Analysis of Variance (ANOVA)

Cancer Research UK – 10^{th} of March 2020

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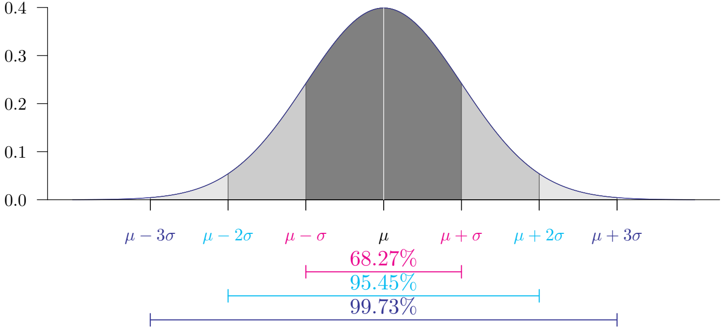
Quick review: Normal distribution

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

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

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

Probability density function of a normal distribution:



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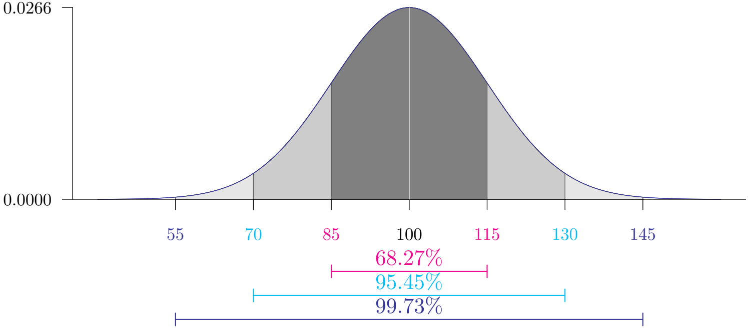
Suitable modelling for a lot of phenomena: IQ $\sim N(100, 15^2)$.



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Central limit theorem (Lindeberg-Lévy CLT)

- Let $(X_1, ..., X_n)$ be n independent and identically distributed (iid) random variables drawn from distributions of expected values given by μ and finite variances given by σ^2,
- then

$$\widehat{\mu} = \overline{X} = \frac{\sum_{i=1}^n X_i}{n} \quad \xrightarrow{d} \quad N\left(\mu, \frac{\sigma^2}{n}\right).$$

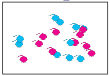
If $X_i \sim N(\mu, \sigma^2)$, this result is true for all sample sizes.

Grand Picture of Statistics

Statistical Hypotheses

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

Sample



Data: Tumour size at day 42

$(x_{T,1}; x_{T,2}; ...; x_{T,n_T})$
$(x_{C,1}; x_{C,2}; ...; x_{C,n_T})$

Point estimation

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

Inference: Under H0

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

Idea:

Tamoxifen represses the progression of ER+ Breast cancer

Grand Picture of Statistics

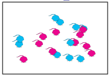
Statistical Hypotheses

H0: $\mu_{Tamoxifen} = \mu_{Control}$

H1: $\mu_{Tamoxifen} < \mu_{Control}$

$\longrightarrow$

Sample



$\downarrow$

Data: Tumour size at day 42

$(x_{T,1}; x_{T,2}; ...; x_{T,n_T})$

$(x_{C,1}; x_{C,2}; ...; x_{C,n_T})$

$\downarrow$

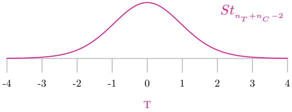
Point estimation

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

$\longleftarrow$

Inference: Under H0

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



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

$\uparrow$

Idea:

Tamoxifen represses the progression of ER+ Breast cancer

Statistical hypothesis testing Process

Several-step process:

- Define H0 and H1 according to a theory
- Set α, the probability of rejecting H0 when it is true (type I error),
- Define n, the sample size, allowing you to reject H0 when H1 is true with a probability $1 - \beta$ (Power),
- Determine the test statistic to be used,
- Collect the data,
- Perform the statistical test, define the p-value, and reject (or not) the null hypothesis.

Statistical hypothesis testing 4 possible outcomes

Conclude:

- if p-value $> \alpha$ $\rightarrow$ do not reject H0.
- if p-value $< \alpha$ $\rightarrow$ reject H0 in favour of H1.

		Test Outcome	
		H0 not rejected	H1 accepted
Unknown Truth	H0 true	$1 - \alpha$	α
	H1 true	β	$1 - \beta$

where

- α is the type I error, the probability of rejecting H0 when it is correct,
- β is the type II error, the probability of not rejecting when H1 is true,
- $1 - \beta$ is the power, the probability of accepting H1 when it is true.

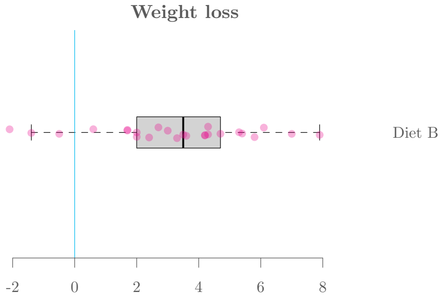
One-sample Student's t-test

- Assumed model

  $Y_i = \mu + \epsilon_i,$

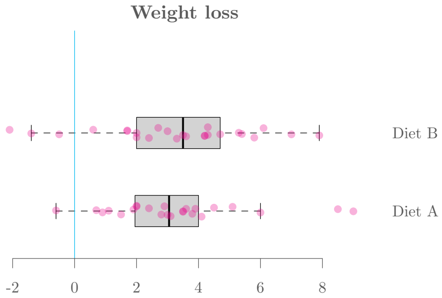
  where $i = 1, ..., n$
  and $\epsilon_i \sim N(0, \sigma^2)$.
- Hypotheses
  - **H0:** $\mu = 0$,
  - **H1:** $\mu > 0$.
- Test statistic's distribution under **H0**

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




```
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
```



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

Two independent sample Student's t-test

- Assumed model

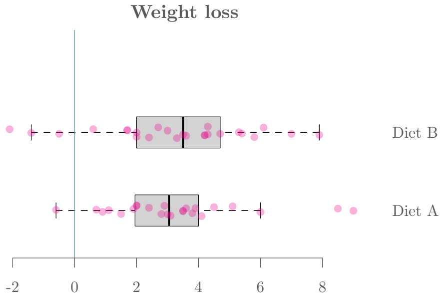
  $$Y_{i(g)} = \mu_g + \epsilon_{i(g)},$$
  $$= \mu + \delta_g + \epsilon_{i(g)},$$

  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**

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




```
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
```

Two independent sample Welch's t-test

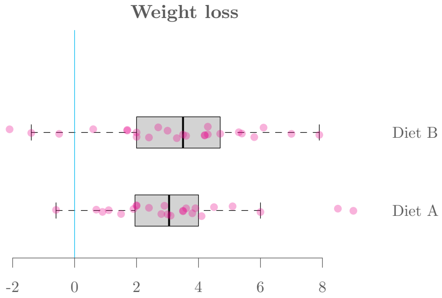
- Assumed model

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

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**

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




```
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
```

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 $\sum n_g \delta_g = 0$.

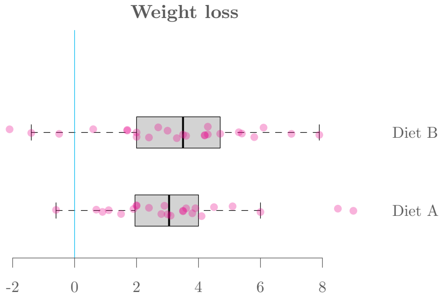
- Hypotheses
  - ▷ **H0:** $\theta_A = \theta_B$,
  - ▷ **H1:** $\theta_A \neq \theta_B$.

- 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.




```
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
```

Model assumptions Normality – Heteroscedasticity

Simulate 2500 samples with

- $X_i \sim Uniform(1.5, 2.5),\ i = 1, ..., n_X,$
- $Y_i \sim Uniform(0, 4),\ i = 1, ..., n_Y,$

so that $\mathrm{E}[X_i] = \mathrm{E}[Y_i] = 2$ (i.e., same mean, same median).

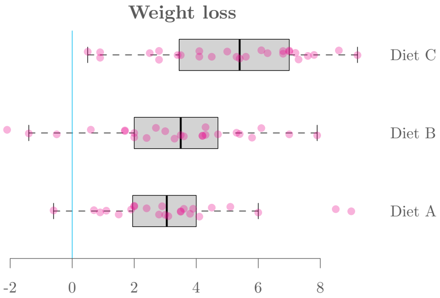
Assume

- $X_i \sim iid(\mu_X, \sigma^2),\ i = 1, ..., n_X,$
- $Y_i \sim iid(\mu_X + \delta, \sigma^2),\ i = 1, ..., n_Y.$

Test **H0**: $\delta = \delta_0$ against **H1**: $\delta \neq \delta_0$, at the 5% level, by means of

- Mann-Whitney-Wilcoxon test (MWW),
- T-test,
- Welch-test.

$\widehat{\alpha}$		Tests		
		MWW	Student's t-test	Welch's test
Sample size	$n_X = 200, n_Y = 70$	0.145	0.202	0.055
	$n_X = 20, n_Y = 7$	0.148	0.240	0.062



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

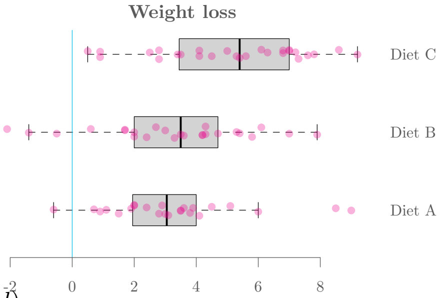
More than two sample case: Fisher's one-way ANOVA

- Assumed model

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

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

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

```
            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 ‘.’ (
```

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:** $\mu_1 = \mu_2 = ... = \mu_G$,
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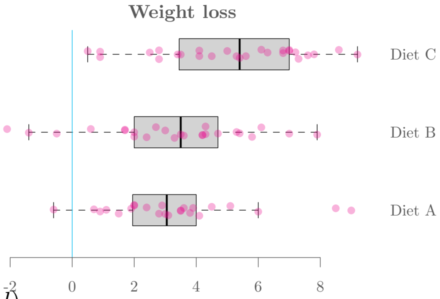


- Test statistic's distribution under **H0**

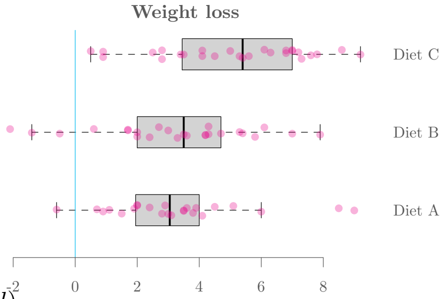
$$F^\star = \frac{s_{\overline{Y}}^{\star 2}}{1 + \frac{2(G-2)}{3\Delta}} \sim Fisher(G-1, \Delta),$$

where

- $s_{\overline{Y}}^{\star 2} = \frac{1}{G-1} \sum_{g=1}^{G} w_g \left(\overline{Y}_g - \overline{\overline{Y}}^\star\right)^2,$
- $\Delta = \left[\frac{3}{G^2-1} \sum_{g=1}^{G} \frac{1}{n_g} \left(1 - \frac{w_g}{\sum w_g}\right)\right]^{-1},$
- $w_g = \frac{n_g}{s_g^2}$, $\overline{\overline{Y}}^\star = \sum_{g=1}^{G} \frac{w_g \overline{Y}_g}{\sum w_g}$.

```
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
```

More than two sample case: Kruskal-Wallis test



- Assumed model

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

  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 $\mathbf{y}$.

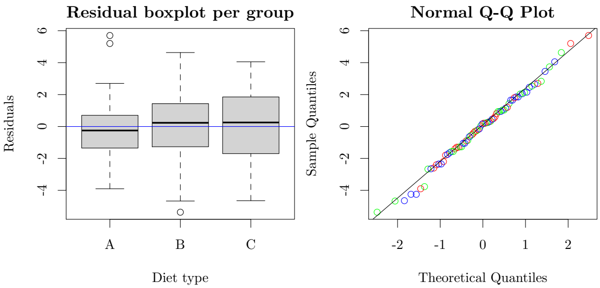
```
Kruskal-Wallis rank sum test

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

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




```
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
```

Finding different pairs: Multiple comparisons

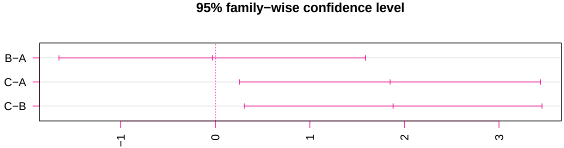
- **All-pairwise comparison problem:**
  Interested in finding which pair(s) are different by testing
  ▷ **H0**$_1$: $\mu_1 = \mu_2$, ▷ **H0**$_2$: $\mu_1 = \mu_3$, ... ▷ **H0**$_K$: $\mu_{G-1} = \mu_G$,
  leading to a total of $K = G(G-1)/2$ pairwise comparisons.

- **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).



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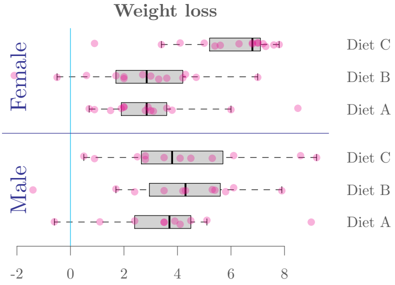


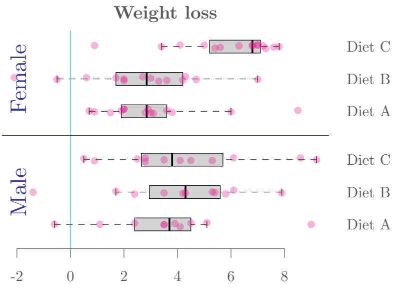
Two or more sample location tests: two-way ANOVA

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

- Assumed model

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

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



- 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.

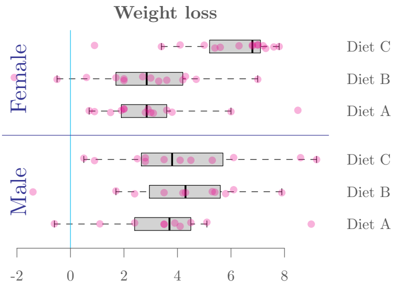
▷ **H0**$_3$: $\delta_{gk} = 0 \; \forall \; g, k$,
▷ **H1**$_3$: **H0**$_3$ is false.

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

- Assumed model

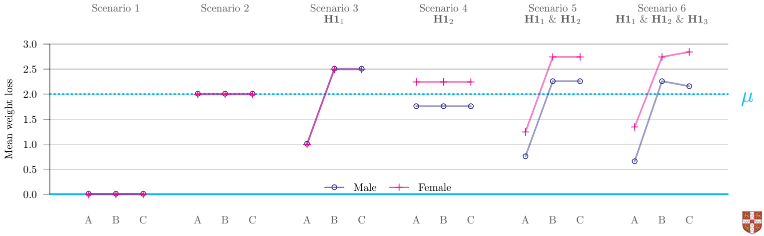
$$Y_{i(g)} = \mu_{gk} + \epsilon_{i(gk)},$$
$$= \mu + \delta_g + \delta_k + \delta_{gk} + \epsilon_{i(gk)},$$

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



- 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.



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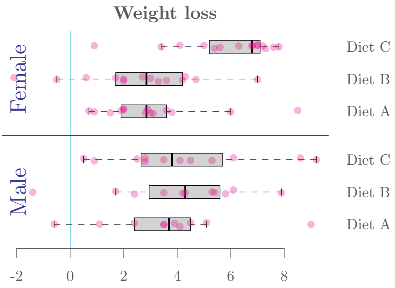
More than one factor: Fisher's two-way ANOVA

- 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.

```
                 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
```

Summary