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EXPLOITING DIFFERENT SCALES OF VARIABILITY IN A GENERIC *EUCALYPTUS* SPECIES

Abstract. This paper tackles a simple question: How should we go about starting a breeding program for a generic eucalypt? I posit that the failure of many attempts is directly related to their lack of focus and small population size, which made very difficult to select and breed trees that showed superiority for multiple-traits. I describe the exploitation of variability at three levels: between populations, within populations and within trees. Taking advantage of within-tree variability would make very early selection for intrinsic wood quality feasible. I use stochastic simulation to study the effect of sample size when dealing with a multiple-trait situation. Arising from this analysis I suggest using a strategy based on open-pollination, enriched with controlled crosses making use of low cost techniques like Artificially Induced Protogyny. Finally, the testing scheme would target very specific environments, considering that we ought to be breeding niche species that take account of a particular site/environment and a specific end-product.

1. INTRODUCTION

We blew it. *Eucalyptus*—and any other species besides radiata pine and Douglas fir for that matter—have disappointed in New Zealand. There are many reasons and excuses for this situation, but at the core of it all there has never been a real belief that we should (and could) succeed growing something else. We followed a contradictory strategy: on one hand we wanted species that could compete with radiata pine, while on the other we dealt superficially with a myriad alternatives. The lack of focus led us to spread our bets too widely, to the point of being unable to identify real winners.

An optimist would say that we have been blowing it for many years but that we still have a chance. This chance should be followed by a big "if we use a different strategy", because one definition of insanity is doing the same thing over and over again expecting different results. Of course we breeders carry our fair share of blame, if not by action then by inaction. We knew that a ridiculously small number of samples of a species is not a good starting point for a breeding program. We also suspected that the selection criteria might have not been appropriate. Finally, we did not act upon this knowledge to help steer these programs in the right direction.

This paper tackles a simple question: if we were starting a breeding program for a new species—let us call it a eucalypt—How would we go about doing it? A good starting point could be to redefine the problem; rather than looking for an overall good performer, we could identify niche opportunities that could be tackled by alternative species, with the following conditions: (i) they should be outside the "optimum" site and product range for radiata pine, and (ii) the application should be a profitable, clearly identified, straightforward forestry investment. Chasing the vagaries of government policies, subsidies, bonuses and credits is more often than not a distraction. These choices have two useful consequences: (i) we reduce the number of environments to be targeted for testing, and (ii) by identifying the production system it lets us define our improvement objectives and selection criteria.

2. A CRASH COURSE ON TREE IMPROVEMENT

Every tree-improvement program is based on the exploitation of variability, for which there are only three essential steps: selection, mating and testing. Selection will be based on an improvement objective that maximizes profit, mating will depend on reproductive biology and economic considerations, and testing will depend on statistical and, again, economic considerations. That is, in theory profit drivers and cost structures are (and should be in practice) at the center of breeding programs.

Breeders have traditionally distinguished two scales of variability that are useful to structure breeding programs: between- and within-populations. Initially most programs rely on differences between populations (provenances, landraces), which are then followed by differences between trees within a population. Further, during the last thirty years there have been numerous studies showing within-tree variation for wood quality, which could also be exploited for early selection purposes. This would be a clear departure from traditional breeding programs and could make a substantial difference on the amount and timing of genetic gain that can be achieved by breeding.

2.1. Variability between populations

During any exploratory stage—that is pre-breeding—foresters target species. Once a promising species is identified they start by looking for differences between populations of that species. This procedure has been extensively documented for growth traits, for example in *E. globulus* (Dutkowski and Potts 1999) and *E. nitens* (Dutkowski *et al*. 2001). There are also population effects for wood properties, with significant differences reported for basic density, fiber length and cellulose content (Apiolaza *et al*. 2005). This variability in population at the trait-level also translates into variability in profit/ha that can be large enough to justify switching provenances or land races in breeding programs. During the 1990s the Southern Tree Breeding Association changed the composition of its *E. globulus* breeding program from one dominated by the King Island subrace to one where the Strzelecki and Otways subraces are now prevalent (P. Gore, personal communication). It is not unusual that existing differences between populations are equivalent to the benefit of one or more cycles of breeding.

2.2. Variability within-populations

Once the most promising populations of a species have been identified, it is important to sample and characterize the variation within them. The number of trees needed for this step is discussed below. This characterization initially encompasses growth and risk traits (drought, frost, disease resistance). Unfortunately, most breeding programs have not characterized differences between families for wood quality beyond basic density, if at all. Nevertheless, there are reports of coefficients of variation for several wood quality traits, mostly applying to samples without a

clear genetic structure or where the structure has been ignored. We have to remember though that those values, for example Chauhan (2009), refer to *phenotypic* variation; that is, they comprise both genetic and environmental variation and only the former can be exploited for breeding purposes. When separating these effects, Apiolaza *et al.* (2005) reported the following genetic coefficients of variation for *Eucalyptus globulus*: 4.7% for basic density, 8.4% for microfibril angle, 3.7% for fiber length and 3% for cellulose content. These values they still pale in comparison with the coefficient of variation for growth: 11.2%. In another study, Raymond *et al.* (2009) report substantial genetic variation for *E. pilularis*.

2.3. Exploiting within-tree variability

Breeders use one-quarter to one-third of rotation as the "rule of thumb" for early selection. There are some implicit assumptions related to this rule: (i) value is generated at rotation end, (ii) we are interested in either the total value or weighted average of a characteristic, and (iii) we have not thought of a better way of doing this. We will tackle these issues one at the time.

In New Zealand current silvicultural practice with regard to our major species generates most value at rotation age, although some intermediate value might be generated by commercial thinnings. Nevertheless, at least one *Eucalyptus* project is targeting a combination of early production of posts and poles for agriculture, and a longer rotation crop for sawn timber (NZDFI, Millen 2009). This approach would create an opportunity for much earlier selection of superior trees.

When improving a trait not all marginal changes have necessarily the same value. This means that for some traits (like volume) more is better, while for others (like wood stiffness) there are value thresholds that result in some improvements having little or no value and others having considerable value.

Rather than tackling an average value, I propose that we use two types of wood quality traits: threshold values (Apiolaza 2009) and gradients. An example of threshold values would be the time taken to start producing heartwood or to achieve structural grade. An example of gradient would be the gradual differences in moving from early corewood to late outerwood with regard to, say, density or growth stress. In general, we should aim for early expression of acceptable quality thresholds and flat or gradual gradients.

3. HOW MANY TREES ARE ENOUGH?

Given that tree breeding relies on exploiting diversity, in general terms the more trees the merrier. When dealing with a single trait, the number of trees to assess in order to find a given number of superior trees is obvious, particularly if one ignores issues like family structure. However, when working with a multiple trait situation—that is, a real life breeding program—things become more complicated. In this case, stochastic simulation provides a straightforward approach to illustrate possible outcomes.

I implemented a simulator using the R statistical software (R Development Core Team 2008), which considered generating random multivariate populations of m families with n progeny each[1]. Then I simulated three traits for varying numbers of families and trees per family, keeping track of the number of superior trees for several traits. The phenotypic observations were generated as:

$$a_p = L'_A z_1 + L'_R z_2$$

where L_A and L_R are the Cholesky decomposition of one-quarter of the additive genetic covariance matrix ($0.25A$) and the residual covariance matrix ($0.75A + R$) respectively, z_1 and z_2 are vectors of normally distributed (0,1) random numbers, and $´$ is the transpose matrix operation.

The simulation considers three traits with heritabilities 0.25, 0.50 and 0.75, representing low (e.g. growth), medium (e.g. wood stiffness) and high (e.g. basic density) genetic control. There were three distinct scenarios of genetic correlation: no association between all three traits (zero correlation, independent traits), intermediate positive correlation (0.50) between all three traits and intermediate negative correlation (-0.5) between the traits. All traits were simulated with a phenotypic variance of one.

Table 1. The number of trees that are in the best 10% and best 20% for all three traits. Results are presented for 100 individuals with 10 to 60 progeny each (for a total of 1000-6000 trees) and three scenarios of genetic correlation.

	Uncorrelated		Positive correlation		Negative correlation	
	Top 10%	Top 20%	Top 10%	Top 20%	Top 10%	Top 20%
1000	0.9	8.4	14.0	46.6	0.1	1.9
2000	2.2	15.2	26.7	91.8	0.1	3.7
3000	3.1	23.5	41.4	138.7	0.2	5.6
4000	4.0	32.4	54.4	185.8	0.3	7.3
5000	5.1	41.0	69.9	229.6	0.5	9.3
6000	5.6	49.0	83.0	275.6	0.5	11.5

The simulation answers the question "How many trees are superior for all traits when testing X number of trees?" Naïve calculations ignore the association between traits: this scenario is presented in Table 1 as "uncorrelated". Trees that are good for one trait can take, on average, any value for others. Positively correlated traits present the simplest and most positive scenario. We are able to select many more superior trees because the best for one trait tend to be within the best for the other traits. In contrast, negative correlations are a curse for breeders: trees that are the best for one trait tend to be within the worst for the other traits. This makes it necessary to use rather large populations to find superior trees across several traits.

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[1] The simulator is completely generic on number of traits, families, trees per family and covariance matrices used.

Table 1 drives home a very simple point: unless we have positively correlated traits, small trials (the norm for alternative species) have very little chance of finding superior trees. We can increase chances by reducing selection intensity—the proportion targeted by selection—but there is a price: lower gain. Herein lies the tragedy of our lack of focus: too many species implies very few trees per species, which means no superior trees—we are deploying something that is close to the original, highly-variable wild resource.

Let us now contrast these results with other theoretical calculations. For example, Danusevicius and Lindgren (2005) point out that a small breeding population of between 30 and 70 individuals (depending on heritability) would maximize group merit—a linear function of genetic value and the associated loss of gene diversity. These values are lower than the 100 individuals tested in Table 1. We should make a distinction, however, between simulated values assuming known parameters and a breeding strategy where those parameters also have to be estimated. That is, we lose "degrees of freedom" because genetic testing has to provide not only material for future generations but a structure broad enough to estimate genetic parameters. Unfortunately, theoretical work often relates to a single trait and to breeding, not deployment (a seed orchard, for example) populations. In the former we are aiming to improve the average and we can afford to make mistakes when selecting and one of the main constraints is the need to maintain diversity. In the latter we are aiming to select specific genotypes, sacrificing diversity and having little room for error, because we will establish thousands of hectares of operational plantations with this material.

An important point to make is that we have to "score some runs" really quickly. There is no point in worrying about inbreeding and viable population sizes if a breeding program will be cancelled next year because of irrelevance. We prove relevance by making available superior material to operational plantations (by seeds or vegetative propagation), and Table 1 gives an indication of how many superior trees we can hope to find in our progeny tests.

It will be difficult to find enough superior trees in natural populations to kick start the testing program for some species: we are struggling with this in the NZDFI. It is possible to partially compensate for this problem by increasing the number of progeny per family. Effective population size (number of unrelated individuals) will be smaller and estimates of genetic parameters may have lower quality, but at least we can have a large number of trees to perform within-family selection, particularly when thinking of deployment populations.

4. THE CORE OF A SIMPLE STRATEGY

It is all well and good to highlight the importance of large numbers when breeding. It is a different story to provide guidelines that will work in a cost-efficient way.

Many small-scale programs start with a very narrow genetic base and "save" money by testing only a few families. However, these experiments tend not to have enough power to provide reliable estimates of genetic parameters and to identify trees that are superior in more than one trait. This is an example of false economy,

because although we keep costs down we get nothing in exchange and any viable breeding program is delayed.

A large number of trees under testing is essential, but it is an insufficient condition for success. From a selection point of view, if the trees are not assessed for a particular trait there is no way to put direct selection pressure on it. To achieve any genetic gain for that trait we must rely on the correlation with other traits that have been assessed. Breeders routinely rely on indirect selection as a valid approach (using dbh to improve stem volume, for example). However, one does not need to go far to find examples of failure: just look at the attempts to improve wood stiffness by selecting for basic density in radiata pine.

I propose that we should rely on broadly applied and cheap very early screening for wood quality. These techniques could include acoustic tools, stem splitting and early introduction of wood quality problems (see Apiolaza *et al.* 2008 and Chauhan 2008, for example). From a sampling point of view, we may have to opt for elliptical assessment using the extremes of the distributions to obtain good quality estimates of the correlations between traits.

Foresters have been tempted to cover many different environments to show that a new species is a good alternative to radiata pine, growing well in many different parts of the country. This approach has thinly spread already too scarce resources, reducing the power of our trials. Indeed, if we are looking for niche species, why should they grow well everywhere? Besides targeting specific products and markets, we should target specific environments for testing. A few good trials trump a large chain of tiny trials.

It is unusual for small-scale breeding programs to rely on controlled-pollination (CP) strategies. CP tends to be expensive, traditionally involving three visits to the trees. First, about-to-open flowers are emasculated and bagged; second, flowers are controlled pollinated at maximum receptivity; finally, bags are removed some time after pollination. Simplified (and cheaper) methods like One Stop Pollination (OSP)—which performs emasculation, style slicing and pollination in a single visit—still struggles with small flowers. However, CP would allow us to exploit non-additive genetic effects (for example, the improved performance of specific crosses), control relatedness between individuals (to avoid inbreeding depression) as well as to emphasize particular combinations of genotypes where looking for trees that are superior across multiple traits, generating more genetic gain.

Assis *et al.* (2005) proposed Artificially Induced Protogyny (AIP), as a very simple, new controlled pollination method that 'involves cutting off the tip of the operculum of the mature flower bud just prior to anthesis, with the cut positioned so as to remove the stigma, and then applying the target pollen to the exposed cut-surface of the upper style'. Their results are comparable to OSP in *E. grandis*, whose small flower buds are of similar size (around 0.8x0.6 cm) to those used in the NZDFI program (*E. bosistoana*, *E. quadrangulata*, *E. globoidea*, *E. macrorrhyncha* and *E. camaldulensis*). A combination of better understanding of reproductive biology and alternative pollination techniques could make feasible the use of CP in a *Eucalyptus* strategy for New Zealand. Using CP lets us improve testing but extends

generation interval. We will minimize this extension by very early screening as discussed above.

Finally, I would emphasise early multiplication and deployment of promising material. The commonest cause of dead for failed breeding programs is 'zero delivery of genetic gain'. We would not like to have those words permanently linked to our names.

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