Module 1: Experimental design

1.1 Why experimental design?

> *Yvonne:* Where were you last night? *Rick:* That's so long ago, I don't remember. *Yvonne:* Will I see you tonight? *Rick:* I never make plans that far ahead. [*Casablanca,* 1942]

We consider some basic principles behind good experimental design, and discuss how valid scientific conclusions can be reached from an appropriately designed experiment.

1.1.1 Themes

- Think before you act!
- A little planning saves a lot of (lab) time.
- Gather information as efficiently and as accurately as possible.
- Make a careful plan, and follow it carefully.
- Consider possible sources of errors and variation.
- Statistics cannot fix a badly designed experiment!
- Poorly collected data lead to poor conclusions.
- If you must break the rules, do so consciously.

1.1.2 Ask the right question

Here are some points that should help you getting started.

- Define a clear hypothesis.
- Do a pilot experiment, and consider previous data.
- Making sure your technique works.
- Observational vs. manipulative study.
- Field/plant or laboratory?
- Observe under a variety of conditions.
- Think bout confounders.
- Think about sample size vs. precision.

Remember that science is not so much about answering the question, but about answering the right question.

1.1.3 Check list

- Clarify the objective
- Summarize beliefs and uncertainties
- Decide on a strategy
- Perform a pilot experiment
- Plan a single trial

- Design the experiment
- Ethical considerations
- Collect the data
- Update beliefs and uncertainties
- Revisit the objective

(Robinson, 2000)

1.2 Motivating examples

1.2.1 Canteen usage

- *Objective:* How big a difference is there between the number of costumers in the canteen during lecture time (15 past to 00) and breaks (00 to 15 past)?
- *Beliefs:* There are much fewer costumers during lecture time.
- *Uncertainties:* The number of costumers varies during the day, during the week and from month to month.
- *Strategy:* Go and count the number of people during lecture time and breaks, and compare.
- *Pilot experiment:* Count on a single day.
- *Single trial:* Count obtained on a specific day and time of day.
- *Design the experiment:*
  - Count during 5 weeks spread out over the semester.
  - Count every day during each week.
  - Count morning, noon and afternoon on each day.
- *Ethical considerations:* Don't disturb the costumers!
- *Collect the data:* Get a team together.
- *Update beliefs and uncertainties*
- *Revisit the objective*

1.2.2 Latin square design

- Rectangular table with two factors and one treatment factor.
- Example: 5×5 Latin square, weeks A, B, C, D, E.

	Mon	**Tue**	**Wed**	**Thu**	**Fri**
8 a.m.	A	B	C	D	E
10 a.m.	B	C	D	E	A
12 p.m.	C	D	E	A	B
2 p.m.	D	E	A	B	C
4 p.m.	E	A	B	C	D

- Note a factor 5 reduction of the work.

1.2.3 Screening chemical compounds

- A certain chemical reaction may depend on the presence of other chemical elements.
- Safety or environmental screenings may involve the response to the presence or not of certain chemical elements.
- There may be interactions between two or more chemical elements.
- Example: 10 elements, low dose versus high dose gives $2^{10} = 1024$ (factorial experiment).
- If each experiment takes 30 minutes, gives a total of 512 hours of lab time!
- Fractional factorial design: $2^{10-6} = 16$, or 8 hours.
- Reduces the about of work by 98.43%!

1.2.4 Example: Proteomics

- Response variable Y: protein expression (size of mass spectrum peak).
- Units: Patients (40)
- Factors that affect the response variable:
  - Tissue samples within patient (2)
  - Spectra within tissue sample (3)
  - Sex (2)
  - Time (5)
  - Malignant (yes/no) (2)
- Continuous variables: age, BMI, cholesterol, ...

1.3 Important notions

Here are some basic notions used in experimental design.

- *Response variable* Y: the outcome to be measured.
- *Experimental unit*: single piece of experimental material.
- *Trial*: measuring Y on a unit.
- *Experimental error:* random variation in the outcome.
- *Replication*: making repeated independent trials under identical conditions.
- *Pilot experiment*: small initial experiment.
- *Experimental factors; covariates*: things that may influence the outcome.
- *Treatment*: factor(s) of main interest.
- *Confounders*: all other important factors (observed or not).
- *Block:* set of units which are similar, e.g. from same batch.
- *Blocking factor:* a factor that distinguishes between blocks.
- *Blinded study:* when the treatment is unknown to the observer.
- **Main strategy**:
  Control what you can, randomize for the rest.
  - *Controlling:* take the factor into account.
    Tends to decrease the experimental error.
  - *Randomization:* assign treatments at random to units.
    Uncontrolled confounders may increase experimental error.
- **TAKE RANDOMIZATION SERIOUSLY!**
- Randomization: every member of the population is equally likely to be included in your sample.

1.4 Variation, replication and sampling

- Replication is crucial for estimating the experimental error (SD, also denoted s).
- Obtain replicates $Y_1, \ldots, Y_n$ (random sample).
- Calculate $\bar{Y}$, SD.
- SD measures the variation.

- Accuracy of $\bar{Y}$ as estimator of μ:

$$\text{SE} = \frac{\text{SD}}{\sqrt{n}}$$

- Small SE requires small SD, large n, or both.
- If in doubt, start with $n = 3$.

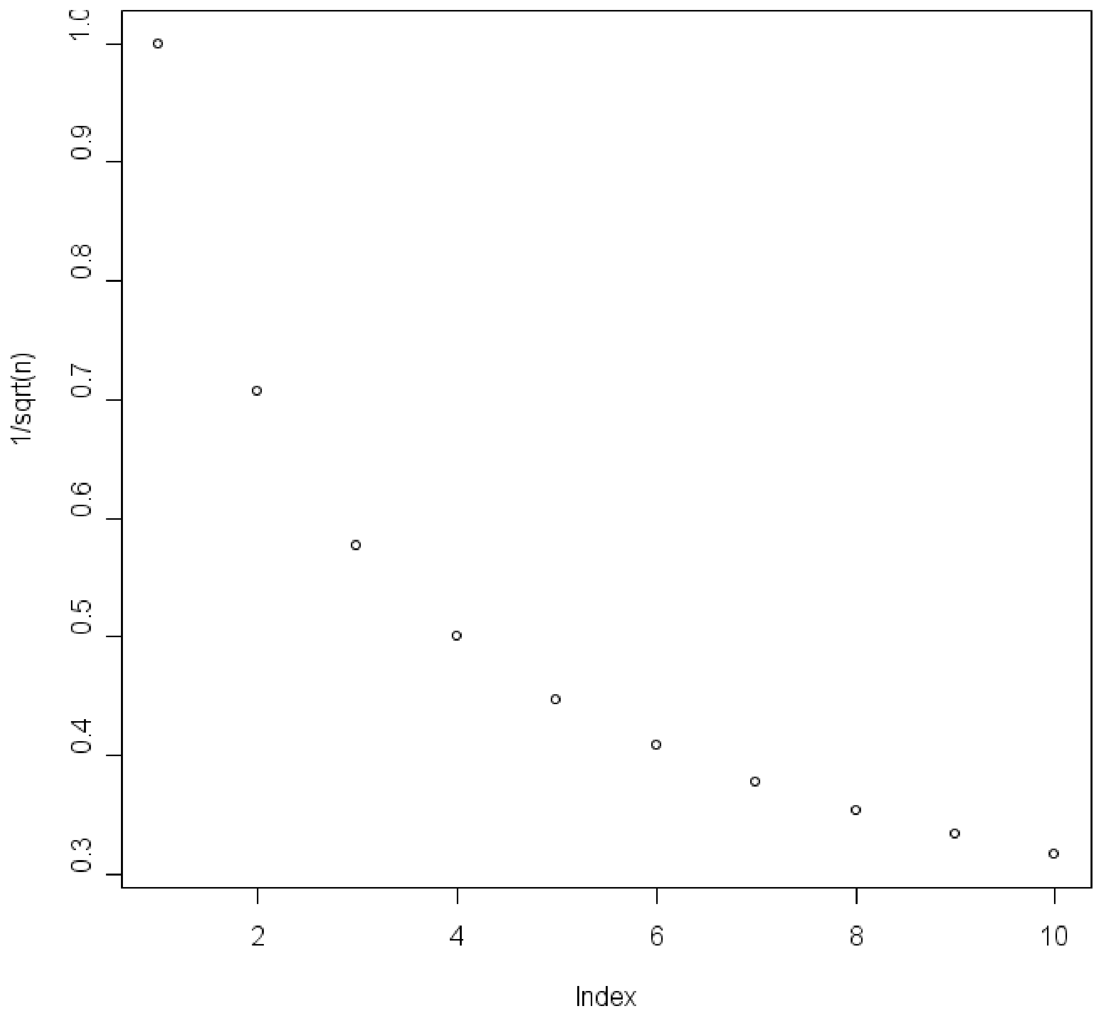

Figure 1: SE as a function of n.

1.5 How large a sample?

- Obtain initial estimate of SD (pilot experiment).

- How many replicates? Enough to bring the SE down!
- Type I and Type II errors when comparing two treatments:

Experiment:	**No difference**	**Difference**
World:		
No difference	Correct	Type I error
Difference	Type II error	Correct

- Fix probability of Type I error ($\alpha = 5\%$).
- Make Type II error small by increasing sample size.

1.6 Pseudo-replication and study population

- Pseudo-replication is a problem that occurs if your replicates are not truly independent.
- Example:
  - We are interested in the between-individual variation.
  - *True replication* means investigating independent individuals, randomly sampled.
  - *Pseudoreplication* means taking several measurements on each individual.
- Think of *prediction*:
  - Sample of individuals allow you to make predictions about *other* individuals.
  - Repeated measurements on one individual allow you to make predictions about future measurements made on *that* individual.
- *Study population:* a random sample from a certain population enables you to make predictions about that specific population.

1.7 Different experimental designs

Here are a few simple (and not so simple) types of experimental design.

- One sample, consisting of n replicates.
- Two independent samples: compare two treatments.
- Completely randomized design, k treatments (preferably balanced).
- Randomized complete block design (k treatments in each block).
- Matched pairs (case/control).

- Two-factor design with no replication.
  - The notion of interaction.
- Two-factor design with equal replication (balanced).
- r-factor design with/without replication, balanced/ unbalanced.
- Cross-over designs.
- Split-plot designs (main-plot factor, sub-plot factor).
- Response surface methodology.

1.7.1 About factors

- Crossed factors:

Malignant:	**Yes**	**No**
Sex:		
Male	$\bar{x}_{11}$	$\bar{x}_{12}$
Female	$\bar{x}_{21}$	$\bar{x}_{22}$

  Sex and disease status are crossed factors.

- Nested factors:

Spectrum:	**1**	**2**	**3**
Tissue sample:			
1	$\bar{x}_{11}$	$\bar{x}_{12}$	
2	$\bar{x}_{21}$	$\bar{x}_{22}$	$\bar{x}_{23}$

  Spectrum is nested within tissue sample.

1.8 Collecting the data

Some consideration about collection data:

- Calibrate your instrument.
- Accuracy: getting it right on average (vs. bias).
- Precision: measurements are repeatable (right or wrong).
- Observer variability:
  - intra-observer;
  - inter-observer.
- Watch out for observer drift.

- Trade-off between precision and cost.
- Randomize to avoid observer variability.
- Be consistent in your data recording.

1.9 Making conclusions

- Each type of design requires a specific type of statistical analysis.
- Know the analysis before carrying out the experiment.
- Make a plot that shows both $\bar{Y}$ and SD.
- Use statistics to confirm what you see in your data.
- Get a small repertoire of simple designs that you feel comfortable using.

1.10 Experimental design flow chart

The following flow chart, adapted from Ruxton and Colegrave (2003), takes you through some important considerations that are relevant for experiments in science.

a) Preliminaries

1. Formulate a clear research question.
2. Form hypotheses that address the question.
3. Form predictions from the hypotheses.
4. Do the predictions follow logically from the hypotheses?
   - If not, return to 3.
5. Are the predictions testable?
   - If not, return to 3.
6. Is each prediction the strongest possible test of the hypotheses?
   - If not, return to 3.
7. Perform a pilot experiment.

b) Structure

1. Observational or manipulative study?

2. Observational study:
    - Problems with reverse causation?
        - If yes, return to 1.
    - Problems with third variables?
        - If yes, return to 1.
3. Manipulative study:
    - Is manipulation possible?
        - If not, return to 1.
    - Is manipulation biologically realistic?
        - If not, return to 1.
4. Do you need controls?
5. Have you selected the best controls?
6. Ethical problems?
    - If yes, return to 1.

c) Measurements

1. Decide about what data to collect.
2. Select a design and statistical analysis.
3. Select an appropriate number of replicates.
4. Is this number practical?
    - If not, return to 3.
5. Does this number raise ethical concerns?
    - If yes, return to 'Structure'.
6. Are your samples truly independent?
    - If not, return to 'Structure'.
7. Consider the reliability of your data.

d) Final checks

1. Ethics still a problem?
   - If yes, return to 'Structure'.
2. Would your design satisfy the devil's advocate?
   - If not, return to 'Preliminaries'.
3. Sleep on it.
4. If in doubt, discuss it with a friend.
   - In case of problems, return to the appropriate point.
5. Have you performed a pilot experiment?
   - If not, return to 'Preliminaries'.
6. Start the experiment with our best wishes.

1.11 Experimental design check list

The following check list, adapted from Robinson (2000), takes you through some of the main steps in the planning of an experiment, including some points that are relevant if you work in a large organization. It complements the above check list.

1. Clarify the objective
   - Consider who to consult.
   - Ask questions.
2. Summarize beliefs and uncertainties
   - Decide whether you need to experiment.
   - Consider relevant science and technology.
   - Consider information from existing data.
   - List response variables and factors.
   - Consider possible transformations of both response variables and factors.
3. Decide on a strategy
   - Beware of the one-factor-at-a-time experimentation.
   - Consider main effects and interactions.
   - Consider strategies for dealing with sampling and measurement errors.

4. Ethical and resource considerations
    - Consider how to reduce the size of the experiment without sacrificing information.
    - Consider doing a laboratory experiment instead of field work or production runs.
5. Perform a pilot experiment
    - Familiarize yourself with the experimental equipment and techniques.
    - Obtain estimates of sampling and measurement variances.
    - Use at least two levels for the main treatment factor.
6. Plan a single trial
    - Decide which response variables to measure.
    - Decide which factors to include and at what levels.
    - Search for efficiencies.
    - Decide when, where and with what materials.
    - Take precautions to avoid biases.
    - Cope with other sources of variation.
7. Design the experiment
    - The concepts of confounding and orthogonality.
    - Choose the appropriate number of runs.
    - Select an experimental design.
    - Seek help from a statistical consulting service.
8. Revisit ethical and resource considerations
    - Revisit previous questions about resources and ethics.
    - Consider the use of resources from the point of view of your group, your company, and society at large.
9. Collect the data
    - Set up a data-recording system.
    - Decide whether to use paper, spreadsheet or some form of electronic collection of data.
    - Try to anticipate possible problems.
    - Perform runs and record data.
    - Make a detailed record of what happened.

10. Update beliefs and uncertainties
    - Cross-examine the data.
    - Focus again on your objective.
    - Describe trends.
    - Describe uncertainties.
    - Check assumptions.
    - Test hypotheses.
    - Seek help from a statistical consulting service.
11. Revisit the objective
    - Reconsider objective and planned strategy.
    - Summarize and communicate results.
    - Help make changes happen.
    - Contribute to improving other things in your group and organization.

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