

# qpCR Transporter Figures

C-T Berezin

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```
library(tidyverse)
library(ggplot2)
library(ggthemes)
library(forcats)
library(ggpubr)
#library(writexl)
library(emmeans)
library(lme4)
library(car)
library(lmerTest)
library(svglite)
```

```
transporters <- read.csv("../data/qRT-PCR/07222022-transporter-gene-study-1inj-only.csv", fileEncoding = "UTF-8")

#transporters <- transporters %>% filter(Sample != "calib")

transporters <- transporters %>% mutate(Tissue = str_sub(Sample, start=-3L, end=-1L),
                                       Treatment = str_extract(Sample, "[:alpha:]+(?:[:digit:])"),
                                       Sample = str_extract(Sample, "[:alnum:]+(?:[-])"))

transporters <- transporters %>% mutate(Treatment = factor(Treatment, c("M", "Flo", "Phi")),
                                       Tissue = factor(Tissue, c("ret", "hyp")))

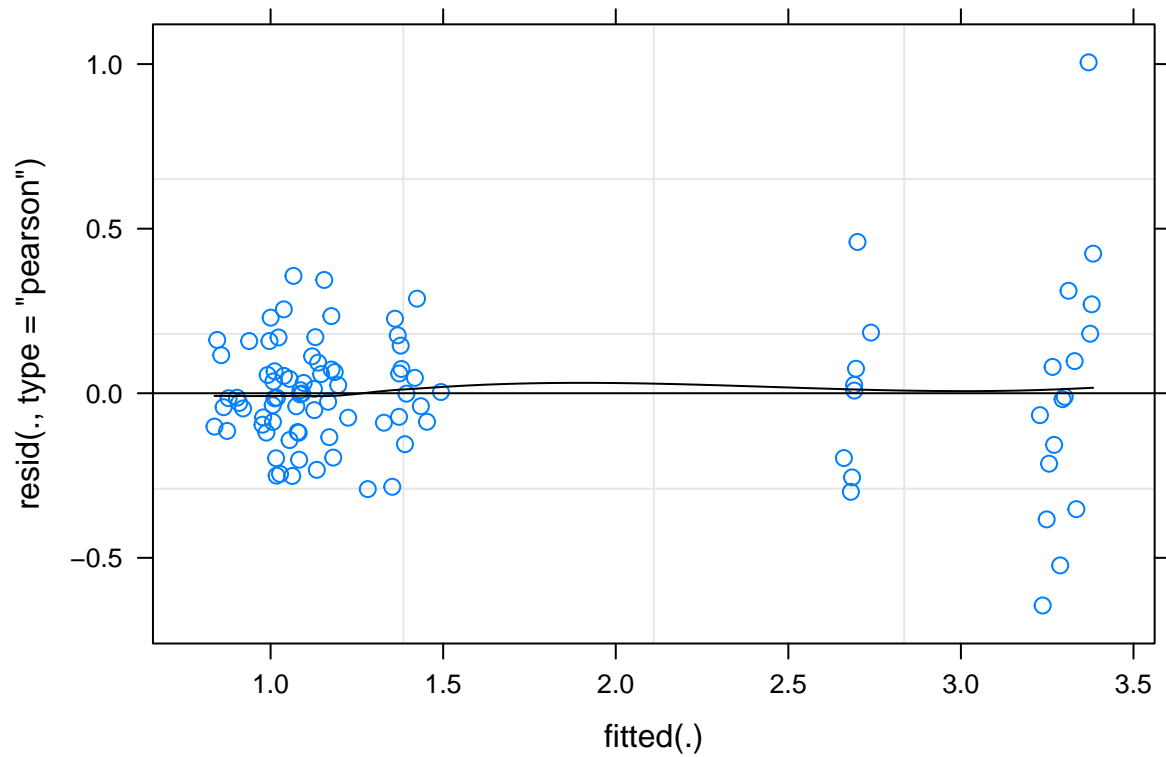
transporters_long <- transporters %>% pivot_longer(cols=c("Pgp.RGE", "Bcrp.RGE"), names_to = "gene", values_to = "rge")

transporters_long <- transporters_long %>% mutate(gene = str_extract(gene, "[:alpha:]+(?:[-])"))
transporters_long <- transporters_long %>% mutate(gene = factor(as.factor(gene), c("Pgp", "Bcrp")))
transporters_long
```

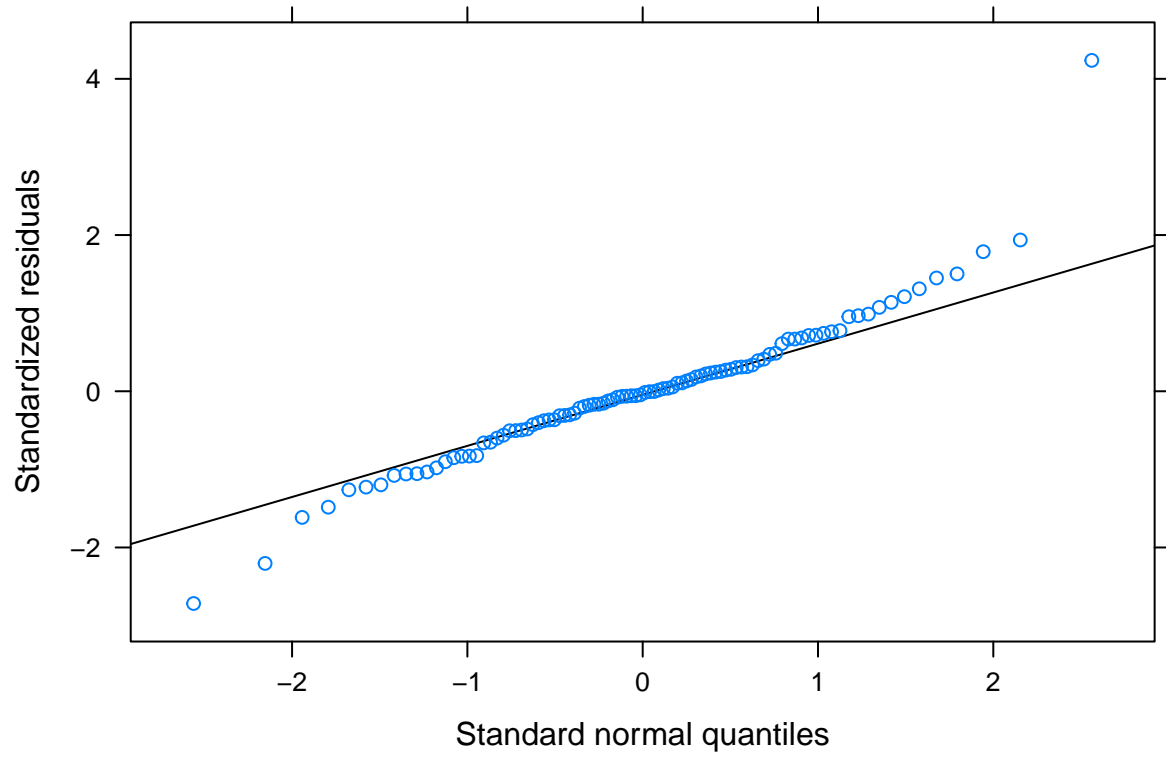
```
## # A tibble: 96 x 5
##   Sample Tissue Treatment gene    rge
##   <chr>   <fct>   <fct>   <fct> <dbl>
## 1 Phi10  hyp    Phi     Pgp   4.38
## 2 Phi10  hyp    Phi     Bcrp  1.50
## 3 Phi10  ret    Phi     Pgp   1.22
## 4 Phi10  ret    Phi     Bcrp  0.905
## 5 Phi11  hyp    Phi     Pgp   3.29
## 6 Phi11  hyp    Phi     Bcrp  1.71
## 7 Phi11  ret    Phi     Pgp   1.08
## 8 Phi11  ret    Phi     Bcrp  0.880
## 9 Phi1   hyp    Phi     Pgp   3.11
```

```
## 10 Fhi1 hyp Fhi Bcrp 1.39
## # ... with 86 more rows
```

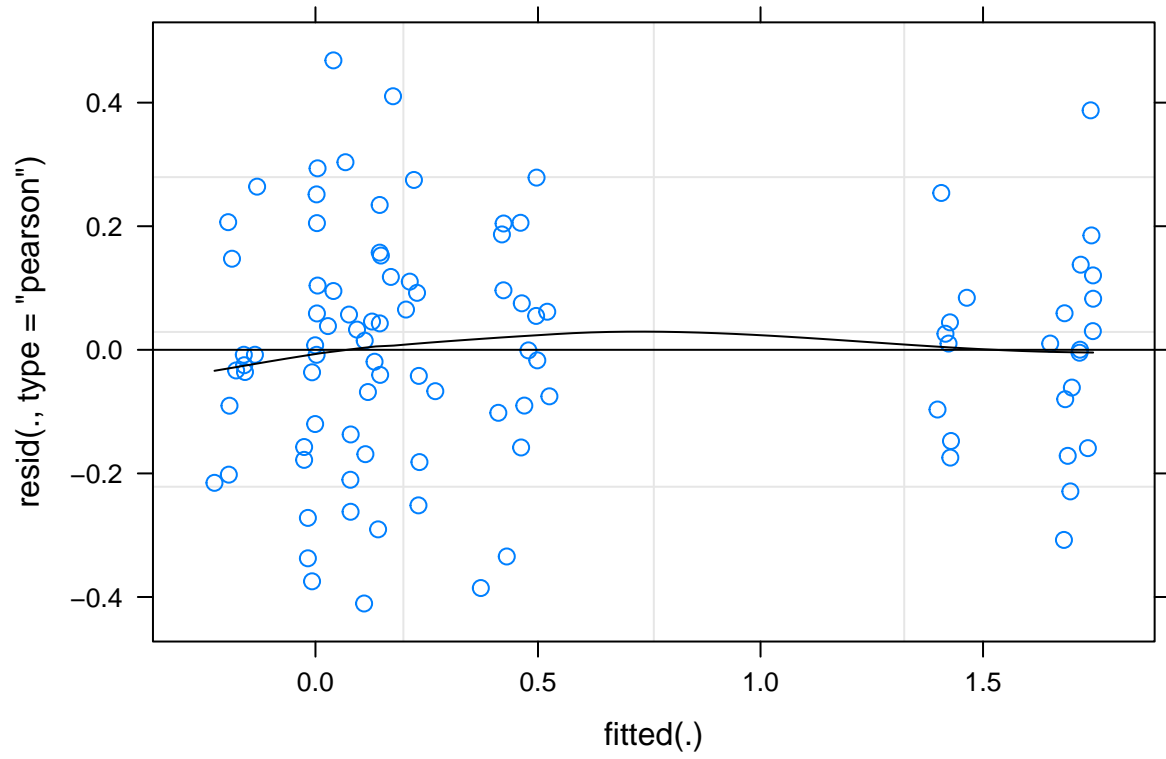
```
transporter_lm <- lmer(rge ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_lm, type=c("p","smooth"), col.line=1)
```



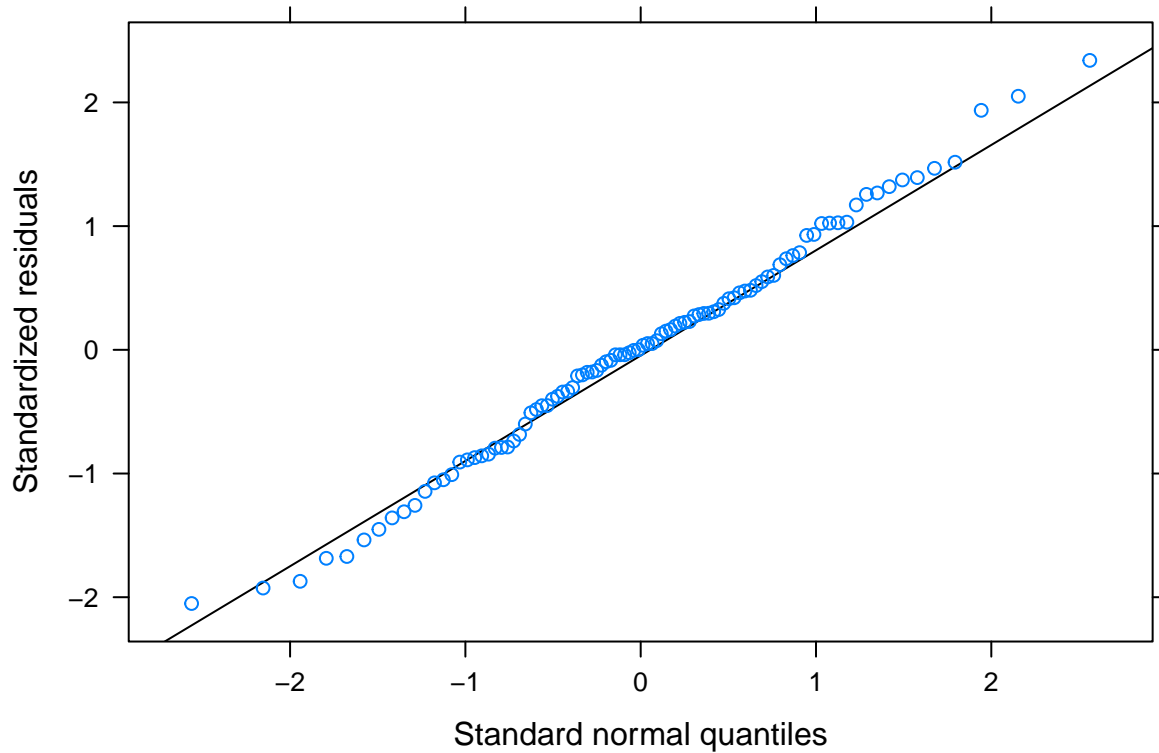
```
lattice::qqmath(transporter_lm)
```



```
transporter_log2_lm <- lmer(log2(rge) ~ gene * Tissue * Treatment + (1|Sample), data=transporters_long)
plot(transporter_log2_lm, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(transporter_log2_lm)
```



```
shapiro.test(transporters_long$rge)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  transporters_long$rge
## W = 0.77877, p-value = 1.044e-10
```

```
shapiro.test(log2(transporters_long$rge))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  log2(transporters_long$rge)
## W = 0.87148, p-value = 1.285e-07
```

```
anova(transporter_log2_lm)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##
```

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
## gene	10.1573	10.1573	1	63	253.4878	< 2e-16 ***
## Tissue	20.4143	20.4143	1	63	509.4664	< 2e-16 ***
## Treatment	0.3434	0.1717	2	21	4.2854	0.02749 *
## gene:Tissue	6.8233	6.8233	1	63	170.2831	< 2e-16 ***

```
## gene:Treatment      0.1278 0.0639    2   63   1.5945 0.21109
## Tissue:Treatment    0.3872 0.1936    2   63   4.8315 0.01117 *
## gene:Tissue:Treatment 0.1405 0.0703    2   63   1.7536 0.18149
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(transporter_log2_lm, pairwise ~ Treatment | Tissue, by="gene")$contrasts
```

```
## Tissue = ret, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.1312 0.1180 83  -1.111  0.5098
## M - Fhi      -0.1306 0.0962 83  -1.357  0.3680
## Flo - Fhi      0.0006 0.1117 83   0.005  1.0000
##
## Tissue = hyp, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.3108 0.1180 83  -2.633  0.0270
## M - Fhi      -0.2798 0.0962 83  -2.909  0.0128
## Flo - Fhi      0.0310 0.1117 83   0.277  0.9585
##
## Tissue = ret, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.0658 0.1180 83  -0.557  0.8430
## M - Fhi       0.1748 0.0962 83   1.818  0.1702
## Flo - Fhi      0.2406 0.1117 83   2.155  0.0852
##
## Tissue = hyp, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## M - Flo      -0.1804 0.1180 83  -1.528  0.2830
## M - Fhi      -0.2530 0.0962 83  -2.630  0.0272
## Flo - Fhi     -0.0726 0.1117 83  -0.651  0.7926
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
emmeans::emmeans(transporter_log2_lm, pairwise ~ Tissue | Treatment, by="gene")$contrasts
```

```
## Treatment = M, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.423 0.1001 63 -14.219  <.0001
##
## Treatment = Flo, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.603 0.1266 63 -12.660  <.0001
##
## Treatment = Fhi, gene = Pgp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -1.572 0.0854 63 -18.422  <.0001
##
## Treatment = M, gene = Bcrp:
## contrast estimate      SE df t.ratio p.value
## ret - hyp     -0.229 0.1001 63  -2.287  0.0255
```

```
##
## Treatment = Flo, gene = Bcrp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.344 0.1266 63 -2.714 0.0086
##
## Treatment = Fhi, gene = Bcrp:
## contrast estimate SE df t.ratio p.value
## ret - hyp -0.657 0.0854 63 -7.695 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

```
emmeans::emmeans(transporter_log2_lm, pairwise ~ gene | Treatment, by="Tissue")$contrasts
```

```
## Treatment = M, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0000 0.1001 63 0.000 1.0000
##
## Treatment = Flo, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.0654 0.1266 63 0.516 0.6073
##
## Treatment = Fhi, Tissue = ret:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 0.3054 0.0854 63 3.578 0.0007
##
## Treatment = M, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.1942 0.1001 63 11.932 <.0001
##
## Treatment = Flo, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.3247 0.1266 63 10.463 <.0001
##
## Treatment = Fhi, Tissue = hyp:
## contrast estimate SE df t.ratio p.value
## Pgp - Bcrp 1.2211 0.0854 63 14.306 <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log2 (not the response) scale.
```

```
trans_sumstats <- transporters_long %>% group_by(Treatment, Tissue, gene) %>% summarise(
  n = n(),
  mean = mean(rge),
  sd = sd(rge),
  se = sd/sqrt(n),
  log2_mean = mean(log2(rge)),
  log2_sd = sd(log2(rge)),
  log2_se = log2_sd/sqrt(n)
)
```

```
## 'summarise()' has grouped output by 'Treatment', 'Tissue'. You can override
## using the '.groups' argument.
```

```
trans_sumstats
```

```
## # A tibble: 12 x 10
## # Groups:   Treatment, Tissue [6]
##   Treatment Tissue gene      n mean    sd    se log2_mean log2_sd log2_se
##   <fct>      <fct> <fct> <int> <dbl> <dbl> <dbl>    <dbl>    <dbl>    <dbl>
## 1 M        ret    Pgp      8 1.02  0.220 0.0779 2.02e-10  0.305  0.108
## 2 M        ret    Bcrp      8 1.01  0.142 0.0501 6.58e-11  0.202  0.0713
## 3 M        hyp    Pgp      8 2.69  0.266 0.0942 1.42e+ 0  0.141  0.0498
## 4 M        hyp    Bcrp      8 1.18  0.133 0.0470 2.29e- 1  0.163  0.0576
## 5 Flo      ret    Pgp      5 1.10  0.143 0.0638 1.31e- 1  0.190  0.0848
## 6 Flo      ret    Bcrp      5 1.06  0.156 0.0699 6.58e- 2  0.209  0.0935
## 7 Flo      hyp    Pgp      5 3.35  0.452 0.202  1.73e+ 0  0.201  0.0897
## 8 Flo      hyp    Bcrp      5 1.35  0.232 0.104  4.09e- 1  0.268  0.120
## 9 Fhi      ret    Pgp     11 1.11  0.187 0.0565 1.31e- 1  0.242  0.0731
## 10 Fhi     ret    Bcrp     11 0.891 0.105 0.0316 -1.75e- 1  0.168  0.0507
## 11 Fhi     hyp    Pgp     11 3.28  0.457 0.138  1.70e+ 0  0.193  0.0580
## 12 Fhi     hyp    Bcrp     11 1.41  0.172 0.0519 4.82e- 1  0.182  0.0548
```

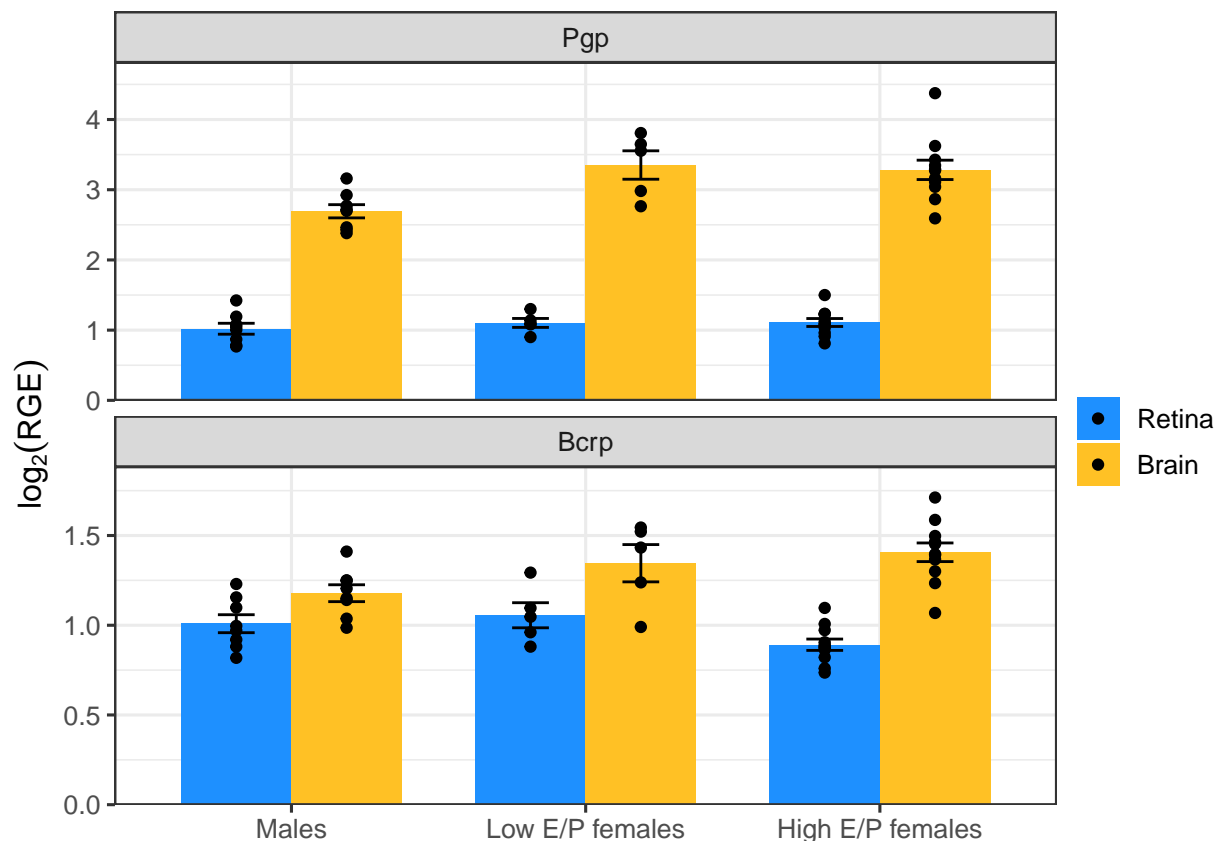
Comparing hyp vs ret within each treatment/gene

```
trans_hyp_ret_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-se, ymax=mean+se, fill=Tissue),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, scales="free_y", ncol=1) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw(12) %+replace%
  theme(legend.title = element_blank(),
    legend.box.spacing = unit(1, "pt"),
    axis.title.x=element_blank()) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
    labels=c("ret" = "Retina", "hyp" = "Brain")) +
  scale_x_discrete(labels=c("M" = "Males", "Flo" = "Low E/P females",
    "Fhi" = "High E/P females")) +
  ylab(expression(log[2](RGE)))
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_hyp_ret_plot
```





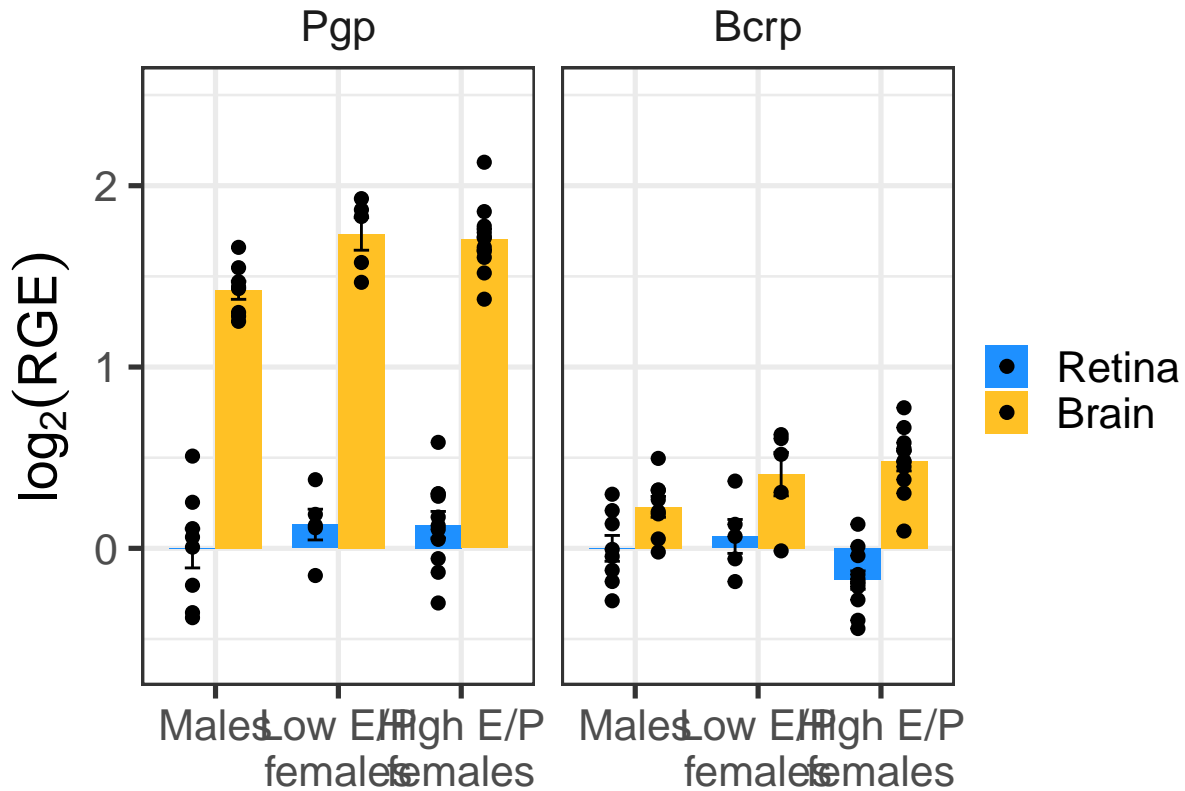
```
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_1inj.png", plot=trans_hyp_ret_plot, height=4, wid
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_1inj.svg", plot=trans_hyp_ret_plot, height=4, wid
```

```
trans_hyp_ret_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=log2_mean-log2_se,
    ymax=log2_mean+log2_se, fill=Tissue),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=Tissue),
    size=2, stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, ncol=2) +
  #scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  ylim(-0.6, 2.5) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw(20) %+replace%
  theme(legend.title = element_blank(),
    legend.box.spacing = unit(1, "pt"),
    axis.title.x=element_blank(),
    strip.background = element_rect(color="transparent", fill="transparent",
      size=0, linetype="solid")
  ) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
    labels=c("ret" = "Retina", "hyp" = "Brain")) +
  scale_x_discrete(labels=c("M" = "Males", "Flo" = "Low E/P\nfemales",
    "Phi" = "High E/P\nfemales")) +
```

```
ylab(expression(log2(RGE)))
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_hyp_ret_log_plot
```



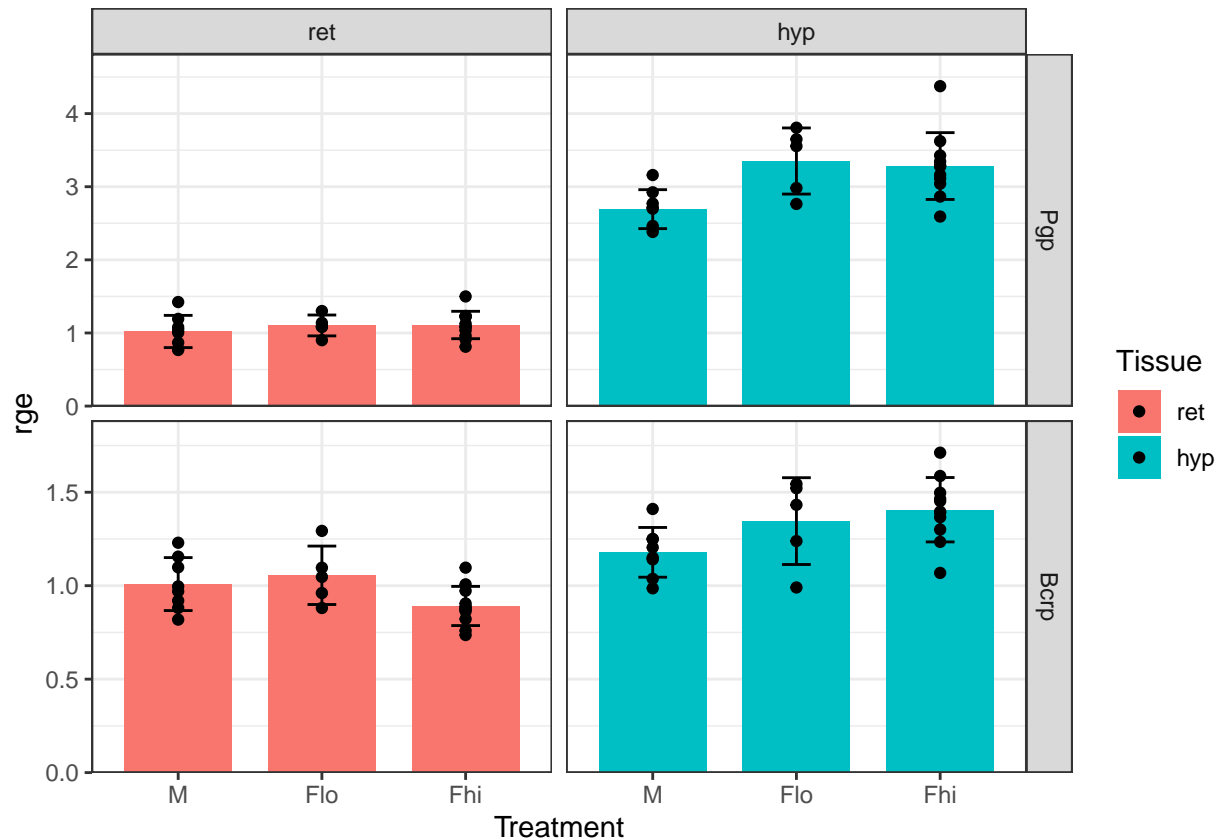
```
ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log_inj_large.png", plot=trans_hyp_ret_log_plot, height=10, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_hyp_ret_log_inj.svg", plot=trans_hyp_ret_log_plot, height=10, width=10)
```

## Comparing treatments within each tissue/gene

```
trans_trt_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=mean-sd, ymax=mean+sd, fill=Tissue),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=rge, fill=Tissue),
    stat="identity", position=position_dodge(width=0.75)) +
  facet_grid(gene~Tissue, scales="free_y") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_trt_plot
```



```
#ggsave(filename="../../figures/qRT-PCR/trans_trt_1inj.png", plot=trans_trt_plot, height=4, width=4)
#ggsave(filename="../../figures/qRT-PCR/trans_trt_1inj.svg", plot=trans_trt_plot, height=4, width=4)
```

```
trans_trt_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Tissue, y=log2(rge), fill=Treatment),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Tissue, ymin=log2_mean-log2_se,
    ymax=log2_mean+log2_se, fill=Treatment),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Tissue, y=log2(rge), fill=Treatment),
    size=2, stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~gene, ncol=2) +
  #scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  ylim(-0.6, 2.5) +
  #scale_fill_manual(values=c("maroon1", "springgreen3")) +
  theme_bw(20) %+replace%
  theme(legend.title = element_blank(),
    legend.box.spacing = unit(1, "pt"),
    axis.title.x=element_blank(),
    strip.background = element_rect(color="transparent", fill="transparent",
```

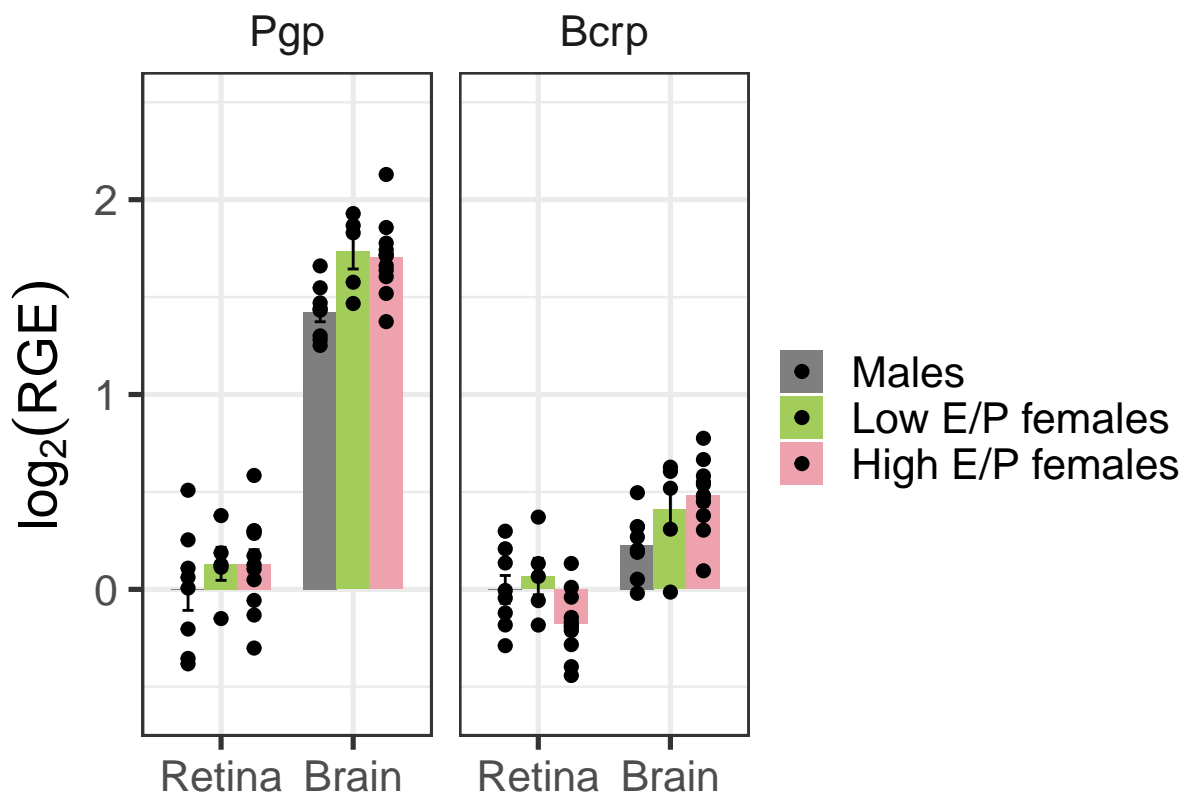
```

    size=0, linetype="solid")
  ) +
  scale_fill_manual(values=c("gray50", "darkolivegreen3", "lightpink2"),
    labels=c("M" = "Males", "Flo" = "Low E/P females",
      "Phi" = "High E/P females")) +
  scale_x_discrete(labels=c("ret" = "Retina", "hyp" = "Brain")) +
  ylab(expression(log[2](RGE)))

```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_trt_log_plot
```



```

ggsave(filename="../../figures/qRT-PCR/trans_trt_log_1inj_large.png", plot=trans_trt_log_plot, height=6
#ggsave(filename="../../figures/qRT-PCR/trans_trt_log_1inj.svg", plot=trans_trt_log_plot, height=4, wid

```

```

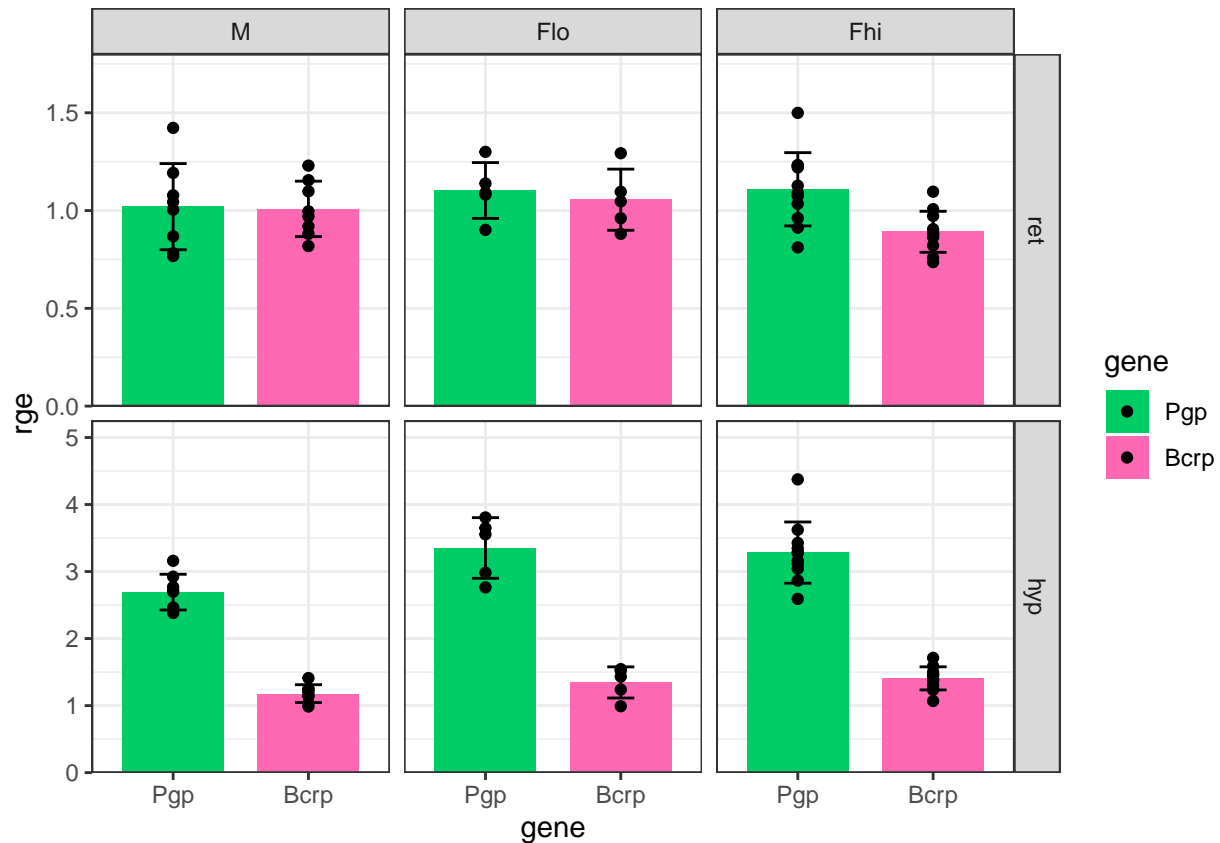
trans_g2g_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=gene, y=rge, fill=gene),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=gene, ymin=mean-sd,
    ymax=mean+sd, fill=gene),
    width=0.2, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=gene, y=rge, fill=gene),
    position=position_dodge(width=0.75)) +
  facet_grid(Tissue~Treatment, scales="free_y") +

```

```
scale_y_continuous(expand = expansion(mult = c(0, 0.2))) +
scale_fill_manual(values=c("springgreen3", "hotpink")) +
theme_bw()
```

```
## Warning: Ignoring unknown aesthetics: fill
```

```
trans_g2g_plot
```



```
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene_1inj.png", plot=trans_g2g_plot, height=4, width=10)
#ggsave(filename="../../figures/qRT-PCR/trans_gene_to_gene_1inj.svg", plot=trans_g2g_plot, height=4, width=10)
```

```
trans_g2g_log_plot <- ggplot() +
  stat_summary(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=gene),
    fun = 'mean', geom="bar", width=0.75, position="dodge") +
  geom_errorbar(data=trans_sumstats, aes(x=Treatment, ymin=log2_mean-log2_se,
    ymax=log2_mean+log2_se, fill=gene),
    width=0.25, position=position_dodge(width=0.75)) +
  geom_point(data=transporters_long, aes(x=Treatment, y=log2(rge), fill=gene),
    size=2, stat="identity", position=position_dodge(width=0.75)) +
  facet_wrap(~Tissue, ncol=2) +
  #scale_y_continuous(expand = expansion(mult = c(0.1, 0.25))) +
  ylim(-0.6, 2.5) +
  theme_bw(20) %+replace%
```

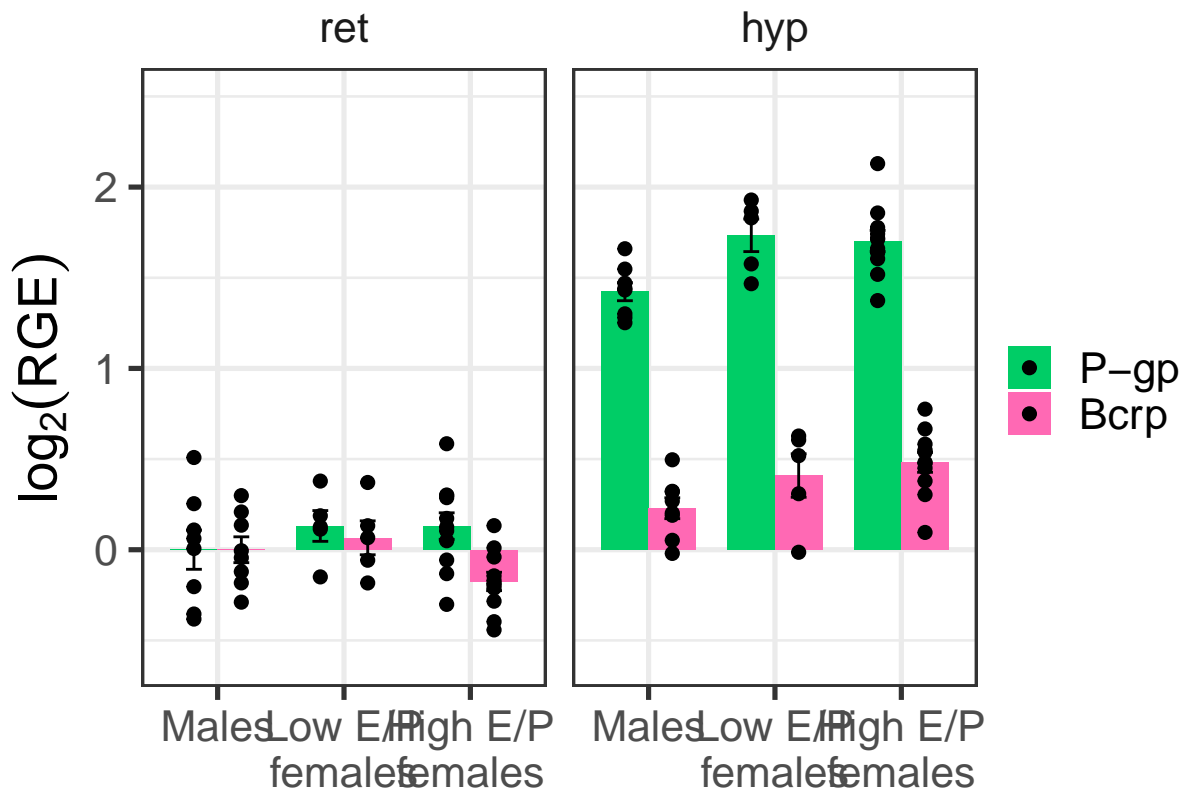
```

theme(legend.title = element_blank(),
      legend.box.spacing = unit(1, "pt"),
      axis.title.x=element_blank(),
      strip.background = element_rect(color="transparent", fill="transparent",
                                      size=0, linetype="solid")
    ) +
scale_fill_manual(values=c("springgreen3", "hotpink"),
                  labels=c("P-gp" = "P-gp", "Bcrp" = "Bcrp")) +
scale_x_discrete(labels=c("M" = "Males", "Flo" = "Low E/P\nfemales",
                          "Phi" = "High E/P\nfemales")) +
ylab(expression(log2(RGE)))

```

## Warning: Ignoring unknown aesthetics: fill

trans\_g2g\_log\_plot



```

ggsave(filename="../../figures/qRT-PCR/trans_g2g_log_1inj_large.png", plot=trans_g2g_log_plot, height=6
#ggsave(filename="../../figures/qRT-PCR/trans_g2g_log_1inj.svg", plot=trans_g2g_log_plot, height=4, wid

```