

Week 3 Problem Based Learning and Practical Solutions

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Problem based learning workshop

Exercise 1

In a genetics experiment on tomatoes, a dihybrid cross was made, with the frequencies of the progeny expected to be in the ratio 9:3:3:1.

The following table gives the observed frequencies

Round/Yellow	Wrinkled/Yellow	Round/Green	Wrinkled/Green
56	19	17	8

1. *What are the expected proportions?*

- Remember that our expected ratio is 9:3:3:1. We can use this to calculate the expected proportions.

Round/Yellow	Wrinkled/Yellow	Round/Green	Wrinkled/Green
$\frac{9}{9+3+3+1} = 0.5625$	$\frac{3}{9+3+3+1} = 0.1875$	$\frac{3}{9+3+3+1} = 0.1875$	$\frac{1}{9+3+3+1} = 0.0625$

2. *What are the expected frequencies?*

- To calculate the expected frequencies, we first need to calculate the number of individuals in the experiment.
- The total number of individuals in the experiment is $56 + 19 + 17 + 8 = 100$.

- We can then multiply the expected proportions (which we calculated in the previous question) by the total number of observed individuals to yield the expected frequencies.

Round/Yellow	Wrinkled/Yellow	Round/Green	Wrinkled/Green
$0.5625 \times 100 = 56.25$	$0.1875 \times 100 = 18.75$	$0.1875 \times 100 = 18.75$	$0.0625 \times 100 = 6.25$

3. What is the χ^2 value?

- We can use the following equation to calculate the χ^2 value: $\chi^2 = \sum_{i=1}^n \frac{(O_i - E_i)^2}{E_i}$.
- In this equation, O_i is the observed frequency for the i 'th combination and E_i is the expected frequency for the i 'th combination.
- We can sub in our observed and expected frequencies into the equation, and calculate the χ^2 value.

$$\begin{aligned}\chi^2 &= \frac{(56 - 56.25)^2}{56.25} + \frac{(19 - 18.75)^2}{18.75} + \frac{(17 - 18.75)^2}{18.75} + \frac{(8 - 6.25)^2}{6.25} \\ \chi^2 &= 0.001 + 0.003 + 0.163 + 0.49 \\ \chi^2 &= 0.657\end{aligned}$$

4. What are the degrees of freedom?

- To help understand how we calculate the degrees of freedom (df) let us first think about our data. We have individuals with combinations of different shape (round vs. wrinkled) and colors (green vs. yellow). We can think of shape and colors as different variables along which individuals can vary. We can use this equation to calculate the degrees of freedom.

$$\begin{aligned}df &= (\text{number of groups in 1st variable} - 1) \times (\text{number of groups in 2nd variable} - 1) \\ df &= (\text{number shapes} - 1) \times (\text{number colors} - 1) \\ df &= (2 - 1) \times (2 - 1) \\ df &= 1\end{aligned}$$

- **If this doesn't make sense, the example in second exercise is easier to understand.**

5. What is the associated p -value?

- Given that we know the χ^2 value is 0.657 and the degrees of freedom is 1, we can calculate the p -value using the following R code, where **x** is the χ^2 and **d** is the degrees of freedom (df).

```
pchisq(x, df=d, lower.tail=FALSE)
```

- We can sub our values into this code, and calculate the p-value.

```
pchisq(0.657, df=1, lower.tail=FALSE)
```

- $P = 0.4176211$

6. *Can you reject the null hypothesis?*

No, given that the p-value is much greater than 0.05, we cannot reject the null hypothesis.

Exercise 2

Here we have a contingency table for case and control individuals genotyped at a diallelic marker locus.

Affection status	AA	AB	BB
Case	23	47	30
Control	12	40	48

1. *Calculate the expected values for each cell?*

- First we need to propose a null hypothesis. Here, our null hypothesis will be that each outcome (eg. Case/AA) has an equal probability of occurring. Since there are six possible outcomes and our null hypothesis is that each outcome has an equal chance of occurring, each outcome has a probability of $\frac{1}{6}$.
- We can express these expected probabilities using a contingency table.

Affection status	AA	AB	BB
Case	$\frac{1}{6} = 0.1667$	$\frac{1}{6} = 0.1667$	$\frac{1}{6} = 0.1667$
Control	$\frac{1}{6} = 0.1667$	$\frac{1}{6} = 0.1667$	$\frac{1}{6} = 0.1667$

- We can then multiply these expected probabilities by the number of replicates in the experiment ($23 + 46 + 30 + 12 + 40 + 45 = 196$) to calculate the expected frequencies

Affection status	AA	AB	BB
Case	$0.167 \times 196 = 32.732$	$0.167 \times 196 = 32.732$	$0.167 \times 196 = 32.732$
Control	$0.167 \times 196 = 32.732$	$0.167 \times 196 = 32.732$	$0.167 \times 196 = 32.732$

2. Perform a χ^2 test on those values.

- Let's refresh our memory on what our observed frequencies are.

Affection status	AA	AB	BB
Case	23	47	30
Control	12	40	48

- Also, let's refresh our memory on what our expected frequencies are.

Affection status	AA	AB	BB
Case	32.732	32.732	32.732
Control	32.732	32.732	32.732

- We can calculate the χ^2 value using this equation $\chi^2 = \sum_{i=1}^n \frac{(O_i - E_i)^2}{E_i}$.
- We can then calculate the χ^2 value by subbing our values into the equation.

$$\begin{aligned}\chi^2 &= \sum_{i=1}^n \frac{(O_i - E_i)^2}{E_i} \\ \chi^2 &= \frac{(23 - 32.732)^2}{32.732} + \frac{(47 - 32.732)^2}{32.732} + \frac{(30 - 32.732)^2}{32.732} + \frac{(12 - 32.732)^2}{32.732} + \\ &\quad \frac{(40 - 32.732)^2}{32.732} + \frac{(48 - 32.732)^2}{32.732} \\ \chi^2 &= 2.894 + 6.219 + 0.228 + 13.131 + 1.614 + 7.122 \\ \chi^2 &= 31.208\end{aligned}$$

3. Find the associated p-value.

- First we need to calculate the degrees of freedom. Since our data is in a contingency table we can do this with **one simple trick**.

$$\begin{aligned}df &= (\text{number of rows} - 1) \times (\text{number of columns} - 1) \\ df &= (2 - 1) \times (3 - 1) \\ df &= 2\end{aligned}$$

- Now that we have the degrees of freedom ($df = 2$) and our χ^2 statistic (31.208) which we calculated earlier, we can calculate the p-value using the following R code.

```
pchisq(31.208, df=2, lower.tail=FALSE)
```

$$P = 1.6721256 \times 10^{-7}$$

$$P < 0.001$$

4. Can you reject the null hypothesis at $\alpha = 0.05$?

- Yes. Yes, we can.

Exercise 3

1. *Are extinction events, as observed in the fossil record, random in time, or do they have cluster (eg. mass extinctions)? In other words, do species go extinct at random intervals, or species tend to go extinct at the same time? To answer this question, use χ^2 to measure the “fit” of the probability model to the data. Significant lack of fit implies rejection of the null hypothesis of “no departure from the model”.*

- Here we have a table that shows the number of time intervals between extinction events. The “number of extinctions” column contains the index (starting at zero) of the extinction (eg. the first extinction has a zero, the second has a one, etc). The “number of time intervals” describes the amount of time that has passed between consecutive extinctions (using a standardised unit of time).

Number of extinctions	Number of Time Intervals
0	0
1	13
2	15
3	16
4	7
5	10
6	4
7	2
8	1
9	2
10	1
11	1
12	0

Number of extinctions	Number of Time Intervals
13	0
14	1
15	0
16	2
17	0
18	0
19	0
20	1

- We will use the Poisson distribution to express our null hypothesis. An expectation (or prediction) from the Poisson distribution can be calculated using the equation $Pr\{Y = k|\mu\} = \frac{e^{-\mu}\mu^k}{k!}$ assuming a given value for μ . This expectation is our expected probability. We can then multiply $Pr\{Y = k|\mu\}$ by the total amount of observed extinction intervals (76) to calculate an expected frequency.
- A different μ value will give us a different expectation for the same input. For example, if we wanted to create an expectation for 5 extinctions, we could chose $\mu = 4$ and this would give us an expected probability of $Pr\{Y = 5|\mu = 4\} = \frac{e^{-4}4^5}{5!} = \frac{0.018 \times 1024}{5 \times 4 \times 3 \times 2 \times 1} = 0.154$. We can then use this to calculate the expected frequency $0.154 \times 76 = 11.704$. Alternatively, if we used $\mu = 8$ we would have an expected probability of $Pr\{Y = 5|\mu = 8\} = \frac{e^{-8}8^5}{5!} = 0.091$ and an expected frequency of $0.091 \times 76 = 6.916$. We can see that with an observed value of 10 for 5, using $\mu = 5$ gives a much closer estimate. So if we want to create a good model for our data using this distribution, we need to pick a good value for μ .
- We will use the following equation to give us a decent μ value, where T_i is the i 'th extinction interval.

$$\mu = \frac{\sum_{i=1}^N (i-1)T_i}{\sum_{i=1}^N T_i}$$

$$\mu = \frac{(0 \times 0) + (1 \times 13) + (2 \times 15) + (3 \times 16) + (4 \times 7) \dots}{0 + 13 + 15 + 16 + 7 \dots}$$

$$\mu = 4.21$$

- Note that if we were feeling particularly clever, we could optimise our μ value to give us the best possible model assuming a Poisson distribution.
- Let's make a quick plot to see how our model compares to the data.

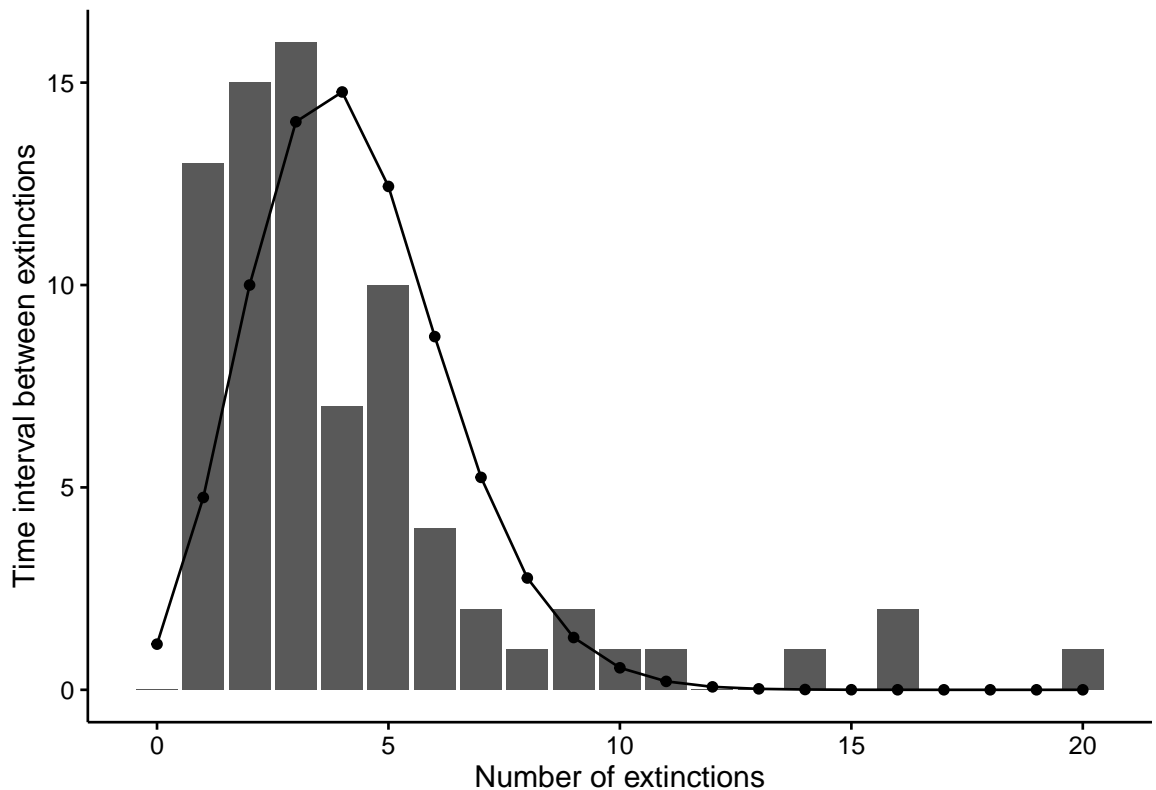
```
# store data in data.frame
ext.DF=data.frame(n.extinctions=0:20,
  n.intervals=c(0,13,15,16,7,10,4,2,1,2,1,1,0,0,1,0,2,0,0,0,1))
```

```

# make column with prediction
ext.DF$predicted.probs <- dpois(ext.DF$n.extinctions, 4.21)
ext.DF$predicted.freqs <- ext.DF$predicted.probs * sum(ext.DF[[2]])

# make plot
library(ggplot2)
ggplot(data=ext.DF) +
  geom_bar(aes(x=n.extinctions, y=n.intervals), stat='identity') +
  geom_line(aes(x=n.extinctions, y=predicted.freqs)) +
  geom_point(aes(x=n.extinctions, y=predicted.freqs)) +
  theme_classic() +
  xlab('Number of extinctions') +
  ylab('Time interval between extinctions')

```



- Now that we can see our data. Let's check to see if the Poisson is appropriate here. Some general rules of thumb for the Poisson distribution are:
 - No expected frequencies should be less than 1.
 - No more than 20% of the expected frequencies should be less than 5.
- **The data breaks one of our rules of thumb for the Poisson distribution.** So, we could either transform the data so that it obeys these rules or pick a different distribution (eg. negative binomial). Here, we will group the data together so that it obeys these rules. Note that the system of grouping we apply can affect the p-value we calculate at

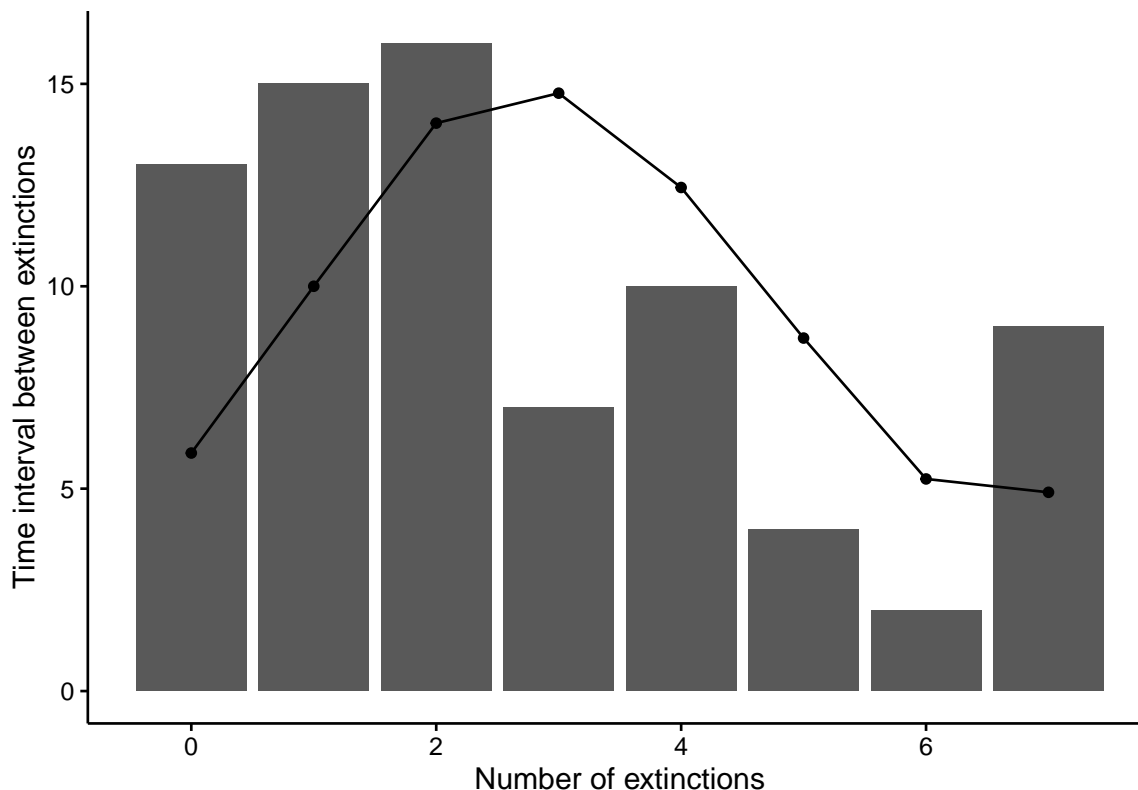
the end. So if you have to group your own data, you should think about how to sensibly group the data. The table below shows the groupings we will use.

Number of extinctions	Number of Time Intervals	Predicted Time Intervals
0 or 1	13	5.88
2	15	10
3	16	14.03
4	7	14.77
5	10	12.44
6	4	8.72
7	2	5.24
≥ 8	9	4.91

- Now, let's plot the grouped data.

```
# store data in data.frame
ext2.DF=data.frame(n.extinctions=0:7,
  n.extinctions.label=c('0 or 1', 2:7, '8+'),
  n.intervals=c(13,15,16,7,10,4,2,9),
  predicted.freqs=c(5.88,10,14.03,14.77,12.44,8.72,5.24,4.91))

ggplot(data=ext2.DF) +
  geom_bar(aes(x=n.extinctions, y=n.intervals), stat='identity') +
  geom_line(aes(x=n.extinctions, y=predicted.freqs)) +
  geom_point(aes(x=n.extinctions, y=predicted.freqs)) +
  theme_classic() +
  xlab('Number of extinctions') +
  ylab('Time interval between extinctions')
```

- Now, let's use the χ^2 test to see if our model adequately describes the data, and in turn, see if extinctions cluster in time.
 - First we will have to calculate our test statistic.

$$\chi^2 = \sum_{i=1}^n \frac{(O_i - E_i)^2}{E_i}$$

$$\chi^2 = \frac{13 - 5.88}{5.88} + \frac{15 - 10}{10} + \frac{16 - 14.03}{14.03} + \frac{7 - 14.77}{14.77} +$$

$$\frac{10 - 12.44}{12.44} + \frac{4 - 8.72}{8.72} + \frac{2 - 5.24}{5.24} + \frac{9 - 4.91}{4.91}$$

$$\chi^2 = 23.929$$

- Second, we will need to calculate the degrees of freedom (df). This can be calculated as the number of samples in our grouped data (8) minus 2. Thus our $df = 6$.
- Finally, at long last, we can calculate our p-value using the R code.

```
pchisq(23.929, df=6, lower.tail=FALSE)
```

- Our p-value is 0.0005382. Therefore our model does not fit the data very well, and we cannot reject the null hypothesis. Thus we do not yet have evidence to suggest that extinctions cluster in time.

R practical session