

Escape analysis

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Statistical analysis for Feng Quan and Claire Wyart

Import data from files

8 kinematic parameters were measured at 10 trials in the slow and fast swim gait: Cbend, Counterbend, Distance, Duration, Latency, mTBF, NumOfOsc and Speed.

Number of values used by Genotype and Clutch

	Cbend	Counterbend	Distance	Duration	Latency	mTBF	NumOfOsc	Speed
WT_2	18	18	18	18	18	18	18	18
WT_3	20	20	20	20	20	20	20	20
WT_4	28	28	28	28	28	28	28	28
WT_5	25	25	25	25	25	25	25	25
Hom_2	17	17	17	17	17	17	17	17
Hom_3	23	23	23	23	23	23	23	23
Hom_4	14	14	14	14	14	14	14	14
Hom_5	26	26	26	26	26	26	26	26

Mean values by Genotype and Clutch

	WT_2	WT_3	WT_4	WT_5	Hom_2	Hom_3	Hom_4	Hom_5
Cbend	103.4	102.5	101.3	92.3	104.9	101.6	102.7	91.5
Counterbend	45.6	46.6	46.2	36.5	42.9	49.2	51.8	40.9
Distance	8.6	9.6	9.1	8.3	9.1	10.3	9.2	8.0
Duration	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3
Latency	6.2	5.0	5.2	3.6	6.6	5.2	5.8	4.0
mTBF	34.8	36.9	37.2	43.6	31.9	37.9	37.2	44.7
NumOfOsc	8.0	9.7	7.5	8.8	7.7	8.5	7.9	8.8
Speed	33.5	34.5	38.1	34.9	33.4	39.3	37.4	34.0

Method

All statistical analyses were conducted using R version 3.6.1 (<https://www.R-project.org/>), and plots were generated with the ggplot2 package.

Between-group comparison based on trial-averaged values was conducted through a linear mixed model using the lmer function in the lme4 package (for multiple-day data collection). Mean difference between the two groups (Hom and WT) was then tested using the emmeans package with the Tukey's method for multiplicity adjustment. To better match the model assumptions (normality and homoscedasticity of residuals), data were transformed by the natural logarithm or the square root function prior to modeling.

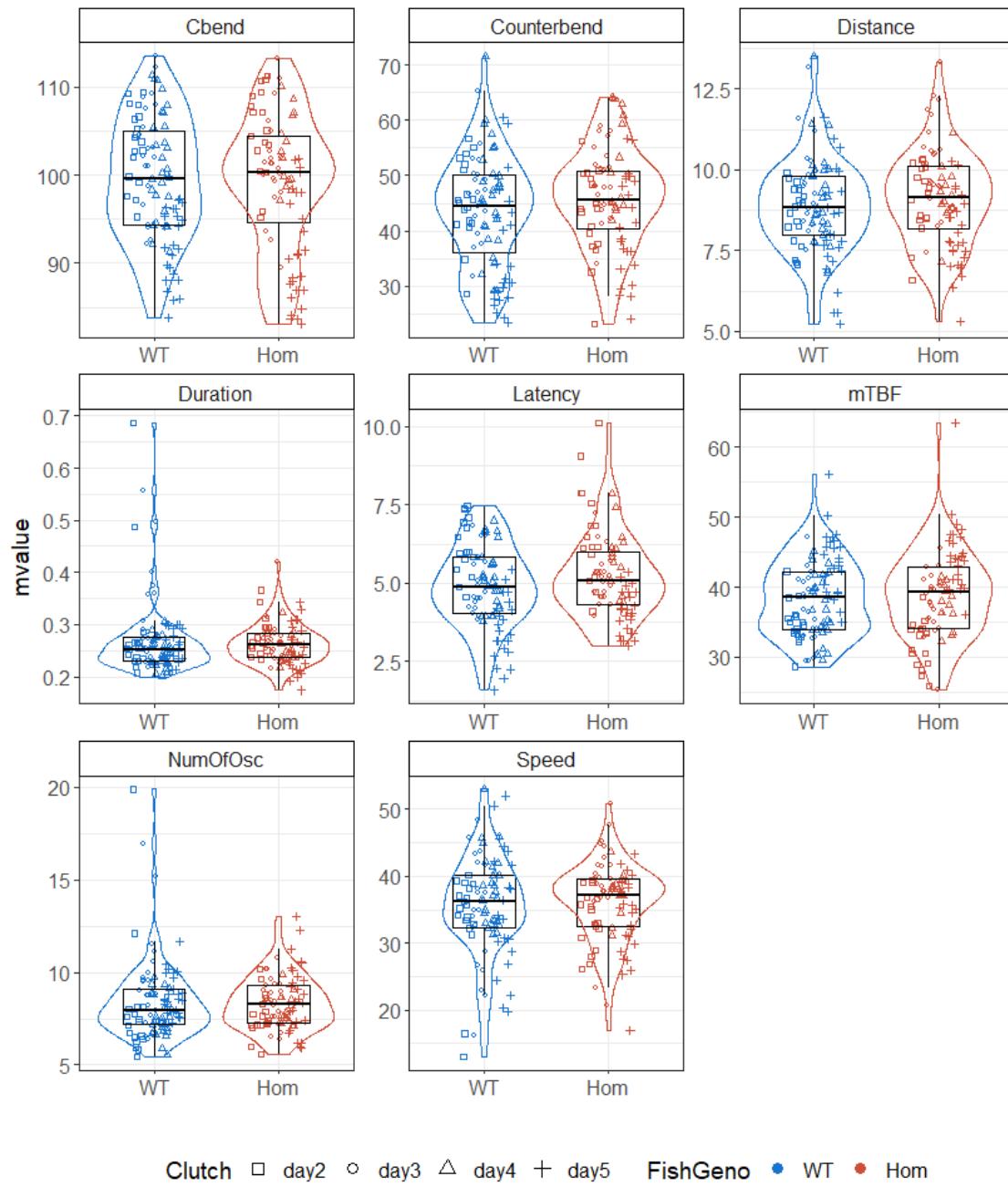
Between-group comparison across the ten trials was performed using a linear mixed model (function lmer in the lme4 package) with the fixed effects of Genotype and Trial, and the random effect of Fish_ID nested within Clutch for repeated measures. Significance for the main effects of genotype (WT vs. Homozygous mutants), Trial and their interaction was then evaluated using the Anova function in the car package with Type II Wald chi-square tests. To improve normality and homoscedasticity of residuals, data were transformed by the natural logarithm or the square root function prior to modeling.

For each kinematic parameter, the estimated marginal means and model-based standard errors on the original scale (non-transformed data) were extracted for each genotype using the emmeans package.

The level of statistical significance was set at $p < 0.05$ for all tests.

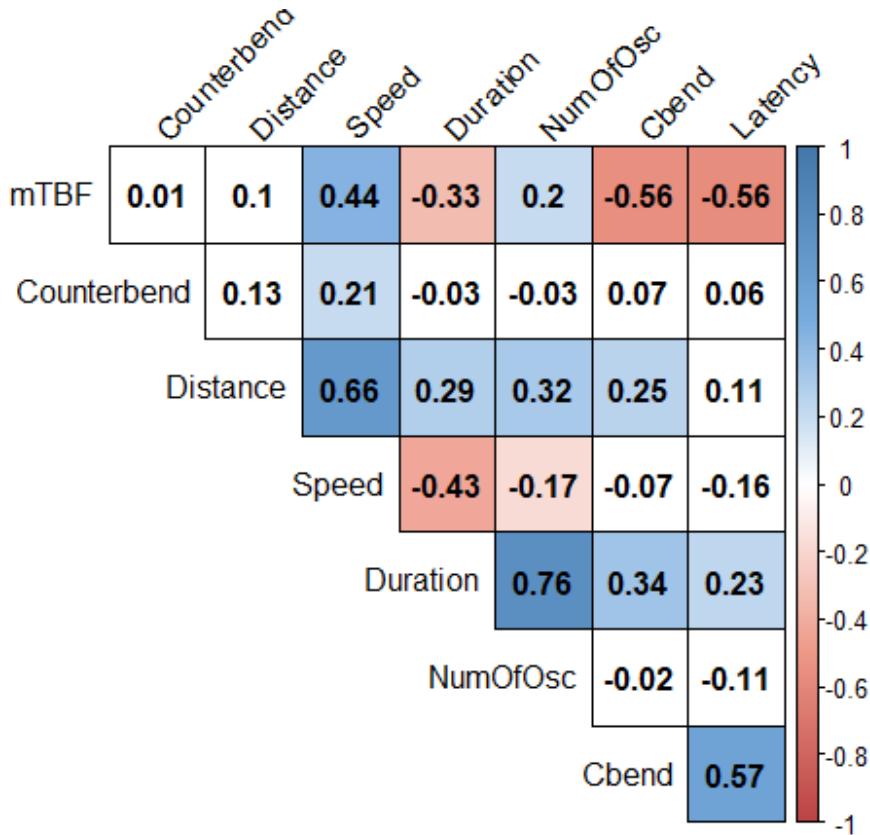
Analysis using one average value per fish

Data visualization



Correlation matrix

Using Spearman's rank correlations on average values:



Based on 8 kinematic parameters, variance of eigenvalues of the correlation matrix is 0.879 leading to an effective number of independent tests (M_{eff}) of 7.230 to be used for multiple testing correction. Method described in Nyholt DR, A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other, Am J Hum Genet, 2004 [PMID: 14997420].

Cbend [log, ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log(mvalue) ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: -481.027
## Random effects:
##   Groups   Name        Std.Dev.
##   Clutch   (Intercept) 0.05604
##   Residual           0.05491
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
## (Intercept) FishGenoHom
##             4.601577    0.001289

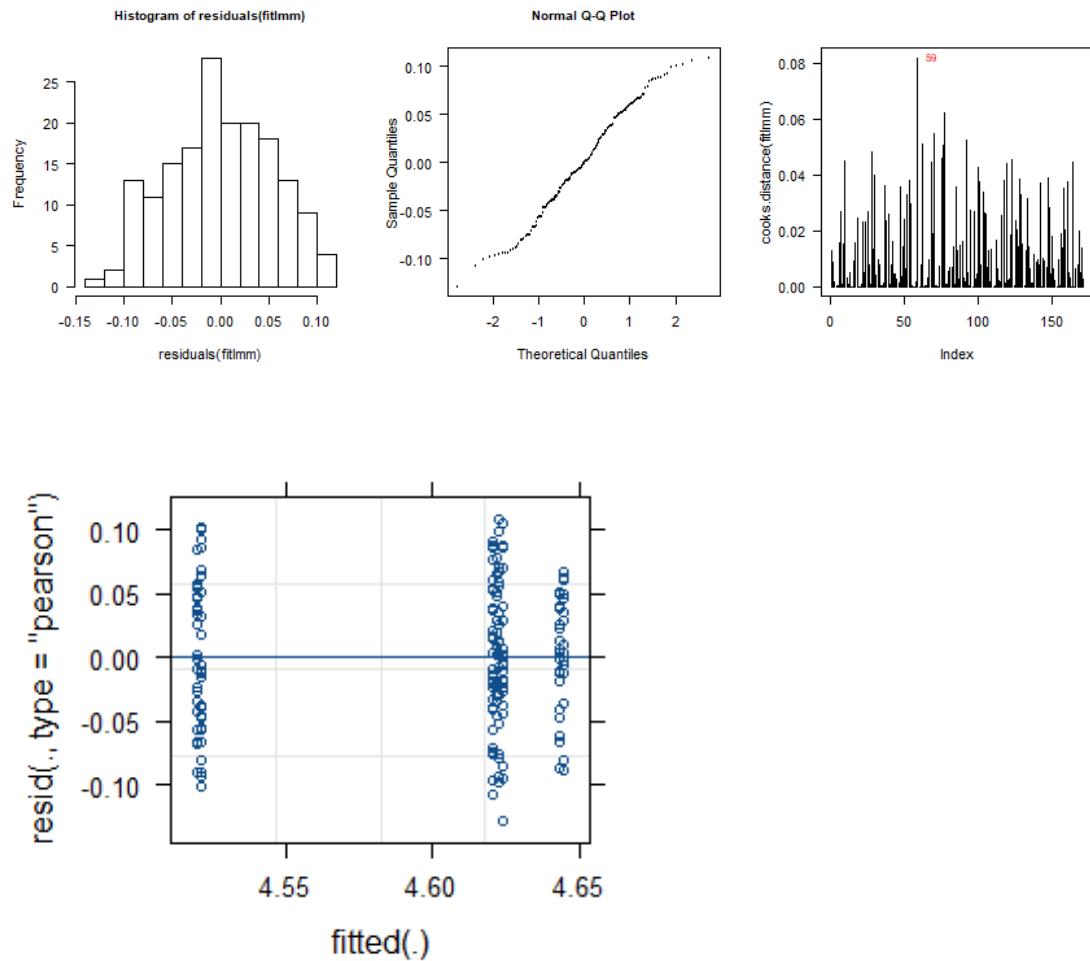
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log(mvalue)
##            Chisq Df Pr(>Chisq)
## FishGeno 0.0229  1    0.8797
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom
## Transformation: "log"

## FishGeno response   SE   df lower.CL upper.CL
## WT          99.6 2.85 3.12    91.1     109
## Hom         99.8 2.86 3.16    91.3     109
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
```

Checking model assumptions



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.880$). Adj. Pvalue: ${}^{ns}p = 1$ (using Meff = 7.230 tests).

Counterbend [sqrt, ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: sqrt(mvalue) ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: 360.0286
## Random effects:
##   Groups   Name      Std.Dev.
##   Clutch   (Intercept) 0.3431
##   Residual           0.6689
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
##   (Intercept) FishGenoHom
##             6.5521      0.2117

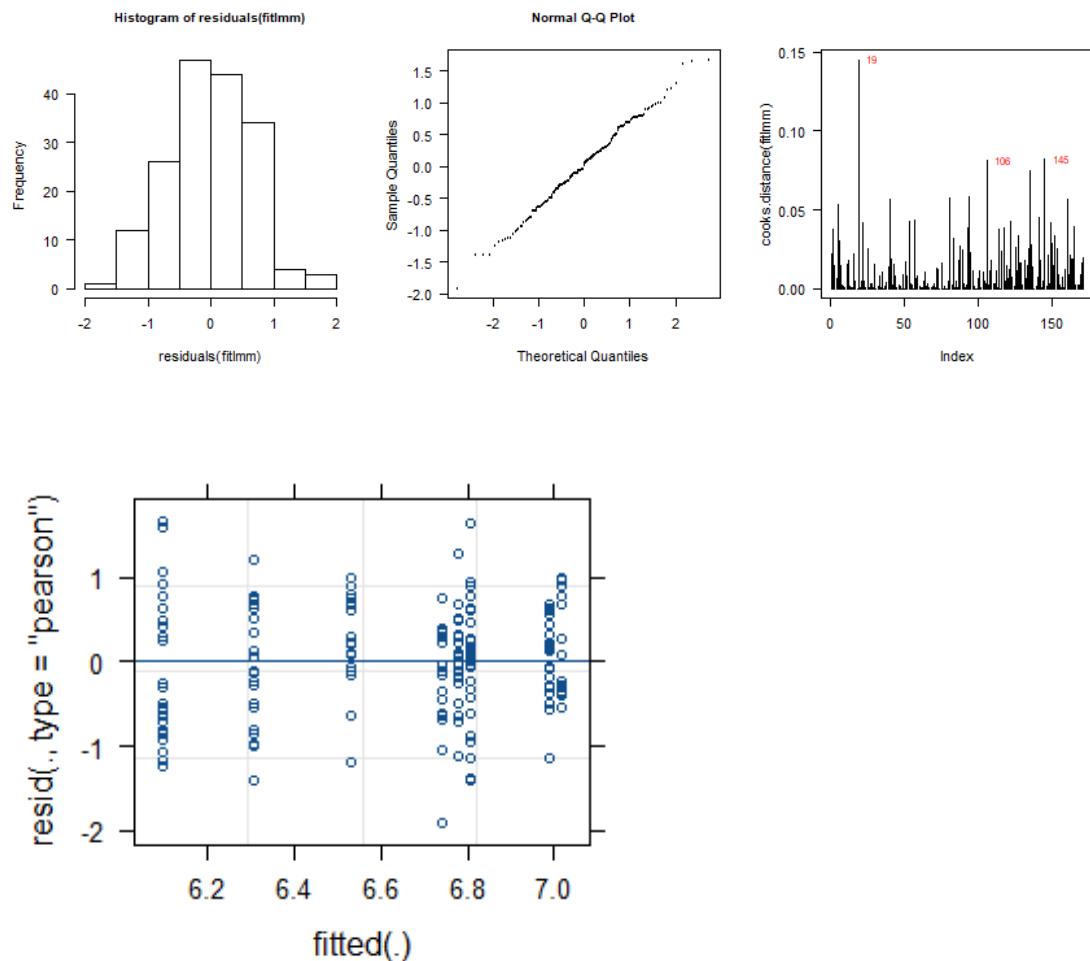
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(mvalue)
##            Chisq Df Pr(>Chisq)
## FishGeno 4.1674  1    0.04121 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom
## Transformation: "sqrt"

##   FishGeno response   SE   df lower.CL upper.CL
##   WT          42.9 2.43 3.45     36.0     50.4
##   Hom         45.7 2.54 3.59     38.7     53.4
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Intervals are back-transformed from the sqrt scale
```

Checking model assumptions



Conclusion

Linear mixed model indicated a significant difference between the two groups (Type II Wald chi-square test; $*p = 0.041$). Adj. Pvalue: $^{ns}p = 0.298$ (using Meff = 7.230 tests). Unadjusted p-value remains significant after removing the most influential data point 19 (see Cook's distance).

Distance [log, ns]

The residuals distribution is skewed without the log transformations.

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log(mvalue) ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: -146.0768
## Random effects:
##   Groups   Name        Std.Dev.
##   Clutch   (Intercept) 0.0846
##   Residual           0.1494
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
## (Intercept) FishGenoHom
##             2.17487    0.02371

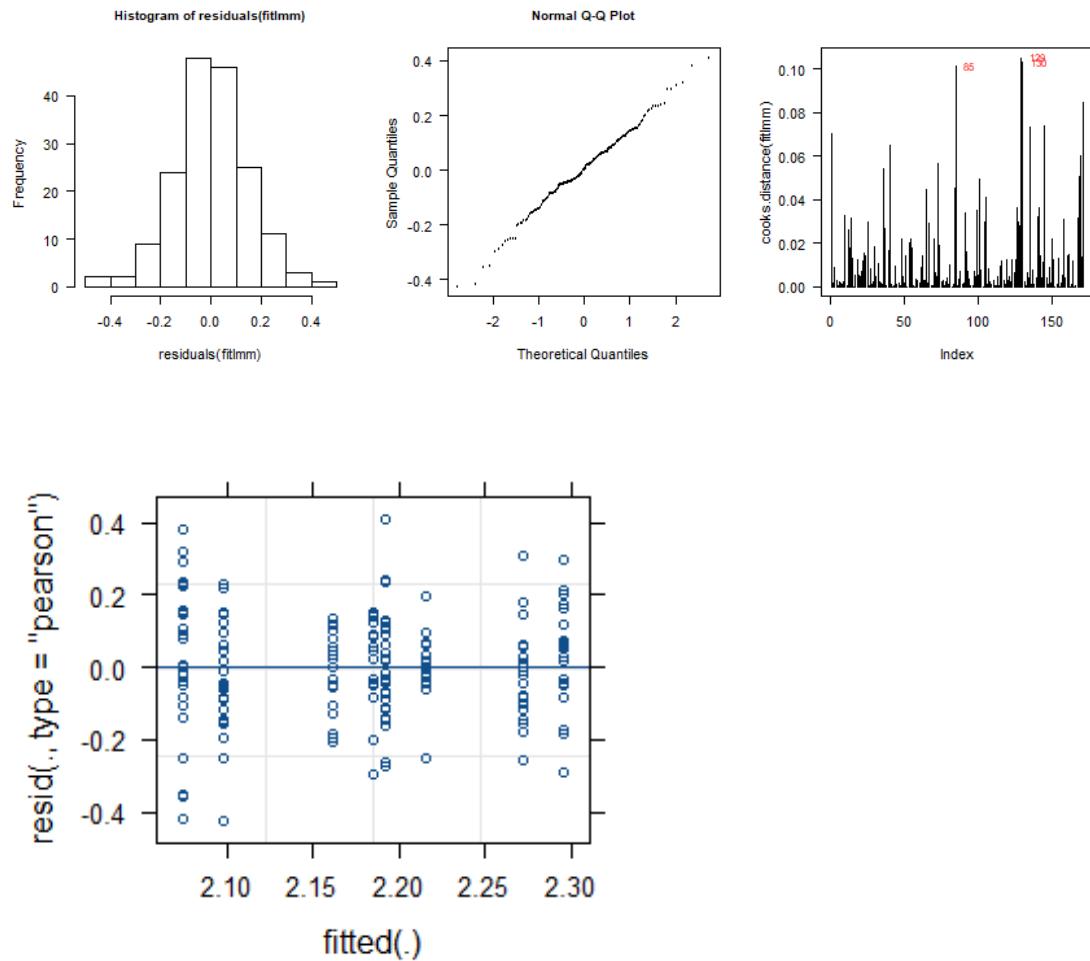
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log(mvalue)
##            Chisq Df Pr(>Chisq)
## FishGeno 1.0477  1     0.306
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom
## Transformation: "log"

## FishGeno response   SE   df lower.CL upper.CL
## WT              8.80 0.397 3.37    7.69    10.1
## Hom             9.01 0.411 3.49    7.88    10.3
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
```

Checking model assumptions



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.306$). Adj. Pvalue: ${}^{ns}p = 1$ (for Meff = 7.230 tests).

Duration [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: mvalue ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: -468.2959
## Random effects:
##   Groups   Name      Std.Dev.
##   Clutch   (Intercept) 0.01968
##   Residual           0.05805
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
## (Intercept) FishGenoHom
##             0.271820  -0.008976

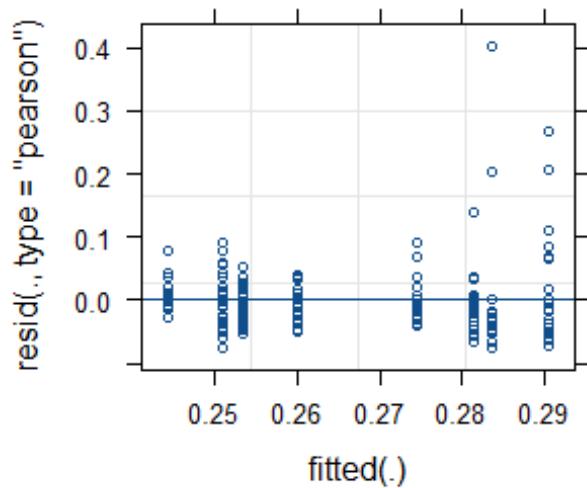
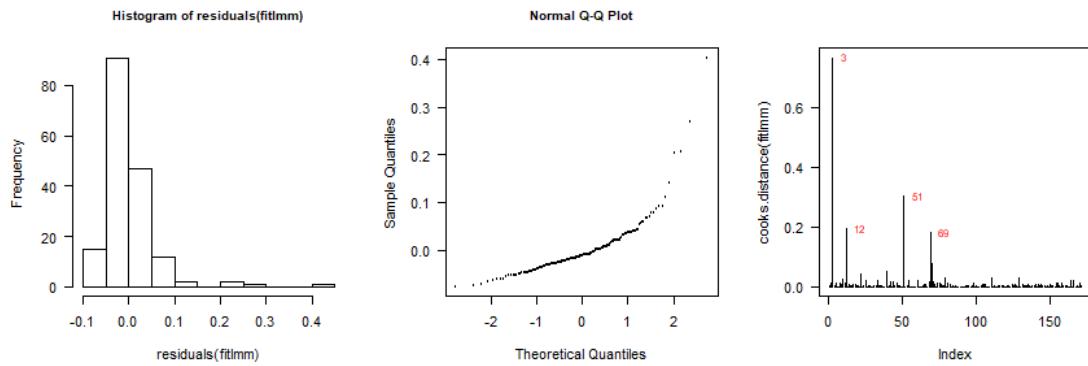
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: mvalue
##            Chisq Df Pr(>Chisq)
## FishGeno 0.9967  1    0.3181
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom

##   FishGeno emmean      SE   df lower.CL upper.CL
##   WT        0.272 0.0116 3.95    0.239    0.304
##   Hom       0.263 0.0118 4.27    0.231    0.295
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

Checking model assumptions



Conclusion

Bad model fit. There does not seem to be any significant effect of the group (Type II Wald chi-square test; $^{ns}p = 0.318$). Adj. Pvalue: $^{ns}p = 1$ (for Meff = 7.230 tests).

Latency [sqrt, ns]

Skewed distribution of residuals without square root transformation.

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: sqrt(mvalue) ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: -15.6549
## Random effects:
##   Groups   Name        Std.Dev.
##   Clutch   (Intercept) 0.2353
##   Residual           0.2173
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
## (Intercept) FishGenoHom
##             2.21005    0.09109

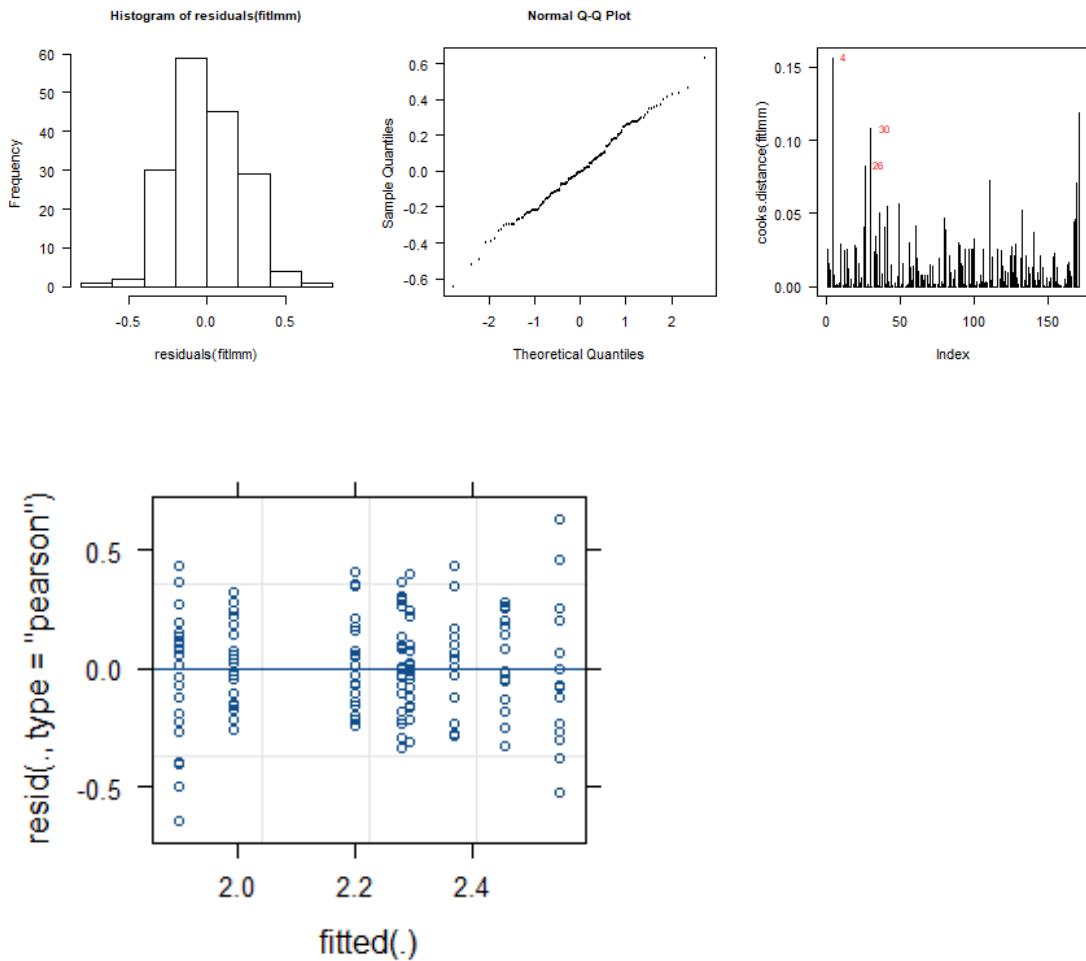
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(mvalue)
##            Chisq Df Pr(>Chisq)
## FishGeno 7.297  1  0.006907 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom
## Transformation: "sqrt"

## FishGeno response   SE   df lower.CL upper.CL
## WT                 4.88 0.530 3.10     3.37    6.68
## Hom                5.30 0.553 3.14     3.72    7.15
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Intervals are back-transformed from the sqrt scale
```

Checking model assumptions



Conclusion

Linear mixed model indicated a significant difference between the two groups (ANOVA Type II test; $**p = 6.9e-3$). Adj. Pvalue: $^{ns}p = 0.050$ (using Meff = 7.230 tests). However, checking for model assumptions, we did not retain the effect as it was no more present after exclusion of one influential observation (point 4) indicated by the Cook's distance.

mTBF [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: mvalue ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: 998.9557
## Random effects:
##   Groups   Name      Std.Dev.
##   Clutch   (Intercept) 4.439
##   Residual           4.378
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
##   (Intercept) FishGenoHom
##       38.07237     -0.02279

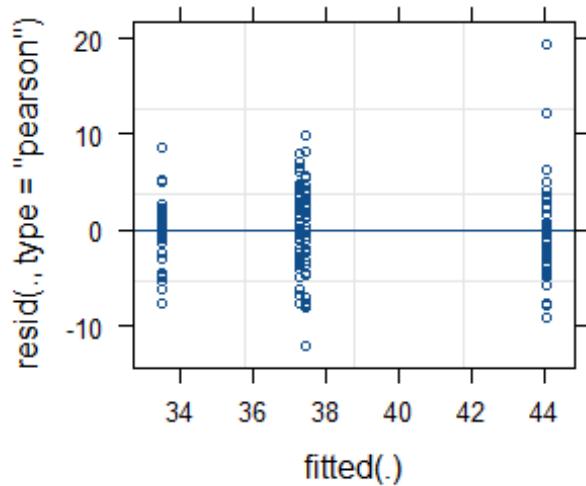
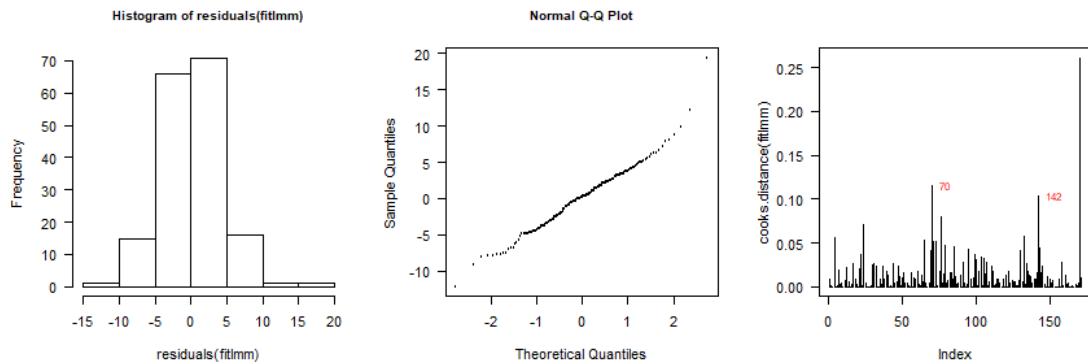
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: mvalue
##             Chisq Df Pr(>Chisq)
## FishGeno 0.0011  1    0.9732
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom

##   FishGeno emmean   SE   df lower.CL upper.CL
##   WT        38.1 2.27 3.12      31     45.1
##   Hom       38.0 2.27 3.16      31     45.1
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

Checking model assumptions



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.973$). Adj. Pvalue: ${}^{ns}p = 1$ (for Meff = 7.230 tests).

Number of oscillations [sqrt, ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: sqrt(mvalue) ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: 71.1761
## Random effects:
##   Groups   Name      Std.Dev.
##   Clutch   (Intercept) 0.1132
##   Residual           0.2857
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
## (Intercept) FishGenoHom
##             2.88692    -0.03236

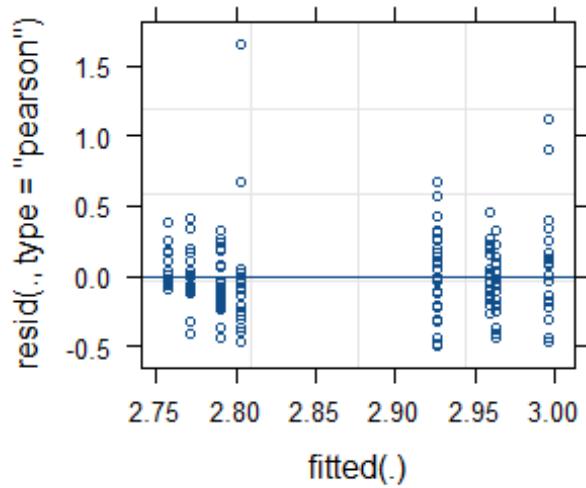
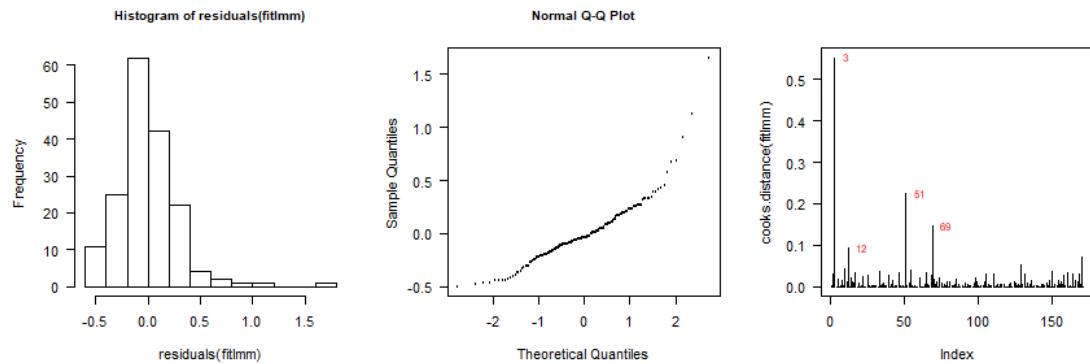
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(mvalue)
##            Chisq Df Pr(>Chisq)
## FishGeno 0.5342  1    0.4648
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom
## Transformation: "sqrt"

## FishGeno response   SE   df lower.CL upper.CL
## WT          8.33 0.370 3.72     7.31    9.43
## Hom         8.15 0.372 3.96     7.14    9.22
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Intervals are back-transformed from the sqrt scale
```

Checking model assumptions



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.465$). Adj. Pvalue: ${}^{ns}p = 1$ (for $M_{eff} = 7.230$ tests).

Speed [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: mvalue ~ FishGeno + (1 | Clutch)
##   Data: tmp
## REML criterion at convergence: 1136.087
## Random effects:
##   Groups   Name      Std.Dev.
##   Clutch   (Intercept) 1.822
##   Residual           6.709
## Number of obs: 171, groups: Clutch, 4
## Fixed Effects:
##   (Intercept) FishGenoHom
##             35.3987     0.7016

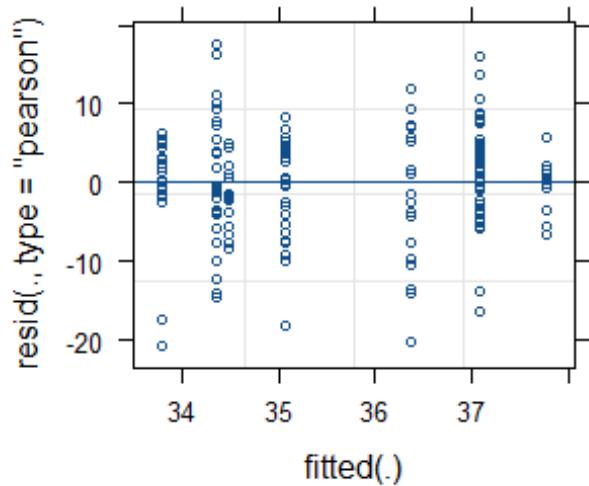
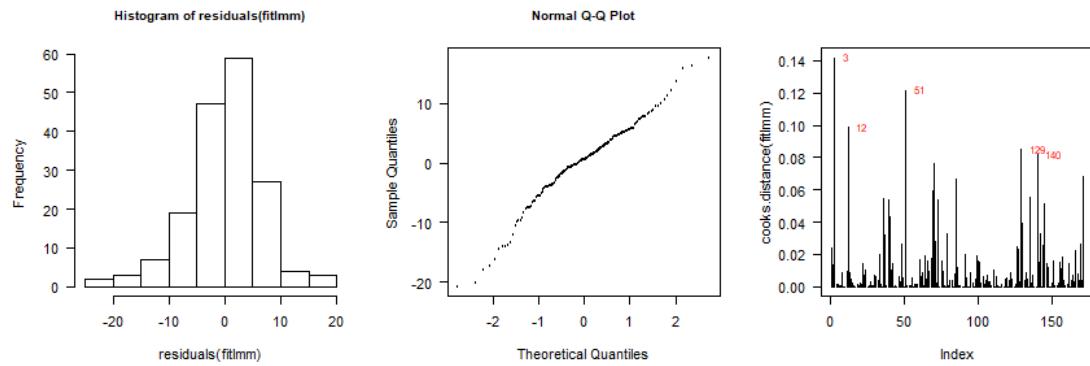
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: mvalue
##             Chisq Df Pr(>Chisq)
## FishGeno 0.4569  1    0.4991
```

Estimated marginal means with model-based standard errors and 95% confidence limits on the original scale (non-transformed data):

```
## 'emmGrid' object with variables:
##   FishGeno = WT, Hom

##   FishGeno emmean   SE   df lower.CL upper.CL
##   WT        35.4 1.15 4.38    32.3    38.5
##   Hom       36.1 1.19 4.86    33.0    39.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

Checking model assumptions

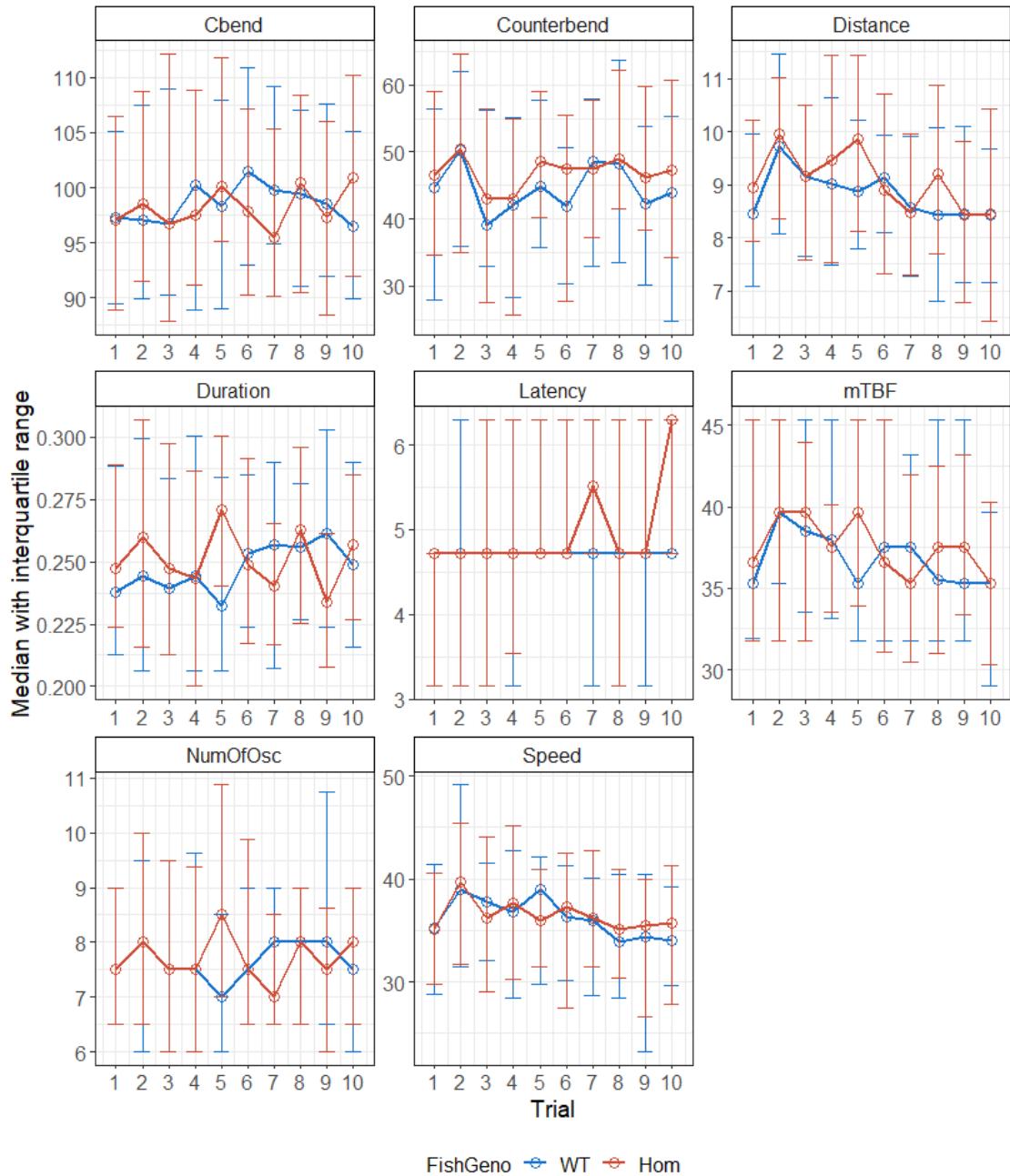


Conclusion

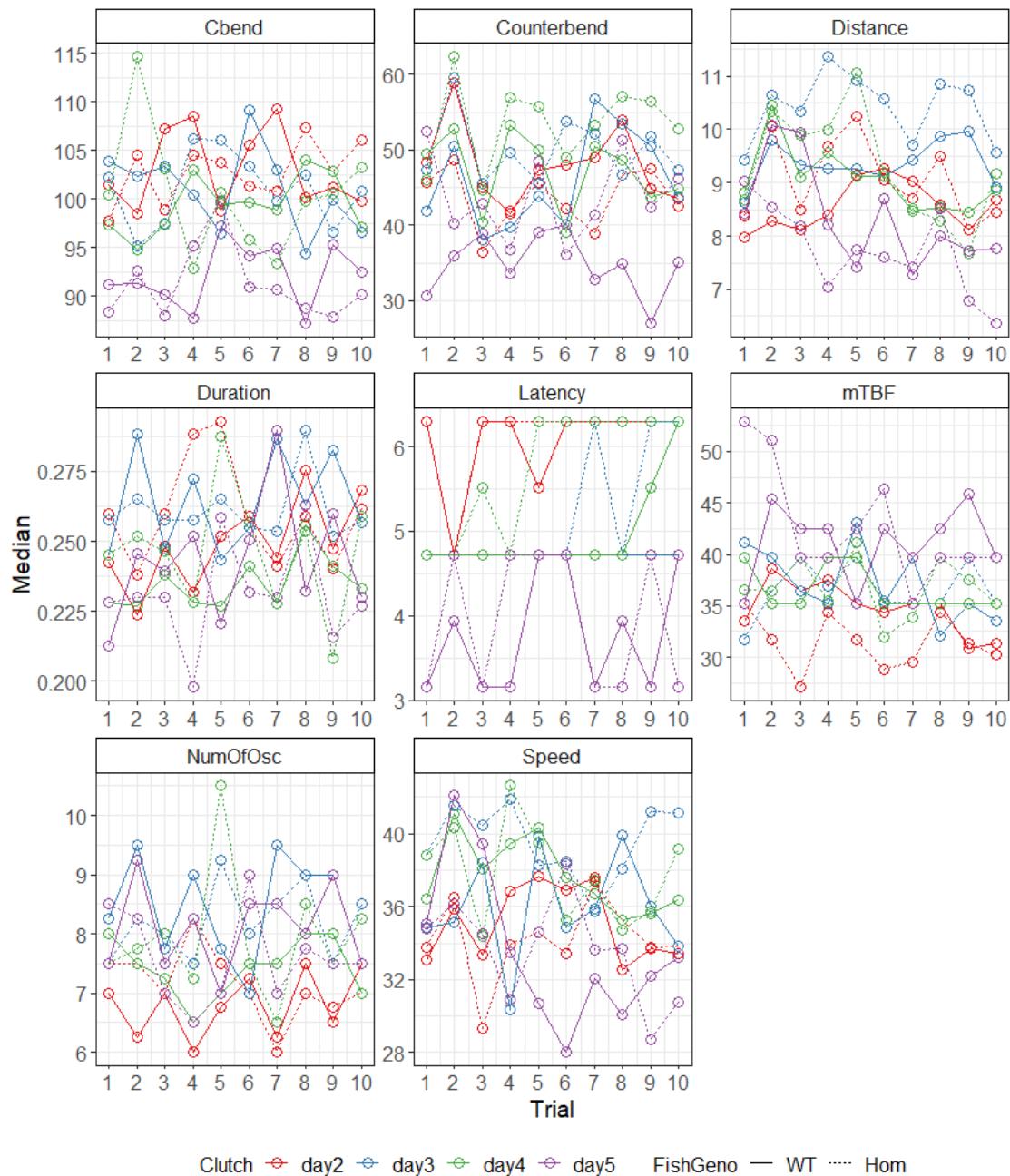
No significant difference was detected between the two groups (Type II Wald chi-square test; $^{ns}p = 0.499$). Adj. Pvalue: $^{ns}p = 1$ (for Meff = 7.230 tests).

Analysis across the 10 trials

Data visualization

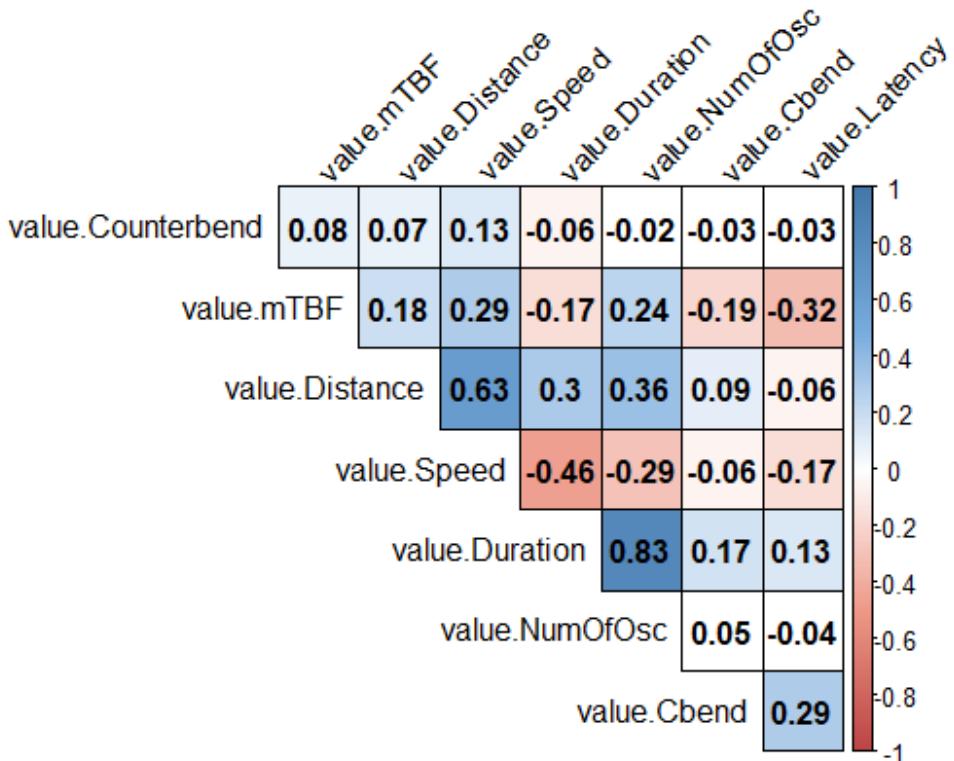


Data visualization with clutch info



Correlation matrix

Using Spearman's rank correlations and all the trials:



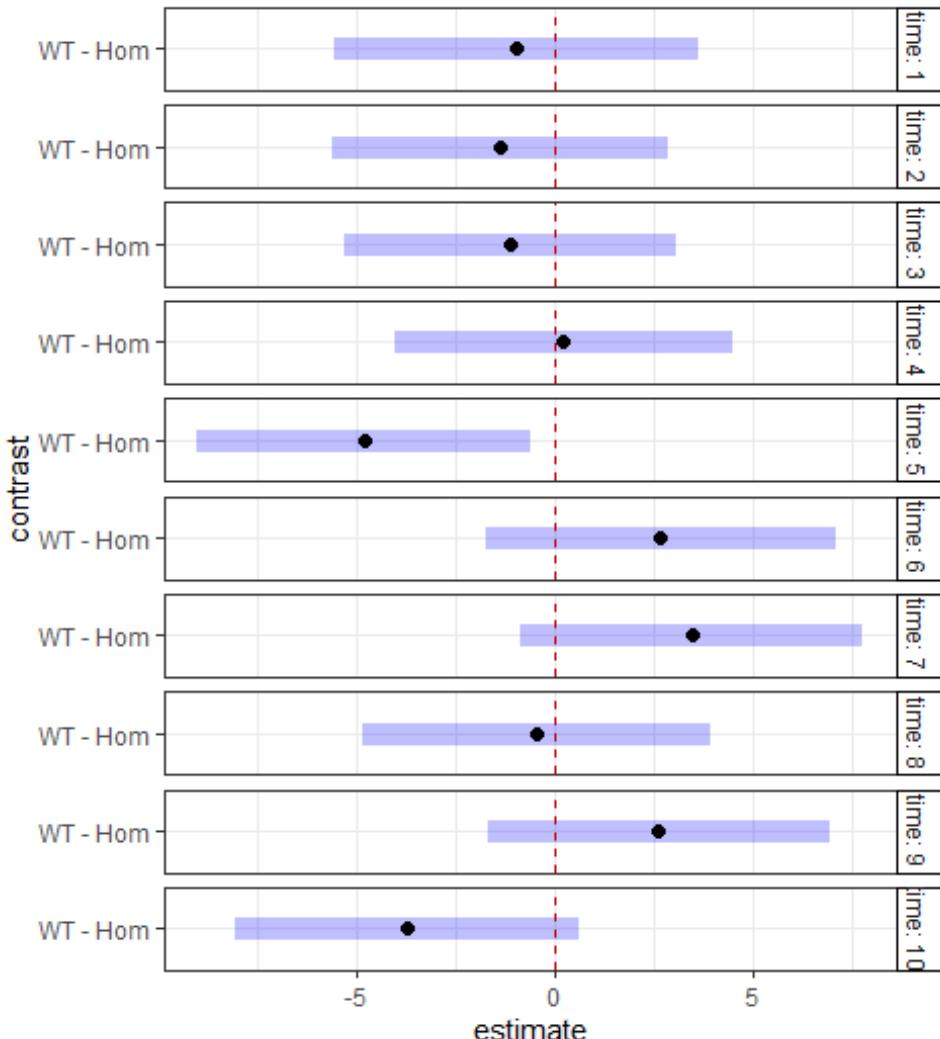
Based on 8 kinetic parameters, variance of eigenvalues of the correlation matrix is 0.622 leading to a Meff value of 7.456 to be used for multiple testing correction. Method described in Nyholt DR, A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other, Am J Hum Genet, 2004 [PMID: 14997420].

Cbend [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: value ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: 11973.44
## Random effects:
## Groups      Name      Std.Dev.
## Fish:Clutch (Intercept) 3.48
## Clutch      (Intercept) 5.08
## Residual          12.88
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##           (Intercept)     FishGenoHom       time  FishGenoHom:time
##             99.53922        1.28642        0.08319       -0.16176
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: value
##              Chisq Df Pr(>Chisq)
## FishGeno    0.1889  1   0.6638
## time       0.0052  1   0.9428
## FishGeno:time 0.4683  1   0.4938
```

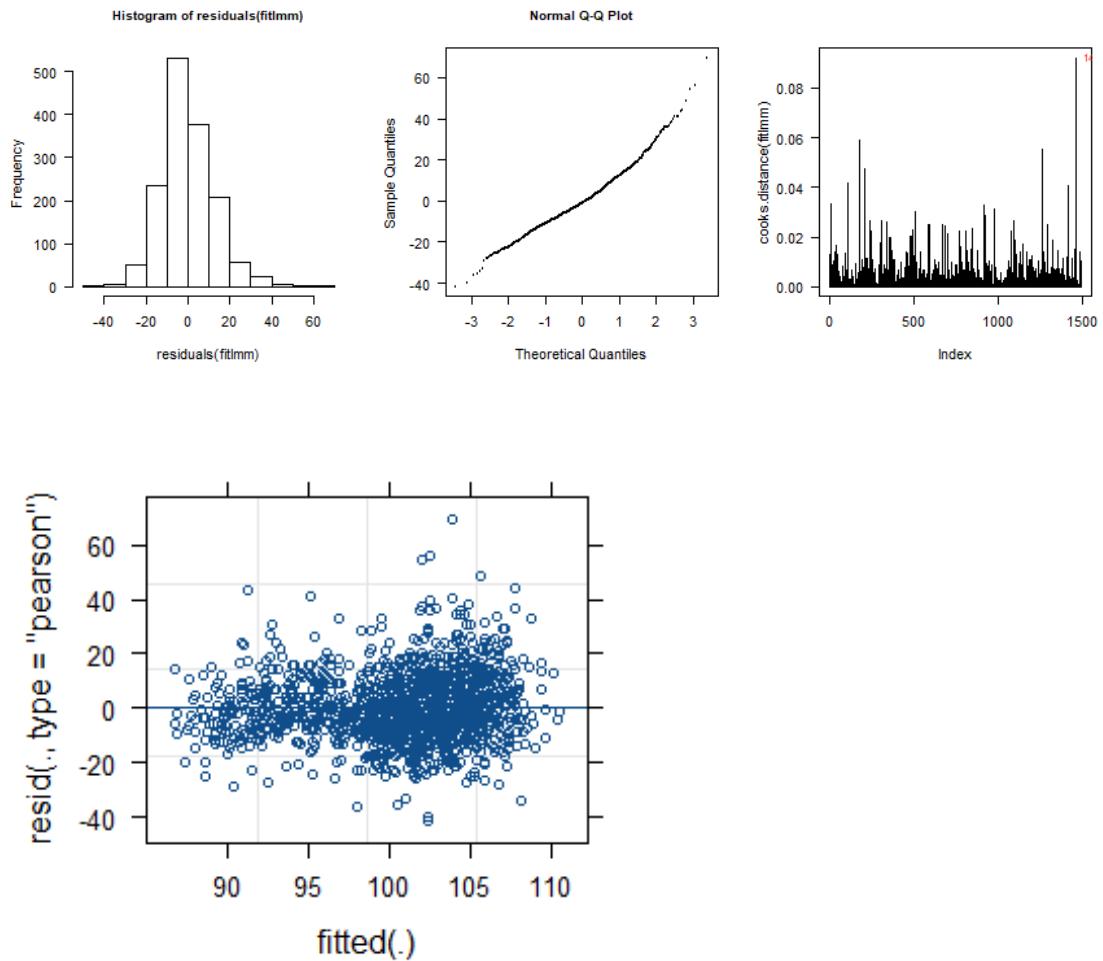
Group comparison at each trial



```
##
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
##
##   contrast    time   estimate      SE      df t.ratio
##   p.value
## -----
##   WT - Hom 1 -0.9461566 2.350722 1384.246 -0.4024961
##   0.6873811
##   WT - Hom 2 -1.3603148 2.164448 1349.969 -0.6284811
##   0.5297951
##   WT - Hom 3 -1.0948662 2.133491 1339.268 -0.5131806
##   0.6079096
##   WT - Hom 4 0.2279092 2.175144 1356.868 0.1047789
##   0.9165667
```

## WT - Hom	5	-4.7921306	2.143612	1348.704	-2.2355398
0.0255450					
## WT - Hom	6	2.6865312	2.253672	1373.360	1.1920681
0.2334405					
## WT - Hom	7	3.4692846	2.199193	1361.850	1.5775265
0.1149067					
## WT - Hom	8	-0.4427511	2.229724	1373.390	-0.1985676
0.8426304					
## WT - Hom	9	2.6471243	2.198675	1360.646	1.2039636
0.2288130					
## WT - Hom	10	-3.7267161	2.216802	1392.952	-1.6811228
0.0929632					

Checking model assumptions



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.664$). Adj. Pvalue: ${}^{ns}p = 1$ (for Meff = 7.456 tests).

Counterbend [ns]

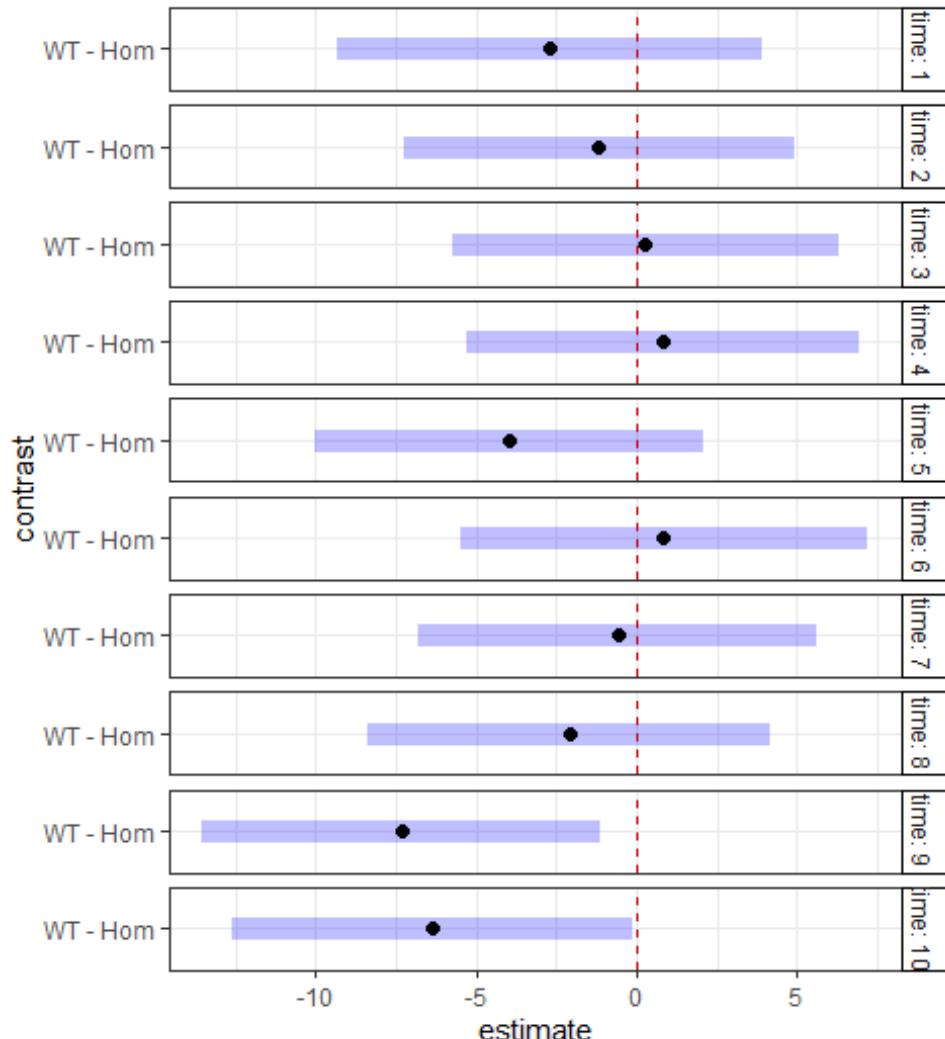
Fitting LMM

```
## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl =
control$checkConv, :
## Model failed to converge with max|grad| = 0.00202687 (tol = 0.002,
component 1)

## Linear mixed model fit by REML ['lmerMod']
## Formula: value ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: 13016.92
## Random effects:
## Groups      Name      Std.Dev.
## Fish:Clutch (Intercept) 6.541
## Clutch      (Intercept) 3.641
## Residual          18.056
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##           (Intercept)     FishGenoHom       time   FishGenoHom:time
##             45.6827        -0.7760        -0.3027         0.5542
## convergence code 0; 1 optimizer warnings; 0 lme4 warnings

## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: value
##              Chisq Df Pr(>Chisq)
## FishGeno    2.4433  1   0.11803
## time       0.0793  1   0.77822
## FishGeno:time 2.7922  1   0.09472 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

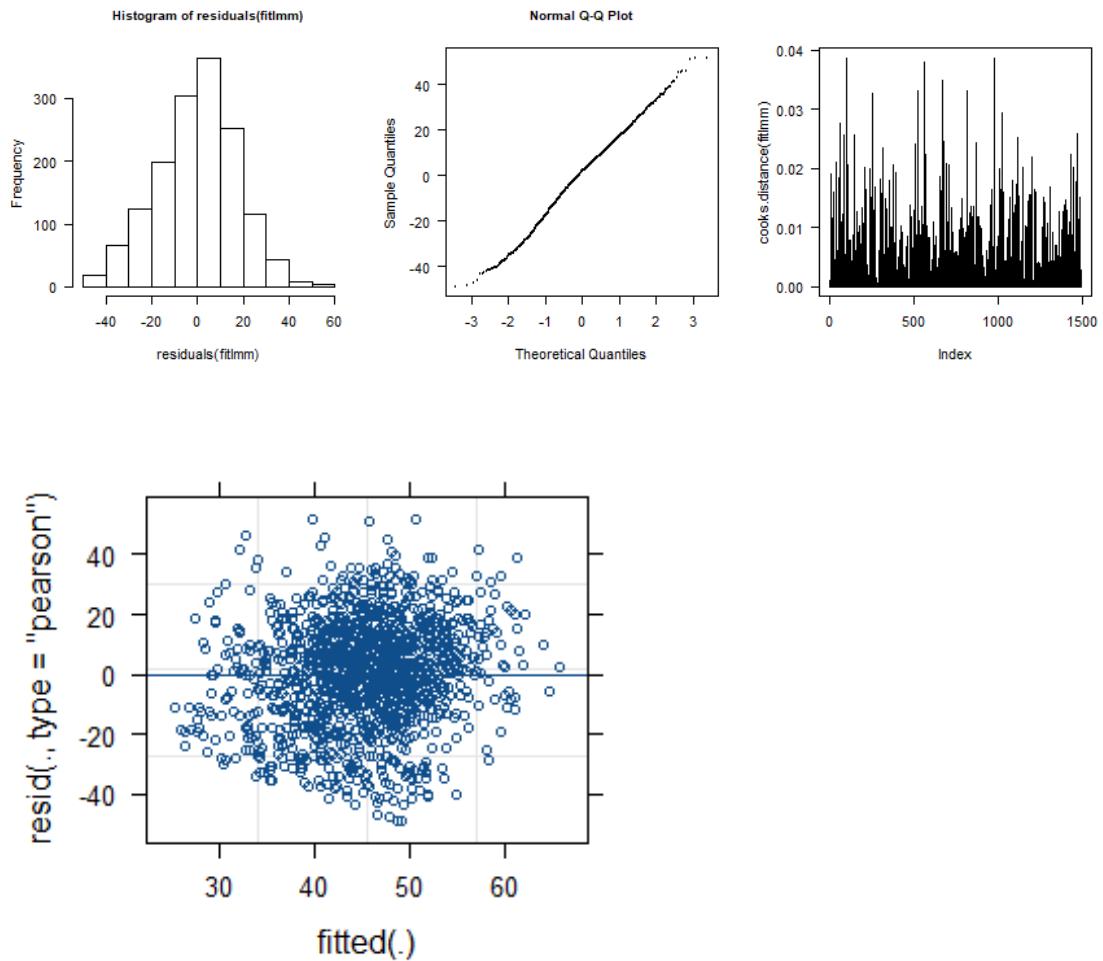
Group comparison at each trial



```
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
## contrast   time   estimate      SE      df t.ratio
## p.value
## -----
## WT - Hom 1 -2.6926632 3.368051 1297.083 -0.7994723
## 0.4241630
## WT - Hom 2 -1.1692153 3.110575 1224.261 -0.3758840
## 0.7070683
## WT - Hom 3 0.2756393 3.068539 1207.292 0.0898275
## 0.9284392
## WT - Hom 4 0.8036688 3.124670 1232.753 0.2572012
## 0.7970664
```

## WT - Hom	5	-3.9833615	3.081405	1217.001	-1.2927097
0.1963568					
## WT - Hom	6	0.8491193	3.233092	1266.458	0.2626338
0.7928755					
## WT - Hom	7	-0.5952959	3.157987	1242.276	-0.1885049
0.8505117					
## WT - Hom	8	-2.1087115	3.199241	1260.283	-0.6591286
0.5099336					
## WT - Hom	9	-7.3305554	3.157560	1241.083	-2.3215889
0.0204163					
## WT - Hom	10	-6.3594786	3.177737	1274.006	-2.0012604
0.0455761					

Checking model assumptions



Conclusion

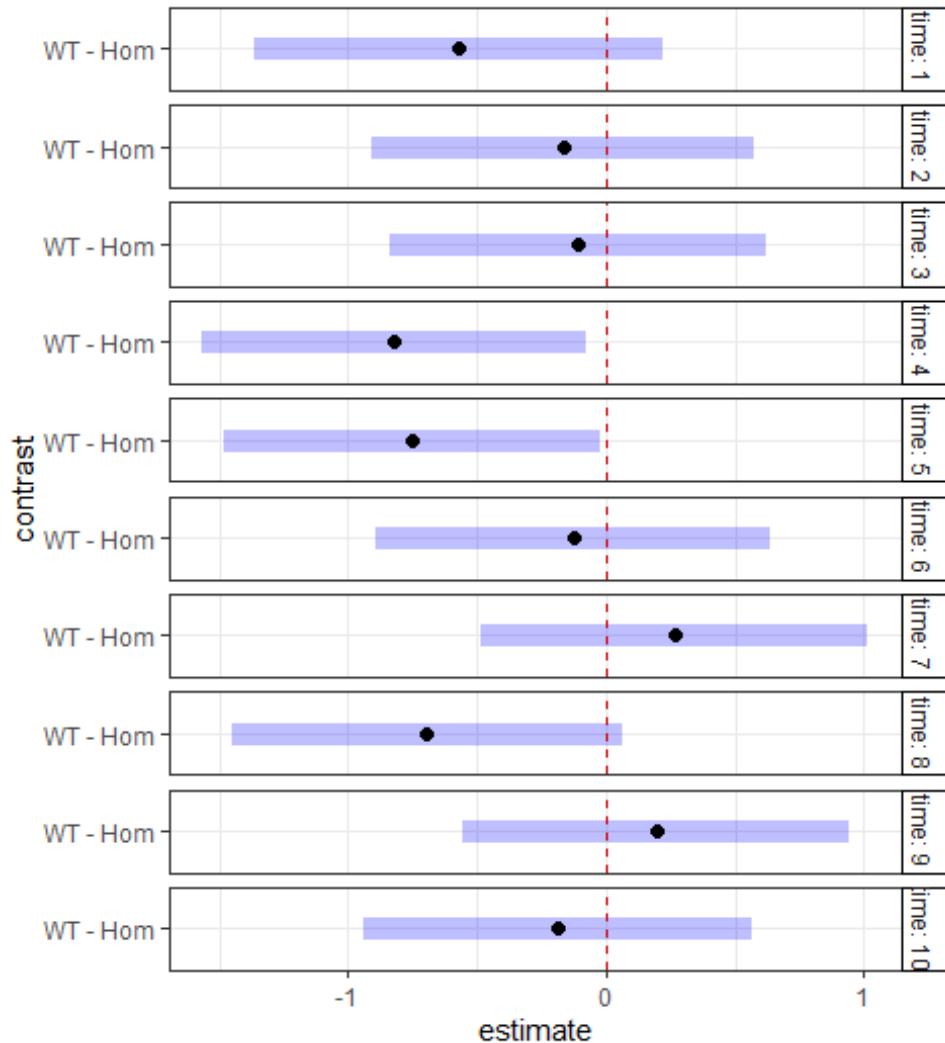
No significant difference was detected between the two groups (Type II Wald chi-square test; $^{ns}p = 0.118$). Adj. Pvalue: $^{ns}p = 0.880$ (for Meff = 7.456 tests).

Distance [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: value ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: 6592.135
## Random effects:
##   Groups      Name      Std.Dev.
##   Fish:Clutch (Intercept) 1.0864
##   Clutch      (Intercept) 0.8443
##   Residual          2.0418
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##   (Intercept)      FishGenoHom      time  FishGenoHom:time
##   9.27267        0.55450       -0.07998      -0.04471
## 
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: value
##             Chisq Df Pr(>Chisq)
## FishGeno      2.0704  1   0.1502
## time         28.8912  1  7.656e-08 ***
## FishGeno:time 1.4173  1   0.2338
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

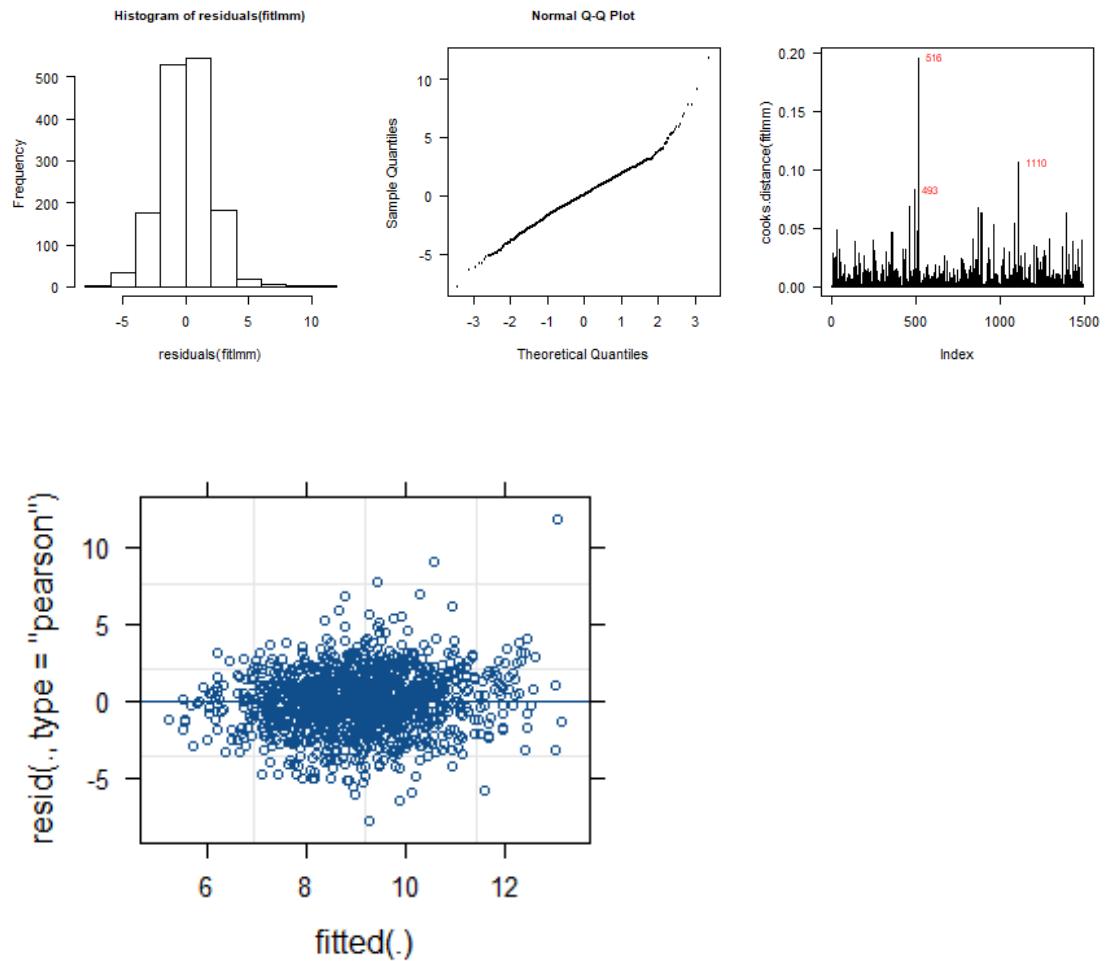
Group comparison at each trial



```
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
## contrast   time   estimate      SE      df t.ratio
## p.value
## -----
## WT - Hom 1 -0.5744247 0.4046534 1039.8291 -1.4195473
## 0.1560392
## WT - Hom 2 -0.1666882 0.3766813 920.3871 -0.4425177
## 0.6582186
## WT - Hom 3 -0.1119769 0.3722008 897.9990 -0.3008508
## 0.7635979
## WT - Hom 4 -0.8228512 0.3781269 929.0854 -2.1761242
## 0.0297973
```

## WT - Hom	5	-0.7528559	0.3734817	906.6431	-2.0157772
0.0441179					
## WT - Hom	6	-0.1289702	0.3898825	981.0622	-0.3307924
0.7408719					
## WT - Hom	7	0.2632344	0.3817751	944.1813	0.6895013
0.4906773					
## WT - Hom	8	-0.6947643	0.3861286	966.7891	-1.7993079
0.0722817					
## WT - Hom	9	0.1939219	0.3817563	943.3995	0.5079731
0.6115910					
## WT - Hom	10	-0.1853175	0.3833664	965.7667	-0.4833952
0.6289247					

Checking model assumptions



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.150$). Adj. Pvalue: ${}^{ns}p = 1$ (for Meff = 7.456 tests). Significant effect of trial (Type II Wald chi-square test; ${}^{ns}p = 7.7e-8$). Adj. Pvalue: ${}^{***}p = 5.7e-7$ (for Meff = 7.456 tests).

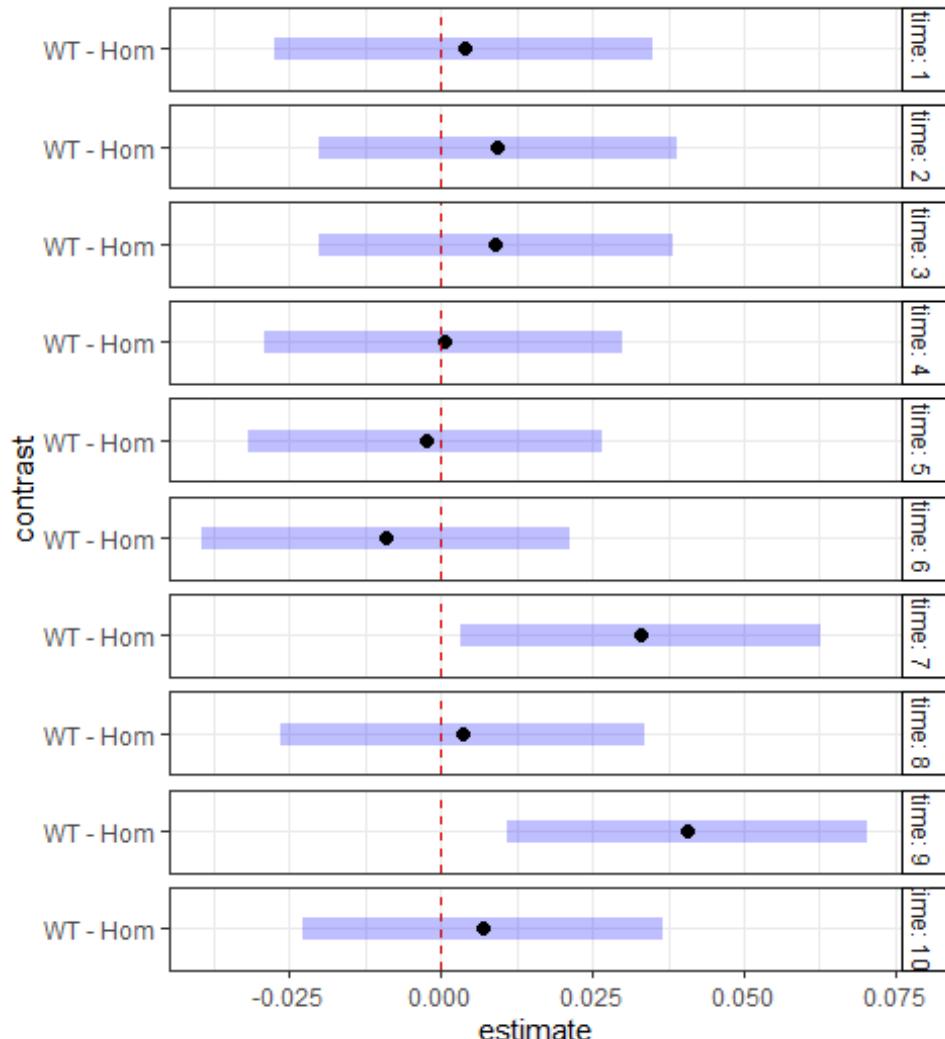
Duration [sqrt, ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: sqrt(value) ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: -3749.684
## Random effects:
## Groups      Name      Std.Dev.
## Fish:Clutch (Intercept) 0.04500
## Clutch      (Intercept) 0.01756
## Residual          0.06226
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##           (Intercept)      FishGenoHom       time  FishGenoHom:time
##             0.505666        0.004041       0.001381       -0.001818

## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(value)
##              Chisq Df Pr(>Chisq)
## FishGeno     0.5334  1    0.4652
## time         0.8942  1    0.3443
## FishGeno:time 2.5174  1    0.1126
```

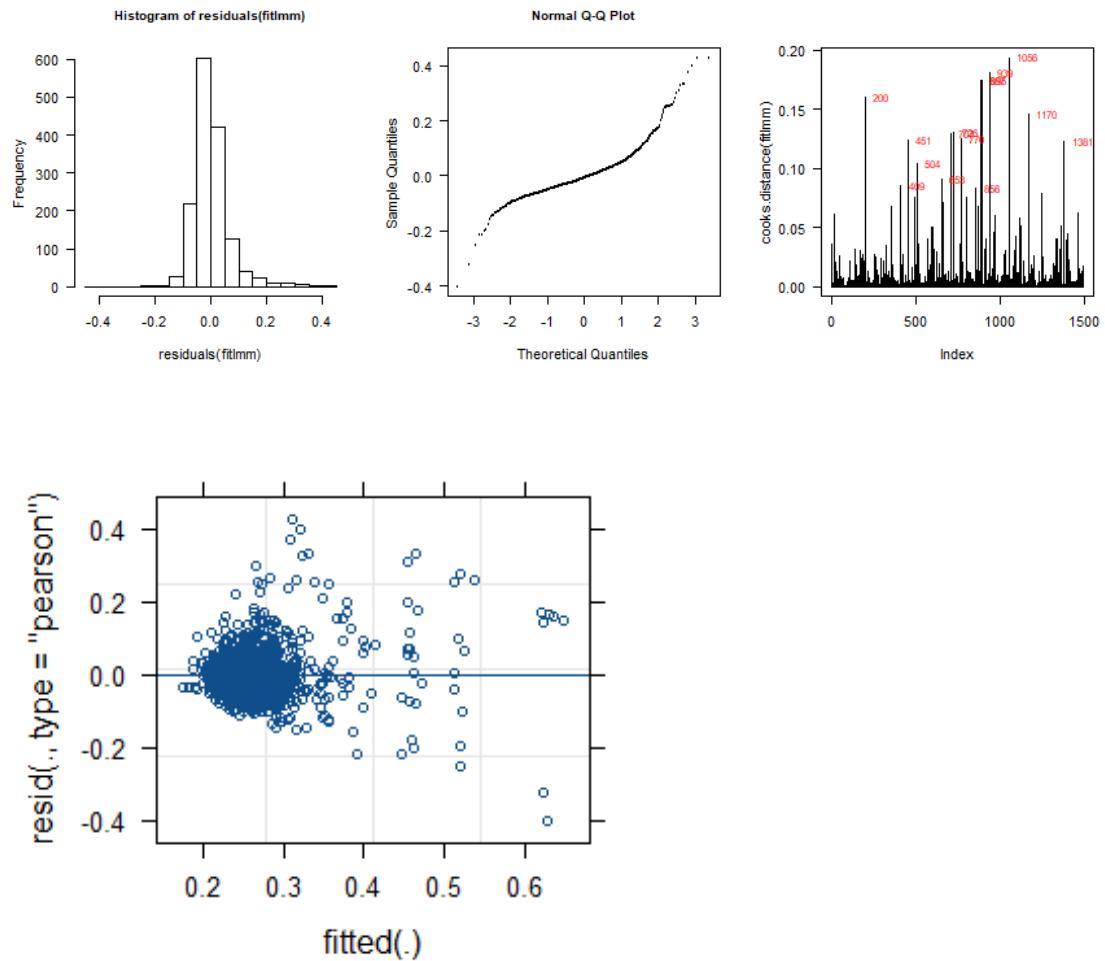
Group comparison at each trial



```
##
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
##
##   contrast   time   estimate      SE      df t.ratio
##   p.value
## -----
##   WT - Hom 1 0.0039350 0.0158774 680.7208 0.2478385
##   0.8043341
##   WT - Hom 2 0.0094118 0.0149821 578.1665 0.6282011
##   0.5301203
##   WT - Hom 3 0.0090667 0.0148419 561.8553 0.6108859
##   0.5415222
##   WT - Hom 4 0.0005141 0.0150262 583.7883 0.0342115
##   0.9727202
```

## WT - Hom	5	-0.0024212	0.0148797	566.7633	-0.1627180
0.8707985					
## WT - Hom	6	-0.0089754	0.0154020	626.6782	-0.5827408
0.5602774					
## WT - Hom	7	0.0329990	0.0151439	596.5782	2.1790312
0.0297199					
## WT - Hom	8	0.0036830	0.0152800	613.2545	0.2410359
0.8096078					
## WT - Hom	9	0.0406323	0.0151442	596.4503	2.6830283
0.0074983					
## WT - Hom	10	0.0069605	0.0151824	605.2845	0.4584559
0.6467895					

Checking model assumptions



Conclusion

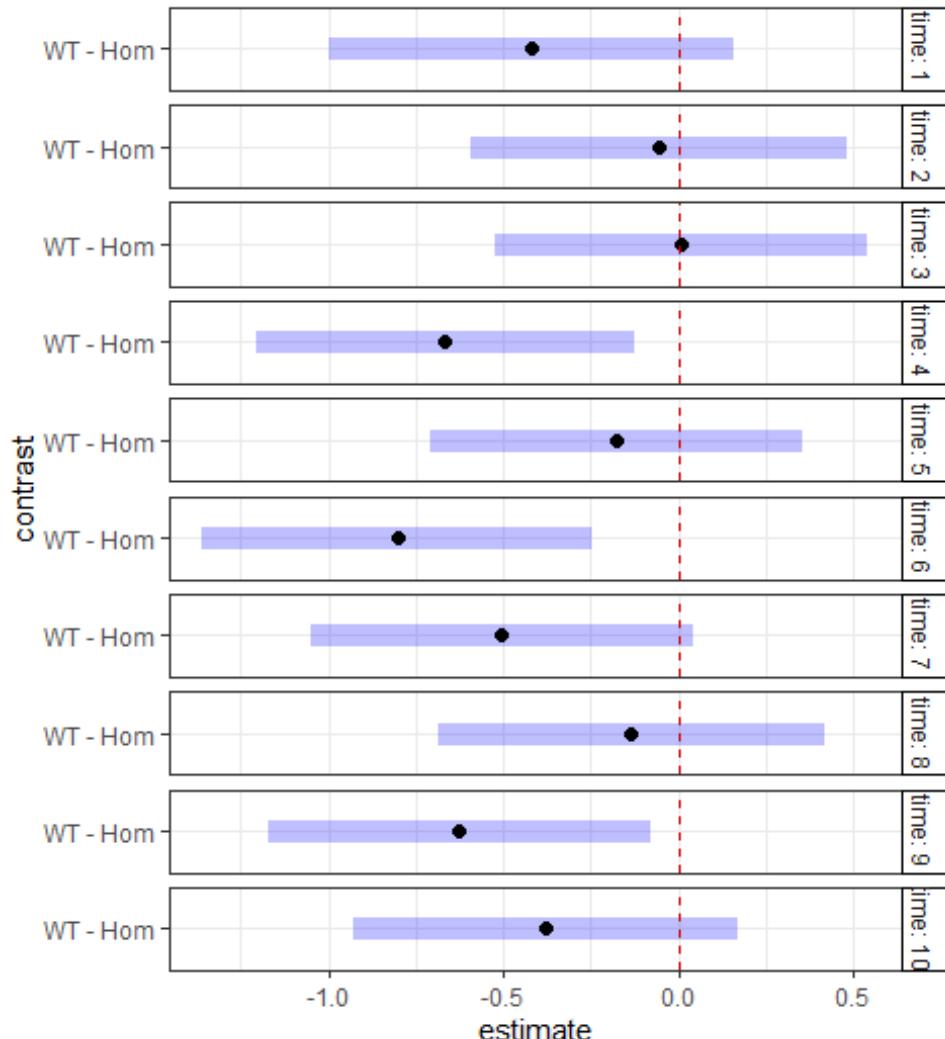
No significant difference was detected between the two groups (Type II Wald chi-square test; $^{ns}p = 0.465$). Adj. Pvalue: $^{ns}p = 1$ (for Meff = 7.456 tests).

Latency [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: value ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: 5584.152
## Random effects:
##   Groups      Name      Std.Dev.
##   Fish:Clutch (Intercept) 0.8489
##   Clutch      (Intercept) 1.0080
##   Residual          1.4444
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##           (Intercept)      FishGenoHom       time  FishGenoHom:time
##           4.74014          0.20958        0.05271        0.02903
## 
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: value
##             Chisq Df Pr(>Chisq)
## FishGeno      5.1803  1  0.02284 *
## time         24.9038  1 6.026e-07 ***
## FishGeno:time 1.1931  1  0.27471
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

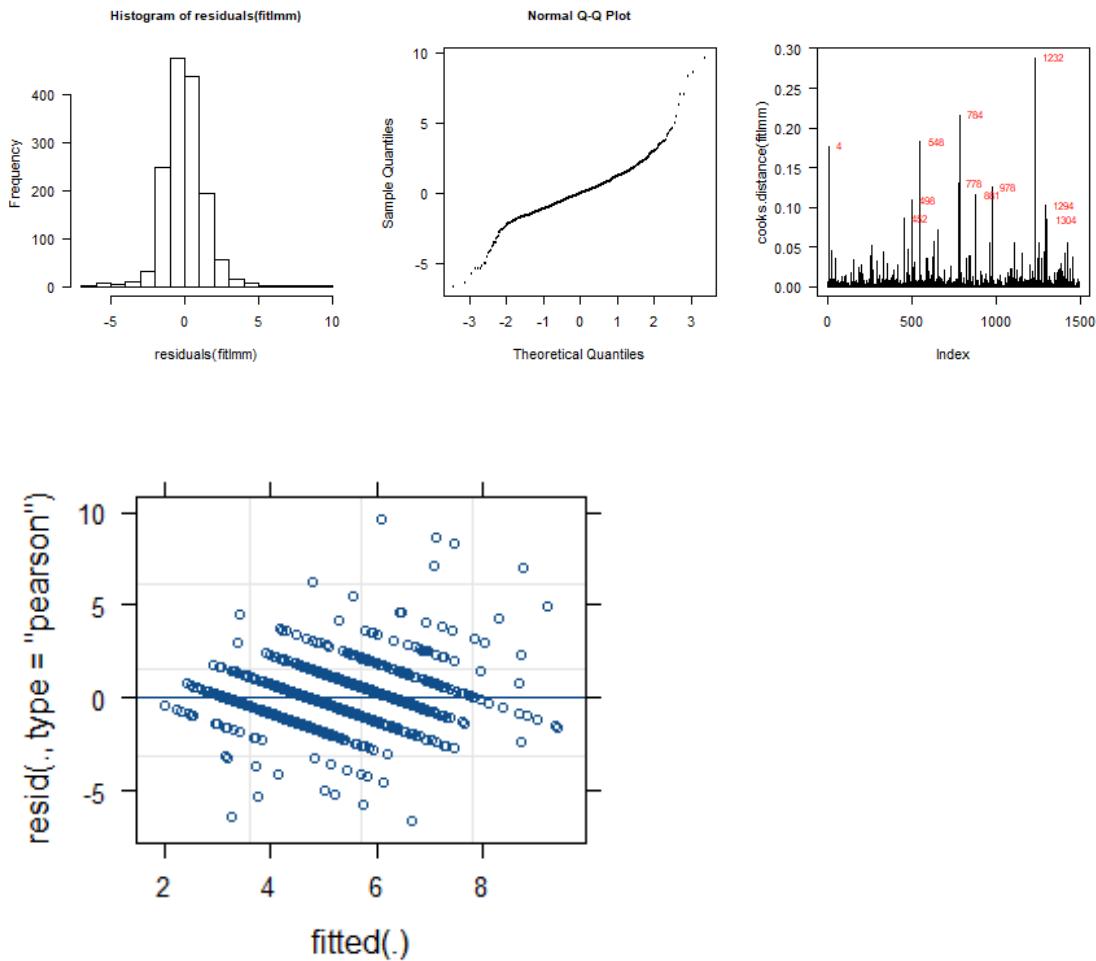
Group comparison at each trial



```
##
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
##
##   contrast   time   estimate      SE      df t.ratio
##   p.value
## -----
##   WT - Hom 1 -0.4191059 0.2942862 954.7892 -1.4241440
##   0.1547315
##   WT - Hom 2 -0.0551944 0.2746788 832.8438 -0.2009416
##   0.8407933
##   WT - Hom 3 0.0078746 0.2715509 810.9336 0.0289985
##   0.9768729
##   WT - Hom 4 -0.6679505 0.2756796 841.0108 -2.4229232
##   0.0156064
```

## WT - Hom	5	-0.1764765	0.2724339	818.8694	-0.6477774
0.5173105					
## WT - Hom	6	-0.8024799	0.2839149	893.3670	-2.8264800
0.0048113					
## WT - Hom	7	-0.5042061	0.2782424	856.3602	-1.8121114
0.0703191					
## WT - Hom	8	-0.1355836	0.2812741	878.3543	-0.4820338
0.6299020					
## WT - Hom	9	-0.6257956	0.2782323	855.7177	-2.2491840
0.0247544					
## WT - Hom	10	-0.3806198	0.2792801	874.4088	-1.3628605
0.1732773					

Checking model assumptions



Conclusion

Linear mixed model indicated a significant difference between the two groups (Type II test chi-square test; $*p = 0.023$). Adj. Pvalue: $^{ns}p = 0.170$ (using Meff = 7.456 tests). Significant effect of trial (Type II test chi-square test; $***p = 6.0\text{e-}7$). Adj. Pvalue: $***p = 4.5\text{e-}6$. But no interaction between trial and genotype (Type II test chi-square test; $^{ns}p = 0.275$). Adj. Pvalue: $^{ns}p = 1$.

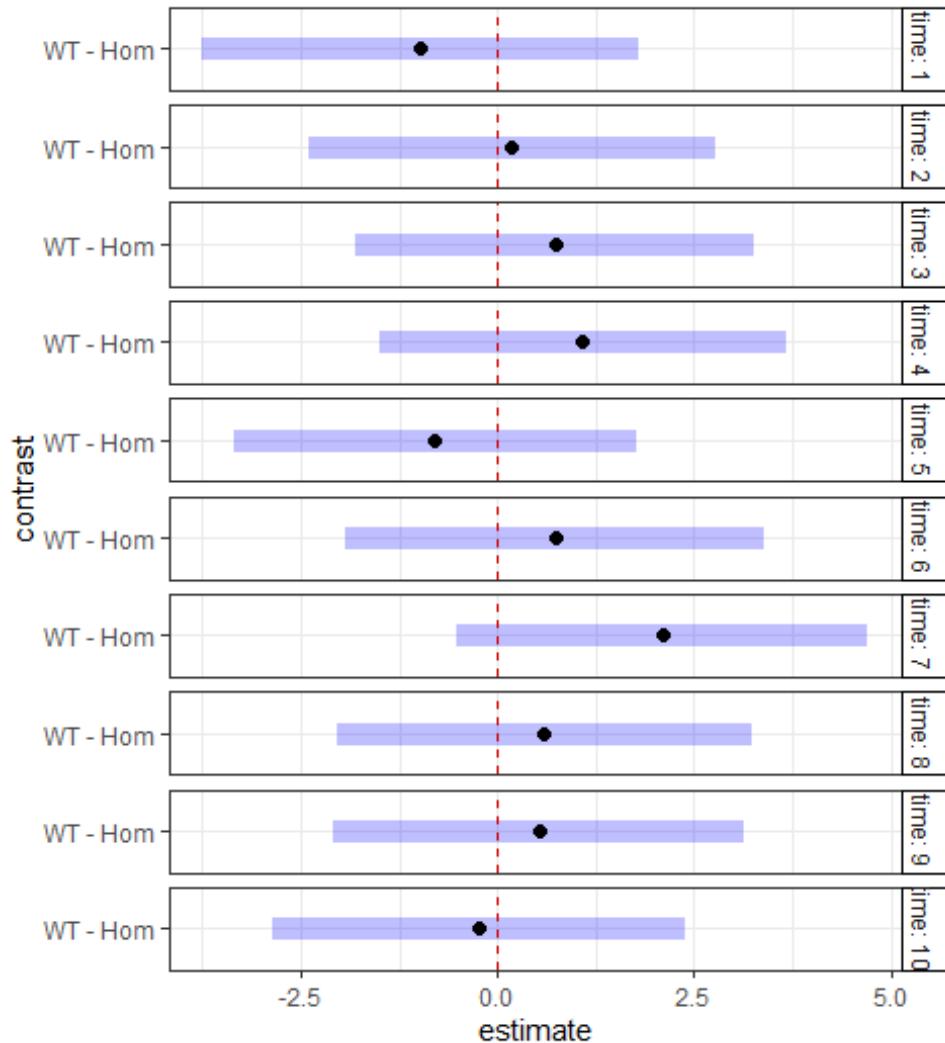
mTBF [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: value ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: 10356.23
## Random effects:
## Groups      Name      Std.Dev.
## Fish:Clutch (Intercept) 3.376
## Clutch      (Intercept) 4.199
## Residual          7.277
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##           (Intercept)     FishGenoHom       time  FishGenoHom:time
##             39.02143        0.03436      -0.15878      -0.08045

## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: value
##              Chisq Df Pr(>Chisq)
## FishGeno      0.3545  1  0.551570
## time         8.6308  1  0.003305 **
## FishGeno:time 0.3616  1  0.547630
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

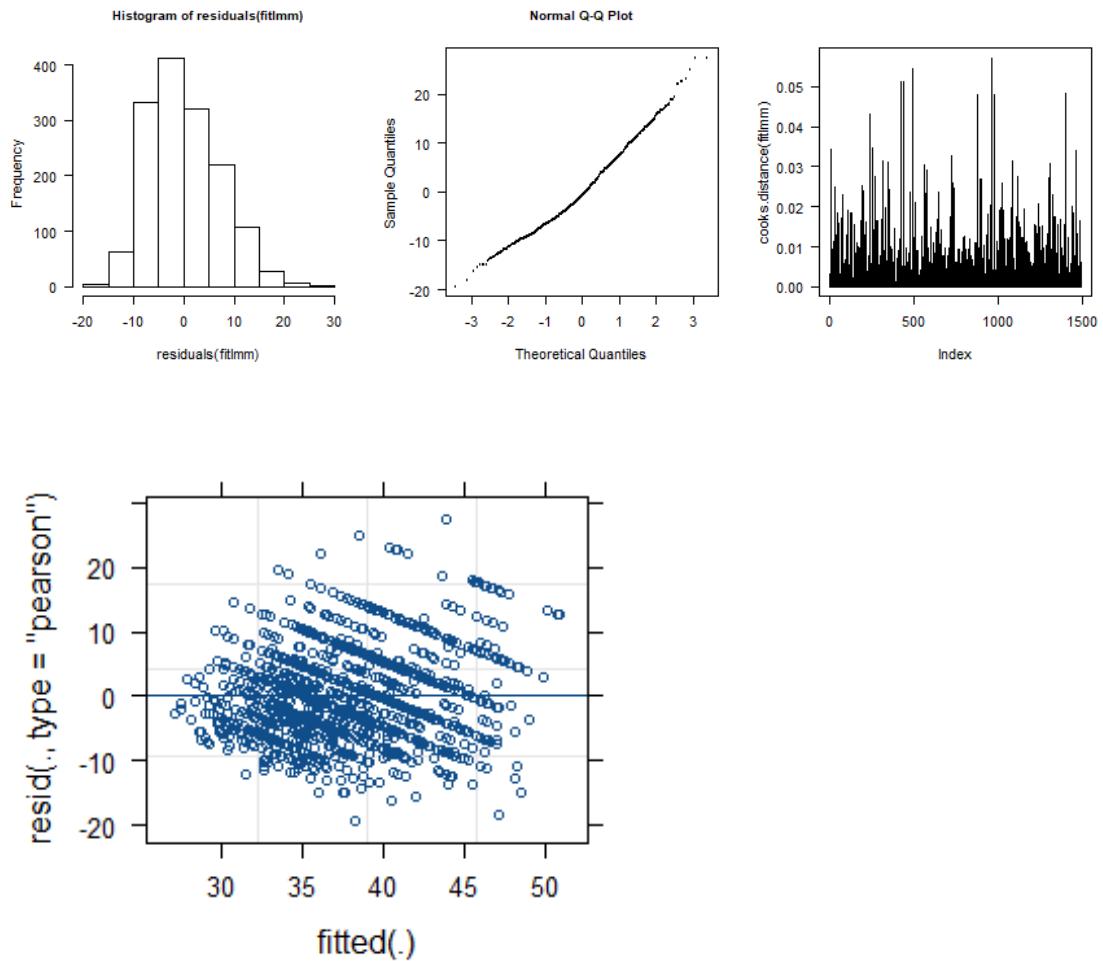
Group comparison at each trial



```
##
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
##
##   contrast   time   estimate      SE      df t.ratio
##   p.value
## -----
##   WT - Hom 1 -0.9734106 1.413502 1159.971 -0.6886517
##   0.4911802
##   WT - Hom 2 0.1873990 1.310815 1054.264 0.1429637
##   0.8863462
##   WT - Hom 3 0.7325100 1.294222 1032.464 0.5659846
##   0.5715272
##   WT - Hom 4 1.0800108 1.316239 1063.311 0.8205277
##   0.4120992
```

## WT - Hom	5	-0.7898452	1.299096	1042.242	-0.6079959
0.5433227					
## WT - Hom	6	0.7280732	1.359414	1110.099	0.5355787
0.5923570					
## WT - Hom	7	2.0921642	1.329584	1076.796	1.5735478
0.1158858					
## WT - Hom	8	0.5925692	1.345742	1098.578	0.4403290
0.6597854					
## WT - Hom	9	0.5256992	1.329468	1075.630	0.3954207
0.6926109					
## WT - Hom	10	-0.2473383	1.336163	1103.474	-0.1851109
0.8531761					

Checking model assumptions



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.552$). Adj. Pvalue: ${}^{ns}p = 1$ (for Meff = 7.456 tests). Significant effect of trial (Type II Wald chi-square test; ${}^{**}p = 3.3e-3$). Adj. Pvalue: $*p = 0.025$ (for Meff = 7.456 tests).

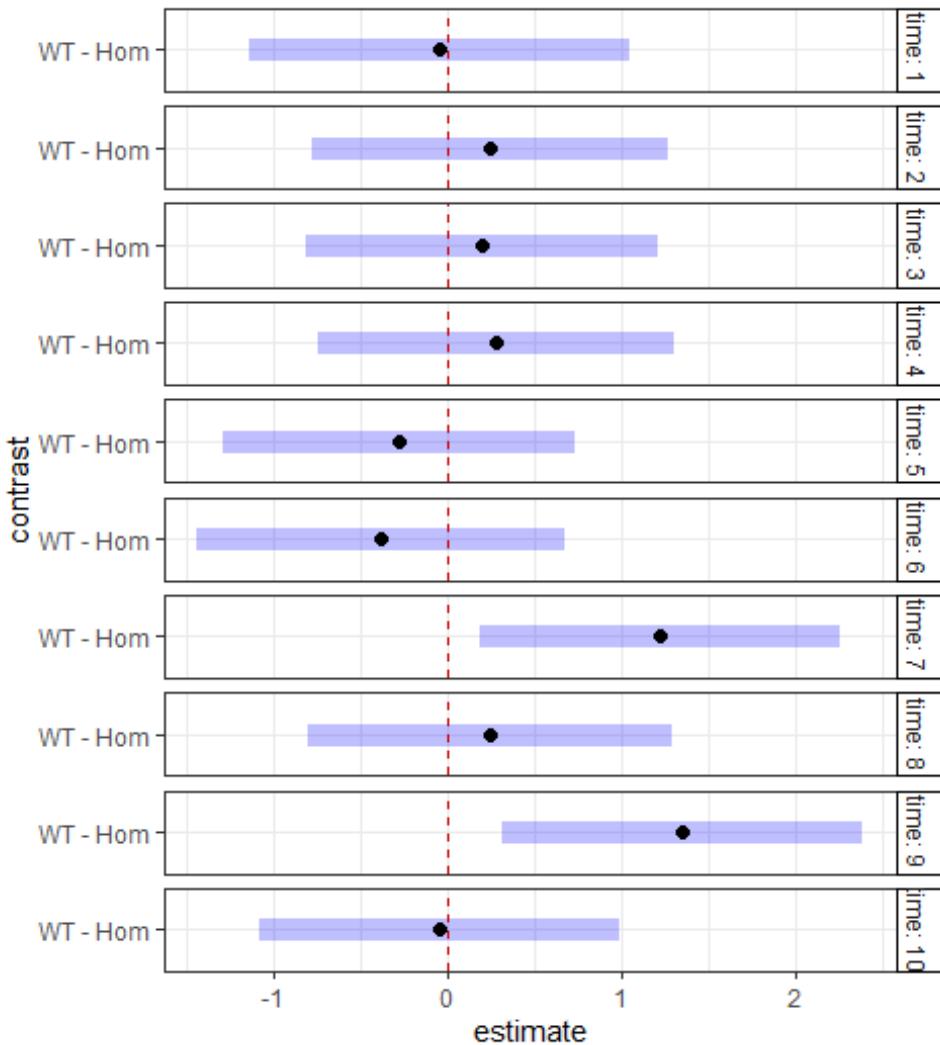
Number of oscillations [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: value ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: 7487.87
## Random effects:
##   Groups      Name      Std.Dev.
##   Fish:Clutch (Intercept) 1.6056
##   Clutch      (Intercept) 0.6045
##   Residual          2.7415
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##           (Intercept)     FishGenoHom       time   FishGenoHom:time
##           8.29158          0.09926        0.02233        -0.06845
## 
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: value
##             Chisq Df Pr(>Chisq)
## FishGeno    0.8302  1   0.3622
## time       0.1371  1   0.7111
## FishGeno:time 1.8414  1   0.1748
```

Group comparison at each trial

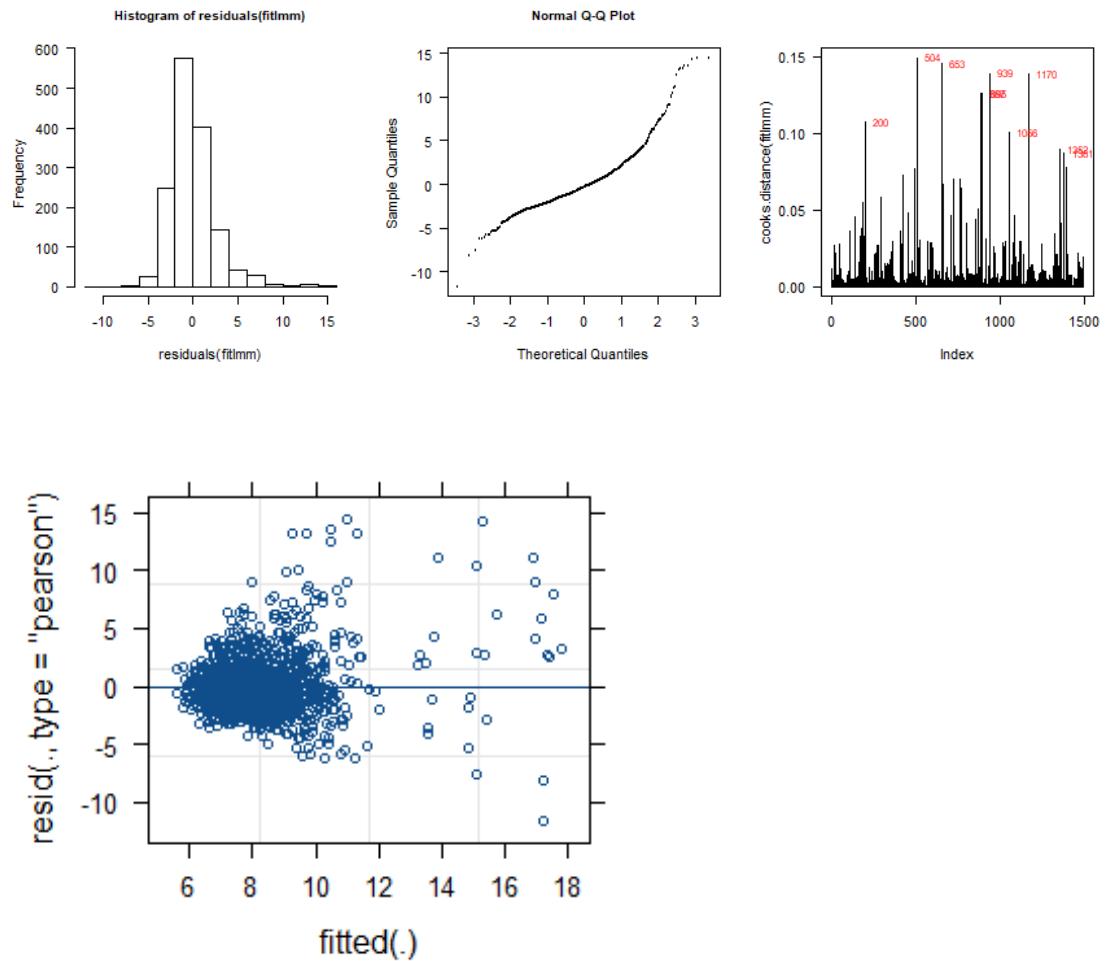
```
## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl =
control$checkConv, :
## Model failed to converge with max|grad| = 0.00422999 (tol = 0.002,
component 1)
```



```
##
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
##
##   contrast     time    estimate      SE      df t.ratio
##   p.value
##   -----
##   -----
##   ##  WT - Hom 1 -0.0403670 0.5575297 964.1214 -0.0724032
##  0.9422960
##   ##  WT - Hom 2 0.2431884 0.5204058 841.6949 0.4673055
##  0.6404023
```

## WT - Hom	3	0.1961259	0.5145035	820.0168	0.3811945
0.7031576					
## WT - Hom	4	0.2783928	0.5223010	849.9094	0.5330123
0.5941644					
## WT - Hom	5	-0.2813587	0.5161586	827.6511	-0.5451011
0.5858308					
## WT - Hom	6	-0.3810672	0.5379080	902.8423	-0.7084246
0.4788645					
## WT - Hom	7	1.2245822	0.5271519	865.2882	2.3230156
0.0204093					
## WT - Hom	8	0.2422537	0.5328998	887.6266	0.4545953
0.6495116					
## WT - Hom	9	1.3529076	0.5271440	864.8153	2.5664856
0.0104408					
## WT - Hom	10	-0.0453025	0.5291317	883.7638	-0.0856166
0.9317906					

Checking model assumptions



Conclusion

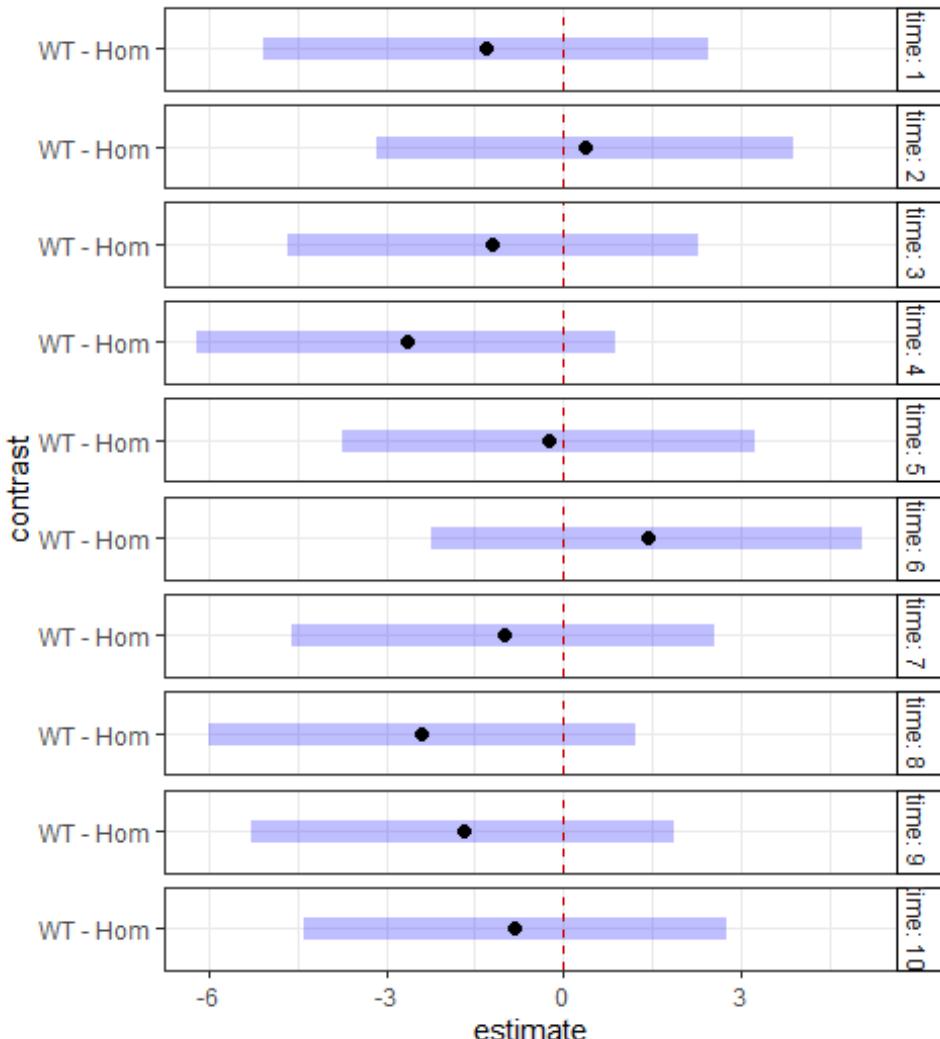
No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.362$). Adj. Pvalue: ${}^{ns}p = 1$ (for Meff = 7.456 tests).

Speed [ns]

Fitting LMM

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: value ~ FishGeno * time + (1 | Clutch/Fish)
##   Data: tmp
## REML criterion at convergence: 11125.9
## Random effects:
##   Groups      Name      Std.Dev.
##   Fish:Clutch (Intercept) 5.888
##   Clutch      (Intercept) 2.041
##   Residual          9.226
## Number of obs: 1495, groups: Fish:Clutch, 147; Clutch, 4
## Fixed Effects:
##           (Intercept)     FishGenoHom       time  FishGenoHom:time
##           38.03022        0.69723      -0.50099        0.05298
## 
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: value
##             Chisq Df Pr(>Chisq)
## FishGeno      0.8035  1    0.3701
## time         31.6810  1  1.817e-08 ***
## FishGeno:time 0.0974  1    0.7550
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

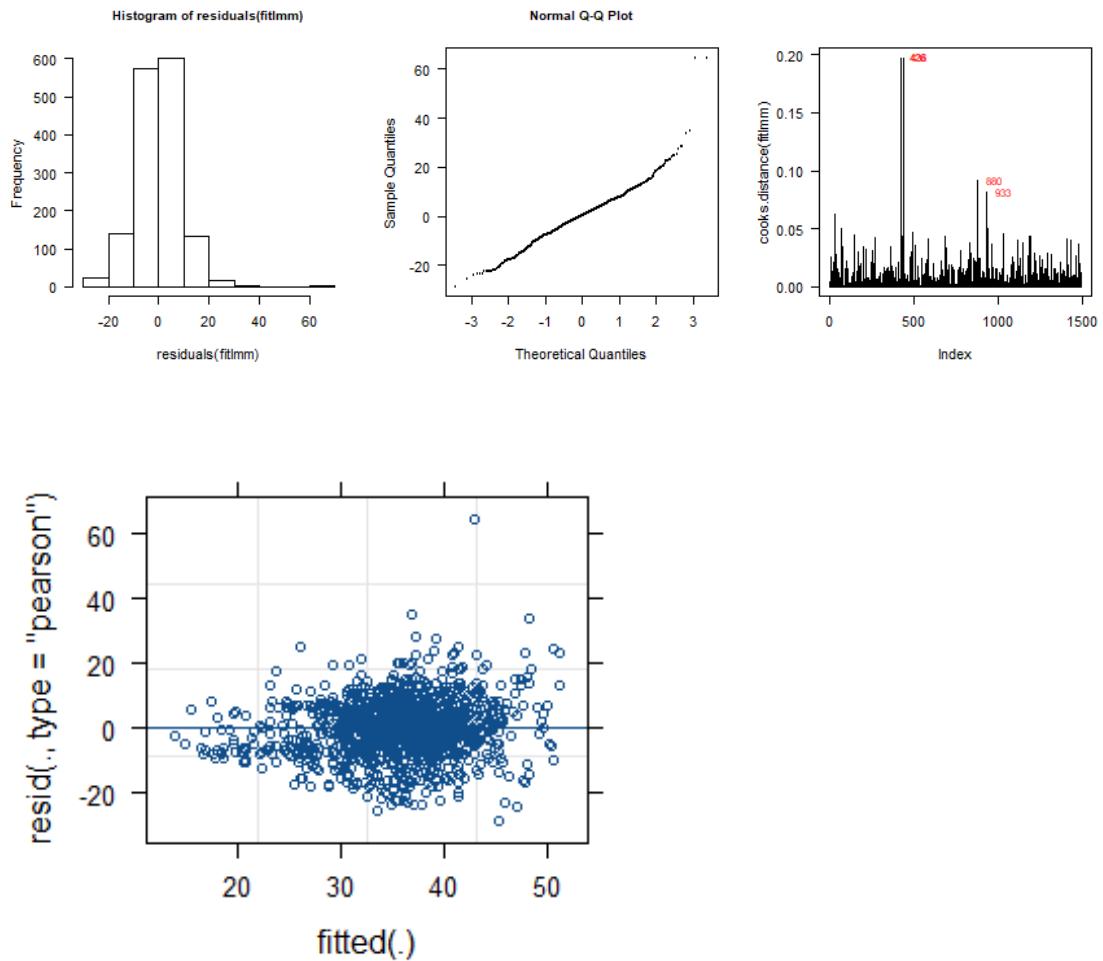
Group comparison at each trial



```
##
##
## Table: Post hoc Tukey's: Mean differences between groups at each trial
##
##   contrast    time   estimate      SE      df t.ratio
##   p.value
## -----
##   WT - Hom 1 -1.3004345 1.918724 882.8071 -0.6777601
##   0.4981014
##   WT - Hom 2 0.3646247 1.796033 761.8937 0.2030167
##   0.8391762
##   WT - Hom 3 -1.1879440 1.776618 741.2595 -0.6686546
##   0.5039240
##   WT - Hom 4 -2.6452924 1.802227 769.4645 -1.4677907
##   0.1425697
```

## WT - Hom	5	-0.2423898	1.781993	748.1265	-0.1360217
0.8918407					
## WT - Hom	6	1.4195334	1.853791	821.1744	0.7657462
0.4440474					
## WT - Hom	7	-1.0073247	1.818289	784.6014	-0.5539959
0.5797395					
## WT - Hom	8	-2.3927226	1.837183	805.8301	-1.3023868
0.1931563					
## WT - Hom	9	-1.6926888	1.818289	784.2603	-0.9309237
0.3521795					
## WT - Hom	10	-0.8185861	1.824428	799.9087	-0.4486810
0.6537833					

Checking model validity



Conclusion

No significant difference was detected between the two groups (Type II Wald chi-square test; ${}^{ns}p = 0.370$). Adj. Pvalue: ${}^{ns}p = 1$ (for Meff = 7.456 tests). Significant effect of trial (Type II Wald chi-square test; ${}^{***}p = 1.8e-8$). Adj. Pvalue: ${}^{***}p = 1.4e-7$ (for Meff = 7.456 tests).

R session information

```
## R version 3.6.1 (2019-07-05)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 17134)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=French_France.1252 LC_CTYPE=French_France.1252
## [3] LC_MONETARY=French_France.1252 LC_NUMERIC=C
## [5] LC_TIME=French_France.1252
##
## attached base packages:
## [1] stats      graphics   grDevices utils      datasets   methods    base
##
## other attached packages:
## [1] corrplot_0.84     emmeans_1.4.5      readxl_1.3.1
## [3] reshape2_1.4.3
## [5] lme4_1.1-21       Matrix_1.2-18      car_3.0-7    carData_3.0-
## [9] plyr_1.8.6        data.table_1.12.8   knitr_1.28
## [11] RColorBrewer_1.1-2
## [13] ggplot2_3.3.0
##
## loaded via a namespace (and not attached):
## [1] Rcpp_1.0.4        mvtnorm_1.1-0     lattice_0.20-41 assertthat_0.2.1
## [5] digest_0.6.25    R6_2.4.1         cellranger_1.1.0 evaluate_0.14
## [9] highr_0.8        pillar_1.4.3     rlang_0.4.5      curl_4.3
## [13] minqa_1.2.4     nloptr_1.2.2.1   rmarkdown_2.1    labeling_0.3
## [17] splines_3.6.1    stringr_1.4.0    foreign_0.8-76  munsell_0.5.0
## [21] compiler_3.6.1   xfun_0.12       pkgconfig_2.0.3 htmltools_0.4.0
## [25] tidyselect_1.0.0  tibble_3.0.0    rio_0.5.16     fansi_0.4.1
## [29] crayon_1.3.4    dplyr_0.8.5     withr_2.1.2    MASS_7.3-51.5
## [33] grid_3.6.1       nlme_3.1-145   xtable_1.8-4   gtable_0.3.0
## [37] lifecycle_0.2.0   magrittr_1.5    scales_1.1.0   zip_2.0.4
## [41] estimability_1.3 cli_2.0.2      stringi_1.4.6  farver_2.0.3
## [45] ellipsis_0.3.0   vctrs_0.2.4    boot_1.3-24   openxlsx_4.1.4
## [49] tools_3.6.1     forcats_0.5.0   glue_1.3.2     purrr_0.3.3
## [53] hms_0.5.3       parallel_3.6.1 pbkrtest_0.4-8.6 abind_1.4-5
## [57] yaml_2.2.1      colorspace_1.4-1 haven_2.2.0
```