Supplemental information for

Honeybee colonies compensate for pesticide-induced effects on royal jelly composition and brood survival with increased brood production

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SFig 1. Sampling scheme. Each week (W1–7), all combs were photographed and samples of worker bees, larvae, and worker jelly were taken. To determine the size of the hypopharyngeal gland, 15 newly-hatched and marked worker bees were introduced into each colony during week 1 and recovered in week 2 (age-defined marked bees, green arrows). In week 7, randomly chosen worker bees of undefined age were taken from brood combs for the same reason (random bees). The total number of eggs, larvae and pupae were recorded for every week and colony (brood quantification). To determine the survival rate of individually tracked larvae, at least 50 individual cells per colony were followed over 4 weeks in the first half of the experiment (first brood cycle, brood survival phase A) and in the second half of the experiment (second brood cycle, brood survival phase B).
**Climate recordings**

**SFig 2. Climate data.** Throughout the study period (July 28 to September 10, 2014), the temperature (°C) and relative humidity (%) were recorded twice daily (8:05 a.m. and 8:05 p.m.). A data logger recorded the temperature and relative humidity within the colonies over the study period. The daily temperature varied between 14.2 and 27.5 °C (8:00–20:00 h) and between 8.9 and 20 °C overnight (20:00–8:00 h). The relative humidity was 52.6–92.6% during the day and 73.8–96.9% overnight. The 20 colonies were all placed within an area of ~20 m². The control and treatment groups were randomly distributed within these colonies and were thus exposed to very similar environmental conditions.
SFig 3A. HPTLC plate from Fig2 showing the *A. fisherii* antimicrobial activity test. Each Sugi strip was cut halved to provide one part without the sample (a) and another with the sample (b). The blank is a fresh Sugi strip from the same batch as the strips used in the experiment, cut into equal halves. The other lanes show the clothianidin doses (Control = no clothianidin). Arrows indicate Peaks. For peak areas see SFig2B.

SFig 3B. HPTLC Peak areas of SFig3A. HPTLC analysed according to by Olech et al. 2012 (Olech, M., Komsta, Ł., Nowak, R., Cieśla, Ł. & Waksmundzka-Hajnos, M. Investigation of antiradical activity of plant material by thin-layer chromatography with image processing. Food Chemistry 132, 549–553 (2012)) and evaluated by Popovic and Sherma2014 (Popovic, N. & Sherma, J. Comparative Study of the Quantification of Thin-Layer Chromatograms of a Model Dye
Using Three Types of Commercial Densitometers and Image Analysis with open source program ImageJ v1.49o (http://rsb.info.nih.gov/ij/index.html). Trends Chromatogr. 9, 21–28 (2014)) with the only difference that we took photos with a TLC vizualizer from CAMAG instead of a mobile phone on a tripod.

**SFig 3C. Full TLC Plate of Fig2A.** Lipid profiles of royal jelly samples at 366 nm, derivatization with primuline. Box indicates the area of Fig2A.
SFig 3D. Peak Profile of Fig 2A. Samples of worker jelly were taken using absorptive filter strips (Sugi strips), extracted with n-hexane and separated by HPTLC. Each Sugi strip was halved: one half without the sample (track a, upper plot) and the other with absorbed sample (track b, lower plot). Numbers on abscissae indicate the peaks shown in Fig. 2A.
**SFig 3E. Full HPTLC Plate of Fig2B.** Lipid profiles of larvae at 366 nm, not derivatized. Box indicates the area of Fig2B.

**SFig 3F. Peak Profile of Fig 2B.** Lipid profiles of larvae, not derivatized. Numbers on abscissae indicate the peaks shown in Fig 2B.
**SFig 3G. Full HPTLC plate of Lipid profiles of larvae.** Same plate as Fig3E at 366 nm after derivatization with primuline. Box indicates the area of Fig2C.

**SFig 3H. Peak Profile of Fig 2C.** Lipid profiles of larvae derivatized. Numbers on abscissae indicate the peaks shown in Fig. 2C.
Protein analysis of worker jelly

For protein analysis, samples of three colonies of each treatment group were analyzed from week 4. Blotting strips were dipped in brood combs and proteins were extracted by incubation in 100 µl RIPA high salt buffer (50 mM Tris HCl pH 7.6, 500 mM NaCl, 10 mM MgCl2, 2 mM EDTA, 1% Triton X-100, 0.25% sodium deoxycholate, 0.1% SDS and Roche EDTA-free Protease cocktail mix) for 30 min at 4 °C. The protein concentration was determined using the DC Protein assay kit (Biorad; mean concentrations: control: 12.82 µg/µL ± 0.17; 1 µg/L clothianidin: 11.97 µg/µL ± 0.21; 10 µg/L clothianidin: 12.68 µg/µL ± 0.13; 100 µg/L clothianidin: 11.67 µg/µL ± 0.31). No statistical significant differences regarding the total protein concentration were found between the treatment groups (Kruskal-Wallis test, p = 0.9832).

To test, whether the concentration of a protein subfraction could be affected in clothianidin exposed bees, 15 µg of protein of each sample were analyzed by SDS-PAGE in 12% polyacrylamide gels followed by staining with Coomassie Brilliant Blue. The concentration and composition of major proteins in worker jelly was unaffected by clothianidin exposure. Although, there was no statistically significant difference in the protein concentration (see above), we observed a decrease in the concentration in major worker jelly proteins for the 100 µg/L treatment group. This could be related to a possible increase of protein degradation in this treatment group. This would lead to an increase of peptides, which appear in the total protein measurement, but are too small to be visualized on this gel. Alternatively, the general decrease of protein levels in the SDS PAGE in the 100 µg/L group could also be due to high molecular weight aggregates that are unable to enter the gel.
Clothianidin uptake

**Suppl. Table 1.** The clothianidin concentrations in spiked sugar syrup were close to the target concentrations, residues were detected in worker bees.

| treatment group | clothianidin (µg/L) in sugar syrup (mean ± SEM) | clothianidin in workers (ng/bee) (mean) |
|-----------------|-----------------------------------------------|---------------------------------------|
| control         | 0.0 ± 0.00                                    | 0.00                                  |
| 1 µg/L          | 1.2 ± 0.11                                    | 0.05                                  |
| 10 µg/L         | 10.1 ± 0.29                                   | 0.20                                  |
| 100 µg/L        | 99.8 ± 0.70                                   | 2.63                                  |

We analyzed the sugar syrup used to feed the experimental colonies to assess its exact clothianidin content. For all four experimental groups, the final spiked pesticide levels were close to the target concentrations (**Suppl. Table 1**). The consumption of the spiked syrup was recorded every week to estimate the total clothianidin uptake over the study period. The provided amount of 400 mL (= 540 g) syrup each week per colony was completely consumed each week with the exception of week 6 in the highest concentration treatment. All five colonies in the 100 µg/L clothianidin treatment group were visibly weakened and did not consume all the sugar syrup, which was provided during week 6 (residual syrup: 76.7, 114.5, 142.2 and 18.0 g, respectively). To determine the clothianidin levels in bees exposed to the different pesticide levels, 10 randomly chosen worker bees from each hive were analyzed on week 7 (**Suppl. Table 1**). Clothianidin was detected in the bee sample from only one colony of the control group (colony I, 0.004 ng/bee).

**Analytical Method**

LC/MS/MS was used for the identification and quantification of the substances in the samples. The system used was a Prominence UFLC XR HPLC (SHIMADZU) coupled to a triple quadrupole mass spectrometer 4000 Q TRAP (AB SCIEX) equipped with an electro spray ionization (ESI) source. Clothianidin and its metabolites clothianidin-metabolite TZMU and clothianidin-metabolite TZNG were identified by their retention time and three MRM transitions. The residues in the samples were quantified with reference standards in matrix (concentrations: 0.1, 0.5, 1, 5, 10, 25, 50 and 100 pg/µL). The quantification was carried out by the internal standard method. The values shown for
the samples are averages of measurements out of duplicate injections of the sample extracts. The limit of detection (LOD) was determined as the lowest concentration tested in which the peak signal of the main MRM, which was used for quantification, was three times higher than the background noise of the chromatogram. The LOD was 0,5 pg/µL for clothianidin, 1 pg/µL for clothianidin-metabolite TZMU and 5 pg/µL for clothianidin-metabolite TZNG.
Number of age-defined worker bees collected for HPG measurements

Suppl. Table 2. 1 Age-defined worker bees retrieved from the colonies. At the beginning of the experiment, 15 newly hatched marked worker bees were introduced into each colony. At the age of 12 days, all marked bees were collected and frozen for HPG preparations. The number of retrieved worker bees differed between colonies. All HPGs were dissected at the same day. Therefore, a maximum of six bees per colony were included in the subsequent analysis.

| treatment group | colony | worker bees |
|-----------------|--------|-------------|
| control         | I      | 7           |
|                 | II     | 8           |
|                 | III    | 6           |
|                 | IV     | 7           |
|                 | V      | 7           |
| 1 µg/L          | I      | 2           |
|                 | II     | 6           |
|                 | III    | 5           |
|                 | IV     | 6           |
|                 | V      | 3           |
| 10 µg/L         | I      | 6           |
|                 | II     | 1           |
|                 | III    | 0           |
|                 | IV     | 6           |
|                 | V      | 6           |
| 100 µg/L        | I      | 6           |
|                 | II     | 6           |
|                 | III    | 6           |
|                 | IV     | 0           |
|                 | V      | 5           |
Technical report for the statistical analysis of
“Honeybee colonies compensate for pesticide-induced
effects on royal jelly composition and brood survival
with increased brood production”

by

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This is a report of the statistical analyses of the article “Honeybee colonies compensate for pesticide-induced
effects on royal jelly composition and brood survival with increased brood production” that is intended to
explain all data analytical and statistical steps used in the article. Furthermore we added the utilized R-
Scripts [1] R Development Core Team] to allow recalculation and usage for own projects.

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R-technical preparations

The current session:

R version 3.6.3 (2020-02-29)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows 10 x64 (build 18363)

Matrix products: default

locale:
[1] LC_COLLATE=German_Germany.1252  LC_CTYPE=German_Germany.1252
[3] LC_MONETARY=German_Germany.1252  LC_NUMERIC=C
[5] LC_TIME=German_Germany.1252

attached base packages:
[1] parallel stats graphics grDevices utils datasets methods base

other attached packages:
[1] RColorBrewer_1.1-2  pbkrtest_0.4-8.6  lme4_1.1-23  Matrix_1.2-18
[5] multcomp_1.4-13  TH.data_1.0-10  MASS_7.3-51.5  mvtnorm_1.1-1
[9] Hmisc_4.4-1  ggplot2_3.3.2  Formula_1.2-3  survival_3.1-8
[13] lattice_0.20-38

loaded via a namespace (and not attached):
[1] statmod_1.4.34  zoo_1.8-8  tidyselect_1.1.0  xfun_0.16
[5] purrr_0.3.4  splines_3.6.3  colorspace_1.4-1  vctrs_0.3.2
[9] generics_0.0.2  htmltools_0.5.0  base64enc_0.1-3  rlang_0.4.7
[13] nloptr_1.2.2.2  pillar_1.4.6  foreign_0.8-75  glue_1.4.1
[17] withr_2.2.0  jpeg_0.1-8.1  lifecycle_0.2.0  stringr_1.4.0
[21] munsell_0.5.0  gttable_0.3.0  htmlwidgets_1.5.1  codetools_0.2-16
[25] latticeExtra_0.6-29  knitr_1.29  htmlTable_2.0.1  Rcpp_1.0.5
[29] backports_1.1.7  scales_1.1.1  checkmate_2.0.0  gridExtra_2.3
[33] png_0.1-7  digest_0.6.25  stringi_1.4.6  dplyr_1.0.2
[37] grid_3.6.3  tools_3.6.3  sandwich_2.5-1  magrittr_1.5
[41] tibble_3.0.3  cluster_2.1.0  crayon_1.3.4  pkgconfig_2.0.3
[45] ellipsis_0.3.1  data.table_1.13.0  minqa_1.2.4  rstudioapi_0.11
[49] R6_2.4.1  rpart_4.1-15  boot_1.3-24  nnet_7.3-12
[53] nlme_3.1-144  compiler_3.6.3

Self-made helper functions are “sourced”:

> source("HelperFcts.R")
1 Raw data

The original raw data had been saved in several MS-Excel-sheets and exported as ‘comma-separated values’ (CSV) files, whose fields have been separated by semicolon (;). The decimal sign is the dot (.). The columns in the CSV-file possess names (in their first rows).

The file names are:

1. "BroodDevelopment_Brood.csv"  BroodDevelopment_Brood_SUM.csv"
2. "BroodDevelopment_Eggs.csv"   BroodDevelopment_Eggs_SUM.csv"
3. "BroodDevelopment_Larvae.csv" BroodDevelopment_Larvae_SUM.csv"
4. "HPGlandsizeS2.csv"           "HPGlandsizeS7.csv"
5. "LarvalSurvivalPhase1.csv"    "LarvalSurvivalPhase2.csv"

After passing an initial, minimal format check each of these 10 CSV-files is imported into a ‘data frame’ in R, Version 3.6.3 [1, R Development Core Team], and those data frames are combined into a list with components according to the file names.

```r
> sapply(Filepaths, function(fn) unique(count.fields(fn, sep = ;)))
> Files <- lapply(Filepaths, read.csv, sep = ;)  # List of imported data frames
```

1.1 Hyperpharingal gland sizes

From the imported files we extract the data on honeybee hyperpharingal gland sizes and create two data frames named "W2HPGland" and "W7HPGland". We check data consistency by controlling the first few rows of the created data frames as well as their summaries.

```r
> Imp <- names(Files)[startsWith(names(Files), "HPG")]
> TMP <- lapply(Files[Imp], function(X) {
+   names(X) <- c("Treatment", "Hive", "Bee", "Size")
+   X <- within(X, {
+     Treatment <- factor(Treatment, levels = 1:4,
+                       labels = Trtmt, ordered = TRUE)
+     Hive <- factor(Hive, levels = 1:5, paste0("H", 1:5))
+     HiveID <- interaction(Treatment, Hive, lex.order = TRUE)
+     Bee <- factor(Bee, levels = 1:6, paste0("B", 1:6))
+     BeeID <- interaction(Treatment, Hive, Bee)
+   })
+   # print(str(X)); print(head(X)); print(tail(X)); print(summary(X))
+   # print(replications(~ Treatment * Hive * Bee, X))
+   droplevels(X)
+ })
> W2HPGland <- TMP[[1]]
> W7HPGland <- TMP[[2]]
> # str(W2HPGland); str(W7HPGland)
>
```
### 1.2 Brood development

Here, we create the three data frames *Eggs*, *Brood* and *Larvae* and check data consistency as before.

```r
> Imp <- names(Files)[endsWith(names(Files), "SUM")]
> for(nfi in Imp)
+ X <- Files[[nfi]]
+ RespName <- unlist(strsplit(nfi,"_"))[2]
+ names(X) <- c("Treatment", "Hive", "Week", RespName)
+ X <- within(X, {
+   Treatment <- factor(Treatment, levels = 1:4,
+     labels = Trtm, ordered = TRUE)
+   Hive <- factor(Hive, levels = 1:5, paste0("H", 1:5))
+   WeekFactor <- factor(Week, levels = 1:7, ordered = TRUE)
+   HiveID <- interaction(Treatment, Hive, lex.order = TRUE)
+ })
+ X <- droplevels(X) # droplevels() added 2017-21-22
+ # print(str(X)); print(head(X)); print(tail(X)); print(summary(X))
+ # print(replications(~ Treatment * Hive * WeekFactor, X))
+ assign(RespName, X)
+ }

> head(Eggs)

| Treatment | Hive | Week | Eggs | HiveID | WeekFactor |
|-----------|------|------|------|--------|------------|
| Ctrl      | H1   | 1    | 180  | Ctrl.H1 | 1          |
| Ctrl      | H2   | 1    | 267  | Ctrl.H2 | 1          |
| Ctrl      | H3   | 1    | 242  | Ctrl.H3 | 1          |
| Ctrl      | H4   | 1    | 268  | Ctrl.H4 | 1          |
| Ctrl      | H1   | 2    | 246  | Ctrl.H1 | 2          |
| Ctrl      | H2   | 2    | 238  | Ctrl.H2 | 2          |

> summary(Eggs)

```
### 1.3 Larval Survival

Here, we create the two data frames *Survival1* and *Survival2*, and again check data consistency by controlling the first few rows of the created data frames as well as their summaries.

```r
> Imp <- names(Files)[startsWith(names(Files), "LarvalSurv")]
> TMP <- lapply(Files[Imp], function(X) {
+   names(X)[1:3] <- c("Treatment", "Hive", "LarvaeAtStart")
+   weeks <- as.numeric(substr(names(X)[-c(1:3)], 2, 2))
+ })
```

#### Larval Survival

| Treatment | Hive | Week | Eggs | HiveID | WeekFactor |
|-----------|------|------|------|--------|------------|
| Ctrl      | H1:21| Min. :1 | 30.0 | Ctrl.H1 | :7 :1:16  |
| 1 ug/l    | H2:21| 1st Qu.:2 | 150.0 | Ctrl.H2 | :7 :2:16  |
| 10 ug/l   | H3:28| Median :4 | 188.5 | Ctrl.H3 | :7 :3:16  |
| 100 ug/l  | H4:21| Mean :4 | 204.1 | Ctrl.H4 | :7 :4:16  |
|           | H5:21| 3rd Qu.:6 | 256.2 | 1 ug/l.H1 | :7 :5:16  |
|           |      | Max. :7 | 386.0 | 1 ug/l.H2 | :7 :6:16  |
|           |      | (Other) :70 | |       | :7 :7:16  |

#### Brood

| Treatment | Hive | Week | Brood | HiveID | WeekFactor |
|-----------|------|------|-------|--------|------------|
| Ctrl      | H1   | 1    | 669   | Ctrl.H1 | 1         |
| 1 ug/l    | H2   | 1    | 806   | Ctrl.H2 | 1         |
| 10 ug/l   | H3   | 1    | 684   | Ctrl.H3 | 1         |
| 100 ug/l  | H4   | 1    | 563   | Ctrl.H4 | 1         |
| Ctrl      | H1   | 2    | 757   | Ctrl.H1 | 2         |
| Ctrl      | H2   | 2    | 830   | Ctrl.H2 | 2         |

#### Larvae

| Treatment | Hive | Week | Larvae | HiveID | WeekFactor |
|-----------|------|------|--------|--------|------------|
| Ctrl      | H1:21| Min. :1 | 0.0   | Ctrl.H1 | :7 :1:16  |
| 1 ug/l    | H2:21| 1st Qu.:2 | 467.0 | Ctrl.H2 | :7 :2:16  |
| 10 ug/l   | H3:28| Median :4 | 625.0 | Ctrl.H3 | :7 :3:16  |
| 100 ug/l  | H4:21| Mean :4 | 597.9 | Ctrl.H4 | :7 :4:16  |
|           | H5:21| 3rd Qu.:6 | 770.5 | 1 ug/l.H1 | :7 :5:16  |
|           |      | Max. :7 | 1408.0 | 1 ug/l.H2 | :7 :6:16  |
|           |      | (Other) :70 | |       | :7 :7:16  |

```r
> > head(Brood)  
| Treatment | Hive | Week | Brood | HiveID | WeekFactor |
|-----------|------|------|-------|--------|------------|
| Ctrl      | H1   | 1    | 669   | Ctrl.H1 | 1         |
| 1 ug/l    | H2   | 1    | 806   | Ctrl.H2 | 1         |
| 10 ug/l   | H3   | 1    | 684   | Ctrl.H3 | 1         |
| 100 ug/l  | H4   | 1    | 563   | Ctrl.H4 | 1         |
| Ctrl      | H1   | 2    | 757   | Ctrl.H1 | 2         |
| Ctrl      | H2   | 2    | 830   | Ctrl.H2 | 2         |

> > summary(Brood)
```

```r
> > head(Larvae)
| Treatment | Hive | Week | Larvae | HiveID | WeekFactor |
|-----------|------|------|--------|--------|------------|
| Ctrl      | H1:21| Min. :1 | 1.0   | Ctrl.H1 | :7 :1:16  |
| 1 ug/l    | H2:21| 1st Qu.:2 | 169.0 | Ctrl.H2 | :7 :2:16  |
| 10 ug/l   | H3:28| Median :4 | 294.5 | Ctrl.H3 | :7 :3:16  |
| 100 ug/l  | H4:21| Mean :4 | 273.6 | Ctrl.H4 | :7 :4:16  |
|           | H5:21| 3rd Qu.:6 | 364.5 | 1 ug/l.H1 | :7 :5:16  |
|           |      | Max. :7 | 560.0 | 1 ug/l.H2 | :7 :6:16  |
|           |      | (Other) :70 | |       | :7 :7:16  |

> > summary(Larvae)

> > Imp <- names(Files)[startsWith(names(Files), "LarvalSurv")]
> > TMP <- lapply(Files[Imp], function(X) {
+   names(X)[1:3] <- c("Treatment", "Hive", "LarvaeAtStart")
+   weeks <- as.numeric(substr(names(X)[-c(1:3)], 2, 2))
+ })

```r
> > Imp <- names(Files)[startsWith(names(Files), "LarvalSurv")]
> > TMP <- lapply(Files[Imp], function(X) {
+   names(X)[1:3] <- c("Treatment", "Hive", "LarvaeAtStart")
+   weeks <- as.numeric(substr(names(X)[-c(1:3)], 2, 2))
+ })
```
X <- within(X, {
  Treatment <- factor(Treatment, labels = Trtmt, ordered = TRUE)
  Hive <- sapply(strsplit(as.vector(Hive), ".\", fixed = TRUE), ":[", 2)
  Hive <- factor(Hive, levels = 1:5, paste0("H", 1:5))
  HiveID <- interaction(Treatment, Hive, lex.order = TRUE)
})

# Compute proportions of larvae
wks <- paste0("S", weeks); wksrel <- paste0(wks, ".rel")
X[wksrel] <- subset(X, select = wks) / X[[wks[1]]]
# print(str(X)); print(head(X)); print(tail(X)); print(summary(X))

# Reshape into "long" format
X <- reshape(X, drop = "LarvaeAtStart", varying = list(wks, wksrel),
              v.names = c("Larvae", "Proportion.of.Larvae"),
              timevar = "Week", times = weeks, direction = "long")

# Create Week factor and delete id column
X$WeekFactor <- factor(X$Week, levels = weeks, ordered = TRUE)
X$id <- row.names(X) <- NULL
droplevels(na.omit(X))
})

> # "Transfer" and control
Survival1 <- TMP[[1]]
> # str(Survival1); head(Survival1); tail(Survival1)
> # replications(~ Treatment * Hive * WeekFactor, Survival1)
>
Survival2 <- TMP[[2]]
> # str(Survival2); head(Survival2); tail(Survival2)
> # replications(~ Treatment * Hive * WeekFactor, Survival2)
>
head(Survival1)

| Treatment | Hive | HiveID | Week | Larvae | Proportion.of.Larvae | WeekFactor |
|-----------|------|--------|------|--------|----------------------|------------|
| Ctrl      | H1   | Ctrl.H1| 1    | 50     | 1                    | 1          |
| Ctrl      | H2   | Ctrl.H2| 1    | 61     | 1                    | 1          |
| Ctrl      | H3   | Ctrl.H3| 1    | 64     | 1                    | 1          |
| Ctrl      | H4   | Ctrl.H4| 1    | 55     | 1                    | 1          |
| 1 ug/l    | H1   | 1 ug/l.H1| 1   | 50     | 1                    | 1          |
| 1 ug/l    | H2   | 1 ug/l.H2| 1   | 78     | 1                    | 1          |

> summary(Survival1)

| Treatment | Hive | HiveID | Week | Larvae | Proportion.of.Larvae |
|-----------|------|--------|------|--------|----------------------|
| Ctrl      | H1   | Ctrl.H1| 4    | 1.00   | 8.00                | 0.1702     |
| 1 ug/l    | H2   | Ctrl.H2| 4    | 1.75   | 32.50               | 0.5428     |
| 10 ug/l   | H3   | Ctrl.H3| 4    | 2.50   | 41.50               | 0.7358     |
| 100 ug/l  | H4   | Ctrl.H4| 4    | 2.80   | 43.54               | 0.7163     |
| 1000 ug/l | H5   | 1 ug/l.H1| 4   | 3.25   | 55.00               | 0.9538     |
|           |     | 1 ug/l.H2| 4   | 4.00   | 98.00               | 1.0000     |

| WeekFactor |
|------------|
| 1          |
| 2          |
| 3          |
| 4          |

> head(Survival2)

| Treatment | Hive | HiveID | Week | Larvae | Proportion.of.Larvae |
|-----------|------|--------|------|--------|----------------------|
| Ctrl      | H1   | Ctrl.H1| 4    | 83     | 10                   | 4          |
|   | Treatment | Hive | HiveID | Week | Larvae | Proportion.of.Larvae |
|---|-----------|------|--------|------|--------|---------------------|
| 2 | Ctrl      | H2   | Ctrl.H2| 4    | 61     | 1 4                 |
| 3 | Ctrl      | H3   | Ctrl.H3| 4    | 63     | 1 4                 |
| 4 | Ctrl      | H4   | Ctrl.H4| 4    | 53     | 1 4                 |
| 5 | 1 ug/l    | H1   | 1 ug/l.H1| 4   | 68     | 1 4                 |
| 6 | 1 ug/l    | H2   | 1 ug/l.H2| 4   | 58     | 1 4                 |

> summary(Survival2)

| Treatment | Hive | HiveID | Week | Larvae | Proportion.of.Larvae |
|-----------|------|--------|------|--------|---------------------|
| Ctrl      | H1   | 12     | 4    | 61     | 1 4                 |
| 1 ug/l    | H2   | 12     | 4    | 63     | 1 4                 |
| 10 ug/l   | H3   | 16     | 4    | 53     | 1 4                 |
| 100 ug/l  | H4   | 12     | 4    | 58     | 1 4                 |
| H5        | 1    | 12     | 4    | 58     | 1 4                 |

WeekFactor

4:16
5:16
6:16
7:16
2 The actual questions

The actual questions, which are going to be examined in the following, are:

1. Does the Clothianidin concentration influence the Hypopharyngeal Gland (HPG) size? [2.1]
   (a) Does the treatment (Clothianidin concentration) significantly influence the Hypopharyngeal Gland (HPG) size in week 2? [2.1.2]
   (b) Does the treatment significantly influence the HPG size in week 7? [2.1.4]
   (c) Which treatments are significantly different to the control (0 ng/l Clothianidin)? (W2Gland and W7Gland have to be examined independent) [2.1.3, 2.1.5]

2. Does the Clothianidin concentration influence the bees development? [2.2]
   (a) Does the treatment (Clothianidin concentration) significantly influence number of eggs during the weeks? [2.2.2]
   (b) Does the treatment significantly influence number of larvae during the weeks? [2.2.4]?
   (c) Does the treatment significantly influence number of capped brood during the weeks? [2.2.6]?
   (d) If there is an treatment effect, which treatment differs significantly from the control? [2.2.5, 2.2.7]?

3. Does the Clothianidin concentration have an impact on the larval survival? [2.3]
   (Exclude week four and week seven from data sets).
   (a) Does the treatment change the larval survival rate in phase 1 (week 1 to 3)? [2.3.2]
   (b) Which treatments differ significantly from the control in phase 1 (week 1 to 3)? [2.3.3]
   (c) Does the treatment change the larval survival rate in phase 2 (week 4 to 7)? [2.3.4]
   (d) Which treatments differ significantly from the control in phase 2 (week 4 to 7)? [2.3.5]

4. How much do the hives in the different treatment compensate for the clothianidin effect? [2.4]
2.1 Question 1: Influence on the HPG size

2.1.1 Exploratory data analysis (EDA)

Figure 1: Per week: Boxplots and raw data of HPG size by treatment; week 2 on the left, week 7 on the right. Within each treatment different colors indicate origin from different hives. (File names: Cloth-Q1_HPG_by_treatment_in_week2_Boxplots.pdf and Cloth-Q1_HPG_by_treatment_in_week7_Boxplots.pdf)

Figure 2: Per week: Barplots of average HPG size by treatment; week 2 on the left, week 7 on the right. Error bars indicate the standard error of the mean (ignoring the grouping structure by hives within each treatment; compare with 95 %-confidence intervals from a mixed-effects ANOVA in fig. 5). Asteriscs indicate significant differences in Dunnett contrasts with “Control” of mixed-effects ANOVAs, in anticipation of the analyses in §2.1.3 and §2.1.5. (File names: Cloth-Q1_HPG_by_treatment_in_week2_Barplot.pdf and Cloth-Q1_HPG_by_treatment_in_week7_Barplot.pdf)
2.1.2 Inferential analysis: Effect of treatment in week 2

Testing a hypothesis about fixed effects in a linear mixed model can always be based on a likelihood ratio or on a Wald test statistic which both have asymptotically (i.e., when the sample size goes to infinity) a $\chi^2$-distribution under the null hypothesis. However, in cases with small and moderate sample sizes using a $\chi^2$-distribution as an approximation of the (unknown true) distribution of the respective test statistic can be quite inappropriate and may lead to wrong conclusions.

For certain factorial model designs, it is alternatively possible to use a test statistic which has an $F$-distribution under the null hypothesis. However, such designs need to be balanced, e.g., with respect to the number of observations in the treatment groups.

In the present case, we unfortunately have neither large sample sizes nor a balanced design, so we have to resort to an alternative of the approximate $\chi^2$-tests. In fact, we shall use two (to double-check the results) alternative, reliable methods for analysing fixed effects:

a) Kenward and Roger (KR) provide a modification of a Wald test statistic which has under the null hypothesis asymptotically an $F$-distribution (whose denominator degrees of freedom need and can be estimated) and is said to yield results more reliable than the $F$-test mentioned above. (This KR approach is for models fitted with restricted maximum likelihood (REML).)

b) A parametric bootstrap (PB) allows to determine the distribution (or moments thereof) of the likelihood ratio test statistic under the null hypothesis. (This is for models fitted with maximum likelihood (ML); so models fitted with REML need to be re-fitted with ML before.)

The R-package `pbkrtest` implements both the KR and the PB approach for tests regarding the fixed effects (in linear mixed models with independent errors). See [5] which describes the methods, their implementation and which contains also examples.

An even more applied description is given in §10.6 in [6, Faraway (2016)] which we shall follow here:

```r
> fit1 <- lmer(Size ~ Treatment + (1|HiveID), data = W2HPGland,
+ contrasts = list(Treatment = "contr.treatment"))
> # print(summary(fit1), cor = FALSE)
> fit2 <- update(fit1, ~ . - Treatment) # Preparing analysis of a treatment effect.
> # KR) Model comparison using an approximate F-test with degrees of freedom based
> # on the Kenward-Roger approach:
> #-------------------------------------------------------------------------------
> (KR <- KRmodcomp(fit1, fit2))
F-test with Kenward-Roger approximation; time: 0.63 sec
large : Size ~ Treatment + (1 | HiveID)
small : Size ~ (1 | HiveID)

| stat    | ndf  | ddf  | F.scaling | p.value |
|---------|------|------|-----------|---------|
| Ftest   | 5.4432 | 3.0000 | 13.0335   | 0.99983 | 0.01199 * |

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

> # PB) Model comparison using the parametric bootstrap (after refitting with
> # maximum likelihood automatically internally if required). (Computations are
> # done with multiple processors in parallel by means of package parallel.)
> #-------------------------------------------------------------------------------
> cl <- makeCluster(rep("localhost", detectCores())) # Create as many clusters as
> # there are cores.
> set.seed(201712) # Ensures reproducibility of simulation-based results.
> # Relevant in the following parametric bootstrap model comparison: outputted
> # p-value in row "PBtest" (others are only for comparison with further methods).
> (PB <- summary(PBmodcomp(fit1, fit2, nsim = nsim.PBmodcomp, cl = cl)))
```
Bootstrap test; time: 317.45 sec; samples: 5000; extremes: 88;
large: Size ~ Treatment + (1 | HiveID)
small: Size ~ (1 | HiveID)

| stat     | df  | ddf | p.value |
|----------|-----|-----|---------|
| LRT      | 3.0000 | 0 | 0.004328 ** |
| PBtest   | 3.0000 | 0 | 0.017796 *   |
| Gamma    | 3.0000 | 0 | 0.017109 *   |
| Bartlett | 3.0000 | 0 | 0.016906 *   |
| F        | 3.0000 | 2.698 | 0.142976 |

> stopCluster(cl) # Stop clusters.

Summary: Treatment has a significant effect on the HPG size with a p-value of 0.01199 from the Kenward-Rogers method, and a p-value of 0.0178 based on the parametric bootstrap.

Model diagnostics for the model in fit1 are found on page 11.

2.1.3 Posthoc tests in week 2 – comparisons with the Control

We perform Dunnett’s multiple comparisons for the one-sided null hypothesis that the Clothianidin treatments do not yield smaller HPG sizes than seen in the Control group.

Multiple Comparisons of Means: Dunnett Contrasts

Fit: lmer(formula = Size ~ Treatment + (1 | HiveID), data = W2HPGland, contrasts = list(Treatment = "contr.treatment"))

Linear Hypotheses:

| Estimate | Std. Error | z value | Pr(>|z|) |
|----------|------------|---------|---------|
| 1 ug/l - Ctrl >= 0 | -51.52 | 13.83 | -3.725 < 0.001 *** |
| 10 ug/l - Ctrl >= 0 | -24.27 | 13.34 | -1.819 0.03447 *   |
| 100 ug/l - Ctrl >= 0 | -39.82 | 13.30 | -2.994 0.00269 ** |

---

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

(Autoadjust p values reported -- Westfall method)

Summary: In week 2 all three Clothianidin treatments show significantly smaller HPG sizes than the Control. (Compare left panel of fig. 1)
Figure 3: Diagnostic plots for the fitted linear mixed-effects model. (Color coding in plots of middle row identical to fig. [II].) Summarizing the findings (without explanation): the fitted model shows some indication against normality and homoscedasticity of the errors, but due to the not-too-small total sample size of 91 we consider the inferential results as reliable. (File names: Cloth-Q1_Week2ModelDiagPlotX.pdf with X = 1,...,6)
2.1.4 Inferential analysis: Effect of treatment in week 7

Analogous to §

```r
> fit1 <- lmer(Size ~ Treatment + (1|HiveID), data = W7HPGland,
+   contrasts = list(Treatment = "contr.treatment"))
+ # print(summary(fit1), cor = FALSE)
> fit2 <- update(fit1, ~ . - Treatment) # Preparing the analysis of a treatment effect.

> # KR) Model comparison using an approximate F-test with degrees of freedom based
> # on the Kenward-Roger approach:
> #---------------------------------------------------------------
> (KR <- KRmodcomp(fit1, fit2))

F-test with Kenward-Roger approximation; time: 0.19 sec
large : Size ~ Treatment + (1 | HiveID)
small : Size ~ (1 | HiveID)

| stat   | ndf  | ddf   | F.scaling | p.value |
|--------|------|-------|-----------|---------|
| Ftest  | 5.0818 | 3.0000 | 13.9955   | 0.01379 * |

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

> # PB) Model comparison using the parametric bootstrap (after refitting with
> # maximum likelihood automatically internally if required). (Computations are
> # done with multiple processors in parallel by means of package parallel.)
> #---------------------------------------------------------------
> cl <- makeCluster(rep("localhost", detectCores())) # Create as many clusters as
> # there are cores.
> set.seed(201712) # Ensures reproducibility of simulation-based results.
> # Relevant in the following parametric bootstrap model comparison: outputted
> # p-value in row "PBtest" (others are only for comparison with further methods).
> (PB <- summary(PBmodcomp(fit1, fit2, nsim = nsim.PBmodcomp, cl = cl)))

Bootstrap test; time: 257.16 sec;samples: 5000; extremes: 87;
large : Size ~ Treatment + (1 | HiveID)
small : Size ~ (1 | HiveID)

| stat     | df     | ddf    | p.value |
|----------|--------|--------|---------|
| LRT      | 12.7833| 3.0000 | 0.00513 ** |
| PBtest   | 12.7833| 0.01760 * |
| Gamma    | 12.7833| 0.01758 * |
| Bartlett | 10.1380| 3.0000 | 0.01743 * |
| F        | 4.2611 | 3.0000 | 2.7187 0.14623 |

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

> stopCluster(cl) # Stop clusters.

Summary: Treatment has a significant effect with a p-value of 0.01379 from the Kenward-Rogers method, and a p-value of 0.0176 based on the parametric bootstrap.
Model diagnostics for the model in fit1:

Figure 4: Diagnostic plots for the fitted linear mixed-effects model. (Color coding in plots of middle row identical to fig. [1]) Summarizing the findings (without explanation): the fitted model does show an indication against normality of the errors (outliers), but due to the not-too-small total sample size of 100 we consider the inferential results as acceptably reliable. (File names: Cloth-Q1_Week7_ModelDiagPlotX.pdf with \( X = 1, \ldots, 6 \))
2.1.5 Posthoc tests in week 7 – comparisons with the Control

Analogous as in 2.1.3, we perform Dunnett’s multiple comparisons for the **one-sided** null hypothesis that the Clothianidin treatments **do not yield smaller** HPG sizes than the Control.

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Dunnett Contrasts

Fit: `lmer(formula = Size ~ Treatment + (1 | HiveID), data = W7HPGland, contrasts = list(Treatment = "contr.treatment"))`

**Linear Hypotheses:**

| Estimate | Std. Error | z value | Pr(<z)  |
|----------|------------|---------|---------|
| 1 µg/l - Ctrl >= 0 | -7.603 | 9.380 | -0.811 | 0.331134 |
| 10 µg/l - Ctrl >= 0 | -2.190 | 9.380 | -0.233 | 0.407710 |
| 100 µg/l - Ctrl >= 0 | -30.899 | 8.806 | -3.509 | 0.000699 *** |

---

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

(Adjusted p values reported -- Westfall method)

**Summary:** In week 7 only the 100 µg/l treatment shows significantly **smaller** HPG sizes than the Control. (Compare right panel of fig. 1.)

Figure 5: Per week and by treatment: Fixed-effects estimates (solid blue circles) with (non-simultaneous!) two-sided 95 %-confidence intervals (blue) of HPG size. (Week 2 on the left, week 7 on the right.) Black error bars indicate the mean HPG size plus/minus one standard error of the mean (ignoring the grouping structure by hives within each treatment). Note that the fixed-effects estimates differ slightly from the treatment specific “simple” mean HPG sizes. They are closer to the overall mean (why they are sometimes also called “shrinkage estimators”). Asterisks indicate significant differences in one-sided Dunnett contrasts with “Control” of the mixed-effects ANOVA in 2.1.3 and 2.1.5 respectively. (File names: Cloth-Q1_HPG_by_treatment_in_week2_CIplot.pdf and Cloth-Q1_HPG_by_treatment_in_week7_CIplot.pdf)
2.2 Question 2: Influence on the brood development

2.2.1 Longitudinal EDA

Figure 6: Numbers of eggs (open circles) in each hive along weeks by treatment, augmented by arithmetic means and medians across hives at each week. Values connected by a black polyline belong to the same hive; different polylines indicate different hives. The blue polylines connect the time specific median values, the green ones the respective means. (File name: Cloth-Q2_Eggs_along_Weeks_by_Treat.pdf)

Figure 7: Larvae along weeks by treatment. Layout as explained in caption of fig. 6 (File name: Cloth-Q2_Larvae_along_Weeks_by_Treat.pdf)

Figure 8: Capped brood cells along weeks by treatment: Layout as explained in caption of fig. 6 (File name: Cloth-Q2_Eggs_along_Weeks_by_Treat.pdf)
2.2 Question 2: Influence on the brood development

Figure 9: Summarizing and overlaying the information in fig. 6 - 8: Mean numbers of eggs, larvae, and capped brood cells along time by treatment: Values at each time point indicate mean values over all hives in the same treatment. The dashed lines in treatments different from “Control” indicate the respective time courses in the control group. (File name: Cloth-Q2_BroodDevelopment.pdf)
2.2.2 Treatment effect on number of eggs

We analyse the number of eggs by a linear mixed-effects model with a fixed effects linear or parabolic time trend along weeks, with fixed treatment main effects, and fixed interaction effects between weeks and treatment as well as between squared weeks and treatment. This is done for weeks centered at 3 – arbitrarily selected – so that the main effect of treatment represents the estimated number of eggs at week 3. Hives are modelled as random shift effects, thus accounting for the within-hive correlation.

For explanations regarding the testing methodology and for comments regarding the R-code see §2.1.2. We again follow, but also extend §10.6 in [6, Faraway (2016)].

```R
> linfit1 <- lmer(Eggs ~ I(Week - 3) + (1 | HiveID), data = Eggs)
> linfit2 <- update(linfit1, ~ . + Treatment,
+ contrasts = list(Treatment = "contr.treatment"))
> linfit3 <- update(linfit2, ~ . + I(Week - 3):Treatment)
> parfit3 <- update(linfit3, ~ . + I((Week - 3)^2) + I((Week - 3)^2):Treatment)
> print(summary(parfit3), cor = FALSE)
```

Linear mixed model fit by REML ['lmerMod']
Formula: Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I((Week - 3)^2) +
I(Week - 3):Treatment + Treatment:I((Week - 3)^2)
Data: Eggs

REML criterion at convergence: 1150.3

Scaled residuals:

| Min | 1Q  | Median | 3Q  | Max  |
|-----|-----|--------|-----|------|
| -2.65724 | -0.52253 | 0.05715 | 0.48153 | 2.51319 |

Random effects:

| Groups   | Name        | Variance | Std.Dev. |
|----------|-------------|----------|----------|
| HiveID   | (Intercept) | 1403     | 37.45    |
|          | Residual    | 2783     | 52.76    |

Number of obs: 112, groups: HiveID, 16

Fixed effects:

|                  | Estimate | Std. Error | t value |
|------------------|----------|------------|---------|
| (Intercept)      | 213.7321 | 23.4417    | 9.118   |
| I(Week - 3)      | -15.2292 | 7.6151     | -2.000  |
| Treatment1 ug/l  | 35.5000  | 33.1515    | 1.071   |
| Treatment10 ug/l | 77.8155  | 35.8077    | 2.173   |
| Treatment100 ug/l| -10.3750 | 31.4503    | -0.330  |
| I((Week - 3)^2)  | -0.6577  | 2.8782     | -0.229  |
| I(Week - 3):Treatment1 ug/l | 33.5982 | 10.7693    | 3.120   |
| I(Week - 3):Treatment10 ug/l | 28.2173 | 11.6322    | 2.426   |
| I(Week - 3):Treatment100 ug/l | 26.2101 | 10.2167    | 2.565   |
| Treatment1 ug/l:I((Week - 3)^2) | -11.2768 | 4.0704     | -2.770  |
| Treatment10 ug/l:I((Week - 3)^2) | -12.4018 | 4.3966     | -2.821  |
| Treatment100 ug/l:I((Week - 3)^2) | -4.8042  | 3.8615     | -1.244  |

> # F-tests with approximated degrees of freedom according to Kenward-Roger:
> #-------------------------------------------------------------------------
> (KR1 <- KRmodcomp(largeModel = linfit3, smallModel = linfit1))
```

F-test with Kenward-Roger approximation; time: 0.11 sec
large : Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I(Week - 3):Treatment
small : Eggs ~ I(Week - 3) + (1 | HiveID)
stat  ndf  ddf F.scaling p.value
Ftest 1.354 6.000 31.621 0.96696 0.2633
> (KR2 <-KRmodcomp(largeModel = parfit3, smallModel = linfit3))

F-test with Kenward-Roger approximation; time: 0.16 sec
large : Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)
small : Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I(Week - 3):Treatment

| stat | ndf | ddf | F.scaling | p.value |
|------|-----|-----|-----------|---------|
| Ftest | 9.2969 | 4.0000 | 88.0000 | 1 | 2.587e-06 *** |
---

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

> (KR3 <-KRmodcomp(largeModel = parfit3, smallM = update(parfit3, ~ . - Treatment)))

F-test with Kenward-Roger approximation; time: 0.16 sec
large : Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)
small : Eggs ~ I(Week - 3) + (1 | HiveID) + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)

| stat | ndf | ddf | F.scaling | p.value |
|------|-----|-----|-----------|---------|
| Ftest | 2.6086 | 3.0000 | 17.7679 | 1 | 0.08367 . |
---

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

> # Parametric bootstrap:
> #-------------------------------------------------------------------------------
> cl <- makeCluster(rep("localhost", detectCores()))
> set.seed(201712) # For reproducibility.
> # Relevant: p-value in row "PBtest"
> (PB1 <- summary(PBmodcomp(largeM = linfit3, smallM = linfit1, nsim = nsim.PBmodcomp, +
+ cl = cl)))

Bootstrap test; time: 240.24 sec; samples: 5000; extremes: 1406;
large : Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I(Week - 3):Treatment
small : Eggs ~ I(Week - 3) + (1 | HiveID)

| stat | df | ddf | p.value |
|------|----|-----|---------|
| LRT | 8.6846 | 6.0000 | 0.1921 |
| PBtest | 8.6846 | 0.2813 |
| Gamma | 8.6846 | 0.2747 |
| Bartlett | 7.5258 | 6.0000 | 0.2749 |
| F | 1.4474 | 6.0000 | 2.3376 | 0.4420 |

> (PB2 <- summary(PBmodcomp(largeM = parfit3, smallM = linfit3, nsim = nsim.PBmodcomp, +
+ cl = cl)))

Bootstrap test; time: 357.50 sec; samples: 5000; extremes: 0;
large : Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I(Week - 3):Treatment
small : Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I(Week - 3):Treatment

| stat | df | ddf | p.value |
|------|----|-----|---------|
| LRT | 33.9631 | 4.0000 | 7.583e-07 *** |
| PBtest | 33.9631 | 0.00020 *** |
| Gamma | 33.9631 | 1.462e-06 *** |
| Bartlett | 31.4881 | 4.0000 | 2.434e-06 *** |
| F | 8.4908 | 4.0000 | 2.6034 | 0.07087 . |
---

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

> (PB3 <- summary(PBmodcomp(largeM = parfit3, smallM = update(parfit3, ~ . - Treatment), +
+ nsim = nsim.PBmodcomp, cl = cl)))
Bootstrap test; time: 349.55 sec; samples: 5000; extremes: 477;
large: Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)
small: Eggs ~ I(Week - 3) + (1 | HiveID) + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)

| stat     | df    | ddf   | p.value |
|----------|-------|-------|---------|
| LRT      | 8.0641| 3.0000| 0.04471 *|
| PBtest   | 8.0641| 0.09558 .|
| Gamma    | 8.0641| 0.09518 .|
| Bartlett | 6.3215| 3.0000| 0.09697 .|
| F        | 2.6880| 3.0000| 2.7075 0.23406 |

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

> stopCluster(cl)

Summary: Allowing (only) a linear time trend along weeks, including an interaction between time and treatment, there is no significant influence of treatment on the time trend of number of eggs (p-value = 0.2633 from the Kenward-Rogers method, and p-value = 0.2813 based on parametric bootstrap).

However, using a parabolic time trend along weeks, including an interaction between squared time and treatment (to account for the visible and different curvatures of the time trends of numbers of eggs), there is a significant influence of treatment on the number of eggs (p-value = 2.587e-06 from the Kenward-Rogers method, and a p-value = 2e-04 based on parametric bootstrap).

This means in particular, that the time trends of the number of eggs are not the same in the four treatment groups.

On top, there is no significant treatment main effect in the parabolic model (p-value = 0.08367 from the Kenward-Rogers method, and p-value = 0.09558 based on parametric bootstrap). This means, the estimated average number of eggs at week 3 (!) are not significantly different between the four treatment groups.
2.2 Question 2: Influence on the brood development

Model diagnostics for the model in linfit3:

| Week | Eggs | Ctrl.H1 | Ctrl.H2 | Ctrl.H3 | Ctrl.H4 | 1 ug/l.H1 | 1 ug/l.H2 | 1 ug/l.H3 | 1 ug/l.H4 | 1 ug/l.H5 |
|------|------|---------|---------|---------|---------|----------|----------|----------|----------|----------|
| 1    | 100  | -       | -       | -       | -       | -        | -        | -        | -        | -        |
| 2    | 200  | -       | -       | -       | -       | -        | -        | -        | -        | -        |
| 3    | 300  | -       | -       | -       | -       | -        | -        | -        | -        | -        |
| 4    | 400  | -       | -       | -       | -       | -        | -        | -        | -        | -        |

Figure 10: Diagnostic plots for the fitted linear mixed-effects model with linear time trends. Summarizing the findings (without explanation): the fitted model appears to fit well and does not show any serious indication against homoscedastic normality of the errors, so the inferential results are considered reliable. (File names: Cloth-Q2_LinFit_EggsModelAugPred.pdf and Cloth-Q2_LinFit_EggsModelDiagX.pdf with X = 1, . . . , 6)
Model diagnostics for the model in `parfit3`:

Figure 11: Diagnostic plots for the fitted linear mixed-effects model with parabolic time trends. Summarizing the findings (without explanation): the fitted model appears to fit quite well and does not show any indication against homoscedastic normality of the errors, so the inferential results are considered reliable. (File names: `Cloth-Q2_ParFit_EggsModelAugPred.pdf` and `Cloth-Q2_ParFit_EggsModelDiagX.pdf` with $X = 1, \ldots, 6$)
2.2 Question 2: Influence on the brood development

2.2.3 Posthoc tests for time trend of eggs – comparisons with the Control

Here, we compare each Clothianidin treatment group to the control group with respect to the time trends in numbers of eggs.

Note: It is actually of little interest to analyse the main effects of treatment since they characterize the situation at only a single point in time, namely here at week 3. Instead, we focus on comparing the treatments with the control with respect to the (local) slope of their trend at week 3 and with respect to their (global) curvature. The first is represented by the interaction effect of treatment and week (centered at 3), and the latter by the by the interaction effect of treatment and squared week.

> fx <- fixef(parfit3)
> K <- diag(length(fx))[-(1:6),]
> rownames(K) <- names(fx)[-1:6]
> CompWCntrl <- glht(parfit3, linfct = K); # summary(CompWCntrl)
> summary(CompWCntrl, test = adjusted(type = "Westfall"))

Simultaneous Tests for General Linear Hypotheses

Fit: lmer(formula = Eggs ~ I(Week - 3) + (1 | HiveID) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2), data = Eggs, contrasts = list(Treatment = "contr.treatment"))

Linear Hypotheses:

|                      | Estimate | Std. Error | z value | Pr(>|z|) |
|----------------------|----------|------------|---------|----------|
| I(Week - 3):Treatment1 ug/l == 0 | 33.598 | 10.769 | 3.120   | 0.00926 ** |
| I(Week - 3):Treatment10 ug/l == 0 | 28.217 | 11.632 | 2.426   | 0.02954 *  |
| I(Week - 3):Treatment100 ug/l == 0 | 26.210 | 10.217 | 2.565   | 0.02656 *  |
| Treatment1 ug/l:I((Week - 3)^2) == 0 | -11.277 | 4.070 | -2.770  | 0.00515 *  |
| Treatment10 ug/l:I((Week - 3)^2) == 0 | -12.402 | 4.397 | -2.821  | 0.00451 *  |
| Treatment100 ug/l:I((Week - 3)^2) == 0 | -4.804 | 3.862 | -1.244  | 0.21346     |

---

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

(Adjusted p values reported -- Westfall method)

Summary: All Clothianidin treatments are significantly different from the control with respect to both their (local) slope at week 3 and their (global) curvature, with the exception of the 100 µg/l group: it does not differ significantly from the control in respect of its curvature.
2.2.4 Treatment effect on number of larvae

In proceeding analogously to §2.2.2 it turned out that the random effects of hives are in both the linear mixed-effects model with a linear time trend and the one with a parabolic time trend not significant. We tested this using exact restricted likelihood ratio tests implemented in package RLRsim. Analysis and results are not shown.

Hence, we decided to analyse the number of larvae by (purely) fixed-effects models with a linear or parabolic time trend along weeks, with treatment main effects, and interaction effects between weeks and treatment as well as between squared weeks and treatment. As in the previous paragraph this is done for weeks centered at 3 so that the main effect of treatment represents the estimated number of larvae at week 3.

```r
> linfit1 <- lm(Larvae ~ I(Week - 3), data = Larvae)
> linfit2 <- update(linfit1, ~ . + Treatment,
+ contrasts = list(Treatment = "contr.treatment"))
> linfit3 <- update(linfit2, ~ . + I(Week - 3):Treatment)
> parfit3 <- update(linfit3, ~ . + I((Week - 3)^2) + I((Week - 3)^2):Treatment)
> print(summary(parfit3), cor = FALSE)

Call:
lm(formula = Larvae ~ I(Week - 3) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2), data = Larvae,
contrasts = list(Treatment = "contr.treatment"))

Residuals:
     Min       1Q   Median       3Q      Max
-150.976  -56.036    -0.614   44.882  176.036

Coefficients:                 Estimate Std. Error t value Pr(>|t|)
(Intercept)          352.5357   20.9082 16.8613 < 2e-16 ***
I(Week - 3)          -6.6369    11.2917 -0.5880  0.558013
Treatment1 ug/l      66.6607    29.5686  2.2539  0.026349 *
Treatment10 ug/l     61.3214    31.9378  1.9199  0.057704 .
Treatment100 ug/l   -162.2500   28.0513 -5.7843  8.37e-08 ***
I((Week - 3)^2)       -9.8512    4.2679 -2.3079  0.023046 *
I(Week - 3):Treatment1 ug/l -3.8988   15.9689 -0.2437  0.807615
I(Week - 3):Treatment10 ug/l 6.4861   17.2484  0.3762  0.707682
I(Week - 3):Treatment100 ug/l -55.0560   15.1494 -3.6334  0.000442 ***
Treatment1 ug/l:I((Week - 3)^2) -6.3810    6.0357 -1.0567  0.292964
Treatment10 ug/l:I((Week - 3)^2) -0.1528    6.5193 -0.0230  0.981350
Treatment100 ug/l:I((Week - 3)^2) 17.3155    5.7259  3.0245  0.003170 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 78.23 on 100 degrees of freedom
Multiple R-squared: 0.7074, Adjusted R-squared: 0.6753
F-statistic: 21.98 on 11 and 100 DF, p-value: < 2.2e-16

> # "Classical" ANOVA
> #-------------------------------------------------------------------------
> (A1 <- anova(linfit1, linfit2, linfit3, parfit3))

Analysis of Variance Table

Model 1: Larvae ~ I(Week - 3)
Model 2: Larvae ~ I(Week - 3) + Treatment
Model 3: Larvae ~ I(Week - 3) + Treatment + I(Week - 3):Treatment
Model 4: Larvae ~ I(Week - 3) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)

 Res.Df    RSS    Df Sum of Sq      F     Pr(>F)
 Model 1: Larvae ~ I(Week - 3)
 Model 2: Larvae ~ I(Week - 3) + Treatment
 Model 3: Larvae ~ I(Week - 3) + Treatment + I(Week - 3):Treatment
 Model 4: Larvae ~ I(Week - 3) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)
 Res.Df    RSS    Df Sum of Sq      F     Pr(>F)
Summary: Allowing (only) a linear time trend along weeks, including an interaction between time and treatment, there is no significant influence of treatment on the time trend of number of larvae (p-value = 0.1771).

Adding a parabolic time trend along weeks, including an interaction between squared time and treatment (to allow for a partly visible curvature of the time trends of numbers of larvae), is a significant contribution to the model (p-value = 5.728e-05).

This means in particular, that the parabolic time trends of the number of larvae are not the same in the four treatment groups.

On top, there is a significant treatment main effect in the parabolic model (p-value = 1.33e-13. This means, there is significant difference in the estimated average number of larvae at week 3 (!) between the four treatment groups.
Model diagnostics for the model in `parfit3`:

![Graphs showing model diagnostics](image)

Figure 12: Diagnostic plots for the fitted linear fixed-effects model with parabolic time trends. Summarizing the findings (without explanation): the fitted model appears to fit very well and does not show any indication against homoscedastic normality of the errors, so the inferential results are considered reliable. (File names: *Cloth-Q2_ParFit_LarvaeModelAugPred.pdf* and *Cloth-Q2_ParFit_LarvaeModelDiagX.pdf* with $X = 3, \ldots, 6$)
2.2.5 Posthoc tests for time trend of larvae – comparisons with the Control

Here, we compare each Clothianidin treatment group to the control group with respect to the time trends in numbers of larvae.

Note: The same “Note” as in 2.2.3 applies!

```r
> fx <- coef(parfit3)
> K <- diag(length(fx))[-(1:6),]
> rownames(K) <- names(fx)[-1:6]
> CompWCntrl <- glht(parfit3, linfct = K); # summary(CompWCntrl)
> summary(CompWCntrl, test = adjusted(type = "Westfall"))

Simultaneous Tests for General Linear Hypotheses
Fit: lm(formula = Larvae ~ I(Week - 3) + Treatment + I((Week - 3)^2) +
I(Week - 3):Treatment + Treatment:I((Week - 3)^2), data = Larvae,
contrasts = list(Treatment = "contr.treatment"))

Linear Hypotheses:

|                        | Estimate | Std. Error | t value | Pr(>|t|) |
|------------------------|----------|------------|---------|----------|
| I(Week - 3):Treatment1 ug/l == 0 | -3.8988  | 15.9689    | -0.244  | 0.96041  |
| I(Week - 3):Treatment10 ug/l == 0 | -6.4861  | 17.2484    | -0.376  | 0.95938  |
| I(Week - 3):Treatment100 ug/l == 0 | -55.0560 | 15.1494    | -3.634  | 0.00226 **|
| Treatment1 ug/l:I((Week - 3)^2) == 0 | -6.3810  | 6.0357     | -1.057  | 0.63482  |
| Treatment10 ug/l:I((Week - 3)^2) == 0 | -0.1528  | 6.5193     | -0.023  | 0.98135  |
| Treatment100 ug/l:I((Week - 3)^2) == 0 | 17.3155  | 5.7259     | 3.024   | 0.01355 *|

---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- Westfall method)

Summary: Only the 100 µg/l group is significantly different from the control with respect to both their (local) slope at week 3 and their (global) curvature.
2.2.6 Treatment effect on number of capped brood cells

In the analysis of capped brood cells we experienced the same phenomenon as with the number of larvae in 2.2.4: the random effects of hives are in both the linear mixed-effects model with a linear time trend and the one with a parabolic time trend not significant (using again exact restricted likelihood ratio tests implemented in package RLRsim; neither analysis nor results shown).

So, the number of caped brood cells is also analysed by (purely) fixed-effects models with a linear or parabolic time trend along weeks, with treatment main effects, and interaction effects between weeks and treatment as well as between squared weeks and treatment. As in the previous paragraph this is done for weeks centered at 3 so that the main effect of treatment represents the estimated number of larvae at week 3.

```r
> linfit1 <- lm(Brood ~ I(Week - 3), data = Brood)
> linfit2 <- update(linfit1, ~ . + Treatment,
+ contrasts = list(Treatment = "contr.treatment"))
> linfit3 <- update(linfit2, ~ . + I(Week - 3):Treatment)
> parfit3 <- update(linfit3, ~ . + I((Week - 3)^2) + I((Week - 3)^2):Treatment)
> print(summary(parfit3), cor = FALSE)

Call:
 lm(formula = Brood ~ I(Week - 3) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment, data = Brood,
    contrasts = list(Treatment = "contr.treatment"))

Residuals:
     Min      1Q  Median      3Q     Max
-345.09  -98.42  -6.42   88.63  499.91

Coefficients:
                        Estimate Std. Error  t value  Pr(>|t|)
(Intercept)         753.036   40.606   18.545  < 2e-16 ***
I(Week - 3)         -15.869   21.930    -0.724   0.470985
Treatment1 ug/l    77.911    57.426     1.357   0.177926
Treatment10 ug/l  -28.179    62.027    -0.454   0.650601
Treatment100 ug/l -296.050   54.479    -5.434  3.89e-07 ***
I((Week - 3)^2)   -13.619    8.289    -1.643   0.103507
I(Week - 3):Treatment1 ug/l -18.607   31.014    -0.600   0.549885
I(Week - 3):Treatment10 ug/l -58.278   33.498    -1.740   0.084986
I(Week - 3):Treatment100 ug/l -193.017  12.661    -15.318  < 2e-16 ***
Treatment1 ug/l:I((Week - 3)^2) -3.375  11.722    -0.288   0.774004
Treatment10 ug/l:I((Week - 3)^2) 22.353  11.261     1.999   0.046464 .
Treatment100 ug/l:I((Week - 3)^2) 42.462  11.120     3.818  0.000233 ***

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

Residual standard error: 151.9 on 100 degrees of freedom
Multiple R-squared:  0.7496,    Adjusted R-squared:  0.722
F-statistic: 27.21 on 11 and 100 DF,  p-value: < 2.2e-16
```

So, the number of caped brood cells is also analysed by (purely) fixed-effects models with a linear or parabolic time trend along weeks, with treatment main effects, and interaction effects between weeks and treatment as well as between squared weeks and treatment. As in the previous paragraph this is done for weeks centered at 3 so that the main effect of treatment represents the estimated number of larvae at week 3.
2.2 Question 2: Influence on the brood development

|   |   |   |   |   |
|---|---|---|---|---|
| 1 | 110 | 5922179 |
| 2 | 107 | 3744238 | 3 | 2177941 | 31.449 | 2.107e-14 *** |
| 3 | 104 | 2836400 | 3 | 907838 | 13.109 | 2.749e-07 *** |
| 4 | 100 | 2308417 | 4 | 527983 | 5.718 | 0.0003471 *** |

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

> (A2 <- anova(update(parfit3, ~ . - Treatment), parfit3))

Analysis of Variance Table

Model 1: Brood ~ I(Week - 3) + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)
Model 2: Brood ~ I(Week - 3) + Treatment + I((Week - 3)^2) + I(Week - 3):Treatment + Treatment:I((Week - 3)^2)

| Res.Df | RSS   | Df    | Sum of Sq | F      | Pr(>F) |
|--------|-------|-------|-----------|--------|--------|
| 1      | 103   | 3592015 |          |        |        |
| 2      | 100   | 2308417 | 3        | 1283599 | 18.535 | 1.225e-09 *** |

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

Summary: Allowing (only) a linear time trend along weeks, including an interaction between time and treatment, there is a significant influence of treatment on the time trend of number of larvae (p-value = 2.749e-07). This means in particular, that the linear time trends of the number of larvae are not the same in the four treatment groups.

Adding a parabolic time trend along weeks, including an interaction between squared time and treatment (to allow for a partly visible curvature of the time trends of numbers of larvae), is a significant contribution to the model (p-value = 0.0003471). This means in particular, that the parabolic time trends of the number of larvae are not the same in the four treatment groups.

On top, there is a significant treatment main effect in the parabolic model (p-value = 1.225e-09). This means, there is significant difference in the estimated average number of larvae at week 3 (!) between the four treatment groups.
Model diagnostics for the model in **parfit3**:

![Diagnostic plots](image)

**Figure 13**: Diagnostic plots for the fitted linear fixed-effects model with *parabolic* time trends. Summarizing the findings (without explanation): the fitted model appears to fit well and does not show any serious indication against normality and homoscedasticity of the errors, so the inferential results can be considered reliable. (File names: *Cloth-Q2_ParFit_BroodModelAugPred.pdf* and *Cloth-Q2_ParFit_BroodModelDiagX.pdf* with $X = 3, \ldots, 6$)
2.2.7 Posthoc tests for time trend of capped brood cells – comparisons with the Control

Here, we compare each Clothianidin treatment group to the control group with respect to the time trends in numbers of capped brood cells.

**Note:** The same “Note” as in §2.2.3 applies!

```r
> fx <- coef(parfit3)
> K <- diag(length(fx))[-(1:6),]
> rownames(K) <- names(fx)[-(1:6)]
> CompWCntrl <- glht(parfit3, linfct = K);  # summary(CompWCntrl)
> summary(CompWCntrl, test = adjusted(type = "Westfall"))
```

Simultaneous Tests for General Linear Hypotheses

|                      | Estimate | Std. Error | t value | Pr(>|t|) |
|----------------------|----------|------------|---------|----------|
| I(Week - 3):Treatment1 ug/l == 0 | -18.607  | 31.014     | -0.600  | 0.72745  |
| I(Week - 3):Treatment10 ug/l == 0 | -58.278  | 33.498     | -1.740  | 0.22119  |
| I(Week - 3):Treatment100 ug/l == 0 | -193.017 | 29.422     | -6.560  | < 0.001 *** |
| Treatment1 ug/l:I((Week - 3)^2) == 0 | -3.375   | 11.722     | -0.288  | 0.77400  |
| Treatment10 ug/l:I((Week - 3)^2) == 0 | 22.353   | 12.661     | 1.765   | 0.22119  |
| Treatment100 ug/l:I((Week - 3)^2) == 0 | 42.462   | 11.120     | 3.818   | 0.00107 ** |

---

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

(Adjusted p values reported -- Westfall method)

**Summary:** Only the 100 µg/l group is significantly different from the control with respect to both their (local) slope at week 3 and their (global) curvature.
2.3 Question 3: Impact on the larval survival

2.3.1 Longitudinal EDA

![Graph showing larval survival across treatments](Cloth-Q3_LarvalSurvivalPhase1.pdf and Cloth-Q3_LarvalSurvivalPhase2.pdf)

Figure 14: Proportions of surviving larvae (filled grey circles) in each hive along weeks by treatment in phase 1 (weeks 1 to 4, top) and in phase 2 (weeks 4 to 7, bottom). Proportions in each hive are calculated relative to hive value in first week of respective phase. The raw values are augmented by arithmetic means and medians across hives at each week. Values connected by a grey polyline belong to the same hive; different polylines indicate different hives. The blue polylines connect the time specific median values, the black ones the respective means. (File names: Cloth-Q3_LarvalSurvivalPhase1.pdf and Cloth-Q3_LarvalSurvivalPhase2.pdf)

Exploratory data analysis using q-q plots revealed that the raw data (displayed in fig. [14]) are neither normally distributed nor homoscedastic. The variance of the data seems to increase with decreasing proportions of surviving larvae. To compensate that we take the logarithm of the proportions of *dead larvae* (= 1 -
proportion of surviving larvae) to obtain (approximately) normally distributed and homoscedastic values.

Figure 15: Logarithms of proportions of dead larvae in each hive along weeks by treatment: For layout details see caption of fig. [14] (Values at first week in each phase are missing since the logarithm of 0 (zero) is undefined.) (File names: Cloth-Q3_LarvalLogSurvivalPhase1.pdf and Cloth-Q3_LarvalLogSurvivalPhase2.pdf)

Note: Since in phase 1 the proportions of dead larvae virtually do not change from the penultimate to the last week in any but a single hive (see the Control group in top part of fig. [14], we exclude the last week of phase 1 from the following analysis. This is similar in phase 2 where proportions remain constant in the last two weeks in all but only two hives (one in the Control group and one in the 1 µg/l treatment; see bottom part of fig. [14]. Since in addition, phase 2 has 100 % dead larvae from week five on in three of the four hives in the 100 µg/l treatment (see bottom part of fig. [14], we exclude this treatment group in phase 2 completely from the following analysis.
2.3.2 Treatment effect on larval survival in phase 1

We analyse the log-proportions of dead larvae using a two-factorial mixed-effects ANOVA with treatment and week as fixed-effects factors including interactions and with hive as random-effects grouping factor. (This model is the same as a two-factorial repeated measures ANOVA with treatment and week as fixed-effects factors and hive as random-effects factor for grouping the repeated measurements.)

We restrict the following analysis to weeks 2 and 3 of phase 1, and we proceed as in §2.1.2; for explanations and comments see also there.

> Data <- droplevels(subset(Survival1, subset = Week > 1 & Week < 4))

> fit0 <- lmer(log(1 - Proportion.of.Larvae) ~ (1 | HiveID), data = Data)
> fit1 <- update(fit0, ~ . + WeekFactor, contrasts = list(WeekFactor = "contr.treatment"))
> fit2 <- update(fit1, ~ . + Treatment, contrasts = list(WeekFactor = "contr.treatment", Treatment = "contr.treatment"))
> fit3 <- update(fit2, ~ . + WeekFactor:Treatment)
> print(summary(fit3), cor = FALSE)

Linear mixed model fit by REML ['lmerMod']
Formula:
  log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment
  Data: Data

REML criterion at convergence: 28.7

Scaled residuals:
     Min  1Q Median  3Q    Max
-1.48971 -0.41749  0.03685  0.35422  1.24429

Random effects:
  Groups   Name     Variance  Std.Dev.
          HiveID (Intercept) 0.25214   0.5021
                 Residual 0.02421   0.1556
Number of obs: 34, groups: HiveID, 17

Fixed effects:
                  Estimate  Std. Error  t value
  (Intercept)  -1.776100  0.2628280 -6.757
  WeekFactor3  0.146378  0.1100380  1.330
  Treatment1 ug/l 0.512183  0.3717470  1.378
  Treatment10 ug/l 0.740020  0.3717470  1.991
  Treatment100 ug/l 0.269917  0.3526580  0.765
  WeekFactor3:Treatment1 ug/l 0.320000  0.1556050  2.056
  WeekFactor3:Treatment10 ug/l 0.186800  0.1556050  1.200
  WeekFactor3:Treatment100 ug/l 0.502397  0.1476020  3.404

> # F-tests with approximated degrees of freedom according to Kenward-Roger:
> #--------------------------------------------------------------
> (KR0 <- KRmodcomp(largeModel = fit1, smallModel = fit0))

F-test with Kenward-Roger approximation; time: 0.13 sec
large : log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor
small : log(1 - Proportion.of.Larvae) ~ (1 | HiveID)
  stat  ndf  ddf  F.scaling p.value
  Ftest 37.826 1.000 16.000 1.396e-05 ***
***
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> (KR1 <- KRmodcomp(largeModel = fit2, smallModel = fit1))
2.3 Question 3: Impact on the larval survival

F-test with Kenward-Roger approximation; time: 0.11 sec
large: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment
small: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor

| stat | ndf | ddf | F.scaling | p.value |
|------|-----|-----|-----------|---------|
| Ftest| 1.9736 | 3.0000 | 13.0000 | 1 | 0.1678 |

> (KR2 <- KRmodcomp(largeModel = fit3, smallModel = fit2))

F-test with Kenward-Roger approximation; time: 0.11 sec
large: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment
small: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment

| stat | ndf | ddf | F.scaling | p.value |
|------|-----|-----|-----------|---------|
| Ftest| 4.125 | 3.000 | 13.000 | 1 | 0.02929 *

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

> # Parametric bootstrap:
> #-------------------------------------------------------------------------
> cl <- makeCluster(rep("localhost", detectCores()))
> set.seed(201712) # For reproducibility.
> # Relevant: p-value in row "PBtest"
> (PB0 <- summary(PBmodcomp(largeM = fit1, smallM = fit0, nsim = nsim.PBmodcomp,
> cl = cl)))

Bootstrap test; time: 377.22 sec; samples: 5000; extremes: 0;
large: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor
small: log(1 - Proportion.of.Larvae) ~ (1 | HiveID)

| stat  | df  | ddf   | p.value   |
|-------|-----|-------|-----------|
| LRT   | 20.639 | 1.000 | 5.544e-06 *** |
| PBtest| 20.639 | 0.0002000 | *** |
| Gamma | 20.639 | 1.201e-05 *** |
| Bartlett | 18.636 | 1.000 | 1.582e-05 *** |
| F     | 20.639 | 1.000 | 20.603 0.0001851 *** |

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

> (PB1 <- summary(PBmodcomp(largeM = fit2, smallM = fit1, nsim = nsim.PBmodcomp,
> cl = cl)))

Bootstrap test; time: 426.21 sec; samples: 5000; extremes: 873;
large: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment
small: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor

| stat  | df  | ddf   | p.value   |
|-------|-----|-------|-----------|
| LRT   | 6.1975 | 3.0000 | 0.1024 |
| PBtest| 6.1975 | 0.1748 |
| Gamma | 6.1975 | 0.1765 |
| Bartlett | 4.9243 | 3.0000 | 0.1774 |
| F     | 2.0658 | 3.0000 | 2.7205 0.2966 |

> (PB2 <- summary(PBmodcomp(largeM = fit3, smallM = fit2, nsim = nsim.PBmodcomp,
> cl = cl)))

Bootstrap test; time: 528.22 sec; samples: 5000; extremes: 142;
large: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment
small: log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment

| stat  | df  | ddf   | p.value   |
|-------|-----|-------|-----------|
| LRT   | 11.5528 | 3.0000 | 0.009083 ** |
|       | PBtest  | 11.5528 | 0.028594* |
|-------|---------|---------|-----------|
|       | Gamma   | 11.5528 | 0.026092* |
|       | Bartlett| 9.2566  | 3.0000    |
|       |         | 0.026066* |
| F     | 3.8509  | 3.0000  | 2.7288    |
|       |         | 0.162266 |

---

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

> stopCluster(cl)

**Summary:** There is a significant main effect of week (p-value = 1.396e-05 from Kenward-Rogers method, and p-value = 2e-04 based on parametric bootstrap), but there is a non-significant main effect of treatment (p-value = 0.1678 from Kenward-Rogers method, and p-value = 0.1748 based on parametric bootstrap).

Since there is a significant interaction effect between treatment and week (p-value = 0.02929 from Kenward-Rogers method, and p-value = 0.02859 based on parametric bootstrap), the main effect of each factor has to be interpreted as averaged across the levels of the other factor: The averages (across the four treatments) of log-proportions of dead larvae are significantly different between the two weeks, but the averaged log-proportions of dead larvae (across the two weeks) are not significantly different between the four treatment groups.

A bit more concrete: Interpreting the week main effect averaged across the levels of treatment implies that we compare the two points in time without separating the treatments. Even more precise: in each week we consider the population mean (of log-proportions of dead larvae) after “pooling” over the four treatment groups, i.e., the data from the four treatments are combined in each week as if there were only a single treatment group.

In turn, interpreting the treatment main effect averaged across the levels of week implies that we compare the four treatments without separating the weeks. Even more precise: in each treatment group we consider the population mean (of log-proportions of dead larvae) after “pooling” week 2 and week 3, i.e., the data from the two weeks are combined in each treatment group as if there were only a single point in time.

Additional remark: the significant interaction effect between treatment and week means that changes (!) in (average) log-proportions of dead larvae along weeks are different between treatments.
2.3 Question 3: Impact on the larval survival

Model diagnostics for the model in fit3:

Figure 16: Diagnostic plots for the fitted linear mixed-effects model. Summarizing the findings (without explanation): the fitted model shows no indication against homoscedastic normality of the errors, so the inferential results are considered as reliable. (File names: Cloth-Q3_Survival1ModelDiagPlotX.pdf with $X = 1, \ldots, 6$)
2.3.3 Posthoc tests in survival phase 1 – comparisons with the Control

We perform Dunnett’s multiple comparisons for treatment (as an “inner” factor) within week (as “outer” factor) for the one-sided null hypothesis that the Clothianidin treatments do not yield higher values than the Control group. (The implementation is an adaption of the approach in chapter 3 of vignette “multcomp-examples” of the R-package multcomp.)

```r
> fm <- fit3  # fm expects the 2-factorial mixed-effects ANOVA-object with interactions.
> # Names of the 2 fixed-effects factors (Note: Order matters!):
> factornames <- list(Outer = "WeekFactor", Inner = "Treatment")
> # Extract factor vector *data* which entered into fm via Data:
> InnerFactor <- Data[[factornames$Inner]]
> OuterFactor <- Data[[factornames$Outer]]

> # 1. Dunnett's multiple comparisons for "inner" factor within "outer" factor:
> #************************************************************************
> # Adapted from ch. 3 of multcomp-vignette "multcomp-examples". In particular the way
> # of generating tmp for the model matrix X was modified to using levels() instead of
> # unique(). (The latter would ignore a potentially non-alphanumerically sorted level
> # order in the "inner" factor and could yield a wrong sign in contrast estimators.)
> # tmp <- expand.grid(levels(InnerFactor), levels(OuterFactor))
> tmp <- expand.grid(levels(InnerFactor), levels(OuterFactor))
> names(tmp) <- c(factornames$Inner, factornames$Outer)
> X <- model.matrix(formula(fm, fixed.only = TRUE)[-2], data = tmp)
> CM <- contrMat(table(InnerFactor), "Dunnett") # Would also work with "Tukey".
> IM <- diag(nlevels(OuterFactor))
> dimnames(IM) <- list(levels(OuterFactor), levels(OuterFactor))
> Kron1 <- kronecker(IM, CM, make.dimnames = TRUE)
> fm.glht <- glht(fm, linfct = Kron1 %*% X, alternative = "greater")
> summary(fm.glht, test = adjusted(type = "Westfall"))
```

### Summary:

In week 2 the log-proportions of dead larvae in hives treated with Clothianidin are not significantly higher than in the Control group, but in week 3 they are significantly higher in all three Clothianidin groups. (Compare top panel in fig. 15.)

Special consideration: Here, we select particular fixed effects coefficients of the fitted model for multiple comparisons with zero, namely coefficients number 6, 7, and 8 which means that we analyse only the interaction effects:

```r
> # 3. Selected fixed effects coefficients of fitted model are specified in
> # vector idx, e.g., for selected interaction effects:
> #************************************************************************
> idx <- c(6, 7, 8)  # Can also be just a scalar, i.e., a single level index.
```
2.3 Question 3: Impact on the larval survival

```r
> fixcoef <- fixef(fm)
> K <- matrix(0, nrow = length(fixcoef), ncol = length(fixcoef),
+   dimnames = list(names(fixcoef), names(fixcoef)))
> diag(K)[idx] <- 1
> K <- K[rowSums(abs(K)) > 0,]
> fm.glht3 <- glht(fm, linfct = K, alternative = "greater")
> # summary(fm.glht3)
> summary(fm.glht3, test = adjusted(type = "Westfall"))
```

Simultaneous Tests for General Linear Hypotheses

Fit: `lmer(formula = log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment, data = Data, contrasts = list(WeekFactor = "contr.treatment", Treatment = "contr.treatment"))`

Linear Hypotheses:

```
Estimate Std. Error z value Pr(>|z|)
WeekFactor3:Treatment1 ug/l <= 0 0.3200 0.1556 2.056 0.0364 *
WeekFactor3:Treatment10 ug/l <= 0 0.1868 0.1556 1.200 0.1150
WeekFactor3:Treatment100 ug/l <= 0 0.5024 0.1476 3.404 <0.001 ***
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- Westfall method)
```

Summary: The changes (!) (here: increases) from week 2 to week 3 in log-proportions of dead larvae are significantly higher in the 1 µg/l and the 100 µg/l Clothianidin group than in the Control group. The change (!) (here increase) from week 2 to week 3 in log-proportions of dead larvae in the 10 µg/l Clothianidin group is not significantly higher than in the Control group. (This does not proof that the change in the 10 µg/l treated hives is not different from the one in the control group, but the sample size is presumably too low to detect a significant difference.) (Compare top panel in fig. [15].)

Another special consideration: We carefully construct a combination of the one-sided tests from above:

```r
> summary(glht(fm, linfct = rbind(Kron1 %*% X, K), alternative = "greater"),
+   test = adjusted(type = "Westfall"))
```

Simultaneous Tests for General Linear Hypotheses

Fit: `lmer(formula = log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment, data = Data, contrasts = list(WeekFactor = "contr.treatment", Treatment = "contr.treatment"))`

Linear Hypotheses:

```
Estimate Std. Error z value Pr(>|z|)
2:1 ug/l - Ctrl <= 0 0.5122 0.3717 1.378 0.21732
2:10 ug/l - Ctrl <= 0 0.7400 0.3717 1.991 0.05810 .
2:100 ug/l - Ctrl <= 0 0.5024 0.3526 1.427 0.15591
3:1 ug/l - Ctrl <= 0 0.7400 0.3717 1.991 0.05810 .
3:10 ug/l - Ctrl <= 0 0.9268 0.3717 2.493 0.01289 *
3:100 ug/l - Ctrl <= 0 0.7723 0.3526 2.190 0.02934 *
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- Westfall method)
```

Summary: If we combine the two test families from above into one family all statements from above hold still true on a significance level slightly higher than 5 %: a level of 6 % would yield the same conclusions.
2.3.4 Treatment effect on larval survival in phase 2

Recall the note on p. 32: Proportions of dead larvae remain constant in the last two weeks in almost all hives and the 100 µg/l treatment group has 100 % dead larvae from week five on in three of the four hives (see bottom part of fig. 14). Therefore, we exclude the 100 µg/l treatment group completely from the following analysis and restrict the analysis to weeks 5 and 6 of phase 2. We proceed as in § 2.3.2.

```r
> Data <- droplevels(subset(Survival2, subset = Week > 4 & Week < 7))
> fit0 <- lmer(log(1 - Proportion.of.Larvae) ~ (1 | HiveID), data = Data)
> fit1 <- update(fit0, ~ . + WeekFactor, contrasts = list(WeekFactor = "contr.treatment"))
> fit2 <- update(fit1, ~ . + Treatment, contrasts = list(WeekFactor = "contr.treatment", Treatment = "contr.treatment"))
> fit3 <- update(fit2, ~ . + WeekFactor:Treatment)
> print(summary(fit3), cor = FALSE)

Linear mixed model fit by REML ['lmerMod']
Formula:
log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment
Data: Data

REML criterion at convergence: 0.6

Scaled residuals:
       Min     1Q  Median     3Q    Max
-1.32234 -0.44419  0.01893  0.50566  1.30689

Random effects:
  Groups     Name        Variance Std.Dev.
   HiveID  (Intercept)  0.141626  0.37633
             Residual     0.004949  0.07035
Number of obs: 32, groups: HiveID, 16

Fixed effects:          Estimate Std. Error t value
 (Intercept)          -2.01225   0.19143  -10.512
 WeekFactor6         0.08708   0.04974   1.751
 Treatment1 ug/l     1.32890   0.27072   4.909
 Treatment10 ug/l    1.24031   0.27072   4.582
 Treatment100 ug/l   1.93471   0.27072   7.147
 WeekFactor6:Treatment1 ug/l 0.12036   0.07035   1.711
 WeekFactor6:Treatment10 ug/l 0.10821   0.07035   1.538
 WeekFactor6:Treatment100 ug/l -0.08708   0.07035  -1.238

> # F-tests with approximated degrees of freedom according to Kenward-Roger:
> #-------------------------------------------------------------------------------------------
> (KRO <- KRmodcomp(largeModel = fit1, smallModel = fit0))

F-test with Kenward-Roger approximation; time: 0.13 sec

|     | Ftest | stat | ndf | ddf | F.scaling | p.value |
|-----|-------|------|-----|-----|-----------|---------|
| 0.001 | 15.387 | 15.000 |    |    | 15.000 | 0.001357 ** |

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

> (KR1 <- KRmodcomp(largeModel = fit2, smallModel = fit1))

F-test with Kenward-Roger approximation; time: 0.09 sec

|     | Ftest | stat | ndf | ddf | F.scaling | p.value |
|-----|-------|------|-----|-----|-----------|---------|
| 0.001 | 15.387 | 15.000 |    |    | 15.000 | 0.001357 ** |

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
2.3 Question 3: Impact on the larval survival

small : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} \)

stat ndf ddf F.scaling p.value
Ftest 18.04 3.00 12.00 1 9.629e-05 ***
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> (KR2 <- KRmodcomp(largeModel = fit3, smallModel = fit2))

F-test with Kenward-Roger approximation; time: 0.20 sec

large : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} + \text{Treatment} + \text{WeekFactor:Treatment} \)
small : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} + \text{Treatment} \)

stat ndf ddf F.scaling p.value
Ftest 3.8761 3.0000 12.0000 1 0.03776 *
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> # Parametric bootstrap:
> #---------------------------------------------------------------
> cl <- makeCluster(rep("localhost", detectCores()))
> set.seed(201712) # For reproducibility.
> # Relevant: p-value in row "PBtest"
> (PB0 <- summary(PBmodcomp(largeM = fit1, smallM = fit0, nsim = nsim.PBmodcomp,
> + cl = cl)))

Bootstrap test; time: 510.44 sec; samples: 5000; extremes: 6;
large : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} \)
small : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) \)

stat df ddf p.value
LRT 11.312 1.000 0.0007699 ***
PBtest 11.312 0.0013997 **
Gamma 11.312 0.0013862 **
Bartlett 10.149 1.000 0.0014434 **
F 11.312 1.000 0.0031823 **
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> (PB1 <- summary(PBmodcomp(largeM = fit2, smallM = fit1, nsim = nsim.PBmodcomp,
> + cl = cl)))

Bootstrap test; time: 528.91 sec; samples: 5000; extremes: 0;
large : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} + \text{Treatment} \)
small : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} \)

stat df ddf p.value
LRT 27.1063 3.0000 5.593e-06 ***
PBtest 27.1063 0.0002000 ***
Gamma 27.1063 0.0001102 ***
Bartlett 21.1325 3.0000 9.881e-05 ***
F 9.0354 3.0000 0.0623470 .
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> (PB2 <- summary(PBmodcomp(largeM = fit3, smallM = fit2, nsim = nsim.PBmodcomp,
> + cl = cl)))

Bootstrap test; time: 455.37 sec; samples: 5000; extremes: 177;
large : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} + \text{Treatment} + \text{WeekFactor:Treatment} \)
small : \( \log(1 - \text{Proportion.of.Larvae}) \sim (1 \mid \text{HiveID}) + \text{WeekFactor} + \text{Treatment} \)
Summary: There is a significant main effect of week (p-value = 0.001357 from Kenward-Rogers method, and p-value = 0.0014 based on parametric bootstrap), and there is a significant main effect of treatment (p-value = 9.629e-05 from Kenward-Rogers method, and p-value = 2e-04 based on parametric bootstrap).

Since there is a significant interaction effect between treatment and week (p-value = 0.03776 from Kenward-Rogers method, and p-value = 0.03559 based on parametric bootstrap), the main effect of each factor has to be interpreted as averaged across the levels of the other factor: The averages (across the four treatments) of log-proportions of dead larvae are significantly different between the two weeks, and the averaged log-proportions of dead larvae (across the two weeks) are significantly different between the four treatment groups.

For help in interpreting the above see the “more concrete” explanations in the summary on page 35.

Recall: the significant interaction effect between treatment and week means that changes (!) in (average) log-proportions of dead larvae along weeks are different between treatments.
Model diagnostics for the model in fit3:

Figure 17: Diagnostic plots for the fitted linear mixed-effects model. Summarizing the findings (without explanation): the fitted model shows no serious indication against homoscedastic normality of the errors, so the inferential results are considered as reliable. (File names: Cloth-Q3_Survival2ModelDiagPlotX.pdf with X = 1, …, 6)
2.3.5 Posthoc tests in survival phase 2 – comparisons with the Control

We proceed completely analogous to §2.3.3.

```r
> fm <- fit3  # fm expects the 2-factorial mixed-effects ANOVA-object with interactions.
> # Names of the 2 fixed-effects factors (Note: Order matters!):
> factornames <- list(Outer = "WeekFactor", Inner = "Treatment")
> # Extract factor vector *data* which entered into fm via Data:
> InnerFactor <- Data[,factornames$Inner]
> OuterFactor <- Data[,factornames$Outer]
> # 1. Dunnett's multiple comparisons for "inner" factor within "outer" factor:
> #************************************************************************
> # Adapted from ch. 3 of multcomp-vignette "multcomp-examples". In particular the way
> # of generating tmp for the model matrix X was modified to using levels() instead of
> # unique(). (The latter would ignore a potentially non-alphanumerically sorted level
> # order in the "inner factor" and could yield a wrong sign in contrast estimators.)
> tmp <- expand.grid(levels(InnerFactor), levels(OuterFactor))
> names(tmp) <- c(factornames$Inner, factornames$Outer)
> X <- model.matrix(formula(fm, fixed.only = TRUE)[-2], data = tmp)
> CM <- contrMat(table(InnerFactor), "Dunnett")  # Would also work with "Tukey".
> IM <- diag(nlevels(OuterFactor))
> dimnames(IM) <- list(levels(OuterFactor), levels(OuterFactor))
> Kron1 <- kronecker(IM, CM, make.dimnames = TRUE)
> fm.glht <- glht(fm, linfct = Kron1 %*% X, alternative = "greater")
> summary(fm.glht, test = adjusted(type = "Westfall"))

Simultaneous Tests for General Linear Hypotheses

Fit: lmer(formula = log(1 - Proportion.of.Larvae) ~ (1 | HiveID) +
  WeekFactor + Treatment + WeekFactor:Treatment, data = Data,
  contrasts = list(WeekFactor = "contr.treatment", Treatment = "contr.treatment"))

Linear Hypotheses:

```
  Estimate Std. Error z value Pr(>|z|)
5:1 ug/l - Ctrl <= 0  1.3289   0.2707  4.909  < 2e-05 ***  
5:10 ug/l - Ctrl <= 0 1.2403   0.2707  4.582  < 2e-05 ***  
5:100 ug/l - Ctrl <= 0 1.9347   0.2707  7.147  < 2e-05 *** 
6:1 ug/l - Ctrl <= 0  1.4493   0.2707  5.353  < 2e-05 ***  
6:10 ug/l - Ctrl <= 0 1.3485   0.2707  4.981  < 2e-05 ***  
6:100 ug/l - Ctrl <= 0 1.8476   0.2707  6.825  < 2e-05 ***  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

(Adjusted p values reported -- Westfall method)

Summary: In both weeks the log-proportions of dead larvae in hives treated with Clothianidin are significantly higher than in the Control group. (Compare bottom panel in fig. [15].)

Special consideration: Here, we select particular fixed effects coefficients of the fitted model for multiple comparisons with zero, namely coefficients number 6, 7, and 8 which means that we analyse only the interaction effects:

```r
> # 3. Selected fixed effects coefficients of fitted model are specified in
> # vector idx, e.g., for selected interaction effects:
> #************************************************************************
> idx <- c(6, 7, 8)  # Can also be just a scalar, i.e., a single level index.
> fixcoef <- fixef(fm)
> K <- matrix(0, nrow = length(fixcoef), ncol = length(fixcoef),
+           dimnames = list(names(fixcoef), names(fixcoef))
> diag(K)[idx] <- 1
> K <- K[rowSums(abs(K)) > 0, ]
```
> fm.glht3 <- glht(fm, linfct = K, alternative = "greater")
> # summary(fm.glht3)
> summary(fm.glht3, test = adjusted(type = "Westfall"))

Simultaneous Tests for General Linear Hypotheses

Fit: lmer(formula = log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment, data = Data, contrasts = list(WeekFactor = "contr.treatment", Treatment = "contr.treatment"))

Linear Hypotheses:

| Estimate | Std. Error | z value | Pr(>z) |
|----------|------------|---------|--------|
| WeekFactor6:Treatment1 ug/l <= 0 | 0.12036 | 0.07035 | 1.711 | 0.104 |
| WeekFactor6:Treatment10 ug/l <= 0 | 0.10821 | 0.07035 | 1.538 | 0.107 |
| WeekFactor6:Treatment100 ug/l <= 0 | -0.08708 | 0.07035 | -1.238 | 0.892 |

(Adjusted p values reported -- Westfall method)

Summary: The changes (!) from week 5 to week 6 in log-proportions of dead larvae are not significantly higher in any of the Clothianidin groups than in the Control group. (Compare bottom panel in fig. 15.)

Another special consideration: We carefully construct a combination of the one-sided tests from above:

> summary(glht(fm, linfct = rbind(Kron1 %*% X, K), alternative = "greater"),
> + test = adjusted(type = "Westfall"))

Simultaneous Tests for General Linear Hypotheses

Fit: lmer(formula = log(1 - Proportion.of.Larvae) ~ (1 | HiveID) + WeekFactor + Treatment + WeekFactor:Treatment, data = Data, contrasts = list(WeekFactor = "contr.treatment", Treatment = "contr.treatment"))

Linear Hypotheses:

| Estimate | Std. Error | z value | Pr(>z) |
|----------|------------|---------|--------|
| 5:1 ug/l - Ctrl <= 0 | 1.32890 | 0.27072 | 4.909 | <0.001 *** |
| 5:10 ug/l - Ctrl <= 0 | 1.24031 | 0.27072 | 4.582 | <0.001 *** |
| 5:100 ug/l - Ctrl <= 0 | 1.93471 | 0.27072 | 7.147 | <0.001 *** |
| 6:1 ug/l - Ctrl <= 0 | 1.44926 | 0.27072 | 5.353 | <0.001 *** |
| 6:10 ug/l - Ctrl <= 0 | 1.34852 | 0.27072 | 4.981 | <0.001 *** |
| 6:100 ug/l - Ctrl <= 0 | 1.84763 | 0.27072 | 6.825 | <0.001 *** |
| WeekFactor6:Treatment1 ug/l <= 0 | 0.12036 | 0.07035 | 1.711 | 0.104 |
| WeekFactor6:Treatment10 ug/l <= 0 | 0.10821 | 0.07035 | 1.538 | 0.107 |
| WeekFactor6:Treatment100 ug/l <= 0 | -0.08708 | 0.07035 | -1.238 | 0.892 |

---

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

(Adjusted p values reported -- Westfall method)

Summary: If we combine the two test families from above into one family all statements from above hold still true (on the same significance level).
2.4 Question 4: Compensation rate

The main idea is to calculate and show the compensation effort for each treatment. The bees have to compensate for the higher larval mortality and therefore expend more energy.

2.4.1 Larvae-to-eggs ratio: Longitudinal EDA

To estimate/assess the development of the hives along time we calculate larvae-to-eggs ratios within each hive for each week. For this ratio to be sensible, the number of larvae in week $w$ has to be compared with the number of eggs of the previous week $w-1$ (as the larvae of week $w$ have been “produced” by the eggs of week $w-1$). The time courses of these “shifted” larvae-to-eggs ratios are then compared among treatments.

One point to consider is, that of all eggs seen in week $w-1$ approximately two thirds have already turned into capped brood at inspection in the subsequent week $w$ because they were already one or two days old at inspection in week $w-1$. In turn, the actually observed number of larvae in week $w$ can approximately be only one third of the number of eggs seen in the previous week.

However, we will not take this into consideration since it would complicate things unnecessarily since the deviation is very likely just a constant factor. We therefore take the raw weekly numbers of eggs and larvae as good estimates.

![Figure 18: "Shifted" larvae-to-eggs ratio (open circles) in each hive along weeks by treatment, augmented by arithmetic means and medians across hives at each week. Values connected by a black polyline belong to the same hive; different polylines indicate different hives. The blue polylines connect the time specific median values, the green ones the respective means.](Cloth-Q4_L2E_along_Weeks_by_Treat.pdf)

Table 1: Per treatment group: Mean and median larvae-to-eggs ratios per week

| Treatment | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 |
|-----------|--------|--------|--------|--------|--------|--------|
| Ctrl      | Mean   | Med.   | Mean   | Med.   | Mean   | Med.   |
| 1 ug/l    | 1.58   | 1.39   | 1.45   | 1.45   | 1.48   | 1.44   |
| 10 ug/l   | 2.93   | 2.68   | 1.94   | 1.98   | 1.44   | 1.44   |
| 100 ug/l  | 6.37   | 2.36   | 1.67   | 1.75   | 1.02   | 1.09   |

Table 2: Per treatment group and across weeks: Average and median of mean larvae-to-eggs ratios

| Ctrl      | 1 ug/l | 10 ug/l | 100 ug/l |
|-----------|--------|---------|----------|
| Means of means | 1.57   | 1.59    | 2.13     | 0.89     |
| Medians of means | 1.53   | 1.48    | 1.38     | 0.60     |
The following two figures display just the mean and median larvae-to-eggs ratio profiles, respectively, i.e., without the raw values.

Figure 19: Means (across hives) of “shifted” larvae-to-eggs ratios along weeks by treatment. As eggs of week \( w - 1 \) are the larvae of week \( w \) the numbers of larvae are shifted by one week. Note that the mean values in the control group stay quite steady at approx. 1.5 until week seven, when environmental conditions got worse. (File name: Cloth-Q4_MeanL2E_along_Weeks_by_Treat.pdf)

Figure 20: Medians (across hives) of “shifted” larvae-to-eggs ratios along weeks by treatment. As eggs of week \( w - 1 \) are the larvae of week \( w \) the numbers of larvae are shifted by one week. Note that the median values in the control group stay quite steady very close to 1.5 until week seven, when environmental conditions got worse. (File name: Cloth-Q4_MedianL2E_along_Weeks_by_Treat.pdf)
2.4.2 Treatment effect on larvae-to-eggs ratio (WITHOUT the extreme outlier)

We again proceed as in §2.1.2, i.e., we follow, but also extend §10.6 in [6 Faraway (2016)]. For explanations regarding the testing methodology and for comments regarding the R-code see §2.1.2.

**Note:** We exclude the extreme outlier above 15 in treatment 10 µg/l from the following analysis!

```r
> EL <- subset(EL0, subset = L2E < 15)
```

We analyse the larvae-to-eggs ratio by a linear mixed-effects model with a fixed effects linear time trend along weeks, with fixed treatment main effects, and fixed interaction effects between weeks and treatment. This is done for weeks centered at 2 – arbitrarily selected – so that the main effect of treatment represents the estimated larvae-to-eggs ratio at week 2. Hives are modelled as random shift effects, thus accounting for the within-hive correlation.

```r
> levels(EL$Treatment) <- olE
> linfit1 <- lmer(L2E ~ I(Week - 2) + (1 | HiveID), data = EL)
> linfit2 <- update(linfit1, ~ . + Treatment,
+ contrasts = list(Treatment = "contr.treatment"))
> linfit3 <- update(linfit2, ~ . + I(Week - 2):Treatment)
> print(summary(linfit3), cor = FALSE)
```

```
Linear mixed model fit by REML ['lmerMod']
Formula: L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
Data: EL

REML criterion at convergence: 222.2

Scaled residuals:
    Min  1Q Median  3Q Max
-1.6475 -0.5277 -0.0943 0.2540 4.5530

Random effects:
  Groups   Name    Variance Std.Dev.  
        HiveID (Intercept) 0.08842  0.2973
            Residual 0.48522  0.6966
Number of obs: 95, groups: HiveID, 16

Fixed effects:                Estimate  Std. Error t value
(Intercept)                 1.66825     0.29265   5.700
I(Week - 2)                -0.03743     0.08326  -0.450
Treatment1 ug/l             0.94119     0.41387   2.274
Treatment10 ug/l            -0.03515     0.46810  -0.075
Treatment100 ug/l           0.05734     0.39263   0.146
I(Week - 2):Treatment1 ug/l  -0.36948     0.11774  -3.138
I(Week - 2):Treatment10 ug/l -0.08292     0.13269  -0.625
I(Week - 2):Treatment100 ug/l -0.29576     0.11170  -2.648

# F-tests with approximated degrees of freedom according to Kenward-Roger:
#-------------------------------------------------------------------------
# (KR1 <- KRmodcomp(largeModel = linfit2, smallModel = linfit1))
# (KR2 <- KRmodcomp(largeModel = linfit3, smallModel = linfit2))
```

F-test with Kenward-Roger approximation; time: 0.20 sec
large : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment

```
Ftest  4.2802  3.0000  75.3889  1 0.007611 ** ---

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
> (KR3 <- KRmodcomp(largeModel = linfit3, smallM = update(linfit3, ~ . - Treatment)))

F-test with Kenward-Roger approximation; time: 0.17 sec
large : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : L2E ~ I(Week - 2) + (1 | HiveID) + I(Week - 2):Treatment

| stat     | ndf   | ddf   | F.scaling | p.value |
|----------|-------|-------|-----------|---------|
| Ftest    | 2.4851| 3.0000| 43.8312   | 0.99993 |

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

> # Parametric bootstrap:
> #-------------------------------------------------------------------------
> cl <- makeCluster(rep("localhost", detectCores()))
> set.seed(201712) # For reproducibility.
> # Relevant: p-value in row "PBtest"
> # (PB1 <- summary(PBmodcomp(largeM = linfit2, smallM = linfit1, nsim = nsim.PBmodcomp,
> #  cl = cl)))
> (PB2 <- summary(PBmodcomp(largeM = linfit3, smallM = linfit2, nsim = nsim.PBmodcomp,
> + cl = cl)))

Bootstrap test; time: 420.33 sec; samples: 5000; extremes: 44;
large : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment

| stat     | df    | ddf   | p.value |
|----------|-------|-------|---------|
| LRT      | 12.6663| 3.0000| 0.005417 ** |
| PBtest   | 12.6663|       | 0.08998 ** |
| Gamma    | 12.6663|       | 0.08019 ** |
| Bartlett | 11.7456| 3.0000| 0.008307 ** |
| F        | 4.2221 | 3.0000| 2.8948 0.138704 |

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

> (PB3 <- summary(PBmodcomp(largeM = linfit3, smallM = update(linfit3, ~ . - Treatment),
> + nsim = nsim.PBmodcomp, cl = cl)))

Bootstrap test; time: 419.61 sec; samples: 5000; extremes: 373;
large : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment

| stat     | df    | ddf   | p.value |
|----------|-------|-------|---------|
| LRT      | 7.9692 | 3.0000| 0.04665 * |
| PBtest   | 7.9692 |       | 0.07479 . |
| Gamma    | 7.9692 |       | 0.07631 . |
| Bartlett | 6.9319 | 3.0000| 0.07410 . |
| F        | 2.6564 | 3.0000| 2.8167 0.23086 |

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

> stopCluster(cl)

Summary: Allowing a linear time trend along weeks with interaction between time and treatment there is a significant interaction between time and treatment (p-value = 0.007611 from the Kenward-Rogers method, and p-value = 0.008998 based on parametric bootstrap).
This means in particular, that the time trends of the larvae-to-eggs ratio are significantly different between the four treatment groups.

However, there is no significant treatment main effect in the model with interaction (p-value = 0.07313 from the Kenward-Rogers method, and p-value = 0.07479 based on parametric bootstrap). This means, the estimated average larvae-to-eggs ratio at week 2 (!) are not significantly different between the four treatment groups.
Model diagnostics for the model in `linfit3`:

![Diagnostic plots for the fitted linear mixed-effects model with linear time trends.](Cloth-Q4_LinFit_L2EModelAugPred.pdf)

Figure 21: Diagnostic plots for the fitted linear mixed-effects model with linear time trends. Summarizing the findings (without explanation): the fitted model does show some indication against homoscedastic normality of the errors, so the inferential results should be interpreted with care. (File names: Cloth-Q4_LinFit_L2EModelAugPred.pdf and Cloth-Q4_LinFit_L2EModelDiagX.pdf with $X = 1, \ldots, 6$)
2.4.3 Posthoc tests for time trend of larvae-to-eggs ratio (WITHOUT the extreme outlier) – comparisons with the Control

Here, we compare each Clothianidin treatment group to the control group with respect to the time trends in larvae-to-eggs ratios.

```r
> fx <- fixef(linfit3)
> K <- diag(length(fx))[-(1:5),]
> rownames(K) <- names(fx)[-1:5]
> CompWCntrl <- glht(linfit3, linfct = K) # summary(CompWCntrl)
> summary(CompWCntrl, test = adjusted(type = "Westfall"))
```

**Simultaneous Tests for General Linear Hypotheses**

Fit: lmer(formula = L2E ~ I(Week - 2) + (1 | HiveID) + Treatment +
I(Week - 2):Treatment, data = EL, contrasts = list(Treatment = "contr.treatment"))

Linear Hypotheses:

| Estimate | Std. Error | z value | Pr(>|z|) |
|----------|------------|---------|----------|
| I(Week - 2):Treatment1 ug/l == 0 | -0.36948 | 0.11774 | -3.138 | 0.00497 ** |
| I(Week - 2):Treatment10 ug/l == 0 | -0.08292 | 0.13269 | -0.625 | 0.53202 |
| I(Week - 2):Treatment100 ug/l == 0 | -0.29576 | 0.11170 | -2.648 | 0.01557 * |

---

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

(Adjusted p values reported -- Westfall method)

**Summary:** The linear time trends of the larvae-to-eggs ratio in the Clothianidin treatments with 1 µg/l and 100 µg/l are significantly different from the one in the control group.
2.4.4 Treatment effect on larvae-to-eggs ratio (WITH the extreme outlier)

Note: Here, we INclude the extreme outlier above 15 in treatment group 10 μg/l in the following analysis!

```r
> EL <- EL0
> levels(EL$Treatment) <- o1E
> linfit2 <- lmer(L2E ~ I(Week - 2) + (1 | HiveID), data = EL)
> linfit2 <- update(linfit1, ~ . + Treatment,
+ contrasts = list(Treatment = "contr.treatment"))
> print(summary(linfit3), cor = FALSE)

Linear mixed model fit by REML ['lmerMod']
Formula: L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
Data: EL

REML criterion at convergence: 359.1

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.9802 -0.3154  0.0671  0.0992  7.3967

Random effects:
     Groups   Name        Variance Std.Dev.
     HiveID  (Intercept)  0.1571   0.3963
        Residual 2.3641   1.5376
Number of obs: 96, groups: HiveID, 16

Fixed effects:                 Estimate Std. Error   t value
(Intercept)                   1.6683  0.5906     2.824
I(Week - 2)                   -0.0374  0.1838    -0.204
Treatment1 ug/l               0.9412  0.8353     1.127
Treatment10 ug/l              2.4567  0.9022     2.724
Treatment100 ug/l             0.0573  0.7924     0.072
I(Week - 2):Treatment1 ug/l   -0.3695  0.2599    -1.422
I(Week - 2):Treatment10 ug/l  -0.7628  0.2807    -2.717
I(Week - 2):Treatment100 ug/l -0.2957  0.2466    -1.200

> linfit3.L2E <- linfit3

# F-tests with approximated degrees of freedom according to Kenward-Roger:
> #-------------------------------------------------------------------------
> KR1 <- KRmodcomp(largeModel = linfit2, smallModel = linfit1))
> (KR2 <- KRmodcomp(largeModel = linfit3, smallModel = linfit2))

F-test with Kenward-Roger approximation; time: 0.14 sec
large : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment
stat ndf ddf F.scaling p.value
Ftest 2.4909 3.0000 76.0000 1 0.06655.
###
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> (KR3 <- KRmodcomp(largeModel = linfit3, smallM = update(linfit3, ~ . - Treatment)))

F-test with Kenward-Roger approximation; time: 0.14 sec
large : L2E ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : L2E ~ I(Week - 2) + (1 | HiveID) + I(Week - 2):Treatment

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Summary: Allowing a linear time trend along weeks with interaction between time and treatment there is NO significant interaction between time and treatment (p-value = 0.06655 from the Kenward-Rogers method, and p-value = 0.07199 based on parametric bootstrap). This means in particular, that the time trends of the larvae-to-eggs ratio ARE NOT significantly different between the four treatment groups.

However, there IS a significant treatment main effect in the model with interaction (p-value = 0.02906 from the Kenward-Rogers method, and p-value = 0.02619 based on parametric bootstrap). This means, the estimated average larvae-to-eggs ratio at week 2 (!) ARE significantly different between the four treatment groups.
Model diagnostics for the model in `linfit3`:

![Diagnostic plots for the fitted linear mixed-effects model with linear time trends.](image)

Figure 22: Diagnostic plots for the fitted linear mixed-effects model with linear time trends. Summarizing the findings (without explanation): the fitted model does show some indication against homoscedastic normality of the errors, so the inferential results should be interpreted with care. (File names: `Cloth-Q4_LinFit_L2EModelAugPred.pdf` and `Cloth-Q4_LinFit_L2EModelDiagX_.pdf` with $X = 1, \ldots, 6$)
2.4 Question 4: Compensation rate

2.4.5 Posthoc tests for time trend of larvae-to-eggs ratio (WITH the extreme outlier) – comparisons with the Control

Here, we compare each Clothianidin treatment group to the control group with respect to the time trends in larvae-to-eggs ratios.

```r
> fx <- fixef(linfit3)
> K <- diag(length(fx))[-(1:5),]
> rownames(K) <- names(fx)[-1:5]
> CompWCntrl <- glht(linfit3, linfct = K) # summary(CompWCntrl)
> summary(CompWCntrl, test = adjusted(type = "Westfall"))
```

Simultaneous Tests for General Linear Hypotheses

Fit: lmer(formula = L2E ~ I(Week - 2) + (1 | HiveID) + Treatment +
          I(Week - 2):Treatment, data = EL, contrasts = list(Treatment = "contr.treatment"))

Linear Hypotheses:

| Estimate | Std. Error | z value | Pr(>|z|) |
|----------|------------|---------|----------|
| I(Week - 2):Treatment1 ug/l == 0 | -0.3695 | 0.2599 | -1.422 | 0.262 |
| I(Week - 2):Treatment10 ug/l == 0 | -0.7628 | 0.2807 | -2.717 | 0.018 * |
| I(Week - 2):Treatment100 ug/l == 0 | -0.2958 | 0.2466 | -1.200 | 0.262 |

---

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

(Adjusted p values reported -- Westfall method)

Summary: The linear time trend of the larvae-to-eggs ratio in the Clothianidin treatments with 10 µg/l IS significantly different from the one in the control group.
2.4.6 Capped-brood-to-larvae ratio

Here the same is done for the capped-brood-to-larvae ratio as was done in §2.4.1 for the larvae-to-eggs ratio.

Table 3: Per treatment group: Mean and median capped-brood-to-larvae ratios per week (shifted by lag 1)

| Treatment | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 |
|-----------|--------|--------|--------|--------|--------|--------|
| Ctrl      | 2.63   | 2.68   | 2.02   | 1.92   | 2.05   | 2.23   |
| 1 ug/l    | 2.63   | 2.59   | 1.74   | 1.76   | 1.92   | 1.93   |
| 10 ug/l   | 2.30   | 1.83   | 1.67   | 1.58   | 1.64   | 1.65   |
| 100 ug/l  | 2.55   | 2.35   | 1.85   | 1.88   | 1.23   | 1.58   |

Table 4: Per treatment group and across weeks: Average and median of mean capped-brood-to-larvae ratios (shifted by lag 1)

|            | Ctrl  | 1 ug/l | 10 ug/l | 100 ug/l |
|------------|-------|--------|---------|----------|
| Means of means | 2.11  | 1.96   | 1.80    | 1.41     |
| Medians of means | 2.03  | 1.92   | 1.71    | 1.28     |
The following two figures display just the mean and median capped-brood-to-larvae ratio profiles (shifted by lag 1), respectively, i.e., without the raw values.

**Figure 24:** Means (across hives) of “shifted” capped-brood-to-larvae ratios along weeks by treatment. As eggs of week \( w - 1 \) are the larvae of week \( w \) the numbers of larvae are shifted by one week. Note that the mean values in the control group are quite consistently the largest values and that all “low-dose”-groups (incl. Control) stay above 1.5. (File name: Cloth-Q4_MeanB2L_along_Weeks_by_Treat.pdf)

**Figure 25:** Medians (across hives) of “shifted” capped-brood-to-larvae ratios along weeks by treatment. As eggs of week \( w - 1 \) are the larvae of week \( w \) the numbers of larvae are shifted by one week. Note that the median values in the control group are quite consistently the largest values and that all “low-dose”-groups (incl. Control) stay above 1.5. (File name: Cloth-Q4_MedianB2L_along_Weeks_by_Treat.pdf)
2.4.7 Treatment effect on capped-brood-to-larvae ratio

We proceed as before in §2.4.2; for details see there.

```r
> levels(BL1$Treatment) <- olE
> linfit1 <- lmer(B2L ~ I(Week - 2) + (1 | HiveID), data = BL1)

> linfit2 <- update(linfit1, ~ . + Treatment,
+ contrasts = list(Treatment = "contr.treatment")
> linfit3 <- update(linfit2, ~ . + I(Week - 2):Treatment)
> print(summary(linfit3), cor = FALSE)
```

Linear mixed model fit by REML ['lmerMod']
Formula: B2L ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
Data: BL1

REML criterion at convergence: 208.4

Scaled residuals:
   Min 1Q Median 3Q Max
-2.0574 -0.5292 -0.0531 0.4513 3.6949

Random effects:
   Groups   Name           Variance Std.Dev.
              HiveID (Intercept) 0.04714 0.2171
              Residual          0.41624 0.6452
Number of obs: 96, groups: HiveID, 16

Fixed effects:
   Estimate Std. Error t value
(Intercept)  2.42479    0.25747   9.418
I(Week - 2) -0.12455    0.07711  -1.615
Treatment1 ug/l -0.17947    0.36412  -0.493
Treatment10 ug/l -0.43654    0.39330  -1.110
Treatment100 ug/l -0.33678    0.34544  -0.975
I(Week - 2):Treatment1 ug/l  0.01170    0.10905   0.107
I(Week - 2):Treatment10 ug/l  0.05090    0.11779   0.432
I(Week - 2):Treatment100 ug/l -0.14593    0.10346  -1.410

> # F-tests with approximated degrees of freedom according to Kenward-Roger:
> #-------------------------------------------------------------------------
> # (KR1 <- KRmodcomp(largeModel = linfit2, smallModel = linfit1))
> (KR2 <- KRmodcomp(largeModel = linfit3, smallModel = linfit2))
F-test with Kenward-Roger approximation; time: 0.12 sec
large : B2L ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : B2L ~ I(Week - 2) + (1 | HiveID) + Treatment
stat  ndf  ddf  F.scaling  p.value
Ftest    1.3626 3.0000 76.0000   1 0.2607

> (KR3 <- KRmodcomp(largeModel = linfit3, smallM = update(linfit3, ~ . - Treatment)))
F-test with Kenward-Roger approximation; time: 0.14 sec
large : B2L ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : B2L ~ I(Week - 2) + (1 | HiveID) + I(Week - 2):Treatment
stat  ndf  ddf  F.scaling  p.value
Ftest    0.5113 3.0000 49.4476   1 0.6764

> # Parametric bootstrap:
> #-------------------------------------------------------------------------
> cl <- makeCluster(rep("localhost", detectCores()))
> set.seed(201712)  # For reproducibility.
> # Relevant: p-value in row "PBtest"
> # (PB1 <- summary(PBmodcomp(largeM = linfit2, smallM = linfit1, nsim = nsim.PBmodcomp,
> > cl = cl)))
> (PB2 <- summary(PBmodcomp(largeM = linfit3, smallM = linfit2, nsim = nsim.PBmodcomp,
> + cl = cl)))

Bootstrap test; time: 498.60 sec; samples: 5000; extremes: 1279;
large : B2L ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : B2L ~ I(Week - 2) + (1 | HiveID) + Treatment

stat df ddf p.value
LRT 4.3121 3.0000 0.2297
PBtest 4.3121 0.2559
Gamma 4.3121 0.2559
Bartlett 4.0565 3.0000 0.2554
F 1.4374 3.0000 2.9137 0.3896

> (PB3 <- summary(PBmodcomp(largeM = linfit3, smallM = update(linfit3, ~ . - Treatment),
> + nsim = nsim.PBmodcomp, cl = cl)))

Bootstrap test; time: 510.00 sec; samples: 5000; extremes: 3548;
large : B2L ~ I(Week - 2) + (1 | HiveID) + Treatment + I(Week - 2):Treatment
small : B2L ~ I(Week - 2) + (1 | HiveID) + I(Week - 2):Treatment

stat df ddf p.value
LRT 1.5730 3.0000 0.6655
PBtest 1.5730 0.7097
Gamma 1.5730 0.7116
Bartlett 1.3876 3.0000 0.7084
F 0.5243 3.0000 2.833 0.6969

> stopCluster(cl)

Summary: Allowing a linear time trend along weeks with interaction between time and treatment there is
no significant interaction between time and treatment (p-value = 0.2607 from the Kenward-Rogers method,
and p-value = 0.2559 based on parametric bootstrap).
This means in particular, that the time trends of the capped-brood-to-larvae ratio are not significantly dif-
ferent between the four treatment groups.

And there is no significant treatment main effect in the model with interaction (p-value = 0.6764 from the
Kenward-Rogers method, and p-value = 0.7097 based on parametric bootstrap). This means, the estimated
average capped-brood-to-larvae ratios at week 2 (!) are not significantly different between the four treatment
groups.
Model diagnostics for the model in `linfit3`:

![Diagnostic plots](image)

Figure 26: Diagnostic plots for the fitted linear mixed-effects model with linear time trends. Summarizing the findings (without explanation): the fitted model appears to fit and does not show a too serious indication against homoscedastic normality of the errors, so the inferential results are considered reliable. (File names: `Cloth-Q4_LinFit_B2LModelAugPred.pdf` and `Cloth-Q4_LinFit_B2LModelDiagX.pdf` with $X = 1, \ldots, 6$)
2.4.8 Posthoc tests for time trend of capped-brood-to-larvae ratio – comparisons with the Control

Here, we compare each Clothianidin treatment group to the control group with respect to the time trends in capped-brood-to-larvae ratios.

```r
> fx <- fixef(linfit3)
> K <- diag(length(fx))[-(1:5),]
> rownames(K) <- names(fx)[-1:5]
> CompWCntrl <- glht(linfit3, linfct = K) # summary(CompWCntrl)
> summary(CompWCntrl, test = adjusted(type = "Westfall"))
```

```
Simultaneous Tests for General Linear Hypotheses

Fit: lmer(formula = B2L ~ I(Week - 2) + (1 | HiveID) + Treatment +
  I(Week - 2):Treatment, data = BL1, contrasts = list(Treatment = "contr.treatment"))

Linear Hypotheses:

| Estimate | Std. Error | z value | Pr(>|z|) |
|----------|------------|---------|----------|
| I(Week - 2):Treatment1 ug/l == 0 | 0.0117 | 0.1090 | 0.107 | 0.915 |
| I(Week - 2):Treatment10 ug/l == 0 | 0.0509 | 0.1178 | 0.432 | 0.876 |
| I(Week - 2):Treatment100 ug/l == 0 | -0.1459 | 0.1035 | -1.410 | 0.356 |

(Adjusted p values reported -- Westfall method)
```

**Summary:** The Clothianidin treatments do not differ significantly from the control group in their linear time trends of the capped-brood-to-larvae ratio.
3 Software & References

All graphics and statistical calculations or analyses have been created or made with the “open-source” software R version 3.6.3 (2020-02-29) [1], a programming language and environment for statistical computing and graphics, including the packages:

- **lattice** (for graphics), see especially [2] for reference,
- **lme4** (for linear mixed-effects models) with [3] as reference, and for whose underlying mathematical-statistical concepts [4] is a valuable source, and whose most recent details can be found at http://lme4.r-forge.r-project.org,
- **pbkrtest** (for testing fixed effects in mixed-effects models using parametric bootstrap, i.e., a simulation-based method, and the Kenward-Roger-method of adjusted degrees of freedom) [5] with examples (and, e.g., [6] for further examples of applications),
- **parallel** (to support computations by parallel computing) [11],
- **multcomp** (for multiple pairwise comparisons) with [7] as main reference and [8] as even more extensive source, and
- **RColorBrewer** (for colors of particular figures) [10].

This report was generated with \LaTeX{}, where [11] as well as [12] are relevant as references for the inclusion of R-code and its results into this report utilizing the R-function Sweave, and where [13] (for the R-package Hmisc) and [14] are references for creating the (few) \LaTeX{}-tables from within R using also Sweave. This all happened in the “integrated development environment” (IDE) RStudio, Version 1.2.1335 [15]. The complete Rnw-files (R- and \LaTeX{}-Code) of this report can be requested by email from the authors.

References

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(There exists a much newer version of this manual as vignette, which can be “ unearthed” by the R-command vignette("Sweave"), or at https://stat.ethz.ch/R-manual/R-devel/library/utils/doc/Sweave.pdf)
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