Spot_natural_pops

Once we have studied how the spot variation reacts plastically to temperature in lab experiments, we look at natural populations to check for similar patterns and therefore to check at what extend this plastic responses are sustained in nature.

Our predictions are that warmer places would present patterns more similar to our lab populations raised at higher temperatures and therefore:

1) Populations in warmer places will show smaller wings and spot sizes.
2) the relative DA in the spot size will increase with temperature.

I start by importing the data and I change the Paris measures because the objective was different:

```r
spot.nat.raw <- read.table(paste('/Users/ceferino_vg/Documents/Drosophila_suzukii/',
                                 'Spot_evolution/data/results_natpops.txt',
                                 sep=''),
                           header=T, sep=',')

spot.nat.raw$POP <- factor(spot.nat.raw$POP)

spot.nat.raw[spot.nat.raw$POP=='PAR',]$Spot <- spot.nat.raw[spot.nat.raw$POP=='PAR',]$Spot*0.885
spot.nat.raw[spot.nat.raw$POP=='PAR',]$Wing <- spot.nat.raw[spot.nat.raw$POP=='PAR',]$Wing*0.885
spot.nat.raw[spot.nat.raw$POP=='PAR',]$Ratio <-
  spot.nat.raw[spot.nat.raw$POP=='PAR',]$Spot/spot.nat.raw[spot.nat.raw$POP=='PAR',]$Wing

spot.nat <- aggregate(Wing ~ IND+POP, FUN = mean, data = spot.nat.raw)

spot.nat$Spot <- aggregate(Spot ~ IND+POP, FUN = mean, data = spot.nat.raw)$Spot

spot.nat$Ratio <- aggregate(Ratio ~ IND+POP, FUN = mean, data = spot.nat.raw)$Ratio

spot.mod <- aov(Spot ~ Wing, data=spot.nat)

spot.nat$Resid <- spot.mod$residuals

head(spot.nat)
```

```
##   IND POP    Wing     Spot      Ratio     Resid
## 1 M01 Bar 1229562 59068.50 0.04804026 -1857.931
## 2 M02 Bar 1727140 94342.50 0.05462352 12156.621
## 3 M03 Bar 1498975 67160.75 0.04480964 -5276.562
## 4 M04 Bar 1364695 74010.50 0.05423226  7310.417
## 5 M05 Bar 1355902 62835.50 0.04634223 -3488.873
## 6 M06 Bar 1298280 73590.50 0.05668309  9728.076
```

For future analyses, we import the libraries we are going to use and we write the function for Cohen's d effect size:

```r
library(ggplot2)
library(ggsignif)
library(car)
library(wesanderson)
library(cvequality)
library(openxlsx)
```

```r
cohens.d <- function(treat1, treat2) {
  d <- (mean(treat1)-mean(treat2))/sqrt(((length(treat1)-1)*var(treat1)+(length(treat2)-1)*var(treat2))/

  return(d)
}
```

We first test whether the population, the wing size and the interaction among the two have an effect over spot size:

```r
summary(aov(Spot ~ Wing*POP, data = spot.nat))
```

```
##              Df    Sum Sq   Mean Sq F value Pr(>F)
## Wing          1 4.664e+10 4.664e+10 525.217 <2e-16 ***
## POP          12 2.642e+09 2.202e+08   2.480 0.0046 **
## Wing:POP     12 1.863e+09 1.552e+08   1.748 0.0583 .
## Residuals   223 1.980e+10 8.879e+07
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
pairwise.t.test(spot.nat$Spot,spot.nat$POP)
```

```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  spot.nat$Spot and spot.nat$POP
##
##     Bar     BRE     Gen     Ita     Lan     Lia     PAR     Sap     Shi
## BRE 1.0000  -       -       -       -       -       -       -       -
## Gen 1.0000  1.0000  -       -       -       -       -       -       -
## Ita 0.0112  0.3265  1.0000  -       -       -       -       -       -
## Lan 0.3642  0.2837  0.0098  4.0e-06 -       -       -       -       -
## Lia 1.0000  1.0000  1.0000  1.0000  0.0091  -       -       -       -
## PAR < 2e-16 1.5e-12 4.7e-10 8.0e-05 < 2e-16 1.2e-11 -       -       -
## Sap 0.0035  0.3114  1.0000  1.0000  4.5e-07 1.0000  8.0e-08 -       -
## Shi 1.0000  1.0000  1.0000  0.0876  0.6610  1.0000  2.2e-14 0.0657 -
## Sok 1.0000  1.0000  1.0000  0.6519  0.0056  1.0000  2.6e-15 0.5747 1.0000
## Tok 1.0000  1.0000  1.0000  0.0211  1.0000  1.0000  1.8e-13 0.0160 1.0000
## Wat 1.0000  1.0000  1.0000  0.0049  1.0000  1.0000  < 2e-16 0.0015 1.0000
## Wis 1.0000  1.0000  1.0000  0.3661  0.2402  1.0000  2.4e-12 0.3642 1.0000
##     Sok    Tok    Wat
## BRE -      -      -
## Gen -      -      -
## Ita -      -      -
## Lan -      -      -
## Lia -      -      -
## PAR -      -      -
## Sap -      -      -
## Shi -      -      -
## Sok -      -      -
## Tok 1.0000 -      -
## Wat 1.0000 1.0000 -
## Wis 1.0000 1.0000 1.0000
##
## P value adjustment method: holm
```

```
summary(lm(Spot ~ POP, data = spot.nat))$r.squared
```

```
## [1] 0.4903924
```

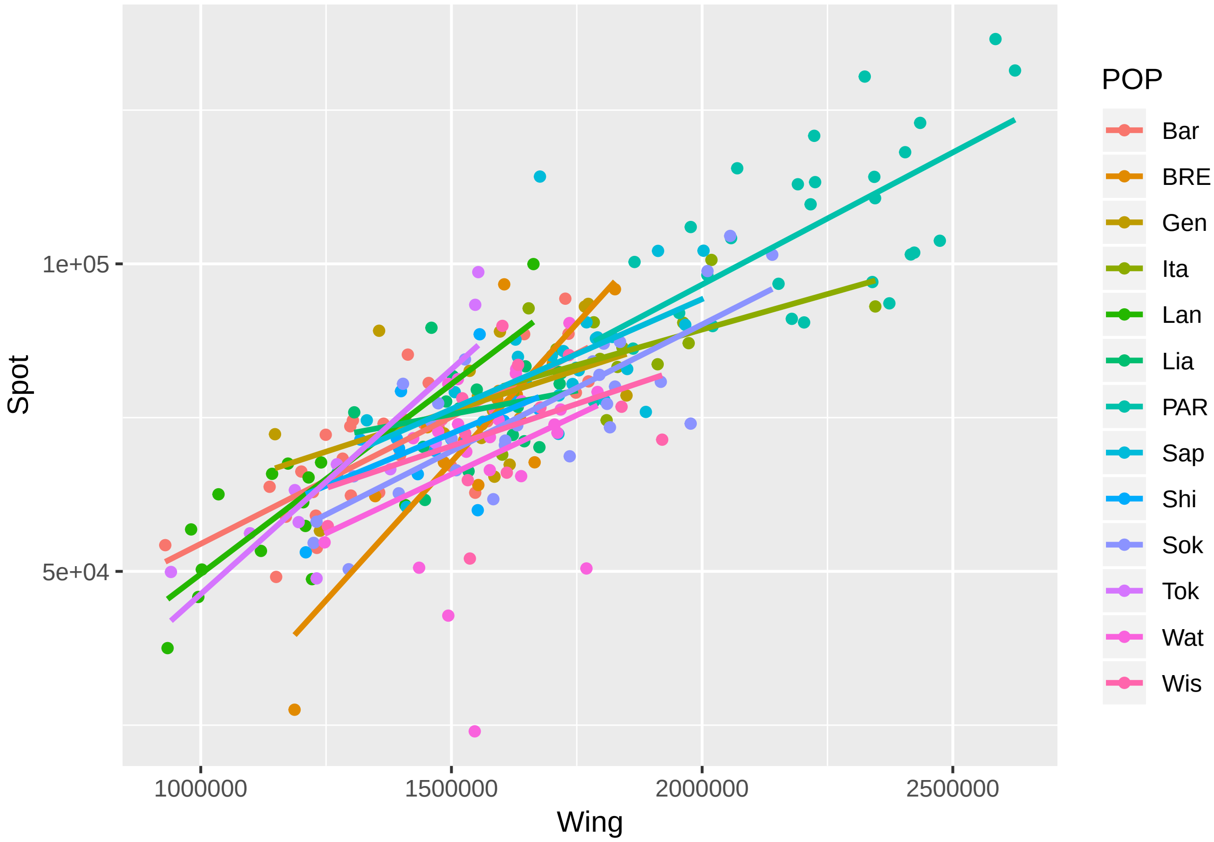
```
summary(lm(Spot ~ Wing, data = spot.nat))$r.squared
```

```
## [1] 0.6573823
```

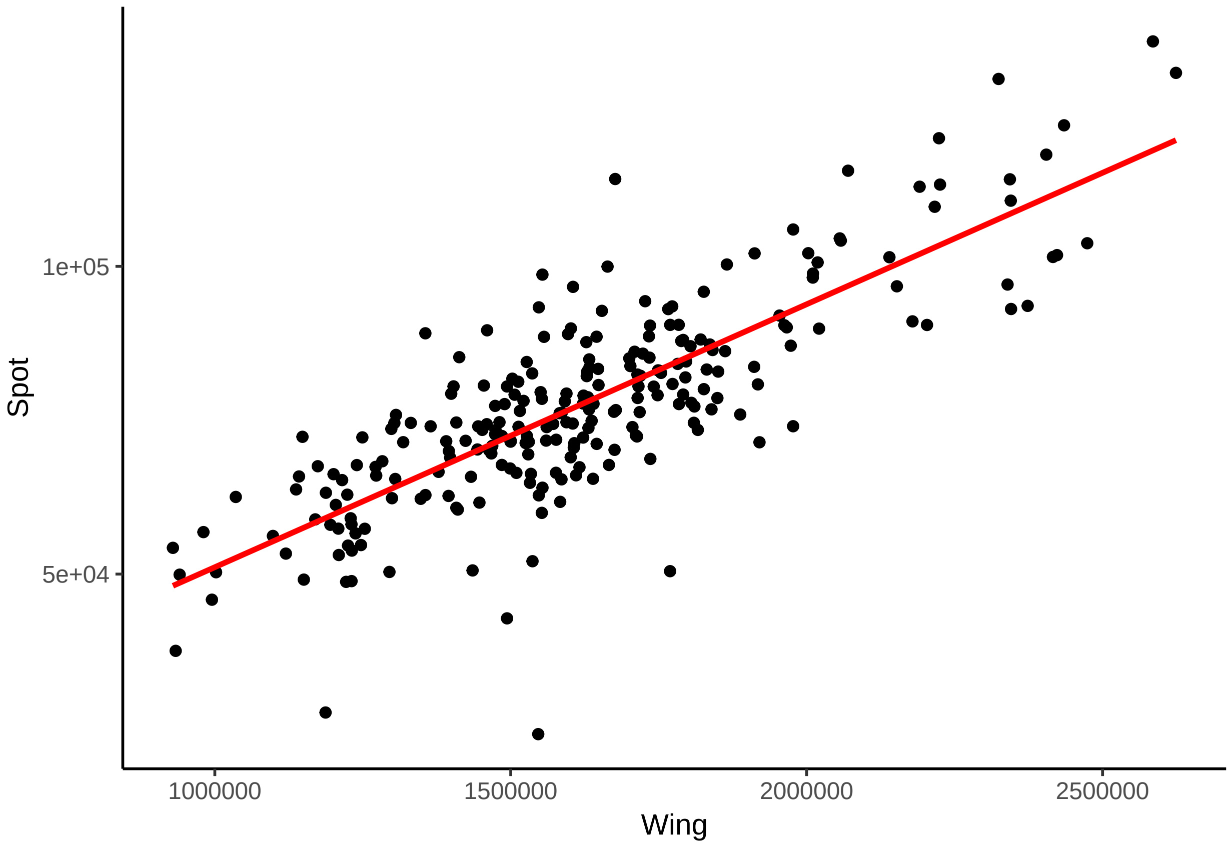
```
summary(lm(Spot ~ Wing:POP, data = spot.nat))$r.squared
```

```
## [1] 0.6953069
```

```
ggplot(data=spot.nat,
       aes(x = Wing, y = Spot, color = POP)) +
  geom_point() +
  geom_smooth(method = 'lm', se = FALSE)
```




```
ggplot(data=spot.nat,
       aes(x = Wing, y = Spot)) +
    geom_point() +
    geom_smooth(method = 'lm', se = FALSE, color = 'red') + theme(panel.grid.major = element_blank(), pa
panel.background = element_blank(), axis.line = element_line(colour = "black"))
```



These results hold for the relative spot size:

```
summary(aov(Ratio ~ POP, data = spot.nat))
```

```
##               Df   Sum Sq   Mean Sq F value   Pr(>F)
## POP           12 0.001388 1.157e-04   3.092 0.000439 ***
## Residuals    236 0.008829 3.741e-05
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm(Ratio ~ POP, data = spot.nat))$r.squared
```

```
## [1] 0.1358539
```

```
summary(lm(Wing ~ POP, data = spot.nat))$r.squared
```

```
## [1] 0.6775546
```

```
summary(aov(Ratio ~ Wing*POP, data = spot.nat))
```

```
##               Df   Sum Sq   Mean Sq F value  Pr(>F)
## Wing           1 0.000317 0.0003174   9.007 0.00300 **
## POP           12 0.001180 0.0000983   2.790 0.00145 **
## Wing:POP      12 0.000862 0.0000719   2.040 0.02209 *
## Residuals    223 0.007857 0.0000352
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.t.test(spot.nat$Ratio,spot.nat$POP)
```

```
##
```

```
##  Pairwise comparisons using t tests with pooled SD
##
## data:  spot.nat$Ratio and spot.nat$POP
##
##     Bar    BRE    Gen    Ita    Lan    Lia    PAR    Sap    Shi    Sok
## BRE 1.0000 -      -      -      -      -      -      -      -      -
## Gen 1.0000 1.0000 -      -      -      -      -      -      -      -
## Ita 1.0000 1.0000 1.0000 -      -      -      -      -      -      -
## Lan 1.0000 1.0000 1.0000 1.0000 -      -      -      -      -      -
## Lia 1.0000 1.0000 1.0000 1.0000 1.0000 -      -      -      -      -
## PAR 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 -      -      -      -
## Sap 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 -      -      -
## Shi 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 -      -
## Sok 0.1630 1.0000 1.0000 1.0000 0.6336 1.0000 1.0000 1.0000 1.0000 -
## Tok 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 0.6600
## Wat 0.0016 1.0000 0.0755 1.0000 0.0189 0.4878 0.9828 0.1577 0.7993 1.0000
## Wis 0.6600 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000
##     Tok    Wat
## BRE -      -
## Gen -      -
## Ita -      -
## Lan -      -
## Lia -      -
## PAR -      -
## Sap -      -
## Shi -      -
## Sok -      -
## Tok -      -
## Wat 0.0315 -
## Wis 1.0000 1.0000
##
## P value adjustment method: holm
```

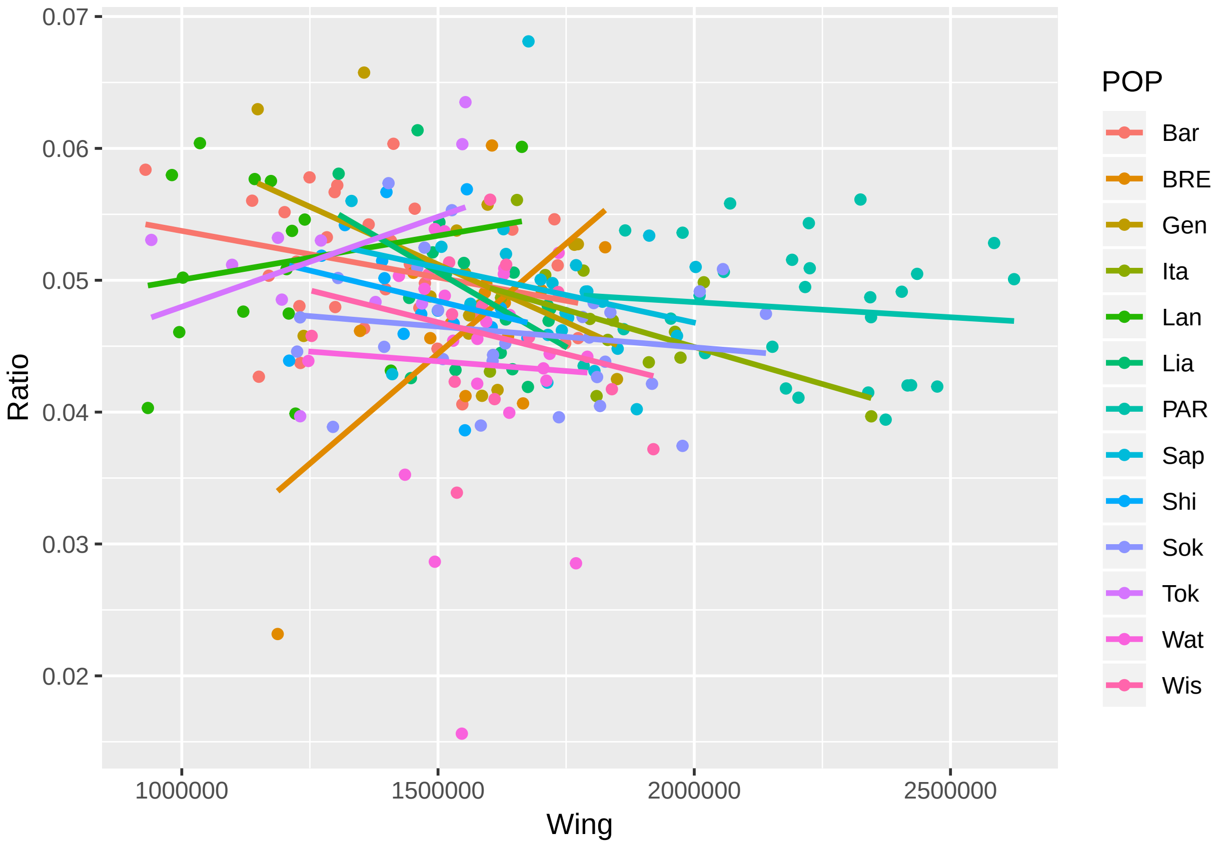
```r
summary(lm(Ratio ~ Wing, data = spot.nat))$r.squared
```

```
## [1] 0.03106388
```

```r
summary(lm(Ratio ~ Wing:POP, data = spot.nat))$r.squared
```

```
## [1] 0.1439055
```

```r
ggplot(data=spot.nat,
       aes(x = Wing, y = Ratio, color = POP)) +
  geom_point() +
  geom_smooth(method = 'lm', se = FALSE)
```




```r
summary(aov(Resid ~ POP, data = spot.nat))
```

```
##              Df    Sum Sq   Mean Sq F value  Pr(>F)
## POP          12 2.637e+09 219737286   2.393 0.00619 **
## Residuals   236 2.167e+10  91816785
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(lm(Resid ~ POP, data = spot.nat))$r.squared
```

```
## [1] 0.1084872
```

Finally, we check the population variation for the wing:

```r
summary(aov(Wing ~ POP, data = spot.nat))
```

```
##              Df    Sum Sq   Mean Sq F value Pr(>F)
## POP          12 1.731e+13 1.442e+12   41.33 <2e-16 ***
## Residuals   236 8.237e+12 3.490e+10
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(lm(Wing ~ POP, data = spot.nat))$r.squared
```

```
## [1] 0.6775546
```

The temperature has any effect on the mean natural variation?

```r
raw.temp <- read.xlsx('/Users/ceferino_vg/Documents/Drosophila_suzukii/Spot_evolution/data/temperatures

temps <- raw.temp[match(spot.nat$POP, raw.temp[,1]),4]
```

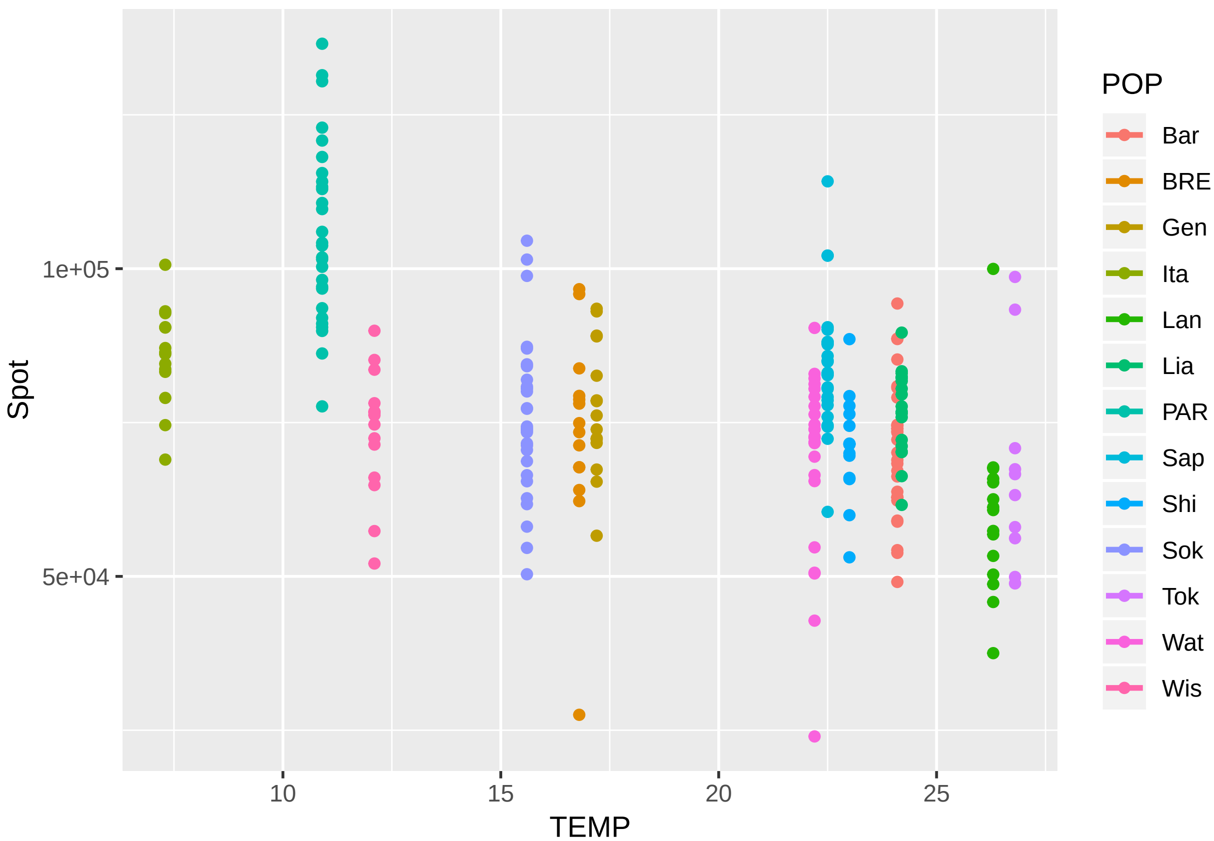
```r
spot.nat$TEMP <- as.numeric(temps)

summary(aov(Spot ~ TEMP, data = spot.nat))
```

```
##              Df    Sum Sq   Mean Sq F value   Pr(>F)
## TEMP          1 1.547e+10 1.547e+10   68.88 6.87e-15 ***
## Residuals   247 5.547e+10 2.246e+08
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

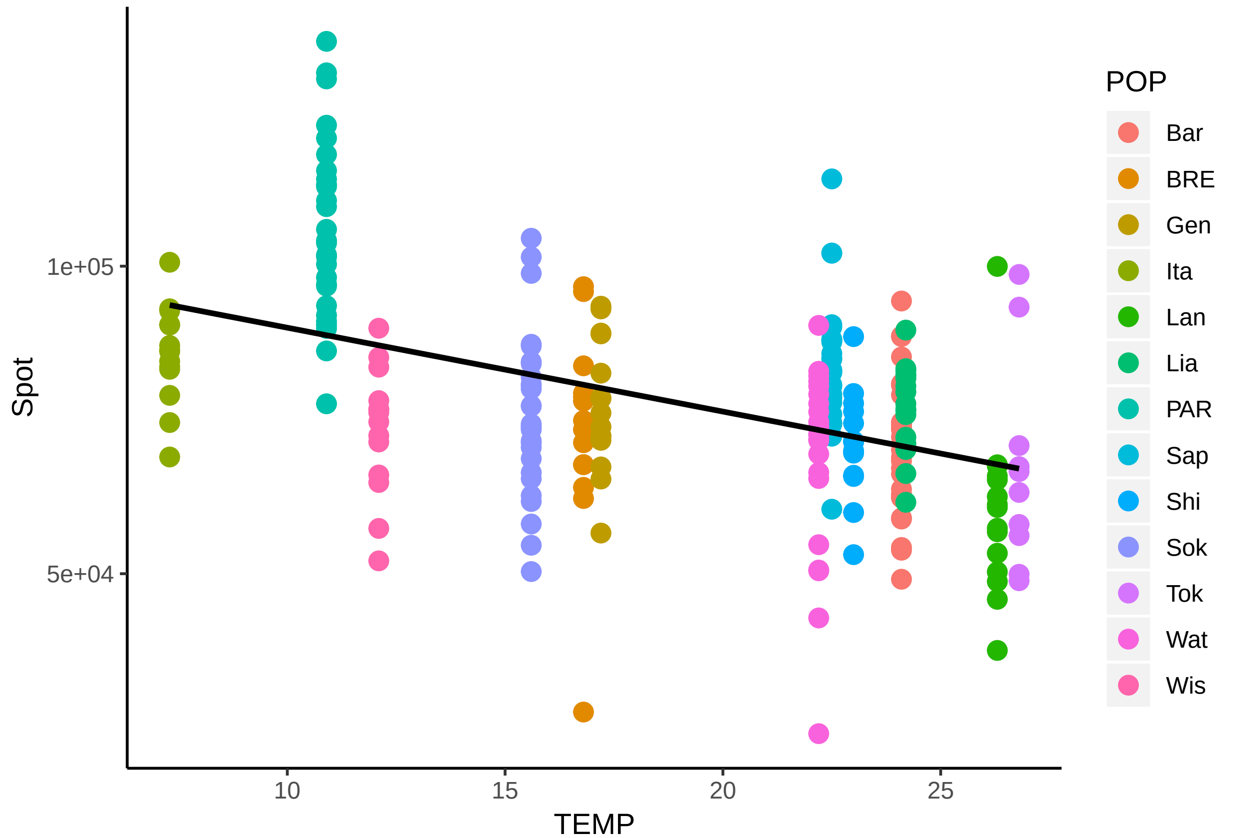
```r
summary(lm(Spot ~ TEMP, data = spot.nat))$r.squared
```

```
## [1] 0.2180535
```

```r
ggplot(data=spot.nat,
       aes(x = TEMP, y = Spot, color = POP)) +
  geom_point() +
  geom_smooth(method = 'lm', se = FALSE)
```




```r
ggplot(data=spot.nat,
       aes(x = TEMP, y = Spot, color = POP)) +
    geom_point(size = 3) +
    geom_smooth(method = 'lm', se = FALSE, color = 'black') +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
          panel.background = element_blank(), axis.line = element_line(colour = "black"))
```



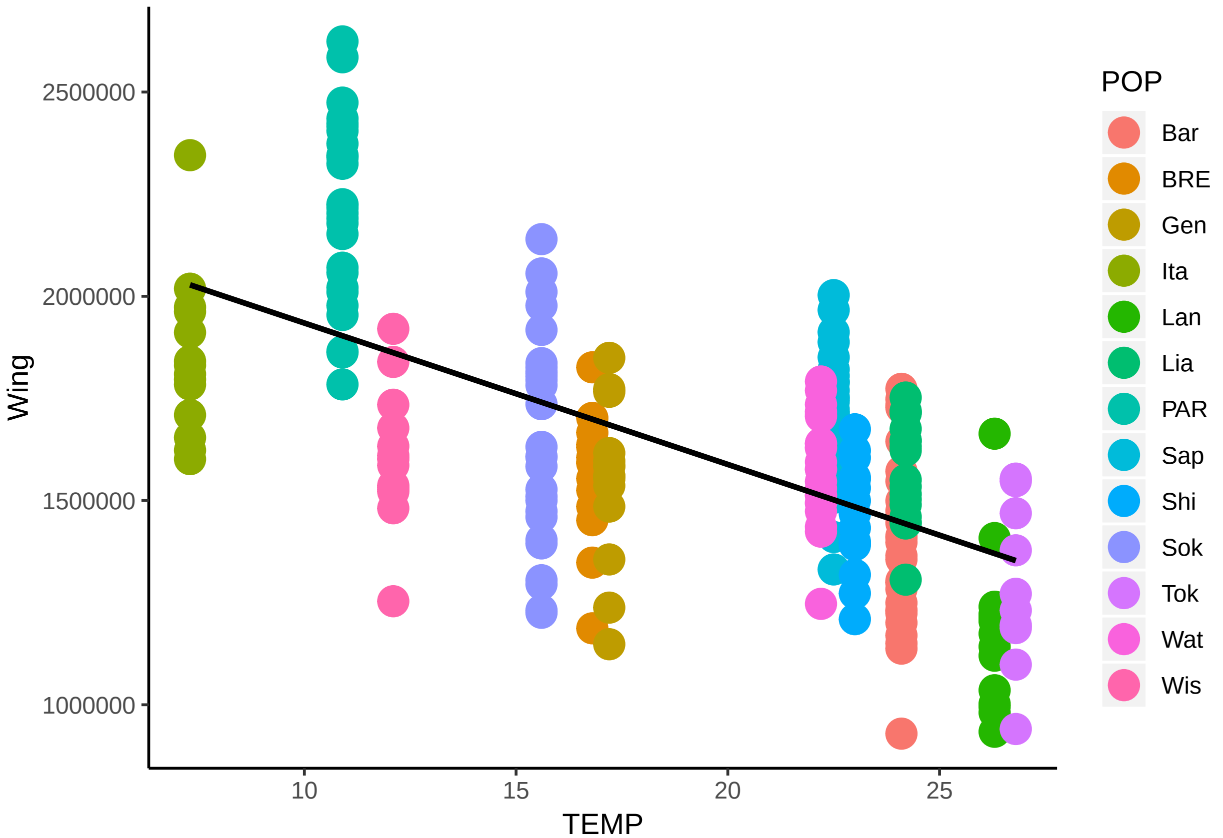

```r
summary(aov(Wing ~ TEMP, data = spot.nat))
```

```
##              Df    Sum Sq   Mean Sq F value Pr(>F)    
## TEMP          1 9.969e+12 9.969e+12   158.1 <2e-16 ***
## Residuals   247 1.558e+13 6.307e+10                   
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(lm(Wing ~ TEMP, data = spot.nat))$r.squared
```

```
## [1] 0.3902096
```

```r
ggplot(data=spot.nat,
       aes(x = TEMP, y = Wing, color = POP)) +
    geom_point(size = 5) +
    geom_smooth(method = 'lm', se = FALSE, color = 'black') +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
          panel.background = element_blank(), axis.line = element_line(colour = "black"))
```



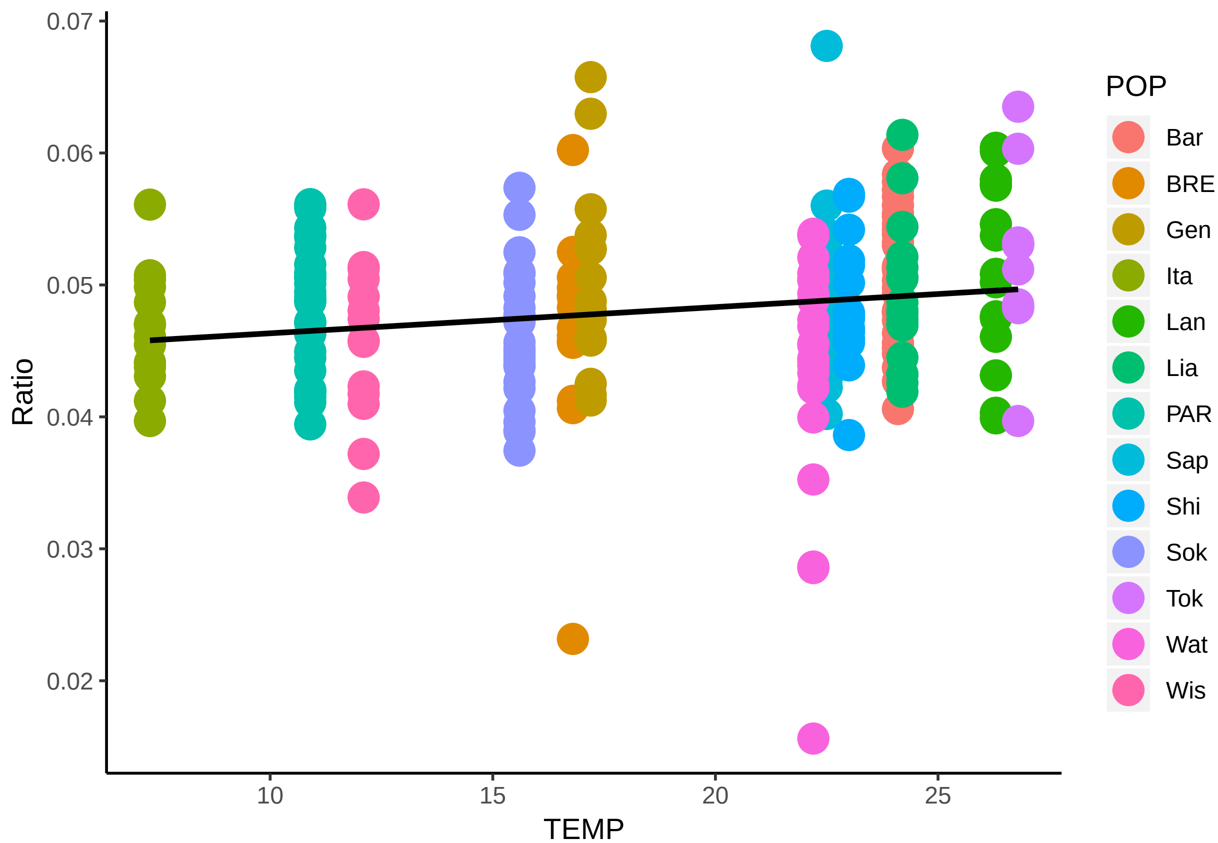

```r
summary(aov(Ratio ~ TEMP, data = spot.nat))
```

```
##              Df   Sum Sq   Mean Sq F value  Pr(>F)
## TEMP          1 0.000328 0.0003276   8.183 0.00459 **
## Residuals   247 0.009889 0.0000400
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(lm(Ratio ~ TEMP, data = spot.nat))$r.squared
```

```
## [1] 0.03206676
```

```r
ggplot(data=spot.nat,
       aes(x = TEMP, y = Ratio, color = POP)) +
    geom_point(size = 5) +
    geom_smooth(method = 'lm', se = FALSE, color = 'black') +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
          panel.background = element_blank(), axis.line = element_line(colour = "black"))
```



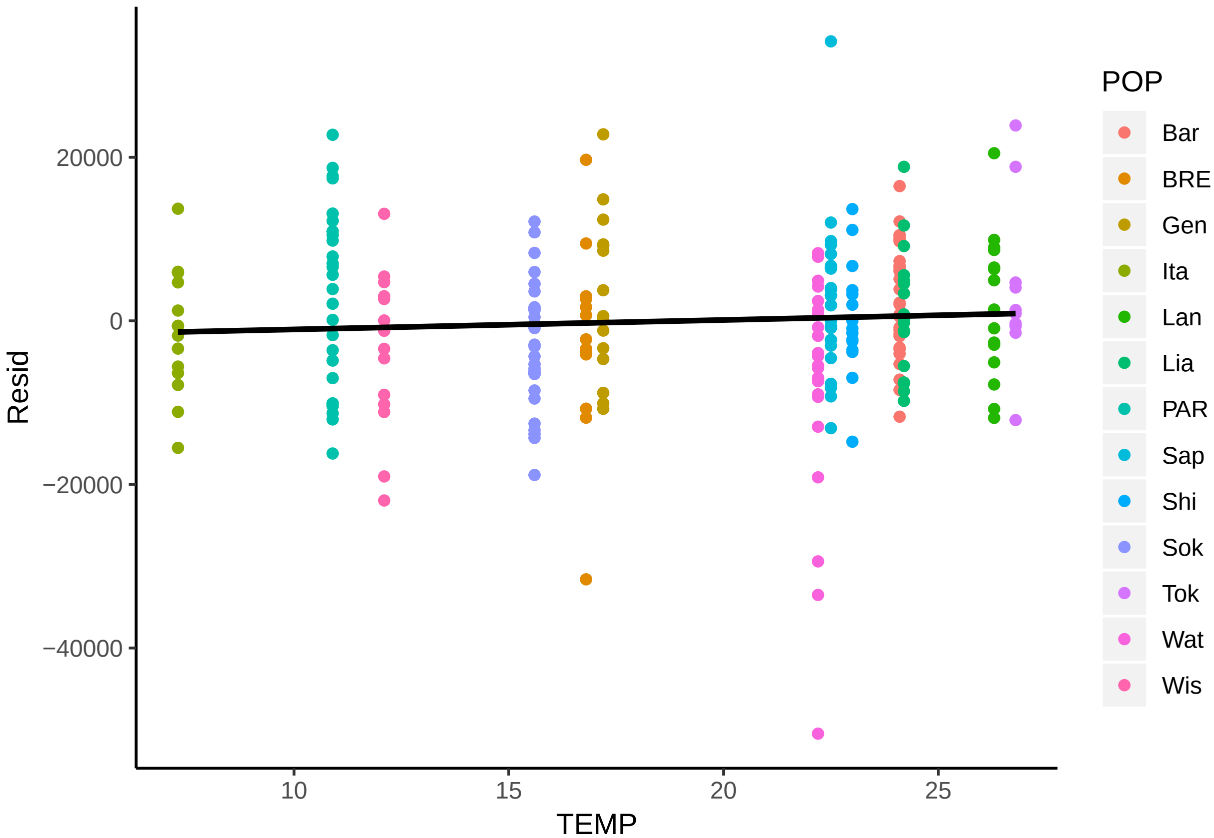

```
summary(aov(Resid ~ TEMP, data = spot.nat))
```

```
##              Df    Sum Sq   Mean Sq F value Pr(>F)
## TEMP          1 1.108e+08 110758423   1.131  0.289
## Residuals   247 2.419e+10  97954860
```

```
summary(lm(Resid ~ TEMP, data = spot.nat))$r.squared
```

```
## [1] 0.004556908
```

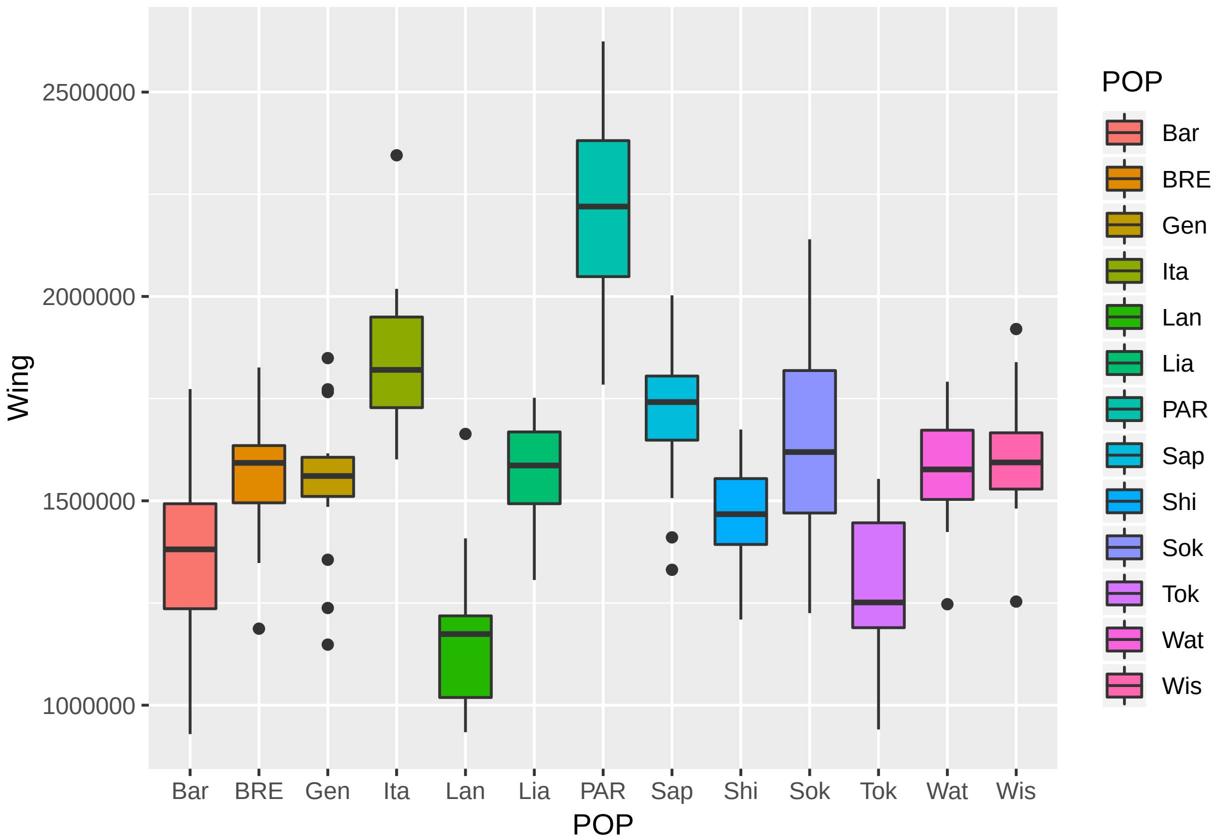
```
ggplot(data=spot.nat,
       aes(x = TEMP, y = Resid, color = POP)) +
    geom_point() +
    geom_smooth(method = 'lm', se = FALSE, color = 'black') +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
          panel.background = element_blank(), axis.line = element_line(colour = "black"))
```



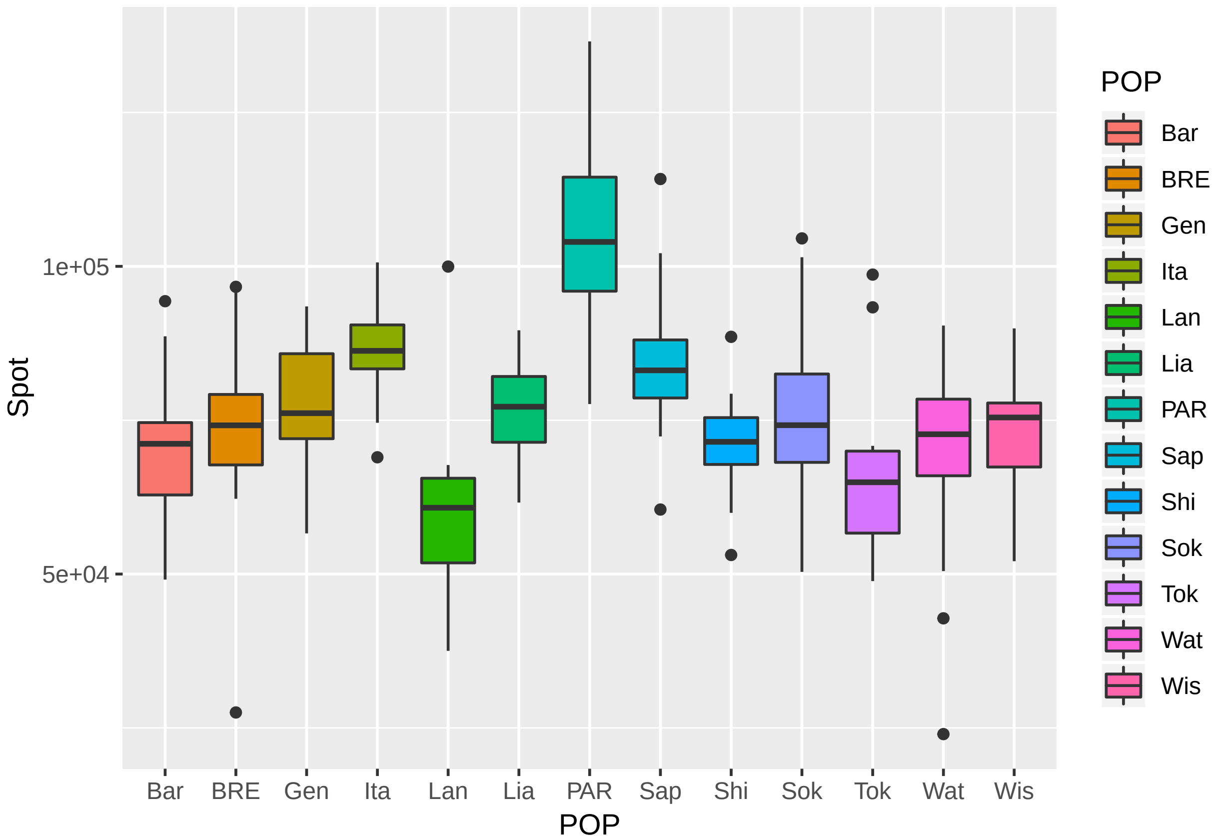
VARIABILITY

We can explore variation in wing size and spot sizes:

```
ggplot(data = spot.nat,
       aes(x = POP, y = Wing, fill = POP)) +
    geom_boxplot()
```

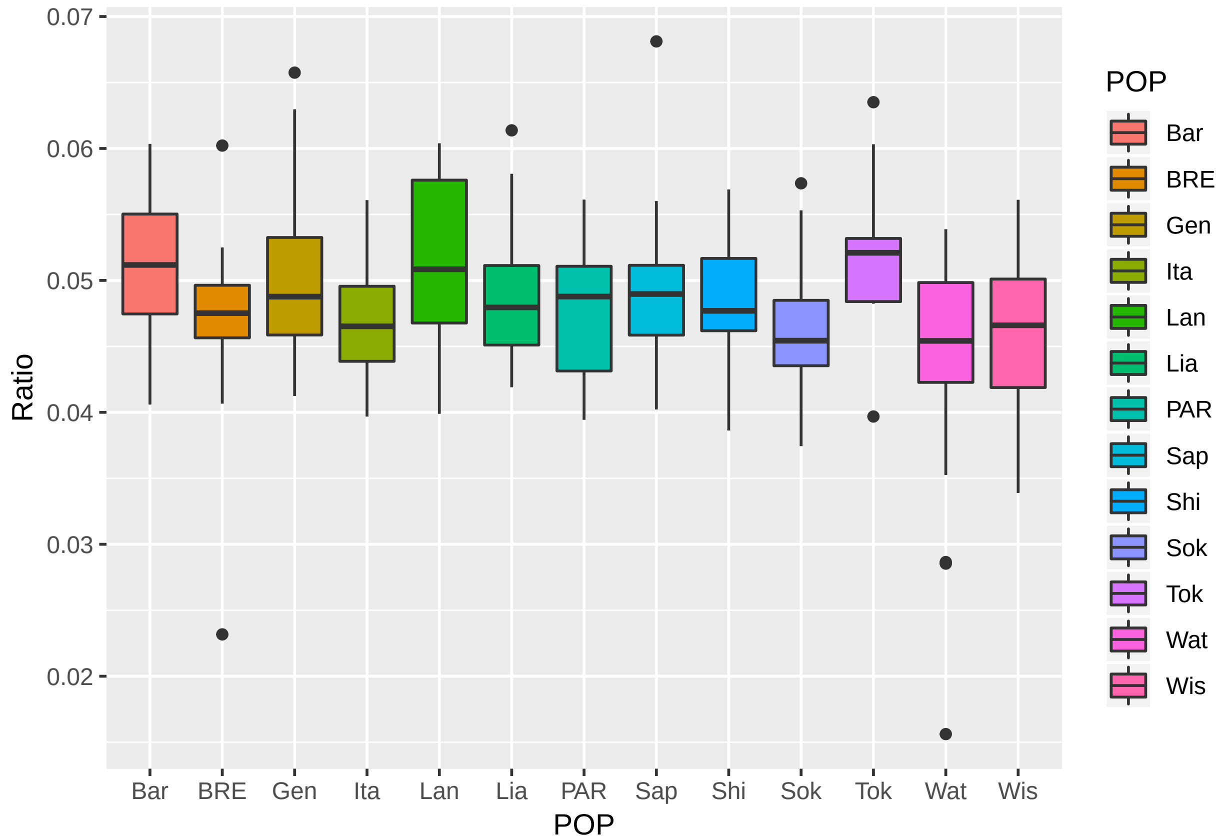



```r
ggplot(data = spot.nat,
       aes(x = POP, y = Spot, fill = POP)) +
    geom_boxplot()
```



Intriguinly, there is an astonishing conservation of the spot size in relation to wing size:

```
ggplot(data = spot.nat,
       aes(x = POP, y = Ratio, fill = POP)) +
    geom_boxplot()
```




```
summary(aov(Resid ~ POP, data = spot.nat))
```

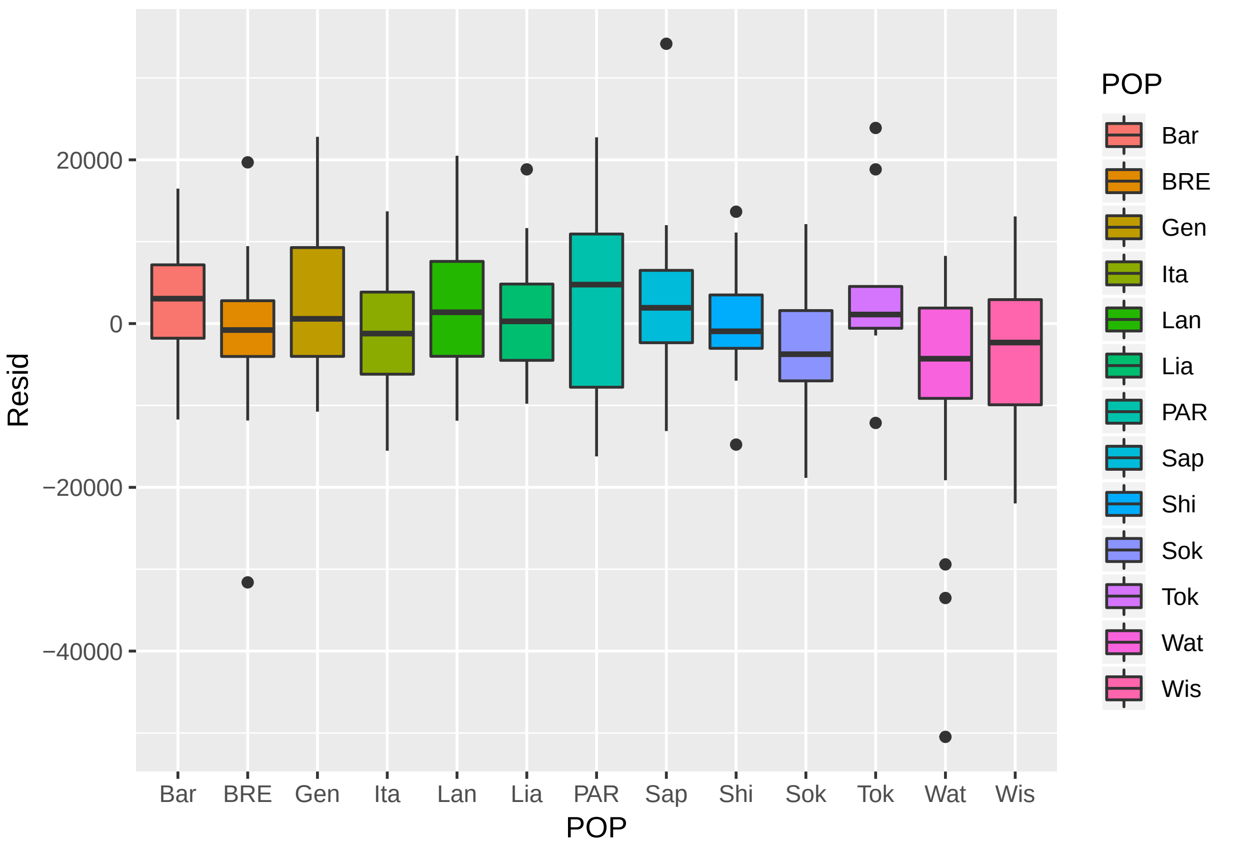
```
##              Df    Sum Sq   Mean Sq F value  Pr(>F)
## POP          12 2.637e+09 219737286   2.393 0.00619 **
## Residuals   236 2.167e+10  91816785
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.t.test(spot.nat$Resid, spot.nat$POP)
```

```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  spot.nat$Resid and spot.nat$POP
##
##     Bar   BRE   Gen   Ita   Lan   Lia   PAR   Sap   Shi   Sok   Tok
## BRE 1.000 -     -     -     -     -     -     -     -     -     -
## Gen 1.000 1.000 -     -     -     -     -     -     -     -     -
## Ita 1.000 1.000 1.000 -     -     -     -     -     -     -     -
## Lan 1.000 1.000 1.000 1.000 -     -     -     -     -     -     -
## Lia 1.000 1.000 1.000 1.000 1.000 -     -     -     -     -     -
## PAR 1.000 1.000 1.000 1.000 1.000 1.000 -     -     -     -     -
## Sap 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -     -     -     -
## Shi 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -     -     -
## Sok 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -     -
## Tok 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -
## Wat 0.020 1.000 0.156 1.000 0.475 0.604 0.023 0.061 1.000 1.000 0.208
## Wis 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
```

```
##     Wat
## BRE -
## Gen -
## Ita -
## Lan -
## Lia -
## PAR -
## Sap -
## Shi -
## Sok -
## Tok -
## Wat -
## Wis 1.000
##
## P value adjustment method: holm
```

```
ggplot(data = spot.nat,
       aes(x = POP, y = Resid, fill = POP)) +
    geom_boxplot()
```



Asymmetry levels:

We first create a matrix with both sides of just the complete individuals of the dataset:

```
non.symet <- c(0)

for (i in 1:length(spot.nat.raw$IND)) {

if (length(intersect(
```

```
    which(as.character(spot.nat.raw$IND[i]) == as.character(spot.nat.raw$IND[-i])),
 which(spot.nat.raw$POP[i] == spot.nat.raw$POP[-i]))) == 0) {

  non.symet <- c(non.symet,i)
  }

}

nat.sym <- spot.nat.raw[-non.symet,]
```

And now we add residuals and temperatures:

```
spot.lm <- aov(Spot ~ Wing, data = nat.sym)

nat.sym$Resid <- spot.lm$residuals

natsym.temps <- raw.temp[match(nat.sym$POP, raw.temp[,1]),4]

nat.sym$TEMP <- as.numeric(natsym.temps)
```

```
Bar_idx <- which(nat.sym$POP == 'Bar')
BRE_idx <- which(nat.sym$POP == 'BRE')
Gen_idx <- which(nat.sym$POP == 'Gen')
Ita_idx <- which(nat.sym$POP == 'Ita')
Lan_idx <- which(nat.sym$POP == 'Lan')
Lia_idx <- which(nat.sym$POP == 'Lia')
PAR_idx <- which(nat.sym$POP == 'PAR')
Sap_idx <- which(nat.sym$POP == 'Sap')
Shi_idx <- which(nat.sym$POP == 'Shi')
Sok_idx <- which(nat.sym$POP == 'Sok')
Tok_idx <- which(nat.sym$POP == 'Tok')
Wat_idx <- which(nat.sym$POP == 'Wat')
Wis_idx <- which(nat.sym$POP == 'Wis')

Nats <- list(Bar_idx, BRE_idx, Gen_idx, Ita_idx, Lan_idx, Lia_idx,
             PAR_idx, Sap_idx, Shi_idx, Sok_idx, Tok_idx, Wat_idx,
             Wis_idx)
```

We start by looking at whether there is a systematic difference between wings (i. e. directional asymmetry):

```
combin <- matrix(c(rep(4:7,length(Nats)), rep(1:length(Nats), each=4)),ncol=2)

for (i in 1:52) {

nat.pop <- nat.sym[Nats[[combin[i,2]]],]

model <- summary(aov(nat.pop[,combin[i,1]] ~ IND*SIDE, data = nat.pop))

DA <- 1 - pf((model[[1]]['Mean Sq'][2,]/model[[1]]['Mean Sq'][3,]),1,model[[1]]['Df'][3,])

cat(as.character(nat.pop[1,2]),' DA results for',
    colnames(nat.pop)[combin[i,1]], "\n")
cat('F value = ', model[[1]]['Mean Sq'][2,]/model[[1]]['Mean Sq'][3,], '; df2 = ',
    model[[1]]['Df'][3,], '; p-val =', DA, "\n")
```

```r
if (DA < 0.05) {
  meanDA <- aggregate(Spot ~ SIDE, data=nat.pop, FUN = mean)
  side_DA <- as.character(meanDA$SIDE[which(meanDA$Spot==max(meanDA$Spot))])
  cat('The larger spot size is in the ',side_DA, ' wing.', "\n")
}

}
```

```
## Bar  DA results for Wing
## F value =  0.03062979 ; df2 =  17 ; p-val = 0.8631364
## Bar  DA results for Spot
## F value =  5.474132 ; df2 =  17 ; p-val = 0.03176105
## The larger spot size is in the  D  wing.
## Bar  DA results for Ratio
## F value =  5.178994 ; df2 =  17 ; p-val = 0.03609035
## The larger spot size is in the  D  wing.
## Bar  DA results for Resid
## F value =  5.545854 ; df2 =  17 ; p-val = 0.030801
## The larger spot size is in the  D  wing.
## BRE  DA results for Wing
## F value =  0.8831537 ; df2 =  6 ; p-val = 0.3836146
## BRE  DA results for Spot
## F value =  9.16585 ; df2 =  6 ; p-val = 0.02317186
## The larger spot size is in the  D  wing.
## BRE  DA results for Ratio
## F value =  8.773348 ; df2 =  6 ; p-val = 0.02521802
## The larger spot size is in the  D  wing.
## BRE  DA results for Resid
## F value =  9.354807 ; df2 =  6 ; p-val = 0.02226599
## The larger spot size is in the  D  wing.
## Gen  DA results for Wing
## F value =  4.63228 ; df2 =  14 ; p-val = 0.04930777
## The larger spot size is in the  D  wing.
## Gen  DA results for Spot
## F value =  26.98449 ; df2 =  14 ; p-val = 0.0001359064
## The larger spot size is in the  D  wing.
## Gen  DA results for Ratio
## F value =  28.583 ; df2 =  14 ; p-val = 0.0001031409
## The larger spot size is in the  D  wing.
## Gen  DA results for Resid
## F value =  30.24054 ; df2 =  14 ; p-val = 7.835032e-05
## The larger spot size is in the  D  wing.
## Ita  DA results for Wing
## F value =  0.06318018 ; df2 =  10 ; p-val = 0.8066282
## Ita  DA results for Spot
## F value =  13.99577 ; df2 =  10 ; p-val = 0.003839641
## The larger spot size is in the  D  wing.
## Ita  DA results for Ratio
## F value =  13.04488 ; df2 =  10 ; p-val = 0.004754128
## The larger spot size is in the  D  wing.
## Ita  DA results for Resid
## F value =  13.30022 ; df2 =  10 ; p-val = 0.004484905
## The larger spot size is in the  D  wing.
## Lan  DA results for Wing
```

```
## F value =  0.06977163 ; df2 =  14 ; p-val = 0.7955182
## Lan  DA results for Spot
## F value =  1.473897 ; df2 =  14 ; p-val = 0.2448149
## Lan  DA results for Ratio
## F value =  0.6935466 ; df2 =  14 ; p-val = 0.4189414
## Lan  DA results for Resid
## F value =  1.27602 ; df2 =  14 ; p-val = 0.2776238
## Lia  DA results for Wing
## F value =  0.04556327 ; df2 =  11 ; p-val = 0.8348764
## Lia  DA results for Spot
## F value =  6.26265 ; df2 =  11 ; p-val = 0.02937405
## The larger spot size is in the  D  wing.
## Lia  DA results for Ratio
## F value =  6.804887 ; df2 =  11 ; p-val = 0.02431738
## The larger spot size is in the  D  wing.
## Lia  DA results for Resid
## F value =  6.345329 ; df2 =  11 ; p-val = 0.02852653
## The larger spot size is in the  D  wing.
## PAR  DA results for Wing
## F value =  7.101591 ; df2 =  21 ; p-val = 0.01449341
## The larger spot size is in the  D  wing.
## PAR  DA results for Spot
## F value =  0.3730445 ; df2 =  21 ; p-val = 0.5479038
## PAR  DA results for Ratio
## F value =  0.8561774 ; df2 =  21 ; p-val = 0.3653224
## PAR  DA results for Resid
## F value =  0.7650035 ; df2 =  21 ; p-val = 0.3916608
## Sap  DA results for Wing
## F value =  0.9923509 ; df2 =  15 ; p-val = 0.3349679
## Sap  DA results for Spot
## F value =  15.26897 ; df2 =  15 ; p-val = 0.001399502
## The larger spot size is in the  D  wing.
## Sap  DA results for Ratio
## F value =  12.43138 ; df2 =  15 ; p-val = 0.003057216
## The larger spot size is in the  D  wing.
## Sap  DA results for Resid
## F value =  14.70617 ; df2 =  15 ; p-val = 0.001623346
## The larger spot size is in the  D  wing.
## Shi  DA results for Wing
## F value =  1.21646 ; df2 =  12 ; p-val = 0.2916844
## Shi  DA results for Spot
## F value =  11.38126 ; df2 =  12 ; p-val = 0.005533125
## The larger spot size is in the  D  wing.
## Shi  DA results for Ratio
## F value =  10.53909 ; df2 =  12 ; p-val = 0.007003076
## The larger spot size is in the  D  wing.
## Shi  DA results for Resid
## F value =  11.17494 ; df2 =  12 ; p-val = 0.005856644
## The larger spot size is in the  D  wing.
## Sok  DA results for Wing
## F value =  6.210043 ; df2 =  19 ; p-val = 0.02211016
## The larger spot size is in the  D  wing.
## Sok  DA results for Spot
## F value =  20.59503 ; df2 =  19 ; p-val = 0.0002248482
```

```
## The larger spot size is in the  D  wing.
## Sok  DA results for Ratio
## F value =  29.35517 ; df2 =  19 ; p-val = 3.15225e-05
## The larger spot size is in the  D  wing.
## Sok  DA results for Resid
## F value =  24.15159 ; df2 =  19 ; p-val = 9.62695e-05
## The larger spot size is in the  D  wing.
## Tok  DA results for Wing
## F value =  6.078108 ; df2 =  2 ; p-val = 0.1325801
## Tok  DA results for Spot
## F value =  0.01680203 ; df2 =  2 ; p-val = 0.9087255
## Tok  DA results for Ratio
## F value =  0.1482581 ; df2 =  2 ; p-val = 0.7372965
## Tok  DA results for Resid
## F value =  0.2242773 ; df2 =  2 ; p-val = 0.6824602
## Wat  DA results for Wing
## F value =  0.005993549 ; df2 =  14 ; p-val = 0.9393866
## Wat  DA results for Spot
## F value =  10.05507 ; df2 =  14 ; p-val = 0.006802038
## The larger spot size is in the  D  wing.
## Wat  DA results for Ratio
## F value =  8.927484 ; df2 =  14 ; p-val = 0.009783523
## The larger spot size is in the  D  wing.
## Wat  DA results for Resid
## F value =  9.559278 ; df2 =  14 ; p-val = 0.007960867
## The larger spot size is in the  D  wing.
## Wis  DA results for Wing
## F value =  0.05218751 ; df2 =  10 ; p-val = 0.8239034
## Wis  DA results for Spot
## F value =  10.75693 ; df2 =  10 ; p-val = 0.008292096
## The larger spot size is in the  D  wing.
## Wis  DA results for Ratio
## F value =  11.71904 ; df2 =  10 ; p-val = 0.006511179
## The larger spot size is in the  D  wing.
## Wis  DA results for Resid
## F value =  11.39146 ; df2 =  10 ; p-val = 0.007060294
## The larger spot size is in the  D  wing.
```

We now build the final symmetric-component dataset:

```r
Sym.comp <- aggregate(Ratio ~ IND + POP,
  data = nat.sym, FUN = mean
)

Sym.comp$Spot <- aggregate(Spot ~ IND + POP,
  data = nat.sym, FUN = mean
)[, 3]

Sym.comp$Wing <- aggregate(Wing ~ IND + POP,
  data = nat.sym, FUN = mean
)[, 3]

Sym.comp$Resid <- aggregate(Resid ~ IND + POP,
  data = nat.sym, FUN = mean
)[, 3]
```

```r
Sym.comp$TEMP <- aggregate(TEMP ~ IND + POP,
  data = nat.sym, FUN = mean
)[, 3]
```

As we have build a matrix where each row is an individual, we use it as a 'model' matrix for the asymmetric component matrix. Then, for each individual (for each name within the first column) we find the position of its two wings in the raw data, we identify right and left wings and then we estimate the asymmetric component as the difference of the right wing minus the left wing. Finally, we discard the NAs (those individuals that have just one wing within the raw dataset):

```r
Asym.comp <- Sym.comp

for (i in 1:nrow(Sym.comp)) {
  indexes <- intersect(
    which(nat.sym$IND == Sym.comp[i, 1]),
    which(nat.sym$POP == Sym.comp[i, 2]))

  if (nat.sym$SIDE[indexes[1]] == "D") {
    ind_droite <- indexes[1]
    ind_gauche <- indexes[2]
  } else {
    ind_gauche <- indexes[1]
    ind_droite <- indexes[2]
  }

  Asym.comp[i, 3] <- nat.sym$Ratio[ind_droite] - nat.sym$Ratio[ind_gauche]
  Asym.comp[i, 4] <- nat.sym$Spot[ind_droite] - nat.sym$Spot[ind_gauche]
  Asym.comp[i, 5] <- nat.sym$Wing[ind_droite] - nat.sym$Wing[ind_gauche]
  Asym.comp[i, 6] <- nat.sym$Resid[ind_droite] - nat.sym$Resid[ind_gauche]
  Asym.comp[i, 7] <- Sym.comp[i,7]
}
```

Now, we can start the analyses on the asymmetric component by looking at the factors influencing the asymmetric mean (i. e. DA):

```r
summary(aov(Wing ~ TEMP*POP, data = Asym.comp))
```

```
##              Df    Sum Sq   Mean Sq F value Pr(>F)
## TEMP          1 5.788e+07  57880062   0.090  0.765
## POP          11 6.445e+09 585883852   0.909  0.533
## Residuals   165 1.064e+11 644670300
```

```r
summary(aov(Spot ~ TEMP*POP, data = Asym.comp))
```

```
##              Df    Sum Sq   Mean Sq F value Pr(>F)
## TEMP          1 2.492e+07  24919584   0.488 0.4859
## POP          11 1.319e+09 119941647   2.348 0.0103 *
## Residuals   165 8.430e+09  51089454
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
pairwise.t.test(Asym.comp$Spot, Asym.comp$POP)
```

```
##
##  Pairwise comparisons using t tests with pooled SD
##
```

```
## data:  Asym.comp$Spot and Asym.comp$POP
##
##     Bar   BRE   Gen   Ita   Lan   Lia   PAR   Sap   Shi   Sok   Tok
## BRE 1.000 -     -     -     -     -     -     -     -     -     -
## Gen 1.000 1.000 -     -     -     -     -     -     -     -     -
## Ita 1.000 1.000 1.000 -     -     -     -     -     -     -     -
## Lan 1.000 1.000 1.000 1.000 -     -     -     -     -     -     -
## Lia 1.000 1.000 1.000 1.000 1.000 -     -     -     -     -     -
## PAR 1.000 1.000 1.000 1.000 1.000 1.000 -     -     -     -     -
## Sap 1.000 1.000 1.000 1.000 1.000 1.000 0.797 -     -     -     -
## Shi 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -     -     -
## Sok 1.000 1.000 1.000 1.000 1.000 1.000 0.595 1.000 1.000 -     -
## Tok 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -
## Wat 1.000 1.000 1.000 1.000 0.596 1.000 0.039 1.000 1.000 1.000 1.000
## Wis 1.000 1.000 1.000 1.000 0.791 1.000 0.079 1.000 1.000 1.000 1.000
##     Wat
## BRE -
## Gen -
## Ita -
## Lan -
## Lia -
## PAR -
## Sap -
## Shi -
## Sok -
## Tok -
## Wat -
## Wis 1.000
##
## P value adjustment method: holm
```

```r
summary(aov(Ratio ~ TEMP*POP, data = Asym.comp))
```

```
##              Df   Sum Sq   Mean Sq F value Pr(>F)
## TEMP          1 0.000003 3.180e-06   0.143 0.7056
## POP          11 0.000505 4.593e-05   2.067 0.0254 *
## Residuals   165 0.003666 2.222e-05
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(lm(Ratio ~ TEMP, data = Asym.comp))$r.squared
```

```
## [1] 0.0007620094
```

```r
pairwise.t.test(Asym.comp$Ratio, Asym.comp$POP)
```

```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  Asym.comp$Ratio and Asym.comp$POP
##
##     Bar  BRE  Gen  Ita  Lan  Lia  PAR  Sap  Shi  Sok  Tok  Wat
## BRE 1.00 -    -    -    -    -    -    -    -    -    -    -
## Gen 1.00 1.00 -    -    -    -    -    -    -    -    -    -
## Ita 1.00 1.00 1.00 -    -    -    -    -    -    -    -    -
## Lan 1.00 1.00 1.00 1.00 -    -    -    -    -    -    -    -
```

```
## Lia 1.00 1.00 1.00 1.00 1.00 -    -    -    -    -    -    -
## PAR 1.00 1.00 1.00 1.00 1.00 1.00 -    -    -    -    -    -
## Sap 1.00 1.00 1.00 1.00 1.00 1.00 1.00 -    -    -    -    -
## Shi 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 -    -    -    -
## Sok 1.00 1.00 1.00 1.00 1.00 1.00 0.87 1.00 1.00 -    -    -
## Tok 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 -    -
## Wat 1.00 1.00 1.00 1.00 1.00 1.00 0.10 1.00 1.00 1.00 1.00 -
## Wis 1.00 1.00 1.00 1.00 0.75 1.00 0.09 1.00 1.00 1.00 1.00 1.00
##
## P value adjustment method: holm
```

```r
summary(aov(Resid ~ TEMP*POP, data = Asym.comp))
```

```
##              Df    Sum Sq   Mean Sq F value Pr(>F)
## TEMP          1 2.825e+07  28253932   0.530 0.4678
## POP          11 1.297e+09 117907880   2.211 0.0161 *
## Residuals   165 8.801e+09  53337875
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(lm(Resid ~ TEMP, data = Asym.comp))$r.squared
```

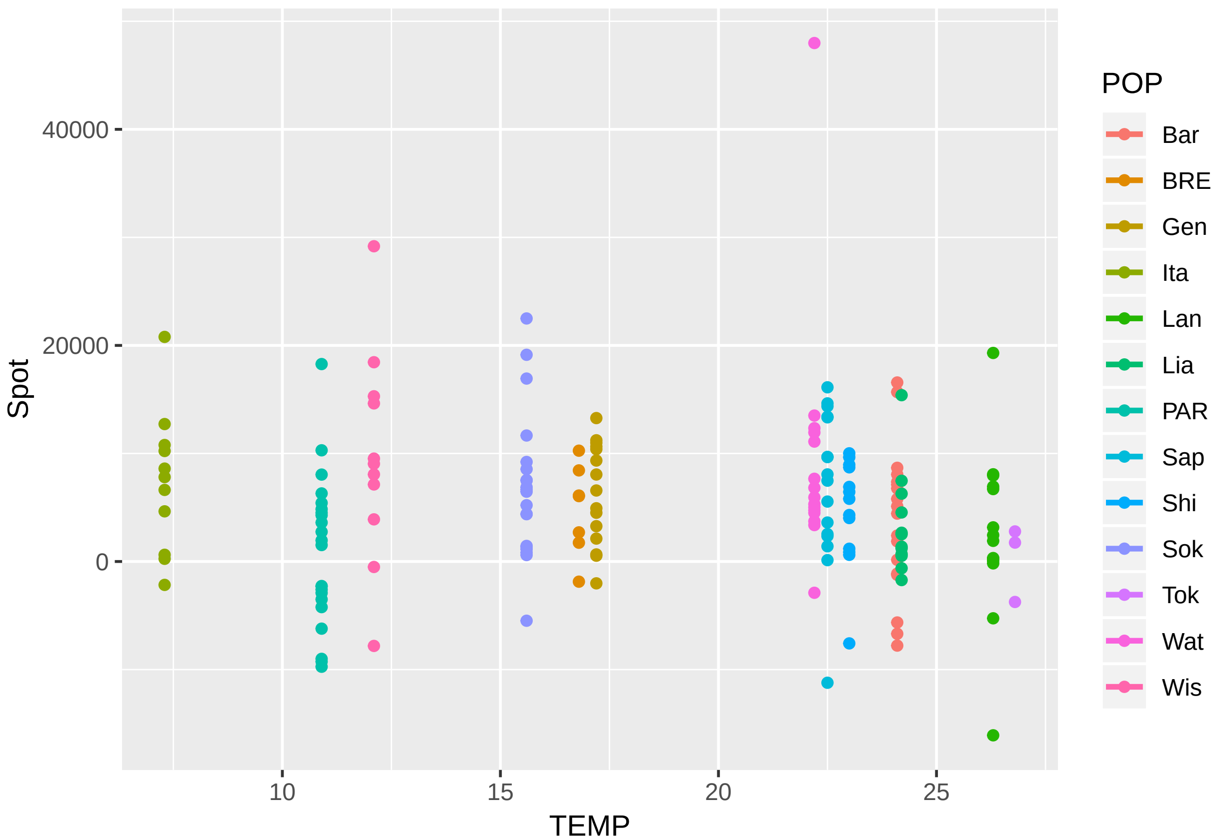
```
## [1] 0.002790239
```

```r
pairwise.t.test(Asym.comp$Resid, Asym.comp$POP)
```

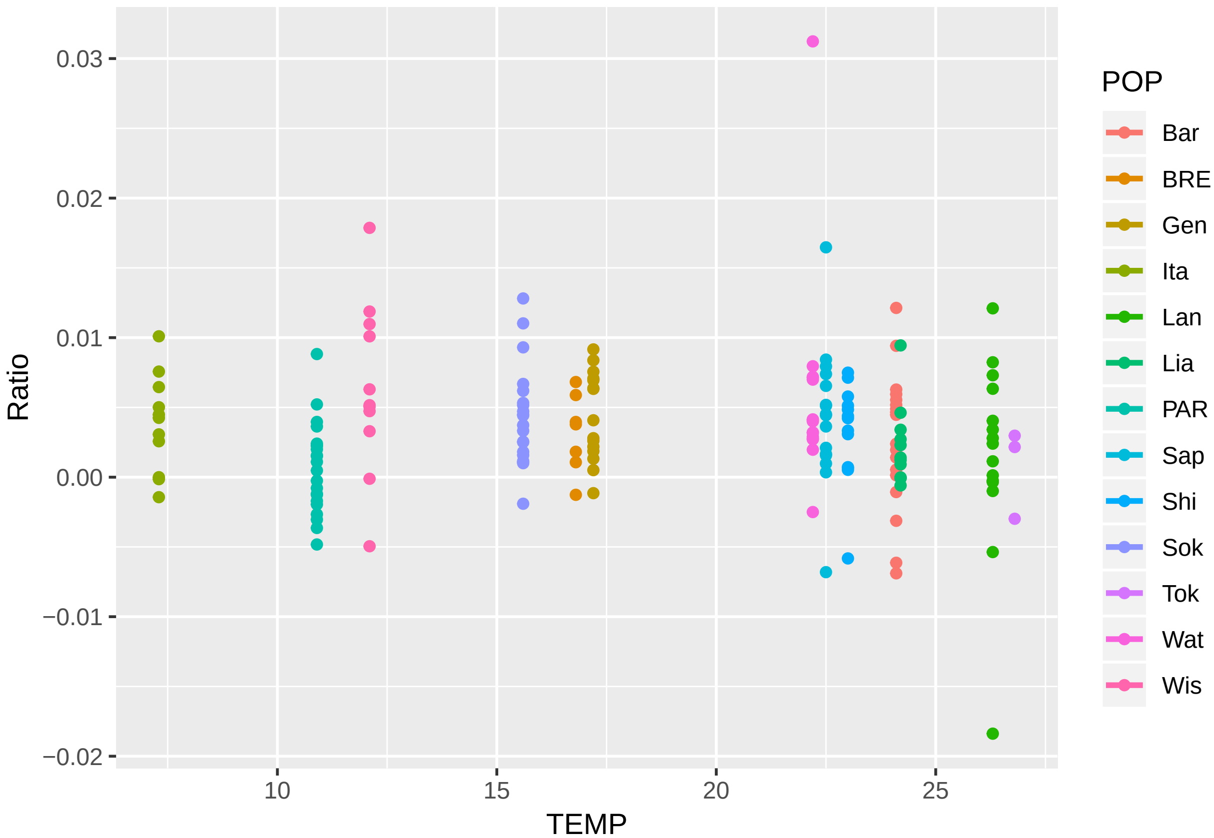
```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  Asym.comp$Resid and Asym.comp$POP
##
##     Bar   BRE   Gen   Ita   Lan   Lia   PAR   Sap   Shi   Sok   Tok
## BRE 1.000 -     -     -     -     -     -     -     -     -     -
## Gen 1.000 1.000 -     -     -     -     -     -     -     -     -
## Ita 1.000 1.000 1.000 -     -     -     -     -     -     -     -
## Lan 1.000 1.000 1.000 1.000 -     -     -     -     -     -     -
## Lia 1.000 1.000 1.000 1.000 1.000 -     -     -     -     -     -
## PAR 1.000 1.000 1.000 1.000 1.000 1.000 -     -     -     -     -
## Sap 1.000 1.000 1.000 1.000 1.000 1.000 0.679 -     -     -     -
## Shi 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -     -     -
## Sok 1.000 1.000 1.000 1.000 1.000 1.000 0.660 1.000 1.000 -     -
## Tok 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 -
## Wat 1.000 1.000 1.000 1.000 0.679 1.000 0.091 1.000 1.000 1.000 1.000
## Wis 1.000 1.000 1.000 1.000 0.841 1.000 0.159 1.000 1.000 1.000 1.000
##     Wat
## BRE -
## Gen -
## Ita -
## Lan -
## Lia -
## PAR -
## Sap -
## Shi -
## Sok -
## Tok -
## Wat -
```

```
## Wis 1.000
##
## P value adjustment method: holm
```

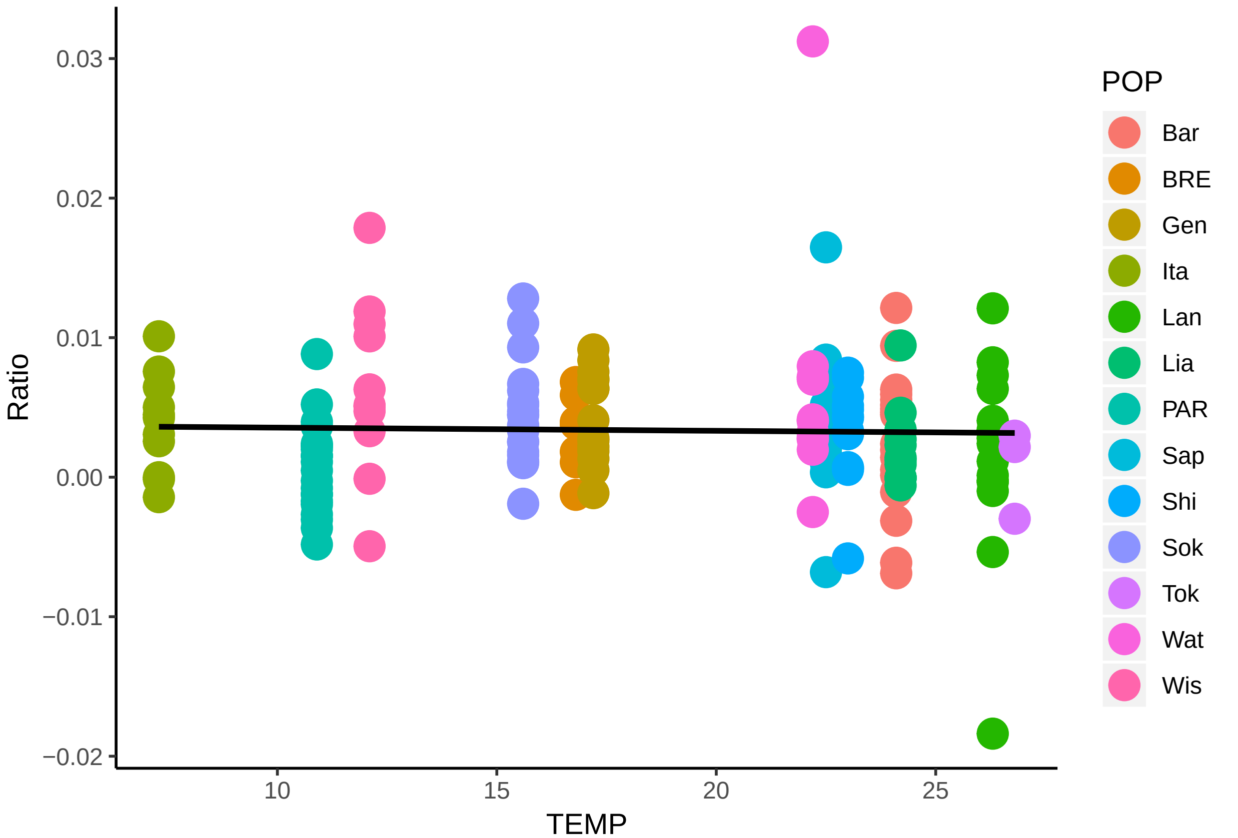
```r
ggplot(data=Asym.comp,
       aes(x = TEMP, y = Spot, color = POP)) +
  geom_point() +
  geom_smooth(method = 'lm', se = FALSE)
```



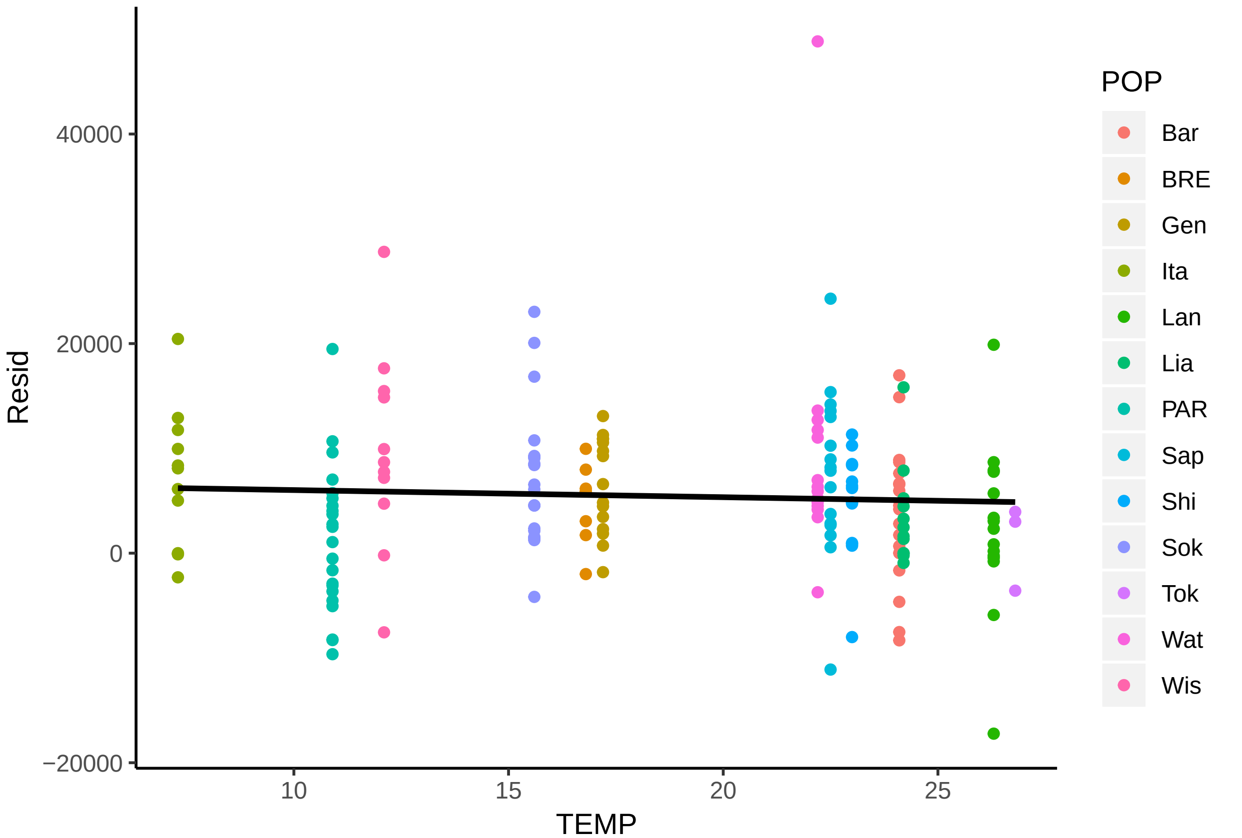

```r
ggplot(data=Asym.comp,
       aes(x = TEMP, y = Ratio, color = POP)) +
  geom_point() +
  geom_smooth(method = 'lm', se = FALSE)
```




```r
ggplot(data=Asym.comp,
       aes(x = TEMP, y = Ratio, color = POP)) +
  geom_point(size = 5) +
  geom_smooth(method = 'lm', se = FALSE, color = 'black') +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        panel.background = element_blank(), axis.line = element_line(colour = "black"))
```




```r
ggplot(data=Asym.comp,
       aes(x = TEMP, y = Resid, color = POP)) +
  geom_point() +
  geom_smooth(method = 'lm', se = FALSE, color = 'black') +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        panel.background = element_blank(), axis.line = element_line(colour = "black"))
```



To study the effect of temperature and population on fluctuating asymmetry we use both Levene tests and tests taking into account the coefficient of variation:

```
leveneTest(Asym.comp$Wing, group = Asym.comp$POP)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value Pr(>F)
## group  12  0.9415 0.5072
##       165
```

```
leveneTest(Asym.comp$Spot, group = Asym.comp$POP)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value Pr(>F)
## group  12  0.5966 0.8428
##       165
```

```
leveneTest(Asym.comp$Ratio, group = Asym.comp$POP)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value Pr(>F)
## group  12  0.8027 0.6474
##       165
```

```
leveneTest(Asym.comp$Resid, group = Asym.comp$POP)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value Pr(>F)
## group  12  0.5732 0.8615
##       165
```

Is FA related to temperature?

```r
vWings <- c()

for (j in 1:length(levels(Asym.comp$POP))) {

st.dev <- var(Asym.comp$Wing[Asym.comp$POP == levels(Asym.comp$POP)[j]])

vWings <- c(vWings, st.dev)

}

summary(aov(vWings ~ unique(Asym.comp$TEMP)))
```

```
##                        Df    Sum Sq   Mean Sq F value Pr(>F)
## unique(Asym.comp$TEMP)  1 4.069e+17 4.069e+17   0.288  0.602
## Residuals              11 1.553e+19 1.411e+18
```

```r
vSpot <- c()

for (h in 1:length(levels(Asym.comp$POP))) {

st.d <- var(Asym.comp$Spot[Asym.comp$POP == levels(Asym.comp$POP)[h]])

vSpot <- c(vSpot, st.d)

}

summary(aov(vSpot ~ unique(Asym.comp$TEMP)))
```

```
##                        Df    Sum Sq   Mean Sq F value Pr(>F)
## unique(Asym.comp$TEMP)  1 2.049e+14 2.049e+14   0.167   0.69
## Residuals              11 1.348e+16 1.226e+15
```

```r
vRatio <- c()

for (h in 1:length(levels(Asym.comp$POP))) {

st.d <- var(Asym.comp$Ratio[Asym.comp$POP == levels(Asym.comp$POP)[h]])

vRatio <- c(vRatio, st.d)

}

summary(aov(vRatio ~ unique(Asym.comp$TEMP)))
```

```
##                        Df   Sum Sq   Mean Sq F value Pr(>F)
## unique(Asym.comp$TEMP)  1 1.98e-10 1.980e-10   0.712  0.417
## Residuals              11 3.06e-09 2.782e-10
```

```r
vResid <- c()

for (h in 1:length(levels(Asym.comp$POP))) {

st.d <- var(Asym.comp$Resid[Asym.comp$POP == levels(Asym.comp$POP)[h]])
```

```r
vResid <- c(vResid, st.d)

}

summary(aov(vResid ~ unique(Asym.comp$TEMP)))
```

```
##                         Df    Sum Sq   Mean Sq F value Pr(>F)
## unique(Asym.comp$TEMP)  1 4.486e+13 4.486e+13   0.034  0.856
## Residuals              11 1.436e+16 1.305e+15
```