

Two-Way ANOVA

We've learned about One-Way Analysis of Variance (ANOVA) previously, it is a regression model for one continuous outcome and one categorical variable. It allows us to compare the means of the groups to detect significant differences.

A one-way fixed-effects ANOVA is an extension of the two-sample t-test. There are **factors**, categorical variables, and **levels**, individual groups of the factor which represent different populations. Balanced design contain the same number of individuals in each level. **This is a special case of linear regression.**

Assumptions of one-way ANOVA:

- The data are random samples from k independent populations
- Within each population the dependent variable is normally distributed
- The observations are independent
- The population variance of the dependent variable is equal in all groups (homoscedasticity)

H_0 : The mean level is independent of the factor

Ex. The mean testosterone level is independent of smoking history. A conclusion might be "there is evidence that testosterone level is significantly different in at least two of the smoking history groups."

We test the hypothesis by decomposing the overall variance into "explained" and "residual" variances and comparing them.

1. **SS Between Groups:** Variability of the group means from the general mean

$$SS_B = \sum_{i=1}^k \sum_{j=1}^{n_i} (\bar{y}_i - \bar{y})^2 = \sum_{i=1}^k n_i (\bar{y}_i - \bar{y})^2$$

2. **SS Within Groups:** Overall variability of the outcome from the group means

$$SS_W = \sum_{i=1}^k \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2$$

SS Total = SS Between Groups + SS Within Groups

Where N is the total sample size and k is the number of groups

SSB represents the variability due to the treatment effect. SSW represents the residual variability.

ANOVA TABLE

Source	Sums of Squares	df	Mean Square	F
Between Groups (Model)	SS Between	k-1	SSB / (k-1)	MSB / MSW
Within Groups (Error)	SS Within	N-k	SSW / (N-k)	
Total	SS Total	N-1		

We compare the F statistic to the critical value of F with [(k - 1), (N - k)] degrees of freedom to test the null hypothesis of equal means.

Multiple Comparisons Procedures

To find which means are different from each other we should make all pairwise comparisons ($k(k-1)/2$). This introduces the multiple tests issues and increases likeliness of type I and II errors. Multiple comparison procedures take into account the total number of comparisons being made by lowering the significance level for each test so that the overall significance level is still fixed at some level alpha.

Tukey's adjustment is appropriate when comparing pairs of means. It tests all pairwise comparisons and provides adjusted p-values to control the family-wise error rate.

Scheffe's adjustment is appropriate for general contrasts.

- ✓ For example we might want to contrast the mean of the combined never/former smokers vs. heavy or light smokers.

Bonferroni's adjustment is appropriate for any situation, but can be too conservative.

- ✓ For all pairwise comparisons, the Bonferroni adjustment divides the α level by the number of possible pairwise comparisons $k(k-1)/2$

All of these adjustments are appropriate when comparing pairs of means. Tukey's is the most powerful and provides exact p-values when the group sizes are equal. Scheffe's procedure is more powerful than Bonferroni's procedure, in general.

We can also write ANOVA as a linear regression model where **the parameter α_i are the group effects** (difference of effect between group i and group 1)

Two Way ANOVA

Two way ANOVA is a regression model for one continuous outcome and **two** categorical variables. We analyze two-way ANOVA experiments using additive and interaction models.

Critical assumptions:

- Observations at different factor levels are samples from normal distributions
- The variances of the different populations are the same

Column factor		Trial									
		Drug	Mutation	y _{i1}	y _{i2}	y _{i3}					
Row factor	No mutation	Drug dosage				1	0	no	4.4	4.5	4.3
		0%	10%	20%	30%	2	10		4.6	4.5	4.8
		4.4	4.6	4.5	4.6	3	20		4.5	4.8	4.8
		4.5	4.5	4.8	4.7	4	30		4.6	4.7	5.1
		4.3	4.8	4.8	5.1	5	0	yes	3.3	3.2	3.1
		3.3	3.8	5.0	5.4	6	10		3.8	3.7	3.6
	Mutation	3.2	3.7	5.3	5.6	7	20		5.0	5.3	4.8
		3.1	3.6	4.8	5.3	8	30		5.4	5.6	5.3

Both are the same data where the two factors are drug dosage and mutation, and we have 3 replications.

We can create summaries of the means:

$$y_{ijk} = \begin{cases} i = 1, \dots, r \text{ row} \\ j = 1, \dots, c \text{ column} \\ k = 1, \dots, n_{ij} \text{ replication} \end{cases}$$

$$n = \sum_{ij} n_{ij} : \text{sample size}$$

$$n_i = \sum_j n_{ij} : \text{sample size of level } i$$

$$n_j = \sum_i n_{ij} : \text{sample size of level } j$$

$$\bar{y}_{ij} = \frac{\sum_k y_{ijk}}{n_{ij}} \quad \bar{y}_{i\cdot} = \frac{\sum_k y_{ijk}}{n_i}$$

treatment mean Row means
joint effect mutation effect

$$\bar{y}_{\cdot j} = \frac{\sum_i y_{ijk}}{n_j} \quad \bar{y} = \frac{\sum_{ijk} y_{ijk}}{n}$$

Column means overall mean
drug effect

Possible Models

Interaction model: The effect of each factor changes as the other factor levels vary

$$y_{ijk} = \mu + \alpha_i + \beta_j + \delta_{ij} + \varepsilon_{ijk}, i = 1, \dots, r, j = 1, \dots, c, k = 1, \dots, n_{ij}$$

$$\alpha_1 = \beta_1 = \delta_{11} = 0$$

α_i =effect of row factor; β_j =effect of column factor; δ_{ij} =interaction

Additive model: The effect of each factor doesn't change as the other factor levels vary

$$y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}, i = 1, \dots, r, j = 1, \dots, c, k = 1, \dots, n_{ij}$$

$$\alpha_1 = \beta_1 = 0$$

α_i =effect of row factor; β_j =effect of column factor;

We test different models using ANOVA to look at the global effect of the factors

Sum of Squares

The traditional one-way ANOVA uses a decomposition of the sum of squares for analysis.

$$\text{Total } SS = \sum_{ijk} (y_{ijk} - \bar{y})^2 = SS(\hat{y}) + RSS$$

$$SS(\hat{y}) = SS(\alpha) + SS(\beta) + SS(\gamma)$$

$$RSS = \sum_{ijk} (y_{ijk} - \bar{y}_{ij})^2$$

Each sum of squares uses a number of degrees of freedom, given by number of different levels - 1

Total SS $n-1$ dof

$SS(\alpha)$ $r-1$;

$SS(\beta)$ $c-1$

$SS(\gamma)$ $(r-1)(c-1)$

$SS(\hat{y})$ $(r-1) + (c-1) + (r-1)(c-1)$

RSS $(n-1) - (r-1) - (c-1) - (r-1)(c-1)$

Source	DF	SS	MS	F=ratio
Model	rc-1	SS(y)	MST=SS(y)/[rc-1]	MST/MSE
Row	r-1	SS(a)	MSR=SS(a)/(r-1)	
Column	c-1	SS(b)	MSC=SS(b)/(c-1)	
Interaction	(r-1)(c-1)	SS(g)	MSI=SS(g)/[(r-1)(c-1)]	MSI/MSE
Error	rc(m-1)	RSS	MSE=RSS/[rc(m-1)]	
Total	n-1	SS		

$$SS(y) = \text{model } SS = SS(a) + SS(b) + SS(g)$$

These sum of squares are computed sequentially, so **the order of terms in the model matters!**

If the design is balanced the order does not matter.

Steps

1. Goodness of fit/Global null hypothesis

H_0 : All parameters = 0

H_a : At least one differs from 0

2. Test interaction

- F test is $MSI/MSE \sim F_{(r-1)(c-1), rc(m-1)}$
- The P-value is $p(F_{(r-1)(c-1), rc(m-1)} > F \mid H_0)$
- If the p-value is smaller than our alpha then we reject the null

- If we do not reject the null, there is no evidence that all interaction terms are not 0.
Fit an additive model and repeat

3. Test main effects

- If we accept the null hypothesis of no interaction, it makes sense to test the significance of the main effects, to do this we pool the RSS with $SS(y)$
- $SS(a)$ and $SS(b)$ are Type III sum of squares

Once a model is accepted one can do mean comparisons to identify the factor levels with different effects (Tukey's).

The balance design means the the design is orthogonal and so there is no need to recompute sum of squares or estimates for different models. If not, analyze the data as a traditional linear model.

Problems

When the design is orthogonal/balanced the decomposition $SS(a) + SS(b) + SS(g)$ is unique, when it is not the decomposition depends on the order.

$Y \sim A + B + AB$

Type I (sequential) - in `aov()`, `anova()`

incremental improvement in the error SS as each effect is added to the model

$SS(A)$, $SS(B|A)$, $SS(AB|A,B)$

Type II (hierarchical)

reduction in error SS due to adding the term to the model after all other terms except those that contain it

$SS(A|B)$, $SS(B|A)$

Type III (orthogonal) – `Anova()` function in `car` package

reduction in error SS due to adding the term after all other terms have been added to the model

$SS(A|B, AB)$, $SS(B|A, AB)$, $SS(AB|A, B)$

R Code

```
##### Two-way ANOVA
##### drug dataset
drug <- read.csv("anova.csv", header=T)
drug

hbf <- c(t(drug[,3:5]))
SNP <- c(rep("No",12), rep("Yes", 12))
Drug <- c(rep(0,3), rep(10,3), rep(20,3), rep(30,3),
         rep(0,3), rep(10,3), rep(20,3), rep(30,3))
data.drug <- data.frame(hbf,SNP, Drug)
head(data.drug)
```

```
### summaries
overall.mean <- mean(hbf); overall.mean
drug.means <- tapply(hbf, Drug, mean); drug.means
snps.means <- tapply(hbf, SNP, mean); snps.means
cell.means <- tapply(hbf, interaction(Drug,SNP), mean); cell.means
```

```
dim(cell.means) <- c(4,2); cell.means
cell.means <- data.frame(cell.means)
row.names(cell.means) <- levels(factor(Drug))
names(cell.means) <- levels(factor(SNP))
cell.means
```

```
## Visualization
#install.packages("gplots")
library(gplots)
plotmeans(hbf~Drug, data=data.drug,xlab="Drug",
  ylab="HbF", main="Mean Plot\n with 95% CI")
plotmeans(hbf~SNP, data=data.drug,xlab="SNP",
  ylab="HbF", main="Mean Plot\n with 95% CI")
plotmeans(hbf~interaction(Drug,SNP), data=data.drug,
  xlab="SNP", connect=list(1:4,5:8),ylab="HbF",
  main="Interaction Plot\nwith 95% CI")
```

```
interaction.plot(factor(Drug), factor(SNP), hbf, type="b",
  xlab="Drug", ylab="hbf", main="Interaction Plot")
```

```
### two-way anova with balanced design
mod <- aov(hbf~as.factor(Drug)*SNP, data=data.drug)
summary(mod)
table(mod$fitted.values)
```

```
# TukeyHSD(mod)
mod <- lm(hbf~as.factor(Drug)*SNP, data=data.drug)
summary(mod)
anova(mod)
table(mod$fitted.values)
```

```
##### Exercise with balance design
```

```
mod.a <- aov(hbf~as.factor(Drug)*SNP, data=data.drug)
summary(mod.a)## anova(mod.a)
mod.lma <- lm(hbf~as.factor(Drug)*SNP, data=data.drug)
anova(mod.lma)
```

```
mod.b <- aov(hbf~SNP*as.factor(Drug), data=data.drug)
summary(mod.b)## anova(mod.b)
mod.lmb <- lm(hbf~SNP*as.factor(Drug), data=data.drug)
anova(mod.lmb)
```

Exercise with unbalance design

```
data.drug.1 <- read.csv("data.drug.1.csv", header=T)
mod.1a <- aov(hbf~as.factor(Drug)*SNP, data=data.drug.1)
summary(mod.1a)
mod.1lma <- lm(hbf~as.factor(Drug)*SNP, data=data.drug.1)
anova(mod.1lma)
```

```
mod.1b <- aov(hbf~SNP*as.factor(Drug), data=data.drug.1)
summary(mod.1b)
mod.1lmb <- lm(hbf~SNP*as.factor(Drug), data=data.drug.1)
anova(mod.1lmb)
```

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