests with natural regeneration) to form the base of material for a conservation strategy.

Climate Change and Tree Breeding

Tree breeders may need to develop breeds today, for climates several decades from now. Assuming climates do change as predicted from global circulation models, it is largely up to the genetic resource manager, with an understanding of genetic variations and patterns of adaptation that the species exhibits, to respond now and generate material that will continue to produce the products we desire. Climate change models, and predictions of changes in biotypes around the world, can only tell us where the problems will be, but do nothing in themselves to offer solutions. The management and movement of the appropriate populations, largely developed and understood by researchers and tree breeders, will be the viable approach for forest managers to consider. Most wild populations of conifers, indeed most tree species, will not be able to migrate enough to maintain much resemblance to current forest ecosystem types. In this respect, climate change research in forest management is first and foremost a problem of genetic resource management.

Biotechnology and its Role in Conifer Breeding

There has been much technical development over the last decade with respect to cloning forest trees and changing the genetics of plants with transgene technologies. Cloning techniques, such as simple grafting, go back as far as the first grape varieties, but with current tissue culture techniques in conifers, such as somatic embryogenesis, genetic transformation using recombinant DNA technology is possible. Still uncertain, however, are the economics and social acceptance of genetically modified trees.

Molecular genetic markers are also becoming more important tools in forest genetics research and breeding, especially as genomes are becoming better understood with more complete molecular analysis. The use of marker technology for marker-assisted selection in applied breeding programs is also possible, but has not yet been applied widely in forestry tree breeding, primarily because of costs. Nevertheless, the information from molecular genetics research is uncovering many interesting aspects of gene evolution and function.

Conclusions

Tree breeding techniques (e.g., modern selection, breeding, and testing methods) are of course of paramount importance in pursuing improved quality of commercial conifers for traits of interest. Important techniques in tree breeding that have

developed over recent years mainly revolve around field-test designs, mating designs for advanced-generation breeding, and many specialized procedures to assess traits of interest. New areas of research and program management are required in the areas of conservation of genetic variation, deployment patterns for improved varieties, special traits that require innovative assessment and selection systems, molecular genetics and genomics, multiple population breeding, and genetic management information systems. Of utmost importance, however, is that tree breeding programs consider the simplest and most effective methods that can be handled by the organization in realistic time frames.

See also: **Genetics and Genetic Resources:** Genetic Aspects of Air Pollution and Climate Change; Genetic Systems of Forest Trees; Molecular Biology of Forest Trees; Population, Conservation and Ecological Genetics. **Tree Breeding, Practices:** A Historical Overview of Forest Tree Improvement; Breeding and Genetic Resources of Scots Pine; Breeding for Disease and Insect Resistance; Breeding Theory and Genetic Testing; Economic Returns from Tree Breeding; Genetics and Improvement of Wood Properties; *Pinus Radiata* Genetics. **Tropical Ecosystems:** Tropical Pine Ecosystems and Genetic Resources.

Further Reading

- Clausen J, Keck DD, and Hiesey WW (1940) *Experimental Studies on the Nature of Species*. Washington, DC: Carnegie Institute.
- Falconer DS and Mackay TFC (1995) *Introduction to Quantitative Genetics*, 4th edn. Harlow, UK: Longman.
- Lambeth CC (1980) Juvenile-mature correlations in Pinaceae and implications for early selection. *Forest Science* 26: 571-580.
- Morgenstern EK (1996) *Geographic Variation in Forest Trees: Genetic Basis and Application of Knowledge in Silviculture*. Vancouver, BC: University of British Columbia Press.
- Muller-Starck G and Schubert R (eds) (2001) *Genetic Responses of Forest Systems to Changing Environmental Conditions*. Dordrecht, The Netherlands: Kluwer.
- Namkoong G, Kang HC, and Brouard JS (1988) *Tree Breeding: Principles and Strategies*. New York: Springer-Verlag.
- White TL and Hodge GR (1989) *Predicting Breeding Values, with Applications in Forest Tree Improvement*. Boston, MA: Kluwer.
- Young A, Boshier DH, and Boyle T (2000) *Forest Conservation Genetics: Principles and Practices*. Collingwood, Australia: CSIRO.
- Zobel BJ and Talbert J (1984) *Applied Forest Tree Improvement*. New York: John Wiley.
- Zobel BJ and van Buijtenen JP (1989) *Wood Variation: Its Causes and Control*. Berlin: Springer-Verlag.