

ETC5521: Exploratory Data Analysis

Making comparisons between groups and strata

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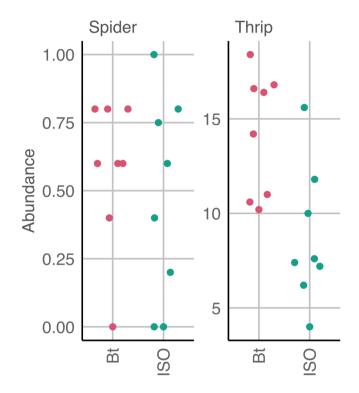
Week 7 - Session 2



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 (thrips)

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

$$\begin{split} H_0 &: \mu_x - \mu_y = 0 \quad \text{vs.} \quad H_1 \,: \, \mu_x - \mu_y \neq 0 \\ T^* &= \frac{\bar{X} - \bar{Y}}{SE(\bar{X} - \bar{Y})} \,. \end{split}$$

- Assuming
$$\sigma_x^2$$
 = σ_y^2 , then $T^* \thicksim t_{n_x+n_y-2}$

• A $100(1 - \alpha)\%$ confidence interval for $\mu_x - \mu_y$ is given as (L, U) such that:

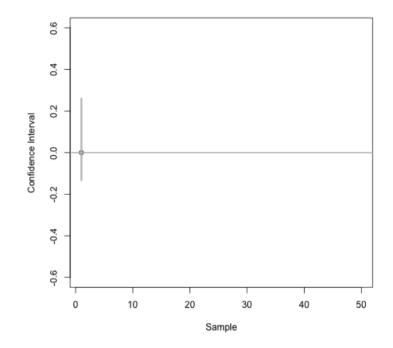
$$P(L < \mu_x - \mu_y < U) = 1 - \frac{\alpha}{2}.$$

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

```
thrip
         spider
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 == "Bt"]
## t = -3.2182, df = 14, p-value = 0.006192
## alternative hypothesis: true difference in means is not \epsilon
## 95 percent confidence interval:
   -9.248813 -1.851187
##
## sample estimates:
## mean of x mean of v
##
       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 4/16

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

 $\bar{\mathbf{X}} - \bar{\mathbf{Y}} \pm \mathbf{t}_{n-2,0.975} \times \mathrm{SE}(\bar{\mathbf{X}} - \bar{\mathbf{Y}})$

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 \boldsymbol{X} and \boldsymbol{Y} are randomly selected values from two populations.

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

vs. H₁ : P(X > Y) \neq P(X < Y)

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

```
thrip
         spider
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 == "Bt"]
## W = 7, p-value = 0.006993
## alternative hypothesis: true location shift is not equal
## 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	IS0	6.2	0.75	
##	10	IS0	10.0	0.20	
##	11	IS0	11.8	1.00	
##	12	IS0	15.6	0.80	
##	13	IS0	7.6	0.00	
##	14	IS0	7.4	0.00	
##	15	IS0	7.2	0.60	
##	16	IS0	4.0	0.40	

$$\begin{split} & \text{thysan}_i = \beta_0 + \beta_1 \mathbb{I}(\text{gen}_i = \text{ISO}) + e_i \\ \text{where } e_i &\sim \text{NID}(0, \sigma^2). \\ \text{one is the squares estimate for } \widehat{\beta}_1 = \bar{X} - \bar{Y}. \\ & \text{Im}(\text{thysan} \sim \text{gen, data} = \text{gathmann.bt}) \ \% > \% \\ & \text{confint}(\text{"genISO", level} = 0.95) \\ & \# \\ & \# \\ & \text{genISO} - 9.248813 - 1.851187 \end{split}$$

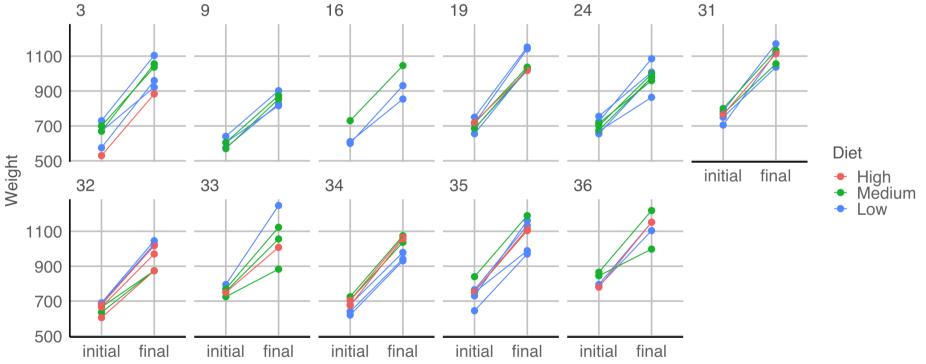
 Notice that the above confidence interval is the same confidence interval from the t-test!

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.
 - 16 9 3 1100

data

R



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

• Modelling the response as weight gain with diet factor:

- 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 = fac
coef(lm((weight2 - weight1) ~ herdf + diet,
## (Intercept) herdf9 herdf16 h
## 354.257353 -91.148529 -51.312039 7.4</pre>
```

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

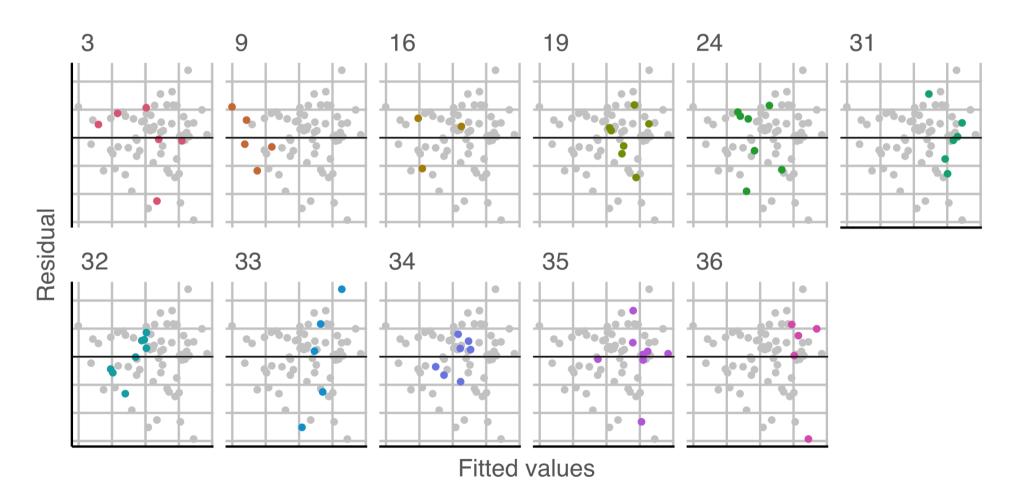
```
coef(lm(weight2 ~ offset(weight1) + herdf +
data = dat4
))
## (Intercept) herdf9 herdf16
## 354.257353 -91.148529 -51.312039 7
```

• 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 Weight gain of calves Part 3/3

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Case study 10 Soil nitrogen Part 1/3

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Soil nitrogen content (%) Growth stage P1 P2 P3 2 T5 T1 T2 T3 T4 **T6** Τ7 **T**8 Fertilizer treatment

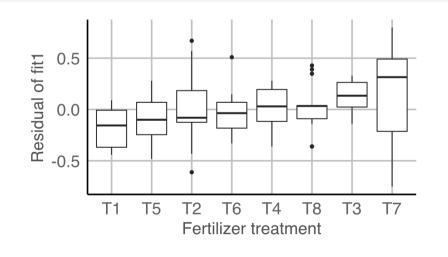
- Soil nitrogen content with 8 different fertilizer treatment is measured at 3 growth stage:
 - P1 = 15 days post transplanting
 - P2 = 40 days post transplanting
 - P3 = panicle initiation
- Clearly the growth stage affects the soil nitrogen content but this makes it hard to compare the fertilizer treatments.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

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



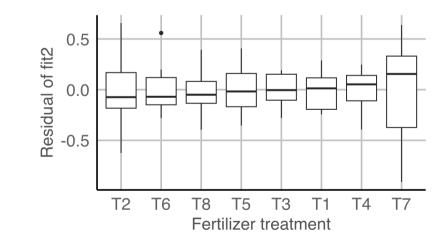
• Considering just the stage effect:

```
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"
```



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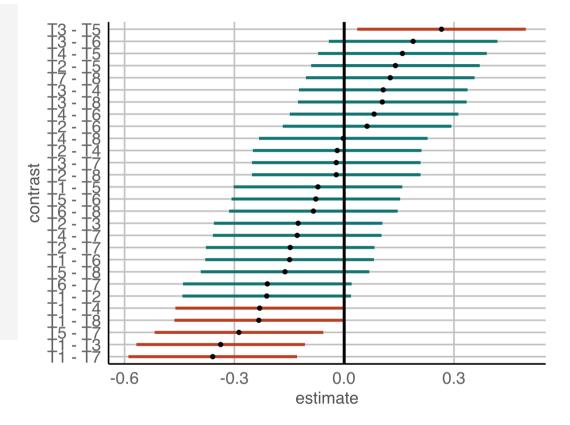


library(emmeans)

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

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- 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. 13/

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?

Bonferroni adjustment

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

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

where m is the number of pairwise comparisons.

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

```
choose(8, 2)
## [1] 28
```

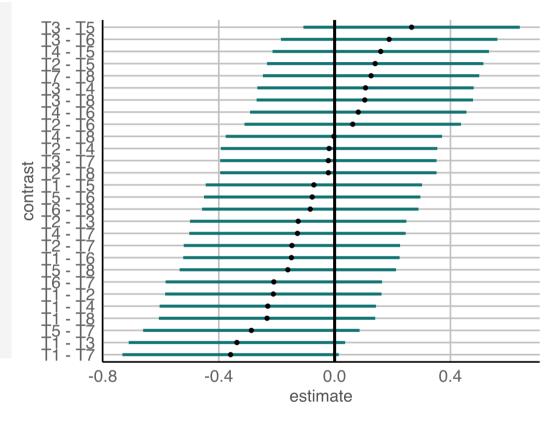
Bonferroni adjusted confidence interval

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



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Heek 7 - Session 2

