

Lecture 6: Comparison of Proportions

GENOME 560, Spring 2017

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Review

- What is α ?

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- The level of significance, which we define and tells us when we will reject H_0

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- The chance of getting a test statistic as extreme as or more extreme as the one we observed, given that the assumptions we made are satisfied

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- What is α ?

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- What is the right way to interpret a p-value?

- The chance of getting a test statistic as extreme as or more extreme as the one we observed, given that the assumptions we made are satisfied

- What is a t-test used for?

Review

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- What is the right way to interpret a p-value?

- The chance of getting a test statistic as extreme as or more extreme as the one we observed, given that the assumptions we made are satisfied

- What is a t-test used for?

- Comparing the mean of a sample to a parametric mean or comparing two sample means

Discussion of ASA statement

- Is it OK to try many different analyses of your data?
- Is it OK to collect more data, after you've analyzed the first set of experiments?
- How should we, as a community, deal with people break the rules/make a mistake?
- What methods besides a p-value are available for evaluating the veracity of a data-based claim in a paper?
- Does bioRxiv help or hurt?

Goals

- Comparing proportions in one sample to a given value
- Chi-square test for comparing proportions between two (or more) samples when n is large
- Fisher's exact test for comparing proportions between two samples when n is small

Proportions

- Previously, given the following data, assumed to have a normal distribution:

$$x_1, x_2, \dots, x_n$$

- We were wondering if the mean of the distribution is equal to a specified value μ_0 .
- Now, let's consider a different situation...
- Say that we have a binary outcome in each of n trials and we know how many of them succeeded
- We are wondering whether the true success rate is likely to be π .

Binomial Distribution

- **$X = x$ # of successes with probability p after n trials**

- **pmf:**

$$P\{X = x\} = \binom{n}{x} p^x (1 - p)^{n-x}$$

- **cdf:**

$$P\{X \leq x\} = \sum_{y=0}^x \binom{n}{y} p^y (1 - p)^{n-y}$$

- **$E(X) = np$**

- **$V(X) = np(1-p)$**

Example

- Say that you're interested in studying a SNV in a gene associated with thrombosis. Its allele frequency overall is $\pi = 0.2$

Example

- Say that you're interested in studying a SNV in a gene associated with thrombosis. Its allele frequency overall is $\pi = 0.2$
- In a new sample of 50 subjects from a previously unstudied population, 5 have the variant

$$\bar{x} = \frac{45 * 0 + 5 * 1}{50} = 0.1 \quad s^2 = \frac{45 * (0 - 0.1)^2 + 5 * (1 - 0.1)^2}{49} = 0.092$$

Example

- Say that you're interested in studying a SNV in a gene associated with thrombosis. Its allele frequency overall is $\pi = 0.2$
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- What range of p is *not* going to surprise you?

Example

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- In a new sample of 50 subjects from a previously unstudied population, 5 having the variant
- What range of p is *not* going to surprise you?
- Then, is $\pi = 0.2$ the “right” frequency?

Example

- Say that you're interested in studying a SNV in a gene associated with thrombosis. Its allele frequency overall is $\pi = 0.2$
- In a new sample of 50 subjects from a previously unstudied population, 5 having the variant
- What range of p is *not* going to surprise you?
 - What operation are we going to do here?
- Then, is $\pi = 0.2$ the “right” population frequency?
 - And here?

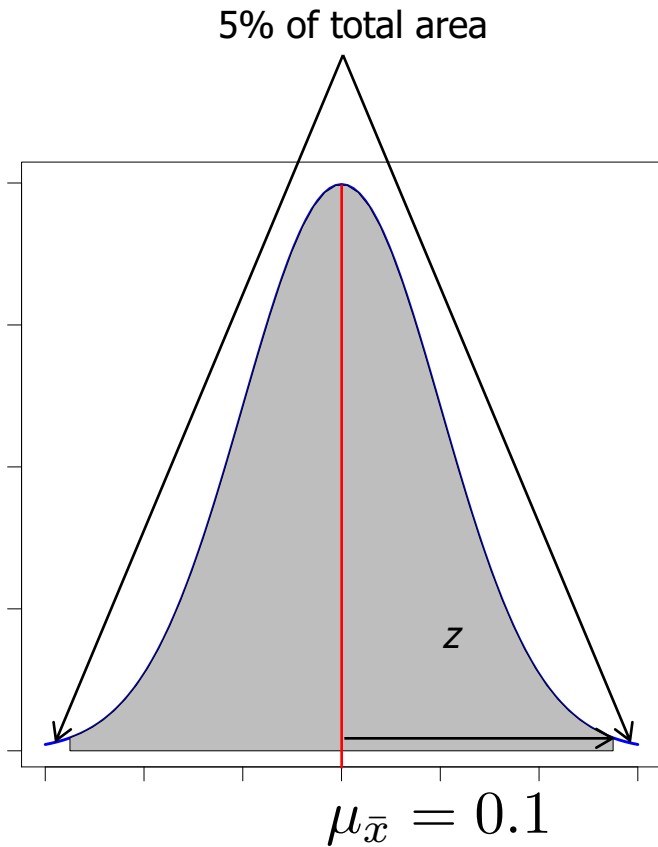
Example

- Say that you're interested in studying a SNV in a gene associated with thrombosis. Its allele frequency overall is $\pi = 0.2$
- In a new sample of 50 subjects from a previously unstudied population, 5 having the variant
- What range of p is *not* going to surprise you?
 - Confidence interval
- Then, is $\pi = 0.2$ the “right” population frequency?
 - Hypothesis test

Confidence Interval on a Proportion

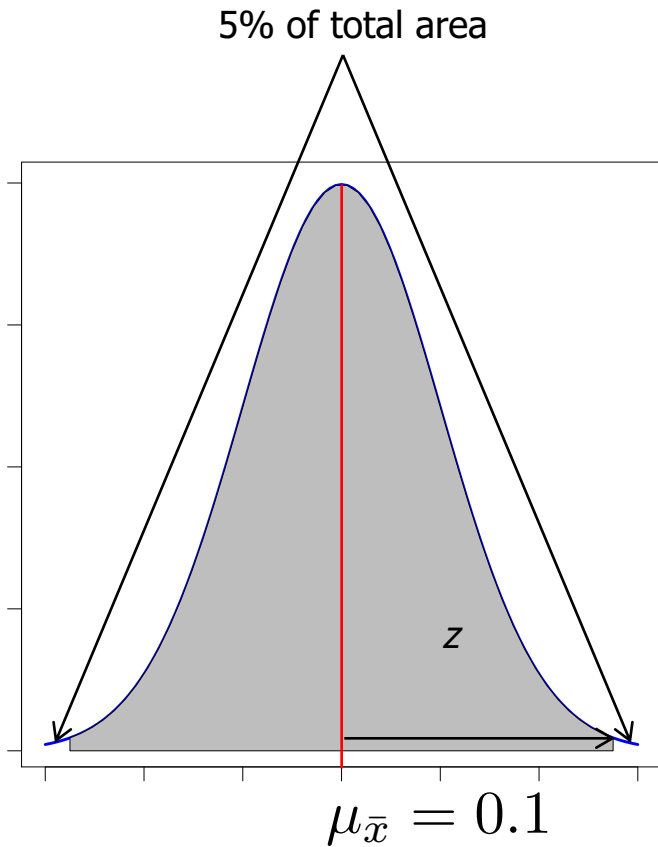
- We would like to attach a confidence interval to our sample mean
- We can assume that sampling dist'n of p is approximately normal if np and $n(1-p)$ are both >5

Confidence Interval on a Proportion



Begin with our assumption of the normality of the sampling distribution

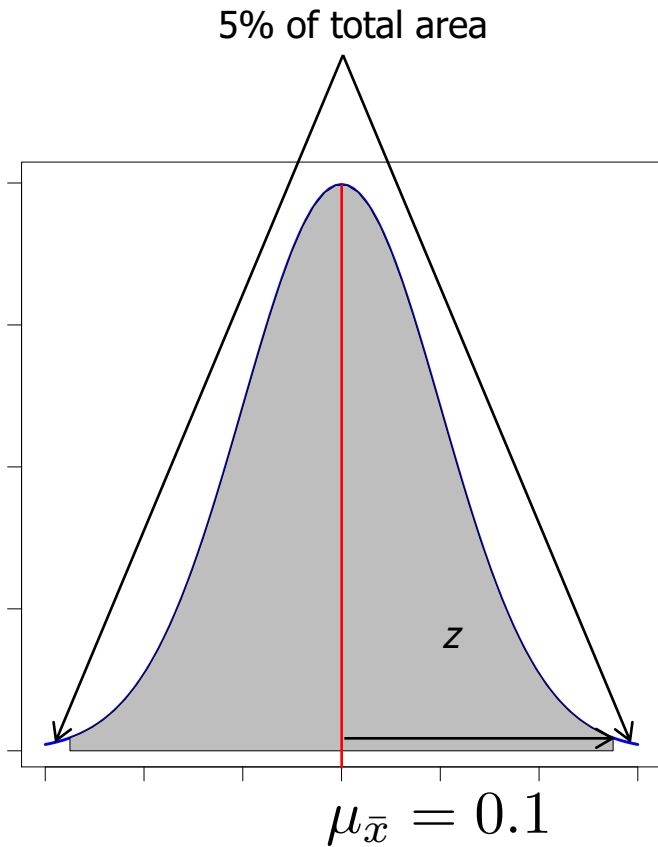
Confidence Interval on a Proportion



Find z such that $\mu_{\bar{x}} \pm z$
encompasses 95% of the
sampling distribution

$$P(-z \leq \mu_{\bar{x}} \leq z) = 0.95$$

Confidence Interval on a Proportion

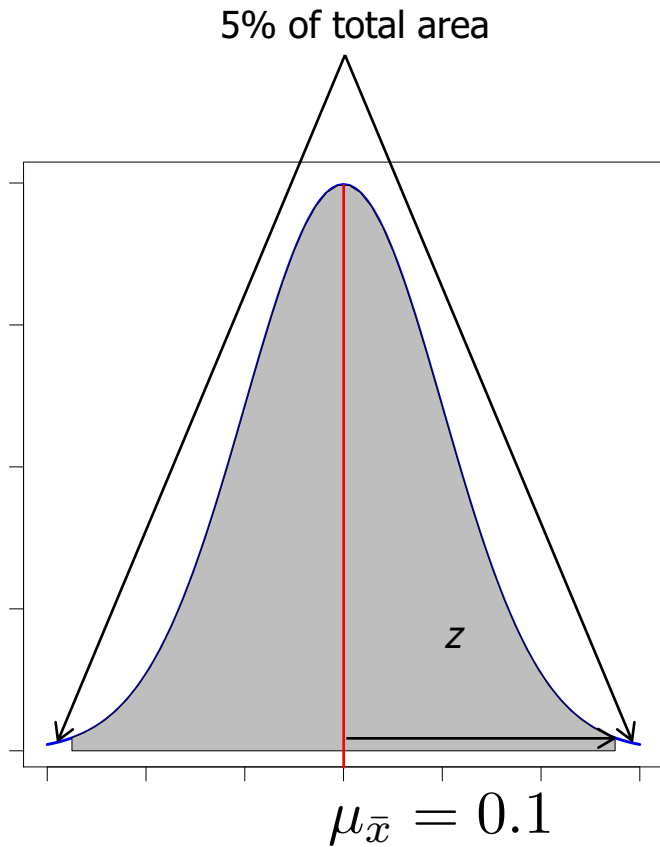


Recall that 95% of a standard normal distribution lies within 1.96 standard deviations of the mean

$$P(-z \leq \mu_{\bar{x}} \leq z) = 0.95$$

$$z = 1.96$$

Confidence Interval on a Proportion



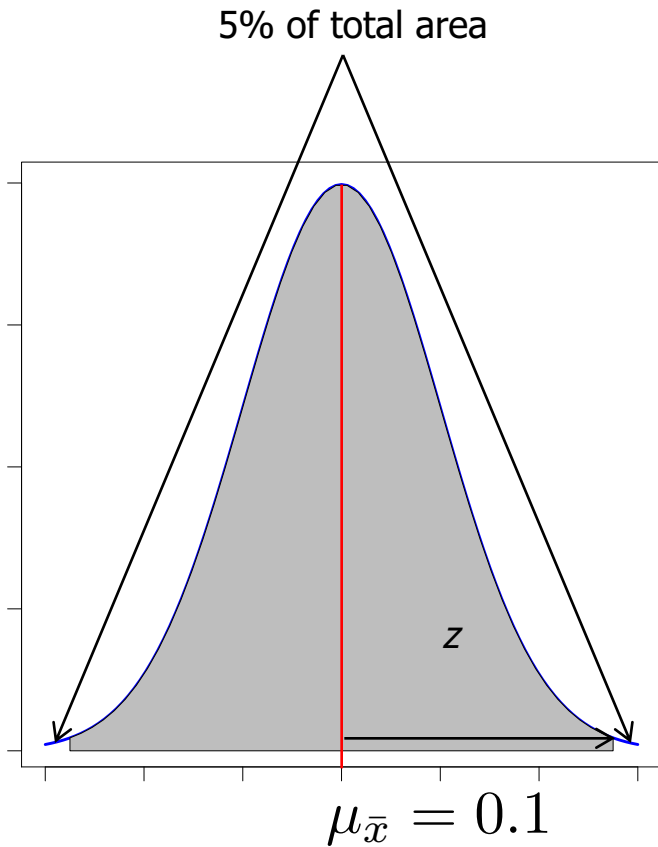
Now we just need $\sigma_{\bar{x}}$, which we can approximate using the sample standard deviation

$$P(-z \leq \mu_{\bar{x}} \leq z) = 0.95$$

$$z = 1.96$$

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}} = \frac{s}{\sqrt{n}}$$

Confidence Interval on a Proportion



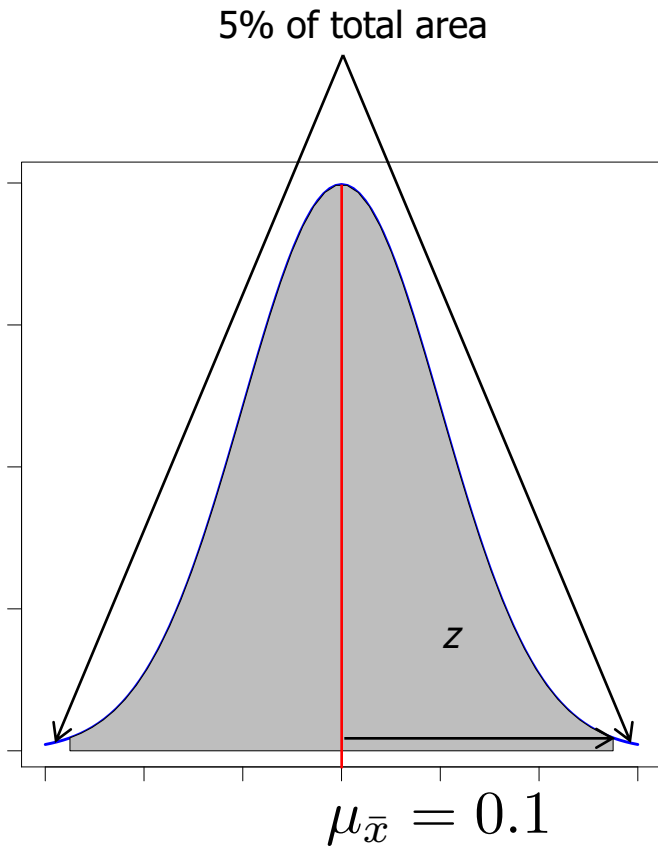
Next we use our SEM to find the value for our confidence interval

$$P(-z \leq \mu_{\bar{x}} \leq z) = 0.95$$

$$z = 1.96$$

$$\mu_{\bar{x}} \pm 1.96 \frac{s}{\sqrt{n}} = 1.96 \frac{\sqrt{0.092}}{\sqrt{50}} = 0.084$$

Confidence Interval on a Proportion



Conclusion?

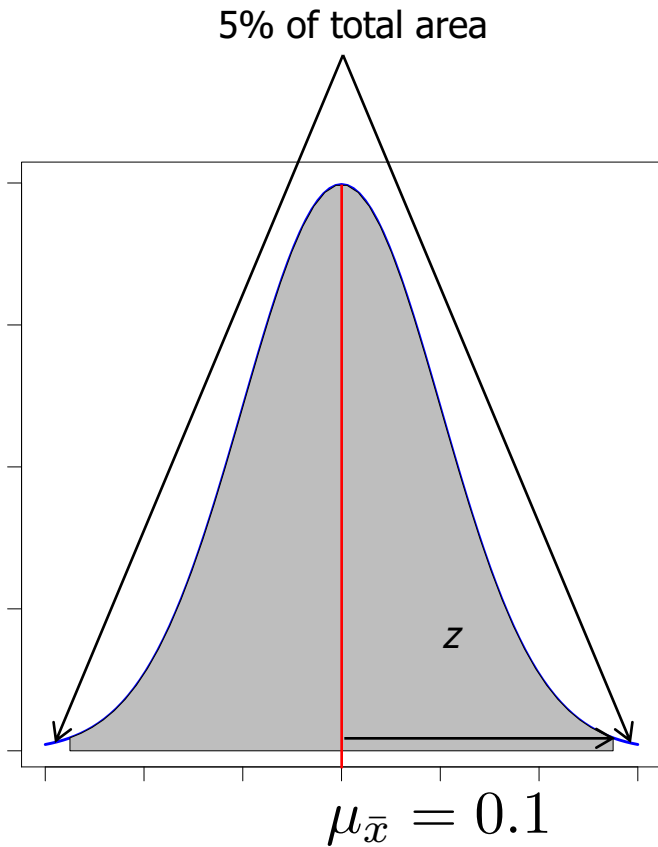
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$$0.1 \pm 0.084$$

Confidence Interval on a Proportion



Conclusion?

$$P(-z \leq \mu_{\bar{x}} \leq z) = 0.95$$

$$z = 1.96$$

$$\mu_{\bar{x}} \pm 1.96 \frac{s}{\sqrt{n}} = 1.96 \frac{\sqrt{0.092}}{\sqrt{50}} = 0.084$$

$$0.1 \pm 0.084$$

Repeated samples from this population would generally not contain our proposed population mean ($\pi = 0.2$)

Hypothesis Testing on a Proportion

- Our question is whether $p=0.2$ would likely result in observing 5 variants (out of 50)
- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$H_0 : p = \mu_p$$

$$H_1 : p \neq \mu_p$$
- What are our hypotheses?

Hypothesis Testing on a Proportion

- Next we would like to test whether $\pi = 0.2$ would likely result in observing 5 mutants in 50
- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$H_0 : p = \mu_p$$
$$H_1 : p \neq \mu_p$$
- What kind of test?
 - Normal dist'n, so z-test

Hypothesis Testing on a Proportion

- Next we would like to test whether $\pi = 0.2$ would likely result in observing 5 mutants in 50
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- How many samples?

Hypothesis Testing on a Proportion

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$$H_0 : p = \mu_p$$

$$H_1 : p \neq \mu_p$$

- How many samples?
 - One

Hypothesis Testing on a Proportion

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- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$H_0 : p = \mu_p$$
$$H_1 : p \neq \mu_p$$
- How many sides/tails?

Hypothesis Testing on a Proportion

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- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$H_0 : p = \mu_p$$

$$H_1 : p \neq \mu_p$$
- How many sides/tails?
 - Two

Hypothesis Testing on a Proportion

- Next we would like to test whether $\pi = 0.2$ would likely result in observing 5 mutants in 50
- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$H_0 : p = \mu_p$$

$$H_1 : p \neq \mu_p$$

- How do we calculate test stat?

Hypothesis Testing on a Proportion

- Next we would like to test whether $\pi = 0.2$ would likely result in observing 5 mutants in 50
- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$Z = \frac{p - \mu_p}{\sigma_p}$$

$$H_0 : p = \mu_p$$

$$H_1 : p \neq \mu_p$$

Hypothesis Testing on a Proportion

- Next we would like to test whether $\pi = 0.2$ would likely result in observing 5 mutants in 50
- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$Z = \frac{\bar{p} - \mu_p}{\sigma_p}$$

$$H_0 : \bar{p} = \mu_p$$

$$H_1 : \bar{p} \neq \mu_p$$

- Of course, we don't know true mean or stdev

Hypothesis Testing on a Proportion

- Next we would like to test whether $\pi = 0.2$ would likely result in observing 5 mutants in 50
- We can assume that sampling dist'n is approximately normal if np and $n(1-p)$ are both >5

$$Z = \frac{p - \mu_p}{\sigma_p} = \frac{p - \mu_p}{\frac{\sigma}{\sqrt{n}}} \approx \frac{p - \mu_p}{\frac{s}{\sqrt{n}}}$$

$$H_0 : \bar{p} = \mu_p$$

$$H_1 : \bar{p} \neq \mu_p$$

- But we approximate σ with s

Hypothesis Testing on a Proportion

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- And we can assume the null hypothesis

Hypothesis Testing on a Proportion

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$$H_0 : \bar{p} = \mu_p$$

$$H_1 : \bar{p} \neq \mu_p$$

$$Z = \frac{0.1 - 0.2}{\frac{\sqrt{0.1*0.9}}{\sqrt{50}}} = 2.38$$

Hypothesis Testing on a Proportion

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Reject H_0

But Wait!

■ In R:

```
> binom.test (5, 50, 0.2)
```

Exact binomial test

data: 5 and 50

number of successes = 5, number of trials = 50, p-value = 0.07883

alternative hypothesis: true probability of success is not equal to 0.2

95 percent confidence interval:

0.03327509 0.21813537

sample estimates:

probability of success

0.1

But Wait!

■ In R:

```
> binom.test (5, 50, 0.2)
```

Exact binomial test

Accept H_0 !

data: 5 and 50

number of successes = 5, number of trials = 50, p-value = 0.07883

alternative hypothesis: true probability of success is not equal to 0.2

95 percent confidence interval:

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sample estimates:

probability of success

0.1

But Wait!

- We were just barely violating our assumptions ($np, n(1-p) > 5$)
- `binom.test` doesn't use the normal approximation method
- Instead, it uses the Clopper-Pearson method which is mathematically more complicated but based on the binomial distribution itself, rather than a normal approximation (hence it is an “exact” test)

But Wait!

- A lesson here is that not all methods are created equal and you can get very different results
- It pays to understand what you are doing when you use an equation or call a function someone else wrote!
- What would you conclude, given these results?

But Wait!

- A lesson here is that not all methods are created equal and you can get very different results
- It pays to understand what you are doing when you use an equation or call a function someone else wrote!
- In the end, I'd conclude that the true mean is *likely* to be 0.2. However, I'd also want to be clear that I don't have a high degree of confidence. I'd want to take more samples (and use ALL of them).

Testing Equality of Proportions

- How do we test whether **two populations** have the same allele frequency?
- There are several ways to do this, one is the **chi-square (χ^2) test**. You set up a 2 x 2 table of numbers of outcomes:

	Mutant allele	WT allele
Population #1	5	45
Population #2	10	35

- In fact, the chi-square test can test bigger tables: R rows by C columns.
- The null hypothesis is that the events are independent (e.g. allele population doesn't influence allele frequency)

The Chi-Square Test

- We draw individuals and classify them in one way, and also another way.

	Mutant allele	WT allele	Total
Population #1	5	45	50
Population #2	10	35	45
Total	15	80	95

An Aside: Contingency Tables

Setting: Let X_1 and X_2 denote categorical variables, X_1 having I levels and X_2 having J levels. There are IJ possible combinations of classifications.

	Level=1	Level=2	Level=J
Level=1				
Level=2				
⋮				
Level=I				

When the cells contain frequencies of outcomes, the table is called a contingency table.

How To Do a Chi-Square Test?

- Assuming independence, calculate the expected number in each class (a cell in a contingency table). For an $m \times n$ contingency table this is (row sum) \times (column sum) / (total)

	Mutant allele	WT allele	Total
Population #1	5	45	50
Population #2	10	35	45
Total	15	80	95

$$\frac{50}{95} = 0.5263$$

$$\frac{15}{95} = 0.1578$$

“Expected” number of subjects in pop #1 AND having mutant allele

$$\frac{15 * 50}{95} = 7.8947$$

How To Do a Chi-Square Test?

- Assuming independence, calculate the expected number in each class (a cell in a contingency table). For an $m \times n$ contingency table this is $(\text{row sum}) \times (\text{column sum}) / (\text{total})$

	Mutant allele	WT allele	Total
Population #1	5	45	50
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Total	15	80	95

“observed”

“Expected” number of subjects in each cell

Calculating the Chi-Square Test Stat

- Assuming independence, calculate the expected number in each class (a cell in a contingency table). For an $m \times n$ contingency table this is (row sum) \times (column sum) / (total)
- Sum over all classes:

$$\chi^2 = \sum_{\text{classes}} \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

- The number of degrees of freedom is $(n-1)(m-1)$.
- In R, use `pchi ()`, which is the distribution of sums of (various numbers of) squares of normally-distributed quantities.

Calculating the Chi-Square Test Stat

	Mutant allele	WT allele	Total
“observed” →	5	45	50
→	10	35	45
	Total	15	80
			95

Expected values (in red): 7.895, 42.105, 7.105, 37.894

“Expected” ←

$$\chi^2 = \sum_{\text{classes}} \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$$\chi^2_1 = \frac{(5-7.895)^2}{7.895} + \frac{(45-42.11)^2}{42.11} + \frac{(10-7.11)^2}{7.11} + \frac{(35-37.89)^2}{37.89}$$

Degrees of freedom = (# rows-1) x (# columns-1) = 1

Calculating the Chi-Square Test Stat

	Mutant allele	WT allele	Total
“observed” →	5	45	50
→	10	35	45
	Total	15	80
			95

Expected values (in red):

- Population #1: 7.895 (Mutant), 42.105 (WT)
- Population #2: 7.105 (Mutant), 37.894 (WT)

“Expected” ←

$$\chi^2 = \sum_{\text{classes}} \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$$\chi_1^2 = 2.65$$

Degrees of freedom = (# rows-1) x (# columns-1) = 1

Calculating the Chi-Square Test Stat

	Mutant allele	WT allele	Total
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→	10	35	45 ← “Expected”
Total	15	80	95

$$\chi^2 = \sum_{\text{classes}} \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

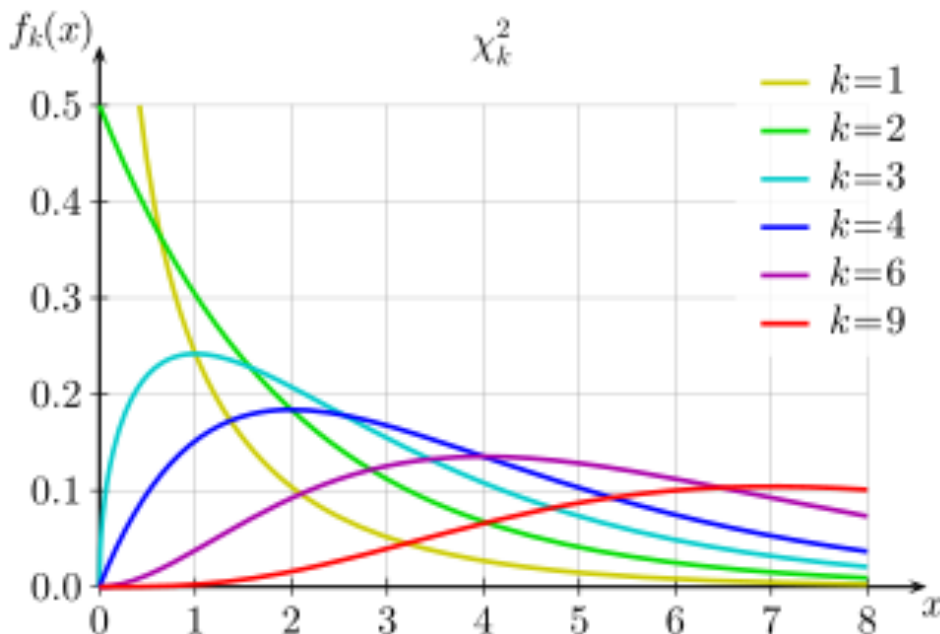
$$\chi_1^2 = 2.65$$

Degrees of freedom = (# rows-1) x (# columns-1) = 1

Chi-Square Test Depends on the Chi-Square Distribution

- The Chi-square distribution is the distribution of the sum of k (=df) squared independent standard normal RVs.

$$\chi_{df}^2 = \sum_{i=1}^{df} Z^2; \text{ where } Z \sim N(0,1)$$

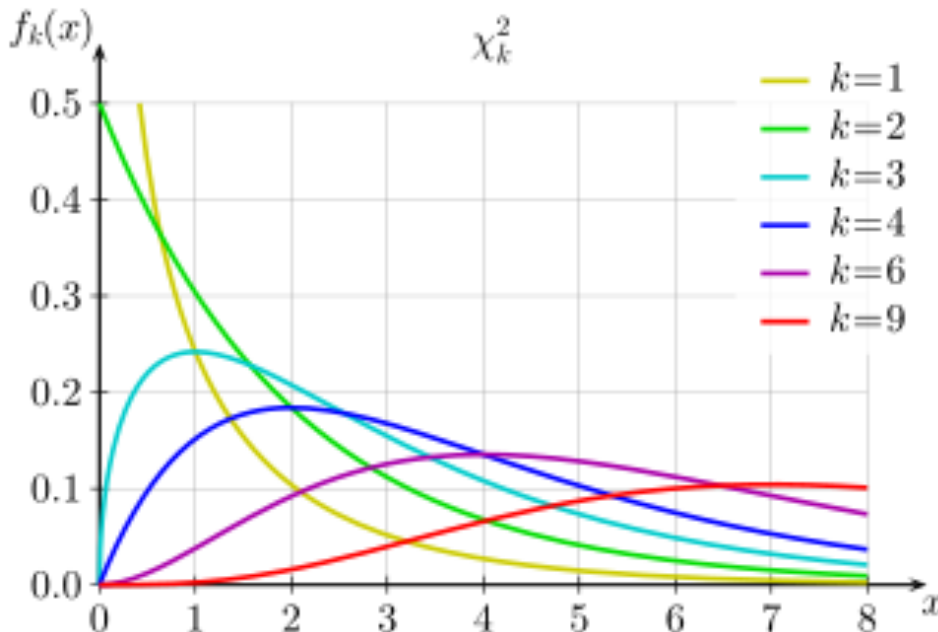


- The expected value and variance of the chi-square
 - $E(x) = df$
 - $Var(x) = 2 * (df)$

The Chi-Square Distribution

- Can be used to test the goodness of fit of an observed distribution or equality of proportions between groups

$$\chi_{df}^2 = \sum_{i=1}^{df} Z^2; \text{ where } Z \sim N(0,1)$$



- The expected value and variance of the chi-square
 - $E(x) = df$
 - $Var(x) = 2 * (df)$

Calculating the Chi-Square Test Stat

	Mutant allele	WT allele	Total
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Total	15	80	95

“observed” →

← “Expected”

Expected values: 7.895, 7.105, 42.105, 37.894

$$\chi^2 = \sum_{\text{classes}} \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$$\chi_1^2 = 2.65$$

$$p \sim 0.1$$

$$\text{Degrees of freedom} = (\# \text{ rows} - 1) \times (\# \text{ columns} - 1) = 1$$

Chi-Square Test - Summary

- Chi-square test can compare proportions between two groups
- Assumptions:
 - Sample data is random, from a fixed population
 - Each cell is assumed to have at least 5 counts. If this assumption is violated, use Fisher's exact test
 - Observations are independent (if you have paired data, use McNemar's test)

Fisher's Exact Test

- Another way of testing for independence between two different ways of classifying individuals
- Based on hypergeometric distribution
- Useful when the number of observations is too small for chi-square test (any $n < 5$)

Fisher's Exact Test

	Men	Women	Row total
Pirates	1	9	10
Ninjas	11	3	14
Column total	12	12	24

Fisher's Exact Test

	Men	Women	Row total
Pirates	1	9	10
Ninjas	11	3	14
Column total	12	12	24

Does sex affect propensity to be a pirate/ninja?
In other words, is the difference in proportions
we observe significant?

Fisher's Exact Test

	Men	Women	Row total
Pirates	1	9	10
Ninjas	11	3	14
Column total	12	12	24

	Men	Women	Row total
Pirates	A	B	A + B
Ninjas	C	D	C + D
Column total	A + C	B + D	A + B + C + D = n

Fisher's Exact Test

	Men	Women	Row total
Pirates	1	9	10
Ninjas	11	3	14
Column total	12	12	24

	Men	Women	Row total
Pirates	A	B	A + B
Ninjas	C	D	C + D
Column total	A + C	B + D	A + B + C + D = n

$$p = \frac{\binom{A+B}{A} \binom{C+D}{C}}{\binom{n}{A+C}}$$

Probability of obtaining the particular observed row and column totals given the number of samples is governed by the hypergeometric distribution

Fisher's Exact Test

	Men	Women	Row total
Pirates	1	9	10
Ninjas	11	3	14
Column total	12	12	24

$$p = \frac{\binom{10}{1} \binom{14}{11}}{\binom{24}{12}}$$

	Men	Women	Row total
Pirates	A	B	A + B
Ninjas	C	D	C + D
Column total	A + C	B + D	A + B + C + D = n

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

$$p = 0.00135$$

Fisher's Exact Test

	Men	Women	Row total
Pirates	1	9	10
Ninjas	11	3	14
Column total	12	12	24

$$p = \frac{\binom{10}{1} \binom{14}{11}}{\binom{24}{12}}$$

	Men	Women	Row total
Pirates	A	B	A + B
Ninjas	C	D	C + D
Column total	A + C	B + D	A + B + C + D = n

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

$$p = 0.00135$$

Gives exact hypergeometric probability of observing this particular arrangement assuming a null of equality between men and women

Fisher's Exact Test - Summary

- Can be used to compare proportions between groups in a 2x2 table
- Assumptions:
 - Samples are independent
 - Row and column totals are fixed before the experiment. This assumption is often violated but FET is *conservative* in this case (e.g. if null is true you will reject it *less* often than you should)

Hypothesis Testing So Far...

- A sample to a parametric mean/value
- Two sample means to each other
- Comparing a proportion to a parametric value
- Comparing two proportions $n > 5$
- Comparing multiple proportions
- Comparing two proportions $n < 5$

Hypothesis Testing So Far...

- A sample to a parametric mean/value
 - t-test; could use z-test if $n > 30$
- Two sample means to each other
- Comparing a proportion to a parametric value
- Comparing two proportions $n > 5$
- Comparing multiple proportions
- Comparing two proportions $n < 5$

Hypothesis Testing So Far...

- A sample to a parametric mean/value
 - t-test; could use z-test if $n > 30$
- Two sample means to each other
 - t-test
- Comparing a proportion to a parametric value
- Comparing two proportions $n > 5$
- Comparing multiple proportions
- Comparing two proportions $n < 5$

Hypothesis Testing So Far...

- A sample to a parametric mean/value
 - t-test; could use z-test if $n > 30$
- Two sample means to each other
 - t-test
- Comparing a proportion to a parametric value
 - Bernoulli/Z
- Comparing two proportions $n > 5$
- Comparing multiple proportions
- Comparing two proportions $n < 5$

Hypothesis Testing So Far...

- A sample to a parametric mean/value
 - t-test; could use z-test if $n > 30$
- Two sample means to each other
 - t-test
- Comparing a proportion to a parametric value
 - Z-test if sample sufficiently large
- Comparing two proportions $n > 5$
 - Chi-square
- Comparing multiple proportions
- Comparing two proportions $n < 5$

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 - Fisher's exact

Parametric Hypothesis Testing - Cautions

- Because parametric hypothesis tests rely on assumptions about how the data is distributed they can easily be misused
- The advantage is that they are easier to calculate, more powerful and often perfectly appropriate

Parametric Hypothesis Testing - Cautions

- When should you decide your critical value/level of significance?
 - Before you start!
- If you collect some data and it fails to reach significance, is it OK to collect more data, test again and ignore the previous data?
 - No – why? This is an example of multiple testing. With $\alpha = 0.05$, we *expect* to reject a true null 5% of the time.

R Goals

- Executing proportion comparisons
- Using `apply()` to vectorize function calls
- Comparing loops and vectorization