

Mixed models as a tool for comparing dynamic changes in chlorophyll fluorescence between different genotypes

TIPS for the supplementary file `tsmeanscomparison.R` for scientists not experienced with R

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- Before you run the code, you need to install all the required R packages. This can be done by the following command (you can copy-paste it on the command line - console):

```
install.packages(c("openxlsx","glmmTMB","forecast"))
```

- In line 9 of the code there is the following command:
 - `df<-read.xlsx("Chlorophyll.xlsx",colNames = T)`

You can replace the name of the file (`Chlorophyll.xlsx`) with your own. The names of the columns must be the same as in `Chlorophyll.xlsx`. The default directory is usually documents but you can use the command `getwd()` (already included in the code) to find exactly where the working directory is.

- In line 31 there is the following command:

```
mdl<-glmmTMB(log(CHL)~1+Genotype+T1+T2+(1+T1+T2|id),data=df, REML = F)
```

Here the form of the model $\log(y)=a_0+a_1T_1+a_2T_2$ is describing a bellshaped curve.

If you want to use a linear model (in case the data have different pattern) then this becomes:

```
mdl<-glmmTMB(CHL~1+Genotype+T1+(1+T1|id),data=df, REML = F)
```

Only exponential increase without the decay second order term:

```
mdl<-glmmTMB(log(CHL)~1+Genotype+T1+(1+T1|id),data=df, REML = F)
```

etc....

- If you want to compare models to see which one is the best fit use the AIC (and BIC) which is given in summary of the model [line 32 of the code: `summary(mdl)`]

The model with the smallest AIC value is the best. For the AIC to have meaning we have to use maximum likelihood estimation (`REML=F`). Alternatively the command `AIC(mdl)` can be used.

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- If the initial residuals do not contain any trend you may delete $d=1$ in line 58.
- In lines 74, 80, 87: Inside the "for" loops replace "WT" "MUT1" "MUT2" with your genotypes' names on the dataset. If you have more than 3 genotypes include more loops in the same way for all the comparisons.

For example: If we have also a 3rd mutant it would be like:

```
for (i in 1:tp){

  t<- t.test(df$fit[which(df$Genotype=="WT" &
df$Time==i)],df$fit[which(df$Genotype=="MUT1" & df$Time==i)])
  test[i]<-t$p.value
}
test2<-0
for (i in 1:tp){

  t<- t.test(s[which(df$Genotype=="WT" & df$Time==i)],s[which(df$Genotype=="MUT2" &
df$Time==i)])
  test2[i]<-t$p.value
}
test3<-0
for (i in 1:tp){

  t<- t.test(s[which(df$Genotype=="WT" & df$Time==i)],s[which(df$Genotype=="MUT3" &
df$Time==i)])
  test3[i]<-t$p.value
}

test4<-0
for (i in 1:tp){

  t<- t.test(s[which(df$Genotype=="MUT1" & df$Time==i)],s[which(df$Genotype=="MUT2" &
df$Time==i)])
  test4[i]<-t$p.value
}
test5<-0
for (i in 1:tp){

  t<- t.test(s[which(df$Genotype=="MUT1" & df$Time==i)],s[which(df$Genotype=="MUT3" &
df$Time==i)])
  test5[i]<-t$p.value
}
test6<-0
for (i in 1:tp){

  t<- t.test(s[which(df$Genotype=="MUT2" & df$Time==i)],s[which(df$Genotype=="MUT3" &
df$Time==i)])
  test6[i]<-t$p.value
}
```

