Nonparametric Statistics

- ✓ Exact test & Sign Test
- ✓ χ^2 test & Contingency Table
- ✓ Fisher's exact test
- ✓ Risk ration & Odd ratio
- ✓ Wilcoxon Signed Rank test
- ✓ Mann-Whitney U test & Kruskal-Wallis test
- ✓ Spearman's Rank Correlation Coefficient

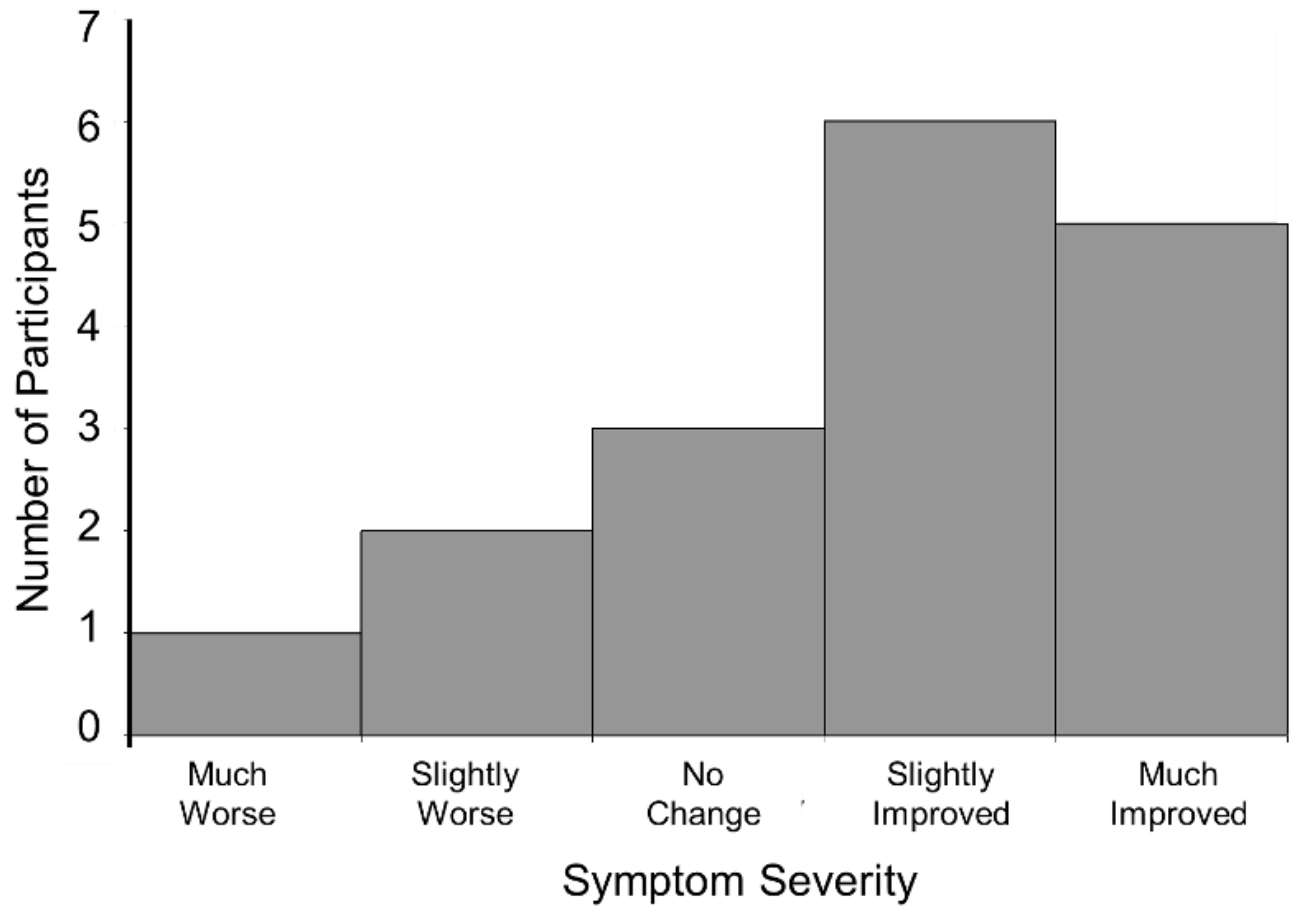
Why do we use parametric tests?

- provide useful parameters for data (e.g. descriptive statistic) and to test hypotheses
- are robust and versatile;
- are capable to tests two or more variable & their interactions;
- can predict the outcomes
- use in several experimental designs;

Why don’t we use parametric tests?

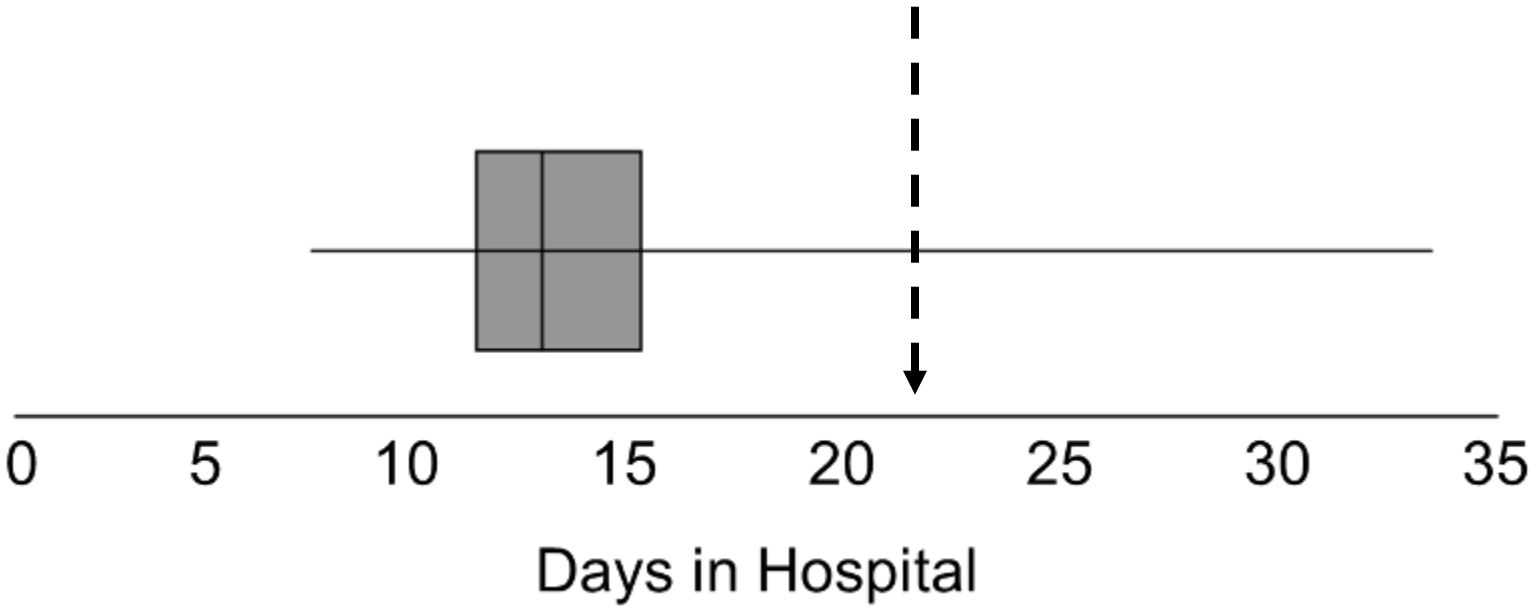
- Normality test, e.g. Kolmogorov-Smirnov test, the Anderson-Darling test, and the Shapiro-Wilk test, suggest data not in normal distribution;
- can’t use when data are extreme violation of normality assumptions, e.g.
  - Skewed data
  - Outliner(s) in data
- is inappropriate if scaling of data is not properly made so.
  - Data are measured in nominal or rank scales

Skewed data



http://sphweb.bumc.bu.edu/otlt/MPH-Modules/BS/BS704_Nonparametric/BS704_Nonparametric2.html

Outliners in data



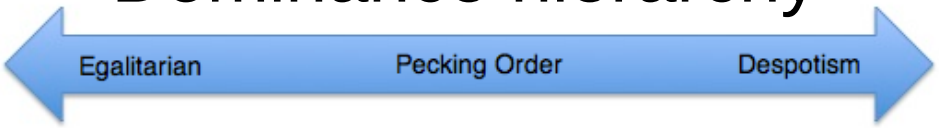
http://sphweb.bumc.bu.edu/otlt/MPH-Modules/BS/BS704_Nonparametric/BS704_Nonparametric2.html

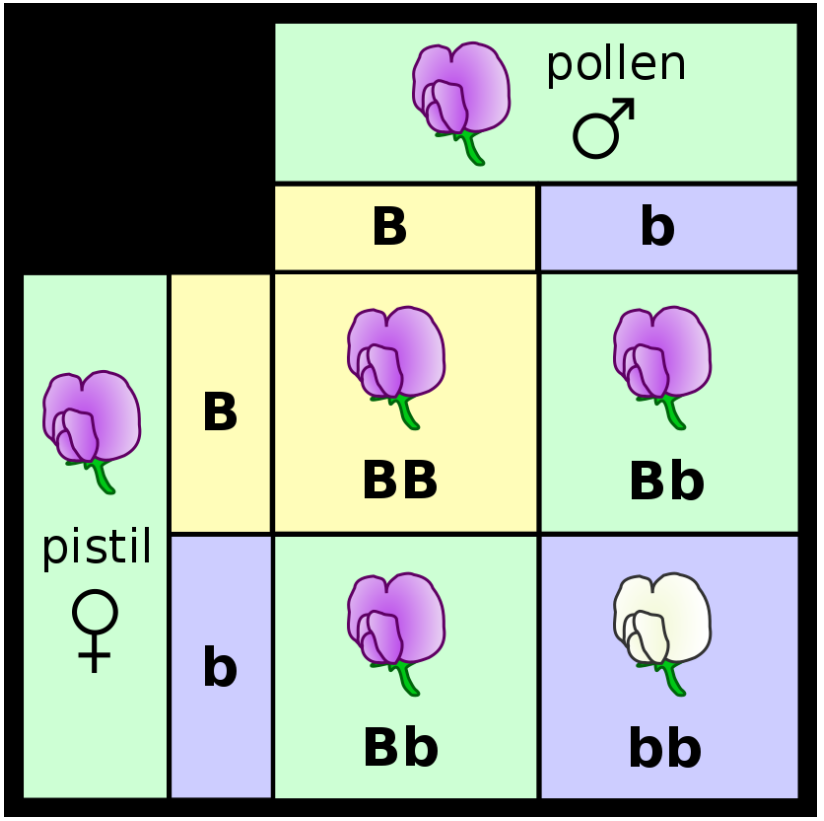
Outcomes from studies being rank or nominal scales

Table 1. The 0–5 scale of the disease severity was classified as follows.

Grade	Infection (%)	Reaction
0	Nil	Immune (I)
1	Upto 5% infection	Resistant (R)
2	Upto 10% infection	Moderately resistant (MR)
3	Upto 20% infection	Moderately susceptible (MS)
4	Upto 30% infection	Susceptible (S)
5	40% or more	Highly susceptible (HS)

Dominance hierarchy





http://www.academicjournals.org/journal/AJMR/article-full-text/D3829A250030
https://en.wikipedia.org/wiki/Dominance_hierarchy
https://en.wikipedia.org/wiki/Phenotype

Exact test for Goodness of Fit

- **ONE nominal variable**
- Two or more mutually exclusive categories
- small N (≤1,000) and independent observations
- Hypotheses
  - Ho : The observed data in each category is equal to that predicted by biological theory
  - H1 : The observed data in each category differs from that the expected
- Example – sex ratio as 1:1 or phenotypes ratio as 3:1

Exact test for Goodness of Fit

- Probability is directly calculated from the observed data under null hypothesis
- For binomial experiment, i.e. only 2 categories, the probability **Y** of *k* successes in *n* trials is obtained:

$$\mathrm{Y} = \frac{p^k (1-p)^{(n-k)!} n!}{k!\,(n-k)!}$$

k = numbers of successes

n = total number of trials

p = the expected proportion of successes if Ho is true

Exact test for Goodness of Fit

- In Excel 2010+, **Y** can be calculated as:

=BINOM.DIST(*k,n,p,FALSE*)

- However, to test hypothesis, the probability ***P*** must include not only as extreme case as in observed data but also more extreme cases than in observed data; that is

***P* = BINOM.DIST(*k,n,p,TRUE*)**

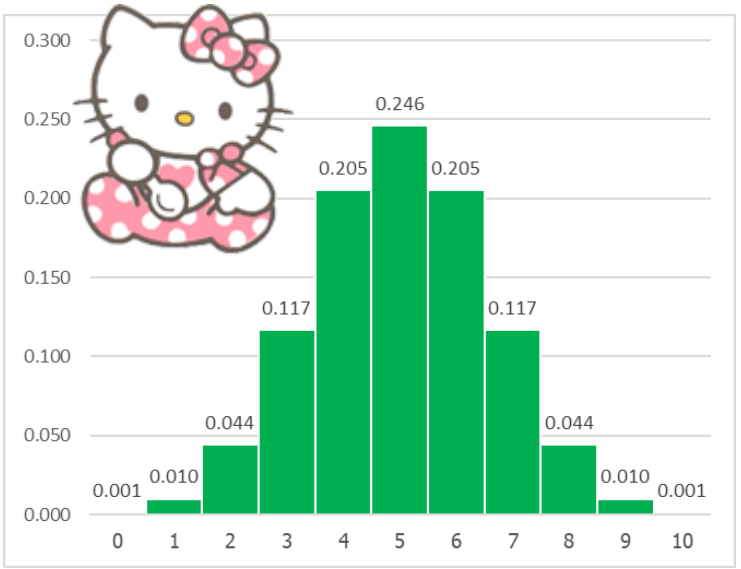
- ***P*** from this function is the sum of ***Y*** for *k*, *k-1*, …, 0 successes and this ***P*** is an one-tailed ***P***
- If *p* is 0.5, the two-tailed ***P*** is 2×(one-tailed ***P***)

Exact test for Goodness of Fit

- A researcher wants to know if his cat use both paws (left & right ones) equally. A cat is irritated by a ribbon, and numbers of a left paws and a right paws used to catch a ribbon are counted
- The result is that a ribbon were caught 8 times by a right paw and only 2 by a left one
- Hypotheses
  - Ho : A cat equally uses both paws
  - H1 : A cat does not equally uses both paws

Exact test for Goodness of Fit

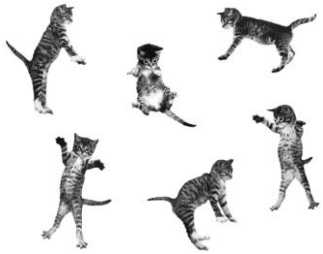
- Use **BINOM.DIST**(k,n,p,TRUE) to generate ***P*** for k=0, 1, …, 10
- For using a *left* paw 2, 1, 0 times, P_L = .044+.010 +.001 =.055
- If we consider the other extreme as well, i.e. using a *right* paws 2, 1, 0 times, it gives another P_R = .044+.010+.001=.055
- As $P = P_L + P_R$ = .11, therefore accept Ho



Exact test for Goodness of Fit

- If $p \neq 0.5$, two-tailed ***P*** must be calculated by another approaches
- A researcher knows that his cats are heterozygous at a hair-length gene, and the short-hair is dominant allele. So she expects to get short-hair kitten 75% and long-hair kittens 25% after crossing them
- The result is that she got 7 short-hair kittens and 5 long-hair kittens
- Hypotheses
  - Ho : The ratio of short-hair kitten to long-hair kittens is 3 to 1
  - H1 : The ratio of short-hair kitten to long-hair kittens is not 3 to 1
- SPSS calculates ***P*** by the “method of small *P* values”
- How, manually?

Exact test for Goodness of Fit



=BINOM.DIST(*k*,*n*,*p*,*FALSE*)

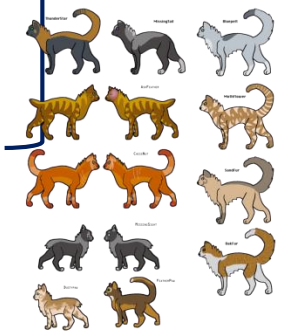
p=0.75 for short-hair allele	**#kittens, *k*, for *n*=12**	*p=0.25 for long-hair allele*
P values for *k*		***P*** values for *k*
0.0000000596	**0**	0.0316763520
0.0000021458	**1**	0.1267054081
0.0000354052	**2**	0.2322932482
0.0003540516	**3**	0.2581036091
0.0023898482	**4**	0.1935777068
0.0114712715	**5**	0.1032414436
0.0401494503	**6**	0.0401494503
0.1032414436	**7**	0.0114712715
0.1935777068	**8**	0.0023898482
0.2581036091	**9**	0.0003540516
0.2322932482	**10**	0.0000354052
0.1267054081	**11**	0.0000021458
0.0316763520	**12**	0.0000000596

0.157643676

0.031676352

0.031676352

0.157643676



P = 0.189320028, <u>thus accept Ho</u>

Multinomial exact test for goodness-of-fit test

- Flower phenotypes from genetic cross in which the expect outcome of a 9:3:3:1 ratio of purple, red, blue, and white
- You get 72 purple, 38 red, 20 blue, and 18 white
- Analyzed in SPSS, you get a sig. of 0.0016 ➔ reject Ho: ratio of genetic cross is 9:3:3:1, then accept **H1**: ratio of genetic cross is *NOT* 9:3:3:1
- Next puzzles: which phenotype(s) is deviated from the expectation?
- Do **Post-hoc test** by conducting binomial exact tests for each category vs. the sum of all others categories with Bonferroni-correction significance level

Post-hoc test for multinomial exact test for goodness-of-fit test

eg. Reject Ho: a ratio of purple, red, blue, and white is 9:3:3:1

- Four tests
  I. Ho: purple : others is a ratio of 9:7
  II. Ho: red : others is a ratio of 3:13
  III. Ho: blue : others is a ratio of 3:13
  IV. Ho: white : others is a ratio of 1:15
- Sig. for four tests
  I. Sig. = 0.068
  II. Sig. = 0.035
  III. Sig. = 0.114
  IV. Sig. = 0.005

Post-hoc test for multinomial exact test for goodness-of-fit test

eg. Reject Ho: a ratio of purple, red, blue, and white is 9:3:3:1

- Bonferroni correction significant level for α=0.05 is

$$\frac{significance\ level}{number\ of\ tests} = \frac{0.05}{4\ tests} = 0.0125$$

- Only test#4 has Sig. < 0.0125 ➔ white is deviated from expected, i.e. more white than expected (18:130 ≈ 1:7.2)

χ^2 test – Goodness of Fit test

- **ONE nominal variable**; two or more mutually exclusive categories, large N (>1000), and independent observations
- Example:
  - number of individuals with genotype ***TT***, ***Tt***, or ***tt***
  - those with pollen phenotype round and elliptic.
- Calculation:

$$\chi^2_{cal} = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

O_i = observed value in category *i*
E_i = expected value in category *i*

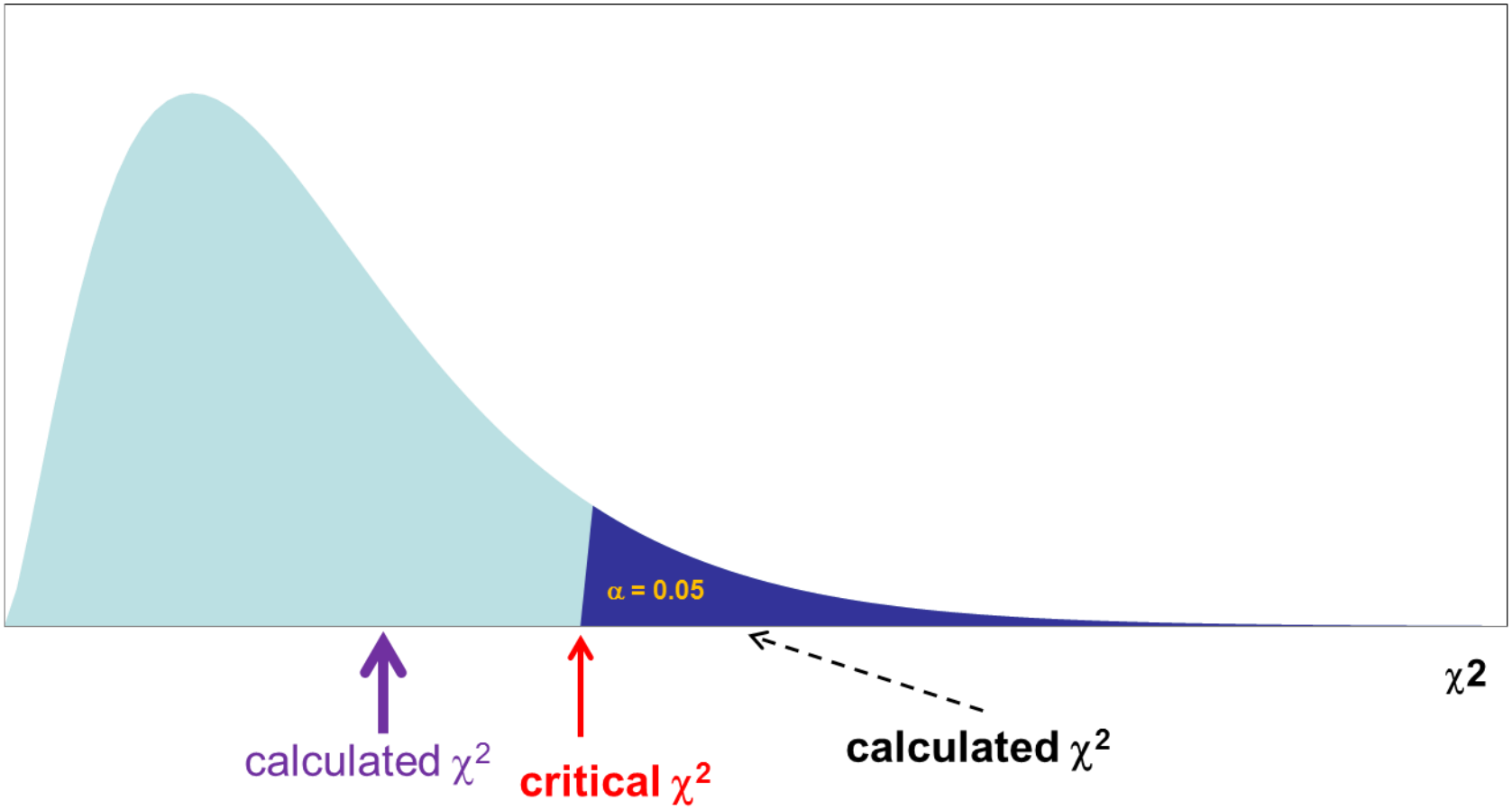
χ^2 test – Goodness of Fit test

- The shape of χ^2 distribution depending on degree of freedom
  - Extrinsic null hypothesis:
    - The predicted proportions are known from the null hypothesis before collecting data.
    - The degree of freedom (d.f.) = *n-1*, where *n* is number of categories in a variable
    - Example:- Sex ratio of male:female = 1:1, d.f. = 2-1 = 1
- Reject H_o if $\chi^2_{cal} \geq \chi^2_{crit}$

χ^2 test – Goodness of Fit test

- The shape of χ^2 distribution depending on degree of freedom
  - Intrinsic null hypothesis:
    - One or more parameters are estimated from the data in order to get the values for the null hypothesis.
    - The degree of freedom
      d.f. = *n-parameter(s)-1*
      - Ex. Genotypes of a codominant gene: ***LL, LS*** & ***SS***, d.f. = 3-1-1 = 1
- Reject H_o if $\chi^2_{cal} \geq \chi^2_{crit}$

Accept / Reject Ho



Example: Extrinsic null hypothesis

	TT	Tt	tt
Observed	42	110	48
Expected			

H_0 : Ratio of genotypes TT : Tt : tt = 1:2:1
H_1 : Ratio of genotypes TT : Tt : tt ≠ 1:2:1

Total = 42 + 110 + 48 = 200
Ratio = 1 + 2 + 1 = 4
Thus, 1 part = 200 ÷ 4 = 50

Example: Extrinsic null hypothesis

	TT	Tt	tt	Total
Observed	42	110	48	**200**
Expected	***50***	***100***	***50***	200
differences	***-8***	***10***	***-2***	
Δ^2	***64***	***100***	***4***	

$$\chi^2 = \frac{(42-50)^2}{50} + \frac{(110-100)^2}{100} + \frac{(48-50)^2}{50} = 3.08, df = 2$$

χ^2_{crit} = 5.991, df = 2, α = 0.05. ***Thus, ACCEPT Ho.***

Example: Intrinsic null hypothesis

	LL	LS	SS
Observed	14	21	25
Expected			

H_0 : Population is in Hardy-Weinberg equilibrium
H_1 : Population is not in Hardy-Weinberg equilibrium

$$p + q = 1$$
$$p^2 + 2pg + q^2 = 1$$

Example: Intrinsic null hypothesis

Allele frequencies (for diploid):

Total alleles = (14 + 21 + 25)×2 = 120

$$\mathrm{L} = \frac{14\times 2 + 21}{120} = 0.408$$

$$\mathrm{S} = 1 - 0.408 = 0.592$$

Thus, genotype frequencies is 0.167*LL*, 0.483*LS*, 0.350*SS*

Total = 14 + 21 + 25 = 60

$\therefore$ *LL* = 0.167 $\times$ 60 = 10.02

LS = 0.483 $\times$ 60 = 28.98

SS = 0.350 $\times$ 60 = 21.00

Example: Intrinsic null hypothesis

	LL	LS	SS
Observed	14	21	25
Expected	**10.02**	**28.98**	**21**

$$\chi^2 = \frac{(14-10.02)^2}{10.02} + \frac{(21-28.98)^2}{28.98} + \frac{(25-21)^2}{21} = 4.56, df = 1$$

χ^2_{crit} = 3.841, df = 1, α = 0.05. ***Thus, REJECT Ho.***

χ^2 test for Contingency Table or R x C table

- **Test of Homogeneity**
  - A test for the determination of whether or not the proportion are the same in <u>two independent samples</u>
    - One set of marginal totals are fixed; the others are free to vary.

χ^2 test for Contingency Table or R x C table

- **Test of Independence** or **Test of association** between/among variables
  - A test for the independence of two [*or more*] characteristics in the same sample when neither characteristic is particularly appropriate as a denominator
    - All marginal totals are free to vary

Test of Homogeneity

	Age at first birth		
Status	≥30	≤29	Total
Case	683	2537	*3,220*
Control	1498	8747	*10,245*
Total	2,181	11,284	13,465

Variable A: Incidence of breast cancer

Case : women with breast cancer

Control : women without breast cancer

Variable B: Age of women giving the first child

≥30 : women with age at first birth ≥30

≤29 : women with age at first birth ≤ 29

Let p_1 = the probability that age at first birth is ≥30 in CASE women with at least one birth

p_2 = the probability that age at first birth is ≥30 in CONTROL women with at least one birth

The problem is whether or not *p1 = p2*, or one want to test these hypotheses:

$H_o : p_1 = p_2$ **vs.** $H_1 : p_1 = p_2$

Test of Independence

	First food-frequency questionnaire		
Second food-frequency questionnaire	High	Normal	Total
High	15	5	20
Normal	9	21	30
Total	24	26	50

Question : Is there any relationship between the two reported measures of dietary cholesterol for the same person?

Variable B	Variable A					
	1	2	3	…	c	
1	n_{11}	n_{12}	n_{13}	…	n_{1c}	$n_{1.}$
2	n_{21}	n_{22}	n_{23}	…	n_{2c}	$n_{2.}$
:	…	…	…	…	…	…
r	n_{r1}	n_{r2}	n_{r3}	…	n_{rc}	$n_{r.}$
	$n_{.1}$	$n_{.2}$	$n_{.3}$	…	$n_{.c}$	n

Where

n = total number of samples

n_{ij} = observed numbers in $(ij)^{th}$ *cell*

$n_{i.}$ = **marginal total** in i^{th} **row**, $I = 1, \ldots, r$

$n_{.j}$ = **marginal total** in j^{th} **column** $j = 1, \ldots, c$

- Calculating of E_{ij}:

$$E_{ij} = \frac{(n_{i\bullet})(n_{\bullet j})}{n}$$

- Reject H_o if $\chi^2_{cal} \geq \chi^2_{crit}$ at df = (r-1)(c-1)

Example: Test of Homogeneity

Let p_1 = the probability that age at first birth is ≥30 in CASE women with at least one birth
p_2 = the probability that age at first birth is ≥30 in CONTROL women with at least one birth

$H_o : p_1 = p_2$ **vs.** $H_1 : p_1 = p_2$

	Age at first birth	
Status	≥30	≤29
Case	683	2537
Control	1498	8747

Example: Test of Homogeneity

	Age at first birth		
Status	≥30	≤29	Total
Case	683 $\frac{3220 \times 2181}{13465} = 521.56$	2537 $\frac{3220 \times 11284}{13465} = 2698.44$	*3,220*
Control	1498 $\frac{10245 \times 2181}{13465} = 1659.44$	8747 $\frac{10245 \times 11284}{13465} = 8585.56$	*10,245*
Total	2,181	11,284	13,465

$\chi^2_{cal} = 78.37$

df = (2-1)(2-1) = 1

$p = 8.54 \times 10^{-19}$

$\chi^2_{crit,\ \alpha = 0.05} = 3.84$

p is function CHISQ.DIST.RT(χ^2_{cal} , df)

Example: Test of Independence

H0: Vaccine inoculation and flu susceptible are independent.
H1: Vaccine inoculation and flu susceptible are NOT independent.

Variable A: Experiencing flu?
- Yes
- No

Variable B: Vaccinated
- Yes
- No

		Experiencing flu		
		Y	N	
inoculated	Y	150	200	
	N	300	250	
				900

		Experiencing flu		
		Y	N	
inoculated	Y	150 175	200 175	350
	N	300 275	250 275	550
		450	450	900

$\chi^2_{cal} = 11.68$

df = (2-1)(2-1) = 1

p = 0.00063

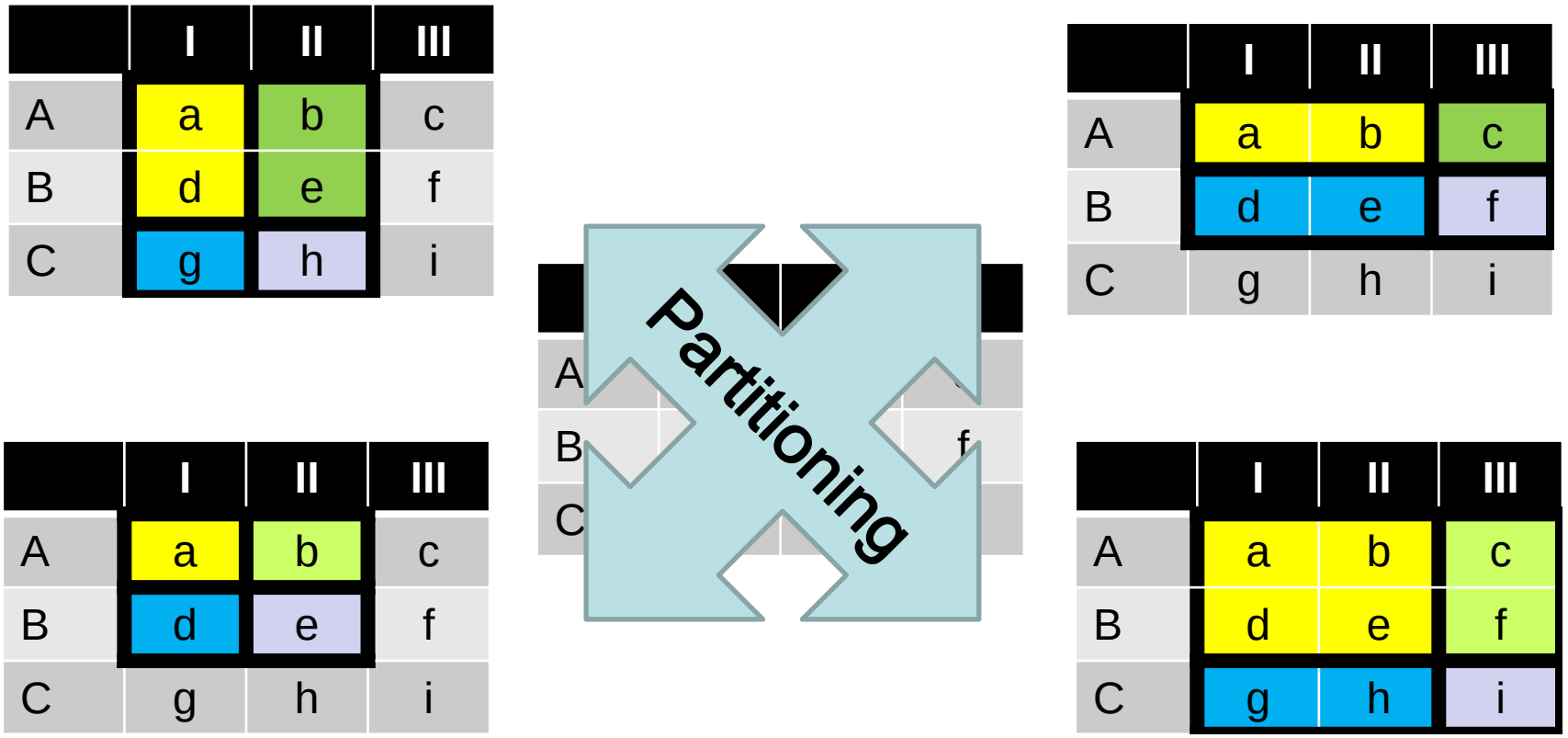
$\chi^2_{crit,\ \alpha = 0.05} = 3.84$

Pos-Hoc test for > 2x2 table

- **Method I** : Calculating residual approach
  - Calculate standardized residuals for each cell: $std\ residual = \frac{O-E}{\sqrt{E}}$
  - More appropriately, *adjusted standardized residuals* should be used
  - If absolute of adjusted standardized residuals are greater than 1.96 ➔ those cell are deviated from expected at sig. level of 0.05
    - This is an uncorrected significance level for number of comparisons

Pos-Hoc test for a table larger than 2x2

- **Method II** : Partitioning approach
  - Analyze 2x2 subtables *orthogonally* partitioned from the original table
  - use χ^2 tests with Bonferroni correction of the P value



Pos-Hoc test for a table larger than 2x2

- Alternatives to Method II
  - Conduct 2x2 tests for each category vs. the sum of all others categories with Bonferroni-correction significance level
  - Conduct 2x2 test for each pair of categories with Bonferroni-correction significance level

Make sense?

$$Bonferroni\ correction = \frac{0.05}{4} = 0.0125$$

$$z_{2-tailed,p=0.0125} = \pm 2.50$$

			Experiencing flu?		
			Yes	No	Total
Vaccine inoculation	Yes	Count	150	200	350
		Expected Count	175.0	175.0	350.0
		% within Vaccine inoculation	42.9%	57.1%	100.0%
		% within Experiencing flu?	33.3%	44.4%	38.9%
		Residual	-25.0	25.0	
		Std. Residual	-1.9	1.9	
		Adjusted Residual	-3.4	3.4	
	No	Count	300	250	550
		Expected Count	275.0	275.0	550.0
		% within Vaccine inoculation	54.5%	45.5%	100.0%
		% within Experiencing flu?	66.7%	55.6%	61.1%
		Residual	25.0	-25.0	
		Std. Residual	1.5	-1.5	
		Adjusted Residual	3.4	-3.4	

Example: 10 x 2 table

H0: Proportion between positive goiter test in men in different province is the same
H1: Proportion between positive goiter test in men in different province is not the same

Variable A: Test result
- Positive
- negative

Variable B: Location
- Bangkok
- Chiangmai
- …

	Goiter test		
	Positive	Negative	
Bangkok	36		500
Chaingmai	17		350
Nan	12		300
Nakornsawan	1		300
Saraburi	4		350
Chonburi	14		500
Udonthani	7		200
Surin	27		500
Srisaket	2		200
Chumpon	4		200

Location	observed		expected		$\frac{(O-E)^2}{E}$	
	Positive	Negative	Positive	Negative	Positive	Negative
Bangkok	36	464	18.23529	481.7647	17.30626	0.65506
Chaingmai	17	333	12.76471	337.2353	1.405259	0.053191
Nan	12	288	10.94118	289.0588	0.102467	0.003878
Nakornsawan	1	299	10.94118	289.0588	9.032574	0.341892
Saraburi	4	346	12.76471	337.2353	6.018162	0.227794
Chonburi	14	486	18.23529	481.7647	0.983681	0.037233
Udonthani	7	193	7.294118	192.7059	0.011860	0.000449
Surin	27	473	18.23529	481.7647	4.212713	0.159456
Srisaket	2	198	7.294118	192.7059	3.842505	0.145443
Chumpon	4	196	7.294118	192.7059	1.487666	0.05631

$\chi^2_{cal} = 46.08$

$df = (10-1)(2-1) = 9$

$p = 5.82 \times 10^{-7}$

$\chi^2_{crit,\ \alpha = 0.05} = 16.919$

$\alpha^* = {}^{0.05}/_{20} = 0.0025;\ z_{2-tailed,\alpha^*=0.0025} = \pm 3.02$

			Goiter test result	
			Positive	Negative
Province	Bangkok	Count	36	464
		Expected Count	18.2	481.8
		Adjusted Residual	4.6	-4.6
	Chaingmai	Count	17	333
		Expected Count	12.8	337.2
		Adjusted Residual	1.3	-1.3
	Nan	Count	12	288
		Expected Count	10.9	289.1
		Adjusted Residual	.3	-.3
	Nakornsawan	Count	1	299
		Expected Count	10.9	289.1
		Adjusted Residual	-3.2	3.2
	Saraburi	Count	4	346
		Expected Count	12.8	337.2
		Adjusted Residual	-2.6	2.6
	Chonburi	Count	14	486
		Expected Count	18.2	481.8
		Adjusted Residual	-1.1	1.1
	Udonthani	Count	7	193
		Expected Count	7.3	192.7
		Adjusted Residual	-.1	.1
	Surin	Count	27	473
		Expected Count	18.2	481.8
		Adjusted Residual	2.3	-2.3
	Srisaket	Count	2	198
		Expected Count	7.3	192.7
		Adjusted Residual	-2.1	2.1
	Chumpon	Count	4	196
		Expected Count	7.3	192.7
		Adjusted Residual	-1.3	1.3

Tests of 2x2 sub-tables one category vs. all others

$$Bonferroni\ correction = \frac{0.05}{10} = 0.005$$

Location	observed		
	Positive	Negative	p-value
Bangkok	36	464	0.000004
all others	88	2812	
Chaingmai	17	333	0.202283
all others	107	2943	
Nan	12	288	0.732711
all others	112	2988	
Nakornsawan	1	299	0.001344
all others	123	2977	
Saraburi	4	346	0.008323
all others	120	2930	

Location	observed		
	Positive	Negative	p-value
Chonburi	14	486	0.273935
all others	110	2790	
Udonthani	7	193	0.908954
all others	117	3083	
Surin	27	473	0.023570
all others	97	2803	
Srisaket	2	198	0.039547
all others	122	3078	
Chumpon	4	196	0.200260
all others	120	3080	

Test of 2x2 sub-tables: two categories

	No disease	Coronary artery disease
ins/ins	268	807
ins/del	199	759
del/del	42	184

χ^2=7.26, df=2, p=0.027

$$Bonferroni\ correction = \frac{0.05}{3} = 0.017$$

	No disease	Coronary artery disease
ins/ins	268	807
ins/del	199	759

χ^2=4.95, df=1, p=0.027

	No disease	Coronary artery disease
ins/ins	268	807
del/del	42	184

χ^2=4.14, df=1, p=0.042

	No disease	Coronary artery disease
ins/del	199	759
del/del	42	184

χ^2=0.54, df=1, p=0.462

Alternative to χ^2 test for RxC table: Fisher's exact test

- A 2×2 contingency table
  (but can be applied to any $m \times n$ table)
- Expected values in any cell is **less than** 5
- members of two independent groups can fall into one of two mutually exclusive categories.
- The test is used to determine ***whether the proportions of those falling into each category differs by groups***
- One- or two-tail *exact* probabilities can be calculated

How to calculate Fisher's p-value

	C_1	C_2	
R_1	*A*	*B*	*A*+*B*
R_2	*C*	*D*	*C*+*D*
	A+*C*	*B*+*D*	*N*

$$p = \frac{(A+B)!(C+D)!(A+C)!(B+D)!}{N!\,A!\,B!\,C!\,D!}$$

In order to calculate the significance of the observed data, i.e. the total probability of observing data as *extreme or more extreme* if the null hypothesis is true, we have to calculate the values of *p* for both these tables, and add them together.

Example: Fisher's p-value

	Cured	Sicked	
Antibiotic treatment	*4*	*9*	*13*
Fecal transfer	*13*	*3*	*16*
	17	*12*	*29*

Observed data

	Cured	Sicked	
Antibiotic treatment	*1*	*12*	*13*
Fecal transfer	*16*	*0*	*16*
	17	*12*	*29*

Extreme data

- Fisher showed that we could deal only with cases where the marginal totals are the same as in the observed table. Thus, there are 11 cases; one extreme data is here!
- In order to calculate the significance of the observed data, i.e. the total probability of observing data as *extreme or more extreme* if the null hypothesis is true, we have to calculate the values of *p* for both these tables, and add them together.

Example: Fisher's p-values

13	0	0.000035
4	12	

9	4	0.177317
8	8	

5	8	0.045135
12	4	

12	1	0.001094
5	11	

8	5	0.283708
9	7	

4	9	0.007715
13	3	

11	2	0.012036
6	10	

7	6	0.264794
10	6	

3	10	0.000661
14	2	

10	3	0.063046
7	9	

6	7	0.144433
11	5	

2	11	0.000024
15	1	

1	12	0.000000
16	0	

One-tail p = 0.007715+[0.000661+0.000024+0.000000]= 0.008401

Two-tail p = [0.007715+0.000661+0.000024+0.000000]+[0.001094+0.000035]

= 0.009530

Testing your mind

Suppose that we have a population of fungal spores which clearly fall into two size categories, large and small. We incubate these spores on agar and count the number of spores that germinate by producing a single outgrowth or multiple outgrowths.

Spores counted:

- 120 large spores, of which 80 form multiple outgrowths and 40 produce single outgrowths
- 60 small spores, of which 18 form multiple outgrowths and 42 produce single outgrowths

You want to know if there is any difference between two classes of spores, what test you should carry out? Why? What hypothesis to set up? How to do the test?

Another one…

In order to test if these two genes are independently segregated, phenotypes in F2 generation were scored. The expected frequencies if genes being independently segregated should be **9:3:3:1**. Here is the result:

Of 1,132 plants, 705 are tall with linear leaves, 145 are tall but broad leaves, 152 are stout with linear leaves, and 130 are stout with broad leaves.

what test you should carry out? Why? What hypothesis to set up? How to do the test?

Another one…

Two types of *Vibrio botanicus* strains have been suspected of causing diseases in squirrels. An experiment was conducted, and found that strain TSSciB54678 caused severe lesion in 9 out of 12 squirrels while strain TSSciB60125 caused mild lesion in 2 out of 20 squirrels.

What kind of test should be carry out to indicate that these 2 strains causing diseases in different manner? Why? Are there any other test?