

QUESTION

16.23 Pure-breeding *Drosophila* with straight wings and grey bodies were crossed with pure-breeding curled-wing, ebony-bodied flies. All the offspring were straight-winged and grey-bodied. Female offspring were then test crossed with curled-wing, ebony-bodied males, giving the following results:

straight wing, grey body 113
 straight wing, ebony body 30
 curled wing, grey body 29
 curled wing, ebony body 115

- State the ratio of phenotypes expected in a dihybrid test cross such as this.
- Explain the discrepancy between the expected result and the results given.
- Calculate the cross over value.
- Is the curled wing locus closer to the ebony locus than is the aristopedia locus? Explain your answer.

us imagine that the two plants produced a total of 144 offspring. If the parents really were both heterozygous, and if the purple stem and cut leaf alleles really are dominant, and if the alleles really do assort independently, then we would expect the following numbers of each phenotype to be present in the offspring:

purple, cut = $\frac{9}{16} \times 144 = 81$
 purple, potato = $\frac{3}{16} \times 144 = 27$
 green, cut = $\frac{3}{16} \times 144 = 27$
 green, potato = $\frac{1}{16} \times 144 = 9$

But imagine that, among these 144 offspring, the results we actually observed were as follows:

purple, cut 86 green, cut 24
 purple, potato 26 green, potato 8

We might ask: are these results sufficiently close to the ones we expected that the differences between them have probably just arisen by chance, or are they so different that something unexpected must be going on?

To answer this question, we can use a statistical test called the **chi-squared (χ^2) test**. This test allows us to compare our observed results with the expected results, and decide whether or not there is a significant difference between them.

The first stage in carrying out this test is to work out the expected results, as we have already done. These, and the observed results, are then recorded in a table like the one below. We then calculate the difference between each set of results, and square each difference. (Squaring gets rid of any minus signs – it is irrelevant whether the differences are negative or positive.) Then we divide each squared difference by the expected value, and add up all of these answers:

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

where: Σ = sum of
 O = observed value
 E = expected value

Phenotypes of plants	purple stems, cut leaves	purple stems, potato leaves	green stems, cut leaves	green stems, potato leaves
Observed number (O)	86	26	24	8
Expected ratio	9	3	3	1
Expected number (E)	81	27	27	9
$O - E$	+5	-1	-3	-1
$(O - E)^2$	25	1	9	1
$(O - E)^2/E$	0.31	0.04	0.33	0.11
$\chi^2 = \sum \frac{(O - E)^2}{E} = 0.79$				

The χ^2 (chi-squared) test

If you look back at the cross between the two heterozygous tomato plants on pages 380–381, you will see that we would expect to see a 9:3:3:1 ratio of phenotypes in the offspring. It is important to remember that this ratio represents the **probability** of getting these phenotypes, and we would probably be rather surprised if the numbers came out absolutely precisely to this ratio.

But just how much difference might we be happy with, before we began to worry that perhaps the situation was not quite what we had thought? For example, let

So now we have our value of χ^2 . Next we have to work out what it means. To do this, we look in a table that relates χ^2 values to probabilities (Table 16.3). The probabilities given in the table are the probabilities that the differences between our expected and observed results are due to chance.

For example, a probability of 0.05 means that we would expect these differences to occur in five out of every 100 experiments, or one in 20, just by chance. A probability of 0.01 means that we would expect these differences to occur in one out of every 100 experiments, just by chance.

In biological experiments, we usually take a probability of 0.05 as being the critical one. If our χ^2 value represents a probability of 0.05 or larger, then we can be fairly certain that the differences between our observed and expected results are due to chance – the differences between them are **not significant**. However, if the probability is smaller than 0.05, then it is likely that the difference is significant, and we must reconsider our assumptions about what was going on in this cross.

There is one more aspect of our results to consider before we can look up our value of χ^2 in Table 16.3. This is the number of degrees of freedom in our results. The degrees of freedom take into account the number of comparisons made. (Remember that to get our value for χ^2 , we added up all our calculated values, so obviously the larger the number of observed and expected values we have, the larger χ^2 is likely to be. We need to compensate for this.) To work out the number of degrees of freedom, simply calculate the number of classes of data minus 1. Here we have four classes of data (the four possible sets of phenotypes), so the degrees of freedom are: $4 - 1 = 3$.

Now, at last, we can look at Table 16.3 to determine whether our results show a significant deviation from what we expected. The numbers in the body of the table are χ^2 values. We look at the third row in the table (because that is the one relevant to 3 degrees of freedom), and find the χ^2 value that represents a probability of 0.05. You can see that this is 7.82. Our calculated value of χ^2 was 0.79. So our value is a much, much smaller value than the one we have read from the table. In fact, we cannot find anything like this number in the table – it would be way off the left-hand side, representing a probability of much more than 0.1 (1 in 10) that the difference in our results is just due to chance. So we can say that the difference between our observed and expected results is almost certainly due to chance, and there is **no significant difference** between what we expected and what we actually got.

Degrees of freedom	Probability greater than			
	0.1	0.05	0.01	0.001
1	2.71	3.84	6.64	10.83
2	4.60	5.99	9.21	13.82
3	6.25	7.82	11.34	16.27
4	7.78	9.49	13.28	18.46

Table 16.3 Table of χ^2 values.

QUESTION

16.24 Look back at your answer to Question 16.18b. In the actual crosses between the animals in this generation, the numbers of each phenotype obtained in the offspring were:

grey, long	54
grey, short	4
white, long	4
white, short	18

Use a χ^2 test to determine whether or not the difference between these observed results and the expected results is significant.

Mutations

You have seen that most genes have several different variants, called alleles. A gene is made up of a sequence of nucleotides, each with its own base. The different alleles of a gene contain slightly different sequences of bases.

These different alleles originally arose by a process called **mutation**. Mutation is an unpredictable change in the genetic material of an organism. A change in the structure of a DNA molecule, producing a different allele of a gene, is a **gene mutation**. Mutations may also cause changes in the structure or number of whole chromosomes in a cell, in which case they are known as **chromosome mutations** (or chromosome aberrations).

Mutations may occur completely randomly, with no obvious cause. However, there are several environmental factors that significantly increase the chances of a mutation occurring. All types of ionising radiation (alpha, beta and gamma radiation) can damage DNA molecules, altering the structure of the bases within them. Ultraviolet radiation has a similar effect, as do many chemicals – for example, mustard gas. A substance that increases the chances of mutation occurring is said to be a **mutagen**.