

Low-Input Tree Breeding Strategies

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Abstract

Low-input strategies for seed production and breeding are discussed. Among such strategies are: phenotypic selection; inefficiency of progeny-testing; absence of testing; unidentified plants; combining objectives; genetic unbalances; seed collection areas; forwarding the breeding population by open pollination instead of controlled crosses; and for some cases vegetative propagation. Sustainable and optimally used low-input strategies require advanced quantitative genetic considerations, in particular concerning the management of relatedness.

Introduction

The development of tree breeding as a science is mainly driven by the needs identified by the large high-input programs. Consequently, methods suitable for high-input programs are too often implemented into low-input programs also. Sophisticated or futuristic tree improvement, which requires large resources, stable funding, efficient monitoring, and control, competent and permanent technical staff and well-developed infrastructure, is well represented in the qualified literature, in scientific research, in available expertise and in formal training programs. In contrast, methods particularly suitable for breeding in resource poor situations may be neglected or used inefficiently, as little thought is given to adequately develop implement and optimize these methods. There is rather little qualified scientific thought given to low-input breeding strategies, and not much is found about it in the more established literature. In the few more known examples of high-lighting low-input breeding (e.g. Namkoong et al. 1980; Shelbourne 1992; Barnes 1995), the focus is still on rather fancy techniques, and little effort is made on optimizing real low-input programs. Tree breeders generally feel more motivated to focus attention on how well things can be done rather than how cheaply (but somewhat sloppy) they can be done. There is no sharp delineation between low-input breeding and high-input breeding and any effort to focus on low-input strategies, as is the purpose of this paper, will unavoidable be hampered by this over-simplification. Low-input breeding can be characterized as cheap, convenient, simple, robust, survives periods of neglect, local, requires little record keeping and central control, limited need of monitoring and control, not dependent on high tech or specialized competence and is possible to run on a small and uncertain budget.

The aim of this lecture is to stimulate implementation of; research about; and development of low-input techniques in forest tree breeding. Similar stuff has been presented in lectures by Lindgren (2000 and 2003). Focus will be made on phenotypic selection, inefficiency of progeny-testing, absence of testing, unidentified plants, combining objectives, genetic unbalances and relatedness control, and forwarding the breeding population by wind pollination instead of controlled crosses.

Short time strategies

Good provenances in natural forests can be identified and seed collections can be guided by such provenance test information. Good stands in a setting similar to where the material is used can be identified and used for seed collection. Stands intended for seed collection can be phenotypically thinned to improve the pollen source. Seed collection can be guided to the best trees. Such techniques are always used in the early stage of forest tree breeding, in low-input situations no further actions may be planned, but in this lecture a situation where continuous improvement is intended is discussed. Probably it is recommendable to always have thoughts about long term strategies when a species is actually used for planting to some extent.

Combine populations!

The breeding stock can be divided into production population, breeding population, candidate population, multiplication population, archives, gene conservation population, mass-multiplication population, testing population. For low input breeding, it is essential to combine different objectives in the same physical groups of trees. Seed collection areas or seedling seed orchards can in the same time be productive forests, candidate population, testing populations and serve gene conservation purposes. Such combinations can be headed for at planting.

Open pollination or controlled cross?

In low-input breeding situations, one of the most important considerations is if controlled crosses are needed. Controlled crosses appear in most circumstances as complicated and demanding operations requiring special skills and subject to unpredictable outcome and mistakes. Grafting is often done and that includes large costs and long time delays. Pollen need to be harvested, extracted and stored. Open-pollination is cheap and simple. It offers the advantage that decision about seed collection can be done when it is known that sufficient amount of seeds can be collected. Open pollinations may increase the breeding efficiency much as generation turn over can be much faster. Open pollination has advantages for gene conservation as it draws genes from many ancestors and preserves diversity (Wei and Lindgren 1995). Open pollination could be applied so it yields higher gain than single-pair mating (Wei and Lindgren 1995) or other crossing designs (Wei et al. 2002).

There are two different advantages in using controlled pollinations in forwarding the generations of the breeding stock. The evident one is to control pedigree and relatedness, but another is to avoid inflow of unimproved, unknown, and variable pollen into the breeding stock. For wind-pollinated species which occupy a large share of the land, it is common that fertilizing pollen originates quite a distance from the mother. E.g. in seed orchards of major wind-pollinated species it is common that half of the fathers are to be found outside the orchard (e.g. Lindgren and Prescher 2005). In such situations the inflow of pollen will reduce the improvement effect considerable, and the open-pollination alternatives may appear inferior even in a low-input situation. It may be possible to isolate the breeding stock from other pollen sources by distance or in other ways, e.g. in a plastic green house or isolation by differences in the phenology. The isolation of the breeding population may not need to be 100%, e.g. gene migration by 5% pollen inflow may be quite tolerable in a low input situation even if 50% inflow usually will be regarded as unacceptable much. If a wind-pollinated species is not dominating (the case for many exotics) it can be easy to find a location where physical isolation is sufficient. For non wind-pollinated species the behavior of the pollen vectors are quite important. Careful

considerations about pollen inflow should be done before deciding to rely on open pollination for continued breeding.

A low input breeding strategy without controlled crosses can utilize the production population as candidates for the next breeding population. That is very cost-efficient. A consequence is that the breeding population is difficult to structure in unrelated lines, and thus in the long run inbreeding and inbreeding depression will occur in the production population. A common tool to create a production population is to create a seed orchard with selected individuals, in the considerations for what trees to place in a seed orchard, the occurrence of relatives is an important factor (Olsson et al 2001), but this can be considered only if there are pedigrees. Considerations about these factors have to be done. It is likely a low level of inbreeding in the production can be tolerated and is optimal compared to the consequences of eliminating it. If multi-function plantations of seedling seed orchard character are used for seed production, some degree of inbreeding is unavoidable. For inbreeding considerations, however, actual calculations are desirable. When low-input breeding is practiced, clonal seed orchards or breeding structured in sublines are seldom considered, thus these possible disadvantages with uncontrolled pedigrees are not important. A general remedy against inbreeding in future production populations is to start with a sufficiently large breeding population. It seems risky to initiate a long term program with less than a hundred open-pollinated families.

An emerging technology is to find out the pedigree of breeding stock by markers. It can be limited to fathers if maternal pedigree is controlled by design. In a more futuristic perspective more distant ancestors can be identified. Parental identification “afterwards” makes it possible to control coancestry, selfing, inbreeding and gene migration without proceeding over generations with controlled crosses. Breeding systems with retro perspective pedigree reconstruction goes well in hand with the low input techniques open pollination and phenotypic selection. Currently it is doubtful if this should be considered in low input breeding, but e.g. if the program heads for creating options for future expansion, when pedigree reconstruction may be an option to consider in the strategy when the input level increases, e.g. if a marginal species becomes more important.

Phenotypic (Mass) Selection has appealing characteristics for low input breeding
Phenotypic selection can be done fast and at a low cost. The generation turn over can be increased. The increase in breeding efficiency by these features is often important enough to compensate for draw-backs.

Phenotypic selection (mass selection) means selection based on the appearance of the individual tree itself, not considering the performance of its relatives. The phenotype of a tree (compared to other trees) gives an estimate of its breeding value (=phenotype*heritability). It may seem an unsophisticated philosophy to rely on the phenotypic appearance, but actually many improved seeds used in plant production today rely mainly on phenotypic selections. E.g. the Swedish plant production was 2005 60% seed orchard origins, but the concerned seed orchard selections are completely dominated by phenotypically selected untested plus trees. Although more advanced seed orchards have been established in Sweden, and some of them come into commercial seed production, their impact on plant production is still marginal. Also genetic thinning or selective cone harvest is uncommon. Thus, those in favor of low-input can be said to be in good company if relying on phenotypic selection. As forests derived from phenotypic

selection of plus trees has existed for many decades, it can be claimed to be the method with which forest tree breeding and forestry has the longest experience, and this experience is generally a positive one. It can thus be argued to be a safe and method based on long experience.

Phenotypic selection is the method used by *Nature* and is the driving force for evolution, and has thus existed as long as Life. Life has evolved by phenotypic selection; it is the fundament Life rests on and the cause of Life. It offers comfort to use a "natural" method; even if it seems unsatisfying that modern developed *Science* has difficulties to do better than *Nature*, as discussed below. The similarity to *Nature* seems a strong argument to believe that it is basically sustainable, natural and environmental friendly. That a method is similar to *Nature* can be regarded as a powerful argument to use it. It is very simple and it seldom requires sophisticated training. The selection situation may be more similar to forestry practice (e.g. random mixture of genotypes planted as operational forestry, which will be more similar to the conditions selected for, than genotypes arranged in plots under experimental conditions). It minimizes or eliminates the need for experimental layout and documentation. It does not use a complicated calculation algorithm that often works like a "black box", where severe mistakes easily may pass undetected. It is cheap. It is easy to keep track of data. In contrast to selections based on genotype testing much of the variation in the characters selected for will remain among the trees selected to constitute the parents of the following generation, at least if heritability is not extremely high. Testing increases the accuracy (it can be viewed as making heritability high), and will considerably reduce the variance in the characters selected for. Phenotypic selection has attractive properties for combining tree improvement and gene conservation in the same operation. This advantage may however be smaller than it may appear at first sight, as much of the variation comes back after recombination even after intensive accurate selection.

Phenotypic selection has effects on gene diversity as selected trees are likely to be more related than unselected. To study the magnitude of this increase in relatedness, a trial with 98 open-pollinated families of Scots pine was (hypothetically) thinned based on tree height (Fedorkov et al 2005). First when less than about 10% of the initial trees remained, notable effects on potential inbreeding, relatedness, status number and diversity was noted. To retain almost all families a somewhat more relaxed thinning leaving about 20% of the trees was needed. The study suggests that rather intense phenotypic thinning (leaving more than 15%) has the ability to raise the genetic gain some percent without many problems with accumulation of relatedness. This can be used as a tool in low input breeding. It seems a reasonable praxis to thin intended seed collection stands. For Scots pine in Sweden it is a rather frequent praxis to leave many trees as seed sources for natural regeneration. When doing this some could be headed for seed collection area, when special attention could be made to leave good trees, and some documentation done. When the trees finally are felled, cones could be collected. The thinning would also improve seed production, and as the stand density is lower there will be less trash on ground to search, thus such praxis has not just genetic advantages.

Phenotypic selection, without knowing or caring about the pedigree of the trees, has in several studies been found to be about as efficient for long-term breeding as if the pedigree was known and used as an aid in selection (e.g. Wei 1995; Andersson 1999). One of things a breeder loses by using phenotypic selection is the deliberate choice of emphases on gain versus gene diversity. It may be possible to mend that by adjusting the selections, e.g. by restrictions, but when it is not

pure phenotypic and probably less efficient. Numerical simulations indicate that phenotypic selection often results in an intuitively appealing compromise and seldom results in a drastic increase in relatedness (e.g. Andersson 1999).

Selection based on the phenotype needs not depend on actual measurements, i.e. a subjective evaluation of all characters of a tree (including its performance compared to its neighbors) can be made in the field and need not be calculated and evaluated in the office at some later time more separated from the actual event. Such an evaluation may actually be more accurate than an “objective measure”, as all characters can be jointly considered including the local environment (thus relating to the performance of the neighbors). A field evaluation and selection does not depend on a long chain of actions on different places and by different people where mistakes or delays may easily occur. In practical selection, there is usually a considerable amount of subjectivity at the final stage in a decision procedure; this cannot be circumvented by using high-input techniques in the office.

Phenotypic thinning for unpedigreed seed production areas and also plus tree selection have been discussed, advocated and applied e.g. by Harwood et al. (1996). As low-input breeding must usually rely on phenotypic selection, it must be pleasing for those applying it to know that it is now acknowledged by modern science as an efficient tool (see below). Thus, seed production areas can be established, and the resulting forests later creamed for the best unpedigreed trees, whose descendants are used for establishing new seed production areas, making low-input breeding sustainable over multiple generations, provided the tree numbers involved are sufficiently large.

In a study of the genetic gains obtainable from a range of alternatives, Shelbourne (1992) found that phenotypic selection in unpedigreed stands, although somewhat lower in genetic gain than more elaborate strategies, still seemed favorable when possibilities for faster generation turn-over and lower cost was taken into account.

Relatedness and Gain

Forest tree breeding can be said to aim for creating options to get regeneration material combining high genetic gain with desired diversity. Here we mainly focus on diversity as gene diversity loss since the improvement operation started. This gene diversity can be quantified as group coancestry or status number.

Relatedness has evident disadvantages and can not be neglected. Relatedness is the likelihood that genes interact, and that is proportional to the square of their frequency, thus the disadvantage of relatedness can be quantified by the square sum of genetic contributions. Gene diversity can be interpreted more or less equivalent to relatedness. It can be measured “absolute” by observations of alleles equal in state by expected average heterozygosity, which is a square sum of contributions of alleles. It can also be measured relative to a reference population (“loss of gene diversity”) as square sum of the genetic contributions. This is the basis of concepts like status number, relative status number and group coancestry, which are measures built on average relatedness. Group coancestry can be interpreted as a measure of *the loss of gene diversity* since the initiation of tree breeding, thus one minus group coancestry. Gain is the sum of the gain of

the genetic contributions; so much of the act of balancing is to consider a sum versus a square sum.

If where exists knowledge about how trees are related, more accurate estimates of breeding value of individual trees can be made which use the information from relatives. If a given number of trees are selected with such more accurate breeding value estimates as a criterion, the genetic gain will be higher. There is a best way of estimating breeding values utilizing the information about how trees are related, for more complex relatedness situations these techniques has developed into an art of its own.

By considering the performance of the relatives, a greater gain can be achieved at the same selection intensity, but the additional gain is accompanied by a correspondingly higher relatedness among the chosen individuals. Actually a philosophy to maximize gain may often result in inoptimal tree breeding programs.

Phenotypic Selection vs. combined index selection

In the simplest situation when where is a simple family structure, the best way of combining values for families and for individuals to get breeding value estimates and when select for them is usually called combined index selection. While selecting among the first generation of offspring, selected trees will be sibs to a higher extent, if sib performance is considered when constructing the selection criteria. In examples relevant for forest tree breeding this overrepresentation was shown to be drastic (Lindgren and Wei 1992) and other ways than combined index selection maximizing gain has to be searched for. The classical selection strategies are compared in Figure 1. The graph is generated by an optimizing selection procedure using the same selection intensity for all diversity values (Lindgren, Wei and Bondesson 1993). There is no way to get a higher gain given the diversity. “Linear deployment” (which is optimal for unrelated clones, Lindgren and Matheson 1986) from unrelated families are usually indistinguishable close to optimal (Wei and Lindgren 1995). Between family selection results in the lowest diversity and in the same time the gain is not at top, so that is an appealing strategy. Although combined index selection maximizes gain, it does so at a dear price in diversity. Within family selection maximizes diversity, but minimizes gain. Phenotypic selection appears as a good compromise between gain and diversity. In the way this curve was drawn phenotypic selection appears as an optimal selection procedure. The calculations assumed an infinite number of large unrelated full-sibs and considered only the first round of selections.

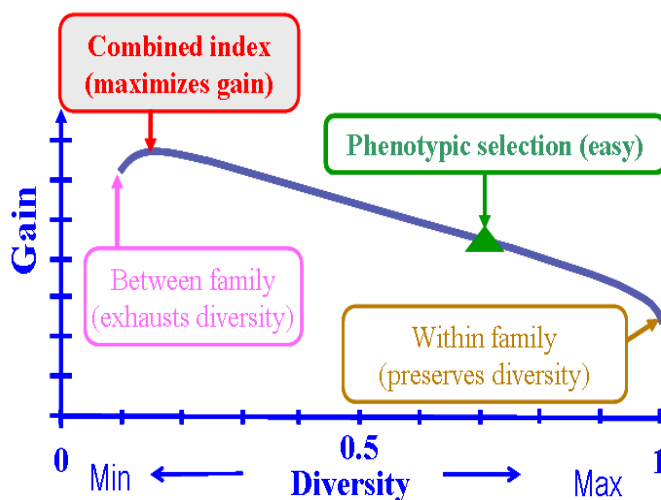


Figure 1. Relation between Gain and Diversity (Relative Status number) following selection in a population with a symmetric family structure. The graph is generated by an optimizing selection procedure. To choose the best families minimizes diversity. Maximal gain is obtained by combined index selection where family and phenotypic values are optimally weighted. But it results often in a very low diversity. Within family selection maximizes diversity, but minimizes gain. Phenotypic selection

appears as a good compromise between gain and diversity. The candidate population was composed of an infinite number of large unrelated full sib families of equal size. Heritability was 0.25 and 10 percent of the individuals were selected. Figure 1 is modified from Lindgren and Wei (1994).

More complex and more realistic situations can be dealt with by simulations. The outcome of breeding programs based on phenotypic versus combined index selection at the same genetic diversity, POPSIM© (developed by Tim Mullin) was used. In practice a sufficient diversity has usually been achieved by restrictions on the number of progeny per parent selected, and such restricted selection has been employed. Figure 2 is based on calculations and results presented in Andersson (1999) and Spanos et al. 1996. The simulated breeding program created a recruiting population by single pair mating and selected the best according to either phenotypic selection or combined index selection over a variety of restrictions. Balanced selection means that 2 individuals in each SPM family were selected (the outmost left in the graphs). The size of the breeding population was kept constant over generations and among compared alternatives.

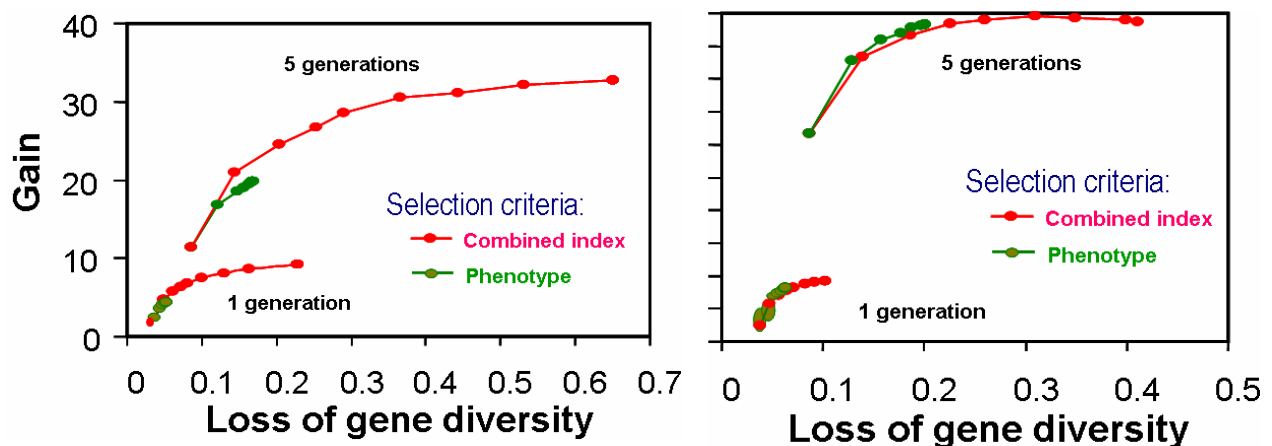


Figure 2. A comparison between selection for combined index (approximately the best estimate of breeding value) versus mass-selection (phenotype). Gain is shown as a function of the loss of gene diversity for simulations of a multigenerational tree breeding program with breeding population size 20 and single pair mating. The points on the graphs correspond to restrictions on the number of offspring selected from each parent. The leftmost point is for 2 selections which corresponds to within family selection or balances selection, where the two top individuals are selected with both criteria. The rightmost point is for unconstrained selection. The left figure is for heritability 0.05 and family size 500, the right figure for heritability 0.5 and family size 20. The figure is based on calculations by Andersson (1999) and others.

When compared at the same loss of gene diversity among the selections and the same selection intensity, phenotypic selection and combined-index selection produce approximately the same genetic gain (Figure 2 and Wei 1995). Phenotypic selection seems to be competitive for achieving genetic gain at a given level of gene diversity even in multi-generational programs generating complex and unbalanced pedigrees, see the upper graphs in Figure 2. Although phenotypic selection appears approximately as good as combined index selection, phenotypic selection is slightly superior when heritability is high and family size small (right part of Figure 2) and slightly inferior when heritability is low and family size large. Clonal testing can be seen as a way of increasing heritability and decreasing family size, thus clonal testing makes phenotypic selection (of the best tested clones) relatively more attractive. The family information gets generally more informative in situations with low heritability and large families, and thus combined index selection is more powerful in such situations (left part of Figure 2).

When only the initial offspring or the development at the first generations is considered, it may be argued that selection for breeding value (combined index selection) are able to produce a much higher genetic gain than phenotypic selection (Figure 1 and 2). Foresters may not care that this is associated with a large reduction in gene diversity; diversity may not be seen as a problem for the production forest. This can be considered a powerful argument against phenotypic selection in the short time perspective. Reduction in gene diversity will, however, lead to reduced gain in later generations, so after around five generations the *maximum* gain obtainable can for some situations (e.g. right part of Figure 2) be about as high for phenotypic selection as for combined-index selection (Andersson 1999). These findings concerned with forest tree breeding are supported by similar results by animal geneticists (e.g. Quinton et al. 1992). Exhaustion of the genetic variation leading to reduced gains by unconstrained selection, in particular if combined index selection or still more powerful estimates of breeding values can occur in a few generations (right part of Figure 2). Reliable breeding value estimates by progeny testing can increase this risk. Such exhaustion is higher in high input breeding and in that sense high input breeding can be seen as a higher risk than low input breeding, hopefully that disadvantage of high input breeding is compensated by a higher competence.

The main alternatives balanced selection, unrestricted phenotypic selection and unrestricted combined index selection (=breeding value estimate) are compared in Figure 3 in a way intended to facilitate comparisons focusing on the effect of early exploitation. It suggests phenotypic selection compared to balanced selection offers a faster accumulation of gain in a shorter time at the cost of a rather small sacrifice in accumulation of gene diversity, while combined index selection erodes the diversity too fast compared to the achievable gain even under conditions chosen to be favorable for combined index selection.

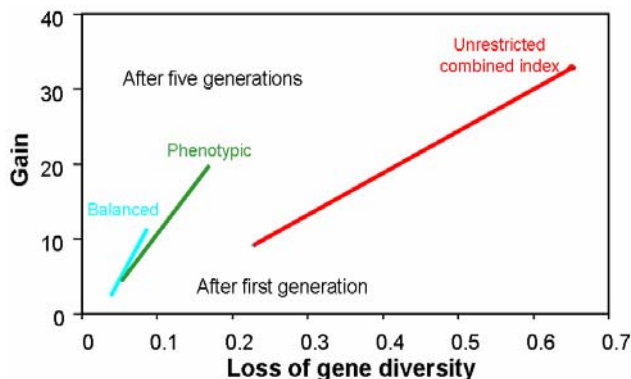


Figure 3. The gain as a function of loss of gene diversity for three strategies (balanced within family selection, phenotypic selection and combined index selection (both without restrictions) during a breeding program spanning over five generations. Heritability is 0.05 and family size 500. The graphs connect the situation after the first generation (leftmost) and five generations (rightmost). The figure is based on calculations by Andersson (1999) and others.

Breeding population size could be dealt with as a variable for fair comparisons

The comparisons in Figure 2 and 3 deal with selection strategies in breeding programs where several factors are dealt with as fixed. The comparisons kept breeding population constant among selection strategies. It can be said to be a common praxis among breeders to first fix the size of the breeding population before giving consideration to other factors in the breeding system. That does not make the basis for fair comparisons, the breeding population size should be considered as a variable the breeder can control and optimize joint with other inputs under the breeders control as done by Danusevicius and Lindgren (2005). Combined index selection tends to erode the diversity fast, but that tendency can be compensated by keeping a larger breeding population size, while balanced or phenotypic selection preserves more of the gene diversity and can thus focus resources on other parts of the breeding system than maintaining a large breeding population size. A comparison between phenotypic selection and combined index selection with breeding population size as a variable and the total number of plants as the fixed resource for the

breeding program was presented by Li and Lindgren (2006). When gain was compared at the same gene diversity under these conditions, the following was noted:

The alternatives became similar when gene diversity (status number) became large. “Sufficient” high diversity to make combined index selection an acceptable alternative seems to be achieved if the breeding population size is above 250. As generations pass phenotypic selection resulted in higher gain. At low heritability, combined index gave more gain.

However, an additional member of the breeding population means added cost and efforts, and this cost is very important for the optimal size of the breeding population (Lindgren et al 1997). Breeders also often feel constrained by earlier decisions about breeding population size. Even if the results by Li and Lindgren (2006) in a way explains why combined index selection appears doubtful in spite of that it can be considered theoretically optimal, it can not be interpreted as a powerful argument against phenotypic selection.

Testing

The simplest way of selecting good individuals is to rely on their phenotypes. Progeny testing or clone testing can be seen as a way of boosting the heritability. A relevant question is if there is place for genotype testing in low-input programs. Testing requires field identities, recorded pedigrees, documentation, long-term planning, long-term co-ordination of activities, organizational stability, and a breeding budget which remains in decades and, often, clone archives. A strategy including testing means a large long-term investment, which seems possible to justify only in situations when it seems certain the results will be utilized and appreciated in the future.

The value of testing vs. phenotypic selection in conditions similar to the Swedish pine and spruce long term breeding was recently analyzed by Danusevičius and Lindgren (2002, 2006). It was concluded that clone testing was the best strategy, followed by phenotypic selection (Figure 4).

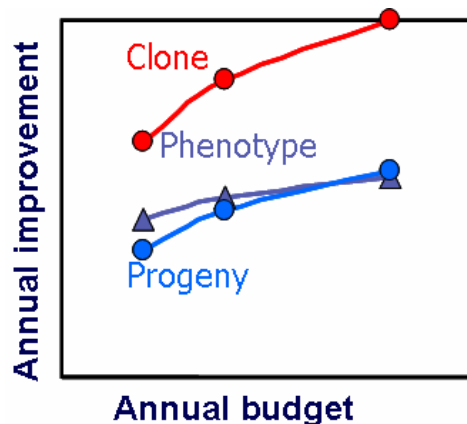


Figure 4. Comparison between different testing strategies as a function of the annual cost per parent. Clone testing was much superior at all cost levels. Phenotype testing was better than progeny testing if the budget was low. The inputs are chosen to be representative for Swedish conifer breeding. The figure is based on calculations by Danusevicius and Lindgren (2002).

It is more favorable to select genotypes for continued long term breeding based on their performance as clones than based on their unreplicated phenotypes or progeny. Clone testing is operative for Norway spruce and used routinely in breeding in Sweden. Phenotypic selection becomes more superior compared to progeny-testing the lower the budget and the higher the heritability. The optimal breeding cycle time with alternatives involving progeny-testing spans over several decades for long-rotation forestry. Thus progeny-testing seems an alternative worth

considering only for breeding programs of economically very important crops with a stable long term breeding commitment.

A study by Ruotsalainen and Lindgren (1998) showed that, with few exceptions, if offspring was generated with pollen as good as the tested population, forward selection was generally superior to backward selection, indicating that phenotypic selection among the progenies is better than progeny-testing the previous generation.

More accurate breeding values can be estimated using information from relatives in procedures like combined index formation, e.g. combining family performance with individual performance or BLUP techniques. These techniques are able to maximize genetic gain when selecting a certain number of individuals, but at the cost of increased relatedness. This cost may be found acceptable in a breeding program of limited duration. Wei and Borralho (2000) found that group coancestry after unrestricted BLUP based selection in three trials with open pollinated progenies of *Eucalyptus urophylla* in southeastern China as less than 1 per cent, thus status number above 50, which is by no way problematic. However, the status number was reduced by a factor of more than 10 by the selection.

Gene Diversity in the Breeding Population Boosts Genetic Gain in the Production Population. Balanced selection may appear inefficient above. The aim of long term forest tree breeding is to supply forestry with best possible genetically improved regeneration material on a sustainable basis. High genetic gain and high gene diversity in the long-term breeding population are just different tools to achieve that goal. To get regeneration material, the breeding population is creamed off to achieve as high an immediate gain combined with an acceptable level of gene diversity. If the breeding population is more diverse, selection for the production population can be done more aggressively, sacrificing a higher share of the gene diversity in the breeding stock. Thus, a more diverse breeding population results in a higher gain in the step when the breeding population is creamed off, thus when selection for the seed orchard is done. This effect partly compensates for the faster advancement of gain in the long-term breeding population, which is possible by caring less about the accumulation of relatedness (Rosvall 1999). Thus it has been decided to use rather balanced designs in Swedish breeding. An implication for low-input breeding may be that a high number of parents is desirable for stands used for long-term population improvement than for commercial seed production. If open pollination families are used, that may not be expensive. To base breeding efforts on a wider genetic base than seed production means that a physical separation is needed between long-term improvement and seed production stands. If both are productive stands, such a separation may be easy and cheap. Long term improvement stands can be harvested for cones for two purposes:
Seed production areas (seedling seed orchards) where progeny (OP seeds) from say the 35 best trees are used to establish a stand;
Long term improvement stands where the selection intensity is lower and OP seeds from say 100 female parents are used.

Clone testing for sexual reproduction

As breeding strategies build on clonal testing are generally superior to unreplicated phenotypes, they should generally be preferred provided it can be done in fast, cheap and uncomplicated ways. It is evident and commonly accepted that clonal testing is important when the end use is

clonal forestry, but it should be wider recognized that it often can be a superior strategy also when production forestry is based on seeds. Those seeds could be obtained from genotypes chosen by clonal testing rather than progeny-testing. Some species are as easy to propagate and handle as vegetative propagules as they are from seed, or vegetative propagation may actually be the only practical way to produce plants. In such clonal testing is very likely to have an important role. Clones are usually thought of as the ultimate tool for high gain, but clone testing may also be a key to low input breeding. If there are difficulties to multiply mature tested clones, that is a powerful argument against clonal forestry, but if sexual progeny can be obtained it is still worth to use clones for

To use clone plantations for seed collection has been suggested as a cheaper alternative to seed orchards of Norway spruce in Sweden (Lindgren and Karlsson 1993). Above it was argued that phenotypic selection often was a competitive alternative. Clonal testing instead of phenotypic testing can be seen as a way as increasing the heritability and thus the associated selection gain. This ought to be true even if when tested clones are unidentified, thus e.g. planting clonal rows and select the good rows for seed harvest ought to have potential for long term breeding or seed collection, even if the clones can not be identified or propagated vegetative.

For low input breeding, it is usually unrealistic to make large efforts to improve the prospects for vegetative propagation, but if the techniques are there it seems wise to use them instead of relying on phenotypic testing or progeny-testing.

Estimates of Accumulation of Relatedness Based on Fertility Variations

Low-input programs must be concerned with inbreeding, relatedness and diversity. These factors must be predicted to manage gene resource plantations and to plan seed collection. In high-input programs, known pedigrees, individual identification and selection algorithms that utilize this knowledge can keep control. In low-input programs, the actual operational control may be relaxed, and thus it becomes more important to forecast what will happen by appropriate use of theoretical predictions and by reasonable estimates of key factors. What happens depends mainly on the gene pool of the population and variations in the contributions of individuals to the next generation. To predict what happens is an advanced operation. The likely consequences may be forecasted by simple heuristic rules, tables and instructions for less-advanced users, but as future low-input breeders will very likely have access to competence and computers, the need of predictions will seldom be bottlenecks.

The gene pool of the offspring is the same as the gene pool of the successful gametes of the parents; this connection links generations. It seems natural to link fertility to successful gametes, but it is not known what gametes will be successful in advance, and there will be stochastic variation, which is high if low-input measures are used. It is thus more useful to define fertility as a characteristic of the parental genotype. Fertility is defined as "a parent's ability to produce successful gametes". The true number of successful gametes per parent is both technically and principally difficult (or impossible) to estimate (e.g. there is no unequivocal definition of how long the zygote that results from a successful gamete must survive to be characterized as "successful"). Quantitative estimates of variation among trees in female or male reproductive structures can be made even for a low-input program, and it seems likely that such counts will be sufficiently accurate for most situations. Anyway, the differences in fertility among considered objects or years are likely to be more important than the inaccuracies of the estimation method.

A quantification of fertility differences among a group of parents in probabilistic terms can be made as a basis for predictions and theoretical development. The sibling coefficient (Kang 2001) refers to the probability that two gametes, chosen randomly from the gene pool of gametes, originate from the same parent, compared to that in the gene pool of the parents. It is associated to the probability that individuals share the same parent, and thus are sibs. Mathematically, sibling coefficient, Ψ , can be defined as $N \sum p_i^2$, in which N is the number of individuals and p_i is the relative fertility of individual i. Relevant theory is developed in the PhD theses by Bila (2000) and Kang (2001) and papers in them. "The effective number of parents" can be expressed as N/Ψ , which can be viewed as identical to the classical "variance effective number". The sibling coefficient is a function of the coefficient of variation for fertility; $\Psi=2$ corresponds to a coefficient of variation of 100%. Sibling coefficient for a forest stand may typically be 2 (Bila 2000), but much higher values has been observed in individual cases. $\Psi=2$ means that there will be twice as many sibs among the seeds as expected if all mating were equally frequent. $\Psi=2$ means that relatedness and later inbreeding will build up over generations twice as fast as if there were equal mating. Reasons to suggest "sibling coefficient" to be useful in predictions of generation shifts are that it is independent of the number of members in the population, that it focuses on probabilities and that it has transparent interpretations as mentioned above. An example of this calculation technique is demonstrated in Bila (2000). The loss over generations is predicted when seeds are collected and a small sample of these seeds is used to replace the stand (a gene conservation stand). If a limited number of offspring is considered, the successful gametes can be seen as obtained by sampling from all gametes. An option to increase the effective number, thus to reduce group coancestry, loss of gene diversity and subsequent inbreeding, is to keep female fertility constant by mixing the same amount of seeds from all trees. This is a rather effective measure. The technique can also be used to trade off gain and gene diversity in the seed crop or gain and gene diversity in the stand itself (low-input breeding). The idea is that inbreeding and the associated phenomenon can be kept manageable and balanced against cost, gain and other desiderata by management technique and numbers, rather than by keeping exact pedigrees. Such calculations are needed for a low-input program, but can be generalized in tables. There can be a tremendous variation in the magnitude of fertility variation. For example, Varghese et al. (2003) reported a sibling coefficient, $\Psi=17.4$ in a first generation progeny trial of *Eucalyptus tereticornis* in south India studied at four years of age with the intention to convert it to a seedling seed orchard. Only 18% trees were fertile out of 200 trees selected for phenotypic superiority. Most cases with high sibling coefficient are objects which are young or flower poorly (Kang et al. 2003) or are not well adapted or it happens to be a special year, and probably not important overall, and even the seeds from the object just described would not have dangerously low gene diversity.

Use More Offspring from the Best Parents

Increase in relatedness and breeding value are the major outcomes of selection, and there are optimal combinations of them in the sense that under given constraints and gene diversity, there exists an optimal strategy that maximizes gain. An optimal strategy may be conservative or aggressive depending on the demand of gene diversity. It is a good breeding practice to allow the better trees to be over-represented in both breeding populations and production populations. A more gradual differentiation in treatment of materials relative to their goodness is more optimal than the either/or truncation selection usually practiced in breeding. Sophisticated algorithms for

identifying such strategies have been developed for deployment. Different variants of linear deployment has been suggested for deployment to seed orchards, candidate populations and the breeding population (e.g. Lindgren and Matheson 1986, Bondesson and Lindgren 1993; Wei and Lindgren 1995, Lindgren and Mullin 1997; Andersson 1999 Lstiburek et al 2005). Different ways of introducing such unbalances were reviewed by Lindgren (2005). While paternal fertility may be unknown, it is still possible to trade off against female fertility, and thus pick more seeds and plant more plants from the best female parents. Fernandez and Toro (2001) applied integer mathematical programming in a selection scheme on an open-pollinated population in *Eucalyptus globulus*, resulting in a large reduction in loss of gene diversity at a small cost of genetic gain compared to truncation selection. There seems to be a need to develop techniques that are simple to handle, but still close to optimal for low-input breeding. Many optimization techniques require plants to be identified by family within gene resource plantations; this is an argument for family identification in the field. These techniques will contribute a better guarantee that diversity is preserved and make the balance between gain and diversity more of a deliberate choice. I doubt it is efficient keeping identifications in a low-input program for this reason only, but if this is done for other reasons, then techniques using this knowledge could be used. Another application could be to consider the family value for low heritability characters or characters such as survival, as a guide to how many to select from each family, but to select phenotypically within family (preferable for other characters). To harvest cones from fewer trees than are left as possible pollen parents can be seen as using more offspring from better trees, the best trees will be used both as seed parents and pollen parents, while more trees will be used at least as pollen parents.

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