Independent Project Statistical Analysis

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Introduction

Thermophilic microorganisms, capable of living at extreme temperatures in habitats like geothermal springs, offer unique insights into the evolution of microbial life and life's potential in harsh environments. (Damer and Deamer, 2020). While microbial evolution has been studied across different environments (Nguyen et al., 2021), the evolution of population genomics and community-level dynamics within geothermal springs remain poorly understood. The Great Boiling Spring (GBS) in Gerlach, Nevada, provides an ideal system to explore these dynamics, as its microbial communities are structured by temperature gradients and micro-environments such as sediment and the water column (Cole et al., 2013). Thermoflexus hugenholtzii, a dominant thermophile across sites in the Great Boiling Spring (GBS) system in Gerlach, Nevada, provides an ideal location to investigate fine-scale genetic variation across environmental gradients. This study uses metagenomic sequencing and population genomics tools to examine the microevolutionary dynamics of T. hugenholtzii, focusing on single codon variations (SCVs) within genes critical to core cellular functions.

Given that temperature is a key factor shaping microbial communities in GBS (Cole et al., 2013; Kees et al., 2022), this study aims to examine how microbial community composition and population genomics evolve in response to seasonal temperature variations. A high-quality metagenome-assembled genome (MAG) generated from the most complete, least contaminated sample (June Site B) served as the reference genome. SCV frequencies across five sample sites were analyzed in relation to site-specific and seasonal temperature fluctuations. This approach allows for the exploration of how environmental variables influence genetic variation within microbial populations, contributing to a broader understanding of microbial genome plasticity and ecological adaptation in extreme ecosystems.

Research Question

How do temperature and site-specific environmental conditions influence single codon variation (SCV) in the genes rpoB, gyrB, and recA of Thermoflexus hugenholtzii across spatial and seasonal gradients in Great Boiling Spring?

Specific Aims

Aim 1: To quantify and compare SCV frequencies in three conserved genes of T. hugenholtzii across five metagenomic samples and assess their correlation with temperature and sampling site.

Dataset Description

Statistical Approach

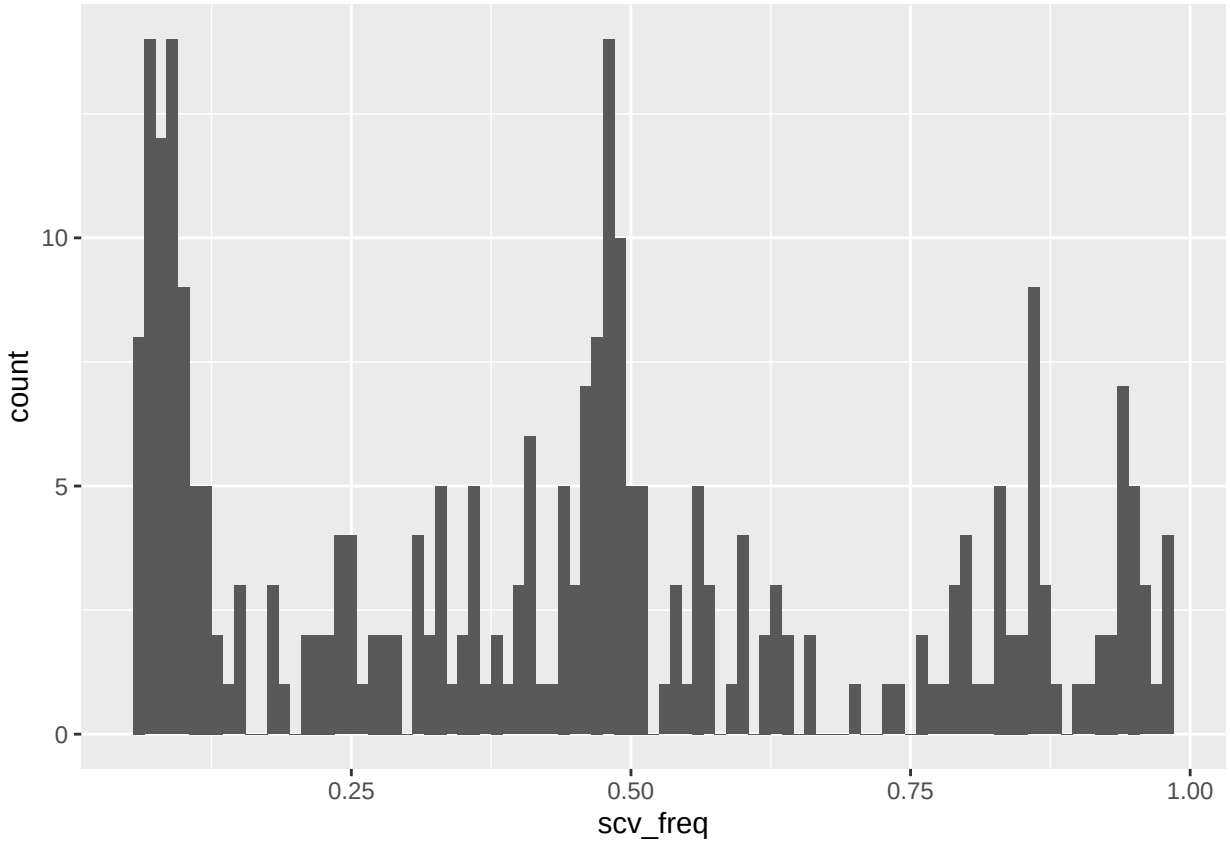
I first checked the distribution of the data for normality to figure out which statistical method was best. All three genes showed non-normality on there distributions. I used a generalized linear mixed model (GLMM) with a beta distribution to analyze single codon variants (SCVs) frequency, where the frequencies all fell between 0 and 1. The model included fixed effects for Site and Month and their interactions on SCV

frequencies within the rpoB, recA, and gyrB genes. This allowed for me to assess both main effects and gene-specific environmental interactions. The model was fit using the glmmTMB package in R.

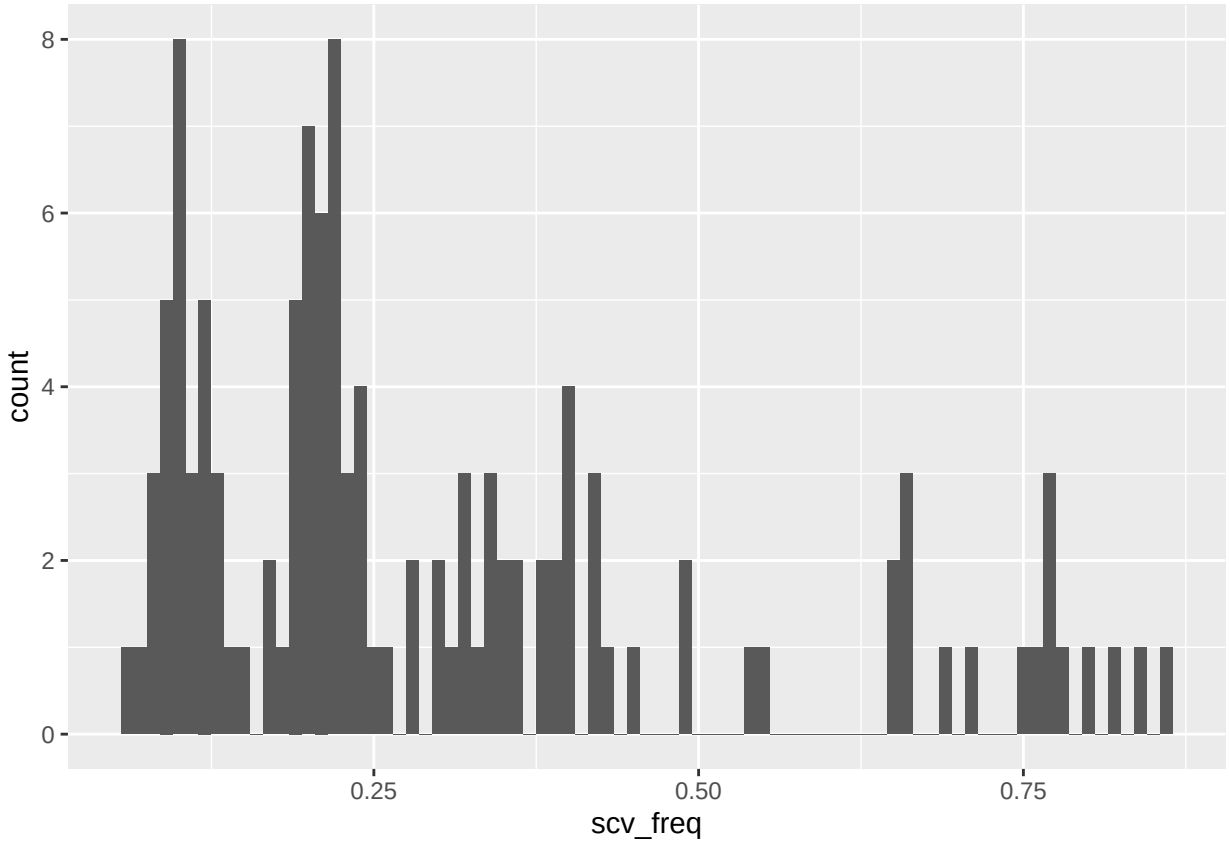
```
# Load SCV dataset
scv <- read.csv("../Data/Thermo_Hugo_SCV.csv")
#str(scv)
```

```
library(tidyverse)
library(viridis)
```

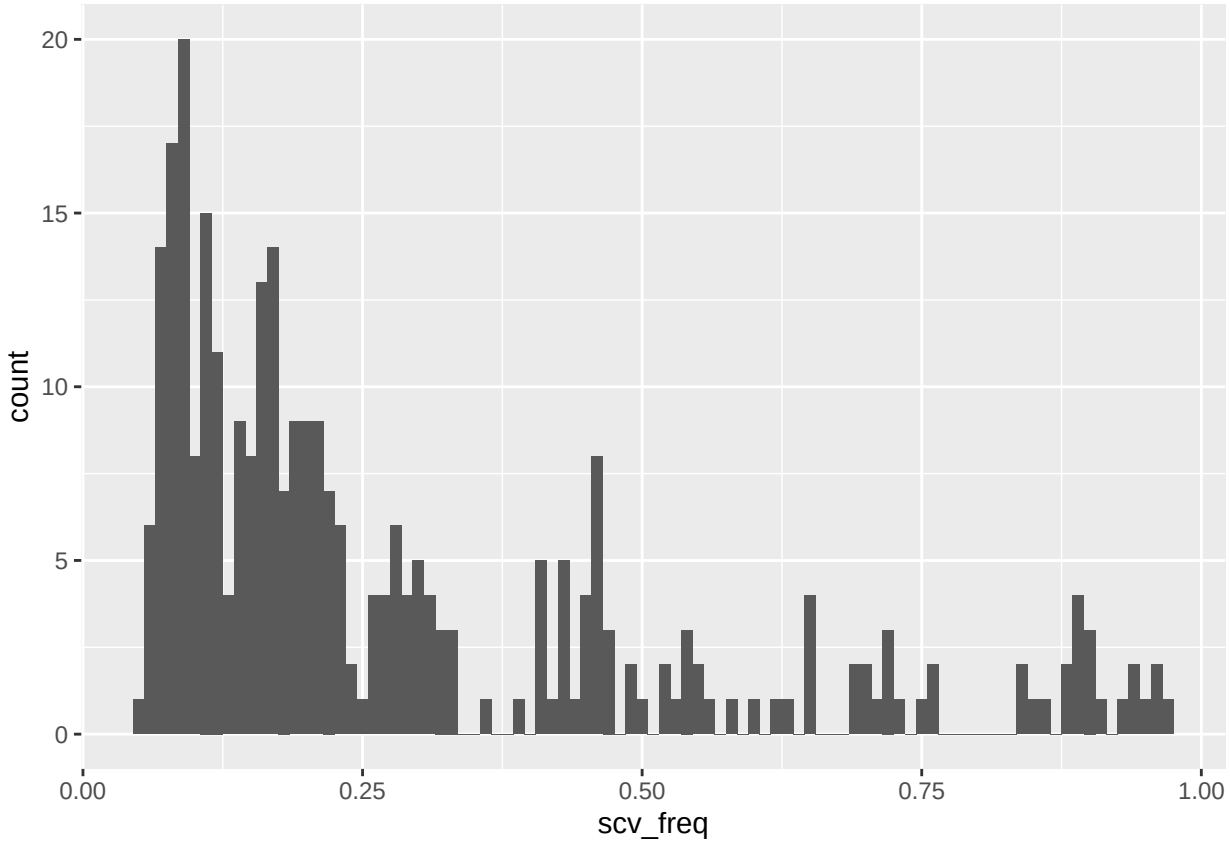
```
# Check Normality
rpoB_scv_data <- subset(scv, Gene_ID == "rpoB")
rpoB_scv_hist <- ggplot(rpoB_scv_data, aes(x = scv_freq)) +
  geom_histogram(binwidth = 0.01)
rpoB_scv_hist
```




```
recA_scv_data <- subset(scv, Gene_ID == "recA")
recA_scv_hist <- ggplot(recA_scv_data, aes(x = scv_freq)) +
  geom_histogram(binwidth = 0.01)
recA_scv_hist
```




```
gyrB_scv_data <- subset(scv, Gene_ID == "gyrB")
gyrB_scv_hist <- ggplot(gyrB_scv_data, aes(x = scv_freq)) +
  geom_histogram(binwidth = 0.01)
gyrB_scv_hist
```




```
# All non-normal
```

Hypotheses

Null hypothesis (H0): Site and Month have no effect on SCV frequency in any of the genes tested.

Alternative hypothesis (HA): SCV frequency varies significantly by Site and/or Month for at least one gene.

```
library(glmmTMB)
```

```
## Warning: package 'glmmTMB' was built under R version 4.4.1
```

```
# SCV

# SCV Frequency GLM
SCV_GLMM <- glmmTMB(
  scv_freq ~ Gene_ID * Site + Gene_ID * Month,
  data = scv,
  family = beta_family()
)

# Add Model Predictions
scv$predicted <- predict(SCV_GLMM, type = "response")
```

```r
# 3. Clean data
scv_clean <- scv %>%
  filter(!is.na(Month)) %>%
  mutate(
    Month = factor(trimws(as.character(Month))),  # Clean text and convert to factor
    SiteMonth = interaction(Site, Month, sep = "_")  # Create grouping variable
  )

# Order of x-axis
scv_clean$SiteMonth <- factor(scv_clean$SiteMonth,
  levels = c("A_February", "B_February", "A_June", "B_June", "C_June")
)
```

```r
# Plot
SCV_Plot <-ggplot(scv_clean, aes(x = SiteMonth, y = scv_freq)) +
  geom_jitter(aes(color = Site), width = 0.2, alpha = 0.6) +
  stat_summary(aes(y = predicted), fun = mean, geom = "point",
               shape = 18, size = 3, color = "black") +
  facet_wrap(~ Gene_ID) +
  scale_color_viridis_d(option = "C") +
  labs(
    title = "SCV Frequency Variance in Thermoflexus hugenholtzii",
    x = "Site and Month",
    y = "SCV Frequency",
    color = "Site"
  ) +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1),
        legend.position = "right")
# Summary Statistics
SCV_Plot
```

```r
summary(SCV_GLMM)
```

```
##  Family: beta  ( logit )
## Formula:          scv_freq ~ Gene_ID * Site + Gene_ID * Month
## Data: scv
##
##       AIC       BIC    logLik -2*log(L)  df.resid
##    -382.3    -323.0     204.1    -408.3       691
##
##
## Dispersion parameter for beta family (): 3.85
##
## Conditional model:
##                       Estimate Std. Error z value Pr(>|z|)
## (Intercept)           -0.73741    0.16495  -4.470 7.81e-06 ***
## Gene_IDrecA           -0.09963    0.21636  -0.460 0.645173
## Gene_IDrpoB            0.20630    0.25127   0.821 0.411627
## SiteB                 -0.29438    0.13802  -2.133 0.032930 *
## SiteC                  0.32127    0.13462   2.387 0.017009 *
## MonthJune             -0.06783    0.15130  -0.448 0.653916
```

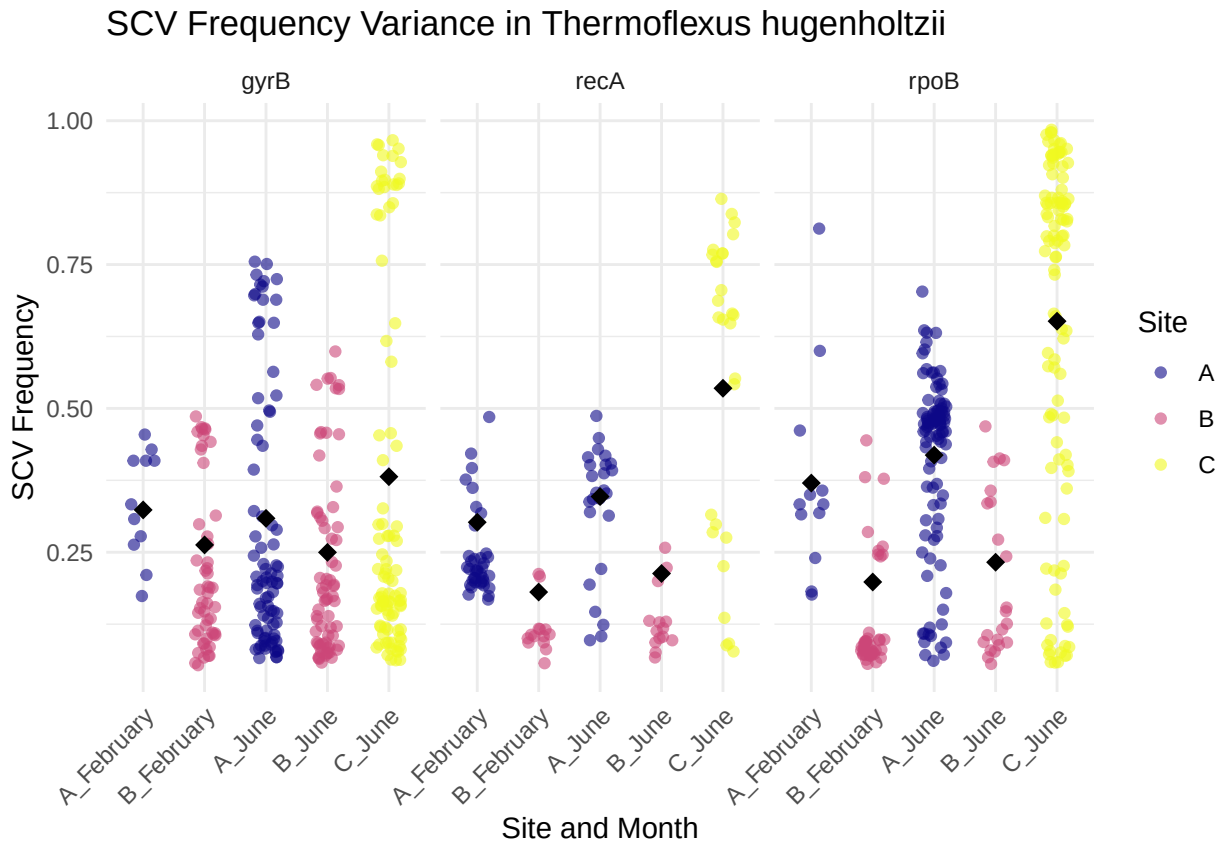


Figure 1: Observed and predicted SCV frequencies by site and sampling month across three genes (gyrB, recA, and rpoB) in *Thermoflexus hugenholtzii*. Points represent individual observations. Black diamonds show model-predicted means from a GLMM with a beta distribution. Data are grouped by site and month and faceted by gene.

```
## Gene_IDrecA:SiteB     -0.37933    0.25813  -1.470 0.141684
## Gene_IDrpoB:SiteB     -0.57132    0.22801  -2.506 0.012220 *
## Gene_IDrecA:SiteC      0.45209    0.27263   1.658 0.097273 .
## Gene_IDrpoB:SiteC      0.63236    0.18331   3.450 0.000561 ***
## Gene_IDrecA:MonthJune  0.27182    0.24979   1.088 0.276519
## Gene_IDrpoB:MonthJune  0.27121    0.24215   1.120 0.262707
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Results

SCV frequency in Thermoflexus hugenholtzii varied significantly by gene and sampling site, with limited influence from sampling month. For the reference gene gyrB in February, SCV frequency was significantly lower at Site B compared to Site A ($p = 0.033$) and significantly higher at Site C ($p = 0.017$). The gene rpoB exhibited strong site-dependent patterns, showing a further significant decrease in SCV frequency at Site B ($p = 0.012$) and a significant increase at Site C ($p < 0.001$), relative to gyrB. In contrast, recA did not display significant variation across sites or months. No significant effects were observed for sampling month or any gene-by-month interactions ($p > 0.26$). These results indicate that SCV frequency is strongly influenced by site and gene identity, with rpoB being particularly sensitive to spatial context.

The observed variation in SCV frequency suggests that codon variation from mutations in Thermoflexus hugenholtzii are both gene-specific and influenced by local environmental conditions. The strong site-dependent differences in SCV frequency for rpoB, a core gene involved in transcription, imply that certain genomic regions may be more susceptible to mutation or subject to different selective pressures depending on the environment. Higher SCV frequencies at Site C, particularly in rpoB and gyrB, may indicate relaxed selection or ecological conditions that promote microdiversity. In contrast, the consistently lower SCV frequencies at Site B could indicate stronger purifying selection or environmental constraints that favor genomic stability. The lack of significant effects associated with sampling month suggests that these patterns are relatively stable over time and driven primarily by spatial, rather than temporal, factors. Together, these results point to localized evolutionary pressures shaping intra-species variation and highlight the potential for certain genes to act as indicators of environmental selection within microbial populations.

References

Cole, J. K., Peacock, J. P., Dodsworth, J. A., Williams, A. J., Thompson, D. B., Dong, H., Wu, G., & Hedlund, B. P. (2013). Sediment microbial communities in Great Boiling Spring are controlled by temperature and distinct from water communities. The ISME Journal, 7(4), 718– 729. https://doi.org/10.1038/ismej.2012.157

Damer, B., & Deamer, D. (2020). The Hot Spring Hypothesis for an Origin of Life. Astrobiology, 20(4), 429–452. https://doi.org/10.1089/ast.2019.2045

Kees, E. D., Murugapiran, S. K., Bennett, A. C., & Hamilton, T. L. (2022). Distribution and Genomic Variation of Thermophilic Cyanobacteria in Diverse Microbial Mats at the Upper Temperature Limits of Photosynthesis. mSystems, 7(5), e00317-22. https://doi.org/10.1128/msystems.00317-22

Nguyen, J., Lara-Gutiérrez, J., & Stocker, R. (2021). Environmental fluctuations and their effects on microbial communities, populations and individuals. FEMS Microbiology Reviews, 45(4), fuaa068. https://doi.org/10.1093/femsre/fuaa068