Analysis of the *Drosophila* Data Set

Age and copulation duration

The Kruskal-Wallis test is the non-parametric analog of one-way ANOVA. Friedman's test generalizes this to a randomized block design (two-way ANOVA without replication). The non-parametric tests relax the normality assumption of analysis of variance, but not the other assumptions. For example, the Kruskal-Wallis test assumes that the unknown distributions of the response differ only in location (mean), but are otherwise identical. If the shape of the distribution is determined by additional parameters those parameters should be identical for all treatments. So, the Kruskal-Wallis test relaxes normality but it doesn't, for example, relax homogeneity of variance.

If we examine the data we see that both the center of the distribution and the spread of the distribution change with treatment (Fig. 1).

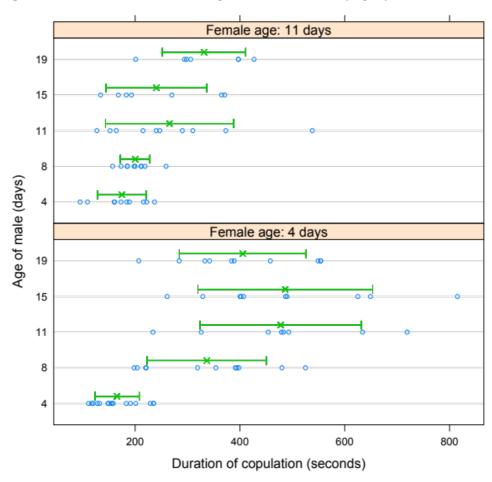


Fig. 1 Distribution of copulation duration times by male and female age. Raw data are shown along with the mean ± 1 std. dev.

As is typical with positive data the mean and the variance appear to be related; observations with larger means tend to be more variable. Treatment groups differ

not only in location but also in spread. This is a violation of a standard analysis of variance assumption. It also violates the non-parametric test assumption that the distributions of the groups differ only in location. There are two ways to proceed.

- 1. Carry out weighted least squares in which the assumption of a common variance for the different normal distributions is relaxed. The reciprocals of the sample estimates of the variance can be used as weights or a variance model that depends on a few parameters can be estimated simultaneously with the regression parameters.
- 2. Use a distribution for the response in which the mean and variance are related. Two common choices are a lognormal and a gamma distribution. The gamma distribution is more attractive because it is implemented in generalized linear model software and doesn't require transforming the response thus avoiding the interpretation problems that transformations cause.

In both the lognormal and the gamma distributions the variance is proportional to the square of the mean. This is equivalent to assuming that the standard deviation and the mean are linearly related. Fig. 2 plots the standard deviation versus the mean copulation duration time for the ten treatment groups. The plot reveals that a linear fit is quite good suggesting that a gamma distribution is a reasonable choice for the response.

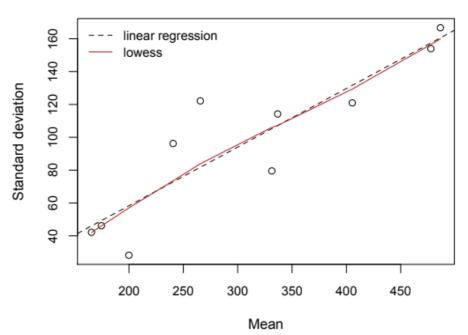


Fig. 2 Plot of egg count standard deviation versus egg count mean

Table 1 compares the fit of ANOVA models with a two-factor interaction between female and male age in which different distributions for copulation duration time are assumed. The fit of a gamma model is better than a normal model. We also see that the choice of link function has no effect on fit.

Table 1 Comparing the fit of two-factor interaction models

Model	Log-likelihood	AIC
Normal	-594.8	1211.6
Gamma (reciprocal link)	-573.9	1169.9
Gamma (log link)	-573.9	1169.9

The default link function for the gamma distribution is the reciprocal link. Because parameter estimates with this link are difficult to interpret the log link is generally used instead. The gamma model with a log link is the following.

$$y_i \sim \text{Gamma}(a', "b_i)$$

$$\log \mu_i = \beta_0 + \sum_{k=1}^4 \beta_k x_{ki} + \beta_5 z_i + \sum_{k=1}^4 \beta_{k+5} x_{ki} z_i$$

The x_k are the four dummy effect terms for male age and z is the dummy effect term for female age. Here a is the shape parameter and b_i is the scale parameter. The shape and scale parameters are related to the mean as follows.

$$b_i = \frac{\mu_i}{a}$$

An examination of the terms reported in a sequential ANOVA table reveals significant main effects as well as a significant interaction.

```
| Df | Deviance | Resid. | Df | Resid. | Dev | Deviance | Resid. | Dev | Deviance | Devi
```

The summary table below displays estimates of the effect terms.

```
printCoefmat(summary(out2a)$coefficients)
                              Estimate Std. Error t value Pr(>|t|)
                              5.108971 0.078279 65.2658 < 2.2e-16 ***
(Intercept)
                                      0.122640 5.7917 1.033e-07 ***
factor (Male.age) 8
                              0.710302
factor (Male.age) 11
                              1.078266 0.126222 8.5426 3.317e-13 ***
factor (Male.age) 15
                             0.895903 0.126222 7.0978 2.951e-10 ***
0.053526 0.126222 0.4241 0.6725406
factor (Male.age) 19
factor(Female.age)11
factor(Male.age)8:factor(Female.age)11 -0.574983 0.186143 -3.0889 0.0026799 **
0.199355 -3.8010 0.0002634 ***
```

The four main effect estimates for male age indicate that young females (females age 4 days) engage in longer copulations with older males (males age 8, 11, 15, and 19 days) than they do with the very youngest males (males age 4). The interaction effect terms describe how this pattern changes for older females (females age 11). Because the female age main effect and its interaction with the male age 19 effect are not significantly different from zero, we conclude that the copulation times of

older and younger females do not differ when they mate with the youngest (4 days) and oldest (19 days) males. Because the other three interaction effects are significant we conclude that old females (11 days) have copulations of significantly shorter duration with males of intermediate age (8, 11, and 15 days) than do young females (4 days).

These results are more clearly represented graphically. Fig. 3 compares the copulation duration time distributions for females age 4 and 11 days when stratified by male age. Box plots of the actual duration time distributions are displayed below the estimated gamma distributions. The two female age groups have distinct distributions only when the males are of intermediate ages (8, 11, and 15 days). In these cases younger females copulate longer than do older females.

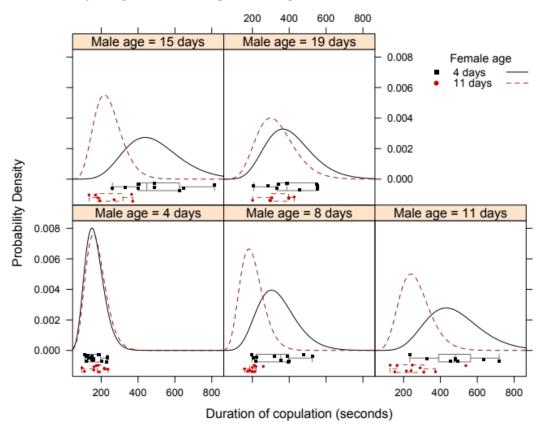


Fig. 3 Estimated gamma distributions that compare the effect of female age separately for each male age. The empirical distributions are shown below the estimated gamma distributions.

It is also informative to compare the individual male results grouped separately by the age of the female (Fig. 4). The narrow bars in Fig. 4 are 95% confidence intervals for the mean copulation duration times. The thick bars are 83.5% confidence intervals. For these data the 83.5% confidence intervals can be used to make pairwise comparisons of the mean copulation duration times for different male ages (Afshartous and Preston 2010; Payton et al. 2003). If the 83.5%

confidence intervals for a pair of male ages fail to overlap then the mean copulation duration times for those male ages are significantly different from each other.

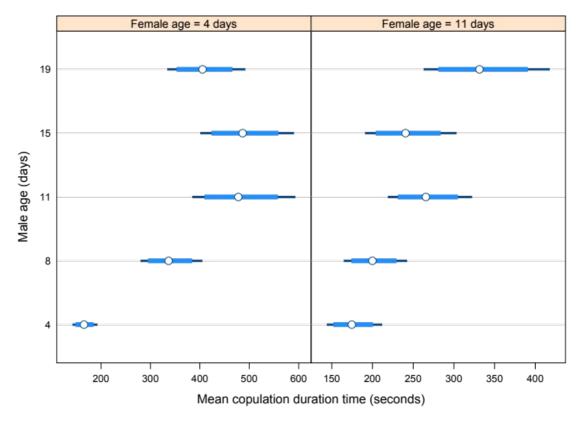


Fig. 4 ANOVA results from a gamma model comparing the male age effect separately for each female age. 95% (thin bar) and 83.5% (thick bar) confidence intervals for the gamma mean are shown. See text for an explanation of how these two intervals should be interpreted.

The left panel displays the results for 4-day old females. Because the 83.5% confidence interval of 4-day old males lies to the left of and does not overlap any other 83.5% confidence interval, 4-day old males have significantly shorter copulation times than do any of the other older males when the males are mated with 4-day old females. We also see that the 83.5% confidence interval of 8-day old males does not overlap that of the 11- and 15-day old males, so the mean copulation duration time of 8-day old males is less than that of 11- and 15-day old males. The remaining 83.5% confidence intervals overlap so no other pairwise comparison in the left panel is significant.

The right panel displays the situation for 11-day old females. Using the 83.5% confidence intervals we see that 4-day old males are not significantly different from 8-day old males, 8-day old males are not significantly different from 15-day old males, 11-day old males are not significantly different from 15- or 19-day old males, and 15-day old males are not significantly different from 19-day old males. All the remaining possible pairwise comparisons are significant.

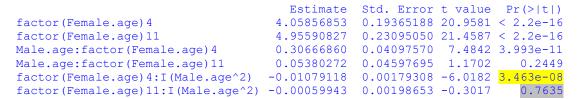
Fig. 4 reveals another interesting pattern. The effect of male age on mean copulation duration time appears to be quadratic for young females but linear for older females. There is a big initial effect of male age on copulation duration time for young females but this eventually reverses itself for the oldest males. As a result the copulation times of the two female age groups are not significantly different when they mate with very young or very old males (which is consistent with Fig. 3).

If we compare a model that treats male age as a factor with a quadratic male age model, allowing separate quadratic models for the two female age groups, AIC ranks the quadratic model as being more parsimonious than the factor model.

 Table 2 Comparing a factor male age model with a quadratic male age model

Model	df	Log- likelihood	AIC
Factor male age	11	-573.9	1169.9
Quadratic male	7	-575.8	1165.5
age			

Examining the model coefficients we see that the quadratic term is significantly different from zero only for females age 4. For females age 11 we just have a linear relationship. The graphs of the two models are shown in Fig. 5.



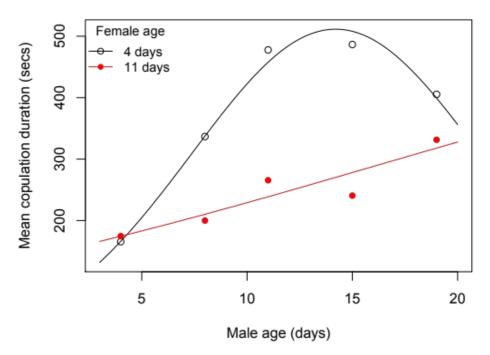


Fig. 5 Quadratic model for mean copulation duration as a function of male age. Empirical mean copulation times at each tested male age are also shown.

The effect of age on egg laying

If we group the egg count data by male and female age and calculate the mean and variance of each group we find that the variance exceeds the mean (Table 3). In a Poisson distribution the mean and variance are equal. If we fit Poisson and negative binomial distributions separately to each group the log-likelihood of the negative binomial fit far exceeds the log-likelihood of the Poisson fit. Thus we have evidence that the egg counts are overdispersed relative to a Poisson distribution.

Table 3	The egg count of	lata are overdisp	ersed relative to	a Poisson distribution
---------	------------------	-------------------	-------------------	------------------------

Female	Male	Mean	Varianc	Variance to Poisson log-		NB1 log-
age	age		e	mean ratio	likelihood	likelihood
4	4	78.7	1916.0	24.4	-287.1	-80.6
4	11	125.1	2645.5	21.1	-86.1	-36.6
4	19	95.0	864.0	9.1	-59.3	-38.2
11	4	45.6	1344.5	29.5	-194.8	-40.5
11	11	71.0	2082.2	29.3	-174.9	-51.7
11	19	53.3	1405.9	26.4	-110.4	-29.7

Alternatives to a Poisson distribution for count data are the two negative binomial distributions, the NB1 model (where the variance is proportional to the square of the mean) and the NB2 model (where the variance is proportional to the mean, a linear relationship). A non-likelihood based approach that is comparable to the NB2 model is the quasi-Poisson. Five of the six variance-to-mean ratios reported in Table 3 are in the 20s suggesting that perhaps a linear relationship is appropriate. However when we plot the variance versus the mean, neither a linear relationship nor a quadratic relationship turn out to be statistically significant. An outlier corresponding to the coupling of 4-year old females with 19-year old males is apparently responsible for this lack of signficance.

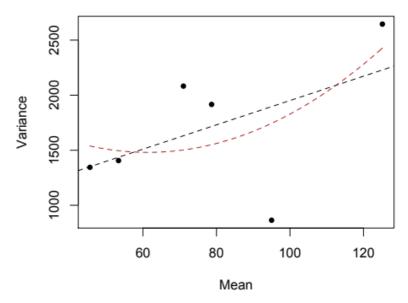


Fig. 6 Mean-variance relationship of the egg count data

If we examine the parameter estimates from the separate NB1 models that were fit in Table 3, we see that the dispersion parameter varies considerably across groups suggesting that a constant dispersion model may not be appropriate (Table 4). Fig. 7 displays the estimated negative binomial distributions contrasted with the empirical distribution of the egg counts for each group.

Table 4 Estimated parameters from individual NB1 models

Female age	Male age	Mean	Dispersion (θ)
4	4	78.67	1.06
4	11	125.14	7.29
4	19	95.00	11.82
11	4	45.56	0.32
11	11	71.00	1.94
11	19	53.33	0.69

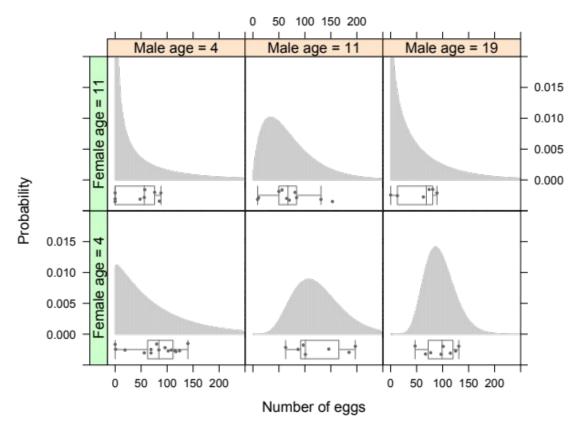


Fig. 7 NB1 distributions for egg counts separately by female and male age

Side-by-side boxplot displays of the egg counts suggest that the ages of males and females affect egg count differently (Fig. 8). The effect of female age is obvious —all the count distributions of young females are shifted upward relative to those of old females. The pattern of male effects appears to be exactly the same in the two female age groups indicating that male age and female age do not interact in their effect on egg count.

Male.age*Female.age

out.g4

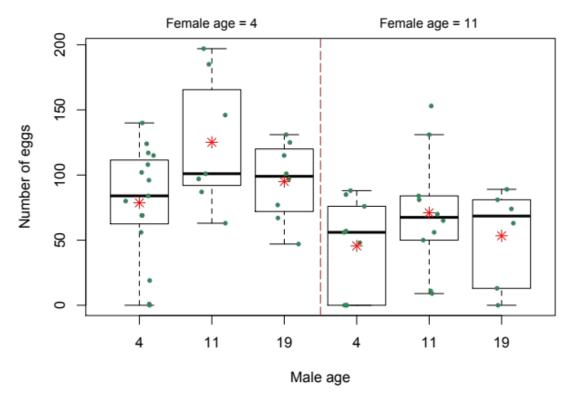


Fig. 8 Box plots showing the distribution of egg counts separately by female and male age. (I denotes the mean).

Although the glm.nb function of the MASS package of R fails to fit negative binomial regression (NB1) models to these data, we can use the gamlss function from the R gamlss package instead (Rigby and Stasinopoulos 2005). Additional references for this package can be found at the gamlss web site (www.gamlss.org). Gamlss extends GLMs to include a number of additional distributions. The package uses a likelihood framework to fit regression models using either the NB1 or NB2 distributions (making the quasi-Poisson model unnecessary). In addition to modeling the mean of the negative binomial distribution, gamlss permits modeling of the reciprocal of the dispersion parameter (which it calls sigma) using a log link.

I fit a sequence of models for the mean of an NB1 distribution using female age and male age as predictors. I fit these models assuming a common value of the dispersion as well as models that allow each male-female combination to have its own value of the dispersion parameter. Table 5 summarizes the results.

Model	Predictor	Dispersion	K	LL	AIC
out.g0	1	common	2	-294.5	593.0
out.g1	Male.age	common	4	-293.9	595.8
out.g2	Female.age	common	3	-292.8	591.6
out.g3	Male.age + Female.age	common	5	-291.6	593.3

common

-291.6

597.3

Table 5 Comparison of NB1 models with male and female age as predictors

out.g0a	1	separate	7	-280.9	575.8	
out.g1a	Male.age	separate	9	-279.8	577.7	
out.g2a	Female.age	separate	8	-279.2	<mark>574.3</mark>	
out.g3a	Male.age + Female.age	separate	10	-277.2	<mark>574.5</mark>	
out.g4a	Male.age*Female.age	separate	12	-277.2	578.5	

According to the AIC rankings each group needs to have its own dispersion. The AIC best-ranked model has separate dispersions and includes only a female age effect on the mean. When we test the female age predictor in this model using a likelihood ratio test it just misses being statistically significant.

```
LR.test(out.g0a,out.g2a)
Likelihood Ratio Test for nested GAMLSS models.

Null model: deviance= 561.7582 with 7 deg. of freedom
Altenative model: deviance= 558.3262 with 8 deg. of freedom

LRT = 3.431973 with 1 deg. of freedom and p-value= 0.06394568
```

The AIC second best model includes both female age and male age as predictors. Although male age is not statistically significant in this model we find that by controlling for male age female age has become statistically significant. This is a legitimate reason for retaining male age in the model regardless of its statistical significance (Kleinbaum et al. 1988, p. 170). This result is consistent with Fig. 8 where we saw evidence of roughly the same female age effect at each male age separately, i.e., when controlling for male age.

Age, copulation duration, and egg counts

The need to have a separate dispersion parameter for each group indicates that even after controlling for age there are still differences between the groups that have not been accounted for. Differences in dispersion indicate heterogeneity within the groups. We already know that copulation duration varies among individuals within groups. Including this variable in the model might account for some of the group heterogeneity and make separate dispersions unnecessary.

I fit a sequence of models that include copulation duration time, male age, and female age as predictors. It turns out that with copulation duration as a predictor, the NB2 models consistently beat the comparable NB1 models in terms of AIC even when the NB1 models include separate dispersions. As a result I focus on the NB2 models exclusively here. Table 6 reports the results of a top-down analysis that starts with the full 3-factor interaction model and tries to simplify it by dropping higher-order interaction terms that are not statistically significant while adhering to the principle of marginality. The likelihood ratio test that is shown compares the model in that row with the model directly above it in the table.

Table 6 Comparison of NB2 models that include copulation duration, male age, and female age as predictors

Model	Predictors	K	LL	AIC	LR test (p- valu e)
3-factor interaction	Copulation.duration* Male.age* Female.age	13	- 277.3	580.6	
Three two-factor interactions	Copulation.duration* Male.age + Copulation.duration* Female.age + Male.age*Female.age	11	- 278.3	578.7 3	0.36
Two two-factor interactions	Copulation.duration* Male.age + Copulation.duration* Female.age	9	- 278.8	575.6	0.63
One two-factor interactions	Copulation.duration* Male.age + Female.age	8	- 279.1	<mark>574.1</mark>	0.48
Main effects only	Copulation.duration + Male.age + Female.age	6	- 284.1	580.2	0.007

The best model contains a main effect of female age and an interaction between copulation duration and male age. This model and its parameter estimates are shown below.

$$y_{i} \sim NB2(\mu_{i}, \sigma)$$

$$\log \mu_{i} = \beta_{0} + \beta_{1}x_{i} + \beta_{2}z_{1i} + \beta_{3}z_{2i} + \beta_{4}w_{i} + \beta_{5}x_{i}z_{1i} + \beta_{6}x_{i}z_{2i}$$

The variables z_1 and z_2 are dummy variables for male age (indicators for age 11 and age 19 respectively), w is a dummy variable for female age (an indicator of age 11),

and *x* is copulation duration time. Fig. 9 displays the estimated mean of the NB2 distribution as predicted by the model separately for the different age combinations.

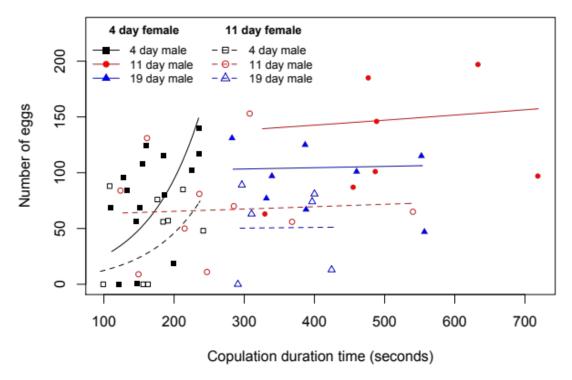


Fig. 9 Negative binomial means from an NB2 regression model containing an interaction between male age and copulation duration time as well as a main effect of female age.

By reparameterizing the regression model we can obtain the individual regression equations at each male age. Doing so we find that the coefficient of the copulation duration term for 11-day and 19-day males is not significantly different from zero. (These tests correspond to the interactions between copulation duration time and male age and are highlighted in the summary table below.)

```
Mu link function:
Mu Coefficients:
                                           Estimate
                                                     Std. Error
                                                                  t value
factor (Male.age) 4
                                           1.924545
                                                        0.697016
                                                                   2.7611
                                                                            8.19e-03
                                                        0.425095
factor (Male.age) 11
                                           4.837343
                                                                  11.3794
                                                        0.778578
factor (Male.age) 19
                                           4.603791
                                                                   5.9131
                                                                            3.64e-07
factor (Female.age) 11
                                          -0.718575
                                                        0.236982
                                                                   -3.0322
                                                                            3.94e - 03
Copulation.duration:factor(Male.age)4
                                           0.013106
                                                        0.003316
                                                                    3.9528
Copulation.duration:factor(Male.age)11
                                           0.000307
                                                        0.000713
                                                                    0.4312
                                                                            6.68e-01
Copulation.duration:factor(Male.age)19
                                           0.000113
                                                        0.001584
                                                                    0.0715
```

The model indicates that when copulations are with 4-day old males there is a positive linear relationship between egg count and copulation duration time. When copulations are instead with 11-day or 19-day old males copulation duration time does not significantly affect egg count. In all cases copulations with 4-day old females result in higher average egg counts than copulations with 11-day old females regardless of the age of the male.

Based on the relative magnitudes of the individual coefficients, the above model could be simplified further by collapsing the 11-day and 19-day male age categories together. If we do so we find that the reduced two-category male age model and the three-category male age model are not significantly different from each other.

```
#compare two-category male model to a three-category male model
LR.test(nb6d, nb6a)
Likelihood Ratio Test for nested GAMLSS models.

Null model: deviance= 560.02 with 6 deg. of freedom
Altenative model: deviance= 558.1 with 8 deg. of freedom

LRT = 1.9201 with 2 deg. of freedom and p-value= 0.38287
```

However when we try to examine the summary table of the reduced model the warning message "vcov has failed, option qr is used instead" is displayed. Rigby and Stasinopoulos (2009) explain this error message as follows (p. 78).

This is because the function vcov which is the default option for obtaining standard errors in summary() has failed, probably because of the peculiarity of the likelihood function at the point of the (possibly local) maximum, a consequence of a kurtotic parameters being less than one. The standard errors given, obtained from the individual fits by assuming that the rest of the parameters are fixed at their point of maximum, should be treated with caution.

The GAMLSS manual (Stasinopoulos et al. 2008) expands on these comments (p. 23).

The summary function has two ways of producing standard errors. The default value is type="vcov". This uses the vcov method for gamlss objects which in turn uses a non linear fitting, see Chapter ??, with only one iteration at the maximum to obtain the Hessian matrix. Standard errors are obtained from the observed information matrix (the inverse of the Hessian). The standard errors obtained this way are reliable since they take into the account the interrelationship between the distribution parameters i.e. μ and σ in the above case. On occasions, when the above procedure fails, the standard errors are obtained from type="qr" which uses the individual fits of the parameters (used in the gamlss() algorithms) and therefore should be used with caution.

The presence of the warning suggests that we should stick with the three-category male age model because the standard errors reported there are obtained using the vcov function and hence are more reliable.

Cited references

Afshartous, D. and R. A. Preston. 2010. Confidence intervals for dependent data: Equating non-overlap with statistical significance. *Computational Statistics and Data Analysis* **54**: 2296–2305.

Kleinbaum, D. G., L. L. Kupper, and K. E. Muller. 1988. *Applied Regression Analysis and other Multivariable Methods*. PWS-Kent Publishing Co., Boston.

- Payton, M. E., M. H. Greenstone, and N. Schenker. 2003. Overlapping confidence intervals or standard error intervals: What do they mean in terms of statistical significance? 6pp. *Journal of Insect Science* **3**: 34.
- Rigby, R. A. and D. M. Stasinopoulos. 2005. Generalized additive models for location, scale and shape (with discussion). *Applied Statistics* **54**: 507–554.
- Rigby, R. A. and D. M. Stasinopoulos. 2009. *A flexible regression approach using GAMLSS in R.* http://www.gamlss.org/images/stories/papers/Lancaster-booklet.pdf [designed for a short course in GAMLSS given at the University of Lancaster]
- Stasinopoulos, D. M., R. A. Rigby, and C. Akantziliotou. 2008. *Instructions on how to use the gamlss package in R, 2nd Edition*.
 - http://www.gamlss.org/images/stories/papers/gamlss-manual.pdf