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#Reactivation Time Analysis
#Riley Morris

#Install packages
install.packages("ggplot2")
install.packages("devtools")
library(devtools)
install_github("easyGgplot2", "kassambara")
install.packages("EnvStats")
install.packages("dplyr")
install.packages("car")

#Call packages
library(readxl)
library(dplyr)
library(EnvStats)
library(easyGgplot2)
library(ggplot2)
library(car)

#Data import
ReactTime <- read_excel("C:/Users/Morris Clan/Dropbox/Academics/Education/03 - Master of Biology/BIOL 5909 -
Masters Thesis/Analysis/Reactivation Time Analyses/ReactTimeData.xlsx")

ReactTime$ReactTemp <- as.factor(ReactTime$ReactTemp)
ReactTime$Genotype <- as.factor(ReactTime$Genotype)
ReactTime$MBO <- as.factor(ReactTime$MBO)
ReactTime$TBO <- as.factor(ReactTime$TBO)

#Break Down
RTimeSplit <- split(ReactTime, ReactTime$ReactTemp)
ReactTime10 <- RTimeSplit$`10`
ReactTime18 <- RTimeSplit$`18`
ReactTime26 <- RTimeSplit$`26`

#Turion production
levels(ReactTime$MBO) <- c("MBO3", "MBO5", "MBO7", "MBO9")
ggplot(ReactTime, aes(as.numeric(TBO), colour = I("black")) +
  geom_histogram(binwidth = 1, show.legend = F, alpha = 0.2) +
  facet_grid( ~ ReactTime$MBO) +
  theme_bw() +
  theme(panel.grid = element_blank()) +
  xlab("Turion Birth Order") +
  xlim("3", "5", "7", "9") +
  ylab("Turion Yield")
levels(ReactTime$MBO) <- c("3", "5", "7", "9")

#1.      Is there variability in reactivation timing?
#2.      Is the variability in timing influenced by reactivation temperature?
#3.      Is the variability within temperature due to birth order?
#4.      Birth order and size are correlated, does reactivation time still vary within temp due to birth order after
correcting for size?
#5.      Is reactivation time within temperature related to the time at which the turion was formed (as
AfterRipeTime)? (need to pay attention to remove birth order formation time from this analysis which will confound
results)

#Item #1: Is there variability?
summary(ReactTime$RTime)
sd(na.omit(ReactTime$RTime))

#Plot
ggplot(ReactTime, aes(RTime)) +
  geom_histogram() +
  ggtitle("Reactivation Time Distribution") +
  xlab("Reactivation Time (hrs)") +
  ylab("Frequency") +
  theme_bw()

#Item #2: Is the variability influenced by temperature?
Fit_Temp <- lm(RTime ~ ReactTemp, data = ReactTime)
AOV_Temp <- aov(Fit_Temp)
summary(Fit_Temp)
summary(AOV_Temp)
TukeyHSD(AOV_Temp)

#Plot
RTimeDays <- ReactTime$RTime/24
ggplot(ReactTime, aes(x = RTimeDays, colour = ReactTemp, ..density..)) +
  geom_histogram(aes(fill = ReactTemp), alpha = 0.6, position = "stack", stat = "bin") +
  theme_bw() +
  xlab("Reactivation Time (Days)") +
  ylab("Normalized Frequency") +
  theme(legend.position = "bottom",
    axis.title.y = element_text(margin = margin(t = 0, r = 10, b = 0, l = 0))) +
  scale_colour_manual("Reactivation Temperature",

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      values = c("#E69F00", "#0072B2", "#009E73"),
      labels = c("10°C", "18°C", "26°C")) +
scale_fill_manual("Reactivation Temperature",
  values = c("#E69F00", "#0072B2", "#009E73"),
  labels = c("10°C", "18°C", "26°C"))

#Item #3: Is variability within temp due to BO?
Fit_BO1 <- lm(RTime ~ ReactTemp*MBO*TBO, data = ReactTime)
AOV_BO1 <- aov(Fit_BO1)
summary(Fit_BO1)
summary(AOV_BO1)
#TukeyHSD(AOV_BO1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_BO2 <- Anova(lm(RTime ~ ReactTemp*TBO+MBO, data = ReactTime), type="III")

#Analysis for BO as continuous variables
Fit_BO3 <- lm(RTime ~ ReactTemp*as.numeric(MBO)*as.numeric(TBO), data = ReactTime)
AOV_BO3 <- aov(Fit_BO3)
summary(Fit_BO3)
summary(AOV_BO3)
#TukeyHSD(AOV_BO3)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_BO4 <- Anova(lm(RTime ~ ReactTemp*as.numeric(TBO)+as.numeric(MBO), data = ReactTime), type="III")

#Plot
levels(ReactTime$ReactTemp) <- c("10°C", "18°C", "26°C")
ggplot(ReactTime, aes(TBO, RTime, colour = MBO)) +
  geom_jitter(position = position_jitter(0.05), alpha = 0.2) +
  geom_smooth(aes(group = MBO, colour = MBO),
    method = "lm",
    formula = y~x,
    se = FALSE,
    fullrange = TRUE) +
  facet_grid( ~ ReactTime$ReactTemp) +
  theme_bw() +
  xlab("Turion Birth Order") +
  xlim("3", "5", "7", "9") +
  ylab("Reactivation Time (hrs)") +
  scale_shape_manual(name = "Mother Birth Order", values = c(1,1,1,1)) +
  scale_color_manual(name = "Mother Birth Order",
    values = c("#E69F00", "#0072B2", "#009E73", "#CC79A7")) +
  theme(legend.position = "bottom") +
  stat_n_text(size = 3)
levels(ReactTime$ReactTemp) <- c("10", "18", "26")

#Item #4: Same result when accounting for size?
Fit_LxW <- lm(Length ~ Width, data = ReactTime)
AOV_LxW <- aov(Fit_LxW)
summary(Fit_LxW)
summary(AOV_LxW)
Fit_TSizeL1 <- lm(Length ~ MBO*TBO, data = ReactTime)
AOV_TSizeL1 <- aov(Fit_TSizeL1)
summary(Fit_TSizeL1)
summary(AOV_TSizeL1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_TSizeL2 <- Anova(lm(Length ~ MBO*TBO, data = ReactTime), type="III")

Fit_TSizeW1 <- lm(Width ~ MBO*TBO, data = ReactTime)
AOV_TSizeW1 <- aov(Fit_TSizeW1)
summary(Fit_TSizeW1)
summary(AOV_TSizeW1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_TSizeW2 <- Anova(lm(Width ~ MBO*TBO, data = ReactTime), type="III")

Fit_TSizeL <- lm(RTime ~ ReactTemp + Width, data = ReactTime)
AOV_TSizeL <- aov(Fit_TSizeL)
summary(Fit_TSizeL)
summary(AOV_TSizeL)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_TSize2 <- Anova(lm(RTime ~ ReactTemp + Width, data = ReactTime), type="III")

Fit_TSizeBO1 <- lm(RTime ~ ReactTemp + TBO + Width, data = ReactTime)
AOV_TSizeBO1 <- aov(Fit_TSizeBO1)
summary(Fit_TSizeBO1)
summary(AOV_TSizeBO1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_TSizeBO2 <- Anova(lm(RTime ~ ReactTemp + TBO + Width, data = ReactTime), type="III")

#Plot

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ggplot(ReactTime, aes(Width, Length)) +
  geom_point(aes(colour = I("grey"))) +
  geom_smooth(aes(colour = 4), method = lm, show.legend = F) +
  theme_bw() +
  ggtitle("Length and Width") +
  xlab("Turion Width (mm)") +
  ylab("Turion Length (mm)")
ggplot(ReactTime, aes(as.numeric(MBO), Width)) +
  geom_jitter(colour = "grey", position = position_jitter(0.1)) +
  geom_smooth(aes(colour = 4), method = loess, show.legend = F) +
  theme_bw() +
  ggtitle("Length with Mother Birth Order") +
  xlab("Mother Birth Order") +
  ylab("Turion Length (mm)")
ggplot(ReactTime, aes(as.numeric(TBO), Width)) +
  geom_jitter(colour = "grey", position = position_jitter(0.1)) +
  geom_smooth(aes(colour = 4), method = loess, show.legend = F) +
  theme_bw() +
  ggtitle("Length with Turion Birth Order") +
  xlab("Turion Birth Order") +
  ylab("Turion Length (mm)")

Fit_SizeFull1 <- lm(RTime ~ ReactTemp + Width + MBO*TBO, data = ReactTime)
AOV_SizeFull1 <- aov(Fit_SizeFull1)
summary(Fit_SizeFull1)
summary(AOV_SizeFull1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_TSizeFull2 <- Anova(lm(RTime ~ ReactTemp + Width + MBO*TBO, data = ReactTime), type="III")

Fit_SizeMain1 <- lm(RTime ~ Width + ReactTemp*MBO*TBO, data = ReactTime)
AOV_SizeMain1 <- aov(Fit_SizeMain1)
summary(Fit_SizeMain1)
summary(AOV_SizeMain1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_TSizeMain2 <- Anova(lm(RTime ~ Width + ReactTemp*TBO+MBO, data = ReactTime), type="III")

#Item #5: Is reactivation time correlated with formation time?
Fit_Form1 <- lm(RTime ~ AfterRipeTime, data = ReactTime)
AOV_Form1 <- aov(Fit_Form1)
summary(Fit_Form1)
summary(AOV_Form1)

Fit_Form2 <- lm(RTime ~ ReactTemp + TBO + AfterRipeTime, data = ReactTime)
AOV_Form2 <- aov(Fit_Form2)
summary(Fit_Form2)
summary(AOV_Form2)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_Form3 <- Anova(lm(RTime ~ ReactTemp + TBO + AfterRipeTime, data = ReactTime), type="III")

#BO3 Only
RTSplit <- split(ReactTime, ReactTime$TBO)
RT3 <- RTSplit$`3`
Fit_ARipe3_1 <- lm(RTime ~ ReactTemp + AfterRipeTime, data = RT3)
AOV_ARipe3_1 <- aov(Fit_ARipe3_1)
summary(Fit_ARipe3_1)
summary(AOV_ARipe3_1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_ARipe3_2 <- Anova(lm(RTime ~ ReactTemp + AfterRipeTime, data = RT3), type="III")

#Other Analyses
#1. Genotype
Fit_Gen1 <- lm(RTime ~ ReactTemp + Genotype, data = ReactTime)
AOV_Gen1 <- aov(Fit_Gen1)
summary(Fit_Gen1)
summary(AOV_Gen1)
#TukeyHSD(AOV_Gen)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_Gen2 <- Anova(lm(RTime ~ ReactTemp + Genotype, data = ReactTime), type="III")

Fit_Gen3 <- lm(RTime ~ ReactTemp*TBO*MBO + Genotype, data = ReactTime)
AOV_Gen3 <- aov(Fit_Gen3)
summary(Fit_Gen3)
summary(AOV_Gen3)
#TukeyHSD(AOV_Gen2)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_Gen4 <- Anova(lm(RTime ~ ReactTemp+TBO+MBO + Genotype, data = ReactTime), type="III")

#Plot
levels(ReactTime$Genotype) = c("Genotype 1", "Genotype 2")

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ggplot(ReactTime, aes(as.numeric(ReactTemp), RTime)) +
  geom_jitter(aes(colour = ReactTime$Genotype), alpha = 0.3, position = position_jitter(0.04)) +
  geom_smooth(method = lm, aes(group = ReactTime$Genotype, colour = ReactTime$Genotype)) +
  theme_bw() +
  xlab("Reactivation Temperature (deg C)") +
  xlim(labels = c("10", "18", "26")) +
  ylab("Reactivation Time (hrs)") +
  scale_colour_discrete("Genotype", labels = c("Genotype 1", "Genogype 2")) +
  theme(legend.position = "bottom")
levels(ReactTime$Genotype) = c("1", "2")

#Mean Differences
mean(ReactTime10$RTime[ReactTime10$Genotype == 1], na.rm = T)
mean(ReactTime10$RTime[ReactTime10$Genotype == 2], na.rm = T)
mean(ReactTime18$RTime[ReactTime18$Genotype == 1], na.rm = T)
mean(ReactTime18$RTime[ReactTime18$Genotype == 2], na.rm = T)
mean(ReactTime26$RTime[ReactTime26$Genotype == 1], na.rm = T)
mean(ReactTime26$RTime[ReactTime26$Genotype == 2], na.rm = T)

#2. Experiment
Fit_Exp <- lm(RTime ~ Exp, data = ReactTime)
AOV_Exp <- aov(Fit_Exp)
summary(Fit_Exp)
summary(AOV_Exp)
#TukeyHSD(AOV_Exp)

#3. Formation Temperature
Fit_FormTemp1 <- lm(RTime ~ FormTemp + ReactTemp, data = ReactTime)
AOV_FormTemp1 <- aov(Fit_FormTemp1)
summary(Fit_FormTemp1)
summary(AOV_FormTemp1)

options(contrasts = c("contr.sum", "contr.poly"))
AOV_FormTemp2 <- Anova(lm(RTime ~ FormTemp + ReactTemp, data = ReactTime), type="III")

#Plot
ggplot(ReactTime, aes(as.numeric(FormTemp), RTime)) +
  geom_jitter(aes(colour = ReactTime$Exp), alpha = 0.3, position = position_jitter(0.04)) +
  geom_smooth(method = lm, aes(group = ReactTime$Exp, colour = ReactTime$Exp)) +
  geom_smooth(method = lm, aes(group = ReactTime$Trial, colour = ReactTime$Trial)) +
  theme_bw() +
  facet_grid(~ ReactTime$Exp) +
  xlab("Formation Temperature (deg C)") +
  ylab("Reactivation Time (hrs)")
#scale_colour_discrete("Genotype", labels = c("Genotype 1", "Genogype 2")) +
#theme(legend.position = "bottom")

#4. Position
ExpSplit <- split(ReactTime, ReactTime$Exp)
ReactTimeLab <- ExpSplit$Lab

#Reactivation Position (Lab only)
Fit_PosTray <- lm(RTime ~ RP_Tray, data = ReactTimeLab)
AOV_PosTray <- aov(Fit_PosTray)
summary(Fit_PosTray)
summary(AOV_PosTray)
Fit_PosRow <- lm(RTime ~ RP_Row, data = ReactTimeLab)
AOV_PosRow <- aov(Fit_PosRow)
summary(Fit_PosRow)
summary(AOV_PosRow)
Fit_PosCol <- lm(RTime ~ RP_Col, data = ReactTimeLab)
AOV_PosCol <- aov(Fit_PosCol)
summary(Fit_PosCol)
summary(AOV_PosCol)

#Plot
Tray <- ggplot(ReactTimeLab, aes(RP_Tray, RTime)) +
  geom_point() +
  geom_smooth(method = lm) +
  facet_grid(~ ReactTimeLab$ReactTemp) +
  theme_bw()
Row <- ggplot(ReactTimeLab, aes(RP_Row, RTime)) +
  geom_point() +
  stat_summary(fun.y = "mean", geom = "line") +
  theme_bw()
Col <- ggplot(ReactTimeLab, aes(RP_Col, RTime)) +
  geom_point() +
  geom_smooth(method = loess) +
  theme_bw()
ggplot2::multiplot(Tray, Row, Col)

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