

# Host protein and MAA concentration-Linear mixed effects model

Code ▼

*Or Ben-Zvi*

## load packages

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```
library(car)
library(ggplot2)
library(lme4)
library(lmerTest)
library(MASS)
library(cowplot)
library(dplyr)
library(doBy)
```

## read data

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```
data_hpmaa <- read.csv(file.choose())
```

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```
str(data_hpmaa)
```

```
'data.frame': 79 obs. of 13 variables:
 $ id      : Factor w/ 79 levels "GA1","GA2","GA3",...: 1 2 3 4 5 6 7 8 9 10 ...
 $ treatment: Factor w/ 2 levels "par","paruv": 1 1 1 2 2 2 1 1 1 2 ...
 $ morph    : Factor w/ 3 levels "green","red",...: 1 1 1 1 1 1 1 1 1 1 ...
 $ colony   : Factor w/ 14 levels "a","b","c","d",...: 1 1 1 1 1 1 2 2 2 2 ...
 $ tank     : int  1 2 3 4 5 6 1 2 3 4 ...
 $ maat0    : Factor w/ 76 levels "#DIV/0!","10.62034265",...: 6 11 10 22 12 14 23 36 65 26
 ...
 $ maat1    : Factor w/ 77 levels "1.049626333",...: 17 15 13 44 40 64 22 11 51 59 ...
 $ dmaa     : Factor w/ 74 levels "-1.15E+00","-1.21E+00",...: 15 46 49 74 56 64 50 28 20 55
 ...
 $ pmaa     : Factor w/ 76 levels "-10.32869565",...: 48 8 13 59 67 74 7 42 17 62 ...
 $ hpt0     : Factor w/ 77 levels "10.25148515",...: 18 44 28 66 57 49 67 69 41 65 ...
 $ hpt1     : Factor w/ 73 levels "1.308888889",...: 62 71 41 13 70 22 69 33 7 17 ...
 $ dhp      : Factor w/ 72 levels "-0.03130911",...: 62 10 7 30 25 11 34 36 52 29 ...
 $ php      : Factor w/ 72 levels "-0.287346704",...: 68 30 2 45 42 26 47 46 24 41 ...
```

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```

data_hpmaa$hpt0 <- as.numeric(as.character(data_hpmaa$hpt0))
data_hpmaa$hpt1 <- as.numeric(as.character(data_hpmaa$hpt1))
data_hpmaa$maat0 <- as.numeric(as.character(data_hpmaa$maat0))
data_hpmaa$maat1 <- as.numeric(as.character(data_hpmaa$maat1))
data_hpmaa$dhp <- as.numeric(as.character(data_hpmaa$dhp))
data_hpmaa$dmaa <- as.numeric(as.character(data_hpmaa$dmaa))
data_hpmaa$php <- as.numeric(as.character(data_hpmaa$php))
data_hpmaa$pmaa <- as.numeric(as.character(data_hpmaa$pmaa))
data_hpmaa$tank <- as.factor(data_hpmaa$tank)
str(data_hpmaa)

```

```

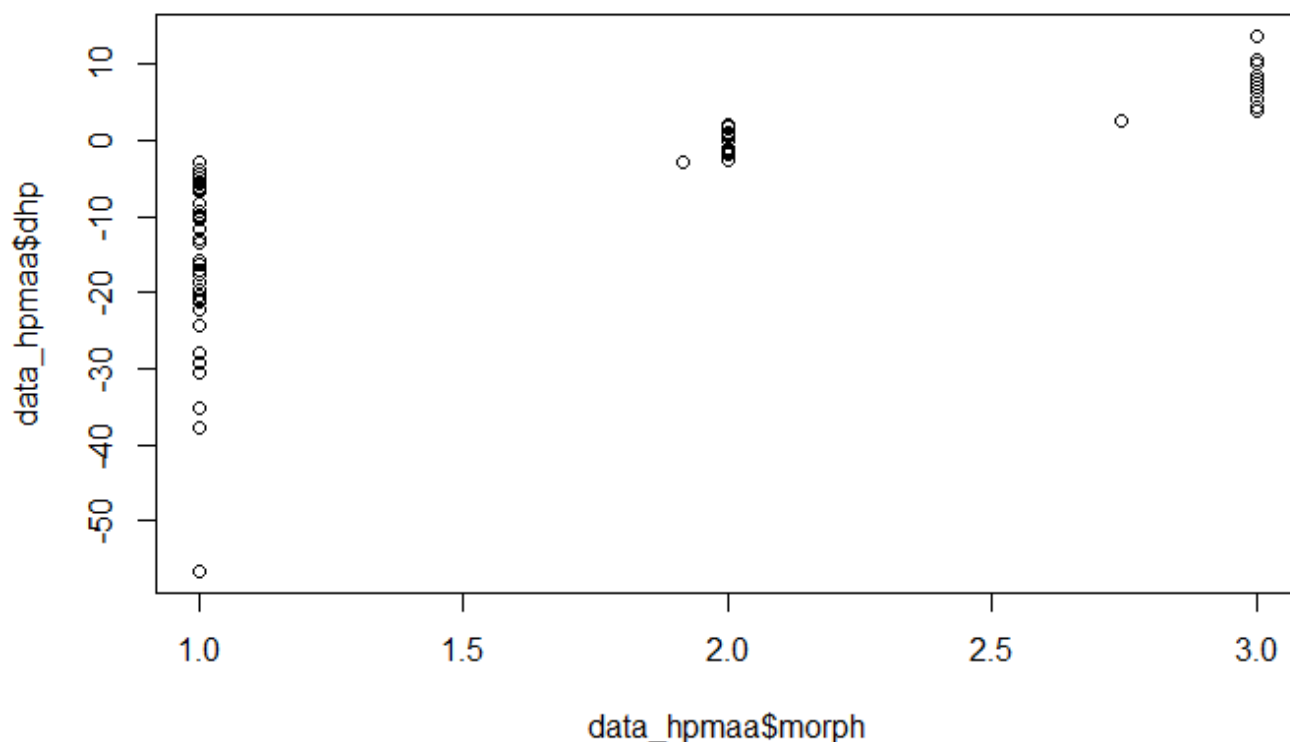
'data.frame': 79 obs. of 13 variables:
 $ id      : Factor w/ 79 levels "GA1","GA2","GA3",...: 1 2 3 4 5 6 7 8 9 10 ...
 $ treatment: Factor w/ 2 levels "par","paruv": 1 1 1 2 2 2 1 1 1 2 ...
 $ morph    : Factor w/ 3 levels "green","red",...: 1 1 1 1 1 1 1 1 1 1 ...
 $ colony   : Factor w/ 14 levels "a","b","c","d",...: 1 1 1 1 1 1 2 2 2 2 ...
 $ tank     : Factor w/ 6 levels "1","2","3","4",...: 1 2 3 4 5 6 1 2 3 4 ...
 $ maat0    : num  2.59 3.03 2.95 3.71 3.04 ...
 $ maat1    : num  2.39 2.29 2.09 4.45 4.17 ...
 $ dmaa     : num  -0.205 -0.744 -0.856 0.743 1.13 2.51 -0.91 -3.1 -2.45 1.12 ...
 $ pmaa     : num  -7.88 -24.51 -29.06 20.06 37.01 ...
 $ hpt0     : num  15.6 21.5 17.4 39.6 30.2 ...
 $ hpt1     : num  26.08 9.93 15.47 11.69 9.87 ...
 $ dhp      : num  10.51 -11.55 -1.89 -27.88 -20.37 ...
 $ php      : num  67.6 -53.8 -10.9 -70.5 -67.4 ...

```

## Visualize data

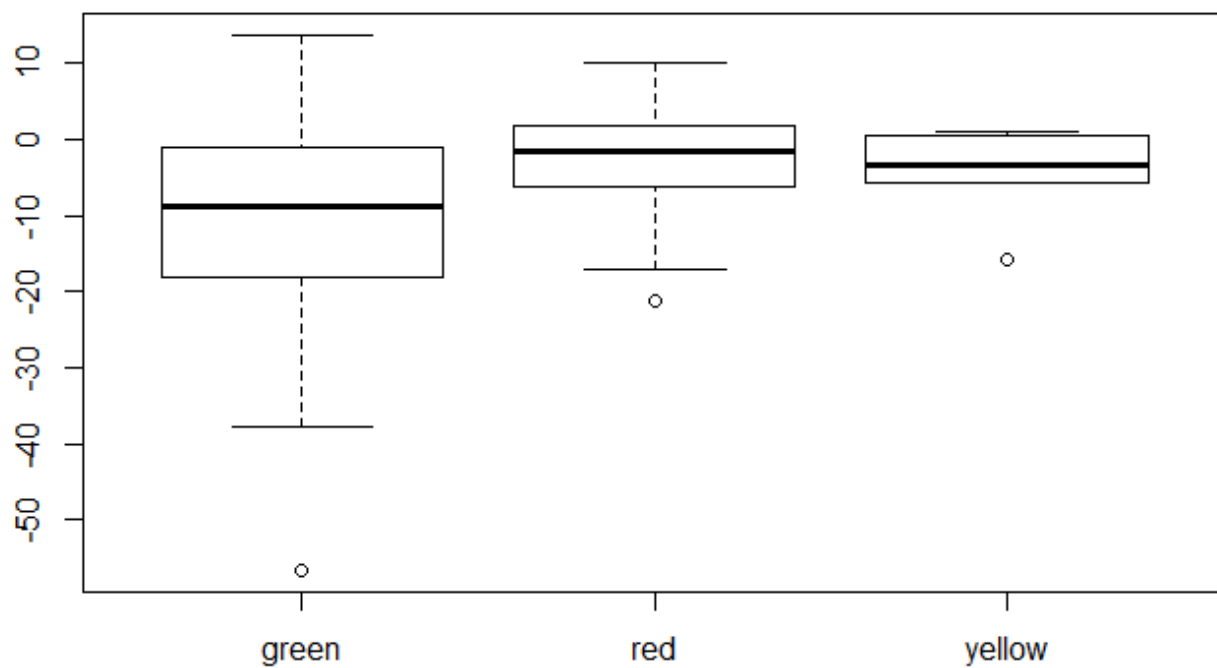
[Hide](#)

```
qqplot(data_hpmaa$morph,data_hpmaa$dhp)
```

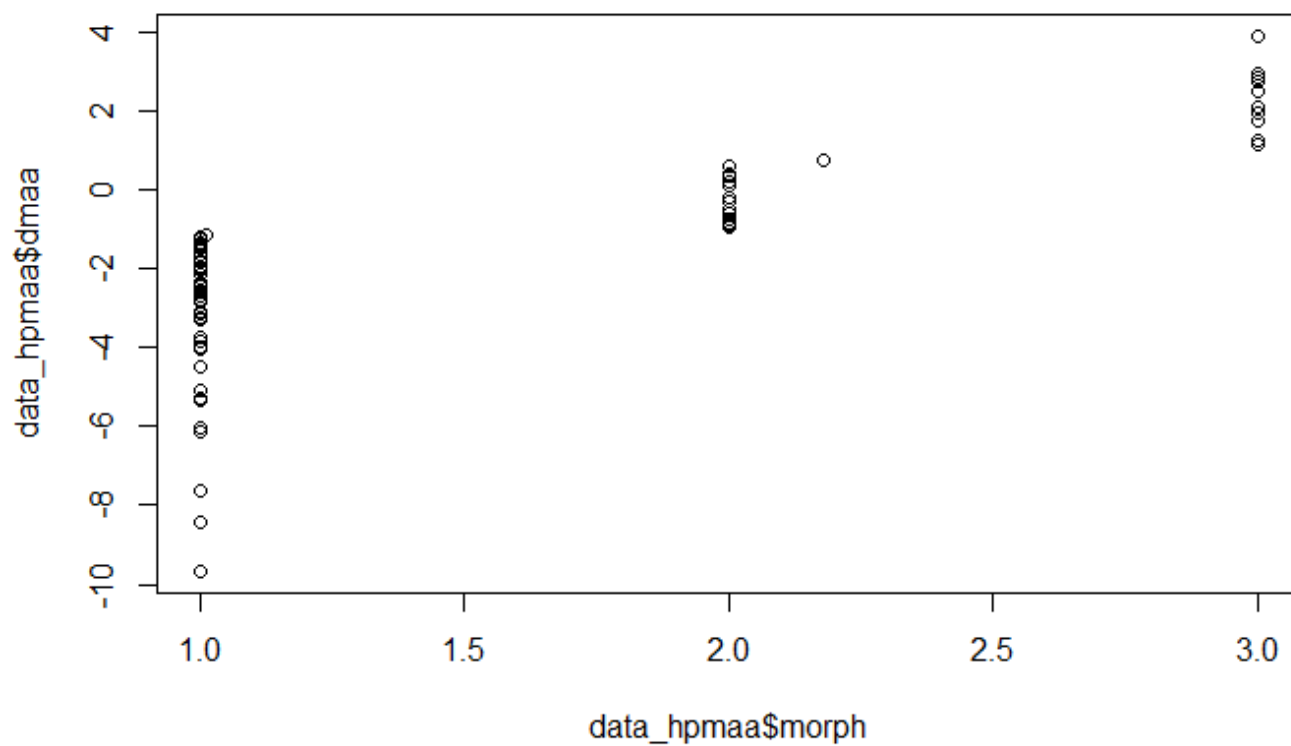


[Hide](#)

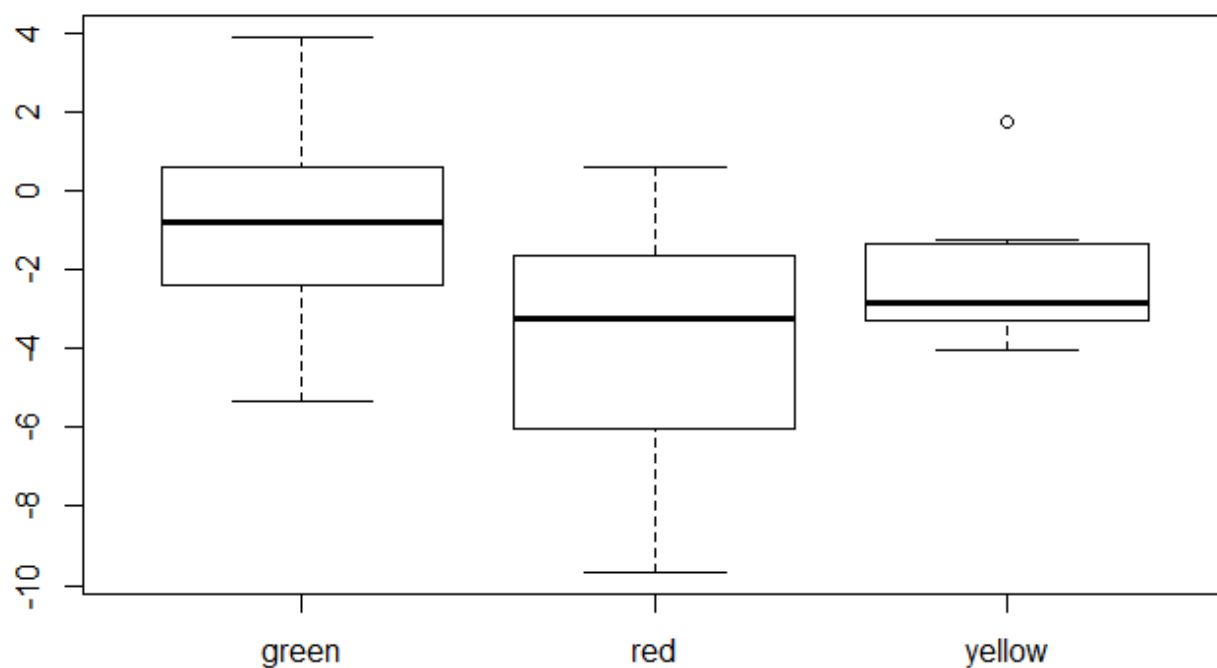
```
plot(data_hpmmaa$morph,data_hpmmaa$dhp)
```

[Hide](#)

```
qqplot(data_hpmmaa$morph,data_hpmmaa$dmaa)
```

[Hide](#)

```
plot(data_hpmaa$morph,data_hpmaa$dmaa)
```



## Test with mixed effect model

[Hide](#)

```
model_hp <- lmer(dhp ~ treatment*morph + (1|colony) + (1|tank) ,  
  data=data_hpmaa, REML=FALSE)
```

```
singular fit
```

[Hide](#)

```
summary(model_hp)
```

Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's method [lmerModLmerTest]

Formula: dhp ~ treatment \* morph + (1 | colony) + (1 | tank)

Data: data\_hpmaa

AIC	BIC	logLik	deviance	df.resid
568.9	589.3	-275.5	550.9	62

Scaled residuals:

Min	1Q	Median	3Q	Max
-3.6089	-0.5205	0.0674	0.5473	1.8754

Random effects:

Groups	Name	Variance	Std.Dev.
colony	(Intercept)	22.34	4.727
tank	(Intercept)	0.00	0.000
Residual		120.82	10.992

Number of obs: 71, groups: colony, 14; tank, 6

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t )
(Intercept)	-9.8052	2.7977	26.2562	-3.505	0.00166 **
treatmentparuv	-0.2481	3.1789	59.3435	-0.078	0.93806
morphred	3.1616	5.3572	26.2562	0.590	0.56013
morphyellow	4.9310	6.3187	40.7946	0.780	0.43968
treatmentparuv:morphred	6.9688	6.2249	59.3931	1.119	0.26744
treatmentparuv:morphyellow	0.4473	12.8195	66.9450	0.035	0.97227

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	trtmnt	mrphrd	mrphyl	trtmntprv:mrphr
treatmntprv	-0.566				
morphred	-0.522	0.296			
morphyellow	-0.443	0.251	0.231		
trtmntprv:mrphr	0.289	-0.511	-0.554	-0.128	
trtmntprv:mrphy	0.140	-0.248	-0.073	-0.351	0.127

convergence code: 0  
singular fit

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anova(model\_hp)

Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
treatment	27.868	27.868	1	66.003	0.2307	0.6326
morph	294.783	147.392	2	20.904	1.2199	0.3154
treatment:morph	152.819	76.409	2	63.040	0.6324	0.5346

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```
model_hp1 <- lmer(dhp ~ treatment + (1|colony) + (1|tank) ,
  data=data_hpmaa, REML=FALSE)
```

singular fit

Hide

```
model_hp2 <- lmer(dhp ~ morph + (1|colony) + (1|tank) ,
  data=data_hpmaa, REML=FALSE)
```

singular fit

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```
anova(model_hp, model_hp1)
```

Data: data\_hpmaa

Models:

model\_hp1: dhp ~ treatment + (1 | colony) + (1 | tank)

model\_hp: dhp ~ treatment \* morph + (1 | colony) + (1 | tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
model_hp1	5	564.51	575.82	-277.25	554.51			
model_hp	9	568.92	589.29	-275.46	550.92	3.5868	4	0.4648

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```
anova(model_hp, model_hp2)
```

Data: data\_hpmaa

Models:

model\_hp2: dhp ~ morph + (1 | colony) + (1 | tank)

model\_hp: dhp ~ treatment \* morph + (1 | colony) + (1 | tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
model_hp2	6	564.48	578.06	-276.24	552.48			
model_hp	9	568.92	589.29	-275.46	550.92	1.5628	3	0.6678

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```
model_maa <- lmer(dmaa ~ treatment*morph + (1|colony) + (1|tank) ,
  data=data_hpmaa, REML=FALSE)
summary(model_maa)
```

Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's method [lmerModLmerTest]

Formula: dmaa ~ treatment \* morph + (1 | colony) + (1 | tank)

Data: data\_hpmaa

AIC	BIC	logLik	deviance	df.resid
337.9	358.6	-159.9	319.9	65

Scaled residuals:

Min	1Q	Median	3Q	Max
-2.6095	-0.5767	0.1880	0.6077	2.0744

Random effects:

Groups	Name	Variance	Std.Dev.
colony	(Intercept)	0.4243	0.6514
tank	(Intercept)	0.1041	0.3226
Residual		3.9744	1.9936

Number of obs: 74, groups: colony, 14; tank, 6

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t )
(Intercept)	-1.74283	0.50332	11.92694	-3.463	0.00473 **
treatmentparuv	1.96226	0.63364	11.21973	3.097	0.00994 **
morphred	-1.78639	0.89535	28.81439	-1.995	0.05556 .
morphyellow	-0.04146	1.02326	38.30825	-0.041	0.96789
treatmentparuv:morphred	-2.53934	1.12886	55.22549	-2.249	0.02849 *
treatmentparuv:morphyellow	-3.37762	1.53059	57.08043	-2.207	0.03137 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	trtmnt	mrphrd	mrphyl	trtmntprv:mrphr
treatmntprv	-0.628				
morphred	-0.485	0.292			
morphyellow	-0.425	0.255	0.239		
trtmntprv:mrphr	0.291	-0.464	-0.601	-0.143	
trtmntprv:mrphy	0.215	-0.342	-0.121	-0.519	0.192

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anova(model\_maa)

Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
treatment	0.001	0.001	1	12.875	0.0002	0.988057
morph	76.640	38.320	2	16.337	9.6416	0.001717 **
treatment:morph	33.103	16.552	2	56.191	4.1646	0.020581 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

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```
model_maa1 <- lmer(dmaa ~ treatment + (1|colony) + (1|tank) ,
                  data=data_hpmaa, REML=FALSE)
model_maa2 <- lmer(dmaa ~ morph + (1|colony) + (1|tank) ,
                  data=data_hpmaa, REML=FALSE)
anova(model_maa, model_maa1)
```

Data: data\_hpmaa

Models:

model\_maa1: dmaa ~ treatment + (1 | colony) + (1 | tank)

model\_maa: dmaa ~ treatment \* morph + (1 | colony) + (1 | tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi	Df	Pr(>Chisq)
model_maa1	5	348.44	359.96	-169.22	338.44				
model_maa	9	337.87	358.60	-159.93	319.87	18.572		4	0.0009538 ***

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

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```
anova(model_maa, model_maa2)
```

Data: data\_hpmaa

Models:

model\_maa2: dmaa ~ morph + (1 | colony) + (1 | tank)

model\_maa: dmaa ~ treatment \* morph + (1 | colony) + (1 | tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi	Df	Pr(>Chisq)
model_maa2	6	342.28	356.11	-165.14	330.28				
model_maa	9	337.87	358.60	-159.93	319.87	10.416		3	0.01534 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

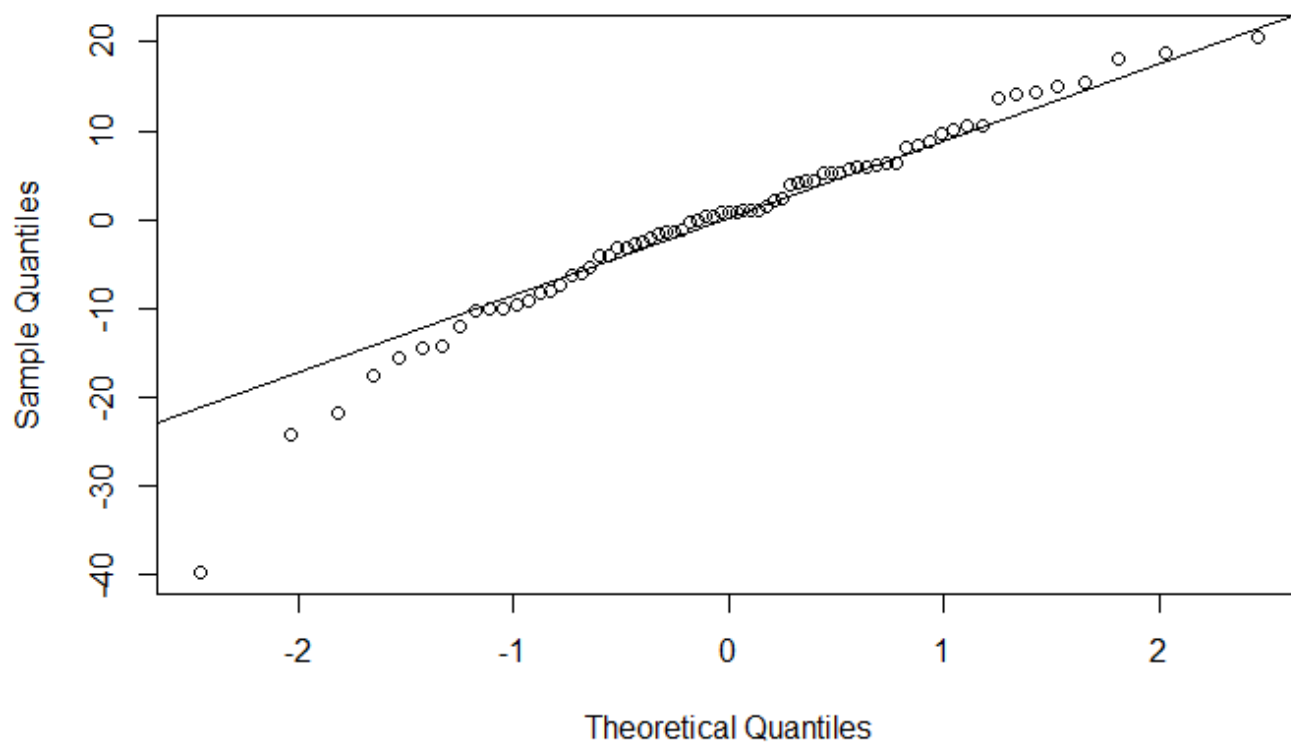
## Check the LMM assumptions

## Check the normality of model residuals

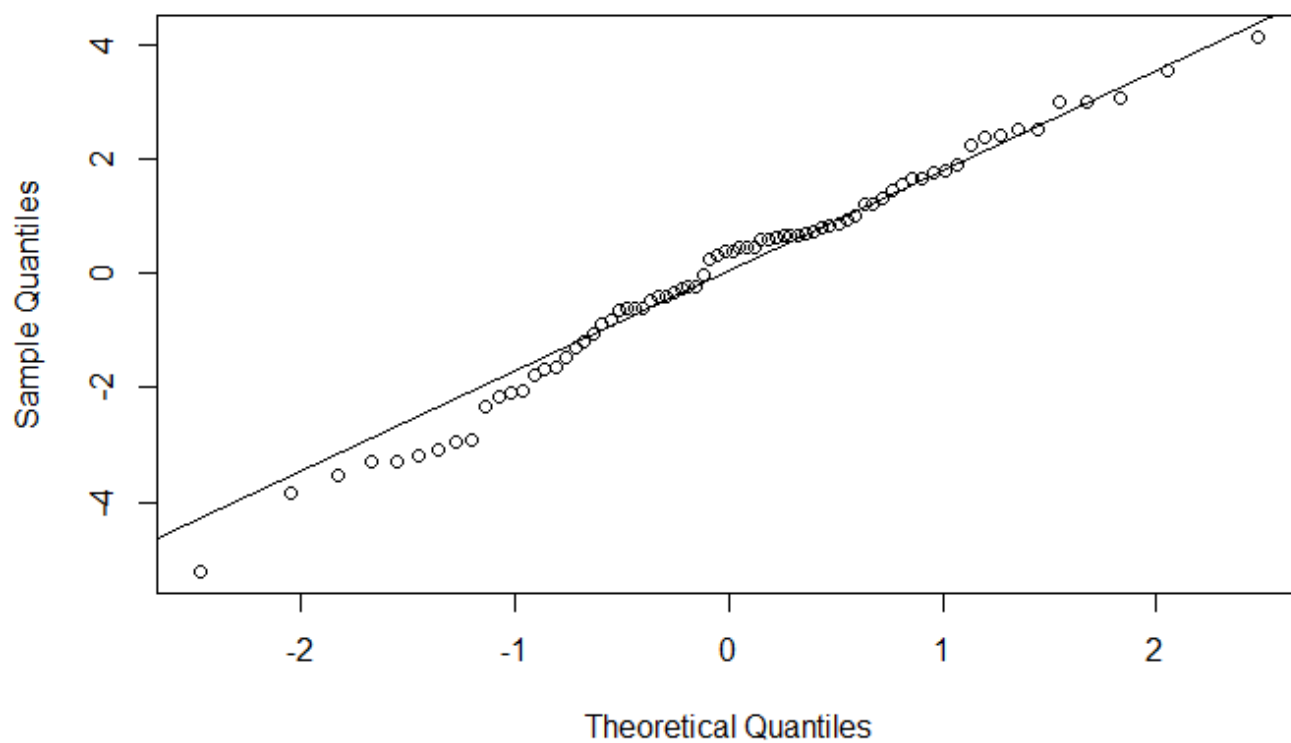
Hide

```
qqnorm(resid(model_hp))
qqline(resid(model_hp))
```



**Normal Q-Q Plot**[Hide](#)

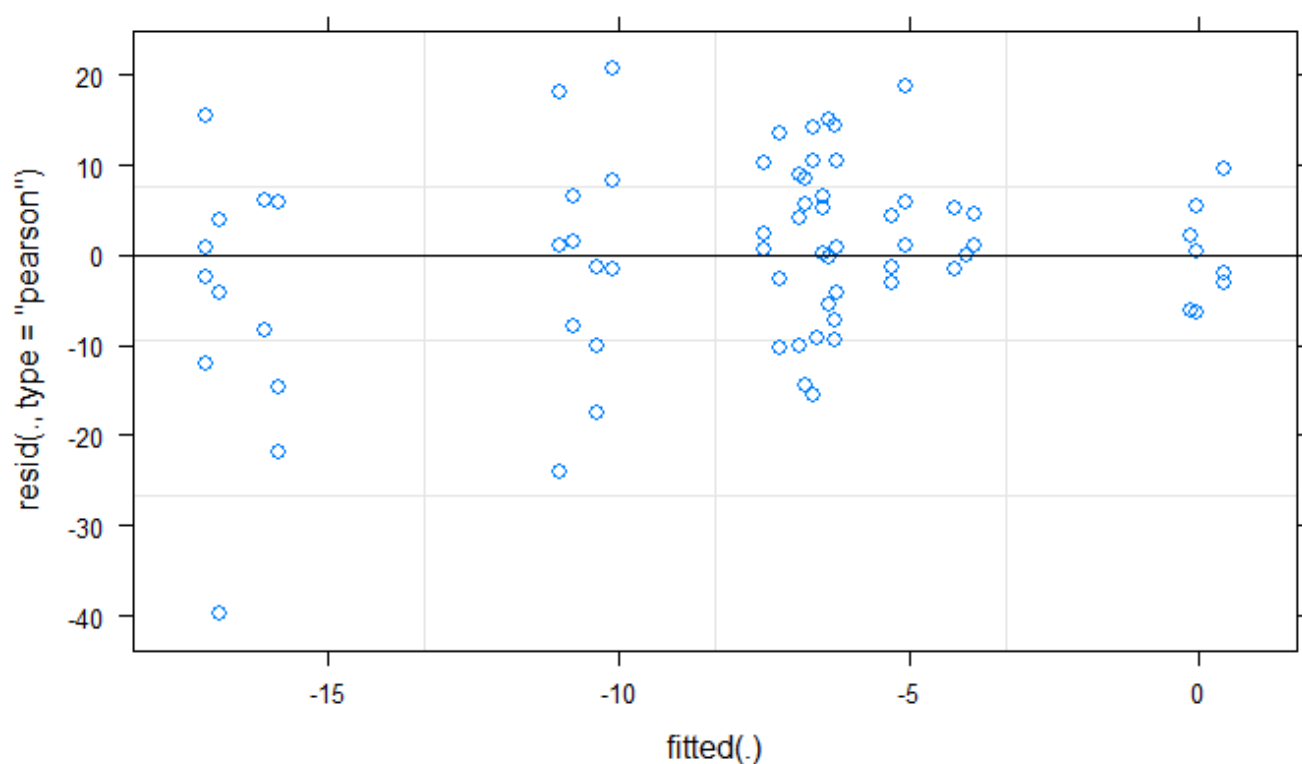
```
qqnorm(resid(model_maa))  
qqline(resid(model_maa))
```

**Normal Q-Q Plot**

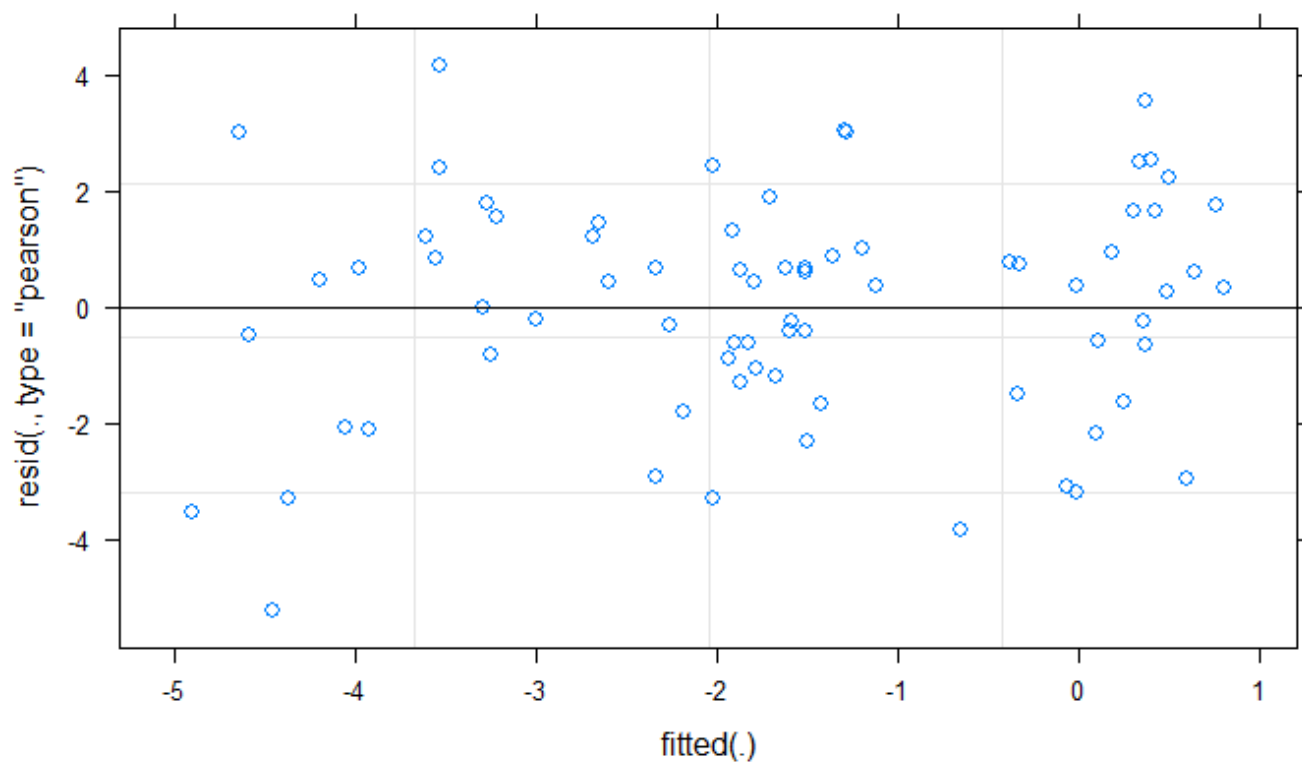
## Check for homogeneity of variance

[Hide](#)

```
plot(model_hp)
```

[Hide](#)

```
plot(model_maa)
```



## Obtain mean and SD

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```
sumfun <- function(x, ...){
  c(mean=mean(x, na.rm=TRUE, ...), sd=sd(x, na.rm=TRUE, ...), l=length(x))
}
mean(data_hpmaa$hpt0, na.rm = TRUE)
```

```
[1] 21.67733
```

Hide

```
sd(data_hpmaa$hpt0, na.rm = TRUE)
```

```
[1] 11.15114
```

Hide

```
mean(data_hpmaa$hpt1, na.rm = TRUE)
```

```
[1] 13.80183
```

Hide

```
sd(data_hpmaa$hpt1, na.rm = TRUE)
```

```
[1] 4.568941
```

Hide

```
summaryBy(hpt0 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

	morph	treatment	hpt0.mean	hpt0.sd	hpt0.l
1	green	par	23.79227	13.165150	24
2	green	paruv	24.08591	11.808555	24
3	red	par	21.83404	9.060078	9
4	red	paruv	13.16696	3.442265	9
5	yellow	par	18.12794	8.097353	7
6	yellow	paruv	17.41583	2.038584	6

Hide

```
summaryBy(hpt0 ~morph, data=data_hpmaa, FUN=sumfun)
```

	morph	hpt0.mean	hpt0.sd	hpt0.l
1	green	23.93909	12.372401	48
2	red	17.75541	8.130852	18
3	yellow	17.86899	6.380917	13

Hide

```
summaryBy(hpt0 ~treatment, data=data_hpmaa, FUN=sumfun)
```

```
treatment hpt0.mean hpt0.sd hpt0.l
1      par  22.36041 11.56206    40
2    paruv  20.91836 10.78772    39
```

Hide

```
summaryBy(hpt1 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

```
      morph treatment hpt1.mean hpt1.sd hpt1.l
1  green      par  13.98704 5.885944    24
2  green    paruv  13.98997 3.510027    24
3   red      par  15.19037 4.967352     9
4   red    paruv  13.34032 3.781468     9
5 yellow      par  10.70346 2.117577     7
6 yellow    paruv  11.98981      NA     6
```

Hide

```
summaryBy(hpt1 ~morph, data=data_hpmaa, FUN=sumfun)
```

```
      morph hpt1.mean hpt1.sd hpt1.l
1  green 13.98850 4.794027    48
2   red 14.26534 4.387113    18
3 yellow 10.91785 1.965475    13
```

Hide

```
summaryBy(hpt1 ~treatment, data=data_hpmaa, FUN=sumfun)
```

```
treatment hpt1.mean hpt1.sd hpt1.l
1      par  13.83999 5.398380    40
2    paruv  13.75917 3.497838    39
```

Hide

```
summaryBy(php ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

```
      morph treatment  php.mean  php.sd php.l
1  green      par -22.637765 58.92197    24
2  green    paruv -25.527091 44.47746    24
3   red      par -18.228766 43.30632     9
4   red    paruv   7.760849 45.01695     9
5 yellow      par -21.855105 28.62351     7
6 yellow    paruv -25.002275      NA     6
```

Hide

```
summaryBy(php ~morph, data=data_hpmaa, FUN=sumfun)
```

```

morph   php.mean   php.sd  php.l
1 green -24.082428 51.66404   48
2 red   -5.998359 44.75625   18
3 yellow -22.379633 25.63386   13

```

Hide

```
summaryBy(php ~treatment, data=data_hpmaa, FUN=sumfun)
```

```

treatment  php.mean   php.sd  php.l
1      par  -21.49055 51.53327   40
2    paruv -17.44138 45.54969   39

```

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```
summaryBy(maat0 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

```

morph treatment maat0.mean maat0.sd maat0.l
1 green      par    5.430055 1.632603     24
2 green    paruv    5.054276 1.971125     24
3 red       par    5.846749 2.595752      9
4 red     paruv    6.894724 3.607765      9
5 yellow    par    7.499501 1.410929      7
6 yellow   paruv    6.240481 2.451593      6

```

Hide

```
summaryBy(maat0 ~morph, data=data_hpmaa, FUN=sumfun)
```

```

morph maat0.mean maat0.sd maat0.l
1 green  5.242166 1.800480     48
2 red    6.339914 3.058458     18
3 yellow 7.041675 1.844183     13

```

Hide

```
summaryBy(maat0 ~treatment, data=data_hpmaa, FUN=sumfun)
```

```

treatment maat0.mean maat0.sd maat0.l
1      par  5.885964 1.963635     40
2    paruv  5.595065 2.511137     39

```

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```
summaryBy(maat1 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

```

morph treatment maat1.mean maat1.sd maat1.l
1 green      par   3.687744 1.366164    24
2 green      paruv 5.270069 1.528755    24
3 red        par   2.317337 1.263014     9
4 red        paruv 2.915283 1.222423     9
5 yellow     par   5.846019 1.413096     7
6 yellow     paruv 5.785419 4.032577     6

```

Hide

```
summaryBy(maat1 ~morph, data=data_hpmaa, FUN=sumfun)
```

```

morph maat1.mean maat1.sd maat1.l
1 green  4.478906 1.642037    48
2 red    2.616310 1.244401    18
3 yellow 5.821779 2.555562    13

```

Hide

```
summaryBy(maat1 ~treatment, data=data_hpmaa, FUN=sumfun)
```

```

treatment maat1.mean maat1.sd maat1.l
1 par      3.703538 1.705366    40
2 paruv    4.752996 2.078780    39

```

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```
summaryBy(pmaa ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

```

morph treatment pmaa.mean pmaa.sd pmaa.l
1 green      par -28.75316 26.64023    24
2 green      paruv 17.69605 52.44962    24
3 red        par -53.98800 33.83796     9
4 red        paruv -53.09773 19.08400     9
5 yellow     par -20.78543 27.50400     7
6 yellow     paruv -48.02251 14.85842     6

```

Hide

```
summaryBy(pmaa ~morph, data=data_hpmaa, FUN=sumfun)
```

```

morph pmaa.mean pmaa.sd pmaa.l
1 green -5.528554 47.37484    48
2 red   -53.569050 27.05644    18
3 yellow -29.864453 26.71051    13

```

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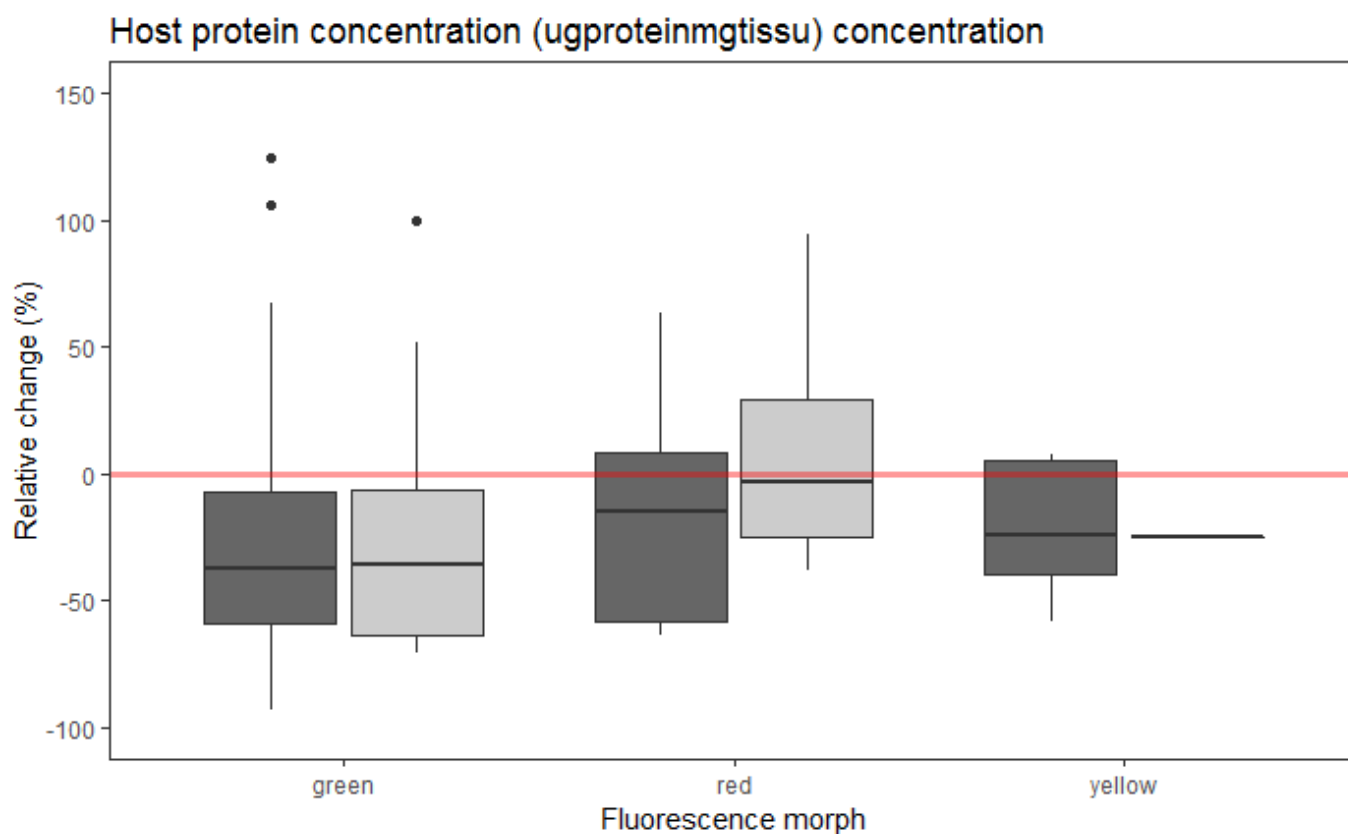
```
summaryBy(pmaa ~treatment, data=data_hpmaa, FUN=sumfun)
```

	treatment	pmaa.mean	pmaa.sd	pmaa.l
1	par	-33.350781	30.15454	40
2	paruv	-4.118402	54.94876	39

## Visualize the data as a box plot

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```
hp_plot <- ggplot(data = data_hpmaa, aes(x=morph, y=php)) +
  geom_boxplot(aes(fill=treatment)) +
  labs(title= "Host protein concentration (ugproteinmgtissu) concentration", x="Fluorescence morph", y="Relative change (%)") +
  geom_hline(yintercept=0, color= "red", size = 1.25, alpha = 0.4) +
  ylim(-100, 150) +
  scale_fill_grey(start = 0.4, end = 0.8, name="Treatment",breaks=c("par", "paruv"), labels=c("PAR","PAR+UV")) +
  theme_bw() +
  theme(legend.position="none")+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
hp_plot
```



Hide

```

maa_plot <- ggplot(data = data_hpmaa, aes(x=morph, y=pmaa)) +
  geom_boxplot(aes(fill=treatment)) +
  labs(title = "MAA concentration (abs320 nmmgtissu)", x="Fluorescence morph", y="Relative ch
ange (%)") +
  geom_hline(yintercept=0, color= "red", size = 1.25, alpha = 0.4) +
  ylim(-100, 150) +
  scale_fill_grey(start = 0.4, end = 0.8, name="Treatment",breaks=c("par", "paruv"), labels=c
("PAR", "PAR+UV")) +
  theme_bw() +
  theme(legend.position="right")+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())

maa_plot

```

