Lattice Designs

Applications in Plant Breeding

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Presentation Outline

- Why use incomplete block designs?
- Distinguishing features
- Lattice designs – basic plans and field layout
- Statistical analysis
- Cyclic designs and α-Lattices
- Meadowfoam breeding example
- Randomization and field plan
- SAS analysis and interpretation

Blocking

- Purpose
  - Reduce experimental error, increase precision
  - Compare treatments (genotypes) under more uniform conditions
- The problem
  - In breeding programs, the number of treatments may be large
  - As blocks get larger, the conditions within blocks become more heterogeneous
  - Other factors may limit the number of experimental units in a block
    - Size of a growth chamber, greenhouse, or available field area
    - Number of samples that can be processed at one time

Incomplete Block Designs

- Plots are grouped into blocks that are not large enough to contain all treatments (genotypes)
- Distinguishing features
  - Number of blocking criteria
  - Balanced or partially balanced
  - Resolvable (or not)
  - Shape (square, rectangular)
  - Process for generating design

Types of Incomplete Block Designs

- Single blocking criterion
  - Randomized incomplete blocks
- Two blocking criteria – based on Latin Squares
  - Latin Square is a complete block design that requires $N=t^2$. May be impractical for large numbers of treatments.
  - Row-Column Designs – either rows or columns or both are incomplete blocks
  - Youden Squares – two or more rows omitted from the Latin Square

Balanced Incomplete Block Designs

- Each treatment occurs together in the same block with every other treatment an equal number of times
  - t = number of treatments
  - k = number of units per block (block size)
  - b = total number of blocks in the experiment
  - r = number of replicates of each treatment
  - λ= number of times that treatments occur together in the same block

$$\lambda = \frac{r(k-1)}{t-1}$$

- All pairs of treatments are compared with the same level of precision even though differences between blocks may be large

Balanced Incomplete Block Designs

- For balance, $r = \lambda(t-1)/(k-1)$
  - t = number of treatments
  - k = number of units per block (block size)
  - b = total number of blocks in the experiment
  - r = number of replicates of each treatment
  - λ = number of times that treatments occur together in the same block
  - N = total number of experimental units
- λ must be an integer; $N = b*k = r*t$
- Example: t = 10 treatments with block size k = 4
  - $r = 6$, $b = 15$, $\lambda = 2$

    ⇨ $N = b*k = r*t = 6*10 = 60$
- In plant breeding, the minimum number of replications required for balance is often too large to be practical

Partially Balanced Incomplete Block Designs

- Different treatment pairs occur in the same blocks an unequal number of times or some treatment pairs never occur together in the same block
  - Mean comparisons have differing levels of precision
    - Greater precision when treatments occur in the same block
  - Statistical analysis more complex
- Common for plant breeding trials due to large number of entries

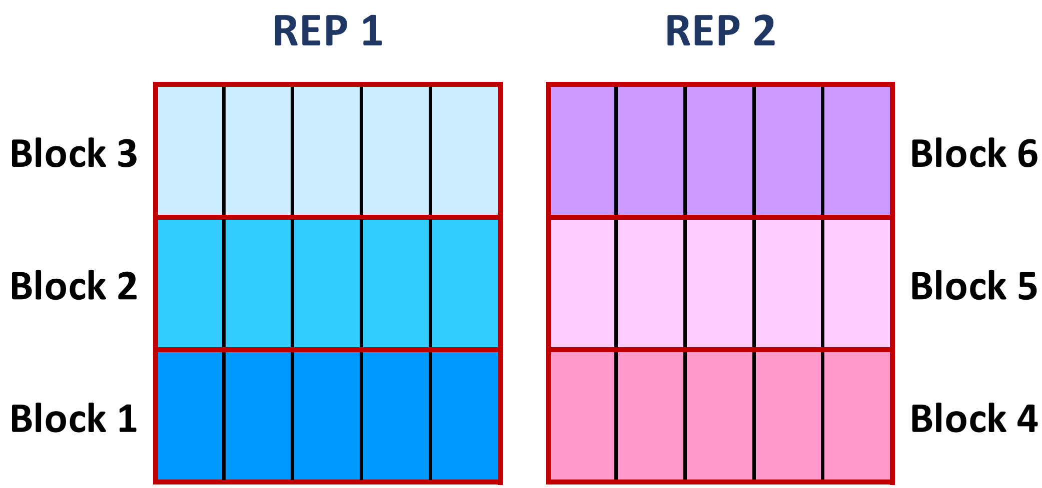
Resolvable Incomplete Block Designs

- Blocks are grouped so that each group of blocks constitute one complete replication of the treatment
  - "block" = incomplete block = "sub-block"
  - replication = "superblock"
- Trials can be managed in the field on a rep-by-rep basis
- Field operations can be conducted in stages (planting, weeding, data collection, harvest)
- Complete replicates can be lost without losing the whole experiment
- If you have two or more complete replications, you can analyze as an RCBD if the blocking turns out to be ineffective

Lattice Designs are Resolvable

- Lattice designs are a well-known type of resolvable incomplete block design

s*r = b



t = number of treatments	**15**
k = number of units per block (block size)	**5**
b = total number of blocks in the experiment	**6**
r = number of replicates of each treatment	**2**
s = number of blocks in each complete replication	**3**

Lattice Designs

- Square lattice designs
  - Number of treatments must be a perfect square ($t = k^2$)
  - Blocks per replicate (s) and plots per block (k) are equal ($s = k$) and are the square root of the number of treatments (t)
  - For complete balance, number of replicates $(r) = k+1$
- Rectangular lattice designs
  - $t = s*(s-1)$ and $k = s-1$
  - Example: 4 x 5 lattice has 4 plots per block, 5 blocks per replicate, and 20 treatments
- Alpha lattices
  - $t = s*k$
  - more flexibility in choice of s and k

The Basic Plan for a Square Lattice

Block	Rep I	Rep II	Rep III	Rep IV
1	1 2 3	1 4 7	1 5 9	1 6 8
2	4 5 6	2 5 8	2 6 7	2 4 9
3	7 8 9	3 6 9	3 4 8	3 5 7

- Balance – each treatment occurs together in the same block with every other treatment an equal number of times
  - Once in this case, so $\lambda = 1$

Basic plans can be found in Cochran and Cox, 1957

Randomization

- Field Arrangement
  - Blocks composed of plots that are as homogeneous as possible
- Randomization of a Basic Plan
  - Randomize order of blocks within replications
  - Randomize the order of treatments within blocks

Example of Randomization of a 3 x 3 Balanced Lattice (t = 9)

1 Assign r random numbers

Random	Sequence	Rank
372	1	2
217	2	1
963	3	4
404	4	3

2 From basic plan

Block	Rep I	Rep II	Rep III	Rep IV
1	1 2 3	1 4 7	1 5 9	1 6 8
2	4 5 6	2 5 8	2 6 7	2 4 9
3	7 8 9	3 6 9	3 4 8	3 5 7

3 Randomize order of replications

Block	Rep I	Rep II	Rep III	Rep IV
1	1 4 7	1 2 3	1 6 8	1 5 9
2	2 5 8	4 5 6	2 4 9	2 6 7
3	3 6 9	7 8 9	3 5 7	3 4 8

4 Randomize blocks within reps

Rep	I	II	III	IV
	3	2	3	1
	2	1	1	3
	1	3	2	2

5 Resulting new plan

Block	Rep I	Rep II	Rep III	Rep IV
1	3 6 9	4 5 6	3 5 7	1 5 9
2	2 5 8	1 2 3	1 6 8	3 4 8
3	1 4 7	7 8 9	2 4 9	2 6 7

Partially Balanced Lattices

- Simple Lattices
  - Two replications – use first two from basic plan
  - 3x3 and 4x4 are no more precise than RCBD because error df is too small
- Triple Lattices
  - Three replications – use first three from basic plan
  - Possible for all squares from 3x3 to 13x13
- Quadruple Lattices
  - Four replications – use first four from basic plan
  - Do not exist for 6x6 and 10x10
    - can repeat simple lattice, but analysis is different

Linear Model for Lattice Design

- Example is for a single blocking factor

$$Y_{ijl} = \mu + \tau_i + \gamma_j + \rho_{l(j)} + \varepsilon_{ijl}$$

Treatment effect
i= 1, 2,…,t

Replicate effect
j= 1, 2,…,r

Block within replicate effect
l= 1, 2,…,s

Random error

ANOVA

- Form of the analysis is the same for simple, triple and quadruple lattices
- Two error terms are computed
  - E_b – Error for block = SSB/r(k-1)
  - E_e – Experimental error = SSE/((k-1)(rk-k-1))

Source	df	SS	MS
Total	rk^2-1	SSTot	
Replications	r-1	SSR	
Treatments (unadj)	k^2-1	SST	
Block in rep (adj)	r(k-1)	SSB	E_b
Intrablock error	(k-1)(rk-k-1)	SSE	E_e

Computing Sums of Squares

- $SSTOT = \sum Y_{ijl}^2 - (G^2 / rk^2)$
  - G is the grand sum of all plots in the experiment
- $SSR = (1/k^2)\sum R_j^2 - (G^2 / rk^2)$
  - R_j is the sum of all plots in the j^{th} replication
- $SSB = \left(1/kr(r-1)\right)\sum C_{jl}^2 - \left(1/k^2r(r-1)\right)\sum C_j^2$ (adjusted)
  - C_{jl} = sum over all replications of all treatments in the l^{th} block of the j^{th} replication minus rB_{jl}
  - B_{jl} = sum of the k plots in the l^{th} block of the j^{th} replication
  - C_j = sum of all C_{jl} in the j^{th} replication
- $SST = (1/r)\sum T_i^2 - (G^2 / rk^2)$ (unadjusted)
  - T_i = sum of the i^{th} treatment across replications
- SSE = SSTot – SSR – SSB – SST

Adjustment factor

- Compare E_b with E_e: If $E_b \leq E_e$
  - Adjustment of treatment means will have no effect
  - Analyze as if it were an RCBD using replications as blocks
- If $E_b > E_e$ then compute an adjustment (weighting) factor A
  - $A = (E_b - E_e)/(k(r - 1)E_b)$
  - used to compute adjusted treatment means

$$\bar{Y}_{i(adj)} = \left(T_i + \sum AC_{jl}\right)/r$$

For all blocks in which the i^{th} treatment occurs

Testing Treatment Differences

- To test significance among adjusted treatment means, compute an adjusted mean square
  - $SSB_u = (1/k)\sum B_{jl}^2 - (G^2/rk^2) - SSR$
  - $SST_{adj} = SST - Ak(r-1)[((rSSB_u)/(r-1)(1+kA)) - SSB]$
  - $MST_{adj} = SST_{adj}/(k^2-1)$
- Finally, compute the F statistic for testing the differences among the adjusted treatment means
  - $F = MST_{adj}/E_e$

    with $k^2 - 1$ and $(k-1)(rk-k-1)$ degrees of freedom

Standard Errors

- Compute the effective error mean square
  - $E_e^{'} = \left[1 + \left((rkA)/(k+1)\right)\right]E_e$
- SE of adjusted treatment mean
  - $= \sqrt{E_e^{'} / r}$
- SE of difference between adjusted means in same block
  - $= \sqrt{(2E_e / r)\left(1 + (r-1)A\right)}$
- SE of difference between adjusted means in different blocks
  - $= \sqrt{(2E_e / r)(1 + rA)}$
- For larger lattices (k > 4) it is sufficient to use
  - $= \sqrt{2E_e^{'} / r}$

Relative Efficiency

- Estimate the error mean square of an RCBD
  - $E_{RCBD} = (SSB+SSE)/((k^2-1)(r-1))$
- Then the relative efficiency of the lattice is
  - $RE = E_{RCBD}/E_e'$

Numerical Example - Simple Lattice

Rep I

Block	Barley Yield kg/plot					B_{jl}	C_{jl}	Adj
1	(19)	(16)	(18)	(17)	(20)			
	18.2	13.0	9.5	6.7	10.1	57.5	17.6	1.54
2	(12)	(13)	(15)	(14)	(11)			
	13.3	11.4	14.2	11.9	13.4	64.2	4.2	0.37
3	(1)	(2)	(3)	(4)	(5)			
	15.0	12.4	17.3	20.5	13.0	78.2	5.3	0.46
4	(22)	(24)	(21)	(25)	(23)			
	7.0	5.9	14.1	19.2	7.8	54.0	-0.5	-0.04
5	(9)	(7)	(10)	(8)	(6)			
	11.9	15.2	17.2	16.3	16.0	76.6	3.9	0.34
Selection number in ()					Sum	330.5	30.5	2.66

Example from Petersen, R.G. 1994. Agricultural Field Experiments

Numerical Example - Simple Lattice

Rep 2

Block	Barley Yield kg/plot					B_{jl}	C_{jl}	Adj
1	(23)	(18)	(3)	(8)	(13)			
	7.7	15.2	19.1	15.5	14.7	72.2	-9.9	-0.86
2	(5)	(20)	(10)	(15)	(25)			
	15.8	18.0	18.8	14.4	20.0	87.0	-13.3	-1.17
3	(22)	(12)	(2)	(17)	(7)			
	10.2	11.5	17.0	11.0	15.3	65.0	-10.4	-0.91
4	(14)	(24)	(9)	(4)	(19)			
	10.9	4.7	10.9	16.6	9.8	52.9	15.5	1.35
5	(6)	(16)	(11)	(21)	(1)			
	20.0	21.1	16.9	10.9	15.0	83.9	-12.4	-1.08
Selection number in ()					Sum	361.0	-30.5	-2.67
					Grand Sum	691.5	0.0	0.0

Initial ANOVA

Source	df	SS	MS
Total	49	805.42	
Replication	1	18.60	
Selection (unadj)	24	621.82	
Block in rep (adj)	8	77.59	$9.70 = E_b$
Intrablock error	16	87.41	$5.46 = E_e$

$E_b > E_e$ so we compute the adjustment factor, A

$$A = (E_b - E_e)/(k(r-1)E_b) = (9.70 - 5.46)/((5)(1)(9.70)) = 0.0874$$

Adjustment of mean for Entry 1

$$Y_{1(adj)} = \left(T_1 + \sum AC_{jl}\right)/r = \left[30 + (0.0874 * 5.3) + (0.0874 * 1.35)\right]/2 = 14.69$$

Intrablock ANOVA of Adjusted Means

Source	df	SS	MS	F
Total	49	805.42		
Replication	1	18.60		
Selection (adj)	24	502.39	20.93	3.83**
Block in rep (unadj)	8	252.18		
Intrablock error	16	87.41	5.46=E_e	

- It may be better to use the effective error in the denominator of the F test
  - $E_e' = (1+(rkA)/(k+1))E_e = (1+(2*5*0.0874)/6)*5.46 = 6.26$
  - $F = 20.93/6.26 = 3.34^{**}$
- See the supplemental Excel spreadsheet for more details

Relative Efficiency

- How does the precision of the Lattice compare to that of a randomized complete block design?
  - First compute MSE for the RCBD as:

    $E_{RCBD} = (SSB+SSE)/(k^2 - 1)(r -1) =$

    $(77.59 + 87.41)/(24)(1) = 6.88$

- Then % relative efficiency =
  - $(E_{RCBD} / E_e^{'})100 = (6.88/6.26)*100 = 110.0\%$
  - There is a 10% gain in efficiency from using the lattice

Cyclic Designs

- Incomplete Block Designs discussed so far
  - Require extensive tables of design plans
  - Need to avoid mistakes when assigning treatments to experimental units and during field operations
- Cyclic designs are a type of incomplete block design
  - Relatively easy to construct and implement
  - Generated from an initial block
  - Example: 6 treatments, block size = 3
  - Add one to each treatment label for each additional block
  - Modulo t=6

Block	Treatment Label
1	0, 1, 3
2	1, 2, 4
3	2, 3, 5
4	3, 4, 0
5	4, 5, 1
6	5, 0, 2

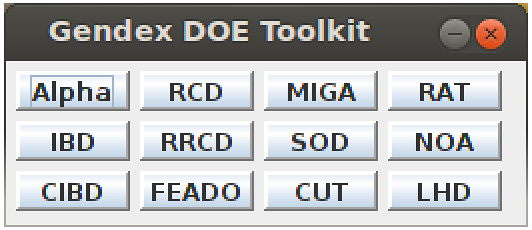
Good reference: Kuehl, 2000, Chapt. 10

Alpha designs

- Patterson and Williams (1976) described a new way to construct cyclic, resolvable incomplete block designs
- α-designs are available for many (r,k,s) combinations
  - r is the number of complete replicates
  - k is the block size
  - s is the number of blocks per replicate
  - *Number of treatments $t = sk$*
- Efficient α-designs exist for some combinations for which conventional lattices do not exist
- Can accommodate unequal block sizes
- Two types: α(0, 1) and α(0, 1, 2)
  - Indicates values of λ that occur in the trial (2 or 3 associate classes)

Alpha Designs - Software

- Gendex http://designcomputing.net/gendex/alpha/
  - Can generate optimal or near-optimal α-designs
  - Up to 10,000 entries
  - Evaluation/academic copy is free and can be downloaded
  - Cost for commercial perpetual license is $299



- CycDesigN
- Agrobase
- R agricolae package
  - design.alpha, design.lattice, design.cyclic, design.bib
- SAS PROC PLAN
  - some code required
  - http://www.stat.ncsu.edu/people/dickey/courses/st711/Demos/

Efficiency Factors for Lattice Designs

- Balanced lattice $$E = \frac{(k+1)(r-1)}{r(k+2)-(k+1)}$$
- Simple lattice $$E = \frac{k+1}{k+3}$$
- Triple lattice $$E = \frac{2k+2}{2k+5}$$
- Alpha lattice (upper bound) $$E = \frac{(t-1)(r-1)}{(t-1)(r-1)+r(s-1)}$$

Use as large a block size as possible while maintaining homogeneity of plots within blocks

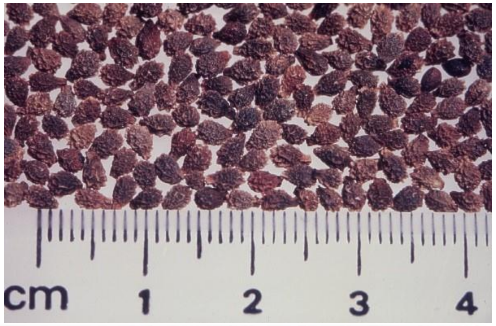
t = # treatments, k = block size, s = # blocks/rep, r = # complete reps

Analysis of Lattice Experiments

- SAS
  - PROC MIXED, PROC LATTICE
  - PROC VARCOMP (random genotypes)
  - PROC GLIMMIX (non-normal)
- ASREML
- GENSTAT
- R
  - stats, lme4 packages
- Agrobase
  - www.agronomix.com/

Meadowfoam (*Limnanthes alba*)

- Native to vernal pools in the PNW
- First produced as a crop in 1980
- Seed oil with novel long-chain fatty acids
  - light-colored and odor free
  - exceptional oxidative stability



- Used in personal care products
- Potential uses
  - fuel additive
  - vehicle lubricants
  - pharmaceutical products

GJ Pool Progeny Trial, 2012 (α-Lattice)

- Plot size 4 ft x 12 ft (planted 2000 seeds/plot)
- Average seed yield 1612 kg/ha

Autofertile Progeny Trial, 2013

- 132 entries
- 127 TC + 5 checks
- 11 x 12 α-lattice
- 2 replications
- 1 location
- 4 ft x 10 ft plots
- 1450 seeds/plot

Average seed yield 879 kg/ha

Meadowfoam Reproduction

- Winter annual, diploid
- Factors that promote outcrossing:
  - Protandrous
  - Heterostylous
  - Many native pollinators
  - Inbreeding depression in *L. alba* ssp *alba*

- Potential for selfing
  - Perfect flowers
  - Self-compatible
  - Flowers close at night

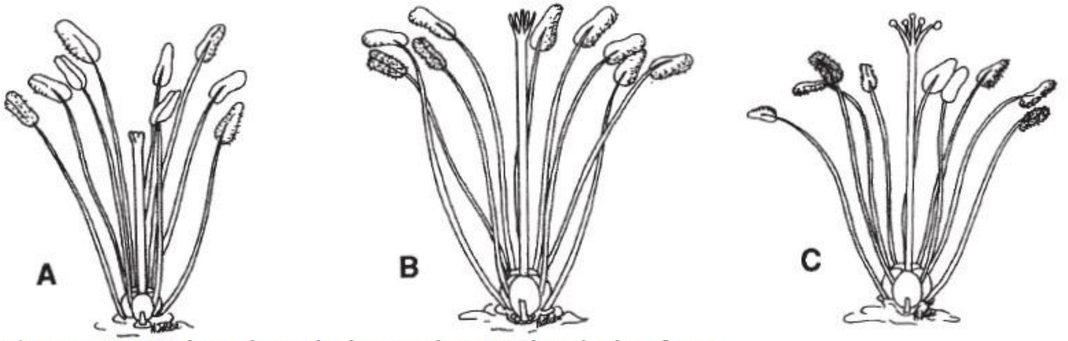


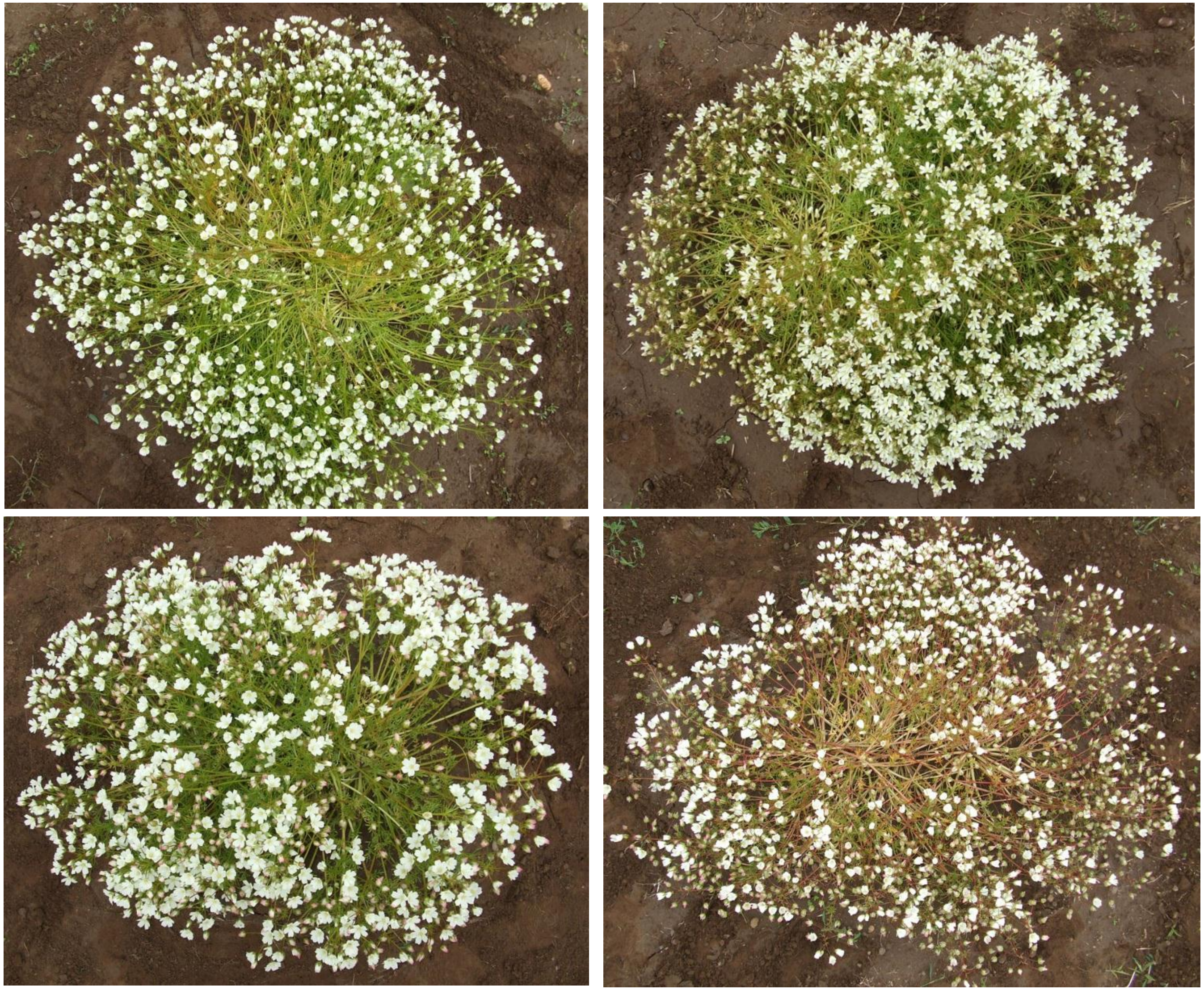
Figure 2.—*Sequential reproductive development of a Mermaid meadowfoam flower:*
A. Flower opening with initial pollen availability (dehiscence) and unreceptive stigmas
B. Maximum pollen shed with unreceptive stigmas
C. Maximum stigmatal receptivity with reduced pollen availability

Autofertile Pool

- Derived from crosses between
  - Outcrossing *L. alba* ssp. *alba* populations
  - Self-pollinating lines from *L. alba* ssp. *versicolor*
- *Inbreeding depression??*
- Development of progeny for evaluation
  - Selfed plants in the greenhouse for two generations, without pollinators
  - Selected for autofertility (seed number)
  - Planted S_2 families in an isolated nursery with honeybees to produce testcross progeny

S_2 Families in Isolated Nursery

- Planted 5 seeds per family in short rows
- 2 replicates
- Randomized blocks
- Honeybee pollinators



ALPHA 7.0: Construct alpha designs of size (r,k,s)

(c) 2013 Design Computing (designcomputing.net/)

Best alpha design for v=132, r=2, k=11, and s=12. **Efficiency 0.8542**

Plan (blocks are rows):

82	83	97	117	48	54	25	2	99	38	107
100	74	86	65	85	94	71	18	126	28	31
84	1	53	120	101	72	104	122	114	66	68
10	112	7	15	33	121	127	59	14	67	130
131	124	118	102	3	39	93	43	109	55	106
103	44	37	32	62	63	108	23	80	70	60
89	27	79	24	4	64	81	29	21	61	128
69	47	41	49	19	98	110	76	75	30	36
105	22	40	9	90	5	91	16	132	95	111
26	113	35	45	12	20	73	11	6	119	34
125	116	13	123	92	51	52	56	88	46	129
57	42	17	78	50	58	8	77	115	96	87
131	59	86	36	114	25	16	119	103	27	88
90	2	23	42	116	65	34	128	67	43	68
93	58	15	64	70	101	82	92	111	100	19
18	5	102	72	73	61	10	49	87	62	99
115	26	1	83	31	30	3	52	40	37	112
85	6	97	121	125	78	66	24	9	47	106
17	109	107	56	60	130	45	84	126	41	29
81	118	132	46	108	28	98	96	38	35	7
51	12	4	76	33	104	44	105	94	39	57
123	54	8	55	32	110	22	71	20	120	21
122	79	69	48	77	129	95	124	11	80	14
75	50	91	113	63	13	117	89	74	53	127

Field Map for the Autofertile Trial (Rep 1)

N

BORDER

10 ft

1132	1131	1130	1129	1128	1127	1126	1125	1124	1123	1122	1121	1120	1119	1118	1117	1116	1115	1114	1113	1112	1111
87	96	115	77	8	58	50	78	17	42	57	129	46	88	56	52	51	92	123	13	116	125
1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110
105	22	40	9	90	5	91	16	132	95	111	26	113	35	45	12	20	73	11	6	119	34
1088	1087	1086	1085	1084	1083	1082	1081	1080	1079	1078	1077	1076	1075	1074	1073	1072	1071	1070	1069	1068	1067
36	30	75	76	110	98	19	49	41	47	69	128	61	21	29	81	64	4	24	79	27	89
1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066
131	124	118	102	3	39	93	43	109	55	106	103	44	37	32	62	63	108	23	80	70	60
1044	1043	1042	1041	1040	1039	1038	1037	1036	1035	1034	1033	1032	1031	1030	1029	1028	1027	1026	1025	1024	1023
130	67	14	59	127	121	33	15	7	112	10	68	66	114	122	104	72	101	120	53	1	84
1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022
82	83	97	117	48	54	25	2	99	38	107	100	74	86	65	85	94	71	18	126	28	31

BORDER

Block 1

Data Set – Variables Included

- Plant height cm
- 1000-seed weight (TSW)
- Seed oil content (%)
- Seed yield kg/ha

SAS Data Input

Other options for data input
- Import Wizard
- Infile statements
- SAS libraries

```
ods html close; ods html;
data AF2013;
Input PLOT REP BLOCK ENTRY NAME$ Height TSW Oil Yield;
Oilyld=yield*oil/100;
datalines;
1001   1    1    82    179-53-2    22.5    9.48    26.16    782
1002   1    1    83    179-56-1    22.5    9.38    27.42    832
1003   1    1    97    179-83-1    23.5    8.56    24.25    944
1004   1    1    117   188-14-1    19.5    7.90    28.11    721
.                                                         .
.                                                         .
.                                                         .
2129   2    24   89    179-63-1    24.0    10.10   26.68    858
2130   2    24   74    179-38-1    22.5    9.35    28.40    631
2131   2    24   53    179-4-2     20.5    9.51    25.76    1127
2132   2    24   127   188-133-2   22.5    9.62    25.37    721
;
```

Mixed Model Analysis of Yield

```
PROC MIXED;
TITLE 'Lattice analysis of yield: PROC MIXED, entries fixed';
CLASS REP BLOCK ENTRY;
MODEL Yield = ENTRY;
RANDOM REP BLOCK(REP) /solution;
ods output solutionr=syield;
/*test for entries that differ from Ross*/
LSMEANS entry/pdiff=CONTROL('128');
ods output lsmeans=Yieldadj diffs=Ylddiff;
RUN;
```

Use export wizard to export

- *syield*
- *Yieldadj*
- *Ylddiff*

Analysis of Yield (fixed entries)

The Mixed Procedure
Covariance Parameter Estimates

Cov Parm	Estimate
REP	2435.58
BLOCK(REP)	9900.50
Residual	12657

Fit Statistics

-2 Res Log Likelihood	1753.2
AIC (smaller is better)	1759.2
AICC (smaller is better)	1759.3
BIC (smaller is better)	1755.2

Type 3 Tests of Fixed Effects

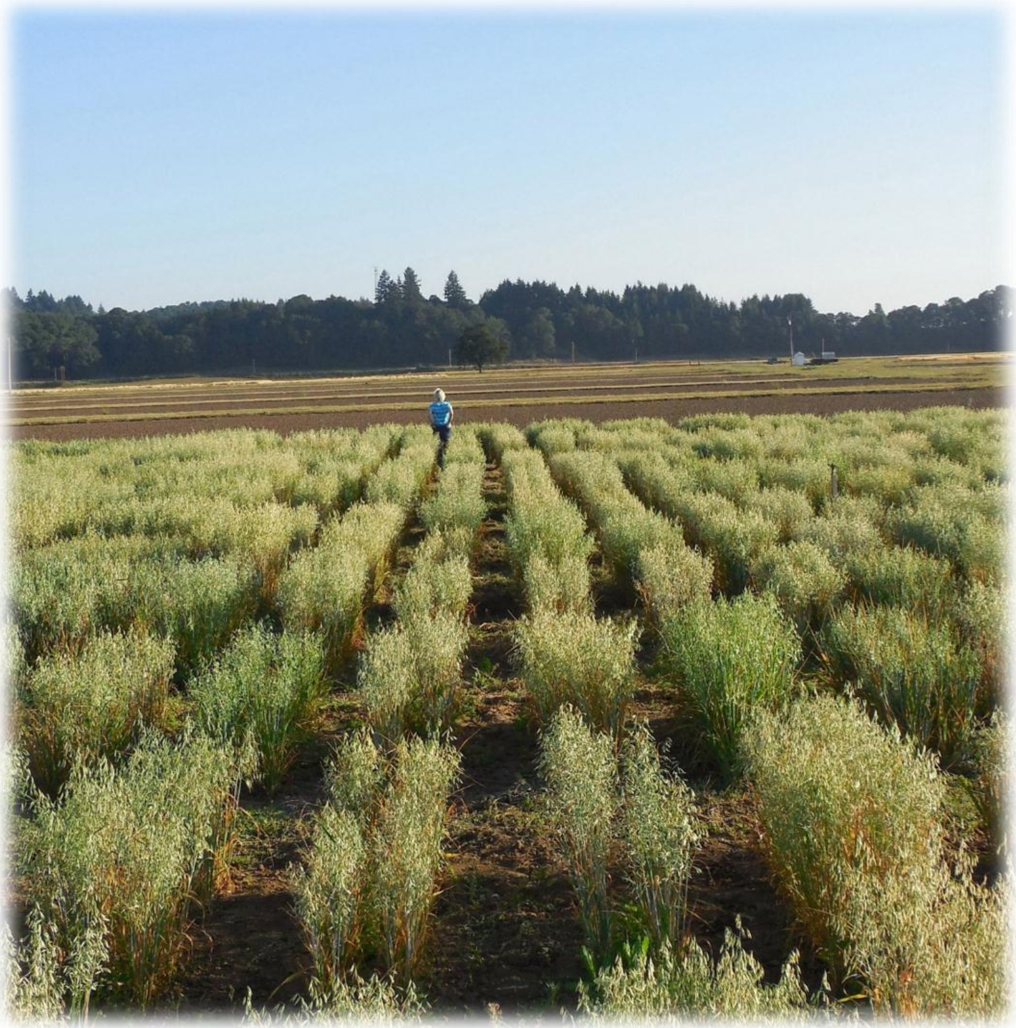
Effect	Num DF	Den DF	F Value	Pr > F
Entry	131	109	6.17	<.0001

Analysis of Yield – Random Effects

Cov Parm	Height	TSW	Oil	Yield
REP	0.191	0.0369	0.4252	2435.6
BLOCK(REP)	1.568	0	0.1696	9900.5
Residual	2.238	0.1972	1.0360	12657.0

Conclusion

- α-Lattice Designs?
- **Yes!**
- You have nothing to lose
- They are most helpful when you need them the most



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- Lattice Design Examples
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  - Roger Petersen
  - Nan Scott