Male gain curve analyses

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1 Introduction

Gain curves describe the relationship between the allocation of resources to a function and the fitness benefits returned by this allocation. Although gain curves can take many different forms, in general they are characterized by power laws of the form $y = ax^b$. Here we present the results of two experiments that quantify the male gain curve for canola.

2 Array-level gain curve

In this experiment we quantified total pollen deposition in arrays with different anther numbers and inflorescence sizes.

2.1 The data

Figure 1 plots the probability that an individual pollen grain reaches a stigma for different anther and flower numbers.

2.2 Linear models

We start with a simple, additive linear model of the probability of pollen export for anther number and inflorescence size.

```
> export.add <- lm(probExport ~ anthers + flowers, data = arrayData)
> summary(export.add)

Call:
lm(formula = probExport ~ anthers + flowers, data = arrayData)

Residuals:
      Min         1Q     Median         3Q        Max
-0.0068616 -0.0024845 -0.0006813  0.0017046  0.0108378

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
```
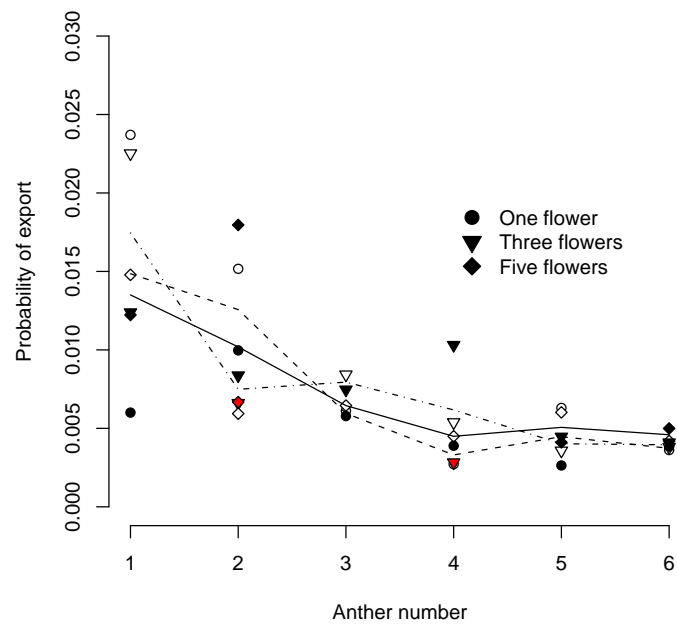
Figure 1: The gain curve for canola based on an array-level analysis of pollen deposition.

```
(Intercept)   0.0148836   0.0022024    6.758 1.06e-07 ***
anthers      -0.0021266   0.0003774   -5.634 2.83e-06 ***
flowers       0.0001103   0.0008145    0.135    0.893
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.003902 on 33 degrees of freedom
Multiple R-Squared: 0.4911,       Adjusted R-squared: 0.4602
F-statistic: 15.92 on 2 and 33 DF,  p-value: 1.445e-05
```

Anthers are a significant effect in this additive model, but an interaction model may be a better explanation for these data.

```
> export.int <- update(export.add, . ~ anthers * flowers)
> summary(export.int)

Call:
lm(formula = probExport ~ anthers + flowers + anthers:flowers,
    data = arrayData)

Residuals:
      Min        1Q    Median        3Q       Max
-0.007626 -0.002452 -0.000759  0.001857  0.010073

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)      0.0170013  0.0039287    4.327 0.000138 ***
anthers         -0.0027394  0.0010120   -2.707 0.010800 *
flowers         -0.0009340  0.0017967   -0.520 0.606769
anthers:flowers  0.0003041  0.0004653    0.654 0.518052
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.003936 on 32 degrees of freedom
Multiple R-Squared: 0.4978,       Adjusted R-squared: 0.4507
F-statistic: 10.57 on 3 and 32 DF,  p-value: 5.492e-05

> anova(export.int, export.add)

Analysis of Variance Table

Model 1: probExport ~ anthers + flowers + anthers:flowers
Model 2: probExport ~ anthers + flowers
  Res.Df        RSS Df   Sum of Sq      F Pr(>F)
1     32  0.00049579
2     33  0.00050241 -1 -0.00000662 0.4272 0.5181
```

There is no evidence for a significant interaction between anther number and inflorescence size for this linear approach. However, if we consider pollinator behaviour by including the number of flowers visited and amount of pollen transferred, we obtain slightly different conclusions.

```
> exp.stp <- stepAIC(export.int, scope = list(upper = ~anthers * flowers *
+     meanFlowerVisits * arrayRemoval, lower = ~1))

Start:  AIC= -394.94
 probExport ~ anthers + flowers + anthers:flowers

                  Df Sum of Sq        RSS     AIC
+ arrayRemoval     1 6.670e-05 4.291e-04 -398.14
- anthers:flowers  1 6.618e-06 5.024e-04 -396.47
<none>                         4.958e-04 -394.94
+ meanFlowerVisits 1 3.653e-08 4.958e-04 -392.95

Step:  AIC= -398.14
 probExport ~ anthers + flowers + arrayRemoval + anthers:flowers

                     Df Sum of Sq        RSS     AIC
<none>                            4.291e-04 -398.14
+ flowers:arrayRemoval 1 1.306e-05 4.160e-04 -397.26
- anthers:flowers      1 4.624e-05 4.753e-04 -396.46
+ meanFlowerVisits     1 2.907e-06 4.262e-04 -396.39
+ anthers:arrayRemoval 1 2.424e-06 4.267e-04 -396.35
- arrayRemoval         1 6.670e-05 4.958e-04 -394.94

> dropterm(exp.stp, test = "F")

Single term deletions

Model:
probExport ~ anthers + flowers + arrayRemoval + anthers:flowers
                Df Sum of Sq        RSS     AIC F Value   Pr(F)
<none>                       4.291e-04 -398.14
arrayRemoval     1 6.670e-05 4.958e-04 -394.94    4.82 0.03576 *
anthers:flowers  1 4.624e-05 4.753e-04 -396.46    3.34 0.07723 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> exp.aov <- aov(probExport ~ anthers * flowers + arrayRemoval, data = arrayData)
> summary(exp.aov)

              Df      Sum Sq    Mean Sq F value     Pr(>F)
anthers        1 0.00048451 0.00048451 35.0037  1.557e-06 ***
flowers        1 0.00000028 0.00000028  0.0202    0.88793
```

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```
arrayRemoval     1 0.00002708 0.00002708   1.9566    0.17180
anthers:flowers  1 0.00004624 0.00004624   3.3405    0.07723 .
Residuals       31 0.00042909 0.00001384
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> coef(exp.aov)

    (Intercept)          anthers          flowers     arrayRemoval anthers:flowers
   1.680757e-02    -2.633648e-03    -5.469398e-03    1.373010e-08     9.823610e-04
```

We find that the total amount of pollen removed in the array is an important predictor of the probability of pollen export. Furthermore, with this addition, the interaction between anther number and inflorescence size becomes marginally significant.

Another approach is to log transform the probability of pollen export to investigate non-linear changes with anther number.

```
> export.log <- update(export.int, log(probExport) ~ .)
> summary(export.log)

Call:
lm(formula = log(probExport) ~ anthers + flowers + anthers:flowers,
    data = arrayData)

Residuals:
     Min       1Q   Median       3Q      Max
-0.71680 -0.22690  0.02449  0.21408  0.65618

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)     -4.05913    0.38610 -10.513 6.57e-12 ***
anthers         -0.32149    0.09945  -3.233  0.00284 **
flowers         -0.05191    0.17657  -0.294  0.77067
anthers:flowers  0.03429    0.04573   0.750  0.45879
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3868 on 32 degrees of freedom
Multiple R-Squared: 0.5955,        Adjusted R-squared: 0.5576
F-statistic:  15.7 on 3 and 32 DF,  p-value: 1.868e-06
```

2.3   Selfing

The count of transgenic pollen on stigmas provides an estimate of the total amount of self pollen deposited within plants.

```
> self.lm <- lm(selfing ~ anthers * flowers, data = arrayData)
> summary(self.lm)

Call:
lm(formula = selfing ~ anthers * flowers, data = arrayData)

Residuals:
     Min       1Q   Median       3Q      Max
-141.691  -40.937   -6.457   54.320   97.264

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      -248.91     178.42  -1.395   0.1905
anthers            86.40      39.70   2.176   0.0522 .
flowers           219.83      74.01   2.970   0.0127 *
anthers:flowers   -45.09      16.66  -2.706   0.0204 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 74.2 on 11 degrees of freedom
Multiple R-Squared: 0.5165,        Adjusted R-squared: 0.3846
F-statistic: 3.917 on 3 and 11 DF,  p-value: 0.03981
```

These results suggest some influence of anther number and inflorescence size on selfing. However, the plot of these data (Figure 2) suggests more analyses are required.

2.4   Non-linear regression

Typically, gain curves are analysed with power-law distributions.

```
Formula: I(probExport/anthers) ~ a * anthers^(b - 1)

Parameters:
   Estimate Std. Error t value Pr(>|t|)
a  0.015056   0.002859   5.266 0.000365 ***
b -0.596431   0.551845  -1.081 0.305164
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.004077 on 10 degrees of freedom

Formula: I(probExport/anthers) ~ a * anthers^(b - 1)

Parameters:
   Estimate Std. Error t value Pr(>|t|)
a  0.017365   0.001588  10.934 3.01e-07 ***
```
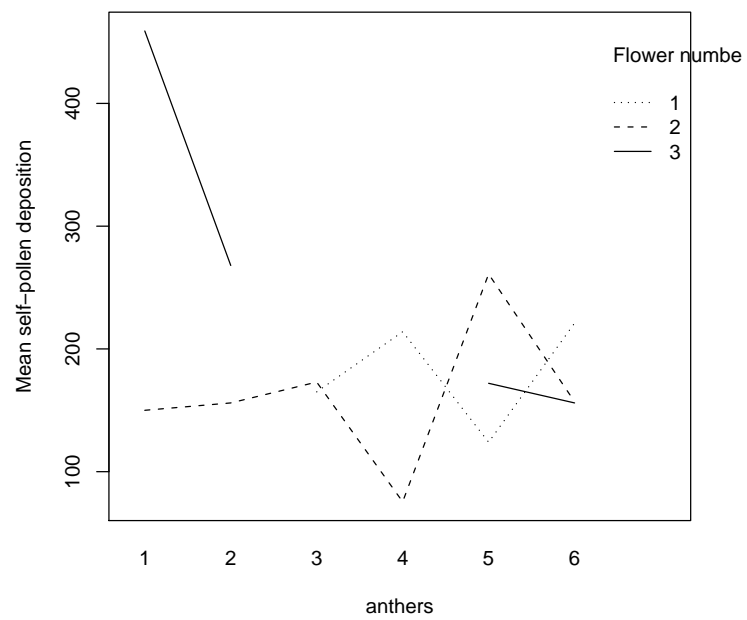
Figure 2: Self pollen deposition.

```
b -0.943255   0.359687  -2.622    0.0237 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.002255 on 11 degrees of freedom

Formula: I(probExport/anthers) ~ a * anthers^(b - 1)

Parameters:
   Estimate Std. Error t value Pr(>|t|)
a  0.013591   0.001212  11.216 1.37e-06 ***
b -0.518122   0.248459  -2.085   0.0667 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.001732 on 9 degrees of freedom
```

With the exception of b for three-flowered plants, the estimate of each parameter is significantly different from zero. However, plotting the predicted and observed values (Figure 3) shows that these power-law distributions are significantly underfitting the values for large anther numbers.

3   Pollen shadow

In the second experiment, a focal plant with transgenic pollen was surrounded by concentric squares of wild-type plants. Pollen export from the focal plant was estimated by counting transgenic pollen on wild-type stigmas.

We'll start with a look at the pollinator visits in each array.

```
      The following object(s) are masked from visits ( position 32 ) :

       array_id arrive bout depart donor_anthers duration plant_id recipient_anthers

Call:
lm(formula = as.integer(duration) ~ array_id/(as.integer(donor_anthers) +
    as.integer(recipient_anthers)), data = visits)

Residuals:
    Min      1Q  Median      3Q     Max
-13.203  -6.342  -3.203   3.092  65.233

Coefficients:
                                 Estimate Std. Error t value Pr(>|t|)
(Intercept)                      9.056408   0.603822  14.998   <2e-16 ***
array_id                        -0.033354   0.068805  -0.485    0.628
array_id:as.integer(donor_anthers)  0.058831   0.031241   1.883    0.060 .
```
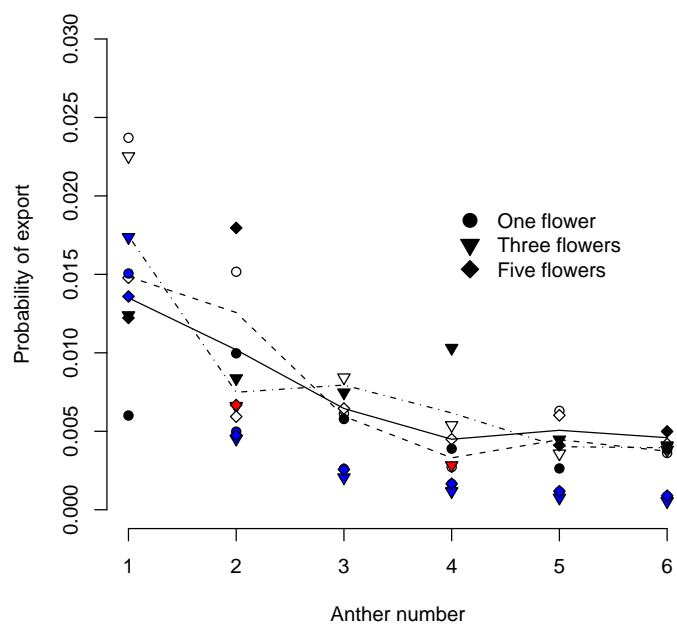
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Figure 3: Predicted values from the non-linear regression.

```
array_id:as.integer(recipient_anthers)  0.005446  0.023742   0.229    0.819
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 9.952 on 968 degrees of freedom
Multiple R-Squared: 0.01903,      Adjusted R-squared: 0.01599
F-statistic:  6.26 on 3 and 968 DF,  p-value: 0.0003293
```

This provides marginal evidence that pollinators respond to the number of donor anthers.

3.1 Selfing

As with the gain curve experiment, we can look at self-pollen deposition for different anther numbers.

```
        The following object(s) are masked from visits :

         array_id donor_anthers recipient_anthers

Call:
lm(formula = gus_pollen ~ donor_anthers, data = selfing)

Residuals:
    Min       1Q   Median       3Q      Max
-474.721 -145.546    3.553  114.841  444.128

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)     189.32     157.10   1.205    0.240
donor_anthers    73.85      43.64   1.692    0.104

Residual standard error: 248.2 on 24 degrees of freedom
Multiple R-Squared: 0.1066,      Adjusted R-squared: 0.06939
F-statistic: 2.864 on 1 and 24 DF,  p-value: 0.1035

Call:
lm(formula = gus_pollen ~ wt_pollen, data = selfing)

Residuals:
    Min      1Q  Median      3Q      Max
-469.54 -154.88  -54.35  175.30   492.30

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 390.4423    88.7636   4.399 0.000192 ***
wt_pollen     0.6961     0.9791   0.711 0.483942
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 259.8 on 24 degrees of freedom
Multiple R-Squared: 0.02063,        Adjusted R-squared: -0.02018
F-statistic: 0.5055 on 1 and 24 DF,  p-value: 0.4839

Call:
lm(formula = gus_pollen ~ donor_anthers + recipient_anthers,
    data = selfing)

Residuals:
     Min       1Q    Median        3Q       Max
-482.906 -137.523     2.289   123.050   436.191

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)        188.474    160.477   1.174    0.252
donor_anthers       82.140     67.912   1.210    0.239
recipient_anthers   -8.042     49.720  -0.162    0.873

Residual standard error: 253.4 on 23 degrees of freedom
Multiple R-Squared: 0.1076,         Adjusted R-squared: 0.03003
F-statistic: 1.387 on 2 and 23 DF,  p-value: 0.2699
```
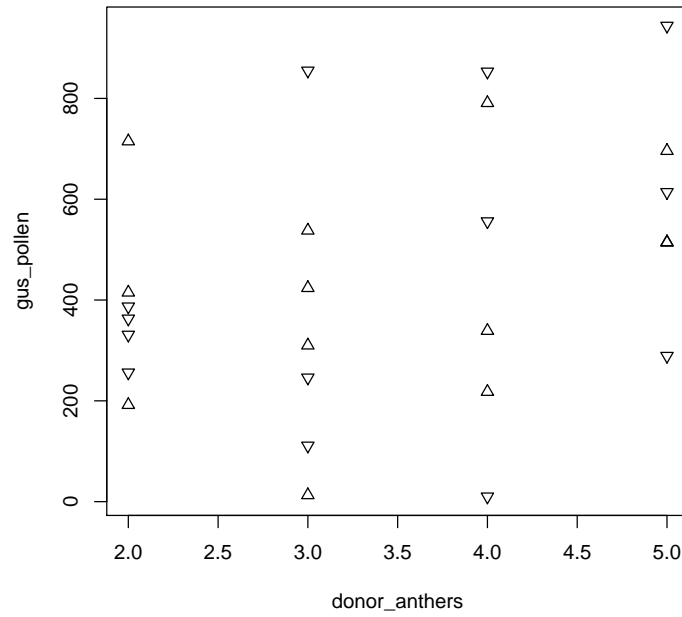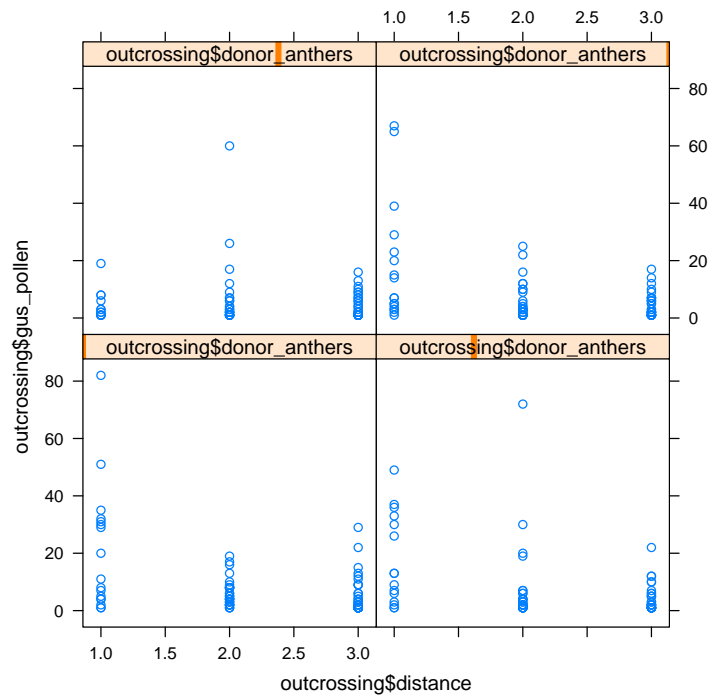
We find no evidence that selfing is correlated with anther number or pollen export.

3.2 Distance

A plot of the distance pollen travels for each number of donor anthers shows significant scatter, but perhaps an interaction.

```
Call:
lm(formula = distance ~ donor_anthers * recipient_anthers, data = outcrossing)

Residuals:
    Min      1Q  Median      3Q     Max
-1.3280 -0.2946 -0.2475  0.7296  0.7537

Coefficients:
                               Estimate Std. Error t value Pr(>|t|)
(Intercept)                     2.47632    0.30889   8.017 1.81e-14 ***
donor_anthers                  -0.05978    0.10752  -0.556    0.579
recipient_anthers              -0.06101    0.09996  -0.610    0.542
donor_anthers:recipient_anthers 0.01611    0.02657   0.606    0.545
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.7545 on 336 degrees of freedom
Multiple R-Squared: 0.001375,       Adjusted R-squared: -0.007542
F-statistic: 0.1542 on 3 and 336 DF,  p-value: 0.927
```
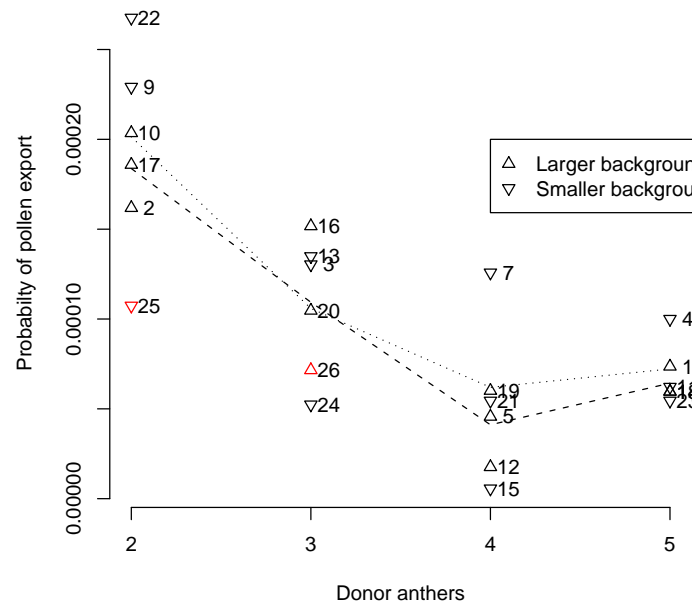
```
Start:  AIC= -472.43
 pollen_export ~ donor_anthers + background + donor_anthers:background


                            Df Sum of Sq       RSS      AIC
- donor_anthers:background  1 1.675e-12 4.860e-08 -474.43
+ mean_visit                1 5.769e-09 4.283e-08 -473.46
+ distance                  1 4.159e-09 4.444e-08 -472.57
<none>                                  4.860e-08 -472.43

Step:  AIC= -474.43
 pollen_export ~ donor_anthers + background


                            Df Sum of Sq       RSS      AIC
- background                1 6.916e-10 4.929e-08 -476.09
+ mean_visit                1 5.748e-09 4.285e-08 -475.45
+ distance                  1 3.891e-09 4.471e-08 -474.43
<none>                                  4.860e-08 -474.43
+ donor_anthers:background  1 1.675e-12 4.860e-08 -472.43
- donor_anthers            1 5.522e-08 1.038e-07 -458.21

Step:  AIC= -476.09
 pollen_export ~ donor_anthers
```

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```
              Df Sum of Sq        RSS      AIC
+ mean_visit    1 5.797e-09 4.349e-08 -477.09
<none>                      4.929e-08 -476.09
+ distance      1 3.336e-09 4.595e-08 -475.77
+ background    1 6.916e-10 4.860e-08 -474.43
- donor_anthers 1 5.522e-08 1.045e-07 -460.05

Step:  AIC= -477.09
 pollen_export ~ donor_anthers + mean_visit


                            Df Sum of Sq        RSS      AIC
<none>                                   4.349e-08 -477.09
+ distance                   1 1.855e-09 4.164e-08 -476.13
- mean_visit                 1 5.797e-09 4.929e-08 -476.09
+ donor_anthers:mean_visit   1 1.730e-09 4.176e-08 -476.06
+ background                 1 6.427e-10 4.285e-08 -475.45
- donor_anthers              1 6.016e-08 1.037e-07 -458.25

Single term deletions


Model:
pollen_export ~ donor_anthers + mean_visit
              Df Sum of Sq        RSS      AIC F Value      Pr(F)
<none>                      4.349e-08 -477.09
donor_anthers  1 6.016e-08 1.037e-07 -458.25   29.05 2.404e-05 ***
mean_visit     1 5.797e-09 4.929e-08 -476.09    2.80    0.1092
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              Df     Sum Sq    Mean Sq F value    Pr(>F)
donor_anthers  1 5.5221e-08 5.5221e-08 26.6620 4.078e-05 ***
mean_visit     1 5.7970e-09 5.7970e-09  2.7988    0.1092
Residuals     21 4.3494e-08 2.0710e-09
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

  (Intercept) donor_anthers     mean_visit
 2.172152e-04 -4.564110e-05   4.320045e-06

                        Df     Sum Sq    Mean Sq F value  Pr(>F)
donor_anthers            1 5.5221e-08 5.5221e-08 24.4978  8.9e-05 ***
background               1 6.9200e-10 6.9200e-10  0.3068   0.5861
mean_visit               1 5.7480e-09 5.7480e-09  2.5499   0.1268
donor_anthers:background 1 2.3000e-11 2.3000e-11  0.0103   0.9204
Residuals               19 4.2828e-08 2.2540e-09
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

So, we find that pollen export declines with the number of donor anthers and increases with pollinator visits.