Model Based Statistics in Biology.

Part V. The Generalized Linear Model.

Chapter 18.6 Two categorical explanatory variables.

ReCap. Part I (Chapters 1,2,3,4), Part II (Ch 5, 6, 7)
ReCap Part III (Ch 9, 10, 11), Part IV (Ch 13, 14)
18 Binomial Response Variables
18.1 Logistic Regression (Dose-Response)
18.2 Single Factor. Prospective Analysis
18.3 Single Factor. Retrospective Analysis
18.4 Single Fixed Factor.
18.5 Single Explanatory Variable. Ordinal Scale.
18.6 Two Categorical Explanatory Variables
18.7 Logistic ANCOVA

Ch18.xls

on chalk board

ReCap Part I (Chapters 1,2,3,4) Quantitative reasoning
ReCap Part II (Chapters 5,6,7) Hypothesis testing and estimation
ReCap (Ch 9, 10,11) The General Linear Model with a single explanatory variable.
ReCap (Ch 12,13,14,15) GLM with more than one explanatory variable
ReCap (Ch 16,17). Generalized Linear Model. Poisson response variables.
ReCap. Response variables of interest in the natural and social sciences are often binomial: a series of trials (cases) that can be scored as yes/no, present/absent, *etc*.

We compared a binomial proportion as a function of a numeric variable (Ch 18.1)

We compared two proportions in both prospective (experimental) and retrospective (observational) analyses (Ch 18,2, 18.3)

Today: Comparison of binomial proportions in a two-way design.

Wrap-up. The generalized linear model permits us to apply what we have learned about two-way classification of data to the analysis of binomial response variables. We compared several proportions in a one way design.

We can extend this to other designs, such as
-more than nested classification variables
-more than one regression variable
-mixtures of classification and regression variables (ANCOVA)

Two-way classification of a binomial response variable.

Count data in two-way classifications have a long history in statistics, going back to contingency tests and goodness of fit tests in the early 20th century. These were generalized later in the century to log-linear models (Bishop et al 1978), recognizing that count data as proportions are multiplied, not added. In generalized linear model terms, we require a log link to compare proportions on a multiplicative scale.

Here is an example from Sokal and Rohlf (2012) Box 17.10, p760,
The data are counts of fruit flies in an experiment to test the effects of the insecticide DDT added to growth medium.

Does the proportion of flies that pupate depend on pupal site (in medium, on margin, on top of medium, away from medium on wall)?
Do male and female flies differ in pupation success?

```
Data a;
 Input Emerge Pupae Site $ Sex $;
  Cards;
   55 61 IM F
   34 51 IM M
   23 24 AM F
   15 20 AM M
    7 11 OW F
    3  8 OW M
    8 11 OM F
    5  8 OM M
;
```

Sokal and Rohlf analyzed this data as Poisson counts classified by 3 factors: sex, site, and healthy (pupated) or not (poisoned). The experiment appears to be cross-sectional (one point in time) in the 3-way classification of counts. They also analyzed it as a binomial response (pupation or not) versus two factors, site and sex. The data become longitudinal (prospective) if we take the time period as that from pupation to emergence. The failed and successful pupae together consist of the trials (beginning of pupation), which are then scored as healthy (emerging) or poisoned (not emerging). This assumes that flies that fail to emerge were poisoned by DDT, rather than failing to emerge because of some other factor.

1. Construct the Model

Verbal.   Survival of fruit flies depends on pupation site, sex, and interactive effects of these two factors.
Graphical   Plot of survival rate of flies relative to site and sex.
Response variable: Odds of survival (for binomial scoring of results)
Explanatory variables: pupation site and sex.

1. Construct the Model

Write formal model

$$Odds(Nemerge) \sim \text{Binomial}\ (Npupae,\ \pi)$$
$$Odds = e^{\eta} + \varepsilon$$
$$\eta = \beta_{intercept} + \beta_{Site}Site + \beta_{Sex}Sex + \beta_{Site\cdot Sex}Site \cdot Sex$$

$e^{\beta_{intercept}}$ = survival odds, reference group (male, on wall OW)

$e^{\beta_{site}}$ = odds ratio, other 3 sites relative to reference group (male, OW)
other sites are at margin AM, in medium IM, on medium OM

$e^{\beta_{sex}}$ = odds ratio, female (OW) relative to ref. group (male, OW)

$e^{\beta_{sex*site}}$ = odds ratio, female relative to male at other 3 sites (not OW)

$\ln Odds = \beta_{ref} + \beta_{site} + \beta_{sex} + \beta_{site*sex}$ The model is linear on a logarithmic scale.

ε = raw (working) residuals

The explanatory model is the same as that for a 2-way ANOVA.
The model has 8 data equations and 8 parameters. Hence the sum of the residuals will fall to zero after all 4 terms are estimated. This is called a saturated model. The model fits the data perfectly. This does not prevent us from evaluating the improvement in fit for each term.

2. Execute model.

The data are already in model format (see above).
We have 8 observations of 4 variables: Emerge Pupae Site Sex
The response variable is a proportion Nemerge/Npupae.
The explanatory variables are categorical.

To execute the model in a GzLM routine, we specify the error distribution (binomial), the link (logit), and the explanatory (structural) model.

Distribution $Odds \sim \text{Binomial}(N,\ \pi)$

$$\eta = \beta_{intercept} + \beta_{Site}{\cdot}Site + \beta_{Sex}{\cdot}Sex + \beta_{Site\cdot Sex}{\cdot}Site \cdot Sex$$

Link $Odds = e^{\eta}$

Binomial parameter π is estimated from the data.

```
Proc Genmod;
  classes Site Sex;
  model Emerge/Pupae = Site Sex Site*Sex/
  link=logit dist=binomial type1 type3;
```

SAS command file

3. Use residuals to evaluate straight line model and error model.

The residual error is zero in this saturated model so we cannot use residuals to evaluate assumptions for estimating Type I error from χ^2 distribution.

4. How good is the evidence?

Calculate the LR from the improvement in fit (ΔG) due to the explanatory variables.

The ANOVA table is replaced by the Analysis of Deviance table.
The ANODEV table shows the degrees of freedom for a sequence of models.
One degree of freedom is lost in fitting the intercept.
Three are lost in a model that includes *Site*
One is lost in fitting a model that includes *Sex*
Three are lost in fitting the interaction term.

ANODEV Table.

Source	df	Δdf	Deviance = *G*	Δ*G*
Intercept	7	1		
Site	4	3 = 4–1		
Sex	3	1		
Sex*Site	0	3 = 3*1		

The statistical package computes improvement in fit Δ*G*) as each term is added (site, sex, and finally site*sex).

```
                          LR Statistics For Type 1 Analysis

                                                          Chi-
            Source          Deviance         DF      Square     Pr > ChiSq

            Intercept        24.2976
            Site             15.3385          3
            Sex               1.3655          1
            Site*Sex          0.0000          3
```

SAS output

As each term is add the deviance decreases and the fit improves.
The fit of the data to the full model is perfect $G = 0.00$ (no deviance)
The improvement in fit for the omnibus model is 24.2976 on 7 degrees of freedom.
LR = 1.9 x 10^5
There is strong evidence for effects on DDT on pupation, so we proceed with analysis of each term, beginning with the interaction term.

5. Choose mode of inference.

This is an experiment and so we will infer first from the data to the model based on a binomial error, then infer from the model to a population.

Population. The sample is count of fruit flies emerging form pupae with DDT in the growth medium. Population to which inference is made is all possible outcomes, given the experimental protocol. We have no basis for inference to a population of enumerable units, such as a population of flies.

In this experiment, with a broad spectrum insecticide with known health and environmental risks, there are good reasons to control Type I error. For the experimenter investigating the behavior that affects mortality odds for flies, the risk of Type I error (false positive) is publication of an effect that is no more than due to chance.

6. State H_A / H_o pair, LR ranges, tolerance for Type I error

Site·Sex term. We begin with the interaction term, same as with two way ANOVA The research hypothesis H_A will be that the odds ratio for *Site·Sex* will differ from 1.

H_A: $\beta_{Site \cdot Sex} \neq 0$ $OR = e^{\beta_{site*sex}} \neq 1$

The null hypothesis H_o will be that the odds ratio for *Site·Sex* will be 1.

H_o: $\beta_{Site \cdot Sex} = 0$ $OR = e^{\beta_{site*sex}} = 1$

If the interaction term is not significant, then we continue by examining the main effects, site and sex.

Site term.

The research hypothesis will be that the odds ratios differs among sites.

H_A: $\beta_{Site} \neq 0$ $OR = e^{\beta_{site}} \neq 1$

The null hypothesis will be that the odds ratios do not differ among sites.

H_0: $\beta_{Site} = 0$ $OR = e^{\beta_{site}} = 1$

Sex term.

The research hypothesis will be that the odds differ for males and females.

H_A: $\beta_{Sex} \neq 0$ hence: $OR = e^{\beta_{sex}} \neq 1$

The null hypothesis will be that the odds do not differ for males and females.

H_0: $\beta_{Sex} = 0$ hence: $OR = e^{\beta_{sex}} = 1$

6. State H_A / H_o pair, LR ranges, tolerance for Type I error

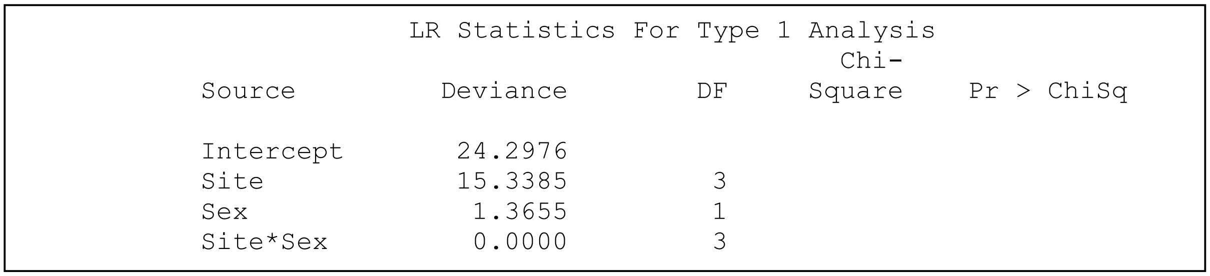
Statistic = ΔG where ΔG is the evidential support (improvement in fit)
for a research model compared to a simpler reference model.
For the analysis of deviance in this example the likelihood ratios are:
LR = likelihood(Model$_{\text{Site·Sex}}$ / Model$_{\text{intercept}}$)
LR = likelihood(Model$_{\text{Site}}$ / Model$_{\text{Site·Sex}}$)
LR = likelihood(Model$_{\text{Sex}}$ / Model$_{\text{Site}}$)$LR = e^{-\Delta G/2}$
ΔG= -2 ln(LR)

Weight of evidence criteria	$LR < 10$	Insufficient evidence
	$10 < LR < 20$	Some evidence
	$20 < LR < 100$	Good evidence
	$100 < LR$	Strong evidence

Probability distribution = Chisquare

Tolerance for Type I error. α = 5%

7. ANODEV - Improvement in fit (ΔG) due to each explanatory variables.



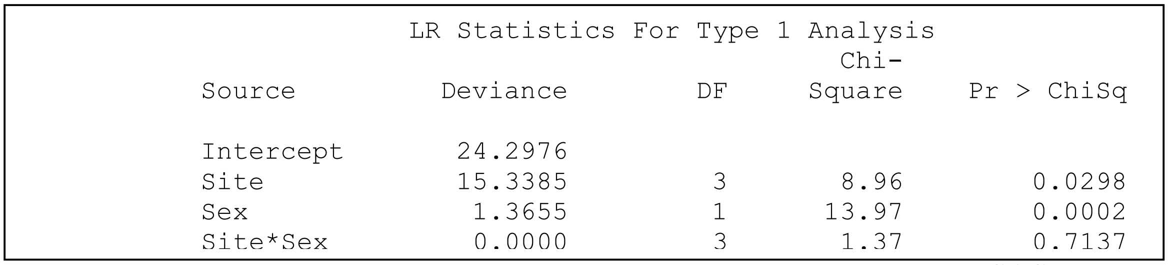
```
                         LR Statistics For Type 1 Analysis
                                                       Chi-
            Source          Deviance         DF      Square     Pr > ChiSq

            Intercept        24.2976
            Site             15.3385          3
            Sex               1.3655          1
            Site*Sex          0.0000          3
```

SAS output

As each term is add the deviance decreases and the fit improves.
The fit of the data to the full model is perfect G = 0.00 (no deviance)

Next we compute the improvement in fit due to each term. This is ΔG, the change in fit after a term is introduced.
The improvement in fit due to the interaction term: ΔG =1.3655 –0.0 = 1.37
The improvement in fit due to the sex term: ΔG =15.34 –1.37 = 13.97
The improvement in fit due to the site term: ΔG =24.298 –15.34 = 8.96

In the SAS output ΔG is labelled Chi-Square.



```
                         LR Statistics For Type 1 Analysis
                                                       Chi-
            Source          Deviance         DF      Square     Pr > ChiSq

            Intercept        24.2976
            Site             15.3385          3        8.96         0.0298
            Sex               1.3655          1       13.97         0.0002
            Site*Sex          0.0000          3        1.37         0.7137
```

SAS output

7. ANODEV. Improvement in fit (ΔG) due to each explanatory variables.

Note 1: The deviance for the interaction term based on the logistic model will differ from the G^2 statistic for the interaction term (model of equal proportions) based on Poisson response classified by 3 factors as shown in Box 17.10 of Sokal and Rohlf 2012

Note 2: Sequential (Type 1) versus adjusted (Type 3) analysis.
In looking at ANOVA tables we considered either Type 1 analysis or Type 3. With Type 1 partitioning, the table shows the SS due to each term in the order in which it is listed in the model. This contrasts with Type 3 (adjusted), where the SS is computed for each term controlled for all others (*i.e.* when it is listed last).

The same holds for the analysis of deviance.
The table above shows Type 1 analysis.
Here is the Type 3 rather than Type 1 (sequential) results.

```
                LR Statistics For Type 3 Analysis

                                      Chi-
          Source            DF      Square    Pr > ChiSq

          Site               3       10.97        0.0119
          Sex                1        8.14        0.0043
          Site*Sex           3        1.37        0.7137
```

SAS output

The improvement due to the interaction term is of course the same as the previous analysis because the interaction term was last in that analysis. The improvement differs for the other two terms differ from previously because now each is last.

8. If assumptions are not met decide whether to recompute p-value.

This is a saturated model, so there are no residuals to evaluate.

The binomial distribution is appropriate, and if observations are made independently of one another, the assumptions for computing p-value from χ^2 distribution are met.

9. Report statistical results.

Survival of females relative to males is independent of location of pupation site.

ΔG =1.3655

LR = likelihood(Model$_{\mathrm{Site \cdot Sex}}$ / Model$_{\mathrm{intercept}}$) = $e^{G/2}$ = 2.0

There is insufficient evidence for the *Site·Sex* term in this model.

Estimate Type I error on decision to reject the null hypothesis.

df = 3, p = 0.7137

The null model, no interactive effect, cannot be rejected.

In the absence of an interactive effect we examine the main effects that were of interest in this experiment.

Pupation success differs among sites, with strong evidence and low uncertainty.

ΔG =10.97, LR = 240, df = 3, p = 0.012

Pupation success differs between males and females, with good evidence and very low uncertainty.

ΔG =8.14, LR = 59, df = 1, p = 0.0043

10. Analysis of parameters of biological interest.

See estimates in step 4, with standard errors and confidence limits.

Fitted values from parameter estimates.

```
                           Analysis Of Parameter Estimates

                                             Standard   Wald 95% Confidence
Parameter             DF    Estimate          Error            Limits

Intercept              1     -0.5108         0.7303     -1.9422      0.9205
Site         AM        1      1.6094         0.8944     -0.1436      3.3625
Site         IM        1      1.2040         0.7884     -0.3413      2.7492
Site         OM        1      1.0217         1.0328     -1.0026      3.0459
```

SAS output file

$e^{\beta_{intercept}} = e^{-0.5108} = 0.6$ Survival odds, males on wall (OW) = 3/5 = 0.6

$e^{\beta_{site=AM}} = e^{1.6094} = 5.0$ Odds ratio, males at margin (AM) $OR = \frac{15}{5} \cdot \frac{5}{3} = 5$

$e^{\beta_{site=IM}} = e^{1.2040} = 3.33$ Odds ratio, males in medium (IM) $OR = \frac{34}{17} \cdot \frac{5}{3} = 3.33$

$e^{\beta_{site=OM}} = e^{1.0217} = 2.78$ Odds ratio, males on medium (OM) $OR = \frac{5}{3} \cdot \frac{5}{3} = 2.78$

10. Analysis of parameters of biological interest.

Parameter estimates from statistical package, compared to calculation by hand.

```
                            Analysis Of Parameter Estimates

                                         Standard   Wald 95% Confidence
 Parameter              DF   Estimate       Error          Limits

 Sex         F           1     1.0704      0.9624    -0.8158      2.9567
 Sex         M           0     0.0000      0.0000     0.0000      0.0000
 Site*Sex    AM    F     1     0.9664      1.4954    -1.9646      3.8974
 Site*Sex    AM    M     0     0.0000      0.0000     0.0000      0.0000
 Site*Sex    IM    F     1     0.4520      1.0951    -1.6944      2.5984
 Site*Sex    IM    M     0     0.0000      0.0000     0.0000      0.0000
 Site*Sex    OM    F     1    -0.6004      1.3849    -3.3147      2.1139
```

SAS output file

Odds ratio, females relative to males (OW)

$$e^{\beta_{site=OW}} = e^{1.0704} = 2.92 \qquad OR = \frac{7}{4}\cdot\frac{5}{3} = 2.92$$

Odds ratio, females relative to males (AM)

$$e^{\beta_{site=AM}} = e^{1.0704\,+\,0.9664} = 7.67 \quad OR = \frac{23}{1}\cdot\frac{5}{15} = 7.67$$

Odds ratio, females relative to males (IM)

$$e^{\beta_{site=IM}} = e^{1.0704\,+\,0.4520} = 4.58 \quad OR = \frac{55}{6}\cdot\frac{17}{34} = 4.58$$

Odds ratio, females relative to males (OM)

$$e^{\beta_{site=OM}} = e^{1.0704\,-\,0.6004} = 1.6 \quad OR = \frac{8}{3}\cdot\frac{3}{5} = 1.6$$

Summary

Pupation odds of females are 1.6 to 7.6 times that of males at1-p = 95%.

Pupation odds of flies were lower on wall than in, on, and at margin of the medium. Odds were circa 3 times higher in or on medium, 5 times higher at edge of medium, relative to survival of pupae on wall.

Evidence for site effects (*LR* = 240) was 4 times that for sex effects (*LR* = 59).
p-values for site effects showed greater uncertainty than sex effects.
The p-value is not a measure of evidence.
We report the p-value (uncertainty) along with the measure of evidence.