Analysis of Variance (ANOVA)

Cancer Research UK – 25th of February 2022
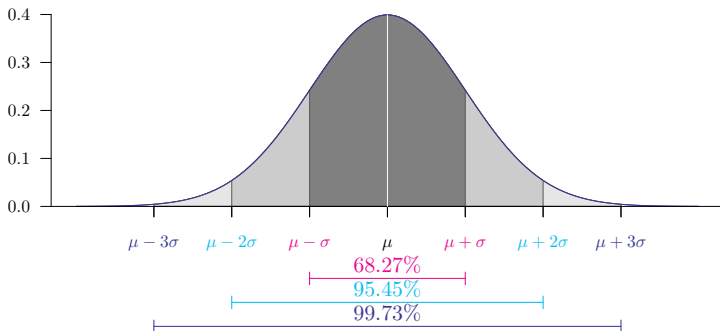
D.-L. Couturier / R. Nicholls / M. Fernandes

Quick review: Normal distribution

$$Y \sim N(\mu, \sigma^2), \qquad f_Y(y) = \frac{1}{\sqrt{2\pi\sigma^2}} \; e^{-\frac{(y-\mu)^2}{2\sigma^2}}$$

$$\mathsf{E}[Y] = \mu, \qquad \mathsf{Var}[Y] = \sigma^2,$$

$$Z = \frac{Y - \mu}{\sigma} \sim N(0, 1), \qquad f_Z(z) = \frac{1}{\sqrt{2\pi}} \; e^{-\frac{z^2}{2}}.$$

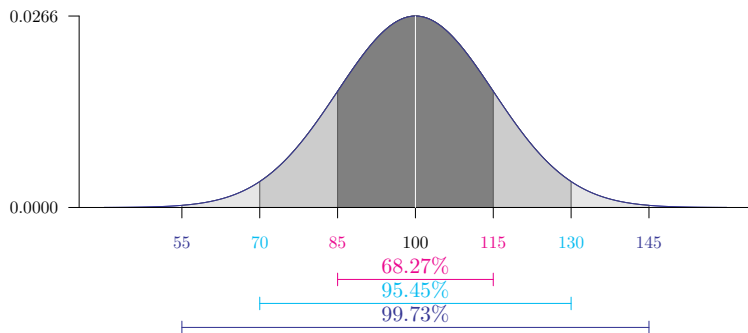Probability density function of a normal distribution:

Quick review: Normal distribution

$$Y \sim N(\mu, \sigma^2), \qquad f_Y(y) = \frac{1}{\sqrt{2\pi\sigma^2}} \; e^{-\frac{(y-\mu)^2}{2\sigma^2}}$$
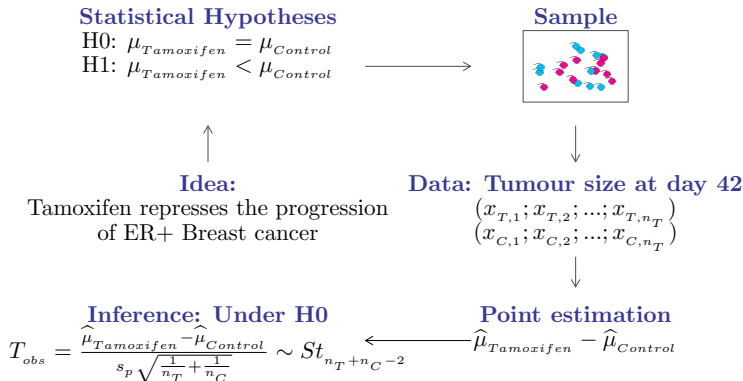
$$\mathsf{E}[Y] = \mu, \qquad \mathsf{Var}[Y] = \sigma^2,$$

$$Z = \frac{Y - \mu}{\sigma} \sim N(0, 1), \qquad f_Z(z) = \frac{1}{\sqrt{2\pi}} \; e^{-\frac{z^2}{2}}.$$

Suitable modelling for a lot of phenomena: $\mathsf{IQ} \sim N(100, 15^2)$.
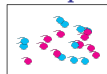
Grand Picture of Statistics

Statistical Hypotheses
H0: $\mu_{Tamoxifen} = \mu_{Control}$
H1: $\mu_{Tamoxifen} < \mu_{Control}$

$\longrightarrow$

Sample

$\uparrow$ $\downarrow$

Idea:
Tamoxifen represses the progression
of ER+ Breast cancer

Data: Tumour size at day 42
$(x_{T,1}; x_{T,2}; ...; x_{T,n_T})$
$(x_{C,1}; x_{C,2}; ...; x_{C,n_T})$

$\downarrow$

Inference: Under H0
$$T_{obs} = \frac{\widehat{\mu}_{Tamoxifen} - \widehat{\mu}_{Control}}{s_p \sqrt{\frac{1}{n_T} + \frac{1}{n_C}}} \sim St_{n_T + n_C - 2}$$

$\longleftarrow$

Point estimation
$\widehat{\mu}_{Tamoxifen} - \widehat{\mu}_{Control}$

Grand Picture of Statistics

Statistical Hypotheses
H0: $\mu_{Tamoxifen} = \mu_{Control}$
H1: $\mu_{Tamoxifen} < \mu_{Control}$

$\longrightarrow$
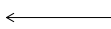
Sample

Idea:
Tamoxifen represses the progression
of ER+ Breast cancer

Data: Tumour size at day 42
$(x_{T,1}; x_{T,2}; ...; x_{T,n_T})$
$(x_{C,1}; x_{C,2}; ...; x_{C,n_T})$

Inference: Under H0

$T_{obs} = \dfrac{\widehat{\mu}_{Tamoxifen} - \widehat{\mu}_{Control}}{s_p \sqrt{\frac{1}{n_T} + \frac{1}{n_C}}} \sim St_{n_T + n_C - 2}$

$\longleftarrow$

Point estimation
$\widehat{\mu}_{Tamoxifen} - \widehat{\mu}_{Control}$

$St_{n_T + n_C - 2}$

$p - value = P(T < T_{obs})$

-4 -3 -2 -1 0 1 2 3 4
T
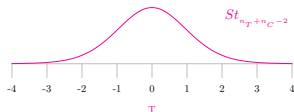
One-sample Student's t-test

Weight loss

- Assumed model

  $Y_i = \mu + \epsilon_i,$

  where $i = 1, ..., n$
  and $\epsilon_i \sim N(0, \sigma^2).$
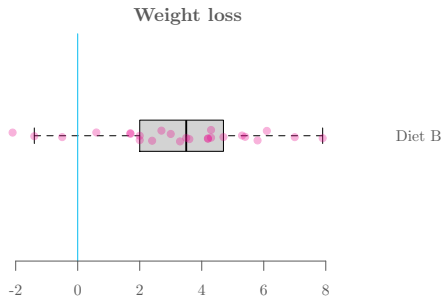
- Hypotheses
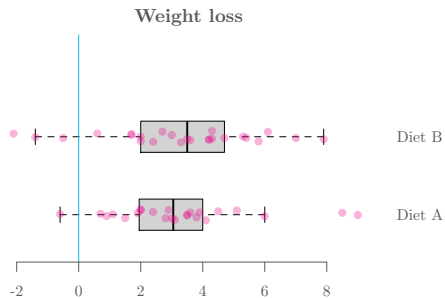 - **H0:** $\mu = 0,$
  - **H1:** $\mu > 0.$

- Test statistic's distribution under **H0**

$$T = \frac{\overline{Y} - \mu_0}{s} \sim Student(n-1).$$

```
One Sample t-test

data:  dietB
t = 6.6301, df = 24, p-value = 3.697e-07
alternative hypothesis: true mean is greater than 0
95 percent confidence interval:
 2.424694      Inf
sample estimates:
mean of x
    3.268
```

4

Weight loss

Two-sample location tests:
t-tests and Mann-Whitney-Wilcoxon's test

Two independent sample Student's t-test

- Assumed model

$$Y_{i(g)} = \mu_g + \epsilon_{i(g)},$$
$$= \mu + \delta_g + \epsilon_{i(g)},$$

where $g = A, B$, $i = 1, ..., n_g$,
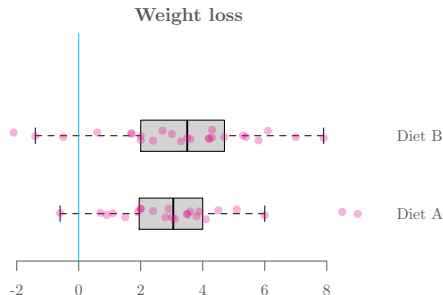$\epsilon_{i(g)} \sim N(0, \sigma^2)$ and $\sum n_g \delta_g = 0$.

- Hypotheses
  - ▷ **H0:** $\mu_A = \mu_B$,
  - ▷ **H1:** $\mu_A \neq \mu_B$.

- Test statistic's distribution under **H0**

$$T = \frac{(\overline{Y}_A - \overline{Y}_B) - (\mu_A - \mu_B)}{s_p \sqrt{n_A^{-1} + n_B^{-1}}} \sim Student(n_A + n_B - 2).$$

Weight loss

Diet B

Diet A

```
Two Sample t-test

data:  dietA and dietB
t = 0.0475, df = 47, p-value = 0.9623
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.323275  1.387275
sample estimates:
mean of x mean of y
    3.300     3.268
```

6

UNIVERSITY OF
CAMBRIDGE

Two independent sample Welch's t-test

- ▶ Assumed model

$$Y_{i(g)} = \mu_g + \epsilon_{i(g)},$$
$$= \mu + \delta_g + \epsilon_{i(g)},$$

where $g = A, B$, $i = 1, ..., n_g$,
$\epsilon_{i(g)} \sim N(0, \sigma_g^2)$ and $\sum n_g \delta_g = 0$.

- ▶ Hypotheses
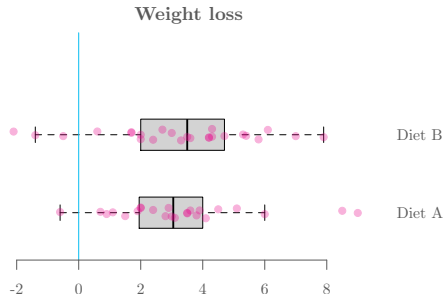 - ▷ **H0:** $\mu_A = \mu_B$,
  - ▷ **H1:** $\mu_A \neq \mu_B$.

- ▶ Test statistic's distribution under **H0**

$$T = \frac{(\overline{Y}_A - \overline{Y}_B) - (\mu_A - \mu_B)}{\sqrt{s_X^2/n_X + s_Y^2/n_Y}} \sim Student(\text{df}).$$

Weight loss

```
Welch Two Sample t-test

data:  dietA and dietB
t = 0.047594, df = 46.865, p-value = 0.9622
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.320692  1.384692
sample estimates:
mean of x mean of y
    3.300     3.268
```

7

UNIVERSITY OF
CAMBRIDGE

Two independent sample Mann-Whitney-Wilcoxon test

▶ Assumed model

$$Y_{i(g)} = \theta_g + \epsilon_{i(g)},$$
$$= \theta + \delta_g + \epsilon_{i(g)},$$

where $g = A, B$, $i = 1, ..., n_g$,
$\epsilon_{i(g)} \sim iid(0, \sigma^2)$ and $\sum n_g \delta_g = 0$.
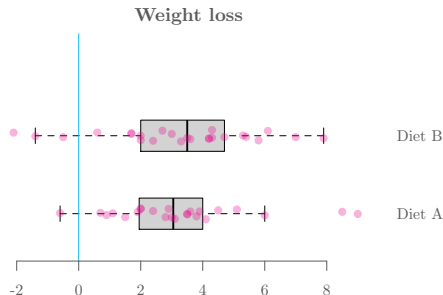
▶ Hypotheses
  ▷ **H0:** $\theta_A = \theta_B$,
  ▷ **H1:** $\theta_A \neq \theta_B$.

▶ Test statistic's distribution under **H0**

$$z = \frac{\sum_{i=1}^{n_B} R_{i(g)} - [n_B(n_A + n_B + 1)/2]}{\sqrt{n_A n_B (n_A + n_B + 1)/12}},$$

where
  ▶ $R_{i(g)}$ denotes the global rank of the ith observation of group g.
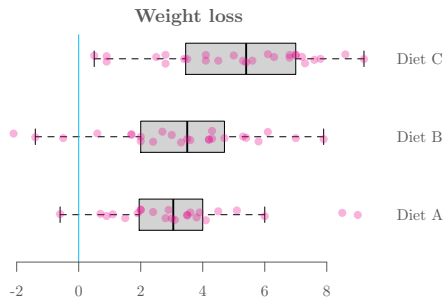

Weight loss

```
Wilcoxon rank sum test with continuity correction

data:  dietA and dietB
W = 277, p-value = 0.6526
alternative hypothesis: true location shift is not equal to 0
```

8

UNIVERSITY OF
CAMBRIDGE

Weight loss

Two or more sample location tests:
one-way ANOVA & multiple comparisons

More than two sample case: Fisher's one-way ANOVA

Weight loss

- Assumed model

  $$Y_{i(g)} = \mu_g + \epsilon_{i(g)},$$
  $$= \mu + \delta_g + \epsilon_{i(g)},$$

  where $g = 1, ..., G$, $i = 1, ..., n_g$,
  $\epsilon_{i(g)} \sim N(0, \sigma^2)$ and $\sum n_g \delta_g = 0$.

- Hypotheses
  ▷ **H0:** $\mu_1 = \mu_2 = ... = \mu_G$,
  ▷ **H1:** $\mu_k \neq \mu_l$ for at least one pair (k, l).

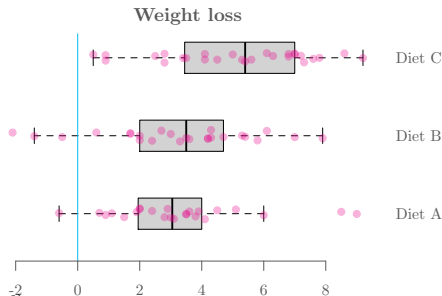- Test statistic's distribution under **H0**

  $$F = \frac{N s_{\overline{Y}}^2}{s_p^2} \sim Fisher(G - 1, N - G),$$

  where

  - $s_{\overline{Y}}^2 = \frac{1}{G-1} \sum\limits_{g=1}^{G} \frac{n_g}{N} \left(\overline{Y}_g - \overline{\overline{Y}} \right)^2$,

  - $s_p^2 = \frac{1}{N-G} \sum\limits_{g=1}^{G} (n_g - 1) s_g^2$,

  - $N = \sum n_g$, $\overline{\overline{Y}} = \frac{1}{N} \sum\limits_{g=1}^{G} n_g \overline{Y}_g$.

```
             Df Sum Sq Mean Sq F value Pr(>F)
diet.type     2   60.5  30.264   5.383 0.0066 **
Residuals    73  410.4   5.622
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' (
```

UNIVERSITY OF CAMBRIDGE

More than two sample case: Welch's one-way ANOVA

- Assumed model

$$Y_{i(g)} = \mu_g + \epsilon_{i(g)},$$
$$= \mu + \delta_g + \epsilon_{i(g)},$$

where $g = 1, ..., G$, $i = 1, ..., n_g$, $\epsilon_{i(g)} \sim N(0, \sigma_g^2)$ and $\sum n_g \delta_g = 0$.

- Hypotheses
  - **H0:** $\mu_1 = \mu_2 = ... = \mu_G$,
  - **H1:** $\mu_k \neq \mu_l$ for at least one pair (k, l).
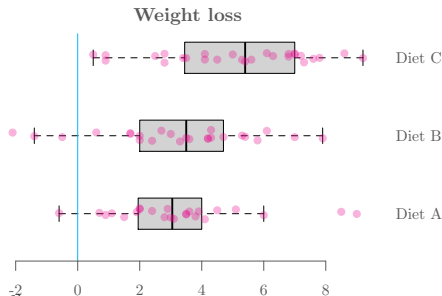
- Test statistic's distribution under **H0**

$$F^{\star} = \frac{s_{\overline{Y}}^{\star 2}}{1 + \frac{2(G-2)}{3\Delta}} \sim Fisher(G-1, \Delta),$$

where

- $s_{\overline{Y}}^{\star 2} = \frac{1}{G-1} \sum\limits_{g=1}^{G} w_g \left(\overline{Y}_g - \overline{\overline{Y}}^{\star} \right)^2$,

- $\Delta = \left[\frac{3}{G^2-1} \sum\limits_{g=1}^{G} \frac{1}{n_g} \left(1 - \frac{w_g}{\sum w_g} \right) \right]^{-1}$,

- $w_g = \frac{n_g}{s_g^2}$, $\overline{\overline{Y}}^{\star} = \sum\limits_{g=1}^{G} \frac{w_g \overline{Y}_g}{\sum w_g}$.

Weight loss


```
One-way analysis of means (not assuming equal variances)

data:  weight.diff and diet.type
F = 5.2693, num df = 2.00, denom df = 48.48, p-value = 0.008497
```

UNIVERSITY OF
CAMBRIDGE

More than two sample case: Kruskal-Wallis test

- **Assumed model**

  $$Y_{i(g)} = \theta_g + \epsilon_{i(g)},$$
  $$= \theta + \delta_g + \epsilon_{i(g)},$$

  where $g = 1, ..., G$, $i = 1, ..., n_g$,
  $\epsilon_{i(g)} \sim iid(0, \sigma^2)$ and $\sum n_g \delta_g = 0$.

- **Hypotheses**
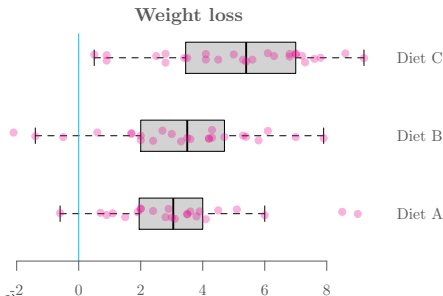 - ▷ **H0:** $\theta_1 = \theta_2 = ... = \theta_G$,
  - ▷ **H1:** $\theta_k \neq \theta_l$ for at least one pair (k, l).

- **Test statistic's distribution under H0**

$$H = \frac{\frac{12}{N(N+1)} \sum_{g=1}^{G} \frac{\overline{R}_g}{n_g} - 3(N-1)}{1 - \frac{\sum_{v=1}^{V} t_v^3 - t_v}{N^3 - N}} \sim \chi(G-1),$$

where
- ▶ $\overline{R}_g = \frac{1}{n_g} \sum_{i=1}^{n_g} R_{i(g)}$ and $R_{i(g)}$ denotes the global rank of the ith observation of group g,
- ▶ V is the number of different values/levels in $\mathbf{y}$ and t_v denotes the number of times a given value/level occurred in $\mathbf{y}$.

```
Kruskal-Wallis rank sum test

data:  weight.loss by diet.type
Kruskal-Wallis chi-squared = 9.4159, df = 2, p-value = 0.009023
```
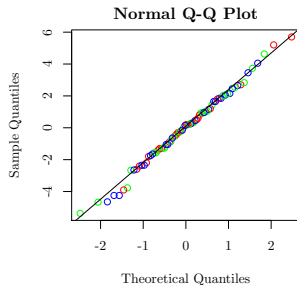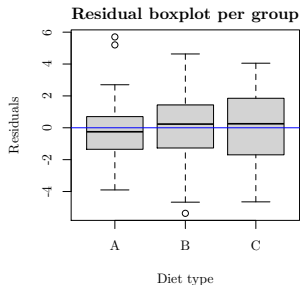

Weight loss

12

UNIVERSITY OF
CAMBRIDGE

Model check: Residual analysis

$$Y_{i(g)} = \theta_g + \epsilon_{i(g)}$$
$$\widehat{\epsilon}_{i(g)} = Y_{i(g)} - \widehat{\theta}_g,$$

where

- $\widehat{\epsilon}_{i(g)} \sim N(0, \widehat{\sigma}^2)$ for Fisher's ANOVA
- $\widehat{\epsilon}_{i(g)} \sim N(0, \widehat{\sigma}_g^2)$ for Welch's ANOVA
- $\widehat{\epsilon}_{i(g)} \sim iid(0, \widehat{\sigma}^2)$ for Kruskal-Wallis' ANOVA

Residual boxplot per group — Residuals vs Diet type (A, B, C)

Normal Q-Q Plot — Sample Quantiles vs Theoretical Quantiles

```
Shapiro-Wilk normality test

data:  diet$resid.mean
W = 0.99175, p-value = 0.9088
```

```
Bartlett test of homogeneity of variances

data:  diet$resid.mean by as.numeric(diet$diet.type)
Bartlett's K-squared = 0.21811, df = 2, p-value = 0.8967
```

13

Finding different pairs: Multiple comparisons

▶ **All-pairwise comparison problem:**
Interested in finding which pair(s) are different by testing
   ▷ **H0$_1$:** $\mu_1 = \mu_2$,   ▷ **H0$_2$:** $\mu_1 = \mu_3$,   ... ▷ **H0$_K$:** $\mu_{G-1} = \mu_G$,
leading to a total of $K = G(G-1)/2$ pairwise comparisons.

▶ **Family-wise type I error for K tests, α_K**
For each test, the probability of rejecting H0 when H0 is true equals α.
For K independent tests, the probability of rejecting H0 at least 1 time
when H0 is true, α_K, is given by
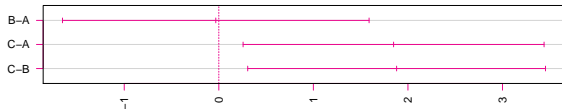
$$\alpha_K = 1 - (1-\alpha)^K.$$
   ▷ $\alpha_1 = 0.05$,
   ▷ $\alpha_2 = 0.0975$,
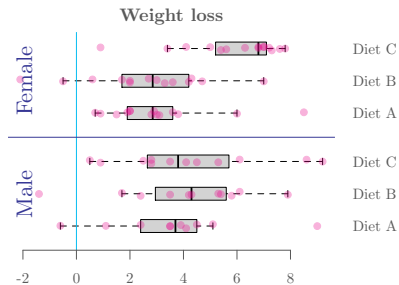   ▷ $\alpha_{10} = 0.4013$.

▶ **Multiplicity correction**
Principle: change the level of each test so that $\alpha_K = 0.05$, for example:
   ▶ Bonferroni's correction (indep. tests): $\alpha = \alpha_K/K$,
   ▶ Dunn-Sidak's correction (indep. tests): $\alpha = 1 - (1-\alpha_K)^{1/K}$,
   ▶ Tukey's correction (dependent tests).

95% family–wise confidence level

14

UNIVERSITY OF
CAMBRIDGE

Two or more sample location tests:
two-way ANOVA

More than one factor: Fisher's two-way ANOVA

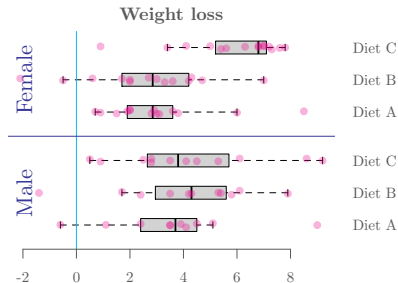- Assumed model

$$Y_{i(g)} = \mu_{gk} + \epsilon_{i(gk)},$$
$$= \mu + \delta_g + \delta_k + \delta_{gk} + \epsilon_{i(gk)},$$

  - $g = 1, ..., G,\ k = 1, ..., K,$
  - $i = 1, ..., n_g,$
  - $\epsilon_{i(gk)} \sim N(0, \sigma^2)$
  - $\sum n_g \delta_g = \sum n_k \delta_k = \sum n_{gk} \delta_{gk} = 0.$

Weight loss

- Hypotheses

  ▷ **H0**$_1$: $\delta_g = 0\ \forall\ g$,
  ▷ **H1**$_1$: **H0**$_1$ is false.

  ▷ **H0**$_2$: $\delta_k = 0\ \forall\ k$,
  ▷ **H1**$_2$: **H0**$_2$ is false.

  ▷ **H0**$_3$: $\delta_{gk} = 0\ \forall\ g, k$,
  ▷ **H1**$_3$: **H0**$_3$ is false.

UNIVERSITY OF
CAMBRIDGE

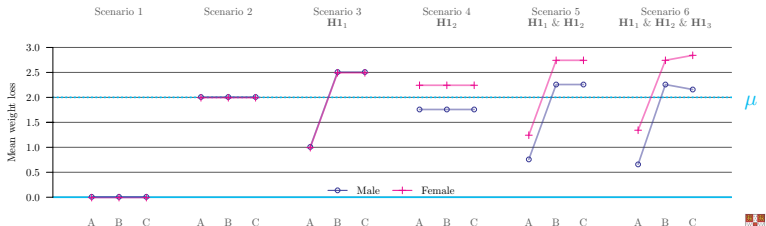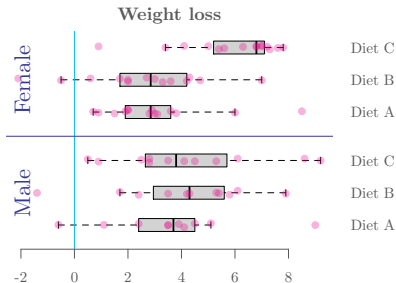# More than one factor: Fisher's two-way ANOVA

▶ Assumed model

$$Y_{i(g)} = \mu_{gk} + \epsilon_{i(gk)},$$
$$= \mu + \delta_g + \delta_k + \delta_{gk} + \epsilon_{i(gk)},$$

    ▶ $g = 1, ..., G, \; k = 1, ..., K,$
    ▶ $i = 1, ..., n_g,$
    ▶ $\epsilon_{i(gk)} \sim N(0, \sigma^2)$
    ▶ $\sum n_g \delta_g = \sum n_k \delta_k = \sum n_{gk} \delta_{gk} = 0.$

▶ Hypotheses

  ▷ **H0$_1$**: $\delta_g = 0 \; \forall \; g$,
  ▷ **H1$_1$**: **H0$_1$** is false.

  ▷ **H0$_2$**: $\delta_k = 0 \; \forall \; k$,
  ▷ **H1$_2$**: **H0$_2$** is false.

  ▷ **H0$_3$**: $\delta_{gk} = 0 \; \forall \; g, k$,
  ▷ **H1$_3$**: **H0$_3$** is false.

UNIVERSITY OF CAMBRIDGE

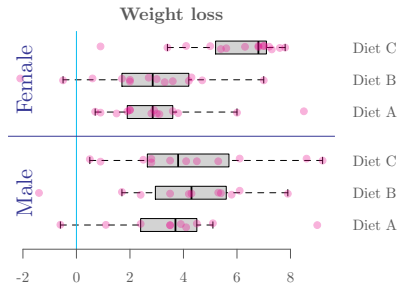# More than one factor: Fisher's two-way ANOVA

- Assumed model

$$Y_{i(g)} = \mu_{gk} + \epsilon_{i(gk)},$$
$$= \mu + \delta_g + \delta_k + \delta_{gk} + \epsilon_{i(gk)},$$

  - $g = 1, ..., G, \ k = 1, ..., K,$
  - $i = 1, ..., n_g,$
  - $\epsilon_{i(gk)} \sim N(0, \sigma^2)$
  - $\sum n_g \delta_g = \sum n_k \delta_k = \sum n_{gk} \delta_{gk} = 0.$

- Hypotheses

  ▷ **H0$_1$**: $\delta_g = 0 \ \forall \ g$,   ▷ **H0$_2$**: $\delta_k = 0 \ \forall \ k$,   ▷ **H0$_3$**: $\delta_{gk} = 0 \ \forall \ g, k$,
  ▷ **H1$_1$**: **H0$_1$** is false.   ▷ **H1$_2$**: **H0$_2$** is false.   ▷ **H1$_3$**: **H0$_3$** is false.

Weight loss

```
                 Df  Sum Sq  Mean Sq  F value  Pr(>F)
diet.type         2    60.5   30.264    5.629  0.00541 **
gender            1     0.2    0.169    0.031  0.85991
diet.type:gender  2    33.9   16.952    3.153  0.04884 *
Residuals        70   376.3    5.376
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

UNIVERSITY OF CAMBRIDGE

Summary