MONASH University

ETC5521: Exploratory Data Analysis

Making comparisons between groups and strata

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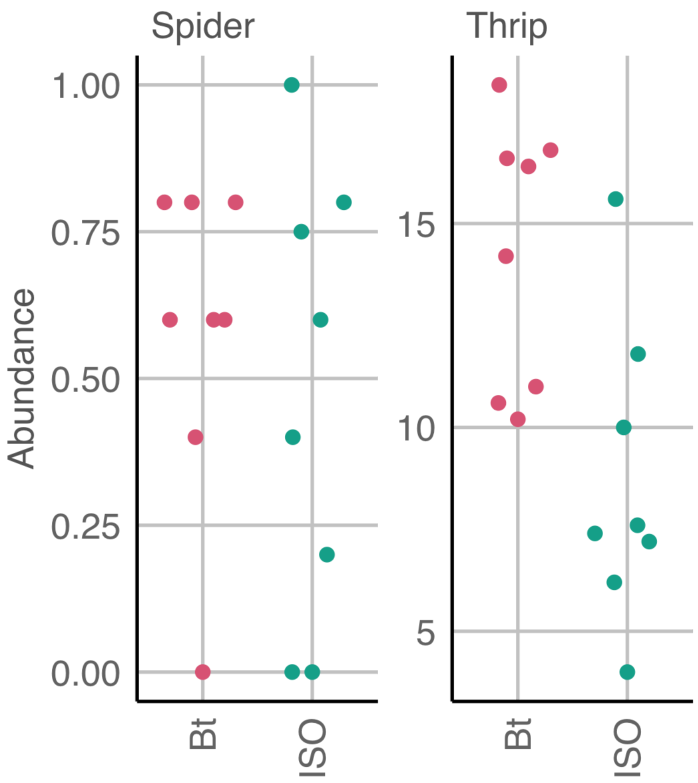
📅 Week 6 - Session 2

Modelling and testing for comparisons

Revisiting Case study 1 Pest resistance maize

📊 data R



- The experiment compared abundance of spiders and thrips on *Bt* variety to the abundance of those on isogenic control variety.
- Would you say that the abundance of spiders and/or thrips are comparable between *Bt* variety and isogenic variety?

Two-sample parametric tests: t-test

- Assumes the two samples are independent and from the $N(\mu_x, \sigma_x^2)$ and $N(\mu_y, \sigma_y^2)$ respectively.

$$H_0 : \mu_x - \mu_y = 0 \text{ vs. } H_1 : \mu_x - \mu_y \neq 0$$

$$T^* = \frac{\bar{X} - \bar{Y}}{SE(\bar{X} - \bar{Y})}.$$

- Assuming $\sigma_x^2 = \sigma_y^2$ then $T^* \sim t_{n_x+n_y-2}$.
- A $100(1-\alpha)\%$ confidence interval for $\mu_x - \mu_y$ is given as (L, U) such that:

$$P(L < \mu_x - \mu_y < U) = 1 - \frac{\alpha}{2}.$$

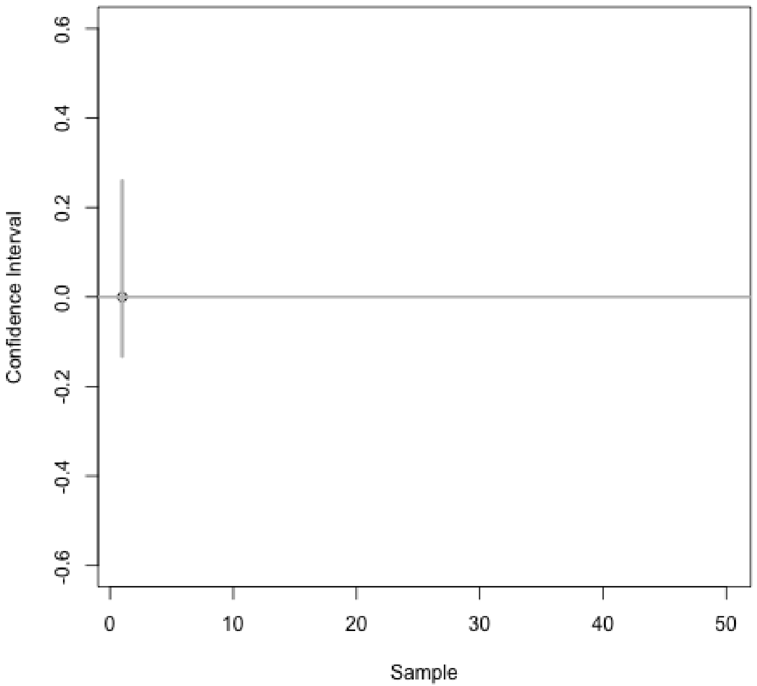
- If $0 \in (L, U)$ consistent with H_0

```
with(gathmann.bt,
     t.test(thysan[gen=="ISO"],
            thysan[gen=="Bt"],
            alternative = "two.sided",
            var.equal = TRUE,
            conf.level = 0.95))
```

```
##
##      Two Sample t-test
##
## data:  thysan[gen == "ISO"] and thysan[gen ==
## t = -3.2182, df = 14, p-value = 0.006192
## alternative hypothesis: true difference in me
## 95 percent confidence interval:
##  -9.248813 -1.851187
## sample estimates:
## mean of x mean of y
##     8.725    14.275
```

Note significance test suggested is different in Achim Gathmann et al. (2006) “Impact of Bt Maize Pollen (MON810) on Lepidopteran Larvae Living on Accompanying Weeds.” Molecular Ecology 15: 2677–85.

Confidence interval for two sample difference



- In the right, a 95% confidence interval for population mean difference is constructed repeatedly, assuming population mean difference is Normally distributed, from 100 samples of the same population.
- The population mean is zero.
- Each confidence interval is calculated as

$$\bar{X} - \bar{Y} \pm t_{n-2,0.975} \times SE(\bar{X}$$

where $t_{n-2,0.975}$ is t^* such that

$$P(t_{n-2} < t^*) = 0.975.$$

Two sample non-parametric tests

Wilcoxon rank-sum test

- Suppose that X and Y are randomly selected values from two populations.

$$H_0 : P(X > Y) = P(X < Y)$$

vs.

$$H_1 : P(X > Y) \neq P(X < Y)$$

- All observations are ranked.
- Test statistic is based on the sum of the ranks of one group.

```
with(gathmann.bt,
     wilcox.test(thysan[gen=="ISO"],
                 thysan[gen=="Bt"],
                 alternative = "two.sided",
                 conf.int = TRUE,
                 conf.level = 0.95))
```

```
##
##     Wilcoxon rank sum exact test
##
## data:  thysan[gen == "ISO"] and thysan[gen ==
## W = 7, p-value = 0.006993
## alternative hypothesis: true location shift i
## 95 percent confidence interval:
##  -9.4 -2.4
## sample estimates:
## difference in location
##                   -6.3
```

Equivalence of tests to testing model parameters

```
##     gen thysan aranei
## 1    Bt   16.6   0.80
## 2    Bt   16.4   0.80
## 3    Bt   11.0   0.60
## 4    Bt   16.8   0.40
## 5    Bt   10.6   0.60
## 6    Bt   18.4   0.80
## 7    Bt   14.2   0.00
## 8    Bt   10.2   0.60
## 9   ISO    6.2   0.75
## 10  ISO   10.0   0.20
## 11  ISO   11.8   1.00
## 12  ISO   15.6   0.80
## 13  ISO    7.6   0.00
## 14  ISO    7.4   0.00
## 15  ISO    7.2   0.60
## 16  ISO    4.0   0.40
```

$$\mathtt{thysan}_i = \beta_0 + \beta_1 \mathbb{I}(\mathtt{gen}_i = \mathtt{ISO}) +$$

where $e_i \sim NID(0, \sigma^2)$

- The least squares estimate for $\hat{\beta}_1 = \bar{X} - \bar{Y}$.

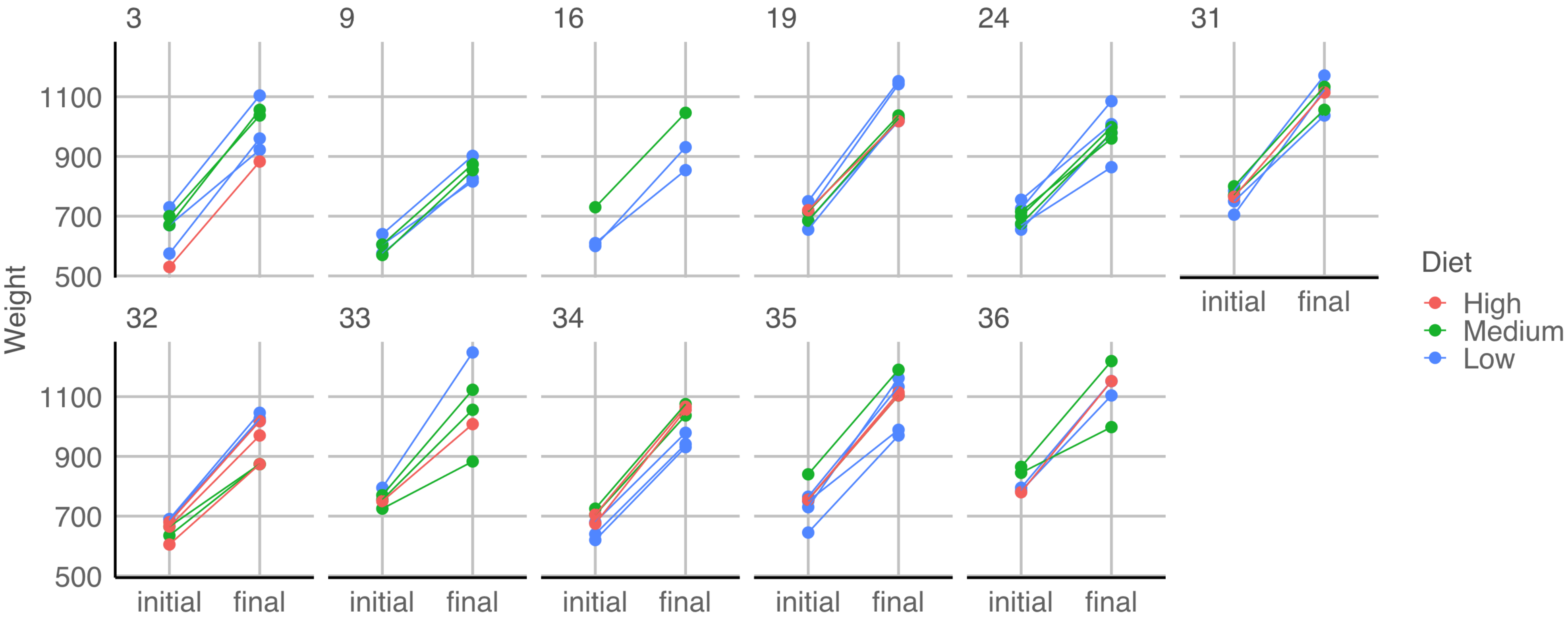
```
lm(thysan ~ gen, data = gathmann.bt) %>%
  confint("genISO", level = 0.95)

##                2.5 %    97.5 %
## genISO -9.248813 -1.851187
```

- Notice that the above confidence interval is the same confidence interval from the $t$test!

Revisiting Case study 4 Weight gain of calves Part 1/3

- 67 calves born in 1975 across 11 herds are fed of one of three diets with low, medium or high energy with their initial and final weights recorded.

📊 data R



Revisiting Case study 4 Weight gain of calves Part 2/3

- Modelling the response as weight gain with diet factor:

```
coef(lm((weight2 - weight1) ~ diet, data = u

## (Intercept)     dietLow  dietMedium
##  332.666667   -4.666667  -33.971014
```

- The herd is thought to be an important factor contributing to the response.
- Modelling the response as weight gain with diet and herd factor:

```
# herd needs to be factor not integer
dat4 <- mutate(urquhart.feedlot,  herdf = fa
coef(lm((weight2 - weight1) ~ herdf + diet,

## (Intercept)      herdf9     herdf16      h
##  354.257353  -91.148529  -51.312039     7.
```

- Last model is the same as modelling the final weight with the initial weight as a covariate with slope fixed to 1:

```
coef(lm(weight2 ~ offset(weight1) + herdf +
    data = dat4))

## (Intercept)      herdf9     herdf16
##  354.257353  -91.148529  -51.312039    7
```

- Estimating slope for initial weight from the data:

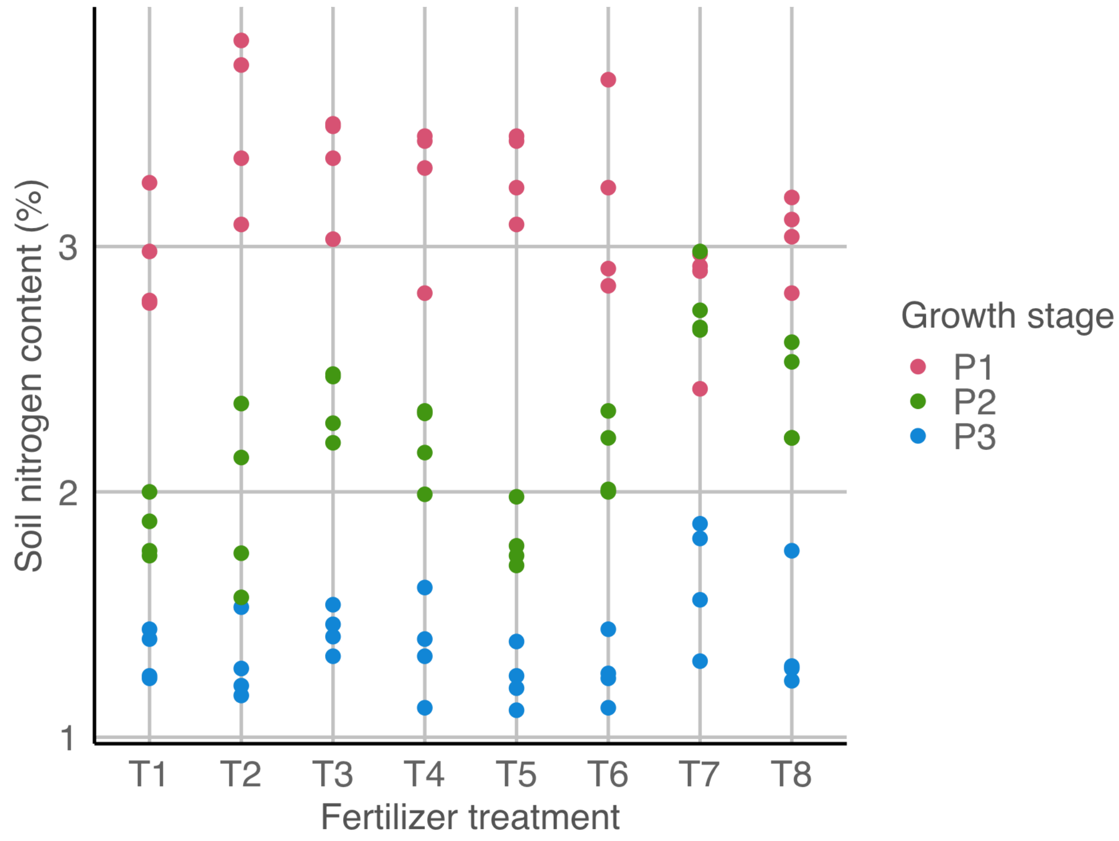
```
coef(lm(weight2 ~ weight1 + herdf + diet,
    data = dat4))

## (Intercept)     weight1      herdf9
##  200.440174    1.243238  -79.102111  -51
```

Revisiting Case study 4 Weight gain of calves Part 3/3

```
dat4 <- lm(weight2 ~ weight1 + herdf + diet, data = dat4) %>% broom::augment()
ggplot(dat4, aes(.fitted, .resid)) +
  geom_point(data = select(dat4, -herdf), size = 2, color = "gray") +
  geom_point(size = 2, aes(color = herdf)) +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "Residual") +
  scale_color_discrete_qualitative() +
  facet_wrap(~herdf, nrow = 2) + guides(color = FALSE)
```

Case study 10 Soil nitrogen Part 1/3

📊 data R



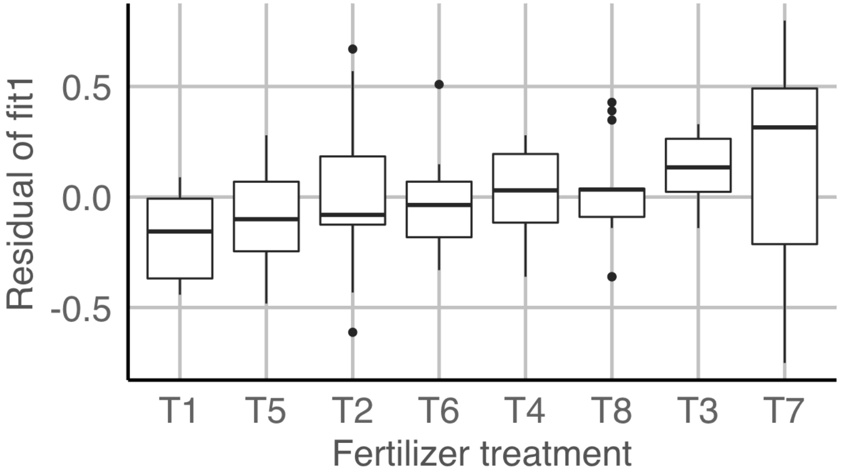
- Soil nitrogen content with 8 different fertilizer treatment is measured at 3 growth stage:
  - P1 = 15 days post transplanting
  - P2 = 40 days post transplanting
  - P3 = panicle initiation
- Clearly the growth stage affects the soil nitrogen content but this makes it hard to compare the fertilizer treatments.
- Let's model the nitrogen content as:

```
lm(nitro ~ stage + trt, data = gomez.nit
```

Case study 10 Soil nitrogen Part 2/3

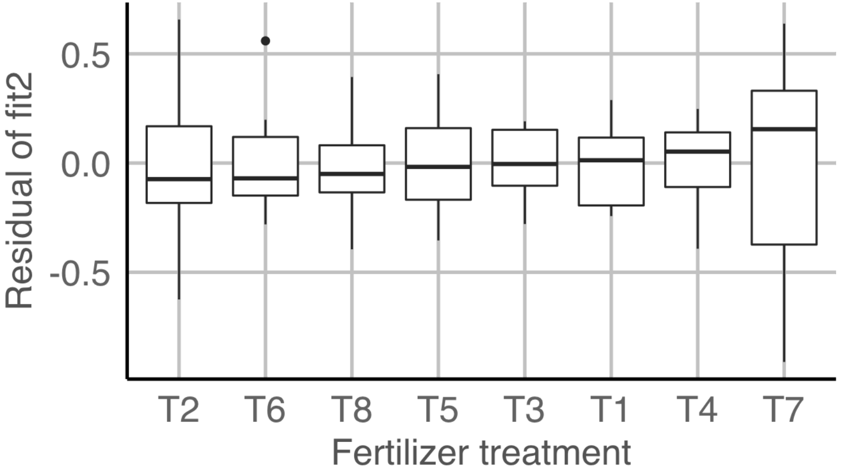
- Considering just the stage effect:

```
fit1 <- lm(nitro ~ stage, data = gomez.nitrogen)
fit1data <- broom::augment(fit1) %>%
left_join(gomez.nitrogen, by=c("nitro", "stage")) %>%
mutate(trt = fct_reorder(trt, .resid))
ggplot(fit1data, aes(trt, .resid)) +
geom_boxplot() +
labs(x = "Fertilizer treatment",
   y = "Residual of fit1")
```



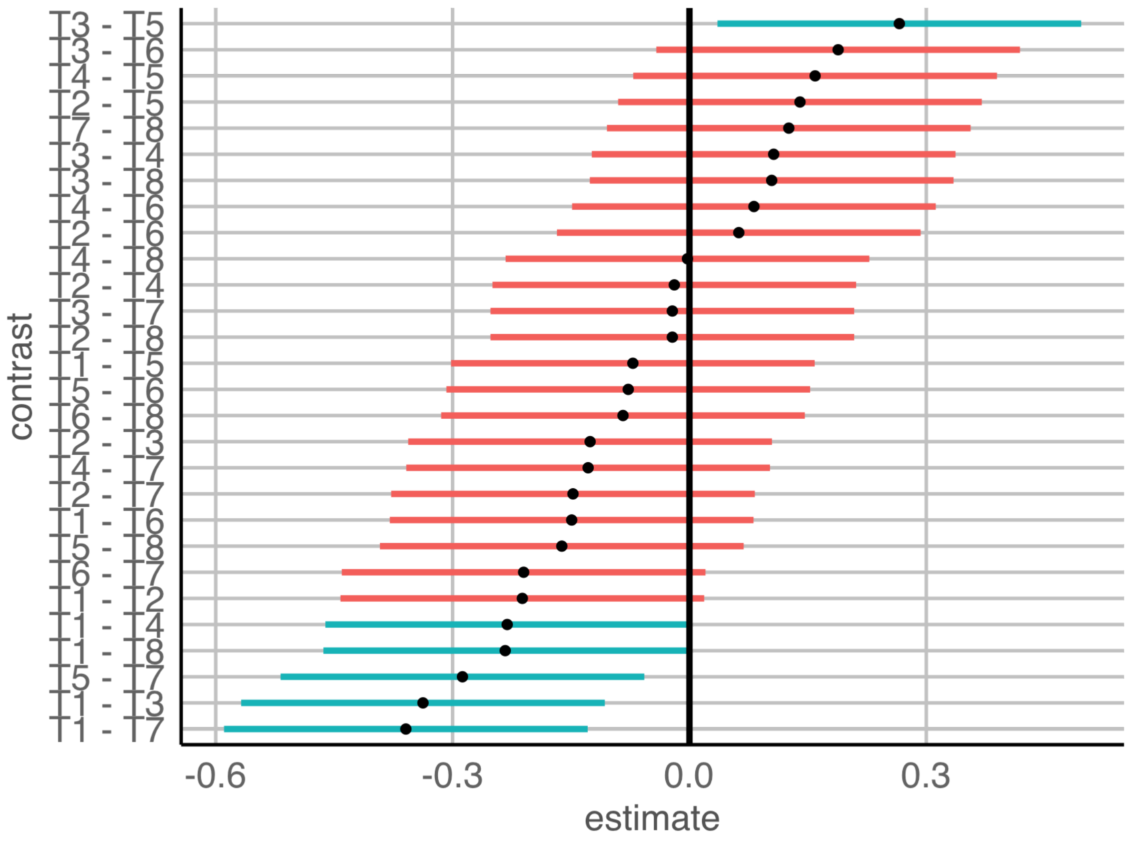
- Here we expect no pattern:

```
fit2 <- lm(nitro ~ stage + trt,
           data = gomez.nitrogen)
fit2data <- broom::augment(fit2) %>%
  mutate(trt = fct_reorder(trt, .resid))
ggplot(fit2data, aes(trt, .resid)) +
  geom_boxplot() +
  labs(x = "Fertilizer treatment",
       y = "Residual of fit2")
```



Case study 10 Soil nitrogen Part 3/3

```
library(emmeans)
confint(pairs(emmeans(fit2, "trt"), adjust="none"))

##  contrast estimate    SE df lower.CL  upper.CL
##  T1 - T2   -0.2117 0.116 86  -0.4420  0.018654
##  T1 - T3   -0.3375 0.116 86  -0.5678 -0.107180
##  T1 - T4   -0.2308 0.116 86  -0.4612 -0.000513
##  T1 - T5   -0.0717 0.116 86  -0.3020  0.158654
##  T1 - T6   -0.1492 0.116 86  -0.3795  0.081154
##  T1 - T7   -0.3592 0.116 86  -0.5895 -0.128846
##  T1 - T8   -0.2333 0.116 86  -0.4637 -0.003013
##  T2 - T3   -0.1258 0.116 86  -0.3562  0.104487
##  T2 - T4   -0.0192 0.116 86  -0.2495  0.211154
##  T2 - T5    0.1400 0.116 86  -0.0903  0.370320
##  T2 - T6    0.0625 0.116 86  -0.1678  0.292820
##  T2 - T7   -0.1475 0.116 86  -0.3778  0.082820
##  T2 - T8   -0.0217 0.116 86  -0.2520  0.208654
##  T3 - T4    0.1067 0.116 86  -0.1237  0.336987
##  T3 - T5    0.2658 0.116 86   0.0355  0.496154
##  T3 - T6    0.1883 0.116 86  -0.0420  0.418654
##  T3 - T7   -0.0217 0.116 86  -0.2520  0.208654
##  T3 - T8    0.1042 0.116 86  -0.1262  0.334487
##  T4 - T5    0.1592 0.116 86  -0.0712  0.389487
##  T4 - T6    0.0817 0.116 86  -0.1487  0.311987
##  T4 - T7   -0.1283 0.116 86  -0.3587  0.101987
##  T4 - T8   -0.0025 0.116 86  -0.2328  0.227820
```



- From above, the 6 pairs of treatments: T3 & T5, T1 & T4, T1 & T8, T6 & T7, T1 & T3, T1 & T7 are significantly different.
- These confidence intervals are constructed *without taking any regard for others*.

Controlling the family-wise error rate

Unadjusted

- Each interval has been constructed using a procedure so that when the model is correct, the probability that the "correct" population contrast is covered is 0.95. . . individually.

$$\bar{X} - \bar{Y} \pm t_{n-t,1-\alpha/2} \times SE(\bar{X} - \bar{Y})$$

where $\alpha = 0.05$ and t is the number of treatments.

- But, what is the probability that all intervals cover their corresponding true values simultaneously?

Bonferonni adjustment

- We can adjust the individual $100(1-\alpha)\%$ confidence intervals so

$$\bar{X} - \bar{Y} \pm t_{n-t,1-\alpha/(2m)} \times SE($$

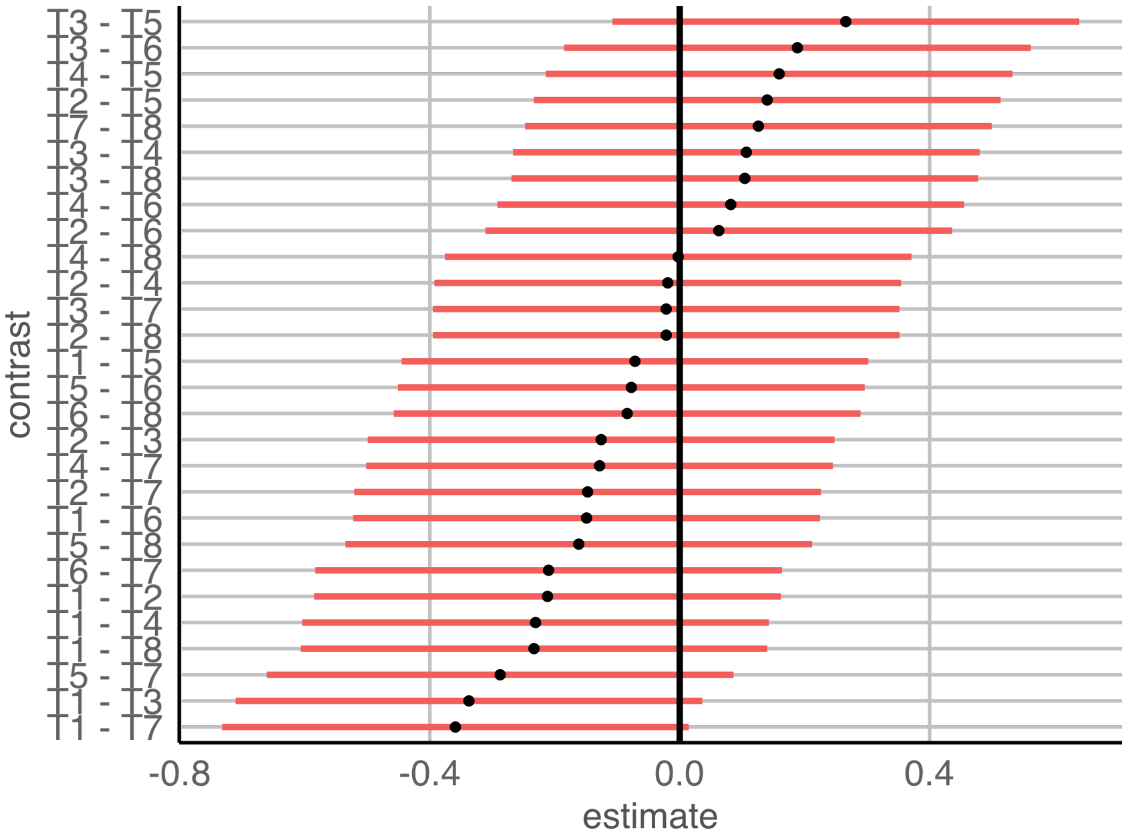
where m is the number of pairwise comparisons.

- So for 8 treatments, the number of pairwise comparisons is

```
choose(8, 2)

## [1] 28
```

Bonferonni adjusted confidence interval

```
confint(pairs(emmeans(fit2, "trt"),
              adjust="bonferroni"))

##  contrast estimate    SE df lower.CL upper.CL
##  T1 - T2   -0.2117 0.116 86   -0.585   0.1619
##  T1 - T3   -0.3375 0.116 86   -0.711   0.0361
##  T1 - T4   -0.2308 0.116 86   -0.604   0.1427
##  T1 - T5   -0.0717 0.116 86   -0.445   0.3019
##  T1 - T6   -0.1492 0.116 86   -0.523   0.2244
##  T1 - T7   -0.3592 0.116 86   -0.733   0.0144
##  T1 - T8   -0.2333 0.116 86   -0.607   0.1402
##  T2 - T3   -0.1258 0.116 86   -0.499   0.2477
##  T2 - T4   -0.0192 0.116 86   -0.393   0.3544
##  T2 - T5    0.1400 0.116 86   -0.234   0.5136
##  T2 - T6    0.0625 0.116 86   -0.311   0.4361
##  T2 - T7   -0.1475 0.116 86   -0.521   0.2261
##  T2 - T8   -0.0217 0.116 86   -0.395   0.3519
##  T3 - T4    0.1067 0.116 86   -0.267   0.4802
##  T3 - T5    0.2658 0.116 86   -0.108   0.6394
##  T3 - T6    0.1883 0.116 86   -0.185   0.5619
##  T3 - T7   -0.0217 0.116 86   -0.395   0.3519
##  T3 - T8    0.1042 0.116 86   -0.269   0.4777
##  T4 - T5    0.1592 0.116 86   -0.214   0.5327
##  T4 - T6    0.0817 0.116 86   -0.292   0.4552
##  T4 - T7   -0.1283 0.116 86   -0.502   0.2452
##  T4 - T8   -0.0025 0.116 86   -0.376   0.3711
```

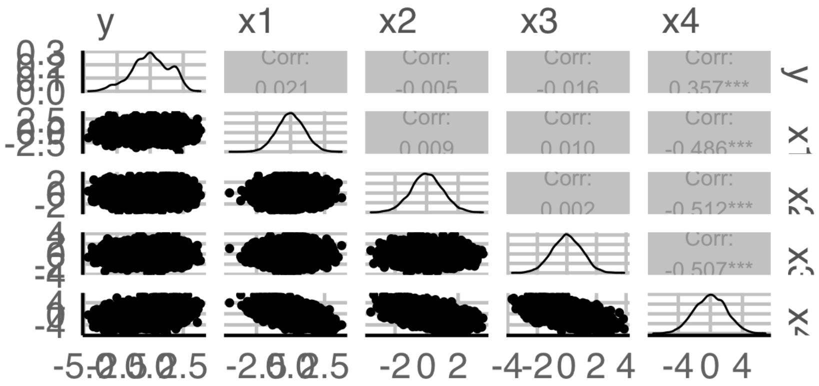


- Now none are significantly different.
- Note: Bonferroni adjustment is quite conservative.

Example 1 Mystery data Part 1/2

- Many inferences, e.g. using confidence intervals or p-values, are based on assumptions being met.
- From the model fit below can we suggest the following model?

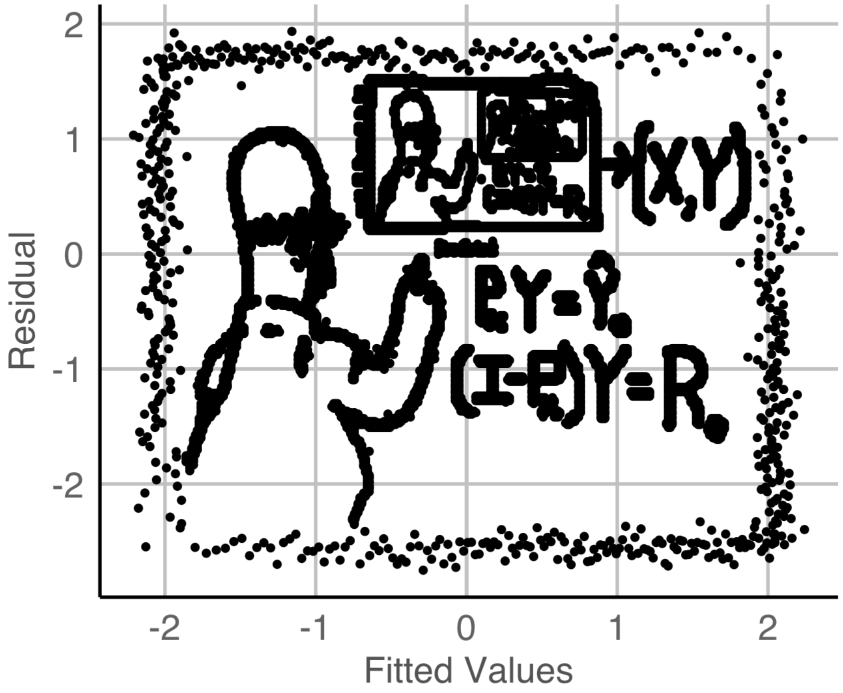
$$\hat{Y} = -0.002 + 0.979x_1 + 0.998x_2 + 0.973x_3 + 0.99$$



```
lm(y ~ x1 + x2 + x3 + x4, data=mystery_data) %>% broom::ti


## # A tibble: 5 × 5
##   term        estimate std.error statistic p.value
##   <chr>          <dbl>     <dbl>     <dbl>   <dbl>
## 1 (Intercept) -0.00204    0.0109    -0.187   0.852
## 2 x1           0.979      0.0151    64.8     0
## 3 x2           0.998      0.0155    64.4     0
## 4 x3           0.973      0.0154    63.1     0
## 5 x4           0.995      0.0109    91.6     0
```

Example 1 Mystery data Part 2/2

```
lm(y ~ x1 + x2 + x3 + x4, data=mystery_
  broom::augment() %>%
  ggplot(aes(.fitted, .resid)) +
  geom_point() +
  labs(x="Fitted Values", y="Residual")
```



Moral of the story:

Don't forget to check model diagnostics.

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