

Module 15:

Statistics with multiple variables

Module 15: Learning Outcomes

- ❑ Use two-way ANOVAs and linear models to test for effects across multiple predictors
- ❑ Use a MANOVA or PERMANOVA in R to test for effects in multiple response variables
- ❑ Interpret model outputs and explain what single and interaction effects mean in two-way models

Previously...

$YVAR \sim XVAR$ [One response, one predictor]

But what if you wanted to have more than one predictor? (e.g. effect of temperature and sampling transect)

or more than one response? (e.g. Hundreds of ASVs changing in response to temperature)

Statistics with multiple variables

| | ONE predictor variable | MULTIPLE predictor variables | | | | | | | | | |
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| MULTIPLE response variables | MANOVA (parametric) PERMANOVA (non-parametric) | | | | | | | | | | |

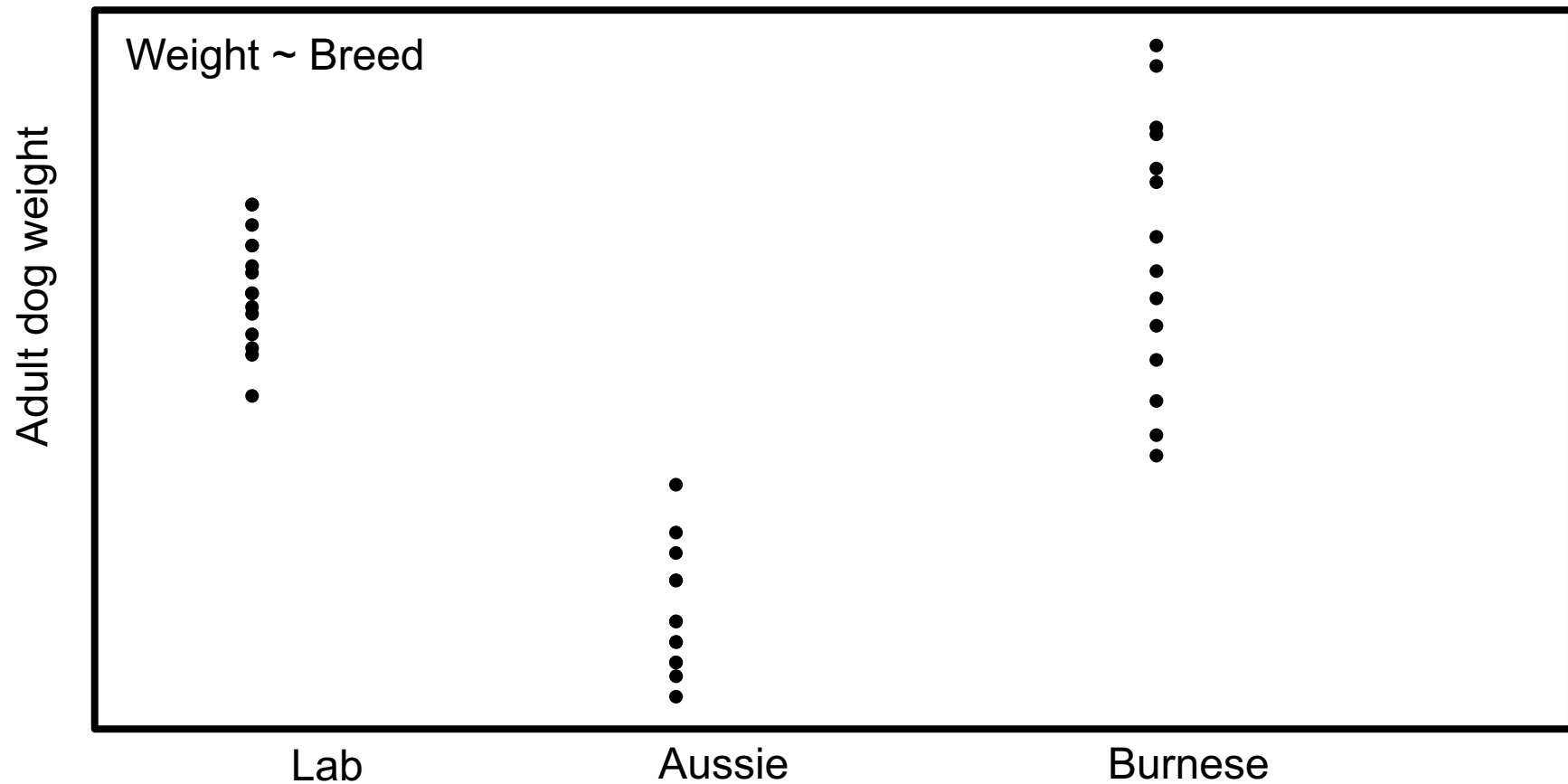
2-way ANOVA

• Like a normal (one-way) ANOVA, except you have two (categorical) predictor variables

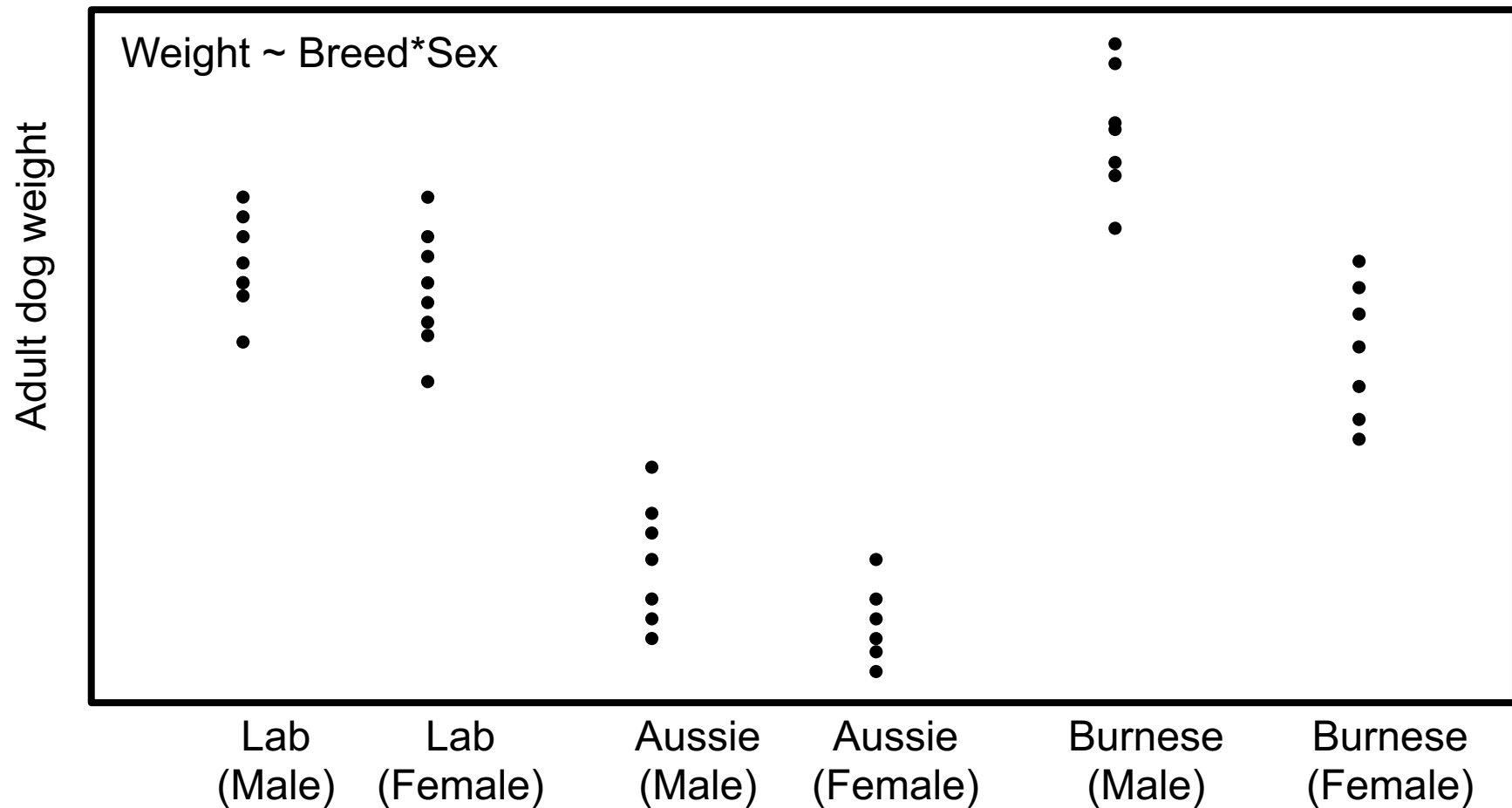
• You can look at independent effects and interactions between effects

- $Y \sim X + Z$ (independent effects only)
- $Y \sim X:Z$ (interactive effects only)
- $Y \sim X*Z$ (independent and interactive effects)

(one-way) ANOVA



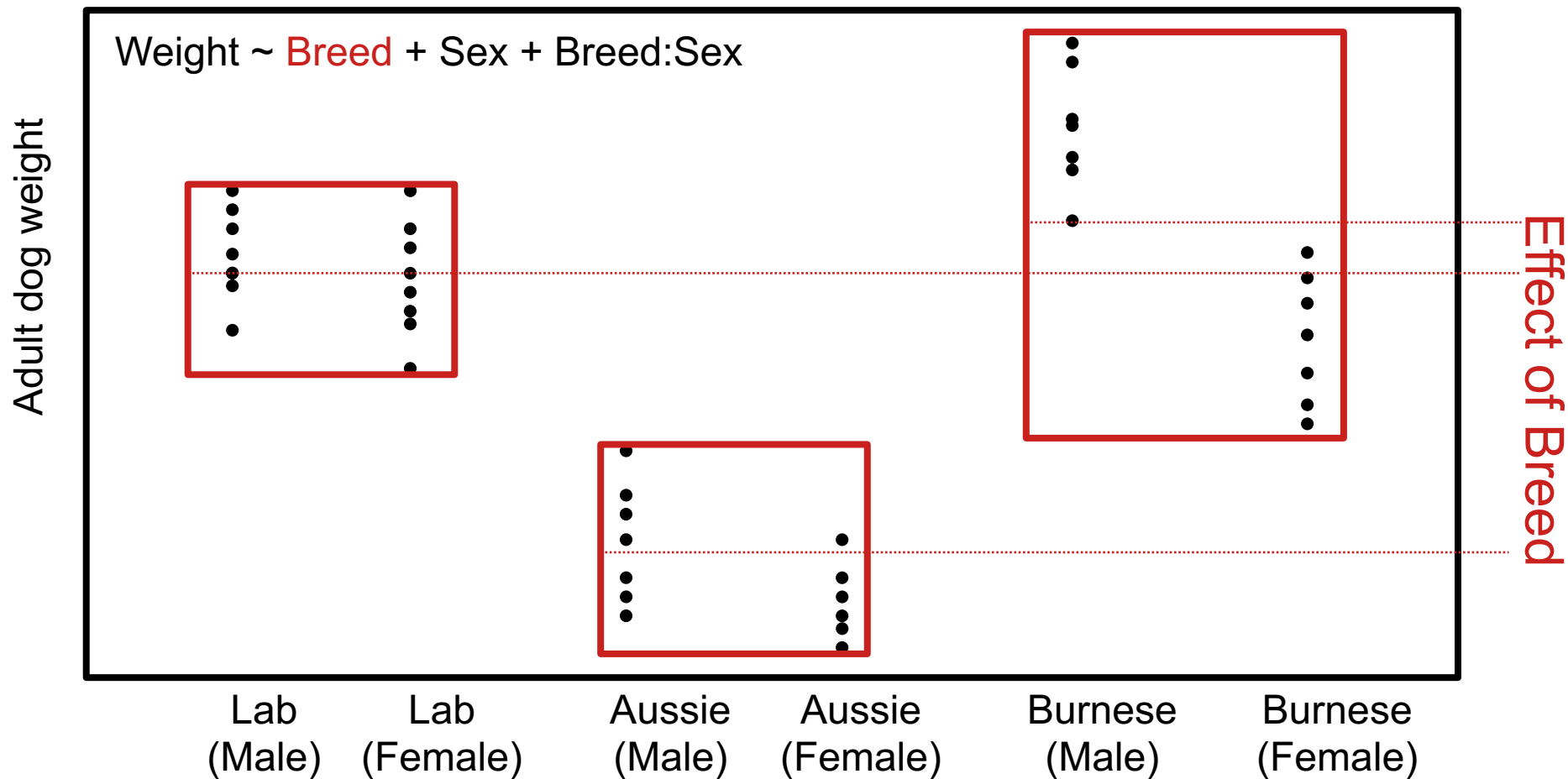
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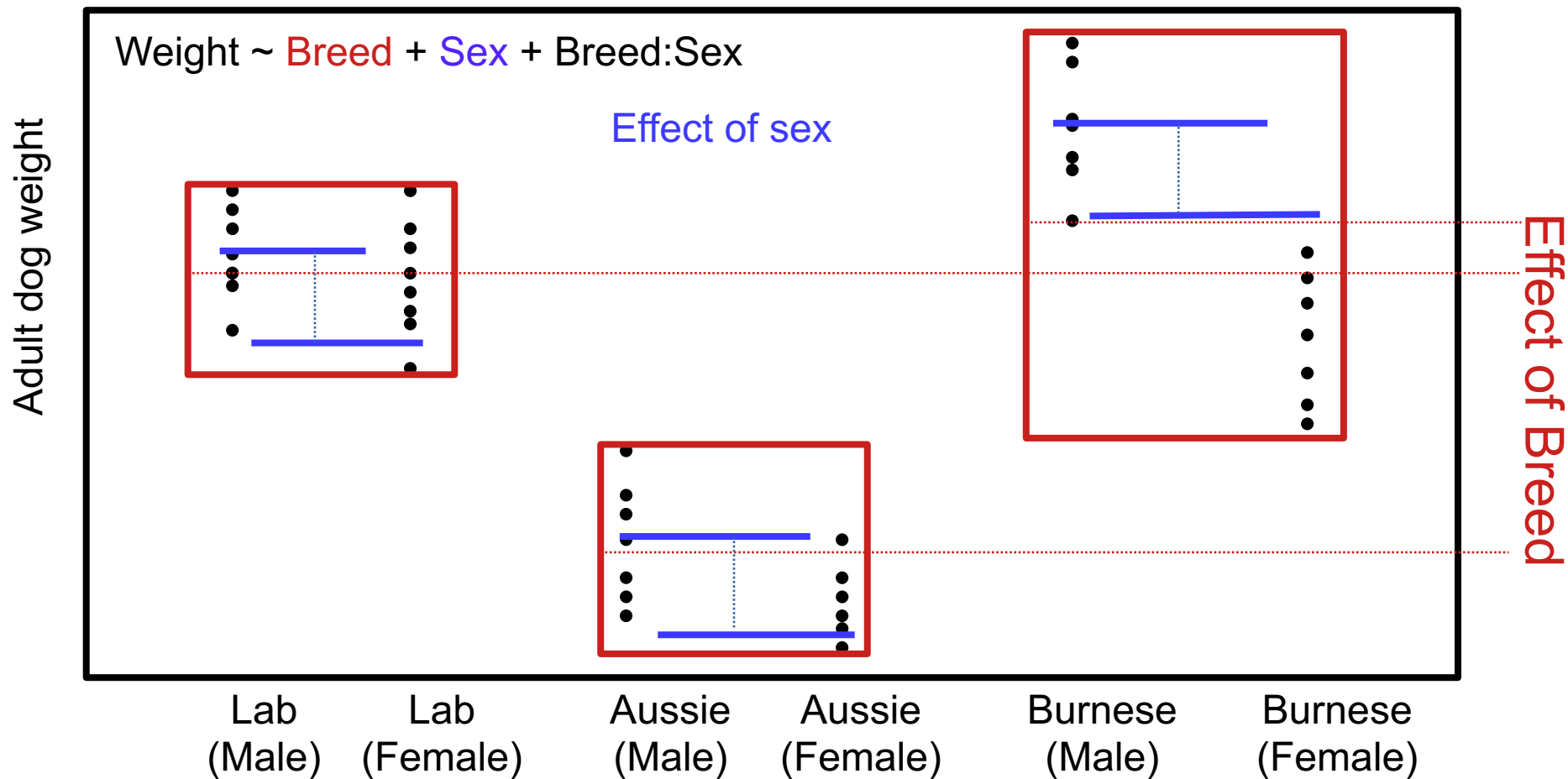
Interpretation of “interactions”



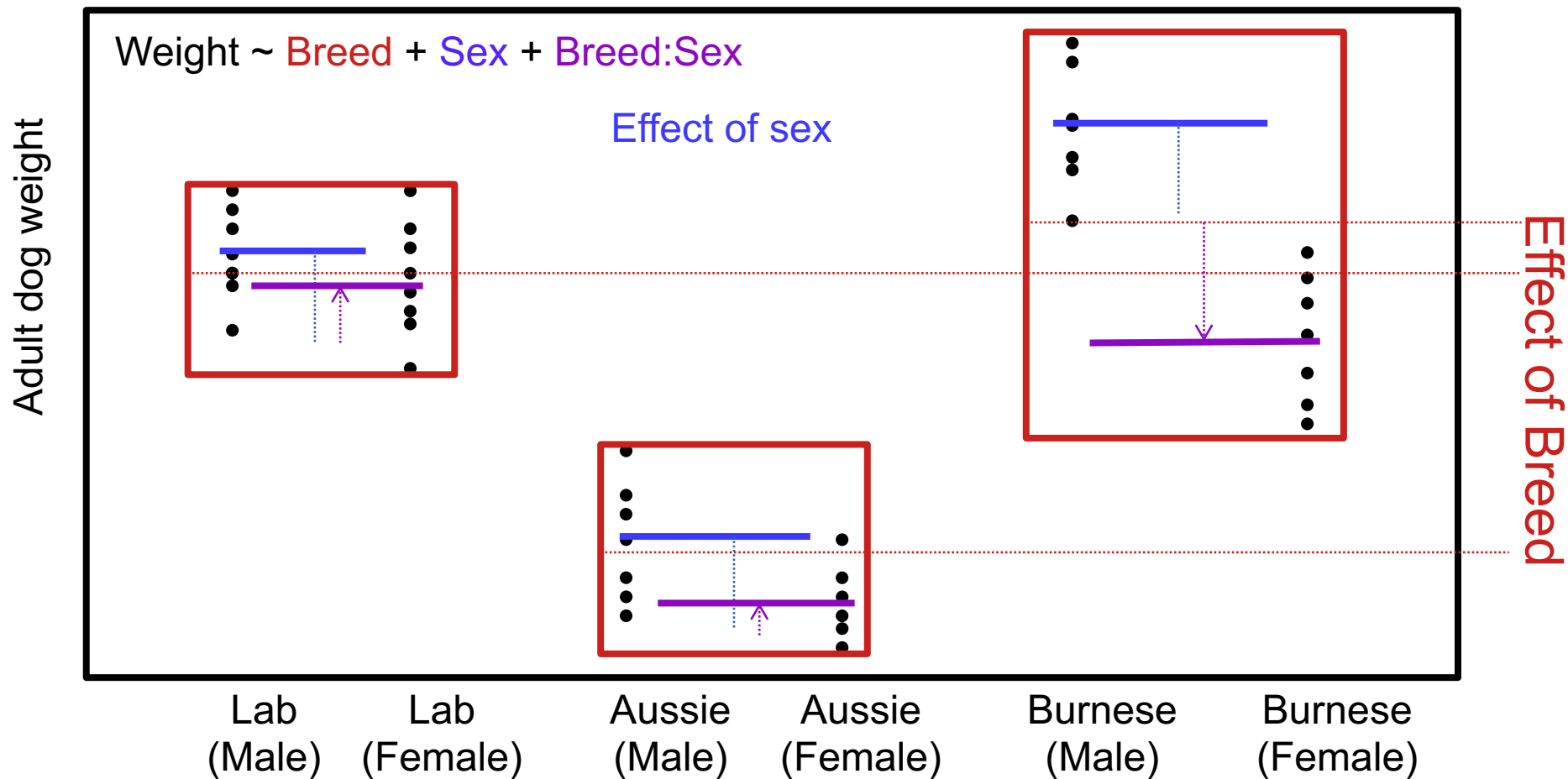
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2-way ANOVA code

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summary(aov(lm( Y ~ X1*X2, data = dat)))
```

```
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```

```
mdl_aov <- aov(mdl)
```

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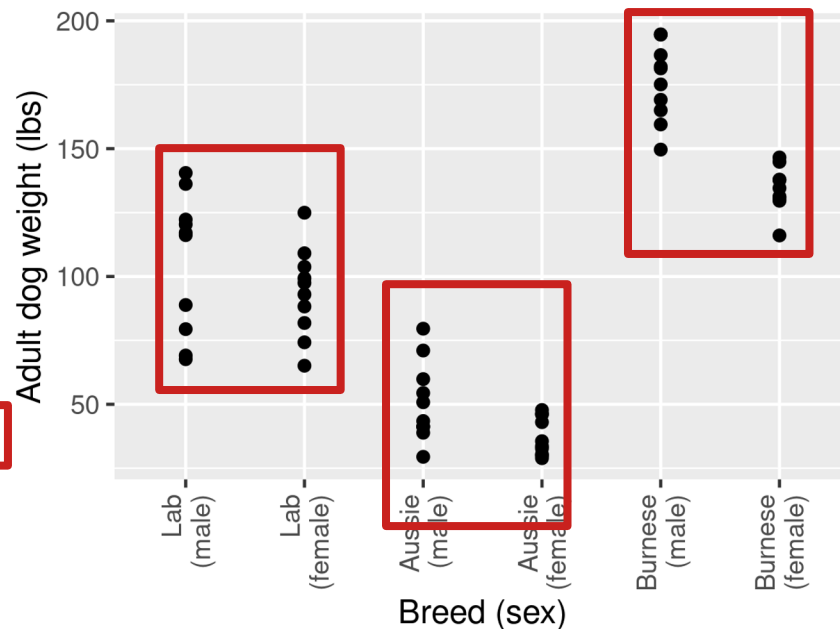
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| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|-----------|----|--------|---------|---------|----------|-----|
| breed | 2 | 122438 | 61219 | 224.513 | < 2e-16 | *** |
| sex | 1 | 7577 | 7577 | 27.787 | 2.43e-06 | *** |
| breed:sex | 2 | 2811 | 1406 | 5.155 | 0.00894 | ** |
| Residuals | 54 | 14724 | 273 | | | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



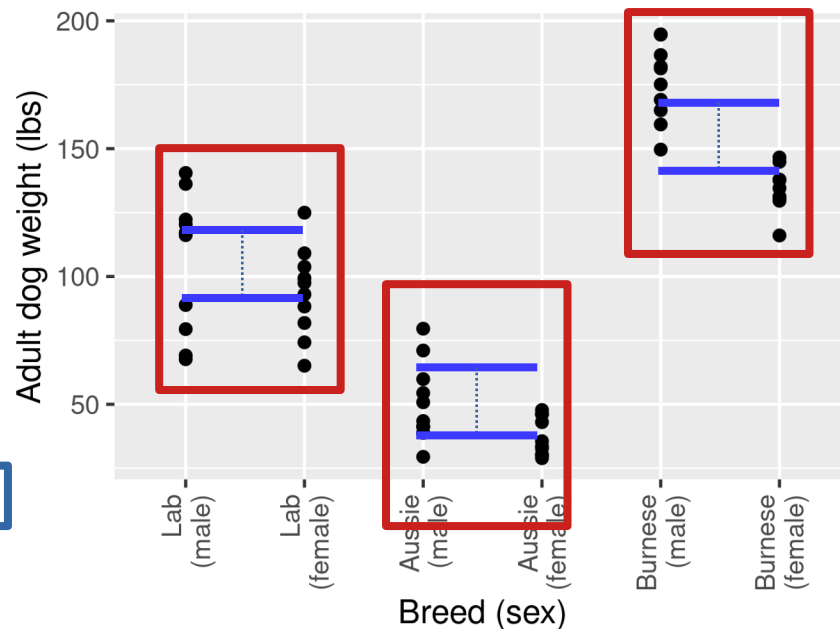
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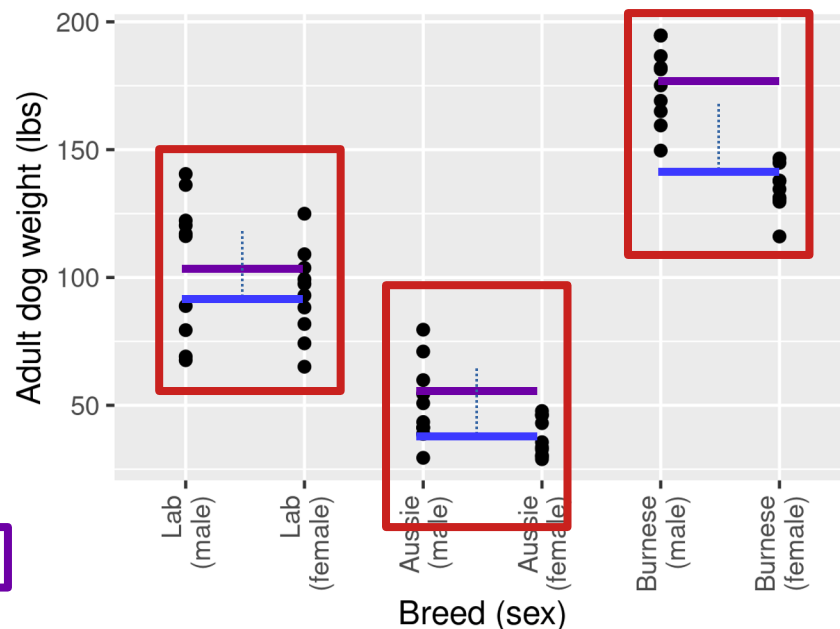
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Statistics with multiple variables

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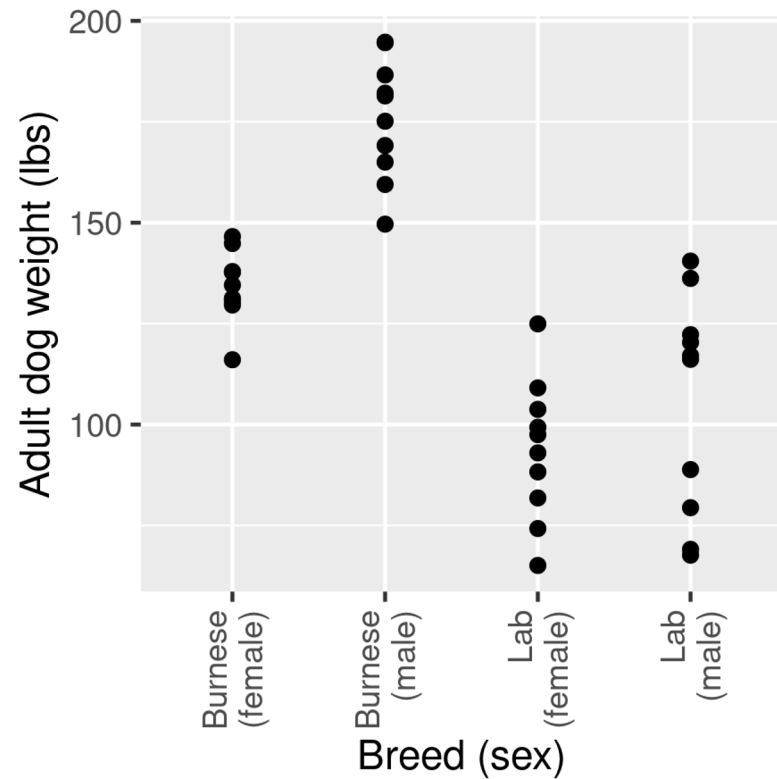
Linear models

- A “better” 2-way ANOVA because it can handle categorical and continuous variables
 - Also more descriptive, but more confusing to interpret
- Computationally identical to t-test and ANOVA in certain conditions

```
mdl <- lm( Y ~ X1*X2..., data = dat)
```

```
summary(mdl)
```

Linear models: how to interpret

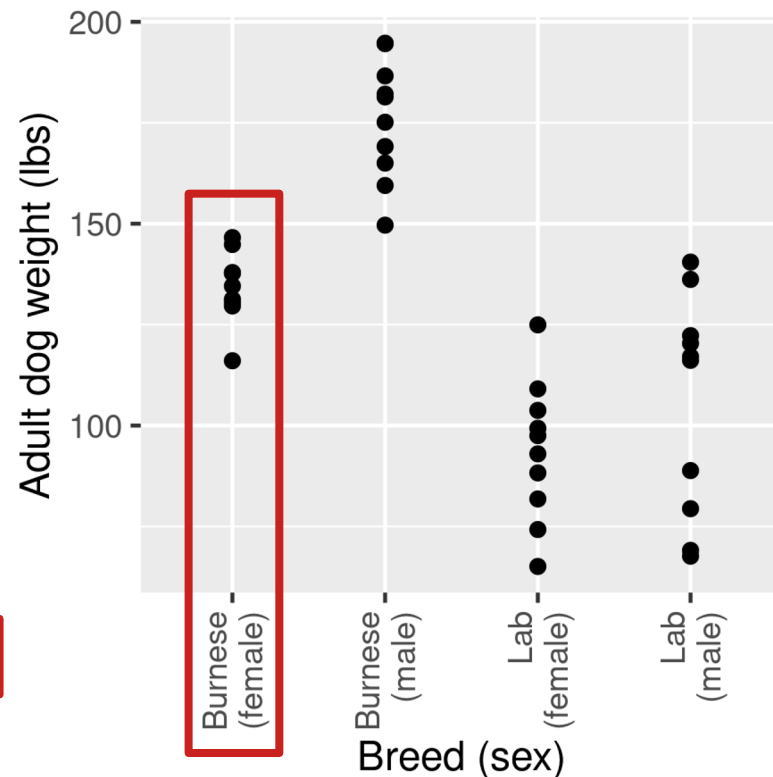


Linear models: how to interpret

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) | |
|------------------|----------|------------|---------|----------|-----|
| (Intercept) | 133.962 | 5.785 | 23.155 | < 2e-16 | *** |
| breedlab | -40.261 | 8.182 | -4.921 | 1.91e-05 | *** |
| sexmale | 41.816 | 8.182 | 5.111 | 1.07e-05 | *** |
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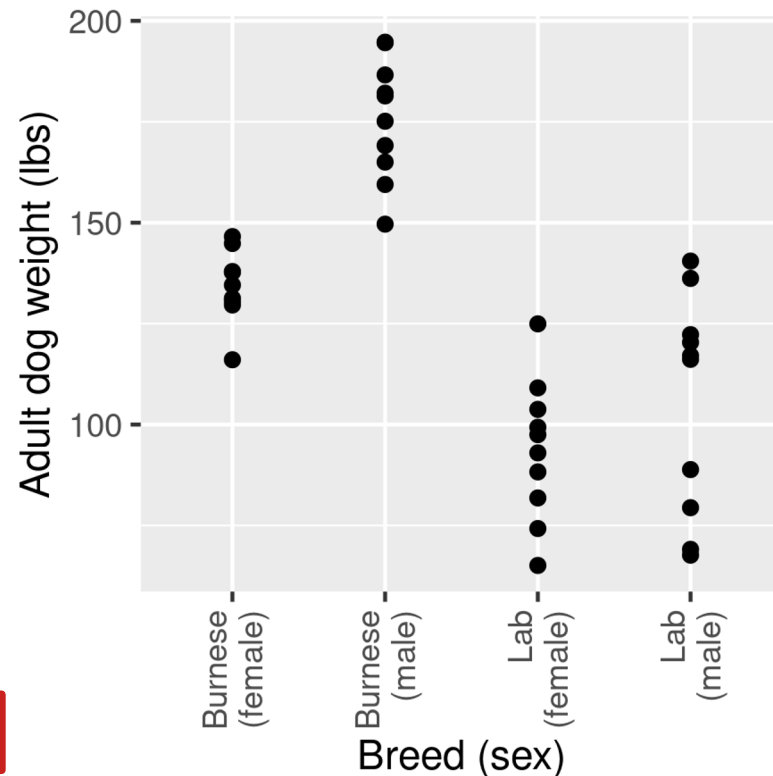
breedburnese, sexfemale

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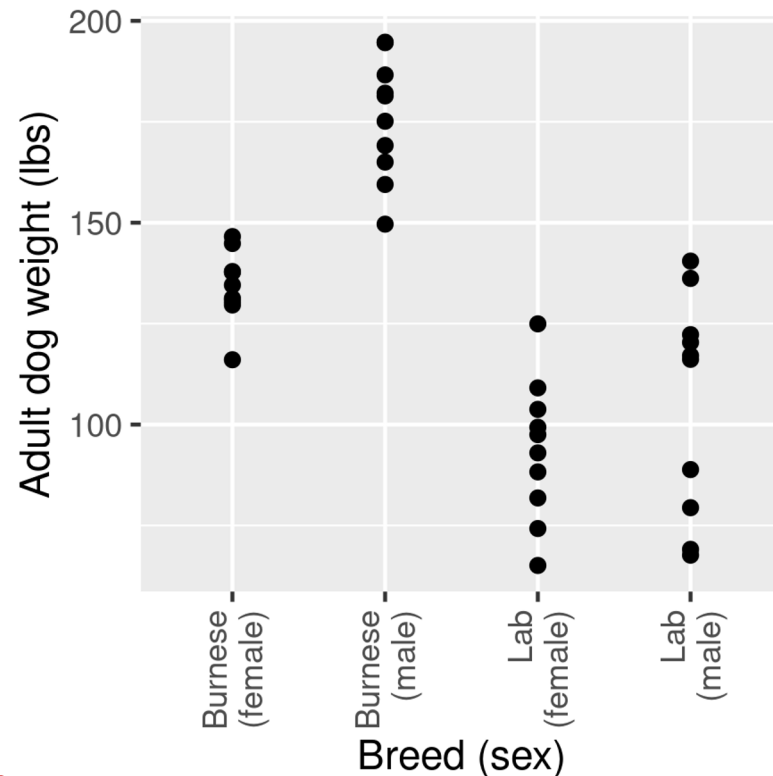


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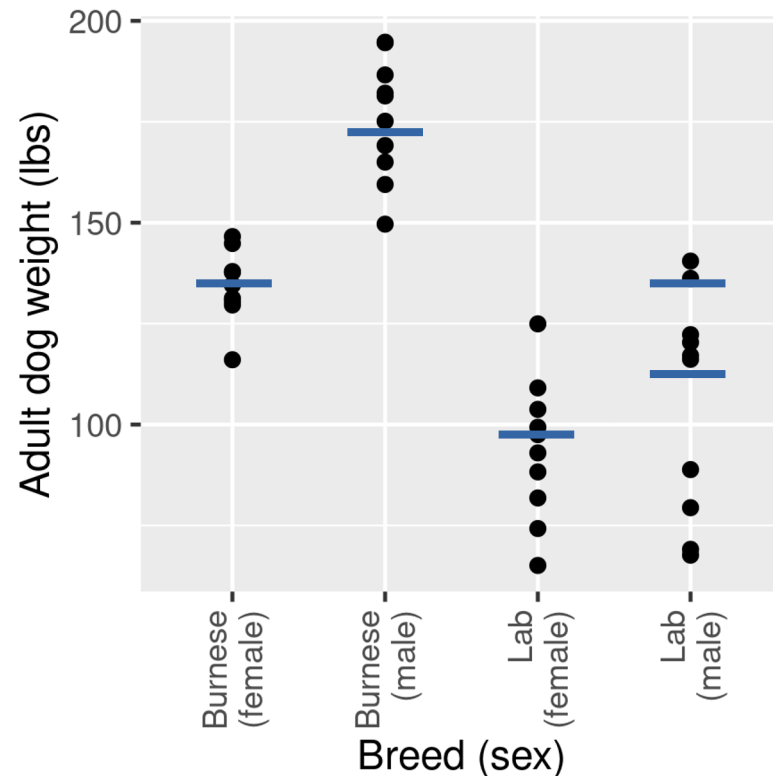


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Multivariate ANOVA

- Like a linear model, but response is matrix (rather than a single vector)

MANOVA

.Like a linear model, but response is matrix (rather than a single vector)

lm(Y1 ~ X)

| | |
|----|---|
| 1 | A |
| 3 | A |
| 16 | A |
| 5 | A |
| 1 | B |
| 4 | B |
| 5 | B |
| 2 | A |

MANOVA

.Like a linear model, but response is matrix (rather than a single vector)

$\text{lm}(Y1, Y2 \sim X)$

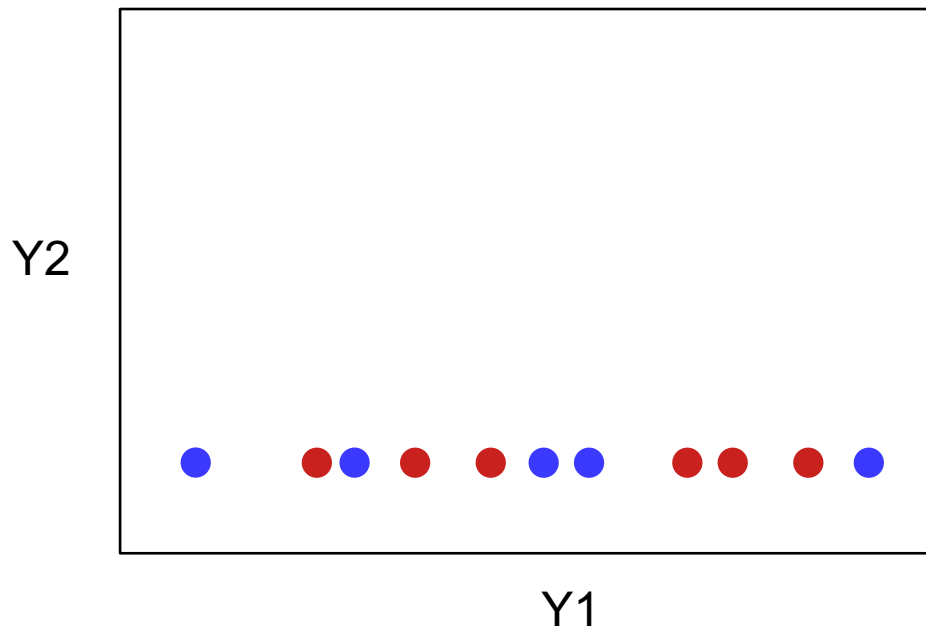
| | |
|---------|---|
| (1 15) | A |
| (3 25) | A |
| (16 12) | A |
| (5 4) | A |
| (1 10) | B |
| (4 90) | B |
| (5 63) | B |
| (2 14) | A |

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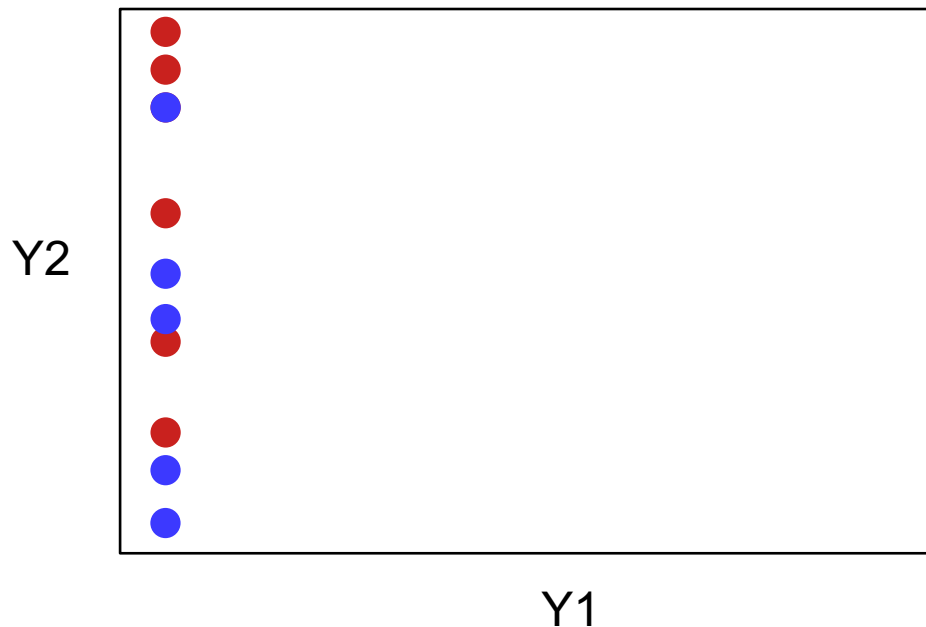


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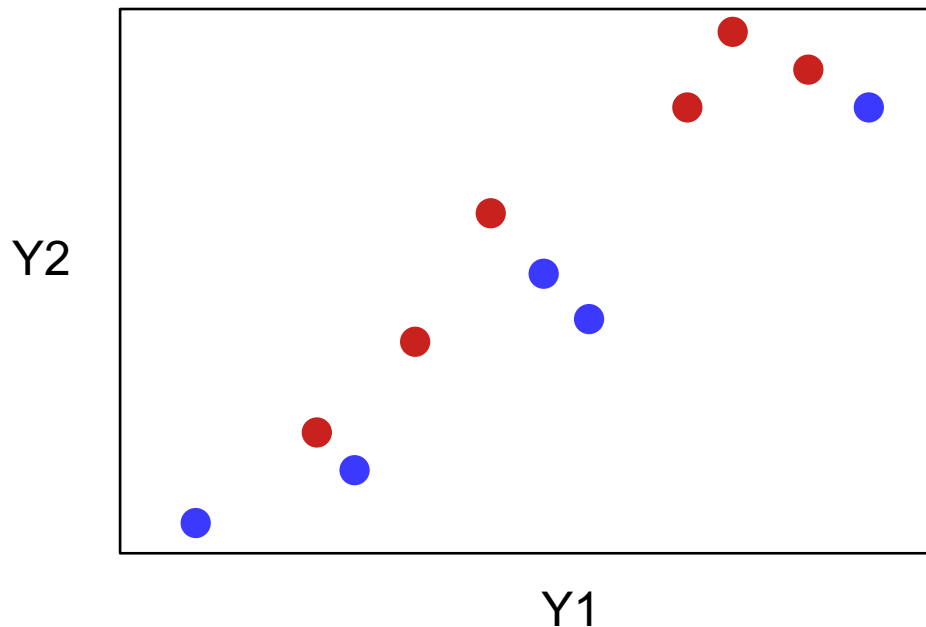


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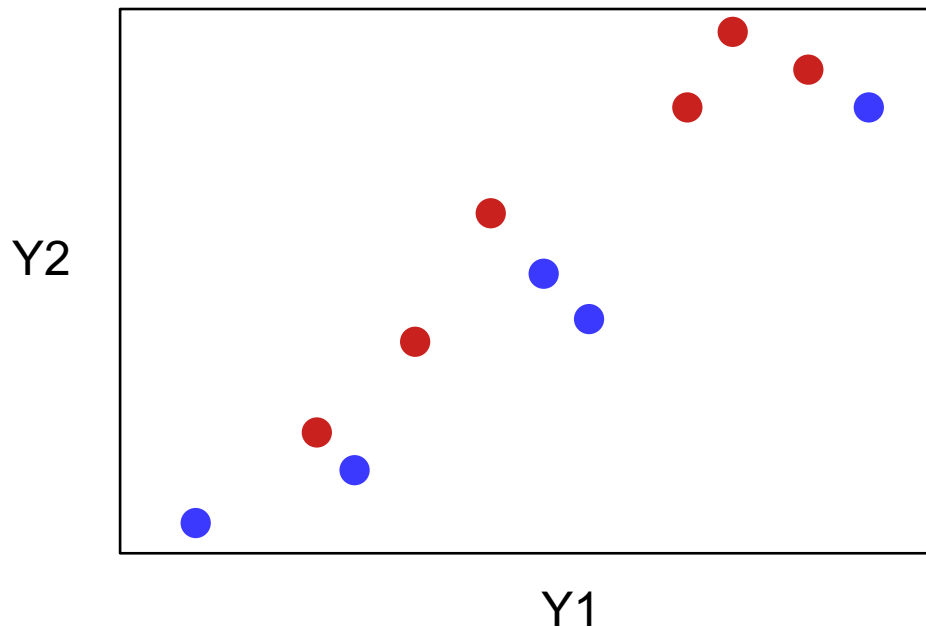
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MANOVA

.Like a linear model, but response is matrix (rather than a single vector)

```
mdl <- lm( MATRIX ~ X )  
manova_mdl <- manova(mdl)  
summary(manova_mdl)
```



Statistics with multiple variables

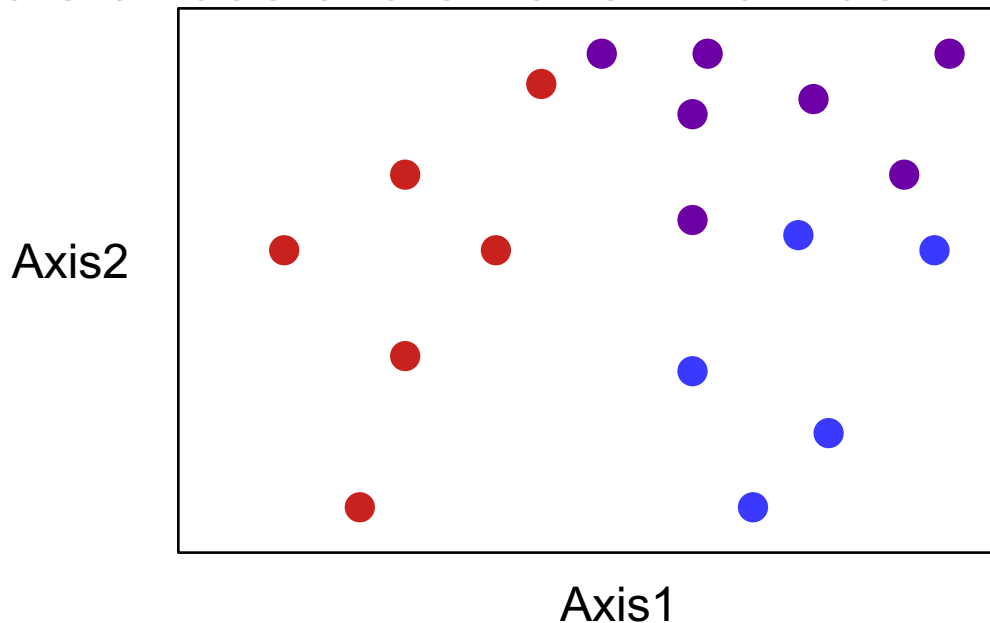
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Permutational ANOVA

- Non-parametric version of MANOVA
- Uses random sampling to test whether within-group distances are smaller than between-group distances

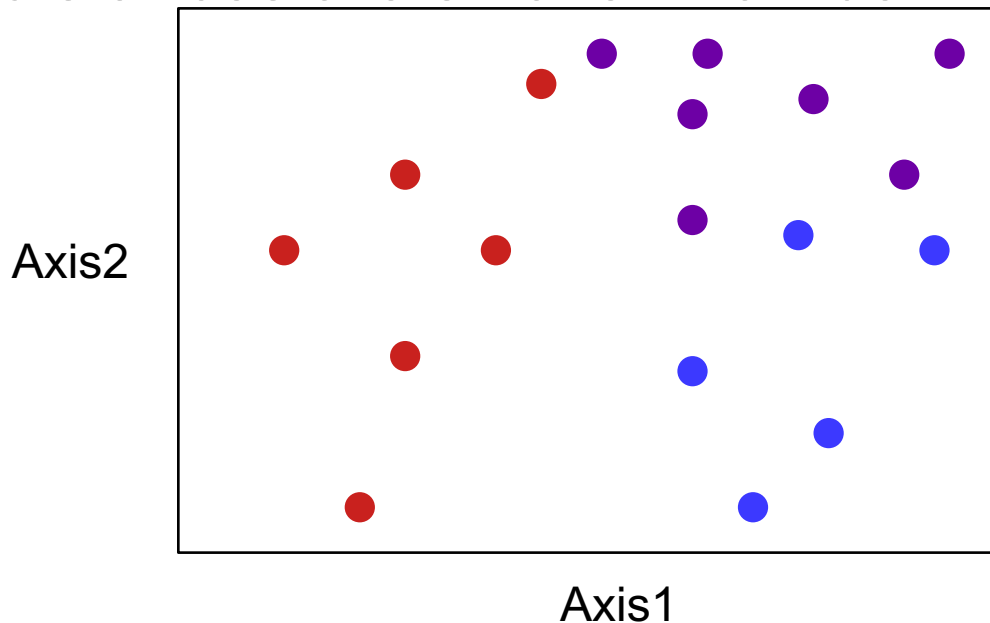
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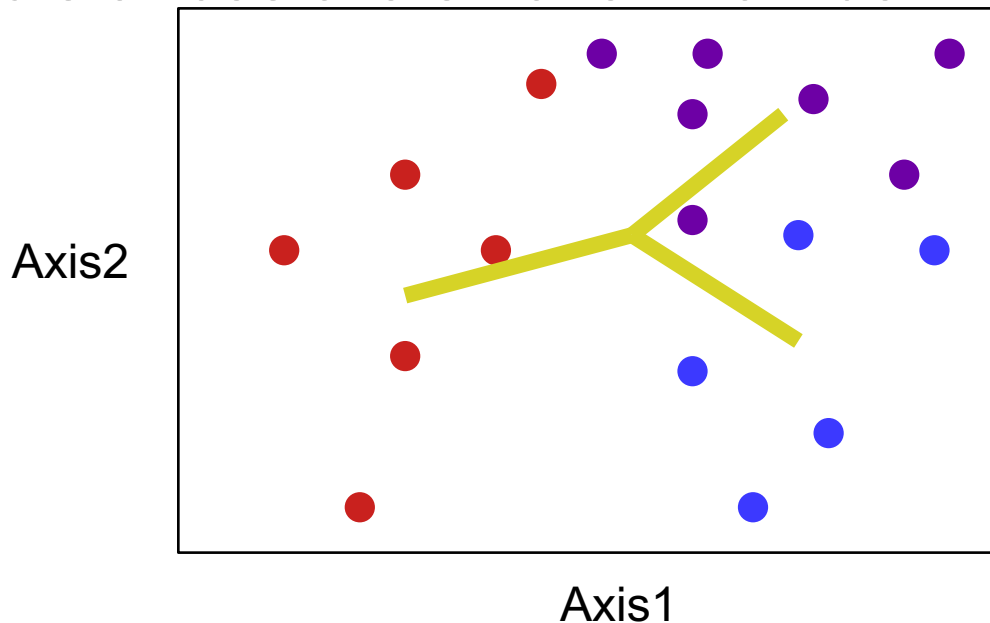
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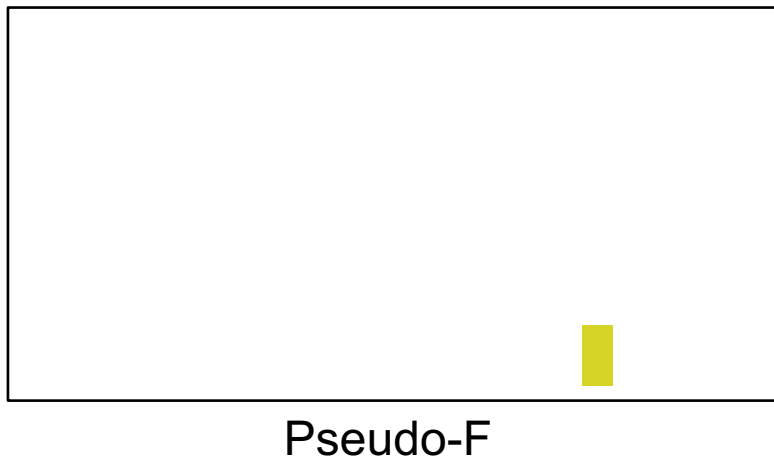


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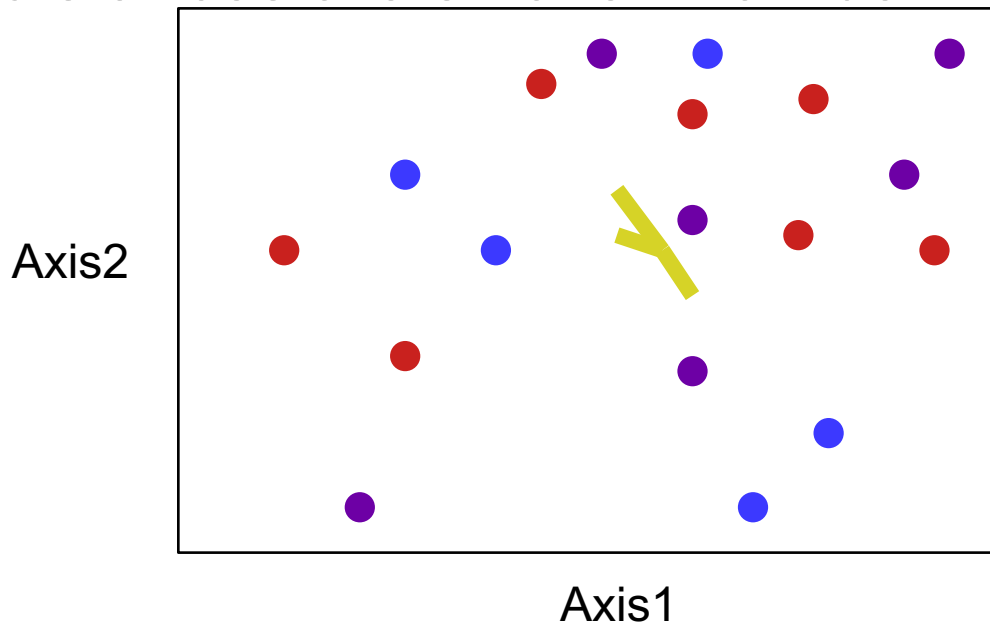


$$\frac{\sum \text{BetweenGroupDistance}^2}{\sum \text{WithinGroupDistance}^2} = \text{Pseudo-F}$$

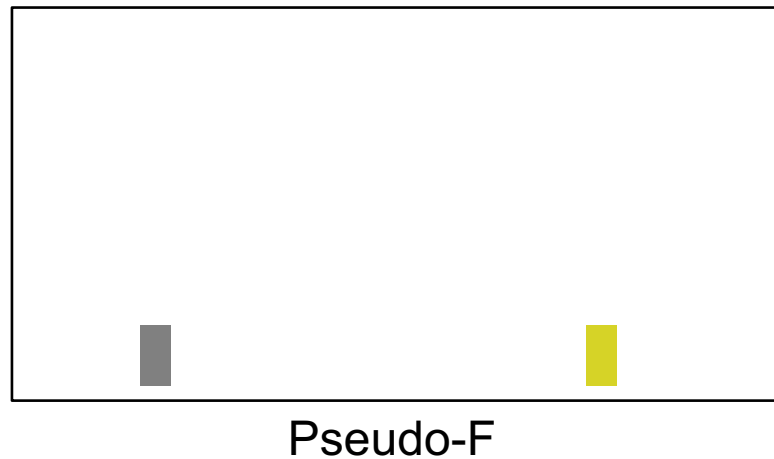


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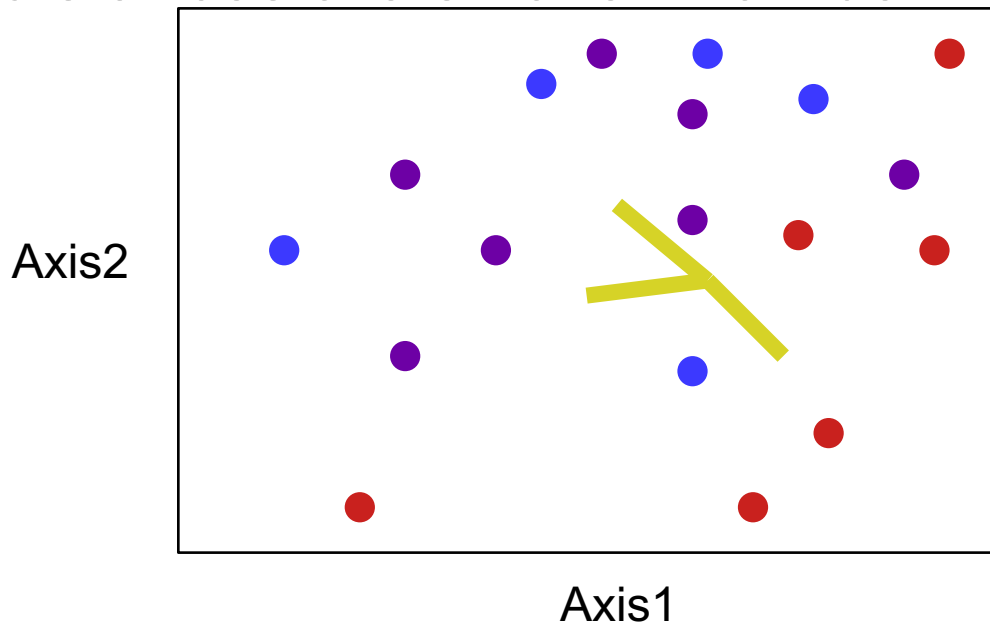


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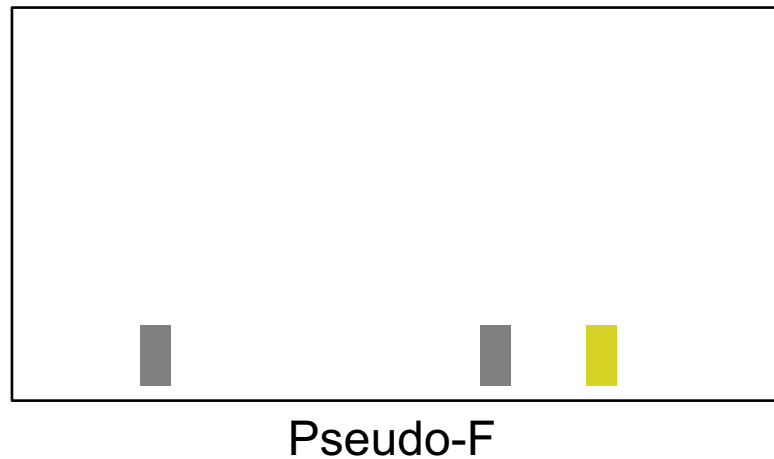


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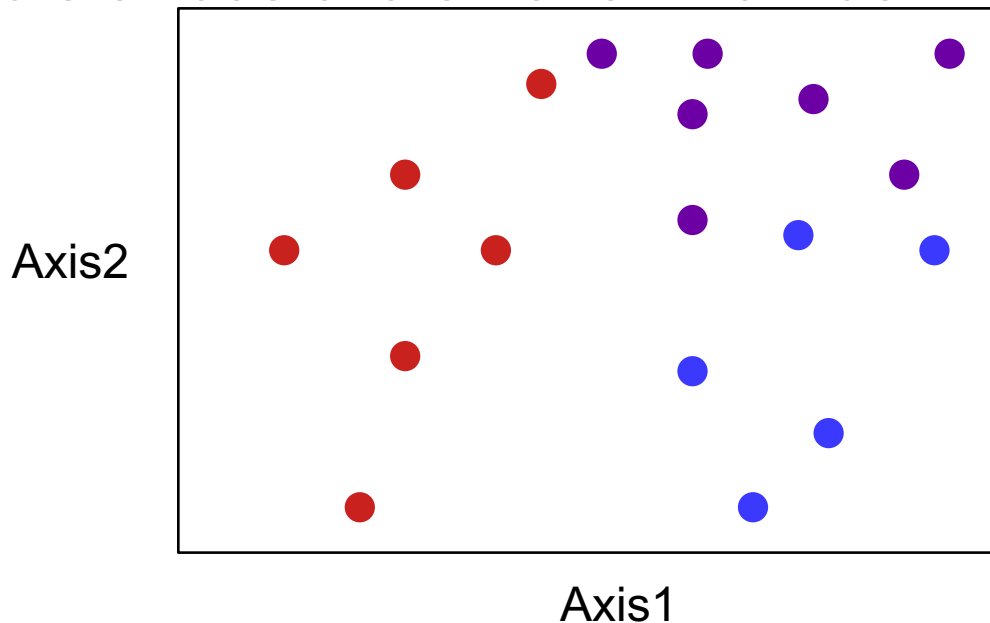


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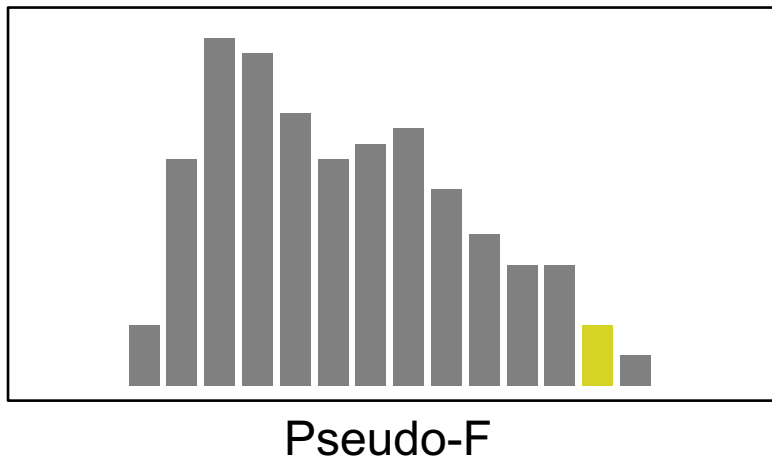


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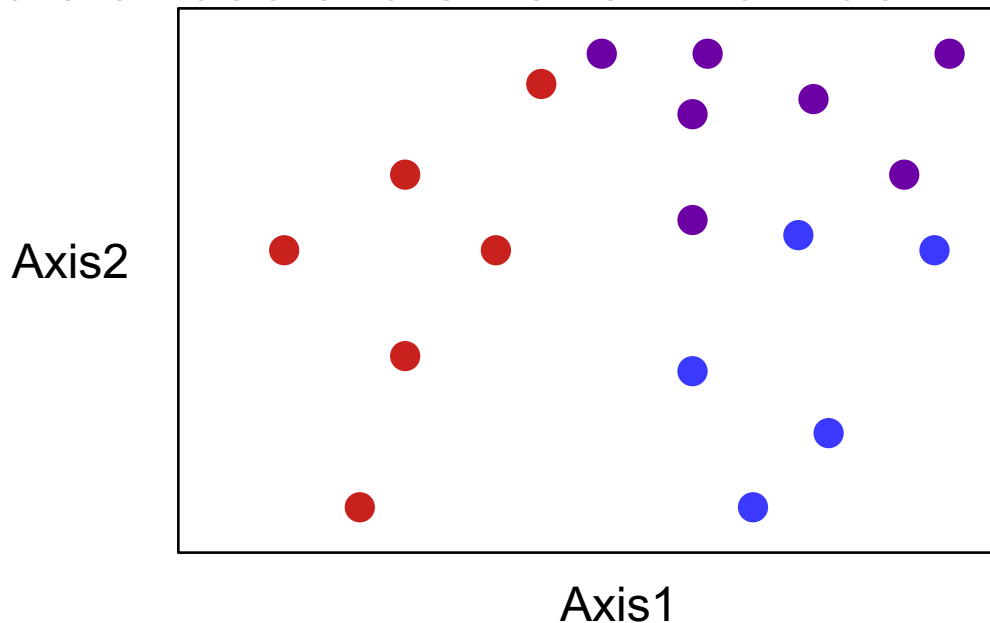


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`adonis2(MATRIX ~ X1*X2, dat=DATA)`

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| | ONE predictor variable | MULTIPLE predictor variables | | | | | | | | | |
|-------------------------------|---|--|----------------------------------|---------------------------------|-------------------------------|--|--|--|---|---|---|
| ONE response variable | <table border="1"> <thead> <tr> <th></th><th>CATEGORICAL independent variable</th><th>CONTINUOUS independent variable</th></tr> </thead> <tbody> <tr> <th>Continuous dependent variable</th><td> <u>T-test</u> (parametric) <u>ANOVA</u> (parametric, 2+ groups) </td><td> <u>Pearson's</u> product-moment correlation (parametric) </td></tr> <tr> <th></th><td> <u>Wilcoxon/Mann-Whitney</u> test (non-parametric) <u>Kruskal-wallis</u> test (non-parametric, 2+groups) </td><td> <u>Spearman's</u> rank correlation (non-parametric) </td></tr> </tbody> </table> | | CATEGORICAL independent variable | CONTINUOUS independent variable | Continuous dependent variable | <u>T-test</u> (parametric) <u>ANOVA</u> (parametric, 2+ groups) | <u>Pearson's</u> product-moment correlation (parametric) | | <u>Wilcoxon/Mann-Whitney</u> test (non-parametric) <u>Kruskal-wallis</u> test (non-parametric, 2+groups) | <u>Spearman's</u> rank correlation (non-parametric) | <p>lm + aov + summary</p> <p>lm + summary</p> |
| | CATEGORICAL independent variable | CONTINUOUS independent variable | | | | | | | | | |
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| | <u>Wilcoxon/Mann-Whitney</u> test (non-parametric) <u>Kruskal-wallis</u> test (non-parametric, 2+groups) | <u>Spearman's</u> rank correlation (non-parametric) | | | | | | | | | |
| MULTIPLE response variables | <p>lm + manova + summary</p> <p>dist + adonis2</p> | | | | | | | | | | |

Summary of all basic statistical tests

`.t.test()` # compare 2 means, parametric

`.wilcox.test()` # compare 2 means, non parametric

`.lm()` + `aov()` + `summary()` + `TukeyHSD()` # compare multiple means, parametric

`.kruskal.test()` # compare multiple means, non parametric

`.cor.test(method= "pearson")` # find correlation, parametric

`.cor.test(method= "spearman")` # find correlation, non parametric

`.lm()` + `summary()` # combine categorical + continuous predictors

`.lm()` + `manova` + `summary()` # multivariate response, any predictors, parametric

`.dist()` + `adonis2()` # multivariate response, any predictors, non-parametric