Statistics for EES

Generalized Linear Models

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July 16, 2020

Contents

1 Poisson GLMs for counting data

1.1 Intro to Poisson GLM

image by Dieter Ebert, Basel
"Female Daphnia magna with a clutch of asexual eggs. The animal is about 4 mm long."

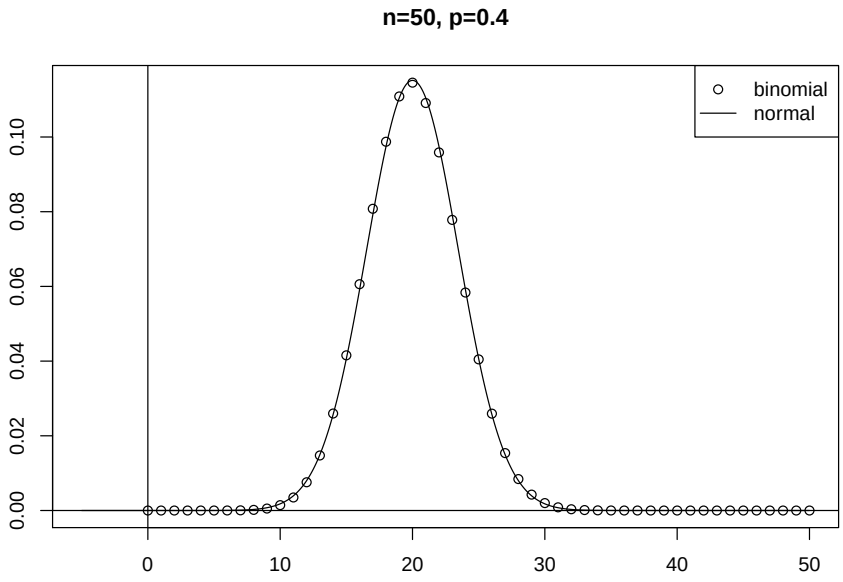
```
> daph <- read.table("daphnia_justina.csv",h=T)
> daph
   counts foodlevel species
1      68      high   magna
2      54      high   magna
3      59      high   magna
4      24      high galeata
5      27      high galeata
6      16      high galeata
7      20       low   magna
8      18       low   magna
9      18       low   magna
10      5       low galeata
11      8       low galeata
12      9       low galeata

> mod1 <- lm(counts~foodlevel+species,data=daph)
> mod2 <- lm(counts~foodlevel*species,data=daph)
> anova(mod1,mod2)
Analysis of Variance Table

Model 1: counts ~ foodlevel + species
Model 2: counts ~ foodlevel * species
  Res.Df    RSS Df Sum of Sq      F   Pr(>F)
1      9 710.00
2      8 176.67  1    533.33 24.151 0.001172 **
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
>
```

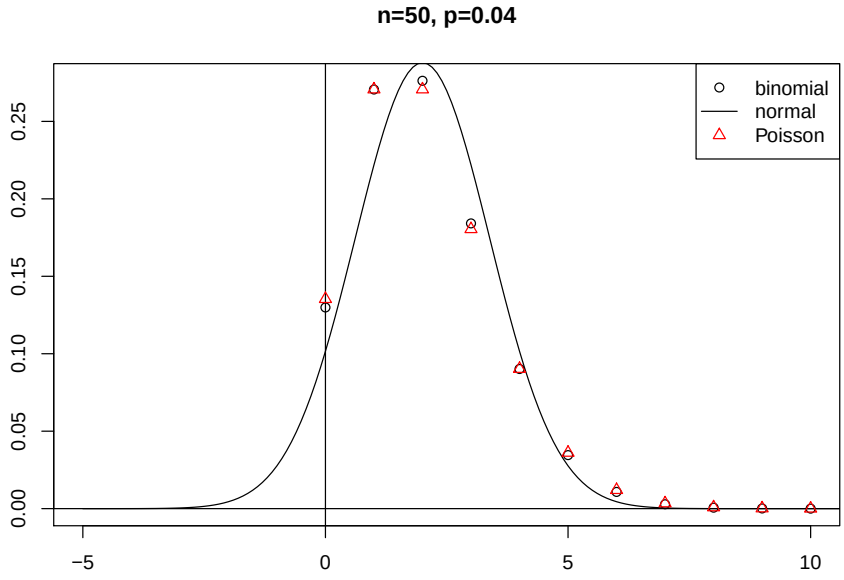
The normal distribution $\mathcal{N}(\mu, \sigma^2)$ is a continuous distribution and thus not suitable to model distributions on small numbers.

The Poisson distribution Pois(λ) is a distribution on $\{0, 1, 2, 3, \dots\}$.

$\mathcal{N}(\mu = n \cdot p, \sigma^2 = n \cdot p \cdot (1-p))$ approximates the binomial distribution Bin(n,p) if $n \cdot p \cdot (1-p)$ is not

too small (rule of thumb: $n \cdot p \cdot (1-p) > 9$), Pois($\lambda = n \cdot p$) gives a better approximation when p is small.





Example: Let X be binomially distributed with $n = 1000$ and $p = 0.002$.

$$\begin{aligned}
\mathbb{E}X &= n \cdot p = 2 \\
\mathsf{Var} &= n \cdot p \cdot (1-p) = 1.996 \approx 2 = n \cdot p \\
\Pr(X=3) &= \binom{n}{3} \cdot p^3 \cdot (1-p)^{997} = \frac{1000 \cdot 999 \cdot 998}{3!} \cdot p^3 \cdot (1-p)^{997} \approx 0.1806 \\
&\approx \frac{1000^3}{3!} \cdot p^3 \cdot (1-p)^{1000} = \frac{(np)^3}{3!} \cdot (1-p)^{1000} \\
&= \frac{(np)^3}{3!} \cdot (1-p)^{1000} \approx \frac{(np)^3}{3!} \cdot 0.13506 \approx \frac{(np)^3}{3!} \cdot 0.13534 \\
&= \frac{(np)^3}{3!} \cdot e^{-n \cdot p} = \Pr(Y=3) \approx 0.1804,
\end{aligned}$$

Where Y is Poisson distributed with $\lambda = np$ (and thus $\mathbb{E}Y = \mathsf{Var}Y = np$).

If Y is Pois(λ)-distributed, then

$$\begin{aligned}
\Pr(Y=k) &= \frac{\lambda^k}{k!} \cdot e^{-\lambda} \qquad \text{for } k = 0, 1, 2, \ldots \\
\mathbb{E}Y &= \lambda \\
\mathrm{Var}(Y) &= \lambda
\end{aligned}$$

Application examples:

- historical: number of Prussian soldiers killed by horse kicks in a year (von Bortkiewitsch, 1898)
- Number of new mutations in the genome of an individual
- Comparing two closely related species: number synonymous nucleotide substitutions in a gene

Is there a linear model with Pois(λ) instead of $\mathcal{N}(\mu, \sigma^2)$?

Yes, the **Generalized Linear Model (GLM) of type Poisson**.
Remeber the normal linear model:

$$Y_i = b_0 + b_1 \cdot X_{1,i} + \cdots + b_k \cdot X_{k,i} + \varepsilon_i \qquad \text{with } \varepsilon_i \sim \mathcal{N}(0, \sigma^2)$$

or equivalently:

$$\begin{aligned} \eta_i &= b_0 + b_1 \cdot X_{1,i} + \cdots + b_k \cdot X_{k,i} \\ Y_i &\sim \mathcal{N}(\eta_i, \sigma^2) \end{aligned}$$

η is called the *linear predictor*.

This also works for the Poisson distribution:

$$\begin{aligned} \eta_i &= b_0 + b_1 \cdot X_{1,i} + \cdots + b_k \cdot X_{k,i} \\ Y_i &\sim \text{Pois}(\eta_i) \end{aligned}$$

(but note that the additional σ^2 is missing!)

Instead of using η directly as parameter of the Poisson distribution, it is common to apply a transformation:

$$\begin{aligned} \ell(\mu_i) = \eta_i &= b_0 + b_1 \cdot X_{1,i} + \cdots + b_k \cdot X_{k,i} \\ Y_i &\sim \text{Pois}(\mu_i) \end{aligned}$$

$\ell(.)$ is called the *link function*.

The default link function for Poisson GLMs is log, the natural logarithm.

Thus,

$$\mathbb{E}Y_i = \mu_i = e^{\eta_i} = e^{b_0 + b_1 \cdot X_{1,i} + \cdots + b_k \cdot X_{k,i}} = e^{b_0} \cdot e^{b_1 \cdot X_{1,i}} \cdots e^{b_k \cdot X_{k,i}}$$

and the Poisson GLM with this default link is multiplicative model rather than an additive one.

1.2 Daphnia and Deviance

```
> pmod1 <- glm(counts~foodlevel+species,data=daph,
                                        family=poisson)
> summary(pmod1)
[...]
Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)    3.1166     0.1105  28.215  < 2e-16 ***
foodlevellow  -1.1567     0.1298  -8.910  < 2e-16 ***
speciesmagna   0.9794     0.1243   7.878 3.32e-15 ***
[...]
```

Note that the Poisson model has log as its default link function. Thus, the model pmod1 assumes that the number of Daphnia in row i is Poisson distributed with mean λ_i, i.e. $\Pr(X = k) = \frac{\lambda_i^k}{k!} e^{-\lambda}$, and

$$\log(\lambda_i) \approx 3.12 - 1.15 \cdot I_{\text{lowfoodlevel}} + 0.979 \cdot I_{\text{magna}}$$

or, equivalently,

$$\lambda_i \approx e^{3.12} \cdot e^{-1.15 I_{\text{lowfoodlevel}}} \cdot e^{0.979 I_{\text{magna}}} \approx 22.6 \cdot 0.317^{I_{\text{lowfoodlevel}}} \cdot 2.66^{I_{\text{magna}}}$$

Thus, this Poisson model assumes multiplicative effects.

```
> pmod1 <- glm(counts~foodlevel+species,
                              data=daph,family=poisson)
> pmod2 <- glm(counts~foodlevel*species,
                              data=daph,family=poisson)
> anova(pmod1,pmod2,test="F")

Analysis of Deviance Table

Model 1: counts ~ foodlevel + species
Model 2: counts ~ foodlevel * species
  Resid. Df Resid. Dev Df Deviance      F Pr(>F)
1         9     6.1162
2         8     6.0741  1 0.042071 0.0421 0.8375
Warning message:
F-Test not appropriate for family 'poisson'
```

Note:

- The anova command gives us an "analysis of deviance" instead of an analysis of variance!
- What is a deviance?
- There is a Warning "F-Test not appropriate for family 'poisson' ".
- Why?
- Which test should we apply?

What is the deviance?

Let $\widehat{b_0}, \ldots, \widehat{b_k}$ be our fitted model coefficients and

$$\widehat{\mu_i} = \ell^{-1}\left(\widehat{b_0} + \widehat{b_1}X_{1i} + \cdots + \widehat{b_k}X_{ki}\right)$$

be the predicted means for all observations. The Likelihood of the fitted parameter values is the probability of the observations assuming the fitted parameter values:

$$L(\widehat{\mu}) = \frac{\widehat{\mu_1}^{Y_1}}{Y_1!}e^{-\widehat{\mu_1}} \cdot \frac{\widehat{\mu_2}^{Y_2}}{Y_2!}e^{-\widehat{\mu_2}} \cdots \frac{\widehat{\mu_n}^{Y_n}}{Y_n!}e^{-\widehat{\mu_n}}$$

Now we compare this to a *saturated* Poisson GLM model, i.e. a model with so many parameters such that we can get a perfect fit of $\widetilde{\mu_i} = Y_i$. This leads to the highest possible likelihood $L(\widetilde{\mu})$.In practice such a model is not desirable because it leads to overfitting.

What is the deviance?

$$\begin{aligned}
\text{our model: } L(\widehat{\mu}) &= \frac{\widehat{\mu_1}^{Y_1}}{Y_1!}e^{-\widehat{\mu_1}} \cdot \frac{\widehat{\mu_2}^{Y_2}}{Y_2!}e^{-\widehat{\mu_2}} \cdots \frac{\widehat{\mu_n}^{Y_n}}{Y_n!}e^{-\widehat{\mu_n}} \\
\text{saturated model: } L(\widetilde{\mu}) &= \frac{Y_1^{Y_1}}{Y_1!}e^{-Y_1} \cdot \frac{Y_2^{Y_2}}{Y_2!}e^{-Y_2} \cdots \frac{Y_n^{Y_n}}{Y_n!}e^{-Y_n}
\end{aligned}$$

The *residual deviance* of our model is defined as

$$2 \cdot \left[\log\left(L(\widetilde{\mu})\right) - \log\left(L(\widehat{\mu})\right)\right].$$

It measures how far our model is away from the theoretical optimum.

- The deviance is approximately χ^2_{df} distributed, where df is the degrees of freedom of our model.
- Thus, the deviance should be of the same order of magnitude as df.
- Check this to assess the fit of the model!

Analysis of deviance: If D_1 and D_2 are the deviances of models M_1 with p_1 parameters and M_2 with p_2 parameters, and M_1 is nested in M_2 (i.e. the parameters of M_1 are a subset of the parameters of M_2), then $D_1 - D_2$ is approximately $\chi^2_{p_2-p_1}$-distributed.

This Test is the classical likelihood-ratio test. (Note that $D_1 - D_2$ is 2x the log of the likelihood-ratio of the two models.)

```
> pmod1 <- glm(counts~foodlevel+species,
                              data=daph,family=poisson)
> pmod2 <- glm(counts~foodlevel*species,
                              data=daph,family=poisson)
> anova(pmod1,pmod2,test="Chisq")

Analysis of Deviance Table

Model 1: counts ~ foodlevel + species
Model 2: counts ~ foodlevel * species
  Resid. Df Resid. Dev Df Deviance    P(>|Chi|)
1         9     6.1162
2         8     6.0741  1 0.042071    0.8375
```

Why not the F-test?

Remember that we did not estimate a variance σ^2 for the Poisson distribution.

There is an F-distribution approximation of a rescaled $D_1 - D_2$ for GLMs in which an extra variance parameter is estimated.

Example: *overdispersed Poisson*, also called *quasipoisson* GLM. Here, $\mathbb{E}Y_i = \mu_i$ but $\text{Var}Y_i = \phi \cdot \mu_i$ with the dispersion parameter $\phi > 1$.

This is often used to model the influence of unknown external factors.

Since the dispersion parameter is estimated, one can apply an F approximation in the analysis of deviance. But also χ^2 is still an option.

```
> qpmod1 <- glm(counts~foodlevel+species,data=daph,
                                  family=quasipoisson)
> qpmod2 <- glm(counts~foodlevel*species,data=daph,
                                  family=quasipoisson)
> anova(qpmod1,qpmod2,test="F")
Analysis of Deviance Table

Model 1: counts ~ foodlevel + species
Model 2: counts ~ foodlevel * species
  Resid. Df Resid. Dev Df Deviance      F Pr(>F)
1         9     6.1162
2         8     6.0741  1 0.042071 0.0572  0.817

> anova(qpmod1,qpmod2,test="Chisq")
Analysis of Deviance Table

Model 1: counts ~ foodlevel + species
```

```
Model 2: counts ~ foodlevel * species
  Resid. Df Resid. Dev Df Deviance P(>|Chi|)
1         9     6.1162
2         8     6.0741  1 0.042071     0.811

> expect <- predict(pmod1,type="response")
> sim <- rpois(12,expect)
> smod1 <- lm(sim~foodlevel+species,data=daph)
> smod2 <- lm(sim~foodlevel*species,data=daph)
> anova(smod1,smod2)

Analysis of Variance Table

Model 1: sim ~ foodlevel + species
Model 2: sim ~ foodlevel * species
  Res.Df     RSS Df Sum of Sq      F    Pr(>F)
1      9 1289.42
2      8  109.33  1    1180.1 86.348 1.464e-05 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

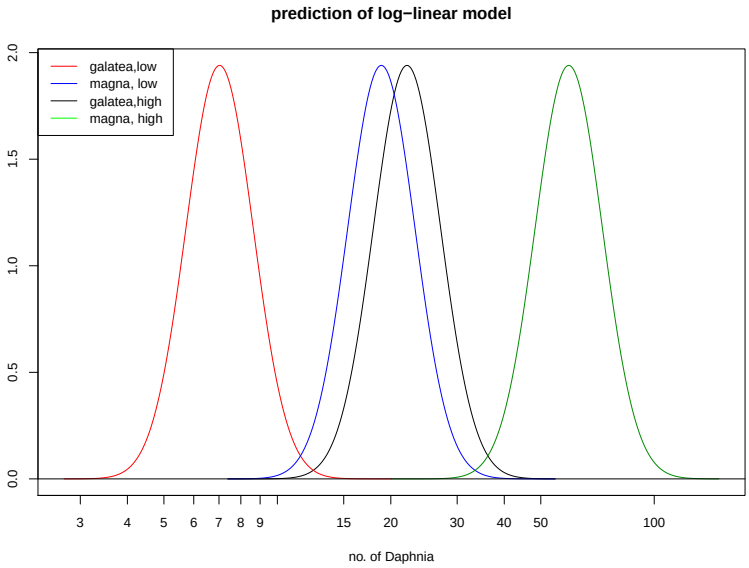
What is the problem? Normal distribution assumption or additivity?

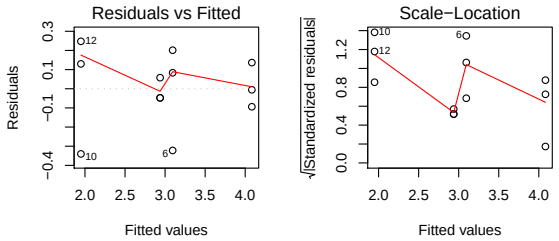
How about a multiplicative linear model?

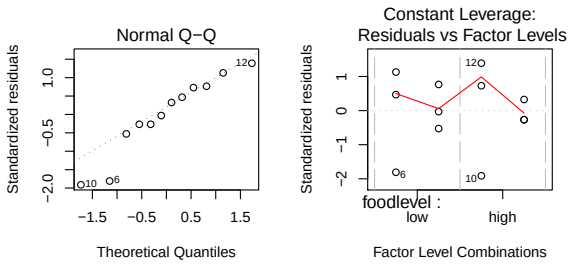
```
> expect <- predict(pmod1,type="response")
> sim <- rpois(12,expect)
> smod1 <- lm(log(sim)~foodlevel+species,data=daph)
> smod2 <- lm(log(sim)~foodlevel*species,data=daph)
> anova(smod1,smod2)

Analysis of Variance Table

Model 1: log(sim) ~ foodlevel + species
Model 2: log(sim) ~ foodlevel * species
  Res.Df     RSS Df Sum of Sq      F Pr(>F)
1      9 0.19216
2      8 0.19115  1 0.0010162 0.0425 0.8418
```

This solves the biggest problem, but what does the model say?

```
> lmod1 <- lm(log(counts)~foodlevel+species,data=daph)
[...]
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    3.0946     0.1028  30.104 2.41e-10 ***
foodlevellow  -1.1450     0.1187  -9.646 4.83e-06 ***
speciesmagna   0.9883     0.1187   8.326 1.61e-05 ***
[...]
Residual standard error: 0.2056 on 9 degrees of freedom
[...]
```

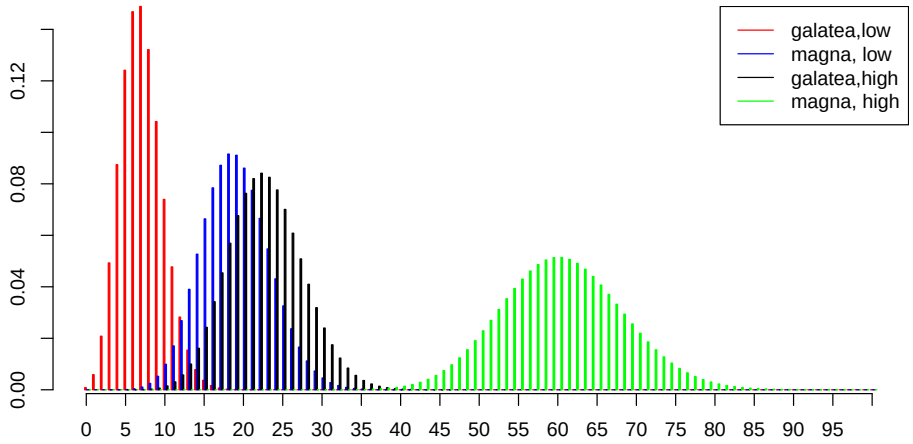


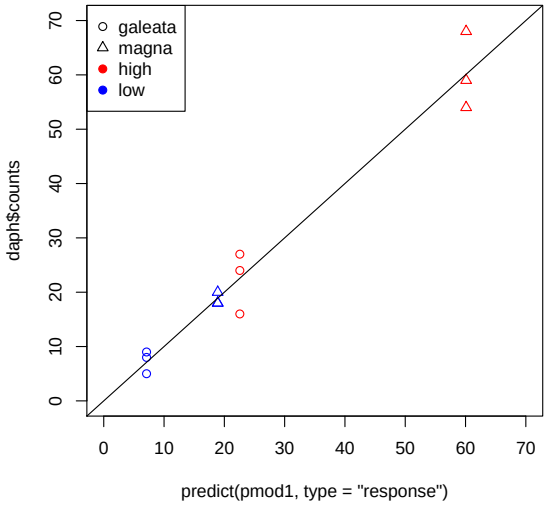





```
> summary(pmod1)
[..]
glm(formula = counts ~ foodlevel + species,
          family = poisson, data = daph)
[..]
             Estimate Std. Error z value Pr(>|z|)
(Intercept)    3.1166     0.1105  28.215  < 2e-16 ***
foodlevellow  -1.1567     0.1298  -8.910  < 2e-16 ***
speciesmagna   0.9794     0.1243   7.878 3.32e-15 ***
[..]
(Dispersion parameter for poisson family taken to be 1)
[..]
```

```
Residual deviance:   6.1162  on  9  degrees of freedom
AIC: 70.497
```





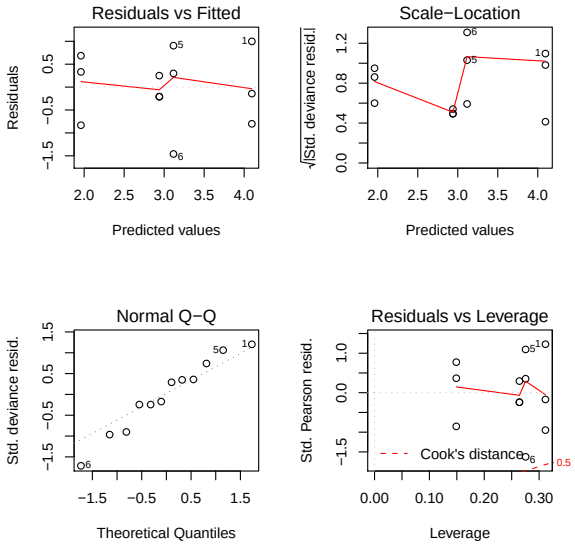
Since the variance is proportional to the expectation value in the Poisson model, usual residuals are not so informatative.

Instead use *deviance residuals*. Let d_i be the contribution of observation i (row i in the data table) to the Deviance, then the deviance residual of observation i is

$$\text{sign}(Y_i - \widehat{\mu}_i) \cdot \sqrt{d_i}.$$

The deviance residuals are the default residuals given by R for GLMs. They have similar properties as the standard residuals in the normal linear model.

In the following plot obtained with plot(pmod1) the word "residual" always refers to deviance residuals.



2 Bionomial GLMs for Ratios

2.1 Intro to binomial GLMs and logistic regression

- Data from EES Master's thesis *Inferences on the evolution of CO_2 avoidance behavioral response in the Drosophila genus* (Ana Catalan, 2010)
- Do male or female Drosophila flies sense and avoid increased CO_2 concentrations?
- Flies of the had the choice between between fresh air or increased CO_2
- Repeated with several Drosophila species

- Some experiments at room temperature, some at 30°C

```
> fly <- read.csv("Flies_AnaCatalan.csv",h=T,sep=";")
> fly
    odorant resp air PI     sex day species
1       CO2    1  29 NA   males   1     mel
2       CO2    2  28 NA   males   1     mel
3       CO2    1  25 NA   males   1     mel
.        .     .   . .      .     .      .
.        .     .   . .      .     .      .
.        .     .   . .      .     .      .
753   30CO2    4   7 NA females   2     vir
754   30CO2    6  12 NA females   2     vir
755   30CO2    6  11 NA females   2     vir
756   30CO2    6  15 NA females   2     vir

> str(fly)
'data.frame': 756 obs. of  7 variables:
 $ odorant: Factor w/ 3 levels "30CO2","CO2",..: 2 2 2 2 2 2 2 2 2 2 ...
 $ resp   : int  1 2 1 2 5 4 9 5 5 11 ...
 $ air    : int  29 28 25 17 36 42 38 13 19 25 ...
 $ PI     : logi  NA NA NA NA NA NA ...
 $ sex    : Factor w/ 2 levels "females","males": 2 2 2 2 2 2 2 2 2 2 ...
 $ day    : int  1 1 1 1 1 1 2 2 2 2 ...
 $ species: Factor w/ 11 levels "ana","atr","ere",..: 5 5 5 5 5 5 5 5 5 5 ...
```

Model

In experiment i (row i of the data table) there are n_i flies. Each of these flies decided independently of all other to go to the odorant with probability p_i and, thus, to go to the fresh air with probability $(1 - p_i)$.

Thus, the number Y_i of flies which went to the odorant is binomially distributed:

$$\begin{aligned} Y_i &\sim \text{bin}(n_i, p_i) \\ \Pr(Y_i = k) &= \binom{n_i}{k} \cdot p_i^k \cdot (1-p_i)^{n_i - k} \\ \mathbb{E}Y_i &= n_i \cdot p_i \\ \text{Var}Y_i &= n_i \cdot p_i \cdot (1-p_i) \end{aligned}$$

How does p_i depend on the odorant and on the species?

Binomial GLM with logit link

Similar as in Poisson GLMs we assume:

$$\ell(p_i) \;=\; \eta_i \;=\; b_0 + b_1 \cdot X_{1,i} + \cdots + b_k \cdot X_{k,i}$$

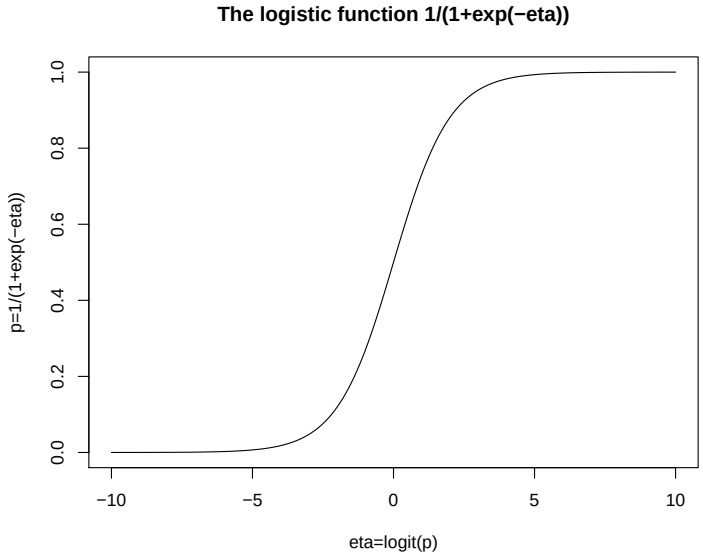
The default link of the Binomial GLM is the logit link:

$$\eta \;=\; \text{logit}(p) \;=\; \log(p/(1-p))$$

Its inverse is the logistic function

$$p \;=\; \frac{1}{1+e^{-\eta}}$$

Binomial GLM with the logit link is also called *logistic regression*.



Likelihood and Deviance

If $\widehat{p_1}, \ldots, \widehat{p_m}$ are the estimated p_i in our model, then the likelihood of the fitted parameters is

$$L(\widehat{p}) \;=\; \binom{n_1}{Y_1} \widehat{p_1}^{\,Y_1} (1-\widehat{p_1})^{n_1 - Y_1} \cdot \binom{n_2}{Y_2} \widehat{p_2}^{\,Y_2} (1-\widehat{p_2})^{n_2 - Y_2} \cdots \\ \cdots \binom{n_m}{Y_m} \widehat{p_m}^{\,Y_k} (1-\widehat{p_m})^{n_m - Y_m}$$

Using this likelihood, the *deviance* and the deviance residuals are defined like in the Poisson GLM.

Analysis of deviance and overdispersion

Note that, like in the Poisson model, $\mathrm{Var} Y_i = n_i \cdot p_i \cdot (1-p_i)$ is fixed for given $\mathbb{E} Y_i = n_i p_i$. Thus, the χ^2 approximation should be used in the analysis of deviance.

There is an overdispersed binomial GLM (available in R with the option family=quasibinomial) with an additional dispersion parameter. For these models one can use both χ^2 approximation and F approximations in analyses of deviance.

2.2 Odorant preferences in flies

```
> fly <- read.csv("Flies_AnaCatalan.csv",h=T,sep=";")
> fly
    odorant resp air PI     sex day species
1       CO2    1  29 NA   males   1     mel
2       CO2    2  28 NA   males   1     mel
3       CO2    1  25 NA   males   1     mel
.        .     .   . .      .     .      .
.        .     .   . .      .     .      .
.        .     .   . .      .     .      .
753   30CO2    4   7 NA females   2     vir
754   30CO2    6  12 NA females   2     vir
755   30CO2    6  11 NA females   2     vir
```

```
756   30CO2    6  15 NA females   2     vir
```

```
> modelbin <- glm(cbind(resp,air)~(sex+species)*odorant+day,
+              subset=odorant!="oct",
+              data=fly,family=binomial)
> summary(modelbin)

Call:
glm(formula = cbind(resp, air) ~ (sex + species) * odorant +
    day, family = binomial, data = fly,
                        subset = odorant != "oct")

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.3735  -0.9693  -0.1187   0.7240   4.4994

Coefficients:
                      Estimate Std. Error z value Pr(>|z|)
(Intercept)          -1.376503   0.123901 -11.110  < 2e-16 ***
sexmales              0.131066   0.053810   2.436 0.014863 *
speciesatr            0.227528   0.145096   1.568 0.116854
speciesere            0.057917   0.150061   0.386 0.699528
speciesmau            0.141718   0.163017   0.869 0.384658

speciesmel           -1.128202   0.164920  -6.841 7.87e-12 ***
speciespse            1.318299   0.143279   9.201  < 2e-16 ***
speciessec           -0.518238   0.143658  -3.607 0.000309 ***
speciessim            0.427407   0.136345   3.135 0.001720 **
speciestei           -0.266130   0.144181  -1.846 0.064921 .
speciesvir            0.424609   0.173881   2.442 0.014608 *
speciesyak           -0.454361   0.170760  -2.661 0.007795 **
odorantCO2           -0.922118   0.171020  -5.392 6.97e-08 ***
day                  -0.008059   0.014922  -0.540 0.589129
sexmales:odorantCO2  -0.023450   0.067791  -0.346 0.729408
speciesatr:odorantCO2  1.180104   0.194524   6.067 1.31e-09 ***
speciesere:odorantCO2  1.473309   0.200023   7.366 1.76e-13 ***
speciesmau:odorantCO2  1.214336   0.222429   5.459 4.78e-08 ***
speciesmel:odorantCO2  1.530291   0.219269   6.979 2.97e-12 ***
speciespse:odorantCO2  0.384300   0.195086   1.970 0.048849 *
speciessec:odorantCO2  2.046612   0.194380  10.529  < 2e-16 ***
speciessim:odorantCO2  1.369519   0.189228   7.237 4.57e-13 ***

speciestei:odorantCO2  1.033078   0.199579   5.176 2.26e-07 ***
speciesvir:odorantCO2  1.262574   0.225086   5.609 2.03e-08 ***
speciesyak:odorantCO2  1.919994   0.215587   8.906  < 2e-16 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 2429.1  on 663  degrees of freedom
Residual deviance: 1187.1  on 639  degrees of freedom
AIC: 3430.7

Number of Fisher Scoring iterations: 4
```

A residual deviance of 1187.1 on 639 degrees of freedom is very high and indicates that the model parameters cannot fully explain the data.

⇒ Fit an overdispersed model!

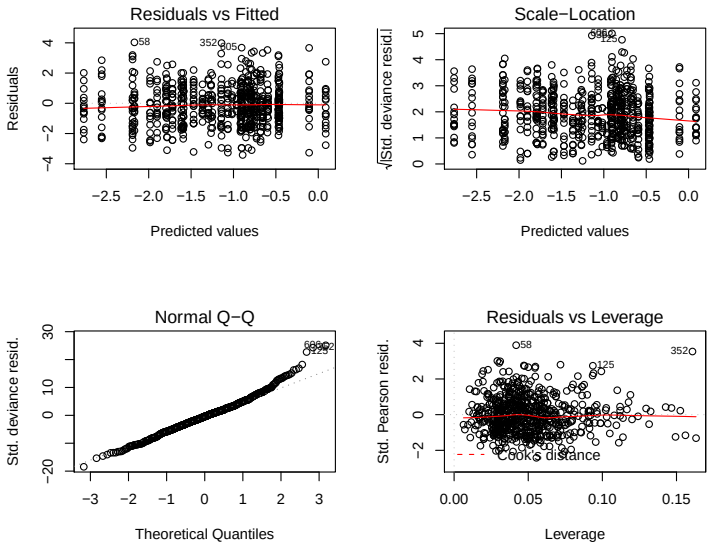
There is a price we have to pay for overdispersion: Since it is not a clearly defined distribution, AIC is not available for model selection.

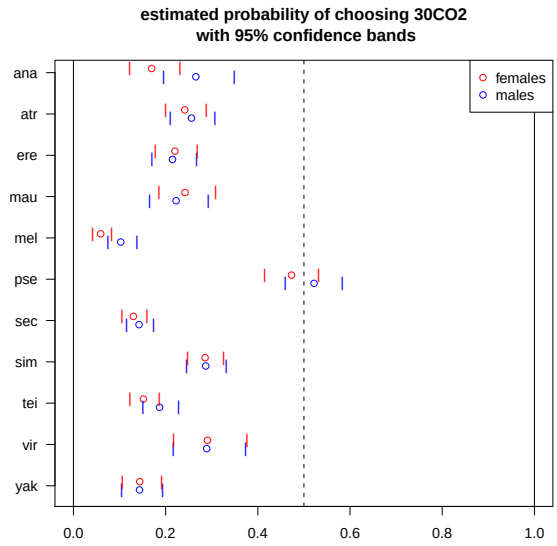
Select parameters

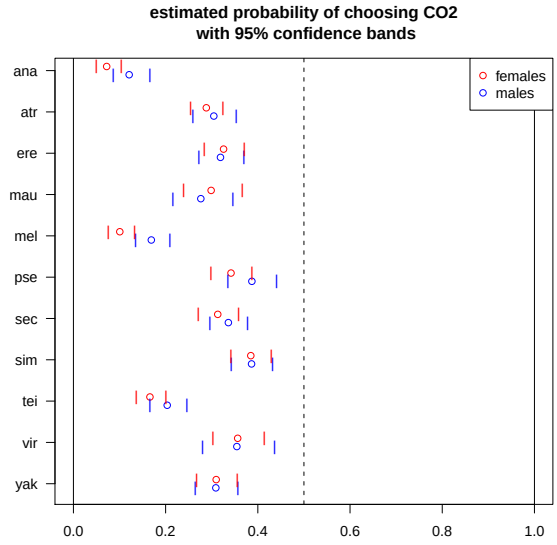
1. that seem important to you from the biological context
2. or have low p-values.

```
> model <- glm(cbind(resp,air)~(sex+species)*odorant+day,
+              subset=odorant!="oct",
+              data=fly,family=quasibinomial)
> drop1(model,test="F")
Single term deletions

Model:
cbind(resp, air) ~ (sex + species) * odorant + day
               Df Deviance F value  Pr(F)
<none>              1187.1
day             1   1187.3  0.1571 0.6920
sex:odorant     1   1187.2  0.0644 0.7997
species:odorant 10  1431.1 13.1365 <2e-16 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

> model2 <- update(model,~.-day)
> drop1(model2,test="F")
Single term deletions

Model:
cbind(resp, air) ~ sex + species + odorant + sex:odorant + species:odorant
               Df Deviance F value  Pr(F)
<none>              1187.3
sex:odorant     1   1187.5  0.0673 0.7953
species:odorant 10  1432.6 13.2215 <2e-16 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

> model3 <- update(model2,~.-sex:odorant)
> drop1(model3,test="F")
Single term deletions

Model:
cbind(resp, air) ~ sex + species + odorant + species:odorant
               Df Deviance F value   Pr(F)
<none>              1187.5
sex             1   1200.0  6.7785 0.00944 **
species:odorant 10  1432.7 13.2366 < 2e-16 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

> model4 <- glm(cbind(resp,air)~sex+species+odorant
+                          +species:odorant+species:sex,
+              subset=odorant!="oct",
+              data=fly,family=quasibinomial)
> anova(model3,model4,test="F")
Analysis of Deviance Table
```

```
Model 1: cbind(resp, air) ~ sex + species + odorant + species:odorant
Model 2: cbind(resp, air) ~ sex + species + odorant + species:odorant +
    species:sex
  Resid. Df Resid. Dev Df Deviance      F Pr(>F)
1       641     1187.5
2       631     1157.1 10   30.395 1.7232  0.072 .
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

> drop1(model4,test="F")
Single term deletions

Model:
cbind(resp, air) ~ sex + species + odorant + species:odorant +
    species:sex
                Df Deviance F value   Pr(F)
<none>               1157.1
species:odorant 10   1402.9 13.4043 < 2e-16 ***
sex:species     10   1187.5  1.6575 0.08708 .
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```








```
> newdata <- data.frame(species=rep(levels(fly$species),4),
+          odorant=rep(levels(fly$odorant)[1:2],rep(22,2)),
+          sex=rep(rep(levels(fly$sex),2),rep(11,4)))
> newdata
   species odorant     sex
1      ana   30CO2 females
2      atr   30CO2 females
3      ere   30CO2 females
4      mau   30CO2 females
5      mel   30CO2 females
6      pse   30CO2 females
7      sec   30CO2 females
8      sim   30CO2 females
9      tei   30CO2 females
10     vir   30CO2 females
```

```
11     yak   30CO2 females
12     ana   30CO2   males
13     atr   30CO2   males
14     ere   30CO2   males
15     mau   30CO2   males
16     mel   30CO2   males
17     pse   30CO2   males
18     sec   30CO2   males
19     sim   30CO2   males
20     tei   30CO2   males
21     vir   30CO2   males
22     yak   30CO2   males

23     ana     CO2 females
24     atr     CO2 females
25     ere     CO2 females
26     mau     CO2 females
27     mel     CO2 females
28     pse     CO2 females
29     sec     CO2 females
30     sim     CO2 females
31     tei     CO2 females
32     vir     CO2 females
33     yak     CO2 females
34     ana     CO2   males
35     atr     CO2   males
36     ere     CO2   males
37     mau     CO2   males
38     mel     CO2   males
39     pse     CO2   males
40     sec     CO2   males
41     sim     CO2   males
42     tei     CO2   males
43     vir     CO2   males
44     yak     CO2   males

> predict(model4,newdata,type="link")
         1          2          3          4          5          6
-1.58789551 -1.14469372 -1.26487696 -1.14101650 -2.76586374 -0.10775557
         7          8          9         10         11         12
-1.90097360 -0.91699408 -1.72012424 -0.89185179 -1.78389658 -1.01728212
        13         14         15         16         17         18
-1.06650110 -1.29566564 -1.25030454 -2.16842944  0.08781449 -1.79595472
        19         20         21         22         23         24
-0.91001993 -1.47044203 -0.89969326 -1.78744176 -2.55428808 -0.90392477
        25         26         27         28         29         30
-0.72774118 -0.85332683 -2.19052045 -0.65510800 -0.78579246 -0.46942549
        31         32         33         34         35         36
-1.61457993 -0.59147161 -0.80167681 -1.98367468 -0.82573216 -0.75852985
        37         38         39         40         41         42
-0.96261487 -1.59308615 -0.45953795 -0.68077358 -0.46245135 -1.36489772
        43         44
-0.59931308 -0.80522198

> predict(model4,newdata,type="response")
         1          2          3          4          5          6          7
0.16968019 0.24145963 0.22013549 0.24213378 0.05919695 0.47308714 0.12999832
         8          9         10         11         12         13         14
0.28557077 0.15185516 0.29072783 0.14382265 0.26555715 0.25606905 0.21489539
        15         16         17         18         19         20         21
0.22264743 0.10262158 0.52193952 0.14234421 0.28699576 0.18687544 0.28911354
        22         23         24         25         26         27         28
0.14338666 0.07213894 0.28824462 0.32569061 0.29873544 0.10060499 0.34183939
        29         30         31         32         33         34         35
0.31307282 0.38475223 0.16595372 0.35629727 0.30966695 0.12092766 0.30454824
```

```
        36         37         38         39         40         41         42
0.31896554 0.27635496 0.16895014 0.38709544 0.33608867 0.38640446 0.20344545
        43         44
0.35450087 0.30890960
```

Compute an approx. 95% confidence range

```
> case <- data.frame(species="mel",odorant="CO2",sex="males")
> (pred <- predict(model4,case,type="link",se.fit=TRUE) )
$fit
-1.593086
$se.fit
[1] 0.1327248
$residual.scale
[1] 1.328106
> invlink <- function(x) {    ## inverse link function
+   1/(1+exp(-x))
+ }
> invlink(pred$fit)     ## prediction
0.1689501
> invlink(pred$fit-2*pred$se.fit)   ## lower bound
0.1348738
> invlink(pred$fit+2*pred$se.fit)   ## upper bound
0.2095506
```

This can be done simultaneously for a whole data frame (e.g. newdata) instead just for one on case (in our example mel/CO2/males)

Should be done on the linear predictor ("link") scale and not on the response scale because it is based on a normal distribution approximation, which is only (more or less) valid on the linear predictor scale. (Remember: for a normal distribution, $> 95\%$ are within the 2σ-bounds around the mean.)

2.3 Sex ratio in ants

Hymenoptera opacior

References

[1] S. Foitzik, I.M. Kureck, M.H. Rüger, D. Metzler (2010) Alternative reproductive tactics and the influence of local competition on sex allocation in the ant *Hypoponera opacior*. *Behavioral Ecology and Sociobiology* **64**:1641-1654

How does the ratio of queens and males produced by an ant nest depend on the nest size?

- Winged sexuals were observed in June, unwinged sexuals in August.
- New queens and workers have more genetic material in common than new males and workers.
- Queens are larger than males and thus more costly to produce.
- Other factors: local resource competition, local mate competition...

Variables in the ants data set.

Nest.size number of workers in the nest

pupae pupae produced by the nest

New.Males new males produced by the nest

New.Queens new queens produced by the nest

month 6=June, 8=August

(Many more variables in full dataset)

```
> str(ants)
'data.frame': 229 obs. of  5 variables:
 $ pupae    : int  71 16 7 6 12 13 330 12 180 0 ...
 $ Nest.size : int  39 6 5 2 5 4 18 9 47 10 ...
 $ New.Males : int  0 1 3 0 0 0 2 2 0 0 ...
 $ New.Queens: int  1 3 9 0 2 0 2 1 0 0 ...
 $ month     : int  6 6 6 6 6 6 6 6 6 6 ...
> attach(ants)
> productivity <-  ( pupae +  New.Males +
                                  New.Queens )/ (Nest.size)
```

```
> M0 <- glm(cbind(New.Queens,New.Males)~(as.factor(month)
+          +Nest.size+productivity)^2,family=binomial)
> summary(M0)
[...]
                          Estimate Std. Error z value Pr(>|z|)
(Intercept)                 -0.428  0.3175  -1.3 0.1776
as.factor(month)8           -0.205  0.3664  -0.5 0.5757
Nest.size                    0.066  0.0177   3.7 0.0001 ***
productivity                 0.002  0.0178   0.1 0.8670
as.factor(month)8:Nest.size -0.030  0.0171  -1.8 0.0710 .
as.factor(month)8:productivity -0.016  0.0165  -0.9 0.3225
Nest.size:productivity      -0.000  0.0007  -0.5 0.5988
[..]
    Null deviance: 494.61  on 138  degrees of freedom
Residual deviance: 354.96  on 132  degrees of freedom
  (10 observations deleted due to missingness)
AIC: 529.5
```

We already have lots of parameters and interactions in the model, but the residual deviance of 354.96 is still to high for 132 degrees of freedom.

⇒ Use *overdispersed* binomial (quasibinomial).

```
> M1 <- glm(cbind(New.Queens,New.Males)~(as.factor(month)
+           +Nest.size+productivity)^2,family=quasibinomial)
> summary(M1)
[..]
                         Estimate Std. Error t value Pr(>|t|)
(Intercept)                -0.4281  0.470  -0.9   0.36
as.factor(month)8          -0.2050  0.542  -0.3   0.70
Nest.size                   0.0667  0.026   2.5   0.01 *
productivity                0.0029  0.026   0.1   0.91
as.factor(month)8:Nest.size    -0.0309  0.025  -1.2   0.22
as.factor(month)8:productivity -0.0164  0.024  -0.6   0.50
Nest.size:productivity         -0.0003  0.001  -0.3   0.72
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

(Dispersion parameter for quasibinomial family 2.190267)

    Null deviance: 494.61  on 138  degrees of freedom
Residual deviance: 354.96  on 132  degrees of freedom
  (10 observations deleted due to missingness)
AIC: NA
```

- Less significance now.
- Residual deviance still the same, but no reason to worry for overdispersed models
- AIC not available anymore; that's a real pity!

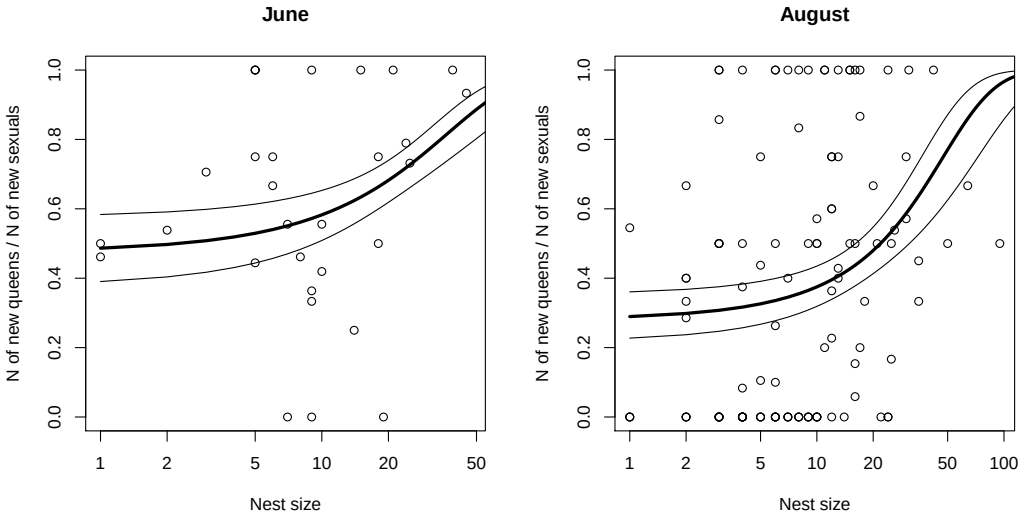
```
> drop1(M1,test="F")
Single term deletions

Model:
cbind(New.Queens, New.Males) ~ (as.factor(month)
       + Nest.size + productivity)^2
                              Df Deviance F value  Pr(F)
<none>                             354.96
as.factor(month):Nest.size     1   358.39  1.2754 0.2608
as.factor(month):productivity  1   355.94  0.3642 0.5472
Nest.size:productivity         1   355.24  0.1035 0.7482
```

Model selection when AIC is not available.

- Apply backward model selection strategy: apply drop1 and remove the variable with the highest p-value. Apply drop1 on the reduced model and repeat this again and again until you only variables are left which are significant or almost significant.
- Variables will not be removed if they are involved in interactions, because drop1 won't show those variables.
- Do not remove a variable if there is a good biological reason why it should be in the model.

```
> M2 <- update(M1,~.-as.factor(month):productivity)
> drop1(M2,test="F")
Single term deletions

Model:
cbind(New.Queens, New.Males) ~ as.factor(month)
    + Nest.size + productivity + as.factor(month):Nest.size
    + Nest.size:productivity
                           Df Deviance F value  Pr(F)
<none>                          355.94
as.factor(month):Nest.size  1   358.86  1.0911 0.2981
Nest.size:productivity      1   355.96  0.0067 0.9349

> M3 <- update(M2,~.-Nest.size:productivity)
> drop1(M3,test="F")
Single term deletions

Model:
cbind(New.Queens, New.Males) ~ as.factor(month) +
    Nest.size + productivity +
    as.factor(month):Nest.size
                           Df Deviance F value  Pr(F)
<none>                          355.96
productivity                1   358.57  0.9832 0.3232
as.factor(month):Nest.size  1   359.40  1.2952 0.2571

> M4 <- update(M3,~.-productivity )
> drop1(M4,test="F")
Single term deletions

Model:
cbind(New.Queens, New.Males) ~ as.factor(month) +
    Nest.size + as.factor(month):Nest.size
                           Df Deviance F value  Pr(F)
<none>                          358.57
as.factor(month):Nest.size  1   360.07  0.5626 0.4545

> M5 <- update(M4,~.-as.factor(month):Nest.size)
> drop1(M5,test="F")
Single term deletions

Model:
cbind(New.Queens, New.Males) ~ as.factor(month) + Nest.size
               Df Deviance F value     Pr(F)
<none>              360.07
as.factor(month)  1   399.32  14.828 0.0001806 ***
Nest.size         1   417.47  21.684 7.559e-06 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

> summary(M5)

Call:
glm(formula = cbind(New.Queens, New.Males) ~ as.factor(month) +
    Nest.size, family = quasibinomial)
```

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.5049  -0.8569   0.0000   0.3521   4.2843

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)       -0.156142   0.236048  -0.661    0.509
as.factor(month)8 -0.839253   0.202793  -4.138 6.10e-05 ***
Nest.size          0.045656   0.009749   4.683 6.76e-06 ***
```




```
plot(Nest.size[month==6],
  New.Queens[month==6]/(New.Males[month==6]+New.Queens[month==6]),
  main="June", log="x", xlab="Nest size",
  ylab="N of new queens / N of new sexuals")

hypotheticaljune <- data.frame(month=6,Nest.size=0:200)

pred <- predict(M5,hypotheticaljune,type="link",se.fit=TRUE)

lines(0:200,1/(1+exp(-pred$fit)),lwd=3)

lines(0:200,1/(1+exp(-(pred$fit+2*pred$se.fit))))

lines(0:200,1/(1+exp(-(pred$fit-2*pred$se.fit))))
```

3 Other GLMs

GLMs and their links (canonical links first)

Poisson $\log(\mu)$, μ, $\sqrt{\mu}$

binomial logit, probit, cloglog

gaussian μ

Gamma $-1/\mu$, μ, $\log(\mu)$

inverse gaussian $-2/\mu^2$

Also interesting: **negative binomial** as alternative to overdispersed Poisson.

4 Application example: Strawberry resistance against *Drosophila suzukii*

Drosophila suzukii male and female. Image by Shane F. McEvey, Australian Museum.
License: Creative Commons Attribution 4.0 International
https://figshare.com/articles/High_resolution_diagnostic_images_of_Drosophila_suzukii_Diptera_Drosophilidae_/4644793/1
https://commons.wikimedia.org/wiki/File:DrosophilasuzukiiphotoMcEvey.jpg

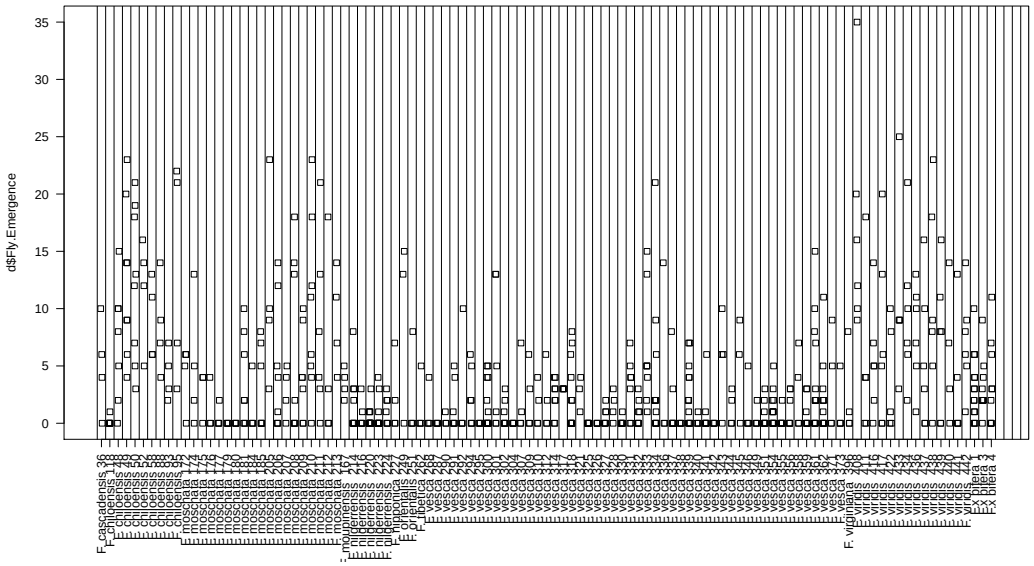
Image by Martin Hauser
License: Creative Commons Attribution 3.0 Germany
https://commons.wikimedia.org/wiki/File:Suzukii_ovi.jpg

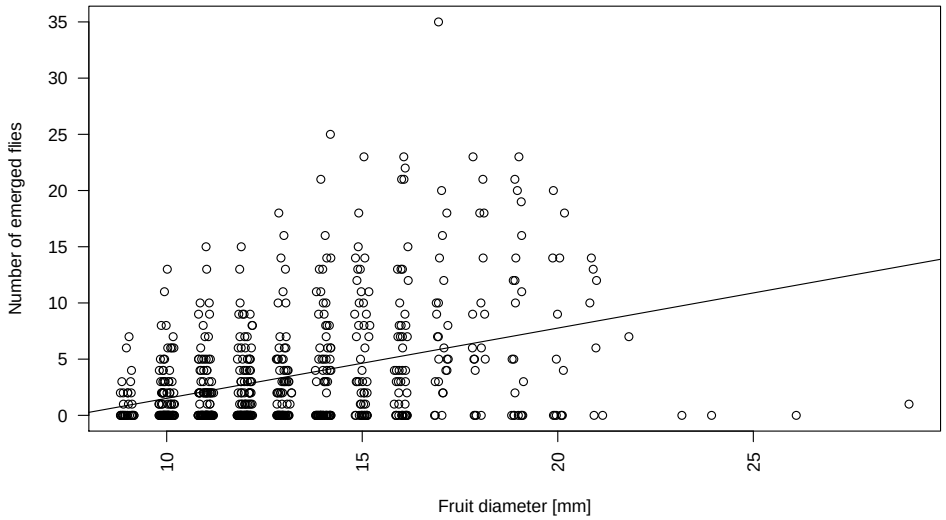
References

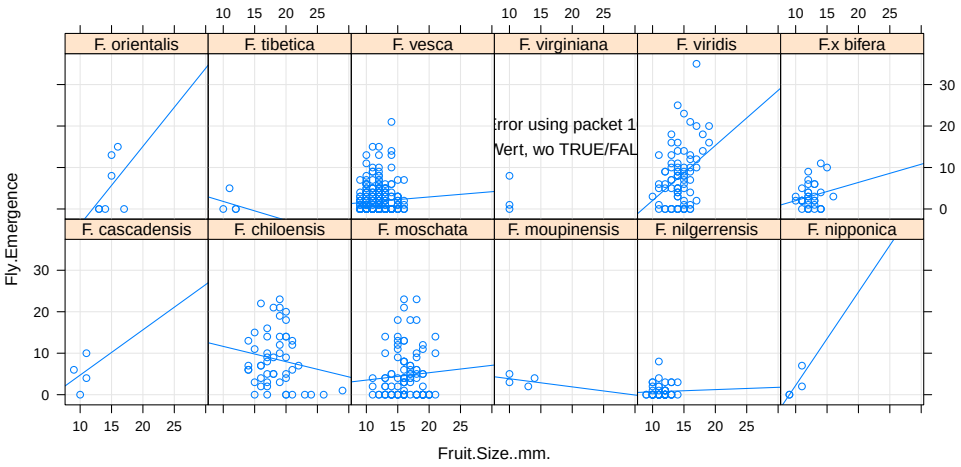
[1] X. Gong, L. Bräcker, N. Bölke, C. Plata, S. Zeitlmayr, D. Metzler, K. Olbricht, N. Gompel, M. Parniske (2016) Identification of strawberry accessions with reduced emergence rates of the pest Drosophila suzukii *Front. Plant Sci.* **7**:1880. doi: 10.3389/fpls.2016.01880

To avoid copyright issues, the data shown in some of the following slides are not original data but simulated data inspired by the data in this study.

```
> str(d)
'data.frame': 681 obs. of  15 variables:
 $ X.1              : int  1 2 3 4 5 6 7 8 9 10 ...
 $ Accession.No.    : Factor w/ 107 levels "1","3","4","36",..: 1 1 1 1 1 1 1 1 1 1 ...
 $ Fly.Emergence    : int  6 2 1 1 0 2 0 10 3 4 ...
 $ Species          : Factor w/ 12 levels "F. cascadensis",..: 12 12 12 12 12 12 12 12 12 12 ...
 $ Ploidy           : Factor w/ 5 levels "decaploid","diploid",..: 2 2 2 2 2 2 2 2 2 2 ...
 $ Fruit.Size..mm.  : int  13 13 13 12 12 13 11 15 16 12 ...
 $ Country          : Factor w/ 23 levels "Aserbaijan","Austria",..: 9 9 9 9 9 9 9 9 9 9 ...
 $ Collection.site  : Factor w/ 63 levels "26D23'01.8\"N 108D12'26.3\"E",..: 21 21 21 21 21 21 21 21 21 21 ...
 $ Colour.1         : num  0.557 0.557 0.557 0.557 0.557 ...
 $ Colour.2         : num  1.26 1.26 1.26 1.26 1.26 ...
 $ Colour.3         : num  3.58 3.58 3.58 3.58 3.58 ...
 $ Day.of.experiment: Factor w/ 7 levels "03/06/15","10/06/15",..: 7 7 7 7 5 5 5 5 5 5 ...
 $ berry            : Factor w/ 681 levels "1","2","3","4",..: 1 2 3 4 5 6 7 8 9 10 ...
 $ emer             : num  1 1 1 1 0 1 0 1 1 1 ...
```


dSFly.Emergence
0
5
10
15
20
25
30
35


Number of emerged flies
0
5
10
15
20
25
30
35
10
15
20
25
Fruit diameter [mm]




```
library(lattice)
xyplot(Fly.Emergence~Fruit.Size..mm.|Species,d,type=c("p","r","g"))
```

4.1 Generalized Linear Mixed Model (GLMM)

```
> modp <- glmer(Fly.Emergence~Fruit.Size..mm.+(1|Species/Accession.No./berry),
+              data=d,family=poisson)
> pr <- profile(modp) ## takes quite long, and gives a warning for orig. data
> confint(pr)
                       2.5 %    97.5 %
.sig01            1.06134062 1.3275719
.sig02            0.60842044 1.0482151
.sig03            0.27681859 1.1030555
(Intercept)      -1.77756133 0.3740383
Fruit.Size..mm.   0.02052691 0.1605697
```

Problem, however: glmer numeric not very accurate for zero-inflated data

Now neglect numbers, just consider emergence yes/no

```
> mod <- glm(emer~Species,family="binomial",data=d)
> mod2 <- glm(emer~Species+Accession.No.,family="binomial",data=d)
> mod3 <- glm(emer~Accession.No.,family="binomial",data=d)
Warnings:
1: glm.fit: algorithms did not converge
2: glm.fit: fitted probabilities with values 0 or 1
>
> anova(mod,mod2,mod3,test="Chisq")
Analysis of Deviance Table

Model 1: emer ~ Species
Model 2: emer ~ Species + Accession.No.
Model 3: emer ~ Accession.No.
  Resid. Df Resid. Dev Df Deviance  Pr(>Chi)
1       669     854.72
2       572     655.27 97   199.45 4.522e-09 ***
3       574     654.85 -2     0.42
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

Note that models 2 and 3 are equivalent

Observation with original data: AIC value contradict likelihood ratio test:

```
> mod6 <- glm(emer~Fruit.Size..mm.+Accession.No.,family="binomial",data=d)
> drop1(mod6,test="Chisq")
Single term deletions

Model:
emer ~ Fruit.Size..mm. + Accession.No.
```

```
               Df Deviance    AIC     LRT  Pr(>Chi)
<none>              676.43 892.43
Fruit.Size..mm.   1   678.66 892.66   2.223     0.136
Accession.No.   106   862.96 866.96 186.525 2.275e-06 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

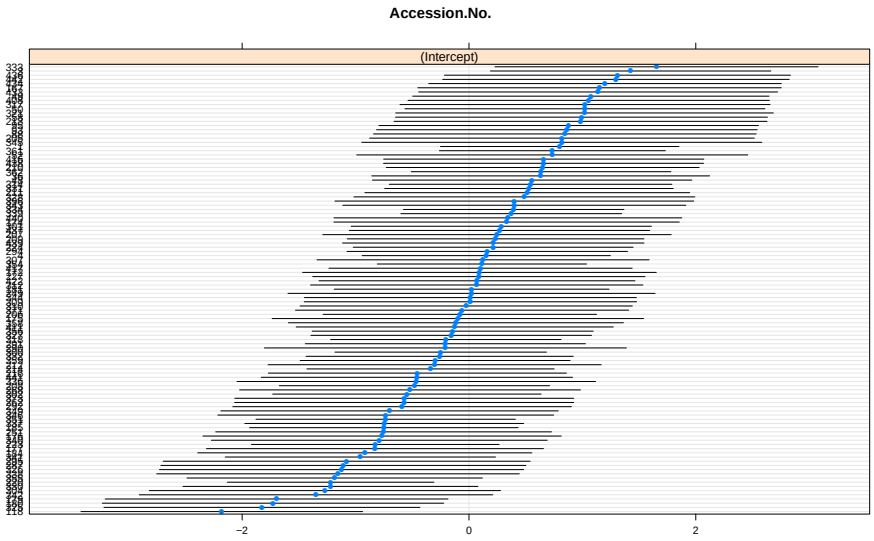
Results of parametric bootstrap challenge results of LRT:

```
> pr <- predict(glm(emer~Fruit.Size..mm.,family="binomial",data=d),type="response")
> p.val <- numeric()
> for(i in 1:100) {
+   random.emer <- rbinom(nrow(d),size=1,prob=pr)
+   rmod <- glm(random.emer~Fruit.Size..mm.+Accession.No.,
+               family="binomial",data=d)
+   p.val[i] <- drop1(rmod,test="Chisq")$"Pr(>Chi)"[3]
+ }
> sum(p.val<0.05)/length(p.val)
[1] 0.42
```

Possible explanation: convergence problems as GLM has many parameters. Better try GLMM approach.

```
> library(lme4)
> library(optimx)
> modm2 <- glmer(emer~Fruit.Size..mm.+(1|Species/Accession.No.),
                 family="binomial",data=d)
> summary(modm2)
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: emer ~ Fruit.Size..mm. + (1 | Species/Accession.No.)
   Data: d

     AIC      BIC   logLik deviance df.resid
   858.0    876.1   -425.0    850.0      665

Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.2321 -0.9739  0.5291  0.7314  1.4723

Random effects:
 Groups                Name        Variance Std.Dev.
 Accession.No.:Species (Intercept) 0.4187   0.6471
 Species               (Intercept) 0.0000   0.0000
Number of obs: 669, groups:  Accession.No.:Species, 107; Species, 12

Fixed effects:
               Estimate Std. Error z value Pr(>|z|)
(Intercept)    -1.27329    0.48181  -2.643  0.00822 **
Fruit.Size..mm.  0.14320    0.03604   3.973 7.09e-05 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

Correlation of Fixed Effects:
            (Intr)
Frt.Sz..mm. -0.972

> modm3 <- glmer(emer~Fruit.Size..mm.+(1|Accession.No.),nAGQ=50,family="binomial",data=d)
> confint(pr)
                     2.5 %     97.5 %
.sig01          0.40196098  0.9856675
(Intercept)    -2.23842728 -0.3029633
Fruit.Size..mm.  0.07195014  0.2165431
> dotplot(ranef(modm3,condVar=TRUE))
```

NOTE: simulated data, only for illustration; see paper for real data



4.2 Zero-Inflated Poisson Model

```
> library(glmmTMB)
> zimod <- glmmTMB(Fly.Emergence~Fruit.Size..mm. +(1|Species/Accession.No.), data=d,
+                  family = poisson(), ziformula = ~ (1|Species/Accession.No.))
> summary(zimod)
 Family: poisson  ( log )
Formula:          Fly.Emergence ~ Fruit.Size..mm. + (1 | Species/Accession.No.)
Zero inflation:                 ~(1 | Species/Accession.No.)
Data: d

     AIC      BIC   logLik deviance df.resid
  3018.4   3049.9  -1502.2   3004.4      662

Random effects:

Conditional model:
 Groups                Name        Variance Std.Dev.
 Accession.No.:Species (Intercept) 0.1535   0.3918
 Species               (Intercept) 0.1040   0.3225
Number of obs: 669, groups:  Accession.No.:Species, 107; Species, 12

Zero-inflation model:
 Groups                Name        Variance Std.Dev.
 Accession.No.:Species (Intercept) 0.7029   0.8384
 Species               (Intercept) 0.6329   0.7956
Number of obs: 669, groups:  Accession.No.:Species, 107; Species, 12

Conditional model:
               Estimate Std. Error z value Pr(>|z|)
(Intercept)      0.73088    0.22376   3.266  0.00109 **
Fruit.Size..mm.  0.06934    0.01345   5.155 2.53e-07 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

Zero-inflation model:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -0.6817     0.3239  -2.105   0.0353 *
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

Final remark on GLMMs and zero-inflated Poisson GLMs

GLMMs and zero-inflated Poisson GLMs models are difficult, even for computers. Make sure that you know what you are doing when you apply them to your data.

Some of what you should be able to explain

- Concept and model assumptions underlying Poisson and binomial GLMs
- Deviance
  - Analysis of deviance: why and how?
  - residual deviance and what it tells us
  - deviance residuals and how to analyse them
- When and how to account for overdispersion
- On which scale to calculate confidence intervals
- When to look into GLMMs or zero-inflated models