

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

# Split-Plot Experimental Designs

	Field								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

Split Plot or Sub Plot

```
#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.

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

```
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
```

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

```
MSBlockGeno = a[4, 3]
```

```
(MSBlockGeno - MSE) / 4
```

```
[1] 67.2981
```

```
#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
```

```
MSGeno = a[2, 3]
```

```
Fstat = MSGeno / MSBlockGeno
```

```
Fstat
```

```
[1] 8.47728
```

```
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
```

```
est = gmeans[2] - gmeans[1]
```

```
names(est) = NULL
```

```
#We showed previously that the variance of  
#the difference between genotype means  
#is  $2 * E(\text{MS\_block} * \text{geno}) / (\text{nblocks} * \text{nferts})$ 
```

```
#Thus, we compute a standard error as
```

```
se = sqrt(2 * MSBlockGeno / (4 * 4))
```

```
lower = est - qt(.975, a[4, 1]) * se
```

```
upper = est + qt(.975, a[4, 1]) * se
```

```
c(estimate = est, se = se, lower = lower, upper=upper)
```

estimate	se	lower	upper
24.881250	6.213881	9.676431	40.086069

```
#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  
[39] 3 4 1 2 3 4 1 2 3 4  
Levels: 1 2 3 4
```

```
g = geno
```

```
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

Random effects:

Formula: ~1 | block  
(Intercept)

StdDev: 11.08399

Formula: ~1 | g %in% block  
(Intercept) Residual

StdDev: 8.203544 6.30128

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.

**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.

**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
}
```

```
#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
#
#   $g_2 + g_2 f_2/4 + g_2 f_3/4 + g_2 f_4/4$ 
#

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
```

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

#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
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