**Chi-Square Test for Significant Difference**

Χ2

**When to use a Chi-square test**

Researchers often need to decide if the results they observe in an experiment are close enough to predicted theoretical results so that the tested hypothesis can be supported or rejected. For example, do a series of coin flips match what you’d expect to get by chance, or is their evidence the coin is unfair? Does the number of women interviewed for a job position match the proportion of women in the applicant pool, or is there evidence of bias? Does the number of white-eyed fruit fly offspring match the number expected if the white-eyed trait is recessive, or are white-eyes inherited in some other way?

Chi-square tests come in two types:

***Chi-square test for independence***

***Chi-square goodness of fit test***: used to test if the observed data match theoretical or expected results. **We will focus on this test**. *Example*: Do the phenotypes you observe in a fruit fly cross match the pattern expected if the trait is dominant?

A Chi-square test is used when:

1. Your response variable is \_\_count\_\_\_\_\_\_\_\_\_\_\_\_\_ data.

2. Your response falls into different \_\_categories\_\_\_\_\_\_\_\_\_\_\_\_\_.

3. You have a hypothesis for the responses you \_\_expect\_\_\_\_\_\_\_\_\_\_\_\_\_.

4. You want to know if the difference between the responses you \_observed\_\_\_\_\_ and the responses you \_\_expect\_\_\_\_\_\_\_\_\_\_\_\_ is significant or not.

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**Steps to the Chi-square test using a Chi-square table**

1. Define the hypothesis you are testing – very important step! This determines your expected values!
2. Identify the categories for your responses (for example, phenotype) and write these in the far left column of your chi-square table.
3. In the following steps we will use the table below to break down each step of the Chi-square (symbol=X2) equation:



Χ2 = Σ

 **(Observed – Expected)2**

 **Expected**

***Example Chi-Square Table***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Observed | Expected | Obs-Exp | (Obs-Exp)2 | (Obs-Exp)2Exp |
| Category 1 |  |  |  |  |  |
| Category 2 |  |  |  |  |  |
| ….. |  |  |  |  |  |
| X2 totalX2 total |  |
| Degrees of Freedom |  |

1. Put your observed data in the “observed” column.
2. Calculate the total for the “observed” column.
3. Identify the ratio among the categories that you expect based on your hypothesis.
4. **Calculate your expected values** for each category by scaling up your expected ratio so that your expected values are in the expected ratio *and* add up to the same total as the observed values. Place these numbers in the “expected” column of your chi-square table.

**Example:** I expect a 9:3:3:1 ratio among the offspring for a plant cross. In that case, I expect the fourth phenotype (the 1 in the 9:3:3:1) to make up 1/(9+3+3+1=16) of the offspring. If I made 63 real observations, then I expect the fourth phenotype to show up in (1/16)\*63 = 3.9375 plants out of the 63.

1. Calculate the Χ2 value for each category and sum (**Σ**) all the categories’ Χ2 values.
2. Determine your **degrees of freedom (df)**. The meaning of your chi-square value depends on the degrees of freedom. The **degrees of freedom is one less than the number of categories.** When using a chi-square table this is the number of rows - 1.

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1. Use a chi-square probability table (below) to determine your **probability (p) value**, or the likelihood your data supports your hypothesis.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **df** | **0.99** | **0.95** | **0.9** | **0.8** | **0.7** | **0.6** | **0.5** | **0.4** | **0.3** | **0.2** | **0.1** | **0.05** | **0.01** |
| 1 | 0.0001 | 0.003 | 0.015 | 0.064 | 0.148 | 0.275 | 0.455 | 0.708 | 1.07 | 1.64 | 2.71 | 3.84 | 6.63 |
| 2 | 0.020 | 0.103 | 0.211 | 0.446 | 0.713 | 1.02 | 1.39 | 1.83 | 2.41 | 3.22 | 4.61 | 5.99 | 9.21 |
| 3 | 0.115 | 0.352 | 0.584 | 1.00 | 1.42 | 1.87 | 2.37 | 2.95 | 3.67 | 4.64 | 6.25 | 7.81 | 11.3 |
| 4 | 0.297 | 0.711 | 1.06 | 1.65 | 2.19 | 2.75 | 3.36 | 4.04 | 4.88 | 5.99 | 7.78 | 9.49 | 13.3 |
| 5 | 0.554 | 1.15 | 1.61 | 2.34 | 3.00 | 3.66 | 4.35 | 5.13 | 6.06 | 7.29 | 9.24 | 11.1 | 15.1 |
| 6 | 0.872 | 1.64 | 2.20 | 3.07 | 3.83 | 4.57 | 5.35 | 6.21 | 7.23 | 8.56 | 10.6 | 12.6 | 16.8 |
| 7 | 1.24 | 2.17 | 2.83 | 3.82 | 4.67 | 5.49 | 6.35 | 7.28 | 8.38 | 9.80 | 12.0 | 14.1 | 18.5 |
| 8 | 1.65 | 2.73 | 3.49 | 4.59 | 5.53 | 6.42 | 7.34 | 8.35 | 9.52 | 11.0 | 13.4 | 15.5 | 20.1 |

*If p>0.05,* ***SUPPORT*** *hypothesis*

*If p<0.05,* ***REJECT*** *hyp.*

**Chi-square probability table**

*Probabilities (top row) 🡪*

**How to use the Chi-square probability table to find the probability (p) range for your calculated total X2**: Find your **df** value in the left column. Look across the row until you find where your X2 value falls. You probably will not see your exact X2, so look for the range where it falls between. Your corresponding probability range can be found in the top row. The **probability range** is expressed as, for example, 0.5 < p < 0.6.



* If the chi-square test shows your p > 0.05, this means the observed values are **not significantly different** from what you expected, you fail to reject (AKA **support)** the hypothesis as an explanation for your data. *Remember, no statistical test can ever* ***prove*** *a hypothesis, only fail to reject it.*
* If the chi-square test shows your p < 0.05, this means the observed values are **significantly different** from what you expected, you **reject** the hypothesis.

Example: Imagine your total Χ2 value is 2.15 for an experiment with 4 categories (df=4- 1=3). Find the row for df=3. Look across the row until you see where 2.15 would fall in that row. Then look up to what probabilities that falls between. In this case it would be 0.5>p>0.6. This means there is a 50-60% probability that the difference between your obs and expect values is due to chance alone. This is a high probability because it is **> 0.05**. So your observations are not significantly different (only difference is random noise) from your expected values (supports hypothesis

*Circle your answer within the () in each statement below.*

* You conclude that there **is a significant difference** between your observed and expected values when the Chi-square probability value is (<0.05 OR >0.05).
* When the p-value is <0.05 you (support OR reject) your hypothesis.

**Example Problem #1**

A university biology department would like to hire a new professor. They advertised the opening and received 220 applications, 25% of which came from women. The department came up with a “short list” of their favorite 25 candidates, 5 women and 20 men, for the job. You want to know if there is evidence for the search committee being biased against women. *Note: If the committee is* ***unbiased*** *the proportion of women in the short list should match the proportion of women in all the applications.*

1. Identify the hypothesis you are testing: I will test the hypothesis that the committee is unbiased—I cannot test a hypothesis that the committee is biased because I do not have a predicted % preference for men over women to use to calculate expected values.

2. Calculate the expected number of candidates in each category based on the hypothesis and the total number of applicants.

Women: 0.25\*25 = 6.25

Men: (1-0.25)\*25=18.75

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Observed | Expected | Obs-Exp | (Obs-Exp)2 | (Obs-Exp) 2Exp |
| Women | 5 | 6.25 | -1.25 | 1.5625 | 0.25 |
| Men | 20 | 18.75 | 1.25 | 1.5625 | 0.08 |
| **Total** |  | X2 total | 0.33 |
| Degree of Freedom | 1 |

3. What is the probability range for your chi-squared value? 0.5 < p < 0.6

4. Based on this probability, do we support or reject the hypothesis above? support

5. Write a statement that interprets this statistical result in the context of the problem.

The observed data are not significantly different from the expected values (p> 0.05). This is evidence in support of the hypothesis that the committee is not biased against women.

**Example Problem #2**

 Wild type *Drosophila* flies’ bodies are gray (*G*) with normal size wings (*W*). The recessive phenotypes are ebony colored bodies (*g*) and vestigial wings (*w*). A researcher hypothesizes that body color and wing size are unlinked traits. To test this, she crossed two heterozygous dihybrid flies (*GgWw* x *GgWw*)—like an F1 cross. She observed the following: 53 Wild Wild : 16 Wild Vestigial : 25 Ebony Wild : 8 Ebony Vestigial. *Do these results support her hypothesis that the genes are* ***unlinked****?*

1. Identify the hypothesis you are testing: Body color and wing shape are unlinked genes.
2. For this type of cross, if the genes for body color and wing size are **unlinked**, what ratio should she expect for the offspring phenotypes? \_\_9:3:3:1\_\_\_\_\_\_\_\_\_\_\_
3. Calculate the expected number of phenotypes in each category based on the hypothesis and the total number of flies observed.

Gray Normal wings (*GgWw)* : 9/16 \* 102 = 57.375

Gray Vestigial wings (*Ggww*) : 3/16 \* 102 = 19.125

Ebony Normal wings (*ggWw*): 3/16 \* 102 = 19.125

Ebony Vestigial wings (*wwgg*) : : 1/16 \* 102 = 6.375

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Observed | Expected | Obs-Exp | (Obs-Exp)2 | (Obs-Exp) 2Exp |
| Wild Wild | 53 | 57.375 | -4.375 | 19.14 | 0.333 |
| Wild Vestigial | 16 | 19.125 | -3.125 | 9.77 | 0.511 |
| Ebony Wild | 25 | 19.125 | 5.875 | 34.52 | 1.805 |
| Ebony Vestigial | 8 | 6.375 | 1.625 | 2.64 | 0.414 |
| **Total** | 102 | X2 total | 3.063 |
| Degree of Freedom | 3 |

1. What is the probability range for your chi-squared value? 0.3 < p < 0.4
2. Based on this probability, do we support or reject the hypothesis above? support

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1. Write a statement that interprets this statistical result in the context of the problem.

There is a 30-40% probability the difference between the observed and expected phenotypic ratios is due to random chance, meaning the obs and exp values are not significantly different. Therefore, the data support the hypothesis that ebony and vestigial are recessive traits for un-linked genes.

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**Example Problem #3**

 A certain squash species’ wild type is long shaped (*L*) and green colored (*G*)—like a zucchini). The recessive shape and color are round (*l*) and orange (*g*)—like a small pumpkin. A plant breeder suspects these two genes are **linked**. To find out he carried out a series of crosses. Like Mendel, he first crossed two true-breeding plants: *LLGG* x *llgg*. This produces the F1 dihybrids, *LlGg*. He then does a special cross, where unlike Mendel who crossed two F1 dihybrids, this plant breeder crossed one F1 dihybrid squash with a squash plant homozygous for both recessive alleles: *LlGg* x *llgg.* He observes 228 *LlGg* : 17 *Llgg* : 21 *llGg* : 243 *llgg*. Do these results support his gene linkage hypothesis? *Note: You may test either hypothesis, that the genes are linked or unlinked, just be sure the offsprings’ expected phenotype ratio matches your chosen hypothesis.*

1. State the hypothesis the plant breeder is testing: Squash color & shape are unlinked / linked genes.

2. Describe the phenotypes for each genotype & **circle the recombinants.**

LlGg: Long green llGg: Round orange

llgg: Round Orange Llgg: Long orange

3. If the two genes are ***not linked*** the expected phenotype ratio is:

\_\_1\_\_\_\_ : \_\_1\_\_\_\_ : \_\_1\_\_\_\_ : \_\_1\_\_\_\_.

4. If the two genes are ***linked*** the expected phenotype ratio is:

\_\_1\_\_\_\_ : \_\_0\_\_\_\_ : \_\_0\_\_\_\_ : \_\_1\_\_\_\_.

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5. Calculate the ***expected*** number of offspring for each phenotype based on the hypothesis and the total number of plants observed. Total number of plants observed = 509

 If hyp. is **unlinked** (1:1:1:1) If hyp is **linked** (1:0:0:1)

Wild Wild (*LlGg)* : 509/4 = 127.25 509/2=254.5

Wild Orange (*Llgg*) : 127.25 0

Round Wild (*llGg*) : 127.25 0

Round Orange (*llgg*) : 127.25 254.5

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| UNLINKED HYP. | Observed | Expected | Obs-Exp | (Obs-Exp)2 | (Obs-Exp) 2Exp |
| Wild Wild | 228 | 127.25 | 100.75 | 10150.56 | 79.8 |
| Wild Orange | 17 | 127.25 | -110.25 | 12155.06 | 95.5 |
| Round Wild | 21 | 127.25 | -106.25 | 11289.06 | 88.7 |
| Round Orange | 243 | 127.25 | 115.75 | 13398.06 | 105.3 |
| **Σ** | 509 | **Σ** X2  | 369.3 |
| Degree of Freedom | 3 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| LINKED HYP. | Observed | Expected | Obs-Exp | (Obs-Exp)2 | (Obs-Exp) 2Exp |
| Wild Wild | 228 | 254.5 | -26.5 | 702.25 | 2.76 |
| Wild Orange | 17 | 0 | 17 | 289 | (Undefined) 0 |
| Round Wild | 21 | 0 | 21 | 441 | (Undefined) 0 |
| Round Orange | 243 | 254.5 | -11.5 | 132.25 | 0.52 |
| **Σ** | 509 | **Σ** X2  | 3.28 |
| Degree of Freedom | 3 |

8. What is the probability range for your chi-squared value? Unlinked: p<0.01 / Linked: 0.3 < p < 0.4

9. Based on this probability, do we support or reject the hypothesis above? Unlinked: Reject / Linked: Support.

10. Write a statement that interprets this statistical result in the context of the problem.

Unlinked: The data are significantly different from the expectation for unlinked genes—there is only a very small % probability that the difference between our observations and expected values is due to random chance. The data suggest we reject the hypothesis, so the phenotypes are significantly different from a 1:1:1:1 ratio. They look more like a 1:0:0:1 ratio, suggesting the genes for squash color and shape are on the same chromosome (they do not sort independently).

Linked: The data are not significantly different from the expected values for linked genes—there is a high (30-40%) probability that the difference between our observed and expected values is merely due to random chance. So, the data support our hypothesis that squash color and shape are linked genes—they do not sort independently.

**Recombination frequency:** [(36+48)\*100] / 485 = 17.3%