Model Based Statistics in Biology.

Part II. Quantifying Uncertainty.

Chapter 7.6 Goodness of fit tests for count data

ReCap. Part I (Chapters 1,2,3,4)
ReCap Part II (Ch 5, 6)
7.0 Statistical Inference
7.1 Three modes of inference
7.2 Inference with Empirical Distributions
7.3 Inference with Probability Models
7.4 Parameter Estimates
7.5 Confidence Limits
7.6 Goodness of fit to count data
Chisquare statistic
G Statistic
Extrinsic hypothesis: Mendelian Ratios
Intrinsic hypothesis: Two-way Contingency Test

Data: Ch16.xls data.

ReCap Likelihood inference yields a measure of strength of evidence. Frequentist inference leads to a decision against a likelihood ratio of 1 (no difference).

Today: Goodness of fit tests

Wrap-up.

Count data are analyzed with goodness of fit tests using either the traditional χ^2 statistic or the *G*-statistic (twice the log of a likelihood ratio)

Goodness of fit tests are used to compare an estimate to a theoretical value, such as a Mendelian ratio.

Goodness of fit tests are used to compare two proportions (row by column contingency test).

Goodness of fit tests can be used to compare an observed frequency distribution to a probability model.

Goodness of fit to count data- the Chisquare statistic.
Count data is common in the life and environmental sciences. Count data are bounded at zero and so we cannot rely on a normal error model to test hypotheses. Instead we will use a normal error model and a $\chi2$ distribution to test hypotheses. We'll begin with the traditional Chisquare statistic. We'll then move to its modern equivalent, the *G*-statistic.

Example: Gregor Mendel (1822-1884), a scientist and Augustinian friar, was one of the founders of modern genetics. He crossed a pure strain of purple flowered pea plants with a pure strain of white flowered plants, to obtain F1 hybrids. He then crossed the F1 hybrids with other F1 hybrids. In one experiment he scored 929 plants as having either white or purple flowers. Does the observed proportion differ from the 3:1 proportion expected in the F2 offspring of the F1 hybrids?

To test data against genetic theory, we calculate the Chisquare statistic X^2, which is defined as the squared difference between the observed and expected value, divided by the expected value, then summed across classes. The X^2 statistic increases as the difference between the observed and expected value decreases toward zero (perfect fit).

The reason for the 3:1 ratio is one of the major ideas in biology. If you have forgotten the concept, or never took a biology course, the idea is easily looked up and easily grasped because you, like Mendel's pea plants, inherit genes from two parents.

	Observed	Expected		Difference^2/Expected		
Purple	705	929*(3/4)	= 696.75	$(-8.25)^2$ / 696.75	=	0.097686
White	224	929*(1/4)	= 232.25	$(+8.25)^2$ / 232.25	=	0.29306
Total	929					0.3907 = X^2

Following convention, we write the Chisquare statistic as X^2 and so distinguish the statistic from the Chisquare distribution denoted by a greek letter as χ^2. We use the χ^2 distribution to evaluate whether a poor fit (large X^2) is too large to be attributed to chance at a pre-set decision criterion, such as $\alpha = 5\%$. The χ^2 distribution, like the *t*- and *F*-distribution, depends on the degrees of freedom.

The X^2 statistic, divided by its degrees of freedom, is a measure of fit similar to the mean squared error MSE used in an ANOVA table.

$$\text{MSE} = \text{SS}_{\text{err}}/\text{df}_{\text{err}} = \text{MS}_{\text{err}}$$
$$\text{MSE} = \text{Var(res)} = \text{Var(Obs - Exp)}.$$

Goodness of fit to theory - The Chisquare statistic.

We use the χ^2 distribution with the appropriate degrees of freedom to compute the Type I error (p-value) when concluding that the observed ratio differs from genetic theory.

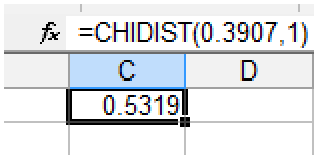
If we could obtain a large number of repetitions of this experiment, would the value of X^2 = 0.3907 be too large to be probable?
The probability of this large a value of X^2 by chance alone is

$p = 1 - 0.4681 = 0.5319$

```
MTB > cdf 0.3907;
SUBC> chisquare 1.
      0.3907   0.4681
```

```
R/S+    > pchisq(0.3907,1)
        [1] 0.4680683
```

Excel



=CHIDIST(0.3907,1)

0.5319

```
Query:  Why 1 df ?
Answer: Once we estimate the proportion of one type
(e.g. purple) we can calculate the proportion of
the other type from the overall proportion of
seeds. We have no degrees of freedom on this
proportion
```

We conclude that the deviation of the data from the 3:1 genetic model cannot be rejected at the conventional criterion of $\alpha = 5\%$.
The difference between the observed ratio of mutant to wild type offspring (705:224) and the theoretically expected value (3 : 1) is due to chance.

In the early 20th century the geneticist W.F.R. Weldon critiqued Mendel's findings as overly simple based on pure strains (purple and white flowers), and improbably good (1902 Biometrika 1, 228-233). R.A. Fisher (1936 Ann. Sci.1, 115) arrived at a similar conclusion based on goodness off fit—improbably good. Fisher argued that Mendel had worked out his theory and that the experiments reported were "a carefully planned demonstration of his conclusions." The so-called Mendel-Fisher controversy emerged later, when Mendel's experiments were construed as an early example of scientific fraud. Improbably good is, however, no evidence of fraud and there is no other evidence of fraud (D. L. Hartl, D. J. Fairbanks 2007 Genetics 175, 975). Weldon's critique remains—Mendel's simple categories omit the interactive effect of environment on genetic expression. Neverthelss, Mendel's simple categories, with their deterministic tilt, remain central in genetics education. For many traits the variance in trait is continuous, where the interactive effects of gene and environment are at least as large as the genetic and the environmental taken separately. We'll see this concept of interactive effects again and again when we look at multiple explanatory variables later in the course.

Goodness of fit to theory. The *G*-statistic

Another measure of goodness of fit is the likelihood ratio chisquare, written either as *G* or as G^2. The *G*-statistic is based on the solid theoretical underpinning of likelihood (Fisher 1935) , which considers the likelihood of the model given of the data.

Unlike the Pearsonian Chisquare statistic that we just computed, the *G*-statistic can be used in complex analyses involving several explanatory variables. The *G*-statistic allows us to compute the improvement in fit of one model relative to another, in complex as well as simple models. It allows us to compare the likelihoods of any two models, using any probability model (Normal, Binomial, *etc*).

The *G* statistic addresses the question "how likely is a parameter, given the data?" For Mendel's pea data we ask " how likely is an observed ratio of 705/224 = 3.15 : 1, compared to a Mendelian ratio of 3:1 purple to white peas?

The likelihood ratio given 705 purple peas is

$$LR_1 = \left(\frac{705/929}{696.75/929}\right)^{705}$$

The likelihood ratio given 224 white peas is

$$L_2 = \left(\frac{224/929}{232.25/929}\right)^{224}$$

In symbolic form the likelihood ratio is $LR = \left(\frac{observed}{expected}\right)^{observed} = \left(\frac{f}{\hat{f}}\right)^{f}$

For all the observed values the likelihood is:

$$LR_{total} = LR_1 \cdot LR_2 \cdot LR_3 \cdot LR_4 \ldots$$

When the fit is perfect ($f\ /\ \hat{f} = 1$) the likelihood ratio becomes *LR*= 1.

Taking the logarithm of both sides will give us a sum to work with, rather than a product. When the fit is perfect ($\ln(f\ /\ \hat{f}) = 0$) the log likelihood ratio is ln*LR* = 0.

$$\ln LR_{total} = \sum\left(observed \cdot \ln\left(\frac{observed}{expected}\right)\right) \qquad \ln LR_{total} = \sum\left(f \cdot \ln\left(\frac{f}{\hat{f}}\right)\right)$$

The *G*-statistic is twice the log-likelihood ratio: *G* = 2ln*LR*

Goodness of fit to theory. The *G*-statistic

Here is the calculation of the *G*-statistic for the pea flower data.
The observed frequency f_i has two values, 705 and 224. The expected frequency from a 3:1 theory is $\hat{f}_i = p_i \cdot N$. It has two value ¾ *N* and ¼ *N*.

	Observed	Expected		$f * \ln(f / \hat{f})$		
✿ Purple	705	929*(3/4)	= 696.75	705*ln(705/696.75)	=	+8.29865
❀ White	224	929*(1/4)	= 232.25	224*ln(224/232.25)	=	- 8.1017
Total	929					+0.1969
				$G^2 = 2\Sigma\ f \ln(f / \hat{f})$	=	+0.394

The evidential support for the data relative to theory is 0.1969, which translates into a likelihood ratio of exp(0.1969) = 1.2, a value well short of LR > 20. The Mendelian ratio of 3:1 is just as likely as the observed ratio: 705/224 = 3.147.

Goodness of fit tests take this result further by calculating the long run probability of this level of evidential support, if we were to repeat the experiment a very large number of times. Either the X^2 statistic or the *G* statistic are used because both are distributed according to the $\chi 2$ probability model.
Often, but not always, the *G*-statistic will be similar in value to the Chisquare statistic. For the Mendel pea data $X^2 = 0.391$ and $G = 0.394$.

G uses the ratio of the observed to fitted values (likelihood ratio). In contrast, the Pearsonian Chisquare statistic uses the squared deviations of the differences between observed and expected values.

The likelihood ratio *LR* or the support ln*LR* are measures of the evidence.
A *p*-value is used to make a decision; it is not a measure of evidence.

Goodness of fit to theory - Likelihood Ratio Test

We'll the the generic recipe for hypothesis testing in the sense of Fisher's interpretation of Mendel's data, as a demonstration of theory.

1. **Population.** All possible outcomes, if the same experiment was carried out repeatedly.
2. **Test statistic.** The statistic is *G*
3. **H₀**: $LR = 1$, $G = 0$ Data supports theory.
4. **H_A**: $LR > 1$, G > 0. Data does not support theory.
5. $\boldsymbol{\alpha} = \mathbf{5\%}$

6. State distribution.

To calculate the probability of the observed value of *G we* need a distribution of all possible outcomes. As always, we have two options. One is to generate a distribution of outcomes by randomly assigning each of the 929 plants to a phenotype (white or purple) by chance. We could do this by flipping a pair of coins: if the outcome is HeadsHeads, then offspring are assigned to the white type. If the outcome is anything else (HT TH or TT) offspring are assigned to the purple type. Obviously we will not obtain exactly the same assignment to the two phenotypes each time we assign the 929 offspring by chance. But if we make the assignment repeatedly (and calculate the *G* each time) then we will obtain a distribution of our *G*-statistic when the data do fit the model of a ratio of 3:1.

The other option is to use the χ^2 distribution. This is less work. We will use this because we know from statistical theory that if we have a binomial (yes/no, purple/white) outcome with probability of $p = 0.25$ successes in 929 independent trials, and we compute *G*, that the statistic will be distributed as χ^2.

7. Calculate statistic. $G = 0.394$ (above).

8. Calculate the p-value.

We have only one degree of freedom because once we compute the expected frequency of white flowers ($p \cdot N = 232.25$) the expected frequency of purple flowers will not be free to vary. It must be 929 - 232.25 = 669.75

The p-value from the χ^2 distribution is p = 1 - 0.4697 = 0.53

What about assumptions for computing p-values from chisquare distributions?
-We have too few residuals to undertake any diagnosis of homogeneity.
-We assume inheritance of flower color in one plant is independent of that of another. We can check the assumption of 929 independent measurements. This could be checked by looking for runs of white or purple flowers in the data, based on neighboring plants. A quick check, if neighbors are known, is to plot scores (0/1, y/n, present/absent etc) against neighbors.

If we found some serious problem we should do the experiment again, as randomization won't solve the problem of non-independent measurements.

9. Compare *p* to α to make decision.

Using the χ^2 distribution (df = 1), we calculate that
$53\% = p < \alpha - 5\%$

10. Report decision with statistic, sample size or df, and *p* value.

We cannot reject the hypothesis that the observed frequencies fit a 3:1 ratio.
$G = 0.394$ df = 1 $p = 0.53$

Goodness of Fit *G*. Intrinsic Hypothesis – Two-way contingency test.
Often we have no theory, such as a Mendelian ratio, to perform a test.
We can always construct an intrinsic hypothesis based on comparisons
The simplest is that one ratio (proportion) differs from another.

Example. Leaf Type.

Data are leaf type of 100 trees found in two soil types in a 400 square mile area. In this two-way table, where the total is fixed ($N = 100$) the statistic of interest is the cross product ratio.

Data from Sokal and Rohlf 2012, Box 17.6

	Pubescent	Smooth	
Serpentine	12	22	34
non-Serpentine	16	50	66
	28	72	100

a	c
b	d

Equal fractions a/b = c/d
Equal fractions a/c = b/d
CPRatio = (a/b) / (c/d) = 1
CPRatio = (a/c) / (b/d) = 1

12	22
16	50

CPRatio = (12/16) / (22/50) = 1.705
CPRatio = (12/22) / (16/50) = 1.705

Does the CPRatio differ from the expected value (null hypothesis) of 1?

1. **Population.** All possible outcomes, if the survey was carried out the same way repeatedly, in the same ecosystem.
2. **Test Statistic.** The statistic is *G*, the non-Pearsonian chisquare.
3. **H_o**: CPRatio = 1 $G = 0$
4. **H_A**: CPRatio ≠ 1 $G > 0$
5. **α = 5%**
6. **State distribution.**

We assume the results were from 100 independent trials, and use the χ^2 distribution to compute Type I error on rejecting the null hypothesis, a CPRatio of 1.

7. Calculate statistic. $G^2 = 1.332$
Texts show several ways of computing $G^2$for a contingency test.

Here is a calculation of the statistic based on proportions of smooth to pubescent (28:72) and serpentine to non-serpentine (34:66) to obtain the expected proportion and expected frequency in each of the 4 cells of the table. For Serpentine/Pubescent, the expected proportion is 100*(28/100)*(34/100)

Leaf*Soil	f	fhat	resid	f*ln(f/fhat
Serp/Pub	12	9.52	2.48	2.778
Serp/Smooth	22	24.5	-2.48	-2.350
NonS/Pub	16	18.5	-2.48	-2.306
NonS/Smooth	50	47.5	2.48	2.544
	100			0.666
			G^2 =	1.332

In a later chapter we will skip the laborious calculations and simply write the model, execute it, and obtain a table displaying the leaf type variability, the soil type variability, and the CPR (interactive) variability.

8. Calculate the Type I error (p-value).
The p-value from the chisquare distribution is

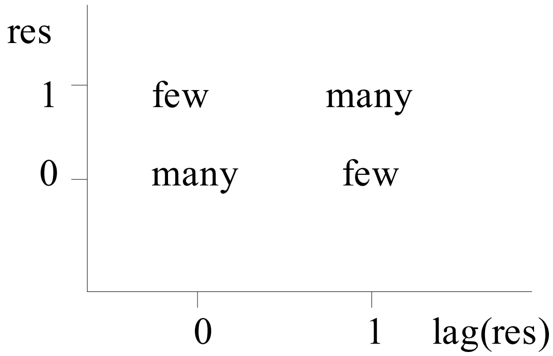
$$p = 1 - 0.752 = 0.248$$

Using the χ^2 distribution with $df = 1$, we calculate that if the data do indeed fit the model of equal proportions (CPRatio = 1), the probability of a G-statistic greater than 1.332 is 25% if we were to run the study many times.

What about assumptions for computing p-values from χ^2 distributions ?
We have too few residuals to undertake any diagnosis of homogeneity. We might be able to check the assumption of 100 independent trials, if we had the data sheets from this experiment. To check independence in the order that data was taken, we would look for runs of one leaf type in the data, based on neighboring observations, or perhaps neighbour *vs* distant trees.

A quick check is to plot scores (0/1, y/n, present/absent *etc*.) against neighbors.

If we found some serious problem we should do the experiment again.



9. Compare p to α to declare decision.

$0.25 = p > \alpha = 5\%$

The Type I error is too high to reject the "null" hypothesis, that CPRatio =1.

10. Report decision with statistic, sample size or df, and *p* value.

We cannot reject the hypothesis of equal proportions, CPRatio = 1.

$G = 1.332$, df = 1, $p = 0.25$

Extensions.

1. Goodman (1964) presents a simple method for computing the confidence limits on a cross-product ratio. Goodman, L.A. 1964. *Journal of the Royal Statistical Society. Series B (Methodological)* 26: 86-102.

Calculate the confidence limits for the leaf type data.
What cross-product ratios can you exclude, even though the null hypothesis cannot be excluded?

2. Set up a structured calculation (as in a spreadsheet) that allows you to calculate the p-value for the leaf type data, holding effect size (leaf type proportion and soil proportion) constant, while allowing sample size to change. What sample size would you need to be able to detect a change in proportion of CPRatio = 1.7 ? [This calculation is the minimum sample size to declare the observed effect size, CPRatio = 1.7, to be statistically significant]

3. The published literature shows G-statistics, which are less immediately interpretable than likelihood ratios. Given that $LR = e^{(G/2)}$ calculate likelihood ratios for the following test statistics. Interpret the resulting ratio in relative to the research question relative to $LR < 20$ for inadequate evidence.

G = 10.965. 80:10 ratio of wild to mutant genotypes, compared to 3:1 Mendelian ratio. Sokal and Rohlf 2012 Box 17.1

G = 1.478. Fit to Mendelian 9:3:3:1 ratio in a dihybird cross:
Tall vs Dwarf at 3:1, Cut-leaf versus Potato at 3:1.
Sokal and Rohlf 2012 Box 17.2 Data from MacArthur (1931).

G = 6.8718. Percent of trees invaded by ant colonies in two species of acacia.
Sokal and Rohlf 2012 Box 17.7