5.7 The chi-squared (χ^2) test

Using χ^2 to test the significance of the difference between observed and expected results

The **chi-squared test** (χ^2 test) is an inferential statistical test which is used to test the **significance** of the difference between expected results and actual observed results. The significance essentially means testing to see if the difference between these two values is small enough to be due to chance alone.

The χ^2 test may be used when a strong, evidence-supported biological theory has been used to generate the expected figures, and when categorical data has been collected. It relies on large sample sizes, also: small tests will not produce a reliable result.

Mendel's pea plants

The 'founding father' of modern genetics, Gregor Mendel, conducted his own crosses of pea plants. He crossed pure-breeding homozygous yellow (dominant) and round (also dominant) pea plants with pure-breeding homozygous green (recessive) and wrinkled (recessive) pea plants. You may be familiar with the cross from 5.6 Genetics.

The box to the right shows the biological explanation for Mendel's dihybrid cross of these two plants. The outcome expected phenotypic ratio is 9:3:3:1, a classic dihybrid cross result.

Firstly, we need some results from an investigation to test. Imagine an experiment just like that in the box to the right was done: two pea plants, each pure-breeding homozygous, were crossed to produce offspring all of **YyRr** and then the F_1 generation were bred to see the proportion of each phenotype observed. Theoretically, there should be the 9:3:3:1 ratio in this experiment.

In the investigation:

- 288 pea plants were grown in the F_2 generation
- in that F_2 generation, the following phenotypes were observed:
  - 169 plants were yellow and round
  - 54 plants were yellow and wrinkled
  - 51 plants were green and round
  - 14 plants were green and wrinkled

This appears fairly close to the 9:3:3:1 prediction. The **expected values** for each phenotype (with a sample size of 288) should have been 162, 54, 54 and 18 respectively, by that ratio. We can now use the χ^2 test to test the significance of the difference.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Note, you do not have to know this formula. If you have a question on this in the exam, it will be given to you

Class	Observed, O	Expected, E	$O-E$	$(O-E)^2$	$\frac{(O-E)^2}{E}$
Yellow round	169	162	7	49	0.30
Yellow wrinkled	54	54	0	0	0.00
Green round	51	54	-3	9	0.17
Green wrinkled	14	18	-4	16	0.88
					χ^2 = **1.35**

Mendel's pea plant dihybrid cross

Y = yellow pea plant colour, **y** = green
R = round pea plants, **r** = wrinkled plants

Parental phenotypes and genotypes:

yellow, round — **YYRR** → meiosis → *Gametes*: YR, YR
green, wrinkled — **yyrr** → meiosis → *Gametes*: yr, yr

F_1 offspring: YyRr

Second generation, parental phenotypes:

yellow, round — **YyRr** → meiosis → YR, Yr, yR, yr
yellow, round — **YyRr** → meiosis → YR, Yr, yR, yr

F_2 offspring:

♀ gametes \ ♂ gametes	YR	Yr	yR	yr
YR	YYRR	YYRr	YyRR	YyRr
Yr	YYRr	YYrr	YyRr	Yyrr
yR	YyRR	YyRr	yyRR	yyRr
yr	YyRr	Yyrr	yyRr	yyrr

F_2 phenotypes:
9 yellow and round
3 yellow and wrinkled
3 green and round
1 green and wrinkled

The bigger the value of χ^2 the more likely it is that there is a significant difference between the observed values and expected values, suggesting that the **experimental hypothesis** will need to be accepted, and vice versa, the smaller the value of χ^2 the less likely it is that there is a significant difference, and so any difference there is will be down to chance. This would mean that the experimental hypothesis can be rejected, and the **null hypothesis** accepted.

Number of classes	**Degrees of freedom**	**χ^2**							
2	1	0.00	0.10	0.45	1.32	2.71	3.84	5.41	6.64
3	2	0.02	0.58	1.39	2.77	4.61	5.99	7.82	9.21
4	3	0.12	1.21	2.37	4.11	6.25	7.82	9.84	11.34
5	4	0.30	1.92	3.36	5.39	7.78	9.49	11.67	13.28
6	5	0.55	2.67	4.35	6.63	9.24	11.07	13.39	15.09
Probability that variation is due to chance alone, *p*=		0.99 (99%)	0.75 (75%)	0.50 (50%)	0.25 (25%)	0.10 (10%)	0.05 (5%)	0.02 (2%)	0.01 (1%)

Accept null hypothesis (any difference is due to chance and not significant)

Critical value of χ^2 where *p*=0.05, the level at which we are 95% certain that the result is not due to chance

Reject null hypothesis, accept experimental hypothesis (difference is significant and not due to chance)

The **degrees of freedom** are always the number of categories, less one. In this case there are four classes, so the *Df* will be 3. The **critical value** found using the probability chosen to accept that the results are not due to chance, and that there is no significance in differences observed. Usually *p*=0.05 will be chosen for this.

We can see that for 0.05 the critical value for 3 degrees of freedom is 7.82.

With our χ^2 value of 1.35, we can tell immediately that this is much less than 7.82, so we can conclude that the difference between the observed values and expected values was not significant. The results are consistent with the Mendelian genetic explanation, and the null hypothesis can be accepted.