Experimental design

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Hint #1: Identify your study design!

Photo: Christian Pansch
Hint #1: Identify your study design!

Data collection and statistical testing

Observational scenario

- hypotheses are formulated *a posteriori* on base of data exploration
- number of data points collected is high
- half of the data should be used for exploration, the other half for testing
- predictors are mostly continuous

Experimental scenario

- *a priori* formulated hypotheses
- number of data points collected is low
- data can be fully used for testing
- predictors are mostly categorical
Hint #1: Identify your study design!
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Observational studies cannot prove causalities!
Carefully screen the literature and discuss your subject with other researchers!
Hint #2: Identify your hypotheses!
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Reinforcement by falsification of the null hypothesis

Important: Perfect complementarity!
Hint #2: Identify your hypotheses!

Job description

Work: design, prepare and run one single experiment

Budget: whole project is 1 billion US Dollars

Time for preparation: 16 years

Distance to experimental site: 300 000 000 km

Mission: Find life on Mars

Problem: The job is done
Is there life on Mars?

The labeled release experiment was positive

The gas chromatography/mass spectroscopy was negative
Think carefully about all possible outcomes of your experiment!
Hint #3: Know the terminology!
Hint #3: Know the terminology!

Population =
    totality of all units characterized by a variable

Sample =
    actually analyzed part of the population

(Experimental) Unit =
    replicate = parallel = sampling unit
Hint #3: Know the terminology!
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- **Treatment levels** = Samples
- **Treatment** = Factor
- **pCO₂**
- **pre-industrial** 2013 → 2100
- Tank 1 → Tank 1
- Tank 2 → Tank 2
- Tank 3 → Tank 3
- Tank 4 → Tank 4

**Sample size** = number of sampling units (n)
In this case n = 4 for each sample

The number of samples is 3

Total number of replicates is N = 12

Since n is the same in all samples, this design is balanced
Put a name to all physical and conceptual components of your experiment!
Hint #4: Know your variables!
Variables in a data set

- Independent
- Predictor
- Grouping
- Explanatory
- Factor
- Covariate

- Dependent
- Response
- Categorical
- Continuous

- Categorical
- Continuous
Variables in a data set

Independent

one

more than one

multifactorial

Dependent

one

multivariate
Hint #4: Know your variables!

Data quality levels
Limpets in intertidal horizons

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Nominal</th>
<th>Ordinal</th>
<th>Derived</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Y = 1</td>
<td>1</td>
<td>61%</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Y = 1</td>
<td>3</td>
<td>1%</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Y = 1</td>
<td>2</td>
<td>38%</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>N = 0</td>
<td>4</td>
<td>0%</td>
<td>0</td>
</tr>
</tbody>
</table>

Possible Transformation
Identify the character of your independent and dependent variables! Count them!
Hint #5: Understand your stats!

Photo: Sarah Piehl
Hint #5: Understand your stats

Why do we need stats?

Sources of variation:

• spatial heterogeneity

• temporal variability (life cycles, rhythms..)

• genotypic variability

• phenotypic plasticity

• methodical problems
Hint #5: Understand your stats

- Resource availability
- Genetic variability
- Phenotypic plasticity
- Parasites & pathogens
- Intra- and interspecific competition
- Predation
- Local climate
- Seasonality

Signal + Noise
A simple statistical model: the sample mean

Hint #5: Understand your stats

= amount of unexplained variance (residuals)

= amount of explained variance (model)
Hint #5: Understand your stats

The t-statistic:

\[ t = \frac{x_1 - x_2}{\sqrt{s_1^2/n_1 + s_2^2/n_2}} \]

where:
- \( x_1 \) and \( x_2 \) are the means
- \( s_1^2 \) and \( s_2^2 \) are the variances
- \( n_1 \) and \( n_2 \) are the sample sizes

The test statistic

\[ \frac{\text{variance explained by the fertilizer}}{\text{variance not explained by the fertilizer}} \]

\( X = \text{mean} \)

\( S = \text{standard deviation} \)

\( n = \text{sample size} \)

\[ t = 8.69 \]
Effect size

Amount of unexplained variation

Sample size

Inside t-test:

$$t = \frac{x_1 - x_2}{\sqrt{s_1^2/n_1 + s_2^2/n_2}}$$

Hint #5: Understand your stats
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Where do p-levels grow?

Where in this distribution is our observed value of t located?

If it is outside the 95% range, we assume that it is not random.
Error? What error?

Type I error: Rejecting the null hypothesis though it is right. We believe there is an effect, but there is not. This is the $\alpha$-level, it is by convention $p \leq 0.05$ (5 %, Fisher’s criterion).

Type II error: Accepting the null hypothesis though it is wrong. We believe there is no effect, but there is one. This is the $\beta$-level, it should not be larger than 0.2.
In simple words….

Type I error: We see something, where there is nothing

Type II error: We overlook the effect

Test power 1 – $\beta$: The probability to detect an existing effect. It should be at 0.8.
Hint #5: Understand your stats

Assumptions of statistical tests

- Independency between samples
  (unless it is a repeated measures study)

- Independency within samples

- Random sampling

- Non-collinearity between independent variables

- Homogenous variances

- Normality of data

Stats are easy, but data can be difficult!
There is no easy way out: Read textbooks, join stats courses!
Hint #6: Avoid pseudoreplication
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Factor: CO$_2$ partial pressure with 4 levels

10 tanks per level

20 mussel individuals per tank

2 valves per mussel

Pseudoreplicated:

$40 \times 20 \times 2 = 1600 = 1596$ df

Replicated:

$4 \times (10-1) = 36$ df
Hint #6: Avoid pseudoreplication

Intermediate Disturbance Hypothesis (IDH) in sensu Grime (1973) und Connell (1978)

- Species richness vs. disturbance
- Low disturbance: competitive exclusion
- High disturbance: exclusion due to harsh environmental conditions
- Intermediate disturbance: coexistence
Hint #6: Avoid pseudoreplication
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**Sources of pseudoreplication:**

1. Shared enclosure
2. Common environment
3. Relatedness
4. Pseudoreplicated stimuli
5. Repeated measures
Discuss your experimental design and set-up with other researchers!
Hint #7: Identify your stats!
Comparing proportions (nominal data)

2 independent samples
- chi-square test

2 dependent samples
- sign test
Hint #7: Identify your stats

Comparing medians (ordinal or interval data)

2 independent samples
Mann-Whitney-U test

> 2 dependent samples
Friedmann‘s ANOVA

2 dependent samples
Wilcoxon signed-rank test

> 2 independent samples
Kruskal-Wallis ANOVA
Comparing means (interval data)

- **2 independent samples**: t-test
- **2 dependent samples**: paired t-test
- **> 2 independent samples**: ANOVA
- **> 2 dependent samples**: Repeated Measures ANOVA

Hint #7: Identify your stats
Find the right stats:

• What are my hypotheses?
• How many samples do I have?
• Are my samples independent or dependent?
• What is my data quality level?
• How are my data distributed (if interval)?
• How many independents do I have?
• Are they categorical or continuous?
• How many dependents do I have?
• Are they categorical or continuous?
Do it before you collect your data! Generate a fake data set and simulate the analysis!
Hint #8: Estimate your test power!
Effect size

$t = \frac{x_1 - x_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$

Sample size

Power analysis can estimate the test power that can be achieved with a given number of replicates!

Hint #8: Estimate your test power!
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Power analysis can estimate the number of replicates that is needed to achieve a certain test power!
A power analysis includes:

1. Observations per group (=n)
2. Delta (=difference between means)
3. Standard deviation
4. Significance level
5. Power level aimed for (should be at least 0.8)
6. The kind of test you want to use
Prospective Power Analysis: how can you know about effects sizes and variability?

- Literature
- Pilot studies
- Academic guess

Hint #8: Estimate your test power!
Do it!
Finally...

Ruxton GD, Colegrave N (2010).
Experimental Design for the Life Sciences.

Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance.
Cambridge University Press