Chapter 11: Linear models with a factor explanatory variable having three or more levels

STATS 201/8

University of Auckland

Learning Outcomes

In this chapter you will learn about:

- Explanatory factor with multiple levels, a.k.a., One-way analysis of variance
- Interpretting the fitted model
- Multiple pairwise comparisons
- Using `emmeans` to solve the multiple comparisons problem
- Alternative model parameterization
- Relevant `R`-code.

Section 11.1
Example with a 5-level explanatory factor variable

Example – Fruit flies

In this case study we look at how the male fruit-fly's longevity is related to his reproductive activity.

~~FYI,~~ fruit flies are a ~~very commonly used animal~~ for laboratory experiments because they are easy to maintain and breed. ~~They have a~~ short lifespan ~~and and so~~ several generations ~~can~~ be observed within a few months. They also have a genome that is very close to that of humans with many genes discovered in humans also found in fruit flies.
See `https://www.yourgenome.org/facts/why-use-the-fly-in-research` for more background on research with fruit flies.

Fruit fly

Studies have shown that the longevity (life span) of female fruit flies decreases with an increase in reproduction, and this leads to a similar question related to males.

The experiment compared the lifespan of males that were divided into 5 treatment groups that varied according to the presence or absence and number of uninterested or interested females.[1] We will see that an adjustment is needed to interpret our fitted model in order to determine if there is a significant difference between the group expected lifespans.

How does one define "interest" in female fruit flies? Here is this study's definition:

> Newly inseminated females will not usually mate again for at least two days. So, the males in the uninterested females treatment groups were always living with newly inseminated females.

[1] Had there been only two treatment groups hen we could have used the ~~two sample~~ two-sample t-test discussed in Chapter 5

Fruit fly...

The response variable measured was:

`days` the number of days the male fly lived

The explanatory variables were:

`group` the group they were allocated to with levels:

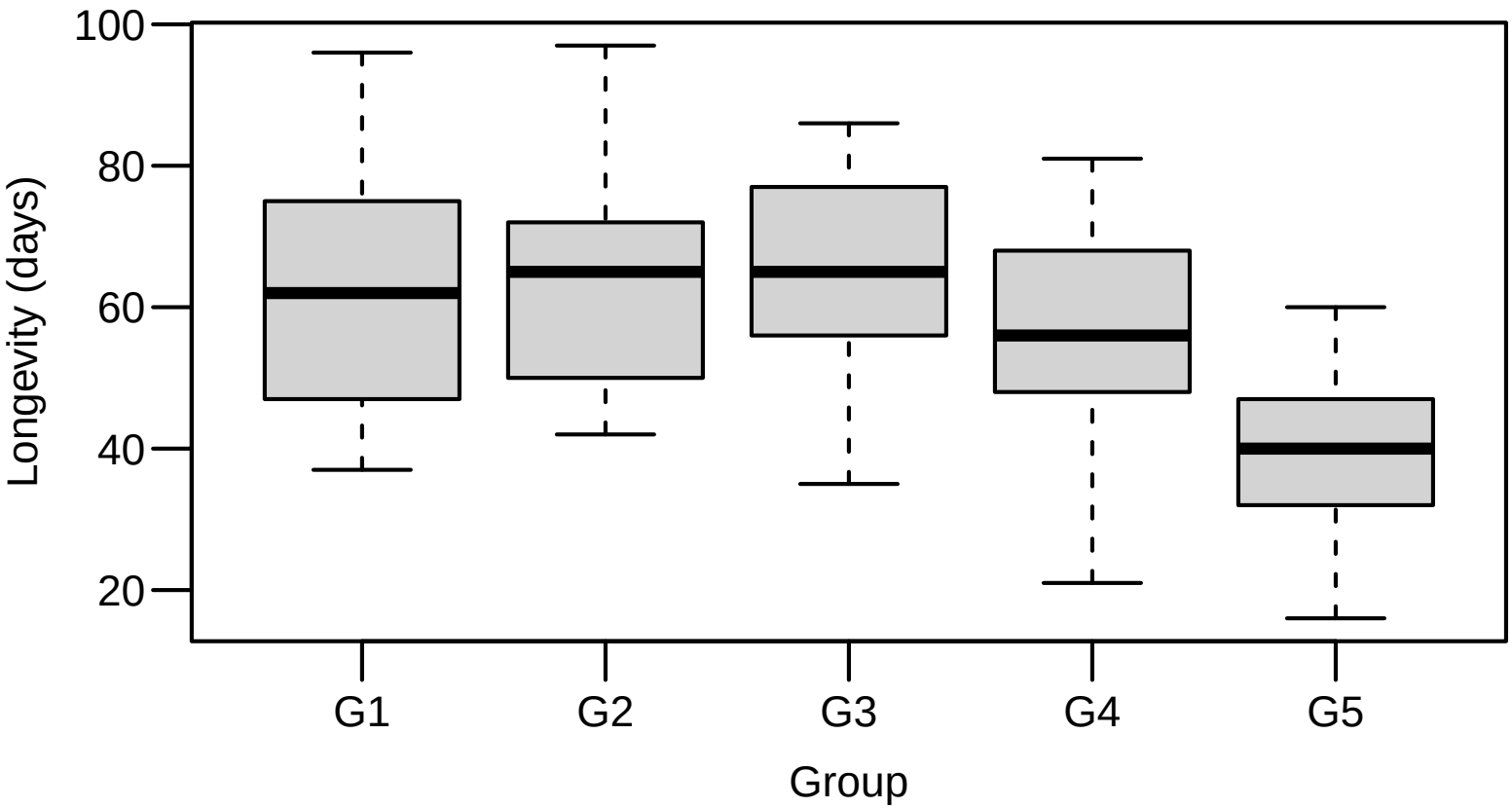
- `G1` males living alone,
- `G2` males living with one interested female,
- `G3` males living with eight interested females,
- `G4` males living with one uninterested female, and
- `G5` males living with eight uninterested females.

There were 25 male flies in each group, for a total sample size of 125.

Fruit fly...

Let us take a look at the data:

```
> Fruitfly.df = read.csv("Data/Fruitfly.csv", header=T)
> Fruitfly.df$group=factor(Fruitfly.df$group)
> boxplot(days ~ group, data = Fruitfly.df)
```



It looks like male fruit flies do not live as long when in the presence of 'uninterested' females (G5), especially when there are several of them.

Fruit fly...

Linear model with multi-level (> 2) explanatory factor

As seen in previous chapters ~~that~~ involved categorical explanatory variables, our model specification uses indicator variables. In this case:

$$\texttt{days} = \beta_0 + \beta_1 \times \texttt{D2} + \beta_2 \times \texttt{D3} + \beta_3 \times \texttt{D4} + \beta_4 \times \texttt{D5} + \epsilon$$

where, as usual $\epsilon \overset{iid}{\sim} N(0, \sigma^2)$, and

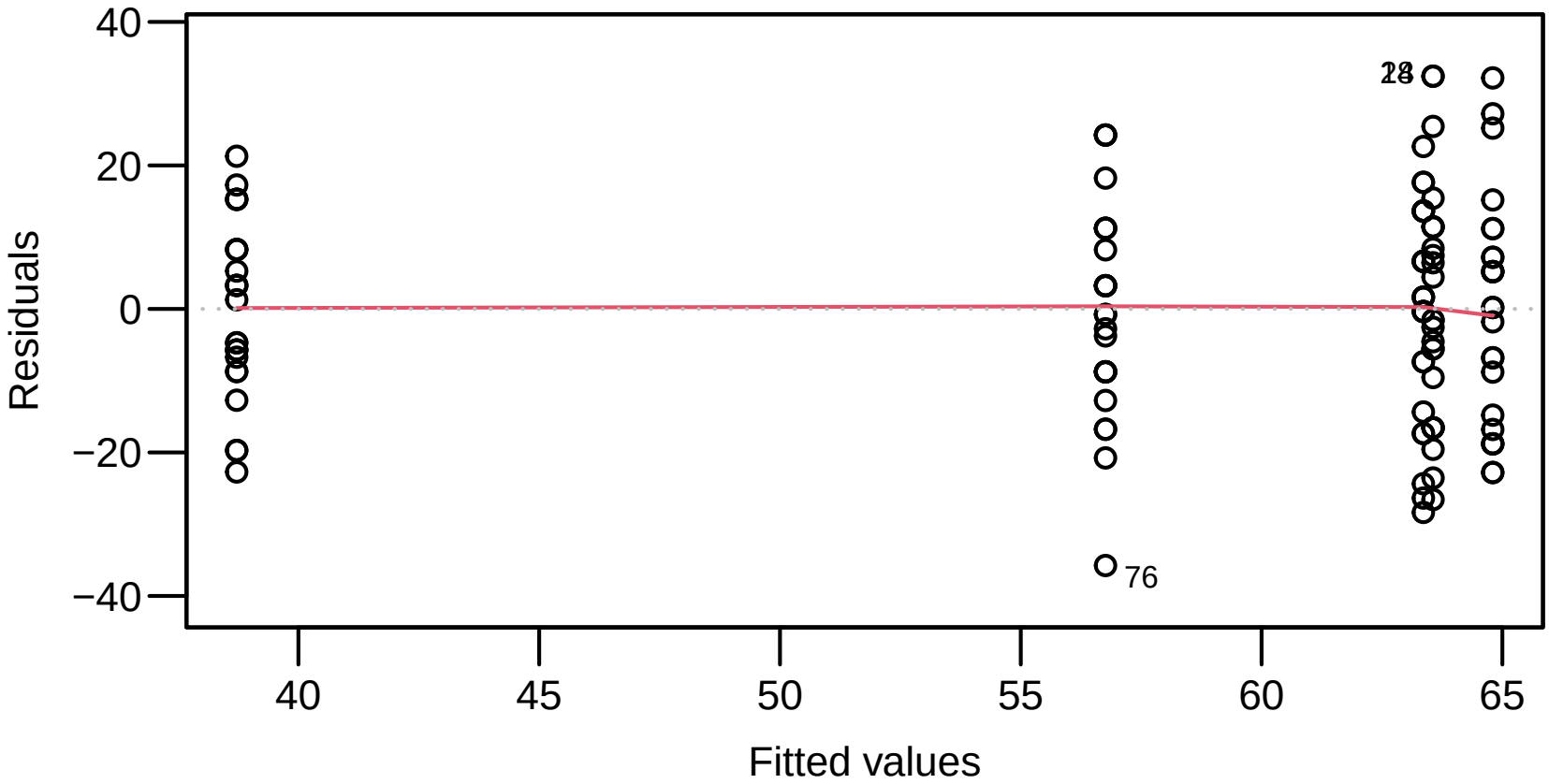
- `D2` is an indicator variable whereby `D2`=1 if the fruit fly is in group 2, otherwise it is 0.
- `D3` is an indicator variable whereby `D3`=1 if the fruit fly is in group 3, otherwise it is 0.
- ... and so on.

For example, β_1 and β_2 represent the differences in expected longevity (`days`) when we compare groups 2 and 3 to group 1 (the baseline).

Fruit fly...

Assumption checks

```
> Fruitfly.fit = lm(days ~ group, data = Fruitfly.df)
> plot(Fruitfly.fit, which=1)
```

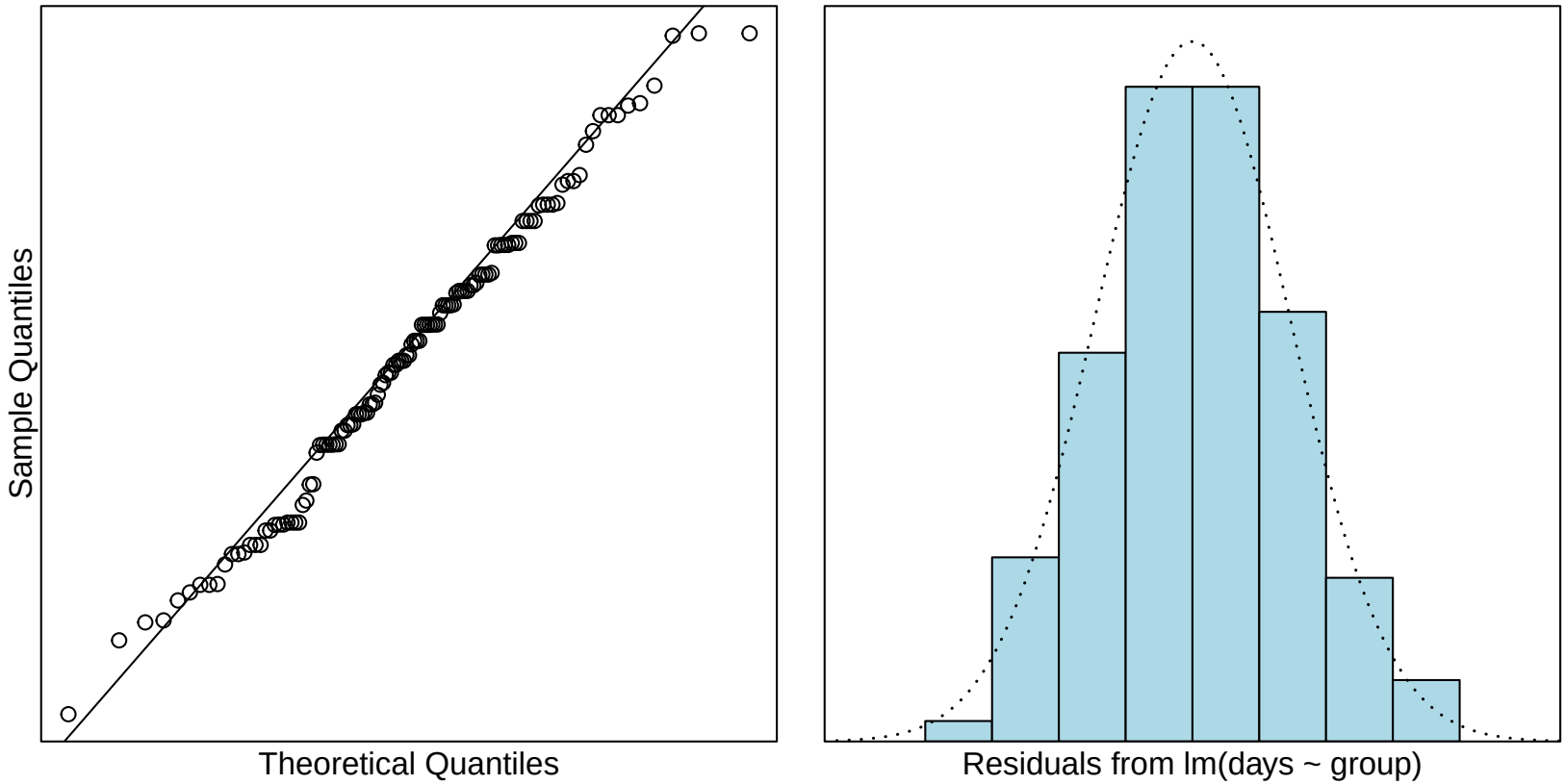


The **EOV** assumption seem to be okay.

Fruit fly...

Assumption checks...

```
> normcheck(Fruitfly.fit)
```

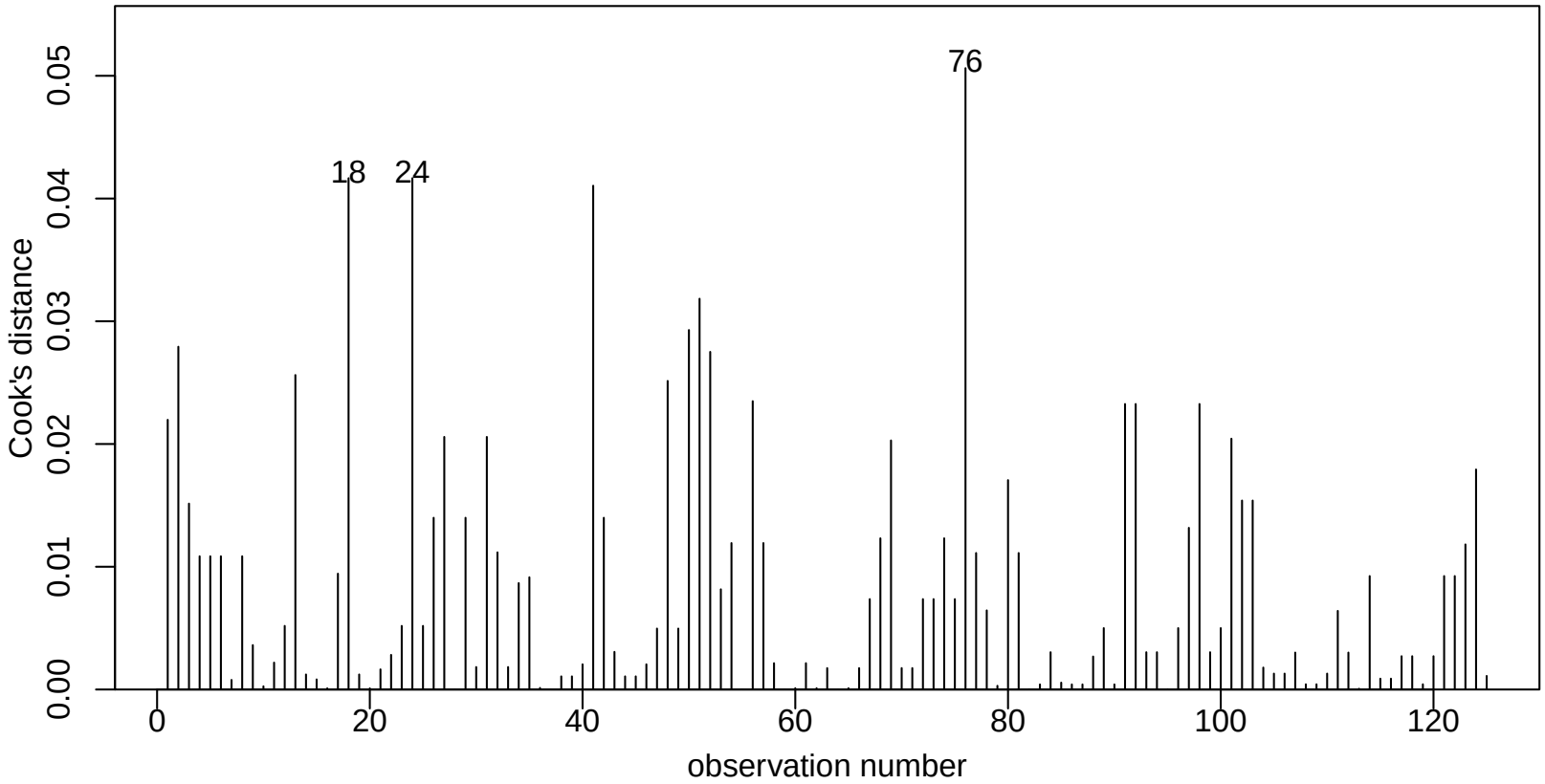


The normality assumption seem to be okay.

Fruit fly...

Assumption checks...

```
> cooks20x(Fruitfly.fit)
```



No unduly influential data points.

Fruit fly...

R^2 and ANOVA table

We can trust the fitted model. What can we conclude?[2]

```
> anova(Fruitfly.fit)
Analysis of Variance Table

Response: days
           Df Sum Sq Mean Sq F value    Pr(>F)
group       4  11939 2984.82  13.612 3.516e-09 ***
Residuals 120  26314  219.28
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

This allows us to say that there is very strong evidence of a difference in expected longevity between the five groups, which was fairly obvious from the boxplot.

A significant result means we should now investigate which groups are different from one another – there is more work to be done.

[2] Recall from Chapter 9 that we have to use the `anova` function to check the significance of a factor variable with more than two levels.

Section 11.2
Interpreting the output

Fruit fly...

The grand mean

Now we know that the variable `group` helps explain longevity, what can we say about these groups? Let us investigate.

Some researchers like to examine the group means and their deviations from the overall (or 'grand') mean.

The estimated grand mean and its confidence interval can be obtained from fitting the iid (i.e., null) model (Chapter 3):

```
> Fruitfly.iidfit = lm(days ~ 1, data = Fruitfly.df)
> coef(summary(Fruitfly.iidfit))
            Estimate Std. Error  t value     Pr(>|t|)
(Intercept)    57.44   1.570962 36.56358 2.877795e-68
> confint(Fruitfly.iidfit)
                2.5 %   97.5 %
(Intercept) 54.33063 60.54937
```

Fruit fly...

The group means

To obtain the individual group means we can refit the one-way ANOVA model, but with the baseline level removed. Recall that the baseline is the intercept term, and this is removed by adding `-1` to the right-hand side of the model formula:

```
> Fruitfly.fit2 = lm(days ~ group-1, data = Fruitfly.df)
> coef(summary(Fruitfly.fit2))
        Estimate Std. Error  t value     Pr(>|t|)
groupG1    63.56   2.961617 21.46125 6.756350e-43
groupG2    64.80   2.961617 21.87994 1.061842e-43
groupG3    63.36   2.961617 21.39372 9.124384e-43
groupG4    56.76   2.961617 19.16521 2.537979e-38
groupG5    38.72   2.961617 13.07394 7.910735e-25
```

The deviations of the group means from the grand mean (rounded to 2 decimal places) are

```
> round( coef(Fruitfly.fit2)-coef(Fruitfly.iidfit),2 )
groupG1 groupG2 groupG3 groupG4 groupG5
   6.12    7.36    5.92   -0.68  -18.72
```

Fruit fly...

Interpretation

We see from above that the overall average longevity of the 125 male flies in the study is about 57.4 days.

We also see that group `G5` has markedly lower longevity (18.72 fewer days) compared to the overall mean.

Note that if `group` does not explain any true underlying variation in longevity, then we expect all these group means to differ at most only moderately from the overall mean. This can be hard to judge informally, since we have to take into account the standard error of each group mean and how many groups there are.

~~That~~ is ~~why we have to~~ rely on the P-value from the `anova` table.

Fruit fly...

Pairwise comparisons

It is natural to ask which of the groups are different.

~~Here is our familiar~~ `summary` output:

```
> summary(Fruitfly.fit)
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   63.560      2.962  21.461  < 2e-16 ***
groupG2        1.240      4.188   0.296    0.768
groupG3       -0.200      4.188  -0.048    0.962
groupG4       -6.800      4.188  -1.624    0.107
groupG5      -24.840      4.188  -5.931 2.98e-08 ***
---
Residual standard error: 14.81 on 120 degrees of freedom
Multiple R-squared:  0.3121,Adjusted R-squared:  0.2892
F-statistic: 13.61 on 4 and 120 DF,  p-value: 3.516e-09
```

Here we see that we have evidence to believe that β_4 (the estimated coefficient for the group 5 indicator variable) is different from from zero.

We estimate that males with 8 uninterested females die, on average, 25 days earlier than males who are by themselves (our baseline group is `G1`).

Fruit fly...

Interpreting the output...

In the output above we are restricted to seeing how each of the groups, G2-G5, differs from the baseline group G1.

If we wish to see how the other groups differed from group G2, for example, then we could achieve this by changing the baseline group to group G2 by reordering the levels of the group factor variable.

This can be done with the R-code below to make G2 the baseline level:

```
> Fruitfly.df$newgroup = relevel(Fruitfly.df$group, ref="G2")
```

But to get all pairwise comparisons (i.e., G3 vs G4, G4 vs G5, ...) we have to do this releveling for G2-G4, and refit the model each time. This is too tedious.

We can get R to do the 'heavy lifting' for us by using the emmeans function from the R package of the same name. Moreover, emmeans solves the multiple comparisons problem discussed below.

Section 11.3
The multiple comparisons problem

Fruit fly...

Multiple comparisons

Note that when we are looking at all pair-wise comparisons of 5 groups, we have a total of 10 different possibilities:

`G1 vs G2`, `G1 vs G3`, `G1 vs G4`, `G1 vs G5`, (4 comparisons)
`G2 vs G3`, `G2 vs G4`, `G2 vs G5`, (3 comparisons)
`G3 vs G4`, `G3 vs G5`, (2 comparisons)
`G4 vs G5`, (1 comparisons).

The number 10 comes from $4+3+2+1=10$ or in fact ${}^5C_2 = 10$ ways of choosing 2 objects from 5 (in no particular order).[3]

Here we are asking 10 questions (comparisons) about our data, as we are looking to test 10 null hypotheses. Of all null hypotheses that are true, 5% are falsely rejected. Equivalently, of all 95% confidence intervals, 5% of them do not contain the true parameter value.

[3] In `R` this is given by `choose(5,2)`.

Erroneous evidence of an effect from multiple testing

The following R code fits a simple linear regression model to iid (independent and identically distributed) normal data.

NOTE: The null hypothesis H_0 : slope $= 0$ is **true**.

```
> x = 1:30 ## Our explanatory variable
> x
 [1]  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
[26] 26 27 28 29 30
> y = rnorm(30) ## y has NO relationship with x
> summary(lm(y~x))$coef ## Print only the coefficient table
               Estimate Std. Error    t value  Pr(>|t|)
(Intercept) -0.256366813 0.39648643 -0.6465967 0.5231583
x            0.003085909 0.02233357  0.1381735 0.8910922
```

If this code is run many times over, then approximately 5% of the time the slope will have P-value < 0.05.[4]

That is, there will be erroneous evidence of an effect of x (i.e., evidence for a non-zero slope) about 1 time in 20!

[4] In fact, it can be shown that the P-value is uniformly distributed between 0 and 1 when H_0 is true.

Erroneous evidence of an effect from multiple testing...

When we do multiple tests (i.e., the 10 paired comparisons in this example) then we greatly increase the probability of obtaining at least one erroneous conclusion[5].

This is known as the multiple comparison problem. It essentially says that if you look at enough things you will find something 'happening', even when there's nothing going on.

Remember, data always have variability, and if we are not careful we can 'discover' false structure that is not really there.

So, when we look at these 10 comparisons we need to adjust so that the overall error rate (the probability of any spurious significance) over all 10 comparison is no more the 5%. This can be done using a Tukey adjustment.

[5] Assuming independent comparisons, if we do 10 95% CIs we have an overall error rate of $1 - (1 - .05)^{10} = 40\%$, which is much higher than our original 5% error rate per comparison.

Example—Fruit fly

Tukey simultaneous confidence intervals

Let's get *simultaneous* 95% confidence intervals[6] for all 10 comparisons via `emmeans`'s `pairs` function.

These confidence intervals are called "simultaneous" since we can be 95% confident that **they all** contain the true group difference simultaneously.

```
> library(emmeans)
> Fruitfly.emm = emmeans(Fruitfly.fit, specs = "group")
> pairs(Fruitfly.emm, infer = TRUE)
 contrast estimate   SE  df lower.CL upper.CL t.ratio p.value
 G1 - G2     -1.24 4.19 120   -12.84     10.4  -0.296  0.9983
 G1 - G3      0.20 4.19 120   -11.40     11.8   0.048  1.0000
 G1 - G4      6.80 4.19 120    -4.80     18.4   1.624  0.4854
 G1 - G5     24.84 4.19 120    13.24     36.4   5.931  <.0001
 G2 - G3      1.44 4.19 120   -10.16     13.0   0.344  0.9970
 G2 - G4      8.04 4.19 120    -3.56     19.6   1.920  0.3127
 G2 - G5     26.08 4.19 120    14.48     37.7   6.227  <.0001
 G3 - G4      6.60 4.19 120    -5.00     18.2   1.576  0.5158
 G3 - G5     24.64 4.19 120    13.04     36.2   5.883  <.0001
 G4 - G5     18.04 4.19 120     6.44     29.6   4.307  0.0003

Confidence level used: 0.95
Conf-level adjustment: tukey method for comparing a family of 5 estimates
P value adjustment: tukey method for comparing a family of 5 estimates
```

[6] By default `infer = c(FALSE, TRUE)` which prints the test statistics but not the CIs.

Fruit fly

Tukey simultaneous confidence intervals...

We see that the majority of these pairwise comparisons are not significantly different. Let's extract only the CIs where the Tukey adjusted *P*-values are less than 0.05.

```
> mc.fruitfly = summary(pairs(Fruitfly.emm, infer = TRUE))
> ## Which entries have a P-value less than 0.05?
> mc.fruitfly[,"p.value"] < 0.05
 [1] FALSE FALSE FALSE  TRUE FALSE FALSE  TRUE FALSE  TRUE  TRUE
> ## Print entries which have a P-value less than 0.05
> signif.fruitfly = mc.fruitfly[mc.fruitfly[, "p.value"] < 0.05, ]
> print(signif.fruitfly, digits = 4)
   contrast estimate    SE  df lower.CL upper.CL t.ratio   p.value
4   G1 - G5    24.84 4.188 120    13.24    36.44   5.931 2.958e-07
7   G2 - G5    26.08 4.188 120    14.48    37.68   6.227 7.232e-08
9   G3 - G5    24.64 4.188 120    13.04    36.24   5.883 3.701e-07
10  G4 - G5    18.04 4.188 120     6.44    29.64   4.307 3.240e-04
```

Fruit fly...

Some conclusions:

- Our model explains 31% of variability in fruit fly longevity.
- We see that the effect of group 5 (males with 8 uninterested females) seems different from all the others.

On average, group 5 males live fewer days than:

- Group 1 (males living alone) by 13 to 36 fewer days.
- Group 2 (males living with one interested female) by 14 to 38 fewer days.
- Group 3 (males living with eight interested females) by 13 to 36 fewer days.
- Group 4 (males living with one uninterested female) by 6 to 30 fewer days.

Fruit fly...

On a lighter note there is little evidence of a difference in longevity if no females or only one uninterested female is about, or if females are there and 'interested' in them — but in the presence of multiple uninterested females they die earlier (they 'drop like flies').

Recall also that in the other studies it was seen that females did not live as long if they reproduced. It appears to be sexual frustration that is killing the males!

For more on this topic see the research article written by Branco et al. (2017, Reproductive activity triggers accelerated male mortality and decreases lifespan: genetic and gene expression determinants in Drosophila. Heredity 118, 221-228 https://doi.org/10.1038/hdy.2016.89) at https://www.nature.com/articles/hdy201689.

Section 11.4
Alternative parameterizations of the 1-way ANOVA model

Alternative parameterizations of the 1-way ANOVA model

The reference cell model

Recall the linear model[7] we used to represent the longevity, in days, of a male fruitfly, i.e.

$$\texttt{days} = \beta_0 + \beta_1 \times \texttt{D2} + \beta_2 \times \texttt{D3} + \beta_3 \times \texttt{D4} + \beta_4 \times \texttt{D5} + \epsilon$$

The *parameters* $\beta_0, \beta_1, \ldots, \beta_4$ denote the true values of some attribute (e.g. longevity) of the population of male fruitflies. Here, β_0 represents the mean longevity of male fruitflies in group `G1`. The parameters $\beta_1, \ldots, \beta_4$ represent the deviations in mean longevity of males in groups `G2`,...,`G5`, respectively, from the mean longevity of males in group `G1`.

The values in the `Estimate` column of the regression summary table[8] result in the following equation for predicted longevity:

$$\widehat{\texttt{days}} = 63.56 + 1.24 \times \texttt{D2} + (-0.20) \times \texttt{D3} + (-6.80) \times \texttt{D4} + (-24.84) \times \texttt{D5}$$

[7] See slide 8.

[8] See slide 17; Coefficients rounded to 2 decimal places.

Alternative parameterizations of the linear model

The reference cell model

Each cell within a column in the table below corresponds to a level of the `Group` factor. One way to 'parametrise' these cells is to use means, i.e. $\mu_1, \mu_2, \ldots, \mu_5$. Another is to select one of the cells as a reference cell (here `Group G1`) and the remaining cells are then parametrised the deviations of the current row's group mean from the reference cell's group mean.

		parameterization			
Group	Data	Means	Estimate[9]	Reference cell	Estimate[10]
G1	$40, 37, \ldots, 44$	μ_1	63.56	$\beta_0 = \mu_1$	63.56
G2	$46, 42, \ldots, 92$	μ_2	64.80	$\beta_1 = \mu_2 - \mu_1$	1.24
G3	$35, 37, \ldots, 77$	μ_3	63.36	$\beta_2 = \mu_3 - \mu_1$	−0.20
G4	$21, 40, \ldots, 68$	μ_4	56.76	$\beta_3 = \mu_4 - \mu_1$	−6.80
G5	$16, 19, \ldots, 44$	μ_5	38.72	$\beta_4 = \mu_5 - \mu_1$	−24.84

The parameterization of the model shown on the previous slide is therefore known as the *reference cell* model.

[9] See estimates of `Group` means on slide 15

[10] See regression coefficients table on slide17

Alternative parameterizations of the linear model

The means model

From the above table we can see that there is an alternative, but equivalent, *means* model parameterization, i.e. linear model for the longevity of the jth ($j = 1, 2, \ldots, 25$) male fruitfly in `Group` i ($i = 1, 2, \ldots, 5$) may be written as

$$days_{ij} = \mu_i + \epsilon_{ij}$$

where μ_i denotes the mean longevity, in days, of a male in `Group` i and, as usual, $\epsilon_{ij} \overset{iid}{\sim} N(0, \sigma^2)$.

Alternative parameterizations of the linear model

The effects model

Another parameterization is to set the overall mean longevity, μ, as the reference and then define the *effect*, τ_i, on longevity due to being in `Group` i as the difference between the `Group` i mean and the overall mean, i.e. $\tau_i = \mu_i - \mu$.

		parameterization			
Group	Data	Means	Estimate	Effects	Estimate[11]
G1	$40, 37, \ldots, 44$	μ_1	63.56	$\tau_1 = \mu_1 - \mu$	6.12
G2	$46, 42, \ldots, 92$	μ_2	64.80	$\tau_2 = \mu_2 - \mu$	7.36
G3	$35, 37, \ldots, 77$	μ_3	63.36	$\tau_3 = \mu_3 - \mu$	5.92
G4	$21, 40, \ldots, 68$	μ_4	56.76	$\tau_4 = \mu_4 - \mu$	-0.68
G5	$16, 19, \ldots, 44$	μ_5	38.72	$\tau_5 = \mu_5 - \mu$	-18.72

The linear *effects* model for the longevity of the jth ($j = 1, 2, \ldots, 25$) male fruitfly in `Group` i ($i = 1, 2, \ldots, 5$) may therefore be written as

$$days_{ij} = \mu + \tau_i + \epsilon_{ij}$$

where, again, $\epsilon_{ij} \overset{iid}{\sim} N(0, \sigma^2)$.

[11] See overall mean (57.44 days) and deviations of group means from overall means on slide 15.

Section 11.5
Closing remarks and relevant R-code

Understanding the anova function output

```
> anova(Fruitfly.fit)
Analysis of Variance Table

Response: days
           Df Sum Sq Mean Sq F value    Pr(>F)
group       4  11939 2984.82  13.612 3.516e-09 ***
Residuals 120  26314  219.28
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

In the above output we see that the variability we observe in our longevity data can be broken down into two components `group` and `residual`.

The amount of variability that the variable `group` (as shown in the `Sum Sq` column) explains is 11939. The residual variability (left over) is 26314. The total variability is $11939 + 26314 = 38253$. The % of variability explained by `group` is therefore

$$100 \times \left(\frac{11939}{11939 + 26314} \right) = 100 \times \left(1 - \frac{26314}{11939 + 26314} \right) = 31\%.$$

Note that we have just calculated the R^2 – the proportion of the variability in the response variable that is explained by the explanatory variables, 0.31.

Most of the R-code you need for this chapter

Use box plots to inspect the data for each level of the factor.

```
> ## Create the pairs plot of the five numeric variables
> boxplot(days ~ group, data = Fruitfly.df)
```

You do not need to create indicator variables - R does that for you. The baseline can be changed if you wish by using the relevel function.

```
> Fruitfly.df$newgroup = relevel(Fruitfly.df$group,ref="G2")
```

Fit the model and use the ANOVA table to see if any of the means differ from one another (regardless of the baseline chosen).

```
> anova(Fruitfly.fit)
```

Adjust confidence intervals for multiple pairwise comparisons by using the Tukey adjustment to obtain simultaneous intervals CIs:

```
> Fruitfly.emm = emmeans(Fruitfly.fit, specs = "group")
> pairs(Fruitfly.emm, infer = TRUE)
```