THE CHI-SQUARE (χ^2) TEST

In some experiments, scientists test the frequency of a certain behaviour by an organism, or the frequency by which a certain characteristic appears in a sample of organisms. The χ^2 test is useful to distinguish between random and non-random behaviour (e.g., preferential movement toward or away from some factor) or distribution of characteristics in a population (e.g., do fish have more spines when raised in the presence of a predator). H_o predicts random behaviour or random distribution (no preference) in a population. You must determine whether your data are "close enough" to the expected values for random behaviour or random distribution and therefore chance alone could have caused the amount of deviation observed. For more information on the chi-square distribution, you can consult any elementary statistics text, e.g., Sokal, R.P. and Rohlf, F.J. 1987. Introduction to Biostatistics. W. H. Freeman and Company, San Francisco, or Zar, J.H. 1999. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J.

- If the data **are in agreement with** those values predicted for random behaviour, you fail to reject H_o.

- If the data **deviate from** the values predicted for random behaviour, you reject H_o and provide support for H_a.

To determine if the data are in agreement with or deviate from the values predicted for random behaviour, calculate the χ^2 value for those data by substituting into the following formula:

$$\chi^2 = \sum \frac{(observed - expected)^2}{expected}$$

To calculate the value of χ^2 follow these steps:

Step 1: Calculate **expected values** based on H_o.

$$\text{Number expected} = \frac{\text{total number of replicates}}{\text{number of items from which to choose}}$$

Example:

You wish to determine if hermit crabs, *Pagarus granosimanus* prefer larger shells when they select new shells for shelter. Your hypotheses would be:

H_o: The size of shell has no effect on shell selection in *Pagarus granosimanus*.

H_a: The size of shell has an effect on shell selection in *Pagarus granosimanus*.

Experiment: Your sample is 20 hermit crabs; **each** is given a choice of two shell sizes, small (internal volume of 2 mL) and larger (internal volume of 4 mL).

Data: number of 4-mL shells chosen = 12, number of 2-mL shells chosen = 8.

Expected values: You have provided a choice of two different items: 4-mL and 2-mL shells. If H_o were true, you would expect equal numbers of each shell size to be chosen; therefore, number expected to choose each type of shell = 20/2 = 10.00.

Note these are the actual numbers expected not the percent of the total. The calculations won't work if you use percentages.

Step 2: Calculate $\frac{(\text{observed} - \text{expected})^2}{\text{expected}}$ for each class (experimental option) and enter these values in Table 1. Keep all decimal places in your calculator.

Table 1. Observed and expected results for the size of shells selected by *Pagarus granosimanus*.

class	observed	expected	$(\text{obs.} - \text{exp.})^2$	$\frac{(\text{obs.} - \text{exp.})^2}{\text{exp.}}$
4-mL shells	12	10.00	4.00	0.40
2-mL shells	8	10.00	4.00	0.40

Step 3: Sum the $\frac{(\text{obs.} - \text{exp.})^2}{\text{exp.}}$ values for all classes.

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$ = 0.40 + 0.40 = **0.80** (two decimal places are required because Table 2 provides critical χ^2 values to two decimal places)

Step 4: Describe the relationship between the factor and the choices made by the organisms. Is there a trend to favour or avoid a certain class? If you did two trials of your experiment, were these trends the same in both trials? In this example the trend is for hermit crabs to select larger shells more frequently than smaller shells.

Are the results significant? Compare the calculated χ^2 value with the critical χ^2 values (see Table 2). **Keep two decimal places** in the calculated χ^2 value because the critical values in the table have two decimal places. A probability of 0.05 is considered an appropriate probability level for biological experiments. This probability means that five times out of 100 experiments, or one time in 20, chance alone would produce your results and you would reject H_o when it is true.

The degrees of freedom represent the number of independent classes of events in your experiment. In most cases, the number of independent classes will be one less than the total number of classes. In this example, there are two classes (4-mL and 2-mL shells); therefore the degrees of freedom are 2 - 1 = 1.

Table 2. Critical values of χ^2 at p = 0.05. (Based on a larger table from P. G. Hoel, 1966. Elementary Statistics, John Wiley and Sons, New York.)

degrees of freedom	critical χ^2 (p = 0.05)
1	3.84
2	5.99
3	7.81
4	9.49
5	11.07

Step 5: Interpret the results; make a decision about your hypotheses:

- If the calculated χ^2 value is < the critical value of χ^2, you fail to reject H_o. Again note the wording, – the researchers (you), not the data, fail to reject H_o.

- If the calculated χ^2 value is ≥ the critical value, you reject H_o and provide support for H_a.

The **critical value** of χ^2 from Table 2 at **p = 0.05** and **degrees of freedom = 1** is 3.84. Your **calculated** χ^2 value is **0.80 which is < the critical χ^2 value**, i.e., 0.80 < 3.84. Therefore you fail to reject H_o.

Note: χ^2 values must be **calculated separately for each trial or experiment**.

Step 6: Explain your results. If you are unable to reject H_o then, **in that trial**, selection was random. However, if you are able to reject H_o and provide support for H_a then you should explain the preference or avoidance behaviour your organism exhibited, e.g., what are the processes that occurred that resulted in this choice? Does this agree with your prediction? If not, why not? The answers to these and other questions will be incorporated into the Discussion portion of your experiment report.

If you have more than two classes in your experiment, e.g., small (2-mL internal volume), medium (4-mL internal volume) and large (6-mL internal volume) shells, your table would look like this:

Table 3. Observed and expected results for the size of shells selected by *Pagarus granosimanus*.

class	observed	expected	(obs.- exp.)^2	$\frac{(\text{obs.- exp.})^2}{\text{exp.}}$
6-mL shells	14	$6.6\overline{6}$	$53.7\overline{7}$	$8.0\overline{6}$
4-mL shells	4	$6.6\overline{6}$	$7.1\overline{1}$	$1.0\overline{6}$
2-mL shells	2	$6.6\overline{6}$	$21.7\overline{7}$	$3.2\overline{6}$

$\chi^2 = 8.0\overline{6} + 1.0\overline{6} + 3.\ \overline{2}6 = 12.41$

Remember, the expected column assumes **equal preference** among all shell types: 20 crabs with a choice of three shell types yields an expected value of 20/3 = 6.66 crabs/shell type. (This is a theoretical value, so fractions of crabs are acceptable.) Keep all decimal places for your intermediate χ^2 calculations; for your final answer, keep the same number of decimal places as used for the critical values (Table 2).

Follow the remaining steps as with the two shell-size choice experiment. When comparing calculated χ^2 values with critical χ^2 values from Table 2, the degrees of freedom = 2 (3 classes -1).

Limitation of the χ^2 test:

If you have more than two classes and your calculated χ^2 is such that you reject H_o, this calculated value will not tell you where the significant difference occurs, i.e., which data are causing the greatest deviation from the expected results. However, in most cases this can be determined by close inspection of the calculated χ^2 values for each data set. If one of the χ^2 terms in the equation is substantially larger than the others, it suggests that that option is preferred or avoided.