

Design for a Gene Recombination Orchard

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Summary

A gene recombination orchard attempts to maximize the spontaneous mating among all clones of interest in a breeding population. An algorithm enables designs for such an orchard to be completed quickly and efficiently.

Key words: gene recombination, mass test cross, polycross, seed orchard.

Zusammenfassung

Es wird versucht, in einer Gen-Rekombinationsplantage die spontan auftretenden Kreuzungen zwischen allen interessanten Klonen einer Züchtungspopulation zu maximieren. Ein Algorithmus ermöglicht es, die Pläne für eine solche Plantage schnell zu vervollständigen und effizient zu machen.

Introduction

In a continuing tree breeding program, it may be desirable to achieve crosses* between all the clones of interest in the breeding population. Where the breeding population consists of more than a few trees, this may be an impossible task, particularly if maintenance of full pedigree is required. To illustrate the complexity of such a breeding programme, consider a population of 300 selected clones, each worthy of inclusion in a continuing breeding program.

The simplest minimal crossing scheme is single-pair crossing which would require 150 matings in order to involve each of the 300 parents in one cross. The most complex maximal crossing scheme would be half diallel mating without selfing, which requires $n(n-1)/2$ or 44 850 crosses. Many variations of schemes intermediate in complexity exist. One scheme currently popular among tree breeders (NIKLES, pers comm) is series of six-tree diallels, which would require 750 crosses. Such schemes are beyond the resources of many tree breeding organizations (NIKLES, pers comm).

Where the full pedigree is not required, more economical schemes become possible. Polycross testing normally requires the artificial pollination of each clone by a balanced mixture of pollen from all other clones (BURDON and SHELBOURNE 1971). However, this too becomes increasingly complex when as many as 300 clones are involved.

Schemes which allow such mating to occur spontaneously by wind pollination have been called 'mass test crosses' by WELLENSIEK (1947) and 'polycross designs' by OLESEN and OLESEN (1973). GIERTYCH (1975) suggested that the term 'polycross' should be reserved for artificial hybridisation studies, and that other terms should be used for spontaneous mating schemes. WELLENSIEK's term 'mass test crossing' has not been widely adopted, and BARNES and NIKLES (pers. comm) have suggested the term 'gene recombination orchard' (GRO). It is not intended that a GRO should spontaneously produce as many matings as a half diallel scheme. If a GRO containing 300 clones resulted in progeny from only 20 000 of the possible 44 850 matings, it would still make a valuable contribution to the tree breeding program.

* In this paper, crossing is defined to be artificial pollination, and mating to be spontaneous natural pollination.

Designs for such mating schemes are reputed to be difficult to compute, to require large numbers of trees, and to yield unsatisfactory results (eg DYSON and FREEMAN 1968, FRANSDEN 1969). However, OLESEN and OLESEN (1973) have developed a formula for a latin square design which will ensure that each clone will have every other clone as a neighbour exactly once in each of the four directions (N, E, S, W). Their design is convenient for small numbers of clones but, as more clones are included, the numbers of trees required becomes unmanageable.

A shortcoming of OLESEN and OLESEN's design is the failure to consider diagonal (NE, SE, SW, NW) relationships which may have a high probability of mating. In the design presented in their paper, many of the diagonal relationships are frequently repeated (eg 3—4 occurs four times on diagonals, as well as once in each of the primary directions), and an unacceptable degree of selfing may be introduced by the large number of identical clones adjacent on diagonals (these occur 18 times).

BELL and FLETCHER (1978) have produced a computer program, COOL, which for a given area, will create a permuted neighbourhood seed orchard design of desirable characteristics. COOL finds for this area, a design which incorporates a specified number of ramets for each clone. No attempt is made to ensure that each clone is adjacent to every other. In contrast, this study aims at developing a computer program which for a given number of clones, will find the design which requires the minimum possible number of trees to ensure that each clone is adjacent to every other clone least once. It is essentially an extension of the work of LA BASTIDE (1967).

Method

Define a link as the relationship between a ramet (or cutting, etc) of any clone and each of its immediate neighbours. In a mass test cross of N clones, each clone must have links with every other clone. Thus we need $(N-1) + (N-2) + \dots + 1 = N(N-1)/2$ links, assuming that none are repeated.

The number of trees required is reduced with a hexagonal arrangement allowing links in six directions, and by requiring links between any pair of clones in only one of these directions.

Figure 1 illustrates how hexagonal arrangement of the design increases the distances between positions not considered adjacent, and may thus decrease the probability of unintended matings occurring (BRAMLETT 1981). The square design fails to consider clones 1.4 times the primary distance from any clone, whereas a hexagonal arrangement increases this distance to 1.7. This arrangement may also encourage more uniform crown development (LANGNER 1953, LANGNER and STERN 1955).

The most efficient design is obtained using a hexagonal shape which minimizes the perimeter with respect to the area. The numbers of trees and links present in hexagonal designs of various sizes are indicated in Table 1.

To find the theoretical minimum size of hexagon which contains the $N(N-1)/2$ links required for N clones, put

$$9r^2 + 3r = N(N-1)/2$$

$$r = (\sqrt{2N^2 - 2N + 1} - 1)/6 \quad (1)$$

$$\lim_N r = 0.24N$$

where r is the radius of the hexagon, or equivalently, the length of the side of the hexagon.

The total number of trees required is

$$t = 3r^2 + 3r + 1$$

$$= (N^2 - N + 4 - 2\sqrt{2N^2 - 2N + 1})/6 \quad (2)$$

$$\lim_N t = 0.17N^2$$

This analysis yields an estimate for the minimum number of trees which could fulfill the requirements, assuming that no links are repeated. However, it is not clear whether it is possible to compile, for any given number of clones, a design in which no links are repeated. LA BASTIDE'S (1967) designs for seed orchards avoiding such repetition required considerable computation. Empirical analysis suggests that for certain values of N , some repetition may be unavoidable.

A computer program has been developed to create a design with a hexagonal arrangement which completes all the required links with minimal repetition. The algorithm is given below, and the FORTRAN program is available from the author.

Algorithm

Number the clones sequentially from 1 to N . Consider each position in the design in turn, commencing at the centre, and working outwards in a spiral, and select a clone to fill the position according to the following criteria

1. For the first N positions, choose any clone not yet represented in the design.
2. For the $N + 1$ and subsequent positions, choose a clone which
 - a. is not identical to any of the adjacent clones;
 - b. will result in the greatest number of new links being created;
 - c. if no links can be created through the selection of a clone for this position, choose a clone for the next position first, and then select a clone for this position using the same criteria;

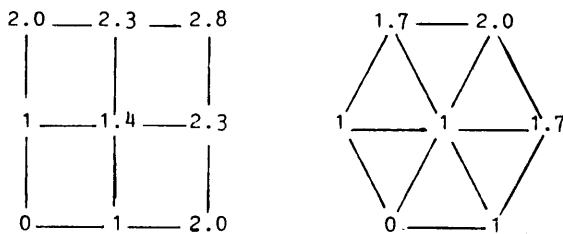


Figure 1. — Distances between Positions.

- 0 = Position under consideration
- 1 = Adjacent position at unit distance
- . = Position considered not adjacent

Table 1. — Characteristics of Hexagonal Design.

Radius of Hexagon	Number of Positions	Number of Links
0	1	0
1	7	12
2	19	42
3	37	90
r	$3r^2 + 3r + 1$	$9r^2 + 3r$

Table 2. — Results from the Program.

No of Clones	Radius of Hexagon	Total Trees	Theoretical Minimum (eqn.2)
10	3	25	21
20	6	93	74
30	8	205	160
40	11	342	280
50	13	522	433
70	18	988	839
100	26	1960	1698
150	38	4328	3797
200	50	7572	6729
250	62	11687	10494

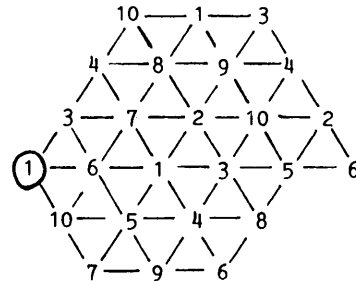


Figure 2. — Design for 10 Clones.

Table 3. — Analysis of 10 Clone Design.

Clone	Number of Links between Clones										No of Ramets
	2	3	4	5	6	7	8	9	10		
1	1	3	1	1	2	1	1	1	2	3	
2	0	1	1	1	1	1	1	1	2	2	
3	.	0	3	1	1	1	1	1	1	3	
4	.	.	0	1	1	1	2	2	2	3	
5	.	.	.	0	2	1	1	1	2	2	
6	0	1	1	1	1	3	
7	0	1	1	1	2	
8	0	1	1	2	
9	0	1	2	
10	0	3	

- d. if more than one clone satisfies (b), choose from these the clone which has fewest links already completed;
- e. if more than one clone satisfies (d), choose the one which has already been included in the design least frequently;
- f. if more than one clone satisfies (e), choose the one with fewest duplicate links;
- g. and if (f) fails to isolate a single clone, any of these may be selected.

3. Repeat 2 until all the required links are completed.

Results

Table 2 indicates some results produced by the program. The algorithm attempts to include the same number of ramets from each clone and to avoid duplication of links. In practice, the number of ramets from each clone may vary by one, and some links may be repeated. The design for 10 clones is given in Figure 2. It can be seen (Table 3) that few links are repeated, and that all clones are represented an (approximately) equal number of times.

The encircled ramet in Figure 2 is redundant, and may be removed from the design to reduce the repetition of links. As such redundancy occurs infrequently in the designs produced, no checks for this are carried out by the program, so as to keep the program simple.

Application to Forest Tree Breeding

The GRO attempts to attain the maximum number of notional crosses among all clones of interest in a breeding population. This can also be achieved through half-diallel mating or polycross schemes, but provided the identity of the pollen parent is not required, the GRO is an economical alternative.

The design takes no account of the direction of the link between neighbours, and may prove unsatisfactory where steady prevailing winds occur during pollination. More serious problems concern practicalities such as location (BROWN and ELDRIDGE 1983), flowering, cone and pollen production (GRIFFIN 1982, KOSKI 1975, SWEET 1975), fertility, self-incompatibility and seed viability (GIERTYCH 1975, SQUILLACE and GODDARD 1982), growth rate, crown size and shape (GIERTYCH 1975), isolation (DENISON and FRANKLIN 1975, GRIFFIN 1980, KOSKI 1975, WERNER 1975), and pest control (MILLER 1983). Unless these and other practical problems are satisfied, it is unlikely that GROs will be useful.

The algorithm may also be applied with success to orchards with an irregular or non-hexagonal shape, but a greater number of trees will be required to complete the necessary links.

Conclusion

This paper has demonstrated that satisfactory designs for gene recombination orchards can be computed without

difficulty. However, the utility of these designs may be limited by practical problems.

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Isoenzymatic Studies of Alcohol Dehydrogenase and Glutamate Oxalacetate Transaminase in four South American Species of *Prosopis* and their Natural Hybrids

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Abstract

The diploid species *Prosopis ruscifolia*, *P. alba*, *P. hassleri* and *P. vinavillo* have two genes coding for the dimeric enzyme alcohol dehydrogenase. Each gene specifies a poly-

peptide with different migration mobilities, and the species have variants resulting from non allelic interaction.

The locus Adh-2 is monomorphic and has a single allele present in *P. alba*, *P. ruscifolia* and *P. hassleri*. The locus