

Can chicks smell their parents? No evidence of olfactory parent recognition in a shorebird

R code

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Required packages

```
library(ggplot2) # for plotting
library(ggpubr) # for plotting (ggqqplot)
library(lemon) # for plotting (coord_capped_cart)
library(dplyr)
library(glmmTMB) # to run beta regressions
library(lme4) # to run mixed models (GLMM)
library(broom.mixed) # to get confidence intervals for fixed effects
library(performance) # to test models and more
```

1 Data

1.1 Data for analysis

In the data used for the analysis, each row corresponds to a trial.

Import data

```
data <- read.csv("ymazeplevers_data_horizontal.csv")
```

Check data

```
str(data)
```

Convert variables that need to be converted

```
data$trial_type <- as.factor(data$trial_type)
data$nest <- as.factor(as.character(data$nest))
data$first_type <- as.factor(data$first_type)
```

For two chicks, trial A was unsuccessful and was repeated one day later. Remove the first trial of each chick (76 trials - 2 = 74 trials) so that we have no repeated data for each chick.

```
data <- data[data$trial != "19A" & data$trial != "20A",]
```

Calculate the proportion of time spent with unrelated adult odour relative to time spent with both odours

```
data$prop_dur_np <- data$np_duration/(data$left_duration+data$right_duration)
```

Chicks participated in two consecutive trials:

- Conspecific odour discrimination (trial A): unrelated adult odour vs no odour
- Parent odour discrimination (trial B): unrelated adult odour vs parent odour

1.1.1 Data for conspecific odour discrimination (trial A)

Subset data on successful trials A (N = 29)

```
data_a <- data[data$trial_type == "A" & data$trial_success == 1,]
```

Subset data excluding trials where the sex of the unrelated adult odour is unknown ($N = 29 - 1 = 28$)

```
data_a_sex <- data_a[!is.na(data_a$np_sex),]
```

Sex of unrelated parent odours used in trials A

```
table(data_a$np_sex)
```

```
##
##  f  m
## 11 17
```

1.1.2 Data for parent odour discrimination (trial B)

Subset data on successful trials B ($N = 24$)

```
data_b <- data[data$trial_type == "B" & data$trial_success == 1,]
```

Subset data excluding trials where the sex of the odour (unrelated adult or parent) is unknown ($N = 24 - 1 = 23$)

```
data_b_sex <- data_b[!is.na(data_b$np_sex) & !is.na(data_b$p_sex),]
```

Sex of odours (unrelated adult or parent) used in trials B

```
table(data_b$p_sex, data_b$np_sex, deparse.level = 2)
```

```
##           data_b$np_sex
## data_b$p_sex  f  m
##           f 10  4
##           m  0  9
```

Create a new variable describing the combination of sex of odours (parent and unrelated adult odour) used in trials B

```
data_b_sex$odour_sex_comb <- paste(data_b_sex$p_sex, data_b_sex$np_sex)
table(data_b_sex$odour_sex_comb)
```

```
##
## f f f m m m
## 10  4  9
```

1.1.3 Preference measures

To measure the preference of the chicks to the different odours (trial A: unrelated adult vs control, trial B: unrelated vs parent), we test used two measures: time spent with odour and odour visited first.

1. Time spent with odour (overall preference): proportion of time spent with unrelated adult odour relative to time spent with both odours

For trial A:

- values > 0.5 indicate a preference (more time spent) for the unrelated adult odour
- values < 0.5 indicate a preference (more time spent) for the control

For trial B:

- values > 0.5 indicate a preference (more time spent) for the unrelated adult odour
- values < 0.5 indicate a preference (more time spent) for the parent odour

2. Odour visited first (first choice)

The odour visited first may indicate a preference for that odour.

Check whether the preference measures are independent:

In trials A, is the time spent with an odour related to whether this odour was visited first?

```
t.test(data_a[data_a$first_type=="np"],$prop_dur_np, data_a[data_a$first_type=="c"],$prop_dur_np)

##
## Welch Two Sample t-test
##
## data: data_a[data_a$first_type == "np", ]$prop_dur_np and data_a[data_a$first_type == "c", ]$prop_dur_np
## t = 3.1075, df = 24.778, p-value = 0.004687
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## 0.08633161 0.42610662
## sample estimates:
## mean of x mean of y
## 0.6593728 0.4031537
```

In trials A, chicks spent more time with the odour they visited first (i.e. the measures are not independent)

In trials B, is the time spent with an odour related to whether this odour was visited first?

```
t.test(data_b[data_b$first_type=="np"],$prop_dur_np, data_b[data_b$first_type=="p"],$prop_dur_np)

##
## Welch Two Sample t-test
##
## data: data_b[data_b$first_type == "np", ]$prop_dur_np and data_b[data_b$first_type == "p", ]$prop_dur_np
## t = -0.47273, df = 23.633, p-value = 0.6407
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.2365998 0.1484728
```

```
## sample estimates:  
## mean of x mean of y  
## 0.3956304 0.4396939
```

In trials B, chicks did not spend more time with the odour they visited first (i.e. measures are independent).

1.1.4 Details on trials

Number of trials

```
nrow(data)
```

```
## [1] 75
```

Success rate of each type of trial

```
table(data$trial_type,data$trial_success)
```

```
##  
##      0  1  
##      0  0  
##   A 15 29  
##   B  6 24
```

Number of chicks tested

```
length(unique(data$chick))
```

```
## [1] 45
```

```
sum(duplicated(data$chick))
```

```
## [1] 30
```

44 chicks were tested in total, among which 30 participated in both trial A and B and 14 participated in trial A only.

Number of families tested

```
length(unique(data$nest))
```

```
## [1] 34
```

From 33 families (1 chick per family in 22 families, 2 chicks per family in 11 families).

Sex of the chicks

```
table(data[data$trial_type == "A",]$sex)
```

```
##  
## f m  
## 19 23
```

Age of the chicks

```
summary(data[data$trial_type == "A",]$age)
```

```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.  
##    1.000   2.000   4.000   8.273  14.750  23.000
```

```
sd(data[data$trial_type == "A",]$age)
```

```
## [1] 7.794941
```

Number of odours used in trials

```
length(unique(unlist(c(data$left_id,data$right_id))))
```

```
## [1] 35
```

Number of times each odour was used (mean & SD)

```
data.frame(mean(c(data$np_used,data$p_used), na.rm = T),sd(c(data$np_used,data$p_used), na.rm = T))
```

```
## mean.c.data.np_used..data.p_used...na.rm...T.  
## 1 2.413462  
## sd.c.data.np_used..data.p_used...na.rm...T.  
## 1 1.39075
```

Number of days before an unrelated adult odour was used in a trial (mean & SD)

```
data.frame(mean(data$trial_np_diffdate), sd(data$trial_np_diffdate))
```

```
## mean.data.trial_np_diffdate. sd.data.trial_np_diffdate.  
## 1 NA NA
```

Number of days before a parent odour was used in a trial (mean & SD)

```
data.frame(mean(data$trial_p_diffdate, na.rm = T), sd(data$trial_p_diffdate,na.rm = T))
```

```
## mean.data.trial_p_diffdate..na.rm...T. sd.data.trial_p_diffdate..na.rm...T.  
## 1 0 0
```

1.2 Data for plot

In the data used for the plot, each row corresponds to an arm of the Y-maze during a trial.

Import data

```
data_plot <- read.csv("ymazeplevers_data_vertical.csv")
```

Prepare data

```
# keep only rows on left and right arm
data_plot <- subset(data_plot, maze_area=="left" | maze_area=="right")
# keep only successful trials
data_plot <- data_plot[data_plot$trial_success==1,]
```

2 Analysis

2.1 Conspecific odour discrimination (trial A)

2.1.1 Time spent with odour

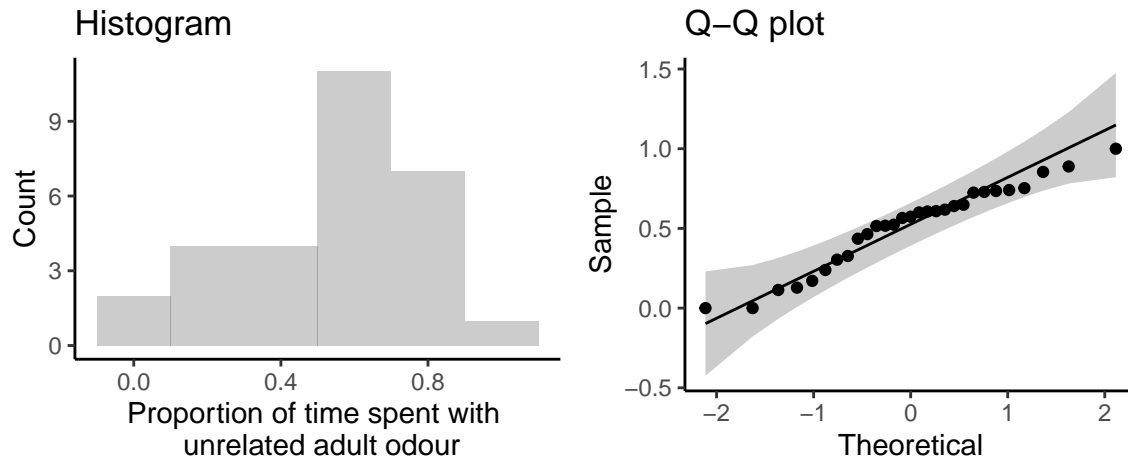
Mean time (and SD) spent by chicks in each arm

```
mean_np <- mean(data_a$np_duration)
sd_np <- sd(data_a$np_duration)
mean_c <- mean(data_a$c_duration)
sd_c <- sd(data_a$c_duration)
data.frame(Variable = c("Time spent with unfamiliar adult odour (s)", "Time spent with no odour (s)"),
            Mean = c(mean_np, mean_c),
            SD = c(sd_np, sd_c))
```

```
##                               Variable      Mean      SD
## 1 Time spent with unfamiliar adult odour (s) 131.0126 101.5941
## 2                Time spent with no odour (s) 122.9225 106.1707
```

Verify that the response variable is normally distributed

```
x <- ggplot(data_a, aes(x=prop_dur_np)) +
  geom_histogram(binwidth=0.2, alpha=.2, fill="black") +
  theme_classic() + ggtitle("Histogram") +
  xlab("Proportion of time spent with \n unrelated adult odour") + ylab("Count")
y <- ggqqplot(data_a$prop_dur_np, color = "black") +
  theme_classic() + ggtitle("Q-Q plot")
ggarrange(x, y, ncol = 2, nrow = 1)
```



Verify that there is no outlier

```
check_outliers(data_a$prop_dur_np)
```

```
## OK: No outliers detected.
## - Based on the following method and threshold: zscore_robust (3.291).
## - For variable: data_a$prop_dur_np
```

T-test (two-tailed one sample)

```
t.test(data_a$prop_dur_np, mu = 0.5, alternative = "two.sided")
```

```
##
## One Sample t-test
##
## data: data_a$prop_dur_np
## t = 0.36908, df = 28, p-value = 0.7148
## alternative hypothesis: true mean is not equal to 0.5
## 95 percent confidence interval:
## 0.4180522 0.6179690
## sample estimates:
## mean of x
## 0.5180106
```

Chicks spent has much time in the arm with the unrelated adult odour as in the arm with no odour, they showed no preference.

To run a beta regression, we need to transform the response variable (proportion of time spent with the unrelated adult odour) so that it does not include any 0 or 1.

Transformation $(y \cdot (n-1) + 0.5) / n$ (y: response variable, n: sample size; Smithson and Verkuilen 2006)

```
data_a_sex$prop_dur_np_transf <- (data_a_sex$prop_dur_np * (nrow(data_a_sex) - 1) + 0.5) / nrow(data_a_sex)
```

Beta regression to test for the effect of sex and freshness of the test odour


```
beta_dur_a <- glmmTMB(prop_dur_np_transf ~ np_sex + trial_np_diffdate, data = data_a_sex,
  family = beta_family(link = "logit"))
summary(beta_dur_a)
```

```
## Family: beta ( logit )
## Formula:      prop_dur_np_transf ~ np_sex + trial_np_diffdate
## Data: data_a_sex
##
##      AIC      BIC    logLik deviance df.resid
##      6.9     12.2      0.6     -1.1      24
##
##
## Dispersion parameter for beta family (): 2.51
##
## Conditional model:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)   -0.23553    0.53542  -0.440   0.660
## np_sex       0.25121    0.47119   0.533   0.594
## trial_np_diffdate 0.02111    0.10210   0.207   0.836
```

Beta estimates (with confidence intervals) of fixed effects

```
broom.mixed::tidy(beta_dur_a, conf.int = TRUE)
```

```
## # A tibble: 3 x 9
##   effect component term      estim~1 std.e~2 stati~3 p.value conf.~4 conf.~5
##   <chr>   <chr>      <chr>      <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 1 fixed   cond      (Intercept) -0.236   0.535   -0.440   0.660   -1.28   0.814
## 2 fixed   cond      np_sex       0.251   0.471   0.533   0.594   -0.672   1.17
## 3 fixed   cond      trial_np_dif~ 0.0211  0.102   0.207   0.836   -0.179   0.221
## # ... with abbreviated variable names 1: estimate, 2: std.error, 3: statistic,
## # 4: conf.low, 5: conf.high
```

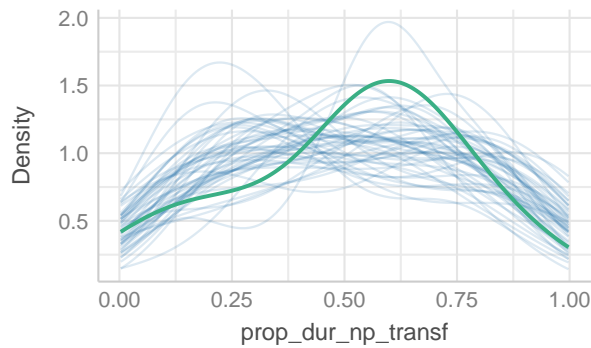
The sex and the freshness of the unrelated adult odour had no effect on the time spent with the unrelated adult odour.

Check model assumptions

```
check_model(beta_dur_a)
```

Posterior Predictive Check

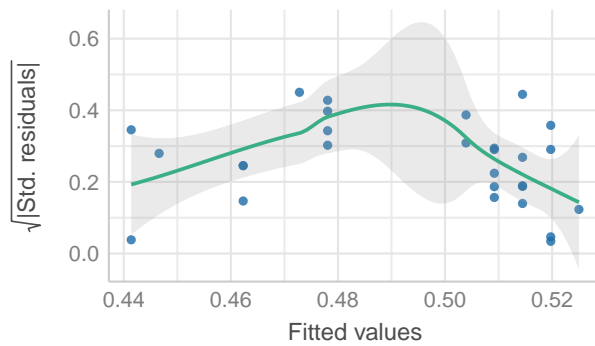
Model-predicted lines should resemble observed data line



— Observed data — Model-predicted data

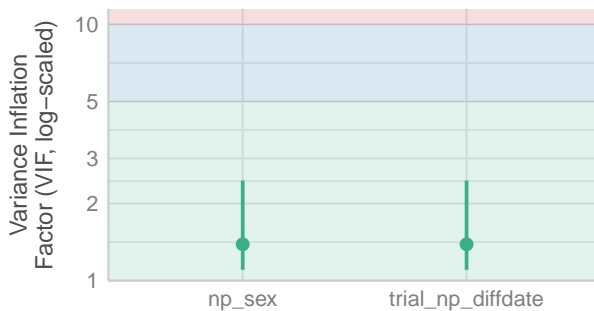
Homogeneity of Variance

Reference line should be flat and horizontal



Collinearity

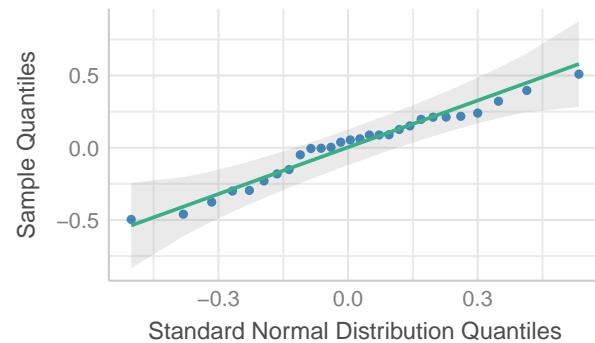
High collinearity (VIF) may inflate parameter uncertainty



● Low (< 5)

Normality of Residuals

Dots should fall along the line



Verify that there is no side bias: create the response variable (proportion of time spent on the left side) and run a one-sample t-test

```
data_a$prop_left <- data_a$left_duration/(data_a$left_duration + data_a$right_duration)
t.test(data_a$prop_left, mu = 0.5, alternative = "two.sided")
```

```
##
## One Sample t-test
##
## data: data_a$prop_left
## t = -0.88376, df = 28, p-value = 0.3844
## alternative hypothesis: true mean is not equal to 0.5
## 95 percent confidence interval:
## 0.3585277 0.5561923
## sample estimates:
## mean of x
## 0.45736
```

There was no significant side bias in the time spent in the test arms.

2.1.2 Odour visited first

Distribution of the response variable (binary)

```
table(data_a$first_type)
```

```
##
##      c np  p
##    0 16 13  0
```

Two-tailed binomial test

```
binom.test(sum(data_a$first_type=="np"), nrow(data_a), p = 0.5, alternative = "two.sided")
```

```
##
## Exact binomial test
##
## data: sum(data_a$first_type == "np") and nrow(data_a)
## number of successes = 13, number of trials = 29, p-value = 0.7111
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval:
##  0.2644553 0.6430613
## sample estimates:
## probability of success
##                0.4482759
```

Chicks did not visit first the arm with the unrelated adult odour more than the arm with no odour.

GLM (binomial)

```
glm_first_a <- glm(first_type ~ np_sex + trial_np_diffdate, data = data_a_sex,
                   family = binomial(link = "logit"))
summary(glm_first_a)
```

```
##
## Call:
## glm(formula = first_type ~ np_sex + trial_np_diffdate, family = binomial(link = "logit"),
##      data = data_a_sex)
##
## Deviance Residuals:
##      Min       1Q   Median       3Q      Max
## -1.3954  -1.0620  -0.5528   1.1027   2.1482
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    -2.5235     1.5858  -1.591   0.112
## np_sex         2.0601     1.2773   1.613   0.107
## trial_np_diffdate 0.3208     0.2662   1.205   0.228
##
## (Dispersion parameter for binomial family taken to be 1)
##
```

```
##      Null deviance: 38.243  on 27  degrees of freedom
## Residual deviance: 34.671  on 25  degrees of freedom
## AIC: 40.671
##
## Number of Fisher Scoring iterations: 4
```

Beta estimates (with confidence intervals) of fixed effects

```
broom.mixed::tidy(glm_first_a, conf.int = TRUE)
```

```
## # A tibble: 3 x 7
##   term                estimate std.error statistic p.value conf.low conf.high
##   <chr>              <dbl>    <dbl>    <dbl>   <dbl>   <dbl>    <dbl>
## 1 (Intercept)       -2.52      1.59     -1.59   0.112   -6.47     0.0220
## 2 np_sex            2.06      1.28      1.61   0.107   -0.0873    5.16
## 3 trial_np_diffdate 0.321     0.266     1.21   0.228   -0.145     0.939
```

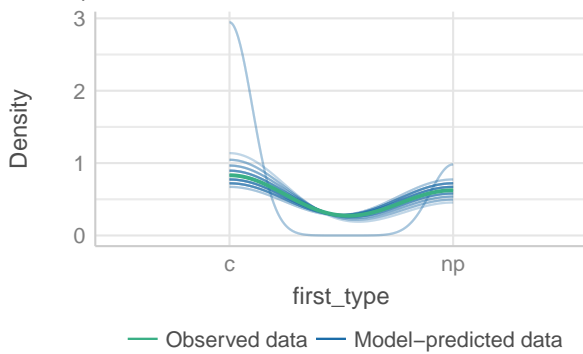
The sex and the freshness of the unrelated adult odour had no effect on which odour they visited first.

Check model assumptions

```
check_model(glm_first_a)
```

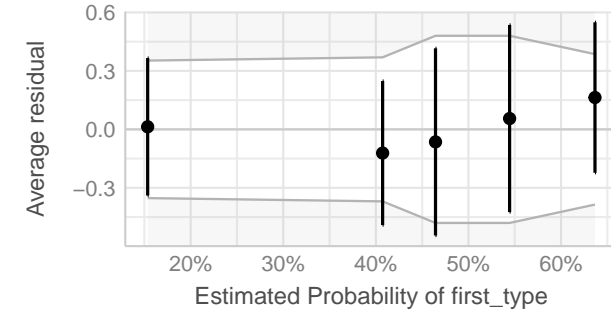
Posterior Predictive Check

Model-predicted lines should resemble observed data line



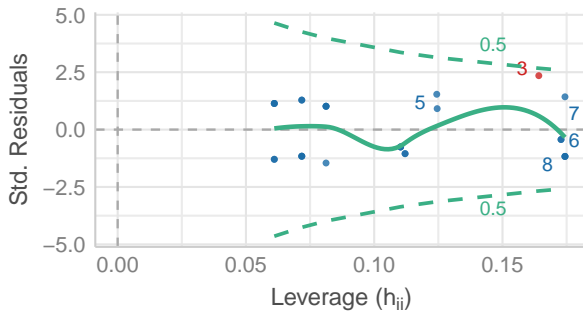
Binned Residuals

Points should be within error bounds



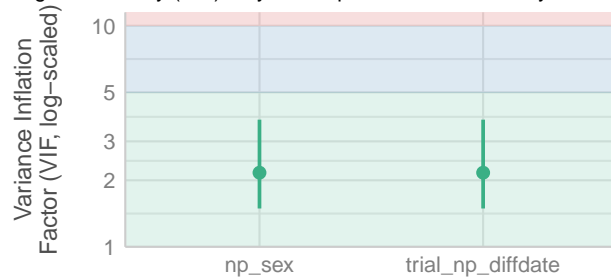
Influential Observations

Points should be inside the contour lines



Collinearity

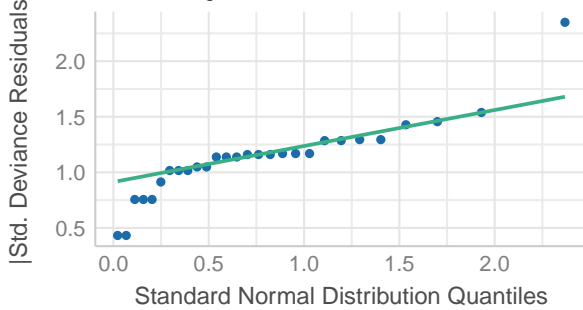
High collinearity (VIF) may inflate parameter uncertainty



Low (< 5)

Normality of Residuals

Dots should fall along the line



Verify that there is no side bias in the test arm visited first

```
binom.test(sum(data_a$first_side=="1"), nrow(data_a), p = 0.5, alternative = "two.sided")
```

```
##
## Exact binomial test
##
## data: sum(data_a$first_side == "1") and nrow(data_a)
## number of successes = 14, number of trials = 29, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval:
##  0.2944856 0.6746850
## sample estimates:
```

```
## probability of success
## 0.4827586
```

There was no side bias in the first arm visited.

2.2 Parent odour discrimination (trial B)

2.2.1 Time spent with odour

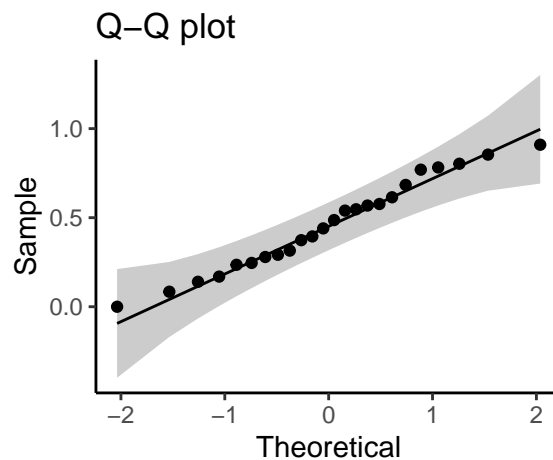
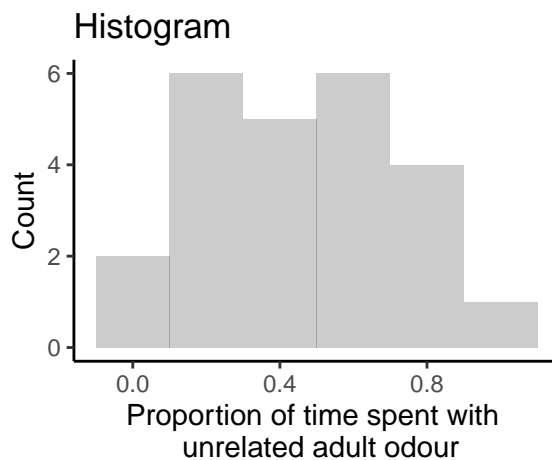
Mean time (and SD) spent by chicks in each arm

```
mean_np <- mean(data_b$np_duration)
sd_np <- sd(data_b$np_duration)
mean_p <- mean(data_b$p_duration)
sd_p <- sd(data_b$p_duration)
data.frame(Variable = c("Time spent with unfamiliar adult odour (s)", "Time spent with parent odour (s)",
                        Mean = c(mean_np, mean_p),
                        SD = c(sd_np, sd_p))
```

```
##
## 1 Time spent with unfamiliar adult odour (s) 134.1160 122.83361
## 2 Time spent with parent odour (s) 114.6987 81.10735
```

Verify that the response variable is normally distributed

```
x <- ggplot(data_b, aes(x=prop_dur_np)) +
  geom_histogram(binwidth=0.2, alpha=.2, fill="black") +
  theme_classic() + ggtitle("Histogram") +
  xlab("Proportion of time spent with \n unrelated adult odour") + ylab("Count")
y <- ggqqplot(data_b$prop_dur_np, color = "black") +
  theme_classic() + ggtitle("Q-Q plot")
ggarrange(x, y, ncol = 2, nrow = 1)
```



Verify that there is no outlier

```
check_outliers(data_b$prop_dur_np)
```

```
## OK: No outliers detected.  
## - Based on the following method and threshold: zscore_robust (3.291).  
## - For variable: data_b$prop_dur_np
```

T-test (two-tailed one sample)

```
t.test(data_b$prop_dur_np, mu = 0.5, alternative = "two.sided")
```

```
##  
## One Sample t-test  
##  
## data: data_b$prop_dur_np  
## t = -0.71163, df = 23, p-value = 0.4839  
## alternative hypothesis: true mean is not equal to 0.5  
## 95 percent confidence interval:  
## 0.3541954 0.5711659  
## sample estimates:  
## mean of x  
## 0.4626806
```

Chicks spent has much time in the arm with the unrelated adult odour as in the arm with the parent odour, they showed no preference.

To run a beta regression, we need to transform the response variable (proportion of time spent with the unrelated adult odour) so that it does not include any 0 or 1.

Transformation $(y*(n-1)+0.5)/n$ (y: response variable, n: sample size; Smithson and Verkuilen 2006)

```
data_b_sex$prop_dur_np_transf <- (data_b_sex$prop_dur_np * (nrow(data_b_sex) - 1) + 0.5) / nrow(data_b_sex)
```

Beta regression

```
beta_dur_b <- glmmTMB(prop_dur_np_transf ~ odour_sex_comb + trial_np_diffdate, data = data_b_sex,  
  family = beta_family(link = "logit"))  
summary(beta_dur_b)
```

```
## Family: beta ( logit )  
## Formula: prop_dur_np_transf ~ odour_sex_comb + trial_np_diffdate  
## Data: data_b_sex  
##  
## AIC BIC logLik deviance df.resid  
## 2.5 8.2 3.8 -7.5 18  
##  
##  
## Dispersion parameter for beta family (): 3.9  
##  
## Conditional model:  
## Estimate Std. Error z value Pr(>|z|)  
## (Intercept) -0.31179 0.34899 -0.893 0.372
```

```
## odour_sex_combf m 0.81731 0.54017 1.513 0.130
## odour_sex_combm m 0.53555 0.41528 1.290 0.197
## trial_np_diffdate -0.08765 0.08386 -1.045 0.296
```

Beta estimates (with confidence intervals) of fixed effects

```
broom.mixed::tidy(beta_dur_b, conf.int = TRUE)
```

```
## # A tibble: 4 x 9
##   effect component term          estim~1 std.e~2 stati~3 p.value conf.~4 conf.~5
##   <chr> <chr> <chr>          <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 fixed cond (Intercept) -0.312 0.349 -0.893 0.372 -0.996 0.372
## 2 fixed cond odour_sex_co~ 0.817 0.540 1.51 0.130 -0.241 1.88
## 3 fixed cond odour_sex_co~ 0.536 0.415 1.29 0.197 -0.278 1.35
## 4 fixed cond trial_np_dif~ -0.0876 0.0839 -1.05 0.296 -0.252 0.0767
## # ... with abbreviated variable names 1: estimate, 2: std.error, 3: statistic,
## # 4: conf.low, 5: conf.high
```

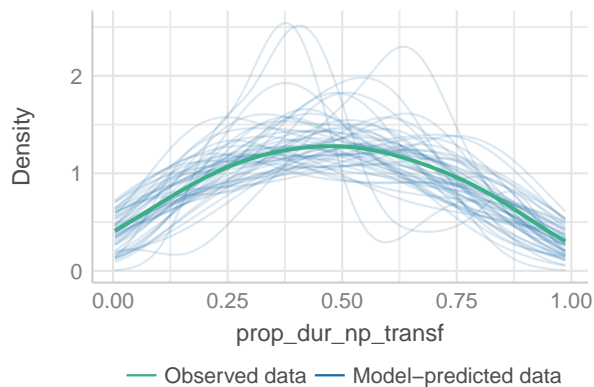
The sex and the freshness of the test odours had no effect on the time chicks spent with the test odours.

Check model assumptions

```
check_model(beta_dur_b)
```

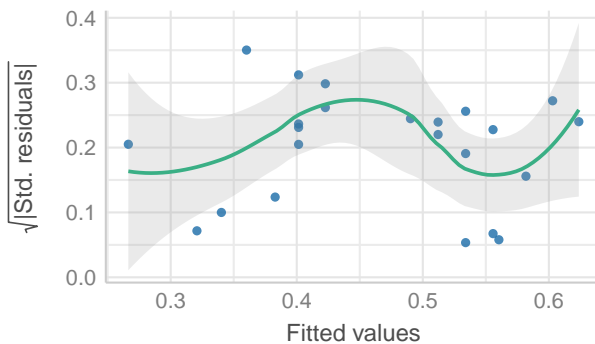
Posterior Predictive Check

Model-predicted lines should resemble observed data line



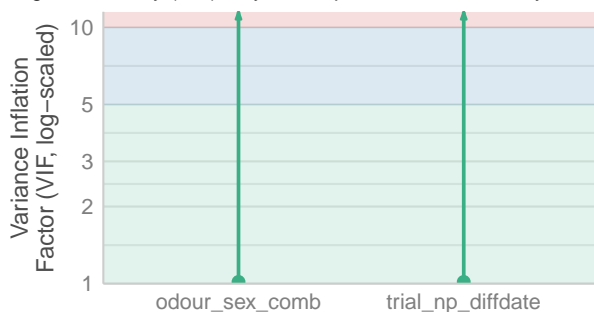
Homogeneity of Variance

Reference line should be flat and horizontal



Collinearity

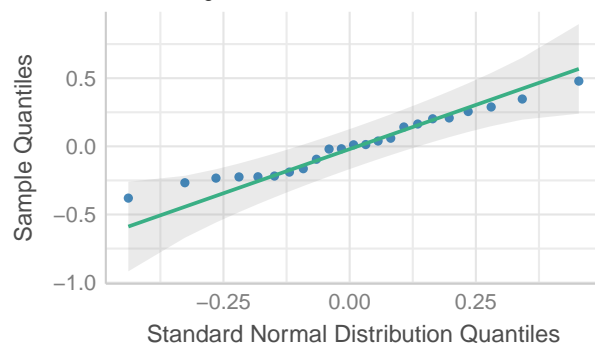
High collinearity (VIF) may inflate parameter uncertainty



Low (< 5)

Normality of Residuals

Dots should fall along the line



Verify that there is no side bias: create the response variable (proportion of time spent on the left side) and run a one-sample t-test

```
data_b$prop_left <- data_b$left_duration/(data_b$left_duration + data_b$right_duration)
t.test(data_b$prop_left, mu = 0.5, alternative = "two.sided")
```

```
##
## One Sample t-test
##
## data: data_b$prop_left
## t = -0.99687, df = 23, p-value = 0.3292
## alternative hypothesis: true mean is not equal to 0.5
## 95 percent confidence interval:
## 0.3408774 0.5556332
## sample estimates:
## mean of x
## 0.4482553
```

There was no significant side bias in the time spent in the test arms.

2.2.2 Odour visited first

Distribution of the response variable (binary)

```
table(data_b$sex$first_type)
```

```
##
##      c np  p
## 0  0 10 13
```

Two-tailed binomial test

```
binom.test(sum(data_b$first_type=="np"), nrow(data_b), p = 0.5, alternative = "two.sided")
```

```
##
## Exact binomial test
##
## data: sum(data_b$first_type == "np") and nrow(data_b)
## number of successes = 11, number of trials = 24, p-value = 0.8388
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval:
## 0.2555302 0.6717919
## sample estimates:
## probability of success
## 0.4583333
```

Chicks did not visit first the arm with the unrelated adult odour more than the arm with parent odour.

GLM (binomial)

```
glm_first_b <- glm(first_type ~ odour_sex_comb + trial_np_diffdate, data = data_b_sex,
  family = binomial(link = "logit"))
summary(glm_first_b)
```

```
##
## Call:
## glm(formula = first_type ~ odour_sex_comb + trial_np_diffdate,
##     family = binomial(link = "logit"), data = data_b_sex)
##
## Deviance Residuals:
##      Min       1Q   Median       3Q      Max
## -1.5891  -1.0592   0.7019   0.9609   1.4799
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    -0.6879     0.8277  -0.831   0.406
## odour_sex_combf m  1.6179     1.3453   1.203   0.229
## odour_sex_combm m  1.1640     0.9765   1.192   0.233
## trial_np_diffdate  0.1151     0.2049   0.562   0.574
##
## (Dispersion parameter for binomial family taken to be 1)
##
##      Null deviance: 31.492  on 22  degrees of freedom
## Residual deviance: 29.087  on 19  degrees of freedom
## AIC: 37.087
##
## Number of Fisher Scoring iterations: 4
```

Beta estimates (with confidence intervals) of fixed effects

```
broom.mixed::tidy(glm_first_b, conf.int = TRUE)
```

```
## # A tibble: 4 x 7
##   term                estimate std.error statistic p.value conf.low conf.high
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>
## 1 (Intercept)        -0.688     0.828    -0.831   0.406    -2.50     0.878
## 2 odour_sex_combf m   1.62      1.35     1.20    0.229    -0.830     4.86
## 3 odour_sex_combm m   1.16      0.977     1.19    0.233    -0.690     3.22
## 4 trial_np_diffdate   0.115     0.205     0.562   0.574    -0.276     0.572
```

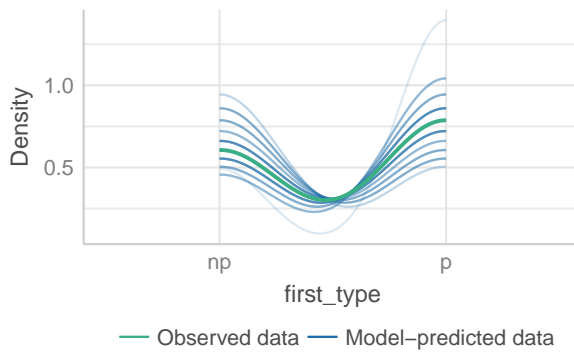
The sex and the freshness of the unrelated adult odour had no effect on which odour they visited first.

Check model assumptions

```
check_model(glm_first_b)
```

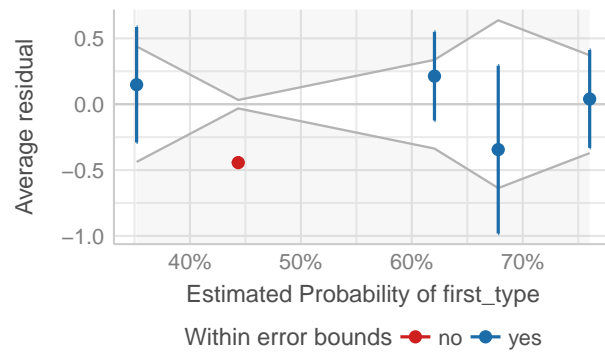
Posterior Predictive Check

Model-predicted lines should resemble observed data line



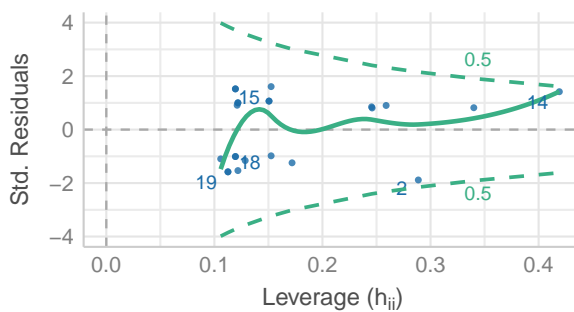
Binned Residuals

Points should be within error bounds



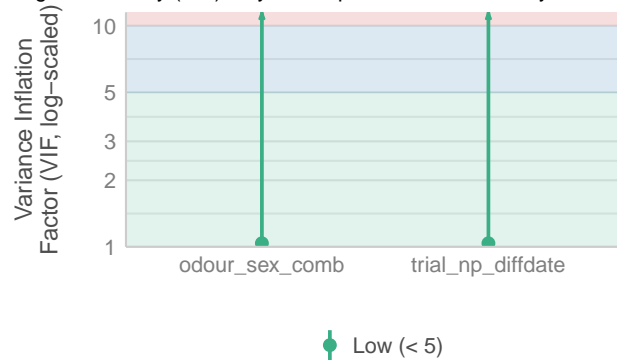
Influential Observations

Points should be inside the contour lines



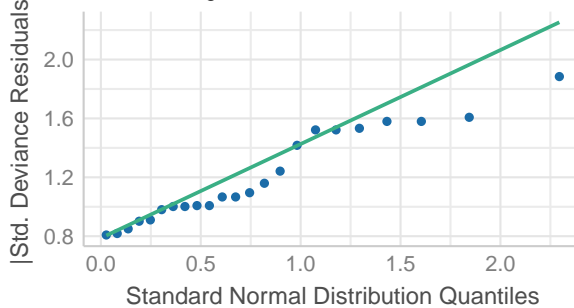
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



Normality of Residuals

Dots should fall along the line



Verify that there is no side bias in the test arm visited first

```
binom.test(sum(data_b$first_side=="1"), nrow(data_b), p = 0.5, alternative = "two.sided")
```

```
##
## Exact binomial test
##
## data: sum(data_b$first_side == "1") and nrow(data_b)
## number of successes = 13, number of trials = 24, p-value = 0.8388
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval:
## 0.3282081 0.7444698
## sample estimates:
```

```
## probability of success
##          0.5416667
```

There was no side bias in the first arm visited.

2.3 Probability and latency to leave the acclimation chamber

In this exploratory analysis, we want to test if the sex and age of the chick has an effect on the probability and latency to leave the acclimation chamber, when they were in the Y-maze for the first time (novel environment).

2.3.1 Probability to leave the acclimation chamber

Subset data on all trials A where the sex of the chick is known (N = 42 chicks)

```
data_prob <- data[data$trial_type == "A" & !is.na(data$sex),]
```

Distribution of the variable (binary)

```
table(data_prob$trial_success, data_prob$sex)
```

```
##
##      f  m
##    0  3 11
##    1 16 12
```

GLMM (binomial)

```
prob <- lme4::glmer(trial_success ~ sex + age + (1|nest), family = binomial(link = "logit"), data = data_prob)
summary(prob)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##   Family: binomial ( logit )
## Formula: trial_success ~ sex + age + (1 | nest)
##   Data: data_prob
##
##      AIC      BIC    logLik deviance df.resid
##    42.9    49.9    -17.5    34.9      38
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.1806 -0.3868  0.2093  0.5054  3.7151
##
## Random effects:
##   Groups Name            Variance Std.Dev.
##   nest    (Intercept) 1.82e-14 1.349e-07
## Number of obs: 42, groups: nest, 32
##
## Fixed effects:
```

```
##           Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.76485    1.12152   3.357 0.000788 ***
## sexm        -2.21884    1.00166  -2.215 0.026749 *
## age         -0.18134    0.05835  -3.108 0.001885 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) sexm
## sexm -0.810
## age  -0.759  0.442
## optimizer (Nelder_Mead) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

Beta estimates (with confidence intervals) of fixed effects

```
broom.mixed::tidy(prob, conf.int = TRUE)
```

```
## # A tibble: 4 x 9
##   effect   group term          estimate std.e~1 stati~2 p.value conf.^3 conf.^4
##   <chr>   <chr> <chr>          <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 1 fixed   <NA> (Intercept)    3.76e+0  1.12     3.36  7.88e-4  1.57    5.96
## 2 fixed   <NA> sexm        -2.22e+0  1.00    -2.22  2.67e-2 -4.18   -0.256
## 3 fixed   <NA> age         -1.81e-1  0.0583  -3.11  1.88e-3 -0.296  -0.0670
## 4 ran_pars nest sd_(Interce~  1.35e-7  NA      NA      NA      NA      NA
## # ... with abbreviated variable names 1: std.error, 2: statistic, 3: conf.low,
## # 4: conf.high
```

Calculate the exponential of the log-odds ratio to get the odds ratio for sex (with 95% confidence interval)

```
exp(summary(prob)$coefficients["sexm",1] +
     qnorm(c(0.025,0.5,0.975)) * summary(prob)$coefficients["sexm",2])
```

```
## [1] 0.01526704 0.10873504 0.77443355
```

Odds of leaving the chamber are 89% (estimate = 0.11, 95% CI = [0.02, 0.77]) lower if the chick is a male (12/23 = 52% of the males left the chamber) compared to a female (16/19 = 84% of females left the chamber).

Calculate the exponential of the log-odds ratio to get the odds ratio for age (with 95% confidence interval)

```
exp(summary(prob)$coefficients["age",1] +
     qnorm(c(0.025,0.5,0.975)) * summary(prob)$coefficients["age",2])
```

```
## [1] 0.7440079 0.8341518 0.9352175
```

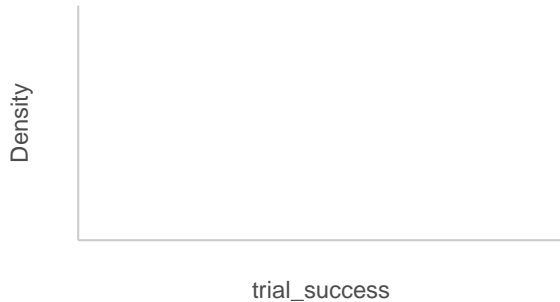
Odds of leaving the chamber were 17% (estimate = 0.83, 95% CI = [0.74, 0.93]) lower if chicks were one day older.

Check model assumptions

```
check_model(prob)
```

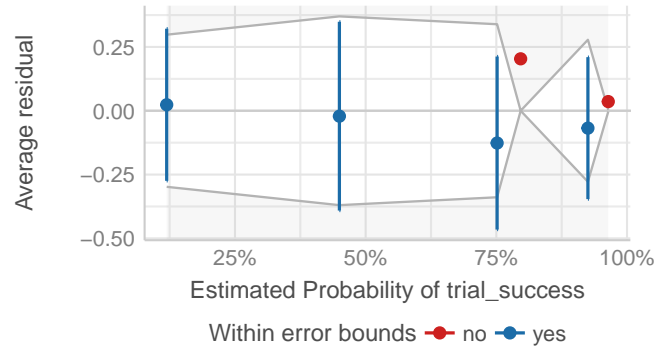
Posterior Predictive Check

Model-predicted lines should resemble observed data line



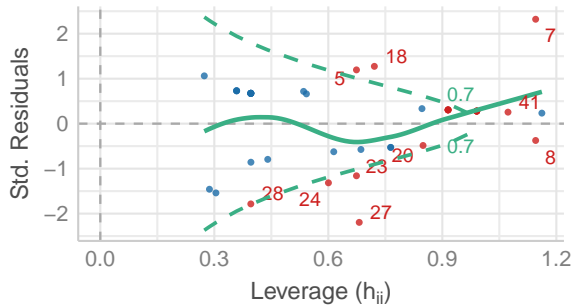
Binned Residuals

Points should be within error bounds



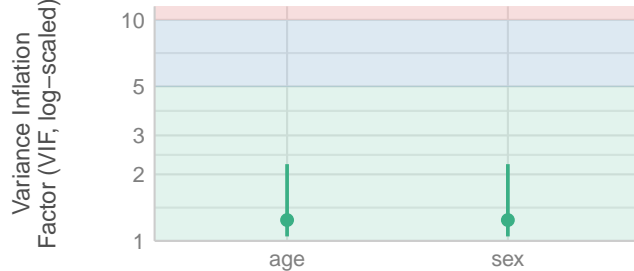
Influential Observations

Points should be inside the contour lines



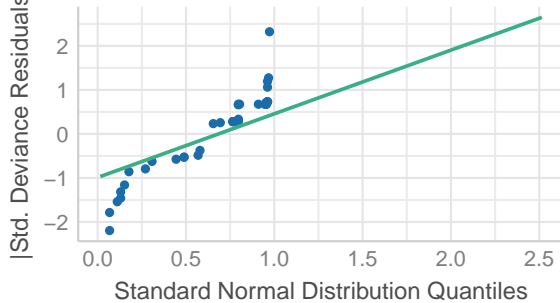
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



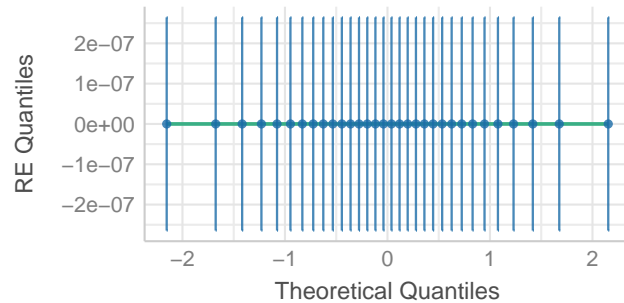
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (nest)

Dots should be plotted along the line



Check overdispersion

```
check_overdispersion(prob)
```

```
## # Overdispersion test
##
##      dispersion ratio = 1.128
##  Pearson's Chi-Squared = 42.849
##                p-value = 0.271
```

2.3.2 Latency to leave the acclimation chamber

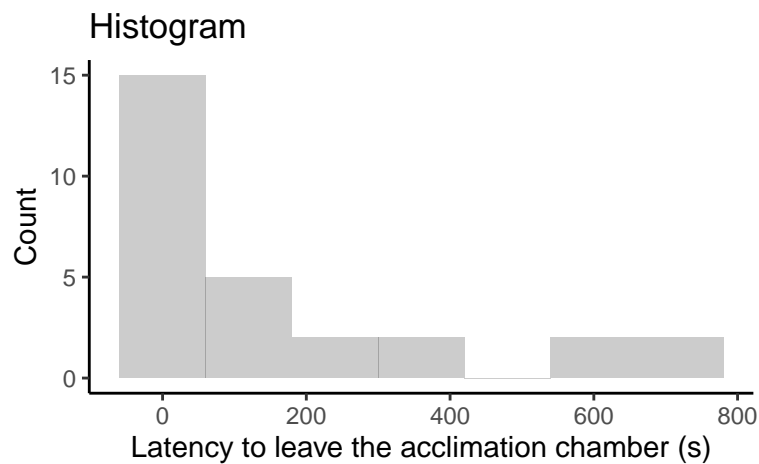
Subset data on all successful trials A where the sex of the chick is known (N = 28, including 16 females and 12 males)

```
data_lat <- data[data$trial_type == "A" & data$trial_success == 1 & !is.na(data$sex),]  
table(data_lat$sex)
```

```
##  
## f m  
## 16 12
```

Distribution of the response variable

```
ggplot(data_lat, aes(x=out_time)) +  
  geom_histogram(binwidth=120, alpha=.2, fill="black") +  
  theme_classic() + ggtitle("Histogram") +  
  xlab("Latency to leave the acclimation chamber (s)") + ylab("Count")
```



GLMM (gamma)

```
lat <- lme4::glmer(out_time ~ sex + age + (1|nest), family = Gamma(link=log), data = data_lat)  
summary(lat)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace  
## Approximation) [glmerMod]  
## Family: Gamma ( log )  
## Formula: out_time ~ sex + age + (1 | nest)  
## Data: data_lat  
##  
##      AIC      BIC    logLik deviance df.resid  
##    306.2    312.8   -148.1    296.2      23  
##  
## Scaled residuals:  
##      Min      1Q    Median      3Q      Max
```

```
## -1.12968 -0.37119 -0.02353 0.33460 1.45751
##
## Random effects:
## Groups Name Variance Std.Dev.
## nest (Intercept) 3.2649 1.8069
## Residual 0.7623 0.8731
## Number of obs: 28, groups: nest, 21
##
## Fixed effects:
## Estimate Std. Error t value Pr(>|z|)
## (Intercept) 1.68178 0.70097 2.399 0.016431 *
## sexm 1.20450 0.58805 2.048 0.040530 *
## age 0.20580 0.06205 3.316 0.000912 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
## (Intr) sexm
## sexm -0.552
## age -0.623 0.208
```

Beta estimates (with confidence intervals) of fixed effects

```
broom.mixed::tidy(lat, conf.int = TRUE)
```

```
## # A tibble: 5 x 9
## effect group term estim~1 std.e~2 stati~3 p.value conf.~4 conf.~5
## <chr> <chr> <chr> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 fixed <NA> (Intercept) 1.68 0.701 2.40 1.64e-2 0.308 3.06
## 2 fixed <NA> sexm 1.20 0.588 2.05 4.05e-2 0.0520 2.36
## 3 fixed <NA> age 0.206 0.0621 3.32 9.12e-4 0.0842 0.327
## 4 ran_pars nest sd__(Inter~ 1.81 NA NA NA NA
## 5 ran_pars Residual sd__Observ~ 0.873 NA NA NA NA
## # ... with abbreviated variable names 1: estimate, 2: std.error, 3: statistic,
## # 4: conf.low, 5: conf.high
```

Exponentiate the log-estimates to get the estimates for sex (with confidence intervals)

```
exp(summary(lat)$coefficients["sexm",1] +
  qnorm(c(0.025,0.5,0.975)) * summary(lat)$coefficients["sexm",2])
```

```
## [1] 1.053325 3.335101 10.559802
```

Males wait 3 times longer (estimate = 3.32, 95% CI = [1.05, 10.56]) before leaving the chamber than females.

Exponentiate the log-estimates to get the estimates for age (with confidence intervals)

```
exp(summary(lat)$coefficients["age",1] +
  qnorm(c(0.025,0.5,0.975)) * summary(lat)$coefficients["age",2])
```

```
## [1] 1.087822 1.228510 1.387392
```

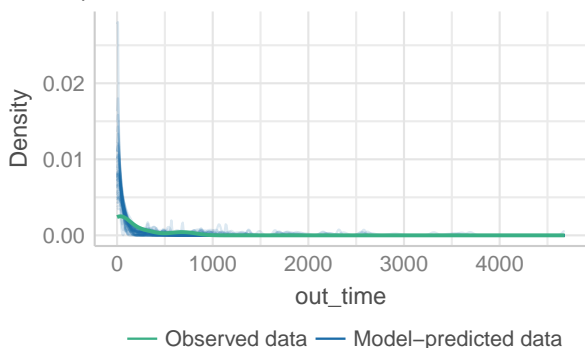

Chicks waited 23% longer (estimate = 1.23, 95% CI = [1.08, 1.39]) before leaving the chamber if they were one day older.

Check model assumptions

```
check_model(lat)
```

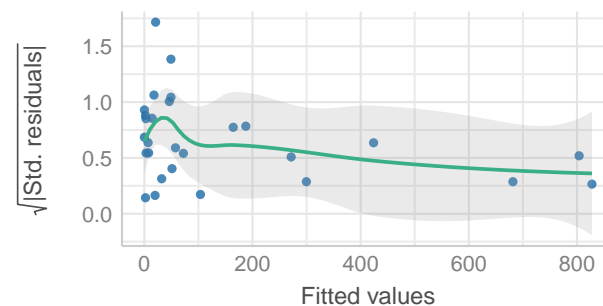
Posterior Predictive Check

Model-predicted lines should resemble observed data line



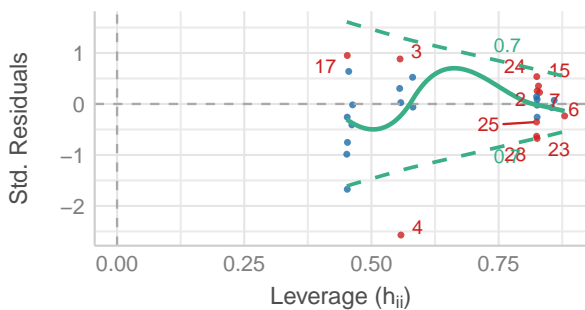
Homogeneity of Variance

Reference line should be flat and horizontal



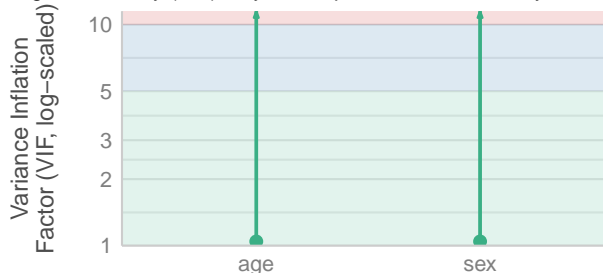
Influential Observations

Points should be inside the contour lines



Collinearity

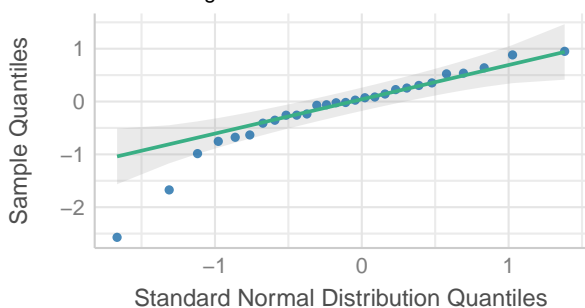
High collinearity (VIF) may inflate parameter uncertainty



Low (< 5)

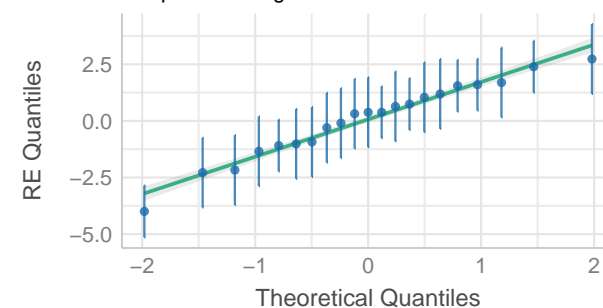
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (nest)

Dots should be plotted along the line



Check for multicollinearity (sex and age)

```
t.test(data_lat[data_lat$sex=="f"],$age, data_lat[data_lat$sex=="m"],$age)
```

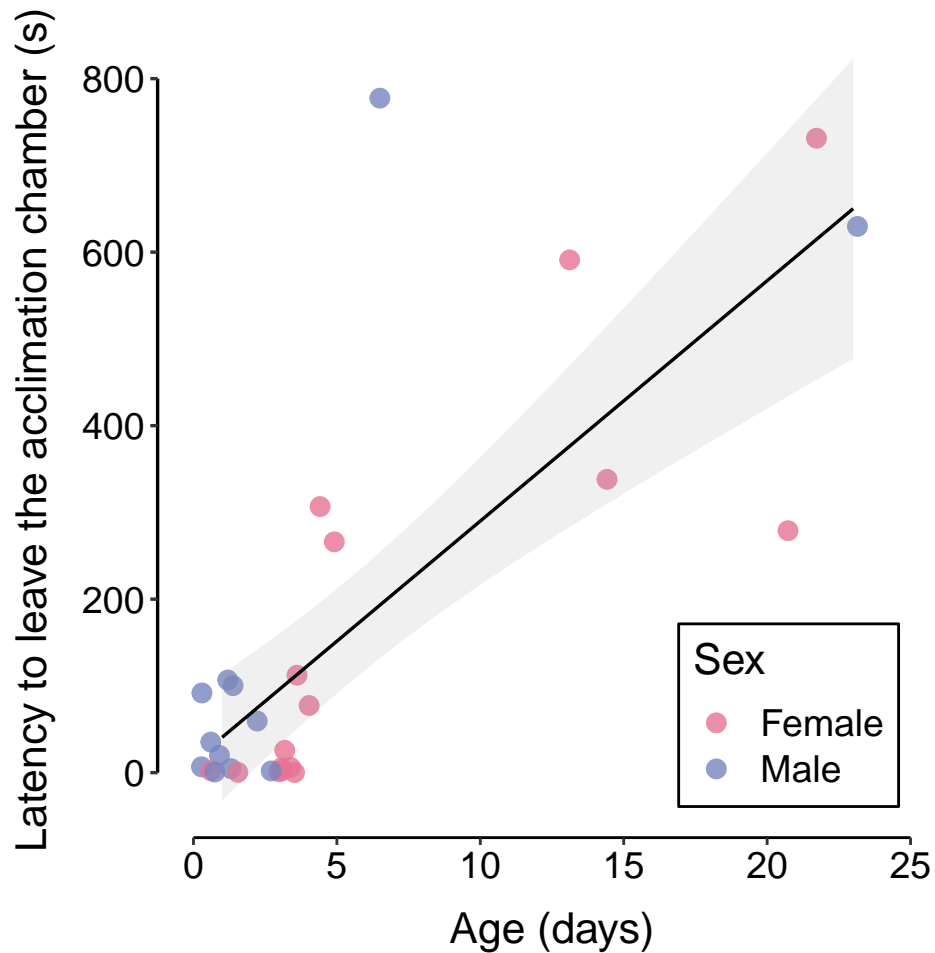
```
##
```

```
## Welch Two Sample t-test
##
## data: data_lat[data_lat$sex == "f", ]$age and data_lat[data_lat$sex == "m", ]$age
## t = 1.2952, df = 24.625, p-value = 0.2073
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -1.909457 8.367791
## sample estimates:
## mean of x mean of y
## 6.812500 3.583333
```

Because the model on the latency to leave included only chicks that left the chamber, and because older males were less likely to leave the chamber, the average age of males (3.6 days old) that left the chamber was somewhat higher than that of females (6.8 days old), although not significantly.

Plot sex and age effect on latency to leave

```
ggplot(data_lat, aes(x = age, y = out_time, colour = sex)) +
  geom_point(alpha = 0.8, cex = 3, position=position_jitter(h=0.75,w=0.75)) +
  xlim(0,25) +
  geom_smooth(method=lm, color = "black", size = 0.6, se=T, alpha = 0.15) +
  scale_color_manual(labels = c("Female", "Male"), values = c("#E67394", "#7785BF")) +
  labs(x="Age (days)", y="Latency to leave the acclimation chamber (s)", color = "Sex") +
  theme_classic()+
  theme(axis.title.y = element_text(size = 16, margin = margin(t = 0, r = 10, b = 0, l = 0)),
        axis.text.y = element_text(size = 14, colour = "black"),
        axis.line.y = element_line(linewidth = 0.6),
        axis.ticks.y = element_line(linewidth = 0.6),
        axis.title.x = element_text(size = 16, margin = margin(t = 10, r = 0, b = 0, l = 0)),
        axis.text.x = element_text(size = 14, colour = "black"),
        legend.title = element_text(size = 16),
        legend.text = element_text(size = 14),
        legend.position = c(0.8, 0.15),
        legend.background = element_rect(size=0.5, linetype="solid", colour="black"))+
  coord_capped_cart(bottom=capped_horizontal(capped="both"), left=capped_vertical(capped="both"))
```



3 Plot

Prepare new variables for the x scale of the plot

```
data_plot$point <- factor(data_plot$odour_type, levels = c("np", "c", "p"))
data_plot <- data_plot %>% mutate(point = recode(point, "np" = 1, "c" = 2, "p" = 2))
data_plot$lp <- data_plot$point + 0.1 * ifelse(data_plot$point == 1, 1.2, -1.2)
data_plot$box <- data_plot$point + 0.1 * ifelse(data_plot$point == 1, -1.2, 1.2)
```

Colour palette

```
fill <- c("#E67394", "#F2C443", "#7785BF")
stroke <- c("black", "black", "black")
```

Plot

```
ggplot(data_plot, aes(x=odour_type, y=duration, fill = odour_type, colour = odour_type)) +
  facet_grid(~trial_type) +
  geom_line(aes(x = lp, group=trial), position = "identity", col="#B7B7B7") +
  geom_point(aes(x = lp, group=trial), shape=21, size=2.5,
```

```

    position = "identity", stroke = NA) +
geom_boxplot(aes(x = box, group=odour_type), width=.25, lwd = 0.6) +
theme_classic() +
theme(strip.background = element_blank(), strip.text.x = element_blank(),
      panel.spacing = unit(1, "lines")) +
scale_x_continuous(breaks = c(1,2), expand=c(0.09,0.09),
                   labels = c("Unrelated adult \n odour","No odour \n Parent odour")) +
scale_fill_manual(values = fill) +
scale_colour_manual(values = stroke) +
labs(x="", y="Time spent with odour (s)") +
coord_capped_cart(left='both') +
theme(axis.title.y = element_text(size = 16, margin = margin(t = 0, r = 10, b = 0, l = 0)),
      axis.text.y = element_text(size = 13, colour = "black"),
      axis.line.y = element_line(linewidth = 0.6),
      axis.ticks.y = element_line(linewidth = 0.6),
      axis.text.x = element_text(size = 13.5, colour = "black"),
      axis.line.x=element_blank(),
      axis.ticks.x =element_blank(),
      legend.position = "none") +
annotate("text", x = 1.5, y=450, label = "ns") +
geom_segment(aes(x = 1.1, y = 435, xend = 1.9, yend = 435), size = 0.6)

```

