

Quantitative genetics and developments in Scion's tree breeding programmes

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Abstract

Quantitative genetics has been the engine behind tree breeding in New Zealand since the 1950s. Put simply, it involves the investigation of variances and the relative proportion of heritable (or genetic) variation, compared with the variation in the observed phenotype (what the tree looks like). Methods are changing rapidly in the field of genetics. With improvements in analytical platforms and techniques, and the change in relative costs of genotyping using DNA-based technologies, there are exciting times ahead.

At Scion, we are very enthusiastic and excited about new synergies between various science disciplines that have recently evolved. Within two to five years we believe that this will also deliver significant value to forest growers and wood processors. This short commentary outlines quantitative genetics and some analytical platforms and techniques that have become very useful, and why. We then discuss what has changed at Scion recently and briefly discuss the breeding programme for Douglas-fir, cypress and fast-growing eucalypts. Radiata pine breeding is undertaken by the Radiata Pine Breeding Company (RPBC) and is therefore not within the scope of this discussion paper.

What is quantitative genetics?

Quantitative genetics is the statistics behind tree breeding. It looks at differences and sizes of variation to understand and estimate the extent of genetic and environmental influence for a given trait and species. It all goes back to a classic formula:

$$\text{Phenotype} = \text{Genotype} + \text{Environment}, \text{ i.e. } P = G + E.$$

The phenotype, or physical appearance and size of a tree, is a result of its genetic make-up (genotype) and the influence of the environment. Quantitative geneticists spend their lives understanding this formula, and how G interacts with E (genotype by environment interaction) in order to determine the best trees for breeding (tree improvement) and for deployment (forests).

Why is estimating genetic and phenotypic variances so important?

This is because the relative proportion of the variance of G to the variance of P represents the intrinsic 'heritability' of a trait. Heritability is a calculated measure – between zero (not heritable) and one (100% heritable)

– of the amount of useful genetic variation that can be passed through DNA to the next generation, where:

$$\text{Heritability} = (\text{genetic variation}) \div (\text{phenotypic variation}).$$

Why is the heritability so important?

This because heritability contributes to 'genetic gain', where:

$$\text{Genetic gain} = \text{heritability} \times \text{selection differential} \times \text{phenotypic standard deviation}.$$

Note that the selection differential represents the intensity of selection, i.e. essentially choosing from more trees gives you a larger selection differential. Phenotypic standard deviation is the square root of the phenotypic variance, or the variation of the phenotype. By manipulating heritability, the selection differential and the phenotypic variance we are able to purposefully increase genetic gain. These three factors can be manipulated by better design of genetic field trials, a greater number of field trials, a greater sample size of trees from which to select and a broader range of genetic material. Furthermore, improved statistical techniques and analytical platforms ensure that heritability is properly estimated and maximised.

Analytical platforms and statistical methodology

Analytical platforms in quantitative genetics are constantly being updated in order to improve the statistical estimation of variances.

Data analysis

A few large steps forward have been made over the last few years. Moving to an ASReml-R (Butler et al., 2009) platform has improved the clarity and analytical workflow for geneticists. ASReml-R is a software package that estimates variance components and is particularly useful for large unbalanced data often found in tree breeding (Butler et al., 2009). Previously, the stand-alone ASReml (without an R platform) was more difficult to code, and data preparation and checking was done separately, often using a different programming language. ASReml-R has significant advantages in singularly capturing workflows from the input of data right through to the estimation of genetic parameters (e.g. heritability) and breeding values (an individual's average genetic worth). These workflows do not need to be totally re-created every time a new data set is added to the analysis, thereby significantly simplifying any re-analysis. Statistical methodology



Unimproved radiata pine (above) was well known for its poor form. Genetic improvement has now yielded better quality, straighter stems

has also been improved, particularly the use of 'spatial analysis' and of 'factor analytic models'.

Spatial analysis

The use of spatial analysis has generally resulted in improved estimates of heritability, thus it has improved estimates of genetic gain. The statistical method is embedded within ASReml-R and requires the field trial trees to be planted on a known grid, so that the tree data is spatially represented by row and column coordinates. An additional row/column spatial effect is fitted to the error term, or residual variance, removing underlying patterns of correlation. By fitting this additional spatial effect to the error variance, the remaining error variance is usually reduced, resulting in improved heritability estimates. There are many other methods for looking at spatial variance, but this is the only one that is embedded within ASReml-R.

Factor analytic models

These models are a statistical methodology that attempts to better estimate the variance components of interest and focuses on better estimation of the error variance. They are based on the 'principal component analysis' framework, where 'factors' are derived in order to describe the overall variation in a meaningful way. The factors are derived from variation of a large number of observations relevant to the trait being analysed, for example, environmental variables. This method is, again, a more effective partitioning of the variance and usually gives rise to either increased estimates of genetic variance, or reduced estimates of phenotypic variance. Both options increase the estimated heritabilities and subsequent predicted genetic gains. These types of models work best when there are many field trial sites (environments) to analyse (e.g. at least 10) and environmental and covariate data are available that can be used to create the factors. This factor analytic model approach has recently been adopted by the RPBC (www.rpbc.co.nz) in the estimation of radiata breeding values (Cullis et al., 2014).

New collaborative connections

Two recent significant connections have been made by the quantitative genetics capability at Scion. The first connection is the joining of forces with Scion's molecular geneticists. At Scion we have enjoyed the challenge of using our combined quantitative and molecular resources and capabilities to work towards genomic selection (genome-wide marker-aided selection). The second connection is our partnering with the RPBC's Genomic Selection programme. Here, we are combining our experience and resources to undertake genotype-based selection within two years, and operational implementation for some traits within five years. This is an exciting and challenging endeavour that is documented in a companion paper in this special journal issue. The venture's future also depends on better understanding the genome, and we are pleased that Scion is also leading the sequencing of the radiata genome, involving multiple national and international collaborators.

In addition to working more closely with molecular genetics, we have also been working with Scion's forest health and radiata productivity teams, including the radiata pine Growing Confidence in Forestry's Future programme. These across-team collaborations have been sparking some new thinking and approaches for disease resistance screening and 'phenotyping the forest'.

Update on individual species breeding programmes

Breeding programmes for different species vary in their complexity and size. Currently at Scion there are active breeding programmes for Douglas-fir, *Eucalyptus fastigata*, *E. nitens*, *E. regnans* and the cypresses, including for example *Cupressus macrocarpa*, *Cupressus lusitanica* and *Chamaecyparis nootkatensis*. Radiata pine breeding is undertaken wholly by the RPBC and is the

largest and most complex of the New Zealand tree breeding programmes. Breeding for the other species is undertaken in partnership with the Forest Growers Levy Trust Board and the Forest Owners' Association.

There is currently no breeding programme for coast redwood (*Sequoia sempervirens*) at Scion, although there are a number of clonal trials and a provenance/progeny trial. If industry supported the development of a breeding population, it would be relatively easy to do. Scion is also grafting selections of *E. muelleriana* and *E. pilularis* from their progeny/provenance trials for the establishment of seed orchards. A number of other species (e.g. Mexican pines, stringybark eucalypts and acacias) have breeding trials and genetic resource plantings that have not been abandoned by Scion, but require interest and funding for continued measurement and analysis.

Douglas-fir

Douglas-fir breeding and genetics has been well documented in several publications (Shelbourne et al. 2007; Maclaren, 2009; Dungey et al., 2012). This species can be more sensitive to the influence of the environment than radiata pine. In the south of the South Island equinoctial gales can cause leader damage, and in the North Island Swiss Needle Cast causes losses in productivity. The breeding of Douglas-fir has stopped and started a number of times due to this needle disease and is currently only in the second generation. Future breeding is aimed at developing resistance or tolerance to Swiss Needle Cast, particularly for the warmer wetter sites where the disease is known to cause reductions of productivity of approximately 32%. Increasing wood stiffness, particularly in the cooler areas, is also an imperative.

Both controlled-cross and open-pollinated trials, as well as multiple progeny/provenance collections from Washington to California, represent the material used in the Douglas-fir breeding programme. It is vital that this is retained as a genetic resource as biosecurity restrictions currently prevent further importation of material from the natural range. The most recent breeding population trials (1996) have been assessed for growth, form and wood stiffness, and one trial was assessed for Swiss Needle Cast. Selections from these trials have been grafted and incorporated into orchards. In 2011, selections that were flowering were incorporated into next-generation trials.

Up to 30% genetic gains for growth are available from the Douglas-fir seed orchards, compared with Washington seedlot material. Seed from selections with superior wood stiffness are also now available. Improved seed can be

purchased from Proseed and Dusky Seed. We encourage growers to try some of this improved seed on their estate.

Eucalypts

Three fast-growing eucalypts are the current focus at Scion, and have all had at least three generations of genetic improvement. All eucalypt breeding programmes have been run on a simple open-pollination forward-selection strategy:

- *Eucalyptus nitens* is currently undergoing fourth generation re-selection and is being remeasured for growth, form and wood quality. Markers have been developed to identify and limit parent contribution to the next generation in order to control in-breeding in our open-pollinated breeding programme of *E. nitens*. Originally, these markers were SSR markers, based on short sequence repeats, developed by Gea et al. (2007). We have recently tested the new SNP (single nucleotide polymorphism) marker panel from the Eucalypt Genome Consortium as an alternative to the SSR marker kits (Telfer et al., submitted). The SNP marker panel was found to be superior in identifying parents, and this technology will be now be used regularly in our breeding programme.
- *Eucalyptus regnans* has just undergone fourth generation re-selection, and a basic genome sequence is planned in collaboration with AgResearch.
- *E. fastigata* is in its third cycle of selection, which will comprise a mix of second and third generation material.
- If there is interest from the forestry industry, we hope to roll-out the genomic selection technology to all eucalypt species.

Predicted genetic gains for eucalypts from the most recent selections have been estimated as in Table 1.

New seed orchards have been established for *E. regnans* and *E. fastigata* in partnership with Proseed. The seed from these orchards will be available soon. The first *E. fastigata* seed is likely to be available in commercial quantities during late summer-to-autumn 2015. Three *E. nitens* seed orchards already exist: Southwood Exports Ltd, Mike Olsen and Proseed. Scion has also trialled many other eucalypts. Recently, we selected trees across a range of provenances from two stringybark species, *E. muelleriana* and *E. pilularis*. These selections are now being grafted for the establishment of new seed orchards. The stringybarks are prized for stable timber with naturally durable heartwood. They

Table 1: Predicted genetic gains for eucalypts

Species	Predicted genetic gain	Comment
<i>E. fastigata</i>	At age 3: height 7–8%, DBH 6%, health 26%, malformation 12%	Based on selecting the top 30% from the third breeding generation
<i>E. nitens</i>	At age 6: height 0.6–1.2%, DBH 3–6%, volume 7–14%	Top 70 individuals, 2 scenarios: 1) selecting for genetic diversity, 2) selecting for gain only. Third generation gains only
<i>E. regnans</i>	At age 3: height 9%, DBH 31%	When selecting the top 30% from the third breeding generation



The 'genetics' walk in the Long Mile next to Scion, which has been felled, demonstrated the benefits of genetic improvement

are slightly slower growing than *E. fastigata* and more susceptible to frost so must be carefully sited.

Cypresses

Recent development of the cypresses (or cedars) has followed a development plan that was written within the Diversified Species Theme of Future Forests Research (2008–2014). The aim of the breeding programme is to develop canker-resistant, germplasm with durable wood that will meet the specifications of the furniture or cladding markets. Essentially, individual breeding programmes of the primary cypress species, *C. macrocarpa* and *C. lusitanica*, will be continued. Selection will be for growth, form, branching, tolerance to canker and, where possible, heartwood durability. The best *Chamaecyparis nootkatensis* (yellow cedar) and *Cupressus guadalupensis* (Guadalupe cypress), will be collected and/or pollen for crossing will be imported from Canadian collaborators. Hybrid crossing will be undertaken between the cypresses to maximise the traits of interest. The hybrids will be clonally tested before release.

A new *C. macrocarpa* breeding orchard has been established with selections chosen for their resilience to canker, and a separate hybridisation orchard has been established for the production of novel hybrids. Hybrid crossing will commence during 2015–2016 for the production of the next round of superior clones. The best hybrid clones that have been produced so far are being tested and remain as stool beds in the Scion Nursery. These clones, pending a field assessment to confirm the final selections, should be available from the nursery for the 2016 planting season.

Superior *C. lusitanica* clones were sent to Appleton's Nursery and should now be available for planting. In partnership with Proseed, seed orchards exist of



Grafted cypress selections ready for installation in the new cypress breeding orchard

C. lusitanica and *C. macrocarpa*. These orchards have now been rogued (thinned) and represent the best seed currently available in New Zealand.

The future

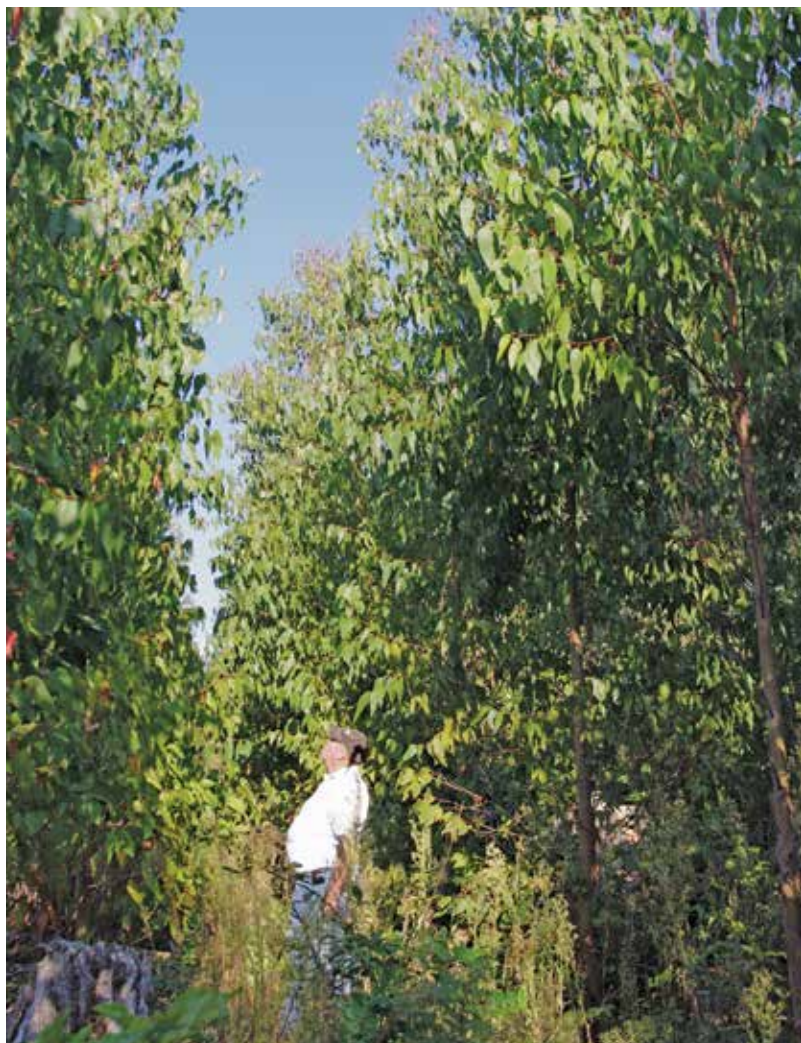
Historically, quantitative genetics and tree breeding (tree improvement) was a slow, phenotypic selection-based process. Speeding up the delivery of genetic gain is vital for reasons of return-on-investment (investment in the breeding programme) and rotation-end productivity, health and wood quality. For these reasons, we clearly see a future for the integration of quantitative and molecular genetics. The combination of these approaches through genomic selection will deliver gain faster and have the ability to deliver across multiple traits. We also believe that many of the next significant outcomes will be achieved through the new thinking and cross-benefits of working in collaboration with researchers in forest health, 'phenotyping the forest' and remote sensing. Collaboration within Scion, nationally and internationally, will be key to unlocking novel approaches to quantitative and molecular genetics for the rapid delivery of resilient and productive trees grown for specific wood product markets.

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Second generation Douglas-fir selections in the Scion nursery (trials from this material were established in 2011)



Charlie Low inspecting a two-and-a-half-year-old progeny test of *Eucalyptus regnans*

many amazing people and contributions that resulted in the resources that are now available for research and which comprise our planted forests.

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