

DNA damage-Linear mixed effects model

Code ▼

Or Ben-Zvi

load packages

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

read data

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

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```
str(data_dna)
```

```
'data.frame':  26 obs. of  5 variables:
 $ treatment: Factor w/ 2 levels "par","paruv": 2 2 2 2 2 2 2 2 2 2 ...
 $ morph    : Factor w/ 3 levels "green","red",...: 1 1 1 1 1 1 1 2 2 2 ...
 $ colony   : Factor w/ 13 levels "a","c","d","e",...: 1 2 3 4 5 6 7 8 9 10 ...
 $ cpd      : num  16.71 7.43 33.86 21 12.07 ...
 $ pp       : num  3.49 4.74 5.52 3.23 4.64 ...
```

Plot the data

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```
qqplot(data_dna$morph,data_dna$cpd)
plot(data_dna$morph,data_dna$cpd)
qqplot(data_dna$morph,data_dna$pp)
plot(data_dna$morph,data_dna$pp)
```

Test with mixed effect model

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```
model_cpd <- lmer(cpd ~ treatment*morph + (1|colony),
                  data=data_dna, REML=FALSE)
summary(model_cpd)
```

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

Formula: cpd ~ treatment * morph + (1 | colony)

Data: data_dna

AIC	BIC	logLik	deviance	df.resid
180.4	190.5	-82.2	164.4	18

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.8034	-0.4268	-0.0516	0.3517	2.8354

Random effects:

Groups	Name	Variance	Std.Dev.
colony	(Intercept)	7.143	2.673
	Residual	26.315	5.130

Number of obs: 26, groups: colony, 13

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t)
(Intercept)	16.8163	2.1863	24.8667	7.692	4.95e-08 ***
treatmentparuv	-0.8163	2.7420	13.0000	-0.298	0.771
morphred	2.2789	3.9915	24.8667	0.571	0.573
morphyellow	2.6361	3.9915	24.8667	0.660	0.515
treatmentparuv:morphred	1.8878	5.0062	13.0000	0.377	0.712
treatmentparuv:morphyellow	1.4116	5.0062	13.0000	0.282	0.782

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

Correlation of Fixed Effects:

	(Intr)	trtmnt mrphrd	mrphyl	trtmntprv:mrphr
treatmntprv	-0.627			
morphred	-0.548	0.343		
morphyellow	-0.548	0.343	0.300	
trtmntprv:mrphr	0.343	-0.548	-0.627	-0.188
trtmntprv:mrphy	0.343	-0.548	-0.188	-0.627
				0.300

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```
anova(model_cpd)
```

Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
treatment	0.447	0.4466	1	13	0.0170	0.8983
morph	45.147	22.5737	2	13	0.8578	0.4468
treatment:morph	4.566	2.2831	2	13	0.0868	0.9174

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```
model_cpd1 <- lmer(cpd ~ treatment + (1|colony),
                  data=data_dna, REML=FALSE)
model_cpd2 <- lmer(cpd ~ morph + (1|colony),
                  data=data_dna, REML=FALSE)
anova(model_cpd, model_cpd1)
```

Data: data_dna

Models:

model_cpd1: cpd ~ treatment + (1 | colony)

model_cpd: cpd ~ treatment * morph + (1 | colony)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi	Df	Pr(>Chisq)
model_cpd1	4	174.23	179.26	-83.115	166.23				
model_cpd	8	180.45	190.51	-82.223	164.45	1.7839		4	0.7754

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```
anova(model_cpd, model_cpd2)
```

Data: data_dna

Models:

model_cpd2: cpd ~ morph + (1 | colony)

model_cpd: cpd ~ treatment * morph + (1 | colony)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi	Df	Pr(>Chisq)
model_cpd2	5	174.62	180.91	-82.310	164.62				
model_cpd	8	180.45	190.51	-82.223	164.45	0.1731		3	0.9818

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```
model_pp <- lmer(pp ~ treatment*morph + (1|colony),
                 data=data_dna, REML=FALSE)
```

```
singular fit
```

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```
summary(model_pp)
```

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

Formula: pp ~ treatment * morph + (1 | colony)

Data: data_dna

AIC	BIC	logLik	deviance	df.resid
70.1	80.2	-27.1	54.1	18

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.94930	-0.39648	-0.02814	0.48214	2.38478

Random effects:

Groups	Name	Variance	Std.Dev.
colony	(Intercept)	0.0000	0.000
Residual		0.4692	0.685

Number of obs: 26, groups: colony, 13

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t)
(Intercept)	3.1153	0.2589	26.0000	12.033	3.95e-12 ***
treatmentparuv	1.2683	0.3661	26.0000	3.464	0.00186 **
morphred	-0.4931	0.4727	26.0000	-1.043	0.30646
morphyellow	-0.3179	0.4727	26.0000	-0.673	0.50715
treatmentparuv:morphred	-0.8989	0.6685	26.0000	-1.345	0.19031
treatmentparuv:morphyellow	-0.6717	0.6685	26.0000	-1.005	0.32426

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

Correlation of Fixed Effects:

	(Intr)	trtmnt	mrphrd	mrphyl	trtmntprv:mrphr
treatmntprv	-0.707				
morphred	-0.548	0.387			
morphyellow	-0.548	0.387	0.300		
trtmntprv:mrphr	0.387	-0.548	-0.707	-0.212	
trtmntprv:mrphy	0.387	-0.548	-0.212	-0.707	0.300

convergence code: 0
singular fit

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anova(model_pp)

Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
treatment	3.0830	3.08300	1	26	6.5708	0.01650 *
morph	4.3666	2.18332	2	26	4.6533	0.01873 *
treatment:morph	1.0350	0.51748	2	26	1.1029	0.34694

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

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```
model_pp1 <- lmer(pp ~ treatment + (1|colony),
  data=data_dna, REML=FALSE)
```

```
singular fit
```

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```
model_pp2 <- lmer(pp ~ morph + (1|colony),
                  data=data_dna, REML=FALSE)
```

```
singular fit
```

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```
anova(model_pp, model_pp1)
```

```
Data: data_dna
Models:
model_pp1: pp ~ treatment + (1 | colony)
model_pp: pp ~ treatment * morph + (1 | colony)
      Df    AIC    BIC logLik deviance Chisq Chi Df Pr(>Chisq)
model_pp1  4 71.641 76.673 -31.820   63.641
model_pp   8 70.110 80.175 -27.055   54.110 9.531     4   0.04911 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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```
anova(model_pp, model_pp2)
```

```
Data: data_dna
Models:
model_pp2: pp ~ morph + (1 | colony)
model_pp: pp ~ treatment * morph + (1 | colony)
      Df    AIC    BIC logLik deviance Chisq Chi Df Pr(>Chisq)
model_pp2  5 75.031 81.321 -32.515   65.031
model_pp   8 70.110 80.175 -27.055   54.110 10.921     3   0.01216 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

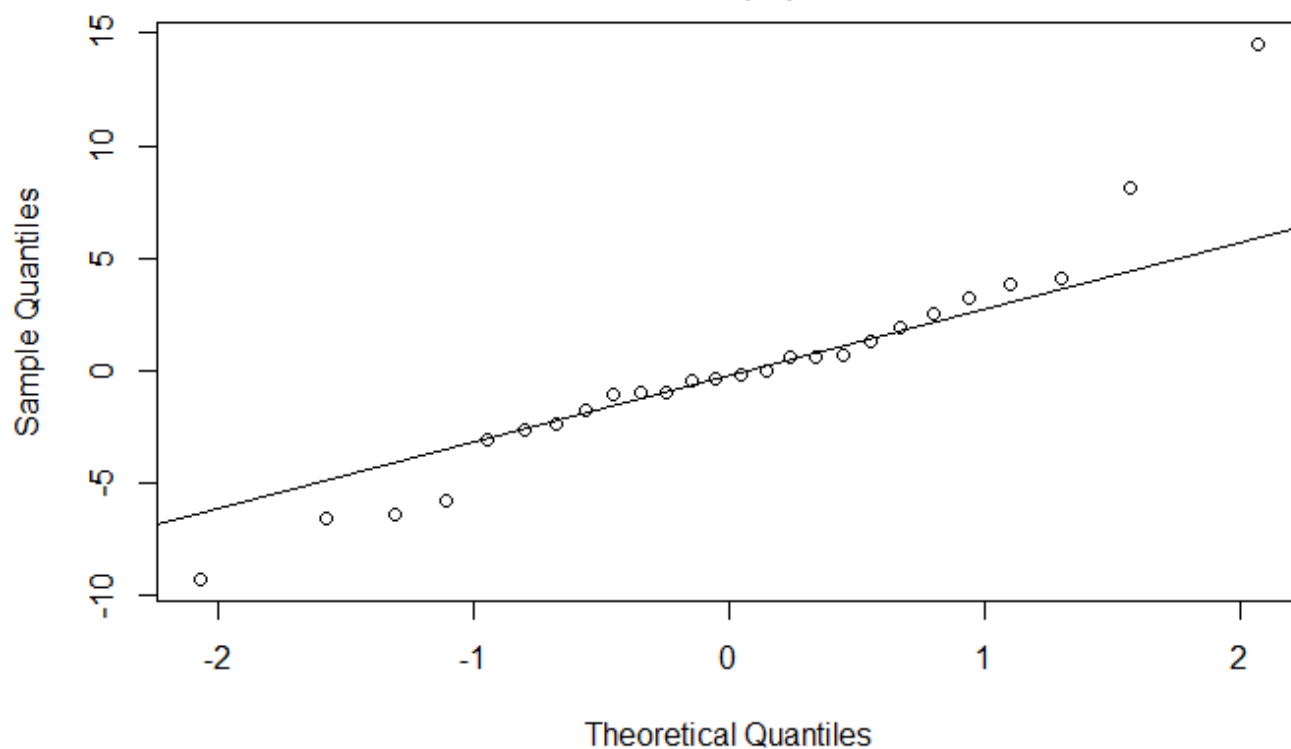
Check the LMM assumptions

Check the normality of model residuals

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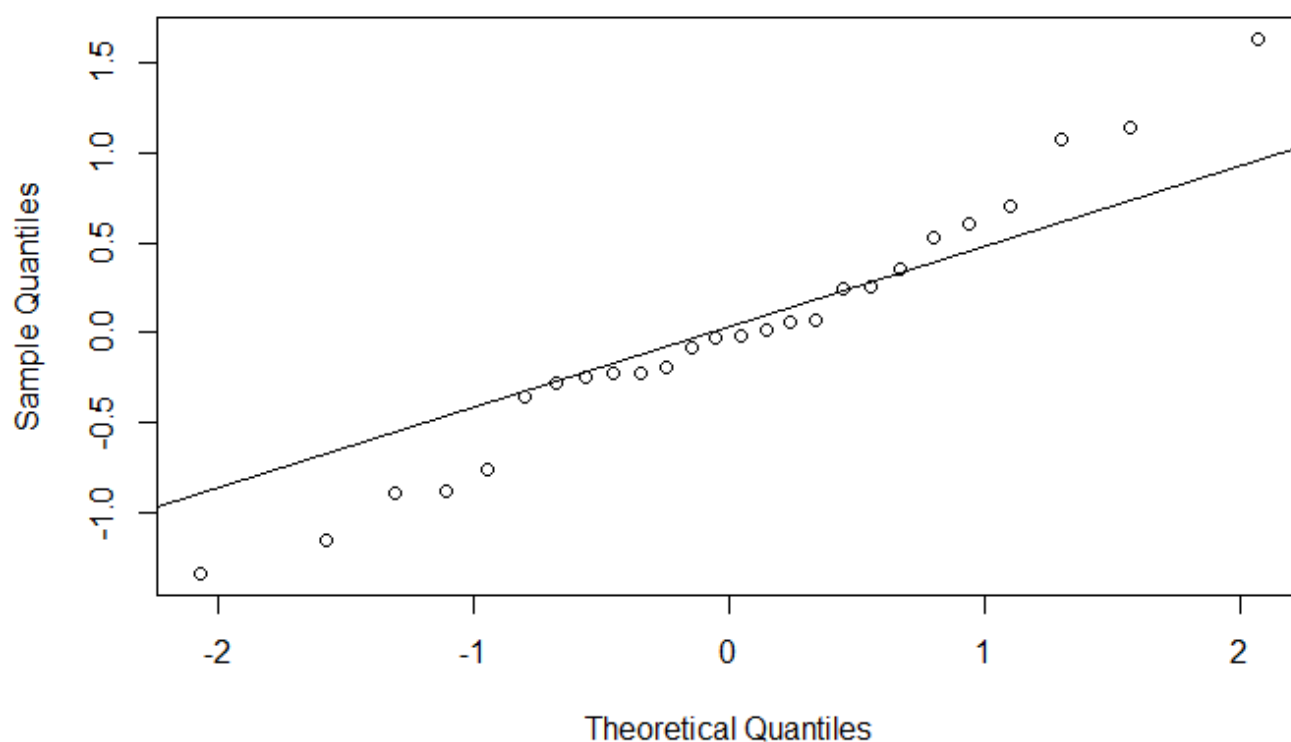
```
qqnorm(resid(model_cpd))
qqline(resid(model_cpd))
```

Normal Q-Q Plot

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```
qqnorm(resid(model_pp))  
qqline(resid(model_pp))
```

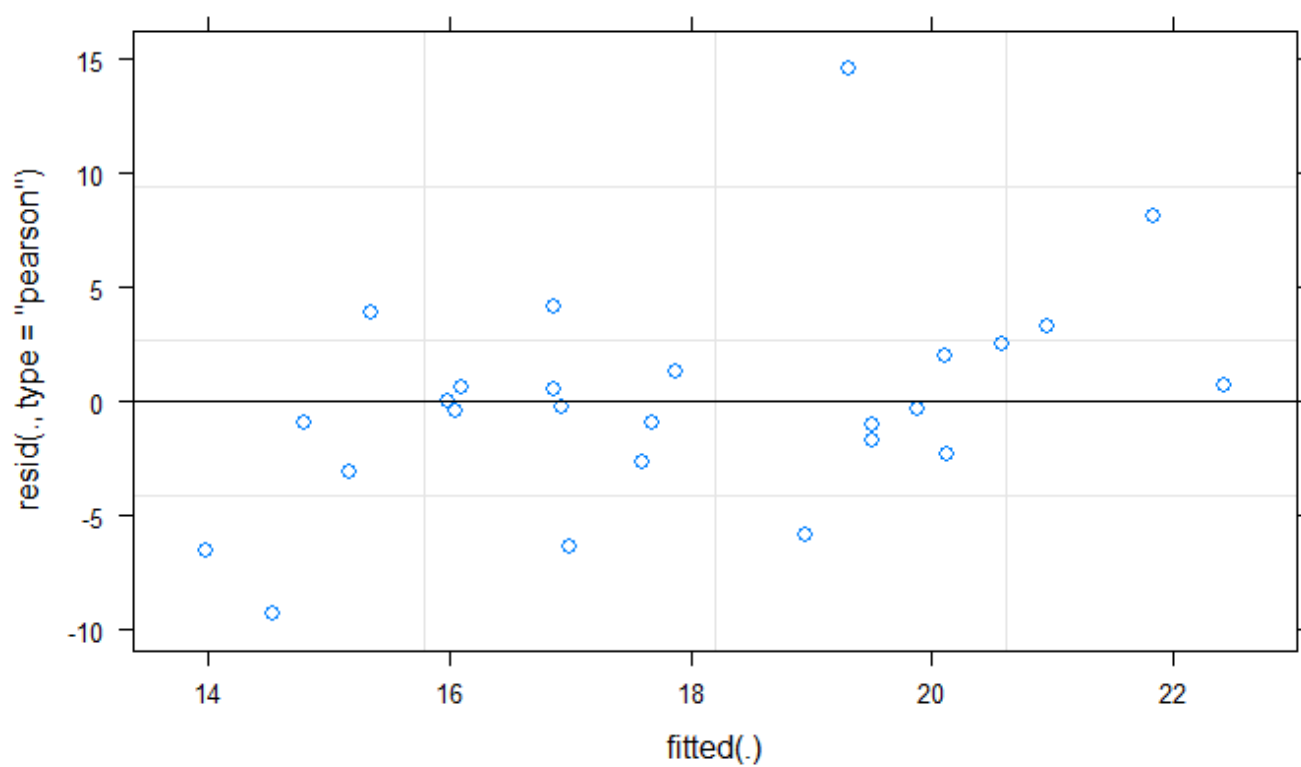
Normal Q-Q Plot



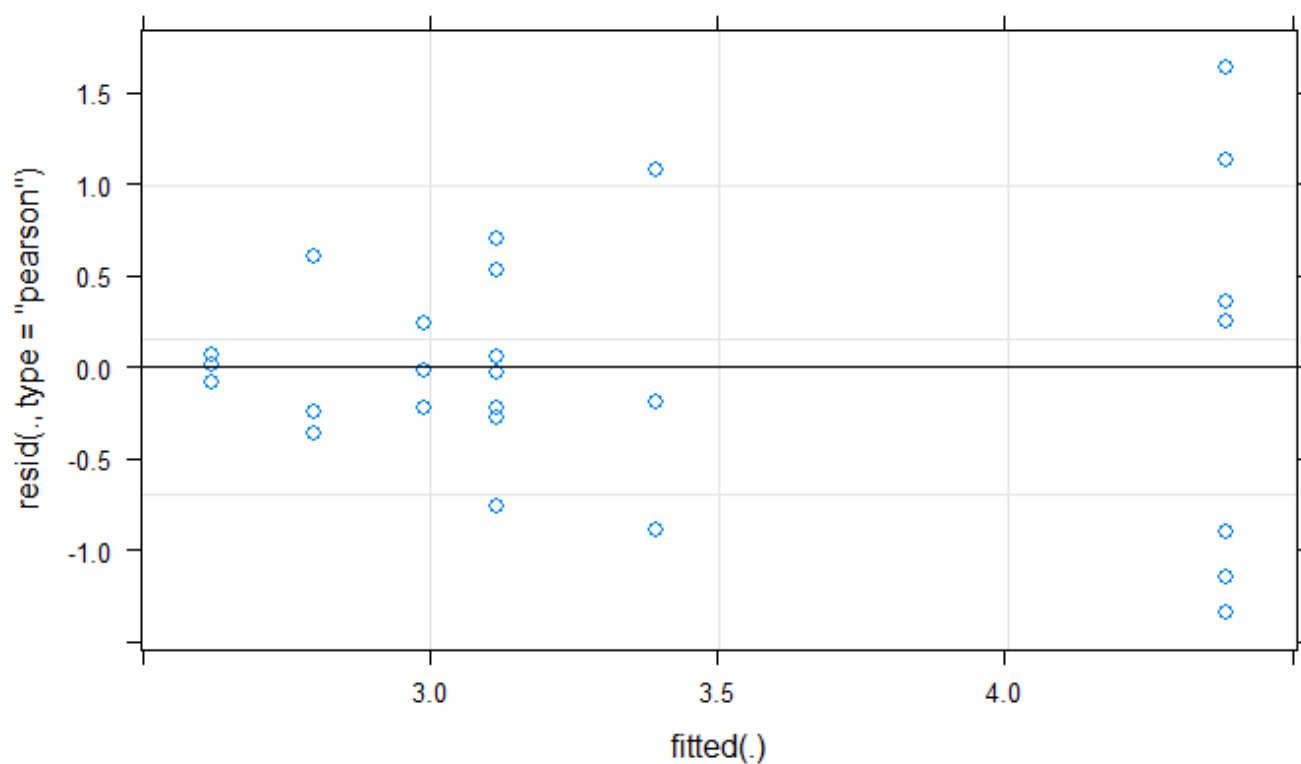
Check for homogeneity of variance

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```
plot(model_cpd)
```

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```
plot(model_pp)
```



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_dna$cpd, na.rm = TRUE)
```

```
[1] 17.92308
```

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```
sd(data_dna$cpd, na.rm = TRUE)
```

```
[1] 6.145412
```

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```
summaryBy(cpd ~ morph * treatment, data=data_dna, FUN=sumfun)
```

	morph	treatment	cpd.mean	cpd.sd	cpd.l
1	green	par	16.81633	1.6587519	7
2	green	paruv	16.00000	9.5587158	7
3	red	par	19.09524	0.5455447	3
4	red	paruv	20.16667	6.1063422	3
5	yellow	par	19.45238	9.7502834	3
6	yellow	paruv	20.04762	4.4654760	3

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```
summaryBy(cpd ~ morph, data=data_dna, FUN=sumfun)
```

	morph	cpd.mean	cpd.sd	cpd.l
1	green	16.40816	6.604517	14
2	red	19.63095	3.921530	6
3	yellow	19.75000	6.790412	6

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```
summaryBy(cpd ~ treatment, data=data_dna, FUN=sumfun)
```

	treatment	cpd.mean	cpd.sd	cpd.l
1	par	17.95055	4.348780	13
2	paruv	17.89560	7.730836	13

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```
mean(data_dna$pp, na.rm = TRUE)
```

```
[1] 3.38101
```

Hide


```
sd(data_dna$pp, na.rm = TRUE)
```

```
[1] 0.9577882
```

Hide

```
summaryBy(pp ~morph * treatment, data=data_dna, FUN=sumfun)
```

	morph	treatment	pp.mean	pp.sd	pp.l
1	green	par	3.115260	0.49754546	7
2	green	paruv	4.383523	1.15892611	7
3	red	par	2.622159	0.07908756	3
4	red	paruv	2.991477	0.23469761	3
5	yellow	par	2.797348	0.52793030	3
6	yellow	paruv	3.393939	0.99374307	3

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```
summaryBy(pp ~morph, data=data_dna, FUN=sumfun)
```

	morph	pp.mean	pp.sd	pp.l
1	green	3.749391	1.0803734	14
2	red	2.806818	0.2558396	6
3	yellow	3.095644	0.7831159	6

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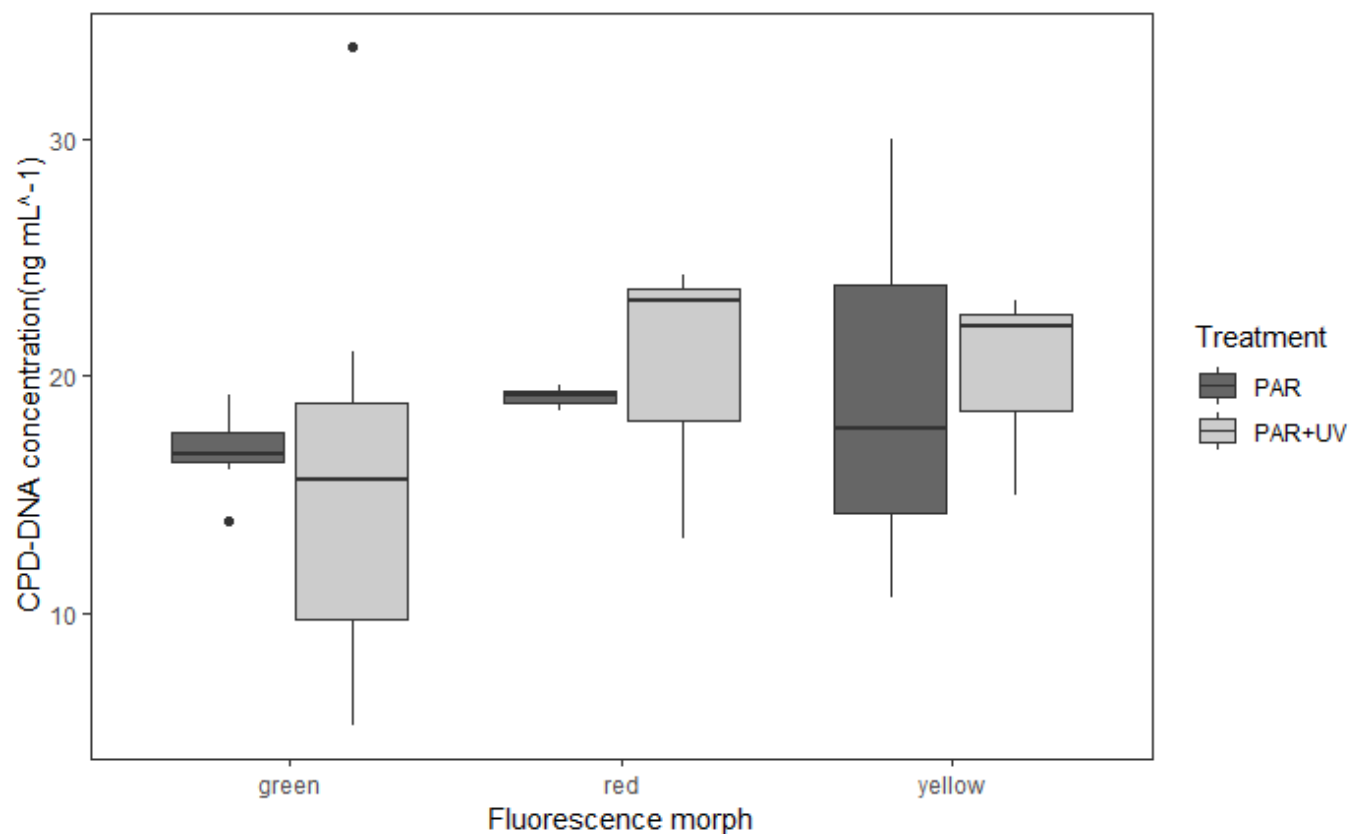
```
summaryBy(pp ~treatment, data=data_dna, FUN=sumfun)
```

	treatment	pp.mean	pp.sd	pp.l
1	par	2.928103	0.4683774	13
2	paruv	3.833916	1.1168481	13

Visualize the data as a box plot

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```
cpd_plot <- ggplot(data = data_dna, aes(x=morph, y=cpd)) +
  geom_boxplot(aes(fill=treatment)) +
  labs(x="Fluorescence morph", y="CPD-DNA concentration(ng mL-1)") +
  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())
cpd_plot
```



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```
pp_plot <- ggplot(data = data_dna, aes(x=morph, y=pp)) +
  geom_boxplot(aes(fill=treatment)) +
  labs(x="Fluorescence morph", y="6-4PP-DNA concentration(ng mL-1)") +
  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())
pp_plot
```

