Examining Different Definitions of Plasticity in Growth and Morphology across Temperatures

Introduction

In the previous document, I showed how outcrossed lines of Paramecium varied in their morphology and movement with temperature, how their growth rates changed with temperature, and how morphology and movement were associated with changes in growth rates within outcrossed lines. One hypothesis that we had that we did not find evidence for, however, was that the overall flexibility or magnitude of plasticity in morphology and movement among outcrossed lines would be related to their degree of plasticity in growth rate. In particular, we hypothesized that outcrossed lines with greater plasticity in morphology/movement across temperature would show less variable growth rates across temperature as, presumably, the morphological/behavioral changes could buffer their growth rate responses to temperature.

In these first analyses of plasticity in morphology/movement and growth, we used the overall magnitude of change in phenotypes as our measure of phenotypic plasticity across the temperature range (the maximum mean value across the temperatures minus the minimum mean value across the temperatures). There were some concerns, however, particularly for the growth rate tpc's, that this metric wasn't really capturing plasticity in the growth rates. In particular, one suggestion was that a better metric might be the thermal breadth – the range of temperatures over which at least some proportion of the maximum in performance is achieved (e.g. in our case, the range of temperatures over which an outcrossed line acheives a growth rate that is at least some proportion of its maximum growth rate). Furthermore, John had also mentioned looking at whether the area under the total curve was related to morphology/movement plasticity. Last, there were also some ideas about measuring plasticity of the morphology and movement variables differently. Specifically, one idea was to use the total amount of change in the phenotype and another was to use the amount of morphological/movement change that occurred between an anchor point (e.g. the thermal optimum (t_{opt} or the temperature at which the maximum of the tpc occurs)) and calculate the total amount of change between the say the edges of the temperature limits of the thermal breadth and the thermal optimum.

In this document, I will try to address these comments and perform analyses to look at whether any of these different definitions of plasticity provide us with a different answer than we arrived at previously.

Calculating Thermal Breadth

The first step we will take is to go ahead and calculate the thermal breadth. To do so, I will first load the growth data, then fit TPC's to the data using GAMs, and then use the predicted growth rates from the GAMs to calculate the TPC metrics of interest including thermal breadth. I will also include a summary of the GAM fits and plots of the GAM fits to the data.

```
### load the growth rate data

tpc_data <- read.csv('StartPop_TPC.csv')

### clean up the data a bit

tpc_data <- tpc_data %>% filter(!is.na(Growth.Rate.Hours) & !is.infinite(Growth.Rate.Hours))

tpc_data <- tpc_data %>% filter(Genotype != 'blank')

tpc_data$Genotype <- as.factor(paste0('G', tpc_data$Genotype))
```

```r
colnames(tpc_data)[which(colnames(tpc_data) == 'Genotype')] <- 'Line'

tpc_data$Date.paramecium.introduced <- as.factor(tpc_data$Date.paramecium.introduced)

### fit a GAM to TPC data (note that this time I have included a random effect
### of experimental day which I forgot to do last time)

tpc_gam <- gam(formula = Growth.Rate.Hours ~ Line + s(Temperature, k = 6, bs = 'tp', by = Line) + s(Date
               method = 'REML')

summary(tpc_gam)
```
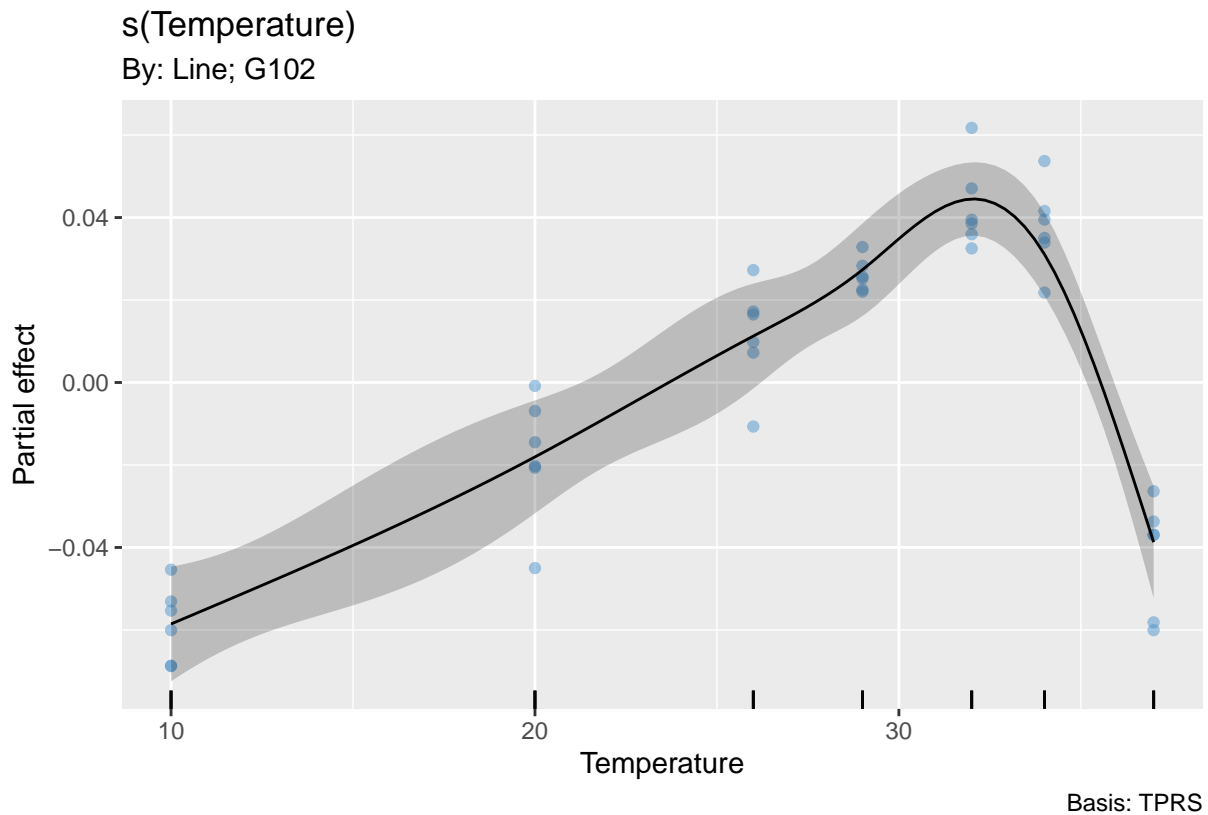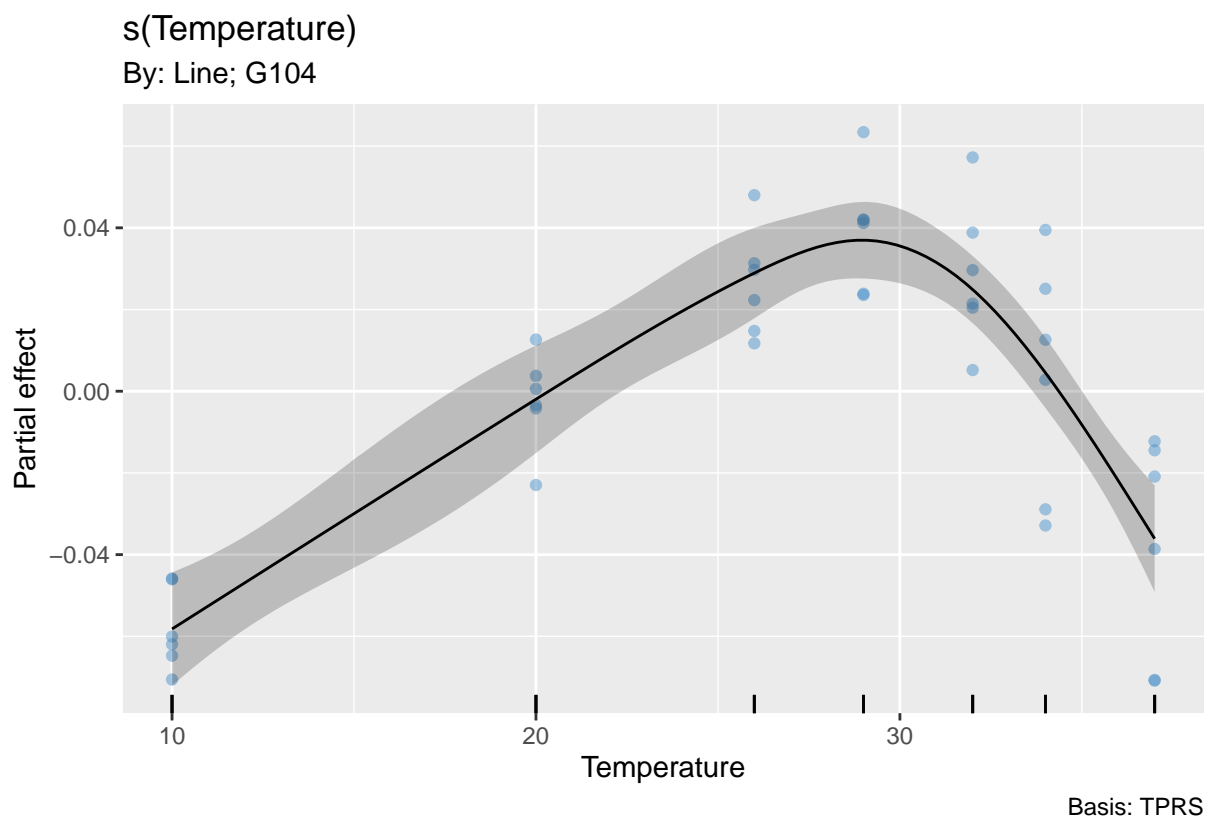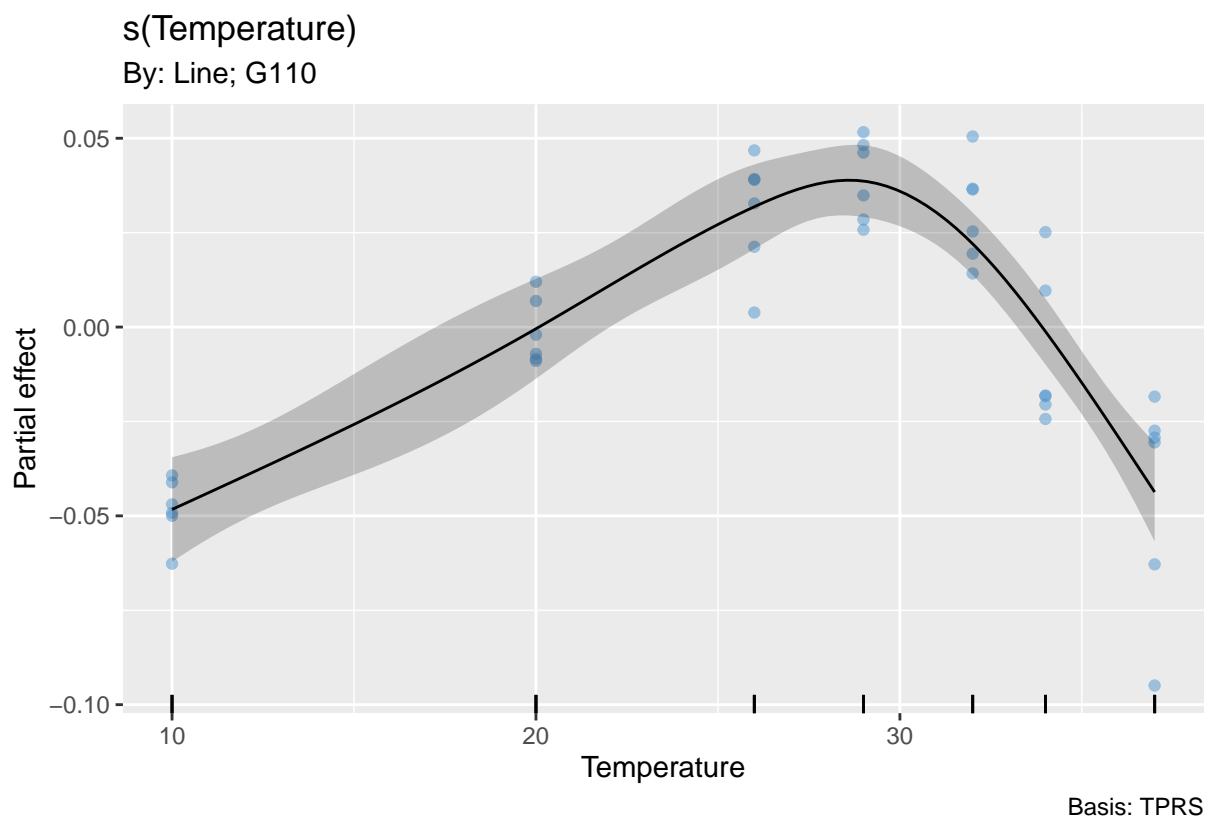
```
##
## Family: gaussian
## Link function: identity
##
## Formula:
## Growth.Rate.Hours ~ Line + s(Temperature, k = 6, bs = "tp", by = Line) +
##     s(Date.paramecium.introduced, bs = "re")
##
## Parametric coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.0665743  0.0039085  17.033  < 2e-16 ***
## LineG104     0.0019021  0.0040764   0.467  0.64091
## LineG110    -0.0060963  0.0040764  -1.496  0.13521
## LineG120    -0.0099190  0.0042187  -2.351  0.01898 *
## LineG16     -0.0222909  0.0040764  -5.468 6.25e-08 ***
## LineG19      0.0003218  0.0040764   0.079  0.93711
## LineG20     -0.0366838  0.0041041  -8.938  < 2e-16 ***
## LineG30     -0.0058367  0.0040764  -1.432  0.15262
## LineG33      0.0030411  0.0040764   0.746  0.45589
## LineG34     -0.0085611  0.0040764  -2.100  0.03606 *
## LineG35     -0.0016298  0.0040767  -0.400  0.68944
## LineG36      0.0050198  0.0040764   1.231  0.21856
## LineG38      0.0063436  0.0041213   1.539  0.12418
## LineG41     -0.0062324  0.0040764  -1.529  0.12672
## LineG44     -0.0108785  0.0040764  -2.669  0.00778 **
## LineG69     -0.0048659  0.0040764  -1.194  0.23299
## LineG72     -0.0011216  0.0040764  -0.275  0.78329
## LineG77     -0.0008309  0.0040764  -0.204  0.83855
## LineG89     -0.0047289  0.0040764  -1.160  0.24640
## LineG90      0.0012594  0.0040764   0.309  0.75745
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Approximate significance of smooth terms:
##                           edf Ref.df     F p-value
## s(Temperature):LineG102 4.573  4.891 31.89  <2e-16 ***
## s(Temperature):LineG104 3.919  4.436 29.92  <2e-16 ***
## s(Temperature):LineG110 3.968  4.477 28.49  <2e-16 ***
## s(Temperature):LineG120 3.813  4.354 15.90  <2e-16 ***
## s(Temperature):LineG16  4.181  4.646 20.28  <2e-16 ***
## s(Temperature):LineG19  3.761  4.298 32.05  <2e-16 ***
```
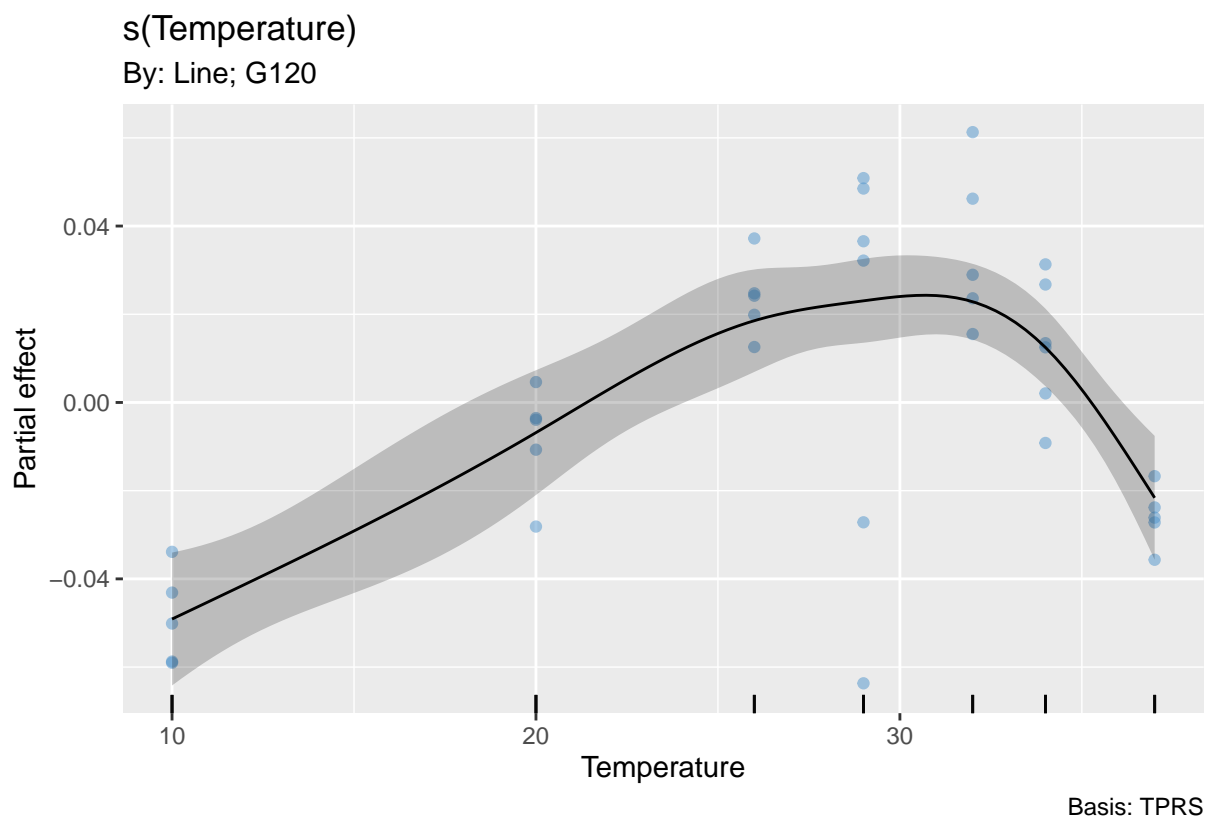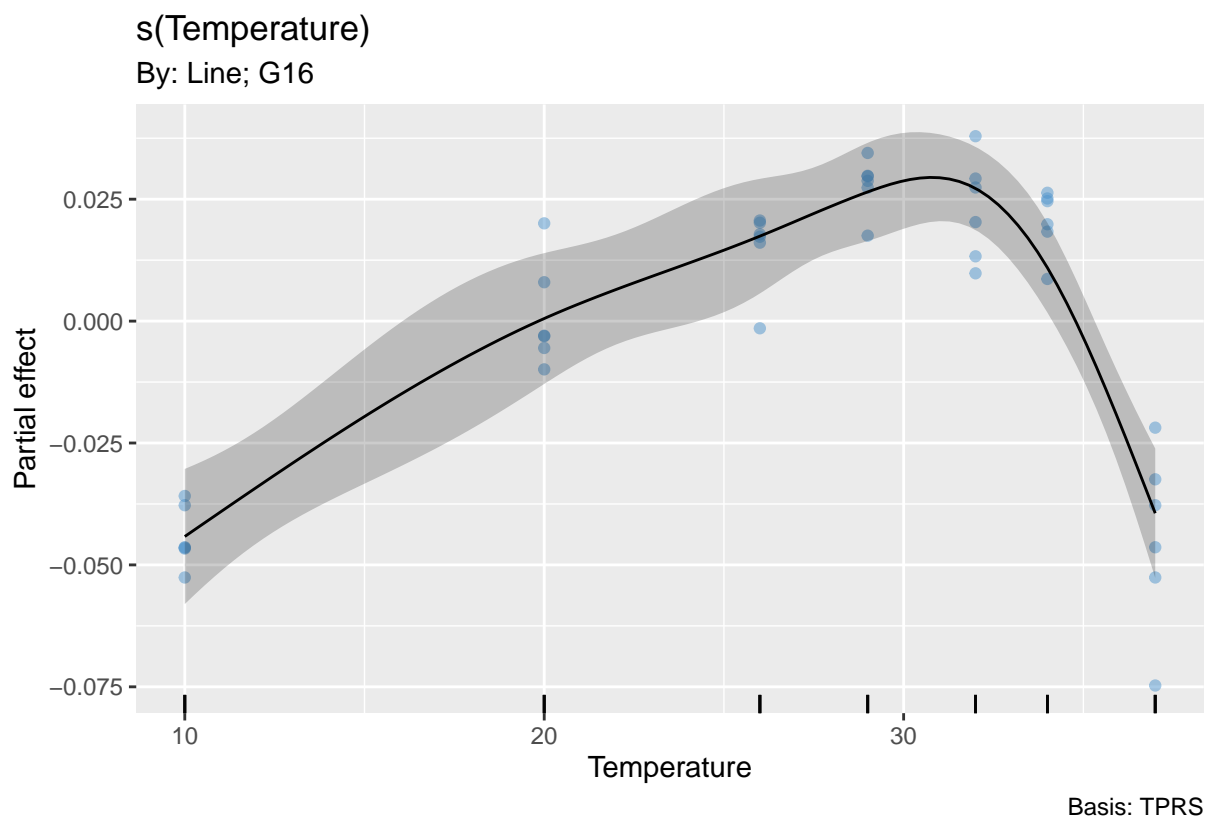
```
## s(Temperature):LineG20      3.594  4.149 13.57  <2e-16 ***
## s(Temperature):LineG30      3.921  4.438 30.39  <2e-16 ***
## s(Temperature):LineG33      3.826  4.356 29.72  <2e-16 ***
## s(Temperature):LineG34      4.354  4.767 29.87  <2e-16 ***
## s(Temperature):LineG35      4.029  4.527 33.83  <2e-16 ***
## s(Temperature):LineG36      4.153  4.624 37.35  <2e-16 ***
## s(Temperature):LineG38      3.914  4.434 32.01  <2e-16 ***
## s(Temperature):LineG41      3.996  4.500 21.93  <2e-16 ***
## s(Temperature):LineG44      4.203  4.662 35.05  <2e-16 ***
## s(Temperature):LineG69      3.774  4.310 23.59  <2e-16 ***
## s(Temperature):LineG72      3.630  4.179 27.73  <2e-16 ***
## s(Temperature):LineG77      4.014  4.515 27.12  <2e-16 ***
## s(Temperature):LineG89      3.386  3.947 15.01  <2e-16 ***
## s(Temperature):LineG90      3.740  4.280 27.53  <2e-16 ***
## s(Date.paramecium.introduced) 4.715  5.000 16.38  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## R-sq.(adj) =  0.764   Deviance explained = 79.3%
## -REML = -1860.8  Scale est. = 0.00034894  n = 833
```

```r
for (i in 1:length(smooths(tpc_gam))) {
  out <- draw(tpc_gam, select = i, residuals = TRUE)
  print(out) }
```
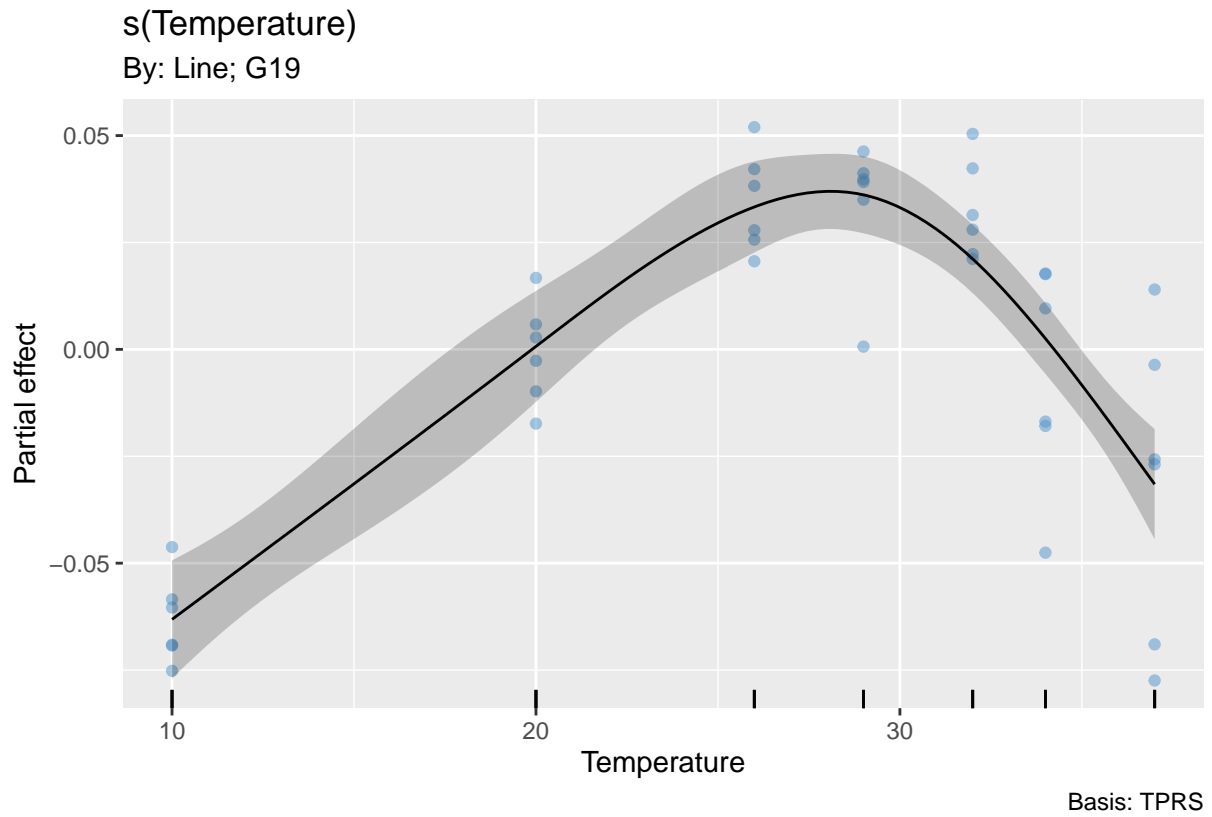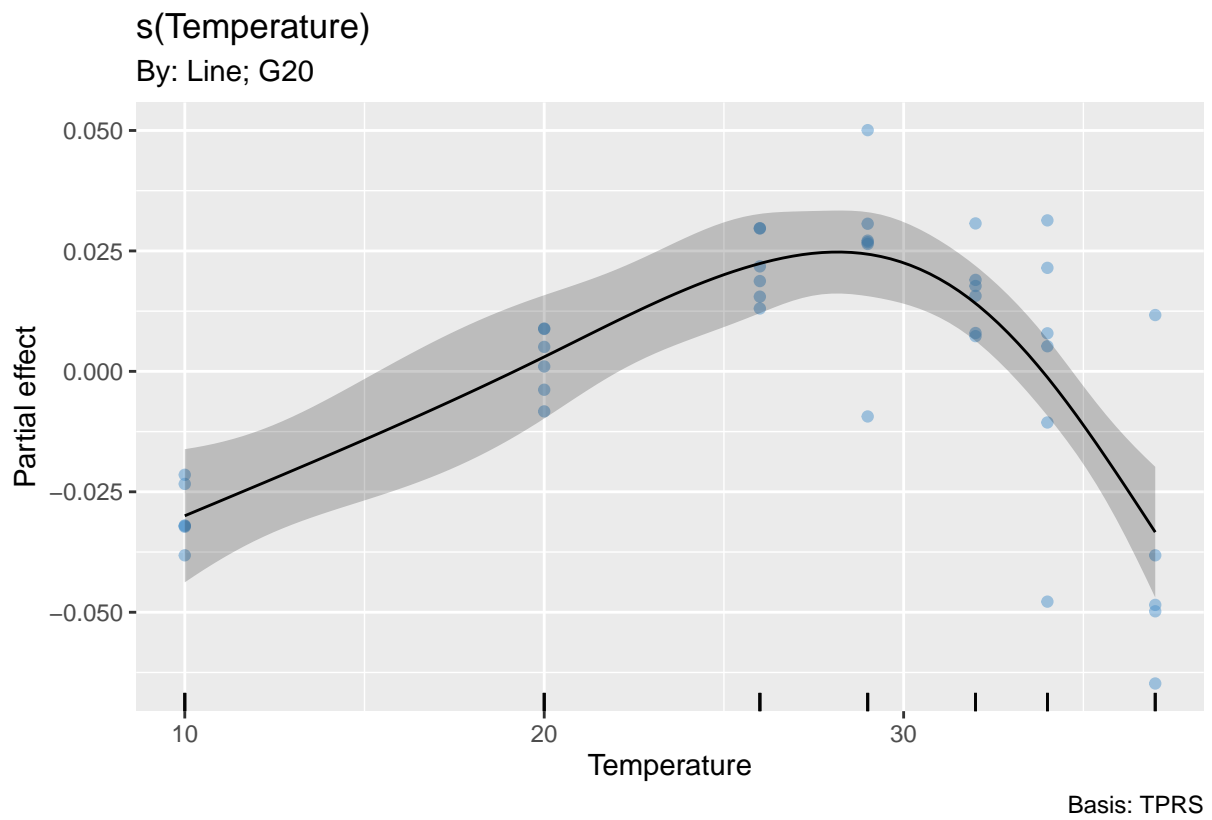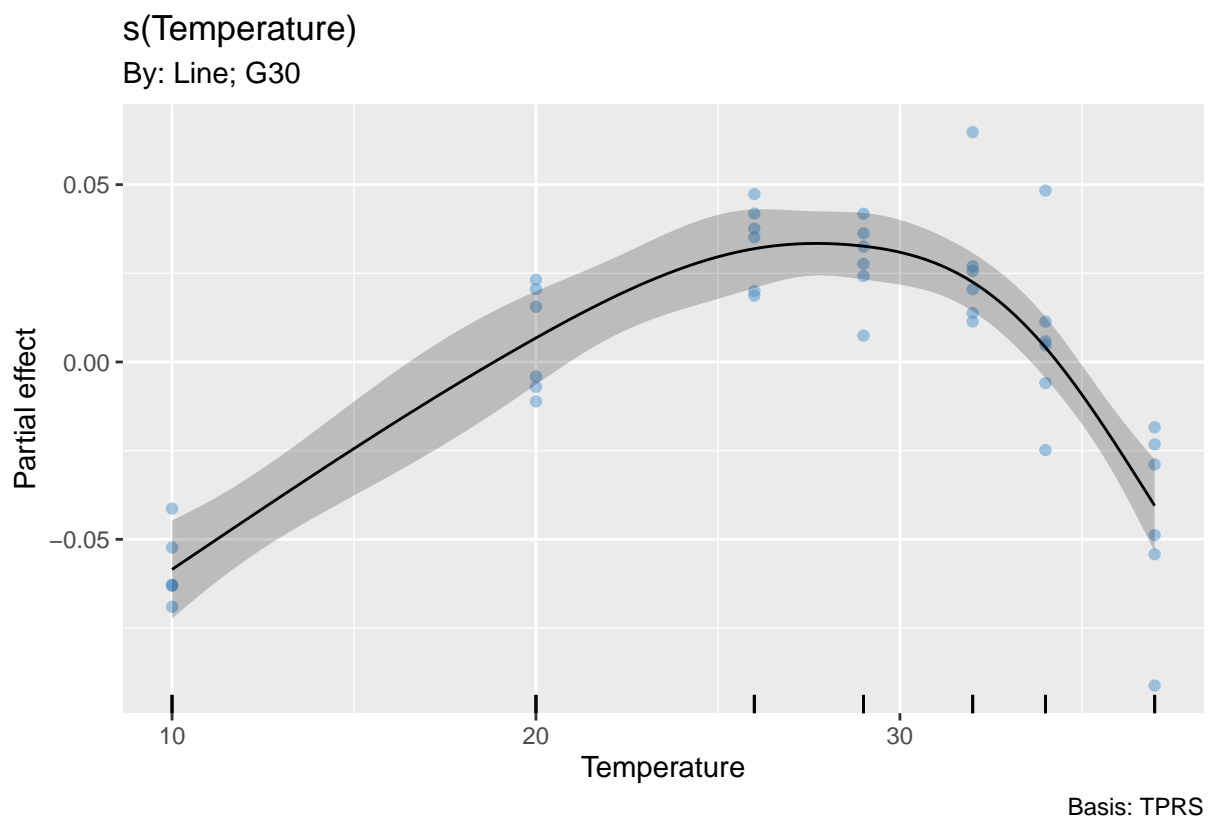

s(Temperature)
By: Line; G102

s(Temperature)
By: Line; G104

Basis: TPRS

s(Temperature)
By: Line; G110

Basis: TPRS

s(Temperature)
By: Line; G120

Basis: TPRS

s(Temperature)

By: Line; G16

Basis: TPRS

s(Temperature)
By: Line; G19

Basis: TPRS

s(Temperature)

By: Line; G20

Basis: TPRS

s(Temperature)

By: Line; G30

Basis: TPRS

s(Temperature)
By: Line; G33

Basis: TPRS

s(Temperature)
By: Line; G34
Basis: TPRS

s(Temperature)

By: Line; G35

Basis: TPRS

13

s(Temperature)
By: Line; G36

Basis: TPRS

s(Temperature)

By: Line; G38

Basis: TPRS

15

s(Temperature)

By: Line; G41

Basis: TPRS

s(Temperature)
By: Line; G44

Basis: TPRS

s(Temperature)
By: Line; G69

Basis: TPRS

s(Temperature)
By: Line; G72
Basis: TPRS

19

s(Temperature)
By: Line; G77
Basis: TPRS

s(Temperature)
By: Line; G89

Basis: TPRS

s(Temperature)
By: Line; G90
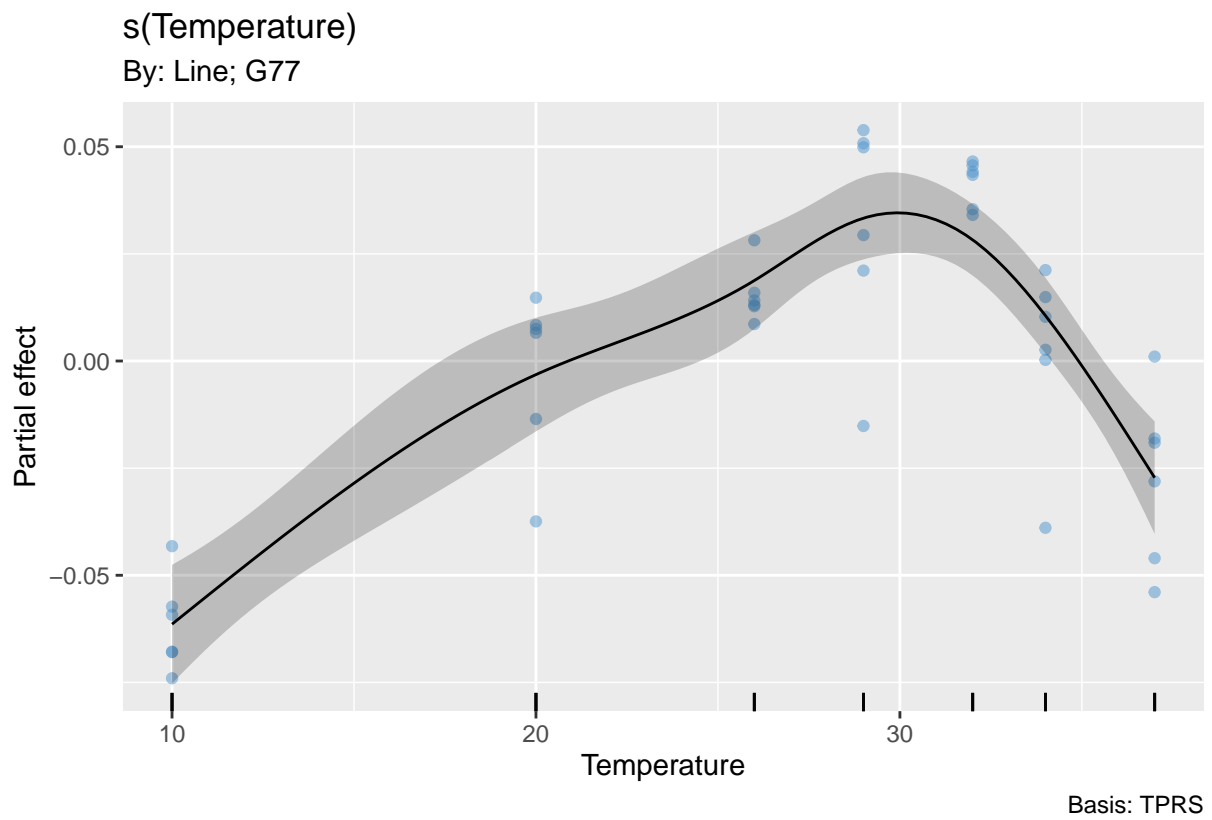Basis: TPRS

22

s(Date.paramecium.introduced)
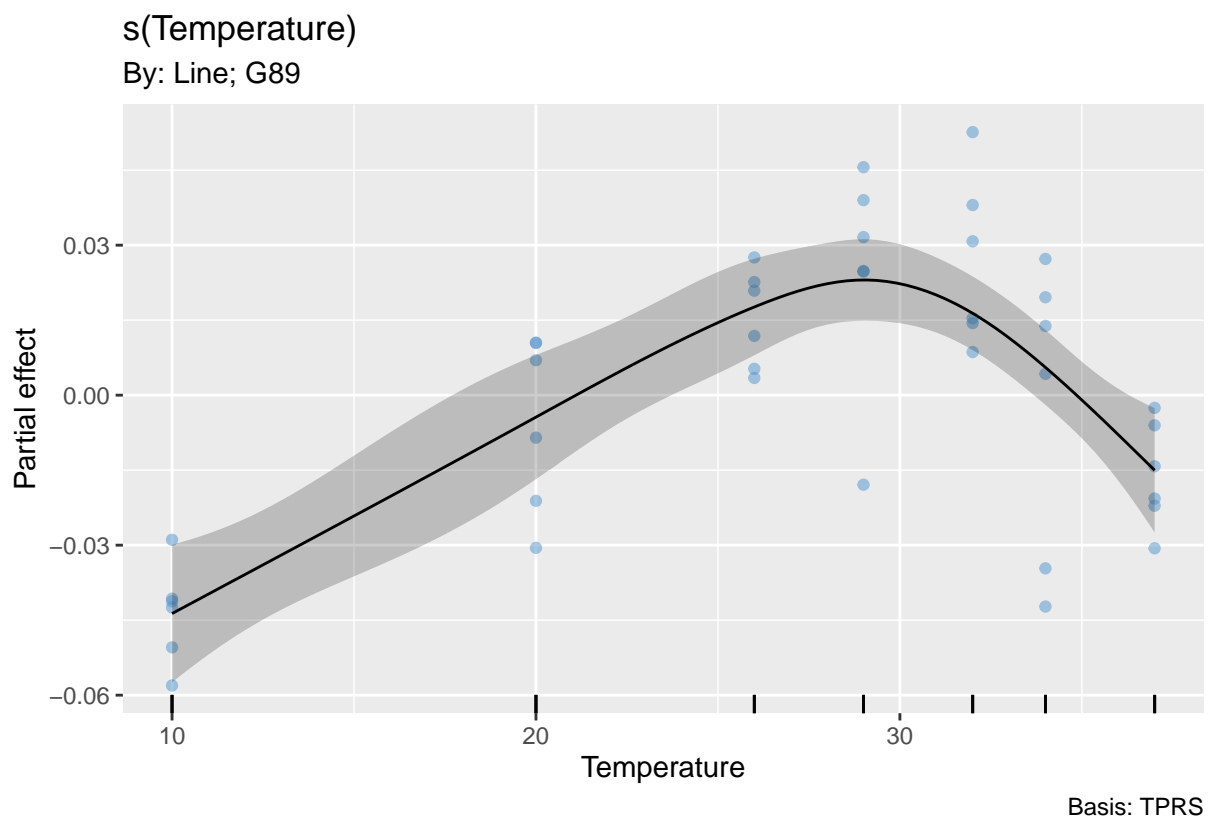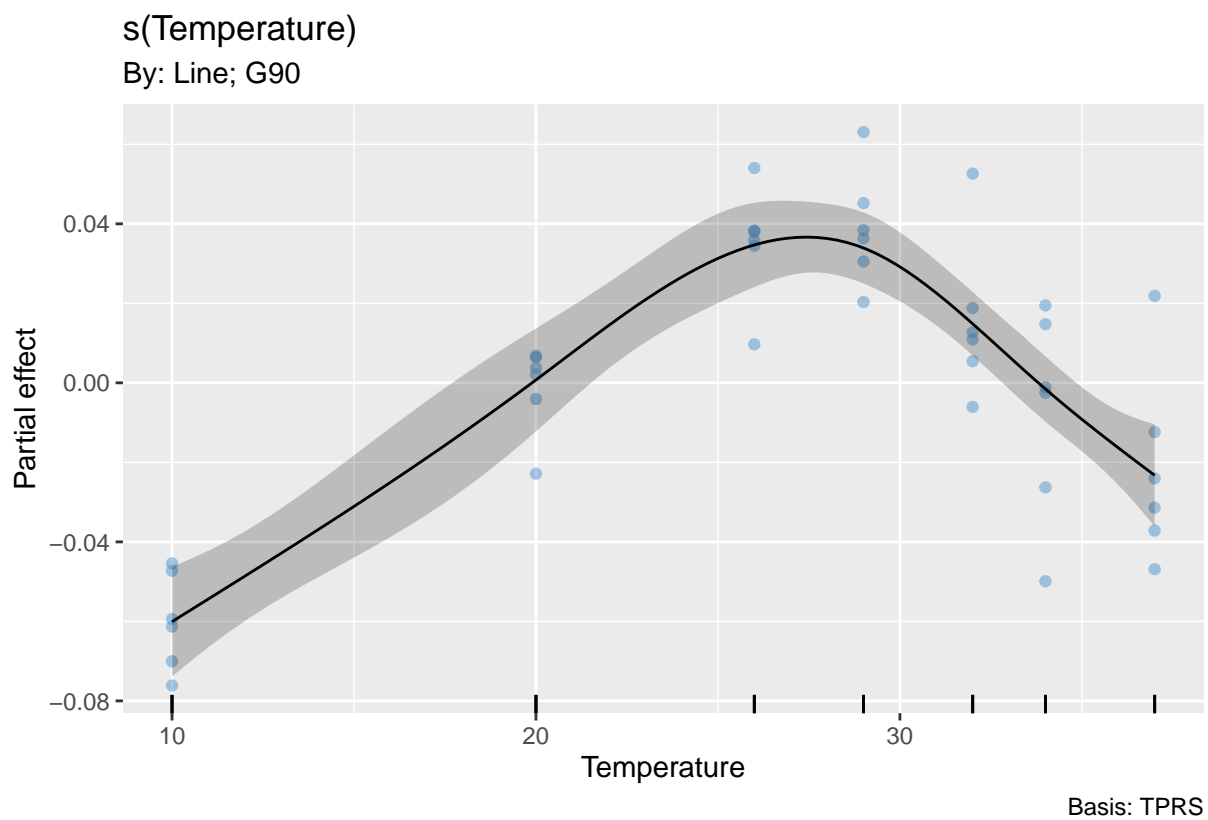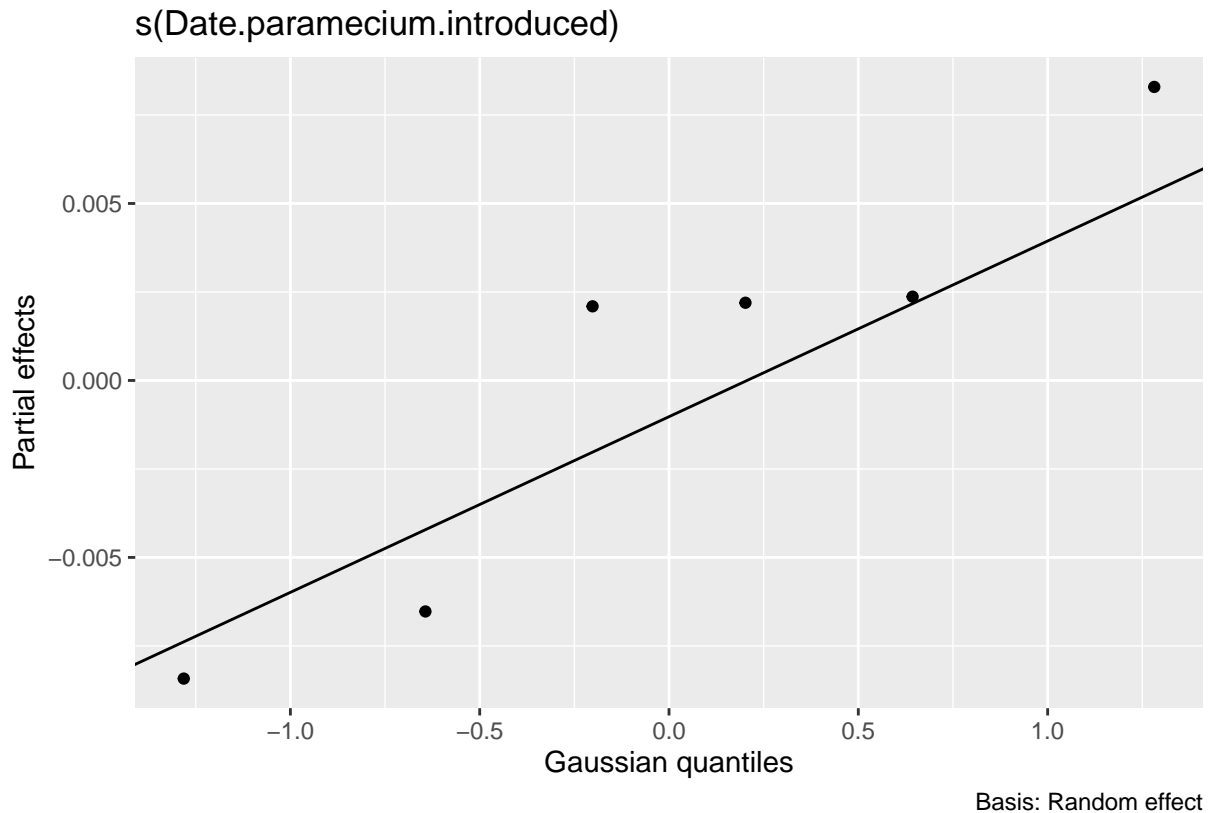

Basis: Random effect

Now we will need to use the fits from the GAMs to get predictions and then use those predictions to calculate the the variables describing the TPC that we might care about (thermal breadth, t_opt, maximum growth rate, etc.). To do so, I'm going to write a little function called tpc_summary that takes the GAM fits, a vector giving the different line names, the range of temperatures to consider, and, for the calculation of thermal breadth, which proportion of the maximum growth rate to consider. The function then runs across all of the lines and calculates summary statistics of the TPCs. I'll also run this function on our GAM fits.

```
tpc_summary <- function(gam_fit, factor, temp_low, temp_high, breadth_proportion){

  out_data <- matrix(nrow = 20, ncol = 8) # columns are Line, max_growth, t_opt, t_min, t_max, thermal_

  for(i in 1:length(unique(factor))){

    line_prediction_data <- data.frame(Line = rep(unique(factor)[i], times = length(seq(temp_low, temp_
                                       Date.paramecium.introduced = rep('2024-02-16', times = length(se
                                       Temperature = seq(temp_low, temp_high, by = 0.1))

    gam_prediction <- predict.gam(gam_fit, newdata = line_prediction_data, exclude = "s(Date.paramecium

    prediction_together <- cbind(line_prediction_data, gam_prediction)

    max_growth <- max(prediction_together$gam_prediction)

    t_opt <- prediction_together$Temperature[which(prediction_together$gam_prediction == max_growth)]

    below_opt <- prediction_together[which(prediction_together$Temperature < t_opt),]
```

```r
    t_min <- below_opt$Temperature[which(abs(below_opt$gam_prediction) == min(abs(below_opt$gam_predict

    above_opt <- prediction_together[which(prediction_together$Temperature > t_opt),]

    t_max <- above_opt$Temperature[which(abs(above_opt$gam_prediction) == min(abs(above_opt$gam_predict

    in_range <- filter(prediction_together, gam_prediction >= breadth_proportion*max_growth)

    thermal_range_min <- in_range$Temperature[1]

    thermal_range_max <- in_range$Temperature[nrow(in_range)]

    thermal_range <- thermal_range_max - thermal_range_min

    out_data[i,1] <- as.character(unique(factor)[i])

    out_data[i,2] <- max_growth

    out_data[i,3] <- t_opt

    out_data[i,4] <- t_min

    out_data[i,5] <- t_max

    out_data[i,6] <- thermal_range_min

    out_data[i,7] <- thermal_range_max

    out_data[i,8] <- thermal_range

    print(i)

  }

  out_data <- as.data.frame(out_data)

  colnames(out_data) <- c("Line", 'max_growth', 't_opt', 't_min',
                          't_max', 'thermal_range_min', 'thermal_range_max', 'thermal_range')

  for(i in 2:8){

  out_data[,i] <- as.numeric(paste(out_data[,i]))

  }

  return(out_data)

}


### run function on our GAM fits

tpc_summary_data <- tpc_summary(gam_fit = tpc_gam, factor = tpc_data$Line, temp_low = 0, temp_high = 45
```

Now we can look at some summaries of the summary, and look at some relationships among some of the TPC descriptions just to get a sense of what is going on with TPCs across the outcrossed lines. First, we can just look at some histograms of the different variables we calculated

```
### max_growth

ggplot(data = tpc_summary_data, aes(x = max_growth)) + geom_histogram()
```

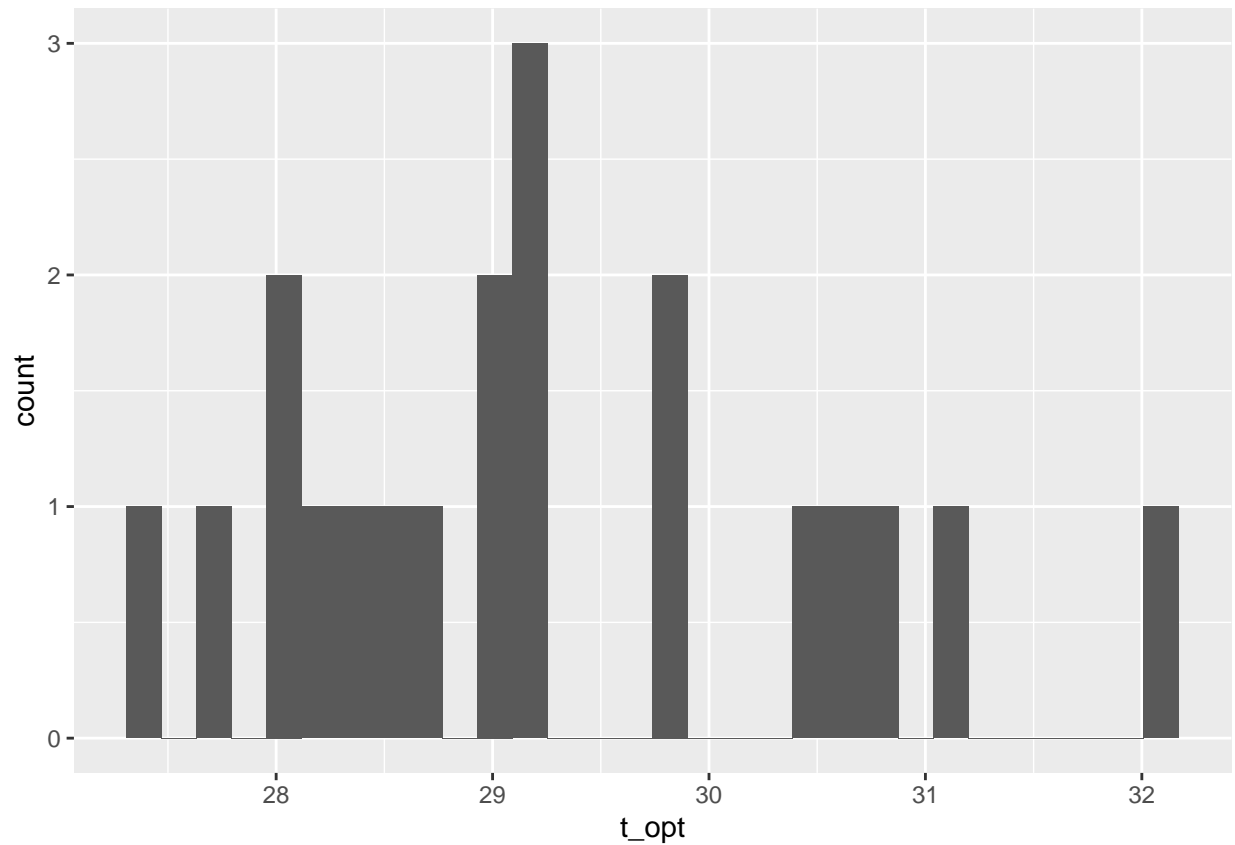## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
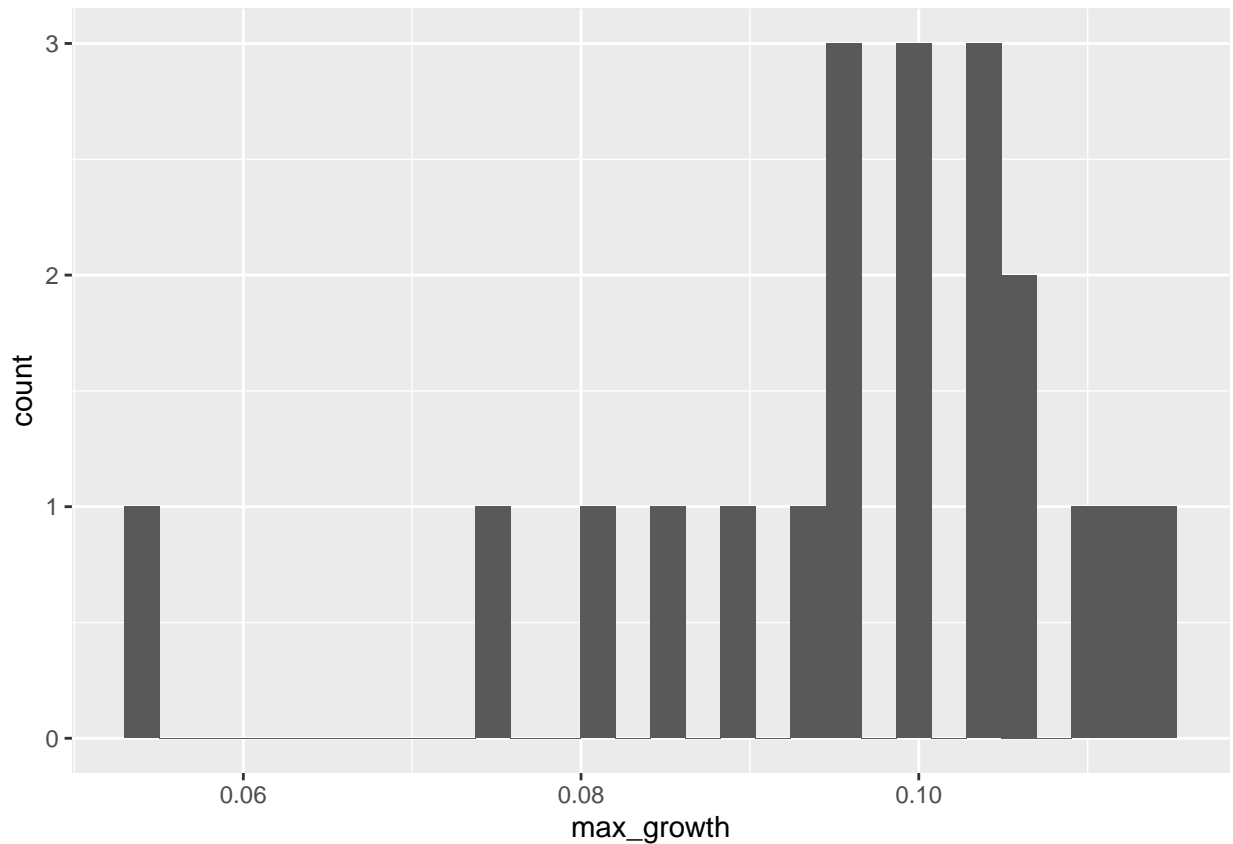

```
### we can definitely see our one outlier outcrossed line G20 here with the
### much lower maximum growth rate relative to all of the other
### outcrossed lines

### t_opt

ggplot(data = tpc_summary_data, aes(x = t_opt)) + geom_histogram()
```

`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
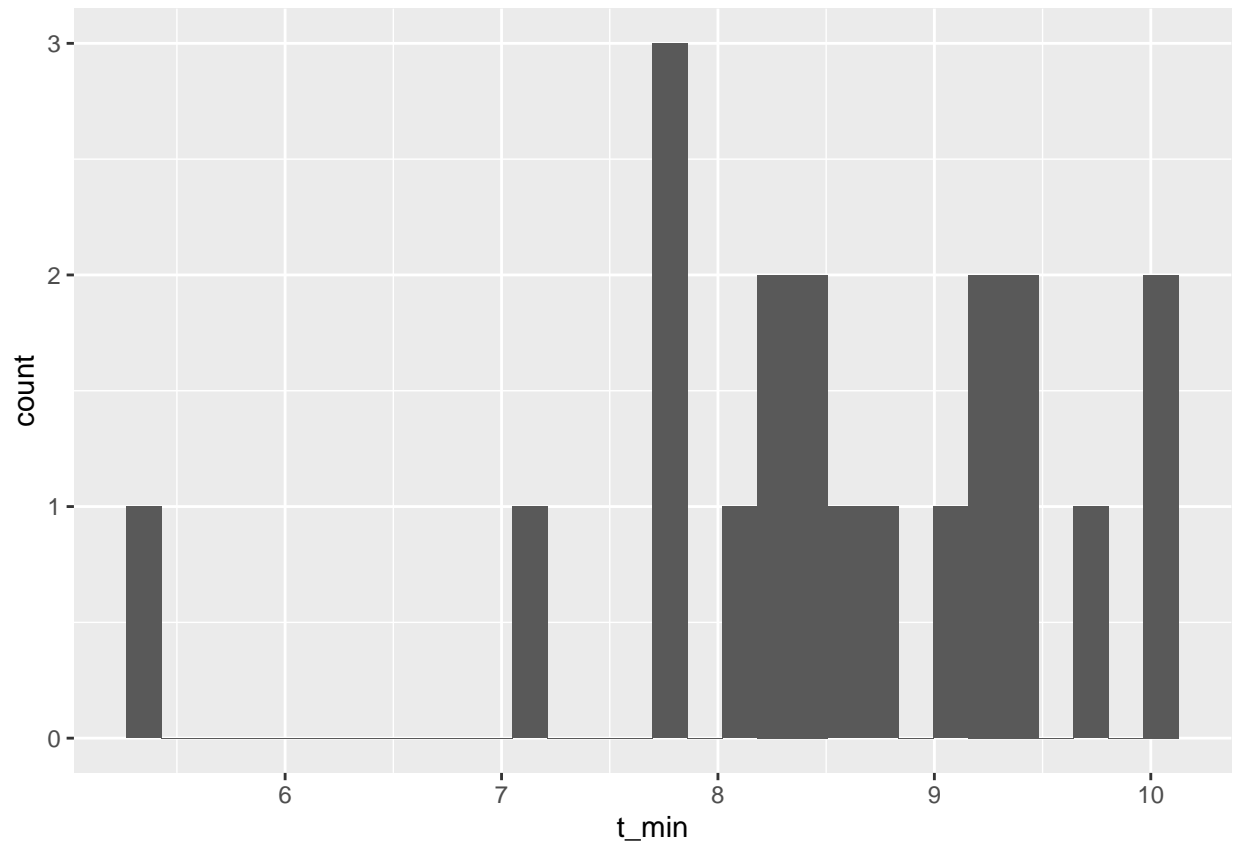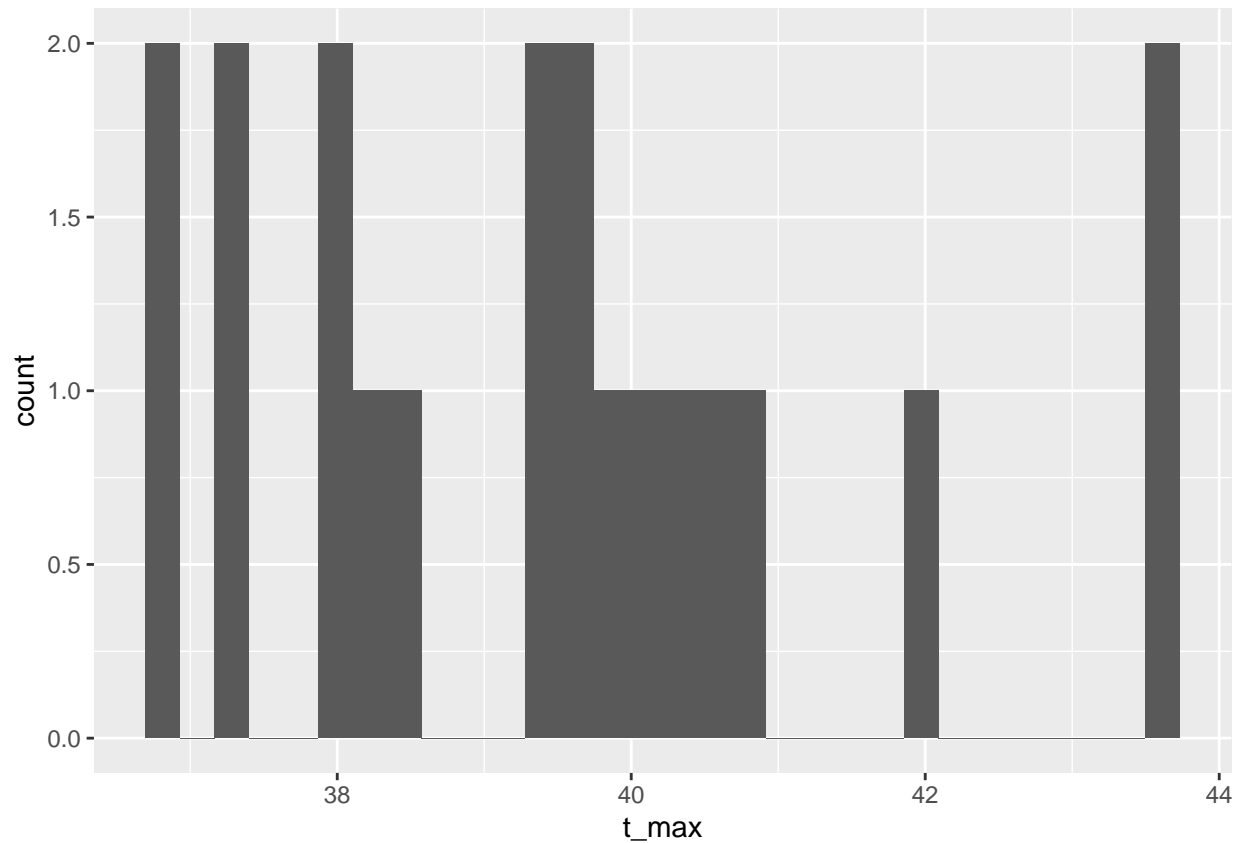
optimal temperatures range from about 27 to 32 degrees celsius. In
fahrenhiet this is about a 9 degree range from 80.6 to 89.6.

t_min

```
ggplot(data = tpc_summary_data, aes(x = t_min)) + geom_histogram()
```

`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.

we also see some variation in predicted minimum temperatures with most
falling between 7 and 10 degrees celsius

t_max

```
ggplot(data = tpc_summary_data, aes(x = t_max)) + geom_histogram()
```

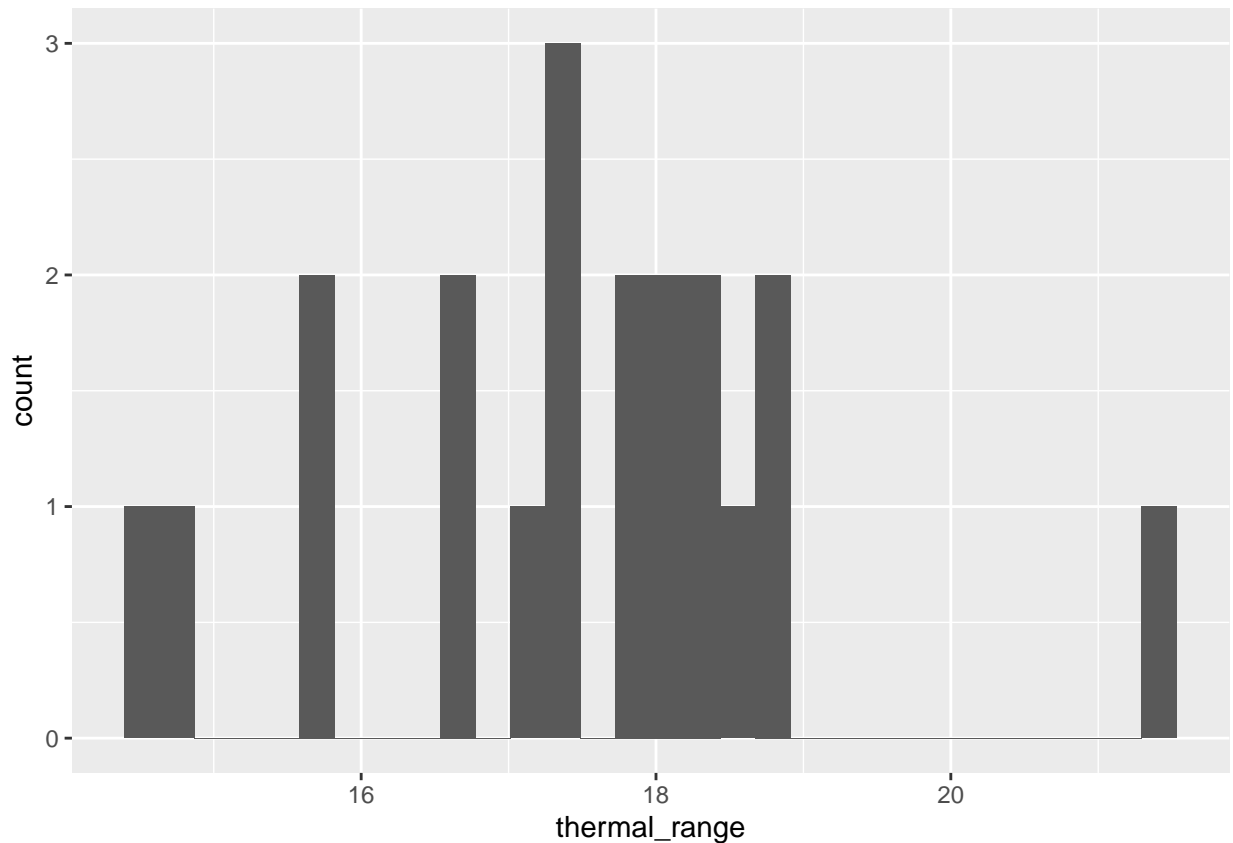## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.

thermal breadth

```r
ggplot(data = tpc_summary_data, aes(x = thermal_range)) + geom_histogram()
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

```
### we also see variation in thermal breadth (here the range of temperatures
### at which the outcrossed line can maintain 50% of its maximum growth rate).
### The thermal breadths range from ~14.5 degrees to ~21.5.
```

Now that we have had a look at the TPC description variables, we can look at some correlations to see if there are any potential relationships among the TPC description variables. I don't really have too many *a priori* hypotheses here. One might be that we would expect a negative relationship between max growth rate and thermal breadth if there is a thermal generalist-specialist trade-off across the outcrossed lines. The other might be that we would expect that outcrossed lines with greater thermal ranges would have lower, lower critical thermal limits and higher upper critical thermal limits.

```
### maximum growth and the other variables

ggplot(data = tpc_summary_data, aes(x = t_opt, y = max_growth)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Thermal Optimum and Max Growth Rate')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```
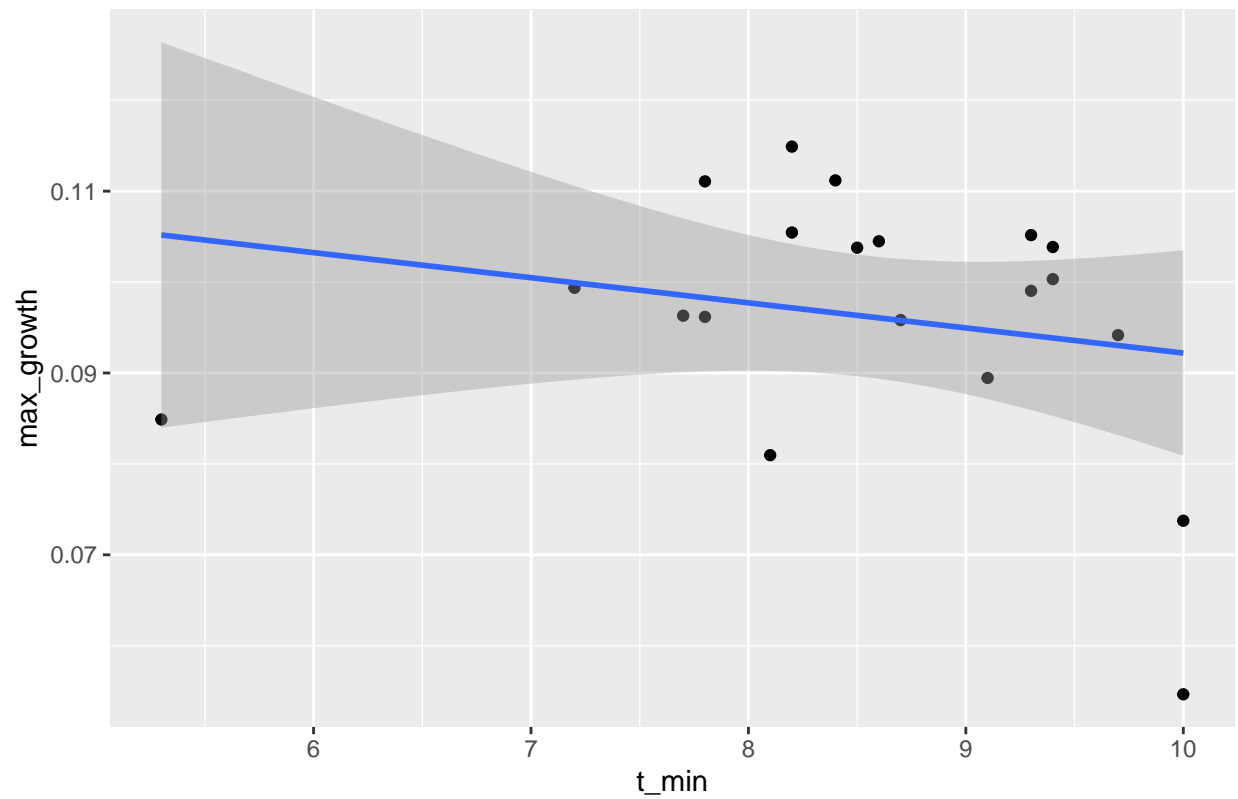
Thermal Optimum and Max Growth Rate
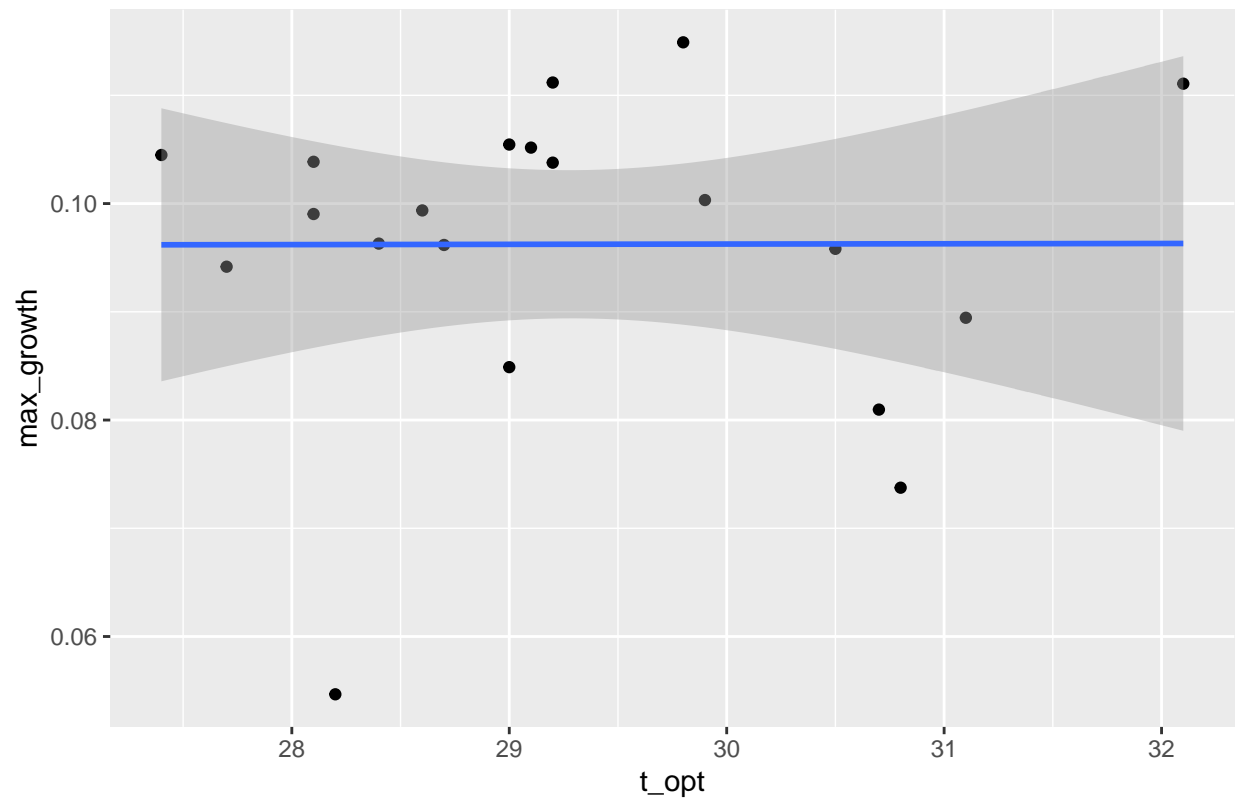

```
### no relationship between maximum growth rate and t_opt

ggplot(data = tpc_summary_data, aes(x = t_min, y = max_growth)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Critical Lower Temperature and Max Growth Rate')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

Critical Lower Temperature and Max Growth Rate
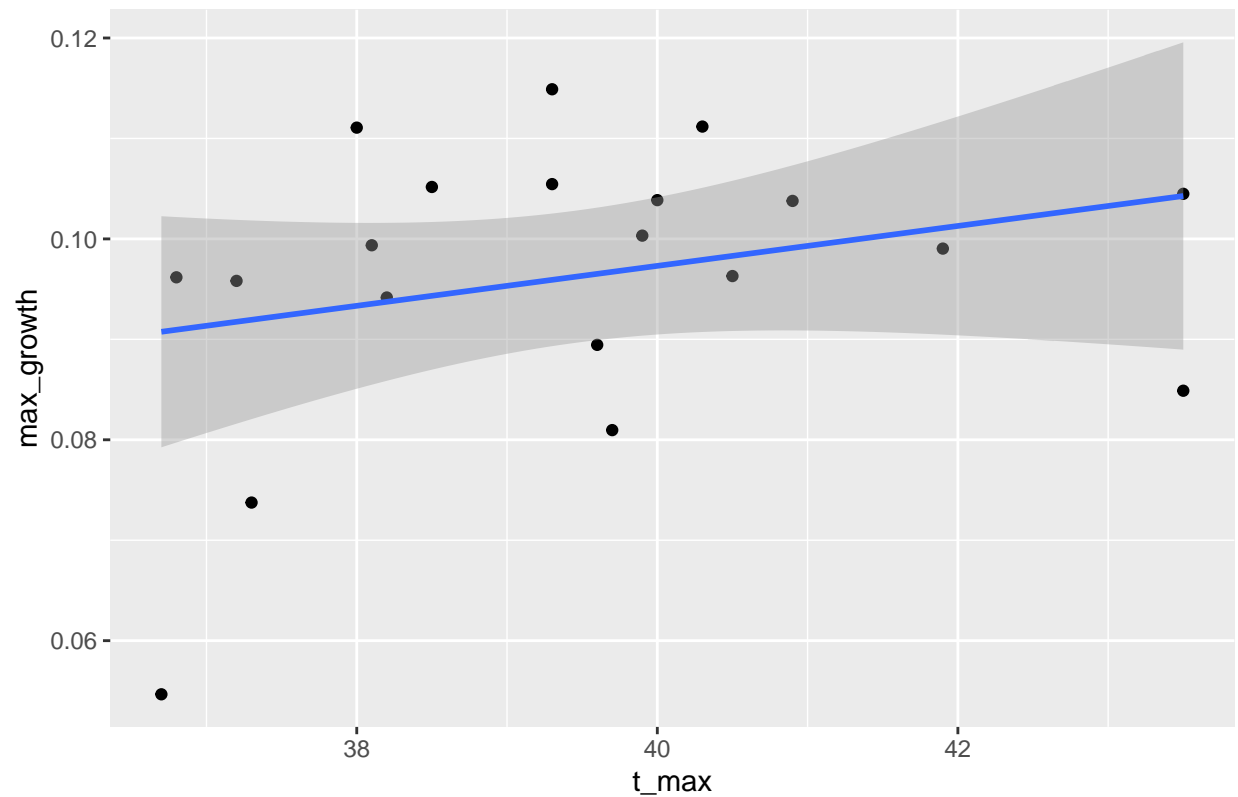


```
### no relationship between maxmimum growth rate and t_min

ggplot(data = tpc_summary_data, aes(x = t_max, y = max_growth)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Critical Upper Temperature and Max Growth Rate')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```
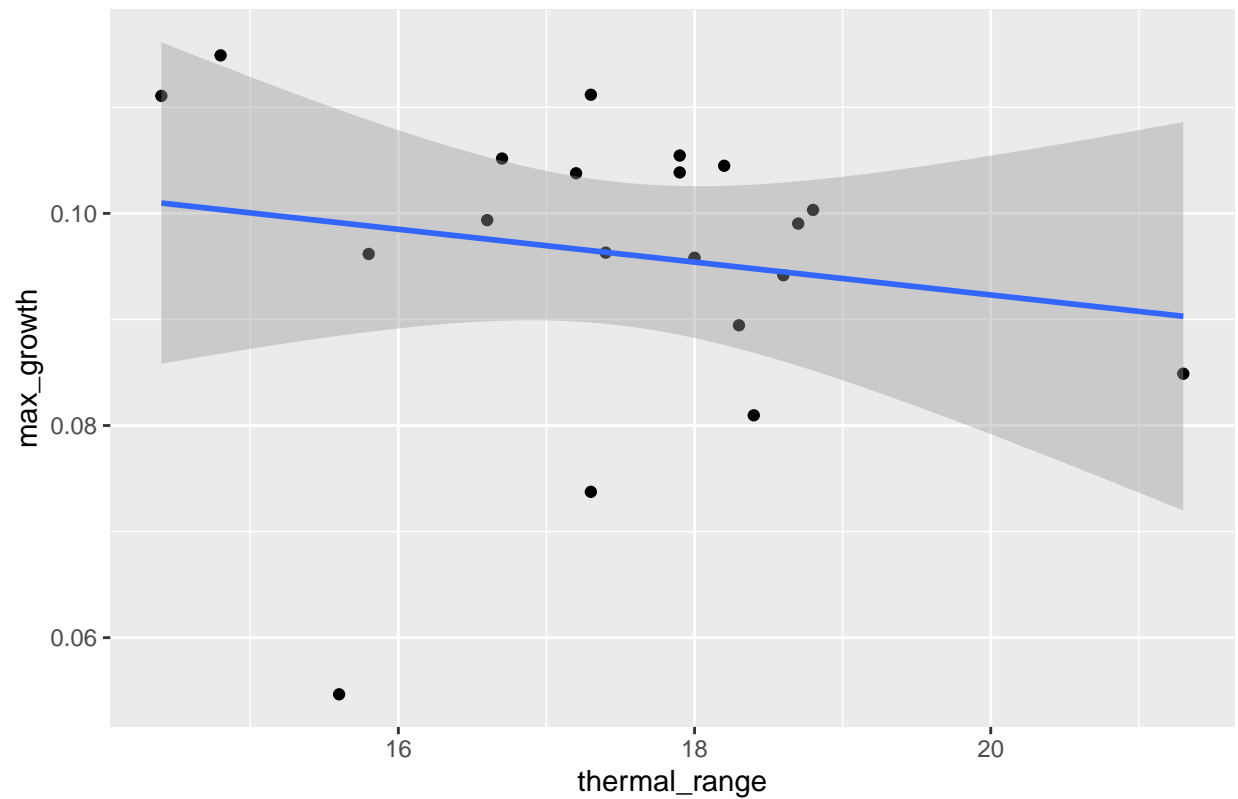
Critical Upper Temperature and Max Growth Rate


```
### no relationship between maximum growth rate and t_max

ggplot(data = tpc_summary_data, aes(x = thermal_range, y = max_growth)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Thermal Breadth and Max Growth Rate')

## `geom_smooth()` using formula = 'y ~ x'
```
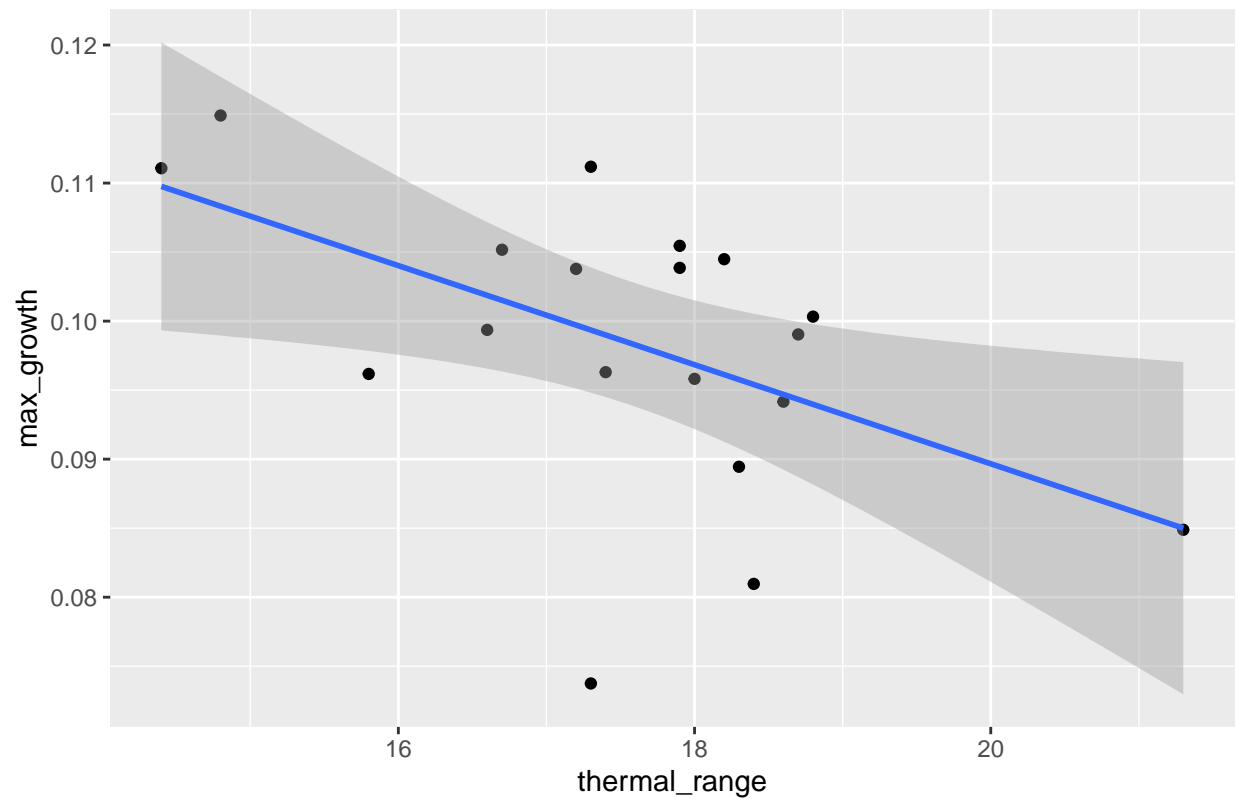
Thermal Breadth and Max Growth Rate


```
### it looks like there might be some influence here of outlier outcrossed line
### G20

ggplot(data = filter(tpc_summary_data, Line != 'G20'), aes(x = thermal_range, y = max_growth)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Thermal Breadth and Max Growth Rate')

## `geom_smooth()` using formula = 'y ~ x'
```

Thermal Breadth and Max Growth Rate

```
### without this outcrossed line, there does appear to be a relationship

### now look at the thermal optimum and the remaining variables

ggplot(data = tpc_summary_data, aes(x = t_min, y = t_opt)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Critical Lower Temperature and Thermal Optimum')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```
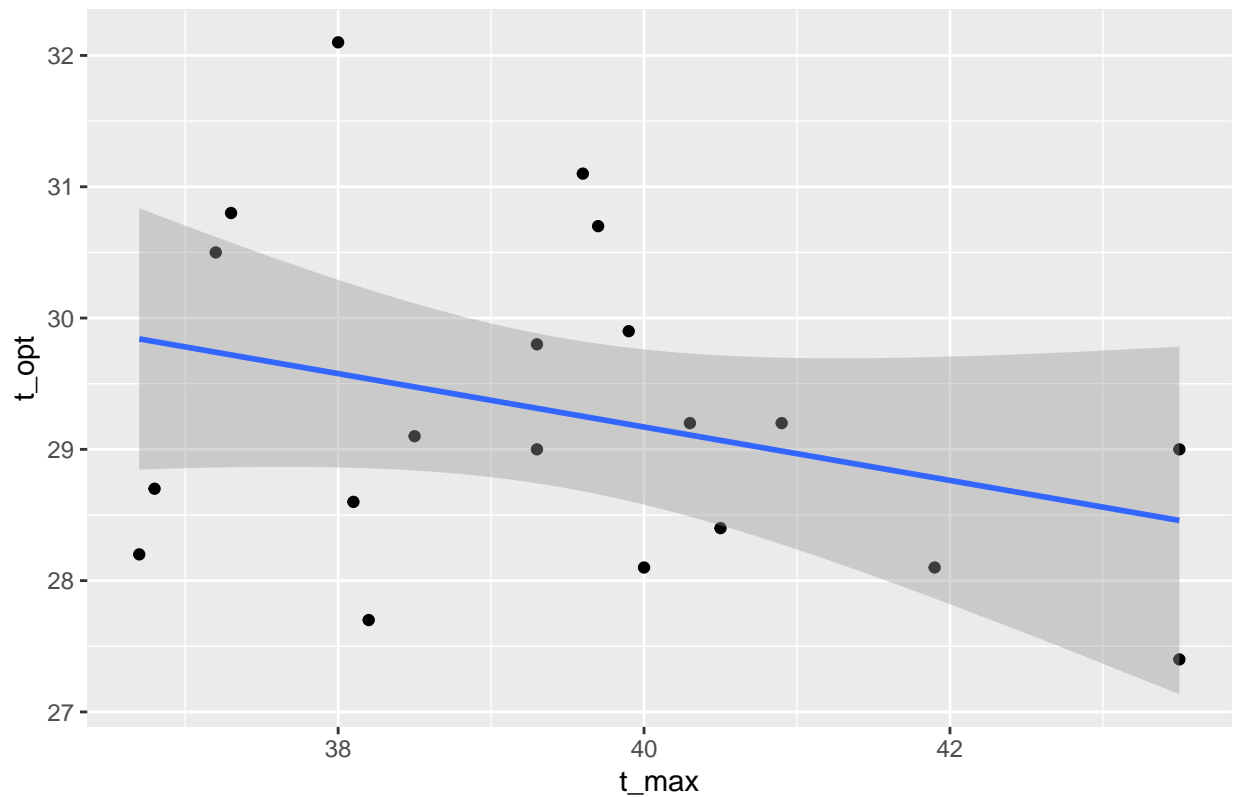
Critical Lower Temperature and Thermal Optimum
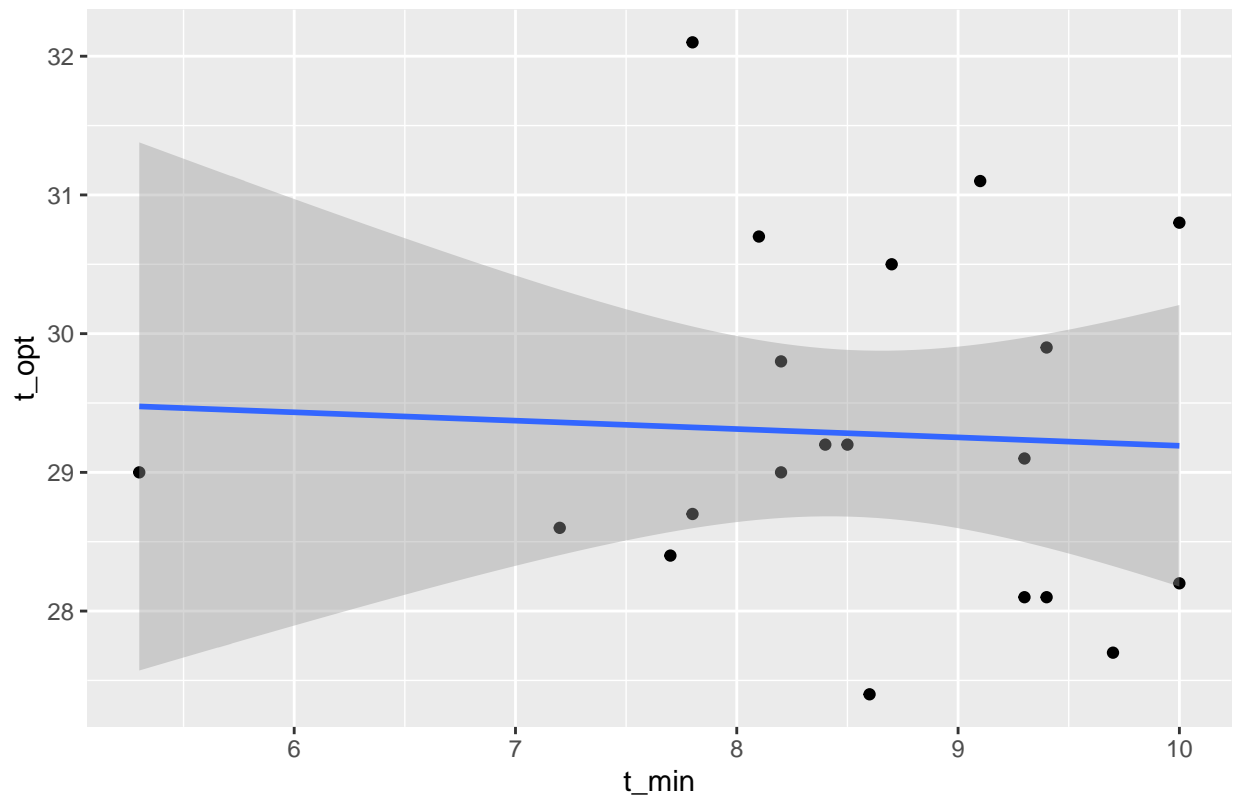

```
### no relationship with the lower critical temperature

ggplot(data = tpc_summary_data, aes(x = t_max, y = t_opt)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Critical Upper Temperature and Thermal Optimum')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

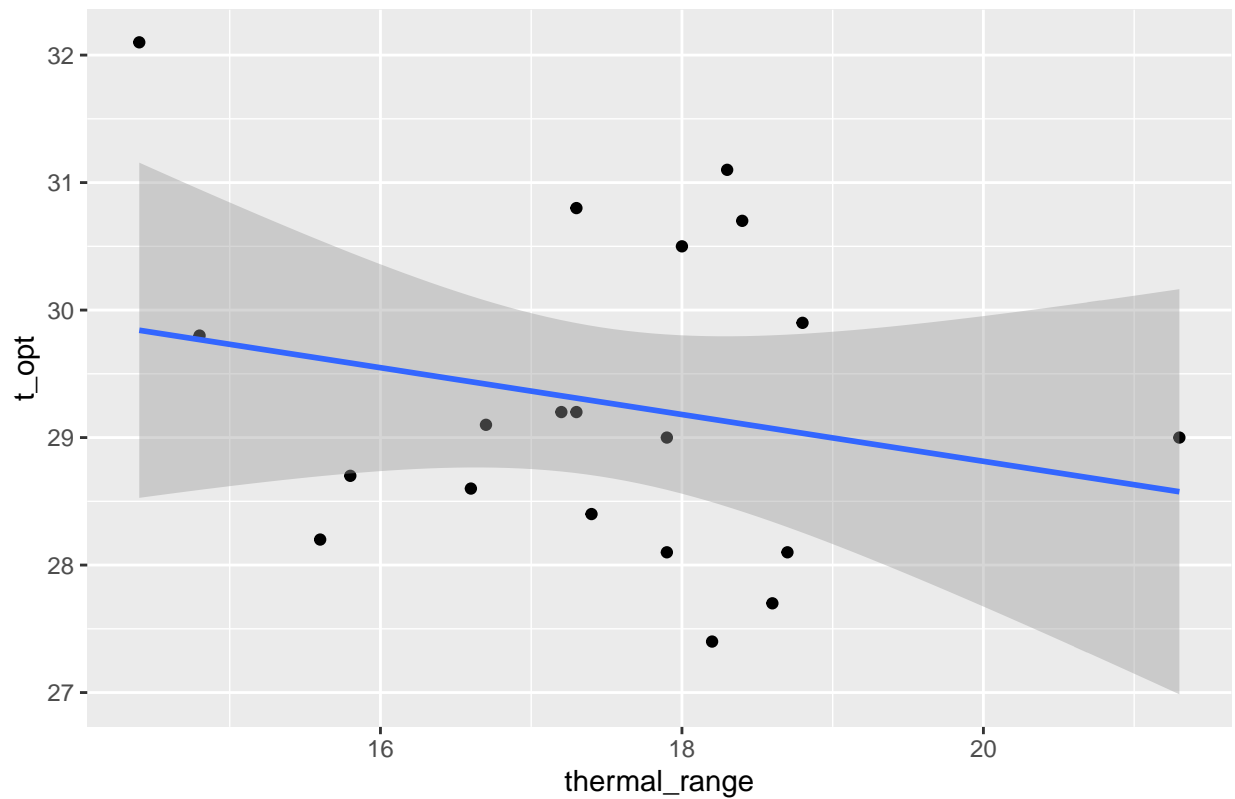## Critical Upper Temperature and Thermal Optimum


```
### no relationship with the upper critical termperature

ggplot(data = tpc_summary_data, aes(x = thermal_range, y = t_opt)) +
  geom_point() + geom_smooth(method = 'lm') + ggtitle('Thermal Breadth and Thermal Optimum')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```
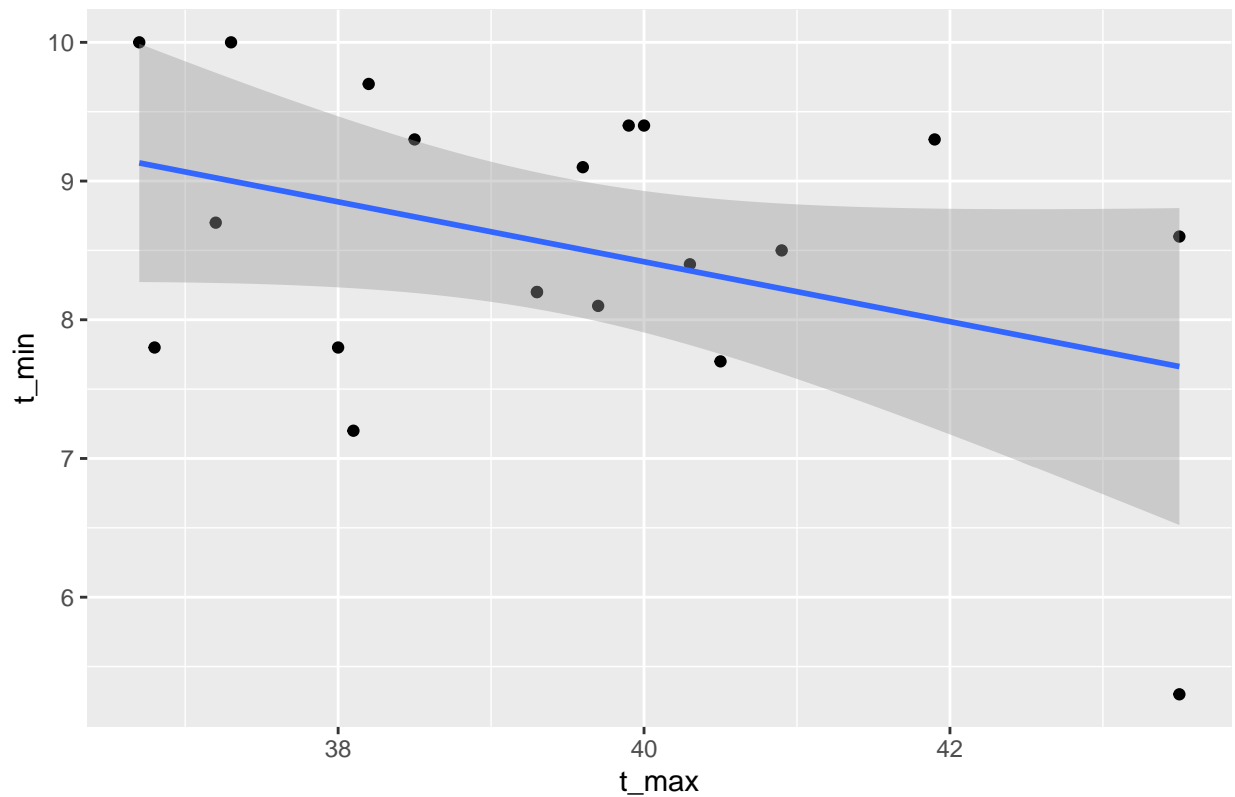
Thermal Breadth and Thermal Optimum


```
### no relationship with thermal range

### look at relationships with t_min and the remaining variables

ggplot(data = tpc_summary_data, aes(x = t_max, y = t_min)) + geom_point() +
  geom_smooth(method = 'lm') + ggtitle('Upper Critical Temperature and Lower Critical Temperature')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

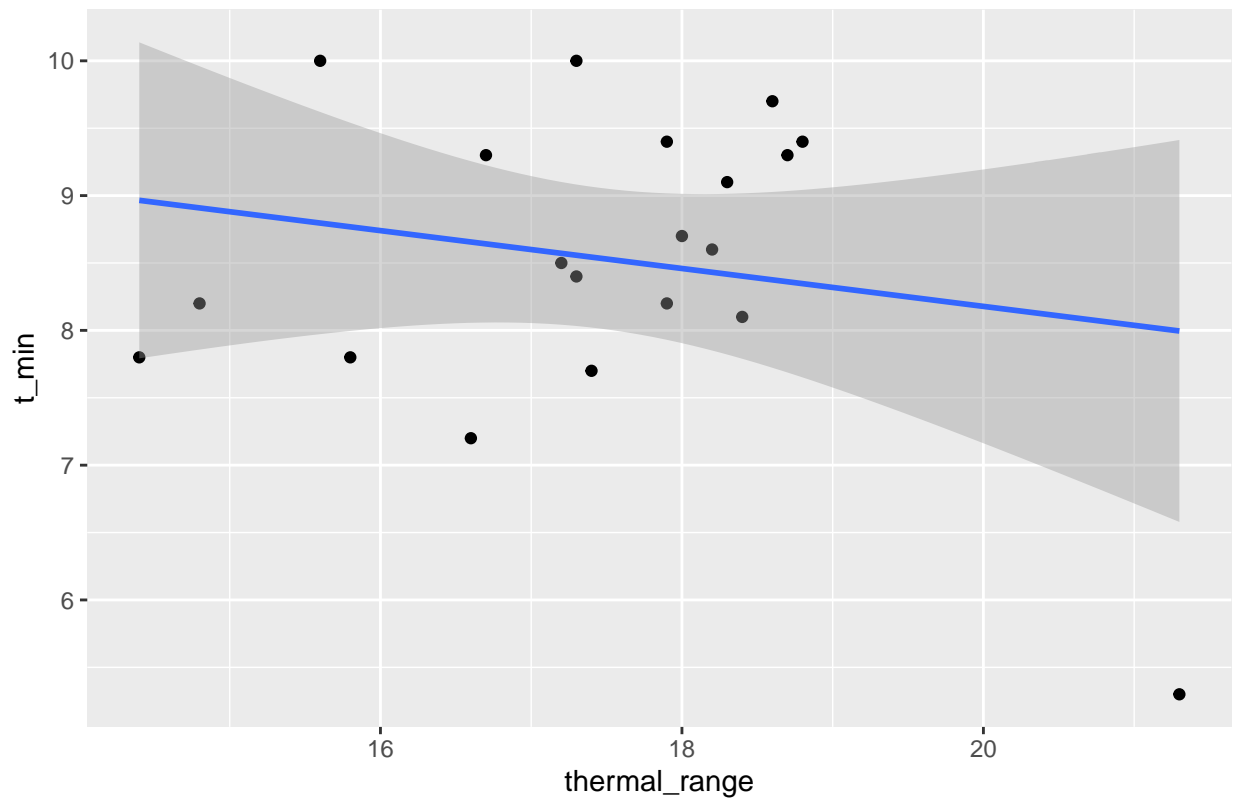## Upper Critical Temperature and Lower Critical Temperature


```
### doesn't really seem to be a relationship with t_max

ggplot(data = tpc_summary_data, aes(x = thermal_range, y = t_min)) + geom_point() +
  geom_smooth(method = 'lm') + ggtitle('Thermal Breadth and Lower Critical Temperature')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

Thermal Breadth and Lower Critical Temperature


```
### no relationship with thermal breadth

### look at the final realtionship between t_max and the thermal range

ggplot(data = tpc_summary_data, aes(x = thermal_range, y = t_max)) + geom_point() +
  geom_smooth(method = 'lm') + ggtitle('Thermal Breadth and Upper Critical Temperature')
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

Thermal Breadth and Upper Critical Temperature
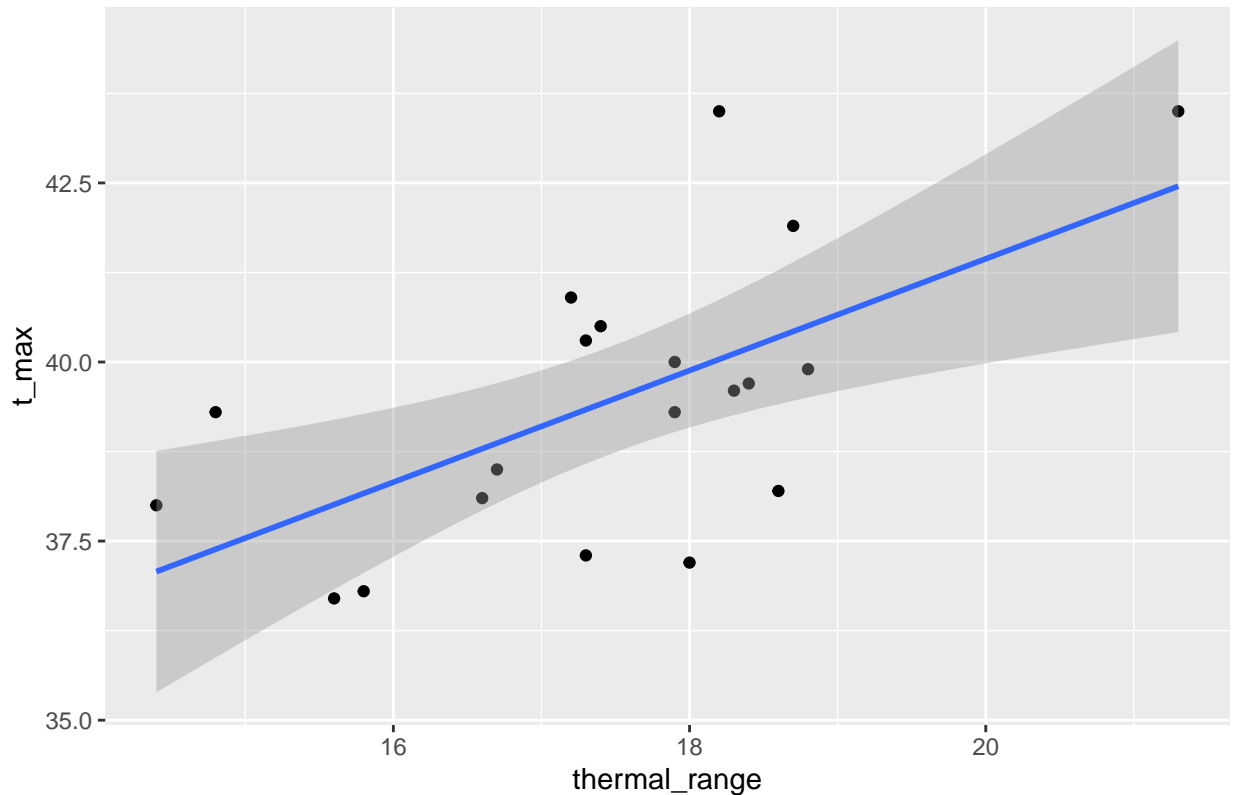
```
### here we do see a relationship
```

So, across all of the TPC descriptors that we got from the GAMs, it seems like there are two potential relationships within the data. First, it looks like we might have some evidence for a bit of a thermal specialist-generalist tradeoff among the outcrossed lines. After removing our one genotype with lower growth rates than everyone else, we see that outcrossed lines with higher maximum growth rates tend to have lower thermal breadths. The other relationship that we see is one between thermal breadth and the upper critical temperature. I think this suggests that what is driving the differences among outcrossed lines in thermal breadth is how well they are able to maintain their growth rates at higher temperatures. We can this somewhat in a plot that has all of the outcrossed lines in one figure.

```
ggplot(data = tpc_data, aes(x = Temperature, y = Growth.Rate.Hours, color = Line)) +
  geom_point() + geom_smooth(method = 'gam', formula = y ~ s(x, k = 6, bs = 'tp'), se = FALSE)
```
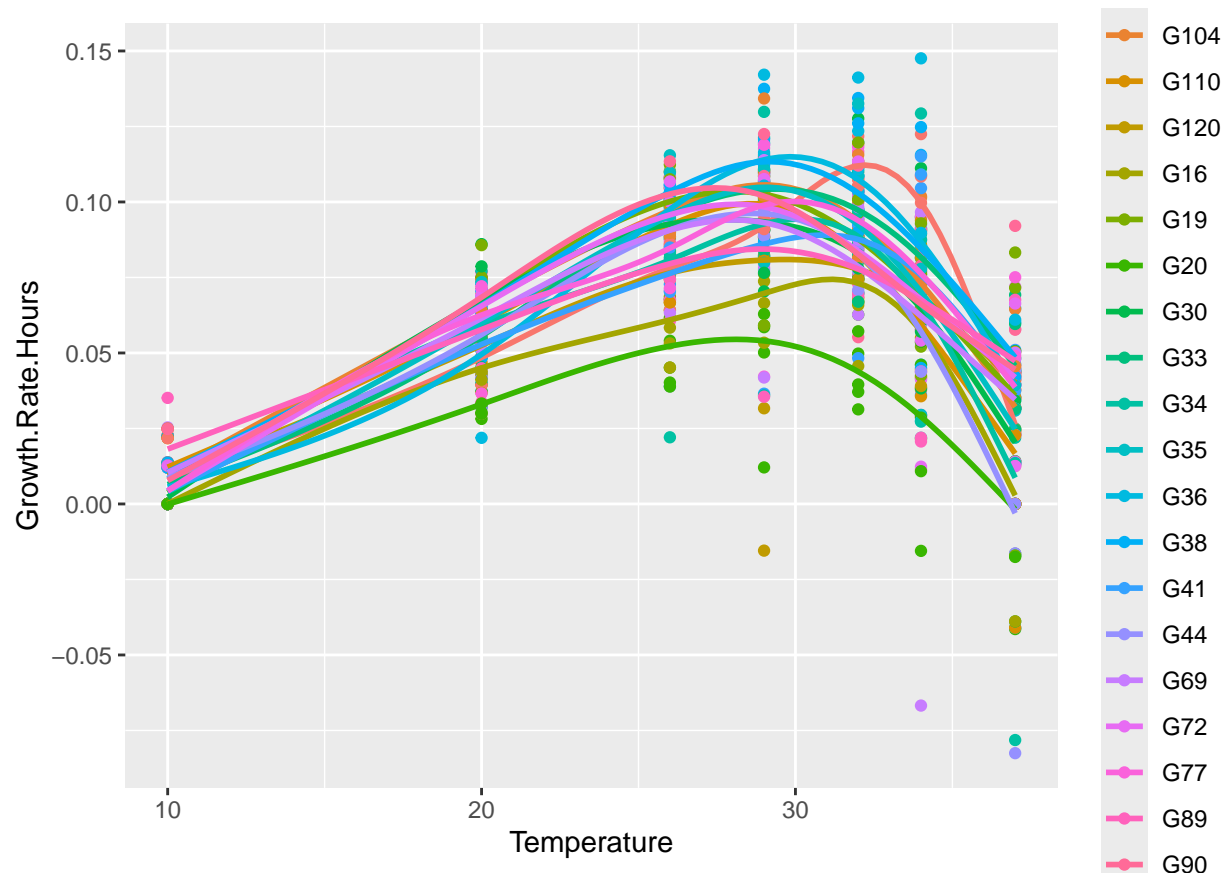
Trying to Link Morphological Plasticity to Thermal Breadth

Now that we have our alternative metric of thermal breadth, we can see if plasticity in morphology and movement is related to