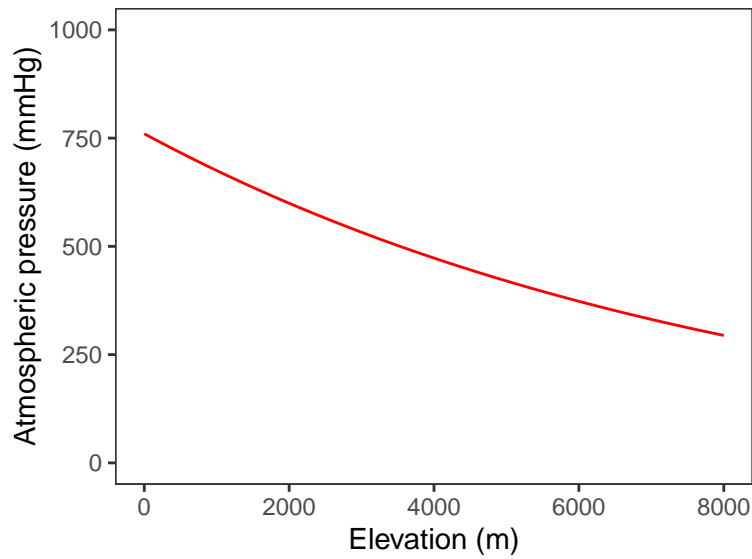


Andean bird blood data exploration

Introduction

What constrains elevational range limits in the absence of an ecotone or obvious biotic constraints? One *abiotic* factor that falls under what Terborgh & Weske (1975) called constraints that vary “continuously and in parallel with the elevational gradient” is the partial pressure of oxygen (PO₂), which declines roughly linearly with altitude and is a strong selective pressure.



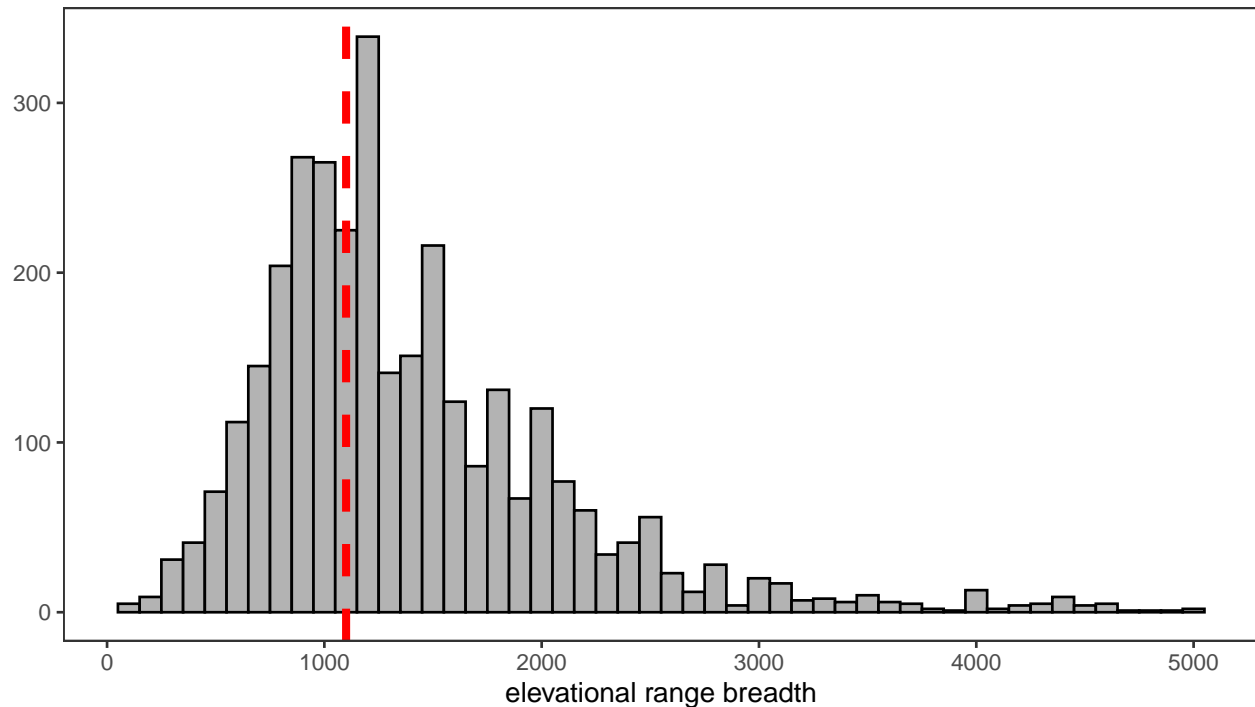
In this analysis, we’re using a large dataset of blood trait values (total blood hemoglobin concentration, haematocrit, or the volume percentage of red blood cells in blood, and MCHC, or mean cellular hemoglobin concentration) to try and understand a little bit better why tropical birds have such narrow elevational ranges. How narrow, you ask? Here’s a visualization of the distribution of elevational range breadth using data from the 3,752 neotropical bird species in Parker et al. 1996 (what Chris calls the “Stotz” data).

```
# load libraries
library(tidyverse, quietly = TRUE)
library(magrittr)
library(ape)
library(phangorn)
library(nlme)
library(phytools)
library(cowplot)
library(mapdata)

# load functions script
source("~/Dropbox/andean_range_limits/scripts/00_functions.R")

# load stotz data
stotz <- read.csv("~/Dropbox/andean_range_limits/data/stotz_elevation_data.csv")
stotz <- cbind.data.frame(stotz$GENUS, stotz$SPECIES,
                          stotz$MIN, stotz$MAX, stotz$MIDPT.ELEV)
colnames(stotz) <- c("genus", "species", "elev_min", "elev_max", "elev_midpt")
stotz$elev_range <- stotz$elev_max - stotz$elev_min
stotz_mod <- stotz[stotz$elev_range > 0,]
```

```
ggplot(stotz_mod, aes(x=elev_range)) +
  geom_histogram(binwidth = 100, color="black",fill="gray70") +
  theme_bw() +
  theme(axis.title.y = element_blank(),
        panel.grid = element_blank()) +
  xlab("elevational range breadth") +
  geom_vline(xintercept = median(stotz_mod$elev_range, na.rm=TRUE),
            linetype="dashed",size=1.5, color="red")
```



Quite narrow, with strong left skew and a median elevational range breadth of 1100 m.

More specifically, we're interested in the following questions:

- 1) Is a species' elevational range breadth associated the rate of change (slope) of its blood trait values a cross elevation?
- 2) Is a species' elevational range breadth associated the total variance of its blood trait values?
- 3) Is the median elevation of a species' range associated with either of these variables?

To begin, we're going to load our data, take a look at it, and make some filtering choices.

```
# load data
blood_df <- read.csv("~/Dropbox/andean_range_limits/data/blood_data.csv",
                    stringsAsFactors = FALSE)

# subset columns of interest
blood_df <- cbind.data.frame(blood_df$Scientific.name,
                             blood_df$Elevation,
                             blood_df$Bursa,
                             blood_df$Mass.for.analysis,
                             blood_df$tHbcorr,
                             blood_df$HctBestEstimate,
                             blood_df$Latitude..degrees.S,
```

```

        blood_df$Latitude.minutes,
        blood_df$Longitude.degrees.W,
        blood_df$Longitude.minutes,
        blood_df$Sex)

colnames(blood_df) <- c("species","elevation","bursa","mass","hb","hct", "lat_degrees",
        "lat_minutes", "long_degrees", "long_minutes", "sex")

# fix longitude minutes error
blood_df$long_minutes <- blood_df$long_minutes %>% as.character() %>% as.numeric()

# fix lat long issue
blood_df$lat <- convert_lat(blood_df)*-1
blood_df$long <- convert_long(blood_df)*-1

# drop sites without locality data
blood_df <- blood_df[!is.na(blood_df$long),]
blood_df <- blood_df[!is.na(blood_df$lat),]

# drop sites beyond plausible limits of sampling
blood_df <- blood_df[blood_df$lat>(-19),]
blood_df <- blood_df[blood_df$long<(-67),]

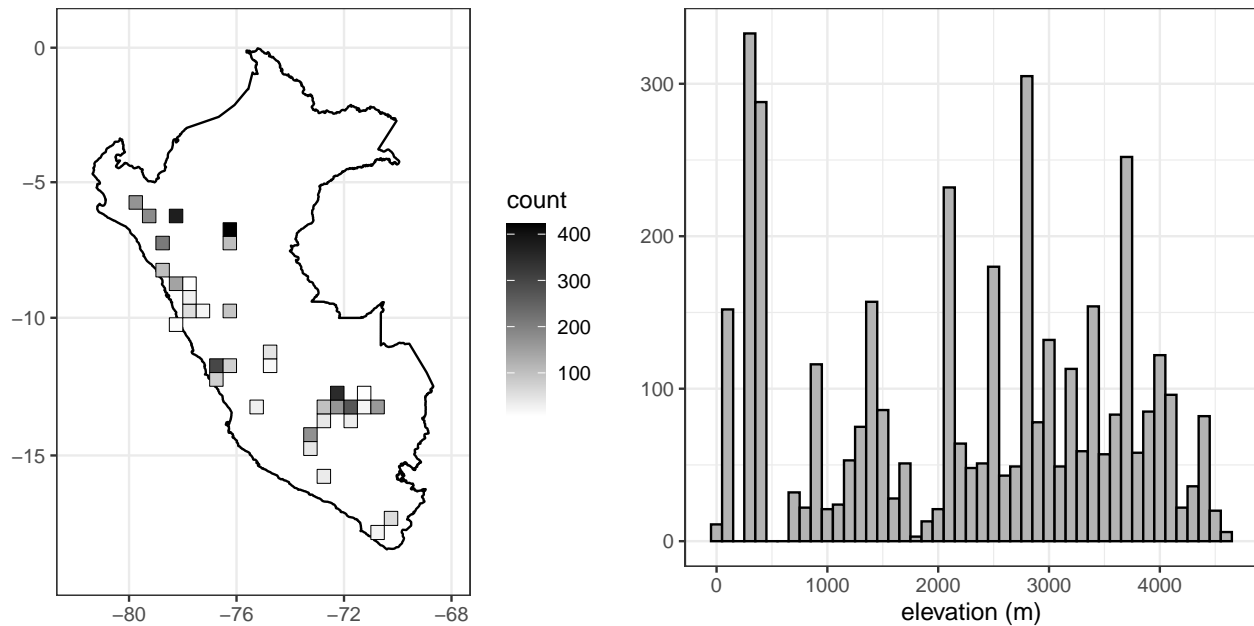
# drop old lat long columns
blood_df <- blood_df[,-c(7:10)]

# factor to character nonsense
blood_df$species <- as.character(blood_df$species)
blood_df$elevation <- as.numeric(as.character(blood_df$elevation))
blood_df$hb <-as.numeric(as.character(blood_df$hb))

# drop all missing records (elevation, haemoglobin, haematocrit)
blood_df <- blood_df[!is.na(blood_df$elevation),]
blood_df <- blood_df[!is.na(blood_df$hb),]
blood_df <- blood_df[!is.na(blood_df$hct),]

```

Before we start, where do these records come from, and how are they distributed across elevation?



Now let's take a look at the head of the dataframe:

```
# simplified column names
head(blood_df)
```

```
##           species elevation      bursa mass hb
## 2    Troglodytes aedon    3750  bursa (mm): 6x4 10.70 8.2
## 4    Myiothlypis coronata  2150 bursa (mm): 4x3 mm 17.19 8.8
## 5    Poospiza hispaniolensis   133    no bursa 11.85 4.3
## 6    Henicorhina leucophrys  2136    no bursa 15.72 7.7
## 8    Mionectes oleagineus  1395    no bursa  9.30 7.1
## 10   Henicorhina leucophrys  2131    no bursa 15.95 7.8
##           hct    sex    lat    long
## 2  0.2083100  male -11.761883 -76.54887
## 4  0.2127072  male  -6.049217 -78.22685
## 5  0.2204536  male  -5.896140 -79.78522
## 6  0.2215403  male  -6.102917 -78.34337
## 8  0.2372900 female -13.055500 -71.54667
## 10 0.2489127  male  -6.103383 -78.34363
```

As you can see, we have columns for species, elevation, presence or absence of a bursa, mass, haemoglobin, haematocrit, collection site longitude and latitude, and sex. As we're also interested in MCHC, let's add a column for that now, using the formula from Campbell and Ellis (thanks, Jessie!)

```
blood_df <- blood_df %>% mutate(hct_percent = hct*100)
blood_df <- blood_df %>% mutate(MCHC_calculated = (hb/hct_percent)*100) # Calculated MCHC
```

Next, let's do some basic filtering, and drop extreme blood parameter values. We'll then drop species with fewer than 8 records:

```
blood_df_sub <- blood_df[which(blood_df$hb >= 14 & blood_df$hb <= 25),]
blood_df_sub <- blood_df_sub[which(blood_df_sub$hct >= 0.35 &
                                   blood_df_sub$hct <= 0.72),]
blood_df_sub <- blood_df_sub[which(blood_df_sub$MCHC_calculated >= 20
                                   & blood_df_sub$hct <= 42),]
```

```

sp_list <- c()
for(i in blood_df_sub$species){
  tmp <- blood_df_sub[blood_df_sub$species==i,]
  records <- nrow(tmp)
  if(records > 7){sp_list[i] <- as.character(tmp$species[1])}
}
sp_list <- as.vector(sp_list)

# subset down to "good" species
blood_df_sub <- blood_df_sub[blood_df_sub$species %in% sp_list,]

length(unique(blood_df$species)) # number of unique species before filtering

## [1] 526

nrow(blood_df) # number of unique records before filtering

## [1] 3962

length(unique(blood_df_sub$species)) # number of unique species after filtering

## [1] 143

nrow(blood_df_sub) # number of unique records after filtering

## [1] 2582

```

We'll now merge these data with the Stotz data. We're using the parameter `all.x=TRUE`, which just means we aren't going to drop blood data if there's not a taxonomy match with the Stotz table.

```

stotz$binomial <- paste0(stotz$genus, " ", stotz$species) # create single col for sp.
blood_df_stotz <- merge(blood_df_sub, stotz, by.x = "species", by.y = "binomial",
                        all.x=TRUE)
head(blood_df_stotz)

```

```

##           species elevation      bursa mass  hb      hct
## 1 Adelomyia melanogenys      2240      not found  NA 21.8 0.6486804
## 2 Adelomyia melanogenys      2111      no bursa 3.78 20.2 0.5595024
## 3 Adelomyia melanogenys      2015 bursa (mm): 5x2 mm 4.21 19.3 0.6050294
## 4 Adelomyia melanogenys      1395      no bursa 3.00 18.6 0.5569300
## 5 Adelomyia melanogenys      2500      no bursa 4.55 18.8 0.6507550
## 6 Adelomyia melanogenys      1227      no bursa 3.20 17.4 0.5566000
##      sex      lat      long hct_percent MCHC_calculated      genus
## 1  male -6.097267 -78.34462   64.86804      33.60669 Adelomyia
## 2  male -6.102800 -78.34302   55.95024      36.10351 Adelomyia
## 3  male -6.098917 -78.33827   60.50294      31.89928 Adelomyia
## 4 female -13.055500 -71.54667   55.69300      33.39737 Adelomyia
## 5  male -7.398033 -78.77827   65.07550      28.88952 Adelomyia
## 6 female -13.039167 -71.52933   55.66000      31.26123 Adelomyia
##      species.y elev_min elev_max elev_midpt elev_range
## 1 melanogenys      1100      2300      1200      1200
## 2 melanogenys      1100      2300      1200      1200
## 3 melanogenys      1100      2300      1200      1200
## 4 melanogenys      1100      2300      1200      1200
## 5 melanogenys      1100      2300      1200      1200
## 6 melanogenys      1100      2300      1200      1200

```

Which species failed to pick up elevational range data?

```
blood_df_stotz[is.na(blood_df_stotz$elev_min),]$species %>% unique() %>% length()
```

```
## [1] 40
```

Bummer. Let's take a look them:

```
missing <- blood_df_stotz[is.na(blood_df_stotz$elev_min),]$species %>% unique()
print(missing)
```

```
## [1] "Agelaiocercus kingii"      "Anairetes nigrocristatus"
## [3] "Anairetes reguloides"     "Arremon assimilis"
## [5] "Arremon brunneinucha"     "Cinclodes albiventris"
## [7] "Cranioleuca antisiensis"  "Diglossa brunneiventris"
## [9] "Diglossa humeralis"      "Diglossa mystacalis"
## [11] "Diglossa sittoides"      "Doryfera ludovicae"
## [13] "Furnarius leucopus"      "Glaucis hirsutus"
## [15] "Heliangelus micraster"    "Isleria hauxwelli"
## [17] "Lepidothrix coeruleocapilla" "Lepidothrix coronata"
## [19] "Myiothlypis chrysogaster" "Myiothlypis coronata"
## [21] "Myiothlypis luteoviridis" "Myiothlypis nigrocristata"
## [23] "Oreotrochilus estella"   "Orochelidon murina"
## [25] "Pheugopedius eisenmanni" "Pipraeidea bonariensis"
## [27] "Premnornis guttuliger"   "Psilopsiagon aurifrons"
## [29] "Pyrrhomyias cinnamomeus" "Spinus magellanicus"
## [31] "Spinus sp."              "Spinus uropygialis"
## [33] "Synallaxis azarae"       "Systellura longirostris"
## [35] "Thamnophilus bernardi"   "Tiaris obscurus"
## [37] "Troglodytes aedon"       "Troglodytes solstitialis"
## [39] "Vireo olivaceus"         "Xiphorhynchus elegans"
```

All can be explained by taxonomic changes and / or typos. I've gone directly to a copy of the spreadsheet and made the taxonomy of the Stotz data match to avoid errors from manually entering elevations. We'll now merge again, the revised data:

```
stotz_rev <- read.csv("~/Dropbox/andean_range_limits/data/stotz_elevation_data_rev.csv")
stotz_rev <- cbind.data.frame(stotz_rev$GENUS, stotz_rev$SPECIES,
                             stotz_rev$MIN, stotz_rev$MAX, stotz_rev$MIDPT.ELEV)
colnames(stotz_rev) <- c("genus", "species", "elev_min", "elev_max", "elev_midpt")
stotz_rev$binomial <- paste0(stotz_rev$genus, " ", stotz_rev$species)
blood_df_stotz <- merge(blood_df_sub, stotz_rev, by.x = "species", by.y = "binomial",
                        all.x=TRUE)
```

Anything still missing?

```
missing <- blood_df_stotz[is.na(blood_df_stotz$elev_min),]$species %>% unique()
length(missing)
```

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

Yep—let's see what it is.

```
blood_df_stotz[is.na(blood_df_stotz$elev_min),]$species %>% unique()
```

```
## [1] "Spinus sp."
```

Unidentified siskins—we'll drop them.

```
blood_df_stotz <- blood_df_stotz[!blood_df_stotz$species=="Spinus sp.",]
```

We'll next apply a filter to drop probable outliers that could have a disproportionate influence on slope estimation, using my custom `outliers_cooks()` function: points with Cook's D of $4/n$, or with a Cook's D of $3.5/n$ if a bursa is present.

```
pass_hb <- outliers_cooks(blood_df_stotz, "hb", 4, 3.5)
pass_hct <- outliers_cooks(blood_df_stotz, "hct", 4, 3.5)
pass_mchc <- outliers_cooks(blood_df_stotz, "MCHC_calculated", 4, 3.5)
blood_df_stotz_pass <- intersect(pass_hb, pass_hct, pass_mchc) # get overlapping spp. set
length(unique(blood_df_stotz_pass$species)) # number of unique species
```

```
## [1] 141
```

```
nrow(blood_df_stotz_pass) # retained records
```

```
## [1] 2511
```

For calculating variance down the road, we also need to records based on their relative position in a species' elevational range.

```
vardf <- list()
for(i in unique(blood_df_stotz_pass$species)){
  tmp <- blood_df_stotz_pass[blood_df_stotz_pass$species==i,]
  if(tmp$elev_max > max(tmp$elevation)){elev_max <- unique(tmp$elev_max)}
  if(tmp$elev_max < max(tmp$elevation)){elev_max <- max(tmp$elevation)}
  if(tmp$elev_min < min(tmp$elevation)){elev_min <- unique(tmp$elev_min)}
  if(tmp$elev_min > min(tmp$elevation)){elev_min <- min(tmp$elevation)}
  elev_range <- elev_max - elev_min
  tmp$range_position <- 1-((elev_max-tmp$elevation)/elev_range)
  tmp$edge_distance <- 0.5-abs(tmp$range_position-0.5)
  tmp$elev_range <- elev_range
  tmp$elev_min <- elev_min
  tmp$elev_max <- elev_max
  bin_number <- elev_range %/% 100
  tmp$binID <- cut(tmp$elevation, bin_number)
  vardf[[i]] <- tmp
}
blood_df_stotz_pass <- do.call(rbind, vardf)
```

We're now going to apply a final set of filters to the data (using the function `outliers_limits()`), removing all species with fewer than 2 unique elevational records at least 200 m apart, and fewer than 2 elevational records in the first and last quantile of their range. (This will create the dataframe we'll use for our analysis of the slope of blood parameters—for variance, we'll begin working with a separate dataframe, as we aren't concerned with how much of the range these data span.)

```
blood_df_slope <- outliers_limits(blood_df_stotz_pass, min_sample=2, min_limit=2, 200)
length(unique(blood_df_slope$species)) # number of unique species
```

```
## [1] 101
```

```
nrow(blood_df_slope) # number of unique records
```

```
## [1] 2054
```

Let's visualize these slope data (printing large .pdfs elsewhere):

```
multispecies_hb <- ggplot(blood_df_slope, aes(x=elevation, y=hb)) +
  facet_wrap(~species,scales="free") +
  geom_point(pch=21,stroke=1,aes(color=species),show.legend = FALSE) +
  geom_smooth(method="lm",se=FALSE,linetype="dashed",color="black") +
```

```

theme_bw() +
xlab("elevation")+
ylab("hb")

pdf("~/Dropbox/andean_range_limits/figures/multispecies_hb.pdf",width=24,height=20)
multispecies_hb
dev.off()

multispecies_hct <- ggplot(blood_df_slope, aes(x=elevation, y=hct)) +
  facet_wrap(~species,scales="free") +
  geom_point(pch=21,stroke=1,aes(color=species),show.legend = FALSE) +
  geom_smooth(method="lm",se=FALSE,linetype="dashed",color="black") +
  theme_bw() +
  xlab("elevation")+
  ylab("hct")

pdf("~/Dropbox/andean_range_limits/figures/multispecies_hct.pdf",width=24,height=20)
multispecies_hct
dev.off()

multispecies_mchc <- ggplot(blood_df_slope, aes(x=elevation, y=MCHC_calculated)) +
  facet_wrap(~species,scales="free") +
  geom_point(pch=21,stroke=1,aes(color=species),show.legend = FALSE) +
  geom_smooth(method="lm",se=FALSE,linetype="dashed",color="black") +
  theme_bw() +
  xlab("elevation")+
  ylab("mchc")

pdf("~/Dropbox/andean_range_limits/figures/multispecies_mchc.pdf",width=24,height=20)
multispecies_mchc
dev.off()

```

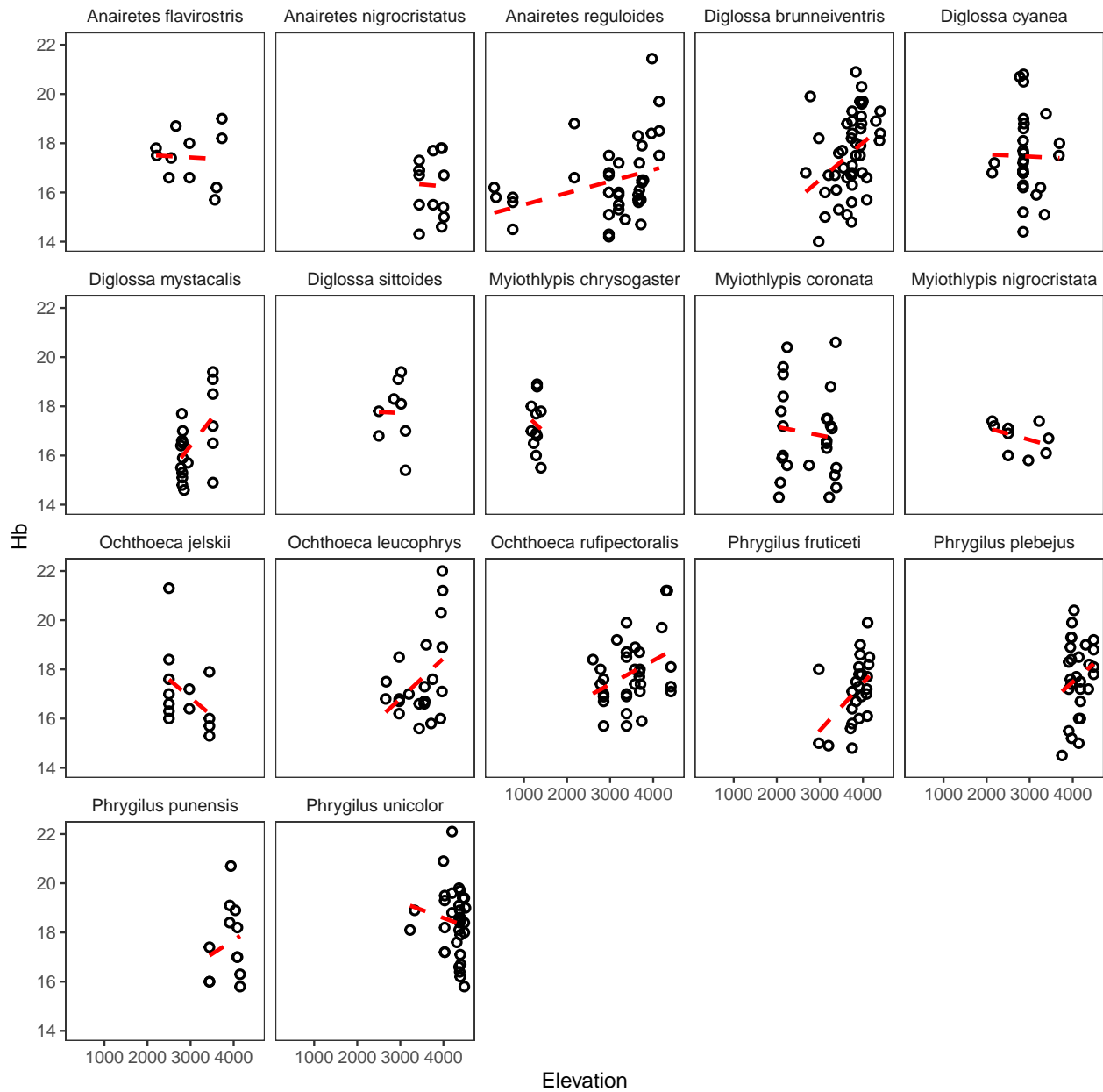
Let's take a quick look at patterns in genera with more than two species. First, we'll subset the dataframe.

```

genus_list <- c()
for(i in unique(blood_df_slope$genus)){
  tmp <- blood_df_slope[blood_df_slope$genus==i,]
  species_num <- unique(tmp$species) %>% length()
  if(species_num>2){genus_list[i] <- as.character(tmp$genus[1])}
}
blood_df_genus <- blood_df_slope[blood_df_slope$genus %in% genus_list,]

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

```

Next, let's calculate the slope of haemoglobin and haematocrit—and the average slope angle for different species—using the `blood_slope()` function I've written.

```
# calculate elevational range and median
blood_df_slope$elev_range <- blood_df_slope$elev_max - blood_df_slope$elev_min

# run function
slope_df <- blood_slope(blood_df_slope)
head(slope_df)
```

##	species	sample_size	unique_elevations	slope_hb
## 1	Adelomyia melanogenys	30	16	1.554223e-03
## 2	Aglaeactis castelnaudii	17	12	-2.202816e-03
## 3	Aglaeactis cupripennis	13	7	3.744381e-06
## 4	Agelaiocercus kingii	15	9	1.184448e-03

```
## 5      Amazilia amazilia      34      7 -9.594754e-04
## 6 Ampelion rubrocristatus      16      9  5.221640e-04
##      r2_hb      error_hb      slope_hct      r2_hct      error_hct
## 1 1.709932e-01 0.0006467317 3.975732e-05 0.12970363 1.946236e-05
## 2 1.234594e-01 0.0015155020 -2.951376e-05 0.03299974 4.125124e-05
## 3 2.109109e-06 0.0007773806 1.641468e-05 0.10743456 1.426541e-05
## 4 2.198264e-01 0.0006188710 2.795618e-05 0.20969694 1.505244e-05
## 5 5.449914e-03 0.0022912757 -6.393651e-05 0.01980297 7.951799e-05
## 6 3.841515e-02 0.0006982090 2.152376e-05 0.13751467 1.440640e-05
##      slope_mchc      r2_mchc      error_mchc      elev_range      median_elevation
## 1 0.0004985206 0.01139720 0.0008774371      1525      1862.5
## 2 -0.0020265795 0.06881669 0.0019248138      1478      3839.0
## 3 -0.0009168391 0.06479625 0.0010502072      1800      3400.0
## 4 0.0005339982 0.03303814 0.0008012444      1558      2079.0
## 5 0.0016833373 0.01808102 0.0021929188      1200      600.0
## 6 -0.0003169037 0.01683834 0.0006471816      1550      3275.0
##      mass
## 1 3.853214
## 2 6.463529
## 3 6.946154
## 4 4.758571
## 5 4.742727
## 6 56.255000
```

We'll create a separate dataframe of variance values, using data from the 100m elevational bin with the most observations for any given species with the `blood_variance()` function:

```
# run function
variance_df <- blood_variance(blood_df_stotz_pass)
variance_df <- variance_df[complete.cases(variance_df),]
head(variance_df)
```

```
##      species      sample_size      unique_elevations      range_position
## 1 Adelomyia melanogenys      30      16      0.67131148
## 2 Aglaeactis castelnaudii      17      12      0.82814614
## 3 Aglaeactis cupripennis      13      7      0.52166667
## 4 Aglaiocercus kingii      15      9      0.05263158
## 5 Amazilia amazilia      34      7      0.30378788
## 6 Amazilia viridicauda      9      2      0.97529691
##      edge_distance      variance_hb      variance_hct      variance_mchc      elev_range
## 1 0.32868852      4.446964      0.0025939529      4.979525      1525
## 2 0.17185386      2.000000      0.0031037275      2.221577      1478
## 3 0.47833333      2.957000      0.0007435695      4.723372      1800
## 4 0.05263158      1.039524      0.0008029394      3.093178      1558
## 5 0.30378788      1.412121      0.0015749576      1.010319      1200
## 6 0.02470309      0.782381      0.0010762322      5.178181      2105
##      median_elevation      mass
## 1      1862.5      3.853214
## 2      3839.0      6.463529
## 3      3400.0      6.946154
## 4      2079.0      4.758571
## 5      600.0      4.742727
## 6      1952.5      5.566667
```

Next, we'll load the Jetz supertree so we can control for phylogeny, and then subset the tree down to only those species we have slope data for:

```

supertree <- read.tree("~/Dropbox/andean_range_limits/data/birds_mcc.tre")
supertree$species <- supertree$tip.label
slope_df$species <- sub(" ", "_", slope_df$species)

```

There are a few taxonomic conflicts, which I'll resolve here:

```

slope_df[grepl("Arremon_assimilis", slope_df$species),]$species <- "Arremon_torquatus"
slope_df[grepl("Myiothlypis_coronata", slope_df$species),]$species <- "Basileuterus_coronatus"
slope_df[grepl("Orochelidon_murina", slope_df$species),]$species <- "Notiochelidon_murina"
slope_df[grepl("Spinus_magellanicus", slope_df$species),]$species <- "Carduelis_magellanica"
slope_df[grepl("Spinus_uropygialis", slope_df$species),]$species <- "Carduelis_uropygialis"
slope_df[grepl("Systellura_longirostris", slope_df$species),]$species <- "Caprimulgus_longirostris"
slope_df[grepl("Agelaiocercus_kingii", slope_df$species),]$species <- "Agelaiocercus_kingi"
slope_df[grepl("Myiothlypis_chrysogaster", slope_df$species),]$species <- "Basileuterus_chrysogaster"
slope_df[grepl("Myiothlypis_nigrocristata", slope_df$species),]$species <- "Basileuterus_nigrocristatus"
slope_df[grepl("Pipraeidea_bonariensis", slope_df$species),]$species <- "Thraupis_bonariensis"
slope_df[grepl("Premnornis_guttuliger", slope_df$species),]$species <- "Premnornis_guttuligera"

# prune tree
slope.tree <- keep.tip(supertree, slope_df$species)

```

And then the same thing for our variance data:

```

variance_df$species <- sub(" ", "_", variance_df$species)
variance_df[grepl("Myiothlypis_coronata", variance_df$species),]$species <- "Basileuterus_coronatus"
variance_df[grepl("Myiothlypis_luteoviridis", variance_df$species),]$species <- "Basileuterus_luteoviridis"
variance_df[grepl("Orochelidon_murina", variance_df$species),]$species <- "Notiochelidon_murina"
variance_df[grepl("Spinus_magellanicus", variance_df$species),]$species <- "Carduelis_magellanica"
variance_df[grepl("Spinus_uropygialis", variance_df$species),]$species <- "Carduelis_uropygialis"
variance_df[grepl("Agelaiocercus_kingii", variance_df$species),]$species <- "Agelaiocercus_kingi"
variance_df[grepl("Pipraeidea_bonariensis", variance_df$species),]$species <- "Thraupis_bonariensis"
variance_df[grepl("Pheugopedius_eisenmanni", variance_df$species),]$species <- "Thryothorus_eisenmanni"
variance_df[grepl("Thamnophilus_bernardi", variance_df$species),]$species <- "Sakesphorus_bernardi"
variance.tree <- keep.tip(supertree, variance_df$species)

```

Now, we'll attempt to fit basic phylogenetic least squares (PGLS) models to our data using Liam Revell's phytools. For now, we're going to ignore possible sex-based confounds, and analyze only the subset of variance estimates we have matching slope estimates for. Specifically, we're going to fit models attempting to predict the rate of change across elevation in Hb, Hct, and MCHC using a species elevational range breadth, median elevational range, and mass as predictors, while weighting the dependent variable by the standard error of the linear regression that generated it, and controlling for phylogeny.

```

# scale variables to be same order of magnitude
slope_df$mass <- slope_df$mass/1000
slope_df$elev_range <- slope_df$elev_range/1000000
slope_df$median_elevation <- slope_df$median_elevation/1000000
slope_df$slope_hct <- slope_df$slope_hct*10

# assign rownames
species1 <- as.vector(as.character(slope_df$species))
rownames(slope_df) <- species1

# get SE of hb measurements
SE1 <- setNames(slope_df$error_hb, slope.tree$tip.label)

# fit model, hb

```

```
fit_hb <- pgls.SEy(slope_hb ~ elev_range + median_elevation + mass,
                  data=slope_df, se=SE1, tree=slope.tree, method="ML")
fit_hb.tidy <- broom.mixed::tidy.gls(fit_hb)
fit_hb.tidy
```

```
## # A tibble: 4 x 5
##   term                estimate std.error statistic p.value
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>
## 1 (Intercept)        0.000345  0.00359    0.0962  0.924
## 2 elev_range        -0.0963    0.281    -0.342   0.733
## 3 median_elevation  0.764      0.369     2.07   0.0411
## 4 mass             -0.0384    0.0281    -1.37   0.175
```

```
# get SE of hct measurements
```

```
SE2 <-setNames(slope_df$error_hct, slope.tree$tip.label)
```

```
# fit model, hct
```

```
fit_hct <- pgls.SEy(slope_hct ~ elev_range + median_elevation + mass,
                  data=slope_df, se=SE2, tree=slope.tree, method="ML")
fit_hct.tidy <- broom.mixed::tidy.gls(fit_hct)
fit_hct.tidy
```

```
## # A tibble: 4 x 5
##   term                estimate std.error statistic p.value
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>
## 1 (Intercept)        0.000900  0.00106     0.849  0.398
## 2 elev_range        -0.201     0.0827    -2.42   0.0172
## 3 median_elevation  0.0489    0.109     0.449  0.654
## 4 mass             -0.0126    0.00829   -1.52   0.133
```

```
# get SE of mchc measurements
```

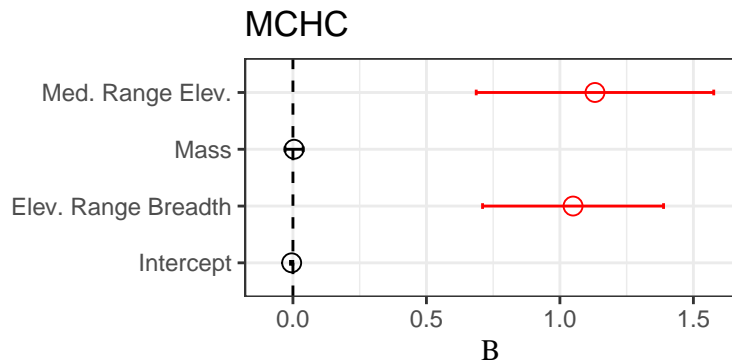
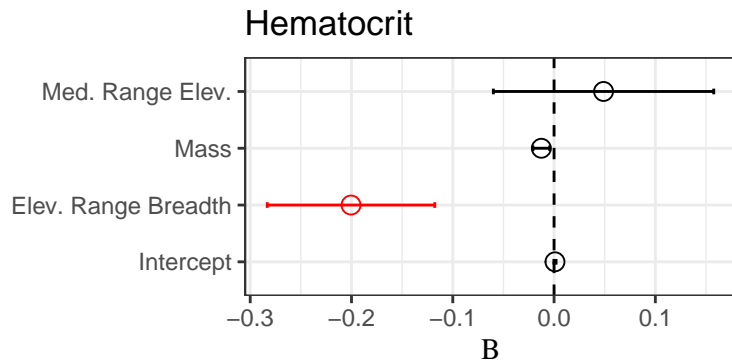
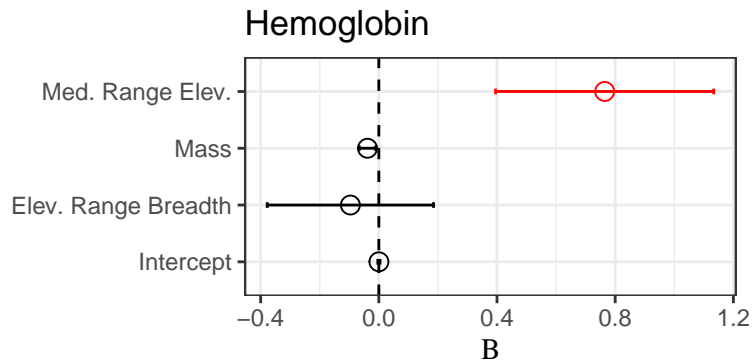
```
SE3 <-setNames(slope_df$error_mchc, slope.tree$tip.label)
```

```
# fit model, mchc
```

```
fit_mchc <- pgls.SEy(slope_mchc ~ elev_range + median_elevation + mass,
                  data=slope_df, se=SE3, tree=slope.tree, method="ML")
fit_mchc.tidy <- broom.mixed::tidy.gls(fit_mchc)
fit_mchc.tidy
```

```
## # A tibble: 4 x 5
##   term                estimate std.error statistic p.value
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>
## 1 (Intercept)       -0.00483  0.00432    -1.12  0.266
## 2 elev_range         1.05      0.339     3.10  0.00257
## 3 median_elevation  1.13      0.445     2.54  0.0126
## 4 mass              0.00433  0.0338     0.128 0.898
```

I printed out tables of coefficients there, but since that's a lot to take in, let's visualize these results:



TL;DR: Median range elevation is a significant positive predictor of the slope of Hb and MCHC; elevational range breadth is a significant negative predictor of Hct and significant positive predictor of MCHC.

Next, let's run models attempting to describe variance in these blood parameters. This time, we're going to use the number of unique elevations we have data for as our weighting criterion:

```
# assign rownames
species2 <- as.vector(as.character(variance_df$species))
rownames(variance_df) <- species2

# get variances in correct order of magnitude; Hct fine
variance_df$variance_hb <- variance_df$variance_hb/1000
variance_df$variance_mchc <- variance_df$variance_mchc/1000
variance_df$mass <- variance_df$mass/1000
variance_df$elev_range <- variance_df$elev_range/1000000
variance_df$median_elevation <- variance_df$median_elevation/1000000
variance_df$range_position <- variance_df$range_position/100
variance_df$edge_distance <- variance_df$edge_distance/100
```

```

# get SE of hb measurements
SE_elev <- setNames(variance_df$unique_elevations, variance.tree$tip.label)

# fit model, hb
fit_vhb <- pglS.SEy(variance_hb ~ elev_range + median_elevation + range_position + edge_distance + mass)
fit_vhb.tidy <- broom.mixed::tidy.gls(fit_vhb)
fit_vhb.tidy

## # A tibble: 6 x 5
##   term                estimate std.error statistic p.value
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>
## 1 (Intercept)        0.00206  0.00118     1.74  0.0861
## 2 elev_range         0.286    0.116     2.47  0.0159
## 3 median_elevation -0.408    0.138    -2.95  0.00419
## 4 range_position     0.0382   0.0425     0.898 0.372
## 5 edge_distance      0.157    0.0797     1.97  0.0520
## 6 mass              -0.0246   0.0155    -1.59  0.115

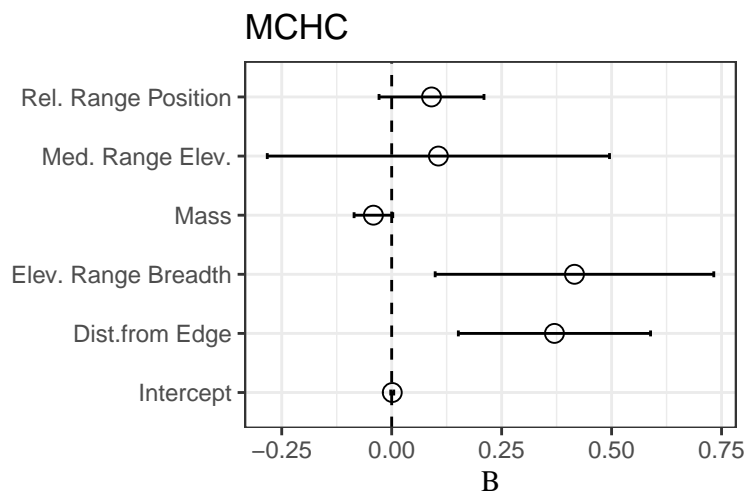
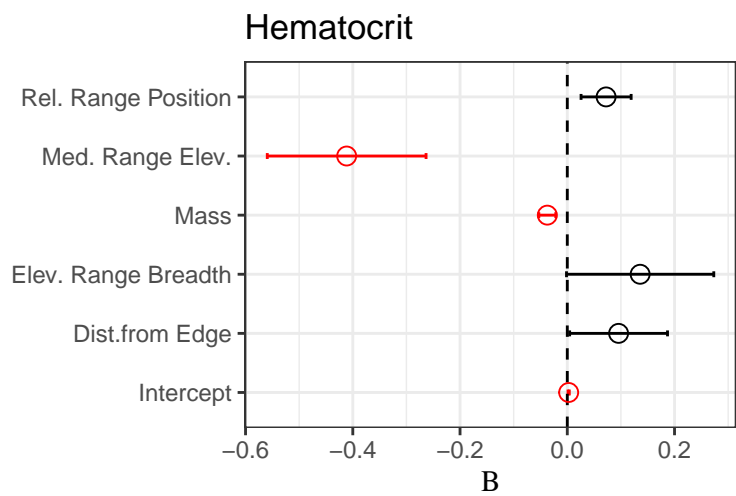
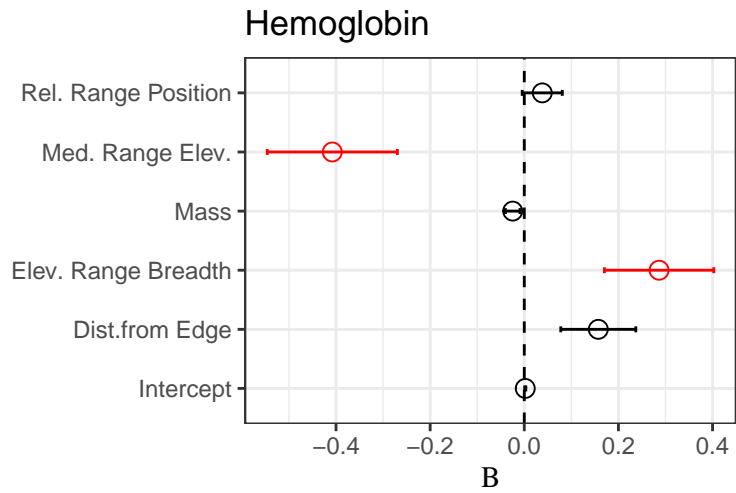
# fit model, hct
fit_vhct <- pglS.SEy(variance_hct ~ elev_range + median_elevation + range_position + edge_distance + mass)
fit_vhct.tidy <- broom.mixed::tidy.gls(fit_vhct)
fit_vhct.tidy

## # A tibble: 6 x 5
##   term                estimate std.error statistic p.value
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>
## 1 (Intercept)        0.00260  0.00118     2.21  0.0300
## 2 elev_range         0.136    0.137     0.989 0.326
## 3 median_elevation -0.411    0.148    -2.78  0.00683
## 4 range_position     0.0724   0.0467     1.55  0.125
## 5 edge_distance      0.0957   0.0913     1.05  0.298
## 6 mass              -0.0373   0.0158    -2.36  0.0207

# fit model, hct
fit_vmchc <- pglS.SEy(variance_mchc ~ elev_range + median_elevation + range_position + edge_distance + mass)
fit_vmchc.tidy <- broom.mixed::tidy.gls(fit_vmchc)
fit_vmchc.tidy

## # A tibble: 6 x 5
##   term                estimate std.error statistic p.value
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>
## 1 (Intercept)        0.00131  0.00338     0.388 0.699
## 2 elev_range         0.416    0.317     1.31  0.193
## 3 median_elevation  0.106    0.389     0.273 0.786
## 4 range_position     0.0906   0.119     0.759 0.450
## 5 edge_distance      0.370    0.218     1.69  0.0943
## 6 mass              -0.0415   0.0439    -0.945 0.348

```



Median range elevation is a significant negative predictor of variance in Hb and Hct; Elevational range breadth is a significant positive predictor of variance in Hb; mass is a significant negative predictor of variance in Hct. There are no good predictors of MCHC.