Sexual selection does not drive hindwing tail elaboration in a moon moth, Actias luna - Supplementary Information

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Defining the parameters

Here are definitions for the parameters in all models:

Date = the date on which trial was run

Cage = the cage identity in which the trial was run. This does not necessarily correlate to the cage size or moth treatments, as the cages were given new identifiers across the data collection seasons based on cage availability, number of moths available to participate in the experiment, etc. Instead, this parameter, in conjunction with the date, can be used to identify which males (rows) were pit against each other in the mating competition.

Tent_type = the model of cage in which trial took place. BA = Big Agnes Tiger Wall 3, Eureka = Eureka Suma, BioQuip = BioQuip collapsible cage.

Cage_category = The size of the tent in which trials were conducted (large vs small – large tents were either Eureka Suma + or Big Agnes Tiger Wall 3 (~180 x 230 x 110cm), small tent was BioQuip (~60 x 60 x 90cm)).

Temp = Temperature reading at a single time point ~3 hours after trial start (units = degree F).

Hum = Humidity reading at a single time point ~3 hours after trial start (units = %).

SQM = An integrated measurement of the nocturnal light environment taken with a Sky Quality Meter (larger numbers are indicative of darker environments). Measurement taken at a single time point ~3 hours after trial start (units = magnitudes/arcsecond).

Size_Rwings = The sum of the surface area of the right fore- and hindwings of each male moth, measured in imageJ. Used as a proxy for moth size (units = cm^2).

Treatment = what experimental treatment the moth was assigned

Success = binomial code for whether the moth mated with the female or not (in each)

dyad = an identifer for the experimental set to which the moth belonged, as described below. Dyad 1 = intact v. ablated males, dyad 2 = sham control v. ablated control males, dyad 3 = intact v. glue control males.

Note: We scaled the numerical variables in the models to allow for comparison across variable type

Defining the experimental sets and building models

Because each dyad was run as a separate experiment, we are dividing the data set up into the dyads that were introduced to females and exploring them separately.

The dyads are as follows:

Intact vs Ablated: male with tails intact vs. male with tails removed

Sham control vs Ablated control: male with tails removed and reglued vs. male with tails removed and glue added to the base of the hindwings

Intact vs glue control: male with tails intact vs. male with tails intact and glue added to the base of the hindwings.

Glue was applied to the underside of the moth wings whenever it was used (see full paper for more detail).

Intact vs. Ablated

We start with the primary test between a male with tails and a male with tails removed.

```
# model set

mod1 <- glm(Success ~ Treatment, data = IvA, family = "binomial")
mod2 <- glm(Success ~ Treatment + scale(Size_Rwings), data = IvA,
    family = "binomial")
mod3 <- glm(Success ~ Treatment + scale(SQM), data = IvA, family = "binomial")
mod4 <- glm(Success ~ Treatment + scale(Temp), data = IvA, family = "binomial")
mod5 <- glm(Success ~ Treatment + scale(Hum), data = IvA, family = "binomial")
mod6 <- glm(Success ~ Treatment + Cage_category, data = IvA,
    family = "binomial")

# Here is the model selection table
model.sel(mod1, mod2, mod3, mod4, mod5, mod6, rank = BIC)
```

```
## Model selection table
##        (Int) Trt scl(Siz_Rwn)    scl(SQM)  scl(Tmp)  scl(Hum) Cag_ctg df  logLik
## mod2 -1.136   +       0.1342                                          3 -22.993
## mod1 -1.153   +                                                      2 -27.554
## mod3 -1.153   +                 -2.611e-16                            3 -27.554
## mod4 -1.153   +                           2.755e-16                  3 -27.554
## mod6 -1.153   +                                             +        3 -27.554
## mod5 -1.153   +                                     1.142e-16        3 -27.554
##       BIC delta weight
## mod2 57.2  0.00  0.918
## mod1 62.9  5.73  0.052
## mod3 66.8  9.65  0.007
## mod4 66.8  9.65  0.007
## mod6 66.8  9.65  0.007
## mod5 66.8  9.65  0.007
## Models ranked by BIC(x)
```

Model 2 has the lowest BIC score, so we will proceed with interpreting this model.

We will first perform model checks to ensure this model does not demonstrate over or under-dispersion. We will then extract parameter estimates and p-values.
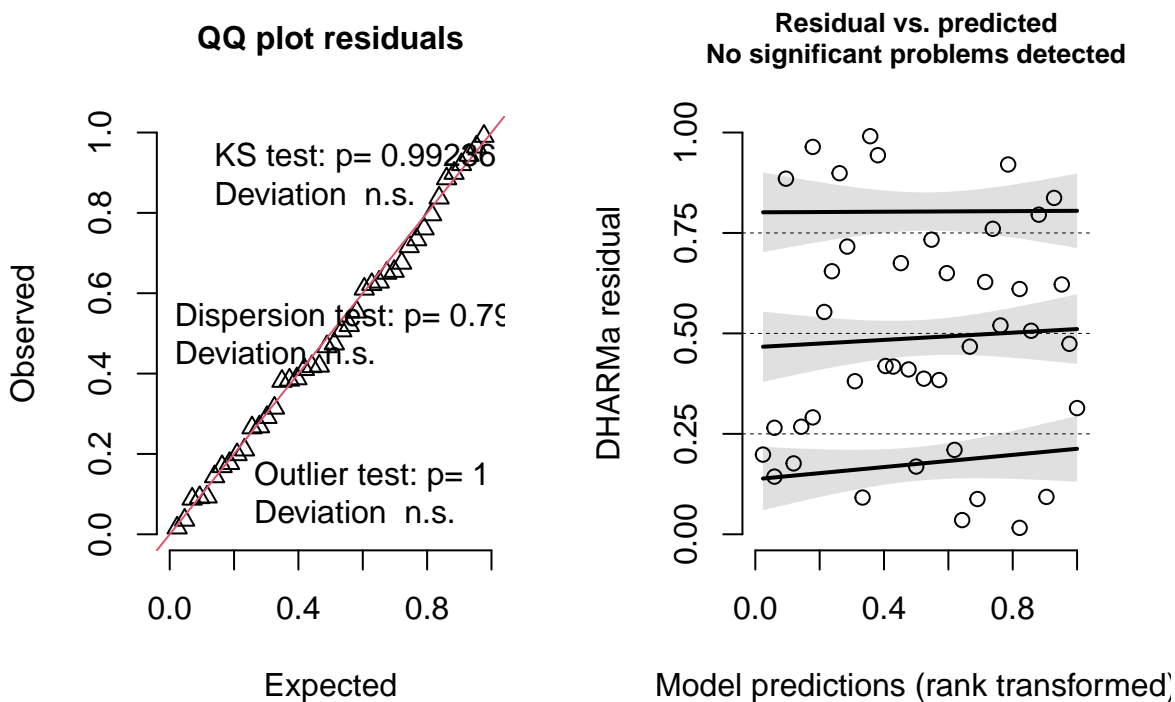
DHARMa residual

Table 1: Table output for binomial model comparing mating success between A. luna males with tails intact and tails removed, accounting for size of each male

Predictor	parameter estimate	SE	t	p
(Intercept)	-1.14	0.518	-2.19	0.028
TreatmentIntact	2.27	0.737	3.09	0.002
scale(Size_Rwings)	0.13	0.394	0.34	0.734

```
## here are the parameter estimates, back-transformed from the logit scale


##  Treatment  prob     SE  df asymp.LCL asymp.UCL
##  Ablated   0.243 0.0953 Inf     0.104     0.470
##  Intact    0.757 0.0948 Inf     0.532     0.896
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale
```

Estimating the intercept – when males are the same size, do females still show a preference?

```
##
## Call:
## glm(formula = Success_Intact ~ size_diff, family = "binomial",
##     data = newIvA)
##
## Deviance Residuals:
##     Min      1Q   Median      3Q      Max
## -1.8263   0.5001   0.7061   0.7639   0.9289
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)  0.97291    0.57351   1.696   0.0898 .
## size_diff    0.08977    0.13927   0.645   0.5192
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 23.053  on 20  degrees of freedom
## Residual deviance: 22.615  on 19  degrees of freedom
##   (4 observations deleted due to missingness)
## AIC: 26.615
##
## Number of Fisher Scoring iterations: 4
```

So, difference in male sizes does not have a significant effect on trial outcome

Sham control vs. Ablated control

We then conducted additional trials to test for the effect of removing the tail - comparing the mating success of males whose tails we removed and reglued and males whose tails we removed and glue only added to the base of the hindwing.

```
# Sham vs ablated control models

# model set
mod7 <- glm(Success ~ Treatment, data = SvAC, family = "binomial")
mod8 <- glm(Success ~ Treatment + scale(Size_Rwings), data = SvAC,
    family = "binomial")
mod9 <- glm(Success ~ Treatment + scale(SQM), data = SvAC, family = "binomial")
mod10 <- glm(Success ~ Treatment + scale(Temp), data = SvAC,
    family = "binomial")
mod11 <- glm(Success ~ Treatment + scale(Hum), data = SvAC, family = "binomial")
mod12 <- glm(Success ~ Treatment + Tent_type, data = SvAC, family = "binomial")


# Here is the model selection table
model.sel(mod7, mod8, mod9, mod10, mod11, mod6, mod12, rank = BIC)
```

```
## Model selection table
##         (Int) Trt scl(Siz_Rwn)  scl(SQM)  scl(Tmp)  scl(Hum) Cag_ctg Tnt_typ df
## mod6  -1.1530   +                                                +          3
## mod8  -0.2860   +       0.2297                                               3
## mod7  -0.2231   +                                                           2
## mod9  -0.2231   +                3.067e-16                                   3
## mod10 -0.2231   +                          2.907e-16                         3
## mod11 -0.2231   +                                    1.963e-16             3
## mod12 -0.2231   +                                                     +    4
##        logLik  BIC delta weight
## mod6  -27.554 66.8  0.00  0.999
## mod8  -35.107 82.1 15.22  0.000
## mod7  -37.096 82.2 15.33  0.000
## mod9  -37.096 86.2 19.31  0.000
## mod10 -37.096 86.2 19.31  0.000
## mod11 -37.096 86.2 19.31  0.000
## mod12 -37.096 90.1 23.30  0.000
## Models ranked by BIC(x)
```

mod7 and mod8 are within 2 BIC of each other. However, mod7 has a simpler structure (treatment as the sole predictor versus treatment + size) and thus, given this limited sample size, we will stick with this simpler model.

We will first present to model checks for mod7 and then proceed to extracting the summary statistics.

DHARMa residual

QQ plot residuals

Within–group deviation from uniformity n.s
Levene Test for homogeneity of variance n.

KS test: p= 0.43448
Deviation n.s.

Dispersion test: p= 0.60
Deviation n.s.

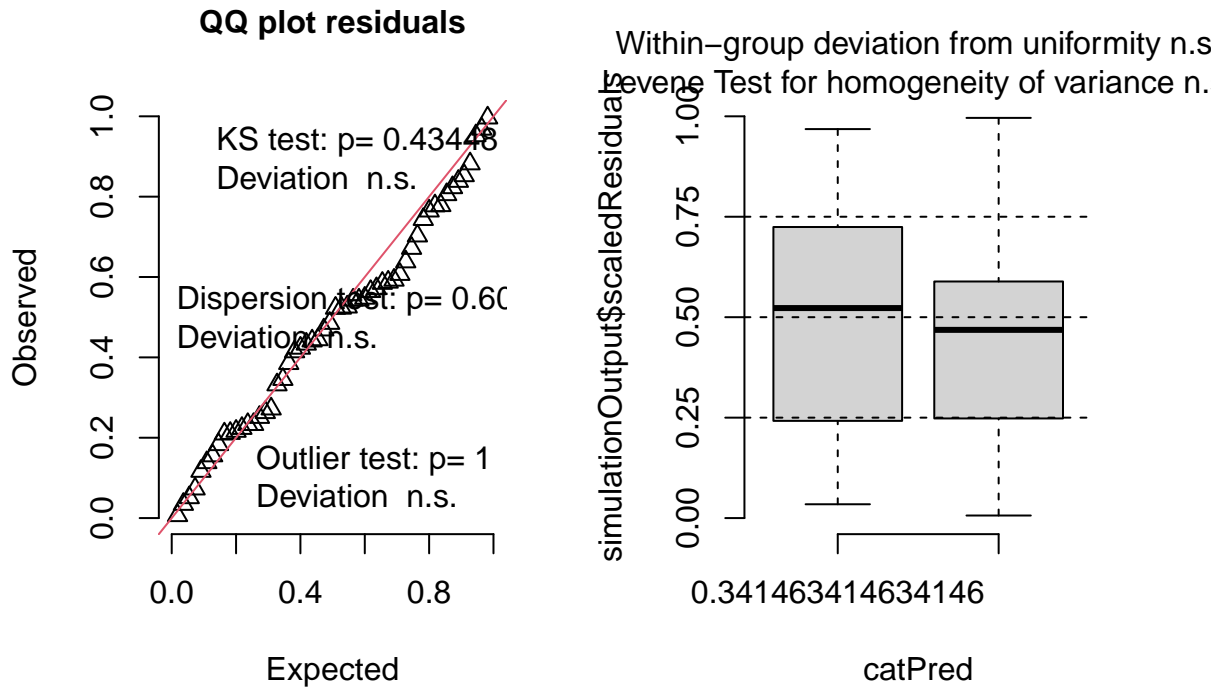Outlier test: p= 1
Deviation n.s.

Table 2: Table output for binomial model comparing mating success between A. luna males with tails removed and reglued (sham control) and A. luna males with tails removed and glue only added (ablated control)

Predictor	parameter estimate	SE	t	p
(Intercept)	-0.22	0.387	-0.58	0.565
TreatmentSham_control	0.45	0.548	0.81	0.415

```
## here are the parameter estimates, back-transformed from the logit scale


##  Treatment       prob     SE  df asymp.LCL asymp.UCL
##  Ablated_control 0.444 0.0956 Inf     0.272     0.631
##  Sham_control    0.556 0.0956 Inf     0.369     0.728
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale
```

Estimating the intercept – when males are the same size, do females still show a preference?

```
##
## Call:
## glm(formula = Success_Sham_control ~ size_diff, family = "binomial",
##     data = newSvAC)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.6104  -1.1957   0.7920   0.9868   1.5930
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)   0.1891     0.4187   0.452    0.651
## size_diff     0.1691     0.1426   1.186    0.236
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 35.426  on 25  degrees of freedom
## Residual deviance: 33.908  on 24  degrees of freedom
##   (1 observation deleted due to missingness)
## AIC: 37.908
##
## Number of Fisher Scoring iterations: 4
```

So again, difference in male sizes does not have a significant effect on trial outcome

Intact vs. glue control

To control for any sensory masking effect that the glue may have had in the damage control trials, we compared the mating success between males with intact hindwings and males with intact hindwings with glue added.

Note: due to tent availability, the smaller tents (BioQuip) were not used for this dyad. Therefore, the Cage_category parameter has been removed.

```
# model set
mod13 <- glm(Success ~ Treatment, data = IvGC, family = "binomial")
mod14 <- glm(Success ~ Treatment + scale(Size_Rwings), data = IvGC,
    family = "binomial")
mod15 <- glm(Success ~ Treatment + scale(Temp), data = IvGC,
    family = "binomial")
mod16 <- glm(Success ~ Treatment + scale(Hum), data = IvGC, family = "binomial")


# Here is the model selection table
model.sel(mod13, mod14, mod15, mod16, rank = BIC)
```

```
## Model selection table
##         (Int) Trt scl(Siz_Rwn)  scl(Tmp)  scl(Hum) df  logLik  BIC delta weight
## mod13 -0.2412   +                                   2 -34.296 76.4  0.00  0.542
## mod14 -0.1562   +      0.1474                        3 -32.979 77.6  1.16  0.304
## mod15 -0.2412   +                9.563e-17          3 -34.296 80.3  3.91  0.077
```

```
## mod16 -0.2412    +                        2.019e-16  3 -34.296 80.3  3.91  0.077
## Models ranked by BIC(x)
```

Here again mod13 and 14, which include Treatment alone and Treatment + size, perform similarly.Given that adding the size term did not significantly improve model fit, we will provide model checks and interpretation for the simplest model only.
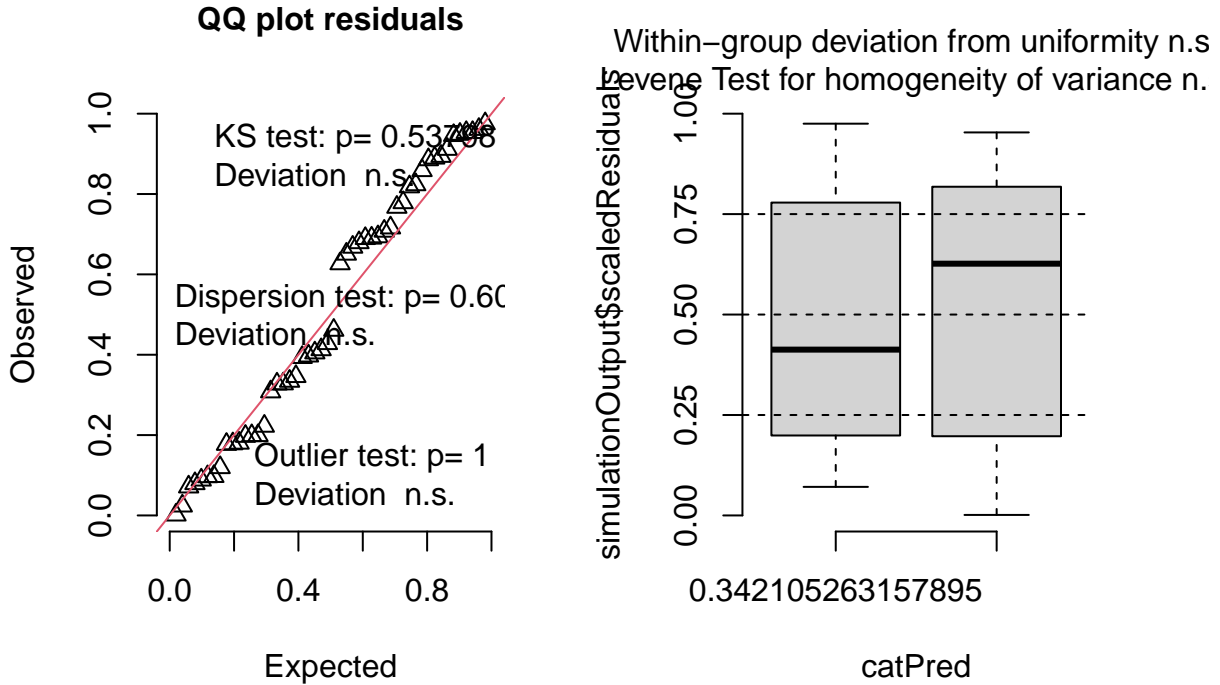
DHARMa residual

Table 3: Table output for binomial model comparing mating success between A. luna males with intact tails and A. luna males with glue added to intact tails

Predictor	parameter estimate	SE	t	p
(Intercept)	-0.24	0.403	-0.60	0.549
TreatmentIntact2	0.48	0.570	0.85	0.397

```
## here are the parameter estimates, back-transformed from the logit scale


##   Treatment   prob     SE  df asymp.LCL asymp.UCL
##   Intact_glue 0.44 0.0993 Inf     0.263     0.634
##   Intact2     0.56 0.0993 Inf     0.366     0.737
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale
```

Estimating the intercept – when males are the same size, do females still show a preference?

```
##
## Call:
## glm(formula = Success_Intact2 ~ size_diff, family = "binomial",
##     data = newIvGC)
##
## Deviance Residuals:
##    Min      1Q  Median      3Q     Max
## -1.456  -1.172   0.713   1.075   1.484
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  0.02843    0.43525   0.065    0.948
## size_diff    0.23554    0.19491   1.208    0.227
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 33.104  on 23  degrees of freedom
## Residual deviance: 31.497  on 22  degrees of freedom
##   (1 observation deleted due to missingness)
## AIC: 35.497
##
## Number of Fisher Scoring iterations: 4
```

Again, difference in male sizes does not have a significant effect on trial outcome

Comparing all experimental sets

To get a sense of whether the outcome of each dyad statistically differed from each other, we compared the mating success of all treatments within one model. Here, we use dyad ID as a random intercept, to account for the grouping arrangements of the treatments.

```
## here is the model summary, with p-values included.
##     You can see that only the intact & ablated dyad vary from the ~50% mean of the other dyads


## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
## Family: binomial  ( logit )
## Formula: Success ~ 0 + Treatment + (1 | dyad)
##    Data: data2
##
##      AIC      BIC   logLik deviance df.resid
##    211.9    233.2    -98.9    197.9      147
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.7795 -0.8944  0.0000  0.8944  1.7795
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  dyad   (Intercept) 0        0
```

```
## Number of obs: 154, groups:  dyad, 3
##
## Fixed effects:
##                          Estimate Std. Error z value Pr(>|z|)
## TreatmentIntact2            0.2412     0.4029   0.599   0.5495
## TreatmentIntact_glue       -0.2412     0.4029  -0.599   0.5495
## TreatmentIntact             1.1527     0.4683   2.461   0.0138 *
## TreatmentAblated           -1.1527     0.4683  -2.461   0.0138 *
## TreatmentSham_control       0.2231     0.3873   0.576   0.5645
## TreatmentAblated_control   -0.2231     0.3873  -0.576   0.5645
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            TrtmI2 TrtmI_ TrtmnI TrtmnA TrtmS_
## TrtmntIntc_ 0.000
## TrtmntIntct 0.000  0.000
## TrtmntAbltd 0.000  0.000  0.000
## TrtmntShm_c 0.000  0.000  0.000  0.000
## TrtmntAblt_ 0.000  0.000  0.000  0.000  0.000
## optimizer (Nelder_Mead) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')


## here are the marginal estimates, back-transformed from the logit scale


##   Treatment        prob     SE  df asymp.LCL asymp.UCL
##   Intact2         0.560 0.0993 Inf     0.366     0.737
##   Intact_glue     0.440 0.0993 Inf     0.263     0.634
##   Intact          0.760 0.0854 Inf     0.558     0.888
##   Ablated         0.240 0.0854 Inf     0.112     0.442
##   Sham_control    0.556 0.0956 Inf     0.369     0.728
##   Ablated_control 0.444 0.0956 Inf     0.272     0.631
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale


## $Treatment
```

So we find that the proportional differences in mating success between the intact and ablated dyad did differ from the outcomes of the other dyads – that is, only in this group was the mating sucess rate different from ~50% for each of the treatments.
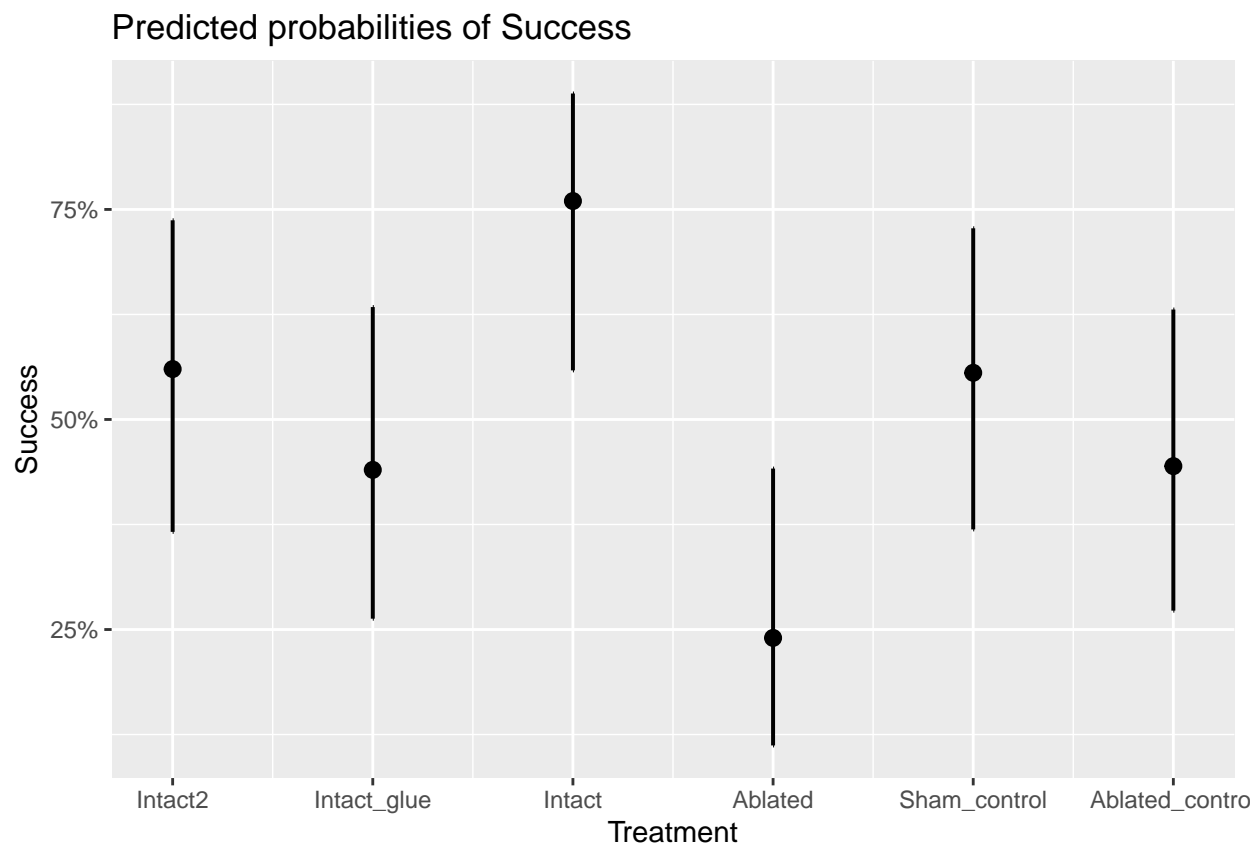
Figure 1: Comparing mating success across all dyads. Geoms are depicted in the true pairs (i.e., Intact - Glue control; Intact - Ablated; Sham control - Ablated control. Central points are marginal estimates and error bars are 95% confidence intervals

Analyzing video recorded behavior

We video recorded a subset of mating trials and here use an exact binomial test to measure whether the male that bopepd the female more was more successful in mating with her than the male that bopped the female less over the course of an experimental night.

```
##
##  Exact binomial test
##
## data:  11 and 18
## number of successes = 11, number of trials = 18, p-value = 0.4807
## alternative hypothesis: true probability of success is not equal to 0.5
## 95 percent confidence interval:
##  0.3574512 0.8270141
## sample estimates:
## probability of success
##              0.6111111
```

p-value > 0.05. We therefore do not find a significant difference in bopping behavior between males who succeeded in mating with the female and males who failed.

**Please see the .rmd file for the full code & outputs. For ease of reading, the code chunks have been hidden from this pdf document.