PART 6

THE USE OF VEGETATIVE PROPAGATION FOR GENETIC AND PHYSIOLOGICAL INFORMATION

THE USE OF VEGETATIVE PROPAGULES FOR OBTAINING GENETIC INFORMATION

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ABSTRACT

Two or more vegetative propagules per clone can provide estimates of genotypic and phenotypic variances and covariances in populations, and of the genotypic values of parent trees. When planted on several sites they can provide information about genotype-site interactions. In theory, at least, vegetative propagules from a set of clones can provide information far more efficiently and/or precisely than a set of seedling progenies. But this information, unless applied to a situation where vegetative propagation is used to produce planting stock, can be seriously biased by such factors as topophysis and non-additive gene effects. Topophysis, which is often irreversible, may be circumvented by the clonal replication of young seedlings. Cuttings are normally much more suitable than grafts for providing genetic information.

INTRODUCTION

When selecting or assessing parent trees in tree breeding work, the phenotype of an individual gives an indication of its genetic value, but the reliability of this indication will depend on how much the environment has affected the expression of the character concerned. The way to separate the effects of the genotype and those of the environment is to study groups of related individuals. The closest relationship that can exist is among identical twins or among vegetative propagules of an individual; it is a special case involving precise genotypic replication. Among the various other cases the relationships between seedling progeny of individual parents have been of most frequent interest in forest genetics and tree breeding. In theory, the complete lack of genetic variation among the ortet (original tree) and the ramets (vegetative propagules) of a clone, in contrast to the considerable genetic variation among the seedlings in a progeny (family), makes for greater precision and efficiency in obtaining genetic information. On the other hand, vegetative propagation often confounds various non-genetic effects which are also clonal with the purely genetic differences. This paper is largely an analysis of this conflict.

There are two types of genetic information that can be obtained from studying propagules:

(a) Estimates of genetic parameters
Total genotypic variance (containing additive and non-additive components), environmental variance, and genotype-environment interaction variance may all be estimated from clonal experiments. From these variances may be derived broad-sense heritability, the ratio of genetic to genetic plus environmental plus interaction variance. The covariances between different characters can furnish estimates of genotypic and phenotypic correlations.

(b) Estimates of the genotypic values of individuals
The mean for the vegetative propagules of a clone can represent an estimate of the clone's total genotypic value. The total genotypic value of an individual is relevant if commercial propagation is to be vegetative, but it may or may not be closely related to the additive genetic value (breeding value or general combining ability) which is relevant if the crop is propagated by seed.

The estimation of genotypic, environmental, and genotype-environment interaction variances makes it possible to investigate the environmental factors that cause the interaction. This can be a major prerequisite to efficient estimation of genetic values. To a large extent environments are permanent effects, as little can be done to change climate, topography, soil, etc., but new genotypes can be easily produced. If the role of environment can be defined, breeding programmes can be effectively regionalised and field experiments can be sited so as to give good resolution of genetic differences.

The genetic information provided by clones and by half-sib progenies will now be considered in order to illustrate the relative merits of vegetative propagules and seedlings in producing estimates of genetic parameters and estimates of genotypic values of individuals. Limited consideration is given also to full-sib progenies. The half-sib progeny, apart from affording an instructive comparison with clones, is of considerable interest in forest genetics and tree breeding. The genetic information which half-sibs do provide is fully relevant to ordinary seed propagation. In favourable circumstances (viz dense plantations of wind-pollinated species) a convenient approximation to half-sibs may be available in open-pollinated progenies.

THE MODEL

In the simple case of propagation by seed the following linear model may be used to describe the relationship of the phenotype of an individual, P, to its genetic constitution, G, and the effect of its environment, E:

$$P = G + E + GE$$

where GE = the effect of genotype-environment interaction.

and G = A + NA, A being the additive genetic value and NA being the non-additive genetic value (dominance and epistasis).*

* It must be noted that subsequent development of this model will involve certain simplifying approximations and assumptions concerning the nature and magnitude of epistasis (interactions between genes at different loci) (cf. Kempthorne, 1956, p. 423). Even large departures from the assumptions should not materially disturb the **comparisons** drawn in this paper.

For a population, the following relationship would hold:

$$V_P = V_G + V_E + V_{GE}$$

where V_P = phenotypic variance, etc.

Alternatively, this relationship may be written:

$$V_P = V_A + V_{NA} + V_E + V_{AE} + V_{NAE}$$

Where vegetative propagules are used the model should be extended to accommodate effects which will be collectively described as "topophysis" (cf. "c" effects, Libby and Jund, 1962). These represent persistent non-genetic characteristics deriving from the condition of the ortet or that part of the ortet from which a propagule is taken. These effects include "physiological ageing", related to the age or the size of the ortet and the part of the ortet from which the cutting or scion is taken. They can include, as an extreme case, the apparently permanent plagiotropic (or horizontal) growth of ramets taken from branches of the Araucariaceae.

Allowing for topophysis, the phenotype of an individual cutting can be expressed as follows:—

$$P = G + m + M + E + Gm + GM + GE + mM + mE + ME$$

where m = "maternal" effect peculiar to the individual propagule, e.g., size of a cutting, its position on the ortet, or presence or absence of flower buds.

M = "maternal" effect common to all ramets of a clone, e.g., the age of an ortet, its vigour, or its particular nutrient status,

Gm = the effect of the interaction between the genotype and the "maternal" effect peculiar to the propagule,
and so on for other interaction terms.

For simplicity we have omitted the second- and third-order interactions, GmM, etc.

The total clonal effect, C, can be described as follows:—

$$C = G + M + GM$$

so the phenotype of an individual ramet can alternatively be described:

$$P = C + m + E + Cm + CE + mE$$

For a population of cuttings we may then assume the relationship:

$$V_P = V_G + V_m + V_M + V_E + V_{Gm} + V_{GM} + V_{GE} + V_{mM} + V_{mE}\ V_{ME}$$

or alternatively:

$$V_P = V_C + V_m + V_E + V_{Cm} + V_{CE} + V_{mE}$$

Although the model is not exhaustive it will suffice for purposes of illustration. All vegetative propagules will be assumed to be cuttings. To consider grafts, which are subject to effects of stock and stock-scion interaction, would demand an even more elaborate model which would not illustrate any additional principles.

ESTIMATION OF GENETIC PARAMETERS

Analyses of variance of progeny and clonal trials will now be considered with the expected mean squares (m.s.) and their compositions in terms of genetic parameters. It is assumed that parents are not inbred. In a half-sib progeny trial (each progeny having one common parent, with the other parents a random population sample), with

n seedlings per progeny, and with genotype-site interactions ignored, the approximate expectations are:—

Between-progenies m.s. $= V_W + nV_F = (V_E + \frac{3}{4}V_A + V_{NA}) + n.\frac{1}{4}V_A$

Within-progenies m.s. $= V_W = V_E + \frac{3}{4}V_A + V_{NA}$

where V_F = between-progenies variance, V_W = within-progenies variance.

In a test of unrelated full-sib progenies (crosses between pairs of parents) the expectations are:—

Between-progenies m.s. $= (V_E + \frac{1}{2}V_A + \frac{3}{4}V_{NA}) + n(\frac{1}{2}V_A + \frac{1}{4}V_{NA})$

Within-progenies m.s. $= V_E + \frac{1}{2}V_A + \frac{3}{4}V_{NA}$

In a clonal trial of the same design, uncomplicated by topophysis effects, the expectations are:—

Between-clones m.s. $= V_E + nV_G = V_E + n(V_A + V_{NA})$

Within-clones m.s. $= V_E$

In the half-sib progeny trial and the clonal trial we can readily solve for V_A and V_G respectively. In turn, the ratios V_A/V_P and V_G/V_P can be obtained, these being narrow-sense and broad-sense heritabilities respectively. If vegetative propagation is used commercially the broad-sense heritability is relevant, but for orthodox seed propagation the narrow-sense heritability will apply and will never exceed the broad-sense heritability, being almost always somewhat lower.

In the absence of topophysis the clonal experiment can give a direct estimate of V_E, which the seedling progeny cannot. It is theoretically possible to estimate V_{NA} by subtracting V_A as estimated in the progeny experiment from V_C i.e., $(V_A + V_{NA})$, as estimated in the clonal experiment. However, V_A and, to a much lesser extent V_C are often subject to considerable errors of estimation, which means that any such estimate of V_{NA} may be very imprecise. In practice, non-additive genetic variance has usually been estimated from elaborate control-cross mating designs such as North Carolina I and II and the diallel cross and its modifications.

It can be seen that the ratio of between-clone to within-clone mean squares will be much greater than the ratio of between-progeny to within-progeny mean squares because $n \times \frac{1}{4}V_A$ is much less than $n \times (V_A + V_{NA})$ This allows $V_A + V_{NA}$ i.e., V_C or V_G, to be estimated more precisely, at least in the relative sense, than V_A can be from the progeny experiment. Alternatively, to achieve a given degree of precision we need far fewer ramets per clone than seedlings per progeny, giving a more compact and economical experiment. If heritability is low the clonal trial will be much more efficient than the progeny test—provided V_{NA} is small, and provided there is no topophysis. A full-sib trial will give a more precisely estimated between-progeny variance than the half-sib trial, but this will be inflated by an appreciable proportion of any non-additive gene effects. However, there is still substantial genetic variance *within* full-sib progenies.

If topophysis does arise, the expected mean squares in the clonal trial become:

Between-clones m.s. $= V_E + V_m + V_{Gm} + V_{mM} + n(V_C)$

$= V_E + V_m + V_{Gm} + V_{mM} + n(V_G + V_M + V_{GM})$

Within-clones m.s. $= V_E + V_m + V_{Gm} + V_{mM}$

It is clear that V_G and V_E are both fully confounded with other effects. The only advantage of this situation is that under vegetative propagation all these components of

V_C the between-clones variance, will contribute to the single-generation gain from selection, although not all this variance is entirely genetic.

To estimate the genotype-environment interaction variance any genetic experiment must be replicated on several sites and the basic model for describing the phenotype must be extended, such that:

$$P = G + E' + E'' + GE'' = A + NA + E' + E'' + AE'' + NAE''$$

where E' = effect of microenvironment (within-site effect)

E'' = effect of macroenvironment (between-site effect)

GE'' = effect of genotype-site interaction.

(GE', the effect of genotype-microenvironment interaction, is ignored, because it is fully confounded with and included in E').

Hence $V_P = V_G + V_{E'} + V_{E''} + V_{GE''}$

For a half-sib seedling progeny trial replicated on s sites, with n seedlings per progeny per site, and k progenies, with a fully random model, the expectations of mean squares become:—

Between-progenies m.s.	$= V_W + nV_{FE''} + nsV_F$
	$= (V_{E'} + \frac{3}{4}V_A + V_{NA}) + n.\frac{1}{4}V_{AE''} + ns.\frac{1}{4}V_A$
Between-sites m.s.	$= V_W + nV_{FE''} + nkV_{E''}$
Progeny-site interaction m.s.	$= V_W + nV_{FE''}$
	$= (V_{E'} + \frac{3}{4}V_A + V_{NA}) + n.\frac{1}{4}V_{AE''}$
Within progeny-site subclasses (error) m.s.	$= V_W = (V_{E'} + \frac{3}{4}V_A + V_{NA})$

and for a corresponding clonal experiment, without topophysis, the expectations become:

Between clones m.s.	$= V_{E'} + nV_{CE''} + nsV_C$
	$= V_{E'} + n(V_{AE''} + V_{NAE''}) + ns(V_A + V_{NA})$
Between-sites m.s.	$= V + nV_{CE''} + nkV_{E''}$
Clone-site interaction m.s.	$= V_{E'} + nV_{CE''}$
	$= V_{E'} + n(V_{AE''} + V_{NAE''})$
Within clone-site subclasses (error)	$= V_{E'}$

It may be noted that the clonal trial confounds $V_{AE''}$ and $V_{NAE''}$, although in general little is known of the importance of this factor. Far more important, probably, is that $V_{GE''}$ will be estimated much more efficiently and/or precisely from the clonal trial than $V_{AE''}$ would be from the progeny trial.

Where topophysis effects are present, the clone-site interaction effect CE'' will correspondingly contain genotypic and non-genotypic interactions with site. The latter interactions are fully relevant if commercial planting stock is propagated vegetatively but would represent bias if the information is to be used in a context of propagation by seed.

ESTIMATING GENOTYPIC VALUES OF PARENT TREES

The precision of estimating the genotypic values of parents from progeny tests or clonal trials can be compared using the heritabilities of progeny and clonal means, with n seedlings or ramets per family or clone, and ignoring site interactions. The heritability of progeny means is the ratio of the additive genetic component of the between-progeny variance to the variance of the progeny means. The heritability of clonal means is the ratio of the additive genetic variance (or clonal variance, if vegetative propagation is used commercially) to the variance of clonal means. The higher the ratio the greater will be the gains from selection on the basis of progeny or clonal means.

For half-sib progenies the ratio is:

$$\frac{V_F}{V_F + (V_W)/n} = \frac{\frac{1}{4}V_A}{\frac{1}{4}V_A + (\frac{3}{4}V_A + V_{NA} + V_E)/n}$$

In a clonal trial without topophysis, the ratio is:

$$\frac{V_A}{V_G + (V_E)/n} = \frac{V_A}{V_A + V_{NA} + (V_E)/n}$$

where propagation is by seed.

Where vegetative propagation is to be practised the non-additive gene effects can be utilised, so the ratio is:

$$\frac{V_A + V_{NA}}{V_A + V_{NA} + (V_E)/n}$$

For clonal tests where topophysis occurs the ratios are:

$$\frac{V_A}{(V_G + V_M + V_{GM}) + (V_m + V_E + V_{Gm} + V_{mM})/n}$$

and:

$$\frac{V_G + V_M + V_{GM}}{(V_G + V_M + V_{GM}) + (V_m + V_E + V_{Gm} + V_{mM})/n}$$

for seed propagation and vegetative propagation respectively.

Hence where equivalent vegetative propagation is practised the clonal trial will always give better information and greater gain from selection on the basis of clonal means (cf. Libby, 1964), but with seed propagation the value of the genetic information will be reduced, or even eliminated, by non-additive effects and by topophysis.

It may be noted that unrelated full-sib families give no valid estimates of the genotypic values of individual parents.

SCREENING ENVIRONMENTS FOR INTERACTIONS

Where an improvement programme operates over a range of sites, the gain from the initial field selection is directly proportional to the heritability (h^2).

$$\text{For seed propagation } h^2 \text{ (narrow-sense)} = \frac{V_A}{V_G + V_{E'} + V_{GE''}}$$

and for vegetative propagation without the complication of topophysis

$$h^2 \text{ (broad-sense)} = \frac{V_G}{V_G + V_{E'} + V_{GE''}}$$

Clearly, the larger $V_{GE''}$ is, the lower h^2 will be, and therefore the lower the gain.

$V_{AE''}$ and $V_{GE''}$ can be estimated for various combinations of sites from progeny or clonal experiments. It is possible thereby to recognise the sites or site categories that inflate $V_{GE''}$ and thereby lower h^2. By treating such sites as separate regional breeding units it is possible to raise the effective heritabilities and the genetic gains. Essential to this process are reasonably precise estimates of genotype-site interaction variance, and it is here that clonal experiments have a major advantage. Only if topophysis or non-additive gene effects show substantial interactions with sites will clonal experiments give an unreliable classification of sites in relation to seed propagation.

Where only a single site is involved $V_{GE''}$ can be treated as part and parcel of V_G (cf. Libby, 1964), and likewise $V_{AE''}$ can be treated as part of V_A.

It may be noted that where different genotypes within seedling progenies are well adapted to different sites, the genotype-site interactions tend to be masked. Naturally, this situation cannot arise with clones.

Closely related to the estimation of genotype-site interaction is the study of differential tolerances and responses in controlled environment experiments. Here the problem is often to obtain precise information with limited numbers of individuals. In this situation the use of clones can be particularly advantageous.

CLONAL EXPERIMENTS IN RELATION TO COMPETITION EFFECTS

With forest trees clonal trials have another important advantage over seedling trials, at least if multi-tree plots are used. In any stand, volume production tends to be governed by the relatively few stems that become dominant. The genetic variation that exists within a progeny means that although a progeny may be ill-adapted, on the average, it will generally contain some individuals that can thrive on the particular site. These individuals may benefit from reduced competition within the plots, so that productivity per unit area varies less than the average genotypic values of the progenies. No such effect of within-group genetic buffering can operate within a clonal trial.

CHARACTERS WITH "ALL-OR-NOTHING" EXPRESSION

Many characters of practical importance show "all-or-nothing" expression, in which a large element of chance is superimposed upon any genetic predisposition. These include survival, forking, leader dieback, stem breakage, and certain types of insect attack. As with low heritability characters in general the alternatives are either to progeny-test prospective parents (which entails going back a generation), or to replicate individual offspring by vegetative propagation. The quantitative theory of selecting for such characters is not fully explored, but vegetative propagation appears a promising technique, provided non-additive gene effects and topophysis effects do not interfere.

VEGETATIVE PROPAGATION OF YOUNG SEEDLINGS

It is now clear that in the context of seed propagation, topophysis and non-additive gene effects adversely affect the genetic information obtainable from vegetative propagules. The desirable goal, of course, is to be able to reverse or eliminate all topophysis effects. Varying success has been achieved by such techniques as applying temperature shock to tissue cultures, propagation of stump sprouts, and hedging of young trees. In some cases the process of physiological ageing has been largely halted,

if not reversed. Nevertheless, many tree breeders must still accept topophysis as a fact of life.

A promising method of circumventing the problem is by vegetative propagation of young seedlings, an approach which appears to have been largely neglected. It has the added advantage of allowing us to test genotypes well before the age of seed production. Libby (1969) has proposed the use of clonally replicated offspring in a seedling progeny trial and has made a theoretical study of some of the potential benefits. However, if the non-additive genetic variance is large relative to the additive, the avoidance of topophysis still does not make the genetic information from vegetative propagules generally acceptable.

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