BIOC461

Research Design and Analysis in Biochemistry

Lecture 2

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Wilcoxon–Mann–Whitney Test (WMW)

- t-test works well for two independent samples when the two populations have normal (or near-normal) distributions.

- Serious problems can occur when the distributions have heavy tails or are skewed.

- Wilcoxon-Mann-Whitney test does not depend on the assumption of normality.

  - H_0: the two populations have same distribution

  - H_A: the two populations have different distributions

- Calculate a test statistic U_s.

Example

- Counting the number of seeds produced by two related species of plant:

    - Do the number of seeds produced by the two plant species have the same distribution (at α = 0.05)?

- No parameters to define:

    - H_0: the two species have the same distribution of seed number

    - H_A: the two species have different distribution of seed number

Example (continued)

- Data should be ordered, if not sort them:

  ```
  Species 1:   19, 23, 25, 28, 28, 34   (n1 = 6)
  Species 2:   14, 18, 19, 20, 25       (n2 = 5)
  ```

- Calculate K_1 : for each observation in sample 1, count the number of observations in sample 2 that are smaller than it; add them up (ties count ½):

  K_1 = 2 ½ + 4 + 4 ½ + 5 + 5 + 5 = 26

- K_2 : for each observation in sample 2, count the number of those in sample 1 that are smaller than it (ties count ½):

  K_2 = 0 + 0+ ½ + 1 + 2 ½ = 4

Example (continued)

- Test statistic: U_s = (the larger of K_1 and K_2)=max(K_1, K_2)=26.

- U_s = max(K_1, K_2) follows the Mann-Whitney null distribution.

- Test at level $\alpha = 0.05$; critical value is $U_{critical}$ = 27.

    - U_s = 26 < 27 = $U_{critical}$ (so P-value > 0.05)

    - *Do not* reject H_0.

- These data do not provide evidence at the 0.05 significance level that the two plant species have different seed number distributions.

Wilcoxon test in R

```
seeds<-read.csv('DataSets2/seedcounts.csv')


View(seeds)


stripchart(SeedCount ~ Species, data=seeds, pch=16,
           ylab="Species", ylim=c(0.5,2.5))
```
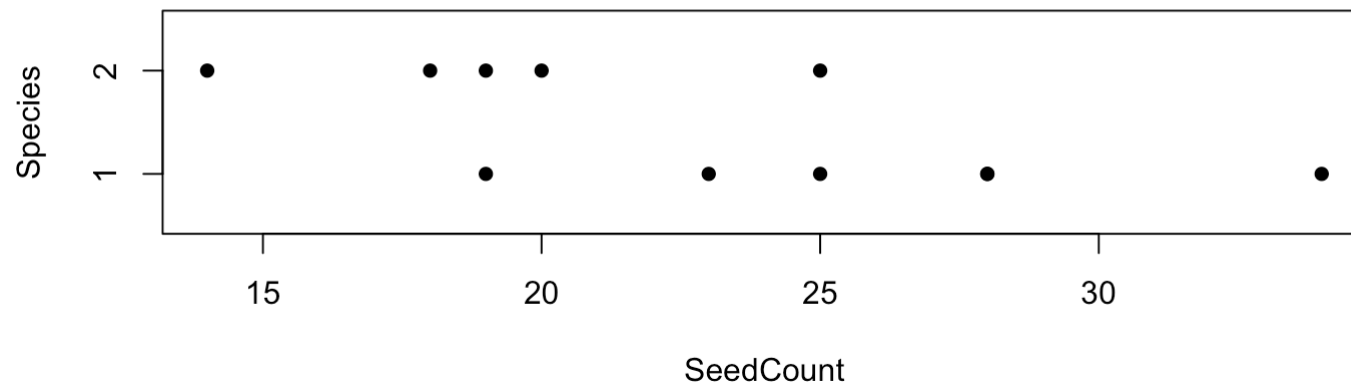
Wilcoxon test in R

```
wilcox.test(SeedCount ~ Species, data=seeds)
```

```
## Warning in wilcox.test.default(x = c(28L, 23L, 19L, 28L, 25L, 34L), y =
## c(19L, : cannot compute exact p-value with ties
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  SeedCount by Species
## W = 26, p-value = 0.05358
## alternative hypothesis: true location shift is not equal to 0
```

Paired Designs

- Sometimes observations occur in pairs:

  - Two observations on the same individual (two days, two sides, before/after…)

  - observing two groups over time

- Examples:

  - subjects occur naturally in pairs, such as identical twins

  - subjects matched in pairs for similar age, sex, profession, disease status, etc (to match extraneous variables)

  - same subject measured on two occasions

Paired sample t-test

- A combination of two things we already know - two-sample and one sample t-tests (also Confidence Intervals):

  1. Recognize that it is a paired design.

  2. Do hypothesis testing steps like a two independent sample t-test

  3. Do t calculations like a 1-sample t-test but for differences of measurements.

Example

- Have 12 fruit flies from 6 different lines (**2 of each**) grown in vials at **two different temperatures**.

- After a specified time for development, count the number of bristles on each fly.

- We think that bristle number has approximately a normal distribution, but flies from the same line should have similar bristle numbers.

- We will look at the **difference** in bristle number **between two flies of the same line**.

Example (continued)

- 6 "blocks" (lines).

- 2 treatments = temperature (cold/warm)

- Have 12 observations but they occur in 6 pairs, so n = 6.

Line	Cold	Warm	difference C – W
1	24	25	−1
2	41	43	−2
3	44	46	−2
4	30	31	−1
5	28	31	−3
6	43	43	0
mean	35	36.5	−1.5
s			1.049
SE			$\dfrac{s}{\sqrt{6}} = -0.428$

- Does temperature affect the number of bristles on fruit flies?

Example (continued)

- Let 1 and 2 be the mean bristle number for flies grown at the colder and warmer temperatures, respectively:

    - H_0: $\mu_1 = \mu_2$ (i.e., $\mu_1 - \mu_2 = 0$): the mean bristle number is the same for both temperatures

    - H_A: $\mu_1 \neq \mu_2$ (i.e., $\mu_1 - \mu_2 \neq 0$): the mean bristle number is different at the two temperatures

- Use a non-directional paired sample t-test.

    - Test statistic: $t_s = \dfrac{\bar{y}_{diff}}{SE_{diff}}$

    - Use t-distribution to find p-value.

- Will reject H_0 if $p < \alpha = 0.05$, otherwise will not reject H_0.

Example (continued)

- Do a one-sample t-test using the difference column only:

  - $\bar{y}_{diff}$ = 35 – 36 = –1.5

  - S_{diff} = 1.049

  - SE_{diff} = $S_{diff}/\sqrt{n}$ = 1.049/$\sqrt{6}$ = 0.428

  - t_s = –1.5/0.428 = –3.503

  - Gives p=0.017 (see next page), so reject H_0.

- This study provides evidence at the 0.05 significance level that flies have different mean bristle numbers at the two temperatures.

Paired sample t-test in R

```
flies<-read.csv('DataSets2/flytemp.csv')


View(flies)


t.test(flies$Cold, flies$Warm, paired=TRUE)


##
##  Paired t-test
##
## data:  flies$Cold and flies$Warm
## t = -3.5032, df = 5, p-value = 0.01722
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2.6006574 -0.3993426
## sample estimates:
## mean of the differences
##                     -1.5
```

Paired tests

- How to know whether to use paired or unpaired t-test to analyze a given data set?

    - Easy: Does each data point in one sample naturally correspond to one specific data point in the other sample? If so, use paired t-test.

- How to know when to use a paired design in your experiment?

    - Harder: Do you expect the extraneous variables to increase variation and want to match on these variables, or pairing is dictated by the problem (e.g. repeated measurements)?

- If so use paired design.

Assumptions

- Paired-sample t-test is based on the idea that the *differences* are approximately normal, so may want to plot the histogram.

- This will automatically be true if the individual groups are both normally distributed.

Sign test

- What if the data are not normally distributed?

    - Recall: for two independent samples did Wilcoxon-Mann-Whitney.

- For paired samples we can do the **sign test**.

- Look at the **sign** of the *difference between each pair of observations*.

- If the two treatments are the same, the sign is equally likely to be positive as negative. That is, if we take a pair at random (from the population of pairs), whether the first or second of the pair is larger is like flipping a coin.

Sign test

- Let π = probability that, for any pair from the population, the first will be larger.

    - H_0: π = 0.5; the two treatments are the same in their effect.

    - H_A: $\pi \neq 0.5$ (this is the non-directional alternative): the two treatments are different in their effect.

1. For each pair of observations, note whether $y_1 - y_2$ is positive (+) or negative (–), that is, + if y_1 is bigger, – if y_2 is bigger

2. count the number of + (= N_+) and – (= N_-) (don't count zeros).

3. test statistic B_s = larger of N_+ and N_-.

4. Reject H_0 if $B_s \geq$ critical value.

Example: skin grafts

- Skin grafts applied to *both sides of body* in 11 recipients.

- One graft has good HLA antigen system match with recipient; the other does not.

- This is paired because we have two observations from each person.

- Observe time to rejection of skin graft (not normally distributed so don't use t-test.)

Does a good HLA match increase graft survival time?

- Let π = probability that good HLA match survives longer in any individual
    - H_0: π = 0.5; HLA match and non-match are equally likely to survive longer.
    - H_A: $\pi > 0.5$; HLA match is more likely to survive longer (directional alternative).
- Use a sign test.
    - B_s = N_+ has a binomial distribution (n=11, π= 0.5) under H_0. If non-directional, Bs would be max(N+, N–).
- Test at level α = 0.05 ; critical value is 9 (from computer).
- Will reject H_0 if $B_s \geq 9$; if $B_s < 9$ we will not reject.

Example (continued)

- Data: survival times (days)

good	37	19	57	93	16	23	20	63	29	60	18
poor	29	13	15	26	11	18	26	43	18	42	19
sign	+	+	+	+	+	+	-	+	+	+	-

- n = 11

- $N_+ = 9$, $N_- = 2$; $B_s = N_+ = 9$; $B_{0.05} = 9$

- So, reject H_0.

- This study provides evidence (P = 0.033) that a good HLA match survives longer than a poor match.

Sign test in R

```r
skin <- read.csv('DataSets2/skingraft.csv')


## Perform Sign Test using binom.test function (1-sided alternative)
## Input the test statistic (Bs=9), and the number of pairs (n=11)
binom.test(9, 11, alternative="greater")



##
##  Exact binomial test
##
## data:  9 and 11
## number of successes = 9, number of trials = 11, p-value = 0.03271
## alternative hypothesis: true probability of success is greater than 0.5
## 95 percent confidence interval:
##  0.5299132 1.0000000
## sample estimates:
## probability of success
##              0.8181818
```

Types of Variables
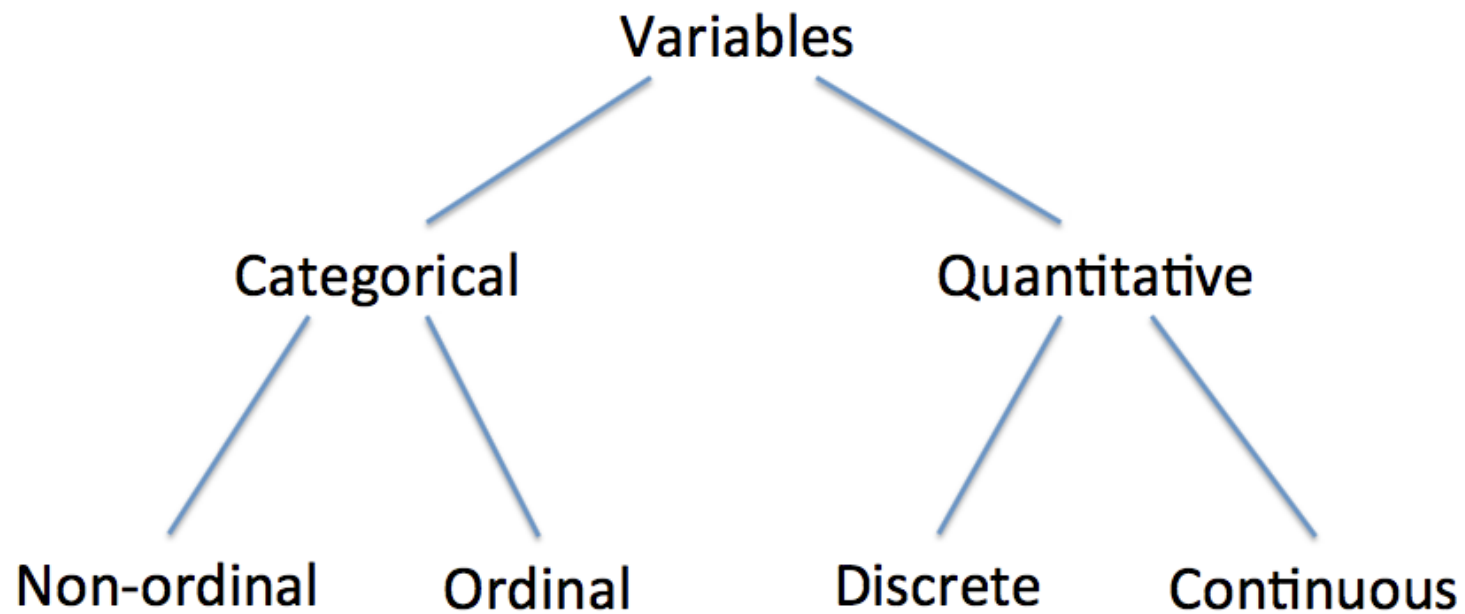
Types of Variables

- Quantitative:
    - Outcome is a number
    - height or concentration (continuous)
    - number of flowers on a plant (discrete)
- Categorical:
    - outcomes fall into categories we can list
    - peas: round & yellow, round & green, wrinkled & yellow, wrinkled & green (not ordinal)
    - choices on a survey: never, rarely, occasionally, often, always (ordinal)

Categorical data

- In order to analyze categorical data we start by counting the number of observations in each category.

- If there are only two categories,

    - the number in one category has a binomial distribution (if the observations in the sample are independent).

    - If there are more than two categories,

    - you can focus on one category and group all the others together; still binomial

    - or, more often, you define different π's for the different categories (π_1, π_2,…)

Binomial distribution

- π is the (unknown) probability of "success", say in category 1.

- n is the number of observations.

- Observe y = # in category 1.

- The proportion in category 1 is $\hat{\pi} = y/n$.

- y has a binomial (n,π) sampling distribution,

- If n and n(1-π) are not too small, this is close to a normal distribution with mean and SD:

$$\mu = n\pi, \quad \sigma = \sqrt{n\pi(1 - \pi)}$$

- This normal approximation is used both explicitly (for confidence intervals) and implicitly (for testing).

Chi-squared distribution

- To test hypotheses, we will use a distribution called a χ^2 distribution (chi-squared).

- Facts about the χ^2 distribution

  - If you have k random variables $y_1, \ldots, y_k$, which are all independent and N(0,1), if you square them all and add them up, the resulting sum has a χ_k^2 distribution. ("a chi-squared distribution with k degrees of freedom").

  - χ^2 random variables only take on positive values.

- We have several different tests which all use the 2 distribution to determine critical values, however, the hypotheses are different.

Contingency tables

- "2x2" means 2 rows and 2 columns in a table: categorical data with 4 groups that are related in pairs.

- Two main contexts (sometimes blurred):

    - Two independent random samples; one binomial variable observed in each sample

    - One sample; observe two different binomial variables on each sample

- Examples of context 1:
    1. samples are "drug" and "placebo" (or any two treatments); observed variable "improve" or "don't improve".

    2. samples are "male" and "female" (any two groups we set up to compare); observed variable "eye color" brown or blue.

Contingency tables

- Examples of context 2:

  - observe whether people smoke, exercise

  - observe response to drug for patients in a randomized clinical trial. Four Categories, observations in 2x2 table:

Observed		treatment		Total
		Drug	Placebo	
Outcome	Improve	15	4	19
	Don't Improve	11	17	28
Total		26	21	47

- Test for independence of row and column variables.

Association testing: example

Observed		treatment		Total
		Drug	Placebo	
Outcome	Improve	15	4	19
	Don't Improve	11	17	28
Total		26	21	47

- π_1 = probability patient improves if they take the drug

- π_2 = probability patient improves if they take the placebo

  - $H_0 : \pi_1 = \pi_2$

  - $H_A : \pi_1 \neq \pi_2$ (or $\pi_1 > \pi_2$)

- H_0 is that the two probabilities are the same (like $\mu_1 = \mu_2$ in a t-test), which can be phrased in terms of independence.

Example (continued)

- H_A is that the probabilities are different, which can be phrased in terms of a *lack of independence*, which is called *"association"*:

  - $\hat{\pi}_1$ = # who improve with drug / # who take drug
    = 15/26 = 0.58

  - $\hat{\pi}_2$ = # who improve with placebo / # who take placebo
    = 4/21 = 0.19

Example (continued)

- What values would we expect under H_0?

  - Total who improve is 19, so under H_0 estimate 19/47 = 40.4% to be the proportion who improve overall.

  - 26 patients took the drug: under H_0 expect 40.4% of them to improve = $(26 \times 0.404) = 10.5 = (26 \times 19)/47$. Similarly, expect number who improve under placebo to be $(21 \times 19)/47 = 8.5$.

  - For the next row, expect $(26 \times 28)/47 = 15.5$ don't improve with drug and $(21 \times 28)/47 = 12.5$ don't improve with placebo.

- Can place these "Expecteds" in a table similar to the observed values. Note that the row and column totals are the same as for observed.

Example (continued)

Expected		Treatment		Total
		Drug	Placebo	
Outcome	Improve	10.5	8.5	19
	Don't Improve	15.5	12.5	28
Total		26	21	47

- In general, for each category:
  - E = (row total)(column total)/(grand total)
- This is the common way to organize the E's and the O's:

Observed (Expected)		Treatment		Total
		Drug	Placebo	
Outcome	Improve	15 (10.5)	4 (8.5)	19
	Don't Improve	11 (15.5)	17 (12.5)	28
Total		26	21	47

Example - questions

- Are patients who take the drug more likely to improve than those who take the placebo?

  - π_1 = probability patient improves if they take the drug

  - π_2 = probability patient improves if they take the placebo

  - $H_0: \pi_1 = \pi_2$; the probability of improving is the same whether drug or placebo is taken (treatment and outcome are independent)

  - $H_A: \pi_1 > \pi_2$; the probability of improving is greater if the drug is taken than if the placebo is taken

- This example is directional; if non-directional, H_A: outcome and treatment are not independent.

Example (continued)

- Use a χ^2 test of independence

  - $X_s^2 = \sum (O - E)^2 / E$ has a χ_1^2 distribution under H_0.

  - Calculate p-value, and reject H_0 if $p < \alpha$.

- Since H_A is directional, must do the following step, but it's a good idea whether directional or not.

  - $\hat{\pi}_1 = \frac{\text{\# who improve with drug}}{\text{\# who take drug}} = \frac{15}{26} = 0.58$

  - $\hat{\pi}_2 = \frac{\text{\# who improve with placebo}}{\text{\# who take placebo}} = \frac{4}{21} = 0.19$

- Check: 0.58 > 0.19, so $\hat{\pi}_1 > \hat{\pi}_2$ is in the same direction as H_A.

Example (continued)

- X_s^2 = (15-10.5)2/10.5 + (4-8.5)2/8.5

    + (11-15.5)2/15.5 + (17-12.5)2/12.5 = 7.2

- Chi-squared P-value is 0.007 (see next page), so reject H_0.

- This study provides evidence at the α= 0.01 significance level that the probability of improving is greater if the drug is taken than if the placebo is taken.

- Degrees of freedom: df = 1 for the 2x2 table.

    - In general it is (#rows-1)(#columns-1)

    - X_s^2 has a χ_1^2 distribution under H_0.

Chi-square test in R

```
drugs<-read.csv('DataSets2/drugs.csv')
table(drugs$Treatment, drugs$Response)
```

```
##
##            Improve NoImprove
##    Drug         15        11
##    Placebo       4        17
```

```
chisq.test(table(drugs$Treatment, drugs$Response), correct=FALSE)
```

```
##
##  Pearson's Chi-squared test
##
## data:  table(drugs$Treatment, drugs$Response)
## X-squared = 7.2037, df = 1, p-value = 0.007275
```

Chi-square test in R

- Note that we used `correct=FALSE` in the `chisq.test` command: this turns off R's default continuity correction, which is used to improve the χ^2 approximation in 2×2 tables.

```
chisq.test(table(drugs$Treatment, drugs$Response))
```

```
##
##  Pearson's Chi-squared test with Yates' continuity correction
##
## data:  table(drugs$Treatment, drugs$Response)
## X-squared = 5.6885, df = 1, p-value = 0.01708
```

What does rejecting H_0 mean?

- Sometimes have to be careful about conclusions in this test. If you reject H_0 with a chi-squared test then that indicates the two variables are **associated**, i.e., not independent; that does not always imply a causal relationship.

- This study provides evidence that patients who take the drug are more likely to improve than patients who take the placebo.

- Here we **controlled** the drug vs. placebo and observed the improvement, so it was reasonable to infer causation. If we had performed a test based on observational data, we could only say that the two variables were associated.

Analysis of Variance (ANOVA)

- How to analyze data when there are k treatments?

  - Same basic assumptions/limitations as t-test:

  - Each population has a normal distribution

  - Samples are independent and random

  - Want to test hypotheses about the population means μ_i

- SD, σ, of each population is the same (or similar) so we can use a pooled SE.

- Goal: test hypotheses such as

  - $H_0 : \mu_1 = \mu_2 = \mu_3 = \ldots = \mu_k$

  - H_A : at least one of the μ_i's is different than the others.

ANOVA - The Global F Test

- $H_0: \mu_1 = \mu_2 = \mu_3 =\ldots= \mu_k$

- H_A: at least one of the μ_i's is different than the others.

- To test this hypothesis, we use the test statistic F_s, which is the ratio of the "between-group variation"'' to the "within-group variation".

- Reject H_0 if observed F_s is "large" (calculate P-value).

- Note: H_A is non-directional for F-Test, but rejection region is one-tailed.

Example

- A random sample of 15 healthy young men are split randomly into 3 groups of 5.

- They receive 0, 20, and 40 mg of the drug Paxil per day for a week, respectively.

- Then their serotonin levels are measured to determine whether Paxil affects serotonin levels.

Hypotheses

- Definitions

  - μ_1 is the mean serotonin level for men on 0 mg of Paxil.

  - μ_2 is the mean serotonin level for men on 20 mg of Paxil.

  - μ_3 is the mean serotonin level for men on 40 mg of Paxil.

- Hypotheses

  - H_0: $\mu_1 = \mu_1 = \mu_3$; mean serotonin levels are the same at all 3 dosage levels (i.e., mean serotonin levels are unaffected by Paxil dose).

  - H_A: The mean serotonin levels of the three groups are not all equal (i.e., serotonin levels are affected by Paxil dose).

One-way ANOVA in R

```
paxil<-read.csv('DataSets2/paxil.csv')
View(paxil)
summary(aov(Serotonin ~ as.factor(Dose), data=paxil))
```

```
##                  Df Sum Sq Mean Sq F value  Pr(>F)
## as.factor(Dose)  2  841.9   420.9   8.358 0.00532 **
## Residuals       12  604.3    50.4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- p = 0.00532, so reject H_0.

- This study provides evidence that Paxil intake affects serotonin levels in young men.

How/Why does this work?

- Remember for t-test,
    - take the difference in means: $\bar{y}_1 - \bar{y}_2$
    - divide by the amount of sampling variation it has: $SE_{\bar{y}_1 - \bar{y}_2}$
    - if $\bar{y}_1 - \bar{y}_2$ is big compared to the amount of variability expected by chance, t_s is large; so reject H_0.
- For F-test,
    - take average squared difference between the group means
    - divide it by the amount of sampling variation it has
    - if the between-group variation is large compared to the amount of variability expected by chance, F is large; so reject H_0

Why not just do t-tests on each pair?

- You could, but the probability of making at least one Type I error is bigger than just the α you use for each test because there can be many possible pairs to test.

- For example when we test $H_0: \mu_1 = \mu_2 = \mu_3$, we are actually testing:

  - $H_{01}: \mu_1 = \mu_2$

  - $H_{02}: \mu_1 = \mu_3$

  - $H_{03}: \mu_2 = \mu_3$

- And we would reject H_0 if any one of H_{01}, H_{02} or H_{03} would be rejected by a t-test with prob of Type I error = 0.05 on first test, 0.05 on second test, etc.

Why not just do t-tests on each pair?

- The more tests you do, the more likely you are to make at least one mistake. (With k > 7 you would expect to make at least one mistake).

- Plus, the tests are not independent so it's not even easy to decide what the true Type I error probability is.

- Also, ANOVA lets you get at more complicated patterns.

- So, if you don't reject H_0, stop. The data provide no evidence in favor of H_A.

Post-hoc testing

- You can perform additional tests to determine which means are different:

- Use the `TukeyHSD` function in R:

```
TukeyHSD(aov(Serotonin ~ as.factor(Dose), data=paxil))
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Serotonin ~ as.factor(Dose), data = paxil)
##
## $`as.factor(Dose)`
##          diff        lwr      upr       p adj
## 20-0   11.678 -0.2961563 23.65216 0.0561323
## 40-0   18.098  6.1238437 30.07216 0.0043776
## 40-20   6.420 -5.5541563 18.39416 0.3571044
```

Post-hoc testing

- There is a significant difference in mean serotonin levels between men receiving a 0mg dose of Paxil, and those receiving a 40mg dose.

```
stripchart(Serotonin ~ as.factor(Dose), data=paxil, pch=16, ylab="Dose")
```
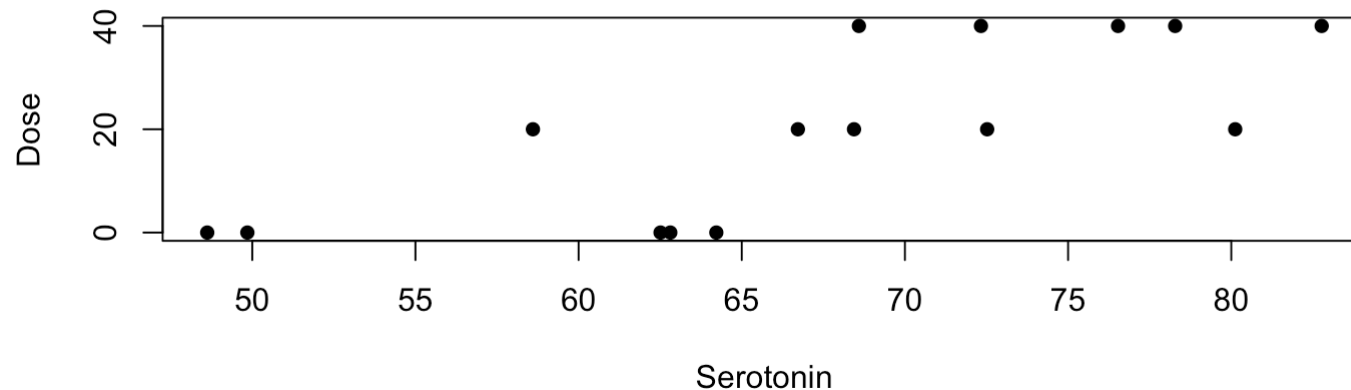
Relationships between quantitative variables

- Often interested in the relationship between two quantitative variables.

- Need methods for:

    - data display

    - describing the relationship

Relationship between variables

- As in the contingency table data, this may be
  - one random sample; observe two quantities
  - one of the variables may be controlled (e.g. dose)
- Examples
  - X = concentration, Y = rate of reaction
  - X = dose, Y = response
  - X = weight, Y = height
  - X = root extension, Y = carbon allocation
  - X = midterm 1 score, Y = midterm 2 score
- If the experimenter controls one of the variables, that is usually labeled X, and the "response" is Y.

Heroin again

- For each cadaver blood taken from heart and from peripheral organs.

- Questions of interest:

  - What is the relationship between the two concentrations?

  - Can we use the heart concentration to predict the peripheral concentration?
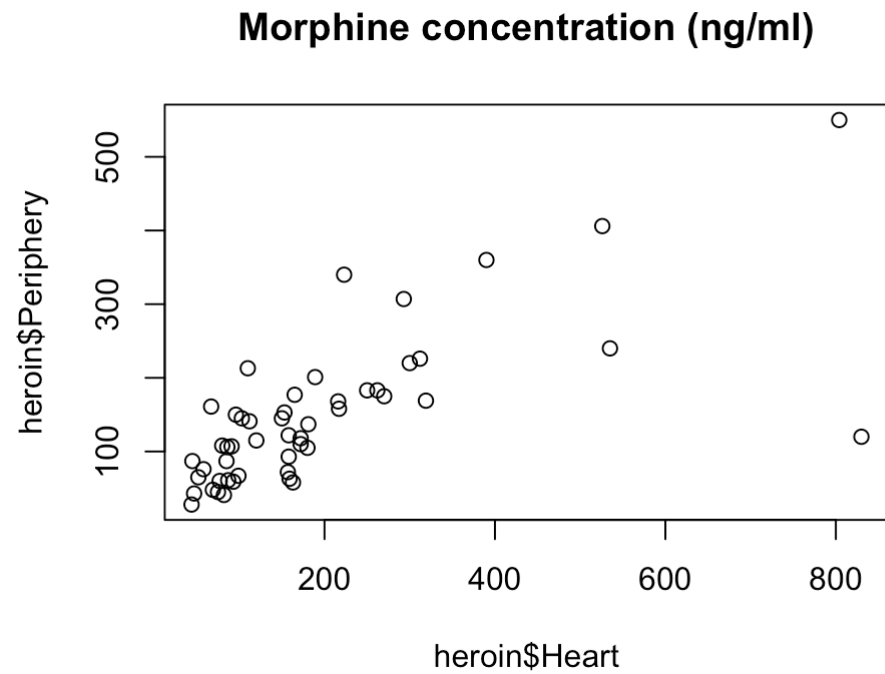
Load heroin data

```
heroin <- read.csv('DataSets2/heroin-regression.csv')
head(heroin)
View(heroin)
```

```
##    Heart Periphery
## 1     67       161
## 2    110       213
## 3     45        87
## 4     96       150
## 5     80       108
## 6    103       145
```
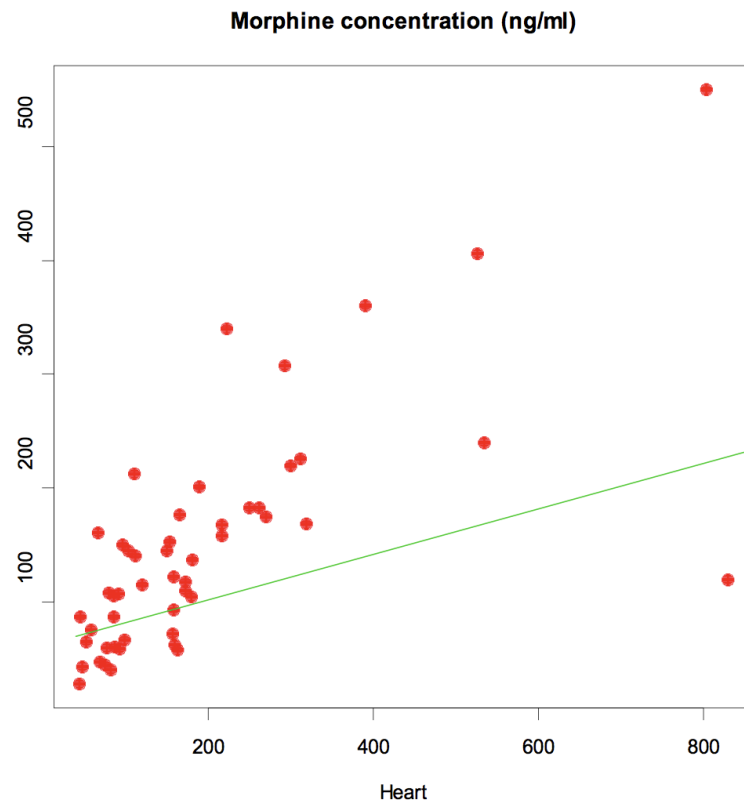
Scatterplot

```
plot(heroin$Heart, heroin$Periphery,
     main="Morphine concentration (ng/ml)")
```
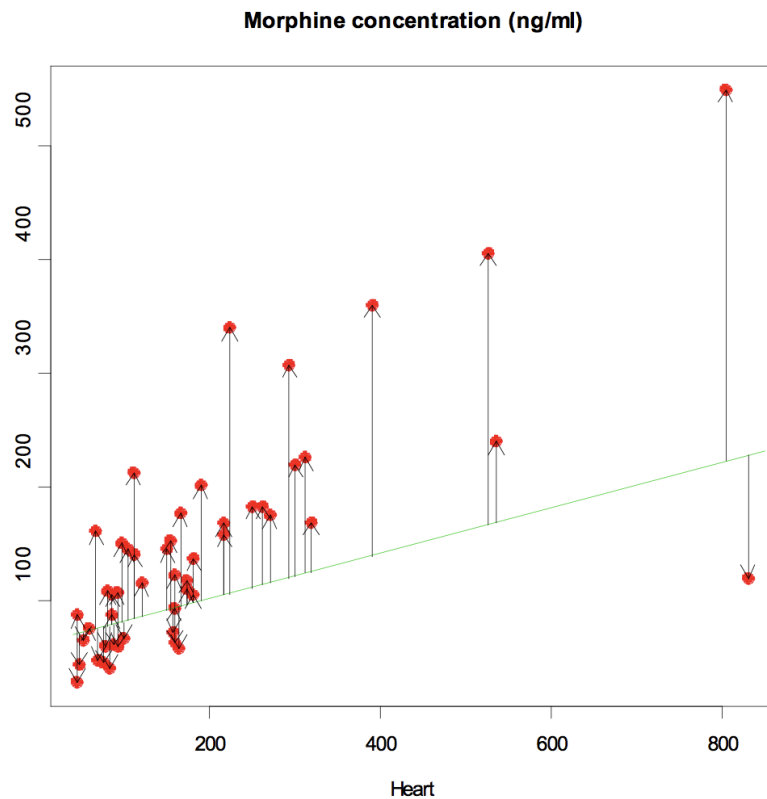

Morphine concentration (ng/ml)

"Line of best fit"

Fit any line: how good is it?

"Line of best fit"

Calculate the distance from each point to the line, square them, and add them all up.

Morphine concentration (ng/ml)

"Line of best fit"

- *Peripheral conc. = 65 + 0.43 Heart conc.*

Morphine concentration (ng/ml)
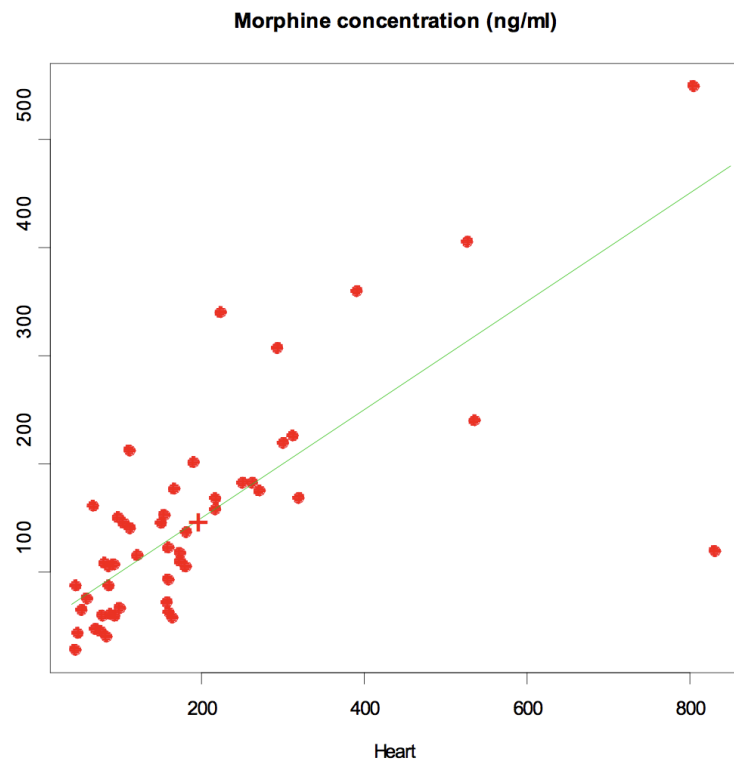
- Line of best fit minimizes these "sums of squared errors".

Linear Regression

- A way to look at the relationship between continuous variables

- Back to quantitative data with assumed normal distributions

- Conceptually related to both ANOVA and contingency tables

- "Finding a straight line" through the data

- Data: pairs of observations (X, Y), so observe:

    - $(x_1, y_1), (x_2, y_2), \ldots, (x_n, y_n)$

What does "best" mean?

- For each observed value of x there is a value of y predicted by the straight line, which we write as

$$\hat{y} = b_0 + b_1 x$$

- This is usually not exactly the same as the observed y for that x.

- Compare the actual vs. predicted y; difference is called residual:

- Residual = y - $\hat{y}$ (one for each pair of observations, i.e., n residuals)

Regression in R

```
summary(lm(Heart ~ Periphery, data=heroin))
```

```
##
## Call:
## lm(formula = Heart ~ Periphery, data = heroin)
##
## Residuals:
##     Min       1Q   Median       3Q      Max
## -196.95   -54.70    -5.40    25.11   668.61
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  20.3604    30.3705    0.670    0.506
## Periphery     1.1753     0.1699    6.916  9.9e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 131.4 on 48 degrees of freedom
```

Regression output

- Coefficients are the intercept and slope of the regression line.
- Significance of the slope means that there is a linear relationship between the variables.

```
summary(lm(Heart ~ Periphery, data=heroin))$coefficients
```

```
##              Estimate Std. Error   t value     Pr(>|t|)
## (Intercept) 20.360409 30.3705099 0.6704006 5.058153e-01
## Periphery    1.175255  0.1699363 6.9158543 9.896584e-09
```

Aside - log transformation

- The relationship between the Heart and Periphery variables is more linear when each variable is log transformed.

```
plot(log(heroin$Heart), log(heroin$Periphery), pch=20, cex=0.8,
    main="Morphine concentration (ng/ml)")
```

Morphine concentration (ng/ml)
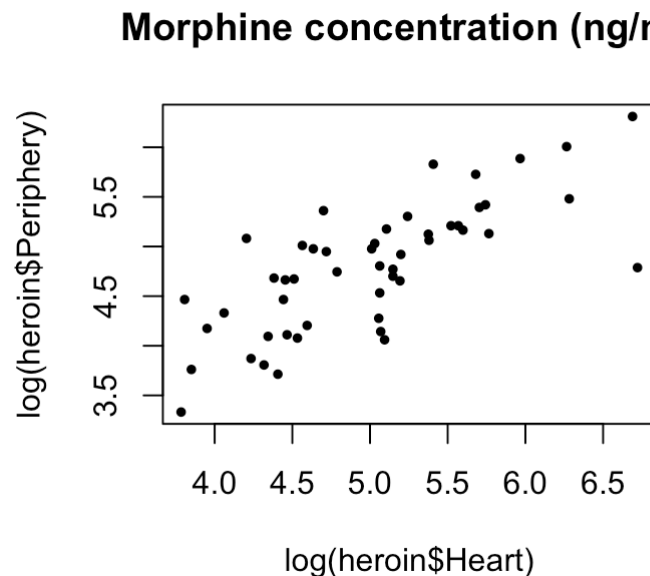
Correlation Coefficient

- The correlation coefficient, ρ, measures the strength of linear association between two normally distributed variables.

- $r = \hat{\rho}$ has the same sign as the slope of the regression model.

- It is always between -1 and +1 and r has no units.

- It will be 1 only if the data points are exactly on a straight line so that all the residuals are 0.

- It will be 0 when there is no linear relationship between X and Y.

- A value "close" to 1 indicates a "tight" linear relationship between X and Y.

Correlation

- If r is large in magnitude (close to 1) we say X and Y are highly "correlated".

    - "Negatively correlated" when r is negative,

    - "Positively correlated" when r is positive.

- If r = 0 there is no linear relationship; we say X and Y are "uncorrelated" (Note: uncorrelated is not the same thing as independent!)

- The word "uncorrelated" is also used to describe data with small but nonzero values of r.

Testing $H_0: \rho = 0$

- Test H_0 the same way (questions, distributions, conclusions) as above:

    - Is there a linear relationship between Y and X?

- Let ρ be the correlation coefficient between X and Y for the population.

    - $H_0: \rho = 0$ There is no linear relationship between Y and X.

    - $H_A: \rho \neq 0$ There is a linear relationship between Y and X. (or H_A could be directional, e.g., $H_A: \rho > 0$).

Correlation in R

- Default is "Pearson": for normally distributed variables

```
cor(heroin$Heart, heroin$Periphery)
```

```
## [1] 0.7064758
```

- "Spearman" is a rank-based approach (doesn't require normality).

```
cor(heroin$Heart, heroin$Periphery, method='spearman')
```

```
## [1] 0.7625885
```

Test for significance

```
cor.test(heroin$Heart, heroin$Periphery)
```

```
##
##  Pearson's product-moment correlation
##
## data:  heroin$Heart and heroin$Periphery
## t = 6.9159, df = 48, p-value = 9.897e-09
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.5329257 0.8229865
## sample estimates:
##       cor
## 0.7064758
```

Assignment: R Markdown

- RStudio provides a framework for producing documents within R: R Markdown

- R Markdown uses a simple syntax to produce documents containing:

  - comments

  - code

  - output

- By deafult, running an R Markdown (.Rmd) file will produce HTML output, although other output formats can be used.

- See the website below for more details, and click the "Get Started" link to get a video overview.

http://rmarkdown.rstudio.com/

Creating an R Markdown document

- In RStudio, select the 'New File' then 'R Markdown…' from the 'File' menu.

- Choose 'Document' and give it a name (leave the output format as "HTML"), then click 'OK'.

- This creates a basic R Markdown document conating some sample code:

  - the top of the file contains some basic information

  - blocks of R code have ```{r} in front, and ``` after: these are run when you click "Knit" (see next slide), and the output appears in the HTML file

  - everything else in the file is treated as text to be displayed

Creating an R Markdown document

- Various text effects can be achieved using markdown syntax (e.g., "##" for headings): see the link below for (many) more options.

- Clicking the "Knit" button in RStudio (above your code window) will run the code, and open a built-in browser that displays the processed document as rendered HTML.

- This file is saved in a folder within your working directory, and can be opened in any web browser, exported to other formats etc.

- A big advantage of R Mardown is *reproducibility* - anyone with the .Rmd file and associated data can *exactly* reproduce the results in the document.

http://rmarkdown.rstudio.com/authoring_basics.html