STUDENT GUIDE TO THE CHI-SQUARE TEST OF INDEPENDENCE

The chi-square test of independence (also called the chi-square contingency test) is a special application of the chi-square goodness of fit test. In the test of independence, the null statistical hypothesis is that two or more categorical variables are independent of each other. In other words, the occurrence of one variable is not *contingent* on the other.

The experiment described below comes from the following published study:

Wullschleger, B., and W. Nentwig. “Influence of venom availability on a spider's prey-choice behaviour.” *Functional Ecology* 16, 6 (2002): 802–807. https://doi.org/10.1046/j.1365-2435.2002.00686.x.

Venomous spiders are known to vary their attack behaviors depending on the type of prey available, a phenomenon called optimality. Venomous spiders can also change their attack behavior depending on how much time has passed since they last used venom to subdue a prey individual; if little time has passed, less venom may be available for the next attack.

Biologists at the Zoological Institute at the University of Bern in Switzerland were interested in testing how the presence or absence of venom in spiders affected their choice of prey. For their study species, they chose the tiger wandering spider (*Cupiennius salei*) from Central America, a well-known hunting spider that is easy to raise in a lab setting. The biologists were interested in testing the **hypothesis** that the spider optimizes its predator behavior (optimality) and changes its prey choice depending on how much venom it has available. They **predicted** that the spiders that had their venom experimentally removed would more often choose prey that were less dangerous and might require less venom to subdue over prey that were more dangerous and might require more venom to subdue.

For the prey species in the experiment, the biologists chose two species of cockroaches, each with a different known sensitivity to spider venom. One species, *Nauphoeta cinerea*, is not very sensitive to spider venom, while the other, *Blatta orientalis*, is over 40 times more sensitive to spider venom. In other words, it takes far more venom to kill *N. cinerea* than it does to kill *B. orientalis*.

The results of the experiment are shown in the table below. The numbers in the table indicate how many times each type of spider (venom/no venom) chose each type of prey species (sensitive or not sensitive) to attack first. There were 45 spiders in each group to begin with, but the numbers of spiders in each group (venom/no venom) in the data table are different because some of the spiders in each group did not attack either cockroach after the 24-hour experimental period.

	Less sensitive prey (*N. cinerea*)	More sensitive prey (*B. orientalis*)
Spider with venom	20	22
Spider without venom	8	25

Your task is to analyze the data in the table with the chi-square test of independence to see if the results support the experimental hypothesis. The following steps will guide you through the quantitative reasoning necessary for calculating the expected values you will use to complete the chi-square test. You will then be shown how to interpret the chi-square results and write a concluding statement.

Step 1: Calculate the total numbers of spiders with venom and spiders without venom that ended up attacking a cockroach. Then calculate the total numbers of less sensitive and more sensitive cockroaches that were attacked.

	Less sensitive prey (*N. cinerea*)	More sensitive prey (*B. orientalis*)	Totals
Spider with venom	20	22	42
Spider without venom	8	25	33
Totals	28	47	75

Step 2: Look at the total number of cockroaches of each type that were attacked out of the 75 total cockroaches. What proportion of all the cockroaches that were attacked were of the less sensitive type, regardless of whether they were attacked by spiders with or without venom? Additionally, what proportion of the more sensitive cockroaches were attacked regardless of the type of spider that attacked them?

	Less sensitive prey (*N. cinerea*)	More sensitive prey (*B. orientalis*)	Totals
Spider with venom	20	22	42
Spider without venom	8	25	33
Totals	28	47	75
Proportions	28/75 = 0.37	47/75 = 0.63	

Step 3: With the proportions you have calculated, you can now determine how many cockroaches of each type you would theoretically expect to be attacked if the attack choice is independent of spider type.

For example, 0.37 of the less sensitive cockroaches were attacked in total. If the attack choice is independent of spider type, you would also expect 0.37 of the 42 cockroaches attacked by spiders with venom to be of the less sensitive type. That expected value is (0.37)(42) = 15.54. For the spiders without venom, you would expect (0.37)(33) = 12.21 of all the cockroaches attacked by those spiders to be of the less sensitive type.

Below are all of the calculations for the expected values. You may get slightly different values if you did not round the proportions in Step 2.

Category	Expected values
Less sensitive cockroaches attacked by spiders with venom	(0.37)(42) = 15.54
More sensitive cockroaches attacked by spiders with venom	(0.63)(42) = 26.46
Less sensitive cockroaches attacked by spiders without venom	(0.37)(33) = 12.21
More sensitive cockroaches attacked by spiders without venom	(0.63)(33) = 20.79

An alternative way to calculate the expected values for each category is by using combinations of probabilities. For example, we first might ask, what is the probability that an attack is carried out by a spider with venom on a less sensitive cockroach? We know that there are 28 less sensitive cockroaches out of 75 total cockroaches. The probability of any attack being carried out on a less sensitive cockroach is 28/75 = 0.37. We also know that there are 42 spiders with venom out of 75 total spiders. The probability that an attack is carried out by a spider with venom is 42/75 = 0.56. The probability that an attack is carried out by a spider with venom on a less sensitive cockroach is thus the product of the two probabilities: (0.37)(0.56) = 0.207. Therefore, the expected number of spiders with venom attacking less sensitive cockroaches is (0.207)(75) total attacks = 15.5. The remaining three expected values can also be calculated using this same logic.

Step 4: With the theoretical expected values now calculated, you can run the chi-square test as is shown below to calculate your chi-square value.

Category	Observed (*O*)	Expected (*E*)	$(O-E)^2/E$
Less sensitive cockroaches attacked by spiders with venom	20	15.54	1.28
More sensitive cockroaches attacked by spiders with venom	22	26.46	0.75
Less sensitive cockroaches attacked by spiders without venom	8	12.21	1.45
More sensitive cockroaches attacked by spiders without venom	25	20.79	0.85
$\chi^2 = \Sigma(O-E)^2/E = 4.33$			

If you did not round your calculations until the end, χ^2 will be 4.32 instead, which is very similar.

Step 5: Determine how many degrees of freedom you have. The method for determining the degrees of freedom for the chi-square test of independence can also be expressed as an equation based on the columns and rows in the original data table: (# of columns – 1)(# of rows – 1) = degrees of freedom.

In the original data table, there are two columns of variables (less sensitive and more sensitive cockroaches) and two rows of variables (spiders with venom and spiders without). Thus, there is one degree of freedom (df = 1).

But how does this equation work? Recall what is stated in the experiment: how many spiders of each type (the rows in the data table) attacked cockroaches and how many cockroaches of each type (the columns in the data table) were attacked. If you count the number of less sensitive cockroaches that were attacked by spiders with venom, you do not need to count the rest of the three categories because they are now set. You can simply subtract from the row and column totals. Therefore, only one of the four categories is free to vary, and once you know it, the other three categories are set and can be determined by subtraction.

Step 6: Any chi-square test you do is a statistical test of the null hypothesis (H_0). For the chi-square test, the null hypothesis is that the observed (*O*) and expected (*E*) values are equal and any difference occurred by chance. In other words, H_0: $O = E$. The larger the differences between the observed and expected values, the more likely it is that the differences are not chance differences but are real differences. In the case of the chi-square test of independence, if the observed and expected differences are large, it is likely that there is a real association between at least two of the variables and not just an accidental association.

Conclusion: From the chi-square critical values table below, you will find that for one degree of freedom (df = 1), the probability (*p*) of obtaining a chi-square value of 4.33 in the spider-cockroach experiment is between 0.05 and 0.03 (gray highlighted squares) assuming the null hypothesis (H_0) is true. This result exceeds (is to the right of) the $p = 0.05$ rejection threshold for the null hypothesis. Thus, you can conclude that, in this sample of 75 spiders and cockroaches, there is a statistically significant association between spiders with and without venom and their choices of cockroach prey. Tiger wandering spiders may be twice as likely (25/12.21 = 2.05) to attack more sensitive and less dangerous cockroaches when they are low on venom compared to what we would expect with less sensitive and more dangerous cockroaches.

Chi-square Critical Values Table

	Probability of Distribution Occurring by Chance (*p*)											
df	0.99	0.95	0.90	0.75	0.70	0.50	0.30	0.25	0.10	0.05	0.03	0.01
	Critical Values											
1	0.00	0.00	0.02	0.10	0.15	0.45	1.10	1.32	2.71	3.84	5.02	6.63
2	0.02	0.10	0.21	0.58	0.71	1.39	2.40	2.77	4.61	5.99	7.38	9.21
3	0.11	0.35	0.58	1.21	1.42	2.37	3.70	4.11	6.25	7.81	9.35	11.34
4	0.30	0.71	1.06	1.92	2.02	3.36	4.90	5.39	7.78	9.49	11.14	13.28

The experiment described below comes from the following published study:

Bohórquez-Alonso, M. L., G. Mesa-Avila, M. Suárez-Rancel, E. Font, and M. Molina-Borja. "Predictors of contest outcome in males of two subspecies of *Gallotia galloti* (Squamata: Lacertidae)." *Behavioral Ecology and Sociobiology* 72, 3 (2018): 63. https://doi.org/10.1007/s00265-018-2480-z.

In many animal species, the color patterns on male bodies are used as warning signals of fighting ability and aggressiveness to other males. For example, Tenerife lizard (*Gallotia galloti*) males have bright cheek and side patches that reflect ultraviolet (UV)-blue light. The patches are larger than similar patches found on females and are brighter during the breeding season.

Ecologists from the University of La Laguna, Spain, were interested in testing the **hypothesis** that the UV-reflecting color patches function as an aggressive and dominance signal to other males. To test this hypothesis, the researchers analyzed experimentally the effect of the presence or absence of the UV-blue patches on the outcome of staged dominance contests between lizard males. The researchers applied sunscreen to the UV-reflecting color patches of several equally sized lizards of the same age. A second group of similar lizards was left without the sunscreen treatment (the control group). The sunscreen treatment reduced the UV reflectance of the UV-blue patches of those lizards and made them appear to other lizard males as having low or no patch reflectance. The researchers **predicted** that the untreated males would win a greater proportion of dominance contests than the sunscreen-treated males. In other words, they predicted that wins would be significantly associated with the lizards exhibiting the bright UV-blue patches. The results of the experiment are shown in the table below.

Task: Analyze the data in the table below with the chi-square test of independence to see if the results support the experimental hypothesis. Use the additional tables that follow to help you organize your calculations and process data. When you have finished your calculations, write a conclusion statement for your analysis and a possible explanation for why the results may have turned out the way they did.

	Winners	Losers	Totals
Control Lizards	9	22	
Sunscreen Lizards	20	7	
Totals			

<u>Calculations</u>

Category	Observed (*O*)	Expected (*E*)	$(O-E)^2/E$
Control Lizard Winners			
Control Lizard Losers			
Sunscreen Lizard Winners			
Sunscreen Lizard Losers			
$X^2=\Sigma(O-E)^2/E =$			

Conclusion Statement

Possible explanation for why the results may have turned out the way they did