

CrossSpecies Plot

Load Necessary Packages

```
#Load the following packages to run the rest of the script.
library(tidyverse)
library(viridis)
library(Rmisc)
library(ggplot2)
library(nlme)
library(arsenal)
library(janitor)
library(ggforce)
library(ggalt)
library(dplyr)
library(ggalt)
library(ggforce)
```

Introduction Plot: Bias in IGF Publication over Time

```
#Read in data set with number of publications per year
pub=read.csv("Timeline_IGFs_pub.csv")
#Remove year "2021" as the year is not yet complete
pub=subset(pub, Year != "2021")

#Plot number of publications per year from ~1970 to 2020 by IGF type
ggplot() +
  #In solid lines, plot the total number of IGF1 and IGF2 publications per year, excluding Reviews
  geom_line(data=pub, aes(x = Year, y = IGF1), color= "orange2", fill = "orange2", size = 1, alpha=1)
  geom_line(data=pub, aes(x = Year, y = IGF2), color= "skyblue4", fill="skyblue4", size = 1, alpha=1)
  #In dashed lines, plot the total number of IGF1 and IGF2 publications per year, excluding Reviews, De
  geom_line(data=pub, aes(x = Year, y = Post.natal_no.cancer_IGF1), color= "orange2", size = 1, linetype="dashed")
  geom_line(data=pub, aes(x = Year, y = Post.natal_no.cancer_IGF2), color= "skyblue4", size = 1, linetype="dashed")
  #In shaded lines, plot the total number of IGF1 and IGF2 publications per year, excluding Reviews, an
  geom_area(data=pub, aes(x = Year, y = Non.model_IGF1), color= "orange2", fill="orange2", size = 1, alpha=0.5)
  geom_area (data=pub, aes(x = Year, y = Non.model_IGF2), color= "skyblue4", fill="skyblue4", size = 1, alpha=0.5)
  #Change axis titles and bold the text
  ylab("Number of Publications") +
  xlab ("Year") +
  theme(axis.title = element_text(face= "bold", size= 12))
```

```
## Warning: Ignoring unknown parameters: fill
```

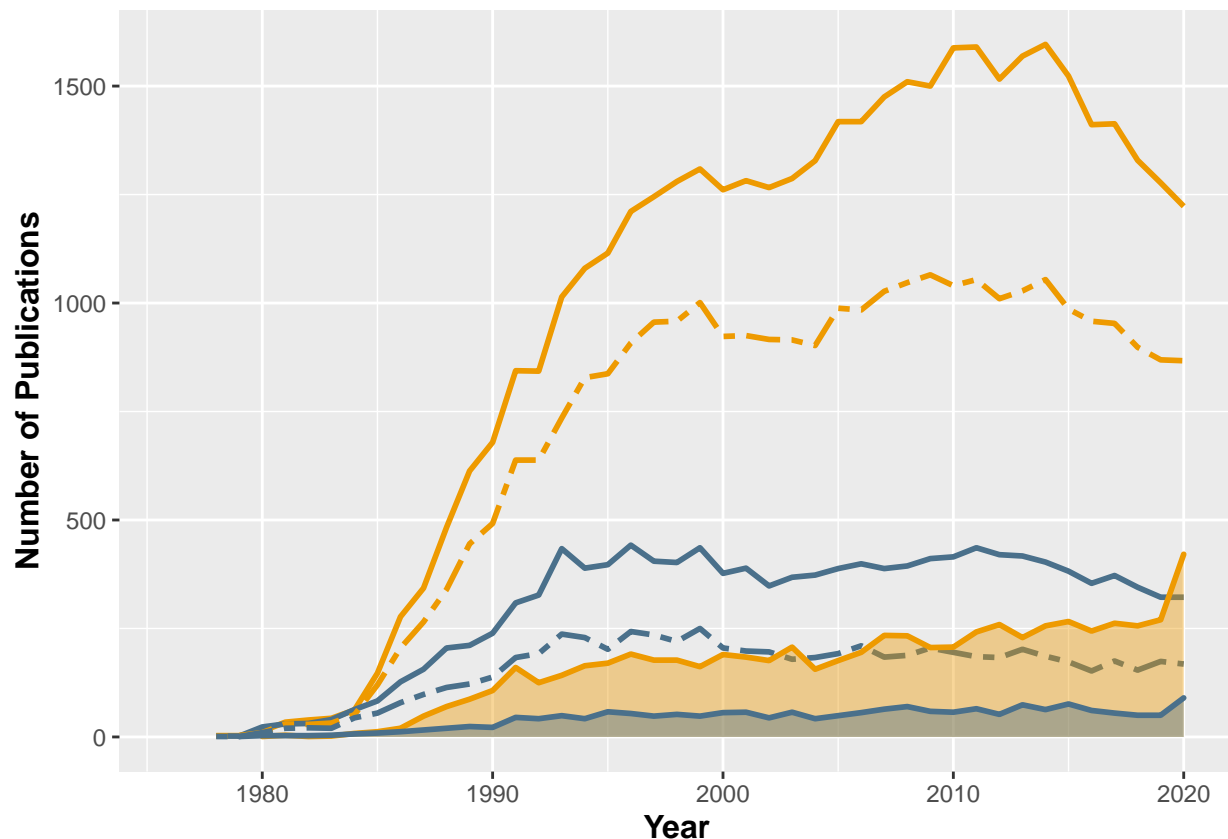
```
## Warning: Ignoring unknown parameters: fill
```

```
## Warning: Removed 4 rows containing missing values (position_stack).
```

```
## Warning: Removed 3 rows containing missing values (position_stack).
```

```
## Warning: Removed 2 row(s) containing missing values (geom_path).
```

```
## Warning: Removed 2 row(s) containing missing values (geom_path).
```



Plylogenetic RNAseq Analysis

Data Curation of RNAseq Data

```
# Upload dataset containing all RNAseq Counts in CSV format
data2 <- read.csv("MetaData_Counts_Cleaned.csv")
#Get a count of the total number of unique species that will be included in the data set.
count=count(unique(data2$Organism))

#aggregate data to get average IGF1 for each species (combining all individuals of the same species)
IGF1=summarySE(data = data2, measurevar="IGF1_Proportion", groupvars = c("Organism", "Common.Name", "Ca
```

```
## Warning in qt(conf.interval/2 + 0.5, datac$N - 1): NaNs produced
```

```
#aggregate data to get average IGF2 for each species (combining all individuals of the same species)
IGF2=summarySE(data = data2, measurevar="IGF2_Proportion", groupvars = c("Organism", "Common.Name", "Ca
```

```
## Warning in qt(conf.interval/2 + 0.5, datac$N - 1): NaNs produced
```

```
#Merge IGF1 and IGF2 files together by the organisms common name.
data=merge(x = IGF1, y = IGF2, by = "Organism")
```

```
#Make new data frame including only the common name, organism, subcategory for organism organization, I
data=data.frame(data$Common.Name.x, data$Organism, data$Category.x, data$Subcategory.x, data$Phylogenetic
```

```
#rename columns for clarity following merge
names(data)[names(data) == "data.Category.x"] <- "Category"
names(data)[names(data) == "data.Common.Name.x"] <- "individual"
names(data)[names(data) == "data.Subcategory.x"] <- "group"
names(data)[names(data) == "data.IGF1_Proportion"] <- "IGF1.plot"
names(data)[names(data) == "data.IGF2_Proportion"] <- "IGF2.plot"
names(data)[names(data) == "data.Organism"] <- "Organism"
names(data)[names(data) == "data.Phylogenetic_Cat.x"] <- "Phylogenetic_Cat"
```

```
#take another count to make sure you did not lose data in the merging process
count2=count(unique(data$Organism))
#compare data frames to see what/if anything has changed
summary(comparedf(count, count2, by = "x"))
```

```
##
##
## Table: Summary of data.frames
##
## version      arg      ncol  nrow
## -----
## x            count      2     82
## y            count2     2     82
##
##
## Table: Summary of overall comparison
##
## statistic                                           value
## -----
## Number of by-variables                               1
## Number of non-by variables in common                 1
## Number of variables compared                       1
## Number of variables in x but not y                   0
## Number of variables in y but not x                   0
## Number of variables compared with some values unequal 0
## Number of variables compared with all values equal    1
## Number of observations in common                     82
## Number of observations in x but not y                 0
## Number of observations in y but not x                 0
## Number of observations with some compared variables unequal 0
## Number of observations with all compared variables equal 82
```

```

## Number of values unequal
##
##
##
## Table: Variables not shared
##
## |
## |:-----|
## |No variables not shared |
##
##
##
## Table: Other variables not compared
##
## |
## |:-----|
## |No other variables not compared |
##
##
##
## Table: Observations not shared
##
## |
## |:-----|
## |No observations not shared |
##
##
##
## Table: Differences detected by variable
##
## var.x   var.y    n   NAs
## -----
## freq    freq     0     0
##
##
##
## Table: Differences detected
##
## |
## |:-----|
## |No differences detected |
##
##
##
## Table: Non-identical attributes
##
## |
## |:-----|
## |No non-identical attributes |

```

```

#Write file to CSV to preserve data set used in all following analyses
write.csv(data,"Concat_Final_Dat2.csv", row.names = FALSE)

```

```

#Format data frame for use in Proportions Plot. Rename columns, and then reformat to have IGF1.plot and

```

```

data3=data.frame(data$individual,data$group,data$IGF1.plot, data$IGF2.plot)
names(data3)[names(data3) == "data.individual"] <- "individual"
names(data3)[names(data3) == "data.group"] <- "group"
names(data3)[names(data3) == "data.IGF1.plot"] <- "IGF1.plot"
names(data3)[names(data3) == "data.IGF2.plot"] <- "IGF2.plot"
names(data)[names(data) == "data.Organism"] <- "Organism"
  #Remove all rows containing NA values
data3=data3[complete.cases(data3), ]
  #this line of code reformats data to have IGF1 and IGF2 plot values in a single column. Note, there c
data3 <- data3 %>% gather(key = "observation", value="value", -c(1,2))

```

Production of Stacked Barplot

```

#produce a CSV file containing the values created in dataframe "data3"
write.csv(data3, "datafile3.csv",row.names = FALSE)

#Using the phylogenetic tree produced representing all species included in the analysis, the CSV file w
p3ID=read.csv("datafile3_plotID.csv")

#Produce stacked barplot showing the proportion of IGF1 and IGF2 expression within each species, and or
p4=ggplot(p3ID, aes(fill=observation, y=Percentage, x=plotID)) +
  geom_bar(position="stack", stat="identity") + scale_fill_manual(values=c("orange2", "skyblue4")) +
coord_flip() +
  scale_x_discrete(lim=rev) +
  theme(axis.text.y = element_blank(), axis.title.y = element_blank())

#Save the image as a PNG file for use in BioRender for final figure production
#ggsave(p4, file="phylo_barplot.nc.png", width=3, height=9, dpi=600)

```

Quantitative Cross-Species Analysis

```

#Read in data and format data/subset data
sp=read.csv("Species_Combined_edited_2.csv")
#Set ages to order chronologically
sp$Age = factor(sp$Age, levels=c('Embryonic','Juvenile','Adult'))
#Set custom dodge location for lining us data with grouped barplots
dodge <- position_dodge(width = 0.6)
#Set species order for facet plotting
sp$Species=factor(sp$Species, levels=c('Zebra Finch', "House Sparrow", "E. Fence Lizard", "Brown Anole")

#Set values to scientific notation on y-axis
fancy_scientific <- function(l) {
  # turn in to character string in scientific notation
  l <- format(l, scientific = TRUE)
  # quote the part before the exponent to keep all the digits
  l <- gsub("^(.*)e", "'\\1'e", l)
  # turn the 'e+' into plotmath format
  l <- gsub("e", "%*%10^", l)
}

```

```

    # return this as an expression
    parse(text=1)
}

```

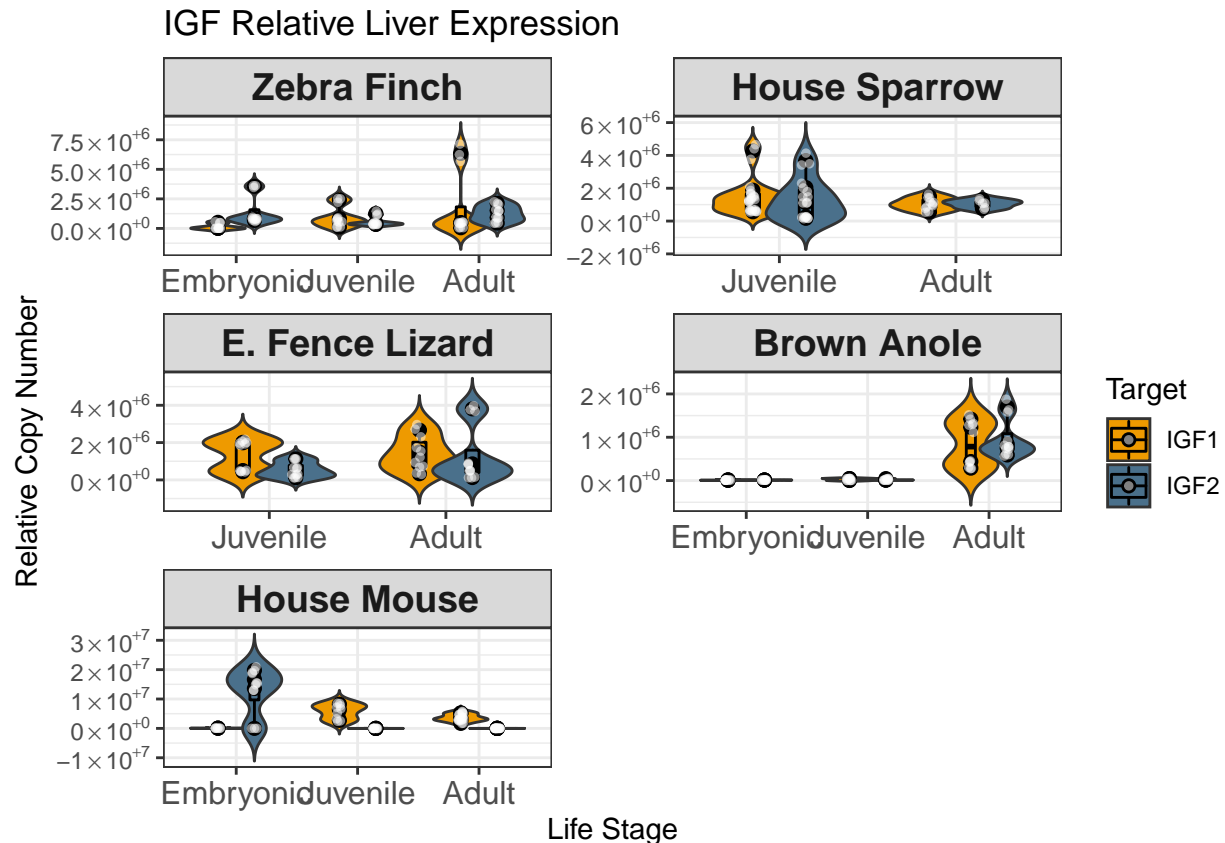
Facet plots of qPCR data

```

#Find average copy number for each age group, at each gene, in each individual for each species. This a
overall.SQ=summarySE(sp, measurevar = "SQ", groupvars = c("Species","Individual", "Target", "Age"))

#Plot IGF1 and IGF2 expression by age group, faceted by species
p1=ggplot(sp, aes(x=Age, y=SQ, fill= Target)) +
  ylab("Relative Copy Number") +
  xlab("Life Stage") +
  ggtitle("IGF Relative Liver Expression") +
  geom_violin(trim=F, position= dodge, scale="width") +
  scale_fill_manual(values = c("orange2", "skyblue4")) +
  geom_boxplot(width=0.15, position= dodge, outlier.shape = NA, color="black") +
  geom_point(data = overall.SQ, size =2, shape = 19, color="black", position=position_dodge(width=0.6)) +
  geom_point(position=position_jitterdodge(jitter.width = 0.05, dodge.width = 0.6), size=1, alpha=0.5, aes(
    scale_y_continuous(labels=fancy_scientific) +
  theme_bw() +
  facet_wrap(~Species, scales="free", nrow=4) +
  theme(strip.text = element_text(size=14, face="bold"),
    axis.text.x = element_text(size=12))
p1

```



```
#Save as a PNG file for final figure production in BioRender
#ggsave(p1, file="qPCR_facet.nc.png", width=6, height=9, dpi=600)
```

Anoles Developmental and Juvenile Inset Plot

```
#Subset data set to include only brown anoles
BA=subset(sp, Species == "Brown Anole")
#Subset data to include developmental and juvenile timepoints (excluding adulthood)
devan=subset(BA, Age != 'Adult')
#Calculate average copy number for each age and gene per individual. This averages across qPCR triplicates
devan.SQ=summarySE(devan, measurevar = "SQ", groupvars = c("Individual", "Target", "Age"))

#plot copy number by age. This plot is used as an inset in the plot including all timepoints. These are
p2=ggplot(devan, aes(x=Age, y=SQ, fill= Target)) +
  ylab("Relative Copy Number") +
  xlab("Life Stage") +
  ggtitle("IGF Relative Liver Expression") +
  geom_violin(trim=F, position= dodge, scale="width") +
  scale_fill_manual(values = c("orange2", "skyblue4")) +
  geom_boxplot(width=0.15, position= dodge, outlier.shape = NA, color="black") +
  geom_point(data = devan.SQ, size =2, shape = 19, color="black", position=position_dodge(width=0.6)) +
  geom_point(position=position_jitterdodge(jitter.width = 0.05, dodge.width = 0.6), size=1, alpha=0.5, aes(
    scale_y_continuous(labels=fancy_scientific) +
    theme(axis.text.x = element_text(size=12))
```

p2



```
#Save file as PNG for final figure production in BioRender
#ggsave(p2, file="BA_dev_inset.nc.png", width=9, height=7, dpi=600)
```

Graph of adult data by sex (when available)

```
#Subset data to include only adult timepoint of species with both male and female individuals
sex=subset(sp, Age == "Adult" & Species != "House Mouse" & Species != "E. Fence Lizard" & Species != "I
#Obtain average copy number of IGF1 and IGF2 for each individual. This accounts for qpcr triplicates an
sex.SQ=summarySE(sex, measurevar = "SQ", groupvars = c("Individual", "Species", "Sex", "Target"))

#Plot IGF1 and IGF2 expression by sex, faceted by species
p7=ggplot(sex, aes(x=Sex, y=SQ, fill= Target)) +
  ylab("Relative Copy Number") +
  xlab("Sex") +
  ggtitle("IGF Relative Liver Expression") +
  geom_violin(trim=F, position= dodge, scale="width") +
  scale_fill_manual(values = c("orange2", "skyblue4")) +
  geom_boxplot(width=0.15, position= dodge, outlier.shape = NA, color="black") +
  geom_point(data = sex.SQ, size =2, shape = 19, color="black", position=position_dodge(width=0.6)) +
```



```
geom_point(position=position_jitterdodge(jitter.width = 0.05, dodge.width = 0.6), size=1, alpha=0.5,
theme_bw() +
theme(axis.text.x = element_text(size=12)) +
facet_wrap(~Species, scales="free") +
theme(strip.text = element_text(size=14, face="bold"))
```

p7



```
#Save image as PNG for final figure production in BioRender
#ggsave(p7, file="Sex_interactions.nc.png", width=9, height=5, dpi=600)
```

Mouse Subset Data Plots by Strain (qPCR Data)

```
#Subset data to include only mouse samples
mouse=subset(sp, Species == "House Mouse")

#Reorder Age in mouse samples for plotting
mouse$Age_sub = factor(mouse$Age_sub, levels=c('Embryonic.Lab', 'Adult.Lab', 'Juvenile.Wild', 'Adult.Wild'))
#Obtain average copy number of IGF1 and IGF2 for each individual. This accounts for qpcr triplicates and
mean.SQ=summarySE(mouse, measurevar = "SQ", groupvars = c("Individual", "Target", "Age_sub"))

#Plot IGF1 and IGF2 expression by age, first the inbred strains, and then the outbred strains
```

```
p5=ggplot(mouse, aes(x=Age_sub, y=SQ, fill= Target)) +
  ylab("Relative Copy Number") +
  xlab("Life Stage") +
  ggtitle("IGF Relative Liver Expression") +
  geom_violin(trim=F, position= dodge, scale="width") +
  scale_fill_manual(values = c("orange2", "skyblue4")) +
  geom_boxplot(width=0.15, position= dodge, outlier.shape = NA, color="black") +
  geom_point(data = mean.SQ, size =2, shape = 19, color="black", position=position_dodge(width=0.6)) +
  geom_point(position=position_jitterdodge(jitter.width = 0.05, dodge.width = 0.6), size=1, alpha=0.5, aes(
    theme_bw() +
    theme(axis.text.x = element_text(size=12))
```

p5



```
#Save image as PNG for final figure production in BioRender
#ggsave(p5, file="Mouse_Strain.nc.png", width=9, height=7, dpi=600)
```

Mouse Subset Data Plots by Strain (RNAseq)

```
#aggregate data to get average IGF1 for each species (combining all individuals of the same species)
IGF1.m=summarySE(data = data2, measurevar="IGF1.plot", groupvars = c("Strain", "Organism", "Category",
```

```
## Warning in qt(conf.interval/2 + 0.5, datac$N - 1): NaNs produced
```

```

IGF2.m=summarySE(data = data2, measurevar="IGF2.plot", groupvars = c("Strain", "Organism", "Category",

## Warning in qt(conf.interval/2 + 0.5, datac$N - 1): NaNs produced

#Merge IGF1 and IGF2 values into a single dataframe by Strain
dat=merge(x = IGF1.m, y = IGF2.m, by = "Strain", all.x=TRUE)

#Isolate columns including strain, organism, IGF1 and IGF2 values
dat=data.frame(dat$Strain, dat$Organism.x ,dat$IGF1.plot, dat$IGF2.plot)
#Remove rows with NA values
dat=dat[complete.cases(dat), ]

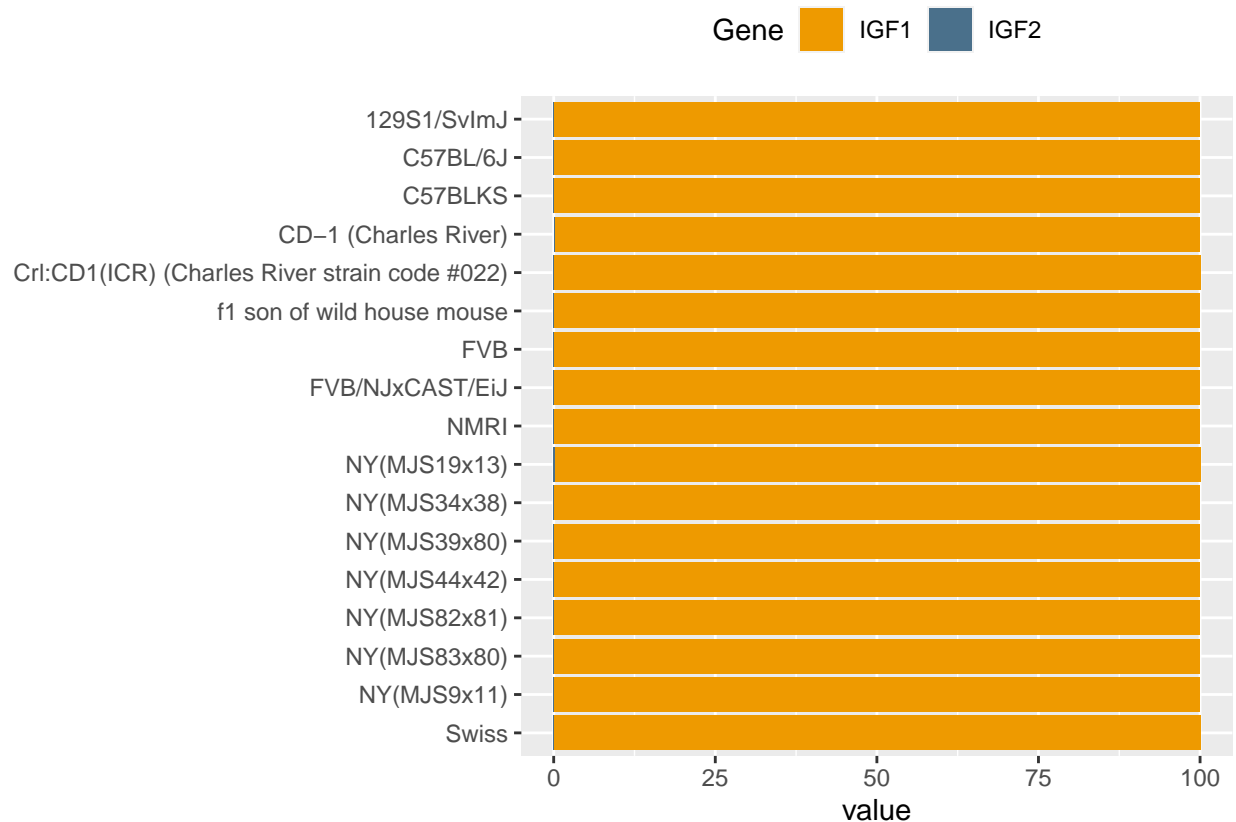
#Rename columns for ease of interpretation
names(dat)[names(dat) == "dat.Strain"] <- "Strain"
names(dat)[names(dat) == "dat.IGF1.plot"] <- "IGF1"
names(dat)[names(dat) == "dat.IGF2.plot"] <- "IGF2"

#Reformat data frame to have IGF1 and IGF2 in a single column named "gene", and the expression levels i
dat3 <- dat %>% gather(key = "Gene", value="value", -c(1,2))

#Plot the IGF expression across strains
p6=ggplot(dat3, aes(fill=Gene, y=value, x=Strain)) +
  geom_bar(position="stack", stat="identity") + scale_fill_manual(values=c("orange2", "skyblue4")) +
  coord_flip() +
  scale_x_discrete(lim=rev) +
  theme(axis.title.y = element_blank(),
        legend.position = "top")

p6

```



```
#Save image as PNG for final figure production in BioRender
#ggsave(p6, file="Mouse_Strain_RNAseq.nc.png", width=5, height=7, dpi=600)
```

Statistical Analysis

ZebraFinch Statistics

```
#Zebra Finch Statistics
sp=read.csv("Species_Combined_edited_2.csv")

#Subset data to include only zebra finch samples
zf=subset(sp, Species == "Zebra Finch")

#Create a data frame for each age class
zf.emb=subset(zf, Age=="Embryonic")
zf.juv=subset(zf, Age=="Juvenile")
zf.ad=subset(zf, Age=="Adult")

#Run linear model on relative IGF1 and IGF2 expression levels at each age class, using a random variable
summary(lme(SQ~Target, random=~1|Content, data=zf.emb))

## Linear mixed-effects model fit by REML
## Data: zf.emb
```

```
##           AIC      BIC    logLik
##    607.7571 611.74 -299.8785
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      812975.3 579977.3
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF   t-value p-value
## (Intercept)  80791.1  446585.8 17  0.180908  0.8586
## TargetIGF2 1391307.0 249475.0 17  5.576939  0.0000
## Correlation:
##      (Intr)
## TargetIGF2 -0.307
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.3047112 -0.5725261 -0.3711238  0.5331178  1.6839173
##
## Number of Observations: 22
## Number of Groups: 4
```

```
summary(lme(SQ~Target, random=~1|Content, data=zf.juv))
```

```
## Linear mixed-effects model fit by REML
## Data: zf.juv
##           AIC      BIC    logLik
##    640.1035 644.4677 -316.0518
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      707675 293621.7
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF   t-value p-value
## (Intercept)  982241.8 363848.1 19  2.699593  0.0142
## TargetIGF2 -379543.5 119870.6 19 -3.166278  0.0051
## Correlation:
##      (Intr)
## TargetIGF2 -0.165
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.69739865 -0.46531676  0.01714984  0.44748453  1.80166374
##
## Number of Observations: 24
## Number of Groups: 4
```

```
summary(lme(SQ~Target*Sex, random=~1|Content, data=zf.ad))
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: zf.ad
##      AIC      BIC    logLik
## 644.2124 650.1868 -316.1062
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      1185535 1357670
##
## Fixed effects: SQ ~ Target * Sex
##              Value Std.Error DF   t-value p-value
## (Intercept)   3424682   1004966 18   3.407757  0.0031
## TargetIGF2    -1602247    783851 18  -2.044071  0.0559
## SexMale       -3163792   1421237  2  -2.226083  0.1559
## TargetIGF2:SexMale 2025798   1108533 18   1.827459  0.0843
## Correlation:
##              (Intr) TrIGF2 SexMal
## TargetIGF2    -0.390
## SexMale       -0.707  0.276
## TargetIGF2:SexMale 0.276 -0.707 -0.390
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.4188018 -0.37255095 -0.01174518  0.37185286  1.94322578
##
## Number of Observations: 24
## Number of Groups: 4
```

```
#Subset adult samples by sex
```

```
zf.ad.m=subset(zf.ad, Sex=="Male")
zf.ad.f=subset(zf.ad, Sex=="Female")
```

```
#Run linear model on relative IGF1 and IGF2 expression levels in each sex, using a random variable "Content"
summary(lme(SQ~Target, random=~1|Content, data=zf.ad.m))
```

```
## Linear mixed-effects model fit by REML
## Data: zf.ad.m
##      AIC      BIC    logLik
## 287.7372 288.9475 -139.8686
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      18.97936 240083.7
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF   t-value p-value
## (Intercept) 260889.4   98013.78  9  2.661763  0.0260
## TargetIGF2  423551.0  138612.41  9  3.055650  0.0137
## Correlation:
##              (Intr)
## TargetIGF2 -0.707
##
## Standardized Within-Group Residuals:
```

```
##           Min           Q1           Med           Q3           Max
## -1.05393796 -0.88101075 -0.03992428  0.83457517  1.22373691
##
## Number of Observations: 12
## Number of Groups: 2
```

```
summary(lme(SQ~Target, random=~1|Content, data=zf.ad.f))
```

```
## Linear mixed-effects model fit by REML
## Data: zf.ad.f
##      AIC      BIC    logLik
##  330.8805 332.0908 -161.4402
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      1679604 1903360
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF   t-value p-value
## (Intercept)  3424682  1419272  9  2.412986  0.0391
## TargetIGF2  -1602247  1098905  9 -1.458040  0.1788
## Correlation:
##      (Intr)
## TargetIGF2 -0.387
##
## Standardized Within-Group Residuals:
##           Min           Q1           Med           Q3           Max
## -1.00997603 -0.80594263 -0.03523639  0.74167308  1.38399265
##
## Number of Observations: 12
## Number of Groups: 2
```

House Sparrow Statistics

```
#Subset data to include only house sparrow samples
hs=subset(sp, Species == "House Sparrow")
```

```
#Create a data frame for each age class
hs.juv=subset(hs, Age=="Juvenile")
hs.ad=subset(hs, Age=="Adult")
```

```
#Run linear model on relative IGF1 and IGF2 expression levels at each age class, using a random variable
summary(lme(SQ~Target, random=~1|Content, data=hs.juv))
```

```
## Linear mixed-effects model fit by REML
## Data: hs.juv
##      AIC      BIC    logLik
##  1260.434 1267.289 -626.2171
##
## Random effects:
```

```
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:    931158.8 808982.4
##
## Fixed effects: SQ ~ Target
##      Value Std.Error DF   t-value p-value
## (Intercept) 1516092.8 368308.2 34  4.116370  0.0002
## TargetIGF2  -346425.4 255461.0 34 -1.356079  0.1840
## Correlation:
##      (Intr)
## TargetIGF2 -0.29
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.5706460 -0.4176272 -0.1484765  0.4173205  2.3969267
##
## Number of Observations: 43
## Number of Groups: 8
```

```
summary(lme(SQ~Target, random=~1|Content, data=hs.ad))
```

```
## Linear mixed-effects model fit by REML
## Data: hs.ad
##      AIC      BIC    logLik
##  424.999 427.8312 -208.4995
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:    278754.5 181198.1
##
## Fixed effects: SQ ~ Target
##      Value Std.Error DF   t-value p-value
## (Intercept) 1023604.8 149861.38 12  6.830344  0.000
## TargetIGF2   50766.3  97480.45 12  0.520785  0.612
## Correlation:
##      (Intr)
## TargetIGF2 -0.214
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.5245467 -0.4003916  0.2043183  0.3575478  1.4939813
##
## Number of Observations: 17
## Number of Groups: 4
```

Sceloporus Statistics

```
#Subset data to include only eastern fence lizard samples
scelop=<subset(sp, Species == "E. Fence Lizard")>
```



```
#Create a data frame for each age class
```

```
sc.juv=subset(scelop, Age=="Juvenile")
```

```
sc.ad=subset(scelop, Age=="Adult")
```

```
#Run linear model on relative IGF1 and IGF2 expression levels at each age class, using a random variable
```

```
summary(lme(SQ~Target, random=~1|Content, data=sc.juv))
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: sc.juv
```

```
##      AIC      BIC    logLik
```

```
## 628.584 632.7621 -310.292
```

```
##
```

```
## Random effects:
```

```
## Formula: ~1 | Content
```

```
##      (Intercept) Residual
```

```
## StdDev:      409614.5 503203.2
```

```
##
```

```
## Fixed effects: SQ ~ Target
```

```
##              Value Std.Error DF   t-value p-value
```

```
## (Intercept) 1304614 255342.9 18  5.109263 1e-04
```

```
## TargetIGF2  -760675 210606.5 18 -3.611830 2e-03
```

```
## Correlation:
```

```
##      (Intr)
```

```
## TargetIGF2 -0.432
```

```
##
```

```
## Standardized Within-Group Residuals:
```

```
##      Min      Q1      Med      Q3      Max
```

```
## -1.6676362 -0.7055741  0.1616344  0.8182156  1.3651213
```

```
##
```

```
## Number of Observations: 23
```

```
## Number of Groups: 4
```

```
summary(lme(SQ~Target, random=~1|Content, data=sc.ad))
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: sc.ad
```

```
##      AIC      BIC    logLik
```

```
## 631.1287 635.3068 -311.5643
```

```
##
```

```
## Random effects:
```

```
## Formula: ~1 | Content
```

```
##      (Intercept) Residual
```

```
## StdDev:      1308213 453037.7
```

```
##
```

```
## Fixed effects: SQ ~ Target
```

```
##              Value Std.Error DF   t-value p-value
```

```
## (Intercept) 1329677.7 668396.3 18  1.9893554 0.0621
```

```
## TargetIGF2   -9152.3 189742.0 18 -0.0482354 0.9621
```

```
## Correlation:
```

```
##      (Intr)
```

```
## TargetIGF2 -0.149
```

```
##
```

```
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.97183374 -0.71010691  0.06204505  0.67744229  1.73007738
##
## Number of Observations: 23
## Number of Groups: 4
```

BA Statistics

```
#Subset data to include only brown anole samples
anole=subset(sp, Species == "Brown Anole")

#Create a data frame for each age class
a.emb=subset(anole, Age=="Embryonic")
a.juv=subset(anole, Age=="Juvenile")
a.ad=subset(anole, Age=="Adult")

#Run linear model on relative IGF1 and IGF2 expression levels at each age class, using a random variable
summary(lme(SQ~Target, random=~1|Content, data=a.emb))
```

```
## Linear mixed-effects model fit by REML
## Data: a.emb
##      AIC      BIC    logLik
##  404.4083 408.5864 -198.2041
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:    3058.422 2274.944
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF  t-value p-value
## (Intercept) 10944.126 1664.2617 18  6.575965  0.000
## TargetIGF2   1047.327  952.5557 18  1.099492  0.286
## Correlation:
##      (Intr)
## TargetIGF2 -0.272
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.72598082 -0.55447779 -0.06286834  0.52304420  1.69476890
##
## Number of Observations: 23
## Number of Groups: 4
```

```
summary(lme(SQ~Target, random=~1|Content, data=a.juv))
```

```
## Linear mixed-effects model fit by REML
## Data: a.juv
##      AIC      BIC    logLik
##  448.6204 452.6034 -220.3102
```

```
##
## Random effects:
## Formula: ~1 | Content
## (Intercept) Residual
## StdDev:      18138.5  10555.9
##
## Fixed effects: SQ ~ Target
## Value Std.Error DF t-value p-value
## (Intercept) 25645.684 9619.142 17 2.666109 0.0163
## TargetIGF2 -5569.075 4501.050 17 -1.237284 0.2328
## Correlation:
## (Intr)
## TargetIGF2 -0.234
##
## Standardized Within-Group Residuals:
## Min Q1 Med Q3 Max
## -1.3758691 -0.7570618 0.1100159 0.6136281 1.6621307
##
## Number of Observations: 22
## Number of Groups: 4
```

```
summary(lme(SQ~Target*Sex, random=~1|Content, data=a.ad))
```

```
## Linear mixed-effects model fit by REML
## Data: a.ad
## AIC BIC logLik
## 560.1316 566.106 -274.0658
##
## Random effects:
## Formula: ~1 | Content
## (Intercept) Residual
## StdDev:      266265.4 156298.4
##
## Fixed effects: SQ ~ Target * Sex
## Value Std.Error DF t-value p-value
## (Intercept) 363400.7 198796.82 18 1.828000 0.0842
## TargetIGF2 308578.4 90238.95 18 3.419569 0.0031
## SexMale 960525.8 281141.16 2 3.416525 0.0760
## TargetIGF2:SexMale -365100.8 127617.14 18 -2.860907 0.0104
## Correlation:
## (Intr) TrIGF2 SexMal
## TargetIGF2 -0.227
## SexMale -0.707 0.160
## TargetIGF2:SexMale 0.160 -0.707 -0.227
##
## Standardized Within-Group Residuals:
## Min Q1 Med Q3 Max
## -1.6037815779 -0.2472233933 0.0005601846 0.2382048527 2.3186639951
##
## Number of Observations: 24
## Number of Groups: 4
```

```

#Subset adult samples by sex
a.ad.m=subset(a.ad, Sex=="Male")
a.ad.f=subset(a.ad, Sex=="Female")

#Run linear model on relative IGF1 and IGF2 expression levels in each sex, using a random variable "Content"
summary(lme(SQ~Target, random=~1|Content, data=a.ad.m))

```

```

## Linear mixed-effects model fit by REML
## Data: a.ad.m
##      AIC      BIC    logLik
## 288.8346 290.0449 -140.4173
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      360122.7 220083.6
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF   t-value p-value
## (Intercept) 1323926.5  270031.4   9   4.902861  0.0008
## TargetIGF2  -56522.5  127065.4   9  -0.444830  0.6669
## Correlation:
##      (Intr)
## TargetIGF2 -0.235
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.13385337 -0.75569209 -0.03853511  0.62701599  1.65177916
##
## Number of Observations: 12
## Number of Groups: 2

```

```

summary(lme(SQ~Target, random=~1|Content, data=a.ad.f))

```

```

## Linear mixed-effects model fit by REML
## Data: a.ad.f
##      AIC      BIC    logLik
## 243.7126 244.9229 -117.8563
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      110030.4 20532.49
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF   t-value p-value
## (Intercept) 363400.7  78253.50   9   4.64389  0.0012
## TargetIGF2  308578.4  11854.44   9 26.03062  0.0000
## Correlation:
##      (Intr)
## TargetIGF2 -0.076
##

```

```
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.4440210 -0.6238506 -0.1488470  0.6935578  1.6278526
##
## Number of Observations: 12
## Number of Groups: 2
```

Mouse Statistics

```
#Subset data to include only mouse samples
mouse=subset(sp, Species == "House Mouse")
```

```
#Create a data frame for each age class
m.emb=subset(mouse, Age=="Embryonic")
m.juv=subset(mouse, Age=="Juvenile")
m.ad=subset(mouse, Age=="Adult")
```

```
#Run linear model on relative IGF1 and IGF2 expression levels at each age class, using a random variable
summary(lme(SQ~Target, random=~1|Content, data=m.emb))
```

```
## Linear mixed-effects model fit by REML
## Data: m.emb
##      AIC      BIC    logLik
##  562.0491 565.382 -277.0245
##
## Random effects:
## Formula: ~1 | Content
##      (Intercept) Residual
## StdDev:      8380785  1674676
##
## Fixed effects: SQ ~ Target
##              Value Std.Error DF  t-value p-value
## (Intercept) -3803299   4247790 14  -0.89536  0.3857
## TargetIGF2  16312472   847373 14  19.25065  0.0000
## Correlation:
##      (Intr)
## TargetIGF2 -0.135
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.21914615 -0.63961092 -0.06928286  0.80910591  1.55551594
##
## Number of Observations: 19
## Number of Groups: 4
```

```
summary(lme(SQ~Target, random=~1|Content, data=m.juv))
```

```
## Linear mixed-effects model fit by REML
## Data: m.juv
##      AIC      BIC    logLik
##  574.3555 577.917 -283.1778
```

```
##
## Random effects:
## Formula: ~1 | Content
## (Intercept) Residual
## StdDev:      1145499 1272705
##
## Fixed effects: SQ ~ Target
## Value Std.Error DF t-value p-value
## (Intercept) 5755705 735305.5 15 7.827637 0
## TargetIGF2 -5648981 606787.2 15 -9.309657 0
## Correlation:
## (Intr)
## TargetIGF2 -0.484
##
## Standardized Within-Group Residuals:
## Min Q1 Med Q3 Max
## -1.61178099 -0.68757465 -0.02427843 0.93291643 1.29370504
##
## Number of Observations: 20
## Number of Groups: 4
```

```
summary(lme(SQ~Target*Sex, random=~1|Content, data=m.ad))
```

```
## Linear mixed-effects model fit by REML
## Data: m.ad
## AIC BIC logLik
## 1161.44 1171.265 -574.7199
##
## Random effects:
## Formula: ~1 | Content
## (Intercept) Residual
## StdDev:      478191.7 720685.6
##
## Fixed effects: SQ ~ Target * Sex
## Value Std.Error DF t-value p-value
## (Intercept) 3685961 349414.2 32 10.548972 0.0000
## TargetIGF2 -3684151 328946.5 32 -11.199850 0.0000
## SexMale 16741 481531.9 6 0.034766 0.9734
## TargetIGF2:SexMale -15786 451778.6 32 -0.034942 0.9723
## Correlation:
## (Intr) TrIGF2 SexMal
## TargetIGF2 -0.565
## SexMale -0.726 0.410
## TargetIGF2:SexMale 0.411 -0.728 -0.540
##
## Standardized Within-Group Residuals:
## Min Q1 Med Q3 Max
## -2.03139347 -0.55419647 0.09384146 0.37740313 2.24847285
##
## Number of Observations: 42
## Number of Groups: 8
```