Experiment 3 – Pupal Development

## Experiment 3–Pupae–Overview

A third experiment examined whether emerged neonates observed in Experiment 2 would continue to develop, thereby damaging their host. Males and females irradiated at 250 and 350 Gy were compare to 0 Gy sham treatments. Response variables examined were the proportion of moths that mated, total fecundity, and the number of F1 that developed to the point of cocooning (prepupa or pupa).

## Mating

Summarize the proportion of moths mated by dose and sex

# Load the data set  
library(readxl)  
df\_mating350 <- read\_excel("Dataset.xlsx", sheet = "Mating\_350Gy")  
  
# Make dose a categorical variable  
df\_mating350$Dose <- as.factor(df\_mating350$Dose)  
df\_mating350

## # A tibble: 30 × 3  
## Dose Sex Mated  
## <fct> <chr> <chr>  
## 1 0 F Yes   
## 2 0 F Yes   
## 3 0 F Yes   
## 4 0 F Yes   
## 5 0 F Yes   
## 6 250 F Yes   
## 7 250 F Yes   
## 8 250 F Yes   
## 9 250 F Yes   
## 10 250 F Yes   
## # ℹ 20 more rows

# Summarize data  
library(dplyr)  
library(tidyr)  
tbl\_mating3 <- df\_mating350 %>%   
 group\_by(Sex,Dose) %>%   
 summarise(nObs = n(),  
 nMated = sum(Mated == "Yes"),  
 pct\_mated = 100\*nMated/nObs)  
tbl\_mating3

## # A tibble: 6 × 5  
## # Groups: Sex [2]  
## Sex Dose nObs nMated pct\_mated  
## <chr> <fct> <int> <int> <dbl>  
## 1 F 0 5 5 100  
## 2 F 250 5 5 100  
## 3 F 350 5 5 100  
## 4 M 0 5 4 80  
## 5 M 250 5 5 100  
## 6 M 350 5 4 80

Determine if there are significant differences with logistic regression

# Apply categorical logistic regression model  
m1 <- glm(cbind(nMated,nObs) ~ Sex + Dose, family = binomial, data = tbl\_mating3)  
  
# Display basic Analysis of Deviance  
summary(m1)

##   
## Call:  
## glm(formula = cbind(nMated, nObs) ~ Sex + Dose, family = binomial,   
## data = tbl\_mating3)  
##   
## Coefficients:  
## Estimate Std. Error z value Pr(>|z|)  
## (Intercept) -3.644e-02 5.226e-01 -0.070 0.944  
## SexM -1.458e-01 5.266e-01 -0.277 0.782  
## Dose250 1.093e-01 6.418e-01 0.170 0.865  
## Dose350 3.609e-17 6.502e-01 0.000 1.000  
##   
## (Dispersion parameter for binomial family taken to be 1)  
##   
## Null deviance: 0.153703 on 5 degrees of freedom  
## Residual deviance: 0.040652 on 2 degrees of freedom  
## AIC: 24.642  
##   
## Number of Fisher Scoring iterations: 3

# Examine model fit using residual deviance and residual degrees of freedom  
resid\_dev <- deviance(m1)  
resid\_df <- df.residual(m1)  
pchisq(resid\_dev, df = resid\_df, lower.tail = FALSE)

## [1] 0.9798792

Far from 0.05–the model fits

library(car)  
# Type II test for predictors  
Anova(m1)

## Analysis of Deviance Table (Type II tests)  
##   
## Response: cbind(nMated, nObs)  
## LR Chisq Df Pr(>Chisq)  
## Sex 0.076718 1 0.7818  
## Dose 0.039051 2 0.9807

No significant effect on mating due to sex or dose

## Effect of 250 and 350 Gy on total fecundity

Total fecundity in this case is actually the total number of eggs laid over 3 days following mating

library(dplyr)  
library(FSA) # for se() function  
  
# Load egg data  
df\_eggs350 <- read\_excel("Dataset.xlsx", sheet = "Oviposition\_350Gy")  
  
# Make dose categorical  
df\_eggs350$dose <- as.factor(df\_eggs350$dose)  
  
# Summarize eggs by sex irradiated and x-ray dose  
df\_eggs350 %>%   
 group\_by(Sex,dose) %>%   
 summarise(nObs = sum(!is.na(Eggs)),   
 mn = mean(Eggs, na.rm = TRUE),  
 sem = se(Eggs))

## # A tibble: 6 × 5  
## # Groups: Sex [2]  
## Sex dose nObs mn sem  
## <chr> <fct> <int> <dbl> <dbl>  
## 1 F 0 5 132. 23.2  
## 2 F 250 5 91.2 34.8  
## 3 F 350 5 39.4 24.7  
## 4 M 0 4 144 45.5  
## 5 M 250 5 84 35.1  
## 6 M 350 4 67.5 35.7

Test for significant effects using a GLM with negative binomial distribution

# Examine GLM for eggs  
m2 <- MASS::glm.nb(Eggs ~ Sex + dose, data = df\_eggs350)  
summary(m2)

##   
## Call:  
## MASS::glm.nb(formula = Eggs ~ Sex + dose, data = df\_eggs350,   
## init.theta = 0.9704783002, link = log)  
##   
## Coefficients:  
## Estimate Std. Error z value Pr(>|z|)   
## (Intercept) 4.8452 0.3808 12.724 <2e-16 \*\*\*  
## SexM 0.1717 0.3875 0.443 0.6578   
## dose250 -0.4511 0.4690 -0.962 0.3361   
## dose350 -0.9914 0.4816 -2.058 0.0396 \*   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## (Dispersion parameter for Negative Binomial(0.9705) family taken to be 1)  
##   
## Null deviance: 36.280 on 27 degrees of freedom  
## Residual deviance: 32.117 on 24 degrees of freedom  
## AIC: 315.26  
##   
## Number of Fisher Scoring iterations: 1  
##   
##   
## Theta: 0.970   
## Std. Err.: 0.237   
##   
## 2 x log-likelihood: -305.263

# Examine model fit  
resid\_dev2 <- deviance(m2)  
resid\_df2 <- df.residual(m2)  
pchisq(resid\_dev2, df = resid\_df2, lower.tail = FALSE)

## [1] 0.1241174

Residual deviance not significantly different from expected value

# Type II test for female eggs  
Anova(m2)

## Analysis of Deviance Table (Type II tests)  
##   
## Response: Eggs  
## LR Chisq Df Pr(>Chisq)  
## Sex 0.1955 1 0.6584  
## dose 4.0857 2 0.1297

Differences in eggs per female between doses are not significant for irradiated females

## Pupae per female

Report mean and standard error of F1 cocoons per female by irradiated sex

df\_pupae350 <- read\_excel("Dataset.xlsx", sheet = "Development\_350Gy")  
  
# Rename irradiation variable and make it categorical  
names(df\_pupae350)[names(df\_pupae350) == "TrtLabel"] <- "Dose"  
df\_pupae350$Dose <- as.factor(df\_pupae350$Dose)  
  
# Get cocoons per female by Sex irradiated and dose  
df\_pupae350 %>%   
 group\_by(Sex, Dose) %>%   
 summarise(nObs = sum(!is.na(Pupae)),  
 mn = mean(Pupae, na.rm = TRUE),  
 sem = se(Pupae))

## # A tibble: 6 × 5  
## # Groups: Sex [2]  
## Sex Dose nObs mn sem  
## <chr> <fct> <int> <dbl> <dbl>  
## 1 F 0 5 48.2 4.91   
## 2 F 250 5 0.2 0.2   
## 3 F 350 5 2.8 0.970  
## 4 M 0 4 60.2 20.2   
## 5 M 250 5 1.8 0.490  
## 6 M 350 4 2.5 1.19

Use GLM with nb to perform Analysis of Deviance examining impact of sex and irradiation

m3 <- MASS::glm.nb(Pupae ~ Sex + Dose, data = df\_pupae350)  
summary(m3)

##   
## Call:  
## MASS::glm.nb(formula = Pupae ~ Sex + Dose, data = df\_pupae350,   
## init.theta = 2.241529273, link = log)  
##   
## Coefficients:  
## Estimate Std. Error z value Pr(>|z|)   
## (Intercept) 3.8034 0.2713 14.020 < 2e-16 \*\*\*  
## SexM 0.3914 0.3302 1.185 0.236   
## Dose250 -4.0544 0.4503 -9.004 < 2e-16 \*\*\*  
## Dose350 -2.9888 0.3780 -7.908 2.62e-15 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
##   
## (Dispersion parameter for Negative Binomial(2.2415) family taken to be 1)  
##   
## Null deviance: 157.257 on 27 degrees of freedom  
## Residual deviance: 28.752 on 24 degrees of freedom  
## AIC: 161.3  
##   
## Number of Fisher Scoring iterations: 1  
##   
##   
## Theta: 2.242   
## Std. Err.: 0.922   
##   
## 2 x log-likelihood: -151.303

# Examine model fit  
resid\_dev3 <- deviance(m3)  
resid\_df3 <- df.residual(m3)  
pchisq(resid\_dev3, df = resid\_df3, lower.tail = FALSE)

## [1] 0.2296217

The ns value indicates no evidence of lack of model fit. Examine type II Analysis of Deviance

# Type II test for female eggs  
Anova(m3)

## Analysis of Deviance Table (Type II tests)  
##   
## Response: Pupae  
## LR Chisq Df Pr(>Chisq)   
## Sex 1.377 1 0.2406   
## Dose 128.154 2 <2e-16 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Examine differences of means for Dose

library(emmeans)  
library(multcomp)  
emm <- emmeans(m3, ~ Dose, type = "response")  
multcomp::cld(emm, Letters = LETTERS, decreasing = TRUE)

## Dose response SE df asymp.LCL asymp.UCL .group  
## 0 54.549 12.400 Inf 34.892 85.28 A   
## 350 2.746 0.829 Inf 1.520 4.96 B   
## 250 0.946 0.366 Inf 0.443 2.02 B   
##   
## Results are averaged over the levels of: Sex   
## Confidence level used: 0.95   
## Intervals are back-transformed from the log scale   
## P value adjustment: tukey method for comparing a family of 3 estimates   
## Tests are performed on the log scale   
## significance level used: alpha = 0.05   
## NOTE: If two or more means share the same grouping symbol,  
## then we cannot show them to be different.  
## But we also did not show them to be the same.