Experimental Designs and Data Analyses

Designing experiments

- Experimental terminology
- Comparing treatments
- Avoiding bias
- Types of randomized designs
- Ethics and experimentation

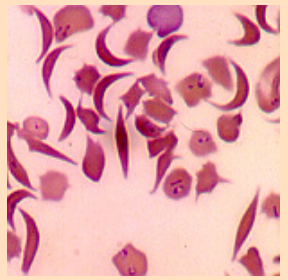
Terminology

- The **individuals** in an experiment are the **experimental units.** If they are human, we call them **subjects.**
- The explanatory variables in an experiment are often called **factors**.
- A **treatment** is any specific experimental condition applied to the subjects. If an experiment has several factors, a treatment is a combination of specific values of each factor.
  - The factor may be the administration of a drug.
  - One group of people may be placed on a diet/exercise program for 6 months (treatment), and their blood pressure (response variable) would be compared with that of people who did not diet or exercise.

- If the experiment involves giving two different doses of a drug, we say that we are testing two **levels** of the factor.
- A response to a treatment is **statistically significant** if it is larger than you would expect by chance (due to random variation among the subjects). We will learn how to determine this later.

In a study of sickle cell anemia, 150 patients were given the drug hydroxyurea, and 150 were given a placebo (dummy pill). The researchers counted the episodes of pain in each subject. Identify:

•The subjects (patients, all 300)
•The factors/treatments (hydroxyurea and placebo)
•And the response variable (episodes of pain)



Comparing treatments

Experiments are comparative in nature: We compare the response to a treatment versus to:

- another treatment
- no treatment (a control)
- a placebo
- or any combination of the above

A **control** is a situation in which no treatment is administered. It serves as a reference mark for an actual treatment (e.g., a group of subjects does not receive any drug or pill of any kind).

A **placebo** is a fake treatment, such as a sugar pill. It is used to test the hypothesis that the response to the treatment is due to the actual treatment and not to how the subject is being taken care of.

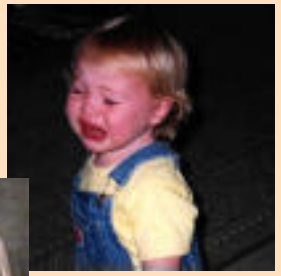
About the placebo effect

The “placebo effect” is an improvement in health due not to any treatment but only to the patient’s belief that he or she will improve.

- The placebo effect is not understood, but it is believed to have therapeutic results on up to a whopping 35% of patients.
- It can sometimes ease the symptoms of a variety of ills, from asthma to pain to high blood pressure and even to heart attacks.
- An opposite, or “negative placebo effect,” has been observed when patients believe their health will get worse.

The most famous and perhaps most powerful placebo is the “kiss,” blow, or hug—whatever your technique.

Unfortunately, the effect gradually disappears once children figure out that they sometimes get better without help, and vice versa.



Avoiding bias

The best way to exclude bias in an experiment is to **randomize** the design. Both the individuals and treatments are assigned randomly.

> Bacterial resistance to antibiotics is studied at several temperatures. Which plates are grown at which temperature is assigned randomly.

When a potentially confounding variable cannot be randomized, it can be fixed or **controlled** instead.

> All experiments on bacterial resistance to antibiotics are performed at the same temperature. Temperature is controlled and will not influence results. However, results may not apply to other temperatures.

Designing "controlled" experiments

Sir Ronald Fisher—The "father of statistics"
He was sent to Rothamsted Agricultural Station in the UK to evaluate the success of various fertilizer treatments.

Fisher found the data from experiments going on for decades to be basically worthless because of poor experimental design.

- Fertilizer had been applied to a field one year and not in another, to compare the yield of grain produced in the two years.
- Or fertilizer was applied to one field and not to a nearby field in the same year.

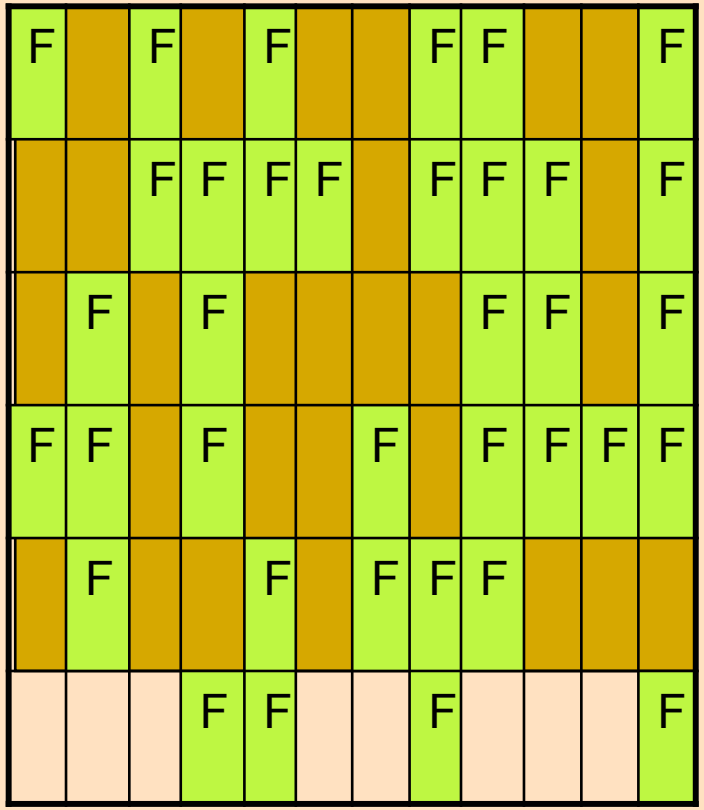
➔ Too many factors affecting the results were "uncontrolled"

Fisher's solution:

Randomized comparative experiments

- In the same field and same year, apply fertilizer to randomly spaced plots within the field. Analyze plants from similarly treated plots together.

- This minimizes the effect of variation of drainage and soil composition within the field on yield as well as controlling for weather.



Bias is a particularly challenging problem when dealing with human subjects because of the placebo effect. A **double-blind** experiment is one in which neither the subjects nor the experimenter know which individuals got which treatment until the experiment is completed.

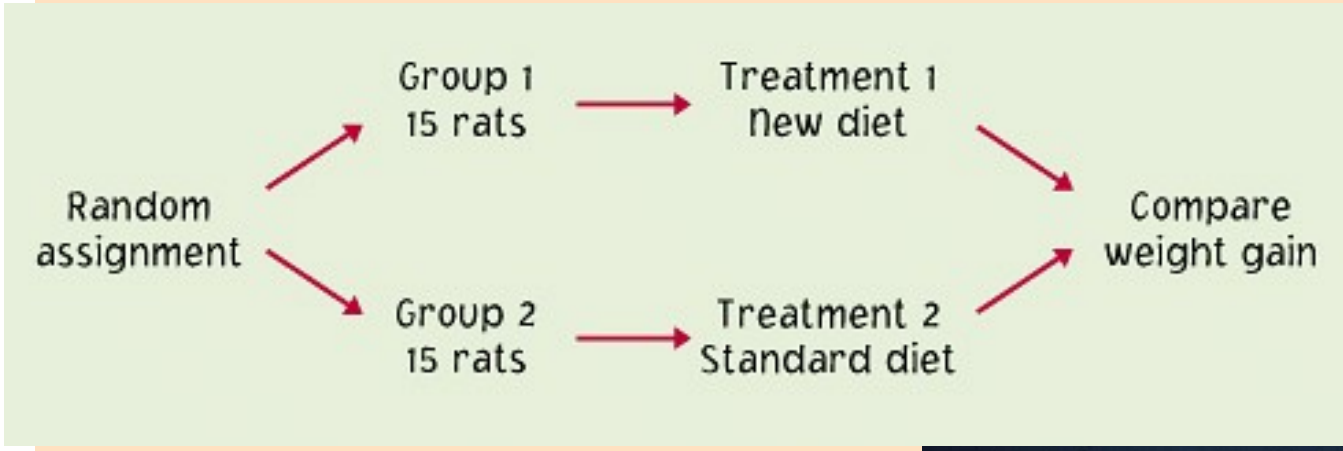
However, subjects must be informed that they will get one of a number of treatments and must consent to that condition (it would be unethical otherwise).

Another way to make sure your conclusions are robust is to **replicate** your experiment—do it over. Replication ensures that particular results are not due to uncontrolled factors or errors of manipulation.

Types of randomized designs

Completely randomized experimental designs

In a **completely randomized experimental design,** individuals are randomly assigned to groups, then the groups are randomly assigned to treatments.



Matched pairs designs

- Choose **pairs of subjects** that are closely matched (like twins). Within each pair, *randomly* assign who will receive which treatment.

- **Or** give the two treatments to a **single person over time**, in *random* order. In this case the "matched pair" is just the same person at different points in time.

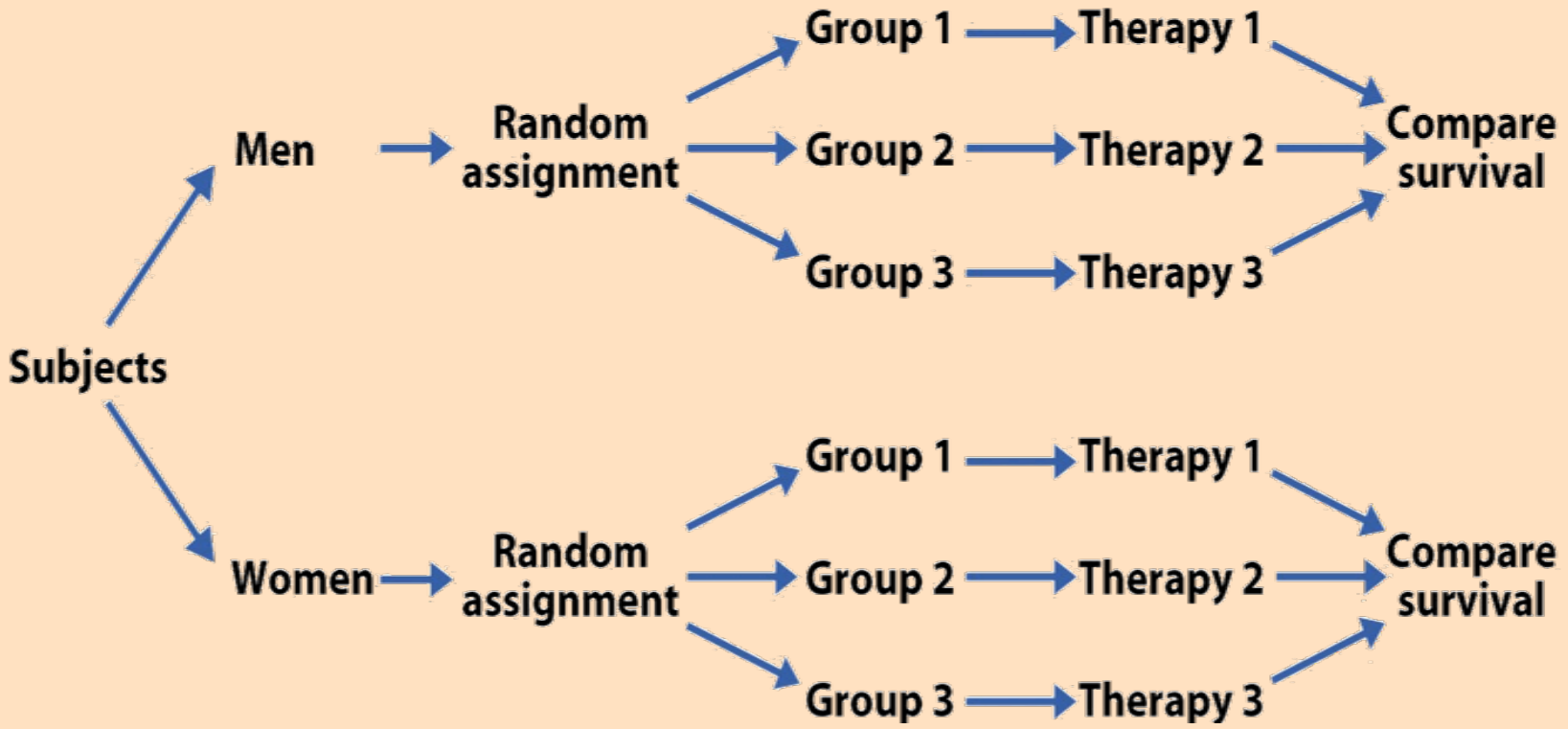
Generics are brand-name drugs manufactured by a different company but with identical active ingredients and properties.

Individuals are given either Brand X or its generic version one day so that drug absorption can be measured. One week later, each individual receives the other drug to measure drug absorption. A difference in absorption extent between Brand X and its generic is then calculated for each individual.

Block designs

In a **block** design, subjects are divided into groups, or blocks, prior to the experiment to test hypotheses about differences between the groups.

The blocking, or stratification, here is by gender.



What experimental design?

A researcher wants to see if there is a significant difference in resting pulse rates for men and women. Twenty-eight men and twenty-four women had their pulse rate measured at rest in the lab.

- One factor, two levels (male and female)
- Stratified/block design (by gender)

Many dairy cows now receive injections of BST, a hormone intended to spur greater milk production. The milk production of 60 Ayrshire dairy cows was recorded before and after they received a first injection of BST.

- Random sample of 60 cows
- Match pair design (before and after)

Issues: Ethics

- Biology deals with life. Experimentations have an impact on live subjects and ecosystems.
- Where do we place the difference between what can physically be done and what can ethically be done.
- What rights do subjects have? Humans, animals, plants, microbes…
- Personal standards vary, and extreme experimentations have been seen.
- Committees have been established to review all research proposals.

Experimental Ethics: Part of the Design

Imagine an experiment testing how well different AIDS drugs work on infected people - should we have placebos and controls? If not, how do we know they are working?

Why do you think many US drug companies do experimental trials in developing countries?

Is it OK to kill thousands of rats to test a new potentially life-saving drug? How about to test a new hair spray?

How are experiments like the Nazi twin experiments; or the Tuskegee Syphilis Study (1932-72) allowed to happen?

(Courtesy National Archives)

Empirical Examples and Mistakes

1. **FEEDING FULL FAT OIL SEEDS TO LAYING HENS: EFFECT ON PRODUCTION PARAMETERS, EGG QUALITY, CHOLESTEROL AND EGG FATTY ACIDS**

An experiment was conducted to evaluate the effects of different dietary sources of poly unsaturated fatty acids (PUFA) on yolk cholesterol, fatty acid profile, egg quality and production performance of layers. Different PUFA sources, such as flax, canola or sunflower seed at three different levels, were fed to 300 white leghorn laying hens from 53 to 62 weeks of age.

Experiment Design

An experiment was conducted, as completely randomized; experimental units were the replicates consisting of ten layers/gp. The production performance and egg quality data thus obtained were analyzed using one way analyses of variance (ANOVA). Analysis of variance was performed by Proc GLM (SAS version 9.1, SAS Institute, USA) for a completely randomized design where treatments were taken as main effects and replicates within treatment as error term.

The following model was used:

$$Yij = \mu + \tau i + \varepsilon ij$$

Where; Yij = variable measured for the jth replicate, μ = overall mean, τi = effect as a result of the ith treatment and εij = CRD error component.

Effect of feeding different full fat oilseeds on production performance of layers

Dietary Treatment[1]	Egg prod (%)	Egg wt. (g)	Egg mass (g/d)	Feed intake (g/d/h)	Feed Conversion
Control	79.26	60.45	47.91	113.38^{abc}	2.367
Flax 5	82.14	61.42	50.42	114.30^{ab}	2.267
Flax 10	81.91	60.37	49.44	109.87^{d}	2.223
Flax 15	81.43	60.33	49.13	110.14^{cd}	2.243
Canola 5	80.14	60.97	48.86	111.89^{bcd}	2.287
Canola 10	82.73	59.74	49.43	111.19^{bcd}	2.253
Canola 15	81.72	61.2	50.01	115.42^{a}	2.307
Sunflower 5	81.9	61.34	50.3	111.84^{bcd}	2.233
Sunflower 10	81.5	61.19	49.87	114.42^{ab}	2.297
Sunflower 15	80.48	60.71	48.86	112.81^{abcd}	2.31
SEM	*1.414*	*0.512*	*1.044*	*1.018*	*0.046*
P Value	*0.822*	*0.385*	*0.825*	*0.011*	*0.556*

Empirical Examples and Mistakes

2. **EFFECT OF FEEDING FLAX AND TWO TYPES OF ANTIOXIDANTS ON EGG PRODUCTION, EGG QUALITY AND LIPID COMPOSITION OF EGGS**

A study was conducted to investigate the effects of feeding flax seed and two types of antioxidants (α-tocopherols [Toc], butylated hydroxy toluene, [BHT]) at three levels (50, 100, 150 IU or mg/kg) on production performance, egg quality, fatty acids profile, Toc, and cholesterol content.

Antioxident

- The experiment employed a completely randomized design and the experimental unit was a replicate consisting of two layers. Hen performance, egg characteristics, egg quality and lipid components were analyzed by one-way ANOVA using Proc GLM (SAS version 9.2, SAS Institute, USA, 2001) with treatments as main effects and replicates within treatment as error term.
- The following model was used: Yij= μ+ Γi + εij, where Yij = variable measured for the jth replicate, μ = overall mean, Γi = effect as a result of the ith treatment and εij = error component. Mean values along with pooled SEM are reported. Values were considered significant if P ≤ 0.05. In case of significant differences, the **Duncan multiple** range test was employed to compare differences among means (Duncan, 1955).

Effect of feeding flax with different antioxidants on production parameters from week 24 through 32

Production parameters	Dietary Treatments[1]									
	Control	Flax	Flax + 50 IU Toc	Flax + 100 IU Toc	Flax + 150 IU Toc	Flax + 50 mg BHT	Flax + 100 mg BHT	Flax + 150 mg BHT	Pooled SEM	*P*-value
Egg production (%)	97.47	95.85	97.28	97.62	96.49	96.23	97.02	96.13	0.602	0.305
Egg mas (g/hen/day)	58.76	58.06	58.23	58.97	57.32	56.89	59.72	59.43	0.724	0.101
Feed intake (g/hen/day)	120.4a	116.1[b]	115.3[b]	116.8[b]	116.4[b]	117.0[b]	117.9[ab]	116.3[b]	0.894	0.011
Feed conversion[2]	2.05	2	1.98	1.98	2.03	2.05	1.98	1.96	0.027	0.098

Empirical Examples and Mistakes

3. EFFECT OF INCORPORATING DESIGNER EGGS IN HUMAN DIET ON LIPID PROFILE, BLOOD GLUCOSE AND BLOOD PRESSURE

Twenty volunteers were selected from the healthy normolipidemic and normotensive students of University of……. All volunteers were female undergraduate students residing in a dorm. Same diet was given to all subjects throughout the experiment. Personal data of volunteers is summarized in the Table…

Data was analyzed by analysis of variance (ANOVA) by using the statistical analysis system (SAS) to check whether there were any significant effects of feeding control or/and designer eggs on the serum lipid profile, blood glucose, and blood pressure (Steel *et al*. 1997).

Female Volunteers

	Group A		Group B		Group C		Group D	
	day 0	*day 21*	*day 0*	*day 21*	*day 0*	*day 21*	*day 0*	*day 21*
				mg/dl				
Serum Cholest	163.4 ±18.72	166.6 ±24.28	168.2 ±22.35	176.8 ±23.76	162 ±24.35	167.2 ±22.39	166.4 ±7.83	169.6 ±21.66
HDL Cholest	51.4 ±6.47	52 ±4.85	52.2 ±6.46	51.6 ±3.13	50 ±6.12	55.8 ±6.42	49.8 ±10.66	54 ±4.85
LDL Cholest	92 ±22.30	95.4 ±20.09	95.6 ±19.92	108 ±21.97	93.2 ±22.43	95 ±16.96	91.2 ±3.27	92.4 ±14.21
Serum Triglyc	119 ±16.66	122.8 ±7.29	112.2 ±14.01	121 ±14.93	118.4 ±15.95	103.8 ±18.30*	116.2 ±11.21	99.6 ±12.86*
Blood Glucose	81.6 ±4.34	83.8 ±3.49	84.2 ±7.19	83 ±4.00	82.4 ±5.55	85.4 ±4.77	83 ±6.20	80.4 ±3.91

Analysis of Variance
ANOVA

ANOVA

- Samples from different populations (treatment groups)
- Any difference among the population means?
- Null hypothesis: no difference among the means

ANOVA Examples

- Effect of different lots of vaccine on antibody titer
- Effect of different measurement techniques on serum cholesterol determination from the same pool of serum

ANOVA Examples

- Water samples drawn at various location in a city
- Effect of antihypertensive drugs and placebo on mean systolic blood pressure

ANOVA

- **Partitioning of the sum of squares**
- The fundamental technique is a partitioning of the total sum of squares into components related to the effects used in the model.

Analysis of Variance

ANOVA is a technique to differentiate between sample means to draw inferences about the presence or absence of variations between populations means.

ANOVA

- The key statistic in ANOVA is the F-test of difference of group means, testing if the means of the groups formed by values of the independent variable (or combinations of values for multiple independent variables) are different enough not to have occurred by chance.

ANOVA

- If the group means do not differ significantly then it is inferred that the independent variable(s) did not have an effect on the dependent variable.
- If the F test shows that overall the independent variable(s) is (are) related to the dependent variable, then *multiple comparison tests* of significance are used to explore just which values of the independent(s) have the most to do with the relationship.

ANOVA

- The overall test for differences among means.
- Used when we wish to determine significance among two or more means. $H_o = \mu_1 = \mu_2 = \mu_3 = M\kappa$.

Analysis of Variance

- Analysis of variance is a technique for testing the null hypothesis that one or more samples were drawn at random from the same population.
- Like “t” or “z” the analysis of variance provides us with a test of significance.
- The “F” test provides an estimate of the experimental effect and an estimate of the error terms.

Analysis of Variance

- A procedure for determining how much of the total variability among scores to attribute to various sources of variation and for testing hypotheses concerning some of the sources.

Analysis of Variance

- A ratio is then made of the two independent variance estimates. This ratio is then compared with the critical f-ratio found in the F table.

One way-Analysis of Variance

- Consider the following experimental design with **one** experimental variable – dietary intervention to reduce body weight.
- ANOVA to evaluate the reduction in weight obtained when volunteer were given 4 dietary treatments.
- Using *COMPLETELY RANDOMIZED DESIGN.*
- 1 classification variable (dietary intervention).
- Randomly assign 5 volunteers to each of the 4 treatments for a total of 20.

Assumptions of ANOVA

- Assume:
  - Observations normally distributed within each *population*
  - Population (treatment) variances are equal
    - Homogeneity of variance or homoscedasticity
  - Observations are independent

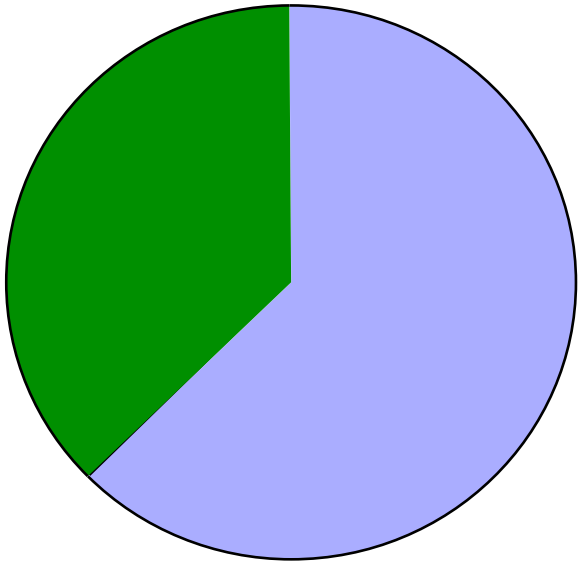
Assumptions--cont.

- Analysis of variance is generally robust
  - A robust test is one that is not greatly affected by violations of assumptions.

Logic of Analysis of Variance

- Null hypothesis (H_o): Population means from different conditions are equal
  - $m_1 = m_2 = m_3 = m_4$
- Alternative hypothesis: H_1
  - Not all population means equal.

Visualize total amount of variance in the Experiment



Total Variance = Mean Square Total

Between Group Differences
(Mean Square Group)

Error Variance
(**Individual Differences + Random Variance**)
Mean Square Error

F ratio is a proportion of the MS group/MS Error.
The larger the group differences, the bigger the F
The larger the error variance, the smaller the F

Logic--cont.

- Create a measure of variability among treatment group means
  - MS_{group}
- Create a measure of variability within treatment groups
  - MS_{error}

Logic--cont.

- Form ratio of MS_{group} /MS_{error}
  - Ratio approximately 1 if null true
  - Ratio significantly larger than 1 if null false

Calculations

- Sum of Squares (SS)
  - SS_{total}
  - SS_{groups}
  - SS_{error}
- Compute degrees of freedom (*df*)
- Compute mean squares and *F-ratio*

Cont.

Degrees of Freedom (*df*)

- Number of "observations" free to vary
  - $df_{total} = N - 1$
    - N observations
  - $df_{groups} = g - 1$
    - g means
  - $df_{error} = (n - 1)-(g-1)$
    - n observations in each group = $n - 1$ *df*
    - times g groups

ANOVA Example

- Efforts to reduce body weight:
- 4 treatment groups:

1. control;
2. diet;
3. physical activity;
4. diet plus physical activity

- After 3 months body weight loss in lbs.

Example

Trt gp	wt loss in lbs						Ti	$x_i.$	T^2_i	$T^2_i/5$
T1:	5	–2	3	2	0	= 8	1.6	64	12.5	
T2:	2	8	4	12	4	= 30	6.0	900	180	
T3:	8	0	2	6	2	= 18	3.6	324	64.8	
T4:	12	6	15	8	10	= 51	0.2	2601	520.2	
Total							107		777.8	
T^2						11449				
$T^2/20$						572.4				

Treatment Mean

ANOVA COMPUTATION

Source	Sum of squares	Degrees of freedom	Mean square	Computing formula	F
Among samples	$\sum_{i=1}^{g} n_i(\bar{x}_{i\cdot} - \bar{\bar{x}})^2$	$g - 1$	$\dfrac{\sum_{i=1}^{g} n_i(\bar{x}_{i\cdot} - \bar{\bar{x}})^2}{g - 1}$	$\dfrac{\sum_{i=1}^{g} \frac{T_i^2}{n_i} - \frac{T^2}{n}}{g - 1}$	$\dfrac{\left(\sum_{i=1}^{g} \frac{T_i^2}{n_i} - \frac{T^2}{n}\right)\Big/(g-1)}{\left(\sum_{i=1}^{g}\sum_{j=1}^{n_i} x_{ij}^2 - \sum_{i=1}^{g} \frac{T_i^2}{n_i}\right)\Big/(n-g)}$
Within samples	$\sum_{i=1}^{g}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i\cdot})^2$	$n - g$	$\dfrac{\sum_{i=1}^{g}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i\cdot})^2}{n - g}$	$\dfrac{\sum_{i=1}^{g}\sum_{j=1}^{n_i} x_{ij}^2 - \sum_{i=1}^{g} \frac{T_i^2}{n_i}}{n - g}$	
Total	$\sum_{i=1}^{g}\sum_{j=1}^{n_i}(x_{ij} - \bar{\bar{x}})^2$	$n - 1$			

Example

$\sum_{j=1}^{5} x_{ij}$ = T1 = 8; T2 = 30; T3 = 18; T4 = 51

$\bar{x}_{i.}$ = 8/5=1.6; 30/5=6; 18/5=3.6; 51/5=10.2

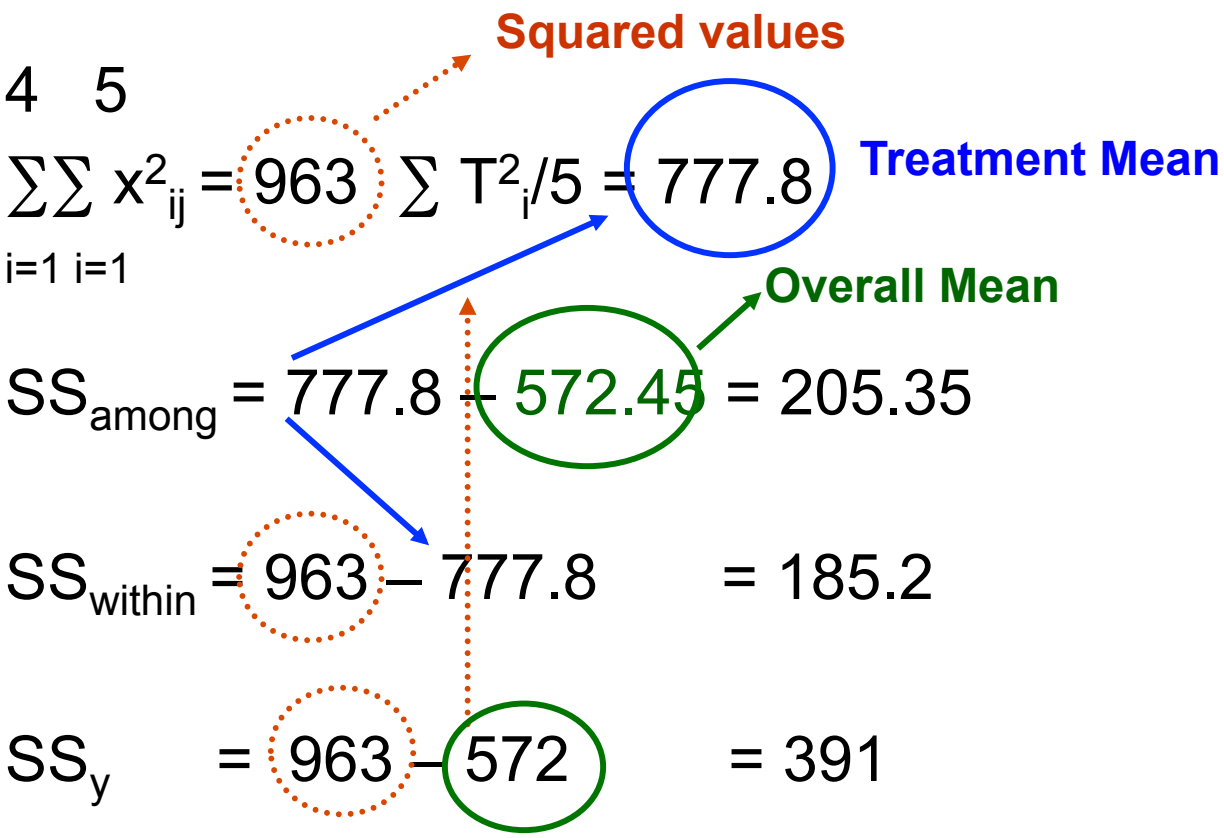
= $T_1^2 = 64$; $T_2^2 = 900$; $T_3^2 = 324$; $T_4^2 = 2601$

T = 107 **T^2** = 11, 449; **$T^2/20$** = 572.45

Overall Mean

$\sum \sum x^2ij = 5^2 + (-2_2) + .. 10^2 =$ 963

Example


Squared values
4 5
∑∑ x²ij = 963 ∑ T²i/5 = 777.8
Treatment Mean
i=1 i=1
Overall Mean
SSamong = 777.8 – 572.45 = 205.35
SSwithin = 963 – 777.8 = 185.2
SSy = 963 – 572 = 391

ANOVA TABLE

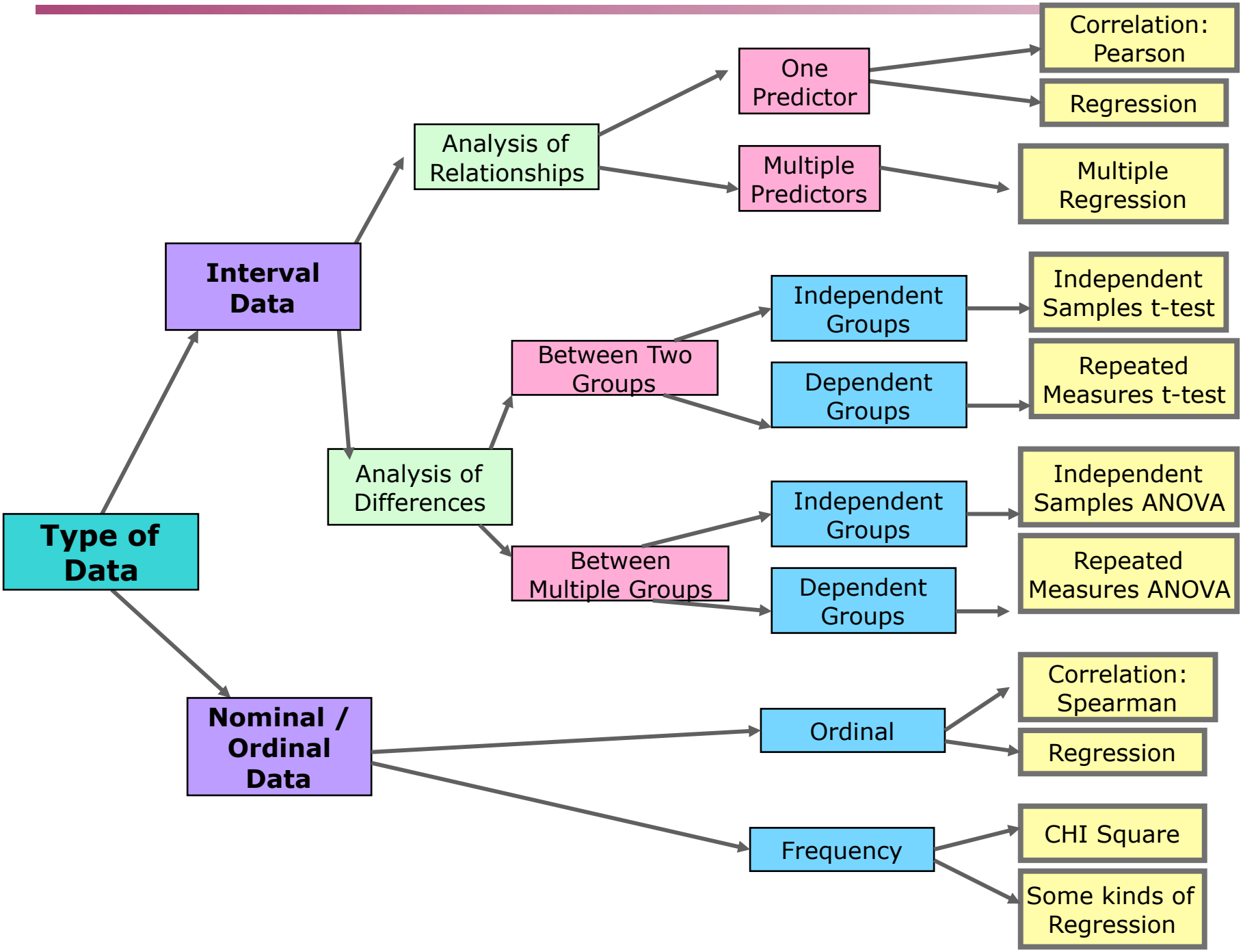
Source	d.f.	SS	MS	F-ratio	p
Among gp	3	205	68	5.7	<.05
Within gp		16	185	12	
Total		19			

$F_{.95(3,16)} = 3.2$

$F_{calculated}$, 5.7 is bigger than Ftabulated,3.2 therefore, reject null hypothesis with less than 5% chance of Type I error.

When there are more than two groups

- Significant *F* only shows that not all groups are equal
  - what groups are different???
  - *Food for Thought*


Type of Data
Interval Data
Analysis of Relationships
One Predictor
Correlation: Pearson
Regression
Multiple Predictors
Multiple Regression
Analysis of Differences
Between Two Groups
Independent Groups
Independent Samples t-test
Dependent Groups
Repeated Measures t-test
Between Multiple Groups
Independent Groups
Independent Samples ANOVA
Dependent Groups
Repeated Measures ANOVA
Nominal / Ordinal Data
Ordinal
Correlation: Spearman
Regression
Frequency
CHI Square
Some kinds of Regression

One Factor-ANOVA (Gill, p148)

Fixed Treatment Effects: $Y_{ij} = \mu + \tau_i + E_{(i)j}$

An experiment was designed to compare t = 5 different media (treatments) for ability to support the growth of fibroblast cells of mice tissue culture. For replication, r = 5 bottles were used for each medium with same number of cells implanted into each bottle and total cell protein (Y) determined after seven days. The results (yij = μg protein nitrogen) are given in the table:

Growth of fibroblast cells in 5 tissue culture media (μ g)

One Factor-ANOVA (Gill, p148)

1	2	3	4	5
102	103	107	108	113
101	105	103	101	117
100	100	105	104	106
105	108	105	106	115
101	102	106	104	116

One Factor-ANOVA (Gill, p148)

- $SS_y = (102^2 + 101^2 + \ldots + 116^2) - [102+101+\ldots 116)^2/25]$

  $= 279{,}985 - 279{,}418 = 567$

- $SS_T = [(102+101+\ldots 101)^2/5 + (103+105+\ldots+102)^2/5+\ldots]$

  $= 279{,}820 - 279{,}418 = 402$

- $SS_E = 567 - 402 = 165$

One Factor-ANOVA (Gill, p148)

Source	d.f	SS	MS	F	$P \leq$
Media	4	402	100.5	12.15	.0001
Error	20	165	8.25		
Total $f_{.01,4,20} = 4.43$	24				

One Factor-ANOVA (Gill, p150)

Random Treatment Effects: $Y_{ij} = \mu + T_i + E_{(i)j}$

Consider the data on daily weight gains, kg, of steer calves sired by 4 different bulls. T = 4 bulls (treatments).

Random Treatment Effects: $Y_{ij} = \mu + T_i + E_{(i)j}$

1	2	3	4
1.46	1.17	0.98	0.95
1.23	1.08	1.06	1.10
1.12	1.20	1.15	1.07
1.23	1.08	1.11	1.11
1.02	1.01	0.83	0.89
1.15	0.86	0.86	1.12
	1.19	0.99	1.15
	0.97		1.10

Random Treatment Effects: $Y_{ij} = \mu + T_i + E_{(i)j}$

- $SS_y = (1.46^2 + 1.23^2 + \ldots + 1.10^2) - [1.46+1.23+\ldots 1.10)^2/29]$

  $= 34.15 - 33.65 = 0.496$

- $SS_T = [(1.46+1.23+\ldots 1.15)^2/6 + (1.17+1.08+\ldots+0.97)^2/8+\ldots]$

  $= 33.79 - 33.65 = 0.1403$

- $SS_E = 0.496 - 0.1403 = 0.3555$

Random Treatment Effects: $Y_{ij} = \mu + T_i + E_{(i)j}$

Source	d.f	SS	MS	F	$P \leq$
Bulls	3	0.1403	0.0468	3.30	.05
Error	25	0.3555	0.0142		
Total $f_{.05,3,25} = 2.99$	28				

```
Data STEER;
INPUT BULLS $ WTGAINK;
CARDS;
B1              1.46
B1              1.23
B1              1.12
B1              1.23
B1              1.02
B1              1.15
B2              1.17
B2              1.08
B2              1.20
B2              1.08
B2              1.01
B2              0.86
B2              1.19
B2              0.97
B3              0.98
B3              1.06
B3              1.15
B3              1.11
B3              0.83
B3              0.86
B3              0.99
B4              0.95
B4              1.10
B4              1.07
B4              1.11
B4              0.89
B4              1.12
B4              1.15
B4              1.10
;
RUN;
PROC PRINT DATA = STEER;
                RUN;
PROC MEANS DATA = STEER;
                RUN;
PROC SORT DATA = STEER OUT = BULLSORT;
                BY BULLS;
                                RUN;
PROC MEANS DATA = BULLSORT;
                BY BULLS;
                                VAR WTGAINK;
                                                RUN;
PROC GLM;
                CLASS  BULLS;
                                MODEL WTGAINK = BULLS;
                                                MEANS BULLS/TUKEY;
                                                                RUN;
QUIT;
```

SAS OUT PUT

The SAS System
The GLM Procedure

Dependent Variable: WTGAINK

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.14026562	0.04675521	3.29	0.0372
Error	25	0.35551369	0.01422055		
Corrected Total	28	0.49577931			

R-Square	Coeff Var	Root MSE	WTGAINK Mean
0.282919	11.06994	0.119250	1.077241

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BULLS	3	0.14026562	0.04675521	3.29	0.0372

ANOVA-3stage Nested Models Gill p201

Fixed effects of treatments:

$$Y_{ij} = \mu + \tau_i + E_{(i)j} + U_{(ij)k}$$

An animal behavior trial was designed to study the potential depressant effects of 2 pharmaceutical products to stimulate response. Thirty (n) rats were randomly assigned, ten (r) to each product and to a control group that received a placebo. On two occasions (u), an observed response was recorded for each animal. The results are given in the table.

Rat no./gp	Treatment 1	Treatment 2	Treatment 3
1	33, 35	37,33	40,42
2	39, 38	31,30	52,50
3	29, 31	43,45	45,44
4	41, 41	36,38	51,53
5	34, 36	30,39	44,41
6	26, 23	38,39	50,52
7	40, 37	43,46	43,43
8	49, 46	32,35	56,53
9	29, 32	44,46	51,50
10	36, 38	30,29	41,43

$$Y_{ij} = \mu + \tau_i + E_{(i)j} + U_{(ij)k}$$

- $SS_y = (33^2 + 35^2 + \ldots + 43^2) - (33+35+\ldots+43)^2/60$
  $= 99551 - 96080 = 3471$
- $SS_T = (33+35+\ldots+38)^2/20 + (37+33+\ldots+29)^2/60$
  $+(40+42+\ldots+43)^2/20 - 96080$
  $= 97652 - 96080 = 1572$
- $SS_E = (33+35)^2/2 + (39+38)^2/2 + \ldots + (41+43)^2/2$
  $- 97652 = 99440 - 97652 = 1788$
- $SS_U = 3471 - 1572 - 1788 = 111$

ANOVA RESPONSE TO STIMULUS

Source of var	df	SS	MS	F
Treatments	2 t-1	1572	786	786/66.2 = 11.9
Exp error (rats/trt)	27 t(r-1)	1788	66.2	
Samples/rats	30 tr(u-1)	111	3.7	
Total $f_{.001,2,27}$=9.02	Tru-1 3*10*2 =60			

2-way ANOVA

TABLE 9-3 TWO-FACTOR ANALYSIS OF VARIANCE WITH m OBSERVATIONS PER CELL

Source	Sum of squares	Sum of squares	d.f.	Mean square*	F (fixed model)	F (random model)	F (rows fixed columns random)
Rows	$cm\sum_{i=1}^{r}(\bar{x}_{i..}-\bar{x})^2$	$\frac{\sum_{i=1}^{r}R_i^2}{cm}-\frac{T^2}{n}$	$r-1$	MS_R	$\frac{MS_R}{MS_W}$	$\frac{MS_R}{MS_I}$	$\frac{MS_R}{MS_I}$
Columns	$rm\sum_{j=1}^{c}(\bar{x}_{.j.}-\bar{x})^2$	$\frac{\sum_{j=1}^{c}C_j^2}{rm}-\frac{T^2}{n}$	$c-1$	MS_C	$\frac{MS_C}{MS_W}$	$\frac{MS_C}{MS_I}$	$\frac{MS_C}{MS_W}$
Interaction	$m\sum_{i=1}^{r}\sum_{j=1}^{c}(\bar{x}_{ij.}-\bar{x}_{i..}-\bar{x}_{.j.}+\bar{x})^2$	$\frac{\sum_{i=1}^{r}\sum_{j=1}^{c}T_{ij}^2}{m}-\frac{\sum_{i=1}^{r}R_i^2}{cm}-\frac{\sum_{j=1}^{c}C_j^2}{rm}+\frac{T^2}{n}$	$(r-1)(c-1)$	MS_I	$\frac{MS_I}{MS_W}$	$\frac{MS_I}{MS_W}$	$\frac{MS_I}{MS_W}$
Within cells	$\sum_{i=1}^{r}\sum_{j=1}^{c}\sum_{k=1}^{m}(x_{ijk}-\bar{x}_{ij.})^2$	$n\sum_{i=1}^{r}\sum_{j=1}^{c}\sum_{k=1}^{m}x_{ijk}^2-\frac{\sum_{i=1}^{r}\sum_{j=1}^{c}T_{ij}^2}{m}$	$rc(m-1)$	MS_W			
Total	$\sum_{i=1}^{r}\sum_{j=1}^{c}\sum_{k=1}^{m}(x_{ijk}-\bar{x})^2$	$\sum_{i=1}^{r}\sum_{j=1}^{c}\sum_{k=1}^{m}x_{ijk}^2-\frac{T^2}{n}$	$n-1$				

* A mean square is a sum of squares divided by the corresponding number of degrees of freedom.

2-way ANOVA Example

- 4 vaccines
- 6 additives
- Response antibody titer in mouse
- 4*6 = 24 treatment combinations
- 72 mouse randomly divided into 24 groups of three mouse each.

Vaccine		Additive I	II	III	IV	V	VI	R_i	$\bar{x}_{i..}$ μ
A		5	2	3	7	3	7	87	4.83
	6	4	3	4	8	8			
	5	4	6	3	6	3			
B		3	3	5	2	6	4	82	4.56
	2	6	7	7	3	7			
	4	3	6	4	4	6			
C		5	5	6	5	9	3	95	5.28
	2	3	7	6	7	6			
	2	6	4	7	4	8			
D		2	4	2	7	5	5	59	3.28
	4	2	2	2	6	2			
	2	3	2	3	2	4			
$\sum (C_j)$		**42**	**45**	**53**	**57**	**63**	**63**	**323 (T)**	
μ $(\bar{x}_{.j.})$		**3.5**	**3.75**	**4.42**	**4.75**	**5.25**	**5.25**	**4.49 ($\bar{x}$)**	

Cell Total (T_{ij})

Vaccine	Additive					
	I	II	III	IV	V	VI
A	16	10	12	14	17	18
B	9	12	18	13	13	17
C	9	14	17	18	20	17
D	8	9	6	12	13	11

$$\sum R_i^2/CM = 87^2/18+82^2/18 +95^2/18+59^2/18$$
$$= 1489$$

$$\sum T^2/N = 323^2/72 = 1449$$

$$SS_R = 1489\text{-}1449 = 40$$
$$MS_R = 40/3 = 13.27$$

$$\sum \mathbf{C}_j^2/RM = (42^2+45^2+53^2+57^2+63^2+63^2)/12$$
$$= 1482$$
$$SS_C = 1482\text{-}1449 = 33/5 = 6.61$$

$\sum \mathbf{T}_{ij}^2/M = 16^2+10^2+\ldots 11^2 = 1560$

$SS_I = 1560-1489-1482+1449 = 38$

$MS_I = 38/15 = 2.52$

Within cell $= \sum \sum \sum x^2_{ijk} = 5^2+2^2+\ldots 4^2 = 1711$

$SS_{within} = 1711- 1560 = 151$

$MS_{within} = 151/48 = 3.15$

2-way ANOVA Table

Source	d.f.	SS	MS	F-ratio	p
Vaccines	3	39.82	13.27	4.21*	
Additives	5	33.07	6.61	2.10^{NS}	
VaccAdd Int.	15	37.76	2.52	0.80^{NS}	
Within cells	48	151	3.15		

$F_{.95(5,48)} = 2.45$

$F_{calculated,}$ 2.1 is smaller than $F_{tabulated}$,2.45 therefore, accept null hypothesis.

SAS DATA SET

```
DATA ABTITER;
INPUT VACCINES $ ADDITIVES MOUSE ABTITER;
DATALINES;
A 1 1 5
A 2 1 2
A 3 1 3
A 4 1 7
A 5 1 3
A 6 1 7
;
RUN;
PROC ANOVA;
        CLASS VACCINES ADDITIVES MOUSE ;
                MODEL ABTITER = VACCINES ADDITIVES MOUSE
                                        VACCINES*ADDITIVES;
                                MEANS VACCINES ADDITIVES /DUNNETT;
RUN;
PROC TABULATE;
        TITLE '2-WAY ANOVA WITH VACCINES AND ADDITIVES MAIN EFFECTS';
                CLASS VACCINES ADDITIVES MOUSE ;
                        VAR ABTITER;
TABLE VACCINES ADDITIVES MOUSE VACCINES*ADDITIVES, ABTITER*MEAN;
RUN;
QUIT;
```

2-WAY ANOVA WITH VACCINES AND ADDITIVES MAIN EFFECTS

The ANOVA Procedure

Class Level Information

Class	Levels	Values
VACCINES	4	A B C D
ADDITIVES	6	1 2 3 4 5 6

Number of Observations Read	72
Number of Observations Used	72

The ANOVA Procedure

Dependent Variable: ABTITER

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	110.6527778	4.8109903	1.53	0.1080
Error	48	151.3333333	3.1527778		
Corrected Total	71	261.9861111			

R-Square	Coeff Var	Root MSE	ABTITER Mean
0.422361	39.58008	1.775606	4.486111

Source	DF	Anova SS	Mean Square	F Value	Pr > F
VACCINES	3	39.81944444	13.27314815	4.21	0.0101
ADDITIVES	5	33.06944444	6.61388889	2.10	0.0819
VACCINES*ADDITIVES	15	37.76388889	2.51759259	0.80	0.6732

The ANOVA Procedure

Dunnett's t Tests for ABTITER

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	48
Error Mean Square	3.152778
Critical Value of Dunnett's t	2.42563
Minimum Significant Difference	1.4357

Comparisons significant at the 0.05 level are indicated by ***.

VACCINES Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
C - A	0.4444	-0.9912	1.8801	
B - A	-0.2778	-1.7134	1.1579	
D - A	-1.5556	-2.9912	-0.1199	***

Randomized Complete Block Design

- Consider this 2x3 factorial design, where 1440 WLH hens were first randomly divided into 4 blocks (cage rows locations) and then within each block were randomly divided into 6 dietary treatments for eight weeks. Each treatments had 8 replicates. Data were collected on several production variables, such as hen day production, egg weight, feed consumption, etc.

RCBD Facts

- A RCBD with factorial ANOVA.
- There were four blocks using cage row locations
- There are two lysine levels and three phosphorus concentrations for a total of (2x3) six treatment groups.
- There were 8 replicates within each treatment.
- For eight weeks, twice a week egg weight data of hens were collected.
- Create weekly egg weight variables and overall average.
- Run ANOVA for Lysine, phosphorus and replicates, if ANOVA is significant run multiple range tests (various)

Two Factor, Fixed Effects

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} E_{(ij)k}$$

Effects of sex and stage of gestation on the activity of fructose-1-phosphate aldolase (n-moles substrate metabolized/min/mg protein) in the upper third of the intestinal mucosa of calves taken by Cesarean section from 18 Holstein heifers undergoing first gestations. The data are shown: (Gill, p225)

Sex (A)	90 d	Stage of 180 d	Gestation 270 d	(B) Total
Males	22.2	35.1	84.6	
	25.4	47.6	108.4	
	38.5	84.9	134.6	
subtotal	86.1	167.6	327.6	581.3
Females	40.5	44.2	81.5	
	76.2	58.8	81.9	
	104.6	125.0	110.7	
subtotal	221.3	228.0	274.1	723.4
Total	307.4	395.6	601.7	1304.7

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{(ij)k}$$

SSy = $(22.2^2 + 25.4^2 + \ldots + 110.7^2) - (22.2 + 25.4 + \ldots + 110.7)^2 / 18$ =

$115{,}379 - 94{,}569$ = **20, 810**

SSA = $(581.3^2 + 723.4^2)/9 - 94{,}569$ = 1122

SSB = $(307.4^2 + 395.6^2 + 601.7^2)/6 - 94{,}569$ = **7604**

SSAB = $(86.1^2 + 167.6^2 + \ldots + 274.1^2)/3 -$

$94{,}569 - 1122 - 7604$ = **3010**

SSE = $20{,}810 - 1122 - 7604 - 3010$ = **9075**

Two Factor, Fixed Effects ANOVA

Source of variation	df	SS	MS	F ratio
Sex (A)	1	1122	1122	1.483^{ns} f.05,1,12=4.75
Gestation (B)	2	7604	3802	5.03* f.05,2,12=3.89
Interaction (AB)	2	3010	1505	1.99^{ns} f.05,2,12=3.89
Expt. Error Total	12 17	9075	756 denom.	

```
DATA SEXGESTATION;
INPUT SEX $ GESTATION $ F1P;
DATALINES;
M               90              22.2
M               90              25.4
M               90              38.5
M               180             35.1
M               180             47.6
M               180             84.9
M               270             84.6
M               270             108.4
M               270             134.6
F               90              40.5
F               90              76.2
F               90              104.6
F               180             44.2
F               180             58.8
F               180             125
F               270             81.5
F               270             81.9
F               270             110.7
;
RUN;
PROC MEANS DATA  = SEXGESTATION;
PROC SORT DATA = SEXGESTATION OUT = SORT;
                BY SEX GESTATION;
PROC MEANS DATA = SORT;
                BY SEX GESTATION;
                                VAR F1P;
PROC ANOVA;
                CLASS SEX GESTATION;
                                MODEL F1P = SEX GESTATION SEX*GESTATION;
                                                                MEANS SEX GESTATION /DUNNETT;
RUN;
PROC TABULATE;
                TITLE '2-WAY ANOVA WITH VACCINES AND ADDITIVES MAIN EFFECTS';
                                CLASS SEX GESTATION;
                                                VAR F1P;
TABLE SEX GESTATION SEX*GESTATION, F1P*MEAN;
RUN;
QUIT;
```

2-WAY ANOVA WITH VACCINES AND ADDITIVES MAIN EFFECTS

The ANOVA Procedure

Dependent Variable: F1P

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	11735.40500	2347.08100	3.10	
Error	12	9074.78000	756.23167		
Corrected Total	17	20810.18500			

R-Square	Coeff Var	Root MSE	F1P Mean
0.563926	37.93930	27.49967	72.48333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SEX	1	1121.800556	1121.800556	1.48	0.2466
GESTATION	2	7603.830000	3801.915000	5.03	0.0259
SEX*GESTATION	2	3009.774444	1504.887222	1.99	0.1793

Factorial Design with Blocking Q5.6v2Gill

- A factorial experiment was planned to study the effects of early feeding of colostrum on serum level of immunoglobulin in dairy calves. Factor *A* was quantity fed (0.5 or 1.5 kg) and factor *B* was time of first feeding (1, 2, 6, or 12 hours after birth). At a later time the experiment was repeated . Consider the two experiments as blocks with random effects. Values recorded are turbidimetric units relative to a barium sulfate standard of 20 when blood was sampled at 48 hours after birth. Colostrum was pooled to eliminate variations among dams.

- Test for interaction and main effects of *A* and *B*.
- Compute the standard errors for A means, B means, and combination means.
- Estimate the relative efficiency of blocking.

Factorial Design with Blocking

Block	Amount	Fed Time 1	Fed Time 2	Fed Time 6	Fed Time 12
1	0.5	7.9	10.2	6.1	2.3
	1.5	11.7	10.7	9.9	5.4
2	0.5	9.5	6.0	7.8	7.1
	1.5	15.0	11.7	9.4	7.2

Factorial Design with Blocking

Source of variation	d.f.	s.s.	m.s.
A, amt fed	1	36.30	36.30
B, time of first feeding	3	66.73	22.24
*A*B*	3	4.78	1.59
Blocks	1	5.64	5.64
Error	7	25.11	3.59

Factorial Design with Blocking

- Test for interaction and main effects of *A* and *B*.
- H: $(\alpha\beta)_{ij} = 0$, $f = (1.59/3.59) = 0.443 < f_{0.50, 3, 7} = 0.871$

  One cannot have even 50% confidence that interaction exists between amount fed and time of first feeding.
- H: $\alpha_i = 0$, $f = (36.30/3.59) = 10.11 > f_{0.05, 1, 7} = 5.59$

  One may have 95% confidence that serum immunoglobulin was higher when 1.5 kg colostrum was fed ($\bar{y}2.. = 10.12$) than when 0.5 kg was fed ($\bar{y}1.. = 7.11$).
- H: $\beta_j = 0$, $f = (22.24/3.59) = 6.19 > f_{0.05, 3, 7} = 4.35$

  One may have 95% confidence that time of first feeding affects serum immunoglobulin. ($\bar{y}.1. = 11.02$, $\bar{y}.2. = 9.65$, $\bar{y}.3. = 8.30$, $\bar{y}.4. = 5.50$

Factorial Analysis, another example Q5.7, Gill v2.

- The pyridoxine content of 10 random lots (blocks) of evaporated milk was determined by microbial assays with *A* = 2 different organisms (*Saccharomyces carlsbergenesis* and *Neurospora sitophilus*) from aliquots taken *B* = before and after sterilizing (Hodson, A. Z., 1956. Vitamin B_6 in sterilized mil abd other milk products. *J. Agr. Food Chem.* 4:876-81). The response is recorded as y = 0.6 + log (mg/L). One value is missing.

- A. Estimate the missing value (y_{126}).

- B. Test for the main effects of organisms(*A*) and sterilization (*B*) and for interaction. What additional conclusions should be drawn?

Block	S.c.		N.s.	
	Before	After	Before	After
1	0.131	0.079	0.214	0.111
2	.206	.207	.167	.129
3	.168	.156	.160	.134
4	.124	.133	.161	.104
5	.159	.201	.180	.135
6	.087	…	.197	.126
7	.109	.161	.189	.076
8	.175	.070	.179	.073
9	.142	.112	.161	.145
10	.197	.174	.156	.090

ANOVA TABLE

Source of Variation	d.f.	SS	MS	f
A= organisms	1	0.000034	0.000034	0.03
B=sterilization	1	0.012709	0.012709	10.76
AB	1	0.008094	0.008094	6.85
Blocks	9	0.011135	0.001237	
Error	26	0.030706	0.001181	

$f_{0.05,\,1,\,26} = 4.23$, $f_{0.01,\,1,\,26} = 7.72$