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## Random non-contiguous plots in interlocking field layouts

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### Summary

Experiments with forest trees are expensive on a per-organism basis. They require large areas, which are generally more environmentally variable than areas used for agricultural research. A proposal is presented to take advantage of the efficiencies of small-plot designs, while minimizing some of their difficulties. Independently-randomized replications are interlocked, with plot members arranged non-contiguously within each block. This design allows small plots that are relatively safe from disqualification due to local causes of mortality or damage.

Such small-plot designs incorporate high levels of genetic dissimilarity between neighboring trees. It is argued that the interactions between such neighboring trees are normal in forest stands and plantations. Lethal competition between neighboring trees can be delayed by systematic thinnings of interlocked replications, with both the cut trees and the remaining plantation fully analysable as balanced and unbiased experiments.

**Key words:** Competition, Double planting, Hexagonal spacing, Mortality, Nursery organization, Quantitative genetics, Replication, Single-tree plots, Statistical efficiency, Thinning.

### Zusammenfassung

Zufallsverteilte zwischengeschaltete Parzellen nichtbenachbarter Versuchsglieder in Feldversuchsplänen

Versuche mit Waldbäumen sind je untersuchte Pflanze teuer. Sie benötigen große Versuchsflächen, die normalerweise größere Umweltvariation aufweisen als Flächen für landwirtschaftliche Versuche. Hier wird ein Vorschlag unterbreitet, der es ermöglicht, die Vorteile der Wirksamkeit (Effektivität) kleiner Parzellen zu nutzen und gleichzeitig einige ihrer Schwierigkeiten zu minimieren. Unabhängig randomisierte Wiederholungen werden zwischengeschaltet mit nichtbenachbarten Parzellen gleicher Versuchsglieder innerhalb eines Blockes. Diese Versuchsanordnung erlaubt kleine Parzellen, die gegen örtlich bedingte Disqualifikation

durch Mortalität oder Schädigung weitgehend gesichert sind.

Versuchspläne mit kleinen Parzellen schließen große genetische Unterschiede zwischen benachbarten Bäumen ein. Es wird argumentiert, daß Interaktionen zwischen solchen benachbarten Bäumen in Naturbeständen und Forsten üblich sind. Tödliche Konkurrenz zwischen benachbarten Bäumen kann durch systematische Durchforstung der zwischengeschalteten Wiederholungen verzögert werden. Dabei bleiben sowohl die herausgenommenen Bäume als auch die verbleibende Pflanzung als balanciertes und fehlerfreies Experiment voll analysierbar.

### Résumé

Parcelles unitaires, non contigües et randomisées, dans des dispositifs expérimentaux de terrain avec emboitement.

Les expériences concernant les arbres forestiers sont chères, si ce coût est rapporté à chaque arbre testé. Elles nécessitent de grandes surfaces qui sont en général plus variables sur le plan écologique que celles utilisées pour les plantes agricoles. Le présent article propose d'utiliser les avantages de l'efficacité des dispositifs comportant des parcelles unitaires de petite taille, tout en minimisant les difficultés inhérentes à ceux-ci. Des répétitions randomisées indépendamment sont imbriquées et les éléments d'une parcelle unitaire sont arrangés de façon non contigüe à l'intérieur de chaque bloc. Le dispositif autorise de petites parcelles qui présentent certaines garanties en cas de mortalité ou de dégâts localisés.

De tels dispositifs avec petite parcelle unitaire entraînent le voisinage d'arbres présentant une forte dissemblance génétique. Les auteurs soulignent que de telles interactions entre arbres voisins sont habituelles dans les peuplements et les plantations forestières; la compétition allant jusqu'à la létalité, entre arbres voisins, peut être retardée par éclaircies systématiques des répétitions emboîtées, tout en autorisant l'analyse des données concernant les arbres restant ou enlevés en éclaircie, comme des dispositifs non biaisés et équilibrés.

### Introduction

Quantitative genetic experiments are usually initiated to serve one of two purposes. One is investigation of the

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genetic architecture of a species, or of some subdivision of a species. The other is selection of a new population, with characteristics in some way changed with respect to populations presently available. When such experiments are conducted with forest trees, they encounter several difficulties that are either unique, or are intensified compared to such experiments with most other organisms.

This paper proposes a non-contiguous field arrangement of the entries in an experiment, suitable for many different kinds of mating designs. Such an arrangement allows the efficient estimation or statistical reduction of the large environmental heterogeneity typical of forest-tree experiments (SALMON, 1953). Interlocked replications provide for systematic thinning, to reduce stand density as the experiment matures. The combination of interlocked non-contiguous plots provides advantages relative to both random and non-random mortality. The effects of interactions between neighboring trees on the design and interpretation of genetic experiments are also discussed.

### Plot Size and Efficiency

The amount of environmental heterogeneity expected within forest-tree experiments differs from that expected with most other organisms for two reasons. Since trees are large, they occupy a much greater area than, say, an equal number of corn plants. Thus, on a comparable site, there will be more environmental variation between the trees because of the greater distance between them (SMITH, 1938). Additionally, a forest-tree site is generally more heterogeneous than an agricultural field, further increasing the relative amounts of environmental variation expected within forest-tree experiments. Thus, perhaps even more than with most other organisms, attention may be profitably focused on reducing the effects of this expected environmental heterogeneity, and thus increasing the statistical efficiency of genetic experiments with forest trees.

The problem of laying out quantitative genetic experiments with forest trees has generally been approached by allocating the  $N$  seedlings per entry (family, provenance, or whatever) to  $p$  contiguous trees per plot and to  $b$  blocks, such that  $pb = N$  (STERN *et al.*, 1967). (Such contiguous plots from each entry are often replicated within each block.)

#### *Advantages of Single-tree Plots*

If, on a per-tree basis, all environmental causes of variation were random within a block, the physical location of plants in the block would make little difference, beyond questions of operational efficiency. However, common sense, observation, and numerous careful investigations (see, for example, SAKAI and HATAKEYAMA, 1963) indicate that, in most field situations, environmental variables are patchy, or change gradually with distance. Under either a patchy or clinal pattern, or a combination of the two, plants growing near each other will share more elements of the environment than will plants growing at some distance from each other. Thus two plots may differ because the entries are genetically different, or because the plot environments are different, or both. One or the other is clearly interpretable, but "both" creates problems.

The plot environmental effect can be treated statistically by replicating the genetic entries within and between blocks. However, with a given  $N$ , the number of such replications is inversely related to plot size ( $1/p$ ).

WRIGHT and FREELAND (1960) argued effectively for one extreme of the allocation of plants to plots, with  $p = 1$  and  $b = N$ . They showed that, compared to larger plots of various sizes and shapes, "single-tree plots" most frequently

gave maximum efficiency with respect to information per tree, to cost, and to ability to distinguish genetically different entries. The single-tree plot is effective because it eliminates the unwanted (biasing) environmental covariances that occur among members of the genetic unit occupying a row-plot, or some-other-shaped-plot, in which the plot members are adjacent to each other (ie, the plot members are contiguous).

#### *Criticisms and Disadvantages of Single-tree Plots*

Single-tree plots have been criticized on three counts: (1) the difficulty of delivering each seedling to its uniquely-assigned location (as opposed to delivering bundles of seedlings to a plot); (2) the problem of intimate competition between unlike entries; and (3) the problem of missing plots due to damage or mortality, and of reasonably thinning such an experiment.

The occurrence of unplanned mortality (or disqualifying damage) among the organisms in an experiment is a difficulty that occurs in almost all biological research. It is intensified with trees, due in part to the long period of risk between initiation and completion of experiments. It is a serious problem with single-tree plots, as such experiments will often have many missing plots, leading to difficulties in analysis and interpretation.

Various schemes for thinning that employ contiguous plots (meaning plots of contiguous trees of the same entry arranged in rows, rectangles, or squares) have been proposed. Thinning can be accomplished within such contiguous plots without eliminating any entries from the block. WRIGHT and FREELAND (1960), as a partial solution to criticism (3), proposed that single-tree-plot experiments be "thinned by variety". Such a thinning would not result in uniform spacing, and it requires redefining the population each time a thinning is made. This is a reasonable procedure for some purposes; for others it is not. An example of a reasonable situation would be if most of the trees in a "variety" were killed or deformed. One might judge that it is too maladapted to the test environment to be realistically evaluated for further characteristics.

If  $N$  seedlings per entry are allocated either to preassigned single-tree plots, or to preassigned multiple-tree contiguous plots, it is likely to be more difficult to deliver each tree to its uniquely assigned location in the field than to deliver a smaller number of bundles to a smaller number of plots (criticism 1). However, if the field layout is random, seedlings or bundles of seedlings need not be delivered to preassigned locations. Instead, the final locations of individually-tagged seedlings can be mapped and checked after planting. It seems to us that there would be little difference between single-tree and multiple-tree plots in delivery, planting, mapping and checking effort in such a random layout. Furthermore, there may be greater opportunity for error with multiple-tree contiguous plots if they must be of a certain shape and composition.

Criticism (2) is valid not only for single-tree plots, but it is also valid for small contiguous plots. Most trees in small or medium-sized contiguous plots are either on an edge or next to an edge. (The smallest plot in which half of the planted trees are at least two trees from the plot border is a  $16 \times 12$  rectangular plot containing 192 trees.) Those on the edge will be in intimate competition with the entry in the adjacent plot; those next to the edge trees directly benefit or suffer from the effects of the interaction of the edge trees with the trees in the adjacent plot. If anything, criticism (2) appears to be more serious with

such small contiguous plots than with single-tree plots. Between-plot competition interactions occur for most or all trees in small contiguous plots. Since all members of a given plot edge compete with the same adjacent entry, the sample of such competition interactions (and thus the ability to statistically reduce their possible biasing effect) is smaller with  $N$  plants allocated to contiguous plots than with the same  $N$  plants allocated to single-tree plots. The question of competition, and of interaction between adjacent genotypes, will be discussed more fully below.

At this point, it seems clear that allocating  $p$  plants per entry to contiguous square or rectangular plots of small or modest size, or to row plots of any size, does not satisfy criticism (2). It appears that, in many of our pioneer genetic experiments with forest trees, the plot sizes used have been too small to reduce competition interactions very much, and have been too large to be very efficient (see, for example, STONECYPHER 1966, pages 118–119).

#### *Non-contiguous Plots*

When most workers use the term “plot” relative to experiment layout, they tacitly imply that the plants in the plot are adjacent to each other. However, there is no requirement that all or any members of a “plot” be physically adjacent to each other, as a prerequisite to statistical analysis of the data in the form of within- and between-plot means and variances. The members of a “plot” may be spread over some large area at random, or perhaps in some systematic manner. Plants from other “plots” may occupy locations between them. The environment affecting the plants in such a plot is not the relatively small, continuous and unique environment associated with a contiguous plot. It is a sample of the larger environment associated with the entire block.

The removal of the requirement that members of a plot be contiguous is crucial to this proposal. Several consequences follow as a result of removing this constraint.

The most important consequence is the avoidance of missing plots. With high anticipated mortality, each non-contiguous plot may consist of many trees, resulting in fewer blocks. Conversely, if mortality is expected to be low,  $p$  can be smaller, block size will thus be reduced, and block number will be increased . . . generally resulting in greater statistical efficiency and a better understanding of the environmental components of variation.

If anticipated mortality is uncertain, temporary sub-blocks can be laid out, each consisting of one member of each plot. The size and number of blocks ( $b$ ), and thus the number of trees per non-contiguous plot ( $p$ ), can be determined at the time data is analysed. If mortality or disqualification has been high, many adjacent sub-blocks can be combined; if low, plot and block size can be kept small by combining just enough sub-blocks to avoid having no data from any such plots. Two consequences of this modification will be to slightly inflate within-plot estimates of environmental variation, and to make selection experiments slightly fairer, and thus more effective.

So far, it may seem that all we have proposed is the removal of boundaries between adjacent blocks of Wright and Freeland's single-tree plots. That is essentially true, particularly if the modification in the preceding paragraph is adopted. By so doing, we have removed the problem of missing plots with low or moderate mortality, and (depending on the purpose of the experiment) have maintained or even increased the efficiency of the experiment.

#### **Planned Mortality**

Planned mortality, which is encountered in many long-term experiments with forest trees, is not a normal feature of experiments with most other organisms. In order to approximate the conditions in most natural stands and in most plantations, the trees in an experiment are often planted at relatively close initial spacing. The space required per tree increases with time, smaller or weaker trees would be suppressed by neighboring trees, and most would eventually die. This may be prevented or delayed by thinning in advance of such suppression.

#### *The Problem of Thinning Randomized Non-contiguous Plots*

In a random layout of single-tree or non-contiguous plots, a uniform thinning is almost impossible to achieve without leaving unequal numbers of trees per plot (entry). To solve this problem, it is helpful to use another statistical term in an unusual context. Most workers are accustomed to “replicating” in space, by repeating permutations of the layout in fields adjacent to each other, or widely separated from each other; or to “replicating” in time, by repeating the layout on the same field in different years. We propose to interlock replications, with such replications being installed at the same time and essentially occupying the same area.

#### *The California Design for Interlocking Replications*

In California, we have used three replications per block. These interlocked replications are each offset along each row at a distance of one spacing unit (say, for example, 3 meters) from the other two (*Figure 1*, top). Like the non-contiguous plots they contain, each replication samples the overall block environment, although no two take the same exact sample.

As an example, suppose we have a clonal experiment (other kinds of entry, such as full-sib families, would serve as well). Within each block, exactly  $r$  members of each clone are allocated to each replication, and they are planted randomly and independently within each replication. The number in  $r = p/3$ , and may vary from 1 to as large as necessary to ensure one or more survivors in each plot. For  $M$  entries, block size is  $3rM$ , with  $N/3r$  blocks. If the sub-block option discussed above is used,  $r = 1$ , sub-block size is  $3M$ , with  $N/3$  sub-blocks. If possible, all three replications within a block are planted on the same day.

Consider tree  $A_{21}$  in *Figure 1*, top. It is one of the  $r$  members of clone 21 in replication A, and its location within that replication has been determined by chance. It is surrounded by, and soon to be competing with, six equally-spaced trees in the configuration of a hexagon. These six trees may be any permutation of the clones in the experiment. The relationship of  $A_{21}$  to its neighbors is random. (A relatively minor point: there is a slightly greater chance that clone 21 will occupy one or more of the six nearest positions to  $A_{21}$  in the interlocked-replicate design than in a completely random design, since no members of clone 21 can be withdrawn from replications B or C to fill the  $A_{21}$  location, and thus all are available to fill the adjacent locations in B or C.)

For analyses of variance, this entire block may be considered to be completely randomized, with  $3r$  trees per clone (plot). Since trees of the same clone are not necessarily adjacent, the within-clone variance measures the environmental component of variance over the entire block.

When the first thinning is appropriate, the C (or B or A) replication is cut. This cut replication may be used for laboratory analyses of wood, chemical composition, or other

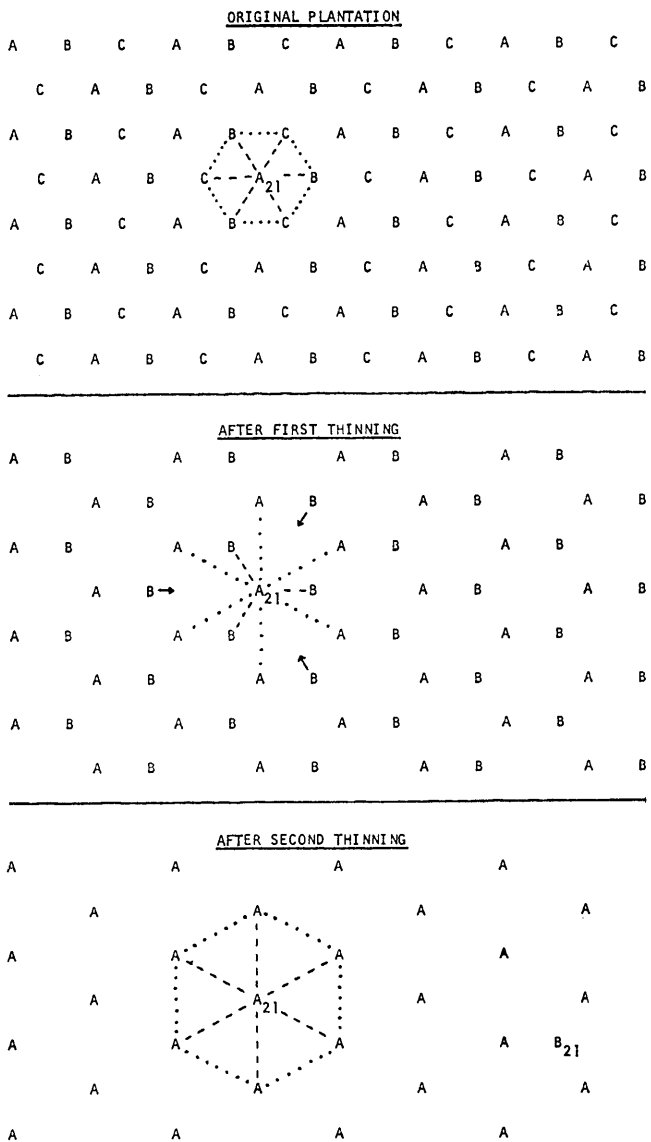


Figure 1. — Part of a California Interlocking Replications Design. Top: Locations of plants belonging to the three replications, designated A, B and C, interlocked within a single block. The distance between plants in a row = 1 unit; between rows =  $\sqrt{3}/2$ , or 0.866, units; and diagonally between nearest plants in adjacent rows = 1 unit.  $A_{21}$  identifies one location of clone 21 in replication A. The average area available for each tree is  $(.866) (\text{unit})^2$ . Center: All members of the C replication have been cut. Each interior plant ( $A_{21}$  for example) is now subject to close competition from three random plants of the alternative (B) replication at distances of 1 unit, to effects of six plants of its own (A) replication at distances of  $\sqrt{3}$  units, and perhaps to effects of three other plants of the alternative (B) replication at distances of 2 units. The average area available for each remaining tree is  $(1.5) (.866) (\text{unit})^2$ . Bottom: Final plantation. The distance between plants in a row = 3 units; between plants in a column and diagonally-nearest plants in adjacent rows =  $\sqrt{3}$  units.  $B_{21}$  indicates a member of clone 21 in the B replication that was not cut. The average area available for each remaining tree (except for  $B_{21}$ ) is  $(.866) (\sqrt{3} \text{ units})^2$ , or  $(3) (.866) (\text{unit})^2$ .

Partie d'un dispositif Californien à répétitions emboîtées en haut: Situation des plants appartenant aux 3 répétitions A, B, C, emboîtées à l'intérieur d'un seul bloc. La distance des plants sur la ligne est d'une unité, entre lignes  $\sqrt{3}/2 = 0,866$  unités, sur la diagonale entre les plants les plus près sur des lignes adjacentes = 1 unité.  $A_{21}$  identifie la position du clone 21 dans la répétition A. La surface moyenne disponible pour chaque plant est  $0,866 \text{ unité}^2$ .

destructive analyses. Such analyses would have  $r$  trees per clone, would have sampled the entire block, and should have data from most or all clones. The remaining plantation would then be as in Figure 1, center. In each row, pairs of trees would be 3 meters (one unit) apart, separated from the next pair by a distance of 6 meters with a stump in the middle. Tree  $A_{21}$  will have been released on three sides, as will every other tree in replications A and B. Analyses of characteristics later measured in the thinned plantation would have  $2r$  trees per clone per block.

As the trees get larger, the B (or A) replication can be cut, and a second set of destructive analyses performed in the lab. The remaining plantation would then be as in Figure 1, bottom. If no mortality has occurred, there would be exactly  $r$  trees of every clone randomly distributed throughout each block. The trees in each row would be 9 meters apart. If we were to walk down a row, we could observe the following sequence: starting from a tree, at 3 meters' distance a fresh stump; at 6 m an old stump; at 9 m a tree; at 12 m a fresh stump; etc. Tree  $A_{21}$  would be surrounded by six equally-spaced trees in the shape of a hexagon. It may be noted that the most important dimension is not the three-unit space between trees in a row, but the  $\sqrt{3}$ -unit distance between trees in a column and diagonally separating trees in adjacent rows.

If mortality has occurred, the laboratory analyses can still be balanced by replacing, say, a  $B_{21}$  that died with an  $A_{21}$ , maintaining a comparable history between the harvested B trees and the replacement  $A_{21}$ . Or, if it is desired to maintain balance in the plantation, a tree of clone 21 in the B replication may be left to replace a dead  $A_{21}$ , but in this option spacing will no longer be equal (Figure 1, bottom).

#### The New Zealand Design for Interlocking Replications

The New Zealand Forest Research Institute designed eight interlocked replicates in a rectangular scheme. Initial planting in a radiata pine experiment was at 9- by 9-foot spacing. Four of the eight replications were scheduled for removal in the first thinning, resulting in a uniform 12.7 by 12.7 spacing. The fifth and sixth replications were scheduled for removal in the second thinning, leaving a uniform 18-foot spacing. By offsetting tiers of the replicates, a third thinning may be obtained, with a 25.4 by 25.4 spacing between trees of the remaining eighth replicate.

Thus, instead of 2/3 of the trees being removed in two equal thinnings, 7/8 of the trees may be removed in three unequal thinnings, a schedule that is more realistic for many forest conditions. Details of installation, generally similar to those described below, are given in BANNISTER'S (1966) report.

au milieu: Tous les éléments de la répétition C ont été coupés. Chaque plant intérieur ( $A_{21}$  par exemple) est maintenant soumis à la compétition de 3 plants au hasard appartenant à la répétition homologue B situés à la distance 1 unité et est soumis aux effets de six plants appartenant à la répétition A situés à une distance  $\sqrt{3}$  unités; il est peut être aussi soumis aux effets de 3 autres plants de la répétition B situés à une distance de 2 unités. La surface moyenne occupée par chaque arbre restant est  $1,5 \times 0,866 \text{ unité}^2$ . en bas: Plantation à densité finale. La distance entre plants sur une ligne est de 3 unités; entre plants sur la perpendiculaire et sur la diagonale dans les lignes adjacentes elle est de  $\sqrt{3}$  unités.  $B_{21}$  indique un ramet du clone 21 dans la répétition B qui n'a pas été coupé. La surface moyenne occupée par chaque arbre restant (excepté  $B_{21}$ ) est  $(0,866) (\sqrt{3} \text{ unités})^2$  ou  $(3) (.866) (\text{unité})^2$ .

### Some Comments on Competition

A major component of a tree's environment is biotic. This consists, in part, of the effects of neighboring trees. With non-contiguous plots, most or all of the neighboring trees belong to different entries, and thus criticism (2), concerning competition, must be considered.

#### *Definitions and Background*

Competition, as strictly defined by BIRCH (1957), occurs when two or more organisms utilize common resources in short supply, or when they in some way cause harm to one another in utilizing an abundant resource. Under this definition, organisms that are most genetically similar will compete most severely with each other (HARPER *et al.*, 1961). Competition in this strict sense would be more severe within a contiguous plot of (say) the same clone, as contrasted to competition between neighboring members of different clones, which would be less similar in their requirements for resources and in their strategies for obtaining them.

BIRCH's definition of competition is important but not comprehensive. At issue in criticism (2) is an interaction in a statistical sense between the performance of trees when their neighbors are genetically similar, compared to their performance when most or all neighbors are genetically dissimilar (FLETCHER, HOWELL and FAULKNER, 1967). This can be viewed as a form of genotype-environment interaction, where the relevant environment is biotic (MATHER, 1955).

That such interactions exist has been amply shown in experiments with many organisms (see, for example, LEE, 1960). That serious mistakes can be made in generalizing the performance of a genotype or variety with genetically similar neighbors to its performance with genetically dissimilar neighbors may be clearly inferred from LEE's experiments.

It is intuitively reasonable that genotypes that are large and vigorous under competition-free conditions would be good competitors when grown with apparently smaller or weaker plants. This generalization seems to be violated about as often as it holds (see, for example, SAKAI, 1955; LEE, 1960; SKINNER, 1961; HELGASON and CHEBIB, 1963; FASOULAS, 1979).

In many cases, one plant's loss is a neighboring plant's gain (HANSON, BRIM and HINSON, 1961), resulting in a balancing minus-plus interaction. BIRCH's (1957) definition of competition implies a minus-minus interaction. If unlike genotypes draw on different subniches, they may make better use of the environment in mixture than in pure culture, giving a plus-plus interaction (see, for example, HARPER, 1968). It seems likely that all of these different forms of competition interaction exist, depending on the genetic composition of the population, on the particular environment of the experiment, and on the characteristics of interest.

An additional question remaining is what a realistic level of such competition interactions is for forest stands, and therefore for research relevant to such stands.

#### *Presence of Genetic Variability in Forests*

Nearly all quantitative genetic experiments include a degree of genetic heterogeneity. For those organisms that are genetically uniform, or for those used in agriculture as pure lines or varieties, the presence of genetic heterogeneity in experiments can be a complicating factor (HANSON, BRIM and HINSON 1961). Most forest-tree species are outcrossing and occupy a variety of ecological conditions. Thus, considerable genetic variation can be expected, both within and

between families and/or population-samples in experiments with forest trees.

Of the reasons for uniformity in agricultural crops, few have merit for forest trees. Where the principal product is wood, trees are "ripe" all year, and both human loggers and mechanical harvesters are capable of handling a considerable range of size and shape of logs and branches. But most important, uniformity with respect to susceptibility to drought, frost, insects or disease can prove disastrous in a forest. Thus, genetic heterogeneity is not only acceptable but desirable, both in forest plantations and in naturally-regenerated stands (RIGHTER, 1946; SNYDER, 1959; HEYBROEK, 1978).

Variance components in natural stands are interesting, but are difficult to study using techniques of quantitative genetics. Populations that do or will exist in plantations, or in genetically-improved plantations, seem both interesting and reasonable to study. The environmental conditions of such populations include the level of competition interactions associated with such plantations. A random arrangement of entries is probably the best way to achieve this level in such a study.

For selection experiments, a case can be made for systematic rather than random arrangement of neighboring genetically dissimilar entries, and schemes for doing this in a hexagonal spacing are available (FASOULAS, 1979). An appropriate level of competition interactions in selection experiments seems difficult to achieve. The problem is that some genotypes may be selected in part due to their ability to take advantage of their environments at the expense of less competitive neighbors. When such selections are then grown together in production plantations, their competitive advantage over their similarly-selected neighbors may have been reduced. However, it seems unlikely that any will then actually fail due to the increase in level of competition. It does appear that selecting trees at low levels of interaction between dissimilar genotypes (for instance, in large contiguous plots of full-sib families), and then releasing the selections for use in genetically heterogeneous plantations, is courting more serious error.

#### *Two-stage Experiments*

Trees of different provenances don't usually grow together in nature, and it is not common practice to mix them in production plantations. It thus seems reasonable that provenance tests be designed with large contiguous plots, minimizing between-provenance competition interactions. Within these large plots, it seems most reasonable to simulate the conditions in a regular plantation, as described above. This involves considerable expenditure of land and seed to evaluate the relative performances of provenances effectively (WRIGHT and FREELAND, 1960). An alternative, used to reduce this expense in crop plants, is to employ a two-stage testing program. Obvious misfits are eliminated in a first-stage test, where a limited number of each entry are, by necessity, grown in intimate competition with each other. The second stage tests only the more promising entries in large contiguous plots. Such a two-stage scheme has been proposed for use with forest trees by SHIUE and PAULEY (1961), with examples and possible exceptions discussed.

#### *Effects of Unplanned Mortality*

Mortality, or some event that disqualifies surviving trees from further meaningful analyses, frequently occurs locally. As examples, we have observed this in a test site containing a droughty area; where a small fire burned a section of a

test; where a freeway was put through a corner of an experiment; and where rabbits clipped trees near cover. In all of these, entire contiguous plots were affected, including most or all members of the families in those plots. A pipeline put through two white fir experiments, laid out in the California interlocking non-contiguous plot design, resulted in little loss of information and no loss of entries from any blocks, although many of the trees were removed or killed.

If members of a few clones (or families, or provenances) are selectively killed or damaged by some event in a random (or systematic) non-contiguous plot design, the interpretation of selective damage can be much more certain than if the damage is concentrated in a few of the contiguous plots containing those entries.

Mortality can also occur at low frequency in a scattered pattern, throughout an experiment. When a tree dies, this normally gives some spacing advantage to its surviving neighbors. With non-contiguous plots, this spacing advantage is bestowed randomly on the clones of families in the experiment. However, in a contiguous-plot design, this spacing advantage is mostly bestowed on other members of the plot, *ie* on other members of the same clone or family. Thus, the performance of the survivors in mortality-prone clones or families will probably be improved, compared to families with the added benefit of good general survival. This could lead to significant errors in selection experiments.

If, during thinning, an uncut replication is balanced by leaving members of a replication being cut, the replacement members do suffer unusual competition ( $B_{21}$ , *Figure 1*, bottom). Whether this is an appropriate penalty for members of mortality-prone entries can be debated. Their competitive effect on their neighbors, however, is imposed at random.

#### *Relationship to a Demonstration Border*

If a tree is assigned its position at random, its position relative to a tree not assigned a position at random is still random. This allows one to treat border rows surrounding an experiment as a useful non-random demonstration planting, without compromising the random nature of the experiment. Several trees of each family (or population, or clone) may be planted in small contiguous plots in the border, and the plots ordered in some sensible way, for instance by location and elevation of origin.

#### **Some Experience to Date**

The interlocking-replication principle, including random non-contiguous plots, has been employed with forest trees in several projects: among the oldest are 1965 and 1966 plantings with 16 blocks in two white fir experiments; in 1965 with several blocks of a redwood provenance study (personal communication, Prof. Jack HANSON, Humboldt State College, Arcata, California); and in a large study of radiata pine, with the first two blocks planted in 1964 (BANNISTER, 1966). An interlocking scheme using single-tree plots had earlier been used in a peach variety trial (PEARCE, 1953, sec.36 . . . sec. 37 in the 1976 edition). A hexagonal design, not interlocked for thinning but sometimes using non-contiguous plots, has been explored for early selection in wheat (FASOULAS, 1973, 1979).

Objection (1) to single-tree plots concerns logistics of planting. The Institute of Forest Genetics (Placerville) and School of Forestry (Berkeley) proceeded as follows in installing 8 blocks of a white fir experiment.

#### *Preparation of Plants at the Nursery*

Seedlings were raised in labeled rows in the nursery, with each open-pollinated family in four random rows. Four locations ( $r$ ) per entry were allocated to each replication, *ie* each block contained  $3r = 12 = p$  locations for each family. However, in order to further reduce early mortality difficulty, two seedlings were assigned to each location. Prior to lifting, a piece of masking tape was attached to each seedling scheduled for lifting. The identifying family letter and number had first been written on the tapes with a laundry pen. Without exception, this identification was clearly readable for two months. The masking tape loosens and rots as the tree grows, so it need not be removed to prevent binding. (Prof. J. W. WRIGHT first brought the masking tape and laundry pen technique to our attention.)

Pairs of seedlings were lifted, one pair per nursery row, checked for identity of marking, and taped together. Thus, four such pairs from each family were placed in a single box, in the random sequence of the rows in the nursery. This procedure was followed three times for each of the eight blocks. The pairs of seedlings were then physically randomized again, in an independent procedure for each box. The rerandomization was just a mixing of the seedling pairs, done indoors under mist to minimize root damage. Each box was labeled as a particular replication in a particular block, and stored until planting.

As an example of an alternative procedure, a radiata pine clonal experiment using rooted cuttings grown in individual containers was prepared as follows. Each non-contiguous plot in each replication was assigned one location in each sub-block, with 270 clones per sub-block. One index card was prepared with a clone identification number for each clone. This 270-card deck was then thoroughly shuffled. Each nursery worker would, in turn, pick off the top card and walk to the (ordered) flat with containers of that clone. Using strong rubber bands, two containers would be fastened together, and the pair of ramets would then be placed in a flat being prepared for the "A" replication of sub-block I. After these flats were loaded with 270 clonal pairs, the card deck was reshuffled and the procedure repeated for sub-block II. This allowed 2 ramets of each clone to be planted at one random location within each of the sub-blocks of replication "A". The same procedure was then followed for replications "B" and "C".

The labeling and randomization at the nursery is the major added cost associated with non-contiguous plots.

#### *Installation at the Planting Site*

At each white fir plantation site, border locations were marked by small plastic flags with family identifications on the flag, while interior locations were marked by red, white and blue plastic pot-labels, designating the "A", "B" and "C" replications, respectively.

The red-replication box was opened first, and the planting crew instructed to take an unselected group of seedlings and plant them, without sorting, at the red pot-labels only. Each planter was assigned a column of red labels. (Note in *Figure 1* that each column consists of members of a single replication. Planting columns required more walking than planting rows, but it reduced possible lapses on the part of the planting crew.) Holes were made about 10 cm east and west of the pot-label, the (unmarked) tape holding a pair of sibs together was easily torn, and the still-labeled sibs were planted 20 cm apart.

After all trees from the red-replication box had been planted, the plantation was quickly checked to see that

seedlings were planted next to all red labels. The white-replication box was then opened and the procedure repeated for the white columns. The blue replication was then similarly planted. Then the families were planted in small contiguous plots in the border.

The final operation was mapping and indexing every planted location as to tree identification, replication, row and column. The immediate compilation of an index is essential to accurate use of this technique. Mapping and indexing was accomplished by three people in about the same amount of time needed for six people to plant one interior replication. Upon completion of the map and index, errors of allocation, planting or mapping were immediately apparent by less-than or more-than 4 plants of a family ( $r$ ) listed per replication. The suspect locations were visited in a few minutes, and if necessary, the newly-planted trees were replaced or replanted in the proper replications.

It was repeatedly found that staking the interior locations with the three colors of pot labels was only slightly slower than staking a similar number of locations with a single type of marker. By comparison, staking the border rows and columns with marked flags took about twice as long per stake as did staking the interior locations with the color-coded but unmarked pot-labels. Similarly, planting the single-color columns went about as fast as planting a production plantation by hand. In the border, even when the entries were reasonably ordered in the box, the planting rate to pre-assigned positions was about half the rate for the randomized interior replications. HANSON (personal communication) reported similar relative efficiencies when installing his redwood tests.

Thus, objection (1) seems to be largely overcome by tagging the seedlings prior to lifting, assigning the seedlings to replications at lifting, physically randomizing at the nursery, color-coding replication locations in the field, planting the replications in sequence, and mapping and indexing immediately upon completion of planting.

Early mortality has not been entirely independent of location. For example, in one experiment with 20 % early mortality, 4% of the double-planted locations might be expected to have had both trees die, while in fact both died at about 6% of the locations. Double-planting has proven to provide substantial benefit, frequently resulting in a low rather than a moderate number of locations with no surviving tree.

#### Thinning

The first thinning occurred two or three years after planting. Where both planted sibs had survived at a location, one was cut before they came into significant competition. A coin was tossed to determine whether the eastern or western tree of all the pairs in a block would be cut.

Thinnings removing interlocked replications have now been completed in five California experiments, and in the radiata pine experiment in New Zealand. The thinnings have been conducted by industry crews, by research staffs, and by students. In none of these six thinnings has a tree from the wrong replication been cut by mistake, or a tree left when it should have been cut. Note in *Figure 1* (center) that diagonals to be thinned are apparent. They were accurately marked and followed by the thinning crews. None of these crews had previous experience with similar designs before conducting the thinning operations. Reasonable, but not excessive, supervision was provided.

#### Analyses

Analyses of data generated by these experiments have been conducted without unusual difficulty. A few examples follow.

A radiata pine experiment, planted in California in 1970, included 3 provenances, each with 10 sampled stands, each with 9 clones. These 270 clones were arranged in 3 interlocked replications of 1-ramet sub-blocks, combined at analysis into 3-ramet and 9-ramet non-contiguous plots. The 2430 locations in the experiment, plus a 3-row border, occupied only 2+ hectares. Calculated theoretical efficiencies were high, and the experiment is working well in practice.

Periodic analyses of such attributes as height, stem diameter, stem volume and crown diameter (personal communication 8 Feb 1980, M. GUINON, U. Calif. Forest Products Lab.) indicate between-clone components accounting for about 9–15% of total variance, statistically highly significant for all analysed attributes. The between-stand components vary from 0.5–2.0% of total variance, depending on the attribute analysed. Those between 1.4–1.9% of total variance are significant at the .05 level, and the one accounting for 2.0% is significant at the .01 level. The provenance component varies from 0–5% of total variance. Provenance components accounting for from 1.2–1.5% are significant at the .05 level; those from 2–5 % at the .01 level.

The red ("A") replication, containing the most mortality, was cut in 1979. Of the 810 locations planted, 666 provided trees that could be analysed for corewood specific gravity. There were no missing plots, i.e. all 270 clones had at least one analysable tree from the three planted locations in the "A" replication. The two provenances with denser wood had statistically significantly greater (3.2% and 2.5% greater) corewood specific gravity than the third provenance, but they were not significantly different from each other.

Data from the older white fir experiments are consistent with these results. Various genetic components accounting for from 1.5–4.0% of total variation are statistically significant, while those accounting for greater than 3–5% are statistically highly significant. Each block consisted of fewer than 400 trees, and occupied about 0.4 hectare.

Thus, with relatively modest expenditures of trees, land, and effort, the design has proved to be statistically sensitive to genetic differences accounting for relatively small percentages of total variation.

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## The Genetic Structure and Levels of Inbreeding in a *Pinus radiata* D. Don Seed Orchard

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### Summary

Thirty clones of a seed orchard of *Pinus radiata* were assayed to determine their genotypes at 22 allozyme loci: 12 loci were monomorphic and 10 polymorphic. The average number of alleles per locus (A) was 1.54 and the estimate of total genetic diversity (H) for the seed orchard 0.108. In three annual seed crops of the orchard the allelic frequencies at eight polymorphic loci, both in the pollen and maternal components of the progeny, were in good agreement with the frequencies in the thirty parental clones. From the progeny arrays in the crops the overall rate of outcrossing in the orchard was at least 90%. There were no significant differences between the rates of outcrossing in the three individual annual crops.

*Key words:* *Pinus radiata* D. DON, seed orchards, allozyme variation, genetic diversity, inbreeding levels.

### Zusammenfassung

Dreißig verschiedene Klone aus einer Samenplantage von *Pinus radiata* wurden im Hinblick auf ihren Genotyp an 22 Allozymloci untersucht, wovon 12 Loci monomorph und 10 polymorph waren. Die durchschnittliche Anzahl der Allele pro Locus (A) betrug 1,54, die geschätzte genetische Gesamtvariation (H) für die Samenplantage 0,108. In den Ernten dreier verschiedener Jahre stimmten die Allelfrequenzen von acht polymorphen Loci sowohl beim Pollen als auch bei den mütterlichen Komponenten der

Nachkommenschaften gut mit den Häufigkeiten in den 30 elterlichen Klonen überein.

Nach den Herkunftsreihen zu schließen, kann die Fremdbefruchtungsrate in der Plantage auf mindestens 90% geschätzt werden.

Es ergaben sich keine signifikanten Unterschiede in den Fremdbefruchtungsraten zwischen den Erntejahren.

### Introduction

An essential requirement for evaluating the status of the genetic resources of forest tree species under various strategies of domestication is the estimation of the genetic variability in populations within tree breeding programs. The better-performing clones in tree improvement programs are usually grown in seed orchards with the aim of producing superior seed for commercial plantations. The measurement of genetic variability in seed orchards would therefore seem a desirable first step in the monitoring of the genetic variability in current and future plantations of tree species. Such estimates would also enable comparisons to be made of the levels of genetic diversity between natural and planted populations of a species. The isozyme technique is the best currently available method for measuring genetic variation (BROWN and MORAN 1980).

Breeding programs commonly used for pine species assume random mating. In particular, in the case of clonal seed orchards the premise is that natural wind-pollination will give virtually complete outcrossing. Progeny tests