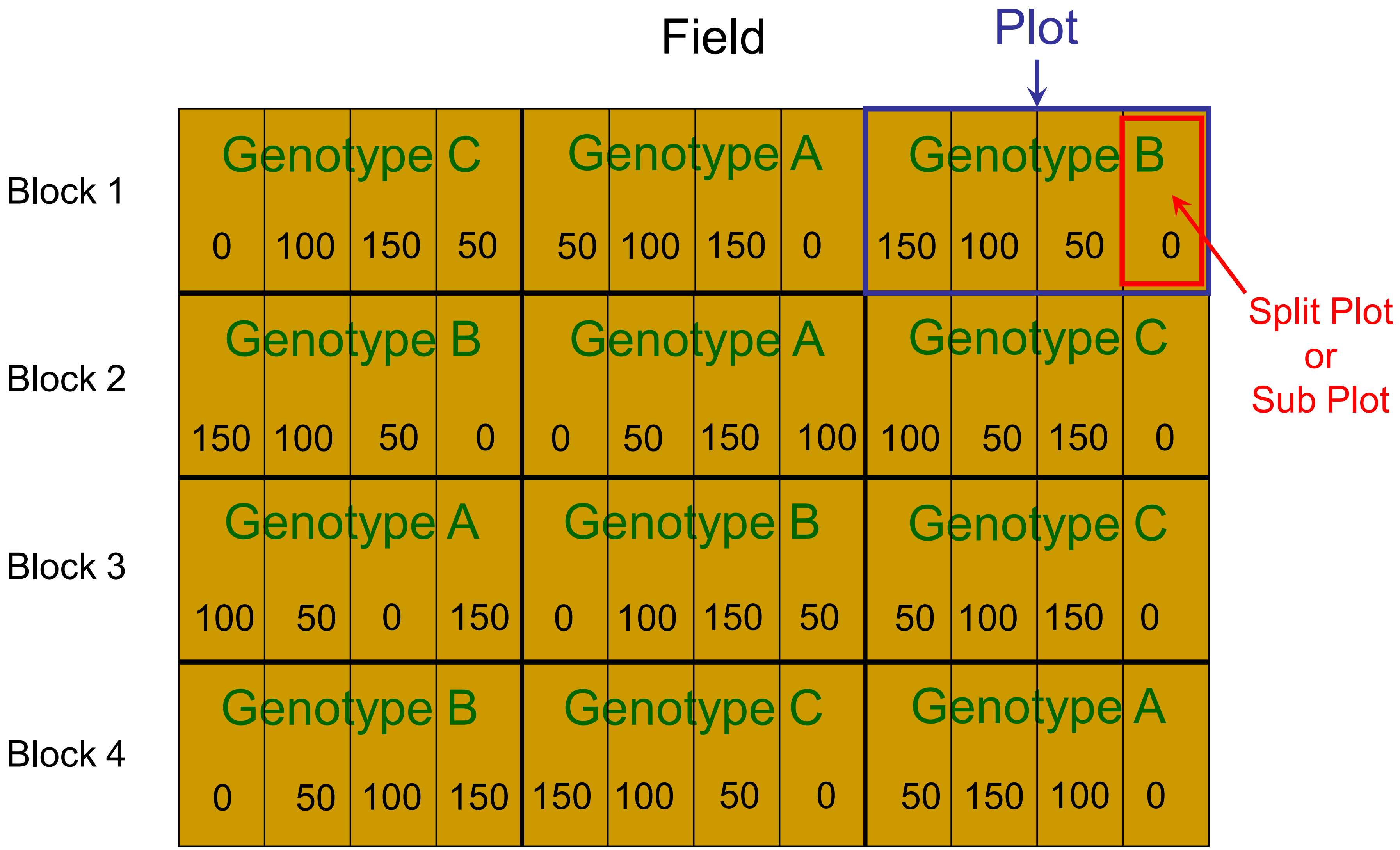
Simulation and Analysis of Data from a Classic Split Plot Experimental Design

Split-Plot Experimental Designs



Field

Plot

Split Plot
or
Sub Plot

Block 1	Genotype C				Genotype A				Genotype B			
	0	100	150	50	50	100	150	0	150	100	50	0
Block 2	Genotype B				Genotype A				Genotype C			
	150	100	50	0	0	50	150	100	100	50	150	0
Block 3	Genotype A				Genotype B				Genotype C			
	100	50	0	150	0	100	150	50	50	100	150	0
Block 4	Genotype B				Genotype C				Genotype A			
	0	50	100	150	150	100	50	0	50	150	100	0

```
#Example code for simulating data from our
#classic split plot example.

block = factor(rep(1:4, each = 12))
geno = factor(rep(rep(1:3, each = 4), 4))
fert = rep(seq(0, 150, by = 50), 12)

X = model.matrix(~geno + fert + I(fert^2) + geno:fert)
beta = c(125, 15, -10, .4, -0.0015, 0, .2)

Z1 = model.matrix(~ 0 + block)
Z2 = model.matrix(~ 0 + geno:block)
Z = cbind(Z1, Z2)
```

```
#The code below generates the random effects
#and random errors and assembles the response
#vector. The function set.seed is used to
#control the random number generator so that
#the same random effects and errors will be
#generated each time this code is called.
```

$u_1,\dots,u_4 \overset{iid}{\sim} N(0,6^2)$ $\quad u_5,\dots,u_{16} \overset{iid}{\sim} N(0,7^2)$

$b_1,\dots,b_4$ $\quad w_1,\dots,w_{12}$

```
set.seed(532)
u = c(rnorm(4, 0, 6), rnorm(12, 0, 7))
e = rnorm(48, 0, 6)
```

$e_1,\dots,e_{48} \overset{iid}{\sim} N(0,6^2)$

$\underline{y} = X\underline{\beta} + Z\underline{u} + \underline{e}$

```
y = X %*% beta + Z %*% u + e
y = round(y, 1)
d = data.frame(block, geno, fert, y)
d
  block geno fert     y
1     1    1    0 148.7
2     1    1   50 150.4
3     1    1  100 166.7
4     1    1  150 156.5
5     1    2    0 162.5
6     1    2   50 168.6
```

```
7       1    2  100 180.2
8       1    2  150 181.1
9       1    3    0 144.5
10      1    3   50 177.3
11      1    3  100 188.1
12      1    3  150 199.1
13      2    1    0 114.2
14      2    1   50 131.5
15      2    1  100 150.8
16      2    1  150 139.8
17      2    2    0 141.6
18      2    2   50 150.9
19      2    2  100 171.8
20      2    2  150 187.4
21      2    3    0 107.9
22      2    3   50 138.0
23      2    3  100 161.8
24      2    3  150 163.5
25      3    1    0 126.5
26      3    1   50 138.8
27      3    1  100 134.5
```

```
28     3    1   150 140.6
29     3    2     0 129.8
30     3    2    50 155.8
31     3    2   100 168.0
32     3    2   150 164.8
33     3    3     0 100.5
34     3    3    50 139.3
35     3    3   100 150.7
36     3    3   150 158.8
37     4    1     0 114.7
38     4    1    50 138.4
39     4    1   100 141.8
40     4    1   150 143.3
41     4    2     0 160.2
42     4    2    50 162.5
43     4    2   100 178.8
44     4    2   150 171.3
45     4    3     0 102.1
46     4    3    50 126.9
47     4    3   100 142.2
48     4    3   150 152.9
```

```
#ANOVA-based analysis
```

WHOLE-PLOT ERROR TERM

WHOLE-PLOT PART

```
o=lm(y~block+geno+block:geno+factor(fert)+geno:factor(fert))

anova(o)
Analysis of Variance Table

Response: y
                  Df Sum Sq Mean Sq F value    Pr(>F)
block              3 5349.5 1783.16 ~~44.9089 1.252e-10~~
geno               2 5237.2 2618.62 ~~65.9500 4.057e-11~~
factor(fert)       3 8737.7 2912.57 73.3531 4.233e-13
block:geno         6 1853.4  308.90  7.7796 6.355e-05
geno:factor(fert)  6 1557.3  259.56  6.5370 0.0002381
Residuals         27 1072.1   39.71
---

a = as.matrix(anova(o))
```

```
#ANOVA estimates of variance components:

#Estimate of sigma^2_e

MSE = a[6, 3]
MSE
[1] 39.70613
```

$$E\left(MS_{ERROR}\right) = \sigma^2_e$$

```
#Estimate of sigma^2_w
```

$$E\left(MS_{BLOCK \times GENO}\right) = 4\sigma^2_w + \sigma^2_e$$

```
MSBlockGeno = a[4, 3]

(MSBlockGeno - MSE) / 4
[1] 67.2981
```

$$E\left(\frac{MS_{BLOCK \times GENO} - MS_{ERROR}}{4}\right) = \sigma^2_w$$

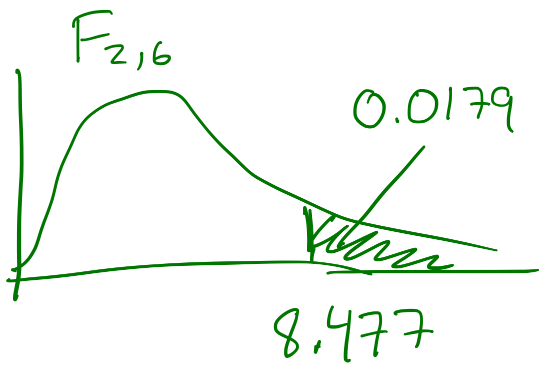
```
#Save the square roots of these estimates
#for comparison with REML estimates computed
#later.

sige = sqrt(MSE)
sigw = sqrt((MSBlockGeno - MSE) / 4)
```

```
#F test for genotype main effects
```

$$F = \frac{MS_{GENO}}{MS_{BLOCK \times GENO}} = \frac{2618.62}{308.90}$$

```
MSGeno = a[2, 3]
Fstat = MSGeno / MSBlockGeno
Fstat
[1] 8.47728
```



2 6

```
pval = 1 - pf(Fstat, a[2, 1], a[4, 1])
pval
[1] 0.01785858
```

```
#95% confidence interval for geno 2 - geno 1

gmeans = tapply(y, geno, mean)
gmeans
       1        2        3
139.8250 164.7063 147.1000
```

$\bar{Y}_{1..}$ $\bar{Y}_{2..}$ $\bar{Y}_{3..}$

```
est = gmeans[2] - gmeans[1]
names(est) = NULL
```

$\bar{Y}_{2..} - \bar{Y}_{1..}$

$Var(\bar{Y}_{2..}-\bar{Y}_{1..}) = 2(4\sigma_w^2+\sigma_e^2)/(4*4)$ ESTIMATED BY $2MS_{BLOCKxGENO}/16$

```
#We showed previously that the variance of
#the difference between genotype means
#is 2 * E(MS_block*geno) / (nblocks * nferts)

#Thus, we compute a standard error as

se = sqrt(2 * MSBlockGeno / (4 * 4))
                                   6
lower = est - qt(.975, a[4, 1]) * se
upper = est + qt(.975, a[4, 1]) * se
                                   6

c(estimate = est, se = se, lower = lower, upper=upper)
 estimate         se      lower      upper
24.881250   6.213881  (9.676431, 40.086069)
```

$\bar{Y}_{2..}-\bar{Y}_{1..} \pm t_{.975,6}\sqrt{\frac{MS_{BLOCKxGENO}}{8}}$

$\bar{Y}_{2..}-\bar{Y}_{1..}$ $\sqrt{\frac{MS_{BLOCKxGENO}}{8}}$

WE WILL LEARN ABOUT THIS LATER

```
#REML analysis via lme

library(nlme)

#Below I create f and g factors to shorten
#code and the names that R assigns to the
#elements of beta hat.

f = factor((fert + 50) / 50)
f
 [1] 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2
[39] 3 4 1 2 3 4 1 2 3 4
Levels: 1 2 3 4

g = geno
```

f	fert
1	0
2	50
3	100
4	150

$g+f+g:f$

```
o = lme(y ~ g * f, random = ~ 1 | block / g)
o
Linear mixed-effects model fit by REML
  Data: NULL
  Log-restricted-likelihood: -137.5281
  Fixed: y ~ g * f
(Intercept)          g2          g3          f2          f3          f4
    126.025      22.500     -12.275      13.750      22.425      19.025

      g2:f2       g3:f2       g2:f3       g3:f3       g2:f4       g3:f4
     -2.825      17.875       3.750      24.525       8.600      35.800
```

$\hat{\beta}_{\hat{\Sigma}} = \hat{\beta}$

FERT

		0	50	100	150
GENO	1	μ	$\mu+f_2$	$\mu+f_3$	$\mu+f_4$
	2	$\mu+g_2$	$\mu+g_2+f_2+g_2f_2$	$\mu+g_2+f_3+g_2f_3$	$\mu+g_2+f_4+g_2f_4$
	3	$\mu+g_3$	$\mu+g_3+f_2+g_3f_2$	$\mu+g_3+f_3+g_3f_3$	$\mu+g_3+f_4+g_3f_4$

```
Random effects:
 Formula: ~1 | block
        (Intercept)
StdDev:    11.08399
```

$\sqrt{\hat{\sigma}_b^2}$

```
 Formula: ~1 | g %in% block
        (Intercept) Residual
StdDev:    8.203544  6.30128
```

$\sqrt{\hat{\sigma}_w^2}$ $\sqrt{\hat{\sigma}_e^2}$

```
Number of Observations: 48
Number of Groups:
       block g %in% block
           4           12

#Note that the REML estimates of standard deviation
#match the ANOVA estimates computed
#from lm output.
sigw
[1] 8.203542
sige
[1] 6.30128
```

```
#The ANOVA table computed from lme output
#automatically gives the correct tests for
#genotype, fertilizer, and
#genotype by fertilizer interaction for
#the balanced data case.

anova(o)
            numDF denDF  F-value p-value
~~(Intercept)     1    27 610.0661  <.0001~~
g               2     6   8.4773  0.0179
f               3    27  73.3531  <.0001
g:f             6    27   6.5370  0.0002
```

```
#The GLS estimate of the fixed effect
#parameter beta is obtained as follows.
```

$$\underline{\hat{\beta}}_{\hat{\Sigma}} = \underline{\hat{\beta}}$$

```
fixed.effects(o)
(Intercept)          g2          g3          f2          f3          f4
    126.025      22.500     -12.275      13.750      22.425      19.025

      g2:f2       g3:f2       g2:f3       g3:f3       g2:f4       g3:f4
     -2.825      17.875       3.750      24.525       8.600      35.800
```

```
#The estimated variance covariance matrix of
#the GLS estimator is obtained as follows.
```

$$\widehat{VAR}(\underline{\hat{\beta}}_{\hat{\Sigma}}) = (X'\hat{\Sigma}^{-1}X)^{-1}$$

```
vcov(o)
            (Intercept)         g2         g3         f2         f3         f4
(Intercept)   57.464798 -26.751067 -26.751067  -9.926532  -9.926532  -9.926532
g2           -26.751067  53.502135  26.751067   9.926532   9.926532   9.926532
g3           -26.751067  26.751067  53.502135   9.926532   9.926532   9.926532
f2            -9.926532   9.926532   9.926532  19.853064   9.926532   9.926532
f3            -9.926532   9.926532   9.926532   9.926532  19.853064   9.926532
f4            -9.926532   9.926532   9.926532   9.926532   9.926532  19.853064
g2:f2          9.926532 -19.853064  -9.926532 -19.853064  -9.926532  -9.926532
g3:f2          9.926532  -9.926532 -19.853064 -19.853064  -9.926532  -9.926532
g2:f3          9.926532 -19.853064  -9.926532  -9.926532 -19.853064  -9.926532
g3:f3          9.926532  -9.926532 -19.853064  -9.926532 -19.853064  -9.926532
g2:f4          9.926532 -19.853064  -9.926532  -9.926532  -9.926532 -19.853064
g3:f4          9.926532  -9.926532 -19.853064  -9.926532  -9.926532 -19.853064
```

```
                g2:f2      g3:f2      g2:f3      g3:f3      g2:f4      g3:f4
(Intercept)  9.926532   9.926532   9.926532   9.926532   9.926532   9.926532
g2         -19.853064  -9.926532 -19.853064  -9.926532 -19.853064  -9.926532
g3          -9.926532 -19.853064  -9.926532 -19.853064  -9.926532 -19.853064
f2         -19.853064 -19.853064  -9.926532  -9.926532  -9.926532  -9.926532
f3          -9.926532  -9.926532 -19.853064 -19.853064  -9.926532  -9.926532
f4          -9.926532  -9.926532  -9.926532  -9.926532 -19.853064 -19.853064
g2:f2       39.706128  19.853064  19.853064   9.926532  19.853064   9.926532
g3:f2       19.853064  39.706128   9.926532  19.853064   9.926532  19.853064
g2:f3       19.853064   9.926532  39.706128  19.853064  19.853064   9.926532
g3:f3        9.926532  19.853064  19.853064  39.706128   9.926532  19.853064
g2:f4       19.853064   9.926532  19.853064   9.926532  39.706128  19.853064
g3:f4        9.926532  19.853064   9.926532  19.853064  19.853064  39.706128
```

```
#We can use the estimate of beta and it's
#variance covariance matrix to construct
#test statistics and confidence intervals
#for testable and estimable quantities.
#This will work in the unbalanced case
#as well. However, care must be taken to
#assign the appropriate degrees of freedom
#and inferences will be only approximate
#for the unbalanced case and whenever
#variance estimates depend on more than
#one mean square.
```

```
#For example, here is a revised version of the
#confidence interval function that we used for the
#normal theory Gauss-Markov linear model.  The test
#function we previously used could be modified in a
#similar way.
ci = function(lmeout, C, df, a = 0.05)
{
  b = fixed.effects(lmeout)
  V = vcov(lmeout)
  Cb = C %*% b
  se = sqrt(diag(C %*% V %*% t(C)))
  tval = qt(1 - a / 2, df)
  low = Cb - tval * se
  up = Cb + tval * se
  m = cbind(C, Cb, se, low, up)
  dimnames(m)[[2]] = c(paste("c", 1:ncol(C), sep=""),
             "estimate","se",
             paste(100 * (1 - a), "% Conf.", sep=""),
             "limits")
  m
}
```

$\hat{\beta}_{\hat{\Sigma}}$

$(X'\hat{\Sigma}^{-1}X)^{-1}$

$C\hat{\beta}_{\hat{\Sigma}}$

$$\widehat{VAR}(C\hat{\beta}_{\hat{\Sigma}}) = C(X'\hat{\Sigma}^{-1}X)^{-1}C'$$

```
#Suppose would like a confidence interval
#for the genotype 2 mean minus the
#genotype 1 mean while averaging over the
#levels of fertilizer.

#The following table shows the cell means
#in terms of the R parameterization.

#                           f
#############################################################
#     1            2                3                4
#############################################################
# g
#
# 1  mu       mu   +f2         mu   +f3         mu   +f4
#
# 2  mu+g2    mu+g2+f2+g2f2    mu+g2+f3+g2f3    mu+g2+f4+g2f4
#
# 3  mu+g3    mu+g3+f2+g3f2    mu+g3+f3+g3f3    mu+g3+f4+g3f4
#
#############################################################
```

```
#The average of row 2 minus the average of row 1 is
#
#  g2 + g2f2/4 + g2f3/4 + g2f4/4
#

C = matrix(c(0,1,0,0,0,0,.25,0,.25,0,.25,0), nrow = 1)

#Note that interval produced below matches
#the interval computed from the lm output.

ci(o, C, 6)

 estimate        se 95% Conf.    limits
 24.88125 6.213883  9.676427  40.08607
```

```
#We can also come up with the coefficients in
#the balanced case using the following code.

X = model.matrix(o)

apply(X[g == 2, ],2,mean) - apply(X[g == 1, ],2,mean)
```

```
(Intercept)          g2          g3          f2          f3          f4 
       0.00        1.00        0.00        0.00        0.00        0.00 

      g2:f2       g3:f2       g2:f3       g3:f3       g2:f4       g3:f4 
       0.25        0.00        0.25        0.00        0.25        0.00 
```

```
#We can obtain the best linear unbiased predictions
#(BLUPs) for the random effects as follows.

random.effects(o)
```

```
Level: block
  (Intercept)
1   14.962791
2   -3.260569
3   -6.781226
4   -4.920996
```

$\hat{u}_1 = \hat{b}_1$

$\hat{u}_2 = \hat{b}_2$

$\hat{u}_3 = \hat{b}_3$

$\hat{u}_4 = \hat{b}_4$

```
Level: g %in% block
     (Intercept)
1/1    0.6860200
1/2   -5.7246507
1/3   13.2350305
2/1   -2.1694371
2/2    1.2891661
2/3   -0.9058214
3/1    1.7919168
3/2   -2.8976223
3/3   -2.6089515
4/1   -0.3084997
4/2    7.3331070
4/3   -9.7202576
```

$\hat{u}_5 = \hat{w}_1$

$\hat{u}_6 = \hat{w}_2$

$\vdots$

$\hat{u}_{16} = \hat{w}_{12}$

```
#Because we have simulated the data, we can compare
#the predictions with the true values of the random
#effects.
cbind(u, unlist(random.effects(o)))
                                u          û
block.(Intercept)1  18.6303551 14.9627915
block.(Intercept)2  -7.9765912 -3.2605692
block.(Intercept)3  -8.7968392 -6.7812260
block.(Intercept)4  -2.0717338 -4.9209963
g.(Intercept)1      -4.5489233  0.6860200
g.(Intercept)2      -1.9147617 -5.7246507
g.(Intercept)3      11.1019481 13.2350305
g.(Intercept)4      -2.3538300 -2.1694371
g.(Intercept)5      14.4051819  1.2891661
g.(Intercept)6       4.7035930 -0.9058214
g.(Intercept)7       3.0466152  1.7919168
g.(Intercept)8       3.8042996 -2.8976223
g.(Intercept)9      -1.0352073 -2.6089515
g.(Intercept)10     -0.8256385 -0.3084997
g.(Intercept)11     10.6835477  7.3331070
g.(Intercept)12    -12.3977860 -9.7202576
```

```
#The same sorts of analyses could be carried out
#using lmer.

library(lme4)
o = lmer(y ~ g * f + (1 | block) + (1 | block:g))
o
Linear mixed model fit by REML ['lmerMod']
Formula: y ~ g * f + (1 | block) + (1 | block:g)
REML criterion at convergence: 275.0563
Random effects:
 Groups   Name        Std.Dev.
 block:g  (Intercept)  8.204
 block    (Intercept) 11.084
 Residual              6.301
Number of obs: 48, groups: block:g, 12; block, 4
Fixed Effects:
(Intercept)        g2          g3          f2          f3          f4
    126.025    22.500     -12.275      13.750      22.425      19.025

  g2:f2        g3:f2        g2:f3        g3:f3        g2:f4        g3:f4
 -2.825       17.875        3.750       24.525        8.600       35.800
```

FIXED EFFECTS → $\boxed{g+f+g:f}$

RANDOM EFFECT FOR EACH BLOCK

RANDOM EFFECT FOR EACH COMBINATION OF BLOCK AND GENOTYPE, i.e., FOR EACH WHOLE-PLOT EXPERIMENTAL UNIT

$\hat{\sigma}_w$

$\hat{\sigma}_b$

$\hat{\sigma}_e$

$$\hat{\underset{\sim}{\beta}}_{\hat{\Sigma}} = \hat{\underset{\sim}{\beta}}$$