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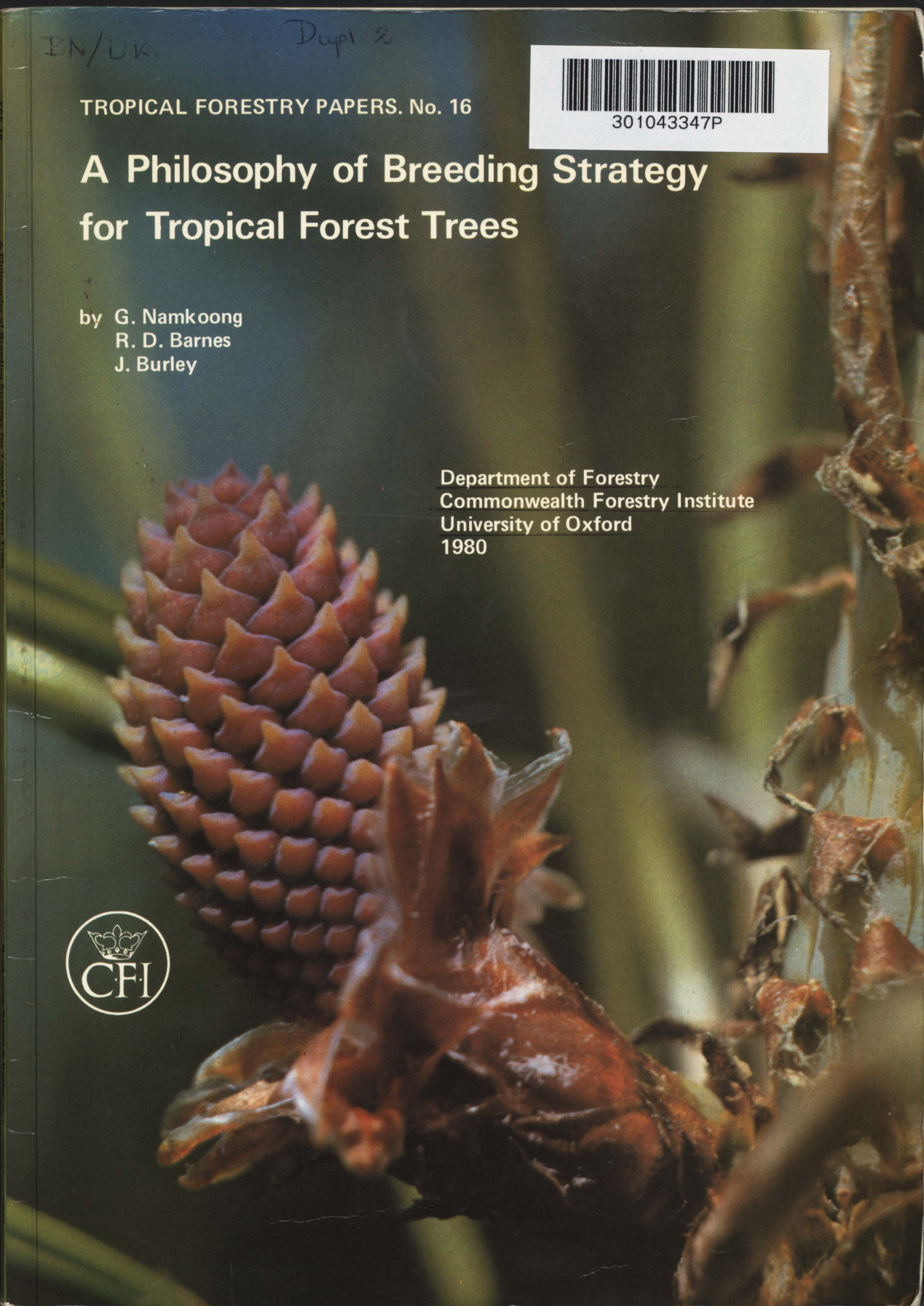


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A Philosophy of Breeding Strategy for Tropical Forest Trees

by G. Namkoong
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Department of Forestry
Commonwealth Forestry Institute
University of Oxford
1980



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A Philosophy of Breeding Strategy for Tropical Forest Trees

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SUMMARY

Economic yields per hectare in forestry are low compared with agriculture and trees must be adapted to a far wider range of conditions. However, the area of potential production for forestry is often large and even small improvements in establishment or productivity may be very significant at the national level both in terms of social benefits and in terms of land values and the production of raw material for industry. There has, therefore, been very wide interest and participation in selective breeding of commercial forest trees in the last few decades when gains as high as 30% in value have been made in the first generation of selection. Breeding strategy, however, has not generally evolved to ensure flexibility and continued advance in future generations. This advance is dependent not only upon imaginative development of methods and materials, but also upon training and the development of skills in the human resource.

Selection starts at the species/provenance/population level. Most species, and particularly those in the tropics, show patterns of variation over their natural range which are indicators of vast genetic variation which can be released by breeders. The problems lie in our ability to observe variation, in the difficulty of recognizing potential useful gene combinations that may only become useful as requirements change in the future, and in the dangers of loss of variation through forest destruction. Planned sampling in programmes of selection may be cursory or intensive depending on information available for the species, on its variability and on its present and predicted economic importance. Because of lack of knowledge, it is almost always impossible to design optimum procedures and these programmes must be planned to yield both materials and information and some redundant sampling must be accepted. A sample of 50 individuals could be expected to provide high security against losing genes and would preserve genetic variances and means in a population.

The key feature of breeding within a population is the recurrent and cumulative nature of genetic improvement. Provided effective population sizes (N_e) are kept over 50, many generations of gain can be achieved and yields increased beyond the extremes of the present population. The rate of gain is dependent upon the amount of information and materials that the breeding method provides. The more rapid growth and shorter breeding cycles found in tropical species, coupled with the use of juvenile-mature relationships, genotype-environment interaction, elegance in experimental design and perceptive data collection, can make progress very rapid indeed. The breeder's dilemma is that, through intensive selection, he can make rapid gains but if N_e is reduced much below 20, future gain and flexibility are shut off. The option of backcrossing to re-introduce variation is not practical for the tree breeder because of the long breeding cycle.

Breeding strategy at the population level has been termed "meta-breeding" and it concerns the process of long-term genetic improvement through management and development of an array of population

varieties. Through sampling variation, differences in selection criteria or uncontrolled error, genetic gain will not be the same in these populations and if there is loss of variation within them, or a change in future requirements, recombination among them would regenerate variation and increase gains.

Administrative and political constraints can be severe hindrances to the development of breeding strategy. Programmes for genetic improvement are long term and there is a need for continuity of staff, facilities and financing. There is also a need for national and international cooperation; real mutual benefits can be realised in the acquisition of materials and information, in obtaining advice and in the development of personnel.

In this book a philosophy of tree breeding is prescribed that will stimulate imaginative initiative by individual breeders to obtain economically acceptable gains while maintaining flexibility for future generations of man, trees, environments and markets.

PREFACE

Forest tree breeding has been practised, particularly in temperate regions, since the Second World War, and some dramatic improvements have been obtained in both qualitative and quantitative characteristics. More recently many tropical and sub-tropical countries have initiated similar programmes for genetic improvement and, in several cases, because of the shorter economic and reproductive rotations that are possible, they have arrived at a similar stage of development and achievement. In all existing programmes the current issue is the development of a strategy that allows rapid exploitation of existing genetic variability, while at the same time providing continuing genetic gains, maintaining flexibility for future changes of site or market, and minimising the risks of monoculture, genetic uniformity and loss of variability.

Because tree breeding is a long term activity with large investment of staff and funds, tree breeders hesitate to embark on innovative programmes, preferring to follow traditional, simple systems. Nevertheless, the authors here try to stimulate innovation within a scientifically reasoned framework that guards against waste of resources, loss of time and loss of genetic variation. In this respect the future is made more secure through international cooperation and the Commonwealth Forestry Institute has long been concerned with the provision of information and material for programmes of tree introduction and improvement in tropical countries. Drs. Barnes and Burley have been active in the initiation and development of breeding programmes in many countries. Under the terms of the U.S. Forest Service scheme for scientist development, Professor Namkoong, an internationally recognised authority on forest genetics, was seconded to the Commonwealth Forestry Institute for three months; the present document is the result of their collaboration.

I hope that their efforts will contribute to a greater understanding of the possibilities of breeding in multiple populations and for many generations, and to more efficient use of resources at the local, national and international levels.



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INTRODUCTION

CURRENT STATUS OF TREE BREEDING STRATEGY

The genetic improvement of agricultural crops has taken place by both unconscious and deliberate selection over many thousands of generations. In contrast, selective breeding of commercial forest trees is a development of the twentieth century with most activity in the second half of this century. Like many other activities within the forestry sector, tree breeding has been practised by some countries, departments or individuals as a "band-wagon" to be ridden without due consideration of the costs, benefits and, above all, long-term procedures.

In many of the early tree breeding programmes, gains of volume or value exceeding 20% or even 30% were predicted or claimed and, indeed, in some cases these are being obtained at the first stage of simple, mass selection. However, there has been relatively little planning of procedures for selection, mating and testing that will allow continued genetic and economic advance over several generations while providing flexibility and security for changing markets, environments and pathogens.

Tree breeding is now an accepted activity in approximately half the countries of the world. However, with a few exceptions in north America, southern Africa and Australasia, the breeding strategy has stopped at the first generation concepts of selection, progeny tests and clonal or seedling seed orchards. Even in these phases of activity there has been for too long a stereotyped format in which (a) selection is phenotypic mass selection based on comparisons of "plus trees" with neighbouring trees, (b) progeny tests are open-pollinated (or, at most, polycross control-pollinated) and planted on few sites with few management conditions tested, and (c) seed orchards are of the fully randomised "polycross" design with constant clonal composition and no provision for using new genetic information or incorporating newly selected or further advanced material.

The gains per generation mentioned above are certainly possible and, in tropical species with fast growth and short reproductive and economic rotations, the gains per year are appreciable. With an optimum strategy using recurrent selection these gains can be compounded for many generations. Preliminary cost-benefit comparisons of tree breeding with silvicultural operations and pulp-mill processing in southern pines of the U.S.A. showed the economic competitiveness of breeding (see e.g. Porterfield, 1974; van Buijtenen and Saitta, 1972); there is no reason to expect a different situation in tropical areas where growth rates of exotic plantation species may be great while the availability of trained man-power may limit silvicultural refinement or pulp and timber technology.

It is because of this promise of tree breeding and the rapid turn-over of generations in the tropics that tree breeders must, from the outset, design research and development programmes that are flexible, dynamic and progressive strategies for improvement over many generations.

PLACE OF TREE BREEDING IN FOREST DEVELOPMENT

This paper is intended to stimulate tree breeders to produce socially useful forest trees quickly and inexpensively. It is hoped that, by presenting the basic concepts and a few models, there will emerge a breeding methodology that will give genetic improvement and create options for future developments. By establishing the means for forest tree breeders to obtain some gain, it is expected that they will then be free to create their own variations and innovate breeding methods better suited to their needs. While all the necessary concepts are presented, little of the detail which inhibits imagination is given and in this way we hope to stimulate new energies to create new breeds. This is a fairly general, conceptual paper which, nevertheless, touches on most aspects of a complete programme of genetics research and development.

The development of materials and data and how they can fit into a forestry development programme are considered. Ultimately, however, that development rests on the people who conduct the research and development programmes. Just as materials and information usually are available at different levels of development, so too are the personnel at different levels of development. Plans for making breeding more useful to forestry must, therefore, include plans for working with the institutional and human resources as they are available, and for developing this human potential. The objective is likely to be to develop trees, information and people in locally controlled and directed ways. While there may exist the necessity to use non-indigenous sources, a reliance on such sources is risky. On the other hand, as advances have been made in non-local sources it would be wasteful not to use the best available resources as a starting point. Therefore, we suggest that wherever material, data, techniques, or personnel are available to generate local programmes, they should also be used in international cooperative development programmes. As various local variants flower, continued cooperation should mutually benefit all participants. This requires a stability of organization and people that is often difficult to ensure. Nevertheless, stable personnel, materials, etc., must be incorporated into any forest development programme and, most critically, in the phases of development of the genetic resource programme. Among the programme options we discuss is one for multiple population development which can help to ensure progress in spite of unstable and uncertain future conditions. Nevertheless, stability can only benefit future people and forests and a certain minimum is necessarily assumed.

OBJECTIVES OF TREE BREEDING AND UNDERLYING ASSUMPTIONS

Forest development

The productivity of forests in village economies, in small and large private holdings, or in national forests is often at such a low level that investments in forestry are difficult to obtain, but the total, potential, social benefits of scientific forestry are too great to ignore. In relation to agriculture, the economic yield per hectare is

low and the time to harvest is long (see Namkoong et al., 1980). On the other hand, forestry is comparatively labour intensive, the area of potential productivity is often large, the value added by processing for diverse products is high, and the intensity of cultivation is low, which all contribute to the value of forestry investments at least at the national level. Thus, costs of planting and genetic improvement, which represent long term investments and hence incur opportunity costs, are usually borne by national or large private investors even though the benefits can be realized locally. In this paper, the assumed objective is to maximize land productivity on as locally adapted a basis as possible, under the constraints of scale required by investment costs. The economies of scale have to be used in forestry to a greater extent than in agriculture but it is the responsibility of scientific foresters to make production so efficient that the scale can be quite refined, and forestry made more intensive. The role of tree breeding in this process is to boost the economic productivity of forest lands through cost or time reduction, income or price increase, or both, within a forest management system. Genetic improvement of growth rate might affect land values directly, or increase allowable cutting rates within sustained yield systems. The effect of tree breeding may be directed on (i) establishment costs (in seed or seedling costs), (ii) juvenile mortality, (iii) rotation age, (iv) conversion costs (in stem form and limb traits), (v) land area reduction (in spacing and tree size uniformity), (vi) resistance to climatic extremes and pathogens and recovery from attacks, or (vii) chemical yields, fibre products, etc. Even with a single species, trees must adapt to environmental variations of a far greater scale than those experienced by agricultural crops. However, one of the effects of breeding for simple survival and moderate growth rates on marginally productive areas is that improvements in those traits can often justify using large areas which would otherwise be economically unproductive. For many areas, such as mine spoil banks or eroded hillsides where amenity values may be high, mere survival is a primary objective and any wood production improvements are bonuses.

Since tree improvement can increase land values on relatively broad scales, breeding operations must be integrated into large afforestation or reforestation schemes; these involve planting seedlings or rooted vegetative propagules or direct seeding. Direct seeding and planting of seedlings will require a large seed production orchard operation while vegetative propagation will require only a small seed production with a large propagule generating operation. The breeding population for the generation of cumulatively improved propagules is necessarily separate from the planted forest in the latter case, but may not be distinct in the other cases. Whether the local forest is established by plantings or by direct sowing, the breeding operations are the same; the problems of seed distribution are different, and the kinds of environmental adaptability required are quite different.

We assume that the forestry programme is well developed, at least at the national level, so that the improved propagules can be used on sites for which the improvement is desired. We assume that planting requirements can be forecast and are adequate to justify a seed production orchard or propagule production operation, and that the extent of site variation is known. We also assume that there is a long term interest in forest

development regardless of the political or economic system such that land productivity is a national objective whether it is in small or large units, in public or private ownership. We assume that some degree of central planning can be effective even if only to assist local agencies in their breeding programmes.

Thus, we assume a level of programme and personnel stability such that long range planning can be effected. While there may occur wide changes in funding and personnel levels and some shifts in forestry objectives, we assume a constant minimal level of technological capability. In this paper we assume that an agency can develop and monitor its own breeding programme and that there are techniques for crossing, planting, measuring, etc., which are or can be well established (see Burley and Wood, 1976). In this paper we shall outline programmes that can provide extraordinary gains with intensive effort and others that can provide minimal levels of assured gain with minimal effort; also we indicate how one may move from one level to another as required by externally controlled factors. We assume each agency has a personnel development programme and appropriate salary and status for technical staff so that stable programmes can be maintained. The quality of personnel and their training have major effects on results.

Investments in personnel are the most vital any nation can make because they are the most critical element in realising the inherent economic productivity of forest lands. Well trained staff are essential to conceive, design and assess experiments efficiently so as to maximise information while minimising expenditure (particularly in the collection and processing of unnecessary data).

Materials

Forest trees are considered rather abstractly in this paper with little concern expressed about our abilities to grow them from seed, or successfully to cross selected trees. We implicitly assume some familiarity with all the species we may be asked to work with in terms of their natural habitat, where and how we will plant them, their natural breeding systems and their economic uses. We assume we know what the species looks like, at least in its important variations if not in all of its potential segregants; further, if the species hybridizes, we assume we know or can soon determine the extent of any introgressions. We assume in this paper that most species are largely cross-pollinating and we will not develop inbreeding systems although such systems can be used. Most trees also require several years to reach sexual maturity from seed and it is assumed that this also imposes some constraint on rate of breeding progress. Since most trees also require several years to reach economic maturity, we assume that there is some difficulty in measuring trees at their age of full value and hence that there is pressure to select at more juvenile stages.

The long time required to harvest a genetically improved crop makes economic evaluation difficult and this factor alone affects our breeding strategies. Tree breeding must also accommodate relatively wide

environmental variations, so there are both ecological and economic uncertainties. For such conditions we will propose some appropriate breeding strategies but it is one of our assumptions that we can define a basic set of sites and traits for which general adaptability is required.

We have assumed the basic requirements for successful breeding and can now consider how intensively we might choose to breed among the various species possible. Most species can be improved by simple breeding procedures if the above assumptions are even approximately valid, and most can also be intensively bred for more rapid advance on more diverse sites for more specific economic objectives. We assume that the breeder cannot devote equal resources to all species; one problem then is to develop an effective programme for handling multiple species with limited time, funds, facilities, and personnel. A further problem is that many such programmes may already exist with some materials and knowledge in hand which are seldom optimally sampled and generated, yet which could form a reasonable base for breeding. We describe systems which assume complete freedom in choosing materials for starting breeding programmes but the actual breeding plans developed by tree breeders must consider starting with whatever prior information and materials are available, and develop the programme from them.

Genetics

The results of 50 years of genetics research have revolutionized the world in our ability to feed, clothe, and house ourselves. Starting with very simple genetic concepts, simple breeding methods were developed which increased agricultural yields dramatically. For much of this time, scientific developments have increased the level of our understanding of the complexities of gene effects and interactions, and this has increased the possibilities of breeding and genetic manipulation. However, some simplifications have begun to emerge which permit us to use simple breeding methods while keeping our options open for more complex operations. While genes are discovered to be even more complex than previously believed, tree breeding can still use simple genetic models for most quantitatively inherited traits. While linkage and epistasis profoundly affect gene action which is complex, much can still be done with the concept of independent loci. These generalities do not mean that substantial gain and new methods may not emerge from a better understanding and use of these factors, but that we can accomplish much by using the simple concepts at the same time that we accumulate information and develop other methods of directing inheritance in the future.

Simple selection concepts based on simple genetic models have led to most genetic improvements in agricultural yields. It is a common experience that in three to five generations of breeding, physical and economic gains in breeding populations have been such that population averages exceed the highest extreme ever observed in unselected populations. It is also a common experience that some six to ten generations of selection on quantitative traits produces populations

whose lowest extreme exceeds the highest extreme of the original population. These results are obtained by choosing a sufficient number of individuals with the desired characteristics which give a better chance of accumulating more of the favourable alleles than otherwise. In most cases, no single individual is likely to have all of the appropriate alleles at the loci affecting even a single trait and it is even less likely to have them for all traits. Therefore the quantitative genetic breeding model is based on crossing the selected trees to allow for recombination among genetic loci in the next generation. This will create new genetic combinations that will vary around the average of the parents and, if an appropriate mating design is used, the genetic variation will be as large as before. Then re-selection of the parents for the next and subsequent generation will provide cumulative improvement. Each genetic locus may have several alleles and the objective of breeding is eventually to accumulate the optimal combination of alleles at all loci.

If each locus were readily observed, the breeding problem would be simpler but still complicated since genes at different loci often interact and this requires observation of gene combinations instead of independent observations of single loci. If the single genes are not readily observed, as is the case for quantitative gene effects, we are a further step removed from simple gene manipulation. The gene actions themselves, whether "quantitative" or easily observed "major" genes, behave no differently and breeding objectives are not different; it is our ability to observe and control them that causes some changes in breeding method.

An additional factor to consider is whether the genes act in a mainly additive manner (the heterozygote is intermediate between the homozygotes), act in a mainly dominant manner (the heterozygote is more like one of the homozygotes), or in a mainly over-dominant manner (the heterozygote exceeds both homozygotes). If the dominance and over-dominance types of gene action and interactions are important, then we try to accumulate genes in different populations and produce the commercial seed from their hybrids. If the additive types of gene action and additive interactions are important, then we accumulate the appropriate genes in a single breeding population.

Most breeding programmes require improvement in many traits simultaneously and, for out-crossing species, we must assume that some of these traits are at least partly dependent on dominant gene action. Hence, most programmes favour crossing essentially unrelated trees, at least for producing commercial seed. In contrast, with additive gene action, it is not so important to use totally unrelated individuals. The breeding population, on the other hand, may be quickly inbred and in any case will ultimately be more highly inbred as optimal gene combinations are developed. In many cases, there will be some distinction between the seed produced for commercial purposes and that for breeding purposes, and most effort will be directed towards developing the parents for the next generation's commercial seed. Types of gene action used can be mixed and the extent to which they can be developed will depend on how much effort we are willing to put into such genetic research.

Even simple programmes can be expected to give substantial genetic gains in the first few generations. The future will then depend on accuracy in selecting the best genotypes and in keeping the parental population size large enough for the creation of the kinds of genetic recombination needed. Thus, there is a basic conflict in trying to select very intensively and still not so intensively that our chances for cumulative progress are foreclosed. There are, usually, very good compromises that can be made and reasonable breeding procedures that can give us satisfactory immediate and long term gains. In this paper we discuss how such plans can be prepared.

A further compromise in breeding must be made between the need to test precisely (so that the selections are made with minimal error) and the need to test among large numbers of entries (*i.e.* families) over a wide variety of sites. The more precisely any one entry is to be tested, the more replicates and testing sites are needed and hence the fewer the entries that can be tested. Furthermore, if breeding is to be continued with progressive adjustment for the changing state of the population's genetic means, variances and covariances, then these genetic parameters require precise estimation. All of these needs can be met but the investment cost may be daunting. Again, compromises can be made among the various objectives and reasonable plans for testing, estimation of variance-covariance structure, and production of the breed population can be made for most forest tree species.

The skill and intuition of the tree breeder is needed to make genetic science useful in forestry. The programmes described in this paper require many skills and the help of many disciplines but they are only a guide and forest tree breeders must adapt them to their particular species and requirements. Since forest tree yields are based on physiological responses in growth of various organs for soil, climate, competition, pathogens, *etc.*, the genetics of all responses require manipulation for maximal improvement. This is obviously a complex and difficult task, but one that only forest geneticists can do. From tree breeders will come the insights into the genetics of forest trees for innovations in breeding.

The individual tree breeder need not feel constrained by a rigid and expensive procedure; the multiple population procedure, as described later in this paper, can allow the breeder freedom to attain breeding objectives rapidly through his own expertise and innovative ability while at the same time minimising the dangers of losing genetic variability and of restricting breeding options.

It maybe a truism but it is nonetheless important to state that, in any programme, genetic and economic gain are proportional to the investment of human, as well as material, resources.

Objectives of this paper

Our objectives are to develop the concepts behind breeding plans and a few models so that the important task of developing new and better

adapted tree populations can be accomplished and so that the options for the future are widened. In the first generation of breeding, much can be accomplished with crude techniques but more refined techniques will be needed if, for example, special gene effects and genotype-environment interactions are to be used and if multiple and specially adapted populations are to be developed. How this can be managed for one species is difficult; how it can be managed for multiple species is more difficult still. We suggest strategies for accomplishing these various objectives.

The paper itself is in four parts. The first part considers the questions of species and provenance trials, their place in breeding programmes, their objectives, and some designs for developing useful populations from them. The second focuses on breeding programmes for a single species and the optimal programmes that might be followed depending on the level of programme intensity chosen. The third part suggests strategies for handling multiple populations in multiple programmes. Finally, the fourth part deals briefly with administrative and logistic considerations for both national and international programmes.

SPECIES, PROVENANCE AND POPULATION SELECTION

OBJECTIVES

In this paper we are concerned with the very practical objectives of choosing one or several populations which are useful for forest production either now or in the future. We are not concerned here with conservation for aesthetic or amenity values separate from utilization of forests for wood, fibre or chemical production, nor with conservation for ecosystem stabilization separate from genetic objectives. These other values are not implied to be less important, nor necessarily in conflict with wood utilization; in fact ecosystem conservation may often be the only way to save genes for future forestry purposes but our end objective is producing trees for human use. For this objective, we wish to find as many genes as there may be in as useful combinations as may exist, and to bring them into more useful combinations through breeding as quickly and as economically as possible.

The problem in selection is three-fold:-

- 1) our ability to observe all variations of all traits in all populations is constrained by limitations of exploration and sampling;
- 2) naturally occurring variations are lost in situ and ex situ;
- 3) for presently perceived needs the most useful combination of genes probably does not exist and is in any case not recognisable. In addition, future needs are poorly perceived by us now and it is not possible to predict with any precision what will be useful gene combinations in future.

This is as true for well known and long studied species as for species just being evaluated; the major differences are our relative experience and familiarity with their distribution, uses and reproduction. In all cases, however, we have barely begun to understand and utilize the potential genetic variations that exist. In some cases we have already lost genetic variations which might have found use but which will never be known. This is almost certainly true of modern Europe, northern Asia and north America and it is now occurring in the tropics.

As we observe our remaining natural legacy and consider the ecological conditions in which we expect trees to produce material of economic value, it becomes apparent that our three problems are substantial. In this section we address these problems and suggest ways to sample, test, and use populations. Various levels of intensity of effort and investment will be seen to provide varying levels of security or precision in results. The objectives of higher levels of investment are to obtain the best materials for immediate use, to provide a base from which to start further breeding with the best chances for developing high yielding varieties, and to ensure cumulative progress in the future.

At this point it might be useful to consider an alternative view which might be forced upon us or which we might sometimes reasonably choose. Consider a species which we feel is well known, all of its ecotypes previously explored, and its potential uses well known. In such a case we might feel that little is to be gained by detailed research, and we can simply choose a reasonably good population for use in a simple breeding programme and ignore effete refinements which are mainly of academic interest. This basic means of operating costs little, is a traditional means of plant breeding, and can always be a programme of last resort. It is quite reasonable to choose this minimal investment option, especially for species about which we can be confident that our knowledge is excellent and that new selections would be almost as good as any which further study may reveal, and which in the future may not be required to change much. The minimum investment option may also be chosen for species about which we know little but for which we can predict little future use. The probable cost of not developing such species is expected to be relatively low. However, for either case, we assume that future needs, which we might not now perceive, are either unimportant or that they can be met by abandoning these species for ones which will be more suitable. The difficulty here is that we may not have a choice of other species or populations, or that they may otherwise not be as useful. In other words our "reasonably good" population may not be as useful at that future time and there will exist no good alternative. We must therefore evaluate the true economic value of minimal cost programmes in terms of their total value over future generations and determine the investments we are willing to make among several species and populations to ensure their value in the future. If species are in the lowest category for investment then we need only select among populations those which can immediately be used in a simple selection and regeneration programme. We discuss below a simple programme for such limited objectives which may be suitable for some species.. We also discuss more complete programmes which include exploration for species about which little is known but which might collectively be significant in future forestry enterprises. We also discuss programmes for those species for which commercial interest is already established and for which there is a concomitant body of knowledge about their structure, possible uses, etc.

MATERIALS

The large number of genera and species of potential value in the tropics precludes any single generalization being made about reproductive behaviour or population structure. To an untrained observer, each species, genus or whole family may appear to be a single, uniform and unchanging whole. To the trained forester, significant variations are perceivable but perhaps are considered to be expressions of environmental variations spread with annoying regularity throughout the forest. To the scientifically attuned, however, the variations occur in patterns, and are cryptic indications of vast potential genetic variations which can be released by tree breeders if the populations can be understood and their behaviour bent to human uses. Some species may actually be genetically uniform, e.g. Pinus resinosa Ait., and others

may be quite variable within populations but show little difference in mean or variation between populations (e.g. P. patula Schiede and Deppe). The majority of conifers show more significant population variations. As we begin to consider more tropical species, particularly hardwoods, uniformities become even rarer as populations are subdivided into smaller groups with shorter breeding cycles and species succession more rapidly culminates in climax communities (see e.g. Stern and Roche, 1974). Hence we actually have considerable need for good provenance research in most forest tree species.

For those species which occur in reasonable density and which have the possibility of inter-mating over long distances, any allelic variations are likely to be widely spread and there is less need to sample among very many stands, since most will hold the same genes but with some minor differences in frequencies. As long as pollination is not restricted (not selfed nor pollinated by insects with limited ranges) and seed fall allows for some distant inter-mixing, the opportunities for genes to be widely dispersed will exist. Then, as stands grow and are replaced by others, or as individual trees develop and disperse their progeny, little differentiation in seedling gene frequencies is likely to occur (Campbell, 1979). Selection may subsequently change the genetic make-up of even closely located neighbours and if selection is consistent over several generations, the frequency of outcrossing may diminish and some sub-divisions may become established e. g. P. thunbergii (Sakai et al., 1971, P. ponderosa (Mitton et al., 1977) and Eucalyptus obliqua (Brown et al., 1975).

In contrast, for species with more restricted gene migration, such as those which are mostly self-pollinated and those which have heavy seed that is rarely transported, the chances for selection or accident to establish genetic differences among stands are increased. Thus, for species such as Liriodendron tulipifera L. and many tropical hardwoods, useful genetic variants exist in some pockets of their distribution and not in others. Since many stands may lack these genes, it becomes more important to sample in widely distributed stands and to save a diversity of genes for their possible future uses.

Both the reproductive biology of the species and the history of diversifying selection can affect the distribution of genes and make the existence of sub-division of the natural populations more significant in the present problem of how to sample for the genes. They may also indicate for us the likely levels of natural inbreeding and the possibility of the existence of special gene combinations, which are more likely to occur in sub-divided populations, and hence the kinds of gene action we may find useful in future breeding programmes. It would be necessary to maintain the distinctiveness of such populations to allow use of their unique gene combinations.

In addition to the natural stands that constitute our usual concept of the distribution and structure of a species, there often exist naturalized populations in non-native areas. These are often of unknown origin but, having been successfully established for more than one generation, they can be considered to be adapted, local provenances

("derived provenances" in the sense of Jones and Burley, 1973) or "land races" (da Vecchi, 1963). They may be more isolated from the rest of the populations of their species but nonetheless they represent an evolutionary adaptation just as any natural population of the species might. In fact, because such exotic populations have adapted somewhat to environments similar to those expected for future planting, they may be prime candidates for initial study and selection. The use and interpretation of such populations in traditional provenance research is made difficult by their more transitory history of selection and inbreeding but such problems should not preclude their use in evolutionary studies nor in breeding programmes. There already exist examples of proof of the genetic changes that occur in introduced populations in even the first generation of environmental and silvicultural selection in their new environment (e.g. P. elliottii in South Africa (Marsh, 1969), P. elliottii and P. taeda in Malawi (Burley et al., 1980), P. patula in Rhodesia (Barnes et al., 1976).

For the variety of species which we may be required to consider in any one national or regional programme, an array of methods for handling the various programme phases would be desirable so that appropriate gene management methods can be applied to each species or population. An optimum programme requires information on the biology of the species in its native habitat as outlined above but also requires information on the habitats in which it is to be used. If a species is to be used within its own area of adaptation, then some degree of trust can be placed on how well individuals or stands perform in their native habitat. If a species is to be used outside its native habitat, however, a different and wider range of adaptations is required of the species than it has previously produced and a different and wider range of biotic agents may be present which can adapt to it than previous evolution has tested. Thus, non-native introductions are made with less secure predictability of results. Absolute security is never possible and even for plantations of pine and hardwood species within their native habitats in North America and Scandinavia, only broad matching of seed source to planting site is possible (IUFRO, 1974). In shifting species to new continents the experience is quite variable. Occasionally, native performance data are good guides, as Douglas fir provenance tests in Europe seem to indicate, but unexpected successes (P. radiata D. Don in Australasia or P. patula in southern and east Africa) and failures (P. radiata in East Africa, E. pilularis and other eucalypts in the sub-genus Monocalyptus) have occurred depending on the unique and unpredictable combinations of adaptabilities to particular environments.

Considerable caution must be exercised in transferring populations without prior information on adaptability or without direct field trials. In some cases, prior experience with the species and planting sites is sufficient to predict reasonable plantation success, and experimentation of the kind we recommend in this paper will develop that information; however, simple assumptions of site comparabilities could be unreliable bases for extensive planting.

One of the great problems tree breeders face is their lack of knowledge not only about adaptabilities of species and populations to the present

variety of possible growing conditions but also their adaptabilities to future conditions. Thus, we can rarely expect to start from a position of having complete knowledge to design an optimum programme. Instead, we can generally expect to start from a position of ignorance and must therefore plan programmes which can yield the information and the materials for future use simultaneously. This entails some redundant sampling and testing but we can rarely expect to be in any more comfortable a position.

PROGRAMMES

An array of programmes is available for sampling species and using them to understand and to breed trees for a future evolution more closely tied to human needs. There is a continuous range of prescriptions for sampling, design and analysis which gives us different capabilities and information, and choices on the amount of time, effort, land area and other initial resources to allocate. For national, regional, or international agencies with an array of species of varying potential value, an allocation of resources will be made. If the agencies responsible for the next generation do not allocate any resources, then only the immediate choices of short-term benefit will be made and the future choices will be limited. The loss of populations and their alleles which may be important will go unabated, plantings of this generation will not be aided by population selection, and future generations will not benefit from having good base populations or the knowledge of their genetics for more rapid improvement.

On the other hand, a panic decision to do everything possible for all species is obviously impossible to accomplish and, by setting no priorities, only immediate exigencies will determine the choices and will also limit future developments, just as if one chose to handle all species with the same total resource distributed at low levels over all the species. Setting priorities on species is a domestic problem which usually involves the consideration of the economic value of the species both now and in the future, the cost-benefit relationship for development and the opportunity costs. However the priority array is made and investments judged, there are likely to emerge three categories of species programmes:-

- (1) The highest value category, which may be very small or even missing for some agencies that choose very low investments in future forest development, would include those species for which both material and informational development is warranted.
- (2) The next value category, which may contain the remaining species of current value and those other species for which hope for future development seems reasonable, would be that for which material development is important, and information is useful but not essential.
- (3) The last category would be for those for which no need is clear, extinction may be disagreeable but otherwise acceptable, and for which only a minimum of investment effort is warranted.

Minimal investment programmes

Let us first consider programmes with minimum investment in time and effort. These are appropriate where the available information is substantial and where little use will be made of any additional information that might be obtained in more involved programmes. These are also appropriate for species of minor current importance and for which the probable future value is relatively low. They are high risk programmes for species or provenances where there is little prior information, as they generate little new information and commit the forester to using particular population selections without background knowledge.

Assuming that we already know the general distribution of the potentially useful populations or that we are willing to risk the programme on the basis of our present and perhaps meagre knowledge, a one-step or at most, a two-step programme can be devised. Simply choose the stand(s) most likely to contain collectively the genes in which we will be interested and directly incorporate the trees into a breeding programme. A collection of 50 trees could conceivably contain most of the genes wanted for use in the near future (see Appendix A) assuming that no negative correlations exist among the frequencies of useful alleles, that there is little epistatic interaction of those genes, that there is no correlation between present values and future uses, and that there is no linkage. If we are forced to use this simplest system by budget or other constraints, then the quality of the programme depends on how well these assumptions approximate the actual gene distribution situation. If the assumptions are valid, then picking the best 50 trees from the best stands will generate an advance and will form a basis for further population breeding. They may be used in the simplest breeding systems in future generations by mating among those original samples and selecting among their progenies. Provided that genes for some new resistances or biotic adaptabilities or any new demands are present in this base population, progress can be made in the future development of this species in its limited, improved form. Thus, we might consider this simplest programme as a starting point for any species; it is quickly operable, costs little, and requires little technical input. Only a single, suitably located planting site for the seedlings or ramets of the representative genotypes is necessary. Many species and populations can be handled in this way, and this method may be reasonable for species which might otherwise receive no attention or for species where more intensive effort would detract from development of more important species.

Obviously, large risks are run for the future of the species so handled and opportunities for present as well as future gains are limited by such minimal programmes since no forest tree species is so well known that it is possible to pick the best trees or the genes of greatest present and future value; complete knowledge is never available either, and hence there is always a high expected rate of missing useful genes or gene combinations. The real question is one of how much effort we are willing to expend to increase our chances of some continued value in the future. More comprehensive sampling and testing programmes reduce risks in choosing for the short and long runs.

In this category are included species which now can only be maintained for non-commercial purposes. In situ stand maintenance is likely to be the safest means of keeping these populations in which natural selection directs the genetic composition. Some of these species may develop into more useful, category 3, species while some category 3 may later drop into category 1. Since some category 1 species may rise to category 2 status, it will always be useful to conserve multiple sources of populations.

The suggestions presented here are minimal programmes to provide a workable system for research and development of forest tree species. The species arrays in the category will shift among agencies in different nations, and with time, as early estimates prove to be correct or otherwise, further shifts will occur. At the international level, different perspectives on species arrays will exist and will require cooperative approaches to tree species management.

Intensive programmes

An intensive programme of species and population selection that can be conducted for forest tree species would include the provision of information as well as genotype development in the immediate and long term. The materials developed would include a sufficient sample of all useful allelic variations for breeding purposes and from enough populations to determine which contained useful genes. This requires that large samples of a large number of populations be tested on a large number of test sites. If the genotype-environment interaction is large and potentially useful, and if each site contains trees from 50 families in each of 50 provenances, an impossibly large programme is required.

One means of increasing efficiency in use of space and materials is to develop the information and materials in several stages. A first stage may represent a crude sample of random seedlings from a few, well dispersed populations grown on a few diversified planting sites. This would not require very large samples since the materials would not be expected to be the basic breeding population and only rough estimates of general performance trends for the whole species would be obtained. A second stage can then be implemented on smaller portions of the species range and more intensive tests conducted to estimate population means and variances more precisely. Then, third and subsequent stages can involve individual tree selections from one or several stands with better assurance that the best available trees are being used. See Burley and Wood (1976) for details of the stages of provenance testing.

However, such programmes take considerable time to determine finally the trees that are the best then available, and this time is a cost in terms of investment accounting and in opportunities lost to generate better genotypes. The investment costs can be very high since no breeding is done until the end of the population testing phase. The opportunity costs can be high because many stands will be lost and therefore will not be available for future sampling and because breeding can often

generate better gene combinations in one generation than can be obtained by any level of refined selection in natural populations. Therefore, there are rapidly diminishing returns to be obtained from extended refinements of testing and an optimum programme requires some compromise between the precision of information, the time it takes to develop the information, and acquiring the materials needed for breeding programmes.

Species in this category require huge samples for analysis and for the use and security of their base populations either in situ, ex situ, or both. The design and analysis of such materials is suggested elsewhere (Namkoong, 1979) and would require large samples from a broad array of natural populations. For most species in this category, a two stage system with a period of exploration, initial sampling and study, followed by more intensive sampling, will be sufficient. Many agencies will be in a situation where some information and some materials are available and they may be forced immediately into designing the second stage with less knowledge than desired. In such cases, it would be reasonable to design a sampling system that obtains enough well structured samples on the basis of the available information, and to supplement the samples with outliers or other replicate population samples. There would not be complete assurance that the best populations for immediate use will be available, nor that genes for future breeding will be readily available, but the chances are good that the immediate loss in not using the best presently available populations is small and that genes of breeding value will be present somewhere in the sample. Populations of effective size $N_e \geq 50$ in perhaps 50 such samples would provide high security against losing genes and would also preserve the genetic variances and means within each. If sacrifice of sample size is needed, then reducing the number of seeds per tree is least harmful for future breeding. This reduces the precision of estimating tree means or population means, but would at least conserve alleles more efficiently. Greater difficulties will occur with some tropical hardwood species which may have much more localized population sub-divisions and higher levels of inbreeding. Even in these cases, however, selection for alleles of general adaptability will be seen more over large areas, and the effect of inbreeding will be random with respect to non-selected alleles, hence sampling among a large number of populations can help ensure against allelic loss. Relatively fewer samples per sub-division need be taken and future breeding with small inbred populations is likely to be useful anyway. Then, $N_e = 20$ in 200 sub-divisions of the populations would be favoured.

If a species or its potentially useful natural population variations are in danger of extinction, the usual safest location is the native habitat and we strongly support the efforts of national and international organizations to ensure the security of such populations in situ. In addition to those, ex situ plantings or land races often display remarkable adaptations to their new habitats and can serve as source populations that have already been selected for new environments. If they were derived from large populations and continue to exist in stands of several hundred or more trees, then the genetic variations within them may not be much less than comparable stands in their native habitats. If such stands are available and their new environments are physically and biotically more similar to the sites for which plantation

performance is desired, they may well be more desirable for our purposes than the native stands and selection is, then, more effective. In either case a multiplicity of populations is desirable; within limits dictated by the needs of management efficiency, the area for sampling is best spread rather than concentrated in a few large stands.

Programmes of intermediate intensity

Regardless of the population's present location, we can design a feasible, moderately intensive programme in which we would determine the patterns of variation in the several traits of interest in controlled experimental plantings and would try to relate the patterns of variation to selective factors of the environment, migration factors, or any other identifiable factor. Each trait may exhibit somewhat different responses to these factors. The detailed analysis of the multivariate, multiple regression responses can provide us with the information needed to select the best one or the best mixture of populations needed for breeding (Namkoong, 1967). If we have already sampled the desired populations in sufficient numbers and can rely on the performances revealed in our tests or other plantings of those populations, we can immediately start breeding. If we have only a small sample or if we believe from the analyses that we can locate more suitable populations elsewhere, then a re-sampling is necessary and should be of sufficient size and distribution to capture the genetic composition desired. In cases where we are transplanting sources or species into non-native situations, selection of individual trees should be more circumspect since we must rely on observations in natural stands of traits which may not be highly correlated with performances in the planting sites in which they will have to grow. Correlated trait selection will be needed and the data from the above multivariate, multiple regression analyses will be useful to design the selection criteria.

In addition to the above practical analyses, genecological studies are vital to our further understanding of the dynamics of forest tree evolution and our future use of the forests. Understanding the natural evolution of our tree species will ultimately lead to more feasible ways of working with natural systems instead of against them and will at least lead to systems of enlightened self-interest. More intensive programmes would provide more exact information and long term species development and, for some species, a continuing series of studies will be important enough to justify continued experimentation. For most species, the moderately intensive programme will be the maximum affordable one and might involve 20-30 provenances on 10-15 sites. If these test plantings are also to serve as the genetic base populations because the original stands are destroyed or their data are not useful, then the genetic variation within each must be preserved in supplemental plantings, or the test itself made large enough for each population to have at least 50-500 trees.

For species in this category, which cannot justify such heavy investments as in the complex programme, we can still save most alleles of use though we would be operating more blindly. If only a few hundred

trees can be sampled, then they can be sub-divided into relatively fewer sub-populations. If the species is widely cross-pollinated, population samples among dispersed marginal populations will be necessary to ensure representation of rare alleles, but replicated samples from the central populations will likely contain all of the useful alleles present on the margins. For more highly sub-divided populations, such as may exist for tropical hardwoods, sampling within a large number of different populations and dispersing sample sites is again more important.

For the last two sets of species (categories 2 and 3), it is assumed that, as much as possible, in situ stands in diverse sites will be maintained for at least one more generation. Samples will represent ex situ populations and all populations will be changing in some manner either directed or not directed. It is also assumed that as multiple populations of the species develop in the following few generations, the undirected stands, either in situ or ex situ, will be less easily used in the more highly developed breeding populations. The diversity and continued development of multiple breeding populations will cause these populations to diverge from the directions followed by the original populations; the original populations may then fall more into the first category of species for which no breeding investments are afforded.

SAMPLING

The intensity of sampling varies with the objective of the different programmes and the structure of the natural populations. In the rare case where the structure is very simple, the species has no sub-division and all genes are present at their average frequencies in all areas, any sample of a few hundred trees (Appendix A) will have a high probability of saving almost all alleles of possible interest. Since such species have no sub-divisions or variations over their range, detailed study of source populations would reveal nothing of interest except that such extraordinary species exist. (Evidence exists for such a lack of variation in the widespread red pine, P. resinosa Ait.). For such species, the sampling of a few hundred trees for gene conservation need not be structured in any way. A few replicate population samples would be useful to assure the continued availability of the genes. However, if the species is structured by its evolutionary history to have different alleles, or different arrays of allelic frequencies, in various parts of its range, then unstructured sampling can easily miss even fairly common alleles. Recent evidence suggests that population differentiation in tree populations occurs over a considerably smaller geographic range than previously thought (see e. g. Mitton et al., 1977; Brown et al., 1975). It is poor strategy to cluster samples according to convenience when different alleles exist in inconvenient locations.

A more satisfactory sampling strategy to follow, if we suspect that subdivisions may exist in the species, is to spread the sampling points over diverse stands which might have diverged because of isolation, selection, limited migration, their small size, or any combination of these factors. In cases where we do not wish, or cannot afford, to develop information on a species structure, a few hundred samples can

still have reasonable chances of conserving useful genes if dispersed over ecotypes or at the extremes of clines. A few replicate populations would still be useful for security reasons. However, the frequencies and even the presence or absence of alleles in the various parts of the range will be subject to variations due to the limited sampling, but at least the various alleles will probably be saved at some unknown frequency, and may be used in breeding programmes. Such sampling carries risks that even high frequency alleles may become low frequency alleles in the sample and also may be associated with other genes of poor value. Then, in future generations, risks of subsequent loss can be high, especially since we know little about which particular sampled trees or which areas of sampling may be useful. For greater utility in the future, the samples from each sub-sampled region or area should be kept separate and, hence, the number of samples per region should be increased to allow for reasonable breeding population sizes per region.

More complete programmes of gene management would be made more efficient by more complete sampling, if it can be afforded, and by the establishment of larger populations from particularly different sub-populations of the species. Larger samples within each population would better maintain the gene frequency arrays present in the natural population, and very large samples would enable us to allow for some natural and silvicultural selection in our planting sites to screen for general adaptability and to establish land races as bases for further breeding. If such larger samples are obtained in a large number of sub-populations, then, even without an elaborate planting design, some information on the extent of inbreeding and natural genetic architecture can be obtained and perhaps some information on the array of gene frequency variations also obtained (by isozyme techniques) and used. With large samples there are also opportunities for planting well designed experiments over a series of environments for information on genotype-environment interaction, information on genetic architecture and, if large enough, they can be used in a breeding programme designed to utilize their different genetic compositions. Replication in such cases serves not only as a security measure but is also necessary for analysis.

If an adequate species-wide sample is available, the types of analyses and uses are suggested in Appendix B.

BREEDING

OBJECTIVES

Breeding within a population, however the original base population may have been generated, simply involves the mating of selected trees (which in themselves or in combination with other trees combine favourable genes) to give higher returns for human uses. In addition to producing combinations of the best available genotypes, breeding involves the attempt to improve the gene combinations in trees of the subsequent generations so that the products of matings in those generations can be improved in a cumulative way over the matings possible at present. The recurrent and cumulative nature of genetic improvement is a key feature of breeding unlike the one-time improvements possible with provenance selection or with finding optimum fertilizer levels. In fact, as the genotypes change and gene combinations are created which have not previously existed, the response to environments will also change and the joint development of breeding populations and silvicultural systems will create new opportunities for improvement.

As our knowledge of genetic systems in trees increases, we may also use specific gene interactions to create phenotypic effects greater than their simple average effects. Specific combining abilities may then be useful in breeding and various kinds of dominance and epistatic gene actions might be used. For the use of these special interactions as between specific genotypes, we require testing and observations of those combinations and hence more complicated programmes than those typically called for in the simple recurrent selection programmes. For long term development of the species, refined sub-divisions of sites and materials may be desirable. In this section, we shall describe those more complicated programmes and some simplified programmes which can be carried out.

The basic means of breeding do not differ with the traits or trait combinations desired. The operations described are valid for any defined objective. However, the choice of traits and their selection intensities will differ among agencies; also, as environments and economic demands change, so too will the directions chosen for breeding. Therefore, there is also a problem of managing breeding populations to accommodate future changes within and among the agencies involved. We address these problems at the end of this section, but throughout we consider the requirement not only of keeping populations in a cumulative improvement system but also of maintaining their flexibility to meet changing demands.

ASSUMPTIONS

Throughout this section it is assumed that for the species with which the breeder is concerned, the reproductive system is well enough known to enable us to handle the crossing and seed development techniques,

that planting, cultivation, measurement, etc., are established, and that the economics of breeding are justified. If afforestation is to be carried out with vegetative propagation instead of seeds, breeding is directed toward the development and choice of ortets rather than that of seed orchard parents. In both cases, the operational production of propagules is assumed to be well worked out or within reach of achievement. This assumption often does not hold since there are enormous practical difficulties in operating seed orchards and obtaining desired matings. By assuming the solution to these problems we are merely placing the burden of discussing those issues on other shoulders.

We might note, however, that the ease and cost of seed production does affect the choice of some phases of the breeding cycle. When crossing cannot be made in the natural forests, then the opportunity for using controlled crosses must await the success of vegetative propagation in seed orchards or experimental plantings, or open-pollinated seed must be used to generate at least the first generation. This is the choice made in some Douglas Fir programmes (Silen, 1966).

Another relationship often overlooked is how the manner of reforestation or afforestation will affect seed demand. If planting is not amenable to large scale machine economies or the timing of planting is critical and difficult to control, then direct seeding in spots may be more useful (e.g. Gmelina arborea L. in Jari, Brazil (pers. comm. G.L. Gibson, CFI, Oxford)), and larger numbers of seed must be used to obtain the tree density desired, the seed orchard programmes must be designed for larger production, and breeding must be more carefully directed towards growth and survival under those conditions. In addition, if many local and small populations are to be developed instead of broadly adapted large populations, breeding and selection for such population structures will have to be adapted to local needs and provision made for the occasional introduction of new germ plasm into the local populations. Low cost forest regeneration systems may require that geneticists adapt to such demands, at least temporarily and only for some forest areas, but assumptions of breeding only for large scale national or industrial operations should not preclude the use of breeding for the smaller scale, less sophisticated, requirements for community and smallholder plantings.

Genetic assumptions

Multiple gene action

The basic genetic theory we use in this paper is that of quantitative genes. We assume that single major gene effects will be recognised and that for them, the appropriate homozygotic or heterozygotic conditions and epistatic interactions can be manipulated. Beyond such genes and traits as may be so recognised and manipulated, we assume that there is a large number of genes affecting each trait, each of small effect relative to the cumulative effect of variations at all the other genetic loci and of environmental variations.

Genes of this latter sort are inherited in exactly the same way as major genes; it is simply more difficult to work with them since they are only indirectly observable. Since they are usually more numerous, however, their cumulative effect may be large. Thus, consider a gene (A) which can affect yield by say 0.1 units, the homozygote $\overline{AA}$ might produce an average of $10.1 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$ growth, the heterozygote $\overline{Aa}$ $10.0 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$, growth, and the homozygote $\overline{aa}$ averaging $9.9 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$; these small genetic differences could well be unnoticed in a forest where there is a total variance of say $10.0 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$. However, if another such simple gene existed at a "B" locus, and its action were similar and its frequencies intermediate, then the genotypes would on the average still be distributed around the average of $10.0 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$ but the genetic variance would be doubled, the range of genetically caused differences extended to $9.8\text{--}10.2 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$ and the total variance slightly increased. With 10, 20 or 50 such genes, the total genetically caused variation would be a substantial part of the total variance, genetic manipulation could vastly increase the average performance, but still no single gene could be recognizably effective. In fact, with genes acting in this way, the original population would have a range of individual tree productivity which would yield between 6.7 and $13.3 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$ (based on a coefficient of variation of 30% as an example) for 95% of the population, while breeding could advance the whole population mean beyond $13.4 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$ in a few generations and, at the same time, still display the same variance and potential for gain.

Recombination and advance

The advances in genetically determined productivity will depend on the selective combination of beneficial genes which act well together in the forest environments in which they will grow. For long term gains, alleles at many loci have to be saved in the breeding population and recombination among them allowed to produce gene combinations which may not exist in wild populations or which may not be useful except for human uses in managed forests. If population sizes are kept large enough, and selection pressure applied, many tens of generations of gain can be achieved and yield increased well beyond any presently available levels. It is not uncommon among agricultural crops for the average yields of selectively bred populations to exceed the extremes of the original populations in a few generations and there appears to be every reason for this also to be possible for forest tree yields.

Changing value of genes and alleles

The actual gene effects on yield in any one generation may be due to relatively few genes at intermediate frequencies which have direct or modifying effects on other genes in the population. Other genes which may be at low frequency may be relatively ineffective at that time but can have greater effects in later generations due to a number of factors. Thus, simply increasing the frequency of the alleles will

increase their visible effect on yields, or the genetic interactions will change and modify the effect of alleles through epistatic interactions, or the environment and economic demands of forestry may change and increase the desirability of alleles not previously recognised as useful. In forestry, we find many traits with high heritability and large amounts of genetic variance. There is thus, a vast potential for substantially changing the forests genetically and, with wise breeding, to continue to evolve new gene combinations for future use.

Breeding dilemma

Effective population size

The basic dilemma for breeders is that with such variability, very intensive selection can achieve rapid gains but can also eliminate alleles which could be useful in the future. If our requirements for tree growth in the present environmental and economic milieu remain constant, there still exists the problem that intensive selection reduces the effective population size (N_e) to such an extent that many alleles can be lost accidentally or have their frequencies reduced to a level at which it becomes difficult to locate them for incorporation in a breeding programme. Even without the complications of linkage and gene interactions, if N_e is much below 20, the population's potential gain is reduced. To some extent, we can increase the population size from which the selections are to be made by inter-crossing among selected parents as thoroughly and in as widely balanced a manner as possible, and by planting many seedlings per cross. Then the selection differential can remain large since the numbers selected for the next generation, which may be constant, will be a smaller proportion of the total population.

Changing needs and multiple populations

However, the problem of selecting populations for economic or environmental requirements which shift in the next generation is not as easily resolved. To some extent, the breeding population may have been misdirected and alleles lost not only by accident, but by incorrect design. In some cases, we can expect that populations can be so misdirected and so many useful alleles lost that the population is best not used at all, or perhaps requires crossing with other populations to restore its use in a breeding programme. We can foresee, for example, that very specific types of wood may be desirable in one generation and undesirable in the next. In both cases, there will continue to exist a basic set of growth and form traits which can be cumulatively improved using the same initial base populations. If selection has moved the population mean prejudicially, the problem will be whether the population can still be improved for the traits of stable value while shifted for the traits of unstable value. Since trees normally take a very long time to complete a breeding cycle, the options of

back-crossing are not generally useful, and hence we might not even be able to use cumulative improvement programmes at all. Thus, over-specifying breeding objectives could lead to breeding with single populations and accomplishing only a marginal improvement over genetically unimproved populations. Breeding for resistances to new biotic enemies falls into this category of shifting trait requirements since the enemy shifts rapidly.

The dilemma arises from the great potential to breed; the more specifically we select for allelic combinations or for adaptabilities to economic and environmental objectives, the more gain we can achieve in one generation, but also the more likely it will be that the population's usefulness is limited. To overcome this problem, and to achieve rapid immediate gains without loss of future gains, we require breeding strategies that can direct multiple populations.

PROGRAMMES

Minimal investment programmes

Primary selection

In the simplest cases, where objectives can be very simply defined and can be expected to remain constant and where little investment can be afforded for breeding programmes, a low cost operation can be designed. If an afforestation or reforestation programme is to be sustained by seeding or planting without recourse to natural reproduction, then reliable quantities of seed are needed. If a native or domesticated species is being used, cheap seed may be available from wild or unimproved stands. Collecting seed from the best trees, using only these to reproduce the next generation of forests, and subsequently collecting seed from the best of that generation, etc., is a breeding method called "mass selection". However, the combined economics of controlled seed production and the greater genetic improvement possible in seed orchards often make clonal or seedling seed orchards economically superior by comparison. Particularly where a non-native species is concerned, domestic control of seed supplies and the cost of importing seed alone may make it advantageous to develop a seed orchard programme.

The selection of parents to insert in the seed production orchard need not be very elaborate though the greater care and intelligence exercised, the greater will be the improvement. Such aids as comparison tree measures, and selecting at ages and in conditions which are comparable to those of commercial stands, increase the potential gain. The difficulty of neighbour tree comparisons in natural stands was discussed by Ledig (1974). Once the trees have been selected and established in a seed orchard, no elaborate care need be taken to control pollination. However the more care taken to ensure that adequate pollination is achieved, that all selected parents contribute as evenly as possible and that there is no external contamination, the more completely will the benefits of selection be realized.

Supplemental pollen application to ensure the adequacy and evenness of pollen genotypic contribution can be operated at low cost. An inexpensive improvement could be brought in by making up a systematic array of pollen mixes so that blocks of males can be placed on designated blocks of females (Burdon and Shelbourne, 1971); this not only ensures good pollen distribution but also makes the ancestry in future selections somewhat easier to control and helps minimise gene loss through relatedness although in any pollen mixing system the male pedigree information is lost.

Selection in subsequent generations

In this minimal programme, no progeny testing is needed and no records need be kept of the seed or pollen parentage of the seeds or seedlings produced for forest planting. If records can be kept, and nurseries or plantations controlled to the extent that the ancestry of the trees in the new forest can be recorded, then inbreeding can be minimized in the future. However, for a simple programme, all that is needed is to ensure that the next generation of parents for developing future, cumulatively improved breeding varieties is selected from stands established from this germ plasm. Continued progress can be expected as long as at least 50 parents are selected in each generation, large numbers of progenies are planted for selection and there has been reasonably balanced genetic representation in the seed orchard. In early maturing tree species with a short reproduction cycle and early economic maturity, the next generation orchard may be brought into production quickly and replace the first either in stages or at a single step as its seed production grows to fulfil planting needs. Cycles of selection can then be generated and the chances of improvement enhanced as experience in selection and in seed orchard technology increases and makes more intensive breeding practices feasible. There is likely to be an increase in realized heritability in the first few generations as selection experience accumulates and selection is carried out in more controlled, managed stands under domestic conditions and with more consistent records and performance appraisals. In addition, the techniques of seed orchard management including the selection of appropriate sites, and treatment for early and prolific reproduction will enable us to go through breeding cycles faster. However, until we can use genetic engineering techniques for gene insertion, and until we can evaluate mature tree performance without dependence upon changeable juvenile-mature tree correlations, there will be a limit to the rate at which fast breeding generations can be cycled. Great improvements can still be reasonably foreseen before those limits are reached.

Problems with vegetative propagation

Such a simple system can be generated for most species, including shy-seeding hardwoods though with greater problems in regard to controlling the effective population size and generating a large enough population for a large selection differential. The production of

seedlings may be so limited for commercial use that vegetative propagation of the best genotypes available in each generation may have to be used. Techniques such as rooted cuttings, tissue culture, or even graft production may have to be resorted to for these purposes. The dangers of monoculture in forests are more of a problem with forests established from such propagation methods than they are for seeded populations. Whereas in most afforestation programmes with large genetic variances the genetic sources of variation will remain large, reductions in the kinds of genetic variants in the population will occur in any clonal programme and particularly if the number of clones is small. Mixtures of large numbers of genotypes should obviously always be used.

Limitations of the simplest system

For almost any species then, this simplest, minimal programme can be used and, in spite of its simplicity, can usually be expected to give some improvements. Thus, for even the lowest priority species, a minimum investment of time, effort, and funds, can be used to develop a breeding programme. However, many opportunities for more rapid and assured progress are foregone in such a programme and we operate blindly with respect to actual genetic benefits; we will more often fail to make gain when it is actually possible to do so, our rate of gain will be slower and gain limits lower, and we will learn little to help improve the situation. More elaborate systems may be desirable for species where heavier investment can be justified; more study can lead to more opportunities for gain. The more elaborate systems create more options for breeding and more information to guide future breeding. We assume that seed or ramet production will be generated in each generation out of the selected parents in seed orchards as for the simpler systems.

At the start of most programmes, few species are involved in these more elaborate systems, partly because we have not made the genetic potential known to forest managers; but, as soon as breeding results can be made known, we may see more species placed in higher priority categories for breeding investment.

Intensive programmes

Intensive breeding methods involve:-

- (1) more precise testing and selection;
- (2) creating more opportunities for using special genetic combinations and genotype-environment interactions;
- (3) gathering information on the genetic parameters of the breeding population.

In an intensive system, these three phases may be carried on more or

less independently and we shall discuss them separately. In actual practice, most programmes would combine at least two of the phases.

Selection and testing

The selection and testing phases involve the original selection from wild or planted stands and use of progeny or other relatives in special tests to help choose the best available genotypes for seed production and for future breeding purposes. In most cases, the selection problem will involve using wild tree performances or juvenile progeny performances which are not exactly correlated with mature tree performance under the competitive conditions experienced in plantations. In addition, the traits we most wish to improve often require more than single, isolated tree observations because of the magnitude of error variation caused by environmental, particularly competitive, effects. Although we can obtain gain by simply observing individual tree phenotypic expressions as in the simpler breeding systems, we cannot observe how well individuals would behave in other conditions nor can we obtain the precision gained by plot experiments.

Thus, replicated plot experiments are often used to observe family performances and to estimate relative genetic value of parents in at least "typical" sites. Complete block experimental designs are used for the sake of simplicity and security but partially balanced replicated designs within sites are usually more efficient and can provide as much precision with fewer replicates (Barnes and Schweppenhauser, 1980). Also, balance is often destroyed by mortality, accidents, etc., and the analyses are often complicated in spite of efforts to maintain simplicity. Therefore, unbalanced designs for replications in sites may be more useful than initially perceived. Furthermore, unbalanced designs for analyses of variance between sites will be far more useful than the traditional balanced factorial designs. These design considerations are detailed more fully in Appendix C. For present purposes we assume that the traits of interest can be well estimated in some reasonably satisfactory experimental design. The materials used may be progeny of crosses among selected parents, open-pollinated progeny of selected females, ramets, or sibs of trees which are being selected in seedling seed orchards, and which themselves may be selected as parents of the next generation.

Mating for the next generation

If we can now assume that at least one consistent set of trait improvement objectives can be well defined and that trees can be well judged according to those objectives, there will be selection among the tested trees. The proven genotypes will be used for seed production and for generating the next generation of improved material for the recurrent selection breeding cycles. It may often occur that the original selections will have been clonally propagated in a seed orchard, or control- or open-pollinated seeds grown in seedling seed

orchards and that clones or families are culled following progeny testing. At this point several problems become obvious which judicious planning could avoid.

First of all, control-pollinated seedling seed orchards or clonal seed orchards will yield more gain if they can be used. Second, several hundred original selections should be made in order that the selection following progeny testing be usefully intensive, otherwise the progeny test information cannot be used effectively for breeding purposes. Third, crossing among selected genotypes for future breeding purposes should begin as soon as possible and in seed orchards in which culling will take no more than half of the population, systematic inter-crossing even before test data is available will be wasteful of effort but economical of time. Therefore, early planning for crossing among selected parents is useful regardless of how they may be culled. Our earlier dictum with respect to the need for large numbers of parents, balanced inter-crossing, and sufficient progeny size, applies. In these more intensive programmes, we can envisage that 20-50 parents in a breeding population would be as nearly completely inter-crossed as possible, though incomplete crossing will usually result. Balanced representation in the progeny generation is desirable and hence square factorial sets, diallel blocks, or other partially controlled factorial mating designs down to single pair matings will give the desired balance. It should be noted, however, that factorial mating designs with a few individuals of one sex or any great departure from square arrangements, carries a heavy penalty in reducing the effective population size of the next generation. Complete plans with controlled crossings will allow for some degree of family selection, wider possibilities for recombination and, with additional control of future inbreeding, and minimal reduction of effective population size. Therefore, wider inter-crossing is desirable and the identity of these families should be recorded in field plantings, which would allow for individual tree selection for the next generation.

Family and individual selection

For selection of individuals within families, large plots of seedlings or many ramets per genotype are desirable to reduce error in judging individual tree merit. For family selection, small plots with many replicates are desirable, and for family testing and other estimation purposes, small plots are also desirable. If these various objectives are kept separate, we can avoid the dilemma but, if not, then a compromise is required. Ignoring the complications of competition factors and efficiency in estimated means, final plot sizes of 10-20 might often be reasonable compromises: juvenile culling will have reduced the selection intensities possible at the final thinning but 10 trees per family will allow for a good selection intensity. Since replicate plots will be used in most cases, selection can be made on the basis of inter-plot adjustments in an index that combines plot and individual tree performance (Henderson, 1963; Namkoong, 1966). Since selection will be based partly on genetic and micro-site environmental differences among individuals within families and within family plots,

the families will vary in their relative performances among replicate blocks and hence will have varying numbers selected in the separate blocks. An index which weights the relative values of individual tree and family plot performances can be constructed to adjust for plot environmental differences (Cockerham and Matzinger, 1966). If this kind of index and adjustments for inter-plot environmental differences can be well made, the plot sizes can be reduced. For clones, individual genotypes can be grown in several environments and hence selection is much more precise if ramets are dispersed. The problem encountered with ramets is that the effort and space required will bring about a reduction in the number of different genotypes available for selection. The optimum number of families, individuals per family, and ramets per individual can be determined for any cost/benefit ratios for these selection purposes using optimising calculus techniques.

Competition factors can bias selection if they are independent of growth under ordinary test conditions. Trees may grow better or more poorly when competing with relatives or other trees of similar growth type and, hence, testing in competition with different genotypes or in specific sets of two and higher order interactions may be desirable, especially when consideration is given to clonal mixtures for forest establishment. In general, however, initial tests are usually directed to favouring good general competitors and, for such tests, many replications of small plots provide a large random sample of all possible competitors for each family and, beyond this, competitive ability has not, to date, received special treatment in practical breeding programmes. The whole concept of genetic variance in competitive ability, as opposed to genetic variance in yield characteristics *per se*, is difficult to describe adequately in qualitative terms and we ignore it here; however quantitative treatments were provided by Huhn (1972; 1973) and Sakai and Mukaide (1963).

Genotype-environment interaction

Among the benefits of using controlled inter-crossing and planting families or ramets over a range of environments are the opportunities to gain information on genotype-environment interactions and to select trees on the basis of their estimated response to environmental conditions. Depending on breeding objectives, selection can favour widely adaptable genotypes or perhaps those that respond particularly well to particular environments. If genotypes behave similarly over most environments of concern both now and in the future, or if those that are widely adaptable also perform well in most environments, then trees with the best mean performance on average sites may be selected. Also if forest sites are highly variable among micro-sites and not between macro-sites, then planting in zones or blocks of differently adapted genotypes is not feasible and sub-dividing the breeding populations will not be useful. It would be surprising if genotype-environment interactions of economic significance did not exist over the wide environmental ranges we predict for present and future forests. While tests on present "typical" sites may show little interaction of this type in southern pines, tests on wider site variations display different genetically determined patterns of response

to environmental gradients (Roberds et al., 1976). The greater question with respect to developing and using these data is whether the forestry planting operations can use differently adapted breeding populations. For intensive programmes of species development, at least regional adaptabilities are commonly assumed to exist and greater efforts are justified to define how environments differ and how different genotypes can be profitably used. For the minimal programme, no such testing is affordable. For such programmes it would seem feasible to define breeding objectives for as many site groups as can be afforded and, even without formal testing, to place unreplicated plantings on extreme as well as "typical" sites. For the more complete programme, testing as described in Appendix B on as many combinations of environmental variables as can be spanned, is desirable. This can be very elaborately designed with incomplete factorial combinations of multiple site variables, or intuitively designed to cover the most important variables. Imbalance with respect to the number of replicates and treatment combinations is desirable, but is distinct from imbalance resulting from unequal genetic sampling in all test sites. For testing purposes, all genotypes or families should be examined on all sites, although the major environmental factors tested by those sites may be unbalanced and possibly unspecified or unknown. In any case, the intensive programmes will include a phase of testing in a variety of environments and selection based partly on the response pattern. Many analytical techniques for the detection and interpretation of genotype-environment interactions exist and some have been used with forest trees (see e.g. Mergen et al., 1974; Shelbourne, 1972).

Specific combining ability

Another benefit of controlled crossing is the opportunity to use specific combining abilities of particular crosses. On an individual or group basis, gene interactions appear to cause the progeny of those matings to perform better than can be predicted by their average parental contributions. These may be due to epistatic or dominance types of gene action and may contribute a bonus to advance through selection in the immediate generation, and may be a basis for cumulative improvement if the breeding operation can develop the specific interactions. At one extreme, specific single pair matings may be especially productive and inbred lines of the parents can be generated to develop higher levels of specific combining ability among the progenies. The various types of inbred line selection programmes can then be followed and, as long as a wide representation of the original lines is maintained, the ultimate gain can be maximised. However, forest trees which are open-pollinated do not generally develop well under intensive inbreeding. There is large variation in the performance of selfed lines varying from equal to or rarely even slightly better than cross performance to almost total failure of selfed seedlings (Orr-Ewing, 1965). Hence, for most species we are likely to use some form of reciprocal recurrent selection for this kind of hybrid or specific combining ability selection if it is used at all. In this system, which is usually reserved for distinct population or species crosses, the two populations are kept in separate breeding programmes

but selection for the parents of the next generation is based on progeny tests in which the progeny are the hybrids of an inter-population cross (Allard, 1964; Shelbourne, 1969). The selected trees are crossed with other selected trees but only within their respective populations, to generate the next two parental breeding populations. The same considerations of size and balance of genetic constitution exist for these populations as for the single population breeding methods. The major differences lie in the nature of the test crosses used for progeny testing and the necessary separation of the two breeding populations from the seed production populations.

Genetic parameters of the breeding population

If we can now assume that proper estimation of genetic values can be made and that the breeding populations can be generated from the selected parents, cumulatively improved recurrent selection programmes can be conducted at any level of intensity desired. The third phase of an intensive programme involves study of the populations as they have originated and are developing to guide the breeding effort. Without this estimation phase, breeding is conducted assuming that simple genetic models are accurate and that the gene actions are unchanging even as generations advance and genetic effects change. To determine the levels of genetic variation among the many traits examined and how it changes from population to population and from generation to generation requires estimation experiments. Not only will genetic means change but genetic correlations among traits can also be expected to change drastically and quickly. Several factors contribute to this including poor estimation of the original parameters, changes in gene frequencies, changes in linkage and linkage disequilibria and changes in the environments in which the trees exist. Thus, while basing breeding on educated guesses in the first generation may be the most reasonable way to operate, in future generations effective selection could well depend on exact knowledge of trait inheritance and its changes.

Fortunately, the considerations for designing estimation experiments are similar to those taken into account when designing experiments for testing and breeding. Large numbers of families are desirable for estimation of genetic variances and covariances; these family variances are the major indicator we have of gene effects and hence good representation of the genetic population is required. Thus, a large number of families is to be preferred even if it means a reduction of family and plot size (Namkoong and Roberds, 1974; Barnes and Schweppenhauser, 1980). If the breeding objectives include estimating site variations, then replication over environmental differences is desirable but balance of all genotypes over all sites is not as necessary as for testing purposes. Various allocations of materials into diallel or square factorial genetic designs are most efficient for estimating genetic variances and can be broken into blocks for ease of field handling and analysis. Since factorials are less useful in the testing or breeding than in the estimation phase, the diallel and its various blocking and incomplete arrangements will often be most suitable though the square factorial should not be rejected completely from

consideration. Variance-covariance matrices can be estimated from these designs and, if the designs are blocked, they can be analyzed even if further unbalanced by missing trees, plots or crosses. These designs also lend themselves to using materials from mixed generations; this will soon be possible when trees from advanced generations become available. The genetic analyses thus afforded will determine the utility of breeding for general or specific combining abilities (depending on the levels of genetic variances for the various traits) and for wide or specific adaptabilities.

Juvenile-mature relationships

There are usually mixed objectives in assessment of traits over the developmental phases of interest. Whereas most traits are best measured at the age for which economic significance is appropriate, it is often desirable to select as early as possible and hence to use juvenile traits. If the same materials are used for juvenile and mature tree measurements, the errors may well be correlated since the juvenile effects have error deviations which will affect the mature tree error deviations. If we select for mature tree performance of relatives or independent ramets of the test trees, these error correlations will bias the statistics. This bias should be recognised as an additional element of concern in estimation experiments; it can be estimated from trait correlations using trees in different plots, or by splitting plots and using the covariance of traits between sub-plots, for clean estimates of the covariance. If the objective of juvenile measurements is to develop juvenile-mature correlations, then some care must also be exercised to consider the changeable nature of genetic correlations in applying the estimates to the next generation. Juvenile traits in themselves may be of interest and worthy of study in their own right especially where juvenile survival and performance are critical to forest management practices. There is a tendency to fix a re-measurement schedule for experiments when there is really no clear necessity for it and there is the danger that over-measurement could so burden a programme that much-needed new mating experiments are not performed; design efficiencies can be wasted through unnecessary measurements as easily as through unnecessary plot replication.

Phase coordination

In a complete programme then, intensive development of one or several populations of a species will include precise testing, development of materials for future breeding options and the accumulation of information on the genetic status of the population to guide the choice of breeding material on a basis of recurrent, cumulative genetic improvement. In such a programme, coordination is required so that information is available for decisions on breeding at the appropriate time and so that adequate materials are available to make the breeding effective. In any finite programme, compromises will have to be made between the various phases and programme objectives and hence any

recurrent selection cycle requires some developmental design. Hopefully, at least one species can be used as an intensive breeding model with the expectation that other species may at first be treated less intensively but will later move into more intensive programmes.

Intermediate programmes - practical compromise

A breeding plan

In a moderately intensive programme, a first stage of development might require a seed or ramet production of, say, four million propagules per year for a single population which can be produced from eight hectares of seed orchard or clone banks made up of, say, 30 parents, the number desired for a recurrent selection programme. An initial selection phase may then take, say, 240 of the best trees available and, as the clones or seedlings are coming into competition and production in the orchard, these selections can be culled to 120 on the basis of juvenile performance in simple progeny or clonal tests established with material from the ortets. As controlled crossings can be made and results from the early tests become more reliable, the final cullings can take place in the orchard and commercial seed can be produced from the remaining trees from then until the next generation seed orchard is in full production. The breeding population from which this next generation is to be selected is made up of the crosses among the finally selected parents and the earlier these crosses were made, the earlier can the next selection cycle be instigated. Early crossing among the parents is important for improving the gain rate as breeding cycles advance and hence crossing on the basis of juvenile test performance is desirable if it can be accurate. Traits which can be observed in the first ten years obviously will have higher correlations with juvenile behaviour than traits which can only be observed in older trees (see e.g. Barnes, 1977; Squillace, 1974). Early selection can be used for such traits as stem form, juvenile insect and disease resistances, some wood quality traits and early survival. Crossings can be made among the parents as they or their tested sibs, progenies, etc., begin to indicate their other growth and behaviour traits more reliably. Difficulties due to deficient flowering should be overcome if possible rather than culling or under-representing a particular parent for this reason. In this phase, rankings can be made of the best 60 parents on the basis of even weakly correlated traits; crossings can begin on these. Every few years thereafter, the rankings can be re-evaluated on the basis of better established correlations and other crosses made as the breeding design might require. The final culling can be made when the additional economic gain that could be made by any further culling equals the opportunity cost of delaying the breeding cycle.

Thus, by establishing early tests from the original ortets, the breeding population in the seed orchard can be culled soon after it reaches reproductive development; by making early crosses among potential final selections, the next generation can be available for initial selection soon after the first generation seed orchard is in its final configuration and in full seed production; and therefore by the time

that the next generation selections are to be made, the information on levels and types of genetic variance and on the size and utility of genotype-environment interactions, should be available.

A timetable

A timetable can look something like Figure 1 where the initial selections (A1) are cloned into a seed orchard and crossed (possibly in the forest but more likely in the orchard itself or in a clone bank) for early test seedlings. (AB1). The tests are established on a wide array of sites to determine the genotype-environment response forms; sites should include those that might be planted 20 years hence. By the tenth year these materials are well established and some early culling in the seed orchard can begin, say to 120 clones (A2). By the fifteenth year early response data are available and culling to around 60 clones is feasible (A3). Crossing for breeding (AB2) should be started as soon as possible and commercial seed production operations begin. By the twentieth year, the early tests are 15 years old, and for rotation ages of 30 years are close enough to maturity for a final culling to take place in the first generation seed orchard (A4). At this time decisions on using genotype-environment interactions are made and the population may be sub-divided into units for replicate population breeding or for site zonations. The final seed orchard set of clones is allowed to produce all commercial seed. By the twenty-fifth year both the early test materials and the crosses of the selected parents have reached sexual maturity. Both sets of materials can then be used for selecting a new breeding population of the next generation (B1), cloned immediately (B2), crossed and outplanted (BC1). By the thirtieth year, a new orchard may start seed production and could be culled to 30 clones (B3) in the thirty-fifth year for commercial seed production.

This next generation can be expected to progress more rapidly than the first one because now reliable data on early juvenile selection can make rapid selection much more feasible. If juvenile selection and reproduction can be reduced to five years by careful study of juvenile-mature relations and by special treatment of seed orchard ramets (to induce flowering), selection of 60 trees or even 30 can be made immediately and, by only 5-10 years after selection, commercial seed can be produced from the 30 selected trees and selections for the next generation can begin. Thus, 10 year breeding cycles can be reasonably foreseen and they may be even shorter for more intensive programmes if improved techniques can eliminate delays between selection and the simultaneous crossing for tests and cloning for seed production. Test localities can be chosen on sites that are to be planted only ten years hence or even less.

Causes of delays in the timetable

Longer initial cycles are required if use of specific combining abilities is desired since that information will not generally be

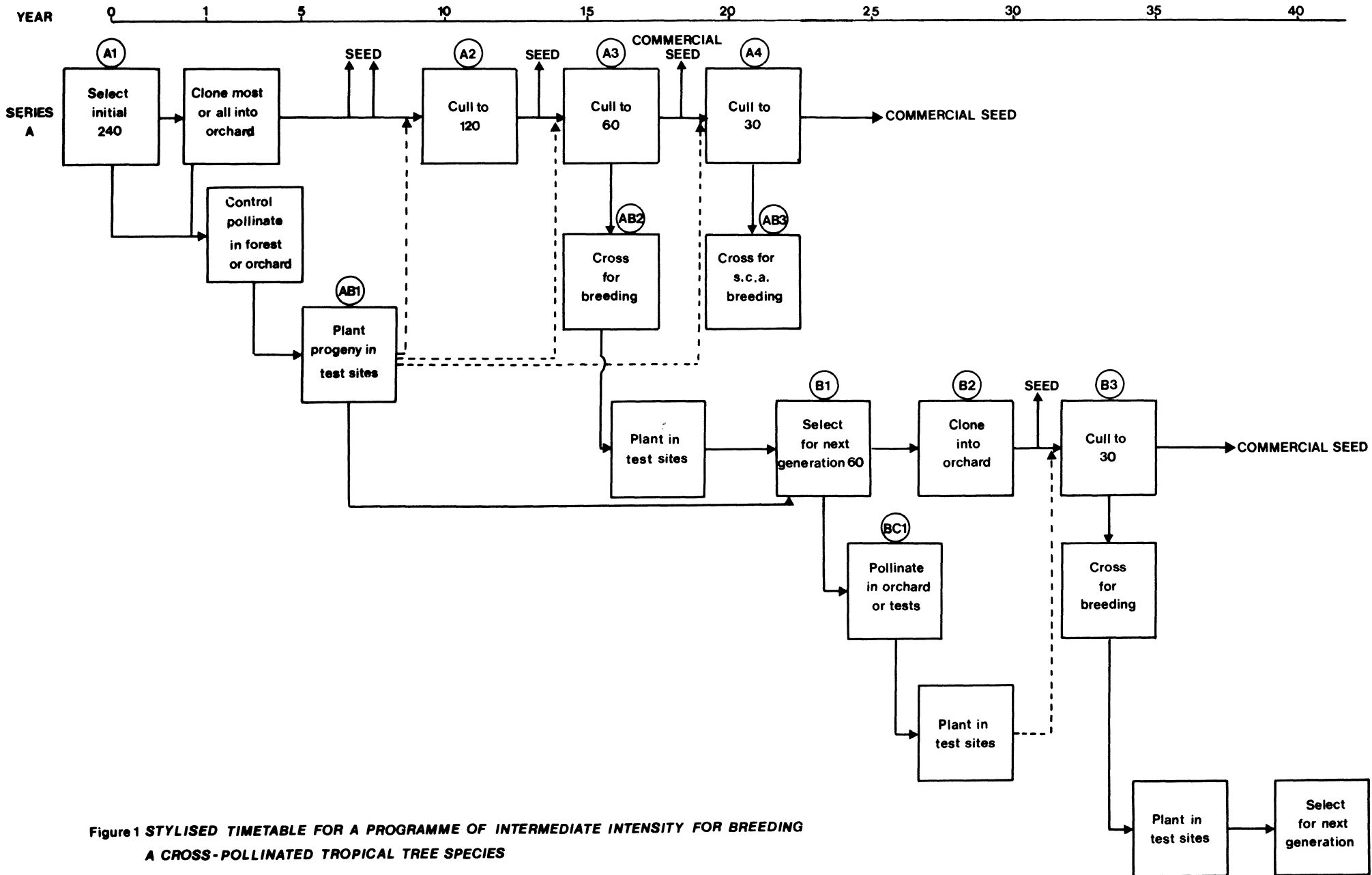


Figure 1 **STYLISTED TIMETABLE FOR A PROGRAMME OF INTERMEDIATE INTENSITY FOR BREEDING
A CROSS-POLLINATED TROPICAL TREE SPECIES**

available until the final selections have been crossed and those progenies tested for their performance. The time between AB2 and B1 is much longer, and AB1 may not feed materials into B1. Thus, the selections for specific combining ability would not be made until the twentieth year and a five year delay would follow (AB3). Thereafter, however, with the earlier selection and crossing possibilities, the cycle time is the same as for any general combining ability programme.

Slower cycles are required if rotation ages are longer and selection is not feasible for, say, 20-40 years. Information from AB1 to cull A2, A3, etc., is delayed. In that case the first breeding cycles can be made somewhat shorter than the selection age if all of the crosses can be made early, that is, if AB2 can be carried out at about age 10. Then, the next generation selections can be made as soon as the trait correlations with full rotation characters are sufficiently high, and inter-crosses made immediately. However, crosses among the first selections would produce many hundreds of families and thousands of seedlings and early inter-crossing among the seedlings would be an impossibly large task. Even if selection is made only in the test cross materials (AB1) and not on the seedlings generated from crosses of the final AB2 a long wait is still required before reliable selection can be made. If there is additional delay in the age to reproduction, then AB1 and BC1 cannot be created early, and very long cycles are required. The use of specific combining ability delays the whole system even further as shown above.

Elaborations possible with tropical species

The shorter maturity times required by many tropical tree species for reproduction and harvesting make these species very attractive for tree breeding. Not only can advances be rapidly accumulated by standard breeding methods, but the opportunities for alternative methods which utilize different kinds of gene action are also much greater than for temperate zone species. The test cross or other mating designs can be used for estimation of parameters and for production of breeding materials. Separate experiments might be justified if breeding advances are sufficiently rapid and refined breeding methods such as reciprocal recurrent selection might be used. Then, the only extra requirement is that the testing be done with inter-population crosses while the breeding population is carried in the within-population crosses (see Figure 2). The initial cycle (C1) is often long, due to the necessity for progeny tests (PT1A and PT1B) which can be generated only from crosses in within-population seed orchards (S01A and S01B). The inter-population crosses (IPC1) cannot be used for testing and crossing for them must await information from PT1A and PT1B. Selection is made in PT1A and PT1B for the next breeding cycle (C2) to produce progeny tests PT2A and PT2B. In these, and in subsequent generations, the initial selections can be crossed with the alternate population very early (e.g. IPC2).

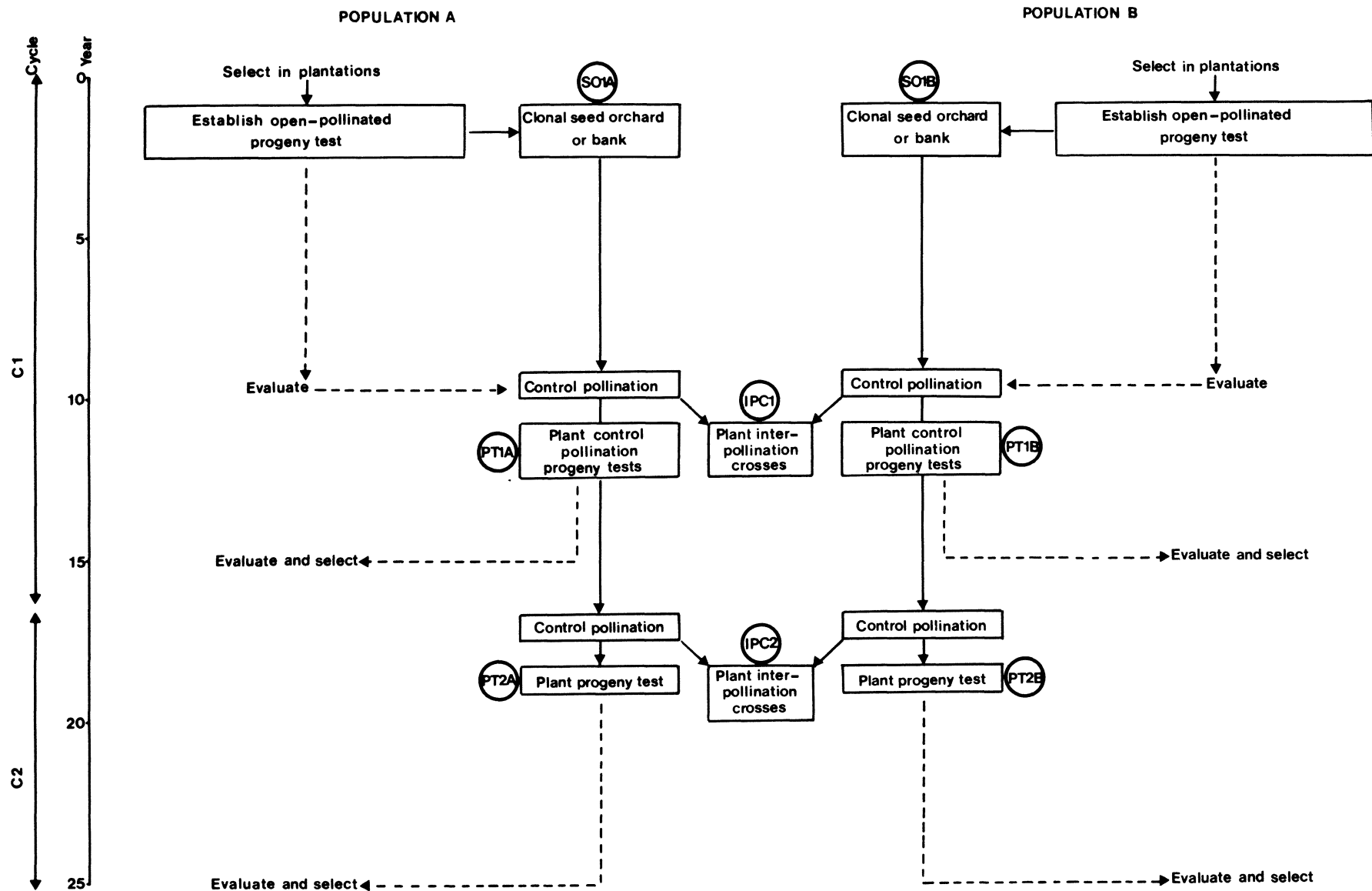


Figure 2: **EXAMPLE OF RECIPROCAL RECURRENT SELECTION SCHEME**

Design considerations for future generations

The principal questions in the next generation will focus more on using the various populations efficiently (through knowledge of their genetic parameters) and designing breeding methods appropriate for the gene actions available at that time. Some form of blocked or partial diallel would probably be most efficient in providing test data for selection, estimating genetic variances and for producing crosses for population development. Small, blocked factorials may also be useful. If variance estimation is not a primary objective, then partially controlled pollinations such as suggested by Shelbourne (1969) in blocked factorials are efficient for breed production and, to some degree, for estimating variances and testing means. Details of the designs and analyses are given in Namkoong (1979). These designs can be used for test crossing among a large number of initial selections, or for breed production among the finally selected parents, and can therefore give the parameter estimates required in time for the final culling and for deciding on the use of general or specific combining ability. If the tests are planted on sites representative of those that will be available for future planting, then the selections will be based on appropriate performance data.

Choice of traits for assessment

Let us consider now the traits which are used in breeding programmes. We might first note that forestry affects land productivity in a way that agronomic crops do not in that it can yield fibre and other products on land which might otherwise be unproductive scrub. Therefore, marginal increases in survival and growth can have large effects on the amount of land which can be profitably managed. In Canada, increases of 20% in survival and 10% in growth of inland Douglas fir could bring many millions of acres of brush land into forest production. Thus breeding for marginal site adaptabilities can have immediate benefits well beyond the percentage increase in individual tree performance.

In most cases, wood production is the primary forestry objective and hence growth rate and dry matter production are sought. Initially, individual tree performance is used as the measure for production under the assumption that it is well correlated with production on a unit of land area. As testing becomes more sophisticated, more direct testing of input-output factors and competitive ability under minimum inputs of fertilizer or other managerial operations will be the basis for selection. This is not simple since wood itself is a complicated set of composite cell traits and not only do mean values affect economic worth but uniformity within trees has high value as well. It is also important to recognise that growth is affected by resistance to biotic, edaphic and climatic exigencies and recovery rates following stresses for short and long periods. Tree form and the pattern of tree growth over the rotation period also affect tree value and hence time effects have to be included also in evaluating trees.

In addition, the pressure to select very early or to select individuals on the basis of performance in other environments requires use of traits only indirectly related to the traits of interest. Such correlated traits may be useful in that they causally relate to, or indicate, some response functions not otherwise observable, or simply by being associated in unknown ways with the directly valuable trait. Thus, juvenile traits are often measured in great detail and extent not only for their own direct value in survival, growth or resistance, but also in the hope that they will be found correlated with other mature tree traits of direct value. Traits which are morphogenetically related are more reliably associated than those related only by genetic linkage which can be expected to change in their correlations. Nevertheless, any high correlations are useful. The use of special tests on sib or progeny families has not been as well developed as it might. For such traits as frost or drought tolerance, or others which can be measured on seedlings or tissue cultures, greenhouse or growth chamber tests can give more information, more rapidly than field tests. Juvenile disease resistance is tested in special inoculation chambers and cold resistance is tested in cold chambers. Close cooperation of silviculturists-physiologists can pay extra dividends for tree breeders if the tests can be arranged in such a way that they accurately screen entries for potential field responses which might otherwise be difficult or more costly to estimate elsewhere.

Two problems exist with this great abundance of traits. The first is that our power of estimating all trait parameters weakens as more traits are added to our list and hence selection is more and more difficult to do accurately and techniques such as selection indices become less and less reliable. The other problem is that the more specific the selection target becomes for some traits, the more likely it is that other traits will change in value incidentally and hence that our population is being mis-directed. Also, less selection pressure is exerted on less variable traits and, hence, cumulative improvement in value is more difficult. For these reasons, only traits which can best or most profitably be genetically improved should be intensively studied for selection purposes. Direct and juvenile selection for these traits can then be more consistently applied and adaptability to site requirement directed to these traits. Other traits should not be totally neglected but selection on them can be very mild and directed only to ensure that they do not decline in value.

If multiple population breeding can be developed as discussed in the next chapter, then selection in the several populations can have different objectives but each population should have an independent set of well studied traits from which to select.

Constraints and modifications

Many optional programmes can be generated from this basic system depending on the biological limitations of the trees and the control which can be administered over crossing, planting, and analysis. It may be impossible to make the early test crosses in the forest. In that

case, early inter-crossing is made only in the seed orchards after they reach a reproductive state. It is then desirable to initiate wide inter-crossing as early as possible and it may not be feasible to select very many more trees in the initial phase than the approximate 30-50 finally desired. In that case, progeny or sib testing is not very useful for improving on the initial selections but may be used for some mild family selection for the next generation. Its primary use will be simply to generate the materials useful for future breeding and to permit estimation of genetic parameters. These parameters, although always obtained for a population that is one generation removed from the current one, can well be used to improve the precision of selection in it. It must be recognised that genetic parameters do change with advancing breeding generations but it is generally assumed that the statistics for populations just one generation apart are good approximations and that it is preferable to use them in this manner rather than to delay breeding until the appropriate statistics are available. To delay breeding would maximise gain per generation but would decrease gain per unit of time. The latter course of action could nevertheless be necessary if a trait that was heavily favoured in selection were found to have lower heritability than expected and selection priorities changed as a result.

Various other difficulties will undoubtedly occur in any one programme, and the imagination of the tree breeder will be called upon to create solutions which will allow more efficient systems to develop. Each species or set of populations will have its unique set of materials and problems and will call for different mating designs, timing, allocation of effort, planting design, *etc.*, which, in combination, will maximise progress. For a low intensity programme, we might choose to forego any attempts to gain genetic information as well as any test crossing and simply allow the breeding population and commercial seed populations to be the same. The initial selections can be cloned into a seed orchard and, as soon as possible, orchard seed distributed for commercial use which would take about the same amount of time as for the intensive programme. The seed may be produced without any supplemental pollination and the next generation then selected without any knowledge of possible ancestral relatedness. The new selections would then be placed in the next generation orchard in about the same time but without the availability of earlier juvenile selection and without the possibilities of selecting for adaptabilities to sites other than the ones on which the new parents grew. Nevertheless, gain is likely to be made and the time for generating the first one or two generations will not be any greater. Many of the minor species may be placed under such simple, recurrent, phenotypic selection systems, and indeed, some populations of the more important species may be treated thus, while the main species development programme is under more intensive management.

To help compensate for some of the short-comings of the simple programme without fully developing the options of the more intensive programme, several low-cost operations can be considered. To reduce the degree of relatedness in subsequent generations, different seed lots from different sets of parents can be designated in field plantings to allow at least some female parentage control. This may be as refined as single seed parents planted in blocks, or as coarse as forming and

planting different sub-populations in seed orchard blocks and keeping their identity in field blocks. On the male side, supplemental dusting with single males or male groups would assume greater balance and could help in ancestral control if field plantings are so designated.

To assist selection for site response, seed from open pollinations in the forest might be collected and planted in an array of sites, and family selections guided by their average responses. Such programmes will be of limited usefulness, however, since individual tree selection will be the more significant contributor to progress in future generations. However, such supplemental aids would be useful if the work with the species is expanded in the future and, for species in which clonal propagation is feasible, replication in wide environmental tests is a direct aid to selection.

It is possible to design an infinite variety of programmes for a variety of situations and constraints. The tree breeder therefore has a range of means to obtain the improvement desired. In some tropical areas, working with adapted species, greater opportunities exist for rapid genetic improvement and virtually any species can at least be placed under the simple recurrent selection systems. Given that basic improvement techniques can be used, the great challenge to breeders is to develop appropriate breeding methods for species of higher priority. The problems and opportunities for breeding are substantial and it would seem likely that we shall witness a blooming of many different programmes that produce gains in unique ways. The potential inherent in rapid growth and early reproduction systems for developing new varieties of trees, in new ways, in a very few years is unexplored. It will be possible to follow established methods but, for most economical gain, new programmes will best be developed as new problems and opportunities are faced.

MULTIPLE POPULATION IMPROVEMENT

OBJECTIVES

Gene conservation and breeding methods can be applied in a variety of forms with a range of objectives for a number of species. As discussed in the two preceding chapters, a programme of gene conservation and breeding involves not only the techniques of optimizing one objective, but also how compromises must be made among the several sub-objectives. Thus, in breeding, the objectives of testing, estimating genetic parameters, and developing the breeding population for selection require time and material allocations for each, and an integration which optimises breeding progress. Similarly, gene and stand conservation for long and short term objectives forces each species or population to accommodate to multiple requirements. While the optimising techniques for the sub-objectives may be known, the breeder must somehow manage the several objectives to produce maximum, overall, long term progress.

A further level of managerial skill is required to consider how, with limited resources but several species and populations to develop, we should allocate time, effort, and materials to advance a general forest development programme. Assuming that we can develop the personnel, techniques and materials for any one population satisfactorily and for any intensity desired, the primary question is how to manage the multiple breeding programme array.

META-BREEDING

Definition

"Meta-" in science, designates a higher science of the same nature but dealing with ulterior or more fundamental problems. Thus meta-breeding deals with the organization of breeding programmes (which vary in form and intensity) and with the overall genetic management of the various breeding populations used now and in the future.

Multiple populations within a species

Even if we can breed for specific objectives, can generate materials and data efficiently, and can produce seed or clones for commercial use, there still exists the problem of specifying breeding directions too narrowly. Given closer definitions of ecological and economic objectives, greater progress in intensive breeding programmes is possible, but this increases the risk that the next generation will require somewhat different kinds of trees. On the other hand, breeding for very broad adaptabilities will result in the selection of trees that are not as suited to particular planting zones or for particular economic uses as it is possible to breed. One solution is to establish

a system of multiple population varieties in which each population is developed for different potential uses and which together form an array of populations for a variety of possible future needs. Genetic management then involves choosing the number and distribution of breeding populations and the means used to develop the population array.

The concept of using multiple population replicates in breeding is not new (Baker and Curnow, 1969). In a set of small populations, instead of a single large one, the genetic gain from breeding will not be the same among them due to sampling variations and other uncontrolled errors. The average of the several populations will not come up to the average of the single large population, even if the same overall selection intensity were kept, because of the smaller effective population size in each. However, two advantages accrue:- (1) the variation among the sub-populations can be expected to be large enough for some of them to exceed the average gain of the large single population, and (2) the different sub-populations, even if the same mean were obtained, will be using and fixing different alleles. Therefore, a selection among the sub-populations in future breeding cycles, and crossing among them, can be expected to give greater gains and to regenerate any lost useful variations in re-combined populations.

In a wider sense, the directions of selection in multiple traits can be more purposefully established for a range of economic or silvicultural objectives. It can be shown that, if there is any variation in future requirements, multiple population breeding for an array of populations around the mean breeding objective gives a higher average gain than single population breeding for the mean expected requirements. Thus, two or more sub-populations can be directed to somewhat divergent combinations of traits or adaptabilities and the one which happens to be closer to the realized future condition can then be used for commercial seed production. At that time, the several other populations would be re-evaluated for use in reaching an optimum array for the subsequent generation. It is possible that several such populations would be similarly directed and be merely replicated populations in the manner of Baker and Curnow (1969). It is also possible that population hybridization would be chosen for immediate use or for future breeding if the new directions required such a genetic merger.

Thus, if breeding can be done with 100 trees, there would be little extra work to breed in 10 units of 10 trees each, as long as particular sets can be rapidly expanded to provide the commercial seed required. As much or as little extra work can be done on the sub-population as on the large single population. In fact, for some of the sub-populations, more intensive development may be pursued than in others if those populations justify more attention. If some sets are directed towards different site adaptabilities, then the separate sub-populations may be used for specific sites.

In the first generation of breeding it might be considered that we already start with multiple populations if we have a number of provenances or source populations. The critical question at this time is the extent to which some of them or some single mixture of trees from a few of them might give better gain in a chosen direction than in

selecting for parallel or divergent development. If there are sufficient time and funds, both approaches would be useful to follow but, lacking in resources, it would often serve best to keep population differences intact, at least for one or two generations. Although population mergers can always be made, coadapted gene complexes become broken up and, once populations are merged, it is very difficult to separate their genes if later desired. In many cases it will be most logical to concentrate effort on the likely sources for intensive development, and to allow for some diversity among others in less intensive breeding programmes.

Multiple species

Within the perspective of a national programme, there would similarly be a choice to make for species development. It is rare that a single species is most satisfactory for all forest sites and all forest products, and the effort is usually sub-divided into discrete species sets. However, it is useful for a nation to reassess its efforts with respect to the needs of future forests and the species or populations which can best meet those needs, especially since both populations and future needs are variable. Even without such a reassessment and with limited resources, national programmes which require work on multiple species need some priority rankings to direct the effort. For most national programmes, two categories of intensity can most easily be established. All species programmes might be placed in a simple recurrent selection scheme and this might provide a perspective in which it could be judged how many species could be effectively put into a high intensity programme.

The first set of species to consider would be those that are highly useful in large areas from which forest management would be significantly aided by genetic improvements. For such species, intensive development of breeding options for economic and environmental variations can generate broad scale gains. The second set of species to consider would be those that are perhaps less valuable now but that are widely planted and are of potentially higher value if genetically improved. Such species can provide forest managers with valuable land use options which can have large national economic impact. The value of genetic improvements in such species can depend more on increasing environmental adaptabilities for survival and general growth than on any very specific market objectives and hence testing for site adaptabilities would be a major emphasis of an intensive programme. While species that are less valuable and are also useful in more limited sites are least likely to justify intensive effort, those species which are of high value on a limited area can be used in modified simple recurrent selection programmes. Since limited site testing is required, some testing or more intensive breeding with fewer trees can be used.

In all cases, the breeder is in the position of directing the evaluation of several forest tree species. The more intensively bred species will have as wide a variation as it might otherwise have had if not bred, but will be directed more usefully for human economic use. The less

intensively bred species can be kept as diverse as can be afforded, but will suffer more from limited effective population size and a limited number of diverse populations. The breeder of course does not operate and create value except within a general forest management and forest development programme. However, the breeder does affect the response potential of forest trees and their inherent capabilities. Within the forest management system, the traits designated for genetic manipulation may be those that other silvicultural systems cannot aid. In most cases, the traits will be mutually affected by genetic and other control means and coordination of efforts is required. Designation of sites for planting and determination of survival and growth characteristics on those sites is similarly a matter for coordinated planning.

Since the genetic situations with the available species will vary, the forestry objectives will vary, and so will the selection intensity, traits, and sites; therefore it is inevitable that local breeding methods will vary even for the same species. For species that are economically important and widely used, it might be expected that a sufficient diversity of breeding programmes will be generated without external efforts. However, many species may deserve more intensive developments than any one nation or agency might decide is justified for their own efforts. For such cases, cooperation among agencies is required to supplement local efforts.

PROGRAMME MANAGEMENT

Throughout this paper we have considered the broad philosophy of tree breeding in the tropics without entering into technical or administrative detail. Nevertheless, whatever approach is taken towards developing breeding strategies, certain administrative constraints must be borne in mind when choosing technical options.

STABILITY AND CONTINUITY

Even in the tropics tree breeding is a long term procedure, particularly when continued beyond the first generation. It is thus essential that permanence of staff and continuity of facilities and financing are guaranteed within the national development and forest research policies. The complexities of, for example, controlled pollination, progeny test design and analysis, and seed orchard design and management, require the increasing and cumulative skill and knowledge of permanent staff if the strategy is to be followed and maximum gains obtained. Rapid changes of indigenous staff (often necessary because of lack of staff throughout the forest service) and short contracts for expatriate advisers do not enhance the technical and economic benefits derived from breeding. Similarly, breeding advances are constrained by uncertainties about buildings, equipment, vehicles and operating budgets.

PERSONNEL TRAINING AND CAREER STRUCTURES

Even if the resources are guaranteed so that permanent posts can be created, successful tree breeding requires dedication by well trained staff at all levels. Untrained staff cannot be expected to design and manage the elegant programmes that are needed; untrained but enthusiastic staff commonly collect vast quantities of data that are frequently unanalysable or uninterpretable. Whereas academic training may be obtained at many Universities (up to M.Sc. and Ph.D. in forest genetics) and practical training in many institutes and departments throughout the world, the dedication of staff is most enhanced by a knowledge that a permanent career is possible in research, without penalty through the loss of promotion prospects (or, in some countries, by the loss of certain fringe benefits that may accrue to field foresters). Only in the largest forest services and companies, with well developed research sections, can a permanent career in tree breeding be assured; yet, anomalously, it is the less developed countries and forest services that most need the benefits that can be derived from tree breeding. The dilemma may not be soluble but attempts must be made to find a solution that will enable the tree breeder to devote a reasonably effective proportion of his career to his subject under conditions of salary, benefits and promotion that stimulate or at least do not mitigate against his technical output.

NATIONAL PROGRAMMES AND INTERNATIONAL COOPERATION

Once reasonably large areas of plantations have been established it is quite feasible for an individual organization to develop a breeding programme based entirely on its own genetic resources and to be completely independent of contacts with other organizations of countries. However, the benefits to be gained from international collaboration should overcome such an insular concept; throughout the world foresters are famous for the degree to which they are prepared to cooperate in the exchange of information, materials and other assistance, often finding means of communication where politicians would establish barriers.

In tree breeding the benefits of international cooperation are in four main groups:- personnel, materials, information and advice (with or without assistance).

Personnel

Personnel in a new breeding programme benefit from formal graduate training and short courses in countries that have well developed programmes and also from attendance at international symposia where shared experience and ideas cover a range of countries, species and problems. (See the proceedings of several IUFRO meetings listed in the bibliography).

Materials

The concept of international cooperation is well established in sharing tropical material for gene conservation (Kemp *et al.*, 1972; Kemp and Burley, 1978) and international provenance trials (see any of the IUFRO proceedings referred to in the bibliography).

At the selective breeding level, interchange of tropical material is relatively new but holds great promise and the possibility of establishing an international tropical pine breeding programme with cooperative selection and testing is currently being examined (see Nikles, 1978). For all of the participants, the programme would provide a broader genetic base, greater amounts of meaningful information and, for new programmes, a rapid start using material selected (and possibly tested) elsewhere.

To date, the material exchanged has been mainly seed but pollen and vegetative propagules also have potential; however, they are usually subject to the same or more rigorous quarantine regulations and are more susceptible to damage by quarantine treatments than seed.

Information

The personnel benefits outlined above clearly include the acquisition of information on latest techniques, equipment, problems and programmes in other countries. However, a major piece of information derived from international provenance and progeny testing is the extent and nature of genotype-environment interactions. By including a far wider range of environments than occurs in one country, the international trial can provide the maximum opportunity for detecting such interaction effects and for identifying genotypes with specific stability or adaptability. The only requirement of collaborators is that they do share information without the political or economic secrecy that is sometimes imposed by Government departments or large companies.

A centrally coordinated programme can often arrange for certain aspects of research to be conducted at one location for the benefit of all collaborators (Burley and Kemp, 1973); for example, in the international provenance trials of tropical pines, the Commonwealth Forestry Institute, Oxford, studies anatomical, botanical and chemical variation among the natural populations tested (the latter in cooperation with the Tropical Products Institute, London - see e.g. Burley and Green, 1977, 1979).

Advice and assistance

Experienced organizations and individuals can provide advice and, in some cases, direct assistance in the planning and implementation of breeding programmes. In particular the design and analysis of progeny tests can be beyond the resources of staff in new or small programmes; the coordinators of international trials are usually pleased to assist with the assessment, analysis and interpretation of the trials and the resultant data. In this way the information on genotype-environment interactions referred to earlier can be obtained and made available to the international community while at the same time assisting the individual country in its own development.

BIBLIOGRAPHY

- ALLARD, R.W. (1964). Principles of plant breeding. Wiley, New York. 485 pp.
- BAKER, L.H., and CURNOW, R.N. (1969). Choice of population size and use of variation between replicate populations in plant breeding selection programs. *Crop. Sci.* 9 : 555-560.
- BARNES, R.D. (1977). Population improvement through selection and hybridization in Pinus patula, P. elliottii and P. taeda in Southern Africa. Third World Consultation on Forest Tree Breeding, Canberra, 21-26 March, 1977. FO-FTB-77-3/3 : 489-505.
- BARNES, R.D., JARVIS, R.F., SCHWEPPEHAUSER, M.A., and MULLIN, L.J. (1976). Introduction, spread and control of the pine woolly aphid, Pineus Pini (L.), in Rhodesia. *Sth. Afr. For. J.*, No. 96 : 1-11.
- BARNES, R.D., and SCHWEPPEHAUSER, M.A. (1980). Genetic control of 1.5-year-old traits in Pinus patula Schiede and Deppe and a comparison of progeny test methods. *Silvae Genetica* 28(4) : 156-167.
- BROWN, A.H.D., MATHESON, A.C., and ELDRIDGE, K.G. (1975). Estimation of the mating system of Eucalyptus obliqua L'Herit. by using allozyme polymorphisms. *Australian J. of Bot.* 23(6) : 931-949.
- BUIJTENEN, J.P., van and SAITTA, W.W. (1972). Linear programming applied to the economic analysis of forest tree improvement. *J. For.* 70(3) : 164-167.
- BURDON, R.D., and SHELBOURNE, C.J.A. (1971). Breeding populations for recurrent selection: conflicts and possible solutions. *N.Z. J. For. Sci.* 1(2) : 174-193.
- BURLEY, J., ANDERSON, P., and HUTTMAN, E. (1980). Juvenile variation among provenances of Pinus elliottii Engelm. and P. taeda L. grown in Malawi. (In preparation, CFI, Oxford.)
- BURLEY, J., BURROWS, P.M., ARMITAGE, F.B., and BARNES, R.D. (1966). Progeny test designs for Pinus patula in Rhodesia. *Silvae Genetica* 15 : 166-173.
- BURLEY, J., and GREEN, C.L. (1977). Variation of gum turpentine between provenances of Pinus caribaea Morelet and P. oocarpa Schiede in Central America. In: *Proc. EEC Symp. For. Tree Biochem.*, Brussels, 1977. (D.T. Seals et al., eds.). 73-108.
- BURLEY, J., and GREEN, C.L. (1979). Relationships of terpenes between exotic and natural populations of Pinus caribaea Morelet and P. oocarpa Schiede. In: *Proc. Conf. Biochem. Genet. For. Trees*, Umea, Sweden, 1978. (D. Rudin, ed.). 118-135.

- BURLEY, J., and KEMP, R.H. (1973). Centralized planning and international cooperation in the introduction and improvement of tropical tree species. *Comm. For. Rev.* 52(4), No. 154 : 335-343.
- BURLEY, J., and NIKLES, D.G. (1972). (Eds.) Selection and breeding to improve some tropical conifers. *Proc. IUFRO Symp. Gainesville, Florida. Commonwealth Forestry Institute, Oxford University, England. Volume 1.* 417 pp.
- BURLEY, J., and NIKLES, D.G. (1973). (Eds.). Selection and breeding to improve some tropical conifers. *Proc. IUFRO Symp. Gainesville, Florida. Commonwealth Forestry Institute, Oxford University, England. Volume 2.* 466 pp.
- BURLEY, J., and NIKLES, D.G. (1973). (Eds.). Tropical provenance and progeny research and international cooperation. *Proc. IUFRO Symp. Nairobi, Kenya. Commonwealth Forestry Institute, Oxford University, England.* 597 pp.
- BURLEY, J., and WOOD, P.J. (1976). A manual on species and provenance research with special reference to the tropics. *Trop. For. Pap.* 10: 226 pp.; and Special Appendices 10A: 63 pp. CFI, Oxford.
- CAMPBELL, R.K. (1979). Genecology of Douglas fir in a watershed in the Oregon Cascades. *Ecology* 60(5) : 1036-1050.
- COCHRAN, W.G., and COX, G.M. (1957). *Experimental designs.* J. Wiley and Sons, London. 611 pp.
- COCKERHAM, C.C., and MATZINGER, D.F. (1966). Simultaneous selfing and partial diallel test crossing. III. Optimum selection procedures. *Austral. J. Biol. Sci.* 19 : 759-805.
- DYSON, W.G. (1974). A note on the conservation of tree species in situ. In: *Rept. 3rd Session Panel of Experts on Forest Gene Resources, Rome, Italy.* FAO Pap. FO:FGR(3) Rep., Rome, 76-80.
- FISHER, W.J. (1978). A basis for the selection and management of scientific areas in Queensland State Forests. *Qld. For. Dept. Tech. Pap. No. 14.* 27 pp.
- HENDERSON, C.R. (1963). Selection index and expected genetic advance. In: *Statistical genetics and plant breeding.* (W.D. Hanson and H.F. Robinson, eds.) *Natl. Acad. Sci., Natl. Res. Council, Washington D.C.* : 141-163.
- HUHN, M. (1971). Untersuchungen zur Konkurrenz zwischen Verschiedenen Genotypen in Pflanzenbeständen. V. Phanotypische Selektion und Konkurrenz. *Silvae Genetica* 20(5-6) : 218-220.
- HUHN, M. (1973). Populationsgenetische Untersuchungen zur phanotypischen Selektion in Pflanzenbeständen mit Konkurrenz. Teil 4. *Silvae Genetica* 22(4) : 93-147.

- IUFRO. (1974). Population and ecological genetics, breeding theory and progeny testing. Proc. Joint Meeting Working Parties S2.04.1-3, Stockholm. Dept. For. Genet., Roy. Coll. For., Stockholm, Sweden. 497 pp.
- JONES, N., and BURLEY, J. (1973). Seed certification, provenance nomenclature and genetic history in forestry. *Silvae Genetica* 22(3) : 53-58.
- KEMP, R.H., and BURLEY, J. (1978). Depletion and conservation of forest genetic resources. *In*: Conservation and agriculture (J.G. Hawkes, ed.). Duckworth, London : 171-185.
- KEMP, R.H., BURLEY, J., KEIDING, H., and NIKLES, D.G. (1972). International cooperation in the exploration, conservation and development of tropical and sub-tropical forest gene resources. Invited Pap. Seventh World For. Congr., 7 CFM/C:V/4G Buenos Aires, Argentina. 16 pp.
- LEDIG, F.T. (1974). An analysis of methods for the selection of trees from wild stands. *For. Sci.* 20(1) : 2-16.
- MARSH, E.K. (1969). Selecting adapted races of introduced species. Second World Consultation on Forest Tree Breeding, Washington. FO-FTB-69-10/4 : 1249-1261.
- MARSHALL, D.R., and BROWN, A.H.D. (1975). Optimum sampling strategies in genetic conservation. *In*: Crop genetic resources for today and tomorrow. (O.H. Frankel and J.G. Hawkes, eds.). IBP2, Cambridge University Press, Cambridge : 53-80
- MERGEN, F., BURLEY, J., and FURNIVAL, G.M. (1974). Provenance-temperature interactions in four coniferous species. *Silvae Genetica* 23(6) : 200-210.
- MITTON, J.B., LINHART, Y.B., HAMRICK, J.L., and BECKMAN, J.S. (1977). Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. *Theoret. Appl. Genet.* 51 : 5-13.
- NAMKOONG, G. (1966). Family indices for family selection. Proc. Second Genetics Workshop of the SAF and the Seventh Lake States For. Tree Impr. Conf., U.S. For. Serv. Res. Pap. NC6 : 7-12.
- NAMKOONG, G. (1967). Multivariate methods for multiple regression provenance analysis. Proc. Sect. 22 XIV IUFRO Congress : 308-318.
- NAMKOONG, G. (1979). Introduction to quantitative genetics in forestry. U.S.D.A. For. Serv. Tech. Bull. No. 1588. 342 pp.
- NAMKOONG, G., BARNES, R.D., and BURLEY, J. (1980). Screening for yield in forest tree breeding. *Commonw. For. Rev.* 59(1) : 61-68.

- NAMKOONG, G., and ROBERDS, J.H. (1974). Choosing mating designs to efficiently estimate genetic variance components for trees. I. Sampling errors of standard analysis of variance estimators. *Silvae Genetica* 23 : 43-53.
- NAMKOONG, G., and ROBERDS, J.H. (1980). Short-term loss of neutral alleles in small population breeding. *Theoret. Appl. Genet.* (In press.)
- NAMKOONG, G., and SNYDER, E.B. (1969). Accurate values for selection intensities. *Silvae Genetica* 18(5-6) : 172-173.
- NIKLES, D.G. (1978). Progress report on international cooperation in genetic improvement of Pinus caribaea var. hondurensis Barrett and Golfari and proposals for further development. In: Progress and problems of genetic improvement of tropical forest trees. (D.G. Nikles, J. Burley, and R.D. Barnes, eds.). Proceedings of a joint workshop of IUFRO W.P.s S2.02-08 and S2.03-01, Brisbane, Australia : 490-508.
- NIKLES, D.G., BURLEY, J., and BARNES, R.D. (1978). (Eds.). Progress and problems of genetic improvement of tropical forest trees. Proceedings of a joint workshop of IUFRO W.P.s S2.02-08 and S2.03-01, Brisbane, Australia. 2 Volumes. 1066 pp.
- ORR-EWING, A.L. (1965). Inbreeding and single crossing in Douglas Fir. *For. Sci.* 11 : 280-290.
- PORTERFIELD, R.L. (1974). Predicted and potential gains from tree improvement programs - a goal programming analysis of program efficiency. N.C. State Univ. School For. Res. Tech. Rept. No. 52. 112 pp.
- ROBERDS, J.H., NAMKOONG, G., and DAVEY, C.B. (1976). Family variation in growth response of loblolly pine to fertilizing with urea. *For. Sci.* 22 : 291-299.
- SAKAI, K.-I., and MUKAIDE, H. (1963). Estimation of genetic, environmental and competition variances in standing forests. *Silvae Genetica* 16 : 149-152.
- SAKAI, K.-I., MIYAZAKI, Y., and MATSUURA, T. (1971). Genetic studies in natural populations of forest trees. I. Genetic variability on the enzymatic level in natural forests of Thujopsis dolabrata. *Silvae Genetica* 20(5/6) : 168-173.
- SCHOENIKE, R.E. (1975). Tree improvement and the conservation of gene resources. In: Forest tree improvement: the third decade. (B.A. Thielges, ed.). Louisiana State University, Baton Rouge. 119-139.
- SHELBOURNE, C.J.A. (1969). Tree breeding methods. N.Z. For. Ser. Tech. Pap. 55. 43 pp.

- SHELBOURNE, C.J.A. (1972). Genotype-environment interaction: its study and its implications in forest tree improvement. In: Proc. IUFRO Genetics - SABRAO Jt. Symp., Tokyo. B-1(I) : 1-28.
- SILEN, R. (1966). A simple progressive tree improvement program for Douglas fir. Pacific North West Forest Exp. Stn. Res. Note 45. 13 pp.
- SOULE, M.E., and WILCOX, B.A. (1980). (Eds.) Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Inc. Publishers. 395 pp.
- SQUILLACE, A.E., and GANSELL, R. (1974). Juvenile:mature correlations in slash pine. For. Sci. 20(3) : 225-229.
- STERN, K., and ROCHE, L. (1974). Genetics of forest ecosystems. Chapman and Hall, London; Springer Verlag, Berlin. 330 pp.
- TODA, R. (1965). Preservation of gene pool in forest tree populations. In: Proc. IUFRO Sect. 22 Spec. Mtg., Zagreb. (Reprinted in Sumarski List No. 1-2, 72-75.)
- VECCHI, E. da. (1963). Evolution and importance of land races in breeding. In: Proc. FAO World Consultn. For. Genet. Stockholm, Sweden. FO-FTB-69-10/5 : 1263-1278.
- WHITMORE, T.C. (1977). A first look at Agathis. Trop. For. Pap. No. 11, CFI, Oxford. 54 pp.
- ZOBEL, B. (1977). Gene conservation - as viewed by a forest tree breeder. For. Ecol. Management 1, 339-44.

Appendix A

PROBABILITIES OF ALLELE LOSS

A single seed or seedling sample, which was drawn at random from a population of hybrid zygotes with the frequency of an allele at a genetic locus, q , has two alleles at this locus in its chromosomes. If the trees are not inbred, the possibility that this random zygote will not contain this allele, the $\text{Pr}(\text{loss})$, equals $(1-q)^2$. Thus, for a single tree, $\text{Pr}(\text{loss}) = (1-q)^2$. For t independently sampled trees, $\text{Pr}(\text{loss}) = (1-q)^{2t}$.

From this, we can derive the minimum number of trees needed in a random sample to reduce this $\text{Pr}(\text{loss})$ as low as desired. This minimum t must exceed

$$\frac{\ln[\text{Pr}(\text{loss})]}{2 \ln[1-q]}$$

The lower the frequency of the allele we wish to save, the lower is $|\ln(1-q)|$, and the larger is the required t . The greater assurance we wish to have against the risk of loss, the larger becomes $|\ln[\text{Pr}(\text{loss})]|$ and the larger t must be.

Thus, for $q = 0.05$ and $\text{Pr}(\text{loss}) \leq 0.05$, we require $t \geq 30$
for $q = 0.05$ and $\text{Pr}(\text{loss}) \leq 0.01$, we require $t \geq 45$
for $q = 0.01$ and $\text{Pr}(\text{loss}) \leq 0.05$, we require $t \geq 150$
for $q = 0.01$ and $\text{Pr}(\text{loss}) \leq 0.01$, we require $t \geq 230$

We are usually concerned with more than one genetic locus when we sample tree populations since we breed for trees which combine many traits and which are quantitative in nature and have many genes affecting each trait. The probability of losing an allele of interest at any of s independent loci

$$\begin{aligned}\text{Pr}(\text{loss at any locus}) &= 1 - \text{Pr}(\text{not losing at all loci}) \\ &= 1 - \prod_{i=1}^s \text{Pr}(\text{not losing at locus } i)\end{aligned}$$

For the sake of deriving some guidelines in sampling, let us consider the model case where all s loci have equal and low frequency for alleles of interest, say $\bar{q}$.

Then: $\text{Pr}(\text{loss at any locus}) = 1 - [1 - (1 - \bar{q})^{2t}]^s$.

From this, we can derive the t required to reduce the probability of loss for any s and $\bar{q}$. The minimum t must exceed

$$\frac{\ln 1 - [(1 - \text{Pr}(\text{loss}))^{1/s}]}{2 \ln(1 - \bar{q})}$$

For large numbers of loci, t must be larger. Thus:

for $\bar{q} = 0.05$, $\text{Pr}(\text{loss}) \leq 0.05$, $s = 1$, we require $t \geq 30$
 for $\bar{q} = 0.05$, $\text{Pr}(\text{loss}) \leq 0.05$, $s = 10$, we require $t \geq 51$
 for $\bar{q} = 0.05$, $\text{Pr}(\text{loss}) \leq 0.05$, $s = 50$, we require $t \geq 77$

The increase in numbers required for many loci is not as severe as for decreasing the frequency of the desired alleles.

A more realistic way to express the probability of loss may be to look at the expected frequency of allele loss for given sizes of $\bar{q}$ and s . If we express this in terms of the sample size necessary to yield an average loss of only one allele, we obtain the results of Table 1.

Table 1. Minimum number of trees required for an average loss of one allele

(one allele per locus)

Allelic frequency ($\bar{q}$)

		0.05	0.01	0.005	0.001
number of loci (s)	10	45	227	455	2278
	50	77	389	780	3906
	100	90	458	919	4601

In many cases, an additional factor will have to be considered, in that each locus may harbour several alleles of relatively low frequencies. If two useful alleles exist at low frequency and we wish to save both, the

$$\text{Pr}(\text{loss of either or both}) = (1-q_1)^{2t} + (1-q_2)^{2t} - (1-q_1-q_2)^{2t}.$$

For three alleles per locus, and if we wish to save all, the

$$\begin{aligned} \text{Pr}(\text{loss of any}) = & (1-q_1)^{2t} + (1-q_2)^{2t} + (1-q_3)^{2t} - (1-q_1-q_2)^{2t} \\ & - (1-q_1-q_3)^{2t} \\ & - (1-q_2-q_3)^{2t} \\ & + (1-q_1-q_2-q_3)^{2t} \end{aligned}$$

For four alleles:

$$\begin{aligned} \text{Pr(loss of any)} &= \sum_{i=1}^4 (1-q_i)^{2t} - \sum_{i,j=1}^4 (1-q_i-q_j)^{2t} \\ &+ \sum_{i,j,k=1}^4 (1-q_i-q_j-q_k)^{2t} \\ &- (1-\sum_{i=1}^4 q_i)^{2t}, \quad k>j>i \end{aligned}$$

(Marshall & Brown, 1975)

From these formulae, we can again derive the requisite sample sizes for various cases. For one locus and for $q_1 = q_2 = \bar{q} = 0.05$, $\text{Pr(loss)} \leq 0.05$, $s=1$, we require $t \geq 35$. For one locus and for three alleles, $q_1 = q_2 = q_3 = \bar{q} = 0.05$, and $\text{Pr(loss)} \leq 0.05$, $s=1$, we require $t \geq 39$. For four or more alleles, modest increases in sample size can assure the savings of alleles with reasonable probabilities. For example, for an average loss of one allele when as many as 50 loci may exist at $\bar{q} = 0.01$, we previously determined that 389 trees would be required. If we now consider that at each of these 50 loci, four alleles exist of $q_1 = q_2 = q_3 = q_4 = \bar{q} = 0.01$, a sample of 459 trees will be sufficient to give an average loss of only one allele.

Table 2. Minimum number of trees required for an average loss of one allele

(four alleles per locus)

Allelic frequency ($\bar{q}$) (per locus)

		0.05	0.01	0.005	0.001
number of loci (s)	10	72	367	735	3681
	50	104	528	1057	5295
	100	117	597	1196	5988

Note

The number of individuals required for conservation of individual alleles has been discussed above; it is a controversial subject only inasmuch as it depends on the object of conservation and on the

knowledge or assumption of gene frequencies and numbers of genes and alleles, and hence effective population size (Fisher, 1978). The numbers of trees recommended vary from 20-30 for a small, single population to be combined with others in a breeding programme (Zobel, 1977), through 300 for long-term breeding seed orchards (Schoenike, 1975), a few hundred for allele conservation (Appendix 1), 200 (Dyson, 1974) or 10,000 (Toda, 1965) for "gene pool conservation", and 5,000-10,000 (Marshall in Whitmore, 1977) for maintenance of heterozygosity. (See also Marshall and Brown, 1975, and several papers in Soulé and Wilcox, 1980).

Appendix B

POTENTIAL INFORMATION AND USES OF AN IDEAL SPECIES-WIDE SAMPLE

If an adequate species-wide sampling is available, we can analyse the differences that may exist among seed sources to determine which ones may be optimum choices for immediate use and which may contain variations of potential future use. Analagous to questions about the best species, we can ask questions about which sub-populations of the chosen species may be best to use. Our first question then is whether sub-specific differences exist in any genes in which we might be interested. There are three ways in which important differences may exist: 1) there may be differences in average performance, 2) there may be differences in genetic variation, and 3) the expression of these differences may change depending on the environment in which the trees are to grow.

For very simple species there may exist such wide and effective gene migration and such little environmental variation over the species range, that all alleles exist in all areas and no differentiation has developed. For such species, any seed source is as good as any other, no one source is likely to be more homeostatic than another, and we can start breeding with any reasonably large sample of trees. At the other extreme, some species may exist with extremely restricted pollen and seed migration and segregated sub-populations. In the absence of gene migration, local variations can result in substantially different genetic compositions among the sub-populations even if selection for the same kinds of environments existed. Natural selection for different environmental adaptabilities would increase divergence among them and breeding results would depend on which seed sources were used as a basis for development. Not only might different average performances exist but populations may differ in their homeostasis and ability to respond to environmental variations.

In this section we consider the kinds of analyses appropriate for determining whether the species' seed sources differ and if the differences are related to measurable features of the source environments. We then consider whether source differences are large in some planting sites and not in others or whether some traits display responses that others do not. We then consider the kinds of trait variation patterns that might have developed by natural evolution and their implications for breeding and gene conservation.

Analyses on one site, for one trait

An analysis of variance (ANOVA) for seed sources when planted in a replicated experiment on one site is a good starting point and is standardized for many forms of planting design (see for example, the designs and analyses in Burley and Wood, 1976). Without defining any relationship among the seed sources, the usual ANOVA provides direct

tests for the existence of significant differences among sources though the statistical test may differ according to whether the set of seed sources is a randomly selected or a fixed set. Whether statistically significant or not, we may wisely select the best sources for our base population provided the sample was sufficiently large. Further information, however, is available if we also know how the source environments differ as for example by latitude, elevation, soil type, etc. We can then determine the extent to which those environmental variables have "caused" different tree responses. In the ANOVA, the degrees of freedom for sources can be partitioned among the contrasts or into single degree of freedom tests for regression coefficients using source environments as independent variables. We may then test for as complicated a regression model as we wish to account for patterns in the observed source means up to the limit of our source sample's degrees of freedom.

The extent to which the variation among sources (as indicated by the relative size of the source mean square) can be accounted for by such regression or contrasts among sets of sources, is the extent to which we can thus identify environmental sources of genetic differentiation among populations and, by inference, causative selection factors in evolution.

In practical tree breeding, this means that we know where to look for specific kinds of growth or response and we could re-sample the source populations for those responses if they are available. The benefits of refined re-selections would have to be weighed against the costs of re-selection and the availability of other sources of genetic variation.

The extent to which we cannot account for all source mean differences is the extent to which population seed sources vary independently of our environmental measures; it makes finding optimal sources more uncertain in spite of the existence of any trends suggested by the regressions investigated. Genetic variation among trees within sources is an important contributor to the total genetic variation. In many cases, especially in temperate zone conifers, the variation in growth traits within sources is at least as large as that between seed sources and indicates that breeding within the better populations can yield substantial gains. For many tropical species, which may contain sets of smaller and more isolated populations, the relative sizes of the genetic variations is expected to favour the source differences more heavily.

In these studies on growth and survival traits, we cannot usually tell whether we are observing the effects of natural selection or migration. On the one hand, if a fair degree of uniformity is observed, extensive pollen and seed migration can be masking selection of locally different types; on the other hand, wide divergence in genetic composition can result from founder effects and restricted gene migration even though environmental selective effects are uniform. Regardless of the results of studies on these traits, it is thus difficult to tell how other genes may be distributed. If we are concerned with genes affecting performance in future breeding generations, then analyses of gene distributions which are not so affected by natural selection can indicate genetic structures more transparently. For this reason, isozyme analyses and studies on non-selective or neutral alleles are useful. In

fact studies on several traits, some more highly selected than others, can provide information on population structure and selection more efficiently than studying them independently.

Analyses on one site, for several traits

If several traits are of interest to the tree breeder, then we can always analyse each trait separately and draw conclusions about the structure of the population. However, the joint analysis of all traits not only unifies the perception of the populations but also allows the recognition of differences in correlated traits that might not be perceived using a separate analysis for each trait. The magnitude of the differences between populations is more precisely measurable by multivariate "distance" statistics as reviewed by Namkoong (1978) for gene frequency measures. The location of discontinuities or gradient changes in allelic types can then be interpreted as for any single trait with the difficulties outlined above.

A statistic useful for testing differences among sources (analogous to the ANOVA F-test) is constructed from the variance-covariance matrix between sources (B) and the variance-covariance matrix of the source error (B + W). The test is:

$$-2n \ln[|W|/|W+B|]$$

where n = total sample size

W = error matrix of sums of squares and cross products

B = source matrix of sums of squares and cross products

and $\ln$ = natural logarithm

This statistic is distributed as a chi-square (χ^2) with $2(p-1)$ degrees of freedom where p = number of sources.

If it is determined by this, or by any univariate test, that sources are different in some important way, regression on source variables can also be analysed for the multiple traits. In these cases, the regressions for each trait can be estimated and the degree to which the regression variables can account for the total variance and covariance between sources can also be defined. Similar to the univariate analyses where

the total variance in the dependent variate (σ_y^2) is reduced by

$$\frac{(\sigma_{xy})^2}{\sigma_x^2} = b\sigma_{xy}, \quad \text{the amount accounted for by regression on x, the}$$

multivariate variance-covariance (Σ_{yy}) is reduced by $(\Sigma_{yx})' \Sigma_{xx}^{-1} (\Sigma_{xy})$,

where Σ_{xx} is the covariance matrix among the independent x variables and Σ_{xy} is the covariance vector of the x's with the y's. The regression coefficients obviously are different for the different traits and account for different amounts of the variance in those traits among the sources.

Analyses on several planting sites

If we are interested in how populations may be differentially adapted to the varying sites on which they may be planted we have an additional problem in allocating the source materials to the available sites. As discussed in the text, the reactions to planting sites can be considered as regressions of yield traits on environmental variables which we can consider as independent variables. These variables, like source environment variables "cause" or affect responses and can be used to account for differences in yield along with any other independent variable.

The major difference that exists with these variables is that there are some particularly interesting effects of the jointly considered variables which we might term "source" and "planting site" environmental variables. Since we can allocate materials to a selected set of planting sites, we can look for particular patterns of relationships in the manner in which trees from certain sources react to certain sites, and we can test hypotheses about the nature of the interaction. If there is no source genotype-site interaction, then any differences among sources are maintained across the planting sites, and any differences in site productivity are maintained for the sources tested. However, if interactions exist, then some sources are better on some sites than on others and we might want to match the appropriate combinations for planting. It is of further interest to see if we can reasonably hypothesize why such interactions may exist and to see if the old adage for native species exists, *i.e.* that "local seed is always best", or if environmental measures of source and site can be matched to give guides to optimum source-site combinations.

These considerations are not new, and even considering multiple traits in a multivariate-multiple regression analysis offers no new concepts (see for example Namkoong, 1967). These analyses merely provide us with explicit statistics and tests for traditional provenance test questions and hypotheses.

For most purposes, it is safest to try to balance the planting design so that all sources are equally represented in all replications on all sites. It often occurs, however, that this requires too much planting area or too large a sample size and some imbalance must be introduced. This can be managed by placing sources in sites according to partially balanced incomplete block designs (see Cochran and Cox, 1957, for design and analysis). We might consider designs such as suggested by Namkoong (1979) where the sources most likely to be good in certain sites are more heavily replicated, while less likely sources are dropped from those sites. Other forms of imbalance might be to plant all sources on a basic set of sites representing the major site variations, and a smaller sample of sources representing major source variations, on a wider sample of all site variations. The analyses of all such unbalanced designs are more complicated than for the standard designs, and there is always a need for replication and careful analysis to avoid statistical bias. Thus greater efficiency of material and space is available but at the cost of more difficult analysis. We recommend such designs only when the analysis is made available at the time of designing the planting.

Breeding and conservation uses

Finally, it should be considered that in addition to informational use, species-wide collections often are made for purposes of creating a gene storage bank for present and future breeding uses. For immediate use, it is sufficient to have enough trees of the species to provide a basis for developing a land race. To the extent that source differences exist, the gain to be achieved by simply choosing any trees from only the best provenances is often substantial and is clearly preferred. However, this gain is a one-time-only gain which gives the tree breeder an initial boost in the favourable gene frequencies. Therefore if the best provenances are not available, the breeder must decide on whether he should delay the subsequent breeding within the available sources to find a better starting source or if he can more quickly advance the breeding population by using the available genetic variance. Either way, a sufficient sample size of 400 or more selected trees is usually required to give a good sampling basis for further breeding.

In the longer run, when environments may be changing, a wider planting of sufficiently sampled sources is useful to ensure that the existing diversity of genes is contained in one or another population and available in a form useful for future breeding. As outlined in Appendix A, this sample need not be any larger than that contained in a reasonable provenance test and if used in a multiple population breeding programme, the separate populations can be started from different provenances.

Appendix C

MATING AND ENVIRONMENTAL DESIGNS

The basic environmental designs and their analyses which are useful in tree breeding are reviewed in Burley and Wood (1976) and detailed in several texts, such as Cochran and Cox (1957). In forestry experiments, as with any plant genetic experiments, we can obtain good estimates of mean performances or regressions by careful distribution of trees over selected sites as outlined in Appendix B. By using many families we can make good estimates of genetic variances and by planting enough trees of any one family on any site we can generate good population sizes for breeding purposes. However, with limited materials to plant, a limited choice of planting sites, and limited funds and personnel, we often require that all objectives of experimental plantings be satisfied in very few designs, or even in one. It then becomes apparent that the objective of precision of estimation of family means, which requires heavy replication of each family, is in some conflict with the object of estimating variances, which is best done using many families at the expense of replication (see e.g. Barnes and Schweppenhauser, 1980). The objectives of mean and variance estimation in turn are well served by using small plot sizes, but the objective of selection within families for future breeding purposes is best served by using large plots. The need for compromise among the objectives is therefore apparent, but unplanned compromises can produce designs which fail to satisfy any objective. We here review the basic design requirements of the multiple objectives and the modifications which can be made, and will see that there often is, in fact, substantial room for compromise to satisfy those multiple objectives.

Testing and estimating family means

The genetic design for family mean estimation requires only that the alternate parents be such that they do not bias the comparisons among the genotypes being tested. This means that the tester parents could be (i) the same for all entries, (ii) tested themselves and their contributions adjusted for, (iii) so numerous and random that their bias can be accounted for, or (iv) not be involved at all. The first implies a factorial mating design, the second a diallel, the third a hierarchical or open pollinated design, and the last requires that we use selfed seed or clones. The third design is often the least expensive to use but is subject to bias in estimating means, while the fourth is often quite simple to analyse but subject to biased behaviour if differences in genotypic performance exist between the test individuals and the genotype acting as a seed orchard parent.

If we can assume a reasonable mating design, then initial plot size must be large enough to allow for competition among cohorts to be reasonably assessed and to allow for thinning and mortality so that final measurements can be made on an adequate number of trees. The smallest

plot size is one, and this may be suitable when inter-family competition is not a biasing factor and when sufficient numbers of one-tree plots per site or replication exist to provide within-site replication. For testing purposes, one- to four-tree plots may often be desirable, at least for the mature tree performance traits, and this may require 10- to 16-tree initial plot sizes. The minimum plot size that we can use and still avoid major bias will generally be the best for test purposes. This can perhaps be most strikingly seen if we consider the objective of testing as making as precise an estimate of family means as possible and hence, of minimizing the error of family means. In a simple randomized block experiment the variance among family means is:

$$\frac{\sigma^2_w + n\sigma^2_p + nr\sigma^2_f}{nr}$$

where σ^2_w = within plot variance

σ^2_p = plot error variance

σ^2_f = family variance

n = number of trees per plot

r = number of replicate blocks

The variance of a family mean is:

$$\frac{\sigma^2_w}{nr} + \frac{\sigma^2_p}{r}$$

Clearly, for a given number of trees per family (nr), the larger r is, the smaller is the error of estimate.

If we expand consideration to include a number of sites, then similar problems with allocation of materials will lead to similar conclusions as to the necessity of maximizing the number of sites sampled and minimizing the number of replicates per site and number of trees per replicate plot. If the sites can be arranged so as to allow us to estimate regression surfaces as suggested in Appendix B, then replicates can be unbalanced with respect to site factors for optimum results. Thus, rotatable designs instead of balanced factorial designs (see Namkoong, 1979) are often to be recommended and will require unequal replications to estimate the regression for these forms of average response. In any case, maximizing the number of sites sampled is a desirable means of optimizing material allocations even if we cannot specify the exact nature of the factors which cause site variations. It is sometimes useful to sample a few years for planting effects, or simply a few elevations, aspects, soil types, etc. We can always reduce our analysis to an analysis of variance (ANOVA) and estimate an average response for each genotype, but we can also later analyse site factor variations and refine the ANOVA into single degree of freedom tests. It may also be possible to allocate a few seedlings to special tests such as laboratory or nursery stress tests for resistance to drought, frost, disease, insect, or toxic substances. No plot design is necessary for these tests.

Variance estimation

In contrast to the above testing objectives where every entry is tested on every site and where the mating design has little effect on efficiency, variance estimation requires a large number of entries with a well structured mating design and site design has little influence on efficiency. Minimum plot sizes are still desirable since these variances will inevitably be well sampled, and similarly, replication numbers are also to be minimized. Site design, however, is of relatively little interest except in so far as genetic variances may change with site (see for example Roberds et al., 1976) or as we may wish to estimate the genetic variance of regression responses. Otherwise, our primary concern is with how the parents are mated to produce full- and half-sib cohorts, the use of parent-offspring or other generational relationships, and the use of self-crosses or clones. The commonly used mating designs are the hierarchical, the factorial, and the diallel which are all discussed by Burley et al. (1966) and diagrammed in Namkoong (1979); their analyses are derived and the estimates of genetic variances are also given in the latter. From these designs, we can derive estimates of the variance among full- and half-sib family groups which are two levels of relationship and which give us two equations with which to estimate genetic variances. To obtain better estimates of the genetic variances we can try to use more levels of relationship, and hence obtain more equations with which to estimate additive, dominance, and epistatic genetic effects. In fact, the more kinds of relationships we can include in a test, the better off we are in estimating genetic components and if we have more relationships and equations than genetic variances to estimate, we can always use least squares estimators.

If we choose to include several kinds of relatives in a test and perhaps even use clones mixed with seedlings, then it is desirable to estimate the variances among those relationship units as accurately as possible. Thus, the error of estimate of these variances is to be reduced by good design and this requires that we maximize the degrees of freedom associated with the estimate of the variance among those relatives. This in turn implies that we include as large a genetic sample as possible by such means as maximizing the number of males and females and different kinds of families as possible, even at the expense of numbers of trees per family and numbers of replications or sites sampled. It is also desirable to balance the degrees of freedom among all those units to obtain errors of estimate among the types of relatives. Thus, designs such as the partial diallels or square factorials give good estimates of full- and half-sib variances and, if equivalent degrees of freedom for other kinds of relatives can be incorporated into the design, the genetic variances can be well estimated.

These design considerations for estimation conflict somewhat with the design considerations for testing, but they are not impossible to reconcile. Efficient mating designs using one hundred or a few hundred crosses can give good estimates of the genetic variances and may not require much wider sampling than most tests would include. On the other hand, planting 5-15 sites can satisfy basic testing needs and not require excessive replication for variance estimation. It may occur, however, that a small basic set of test sites is planted with all

genetic entries and that a wider set of test sites is planted with a reduced set of genetic entries perhaps by reducing the numbers of full-sib families per half-sib, or the number of auxiliary relatives. Alternatively, we may block sub-sets of relatives for planting on sub-sets of sites. In all cases of unbalanced designs, the analyses can be quite complicated and we therefore recommend that the analyses be written and made available at the time that such designs are initiated.

Breeding and selection

For breeding and selection purposes, all considerations on plot design and mating design affect the progress that can be obtained. Plots should be large enough for selection among sibs within plots to be effective, site replication should be extensive enough for family selection to be accurate, and the mating design should be balanced and complete enough to allow selection for specific effects while the breeding population size is kept large.

Plot size need not be much larger than 10-20 since the selection differential is less sensitive to change in larger plots (Namkoong and Snyder, 1969). Similarly, good test design will accomplish the same purposes for selection among families as for testing parental entries. Therefore the compromises discussed above will accommodate most of these considerations. For future breeding, the population sizes may require some expansion over that needed for variance estimation depending on the breeding design as discussed in the text. Balanced genetic representation is important for breeding purposes to reduce the rates of accidental allelic loss, but this can be accommodated in most designs without loss of efficiency for other purposes.

For immediate selection and especially for use of specific combining abilities in specific family crosses, the more completely the crossing design samples all possible crosses, the better off we are. This is not efficient design for estimation or for most testing purposes, and its utility will have to be judged by the expected gain and likelihood of breeding for enhanced specific combining ability.

Other uses

In addition to testing, estimation, and breeding purposes, it is worthwhile considering that experimental plantings have other uses, none of which necessarily compromise the three primary objectives. Obviously, any well designed planting serves a genetic conservation purpose and, to the extent to which the germ plasm is well sampled for other purposes, conservation is also well served. Obviously also, any planting serves as a source of seed and as a source of trees for future harvest when the other objectives are fulfilled. Plantings, therefore, are not an expense without profit and can often serve as examples of well managed forests. In fact, one of their more important functions

will often be as demonstration forests, not only to exhibit good management, but to exhibit the kinds of yield differences achievable by genetic selection in just one generation. To the extent possible, access to such plantings should be made available to those who will administer forests and at least one replication should be located where the public can see how we can manage trees and their genes.

