Biostatistics

Chapter 9 Analysis of binary/categorical outcomes: Chi-square test

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Continuous outcome (means)

Parametric statistics

Non-parametric statistics

Outcome	Are the observations co	Alternatives if the	
Variable	independent	correlated	violated (and small n):
Continuous (e.g. blood pressure,	Ttest: compares means between two independent groups	Paired ttest: compares means between two related groups (e.g., the same subjects before and after)	Wilcoxon sign-rank test: non-parametric alternative to paired ttest Wilcoxon sum-rank test
score)	ANOVA: compares means between more than two independent groups	Repeated-measures ANOVA: compares changes over time in the means of two or	(=Mann-Whitney U test): non- parametric alternative to the ttest Kruskal-Wallis test: non- parametric alternative to ANOVA
	Pearson's correlation coefficient (linear correlation): shows linear correlation between two continuous variables	more groups (repeated measurements) Mixed models/GEE modeling: multivariate regression techniques to compare	Spearman rank correlation coefficient: non-parametric alternative to Pearson's correlation coefficient
	Linear regression: multivariate regression technique when the outcome is continuous; gives slopes or adjusted means	changes over time between two or more groups	

Review Questions (5 min)

List the pros and cons (优缺点) of non-parametric tests

Types of Data



Genetics story

A fundamental problem in genetics is determining whether the experimentally determined data fits the results expected from theory.



Gregor Mendel (1822-1884) "Father of Modern Genetics"



(the Punnett square 旁氏表)

Chi-Square Test for Goodness of Fit

- For example, do a cross and see 290 purple flowers and 110 white flowers in the offspring.
- This is pretty close to a 3/4 : 1/4 ratio, but how do you formally define "pretty close"?
- What about 250:150?



Law of Segregation

Mendel Pea Plant Experiment





Another Example from Mendel

A fruit fly cross





Black body, eyeless





X

FI: all wild



Analysis of the results

• What is the expected outcome of this cross?

Law of Independent Assortment

 <u>9/16</u> wild type: <u>3/16</u> normal body eyeless: <u>3/16</u> black body wild eyes: <u>1/16</u> black body eyeless.

Goodness of Fit

Mendel has no way of solving this problem (1865).

- Shortly after the rediscovery of his work in 1900, Karl Pearson and R.A. Fisher developed the "chisquare" test for this purpose.
- The chi-square test is a "goodness of fit" test: it answers the question of how well do experimental data fit expectations.

Versuche über Pflanzen-Hybriden.

Gregor Mendel.

(Vorgelegt in den Steengen vom 8. Februar und 8. Rot 1865.)

Einleitende Bemerkungen

Künstliche Befruchtungen, welche an Zierpflanzen desshalb vorgenommen wurden, um neue Farben-Varianten zu erzielen, waren die Veranlassung zu den Versuchen, die her besprochen werden sollen. Die auffallende Regelmässigkeit, mit welcher die selben Hybridformen immer wiederkchrten, so oft die Befruchtung zwischen gleichen Arten geschah, gab die Auregung zu weiteren Experimenten, deren Aufgabe es war, die Entwicklung der Hybriden in ihren Nachkommen zu verfolgen.

Dieser Aufgabe haben sorgfültige Beobachter, wie Kölreuter, Gärtner, Horbort, Leeooq, Wichura u. a. einen Theil ihres Lebens mit unermüdlicher Ausdauer geopfert. Namentlich hat Gärtner in seinem Werke "die Bastarderzeugung im Pflanzenreiche" sehr schätzbare Beobachtungen niedergelegt, und in neuester Zeit wurden von Wichura gründliche Unterschaugen über die Bastarde der Weiden veröffentlicht. Wenn es nech nicht gelungen ist, ein allgemein gilfiges Gesetz für die Bildung und Entwicklung der Hybriden aufzustellen, so kaun das Niemanden Wunder nehmen, der den Umfaug der Aufgabe kennt und die Schwierigkeiten zu wirdigen weiss, mit denen Versuche dieser Art zu kämpfen haben. Eine eudgiltige Entsteidung kaun erst dann erfolgen, bis Detail-Versuche aus den verschiedensten Pflanzen-Familien vorliegen. Wer die Ar-



Karl Pearson

R.A. Fisher

Chi-Square Distribution

In probability theory and statistics, the **chi-square distribution** (χ^2 -distribution) with k degrees of freedom is the distribution of a sum of the squares of k independent standard normal random variables. It is one of the most widely used probability distributions in inferential statistics.

Chi-Square Distribution

Characteristics of the Chi-Square Distribution

- I. It is not symmetric.
- 2. The shape of the chi-square distribution depends upon the degrees of freedom, just like Student's *t*distribution.
- 3. As the number of degrees of freedom increases, the chi-square distribution becomes more symmetric.
- The values are non-negative. That is, the values of are greater than or equal to 0.



Chi-Square Test

The chi-square distribution is used in the common chisquared tests for <u>goodness of fit</u> of an observed distribution to a theoretical one, the <u>independence</u> of two criteria of classification of qualitative data

- I. Chi-square test for goodness of fit
- 2. Chi-square test for independence

Chi-square goodness-of-fit test (拟合优度检验)

A <u>goodness-of-fit test</u> is an inferential procedure used to determine whether a frequency distribution follows a <u>claimed</u> distribution.

Let's look at a fruit fly cross



Chi-Square Analysis

- The chi-square analysis allows you to use statistics to determine if your data "good" or not.
- We are using laws of probability to determine possible outcomes for genetic crosses.
- How will we know if our flower or fruit fly data is "good"?
- Do observed and expected differ more than expected due to chance?

Let O_i represent the <u>observed counts</u> of category *i*, \underline{E}_i represent the <u>expected counts</u> of an category *i*, **k** represent the number of categories, and *n* represent the number of independent trials of an experiment. Then,

$$\chi^{2} = \sum \frac{(O_{i} - E_{i})^{2}}{E_{i}}$$
 $i = 1, 2, ..., k$

approximately follows the chi-square distribution with

k - I degrees of freedom provided. $E_i = np_i$ for i = 1, 2, ..., k.

NOTE:

I. All expected frequencies are greater than or equal to 1 (all $E_i \ge 1$)

2. No more than 20% of the expected frequencies are less than 5.

Test for Gooness-of-fit

If your hypothesis is supported by data

•you are claiming that mating is random and so is segregation and independent assortment (分离与自由组合定律).

If your hypothesis is not supported by data

•you are seeing that the deviation between observed and expected is very far apart...something nonrandom must be occurring....

The Chi-Square Goodness-of-Fit Test

Step I: A claim is made regarding a distribution. The claim is used to determine the null and alternative hypothesis.

 H_{o} : the random variable follows the claimed distribution

 H_A : the random variable does not follow the claimed distribution

Step 2: Calculate the expected frequencies for each of the k categories. The expected frequencies are np_i for i = 1, 2, ..., k where n is the number of trials and p_i is the probability of the *i*th category assuming the null hypothesis is true.

Step 3: Verify the requirements fort he goodness-of-fit test are satisfied.

(1) all expected frequencies are greater than or equal to 1 (all $E_i \ge 1$)

(2) no more than 20% of the expected frequencies are less than 5.

Now Conduct the Analysis:

Phenotype	Observed	Hypothesis
Wild	5610 <mark>9/</mark> 1	16
Eyeless	1881 <mark>3/</mark> 1	16
Black body	1896 <mark>3/</mark> 1	16
Eyeless, black body	622 1/ 1	L <mark>6</mark> 626
Total	10009	

To compute the hypothesis value take 10009/16 = 626

Now Conduct the Analysis:

Phenotype	Observed	Hypothesis
Wild	5610	5634
Eyeless	1881	1878
Black body	1896	1878
Eyeless, black body	622	626
Total	10009	

Step 4: Select a level of significance α based upon the seriousness of making a Type I error. The level of significance is used to determine the critical value. All chi-square goodness-of-fit tests are right tests, the shaded region represents the critical region



Step 5: Compute the test statistic

$$\chi^2 = \sum \frac{\left(O_i - E_i\right)^2}{E_i}$$

Phenotype	Observed	Hypothesis	
Wild	5610	5634	
Eyeless	1881	1878	
Black body	1896	1878	
Eyeless, black body	622	626	
Total	10009		

- $(5610 5630)^2 / 5630 = .07$
- $(|88| |877)^2 / |877 = .0|$
- $(1896 1877)^2 / 1877 = .20$
- $(622 626)^2 / 626 = .02$
- χ²=.30
- How many degrees of freedom? 4-1=3

STEP 6: Compare the critical value to the test statistic. If $\chi^2 > \chi^2_{\alpha}$ reject the null hypothesis

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CHI-SQUARE DISTRIBUTION TABLE

Accept Hypothesis						Reject Hypothesis					
				-	Probab	ility (p)					
Degrees of Freedom	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.38	18.47
5	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.63	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	16.81	22.46
7	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.12
9	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59

Conclusions

- the P value read off the table places our chi square number of .30 close to .95 or 95%
- This means that 95% of the time when our observed data is this close to our expected data, this deviation is due to random chance.
- We therefore fail to reject our null hypothesis.
 We accept Mendel's conclusion that the observed results for a 9/16 : 3/16 : 3/16 : 1/16 ratio.

Practice

phenotype	observed	Expected proportion	Expected number
round yellow	315		
round green	101		
wrinkled yellow	108		
wrinkled green	32		
total	556		

Practice

phenotype	observed	Expected proportion	Expected number
round yellow	315	9/16	312.75
round green	101	3/16	104.25
wrinkled yellow	108	3/16	104.25
wrinkled green	32	1/16	34.75
total	556	1	556

X² =0.47, df=3

Chi-Square Test

The chi-square distribution is used in the common chisquared tests for <u>goodness of fit</u> of an observed distribution to a theoretical one, the <u>independence</u> of two criteria of classification of qualitative data

I. Chi-square test for goodness of fit

2. Chi-square test for independence

Chi-square test of Independence

Chi-square test allows you to compare **proportions** between 2 or more groups (ANOVA for means; chi-square for proportions).

Infant HIV/ AZT Study





葛兰素威康制药公司生产

Infant HIV/ AZT Study

We will motivate by using data from the Pediatric AIDS Clinical Trial Group (ACTG) Protocol 076 Study Group

Study design

- "We conducted a randomized, double-blinded, placebo- controlled trial of the efficacy and safety of Zidovudine (AZT) in reducing the risk of maternal-infant HIV transmission"

- 363 HIV infected pregnant women were randomized to AZT or placebo



Note

Random assignment of treatment

Helps insure two groups are comparable
 Patient and physician could not request a particular treatment

Double-blind

 \checkmark Patient and physician did not know treatment assignment
Observed HIV Transmission

Results

- Of the 180 women randomized to AZT group, 13 gave birth to children who tested positive for HIV within 18 months of birth

Of the 183 women randomized to the placebo group,
 40 gave birth to children who tested positive for HIV
 within 18 months of birth

	HIV+	HIV-	Total
AZT	13		180
Placebo	40		183
Total			

Observed HIV Transmission Proportions

$$\hat{p}_{AZT} = \frac{13}{180} = 0.07 = 7\%$$

Placebo

$$\hat{p}_{PLAC} = \frac{40}{183} = 0.22 = 22\%$$

Is the difference significant, or can it be explained by chance?

Two sample Z-test (Compare Two Proportions)

Hypothesis Test to Compare Two Proportions

- Two sample z-test
- Are the proportions of infants contracting HIV within 18 months-ofbirth equivalent at the population level for those whose mothers are treated with AZT versus untreated (placebo)?

$$-H_0: p_1 = p_2$$

- $H_A: p_1 \neq p_2$
- In other words, is the expected difference in proportions zero?

$$\begin{array}{rcl} - & H_{o}: p_{1} - p_{2} = 0 \\ - & H_{A}: p_{1} - p_{2} \neq 0 \end{array}$$

Hypothesis Test to Compare Two Proportions

- Recall, general "recipe" for hypothesis testing . . .
 - 1. Start by assuming H_o true
 - 2. Measure distance of sample result from μ_0 (here again its 0)
 - 3. Compare test statistic (distance) to appropriate distribution to get p-value

$$z = \frac{(observed \ dif \ f) - (null \ dif \ f)}{SE \ of \ observed \ dif \ f \ erenc_i}$$

$$z = \frac{\hat{p}_1 - \hat{p}_2}{S\hat{E}(\hat{p}_1 - \hat{p}_2)} = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\frac{\hat{p}_1 \times (1 - \hat{p}_1)}{n_1} + \frac{\hat{p}_2 \times (1 - \hat{p}_2)}{n_2}}}$$

Infant HIV/ AZT Study

In the infant HIV/AZT study, recall:

$$\hat{p}_1 - \hat{p}_2 = -0.15$$

$$S\hat{E}(\hat{p}_1 - \hat{p}_2) = 0.036$$

• So in this study:

$$z = \frac{-0.15}{0.36} \approx -4.2$$

 So this study result was 4.2 standard errors below the null mean of 0 (i.e., 4.2 standard errors from the difference in the proportion of HIV+ infants between the AZT and placebo groups expected if null was true)

Infant HIV/ AZT Study

If we were to look this up on a normal table, we would find a very low p-value (p < .001)

Infant HIV/ AZT Study

- If we were to look this up on a normal table, we would find a very low p-value (p < .001)
- We can also using **chi-square test** in this study
 - (Pearson's) Chi-Square Test (x²)
 - Calculation is easy (can be done by hand)
 - Works well for "big" sample sizes
 - Gives (essentially) the same p-value as z-test for comparing two proportions
 - Unlike z-test, can be extended to compare proportions between more than two independent groups in one test

Recall: HIV/AZT Example



The procedure to test for the independence:

I. State a hypotheses based on the fit of the data

2. Make a table of the observed and expected values. You will most likely be given the observed values.

3. Calculate the chi-squared test statistic, this is

 $\chi^{2}_{test} = \sum \frac{\left(O_{i} - E_{i}\right)^{2}}{E_{i}}$

4. Look up the chi-squared critical value from your chi-squared tables in the information booklet.

5. Compare your test statistic with your critical value and make a conclusion.

If the test statistic lies in the critical region then reject H0 in favour of H1. Otherwise do not reject H0.

Expected values



For the goodness of fit test, the expected frequency for each category is obtained by expected frequency = p*n

(p is the proportion from the null hypothesis and n is the size of the sample)

Expected values



For the test for independence, the expected frequency for each cell in the matrix is obtained by

expected frequency = (row total)(column total) / n

Degrees of freedom, v.

The degrees of freedom will be defined as:

v=(number of rows-I)(number of columns-I)



Drug Group

Expected values

$$\chi^{2} test = \sum \frac{\left(O_{i} - E_{i}\right)^{2}}{E_{i}}$$

$$HIV \qquad Yes \qquad 13 \qquad 40 \qquad 53$$

$$HIV \qquad Transmission \qquad No \qquad 167 \qquad 143 \qquad 310$$

$$180 \qquad 183 \qquad 363$$

$$E = \frac{RC}{N} = \frac{53(180)}{363} = 26.3$$

Expected values

• We could do the same for the other three cells:



Expected values

• We could do the same for the other three cells:



$$=\frac{(13-26.3)^2}{26.3}+\frac{(40-26.7)^2}{26.7}+\frac{(167-153.7)^2}{153.7}+\frac{(143-156.3)^2}{156.3}$$

= 15.6

P value

• P=0.0001



More example

Vaccine& Influenza











Example 2×2 table

Influenza						
yes No Total						
Vaccine	20	220	240			
Placebo	80	140	220			
Total	100	360	460			

Example 2×2 table

Influenza							
yes No Total							
Vaccine	20	220	240				
Placebo	80	140	220				
Total	100	360	460				

Expected numbers	Influenza					
		yes	No	Total		
	Vaccine	52.2	187.8	240		
	Placebo	47.8	172.2	220		
	Total	100	360	460		

Example 2×2 table

$$\chi^{2} = \sum \frac{(O_{i} - E_{i})^{2}}{E_{i}} = 53.09$$

df=1 for 2×2 table

Yates's continuity correction (连续性矫正)

$$\chi^{2} = \sum \frac{(|O - E| - 0.5)^{2}}{E}, d.f. = 1$$

The corrected value is **51.46**

Yates's continuity correction (连续性矫正)

Yates's continuity correction is needed, when

df=1, and total sample size (total of numbers in the table) is less than 40

or, df=1, and the smallest expected number is less than 5

Cochran (1954) recommended

It reduces the size of the chi-square value and so reduces the chance of finding a statistically significant difference, so that correction for continuity makes the test more conservative.

Yates's continuity correction (连续性矫正)

When df>1, **NO** Yates's continuity correction !

When df>1, the chi-squared test is valid when less 20% of the expected numbers are under 5 and none is less than 1; data is randomly selected.

Cochran (1954) recommended

Summary: the Chi-Square Test

- Both chi-square tests use the same statistic. The calculation of the chi-square statistic requires two steps:
- I. The null hypothesis is used to construct an idealized sample distribution of **expected frequencies** that describes how the sample would look if the data were in perfect agreement with the null hypothesis.

Expected Frequency in The Chi-Square Test

For the goodness of fit test, the expected frequency for each category is obtained by
expected frequency = p*n

(p is the proportion from the null hypothesis and n is the size of the sample)

For the test for independence, the expected frequency for each cell in the matrix is obtained by

expected frequency = (row total)(column total) / n

Caveat

**When the sample size is less than 40 or the expected frequency is very small in any cell (<1), Fisher's exact test is used as an alternative to the chi-square test.

What do we do if the expected values in any of the cells in a 2x2 table is below 5?

For example, a sample of teenagers might be divided into male and female on the one hand, and those that are and are not currently dieting on the other. We hypothesize, perhaps, that the proportion of dieting individuals is higher among the women than among the men, and we want to test whether any difference of proportions that we observe is significant. The data might look like this:





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	men	women	total
dieting	1	9	10
not dieting	11	3	14
totals	12	12	24

What do we do if the expected values in any of the cells in a 2x2 table is below 5?

For example, a sample of teenagers might be divided into male and female on the one hand, and those that are and are not currently dieting on the other. We hypothesize, perhaps, that the proportion of dieting individuals is higher among the women than among the men, and we want to test whether any difference of proportions that we observe is significant. The data might look like this:

The expected values:

	men	women	total
dieting	5	5	10
not dieting	7	7	14
totals	12	12	24

Binary or categorical outcomes (proportions)

Outcome	Are the observations	Alternative to the chi-	
Variable	independent	correlated	cells:
Binary or categorical (e.g. fracture,	Chi-square test: compares proportions between more than two groups	McNemar's chi-square test: compares binary outcome between correlated groups (e.g., before and after)	Fisher's exact test: compares proportions between independent groups when there are sparse data (some cells <5).
yes/no)	Relative risks: odds ratios or risk ratios (for 2x2 tables) Logistic regression: multivariate technique used when outcome is binary; gives multivariate-adjusted odds ratios	Conditional logistic regression: multivariate regression technique for a binary outcome when groups are correlated (e.g., matched data) GEE modeling: multivariate regression technique for a binary outcome when groups are correlated (e.g., repeated measures)	McNemar's exact test: compares proportions between correlated groups when there are sparse data (some cells <5).

The exact test is recommended for a 2×2 table, when

- 1. the overall total of the table is less than 20, or
- 2. the overall total is between 10 and 40 and the smallest of the four expected numbers is less than 5

The chi-squared test is valid when the overall total is more than 40

The question we ask about these data is: knowing that 10 of these 24 teenagers are dieters, what is the probability that these 10 dieters would be so unevenly distributed between the girls and the boys? If we were to choose 10 of the teenagers at random, what is the probability that 9 of them would be among the 12 girls, and only 1 from among the 12 boys?

--Hypergeometric distribution!

a discrete probability distribution that describes the probability of k successes in n draws from a finite population of size N without replacement.

--Fisher's exact test uses hypergeometric distribution to calculate the "exact" probability of obtaining such set of the values.

Fisher's exact test

Before we proceed with the Fisher test, we first introduce some notation. We represent the cells by the letters *a*, *b*, *c* and *d*, call the totals across rows and columns *marginal totals*, and represent the grand total by *n*. So the table now looks like this:



	men	women	total
dieting	а	b	a+b
not dieting	С	d	c + d
totals	a + c	b+d	n

Fisher showed that the <u>probability</u> of obtaining any such set of values was given by the <u>hypergeometric distribution</u>:

$$p = \binom{a+b}{a} \binom{c+d}{c} / \binom{n}{a+c}$$

$$= \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!a!b!c!d!}$$

As extreme as observed	5	In our example			In our example More extra than observed		More extre than observed	treme d	
	men	women	total			me	women	total	
dieting	1	9	10		dieting	0	10	10	
not dieting	11	3	14		not dieting	12	2	14	
totals	12	12	24		totals	12	12	24	

$$p = \frac{10!14!12!12!}{24!1!9!11!3!} = 0.00134$$

 $p = \frac{10!14!12!12!}{24!0!10!12!2!} = 0.00003$

Recall that p-value is the probability of observing data as extreme or more extreme if the <u>null hypothesis</u> is true. So the p-value is this problem is 0.00137.

The fisher Exact Probability Test

- Used when one or more of the expected counts in a contingency table is small.
- Fisher's Exact Test is based on exact probabilities from a specific distribution (the hypergeometric distribution).
- There's really no lower bound on the amount of data that is needed for Fisher's Exact Test. You can use Fisher's Exact Test when one of the cells in your table has a zero in it. Fisher's Exact Test is also very useful for highly imbalanced tables. If one or two of the cells in a two by two table have numbers in the thousands and one or two of the other cells has numbers less than 5, you can still use Fisher's Exact Test.
- Fisher's Exact Test has no formal test statistic and no critical value, and it only gives you a p-value.

END