Distribution, RS ratio and patterns of SHM

Mikhail Shugay, Anna Obraztsova

11/17/2016

Analysis of substitution type and frequency

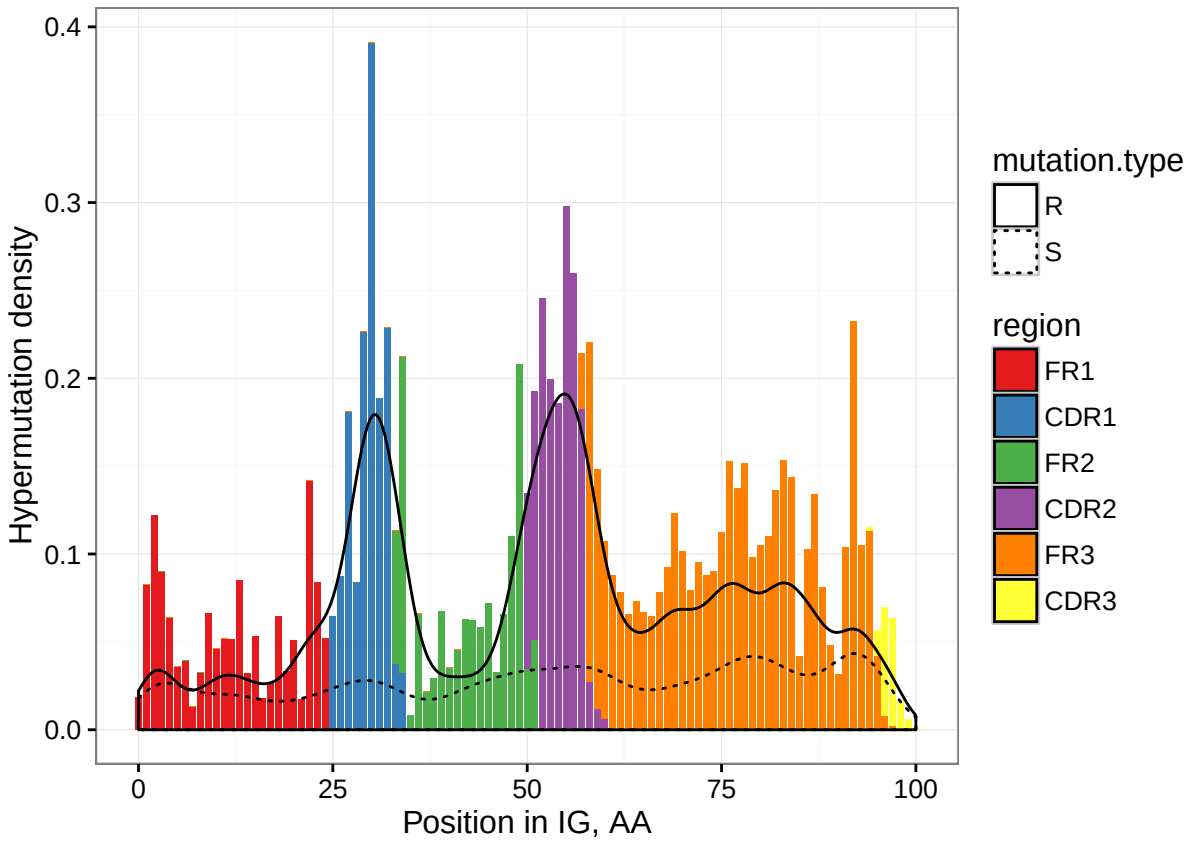
Load preprocessed data

```
library(plyr)
library(ggplot2)
load("sp.Rda")
df <- subset(df, type == "RNA")
df$region <- factor(df$region, c("FR1","CDR1","FR2","CDR2","FR3","CDR3"))
df$mutation.type <- ifelse(df$from.aa == df$to.aa, "S", "R")
df <- ddply(df, .(proj, type, sample, replica), transform,
            weight = total.clonotypes / sum(total.clonotypes))
```

Check if we observe well-documented increase in replacement:synonimic hypermutation ratio in CDR regions:

```
ggplot(df) +
  geom_bar(aes(x=pos.aa, weight=weight, fill=region)) +
  geom_density(aes(x=pos.aa, weight=weight, linetype = mutation.type)) +
  ylab("Hypermutation density") +
  xlab("Position in IG, AA") +
  scale_fill_brewer(palette = "Set1") +
  theme_bw()
```

```
## Warning in density.default(x, weights = w, bw = bw, adjust = adjust, kernel
## = kernel, : sum(weights) != 1 -- will not get true density

## Warning in density.default(x, weights = w, bw = bw, adjust = adjust, kernel
## = kernel, : sum(weights) != 1 -- will not get true density
```



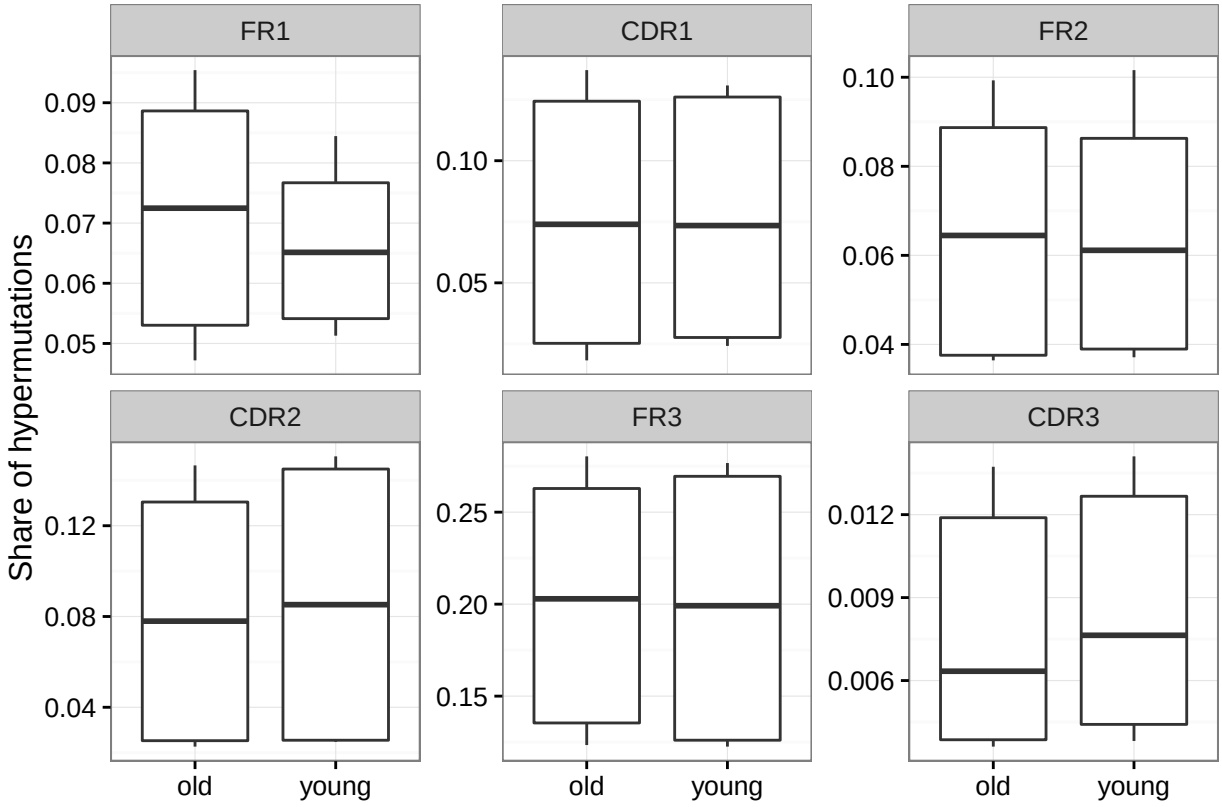
Comparative analysis

Summarize by region and type, compute frequencies and R:S ratio

```
df.1 <- ddply(df, .(proj, sample, type, replica, mutation.type, region), summarize,
              count = sum(total.clonotypes))

df.1 <- ddply(df.1, .(proj, sample, type, replica), transform,
              freq = count / sum(count))

df.1 <- ddply(df.1, .(proj, sample, type, replica, region), transform,
              ratio = count / (sum(count) - count))
```

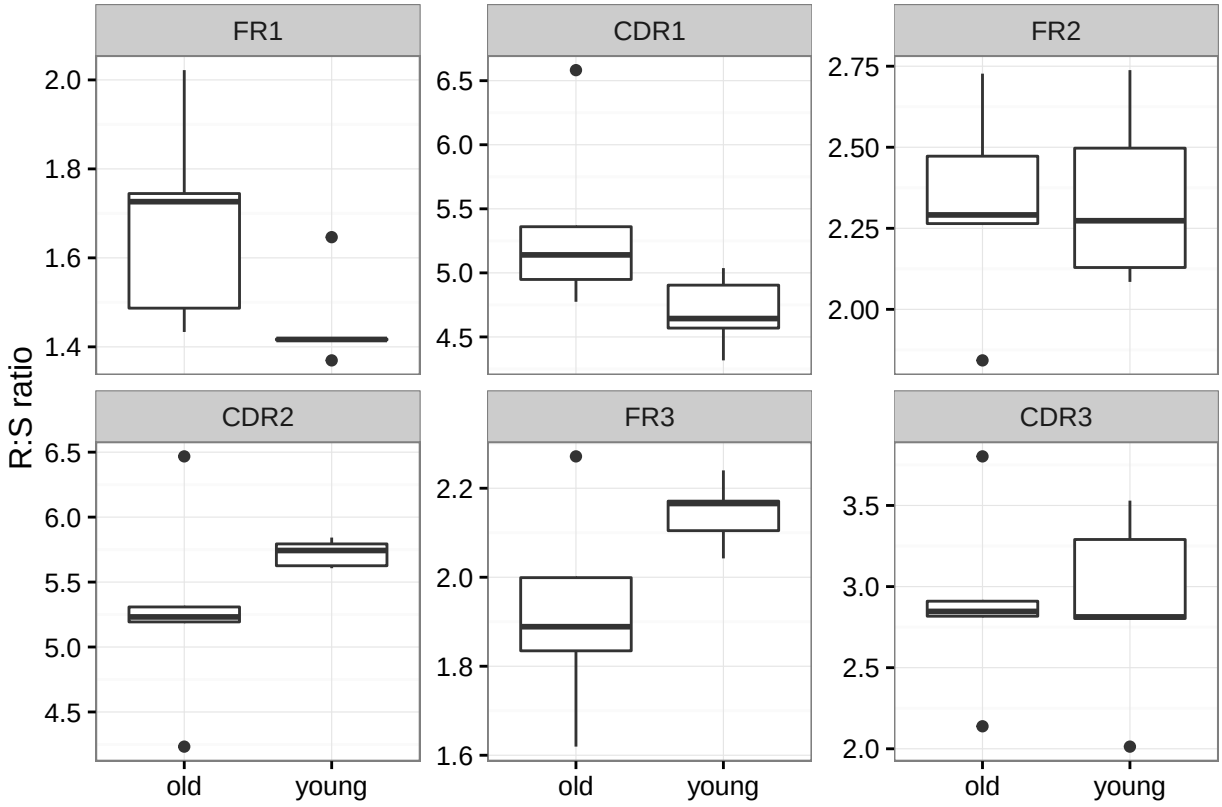
Fraction of errors in each region

```
ggplot(df.1, aes(x=proj, y = freq)) + geom_boxplot() +
  facet_wrap(~region, scales = "free_y") +
  xlab("") + ylab("Share of hypermutations") +
  theme_bw()
```



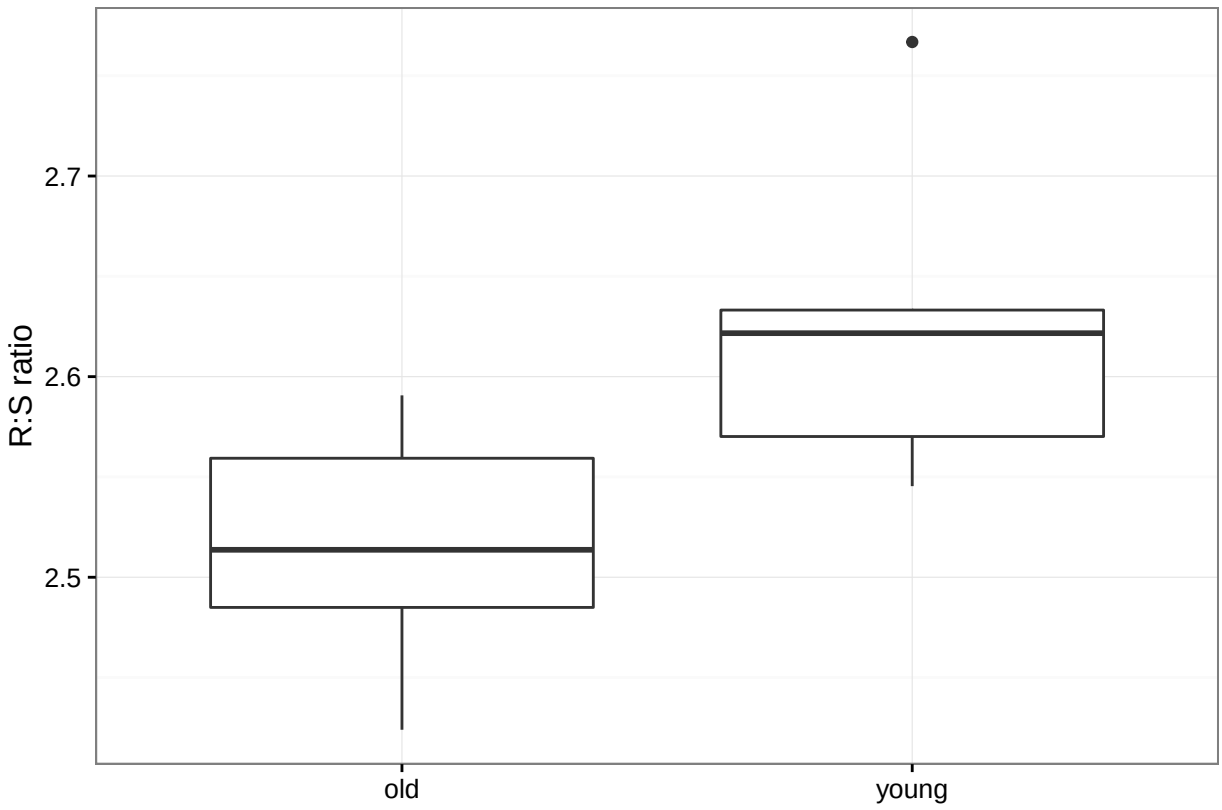
R:S ratio varies greatly by region

```
ggplot(subset(df.1, mutation.type == "R"), aes(x=proj, y = ratio)) +
  geom_boxplot() +
  facet_wrap(~region, scales = "free_y") +
  xlab("") + ylab("R:S ratio") +
  theme_bw()
```



Overall R:S ratio is higher in young

```
df.2 <- ddply(df, .(proj, sample, type, replica, mutation.type), summarize,
              count = sum(total.clonotypes))

df.2 <- ddply(df.2, .(proj, sample, type, replica), transform,
              ratio = count / (sum(count) - count))

ggplot(subset(df.2, mutation.type == "R"), aes(x=proj, y = ratio)) +
  geom_boxplot() +
  xlab("") + ylab("R:S ratio") +
  theme_bw()
```




```
t.test(ratio ~ proj, subset(df.2, mutation.type == "R"))
```

```
##
##  Welch Two Sample t-test
##
## data:  ratio by proj
## t = -2.3449, df = 7.4479, p-value = 0.04934
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.2254143215 -0.0004259294
## sample estimates:
##   mean in group old mean in group young
##            2.514525            2.627445
```

Role of age factor can be deduced using generalized linear model for replacement hypermutation probability (binomial family). Probability of replacement hypermutations is increased by $7 \pm 1\%$ in young compared to old ($P < 10^{-6}$)

```
df$R <- ifelse(df$mutation.type == "R", 1, 0)
fit <- glm(R ~ region + proj - 1, df, family = binomial())

summary(fit)
```

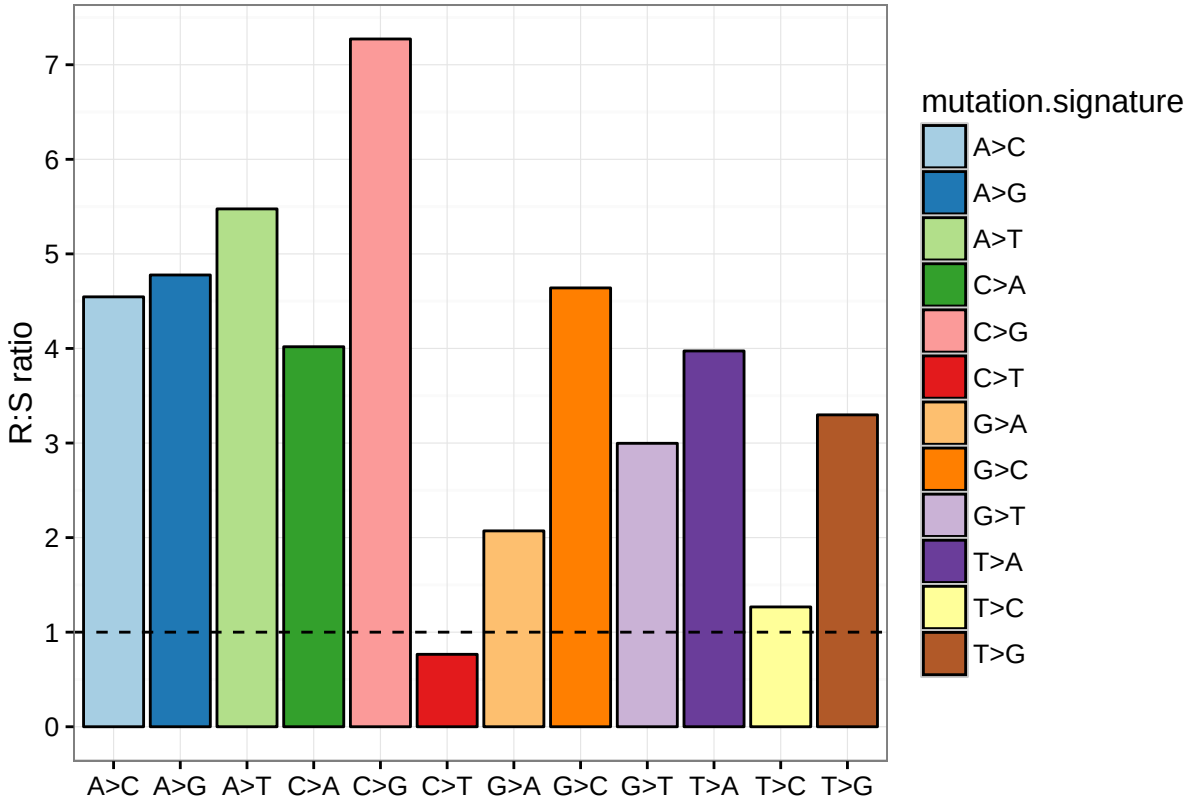
```
##
## Call:
## glm(formula = R ~ region + proj - 1, family = binomial(), data = df)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -2.0091   0.5340   0.5869   0.7584   0.9266
```

```
##
## Coefficients:
##            Estimate Std. Error z value Pr(>|z|)
## regionFR1   0.62327    0.01715  36.340  < 2e-16 ***
## regionCDR1  1.67169    0.02189  76.374  < 2e-16 ***
## regionFR2   0.90704    0.01953  46.433  < 2e-16 ***
## regionCDR2  1.80560    0.02179  82.861  < 2e-16 ***
## regionFR3   1.09888    0.01306  84.153  < 2e-16 ***
## regionCDR3  0.67021    0.04672  14.345  < 2e-16 ***
## projyoung   0.07011    0.01376   5.094 3.52e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 169990  on 122622  degrees of freedom
## Residual deviance: 129749  on 122615  degrees of freedom
## AIC: 129763
##
## Number of Fisher Scoring iterations: 4
```

Substitution patterns

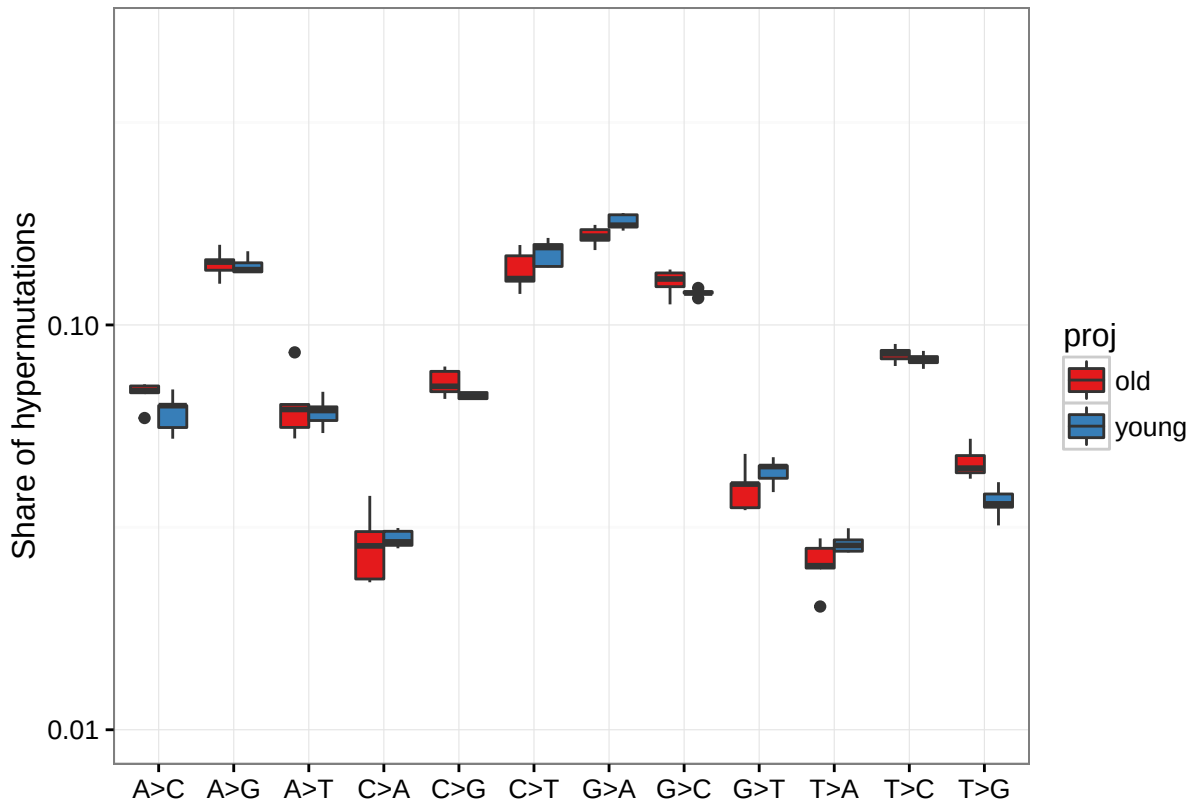
R:S ratio across different substitution patterns at nucleotide level

```
df$mutation.signature <- paste(df$from.nt, df$to.nt, sep = ">")

df.3 <- ddply(df, .(mutation.signature, mutation.type), summarize, count = sum(total.clonotypes))
df.3 <- ddply(df.3, .(mutation.signature), transform, ratio = count / (sum(count) - count))
df.3 <- subset(df.3, mutation.type == "R")

ggplot(df.3, aes(x = mutation.signature, y = ratio, fill = mutation.signature)) +
  geom_bar(stat="identity", color="black") +
  geom_hline(yintercept = 1, linetype="dashed") +
  scale_fill_brewer(palette = "Paired") +
  scale_y_continuous("R:S ratio", breaks=0:8) +
  xlab("") +
  theme_bw()
```



Share of different substitution patterns compared between young and old. Age-related difference observed for certain substitution patterns aross hypermutations.

```
df.4 <- ddply(df, .(proj, sample, type, replica, mutation.signature), summarize,
              count = sum(total.clonotypes))

df.4 <- ddply(df.4, .(proj, sample, type, replica), transform,
              share = count / sum(count))

ggplot(df.4, aes(x = mutation.signature, group = interaction(mutation.signature, proj),
                 y = share, fill = proj)) +
  geom_boxplot() + scale_y_log10(limits = c(0.01, 0.5)) +
  scale_fill_brewer(palette = "Set1") +
  xlab("") + ylab("Share of hypermutations") +
  theme_bw()
```



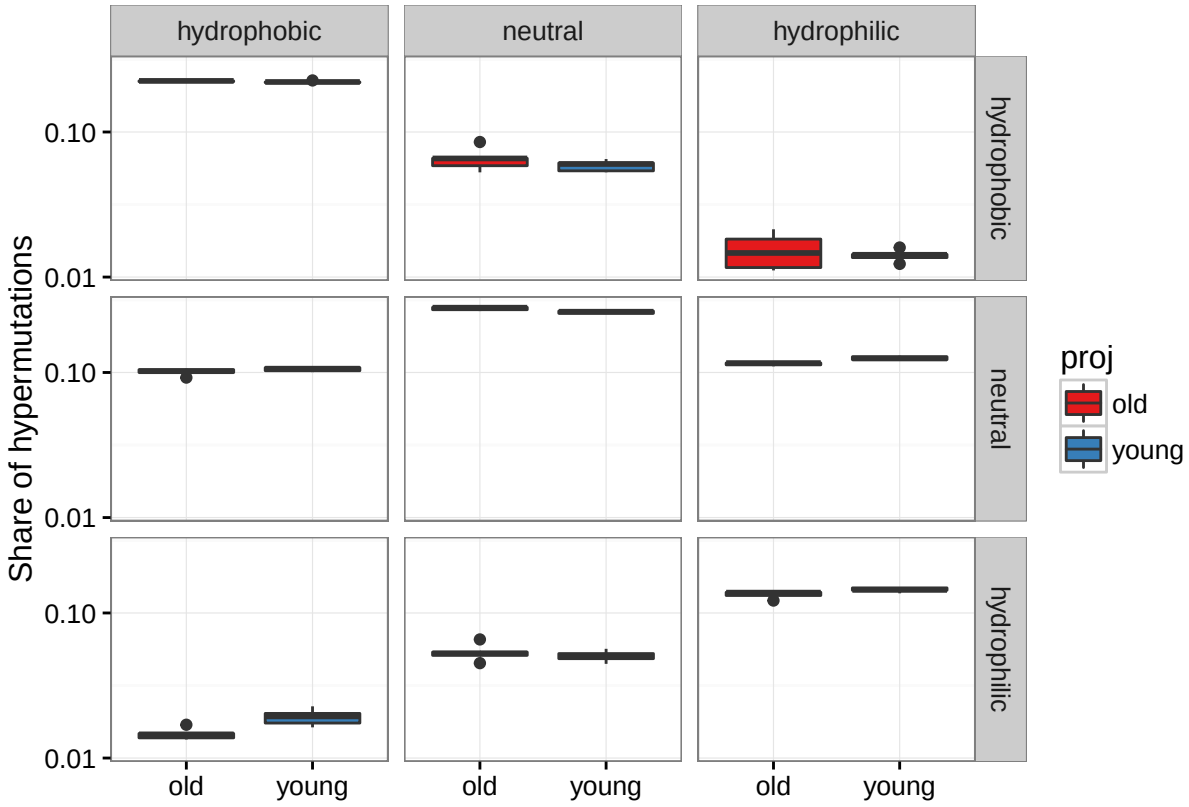

```
a <- aov(share ~ mutation.signature * proj, df.4)
summary(a)
```

```
##                          Df  Sum Sq  Mean Sq F value  Pr(>F)
## mutation.signature       11 0.27207 0.024734 472.036 < 2e-16 ***
## proj                      1 0.00000 0.000000   0.000 1.00000
## mutation.signature:proj 11 0.00164 0.000149   2.838 0.00296 **
## Residuals                96 0.00503 0.000052
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

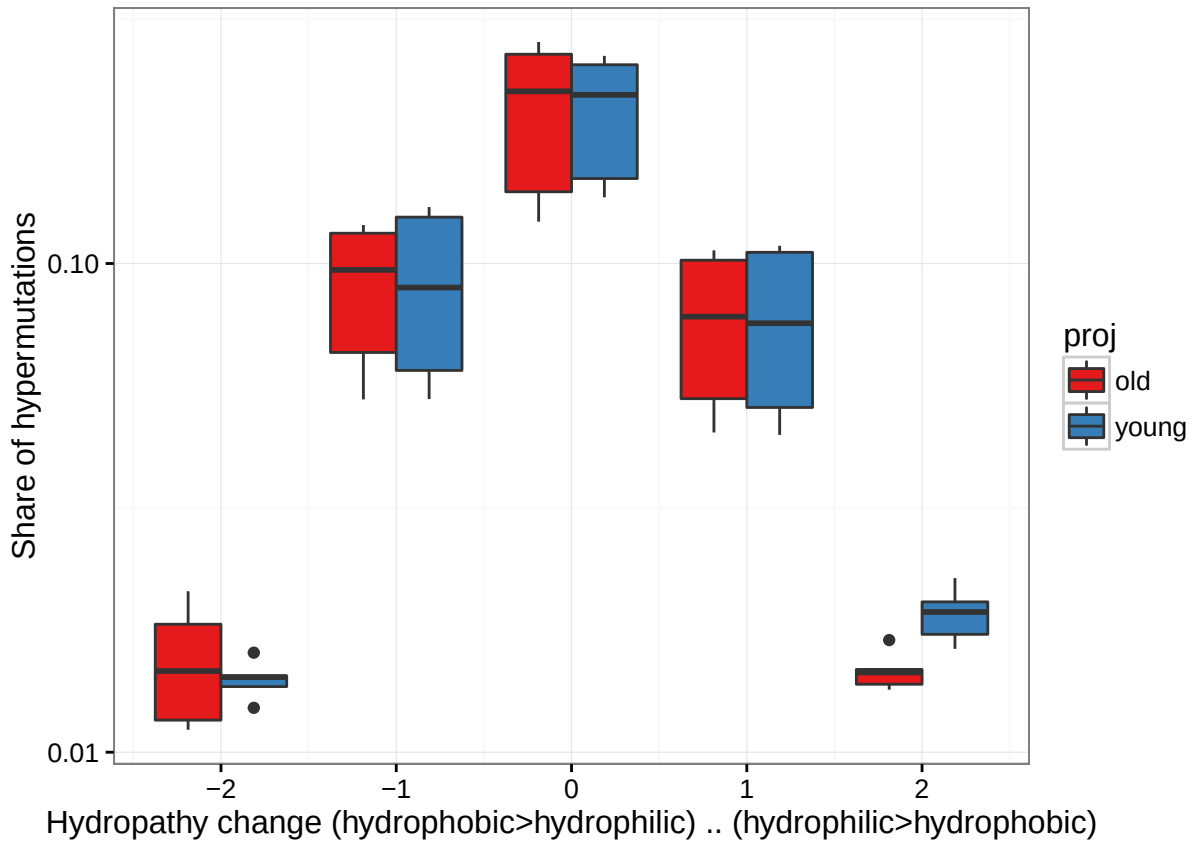
Hydropathy change patterns observed at amino acid level. More hydrophilic -> hydrophobic amino acid hypermutations in young compared to old.

```
aa.classes <- data.frame(aa = strsplit("I,V,L,F,C,M,A,W,G,T,S,Y,P,H,N,D,Q,E,K,R", ",")[[1]],
                         hydrop = c(rep("hydrophobic", 8), rep("neutral", 6),
                                    rep("hydrophilic", 6)))

aa.classes$hydrop <- factor(aa.classes$hydrop, c("hydrophobic", "neutral", "hydrophilic"))

df.5 <- ddply(df, .(proj, sample, type, replica, from.aa, to.aa), summarize,
              count = sum(total.clonotypes))

df.5 <- merge(df.5, aa.classes, by.x = "from.aa", by.y = "aa")
df.5 <- merge(df.5, aa.classes, by.x = "to.aa", by.y = "aa")
#df.5$signature <- paste(df.5$hydrop.x, df.5$hydrop.y, sep = ">")

df.5 <- ddply(df.5, .(proj, sample, type, replica, hydrop.x, hydrop.y), summarize,
              count = sum(count))
```

```
df.5 <- ddply(df.5, .(proj, sample, type, replica), transform,
              share = count / sum(count))

ggplot(df.5, aes(x=proj, group=proj, y=share, fill = proj)) + geom_boxplot() +
  facet_grid(hydrop.x~hydrop.y) + scale_y_log10() +
  scale_fill_brewer(palette = "Set1") +
  xlab("") + ylab("Share of hypermutations") +
  theme_bw()
```




```
hydrop_toint <- function(x) {
  ifelse(x == "hydrophobic", 1, ifelse(x == "neutral", 0, -1))
}

df.5$hydrop.change <- with(df.5, hydrop_toint(hydrop.y) - hydrop_toint(hydrop.x))

ggplot(df.5, aes(x=hydrop.change, group = interaction(hydrop.change, proj), y=share,
                 fill=proj)) +
  geom_boxplot() + scale_y_log10() +
  xlab("Hydropathy change (hydrophobic>hydrophilic) .. (hydrophilic>hydrophobic)") +
  ylab("Share of hypermutations") +
  scale_fill_brewer(palette = "Set1") +
  theme_bw()
```




```
a <- aov(share ~ hydrop.x : hydrop.y + hydrop.x : hydrop.y : proj, df.5)
summary(a)
```

```
##                       Df Sum Sq Mean Sq  F value   Pr(>F)
## hydrop.x:hydrop.y      8 0.6215 0.07769 2534.676  < 2e-16 ***
## hydrop.x:hydrop.y:proj  9 0.0014 0.00016    5.072 2.65e-05 ***
## Residuals              72 0.0022 0.00003
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
t.test(share ~ proj,
       subset(df.5, hydrop.y == "hydrophobic" & hydrop.x == "hydrophilic"))
```

```
##
##  Welch Two Sample t-test
##
## data:  share by proj
## t = -3.5222, df = 6.2061, p-value = 0.01181
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.007634405 -0.001404925
## sample estimates:
##   mean in group old mean in group young
##          0.01469946          0.01921912
```