

STAT 516 Lec 09

Randomized complete block designs

Karl Gregory

2026-04-13

Nitrogen fertilization data from Kuehl (2000)

Six nitrogen timing schedules in each of four plots in a field of wheat. Response is the nitrate content in stem tissue samples.

Display 8.1 Arrangement of Experimental Plots for the Wheat Experiment in a Randomized Complete Block Design

Irrigation Gradient
↓

<i>Block 1</i>	2 40.89	5 37.99	4 37.18	1 34.98	6 34.89	3 42.07
<i>Block 2</i>	1 41.22	3 49.42	4 45.85	6 50.15	5 41.99	2 46.69
<i>Block 3</i>	6 44.57	3 52.68	5 37.61	1 36.94	2 46.65	4 40.23
<i>Block 4</i>	2 41.90	4 39.20	6 43.29	5 40.45	3 42.91	1 39.97

Source: Dr. T. Doerge, Department of Soil and Water Science, University of Arizona.

```
ntr <- c(40.89,37.99,37.18,34.98,34.89,42.07,  
        41.22,49.42,45.85,50.15,41.99,46.69,  
        44.57,52.68,37.61,36.94,46.65,40.23,  
        41.90,39.20,43.29,40.45,42.91,39.97)  
block <- as.factor(c(1,1,1,1,1,1,2,2,2,2,2,3,3,3,3,3,4,4,4,4,4,4))  
trt <- as.factor(c(2,5,4,1,6,3,1,3,4,6,5,2,6,3,5,1,2,4,2,4,6,5,3,1))
```

Randomized complete block design (RCBD)

- ▶ EUs belong to blocks—groups of EUs homogeneous in some way.
- ▶ Each EU in a block is randomly assigned to a fixed treatment.
- ▶ All treatments appear exactly once¹ in each block.
- ▶ Purpose is to capture the between-block variability among the EUs.
- ▶ This helps us detect treatment effects with greater power.

¹Can have replication, but many RCBDs do not.

Treatment effects model for the RCBD

Assume

$$Y_{ij} = \mu + \tau_i + B_j + \varepsilon_{ij}, \quad \text{for } i = 1, \dots, a, \quad j = 1, \dots, b,$$

where

- ▶ Y_{ij} is the response of the EU in block j receiving treatment i .
- ▶ the τ_i are the fixed effects of the treatment.
- ▶ the B_j are independent $\text{Normal}(0, \sigma_B^2)$ random block effects.
- ▶ the ε_{ij} are independent $\text{Normal}(0, \sigma_\varepsilon^2)$ error/interaction terms.
- ▶ μ is an overall or baseline mean.

Define the cell means as

$$\mu_i = \mu + \tau_i, \quad i = 1, \dots, a.$$

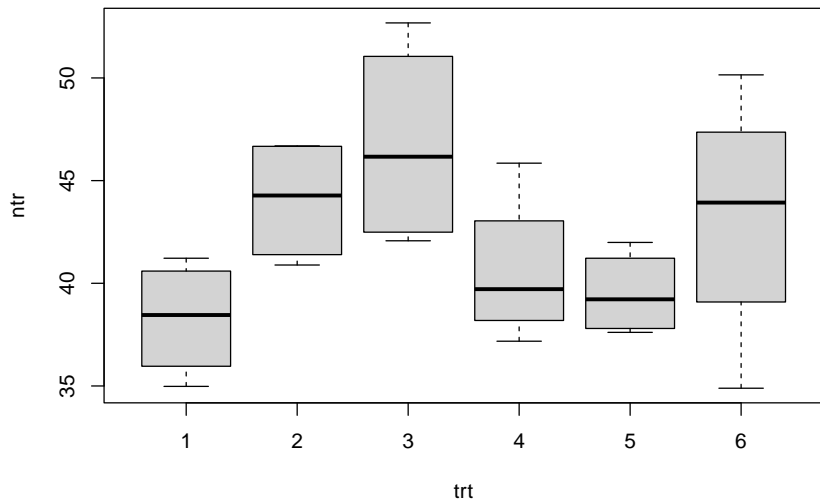
Goals in the RCBD

In the randomized complete block design we wish to

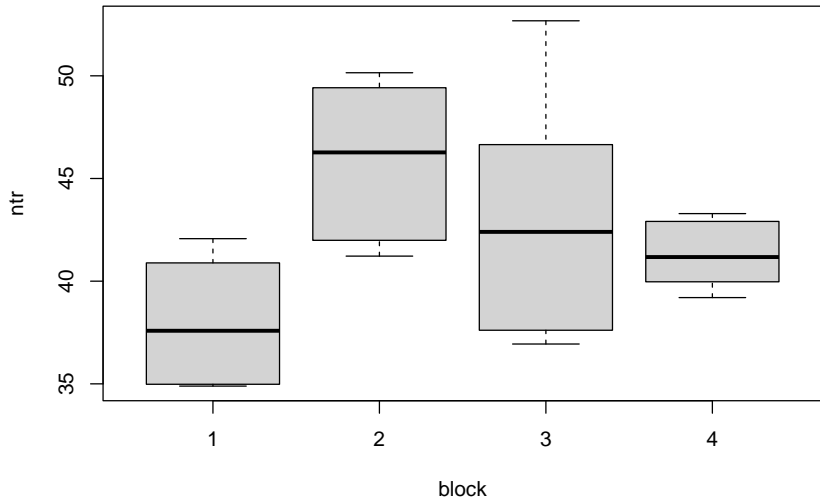
1. Visualize the data.
2. Decompose the variability in the Y_{ij} into its sources.
3. Estimate the variance components σ_B^2 and σ_ε^2 .
4. Test whether the treatment has any effect.
5. Make comparisons between treatment means.
6. Check whether the model assumptions are satisfied.

Nitrogen fertilization data (cont)

```
boxplot(ntr~trt)
```



```
boxplot(ntr~block)
```



Sums of squares for the RCBD

Sum of squares	Symbol	Formula
Total	SS_{Tot}	$\sum_{i=1}^a \sum_{j=1}^b (Y_{ij} - \bar{Y}_{..})^2$
Treatment	SS_A	$b \sum_{i=1}^a (\bar{Y}_{i.} - \bar{Y}_{..})^2$
Block	SS_B	$a \sum_{j=1}^b (\bar{Y}_{.j} - \bar{Y}_{..})^2$
Error	SS_{Error}	$\sum_{i=1}^a \sum_{j=1}^b (Y_{ij} - (\bar{Y}_{i.} + \bar{Y}_{.j} - \bar{Y}_{..}))^2$

- ▶ We can make the decomposition $SS_{\text{Tot}} = SS_A + SS_B + SS_{\text{Error}}$.
- ▶ The SS_{Error} is really the interaction sum of squares SS_{AB} .
- ▶ But without replication, we cannot estimate an interaction.
- ▶ So the interaction serves as the error term.

ANOVA table for RCBD

Source	Df	SS	MS	F value
A	$a - 1$	SS_A	MS_A	$F_A = MS_A / MS_{\text{Error}}$
B	$b - 1$	SS_B	MS_B	$F_B = MS_B / MS_{\text{Error}}$
Error	$(a - 1)(b - 1)$	SS_{Error}	MS_{Error}	
Total	$ab - 1$	SS_{Tot}		

1. Reject $H_0: \mu_1 = \dots = \mu_a$ if $F_A > F_{a-1, (a-1)(b-1), \alpha}$.
2. Reject $H_0: \sigma_B^2 = 0$ if $F_B > F_{b-1, (a-1)(b-1), \alpha}$.

Nitrogen fertilization data (cont)

```
lm_out <- lm(ntr ~ trt + block) # do not include the interaction
anova_out <- anova(lm_out)
anova_out
```

Analysis of Variance Table

Response: ntr

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
trt	5	201.32	40.263	5.5917	0.004191	**
block	3	197.00	65.668	9.1198	0.001116	**
Residuals	15	108.01	7.201			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
y <- ntr
y.. <- predict(lm(ntr ~ 1))
yi. <- predict(lm(ntr ~ trt))
y.j <- predict(lm(ntr ~ block))

SSA <- sum((yi. - y..)^2)
SSB <- sum((y.j - y..)^2)
SSE <- sum((y - (yi. + y.j - y..))^2)

a <- 6
b <- 4

MSA <- SSA/(a-1)
MSB <- SSB/(b-1)
MSE <- SSE/((a-1)*(b-1))

FA <- MSA / MSE
FB <- MSB / MSE

pA <- 1 - pf(FA, a-1, (a-1)*(b-1))
pB <- 1 - pf(FB, b-1, (a-1)*(b-1))
```

Expected mean squares in the RCBD

Source	Df	Expected mean square
A	$a - 1$	$b\theta_A^2 + \sigma_\varepsilon^2$
B	$b - 1$	$a\sigma_B^2 + \sigma_\varepsilon^2$
Error	$(a - 1)(b - 1)$	σ_ε^2

In the above $\theta_A^2 = (a - 1)^{-1} \sum_{i=1}^a (\mu_i - \bar{\mu})^2$.

Method of moments for variance components in RCBD

Equating MS_B and MS_{Error} with their expectations gives

$$\blacktriangleright \dot{\sigma}_\varepsilon^2 = MS_{\text{Error}}.$$

$$\blacktriangleright \dot{\sigma}_B^2 = \frac{MS_B - MS_{\text{Error}}}{a}$$

May obtain $\dot{\sigma}_B^2 < 0$, so one should use REML estimation.

Nitrogen fertilization data (cont)

Obtain REML estimators of σ_B^2 and σ_ε^2 on the fertilization data.

```
library(lmerTest) # first time run install.packages("lmerTest")
lmer_out <- lmer(ntr ~ trt + (1|block))
lmer_out
```

Linear mixed model fit by REML ['lmerModLmerTest']

Formula: ntr ~ trt + (1 | block)

REML criterion at convergence: 101.5658

Random effects:

Groups	Name	Std.Dev.
block	(Intercept)	3.122
	Residual	2.683

Number of obs: 24, groups: block, 4

Fixed Effects:

(Intercept)	trt2	trt3	trt4	trt5	trt6
38.278	5.755	8.492	2.337	1.232	4.947

Obtain MoMs estimators for σ_B^2 and σ_ε^2 on the fertilization data.

```
sg_B <- sqrt((MSB - MSE)/a)
sg_e <- sqrt(MSE)
```

We have $\hat{\sigma}_B = 3.122$ and $\hat{\sigma}_\varepsilon = 2.683$.

Variances of some means and difference in means

Contrast	Variance	MoM variance estimator
$\bar{Y}_i.$	$\frac{1}{b}(\sigma_B^2 + \sigma_\varepsilon^2)$	$\frac{2}{ab}[\text{MS}_B + (b - 1) \text{MS}_{\text{Error}}]$
$\bar{Y}_i. - \bar{Y}_{i'}. $	$\frac{2}{b}\sigma_\varepsilon^2$	$\frac{2}{b} \text{MS}_{\text{Error}}$

Some (unadjusted) CIs in RCB split plot design

Target	$(1 - \alpha)100\%$ confidence interval
--------	---

$$\mu_i \quad \bar{Y}_{i.} \pm t_{\nu^*, \alpha/2} \sqrt{MS_B + (b-1) MS_{\text{Error}}} \sqrt{\frac{2}{ab}}$$

$$\mu_i - \mu_{i'} \quad \bar{Y}_{i.} - \bar{Y}_{i'}. \pm t_{(a-1)(b-1), \alpha/2} \sqrt{MS_{\text{Error}}} \sqrt{\frac{2}{b}}$$

In the above $\nu^* = \frac{MS_B + (b-1) MS_{\text{Error}}}{\frac{MS_B^2}{(b-1)} + \frac{(b-1)^2 MS_{\text{Error}}^2}{(a-1)(b-1)}}$ à la Satterthwaite².

²a degrees of freedom approximation when one has not exactly a t-distribution.

Nitrogen fertilization data (cont)

Unadjusted CIs with `ls_means()` from R package `lmerTest`

```
ls_means(lmer_out)
```

Least Squares Means table:

	Estimate	Std. Error	df	t value	lower	upper	Pr(> t)	
trt1	38.2775	2.0582	6.8	18.597	33.3789	43.1761	4.505e-07	***
trt2	44.0325	2.0582	6.8	21.393	39.1339	48.9311	1.767e-07	***
trt3	46.7700	2.0582	6.8	22.724	41.8714	51.6686	1.179e-07	***
trt4	40.6150	2.0582	6.8	19.733	35.7164	45.5136	3.033e-07	***
trt5	39.5100	2.0582	6.8	19.196	34.6114	44.4086	3.646e-07	***
trt6	43.2250	2.0582	6.8	21.001	38.3264	48.1236	2.000e-07	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Confidence level: 95%

Degrees of freedom method: Satterthwaite

```
ls_means(lmer_out, pairwise = TRUE)
```

Least Squares Means table:

	Estimate	Std. Error	df	t value	lower	upper	Pr(> t)	
trt1 - trt2	-5.7550	1.8974	15	-3.0330	-9.7993	-1.7107	0.0083887	**
trt1 - trt3	-8.4925	1.8974	15	-4.4758	-12.5368	-4.4482	0.0004443	***
trt1 - trt4	-2.3375	1.8974	15	-1.2319	-6.3818	1.7068	0.2369413	
trt1 - trt5	-1.2325	1.8974	15	-0.6496	-5.2768	2.8118	0.5257996	
trt1 - trt6	-4.9475	1.8974	15	-2.6075	-8.9918	-0.9032	0.0198026	*
trt2 - trt3	-2.7375	1.8974	15	-1.4427	-6.7818	1.3068	0.1696521	
trt2 - trt4	3.4175	1.8974	15	1.8011	-0.6268	7.4618	0.0918200	.
trt2 - trt5	4.5225	1.8974	15	2.3835	0.4782	8.5668	0.0308031	*
trt2 - trt6	0.8075	1.8974	15	0.4256	-3.2368	4.8518	0.6764617	
trt3 - trt4	6.1550	1.8974	15	3.2438	2.1107	10.1993	0.0054516	**
trt3 - trt5	7.2600	1.8974	15	3.8262	3.2157	11.3043	0.0016523	**
trt3 - trt6	3.5450	1.8974	15	1.8683	-0.4993	7.5893	0.0813776	.
trt4 - trt5	1.1050	1.8974	15	0.5824	-2.9393	5.1493	0.5689729	
trt4 - trt6	-2.6100	1.8974	15	-1.3755	-6.6543	1.4343	0.1891597	
trt5 - trt6	-3.7150	1.8974	15	-1.9579	-7.7593	0.3293	0.0691104	.

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Confidence level: 95%

Degrees of freedom method: Satterthwaite

Multiple comparisons of treatment means in the RCBD

- ▶ Tukey's for comparing all pairs of means among μ_1, \dots, μ_a :

$$\bar{Y}_{i.} - \bar{Y}_{i' .} \pm q_{a,(a-1)(b-1),\alpha} \sqrt{\text{MS}_{\text{Error}}} \sqrt{\frac{1}{b}}, \quad 1 \leq i < i' \leq a.$$

- ▶ Dunnett's for comparing μ_2, \dots, μ_a to a baseline μ_1 :

$$\bar{Y}_{i.} - \bar{Y}_{1.} \pm d_{a,(a-1)(b-1),\alpha} \sqrt{\text{MS}_{\text{Error}}} \sqrt{\frac{2}{b}}, \quad i = 2, \dots, a.$$

Nitrogen fertilization data (cont)

Compare all pairs of fertilizers with Tukey's CIs for mean differences.

```
alpha <- 0.05
a <- 6
b <- 4
MSE <- anova_out$`Mean Sq`[3]
se <- sqrt(MSE) * sqrt(2/b)
me <- qtuikey(1-alpha,a,(a-1)*(b-1)) / sqrt(2) * se
ntr_means <- aggregate(ntr, by = list(trt), mean)$x

CIs <- matrix(NA,choose(a,2),2)
comp <- numeric(choose(a,2))

k <- 1
for(i in 1:(a-1))
  for(j in (i+1):a){ # double loop takes us through all pairs

    dij <- ntr_means[i] - ntr_means[j]
    CIs[k,] <- c(dij - me, dij + me)
    comp[k] <- paste(i,"-",j)
    k <- k + 1

  }

colnames(CIs) <- c("lower","upper")
rownames(CIs) <- comp
```

```
round(CIs,3)
```

	lower	upper
1 - 2	-11.920	0.410
1 - 3	-14.657	-2.328
1 - 4	-8.502	3.827
1 - 5	-7.397	4.932
1 - 6	-11.112	1.217
2 - 3	-8.902	3.427
2 - 4	-2.747	9.582
2 - 5	-1.642	10.687
2 - 6	-5.357	6.972
3 - 4	-0.010	12.320
3 - 5	1.095	13.425
3 - 6	-2.620	9.710
4 - 5	-5.060	7.270
4 - 6	-8.775	3.555
5 - 6	-9.880	2.450

Compare all fertilizers to fertilizer 1 using Dunnett's method.

```
alpha <- 0.05
a <- 6
b <- 4
MSE <- anova_out$`Mean Sq`[3]
me <- 2.82 * sqrt(MSE) * sqrt(2/b) # value 2.82 from Dunnett's table
ntr_means <- aggregate(ntr, by = list(trt), mean)$x

CIs <- matrix(NA,a-1,2)

k <- 1
for(i in 2:a){

  di <- ntr_means[i] - ntr_means[1]
  CIs[k,] <- c(di - me, di + me)
  k <- k + 1

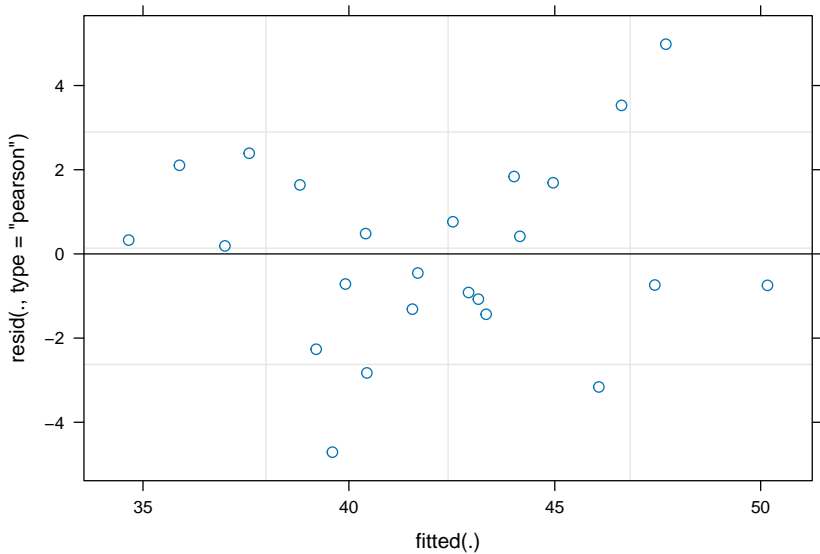
}

colnames(CIs) <- c("lower", "upper")
rownames(CIs) <- paste(2:a, "- 1")
```

```
round(CIs,3)
```

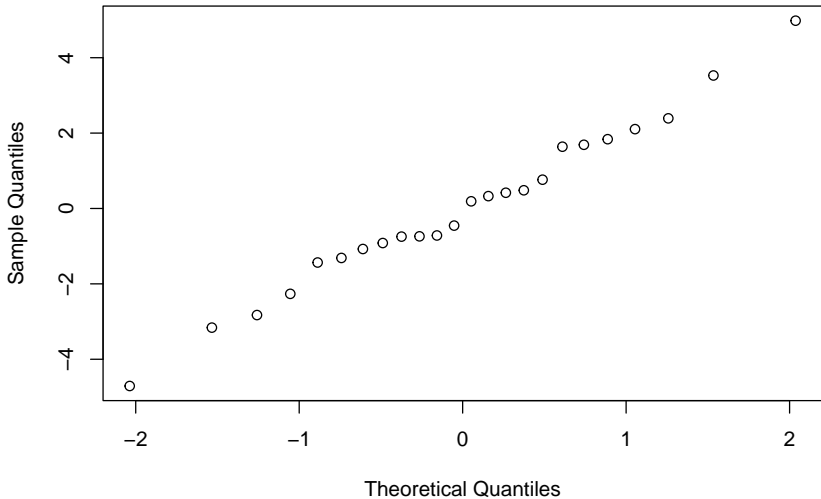
```
      lower upper  
2 - 1  0.404 11.106  
3 - 1  3.142 13.843  
4 - 1 -3.013  7.688  
5 - 1 -4.118  6.583  
6 - 1 -0.403 10.298
```

```
plot(lmer_out)
```



```
yhat <- predict(lmer_out)
ehat <- ntr - yhat
qqnorm(ehat)
```

Normal Q-Q Plot



Ignoring the blocks in the nitrogen data

If we ignore the blocks, the design looks like a one-way ANOVA:

Treatment	1	2	3	4	5	6
	34.98	40.89	42.07	37.18	37.99	34.89
	41.22	46.69	49.42	45.85	41.99	50.15
	36.94	46.65	52.68	40.23	37.61	44.57
	39.97	41.90	43.29	39.20	40.45	43.29

Suppose we fit $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, $i = 1, 2, 3, 4, 5, 6$, $j = 1, 2, 3, 4$.

We lose power to detect a treatment effect!

```
anova(lm(ntr ~ trt))
```

Analysis of Variance Table

Response: ntr

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	5	201.32	40.263	2.3761	0.08024 .
Residuals	18	305.01	16.945		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Cabbage counts versus nitrogen, Kuehl (2000)

Heads of cabbage in subplots of two field plots.

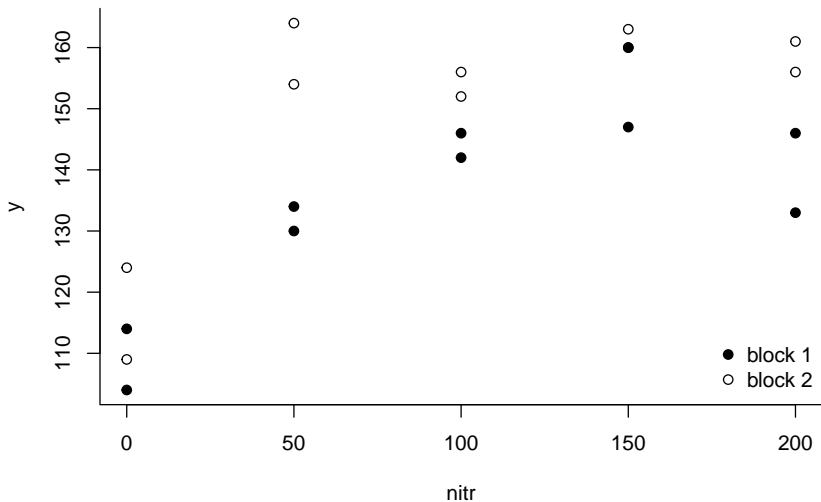
<i>Nitrogen</i>	<i>Block 1</i>		<i>Block 2</i>	
0	104	114	109	124
50	134	130	154	164
100	146	142	152	156
150	147	160	160	163
200	133	146	156	161

Source: Dr. W.D. Pew, Department of Plant Sciences,
University of Arizona.

Subplots in each plot randomly assigned to nitrogen levels.

```
y <- c(104,114,109,124,  
      134,130,154,164,  
      146,142,152,156,  
      147,160,160,163,  
      133,146,156,161)  
  
nitr <- as.factor(c(0,0,0,0,  
                   50,50,50,50,  
                   100,100,100,100,  
                   150,150,150,150,  
                   200,200,200,200))  
  
blk <- as.factor(c(1,1,2,2,  
                  1,1,2,2,  
                  1,1,2,2,  
                  1,1,2,2,  
                  1,1,2,2))
```

```
ylims <- range(y)
par(bty = "l")
stripchart(y~nitr, subset=blk==1, vertical=TRUE, pch=19, ylim=ylims,xlab="nitr")
stripchart(y~nitr, subset=blk==2, vertical=TRUE, pch=1, add=TRUE)
legend("bottomright",legend = c("block 1", "block 2"),pch = c(19, 1),bty="n")
```



Treatment effects model for RCBD with replication

Assume

$$Y_{ijk} + \mu + \tau_i + B_j + (\tau B)_{ij} + \varepsilon_{ijk}$$

for $i = 1, \dots, a$, $j = 1, \dots, b$, and $k = 1, \dots, n_{ij}$, where

- ▶ Y_{ijk} is response of EU k in block j receiving treatment i .
- ▶ the τ_i are the fixed effects of the treatment.
- ▶ the B_j are independent $\text{Normal}(0, \sigma_B^2)$ random block effects.
- ▶ the $(\tau B)_{ij}$ are indep. $\text{Normal}(0, \sigma_{AB}^2)$ random interaction effects.
- ▶ the ε_{ijk} are independent $\text{Normal}(0, \sigma_\varepsilon^2)$ error terms.
- ▶ μ is an overall or baseline mean.

Define the cell means as $\mu_i = \mu + \tau_i$, $i = 1, \dots, a$.

Assume for now a balanced design: $n_{ij} = n$ for all i, j .

Sums of squares for RCBD with replication

Sum of squares	Symbol	Formula
Total	SS_{Tot}	$\sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - \bar{Y}_{...})^2$
Treatment	SS_A	$nb \sum_{i=1}^a (\bar{Y}_{i..} - \bar{Y}_{...})^2$
Block	SS_B	$na \sum_{j=1}^b (\bar{Y}_{.j.} - \bar{Y}_{...})^2$
Block \times Treatment	SS_{AB}	$n \sum_{i=1}^a \sum_{j=1}^b (\bar{Y}_{ij.} - (\bar{Y}_{i..} + \bar{Y}_{.j.} - \bar{Y}_{...}))^2$
Error	SS_{Error}	$\sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - \bar{Y}_{ij.})^2$

► Can make the decomposition $SS_{\text{Tot}} = SS_A + SS_B + SS_{AB} + SS_{\text{Error}}$.

ANOVA table for RCBD with replication

Source	Df	SS	MS	F value
A	$a - 1$	SS_A	MS_A	$F_A = MS_A / MS_{AB}$
B	$b - 1$	SS_B	MS_B	$F_B = MS_B / MS_{AB}$
AB	$(a - 1)(b - 1)$	SS_{AB}	MS_{AB}	$F_{AB} = MS_{AB} / MS_{Error}$
Error	$ab(n - 1)$	SS_{Error}	MS_{Error}	
Total	$abn - 1$	SS_{Tot}		

1. Reject $H_0: \mu_1 = \dots = \mu_a$ if $F_A > F_{a-1, (a-1)(b-1), \alpha}$.
2. Reject $H_0: \sigma_B^2 = 0$ if $F_B > F_{b-1, (a-1)(b-1), \alpha}$.
3. Reject $H_0: \sigma_{AB}^2 = 0$ if $F_{AB} > F_{(a-1)(b-1), ab(n-1), \alpha}$.

Expected MS in RCBD with replication

Source	Df	Expected mean square
A	$a - 1$	$nb\theta_A^2 + n\sigma_{AB}^2 + \sigma_\varepsilon^2$
B	$b - 1$	$na\sigma_B^2 + n\sigma_{AB}^2 + \sigma_\varepsilon^2$
AB	$(a - 1)(b - 1)$	$n\sigma_{AB}^2 + \sigma_\varepsilon^2$
Error	$ab(n - 1)$	σ_ε^2

In the above $\theta_A^2 = (a - 1)^{-1} \sum_{i=1}^a (\mu_i - \bar{\mu})^2$.

Check expected MS values under each H_0 on previous slide.

ANOVA table for cabbage count data

```
a <- nlevels(nitr)
b <- nlevels(blk)
n <- 2

y... <- predict(lm(y ~ 1))
yi.. <- predict(lm(y ~ nitr))
y.j. <- predict(lm(y ~ blk))
yij. <- predict(lm(y ~ nitr + blk + nitr:blk))

SST <- sum((y - y...)**2)
SSA <- sum((yi.. - y...)**2)
SSB <- sum((y.j. - y...)**2)
SSAB <- sum((yij. - (yi.. + y.j. - y...))**2)
SSE <- sum((y - yij. )**2)

MSA <- SSA / (a-1)
MSB <- SSB / (b-1)
MSAB <- SSAB / ((a-1)*(b-1))
MSE <- SSE / (a*b*(n-1))

FA <- MSA / MSAB
FB <- MSB / MSAB
FAB <- MSAB / MSE

pA <- 1 - pf(FA, a-1, (a-1)*(b-1))
pB <- 1 - pf(FB, b-1, (a-1)*(b-1))
pAB <- 1 - pf(FAB, (a-1)*(b-1), a*b*(n-1))
```

ANOVA table for cabbage counts data

Source	Df	SS	MS	F value	p value
A	4	4813.00	1203.25	16.7234	0.0092
B	1	1022.45	1022.45	14.2106	0.0196
AB	4	287.80	71.95	1.7030	0.2253
Error	10	422.50	42.25		
Total	19	6545.75			

ANOVA table for cabbage count data

Note that `anova()` on `lm()` output does not give desired output:

```
anova(lm(y ~ nitr + blk + nitr:blk))
```

Analysis of Variance Table

Response: y

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
nitr	4	4813.0	1203.25	28.479	1.915e-05 ***
blk	1	1022.5	1022.45	24.200	0.0006054 ***
nitr:blk	4	287.8	71.95	1.703	0.2253096
Residuals	10	422.5	42.25		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Method of moments for estimating σ_B^2 , σ_{AB}^2 , and σ_ε^2

Set the mean squares equal to their expectations and solve:

$$MS_B \stackrel{\text{set}}{=} na\sigma_B^2 + n\sigma_{AB}^2 + \sigma_\varepsilon^2$$

$$MS_{AB} \stackrel{\text{set}}{=} n\sigma_{AB}^2 + \sigma_\varepsilon^2$$

$$MS_{\text{Error}} \stackrel{\text{set}}{=} \sigma_\varepsilon^2$$

Solve for σ_B^2 , σ_{AB}^2 , and σ_ε^2 :

$$\dot{\sigma}_B^2 = \frac{1}{na}(MS_B - MS_{AB})$$

$$\dot{\sigma}_{AB}^2 = \frac{1}{n}(MS_{AB} - MS_{\text{Error}})$$

$$\dot{\sigma}_\varepsilon^2 = MS_{\text{Error}}$$

Possible to obtain negative values for $\dot{\sigma}_B^2$ and $\dot{\sigma}_{AB}^2$. Then use REML.

MOM estimates of cabbage data variance components

```
sigma_B_sq <- (MSB - MSAB) / (n*a)
sigma_AB_sq <- (MSAB - MSE) / n
sigma_e_sq <- MSE

sigma_B <- sqrt(sigma_B_sq)
sigma_AB <- sqrt(sigma_AB_sq)
sigma_e <- sqrt(sigma_e_sq)
```

We obtain $\hat{\sigma}_B = 9.749$, $\hat{\sigma}_{AB} = 3.854$, and $\hat{\sigma}_\epsilon = 6.500$.

REML estimates of cabbage data variance components

```
library(lmerTest) # first time run install.packages("lmerTest")
lmer_out <- lmer(y ~ nitr + (1|blk) + (1|nitr:blk))
lmer_out
```

Linear mixed model fit by REML ['lmerModLmerTest']

Formula: y ~ nitr + (1 | blk) + (1 | nitr:blk)

REML criterion at convergence: 110.9695

Random effects:

Groups	Name	Std.Dev.
	nitr:blk (Intercept)	3.854
	blk (Intercept)	9.749
	Residual	6.500

Number of obs: 20, groups: nitr:blk, 10; blk, 2

Fixed Effects:

(Intercept)	nitr50	nitr100	nitr150	nitr200
112.75	32.75	36.25	44.75	36.25

CIs (unadjusted) for diffs in means in RCBD with rep.

- ▶ A $(1 - \alpha)100\%$ confidence interval for $\mu_i - \mu_{i'}$ is

$$\bar{Y}_{i..} - \bar{Y}_{i'..} \pm t_{(a-1)(b-1), \alpha/2} \sqrt{MS_{AB}} \sqrt{\frac{2}{nb}}$$

for $1 \leq i < i' \leq a$.

- ▶ If $(a - 1)(b - 1) = 1$, this interval will be frightfully wide.
- ▶ In this case one should drop the interaction term from the model!

CI for difference in means for cabbage count data

Compare the means of the nitrogen level 50 and 0 groups:

```
y50.. <- mean(y[nitr == "50"])
y0.. <- mean(y[nitr == "0"])
alpha <- 0.05
tval <- qt(1 - alpha/2, (a-1)*(b-1))
me <- tval * sqrt(MSAB) * sqrt(2/(n*b))
lo <- y50.. - y0.. - me
up <- y50.. - y0.. + me
c(lo,up)
```

```
[1] 16.09711 49.40289
```

CI (unadjusted) for parameters in RCBD with replication

Use `confint()` on output of `lmer()`:

```
confint(lmer_out)
```

	2.5 %	97.5 %
.sig01	0.000000	6.253288
.sig02	2.715017	29.859582
.sigma	4.422824	9.062168
(Intercept)	95.101400	130.398323
nitr50	23.245458	42.254543
nitr100	26.745458	45.754543
nitr150	35.245458	54.254543
nitr200	26.745458	45.754543

These CIs are *not* adjusted to achieve a familywise coverage probability!

RCBD with replication *without* an interaction term

If we omit the interaction term in the RCBD with replication, we have

$$Y_{ijk} + \mu + \tau_i + B_j + \varepsilon_{ijk}$$

for $i = 1, \dots, a$, $j = 1, \dots, b$, and $k = 1, \dots, n_{ij}$, where

- ▶ Y_{ijk} is response of EU k in block j receiving treatment i .
- ▶ the τ_i are the fixed effects of the treatment.
- ▶ the B_j are independent $\text{Normal}(0, \sigma_B^2)$ random block effects.
- ▶ the ε_{ijk} are independent $\text{Normal}(0, \sigma_\varepsilon^2)$ error terms.
- ▶ μ is an overall or baseline mean.

Assume for now a balanced design: $n_{ij} = n$ for all i, j .

Use this model if $a = b = 2$, under which $(a - 1)(b - 1) = 1$.

Sums of squares for RCBD with rep., no interaction

SS	Symbol	Formula
Total	SS_{Tot}	$\sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - \bar{Y}_{...})^2$
Treatment	SS_A	$nb \sum_{i=1}^a (\bar{Y}_{i..} - \bar{Y}_{...})^2$
Block	SS_B	$na \sum_{j=1}^b (\bar{Y}_{.j.} - \bar{Y}_{...})^2$
Error	SS_{Error}	$\sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - (\bar{Y}_{i..} + \bar{Y}_{.j.} - \bar{Y}_{...}))^2$

► Can make the decomposition $SS_{\text{Tot}} = SS_A + SS_B + SS_{\text{Error}}$.

ANOVA table for RCBD with rep., omitting interaction

Source	Df	SS	MS	F value
A	$a - 1$	SS_A	MS_A	$F_A = MS_A / MS_{\text{Error}}$
B	$b - 1$	SS_B	MS_B	$F_B = MS_B / MS_{\text{Error}}$
Error	$abn - a - b + 1$	SS_{Error}	MS_{Error}	
Total	$abn - 1$	SS_{Tot}		

1. Reject $H_0: \mu_1 = \dots = \mu_a$ if $F_A > F_{a-1, abn-a-b+1, \alpha}$.
2. Reject $H_0: \sigma_B^2 = 0$ if $F_B > F_{b-1, abn-a-b+1, \alpha}$.

Expected MS in RCBD with rep., omitting interaction

Source	Df	Expected mean square
A	$a - 1$	$nb\theta_A^2 + \sigma_\varepsilon^2$
B	$b - 1$	$na\sigma_B^2 + \sigma_\varepsilon^2$
Error	$abn - a - b + 1$	σ_ε^2

In the above $\theta_A^2 = (a - 1)^{-1} \sum_{i=1}^a (\mu_i - \bar{\mu})^2$.

ANOVA table for cabbage counts, omitting interaction

```
a <- nlevels(nitr)
b <- nlevels(blk)
n <- 2

y... <- predict(lm(y ~ 1))
yi.. <- predict(lm(y ~ nitr))
y.j. <- predict(lm(y ~ blk))

SST <- sum((y - y...)**2)
SSA <- sum((yi.. - y...)**2)
SSB <- sum((y.j. - y...)**2)
SSE <- sum((y - (yi.. + y.j. - y...))**2)

MSA <- SSA / (a-1)
MSB <- SSB / (b-1)
MSE <- SSE / (a*b*n - a - b + 1)

FA <- MSA / MSE
FB <- MSB / MSE

pA <- 1 - pf(FA, a-1, a*b*n - a - b + 1)
pB <- 1 - pf(FB, b-1, a*b*n - a - b + 1)
```

ANOVA table for cabbage counts, omitting interaction

Source	Df	SS	MS	F value	p value
A	4	4813.00	1203.25	23.7160	0.0000
B	1	1022.45	1022.45	20.1525	0.0005
Error	14	710.30	50.74		
Total	19	6545.75			

ANOVA table for cabbage counts, omitting interaction

With the interaction term omitting, `anova()` on `lm()` output is correct:

```
anova(lm(y ~ nitr + blk))
```

Analysis of Variance Table

Response: y

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
nitr	4	4813.0	1203.25	23.716	4.13e-06 ***
blk	1	1022.5	1022.45	20.152	0.0005097 ***
Residuals	14	710.3	50.74		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

M.o.M. for σ_B and σ_ε^2 when omitting interaction

Set the mean squares equal to their expectations and solve:

$$\begin{aligned}MS_B &\stackrel{\text{set}}{=} na\sigma_B^2 + \sigma_\varepsilon^2 \\MS_{\text{Error}} &\stackrel{\text{set}}{=} \sigma_\varepsilon^2\end{aligned}$$

Solve for σ_B^2 and σ_ε^2 :

$$\begin{aligned}\dot{\sigma}_B^2 &= \frac{1}{na}(MS_B - MS_{\text{Error}}) \\ \dot{\sigma}_\varepsilon^2 &= MS_{\text{Error}}\end{aligned}$$

Possible to obtain a negative value for $\dot{\sigma}_B^2$. In this case use REML.

M.o.M.s for cabbage data when omitting interaction

```
sigma_B_sq <- (MSB - MSE) / (n*a)
sigma_e_sq <- MSE

sigma_B <- sqrt(sigma_B_sq)
sigma_e <- sqrt(sigma_e_sq)
```

We obtain $\hat{\sigma}_B = 9.858$ and $\hat{\sigma}_\varepsilon = 7.123$.

REML for cabbage data when omitting interaction

```
library(lmerTest) # first time run install.packages("lmerTest")
lmer_out <- lmer(y ~ nitr + (1|blk))
lmer_out
```

Linear mixed model fit by REML ['lmerModLmerTest']

Formula: y ~ nitr + (1 | blk)

REML criterion at convergence: 111.4024

Random effects:

Groups	Name	Std.Dev.
blk	(Intercept)	9.858
	Residual	7.123

Number of obs: 20, groups: blk, 2

Fixed Effects:

(Intercept)	nitr50	nitr100	nitr150	nitr200
112.75	32.75	36.25	44.75	36.25

CI (unadjusted) for diffs in means in RCBD with rep. without interaction

- ▶ A $(1 - \alpha)100\%$ confidence interval for $\mu_i - \mu_{i'}$ is

$$\bar{Y}_{i..} - \bar{Y}_{i'..} \pm t_{abn-a-b+1, \alpha/2} \sqrt{MS_{\text{Error}}} \sqrt{\frac{2}{nb}}$$

for $1 \leq i < i' \leq a$.

CI for difference in means for cabbage count data when omitting interaction

Compare the means of the nitrogen level 50 and 0 groups:

```
y50.. <- mean(y[nitr == "50"])
y0.. <- mean(y[nitr == "0"])
alpha <- 0.05
tval <- qt(1 - alpha/2, a*b*n - a - b + 1)
me <- tval * sqrt(MSE) * sqrt(2/(n*b))
lo <- y50.. - y0.. - me
up <- y50.. - y0.. + me
c(lo, up)
```

```
[1] 21.94746 43.55254
```

CI for difference in means for cabbage count data when omitting interaction

Can use `ls_means()` as below:

```
ls_means(lmer_out, pairwise=T)
```

Least Squares Means table:

	Estimate	Std. Error	df	t value	lower	upper	Pr(> t)
nitr0 - nitr50	-32.7500	5.0367	14	-6.5023	-43.5525	-21.9475	1.396e-05
nitr0 - nitr100	-36.2500	5.0367	14	-7.1972	-47.0525	-25.4475	4.585e-06
nitr0 - nitr150	-44.7500	5.0367	14	-8.8849	-55.5525	-33.9475	3.957e-07
nitr0 - nitr200	-36.2500	5.0367	14	-7.1972	-47.0525	-25.4475	4.585e-06
nitr50 - nitr100	-3.5000	5.0367	14	-0.6949	-14.3025	7.3025	0.49849
nitr50 - nitr150	-12.0000	5.0367	14	-2.3825	-22.8025	-1.1975	0.03192
nitr50 - nitr200	-3.5000	5.0367	14	-0.6949	-14.3025	7.3025	0.49849
nitr100 - nitr150	-8.5000	5.0367	14	-1.6876	-19.3025	2.3025	0.11362
nitr100 - nitr200	0.0000	5.0367	14	0.0000	-10.8025	10.8025	1.00000
nitr150 - nitr200	8.5000	5.0367	14	1.6876	-2.3025	19.3025	0.11362

```
nitr0 - nitr50    ***
nitr0 - nitr100   ***
nitr0 - nitr150   ***
nitr0 - nitr200   ***
nitr50 - nitr100
nitr50 - nitr150  *
nitr50 - nitr200
nitr100 - nitr150
nitr100 - nitr200
nitr150 - nitr200
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Confidence level: 95%

Degrees of freedom method: Satterthwaite

CI for difference in means for cabbage count data when omitting interaction

Compare above to `confint()` on output from `lmer()`.

```
confint(lmer_out)
```

	2.5 %	97.5 %
.sig01	2.715987	29.862871
.sigma	4.678640	9.062171
(Intercept)	95.101978	130.398015
nitr50	23.558217	41.941783
nitr100	27.058217	45.441783
nitr150	35.558217	53.941783
nitr200	27.058217	45.441783

Skin response data, Mohr, Wilson, and Freund (2021)

Galvanic skin responses of five subjects under shock and noise stimuli.

		SUBJECT				
Noise	Shock	1	2	3	4	5
40	0.25	3	7	9	4	1
40	0.50	5	11	13	8	3
40	0.75	9	12	14	11	5
40	1.00	6	11	12	7	4
80	0.25	5	10	10	6	3
80	0.50	6	12	15	9	5
80	0.75	18	18	15	13	9
80	1.00	7	15	14	9	7

```
skin <- data.frame(resp = c(3,7,9,4,1,5,11,13,8,3,9,12,14,11,5,6,11,12,7,4,5,  
                           10,10,6,3,6,12,15,9,5,18,18,15,13,9,7,15,14,9,7),  
                  noise = as.factor(c(rep(40,20),rep(80,20))),  
                  shock = as.factor(rep(c(rep(.25,5),rep(.5,5),  
                                          rep(.75,5),rep(1,5)),2)),  
                  subj = as.factor(rep(1:5,8)))
```

```
head(skin,n=20)
```

	resp	noise	shock	subj
1	3	40	0.25	1
2	7	40	0.25	2
3	9	40	0.25	3
4	4	40	0.25	4
5	1	40	0.25	5
6	5	40	0.5	1
7	11	40	0.5	2
8	13	40	0.5	3
9	8	40	0.5	4
10	3	40	0.5	5
11	9	40	0.75	1
12	12	40	0.75	2
13	14	40	0.75	3
14	11	40	0.75	4
15	5	40	0.75	5
16	6	40	1	1
17	11	40	1	2
18	12	40	1	3
19	7	40	1	4
20	4	40	1	5

Treatment effects model for two-way factorial RCBD

Assume

$$Y_{ijk} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij} + C_k + \varepsilon_{ijk},$$

for $i = 1, \dots, a$, $j = 1, \dots, b$, and $k = 1, \dots, c$, where

- ▶ Y_{ijk} is the response in block k under treatment combination $i \times j$.
- ▶ μ is an overall or baseline mean.
- ▶ the τ_i are fixed effects for factor A.
- ▶ the γ_j are fixed effects for factor B.
- ▶ the $(\tau\gamma)_{ij}$ are effects for the A×B interaction.
- ▶ the C_k are independent $\text{Normal}(0, \sigma_C^2)$ block effects.
- ▶ the ε_{ijk} are independent $\text{Normal}(0, \sigma_\varepsilon^2)$ error terms.

Define the cell means as

$$\mu_{ij} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij}, \quad i = 1, \dots, a, \quad j = 1, \dots, b.$$

Sums of squares for the two-way factorial RCBD

Sum of squares	Symbol	Formula
Total	SS_{Tot}	$\sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^c (Y_{ijk} - \bar{Y}_{...})^2$
A	SS_A	$bc \sum_{i=1}^a (\bar{Y}_{i..} - \bar{Y}_{...})^2$
B	SS_B	$ac \sum_{j=1}^b (\bar{Y}_{.j.} - \bar{Y}_{...})^2$
AB	SS_{AB}	$c \sum_{i=1}^a \sum_{j=1}^b (Y_{ij.} - (\bar{Y}_{i..} + \bar{Y}_{.j.} - \bar{Y}_{...}))^2$
C	SS_C	$ab \sum_{k=1}^c (\bar{Y}_{..k} - \bar{Y}_{...})^2$
Error	SS_{Error}	$SS_{\text{Tot}} - (SS_A + SS_B + SS_{AB} + SS_C)$

- ▶ Then we have $SS_{\text{Tot}} = SS_A + SS_B + SS_{AB} + SS_C + SS_{\text{Error}}$.
- ▶ The error SS_{Error} is really the interaction sum of squares $SS_{\text{Trt} \times C}$.
- ▶ Again, without replication, we cannot estimate this interaction.
- ▶ So the interaction serves as the error term.

ANOVA table for two-way factorial RCBD

Source	Df	SS	MS	F value
A	$a - 1$	SS_A	MS_A	$F_A = MS_A / MS_{\text{Error}}$
B	$b - 1$	SS_B	MS_B	$F_B = MS_B / MS_{\text{Error}}$
AB	$(a - 1)(b - 1)$	SS_{AB}	MS_{AB}	$F_{AB} = MS_{AB} / MS_{\text{Error}}$
C	$c - 1$	SS_C	MS_C	$F_C = MS_C / MS_{\text{Error}}$
Error	$(ab - 1)(c - 1)$	SS_{Error}	MS_{Error}	
Total	$abc - 1$	SS_{Tot}		

1. Reject $H_0: \mu_{1.} = \dots = \mu_{a.}$ if $F_A > F_{a-1, (ab-1)(c-1), \alpha}$.
2. Reject $H_0: \mu_{.1} = \dots = \mu_{.b}$ if $F_B > F_{b-1, (ab-1)(c-1), \alpha}$.
3. R. $H_0: \mu_{ij} = \bar{\mu}_{i.} + \bar{\mu}_{.j} - \bar{\mu}_{..} \forall ij$ if $F_{AB} > F_{(a-1)(b-1), (ab-1)(c-1), \alpha}$.
4. Reject $H_0: \sigma_C^2 = 0$ if $F_C > F_{c-1, (ab-1)(c-1), \alpha}$.

Skin response data (cont)

```
lm_out <- lm(resp ~ noise + shock + noise:shock + subj, data = skin)
anova(lm_out)
```

Analysis of Variance Table

Response: resp

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
noise	1	65.02	65.025	28.3819	1.134e-05	***
shock	3	219.27	73.092	31.9028	3.564e-09	***
subj	4	361.85	90.463	39.4848	3.975e-11	***
noise:shock	3	12.67	4.225	1.8441	0.1621	
Residuals	28	64.15	2.291			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
y <- skin$resp
y... <- predict(lm(resp ~ 1,data = skin))
yi.. <- predict(lm(resp ~ noise,data=skin))
y.j. <- predict(lm(resp ~ shock,data=skin))
y..k <- predict(lm(resp ~ subj,data=skin))
yij. <- predict(lm(resp ~ noise + shock + noise:shock,data=skin))
```

```
SSA <- sum((yi.. - y...)^2)
SSB <- sum((y.j. - y...)^2)
SSC <- sum((y..k - y...)^2)
SSAB <- sum((yij. - (yi.. + y.j. - y...))^2)
SST <- sum((y - y...)^2)
SSE <- SST - (SSA + SSB + SSC + SSAB)
```

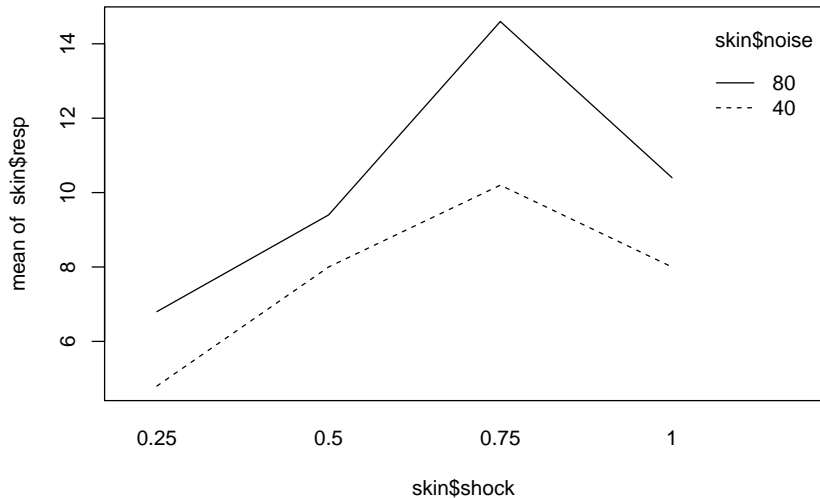
```
a <- 2
b <- 4
c <- 5
```

```
MSA <- SSA/(a-1)
MSB <- SSB/(b-1)
MSC <- SSC/(c-1)
MSAB <- SSAB/((a-1)*(b-1))
MSE <- SSE/((a*b-1)*(c-1))
```

```
FA <- MSA / MSE
FB <- MSB / MSE
FAB <- MSAB / MSE
FC <- MSC / MSE
```

```
pA <- 1 - pf(FA,a-1,(a*b-1)*(c-1))
pB <- 1 - pf(FB,b-1,(a*b-1)*(c-1))
pAB <- 1 - pf(FAB,(a-1)*(b-1),(a*b-1)*(c-1))
pC <- 1 - pf(FC,c-1,(a*b-1)*(c-1))
```

```
interaction.plot(skin$shock,skin$noise,skin$resp)
```



Expected mean squares in factorial RCBD

Source	Df	Expected mean square
A	$a - 1$	$bc\theta_A^2 + \sigma_\varepsilon^2$
B	$b - 1$	$ac\theta_B^2 + \sigma_\varepsilon^2$
AB	$(a - 1)(b - 1)$	$c\theta_{AB}^2 + \sigma_\varepsilon^2$
C	$c - 1$	$ab\sigma_C^2 + \sigma_\varepsilon^2$
Error	$(ab - 1)(c - 1)$	σ_ε^2

In the above

$$\blacktriangleright \theta_A^2 = (a - 1)^{-1} \sum_{i=1}^a (\bar{\mu}_{i.} - \bar{\mu}_{..})^2$$

$$\blacktriangleright \theta_B^2 = (b - 1)^{-1} \sum_{j=1}^b (\bar{\mu}_{.j} - \bar{\mu}_{..})^2$$

$$\blacktriangleright \theta_{AB}^2 = [(a - 1)(b - 1)]^{-1} \sum_{i=1}^a \sum_{j=1}^b (\mu_{ij} - (\bar{\mu}_{i.} + \bar{\mu}_{.j} - \bar{\mu}_{..}))^2$$

MoMs for variance components in two-way factorial RCBD

- ▶ Equating MS_C and MS_{Error} with their expectations gives

$$\hat{\sigma}_C^2 = \frac{MS_C - MS_{\text{Error}}}{ab} \quad \text{and} \quad \hat{\sigma}_\varepsilon^2 = MS_{\text{Error}}.$$

- ▶ May obtain $\hat{\sigma}_C^2 < 0$, so one should use REML estimation.

Skin response data (cont)

Obtain REML estimators of σ_C^2 and σ_ε^2 on the skin response data.

```
lmer_out <- lmer(resp ~ noise + shock + noise:shock + (1|subj), data = skin)
lmer_out
```

Linear mixed model fit by REML ['lmerModLmerTest']

Formula: resp ~ noise + shock + noise:shock + (1 | subj)

Data: skin

REML criterion at convergence: 144.9199

Random effects:

Groups	Name	Std.Dev.
subj	(Intercept)	3.320
	Residual	1.514

Number of obs: 40, groups: subj, 5

Fixed Effects:

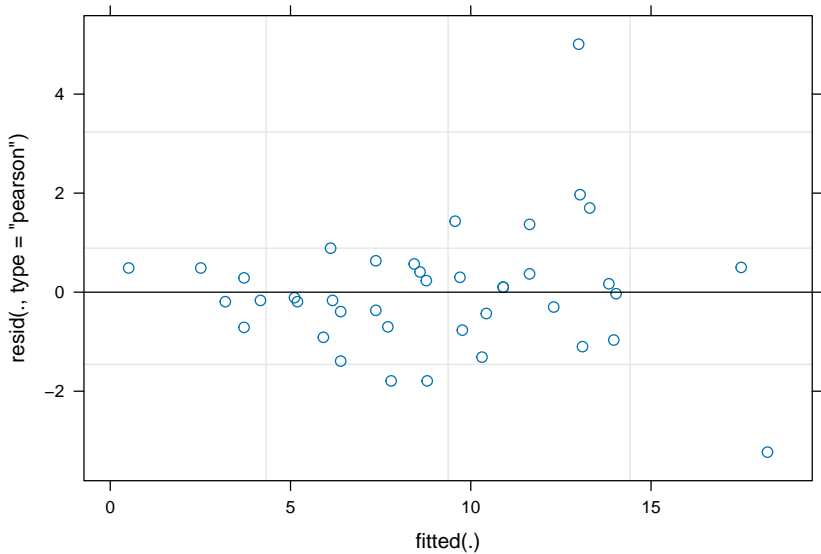
(Intercept)		noise80	shock0.5	shock0.75
4.8		2.0	3.2	5.4
shock1	noise80:shock0.5	noise80:shock0.75		noise80:shock1
3.2	-0.6	2.4		0.4

Obtain MoMs estimators for σ_C^2 and σ_ε^2 on the skin response data.

```
sg_C <- sqrt((MSC - MSE)/(a*b))  
sg_e <- sqrt(MSE)
```

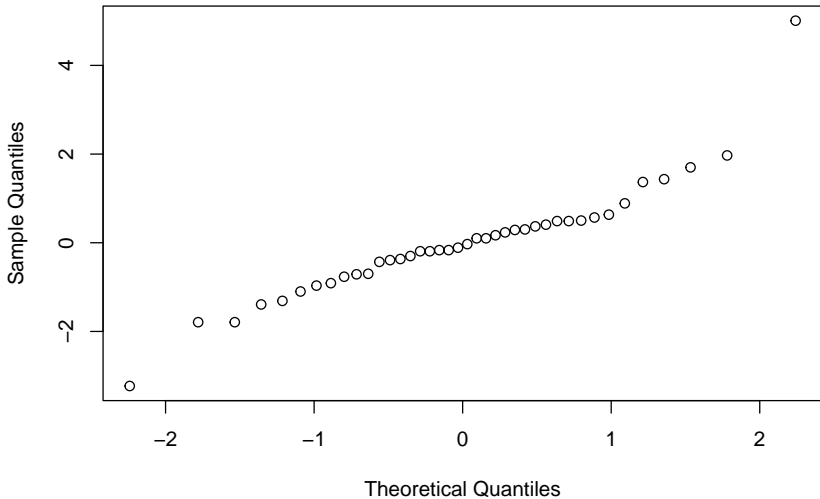
We have $\hat{\sigma}_C = 3.320$ and $\hat{\sigma}_\varepsilon = 1.514$.

```
plot(lmer_out)
```



```
yhat <- predict(lmer_out)
ehat <- skin$resp - yhat
qqnorm(ehat)
```

Normal Q-Q Plot



References

Kuehl, R. O. 2000. *Design of Experiments: Statistical Principles of Research Design and Analysis*. Duxbury/Thomson Learning.

Mohr, Donna L, William J Wilson, and Rudolf J Freund. 2021. *Statistical Methods*. Academic Press.