Touch Paper

The analysis we did of the stats behind figure 1 was sub-standard. The model fitted should be a two-factor linear mixed effect model (Genotype * treatment are the two factors, w/ interaction), with a random effect term for the tray (batch) effect. This removes the error in each of the two model factors caused by tray effect whilst fitting the model.

So, lets get the data:

```
fig1_data <- read.csv("TouchData.csv")
fig1_data$Genotype <- relevel(fig1_data$Genotype, ref="WT")
summary(fig1_data)
```

```
##    Genotype        Treatment        Tray           Rep
##  WT    :252   NotTouched:215   Min.   :1.00   Min.   : 1.0
##  ccr1.1:197   Touched   :234   1st Qu.:1.00   1st Qu.:10.0
##                                Median :2.00   Median :19.0
##                                Mean   :2.01   Mean   :19.6
##                                3rd Qu.:3.00   3rd Qu.:29.0
##                                Max.   :3.00   Max.   :42.0
##         Measurement      Value
##  LeafLength   :150   Min.   : 4.05
##  LeafWidth    :149   1st Qu.:10.35
##  PetioleLength:150   Median :12.58
##                      Mean   :14.10
##                      3rd Qu.:18.33
##                      Max.   :27.17
```

Now, for each phenotype, we want to fit the linear mixed effects model. We'll need the package `nlme`, which contains the `lme` function we use below.

```
library(nlme)

phenotypes <- unique(as.character(fig1_data$Measurement))
for (pheno in phenotypes) {
  pdata <- fig1_data[fig1_data$Measurement == pheno,]
  fit <- lme(Value ~ Genotype * Treatment, data = pdata, random =~ 1|Tray)
  print(pheno)
  print(summary(fit))
#   g.fit <- tapply(predict(fit),paste(gp$Genotype,gp$Treatment),mean)
#   oo <-  barplot(g.fit,ylim = c(0,20),main = "PetLength")
#   segments(oo,g.fit,oo,g.fit+fit$sigma)
#   segments(oo,g.fit,oo,g.fit-fit$sigma)
}
```

```
## [1] "LeafLength"
## Linear mixed-effects model fit by REML
##  Data: pdata
##     AIC BIC logLik
##   814.1 832 -401.1
##
## Random effects:
##  Formula: ~1 | Tray
```

```
##         (Intercept) Residual
## StdDev:    0.000173    3.592
##
## Fixed effects: Value ~ Genotype * Treatment
##                                  Value Std.Error  DF t-value p-value
## (Intercept)                     22.302    0.5542 144   40.24  0.0000
## Genotypeccr1.1                  -6.132    0.8585 144   -7.14  0.0000
## TreatmentTouched                -1.301    0.7837 144   -1.66  0.0990
## Genotypeccr1.1:TreatmentTouched  3.438    1.1843 144    2.90  0.0043
##  Correlation:
##                                 (Intr) Gnt1.1 TrtmnT
## Genotypeccr1.1                  -0.645
## TreatmentTouched                -0.707  0.456
## Genotypeccr1.1:TreatmentTouched  0.468 -0.725 -0.662
##
## Standardized Within-Group Residuals:
##      Min       Q1      Med       Q3      Max
## -3.18435 -0.56608  0.08486  0.62879  2.01400
##
## Number of Observations: 150
## Number of Groups: 3
## [1] "PetioleLength"
## Linear mixed-effects model fit by REML
##  Data: pdata
##     AIC   BIC logLik
##   661.6 679.5 -324.8
##
## Random effects:
##  Formula: ~1 | Tray
##         (Intercept) Residual
## StdDev:      0.8381    2.098
##
## Fixed effects: Value ~ Genotype * Treatment
##                                  Value Std.Error  DF t-value p-value
## (Intercept)                     15.098    0.5823 144  25.928  0.0000
## Genotypeccr1.1                  -3.706    0.5030 144  -7.368  0.0000
## TreatmentTouched                -4.568    0.4580 144  -9.975  0.0000
## Genotypeccr1.1:TreatmentTouched  1.998    0.6937 144   2.880  0.0046
##  Correlation:
##                                 (Intr) Gnt1.1 TrtmnT
## Genotypeccr1.1                  -0.358
## TreatmentTouched                -0.393  0.456
## Genotypeccr1.1:TreatmentTouched  0.260 -0.726 -0.661
##
## Standardized Within-Group Residuals:
##      Min       Q1      Med       Q3      Max
## -3.05788 -0.50321  0.02794  0.67962  2.54746
##
## Number of Observations: 150
## Number of Groups: 3
## [1] "LeafWidth"
## Linear mixed-effects model fit by REML
##  Data: pdata
##     AIC   BIC logLik
```

```
##   556.5 574.3 -272.2
##
## Random effects:
##  Formula: ~1 | Tray
##         (Intercept) Residual
## StdDev:   4.082e-05    1.505
##
## Fixed effects: Value ~ Genotype * Treatment
##                                  Value Std.Error  DF t-value p-value
## (Intercept)                     12.731    0.2322 143   54.82   0e+00
## Genotypeccr1.1                  -3.383    0.3633 143   -9.31   0e+00
## TreatmentTouched                -1.366    0.3284 143   -4.16   1e-04
## Genotypeccr1.1:TreatmentTouched  1.801    0.4989 143    3.61   4e-04
##  Correlation:
##                                 (Intr) Gnt1.1 TrtmnT
## Genotypeccr1.1                  -0.639
## TreatmentTouched                -0.707  0.452
## Genotypeccr1.1:TreatmentTouched  0.465 -0.728 -0.658
##
## Standardized Within-Group Residuals:
##     Min      Q1     Med      Q3     Max
## -2.9119 -0.5388  0.1646  0.7409  2.0657
##
## Number of Observations: 149
## Number of Groups: 3
```

So, we have the following "ANOVA" table (though this is NOT an anova). The values are the size of the effect (in mm) and the p-value assoicated with the effect. This is all relative to non-touched wild-type plants.

Phenotype	Genotype	Treatment	Genotype:Treatment
Leaf Len	-6.1 (0.00)	-1.3 (0.09)	3.4 (0.00)
Leaf Width	-3.4 (0.00)	-1.4 (0.00)	1.8 (0.00)
Petiole Len	-3.7 (0.00)	-4.6 (0.00)	2.0 (0.00)

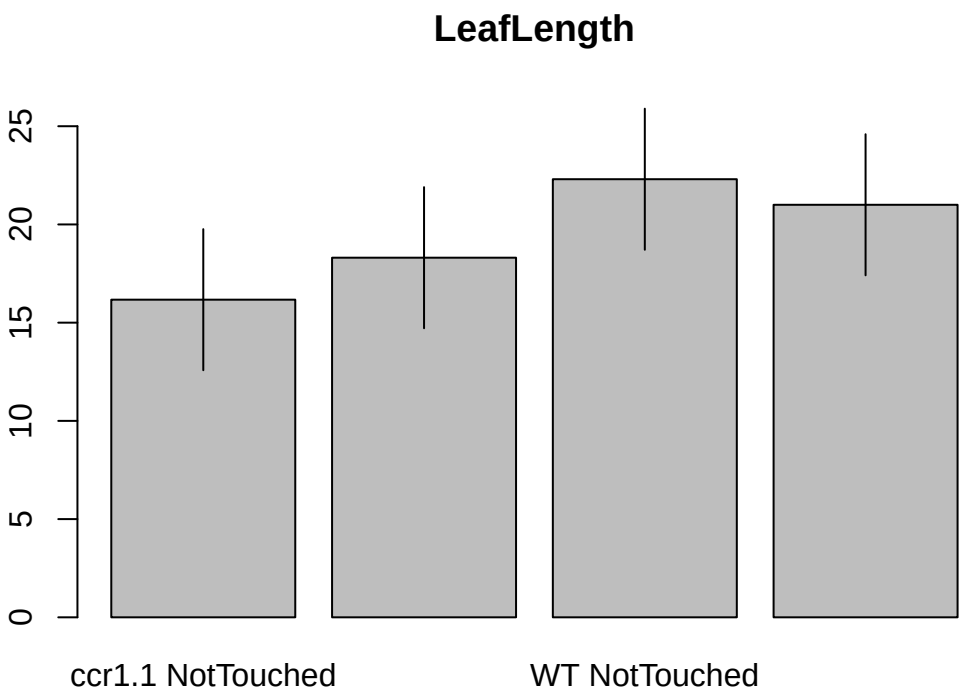
Which is pretty sweet.

What does this mean?

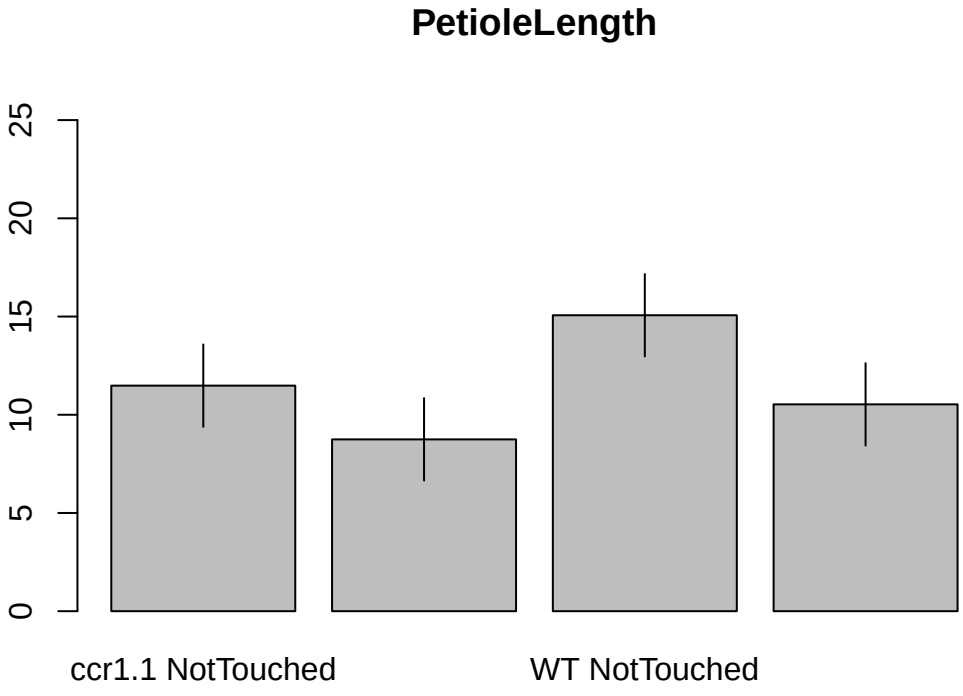
Basically, this fits a linear model. If we go back to old school regression, it's like fitting y = ax + b type formulae over the model "Phenotype depends on Genotype + Treatement + an interaction between the two + a random effect from the tray". So, in the above table, the effects (relative to WT Non-touched) indicated a model estimation of roughly how large the effect of genotype, treatment, and the interaction is the size and direction of the interaction. Positive interactions bascially mean that the effect is exaggerated in the interaction, i.e. in this case, for Leaf Len, both genotype and treatment have negative effects (SDG8 reduces leaf length, as does touching). Therefore, SDG8 touched plants have shorter leaves than just the addition of the effects of genotype and treatment.

OK, now some plots. These are NOT actual values.

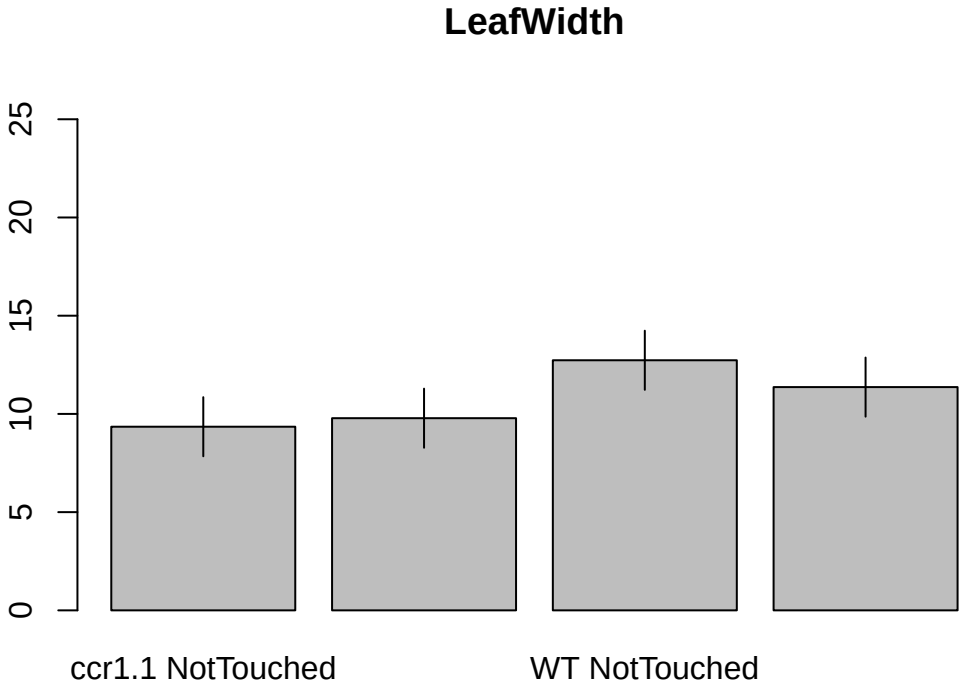
```
for (pheno in phenotypes) {
  pdata <- fig1_data[fig1_data$Measurement == pheno,]
  fit <- lme(Value ~ Genotype * Treatment, data = pdata, random =~ 1|Tray)
  fit.data <- tapply(predict(fit),paste(pdata$Genotype,pdata$Treatment),mean)
  xx <-  barplot(fit.data,ylim = c(0,26),main = pheno)
  segments(xx,fit.data,xx,fit.data+fit$sigma)
  segments(xx,fit.data,xx,fit.data-fit$sigma)
  print(paste("Predicted values for", pheno))
}
```




```
## [1] "Predicted values for LeafLength"
```




```
## [1] "Predicted values for PetioleLength"
```




```
## [1] "Predicted values for LeafWidth"
```