P-values and statistical tests
2. Contingency tables

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Hand-outs available at http://is.gd/statlec

Contingency tables

Drug treatment

	No treatment	Drug X
No improvement	57	32
Improvement	13	46

Enrichment

	In cluster	Outside cluster
With GO-term	6	1
Without GO-term	38	623

Cell counting

	WT	KO
G1	50	61
S	172	175

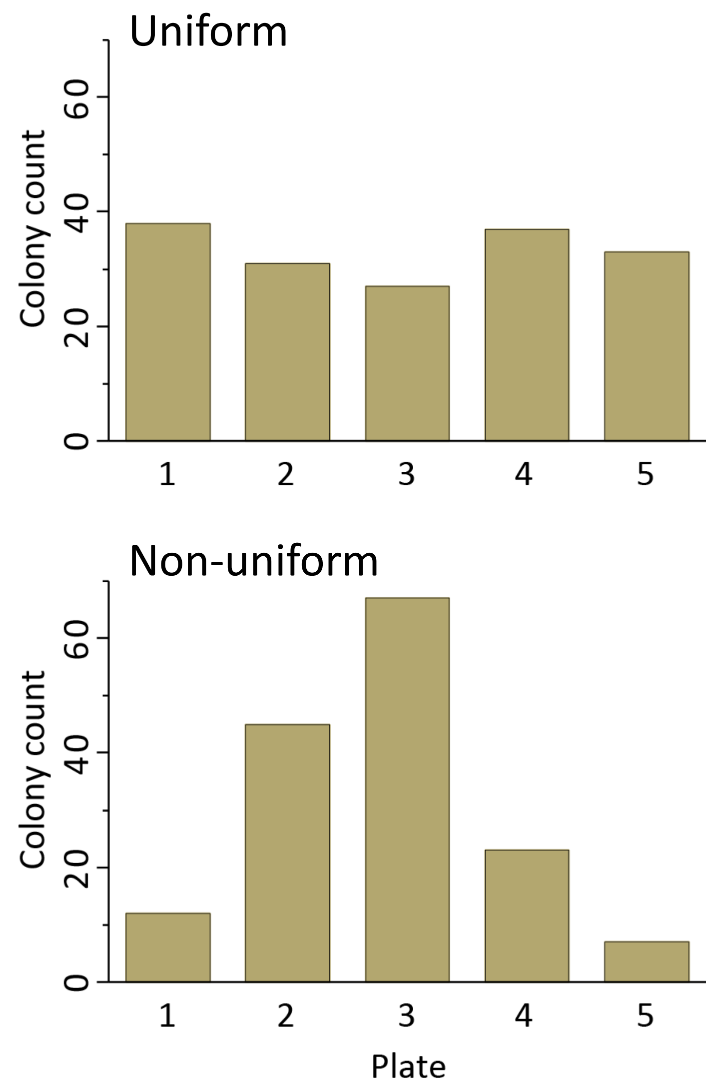
	WT	KO1	KO2	KO3
G1	50	61	78	43
S	172	175	162	178
G2	55	45	47	59

Chi-square test

Goodness-of-fit test

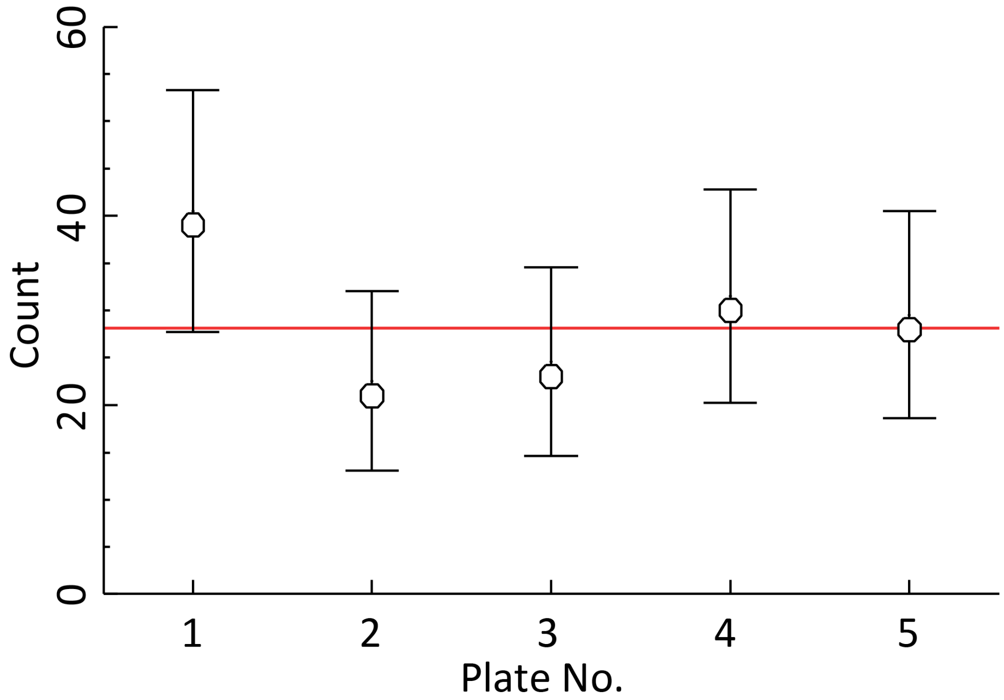
Pipetting experiment

- Dilution plating over five plates
- Aliquots taken from the same culture
- Good pipetting: uniform distribution of counts



Chi-square goodness-of-fit test

	Plate				
	1	2	3	4	5
Observed	39	21	23	30	28
Expected	28.2	28.2	28.2	28.2	28.2



Chi-square goodness-of-fit test

	Plate				
	1	2	3	4	5
Observed	39	21	23	30	28
Expected	28.2	28.2	28.2	28.2	28.2
$\chi_i = \frac{O_i - E_i}{\sqrt{E_i}}$	2.03	-1.36	-0.98	0.34	-0.04

- We have observed (O_i) and expected (E_i) counts, $i = 1, 2, \ldots, n$
- Test statistic is

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

- Distributed as χ^2 with $n - 1$ degrees of freedom

Chi-square goodness-of-fit test

	Plate				
	1	2	3	4	5
Observed	39	21	23	30	28
Expected	28.2	28.2	28.2	28.2	28.2
$\chi_i = \frac{O_i - E_i}{\sqrt{E_i}}$	2.03	-1.36	-0.98	0.34	-0.04

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$
$$= \frac{(39 - 28.2)^2}{28.2} + \frac{(21 - 28.2)^2}{28.2} + \frac{(23 - 28.2)^2}{28.2} + \frac{(30 - 28.2)^2}{28.2} + \frac{(28 - 28.2)^2}{28.2}$$
$$= 7.05$$

- For 4 d.o.f. we find $p = 0.13$

Chi-square goodness-of-fit test

$\chi_i = \dfrac{O_i - E_i}{\sqrt{E_i}}$	2.03	-1.36	-0.98	0.34	-0.04

- Null hypothesis: $E_1 = E_2 = E_3 = E_4 = E_5$
- Observed random variable: $O_i = E_i + \text{noise}$
- Poisson distribution with standard deviation $\sqrt{E_i}$

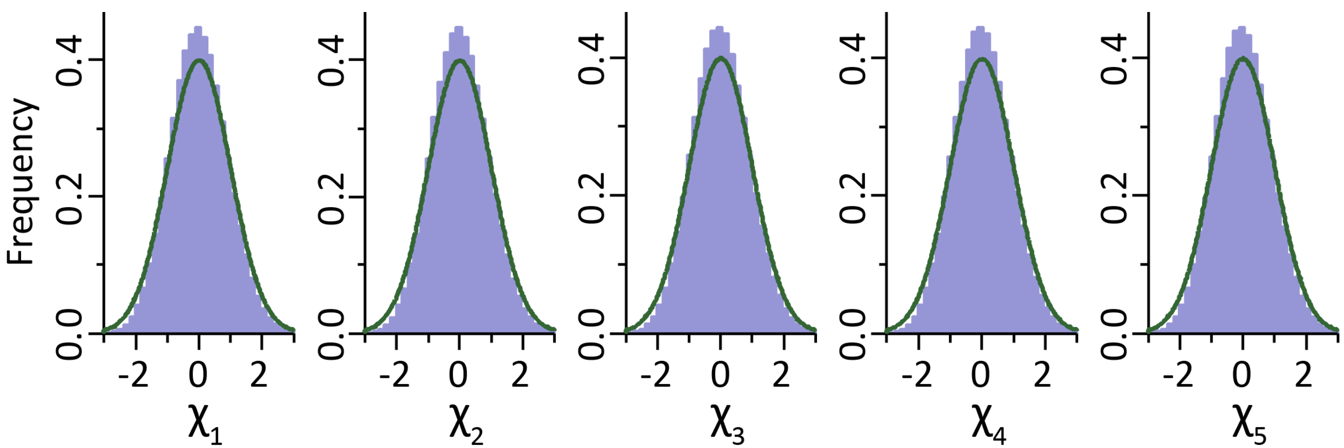
$\chi_i = \dfrac{O_i - E_i}{\sqrt{E_i}}$ looks very much like $Z = \dfrac{X - \mu}{\sigma}$

- Then χ_i roughly follow standardized normal distribution (i.e., centred at 0 and with standard deviation of 1)

Gedankenexperiment

- Simulate dilution plating experiment 1 million times
- Generate random counts with the same total count (141) as the original data
- Uniform distribution between plates: null hypothesis

	Plate				
	1	2	3	4	5
Observed	**39**	**21**	**23**	**30**	**28**
Sim 1	33	23	32	22	31
Sim 2	30	22	25	28	36
Sim 3	29	30	32	18	32
...	...	...	...	...	...

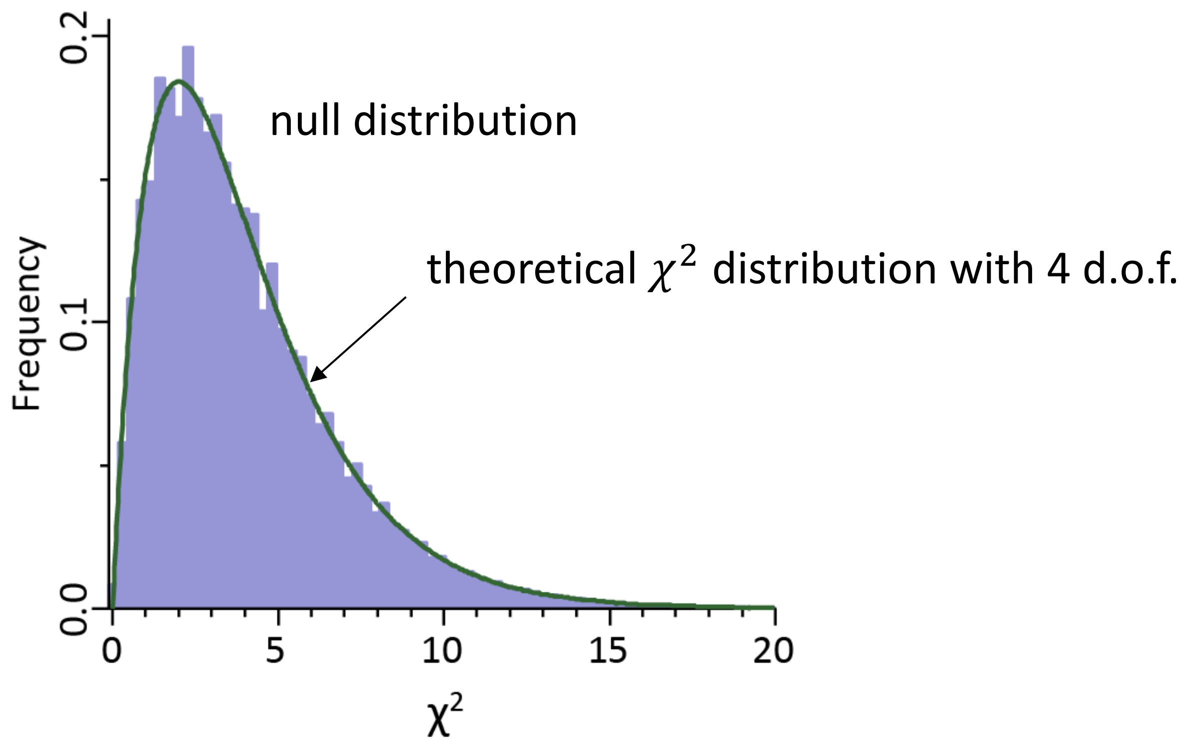


Chi-square distribution

- Definition: a sum of squares of independent standard normal variables

$$\chi^2 = \sum_{i=1}^{n} \chi_i^2$$

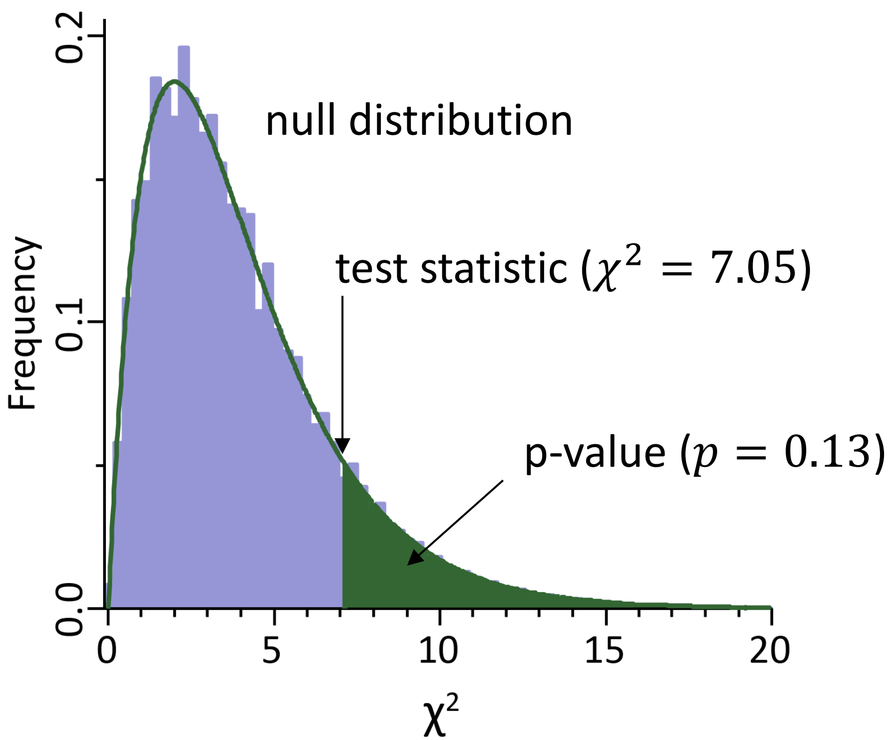
- is distributed with χ^2 distribution with $n - 1$ degrees of freedom



Chi-square test

- Null hypothesis: counts are uniformly distributed
- Test statistic (from data): $\chi^2 = 7.05$

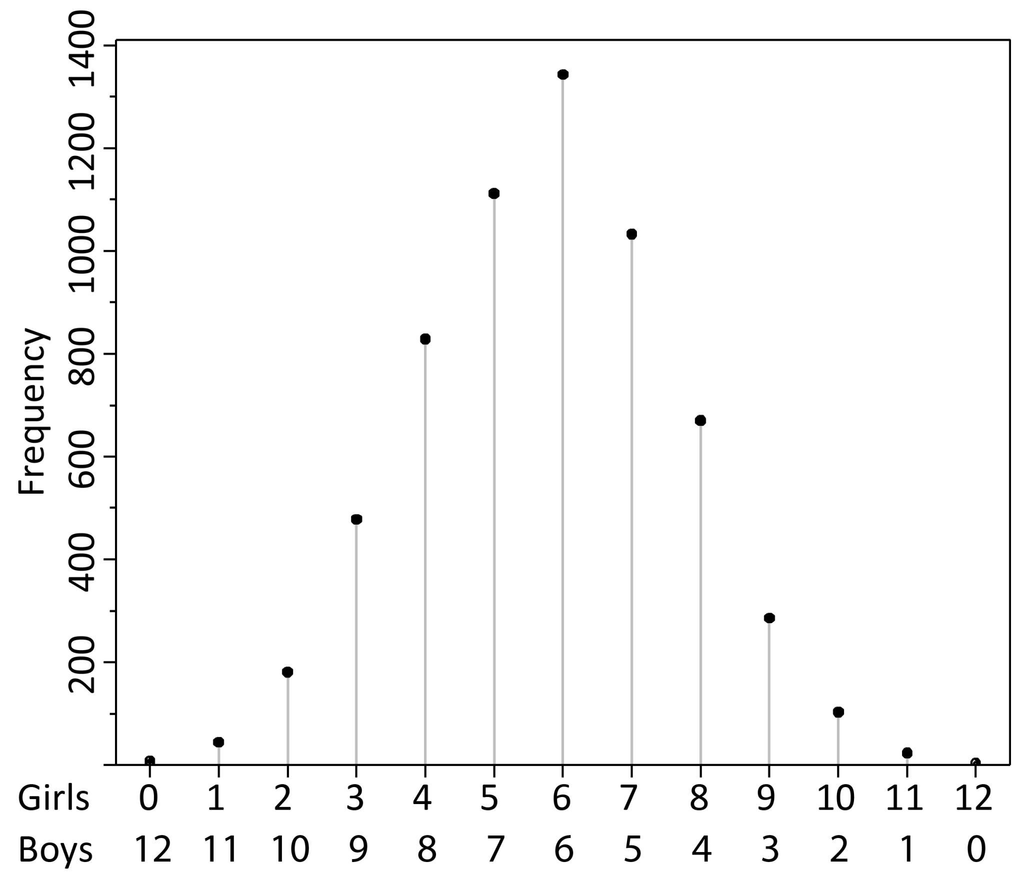
```
> counts = c(39, 21, 23, 30, 28)
> mean = mean(counts)
> chi2 = sum((counts - mean)^2 / mean)
> chi2
[1] 7.049645
> 1 - pchisq(chi2, df = length(counts) - 1)
[1] 0.1332878
```



Geissler (1889)

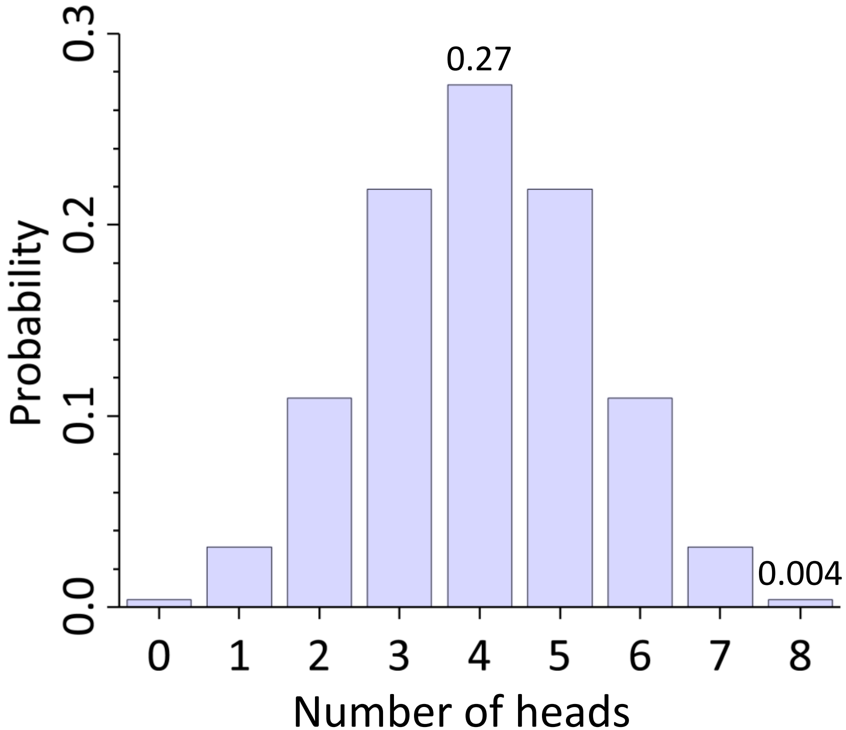
- Birth data from a hospital in Saxony, 1876-1885
- Includes 6115 sibships of 12 children
- Girl/boy ratio $\hat{p} = 0.481 \pm 0.004$ (95% CI)
- Does it follow binomial distribution?

No. girls	Observed
0	7
1	45
2	181
3	478
4	829
5	1112
6	1343
7	1033
8	670
9	286
10	104
11	24
12	3



Remainder: binomial distribution

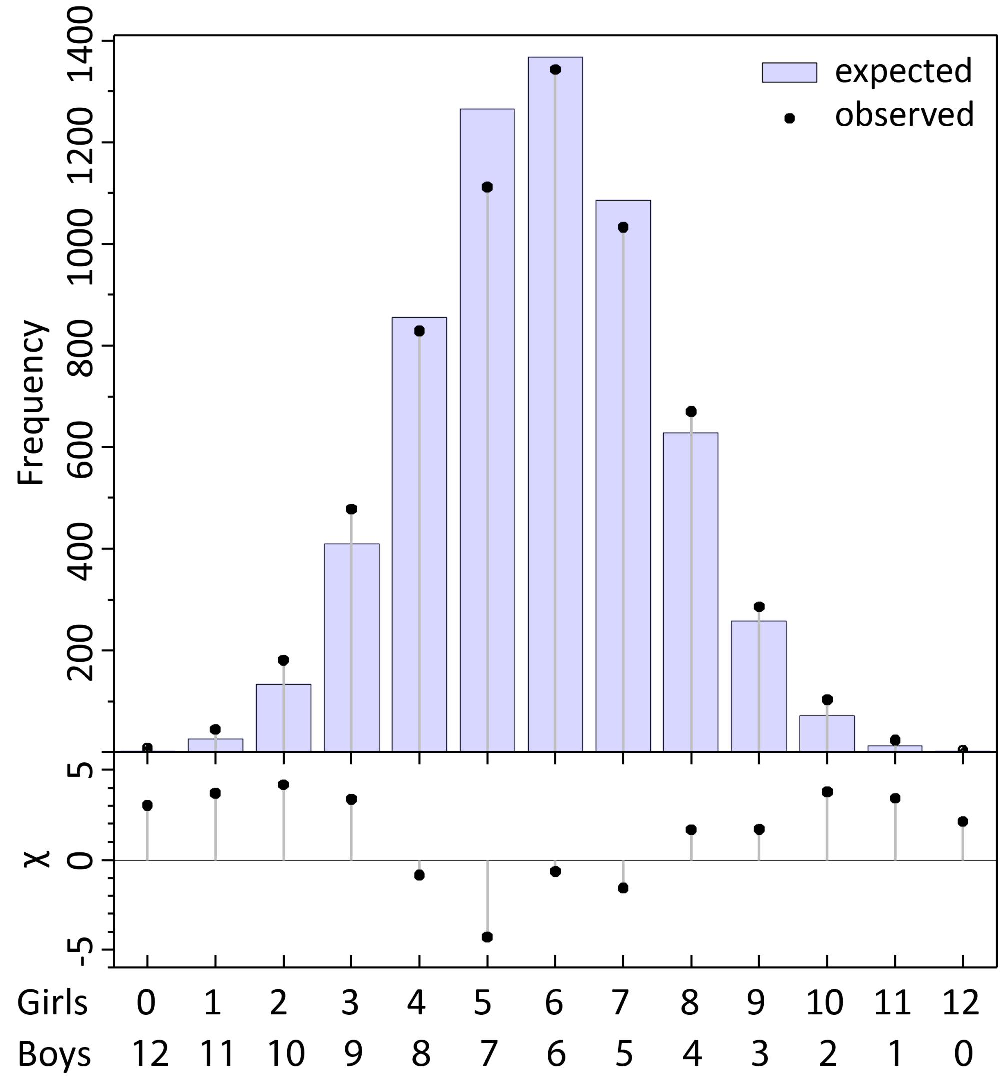
- n repeated trials
- Two possible outcomes, probability p and $1 - p$
- Example: toss a coin ($p = 0.5$)



Geissler (1889)

# girls	O_i	E_i	χ_i	χ_i^2
0	7	2.3	3.04	9.2
1	45	26.1	3.70	13.7
2	181	132.8	4.18	17.5
3	478	410	3.36	11.3
4	829	854.2	-0.86	0.8
5	1112	1265.6	-4.32	18.7
6	1343	1367.3	-0.66	0.4
7	1033	1085.2	-1.58	2.5
8	670	628.1	1.67	2.8
9	286	258.5	1.71	2.9
10	104	71.8	3.80	14.4
11	24	12.1	3.43	11.7
12	3	0.9	2.14	4.6
				110.5

$$\chi^2 = 110.5$$
$$\mathrm{d.o.f.} = 11$$
$$p = 0$$



Degrees of freedom

- Degrees of freedom = independent pieces of available information

- Input data consists of n numbers
- The total number of counts is fixed

33	23	32	22	31	**141**

- We lose one degree of freedom, $n - 1$ left

- Lose one degree of freedom per each model parameter found
- Binomial proportion from the input data ($\hat{p} = 0.481$)
- We use up more information and have $n - 2$

Chi-square goodness-of-fit d.o.f. = $n - 1 - m$
n is size of data
m is the number of model parameters

Chi-square goodness-of-fit test: summary

Input	Counts from n categories
Assumptions	Observations are random and independent Mutual exclusivity (no overlap between categories) Errors are normal
Usage	Compare the observed counts with a theoretical distribution
Null hypothesis	Number of observations in each category is equal to that predicted by the theoretical distribution
Comments	Approximate test Breaks down for small numbers (total count < 100) For small numbers use the exact multinomial (or binomial) test Be careful with the number of degrees of freedom!

Chi-square test

Test of independence

Chi-square test of independence

- Comparing observed (O_{ij}) with expected (E_{ij}) values
- Expected values are

$$E_{ij} = N p_i p_j$$

  - p_i – proportion in row i
  - p_j – proportion in column j
  - N – total number

- Test statistic

$$\chi^2 = \sum_{i,j} \frac{\left(O_{ij} - E_{ij}\right)^2}{E_{ij}}$$

- $\nu = (n_{\mathrm{rows}} - 1)(n_{\mathrm{columns}} - 1)$
- P-value is from χ^2 distribution with 1 d.o.f.
- Corresponds to two-sided Fisher's test

	Drug A	Drug B	Total	Proportion
Improvement	12 18.6	30 23.3	42	23.3%
No improvement	68 61.3	70 76.8	138	76.7%
Total	80	100	180	
Proportion	44.4%	55.6%		

Observed

Estimated
$180 \times 0.767 \times 0.556 = 76.8$

$$\chi^2 = \frac{(12 - 18.6)^2}{18.6} + \frac{(30 - 23.3)^2}{23.3} + \frac{(68 - 61.3)^2}{61.3} + \frac{(70 - 76.8)^2}{76.8} = 5.59$$

$$p_{\mathrm{chi2}} = 0.018$$
$$p_{\mathrm{Fisher}} = 0.013$$

Chi-square test for independence

- Flow cytometry experiment
- WT and three KOs
- Take about 280 cells in each condition
- Establish cell cycle stage

- Are the any differences between the WT and KOs?

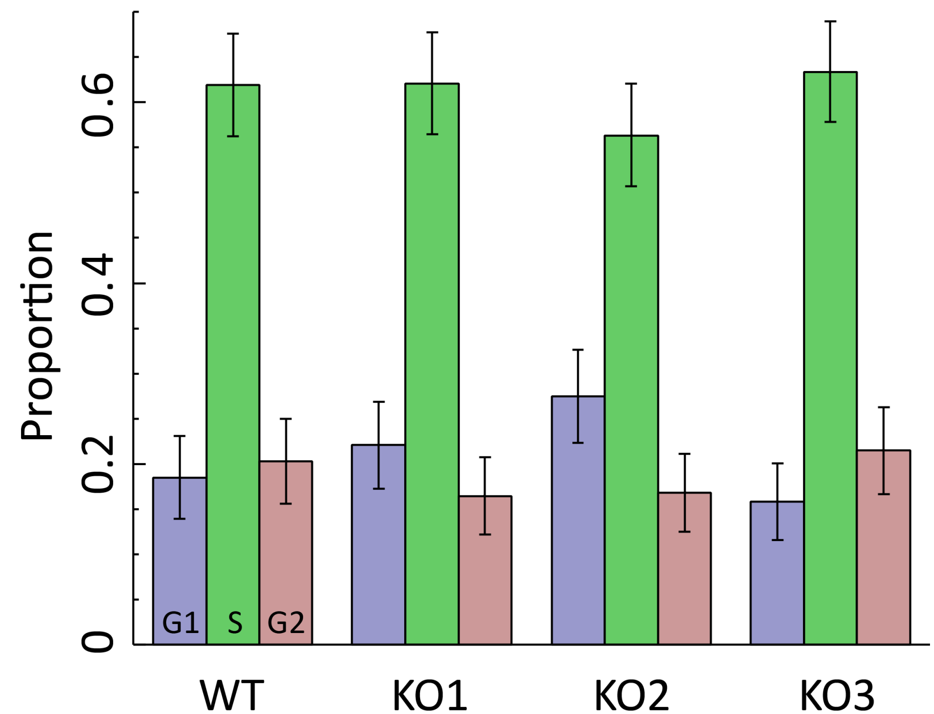
$$\chi^2 = 15.1$$

$$\nu = (4-1)(3-1) = 6$$

$$p = 0.02$$

- But what does it mean?

	WT	KO1	KO2	KO3
G1	50	61	78	43
S	172	175	162	178
G2	55	45	47	59



Independence of proportions

- Like in Fisher's test

- Rows and columns are independent
- Proportions between rows do not depend on the choice of column
- Proportions between columns do not depend on the choice of row

- Proportions in each row are 1:2:3:4
- Proportions in each column are 1:2

- This contingency table is consistent with the null hypothesis

	C1	C2	C3	C4
G1	10	20	30	40
G2	20	40	60	80

Pairwise comparison

- Null hypothesis: proportions of cells in G1-S-G2 stages are the same for each condition
- $p = 0.02$, reject the null hypothesis

- Pairwise comparison
- WT vs. KO1

	WT	KO1
G1	50	61
S	172	175
G2	55	45

$\chi^2 = 2.09$
$\nu = 2$
$p = 0.35$

	WT	KO1	KO2	KO3
G1	50	61	78	43
S	172	175	162	178
G2	55	45	47	59

Comparison	p-value	Adj. p-value
WT vs. KO1	0.35	1
WT vs. KO2	0.03	0.19
WT vs. KO3	0.69	1
KO1 vs. KO2	0.28	1
KO1 vs. KO3	0.08	0.49
KO2 vs. KO3	0.002	0.01

One versus others

- Compare each column vs. the sum of others
- WT vs. others

	WT	others
G1	50	182
S	172	515
G2	55	151

$$\chi^2 = 1.72$$
$$\nu = 2$$
$$p = 0.42$$

	WT	KO1	KO2	KO3
G1	50	61	78	43
S	172	175	162	178
G2	55	45	47	59

Comparison	p-value	Adj. p-value
WT	0.42	1
KO1	0.50	1
KO2	0.006	0.02
KO3	0.03	0.12

Chi-square test of independence: summary

Input	$n_r \times n_c$ contingency table table contains counts
Assumptions	Observations are random and independent (no before-after) Mutual exclusivity (no overlap between categories) Errors are normal
Usage	Examine if there is an association (contingency) between two variables; whether the proportions in "groups" depend on the "condition" (and vice versa)
Null hypothesis	The proportions between rows do not depend on the choice of column
Comments	Approximate test Use when you have large numbers For small numbers use Fisher's test (2x2 only) For before-after data use McNemar's test

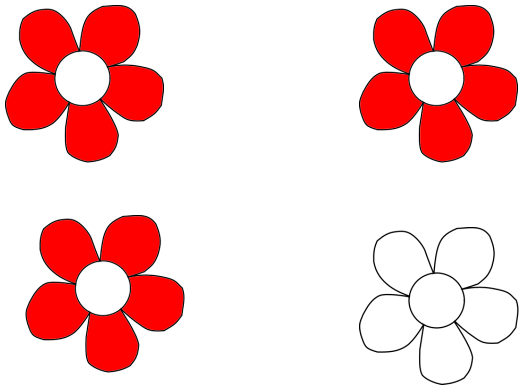
How to do it in R?

```
# Colony count test
> counts = c(39, 21, 23, 30, 28)
> chisq.test(counts, p=rep(1/5, 5))

        Chi-squared test for given probabilities

data:  counts
X-squared = 7.0496, df = 4, p-value = 0.1333



# Drug comparison
> chisq.test(rbind(c(12, 30), c(68, 70)), correct=FALSE)

        Pearson's Chi-squared test

data:  rbind(c(12, 30), c(68, 70))
X-squared = 5.5901, df = 1, p-value = 0.01806
```

G test

Likelihood ratio

- Red-to-white flowers = 3:1
- Null hypothesis, H_0: $p = 0.75$
- Sample of $m = 200$ flowers
  - 140 red
  - 60 white
- Observed proportion, $\hat{p} = 0.70$
- Binomial distribution



$$P(X = k) = \binom{m}{k} p^k (1-p)^{m-k}$$

Null hypothesis: $p = 0.75$

$$P(X = 140 | H_0) = 0.017$$

Alternative hypothesis: $p = 0.70$

$$P(X = 140 | H_1) = 0.061$$

Likelihood ratio

$$\frac{P(X = 140 | H_0)}{P(X = 140 | H_1)} = 0.28$$

G-test

$$G = -2 \ln \frac{P(X = k|H_0)}{P(X = k|H_1)}$$

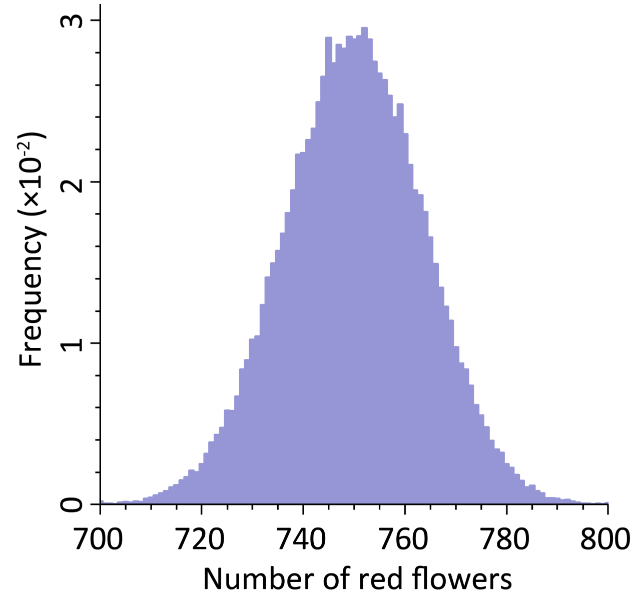
- Statistic G is chi-square distributed with $n - 1$ degrees of freedom (n categories)

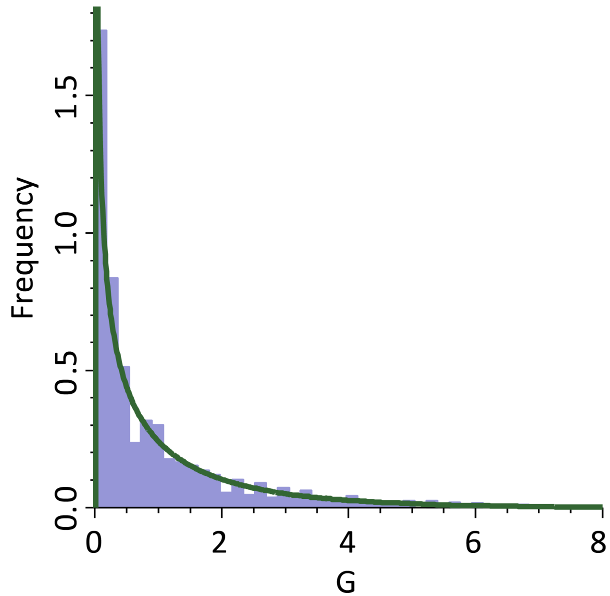
- Factorials cancel out and G simplifies a lot

$$G = 2 \sum_{i=1}^{n} O_i \ln \frac{O_i}{E_i}$$

- We can use it just like chi-square test: goodness-of-fit and independence

- For large numbers chi-square test and G-test give very similar results





G test is like chi-square test

- You can use G test just like chi-square test

- Goodness-of-fit test
- Test of independence

- Results are very similar
- Chi-square test is an approximation of the G test

- G is additive, chi-square is not

	Plate				
	1	2	3	4	5
Obs	39	21	23	30	28
Exp	28.2	28.2	28.2	28.2	28.2

$\chi^2 = 7.05$
$p = 0.13$

$G = 6.85$
$p = 0.14$

	WT	KO1	KO2	KO3
G1	1	61	78	43
S	172	175	162	178
G2	55	45	47	59

$\chi^2 = 15.1$
$p = 0.02$

$G = 15.0$
$p = 0.02$

G test for replicated experiments

	WT	KO1	KO2	KO3
G1	50, 54, 48	61, 75, 69	78, 77, 80	43, 34, 49
S	172, 180, 172	175, 168, 166	162, 167, 180	178, 173, 168
G2	55, 50, 63	45, 41, 38	47, 49, 43	59, 50, 45

Replicate 1

	WT	KO1	KO2	KO3
G1	50	61	78	43
S	172	175	162	178
G2	55	45	47	59

$G = 15.0 \quad p = 0.02$

Replicate 1

	WT	KO1	KO2	KO3
G1	54	75	77	34
S	180	168	167	180
G2	50	41	49	50

$G = 21.1 \quad p = 0.002$

Replicate 1

	WT	KO1	KO2	KO3
G1	48	69	80	49
S	172	166	180	168
G2	63	38	43	45

$G = 16.5 \quad p = 0.01$

Pooled data

	WT	KO1	KO2	KO3
G1	152	205	235	126
S	524	509	509	519
G2	168	124	139	154

$G = 44.9 \quad p = 5{\times}10^{-8}$

G test for replicated experiments

- Perform G test for each replicate
- Find the total G

$$G_{\text{tot}} = G_1 + G_2 + \cdots + G_n$$

$$\nu_{\text{tot}} = \nu_1 + \nu_2 + \cdots \nu_n$$

- Find G_{pool} and ν_{pool} from pooled data
- Find heterogeneity G

$$G_{\text{het}} = G_{\text{tot}} - G_{\text{pool}}$$

$$\nu_{\text{het}} = \nu_{\text{tot}} - \nu_{\text{pool}}$$

- Find p-value for $G_{\text{tot}}, \nu_{\text{tot}}$ and $G_{\text{het}}, \nu_{\text{het}}$ from χ^2 distribution

	G	d.o.f	p-value
Replicate 1	15.0	6	0.02
Replicate 2	21.1	6	0.002
Replicate 3	16.5	6	0.01
Total	52.6	18	3×10^{-5}
Pooled	44.9	6	5×10^{-8}
Heterogeneity	7.7	12	0.8

G test for replicated experiments

- G represents deviation from the null hypothesis
- We can split total G into

$$G_{\text{tot}} = G_{\text{het}} + G_{\text{pool}}$$

variation among replicates

deviation of the pooled data from H_0

	G	d.o.f	p-value
Replicate 1	15.0	6	0.02
Replicate 2	21.1	6	0.002
Replicate 3	16.5	6	0.01
Total	52.6	18	3×10^{-5}
Pooled	44.9	6	5×10^{-8}
Heterogeneity	7.7	12	0.8

- Use G_{tot} to test the null hypothesis
- However, if G_{het} is large (and p_{het} significant), the deviation from H_0 is due to variation between replicates

G test: summary

Input	$n_r \times n_c$ contingency table table contains counts possible replicates in cells
Assumptions	Observations are random and independent Mutual exclusivity (no overlap between categories) Errors are normal
Usage	Examine if there is an association (contingency) between two variables; whether the proportions in “groups” depend on the “condition” (and vice versa)
Null hypothesis	The proportions between rows do not depend on the choice of column
Comments	Very similar to chi-square test G and d.o.f. are additive Can be used for replicated experiments Not to be confused with ANOVA!

How to do it in R?

```
# Flow cytometry experiment, first replicate
> library(DescTools)
> flcyt = rbind(c(50,61,78,43), c(172,175,162,178), c(55,45,47,59))
> GTest(flcyt)

        Log likelihood ratio (G-test) test of independence without correction

data:  flcyt
G = 14.994, X-squared df = 6, p-value = 0.0203

# The remaining replicates and the pooled value are found in the same fashion

# Finding p-value for total and heterogeneity G
> 1 - pchisq(52.6, 18)
[1] 3.024812e-05

> 1 - pchisq(7.7, 12)
[1] 0.8081131
```

Contingency table tests

Test	Table	To test if...	Comments
Fisher's exact	2×2	rows and columns are independent; proportions are equal	Works for small numbers, some consider it too conservative
Chi-square goodness-of-fit	$1\times n$	Observed counts follow a theoretical distribution	Requires categorical data, doesn't work for continuous distributions
Chi-square test of independence	$n_r\times n_c$	rows and columns are independent; proportions are equal	Similar to Fisher's works better with large numbers
G-test of independence	$n_r\times n_c$	rows and columns are independent; proportions are equal	Similar to chi-square test, more powerful, can take replicates into account

Hand-outs available at http://tiny.cc/statlec