

Supplementary material for “Building a beetle: how larval environment leads to adult fitness in a horned beetle”

*Rob Knell & Leeann Reaney
School of Biological and Chemical Sciences
Queen Mary University of London*

24 July, 2015

Contents

1	Introduction	2
2	Sexual dimorphism in pronotum length	3
3	Comparisons of male and female beetles	4
4	Path analysis for male performance	7
4.1	Data transformations used:	7
4.2	Initial model	7
4.3	Final model	10
4.4	Notes	12
5	Path analysis for female performance	12
5.1	Data transformations used:	12
5.2	Initial model	13
5.3	Final model	15
6	Path analysis for male testes mass and fat content	17
6.1	Initial model	17
6.2	Final model	20
7	Path analysis for female fat content	24
7.1	Initial model	24
7.2	Final model	26
7.3	Notes	28
	References	28

1 Introduction

This document contains supplementary information and full details of the analysis, including all the R (R Core Team 2014) code necessary, for the paper named above. To run the R code below you will need the lavaan (Rosseel 2012), scales and ggplot2 (Wickham 2009) packages installed, and the data files Beetle_data_exploratory.txt, maledata.txt and femaledata.txt which can be obtained from the [Dryad](#) archive. This document was prepared in R markdown and rendered to pdf using the [rmarkdown v2](#) package.

2 Sexual dimorphism in pronotum length

```
Beetle <- read.table("Beetle_data_exploratory.txt", header = TRUE)
library(ggplot2)
library(scales)
attach(Beetle)
p1 <- ggplot(data = Beetle, aes(x = Elytra, y = Pronotum, shape = Sex,
  colour = Sex))
p1 <- p1 + geom_point() + scale_shape_manual(values = c(16, 17)) +
  scale_color_manual(values = c("darkred", "steelblue")) +
  theme_bw()
p1 <- p1 + xlab("Elytra length (mm)") + ylab("Pronotum length (mm)")
p1
```

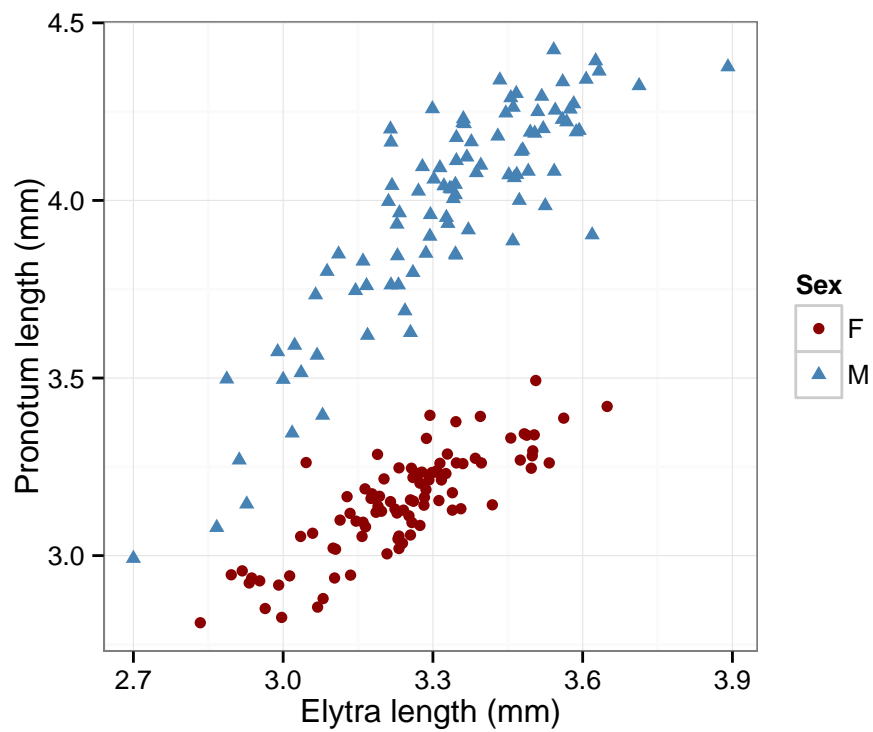


Figure 1:

Figure A: Pronotum length plotted against elytra length for male and female beetles.

3 Comparisons of male and female beetles

```
p1 <- ggplot(data = Beetle, aes(x = Elytra, y = MaxPerform, shape = Sex,
  colour = Sex)) + geom_point()
p1 <- p1 + scale_shape_manual(values = c(16, 17))
p1 <- p1 + scale_y_continuous(breaks = c(20, 30, 40, 50, 60,
  70, 90), trans = log_trans())
p1 <- p1 + scale_color_manual(values = c("darkred", "steelblue")) +
  theme_bw()
p1 <- p1 + xlab("Elytra length (mm)") + ylab("Maximum strength (N)")
p1
```

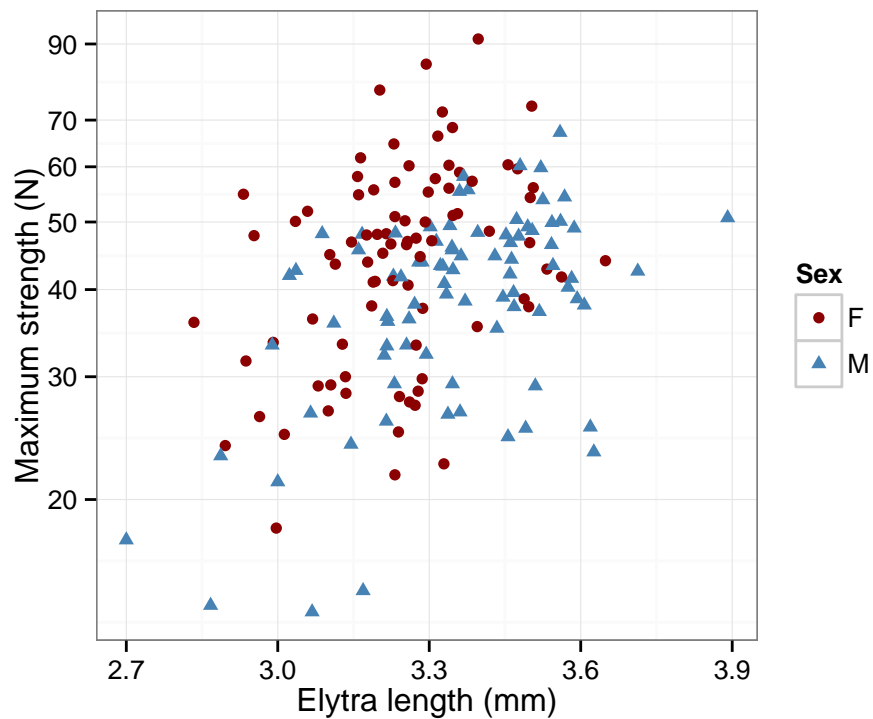


Figure 2:

Figure B: Log maximum strength plotted against elytra length for male and female beetles.

```
# Comparison of size
```

```
t.test(Elytra ~ Sex, var.equal = TRUE)
```

```
##
```

```
## Two Sample t-test
```

```
##
```

```
## data: Elytra by Sex
```

```
## t = -3.4842, df = 185, p-value = 0.0006162
```

```
## alternative hypothesis: true difference in means is not equal to 0
```

```
## 95 percent confidence interval:
```

```
## -0.14868766 -0.04117847
```

```
## sample estimates:
## mean in group F mean in group M
##      3.240120      3.335053
```

Comparison of strength

```
strengthmod1 <- lm(log(MaxPerform) ~ Sex * Elytra)
## Fit initial model
drop1(strengthmod1, sex = "F")
```

```
## Single term deletions
##
## Model:
## log(MaxPerform) ~ Sex * Elytra
##      Df Sum of Sq    RSS    AIC
## <none>            14.558 -402.90
## Sex:Elytra  1  0.001018 14.559 -404.89
```

```
strengthmod2 <- update(strengthmod1, ~. - Sex:Elytra)
## No significant interaction so refit model only main effects

drop1(strengthmod2, test = "F")
```

```
## Single term deletions
##
## Model:
## log(MaxPerform) ~ Sex + Elytra
##      Df Sum of Sq    RSS    AIC F value    Pr(>F)
## <none>            14.559 -404.89
## Sex      1      1.8125 16.371 -387.18  20.542 1.116e-05 ***
## Elytra  1      3.4897 18.049 -370.79  39.550 2.758e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
detach(Beetle)
```

```
## Both main effects highly significant
```

```
summary(strengthmod2)
```

```
##
## Call:
## lm(formula = log(MaxPerform) ~ Sex + Elytra)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.80386 -0.17680  0.06066  0.18730  0.62068
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   1.2057     0.4095   2.944  0.0037 **
## SexM          -0.2166     0.0478  -4.532 1.12e-05 ***
```

```
## Elytra          0.7919      0.1259    6.289 2.76e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.297 on 165 degrees of freedom
## (23 observations deleted due to missingness)
## Multiple R-squared:  0.2245, Adjusted R-squared:  0.2151
## F-statistic: 23.88 on 2 and 165 DF,  p-value: 7.763e-10
```

```
rm(Beetle)
```

4 Path analysis for male performance

```
# setwd('~/.Dropbox/Current/Leeann's resource  
# availability/Analysis 2014/path analysis 2014')  
library(lavaan)
```

```
## Warning: package 'lavaan' was built under R version 3.1.3
```

```
## This is lavaan 0.5-18  
## lavaan is BETA software! Please report any bugs.
```

```
maledata <- read.table("maledata.txt")
```

4.1 Data transformations used:

Haemolymph protein and maximum strength were both log transformed to reduce heteroscedasticity Weight gain was log transformed to reduce curvature in the relationship between weight gain and maximum strength Eclosion weight and fat content were rescaled by dividing by 100 in order to make the scales the variables are measured on more equivalent.

4.2 Initial model

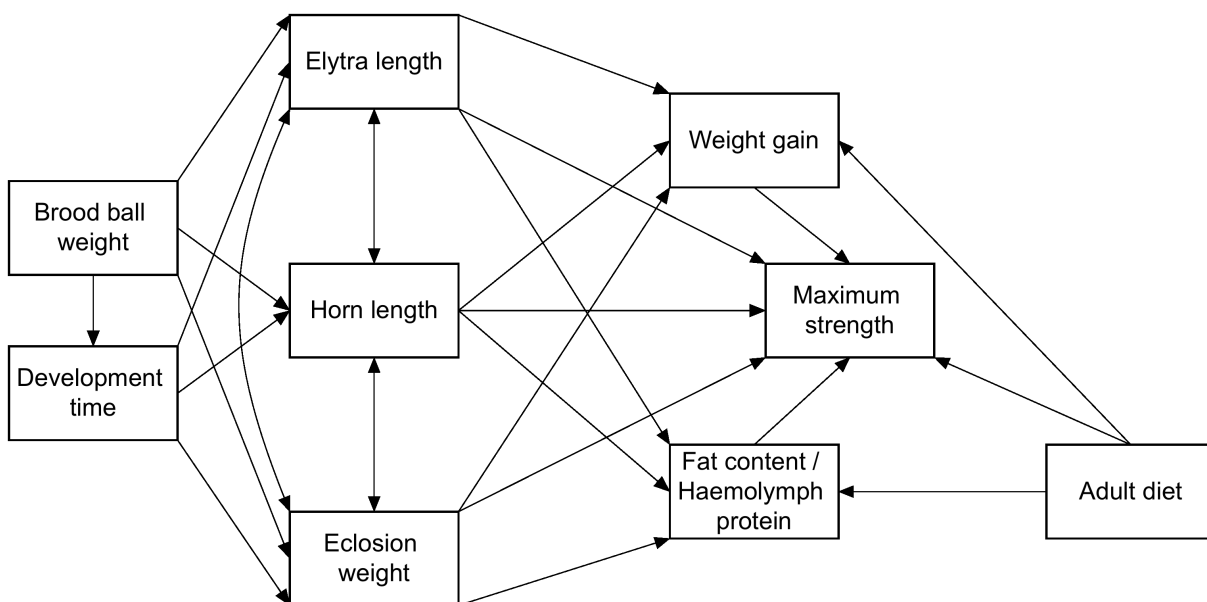


Figure C: Path diagram showing the original model fitted to explain male performance. Double headed arrows indicate correlation (i.e. no assumptions made about causality)

```
##Specify the model
```

```
Path.full.model.perform.male<-'#Regressions
```

```

lnPerform~lnProt+Fat.trans+lnWtgain+Elytra+Horn+Ec.Weight.trans+Adult.diet
lnProt~Elytra+Ec.Weight.trans+lnWtgain+Horn+Adult.diet
Fat.trans~Elytra+Ec.Weight.trans+lnWtgain+Horn+Adult.diet
lnWtgain~Elytra+Ec.Weight.trans+EndBBweight+Horn+Adult.diet
Ec.Weight.trans~EndBBweight +Developmenttime
Elytra~EndBBweight +Developmenttime
Developmenttime~EndBBweight
Horn~ EndBBweight+Developmenttime

#covariances
Horn~~Elytra
Horn~~Ec.Weight.trans
lnProt~~Fat.trans
Elytra~~Ec.Weight.trans

```

```
## Fit the model and check the summary
```

```

Male.performance.full.model <- sem(Path.full.model.perform.male,
  data = maledata, missing = "fiml")
summary(Male.performance.full.model, standardized = TRUE)

```

```
## lavaan (0.5-18) converged normally after 156 iterations
```

```

##
##   Number of observations                98
##
##   Number of missing patterns           8
##
##   Estimator                           ML
##   Minimum Function Test Statistic      13.481
##   Degrees of freedom                   11
##   P-value (Chi-square)                 0.263
##
## Parameter estimates:
##
##   Information                        Observed
##   Standard Errors                   Standard
##
##           Estimate  Std.err  Z-value  P(>|z|)  Std.lv  Std.all
## Regressions:
##   lnPerform ~
##     lnProt      -0.006   0.068   -0.094   0.925   -0.006   -0.008
##     Fat.trans    0.102   0.245    0.418   0.676    0.102    0.054
##     lnWtgain     0.543   0.280    1.938   0.053    0.543    0.385
##     Elytra       0.281   0.264    1.063   0.288    0.281    0.168
##     Horn         0.647   0.378    1.714   0.086    0.647    0.377
##     Ec.Weght.trns -0.163   0.088   -1.862   0.063   -0.163   -0.300
##     Adult.diet   0.009   0.069    0.130   0.897    0.009    0.012
##   lnProt ~
##     Elytra      -0.750   0.463   -1.620   0.105   -0.750   -0.353
##     Ec.Weght.trns 0.114   0.146    0.781   0.435    0.114    0.165
##     lnWtgain    -0.028   0.418   -0.067   0.946   -0.028   -0.016
##     Horn        0.157   0.670    0.234   0.815    0.157    0.072

```



```

##      Adult.diet      0.087      0.099      0.871      0.384      0.087      0.095
##      Fat.trans ~
##      Elytra      0.050      0.127      0.393      0.694      0.050      0.057
##      Ec.Weght.trns      0.031      0.040      0.776      0.438      0.031      0.108
##      lnWtgain      0.527      0.109      4.830      0.000      0.527      0.714
##      Horn      -0.224      0.179      -1.252      0.211      -0.224      -0.250
##      Adult.diet      -0.164      0.027      -6.061      0.000      -0.164      -0.438
##      lnWtgain ~
##      Elytra      0.353      0.120      2.939      0.003      0.353      0.298
##      Ec.Weght.trns      -0.095      0.041      -2.315      0.021      -0.095      -0.246
##      EndBBweight      -0.027      0.045      -0.610      0.542      -0.027      -0.041
##      Horn      1.015      0.137      7.395      0.000      1.015      0.834
##      Adult.diet      -0.008      0.027      -0.281      0.779      -0.008      -0.015
##      Ec.Weight.trans ~
##      EndBBweight      1.109      0.136      8.145      0.000      1.109      0.641
##      Developmenttm      -0.019      0.020      -0.938      0.348      -0.019      -0.074
##      Elytra ~
##      EndBBweight      0.337      0.046      7.333      0.000      0.337      0.599
##      Developmenttm      -0.019      0.007      -2.783      0.005      -0.019      -0.230
##      Developmenttime ~
##      EndBBweight      0.895      0.679      1.319      0.187      0.895      0.132
##      Horn ~
##      EndBBweight      0.305      0.045      6.811      0.000      0.305      0.557
##      Developmenttm      -0.026      0.007      -3.952      0.000      -0.026      -0.327
##
## Covariances:
##      Elytra ~~
##      Horn      0.021      0.004      5.740      0.000      0.021      0.740
##      Ec.Weight.trans ~~
##      Horn      0.065      0.011      6.021      0.000      0.065      0.781
##      lnProt ~~
##      Fat.trans      -0.011      0.006      -1.787      0.074      -0.011      -0.208
##      Ec.Weight.trans ~~
##      Elytra      0.059      0.011      5.497      0.000      0.059      0.686
##
## Intercepts:
##      lnPerform      -0.592      1.400      -0.423      0.672      -0.592      -1.658
##      lnProt      10.255      2.008      5.106      0.000      10.255      22.591
##      Fat.trans      -2.356      0.533      -4.420      0.000      -2.356      -12.620
##      lnWtgain      3.970      0.295      13.464      0.000      3.970      15.688
##      Ec.Weght.trns      2.574      0.621      4.144      0.000      2.574      3.918
##      Elytra      3.419      0.212      16.153      0.000      3.419      16.021
##      Developmenttm      29.224      1.016      28.751      0.000      29.224      11.361
##      Horn      1.412      0.206      6.850      0.000      1.412      6.785
##
## Variances:
##      lnPerform      0.065      0.010      0.065      0.511
##      lnProt      0.195      0.031      0.195      0.947
##      Fat.trans      0.015      0.002      0.015      0.429
##      lnWtgain      0.015      0.002      0.015      0.234
##      Ec.Weght.trns      0.257      0.037      0.257      0.596
##      Elytra      0.028      0.004      0.028      0.625
##      Developmenttm      6.502      0.929      6.502      0.983
##      Horn      0.027      0.004      0.027      0.631

```

4.3 Final model

A final model can be produced by removing non-significant terms, adding in ones indicated by modification indices and by removing terms which have no direct or indirect effect on performance. Modification indices are used for model refinement: they give an index of the change in goodness of fit from adding a term into a model. The final path model for male performance is:

```
Path.reduced.model.perform.male<-'#Regressions
```

```
lnPerform~lnWtgain+Elytra+EndBBweight
lnWtgain~Elytra+Ec.Weight.trans+Horn
Ec.Weight.trans~EndBBweight
Elytra~EndBBweight+Developmenttime
Horn~ EndBBweight+Developmenttime
```

```
#covariances
Horn~~Ec.Weight.trans
Elytra~~Ec.Weight.trans
Elytra~~Horn
'
```

```
Male.performance.reduced.model <- sem(Path.reduced.model.perform.male,
  data = maledata, missing = "fiml")
summary(Male.performance.reduced.model, standardized = TRUE,
  fit.measures = TRUE)
```

```
## lavaan (0.5-18) converged normally after 91 iterations
##
##   Number of observations                98
##
##   Number of missing patterns            4
##
##   Estimator                            ML
##   Minimum Function Test Statistic       4.931
##   Degrees of freedom                    6
##   P-value (Chi-square)                  0.553
##
## Model test baseline model:
##
##   Minimum Function Test Statistic       453.425
##   Degrees of freedom                    20
##   P-value                               0.000
##
## User model versus baseline model:
##
##   Comparative Fit Index (CFI)           1.000
##   Tucker-Lewis Index (TLI)             1.008
##
## Loglikelihood and Information Criteria:
##
##   Loglikelihood user model (H0)         -137.508
##   Loglikelihood unrestricted model (H1)  -135.042
##
##   Number of free parameters             24
```

```

## Akaike (AIC) 323.016
## Bayesian (BIC) 385.055
## Sample-size adjusted Bayesian (BIC) 309.266
##
## Root Mean Square Error of Approximation:
##
## RMSEA 0.000
## 90 Percent Confidence Interval 0.000 0.118
## P-value RMSEA <= 0.05 0.688
##
## Standardized Root Mean Square Residual:
##
## SRMR 0.036
##
## Parameter estimates:
##
## Information Observed
## Standard Errors Standard
##
## Estimate Std.err Z-value P(>|z|) Std.lv Std.all
## Regressions:
## lnPerform ~
## lnWtgain 0.770 0.201 3.826 0.000 0.770 0.551
## Elytra 0.455 0.227 2.007 0.045 0.455 0.275
## EndBBweight -0.221 0.087 -2.547 0.011 -0.221 -0.241
## lnWtgain ~
## Elytra 0.343 0.118 2.896 0.004 0.343 0.290
## Ec.Weght.trns -0.105 0.038 -2.781 0.005 -0.105 -0.277
## Horn 1.025 0.137 7.500 0.000 1.025 0.839
## Ec.Weight.trans ~
## EndBBweight 1.092 0.136 8.056 0.000 1.092 0.631
## Elytra ~
## EndBBweight 0.333 0.046 7.266 0.000 0.333 0.598
## Developmenttm -0.015 0.005 -2.902 0.004 -0.015 -0.180
## Horn ~
## EndBBweight 0.301 0.045 6.734 0.000 0.301 0.558
## Developmenttm -0.022 0.004 -5.039 0.000 -0.022 -0.271
##
## Covariances:
## Ec.Weight.trans ~~
## Horn 0.066 0.011 6.021 0.000 0.066 0.783
## Elytra 0.059 0.011 5.497 0.000 0.059 0.687
## Elytra ~~
## Horn 0.021 0.004 5.734 0.000 0.021 0.742
##
## Intercepts:
## lnPerform -2.061 0.830 -2.483 0.013 -2.061 -5.897
## lnWtgain 3.980 0.286 13.909 0.000 3.980 15.905
## Ec.Weght.trns 2.023 0.203 9.960 0.000 2.023 3.080
## Elytra 3.294 0.164 20.091 0.000 3.294 15.575
## Horn 1.272 0.142 8.962 0.000 1.272 6.209
##
## Variances:
## lnPerform 0.064 0.010 0.064 0.520

```

##	lnWtgain	0.015	0.002	0.015	0.241
##	Ec.Weght.trns	0.259	0.037	0.259	0.602
##	Elytra	0.029	0.004	0.029	0.639
##	Horn	0.027	0.004	0.027	0.655

4.4 Notes

- The “std.all” column gives the coefficient estimates when all predictors are standardised, meaning you can make meaningful comparisons between them (i.e. horn length has a bigger influence on weight gain than elytra length)
- Diet drops out of the path model so there’s no problem with the binomial predictor variables in this case.
- In the fit measures section, the first p-value under “Minimum Function Chi Square” should be close to 1 for a well fitted model. In this case it is.
- RMSEA values close to zero are indicative of a well fitted model, again this meets the criterion.
- Brood ball weight added as a predictor for performance after checking modification indices.

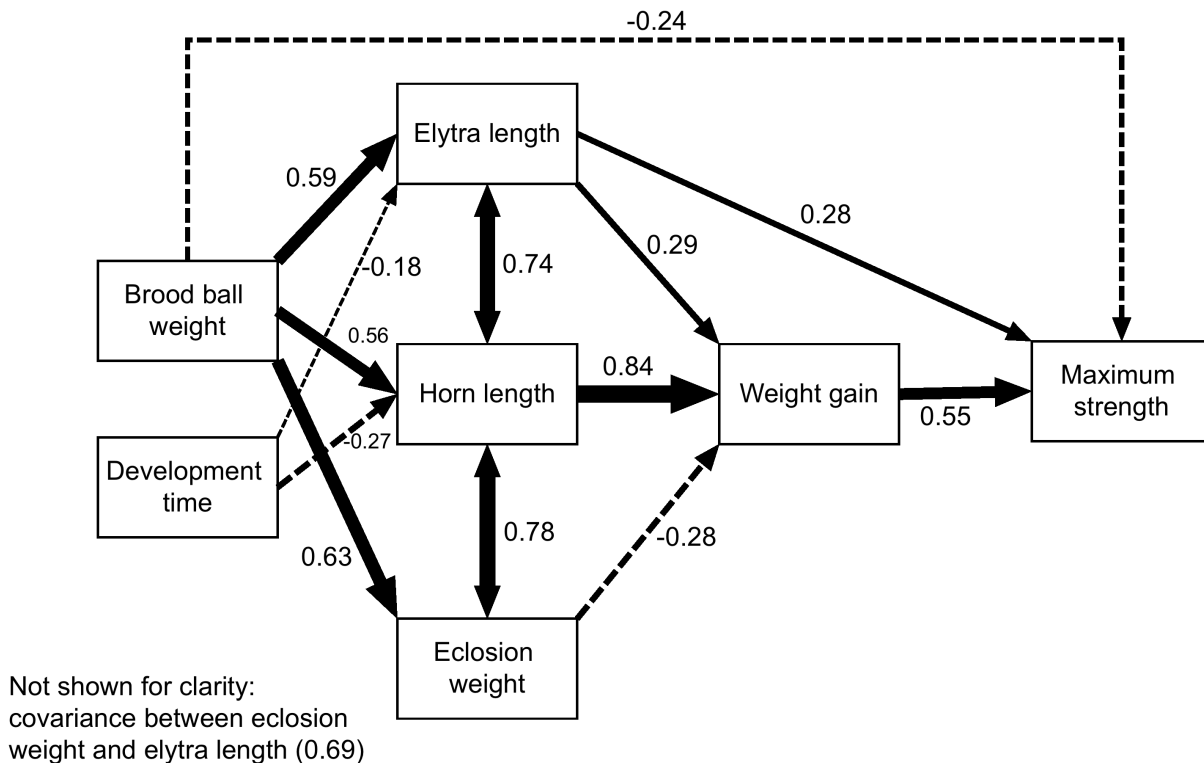


Figure D: Path diagram for the final model explaining male performance. Solid lines are positive relations, dashed lines negative, double headed arrows indicate correlation (i.e. no assumptions about causality), line width is proportional to the strength of the relationship.

5 Path analysis for female performance

5.1 Data transformations used:

The same transformations were used as for the analysis of male performance:

Haemolymph protein and maximum strength were both log transformed to reduce heteroscedasticity Weight gain was log transformed to reduce curvature in the relationship between weight gain and maximum strength Eclosion weight and fat content were rescaled by dividing by 100 in order to make the scales the variables are measured on more equivalent.

5.2 Initial model

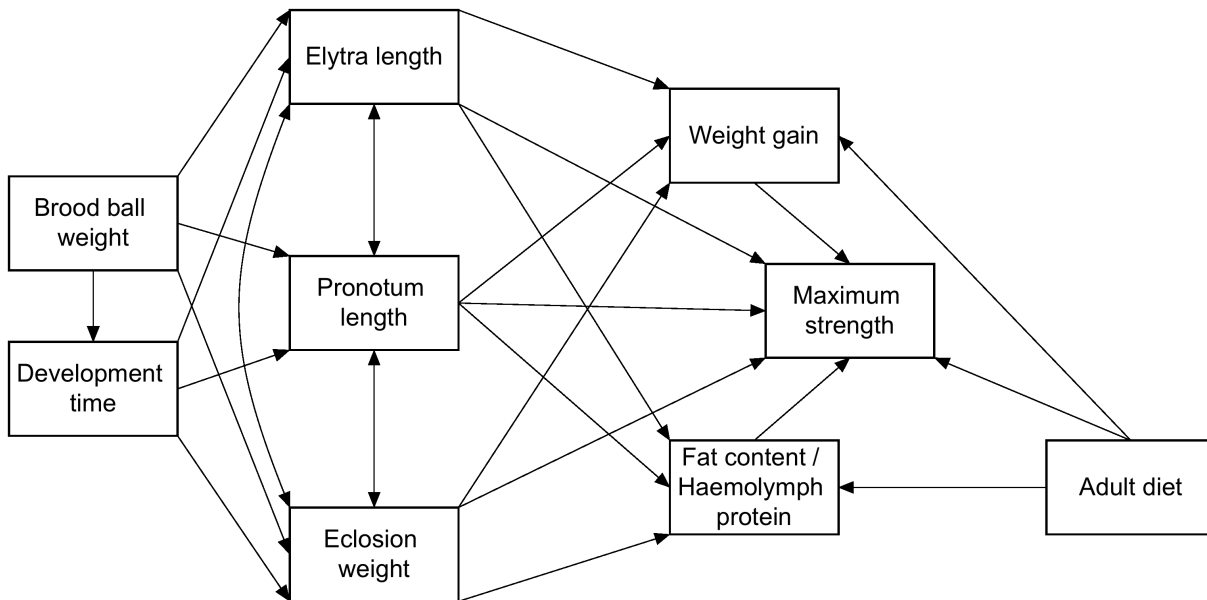


Figure E: Path diagram showing the original model fitted to explain female performance. Double headed arrows indicate correlation (i.e. no assumptions made about causality)

```

#Load data
femaledata<-read.table("femaledata.txt",header=TRUE)

#Specify full model
Path.full.model.perform.female<-'#Regressions

lnPerform~lnProt+Fat.trans+lnWtgain+Elytra+Ec.Weight.trans+Adult.diet
lnProt~Elytra+Ec.Weight.trans+lnWtgain+Adult.diet
Fat.trans~Elytra+Ec.Weight.trans+lnWtgain +Adult.diet
lnWtgain~Elytra+Ec.Weight.trans+EndBBweight +Adult.diet
Ec.Weight.trans~EndBBweight +Developmenttime
Elytra~EndBBweight +Developmenttime
Developmenttime~EndBBweight

#covariances
lnProt~~Fat.trans
Elytra~~Ec.Weight.trans'

## Fit the model and check the summary

Female.performance.full.model <- sem(Path.full.model.perform.female,
  data = femaledata, missing = "fiml")
summary(Female.performance.full.model, standardized = TRUE)

```

```

## lavaan (0.5-18) converged normally after 135 iterations
##
##   Number of observations                93
##
##   Number of missing patterns           5
##
##   Estimator                           ML
##   Minimum Function Test Statistic      12.009
##   Degrees of freedom                   10
##   P-value (Chi-square)                 0.284
##
## Parameter estimates:
##
##   Information                        Observed
##   Standard Errors                   Standard
##
##           Estimate  Std.err  Z-value  P(>|z|)  Std.lv  Std.all
##
## Regressions:
##   lnPerform ~
##     lnProt      -0.032   0.105   -0.307   0.759   -0.032   -0.032
##     Fat.trans    0.148   0.109    1.362   0.173    0.148    0.200
##     lnWtgain     0.107   0.166    0.644   0.519    0.107    0.097
##     Elytra       0.010   0.151    0.063   0.950    0.010    0.011
##     Ec.Weght.trns 0.083   0.052    1.605   0.109    0.083    0.262
##     Adult.diet   0.026   0.034    0.780   0.436    0.026    0.091
##   lnProt ~
##     Elytra      -0.305   0.153   -1.998   0.046   -0.305   -0.342
##     Ec.Weght.trns 0.127   0.050    2.514   0.012    0.127    0.397
##     lnWtgain     0.223   0.151    1.470   0.142    0.223    0.203
##     Adult.diet   0.062   0.029    2.128   0.033    0.062    0.216
##   Fat.trans ~
##     Elytra       0.031   0.149    0.207   0.836    0.031    0.026
##     Ec.Weght.trns 0.099   0.049    2.015   0.044    0.099    0.232
##     lnWtgain     0.661   0.147    4.499   0.000    0.661    0.448
##     Adult.diet   -0.158   0.029   -5.501   0.000   -0.158   -0.406
##   lnWtgain ~
##     Elytra       0.396   0.101    3.922   0.000    0.396    0.487
##     Ec.Weght.trns 0.079   0.037    2.125   0.034    0.079    0.272
##     EndBBweight  -0.012   0.035   -0.339   0.735   -0.012   -0.031
##     Adult.diet   0.000   0.021    0.014   0.989    0.000    0.001
##   Ec.Weight.trans ~
##     EndBBweight   0.641   0.118    5.410   0.000    0.641    0.485
##     Developmenttm -0.020   0.013   -1.476   0.140   -0.020   -0.132
##   Elytra ~
##     EndBBweight   0.184   0.044    4.222   0.000    0.184    0.389
##     Developmenttm -0.012   0.005   -2.436   0.015   -0.012   -0.225
##   Developmenttime ~
##     EndBBweight  -1.432   0.917   -1.563   0.118   -1.432   -0.160
##
## Covariances:
##   lnProt ~~
##     Fat.trans     -0.001   0.002   -0.727   0.467   -0.001   -0.079
##   Ec.Weight.trans ~~
##     Elytra        0.036   0.007    5.308   0.000    0.036    0.659

```

```
##
## Intercepts:
##   lnPerform      0.722    0.840    0.860    0.390    0.722    5.033
##   lnProt         3.044    0.720    4.230    0.000    3.044   21.219
##   Fat.trans      -3.328    0.698   -4.765    0.000   -3.328  -17.250
##   lnWtgain       4.167    0.247   16.874    0.000    4.167   31.901
##   Ec.Weght.trns  2.940    0.448    6.557    0.000    2.940    6.537
##   Elytra         3.335    0.166   20.098    0.000    3.335   20.734
##   Developmenttm  31.446    1.314   23.936    0.000   31.446   10.323
##
## Variances:
##   lnPerform      0.016    0.002                0.016    0.777
##   lnProt         0.017    0.003                0.017    0.831
##   Fat.trans      0.016    0.002                0.016    0.433
##   lnWtgain       0.009    0.001                0.009    0.513
##   Ec.Weght.trns  0.147    0.022                0.147    0.727
##   Elytra         0.020    0.003                0.020    0.769
##   Developmenttm  9.042    1.326                9.042    0.974
```

5.3 Final model

Model reduction leads to this:

```
#Specify reduced model
Path.reduced.model.perform.female<-'#Regressions

lnPerform~Ec.Weight.trans
Ec.Weight.trans~EndBBweight'

# Fit the model and check the summary

female.performance.reduced.model <- sem(Path.reduced.model.perform.female,
  data = femaledata, missing = "fiml")
summary(female.performance.reduced.model, standardized = TRUE,
  fit.measures = TRUE)
```

```
## lavaan (0.5-18) converged normally after 25 iterations
##
##   Number of observations                93
##
##   Number of missing patterns           2
##
##   Estimator                           ML
##   Minimum Function Test Statistic      1.716
##   Degrees of freedom                   1
##   P-value (Chi-square)                 0.190
##
## Model test baseline model:
##
##   Minimum Function Test Statistic      46.424
##   Degrees of freedom                   3
##   P-value                             0.000
##
```

```

## User model versus baseline model:
##
##   Comparative Fit Index (CFI)                0.984
##   Tucker-Lewis Index (TLI)                  0.951
##
## Loglikelihood and Information Criteria:
##
##   Loglikelihood user model (H0)              -23.327
##   Loglikelihood unrestricted model (H1)      -22.469
##
##   Number of free parameters                  6
##   Akaike (AIC)                             58.653
##   Bayesian (BIC)                           73.849
##   Sample-size adjusted Bayesian (BIC)       54.908
##
## Root Mean Square Error of Approximation:
##
##   RMSEA                                     0.088
##   90 Percent Confidence Interval            0.000  0.306
##   P-value RMSEA <= 0.05                    0.241
##
## Standardized Root Mean Square Residual:
##
##   SRMR                                     0.036
##
## Parameter estimates:
##
##   Information                                Observed
##   Standard Errors                          Standard
##
##           Estimate  Std.err  Z-value  P(>|z|)  Std.lv  Std.all
## Regressions:
##   lnPerform ~
##     Ec.Weght.trns    0.136    0.031    4.373    0.000    0.136    0.427
##     Ec.Weight.trans ~
##       EndBBweight    0.669    0.118    5.654    0.000    0.669    0.506
##
## Intercepts:
##   lnPerform          1.197    0.102   11.733    0.000    1.197    8.334
##   Ec.Weght.trns      2.327    0.170   13.725    0.000    2.327    5.173
##
## Variances:
##   lnPerform          0.017    0.003             0.017    0.818
##   Ec.Weght.trns      0.151    0.022             0.151    0.744

```

This model is not as good a fit as the one for males but it is acceptable.

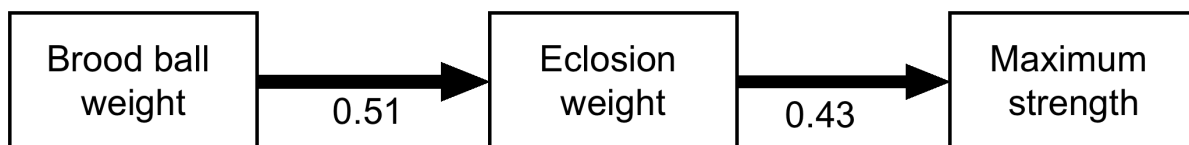


Figure F: Path diagram for the final model explaining female performance. Solid lines are positive relations, line width is

proportional to the strength of the relationship.

6 Path analysis for male testes mass and fat content

6.1 Initial model

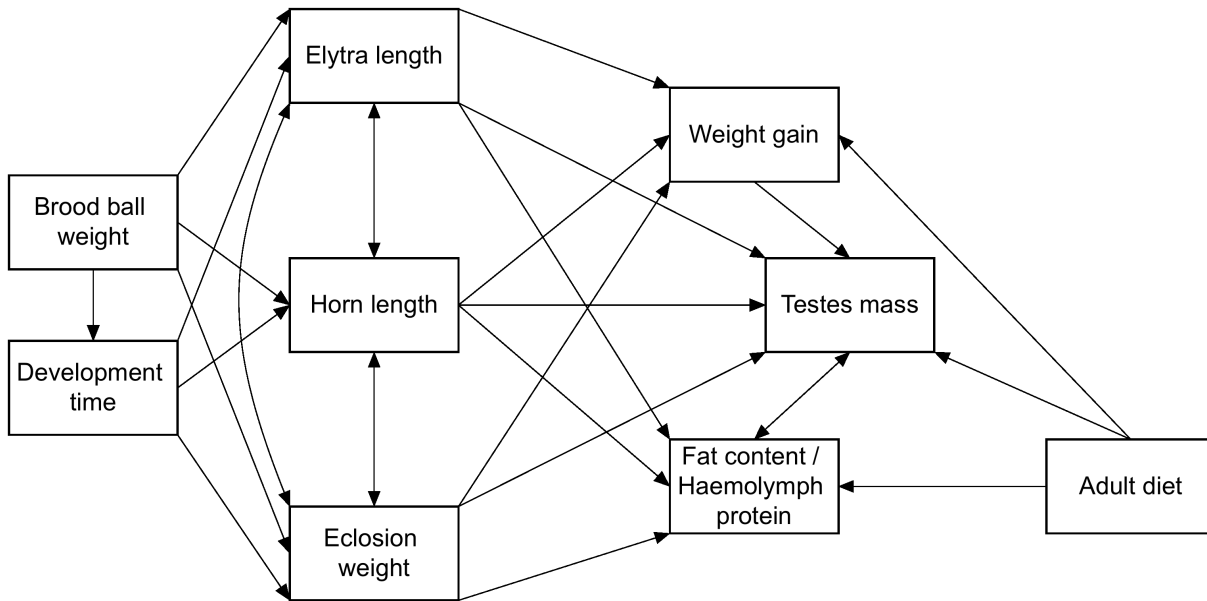


Figure G: Path diagram showing the original model fitted to explain testes mass. Double headed arrows indicate correlation (i.e. no assumptions made about causality)

#Specify full model

```
Path.full.model.testes<-'#Regressions
```

```

lnTestes~Elytra+Ec.Weight.trans+lnWtgain+Horn+Adult.diet
lnProt~Elytra+Ec.Weight.trans+lnWtgain+Horn+Adult.diet
Fat.trans~Elytra+Ec.Weight.trans+lnWtgain+Horn+Adult.diet
lnWtgain~Elytra+Ec.Weight.trans+EndBBweight+Horn+Adult.diet
Ec.Weight.trans~EndBBweight +Developmenttime
Elytra~EndBBweight +Developmenttime
Developmenttime~EndBBweight
Horn~ EndBBweight+Developmenttime

```

```
#covariances
```

```

lnTestes~~Fat.trans
Elytra~~Ec.Weight.trans
Horn~~Elytra
Horn~~Ec.Weight.trans'

```

```
# Fit the model and check the summary
```

```
male.testes.full.model <- sem(Path.full.model.testes, data = maledata,  
  missing = "fiml")  
summary(male.testes.full.model, standardized = TRUE, fit.measures = TRUE)
```

```
## lavaan (0.5-18) converged normally after 170 iterations  
##  
##   Number of observations                  98  
##  
##   Number of missing patterns              8  
##  
##   Estimator                               ML  
##   Minimum Function Test Statistic         11.983  
##   Degrees of freedom                      11  
##   P-value (Chi-square)                    0.365  
##  
## Model test baseline model:  
##  
##   Minimum Function Test Statistic         525.548  
##   Degrees of freedom                      44  
##   P-value                                0.000  
##  
## User model versus baseline model:  
##  
##   Comparative Fit Index (CFI)             0.998  
##   Tucker-Lewis Index (TLI)               0.992  
##  
## Loglikelihood and Information Criteria:  
##  
##   Loglikelihood user model (H0)           -174.026  
##   Loglikelihood unrestricted model (H1)    -168.035  
##  
##   Number of free parameters               49  
##   Akaike (AIC)                           446.052  
##   Bayesian (BIC)                         572.716  
##   Sample-size adjusted Bayesian (BIC)     417.981  
##  
## Root Mean Square Error of Approximation:  
##  
##   RMSEA                                0.030  
##   90 Percent Confidence Interval          0.000  0.113  
##   P-value RMSEA <= 0.05                  0.569  
##  
## Standardized Root Mean Square Residual:  
##  
##   SRMR                                0.041  
##  
## Parameter estimates:  
##  
##   Information                            Observed  
##   Standard Errors                        Standard
```

```

##
##           Estimate Std.err Z-value P(>|z|) Std.lv Std.all
## Regressions:
##   lnTestes ~
##     Elytra      0.136   0.202   0.673   0.501   0.136   0.129
##     Ec.Weght.trns 0.073   0.064   1.140   0.254   0.073   0.212
##     lnWtgain     0.457   0.176   2.596   0.009   0.457   0.515
##     Horn        -0.412   0.291  -1.416   0.157  -0.412  -0.382
##     Adult.diet  -0.073   0.043  -1.688   0.091  -0.073  -0.163
##   lnProt ~
##     Elytra      -0.748   0.463  -1.617   0.106  -0.748  -0.352
##     Ec.Weght.trns 0.114   0.146   0.779   0.436   0.114   0.165
##     lnWtgain    -0.030   0.417  -0.072   0.942  -0.030  -0.017
##     Horn         0.160   0.668   0.240   0.810   0.160   0.073
##     Adult.diet   0.088   0.099   0.889   0.374   0.088   0.097
##   Fat.trans ~
##     Elytra       0.057   0.126   0.448   0.654   0.057   0.065
##     Ec.Weght.trns 0.030   0.040   0.761   0.447   0.030   0.106
##     lnWtgain     0.529   0.109   4.852   0.000   0.529   0.715
##     Horn        -0.230   0.179  -1.285   0.199  -0.230  -0.256
##     Adult.diet  -0.165   0.027  -6.100   0.000  -0.165  -0.439
##   lnWtgain ~
##     Elytra       0.353   0.120   2.938   0.003   0.353   0.298
##     Ec.Weght.trns -0.095   0.041  -2.315   0.021  -0.095  -0.246
##     EndBBweight  -0.027   0.045  -0.610   0.542  -0.027  -0.041
##     Horn         1.015   0.137   7.396   0.000   1.015   0.834
##     Adult.diet  -0.008   0.027  -0.280   0.779  -0.008  -0.015
##   Ec.Weight.trans ~
##     EndBBweight   1.109   0.136   8.145   0.000   1.109   0.641
##     Developmenttm -0.019   0.020  -0.938   0.348  -0.019  -0.074
##   Elytra ~
##     EndBBweight   0.337   0.046   7.333   0.000   0.337   0.599
##     Developmenttm -0.019   0.007  -2.783   0.005  -0.019  -0.230
##   Developmenttime ~
##     EndBBweight   0.895   0.679   1.319   0.187   0.895   0.132
##   Horn ~
##     EndBBweight   0.305   0.045   6.811   0.000   0.305   0.557
##     Developmenttm -0.026   0.007  -3.952   0.000  -0.026  -0.327
##
## Covariances:
##   lnTestes ~~
##     Fat.trans      0.006   0.003   2.211   0.027   0.006   0.251
##   Ec.Weight.trans ~~
##     Elytra         0.059   0.011   5.497   0.000   0.059   0.686
##   Elytra ~~
##     Horn           0.021   0.004   5.740   0.000   0.021   0.740
##   Ec.Weight.trans ~~
##     Horn           0.065   0.011   6.021   0.000   0.065   0.781
##   lnTestes ~~
##     lnProt         -0.001   0.010  -0.112   0.911  -0.001  -0.013
##   lnProt ~~
##     Fat.trans      -0.011   0.006  -1.781   0.075  -0.011  -0.207
##
## Intercepts:

```

```
##      lnTestes      0.087      0.858      0.101      0.919      0.087      0.388
##      lnProt       10.256      2.001      5.125      0.000     10.256     22.592
##      Fat.trans     -2.380      0.532     -4.469      0.000     -2.380    -12.721
##      lnWtgain       3.970      0.295     13.465      0.000      3.970     15.688
##      Ec.Weght.trns  2.574      0.621      4.144      0.000      2.574      3.918
##      Elytra         3.419      0.212     16.153      0.000      3.419     16.021
##      Developmenttm 29.224      1.016     28.751      0.000     29.224     11.361
##      Horn           1.412      0.206      6.850      0.000      1.412      6.785
##
## Variances:
##      lnTestes      0.039      0.006                      0.039      0.768
##      lnProt       0.195      0.031                      0.195      0.946
##      Fat.trans     0.015      0.002                      0.015      0.427
##      lnWtgain       0.015      0.002                      0.015      0.234
##      Ec.Weght.trns 0.257      0.037                      0.257      0.596
##      Elytra         0.028      0.004                      0.028      0.625
##      Developmenttm 6.502      0.929                      6.502      0.983
##      Horn           0.027      0.004                      0.027      0.631
```

6.2 Final model

```
#Specify reduced model

Path.reduced.model.testes<-'#Regressions

lnTestes~lnWtgain+Developmenttime
Fat.trans~lnWtgain+Adult.diet
lnWtgain~Elytra+Ec.Weight.trans+Horn
Ec.Weight.trans~EndBBweight
Elytra~EndBBweight +Developmenttime
Horn~ EndBBweight+Developmenttime

#covariances
lnTestes~~Fat.trans
Elytra~~Ec.Weight.trans
Horn~~Elytra
Horn~~Ec.Weight.trans'

# Fit the model and check the summary

male.testes.reduced.model <- sem(Path.reduced.model.testes, data = maledata,
  missing = "fiml")
summary(male.testes.reduced.model, standardized = TRUE, fit.measures = TRUE)

## lavaan (0.5-18) converged normally after 116 iterations
##
##      Number of observations                      98
##
##      Number of missing patterns                      7
##
```

```

## Estimator ML
## Minimum Function Test Statistic 11.606
## Degrees of freedom 17
## P-value (Chi-square) 0.823
##
## Model test baseline model:
##
## Minimum Function Test Statistic 513.155
## Degrees of freedom 33
## P-value 0.000
##
## User model versus baseline model:
##
## Comparative Fit Index (CFI) 1.000
## Tucker-Lewis Index (TLI) 1.022
##
## Loglikelihood and Information Criteria:
##
## Loglikelihood user model (H0) -128.717
## Loglikelihood unrestricted model (H1) -122.914
##
## Number of free parameters 28
## Akaike (AIC) 313.434
## Bayesian (BIC) 385.813
## Sample-size adjusted Bayesian (BIC) 297.393
##
## Root Mean Square Error of Approximation:
##
## RMSEA 0.000
## 90 Percent Confidence Interval 0.000 0.057
## P-value RMSEA <= 0.05 0.933
##
## Standardized Root Mean Square Residual:
##
## SRMR 0.057
##
## Parameter estimates:
##
## Information Observed
## Standard Errors Standard
##
## Estimate Std.err Z-value P(>|z|) Std.lv Std.all
## Regressions:
## lnTestes ~
## lnWtgain 0.445 0.093 4.781 0.000 0.445 0.492
## Developmenttm 0.019 0.008 2.354 0.019 0.019 0.222
## Fat.trans ~
## lnWtgain 0.459 0.057 8.085 0.000 0.459 0.626
## Adult.diet -0.155 0.027 -5.830 0.000 -0.155 -0.421
## lnWtgain ~
## Elytra 0.342 0.118 2.893 0.004 0.342 0.289
## Ec.Weght.trns -0.105 0.038 -2.782 0.005 -0.105 -0.277
## Horn 1.026 0.136 7.522 0.000 1.026 0.840
## Ec.Weight.trans ~

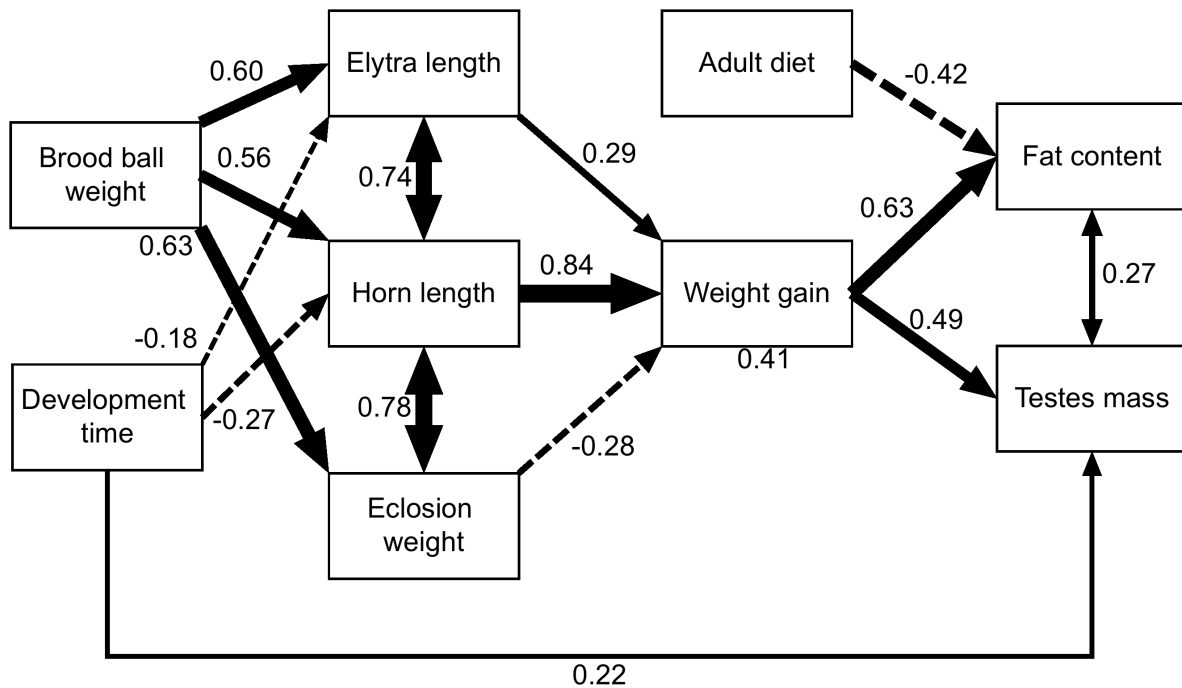
```

```

##      EndBBweight      1.092      0.136      8.056      0.000      1.092      0.631
##      Elytra ~
##      EndBBweight      0.333      0.046      7.266      0.000      0.333      0.598
##      Developmenttm -0.015      0.005      -2.902      0.004      -0.015      -0.180
##      Horn ~
##      EndBBweight      0.301      0.045      6.734      0.000      0.301      0.558
##      Developmenttm -0.022      0.004      -5.039      0.000      -0.022      -0.271
##
## Covariances:
##      lnTestes ~~
##      Fat.trans      0.007      0.003      2.352      0.019      0.007      0.272
##      Ec.Weight.trans ~~
##      Elytra      0.059      0.011      5.497      0.000      0.059      0.687
##      Elytra ~~
##      Horn      0.021      0.004      5.734      0.000      0.021      0.742
##      Ec.Weight.trans ~~
##      Horn      0.066      0.011      6.021      0.000      0.066      0.783
##
## Intercepts:
##      lnTestes      -0.269      0.654      -0.411      0.681      -0.269      -1.190
##      Fat.trans      -1.935      0.336      -5.766      0.000      -1.935      -10.540
##      lnWtgain      3.982      0.286      13.938      0.000      3.982      15.913
##      Ec.Weght.trns      2.023      0.203      9.960      0.000      2.023      3.080
##      Elytra      3.294      0.164      20.091      0.000      3.294      15.575
##      Horn      1.272      0.142      8.962      0.000      1.272      6.209
##
## Variances:
##      lnTestes      0.039      0.006      0.039      0.756
##      Fat.trans      0.015      0.002      0.015      0.454
##      lnWtgain      0.015      0.002      0.015      0.241
##      Ec.Weght.trns      0.259      0.037      0.259      0.602
##      Elytra      0.029      0.004      0.029      0.639
##      Horn      0.027      0.004      0.027      0.655

```

Once again this is a well-fitted model. NB development time has been added in as a direct predictor of testes mass, this was a consequence of inspecting modification indices. The final path model looks like this:



Not shown for clarity:
covariance between eclosion
weight and elytra length (0.69)

Figure H: Path diagram for the final model explaining testes mass. Solid lines are positive relations, dashed lines negative, double headed arrows indicate correlation (i.e. no assumptions about causality), line width is proportional to the strength of the relationship.

7 Path analysis for female fat content

7.1 Initial model

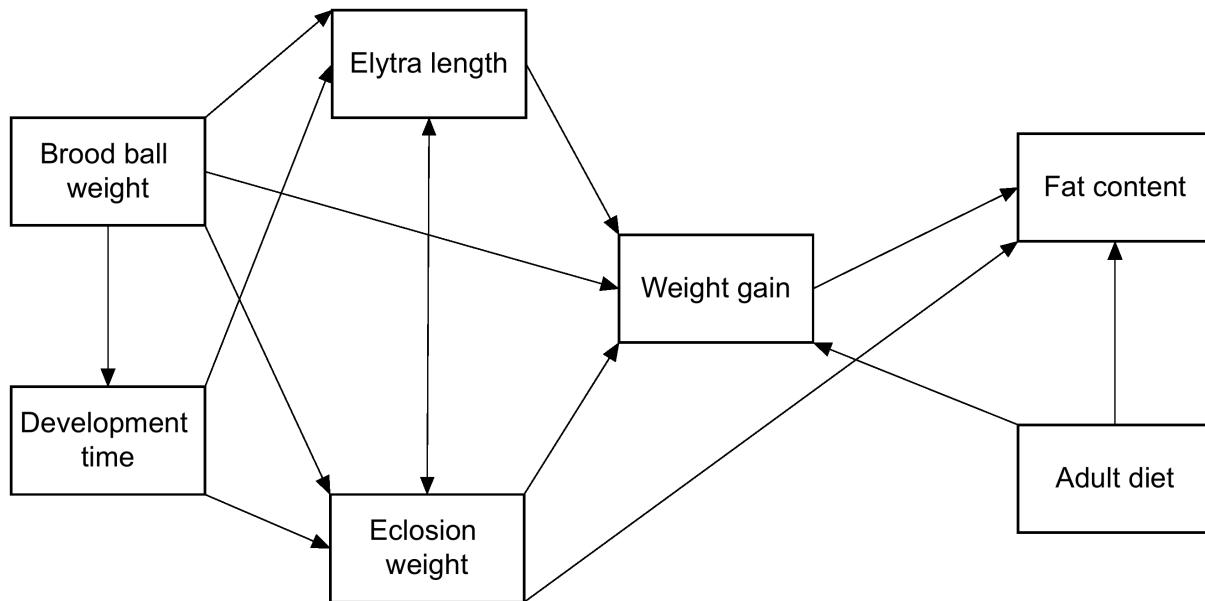


Figure 1: Path diagram showing the original model fitted to explain fat content in females. Double headed arrows indicate correlation (i.e. no assumptions made about causality)

#Specify the full model

```
Path.full.model.fat<-'#Regressions
```

```
lnProt~Elytra+Ec.Weight.trans+lnWtgain+ Adult.diet
Fat.trans~Elytra+Ec.Weight.trans+lnWtgain +Adult.diet
lnWtgain~Elytra+Ec.Weight.trans+EndBBweight +Adult.diet
Ec.Weight.trans~EndBBweight+Developmenttime
Elytra~EndBBweight+Developmenttime
Developmenttime~EndBBweight
```

```
#covariances
```

```
lnProt~~Fat.trans
```

```
Elytra~~Ec.Weight.trans'
```

Fit the model and check the summary

```
female.fat.full.model <- sem(Path.full.model.fat, data = femaledata,
  missing = "fiml")
summary(female.fat.full.model, standardized = TRUE)
```

```
## lavaan (0.5-18) converged normally after 119 iterations
```

```
##
```

```
## Number of observations
```

```
93
```



```

##
## Number of missing patterns          4
##
## Estimator                          ML
## Minimum Function Test Statistic    8.459
## Degrees of freedom                 8
## P-value (Chi-square)               0.390
##
## Parameter estimates:
##
## Information                        Observed
## Standard Errors                   Standard
##
## Estimate Std.err Z-value P(>|z|) Std.lv Std.all
## Regressions:
## lnProt ~
## Elytra          -0.305  0.153  -1.998  0.046  -0.305  -0.342
## Ec.Weght.trns   0.127  0.050   2.514  0.012   0.127   0.397
## lnWtgain        0.223  0.151   1.470  0.142   0.223   0.203
## Adult.diet      0.062  0.029   2.128  0.033   0.062   0.216
## Fat.trans ~
## Elytra          0.029  0.149   0.193  0.847   0.029   0.024
## Ec.Weght.trns   0.100  0.049   2.032  0.042   0.100   0.234
## lnWtgain        0.661  0.147   4.500  0.000   0.661   0.448
## Adult.diet     -0.157  0.029  -5.483  0.000  -0.157  -0.405
## lnWtgain ~
## Elytra          0.396  0.101   3.922  0.000   0.396   0.487
## Ec.Weght.trns   0.079  0.037   2.125  0.034   0.079   0.272
## EndBBweight    -0.012  0.035  -0.339  0.735  -0.012  -0.031
## Adult.diet      0.000  0.021   0.014  0.989   0.000   0.001
## Ec.Weight.trans ~
## EndBBweight     0.641  0.118   5.410  0.000   0.641   0.485
## Developmenttm  -0.020  0.013  -1.476  0.140  -0.020  -0.132
## Elytra ~
## EndBBweight     0.184  0.044   4.222  0.000   0.184   0.389
## Developmenttm  -0.012  0.005  -2.436  0.015  -0.012  -0.225
## Developmenttime ~
## EndBBweight    -1.432  0.917  -1.563  0.118  -1.432  -0.160
##
## Covariances:
## lnProt ~~
## Fat.trans       -0.001  0.002  -0.735  0.462  -0.001  -0.080
## Ec.Weight.trans ~~
## Elytra          0.036  0.007   5.308  0.000   0.036   0.659
##
## Intercepts:
## lnProt          3.044  0.720   4.230  0.000   3.044  21.219
## Fat.trans      -3.326  0.699  -4.762  0.000  -3.326 -17.242
## lnWtgain        4.167  0.247  16.874  0.000   4.167  31.901
## Ec.Weght.trns   2.940  0.448   6.557  0.000   2.940   6.537
## Elytra          3.335  0.166  20.098  0.000   3.335  20.734
## Developmenttm  31.446  1.314  23.936  0.000  31.446  10.323
##
## Variances:

```

##	lnProt	0.017	0.003	0.017	0.831
##	Fat.trans	0.016	0.002	0.016	0.433
##	lnWtgain	0.009	0.001	0.009	0.513
##	Ec.Weght.trns	0.147	0.022	0.147	0.727
##	Elytra	0.020	0.003	0.020	0.769
##	Developmenttm	9.042	1.326	9.042	0.974

7.2 Final model

This leads to a reduced model as follows:

```
#Specify the reduced model
```

```
Path.reduced.model.fat<-'#Regressions
```

```
Fat.trans~Ec.Weight.trans+lnWtgain +Adult.diet
```

```
lnWtgain~Elytra+Ec.Weight.trans
```

```
Ec.Weight.trans~EndBBweight
```

```
Elytra~EndBBweight
```

```
#covariances
```

```
Elytra~~Ec.Weight.trans'
```

```
# Fit the model and check the summary
```

```
female.fat.reduced.model <- sem(Path.reduced.model.fat, data = femaledata,
```

```
  missing = "fiml")
```

```
summary(female.fat.reduced.model, standardized = TRUE, fit.measures = TRUE)
```

```
## lavaan (0.5-18) converged normally after 73 iterations
```

```
##
```

```
##   Number of observations                93
```

```
##
```

```
##   Number of missing patterns              4
```

```
##
```

```
##   Estimator                            ML
```

```
##   Minimum Function Test Statistic       6.024
```

```
##   Degrees of freedom                    6
```

```
##   P-value (Chi-square)                  0.420
```

```
##
```

```
## Model test baseline model:
```

```
##
```

```
##   Minimum Function Test Statistic       236.467
```

```
##   Degrees of freedom                    14
```

```
##   P-value                               0.000
```

```
##
```

```
## User model versus baseline model:
```

```
##
```

```
##   Comparative Fit Index (CFI)           1.000
```

```
##   Tucker-Lewis Index (TLI)             1.000
```

```
##
```

```

## Loglikelihood and Information Criteria:
##
##   Loglikelihood user model (H0)                66.268
##   Loglikelihood unrestricted model (H1)         69.280
##
##   Number of free parameters                    16
##   Akaike (AIC)                                -100.536
##   Bayesian (BIC)                              -60.014
##   Sample-size adjusted Bayesian (BIC)          -110.523
##
## Root Mean Square Error of Approximation:
##
##   RMSEA                                0.007
##   90 Percent Confidence Interval      0.000  0.135
##   P-value RMSEA <= 0.05              0.563
##
## Standardized Root Mean Square Residual:
##
##   SRMR                                0.037
##
## Parameter estimates:
##
##   Information                                Observed
##   Standard Errors                          Standard
##
##           Estimate  Std.err  Z-value  P(>|z|)  Std.lv  Std.all
##
## Regressions:
##   Fat.trans ~
##     Ec.Weght.trns    0.106    0.039    2.726    0.006    0.106    0.247
##     lnWtgain         0.673    0.135    4.967    0.000    0.673    0.456
##     Adult.diet       -0.156    0.028   -5.579    0.000   -0.156   -0.403
##   lnWtgain ~
##     Elytra           0.394    0.098    4.011    0.000    0.394    0.485
##     Ec.Weght.trns    0.075    0.034    2.170    0.030    0.075    0.258
##   Ec.Weight.trans ~
##     EndBBweight      0.669    0.118    5.654    0.000    0.669    0.506
##   Elytra ~
##     EndBBweight      0.202    0.044    4.544    0.000    0.202    0.426
##
## Covariances:
##   Ec.Weight.trans ~~
##     Elytra           0.038    0.007    5.357    0.000    0.038    0.669
##
## Intercepts:
##   Fat.trans         -3.316    0.696   -4.761    0.000   -3.316   -17.210
##   lnWtgain          4.171    0.243   17.155    0.000    4.171   31.944
##   Ec.Weght.trns     2.327    0.170   13.725    0.000    2.327    5.173
##   Elytra            2.960    0.064   46.509    0.000    2.960   18.406
##
## Variances:
##   Fat.trans         0.016    0.002                0.016    0.434
##   lnWtgain          0.009    0.001                0.009    0.515
##   Ec.Weght.trns     0.151    0.022                0.151    0.744
##   Elytra            0.021    0.003                0.021    0.818

```

Once again the fit measures are fine. The final path model looks like this.

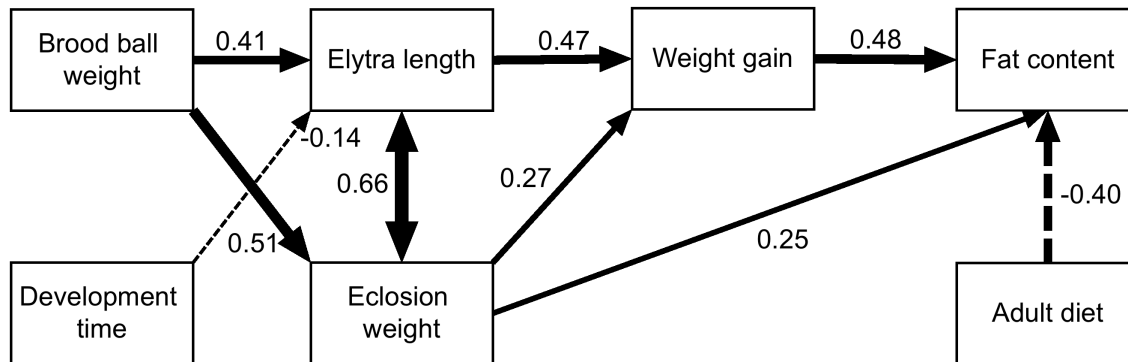


Figure J: Path diagram for the final model explaining fat content in females. Solid lines are positive relations, dashed lines negative, double headed arrows indicate correlation (i.e. no assumptions about causality), line width is proportional to the strength of the relationship.

7.3 Notes

Development time was very close to being retained in the final model as a predictor of elytra length ($p=0.054$, standardised regression coefficient = -0.127).

Haemolymph protein is not related to fat content in this case and was removed from the model because the fit was poor if it was retained, however note that haemolymph protein in females is significantly correlated with eclosion weight.

```
cor.test(femaledata$Ec.Weight.trans, femaledata$lnProt)
```

```
##
## Pearson's product-moment correlation
##
## data: femaledata$Ec.Weight.trans and femaledata$lnProt
## t = 2.536, df = 83, p-value = 0.01309
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
## 0.05837398 0.45526668
## sample estimates:
##      cor
## 0.2681619
```

References

- R Core Team. 2014. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org/>.
- Rosseel, Yves. 2012. "lavaan: An R Package for Structural Equation Modeling." *Journal of Statistical Software* 48 (2): 1–36. <http://www.jstatsoft.org/v48/i02/>.
- Wickham, Hadley. 2009. *Ggplot2: Elegant Graphics for Data Analysis*. Springer New York. <http://had.co.nz/ggplot2/book>.