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Dealing with "some" important statistical assumptions.

**1) The issue of normality (today):**

- Parametric (e.g., ANOVA): assume parametrized families of probability distributions (e.g., normal defined by two parameters, i.e., mean and variance). Parameter estimates tend to be sensitive to non-normality (e.g., issue in regression slopes), but not necessarily in statistical hypothesis testing (P-values may be not as sensitive).
- Non-parametric: either distribution free (e.g., permutation tests) or ranked based tests.

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Dealing with "some" important statistical assumptions.

**2) The issue of homogeneity of variances (later in the course):**

- Standard (e.g., ANOVAs, regressions) assume homoscedasticity.
- Robust approaches (Welch's ANOVA, Weighted least squares) are good to deal with heteroscedasticity.

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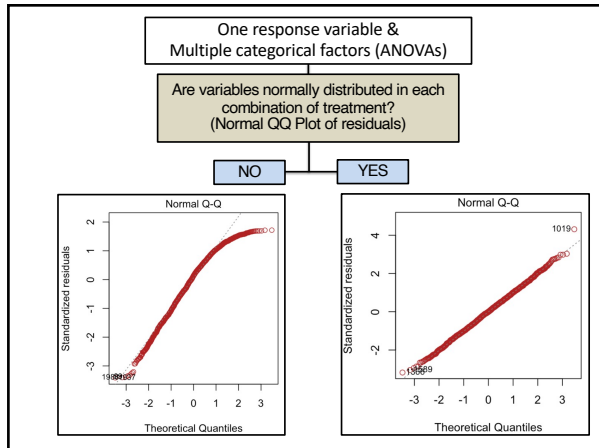
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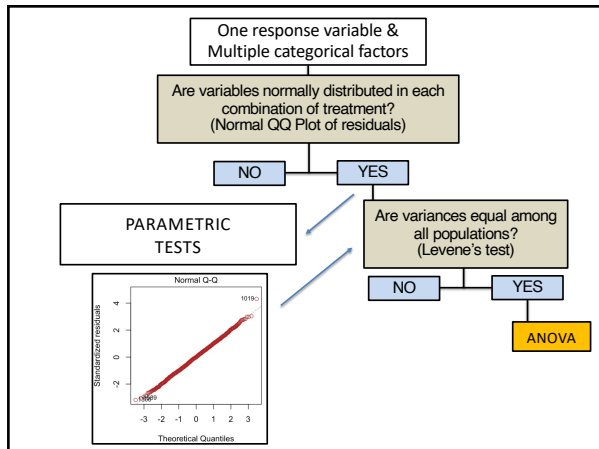
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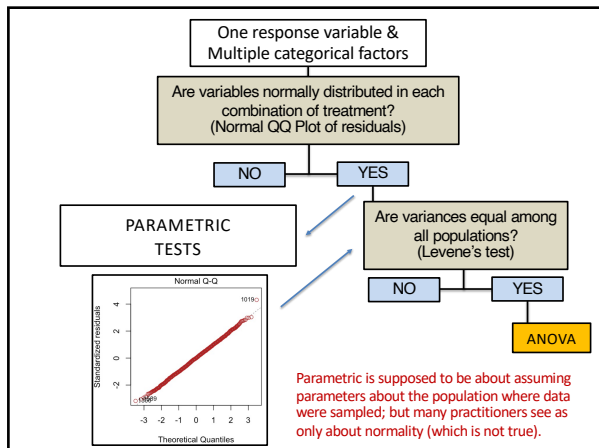
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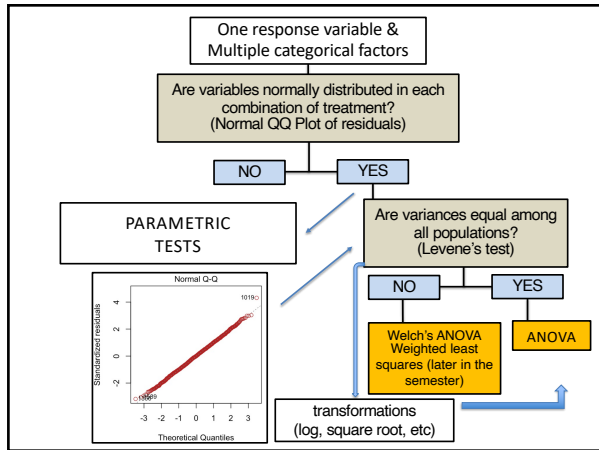
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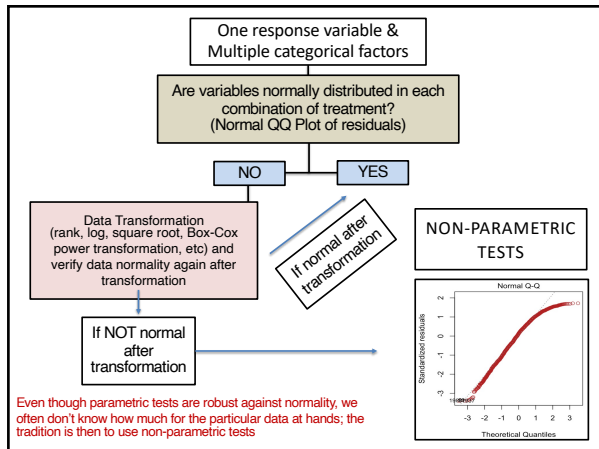
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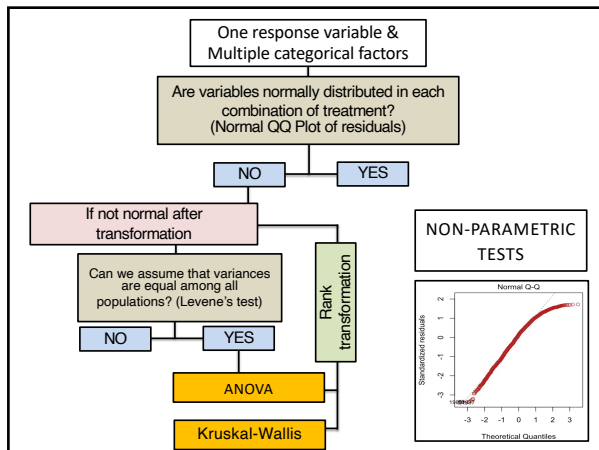
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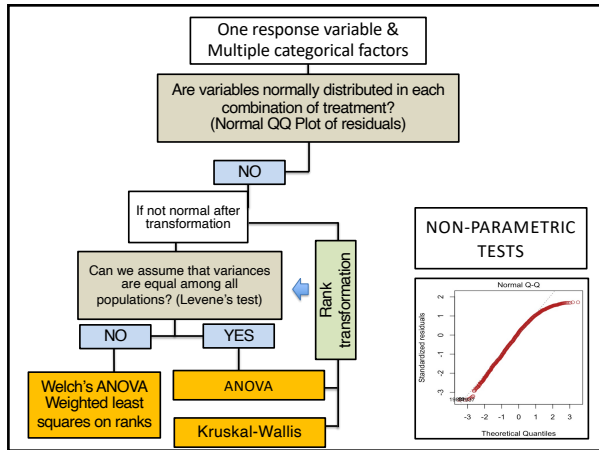
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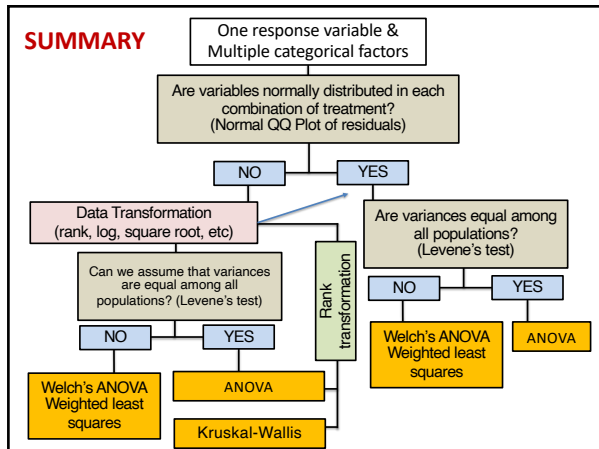
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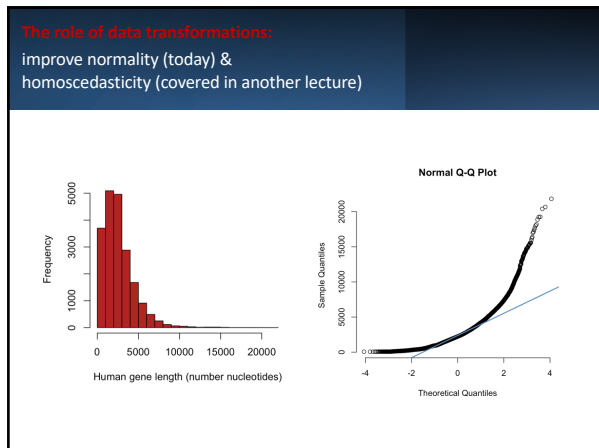
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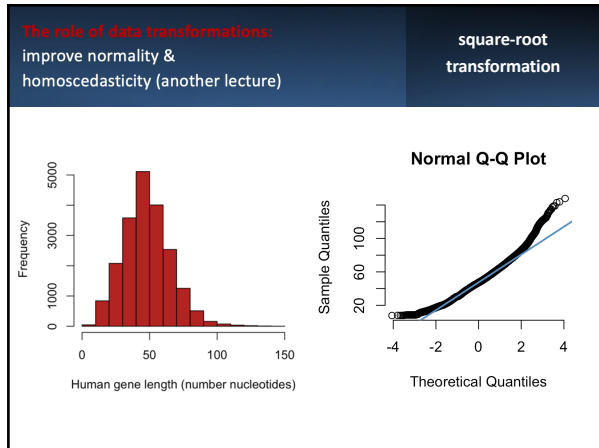
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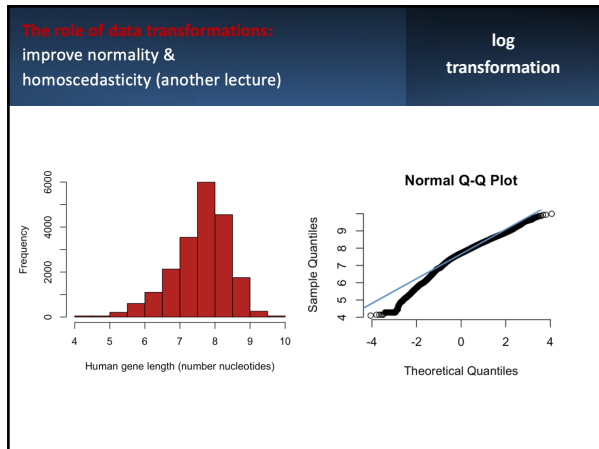
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**A few words on data transformation**

One size may not fit all:

- 1) One transformation may help approximate normality, but another transformation may be required to approximate homoscedasticity (e.g.,  $\log(\sqrt{\text{data}})$ ).
- 2) One transformation may negate (reverse) the other – the one that makes the data approximate homoscedasticity may make data non-longer normal.
- 3) If data are complex (e.g., several predictors in a regression model), it may not be possible that one single transformation will allow data to behave properly under assumptions.

Possible solution: focus on analytical solutions (many covered in this course) and not always transformations; or combine different transformation.

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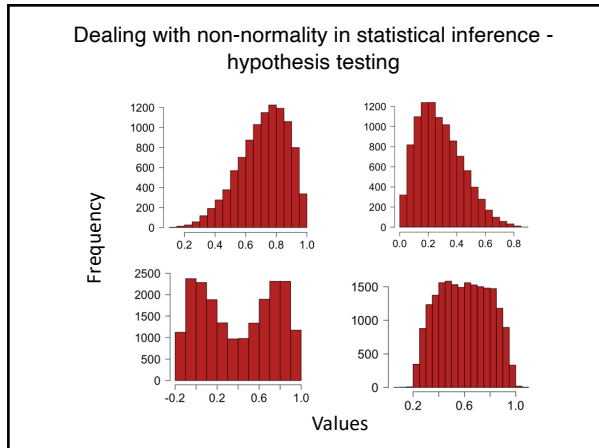
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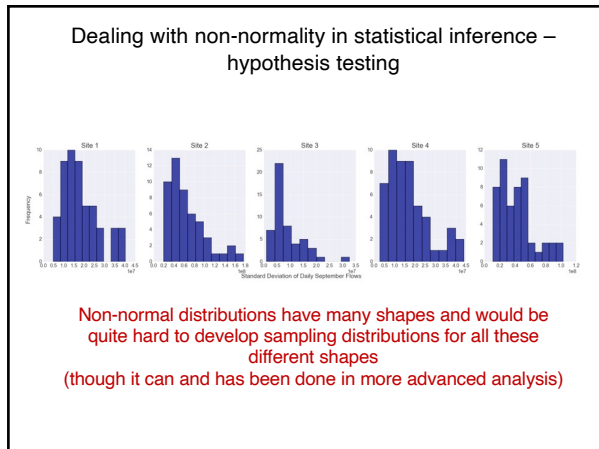
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**The effects of non-normality on statistical test**

Parametric tests assuming normality (e.g., t-test & ANOVA) are often robust against non-normality; but depending on the type of non-normality (shape), parametric tests can have type I errors different (often greater) from alpha; and low power (increased type II error).

One challenge is to separate normality from heteroscedasticity issues (even in simulations).

The other challenge is when samples come all from populations with different distributions (even though they could have the same means, i.e.,  $H_0$  is true).

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The effects of non-normality on statistical test

Parametric tests assuming normality (e.g., t-test & ANOVA) are often robust against non-normality; but depending on the type of non-normality (shape of the distribution), parametric tests can have type I errors (false positives) that differ (often greater) from alpha; and low power (increased type II error; false negatives).

Br. J. Math. Stat. Psychol., 2013 May 6(2):224-44. doi: 10.1111/2044-8317.12024. Epub 2012 May 24.  
The impact of sample non-normality on ANOVA and alternative methods.  
Larsz B.

Author information

Abstract

In this journal, Zimmerman (2004, 2011) has discussed preliminary tests that researchers often use to choose an appropriate method for comparing locations when the assumption of normality is doubtful. The conceptual problem with this approach is that such a two-stage process makes both the power and the significance of the entire procedure uncertain, as type I and type II errors are possible at both stages. A type I error at the first stage, for example, will obviously increase the probability of a type II error at the second stage. Based on the idea of Schmidt et al. (2010), which proposes that simulated sets of sample data be ranked with respect to their degree of normality, this paper investigates the relationship between population non-normality and sample non-normality with respect to the performance of the ANOVA, Brown-Forsythe test, Welch test, and Kruskal-Wallis test when used with different distributions, sample sizes, and effect sizes. The overall conclusion is that the Kruskal-Wallis test is considerably less sensitive to the degree of sample normality when populations are distinctly non-normal and should therefore be the primary tool used to compare locations when it is known that populations are not at least approximately normal.

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The effects of non-normality on statistical test

Parametric tests assuming normality (e.g., t-test & ANOVA) are often robust against non-normality; but depending on the type of non-normality (shape), parametric tests can have type I errors different (often greater) from alpha and also low power (increased type II error).

What happens if the Type I error probability (rate) is **greater** than alpha? i.e., **increase number of False Positives.**

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The effects of non-normality on statistical test

Parametric tests assuming normality (e.g., t-test & ANOVA) are often robust against non-normality; but depending on the type of non-normality (shape), parametric tests can have type I errors different (often greater) from alpha and also low power (increased type II error).

What happens if the Type I error probability (rate) is **greater** than alpha? i.e., **increase number of False Positives.**

What happens if the Type I error probability (rate) is **smaller** than alpha? **decrease False Positives but also decrease True Positives (i.e., lower statistical power).**

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**Type I versus Type II errors – the “common” view**

A **Type I error (false positive)** is an **error** in every sense of the word. A conclusion is drawn that the null hypothesis is false when, in fact, it is true.

Therefore, **Type I** errors are generally considered more serious than **Type II** errors (false negatives).

Type II errors are often considered as “oh well, we were not able to detect an effect”...perhaps increase sample size!

Adapted from <http://davidmlane.com/hyperstat/A2917.html>

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**Type I versus Type II errors – the “common” view**

A **Type I error (false positive)** is an **error** in every sense of the word. A conclusion is drawn that the null hypothesis is false when, in fact, it is true.

Therefore, **Type I** errors are generally considered more serious than **Type II** errors (false negatives). **Type II** errors are often considered as “oh well, we were not able to detect an effect”...perhaps increase sample size!

Adapted from <http://davidmlane.com/hyperstat/A2917.html>

**When committing a type I error, you are stating that something that is false to be true.**

**CONFUSING: When committing a type II error, you are NOT stating that something that is true to be false (you are just not discovering something new).**

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**Non-parametric tests based on ranks are those that can handle non-normal data**

These are the main tests traditionally used in **Biology** for comparing samples:

1) For comparing two samples (analogue of the parametric two sample t-test) – **The Mann-Whitney U-test** (also known as the Mann-Whitney-Wilcoxon test, the Wilcoxon rank-sum test, or the Wilcoxon two-sample test).

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**Non-parametric tests based on ranks are those that can handle non-normal data**

These are the main tests traditionally used in Biology for comparing samples:

- 1) For comparing two samples (analogue of the parametric two sample t-test) – *The Mann-Whitney U-test* (also known as the Mann-Whitney-Wilcoxon test, the Wilcoxon rank-sum test, or the Wilcoxon two-sample test).
- 2) For comparing multiple samples (analogue of the parametric ANOVA) – *The Kruskal-Wallis test* (generalization of the U-test)

The P-value for the *The Mann-Whitney U-test* and the *The Kruskal-Wallis test* is mathematically the same; as such, we will cover only the latter.

**Note: remember that  $t^2 = F$ ;** we often cover t-tests (and not only ANOVAs) in courses for two main reasons – [1] one sample t-tests; [2] understand the nature of post-hoc testing (e.g., post-hoc pairwise comparisons of means after ANOVA and because there is a t-test dealing with samples when their populations differ in their variances).

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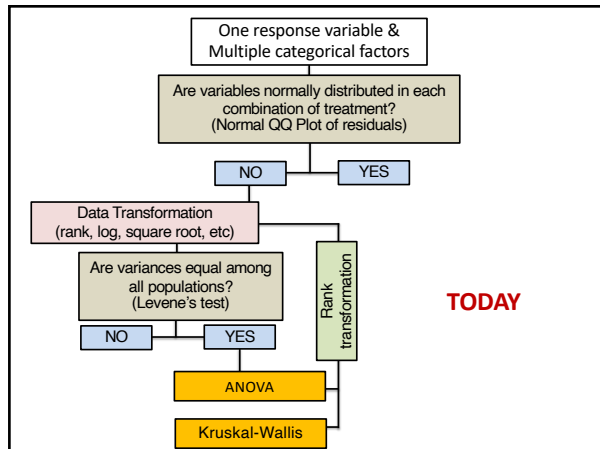
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**Many non-parametric tests are based on rank transformations**

gene	class	F <sub>ST</sub>
CVJ5	DNA	-0.006
CVB1	DNA	-0.005
6Pgd	protein	-0.005
Pgi	protein	-0.002
CVL3	DNA	0.003
Est-3	protein	0.004
Lap-2	protein	0.006
Pgm-1	protein	0.015
Aat-2	protein	0.016
Adk-1	protein	0.016
Sdh	protein	0.024
Acp-3	protein	0.041
Pgm-2	protein	0.044
Lap-1	protein	0.049
CVL1	DNA	0.053
Mpi-2	protein	0.058
Ap-1	protein	0.066
CVJ6	DNA	0.095
CVB2m	DNA	0.116
Est-1	protein	0.163

**Example:** F<sub>ST</sub> is a measure of the amount of geographic variation in a genetic polymorphism. Here, McDonald et al. (1996) compared two populations of the American oyster regarding the F<sub>ST</sub> based on six anonymous DNA polymorphisms (variation in random bits of DNA of no known function) and compared them to F<sub>ST</sub> values on 13 proteins.

**Question:** Do protein differ in F<sub>ST</sub> values in contrast to anonymous DNA polymorphisms?

**Zero F<sub>ST</sub>** = no genetic variation (panmictic)

**negative F<sub>ST</sub>** = more genetic variation within populations than between the two populations being compared.

**positive F<sub>ST</sub>** = more variation between populations than within the two populations being compared.

<http://www.biostathandbook.com/kruskalwallis.html>

Data from McDonald et al. (1996)

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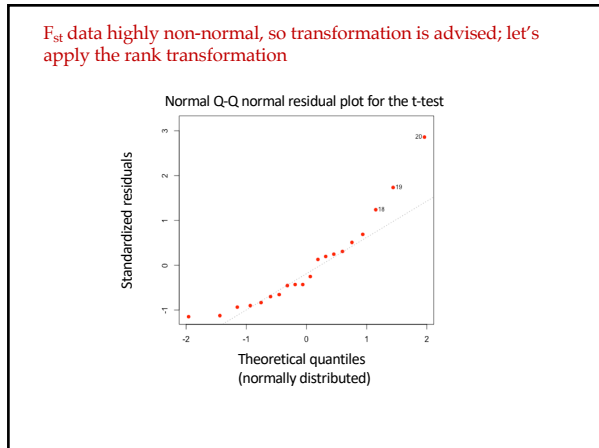
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Many non-parametric tests are based on rank transformations

gene	class	$F_{ST}$	Rank	Rank
CVJ5	DNA	-0.006		1
CVB1	DNA	-0.005	2.5	(2+3)/2=2.5
6Pgd	protein	-0.005	2.5	
Pgi	protein	-0.002		4
CVL3	DNA	0.003		5
Est-3	protein	0.004		6
Lap-2	protein	0.006		7
Pgm-1	protein	0.015		8
Aat-2	protein	0.016	9.5	(9+10)/2=9.5
Adk-1	protein	0.016	9.5	
Sdh	protein	0.024		11
Acp-3	protein	0.041		12
Pgm-2	protein	0.044		13
Lap-1	protein	0.049		14
CVL1	DNA	0.053		15
Mpi-2	protein	0.058		16
Ap-1	protein	0.066		17
CVJ6	DNA	0.095		18
CVB2m	DNA	0.116		19
Est-1	protein	0.163		20

<http://www.biostathandbook.com/kruskalwallis.html> Data from McDonald et al. (1996)

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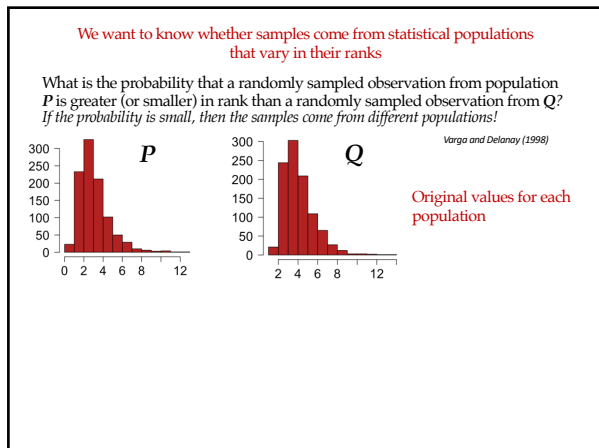
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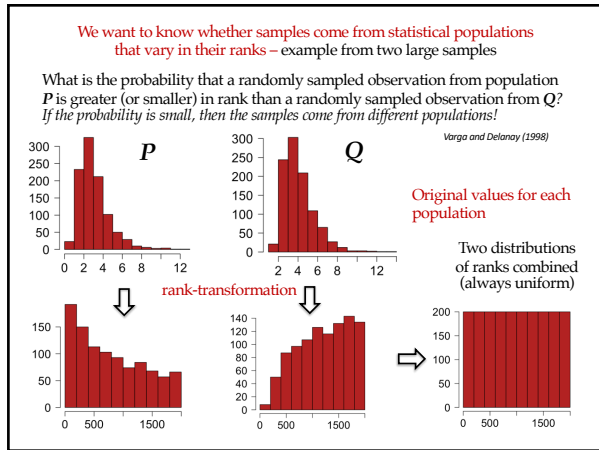
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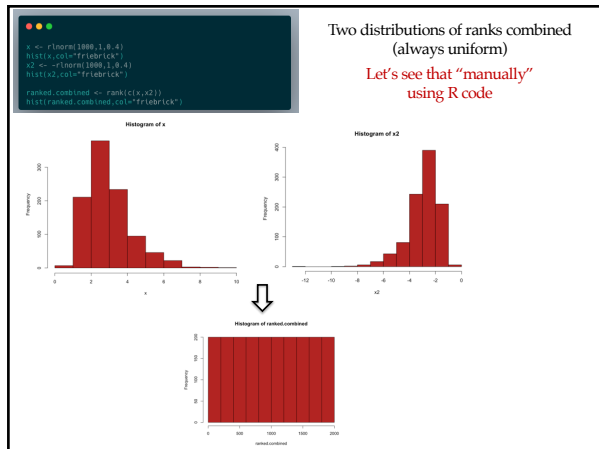
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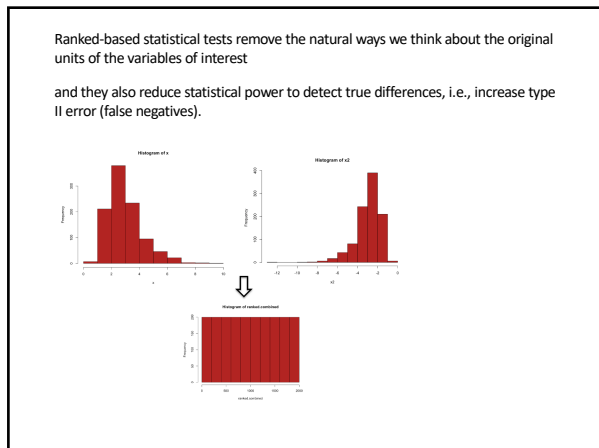
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# Rank based tests



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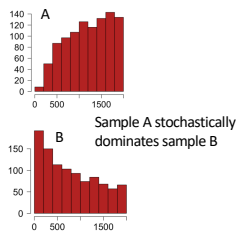
## Kruskal-Wallis test (akin to one-factorial ANOVA but based on ranks)

**H<sub>0</sub>:** no population from where the samples were taken stochastically dominates another population (stochastic homogeneity).

**H<sub>a</sub>:** at least one population from where the sample was taken stochastically dominates another population (stochastic heterogeneity).



Which sample? Post-hoc tests (based on ranks)



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## Kruskal-Wallis test (akin to one-factorial ANOVA but based on ranks)

**H<sub>0</sub>:** no population from where the samples were taken stochastically dominates another population (stochastic homogeneity).

**H<sub>a</sub>:** at least one population from where the sample was taken stochastically dominates another population (stochastic heterogeneity).

————— F<sub>STS</sub> data —————  
**H<sub>0</sub>:** DNA and protein do not stochastically dominate each other in their F<sub>STS</sub>.

**H<sub>a</sub>:** Either DNA or protein stochastically dominate each other in their F<sub>STS</sub>.

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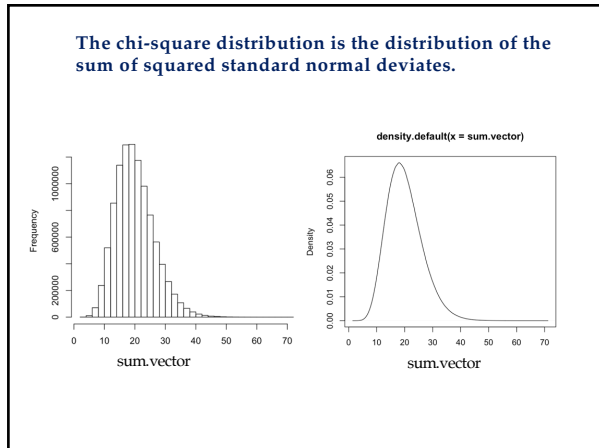
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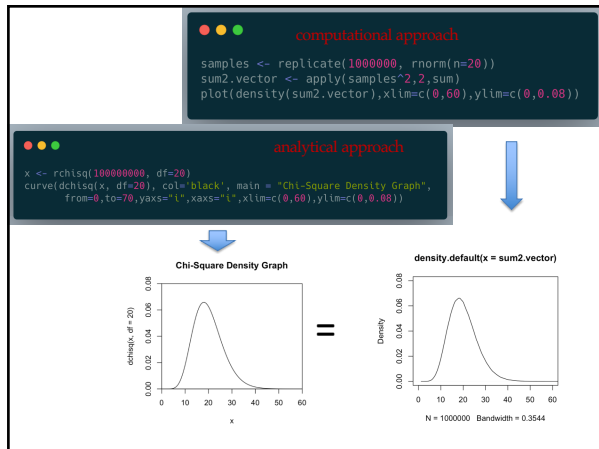
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The chi-square distribution is the distribution of the sum of squared standard normal deviates.

*fun fact:* The F distribution is the ratio of two (scaled) chi-square distributed values. The scaling is done by appropriate division of degrees of freedom.

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# A general solution to rank-based tests



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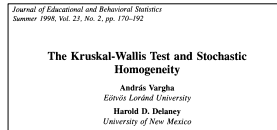
## Kruskal-Wallis test is equivalent (close enough) to an ANOVA on ranks

**Ho:** no sample stochastically dominates another sample (stochastic homogeneity).

**Ha:** at least one sample stochastically dominates one other sample (stochastic heterogeneity).

*“Stochastic homogeneity is equivalent to the equality of the expected values of the rank sample means. This finding implies that the null hypothesis of stochastic homogeneity can be tested by an ANOVA performed on the rank transforms, which is essentially equivalent to doing a Kruskal-Wallis H test.”*

Varga and Delaney (1998)



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## Kruskal-Wallis test = ANOVA on ranks

### Kruskal-Wallis:

**Ho:** no sample stochastically dominates another sample (stochastic homogeneity).

**Ha:** at least one sample stochastically dominates one other sample (stochastic heterogeneity).



Varga and Delaney (1998)

### ANOVA:

**Ho:** no mean differences in ranked values

**Ha:** at least one sample differs in mean ranked values from another sample

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**Kruskal-Wallis test = ANOVA on ranks**

```
> Fst.values <- c(-0.006,-0.005,-0.005,-0.002,0.003,0.004,
0.006,0.015,0.016,0.016,0.024,0.041,0.044,
0.049,0.053,0.050,0.066,0.095,0.116,0.163)
> Fst.rank <- rank(Fst.values)
> hist(Fst.rank,col="firebrick")
> Fst.group <- c(1,1,2,2,1,2,2,2,2,2,2,2,2,1,2,1,1,2)
> kruskal.test(Fst.values~Fst.group)
```

```
> kruskal.test(Fst.values~Fst.group)
Kruskal-Wallis rank sum test

data:  Fst.values by Fst.group
Kruskal-Wallis chi-squared = 0.042581, df = 1, p-value = 0.8365
```

They are slightly different, no?

```
> summary(aov(Fst.rank~Fst.group))
          Df Sum Sq Mean Sq F value Pr(>F)
Fst.group  1  1.5    1.49    0.04  0.843
Residuals 18 662.5   36.81
```

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**Kruskal-Wallis test = ANOVA on ranks**

Kruskal-Wallis and ANOVA are “asymptotically equivalent” (i.e., the two functions “eventually” become “essentially equal”) and so P-values are exactly the same for very large samples and they do not differ by much for small sample size.

Two sample Kruskal-Wallis P-values (chi-square based) and F-based P values

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Kruskal-Wallis and ANOVA are “asymptotically equivalent” and so P-values are the same for very large samples and they do not differ by much for small sample size. Using R code to demonstrate the asymptotic equivalence.

```
n.simul <- 200
Pvector <- matrix(0,n.simul,2)
n <- 10
n.vector <- matrix(0,n.simul,1)
for (i in 1:n.simul){
  groups <- c(rep(1,n),rep(2,n))
  x <- rnorm(n*2)
  Pvector[i,1] <- kruskal.test(x~groups)$p.value
  Pvector[i,2] <- anova(lm(rank(x)~groups))$'Pr(>F)'[1]
  n <- n + 10
  n.vector[i] <- n
}

plot(n.vector/2,abs(Pvector[,1]-Pvector[,2]))
abline(h=0,col="red")
```

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Kruskal-Wallis and ANOVA are “asymptotically equivalent”

```

n.simul <- 200
Pvector <- matrix(0,n.simul,2)
n <- 10
n.vector <- matrix(0,n.simul,1)
for (i in 1:n.simul){
  groups <- c(rep(1,n),rep(2,n))
  x <- rnorm(n*2)
  Pvector[i,1] <- kruskal.test(x~groups)$p.value
  Pvector[i,2] <- anova(lm(rank(x)~groups))$Pr(>F)[1]
  n <- n*10
  n.vector[i] <- n
}
plot(n.vector/2,abs(Pvector[,1]-Pvector[,2]))
abline(h=0,col="red")
    
```

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**Kruskal-Wallis test = ANOVA on ranks**

Kruskal-Wallis and ANOVA are “asymptotically equivalent” and so P-values are exactly the same for very large samples and they do not differ by much for small sample size.

Because of the equivalence, we can then expand non-parametric analysis based on ranks to any multi-factorial ANOVAs, regressions, MANOVA, ANCOVA, etc

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**NOTE: Non-parametric tests are those that can handle non-normal data**

There is a common misunderstanding in the statistical literature and among practitioners, including many biostatistics books, that non-parametric tests can also handle differences in variances among samples.

THIS IS NOT TRUE! They are also affected by variance differences among groups/ treatments (i.e., homoscedasticity).

Test variance differences in ranks (almost never done in the literature)!

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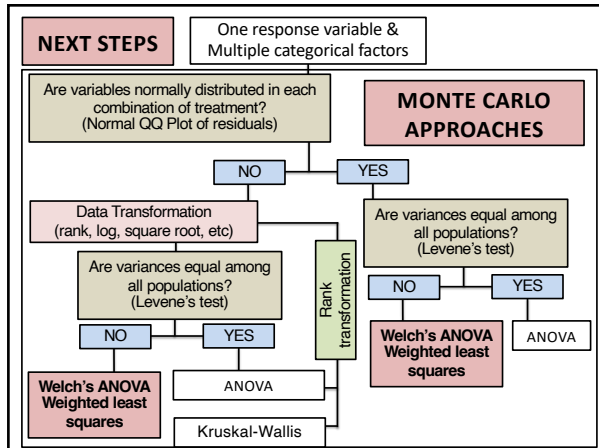
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