LATTICE AND COMPACT FAMILY BLOCK DESIGNS IN FOREST GENETICS

by E. Bayne Snyder'

One of the principles of experimental design is that replicates be relatively homogeneous. Thus, in forest research a replicate is often assigned to a single crew for planting in a single day on a uniform site. When treatments are numerous, a large area is required per replication, and homogeneity of site is difficult to achieve. In this situation, crop scientists (LeClerg *et al.* 1962) frequently divide the replicate into sub-blocks. The most used of the incomplete block designs are the lattices. Another type of incomplete block designs, the compact family block (Hutchinson and Panse 1937; Federer 1955) — essentially a split-plot design with genetic rather than cultural whole plots — has also been advocated for certain genetic materials.

Such designs are frequently used in forestry abroad, e.g., Jeffers (1959), Langner (1961), and Schober (1961). This paper reports results from 16 current experiments in the United States. It also reviews literature dealing with specific designs and with "efficiency" as a means of comparing them. Many tree breeding programs are entering an era of comparing numerous genotypes, and it is hoped that this presentation will aid in choosing among designs.

"Efficiency" for Design Evaluation

Essentially, efficiency is the ratio, in percent, of the error variance of the randomized block design to the error variance of the design being compared. Various texts show how to calculate average effective error variances and efficiencies for lattice designs. In this presentation, it has been assumed that the within-block efficiency of the compact family block may be calculated as detailed by Federer (1955) for split plots.

Relative efficiency is often used to indicate how much saving in cost and land can be envisaged from a design. Thus, if an incomplete design had six replications and an efficiency of 150 per cent, nine replications of a randomized block would be needed for equal accuracy. Day and Austin (1939) estimated that a 729-entry cubic lattice had an efficiency of 205 percent for ponderosa pine germination time. Beard (1954) calculated that, for wattle tree bark yield, à 10 by 10 lattice had an efficiency of 329 percent. Johnsson (1963) found a 5 by 6 rectangular lattice to have an efficiency of 133 percent for the heights of 10-yearold Scotch pine. Lester and Barr (1965), in a series of provenance tests for heights of 9- to 11-year-old red pine trees in rectangular lattices with replica tion sizes of approximately 1 to 2 acres, found that efficiencies varied according to planting site: 172, 179, 110, 105, 102, and 101 percent.

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Description of Designs

Limitation of this presentation to the two types of design does not imply that others are of no value. For example, the new augmented incomplete block designs (Federer 1961; Corsten 1962) should be useful where some treatments are to be more highly replicated than others. Even the discussion of the two designs is necessarily limited, so that full understanding of their principles and methodology will require additional study by the reader.

Compact family blocks — The compact family block is similar to a standard split-plot design in that comparisons among subplots are more accurate than those among whole plots. It is advantageous where genotypes divide themselves naturally into groups within which variation is smaller than between groups — say a provenance test where the progenies from 10 trees are collected per source and planted in a single sub-block (family block). Since there are no block adjustments as for lattices, comparison of entries in different sub-blocks is less precise than with a lattice. If there are sufficient whole-plot treatments, a lattice should be imposed on the whole plots.

The analysis, somewhat different from that for an ordinary split plot, is outlined by Panse and Sukhatme (1954). An equal number of subplots per family is not required, though it is desirable, and entries may be dropped from the experiment without complicating the analysis. Federer (1955) gives formulas for standard error. Cochran and Cox (1957) and Cockerham (1963) offer general discussion, and Johnsson (1952) reports a way of buffering against interfamily competition. John (1963) and Gates and Wilcox (1965) show how these designs may be used to obtain the genetic variance components of general and specific combining abilities and reciprocal effects.

Lattices — The array of lattices shown below allows for selection of a design over a wide range of treatments and replications. The size of the subblock is designated as k. Thus, a 6 by 6 lattice has six sub-blocks per replication, each containing six entries. Another point shown is the increased replication required to achieve balance. Balance permits all comparisons at equal accuracy. When two designs are possible, the more balanced is preferred, but this is not to say that the investigator must adopt a balanced design when a partially balanced one fits his needs. Schutz and Cockerham (1962) suggest that an overall balanced design be used where the combination of locations and replications per location equals the required total number of replications for balance.

Design	Minimum number of replications				
k ² entries					
Simple lattice (partially h		2			
Triple lattice (partially b		3			
Quadruple lattice (partially	y balanced)	4			
•					
Balanced lattice	k	+ 1			
k (k + 1) entries					
Rectangular lattice (partial	lly balanced)	2			
		12			
and the second second second					
Near balanced lattices k ³ entries		k			
Cubic lattice (partially ba	lanced)	3			
	1				
		*			
Balanced lattice	k² -	$+\dot{k}+1$			

Lattice Design Problems and Their Amelioration

Additional stratification within replicates necessitates more work and complications than are encountered for randomized complete block designs. The disadvantages must be weighed against the gains and minimized to the extent possible. Five alleged disadvantages are discussed below.

1. Lattice designs take more randomizations and bookkeeping at the planning stage than randomized blocks. First, to attain the proper number of entries for a design, the investigator may need to modify his original number. Then there is the chore of randomization. Clem and Federer (1950) supply random arrangements and Carmer (1965) furnishes a Fortran program for randomizations by an IBM 7094. Thompson (1958) shows how seed envelopes can be run through IBM accounting machines to be labeled. The advent of computers and automatic data processing equipment is thus minimizing the drudgery of planning and layout.

2. Wright (1962) claims that "... with sophisticated designs, it is necessary to search for the correct bundle to match the plot to be planted. This results in exposure of seedling roots to the air and increases variability." It is true that entries must be assigned and bundled by block within replication. This can be done at the time of packaging. Also, the field must be staked by block, but it is not necessary to number the blocks or the order of the plots within them, since both are at random. Thus, the bundle numbers that match field plot numbers may be recorded after planting just as with a randomized complete block design. Depending on the experiment, I often prefer to keep track of plot assignments from the time of seed packeting.

3. According to Cochran and Cox (1957), hand calculations of the lattices may exceed those for randomized blocks by 20 to 150 percent. However, various developments ease the difficulty. Color coding of the III — or Z-blocks — in the treatment totals of some designs speeds up adjustment procedures. Staude (1963) supplies precalculated factors necessary for Duncan's Multiple Range Test. Carmer et al. (1963) supply automatic data processing methods for these designs. Homeyer et al. (1947) advocate that computers be used for 150 or more plots, or for a lesser total number if more than one character is being evaluated. In any event, the rapid development in computer technology has virtually eliminated the difference in calculation time.

4. The "insecurity" which condemns the lattices according to Evans *et al.* (1961) means, I assume, complications caused by missing plots. It is true that perennials grown in the rough are far more subject to losses than intensively cultivated annual crops. It is also true that missing-plot formulas are more complicated and hence more timeconsuming for lattices than for randomized blocks.

However, these designs can be analyzed as randomized blocks and therefore are never appreciably less accurate than randomized blocks. The experimenter may choose whether to analyze them as randomized blocks or to complete the full analysis after data have been collected. Indeed, even without missing plots, the experimenter usually reverts to randomized block analysis if the efficiency is less than 105 or 110 percent. Cochran and Cox (1957) state that if there is any criterion for forming incomplete blocks, such a design is worth a trial in preference to a randomized block design.

I prefer to proceed with the full analysis in spite of missing plots. Missing-plot formulas are available in texts for most designs; Healy (1952) gives the procedure when an entire variety is missing. I have no information as to the percentage of missing plots that makes either randomized block or incomplete block analysis inadvisable. With experiments having few treatments, missing plots cause biases partly because of appreciable decreases in degrees of freedom, but where lattice designs are employed for 25 or more treatments, as is generally true, this effect is small. Healy and Westmacott (1956) and Yates (1960) have worked out computer analysis-of-variance methods for experiments with missing plots. The analyses of variances include lattices and are said to be suitable even if 10 percent or more of the plots are missing. I have a similar IBM 7094 program in Fortran for randomized blocks. In large experiments with missing values too numerous for hand calculations, my program will estimate missing-plot values for randomized blocks; these can be used in the lattice analysis (Goulden 1952). It is only a matter of time until improved computer programs are available.

5. The propriety of lattices for selection and studies of genetic variance components has been questioned. The subject was partially investigated by Schultz and Cockerhan (1962). They found that optimizing efficiency based on average error variances also optimizes efficiency based on expected gains. This was not true for some other types of incomplete block designs where additional computations are necessary to optimize gain. They also said that genotype-by-environment interactions caused by testing at different locations and years should not affect the superiority of any of the designs, since the interactions affect each of the expected gains in a similar fashion. Their study indicates that lattices are appropriate for selection studies or mean separation studies such as those for estimating combining abilities or testing provenances.

They further stated that gain from selection is not the only consideration, since simultaneous estimation of genetic variances is often desired, and that blocking may be desirable since it permits distribution of degrees of freedom more evenly among the mean squares as well as reduction in the error variances.

In further discussing genetic variances, Cockerham (1963) stated:

Increase in land area generally increases the environmental variance because of soil heterogeneity which also reduces the reliability of components of variance per unit of land area. A solution ... is ... to use incomplete blocks. Other features, however, such as the distribution of degrees of freedom . . . are more pertinent to the reliability of the components of variance . . . of the joint mating-environment design.... One cannot accomplish anything by indiscriminately throwing the progenies into just any incomplete block environmental design which may fit. Care must be taken that the incomplete block design allows one to estimate the desired components of variance unconfounded with environmental components of the design.

Lester and Barr (1965) show that, for a series of provenance plantings, ordinary mean squares analysis is suitable. However, the breeder should consult a quantitative geneticist before he tests structured genetic materials. Namkoong and Stern, while commending incomplete block designs for obtaining genetic components, have cautioned me that least squares computer analysis may be necessary to obtain "clean" components.

Standard Controls as Alternatives to Incomplete Blocks

Evans *et al.* (1961) proposed a series of smaller randomized block tests with standard controls in preference to incomplete blocks. The design was used by Cech *et al.* (1963) in a study whose primary goal was to estimate components of variance. For varietal testing, however, Cochran and Cox (1957) and Schutz and Cockerham (1962) found that the control system is theoretically likely to be inferior in accuracy to a comparable incomplete design. Wishart and Sanders (1955), from results with crop plants, stated: "... but [the use of standard controls] has not proved to be a very satisfactory arrangement, even where it is arranged to have the same standard variety represented in all the experiments."

Results from Experiments in the United States

The efficiencies of the 10 nursery or greenhouse experiments in table 1 vary from 100 to 274 percent for the lattices and from 92 to 163 percent for the compact family blocks. In general, the designs were not efficient for plants up to 3 weeks old and for characters such as Melampsora rust or fiber length. These limited results indicate that such characters are not affected by site heterogeneity. If they are the only characters studied, incomplete design would not be warranted. The designs were efficient for heights and weights of tor 2-year-old seedlings. In experiment 6, a chlorotic stunting occurred in part of one replication. The lattice design enabled use of all the data; otherwise, it would have been necessary to abandon this replication. If 110 percent is arbitrarily set as the efficiency required before a design is worthwhile, incomplete block designs were justifiable for one or more characters in 7 of the 10 nursery experiments.

The efficiencies of the seven field experiments in table 2 vary from 100 to 152 percent for the lattices and from 114 to 275 percent for the compact family blocks. Experiment 11, where the lattice was efficient, was on rolling land. Experiment 12, where efficiency was not attained, was with the same nursery stock but on a level crayfish flat. Two of the three experiments (11, 12, 13) showed no increased efficiency for fusiform rust data. The brown spot disease often infects fields irregularly for the first 2 years, and in experiment 15 the use of the lattice during this time was helpful. By the third year, inoculum had built up sufficiently to provide uniform infection, so that the lattice was ineffective.

Experiments 11 and 13 provide conflicting evidence as to whether incomplete blocks become more efficient as the trees grow older. In 11, efficiency for heights decreased from 138 percent at 3 years to 113 at 7 years, and in 13 it increased from 139 percent at 2 years to 275 at 5 years. It is suspected the two sites differ in the relative homogeneity of surface and subsurface conditions. Thus, in the field, incomplete blocks were valuable for one or more characters, i.e, had efficiencies of at least 110 percent in five of the seven experiments.

These results are confirmed by K. Stern (personal correspondence). He stated that more than 50 experiments at Schmalenbeck, West Germany, utilize incomplete block designs and that these designs are usually more efficient than randomized blocks.

Summary

Testing numerous treatments often requires large replications on heterogeneous sites. Subdividing replications into smaller, more homogeneous incomplete blocks results in more precise comparisons. The compact family block (split-plot) is appropriate where genotypes divide themselves naturally into groups and where the within-group differences are smaller or of more interest than those between groups. Provenance studies with individual parents kept separate are examples. Lattices are appropriate when testing many genotypes whose differences are unknown or are of equal size or interest — for example, in the estimation of genetic gains, combining abilities, and components of variance.

Lattice layout and analyses may be speeded in various ways. Newly developed computer programs simplify and minimize the time of operations at all stages. Computers also minimize missing-plot problems, or the researcher can analyze lattices with missing plots as if they were randomized blocks. Therefore, whenever a large number of treatments necessitates large replication size, an incomplete block design is worth a trial.

Of 10 incomplete-block nursery or greenhouse experiments analyzed, 7 had efficiencies exceeding 110 percent in one or more characters, the range being 92 to 274 percent. The designs were efficient for heights and weights of 1- or 2-year-old seedlings. They were generally not efficient for nursery data up to 3 weeks, nor for such characters as fiber length or *Melampsora rust*. In the field, five of seven experiments were efficient, the range being 100 to 275 percent. In one experiment, efficiency for height increased during the period 2 to 5 years after planting; in another, it decreased during the interval 3 to 7 years.

			41. (4		: Number of-			
Exper- : iment number 1/:					Blocks : : :			
	Species	: Character		: Design	:per replica -:	Replica-:	Entries :	Efficiency
				1	: tion :		1	
1	elliottii	Germination	2 wk.	Rectangular lattice	9	4	72	100
1	ernoun	percentage						
		Hypocotyl height	3 wk,	do,	9	4	72	103
		Seedcoat retention	3 wk.	do.	9	4	72	106
		Within-plot height variation	l yr.	do.	9	4	72	106
		Weight	1 yr.	do,	.9	4	72	116
		Height	1 yr.	do,	9	4	72	117
2	elliottii	Seedcoat retention	3 wk.	Compact family block	s 8	3	23	92
		Within-plot height			100		1000	1.5.5
		variation	1 yr.	do,	8	3	23	161
		Weight	1 yr.	do.	8	3	23	150
		Height	1 yr.	do.	8	3	23	148
3	<u>elliottii</u> X echinata	Height	1 yr.	Compact family block	s. 11	5	117	93
4	palustris	Weight	1 yr.	Rectangular lattice	9	3	72	124
		Height	1 yr.	do,	9	3	72	159
5	palustris	Germination						
		percentage	2 wk.	Simple lattice	10	2	100	101
		Weight	l yr.	do.	10	2	100	126
6	palustris	Weight	l yr.	Simple lattice	10	4	100	274
7	deltoides	Reight	s mo.	Triple lattice	10	3	100	178
		Height	1 yr.	do.	10	3	100	198
		Diameter	1 yr.	do.	10	3	100	1 62
		Specific gravity	1 yr.	do.	10	3	100	117
		Fiber length	l yr.	do.	10	3	100	104
		Melampsora rust	1 yr.	do.	10	3	100	100
8	deltoides	Height	6 mo.	Triple lattice	10	3	100	108
		Height	1 yr.	do.	10	3	100	105
		Diameter	1 yr.	do.	10	3	100	104
		Specific gravity	l yr,	do.	10	3	100	104
		Fiber length	1 yr.	do.	10	з	100	102
		Melampsora rust	1 yr.	do.	10	3	100	103
9	ponderosa	Height	2 yr.	Compact family block	x 22	3	110	163
10	menziesii	Photosynthetic efficiency	1 yr.	Simple lattice	10	2	100	102

Table 1.--Efficiencies of lattice and compact family block designs for Pinus species in the nursery or greenhouse

1/ Experiments 1 through 8 are from nursery of Southern Forest Experiment Station, U.S. Forest Service. Data for experiments 7 and 8 were contributed by J. R. Wilcox and R. E. Farmer. They are from two closely spaced, short-term Populus clonal tests, one on a nursery site (7), and one on a forest site (8).

Data for experiment 9 are from Wells, O. O. Geographic variation in ponderosa pine (Pinus ponderosa Dougl. ex Laws). 1962. (Ph. D. thesis, Michigan State Univ., 112 pp.) The <u>Pseudotsuga</u> of experiment 10 were grown in the greenhouse; data were contributed by R. K. Campbell, Weyerhaeuser Forestry Research Center, Centralia, Washington.

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xperiment	Species Pinus-	: Character	Years after outplanting	: Design			ations :	Entries	: Efficiency
Trainin/or	:	1	:	1	: cation	:	:		1
					Number	Number	Acres	Number	Percent
11	elliottii	Survival	1	Triple lattice	8	3	1.0	64	152
		Survival	7	do.	8	3	1.0	64	109
		Height	3	do.	8	3	1.0	64	138
		Height	5	do.	8	3	1.0	64	129
		Height	7	do.	8	3	1.0	64	113
		Fusiform rust	7	do,	8	3	1.0	64	100
12 <u>elliottii</u>	elliottii	Survival	7	Triple lattice	8	3	1.0	64	100
		Height	3	do,	8	3	1.0	64	104
		Height	5	do.	8	3	1.0	64	106
		Height	7	do.	8	3	1.0	64	102
		Fusiform rust	7	do.	8	3	1,0	64	101
13 <u>elliott</u>	elliottii	Height	2	Compact family block	8	3	1.0	23	139
		Height	5	do.	8	з	1.0	23	275
		Fusiform rust	5	do.	8	3	1.0	23	114
							2.		
14	<u>palustris</u> X taeda	Height	5	Compact family block	7	6	1,0	25	105
15	palustris	Brown spot	2	Simple lattice	10	2	0.4	100	128
			3	do.	10	2	0.4	100	103
16	palustris	Survival	5	Triple lattice	10	6	1.4	100	110
17	palustris	Height	F	Rectangular lattice	9	3	0.6	72	114

Table 2.--Efficiencies of lattice and compact family block designs for Pinus species in field at Southern Forest Experiment Station

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