

Advanced Higher Biology



Unit 3: Investigative Biology

Revision Notes

Valid Experiments

Valid Experiments

For conclusions to be valid there must have been

1. Adequate control of all **confounding variables**
2. Necessary **controls** to be carried out (positive/negative)

Controls

Needed for **valid results** to provide a **comparison** with treatment.

Positive Controls

A positive control is a treatment that is included to check that the **system can detect a positive result** when it occurs

Negative Controls

Exact same set up but with absence of treatment (independent variable) & replace with water (if appropriate)

This proves the independent variable is responsible for **altering the dependent variable**.

In **human clinical trials** control groups **placebo group** help account for the placebo effect

Placebo effect: expectation of being treated affects dependent variable measurement even by sugar pill/saline injection.

Confounding Variables

Due to the complexities of biological systems, **other variables** besides the independent variable may **affect the dependent variable**.

1. Held constant ideally
2. Monitored if not so that their effect on the results can be accounted for in the analysis.
3. Random block design to eliminate bias

Example

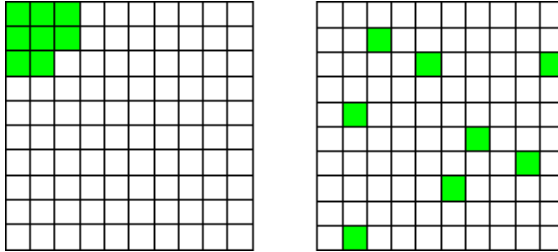
Reaction at room temperature as not possible for water bath e.g. in colorimetry measure temperature of

Types of Sampling

1. Random sampling

Members of the population have an **equal chance** of being selected.

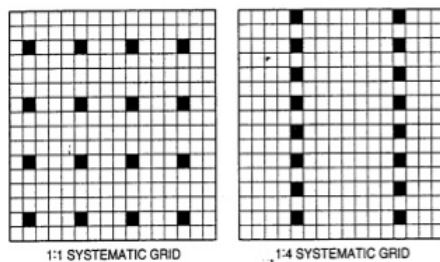
Difficult to achieve—use number generator to ensure truly random



2. Systematic sampling

Members of a population are selected at **regular intervals** (non random sampling)

Example line/belt transect quadrats every 2m OR every 3rd person etc



3. Stratified sampling

The population is **divided into categories**—some categories make up a greater proportion than others so that each category is **sampled proportionally** to the total.

Use to sample **habitats that are not uniform** using a formula to calculate the number of samples from each area.

Human Example

More S1 pupils than S6 pupils in the school; sample proportional to total not random or 20 for each year group



Reliability & Representative Sampling

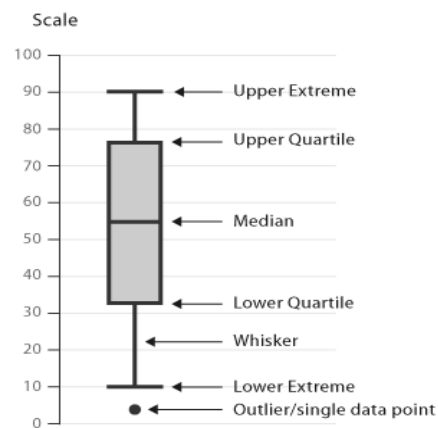
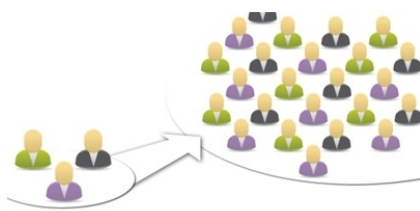
When to use representative Sampling

When it is **impractical to measure every individual**, a representative sample is selected.

What is representative Sampling

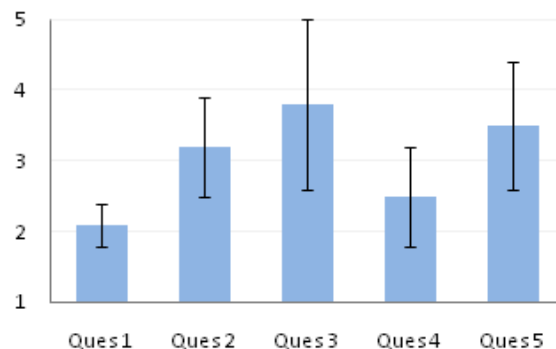
A representative sample should share the **same mean** and the **same degree of variation** about the mean as the population as a whole.

Shown via **descriptive statistical analysis** of measures of central tendency via box plot analysis of mean, median, mode, range & interquartile range & error whiskers (not error bars)



Sample Size & Reliable Results

The extent of the natural variation within a population determines the appropriate sample size as shown by SEM error bars.



More variable populations (Ques 3 & 5) have larger SEM error bars & **require more samples** to be **representative**

Less variable populations (Ques 1) have smaller SEM error bars & require a smaller sample size

Importance of Representative sampling

If a representative sample has not been selected there is **sampling bias**

i.e. sample size too small

The data and therefore conclusions are not reliable and cannot be accepted

Variation in results

Variation in results attributable to

- Inherent variation in the specimens.
- Reliability of measurement methods (precision error issues)

**** Remember widely variable results are not reliable*****

Inherent Variation

Some of the variation in results is due to inherent variation that exists in nearly all biological organism studied due to **sexual reproduction**.

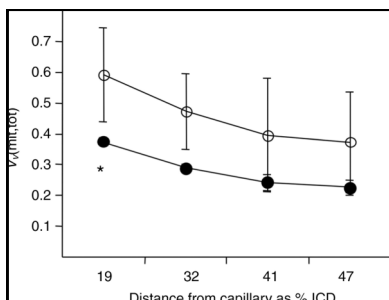
Exception—bacteria reproduce asexually/some plants reproduce by vegetative propagation e.g runners

Working Out Natural Variation

Natural variation can be determined by **measuring a sample of individuals** from the population and analysing the spread to see if the sample is representative

Analysing Variation in Sample

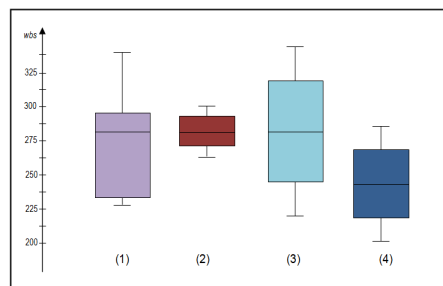
1. SEM—size of error bars



Large variation in experimental results is shown by **larger SEM error bars**

(white circles larger variation than black circles)

2. Box plot analysis



Data 1 shows skewed data to lower quartiles
Compared to **data 2, 3 & 4**

2. Precise Readings

Determined by **repeated readings** of an individual datum point.

The variation observed indicates the **precision** of the measurement instrument

Reliability & Independent Sampling

Importance of Independent Replication

Overall results can only be considered reliable if they come from two independent replicates (two fields or two clinical trials) showing a similar result with at least two samples in each replicate

Results not reliable

If all data comes from **single** source then no independent replication has occurred and data is not reliable.

OR

If the two independent replicates contradict each other the results are not reliable and the conclusions can not be accepted.

OR

Sample size too small

Reliability of Measurement Methods

Reliability Of Measurement Methods

Some of the variation observed in experimental results is due to the **reliability of measurement methods** which are in turn affected by the **precision & accuracy of the data**.

Precision (repeated measures)

How **close repeated measures** are to one another on a single datum point in a sample.

Necessary for each sample to be **measured more than once** to comment on precision of equipment which affects reliability of data.

Accuracy (calibrate against standard)

How **close the reading is to the actual value**.

Often not **possible to work out “actual value”** in biological systems unless **calibration** against a known standard is feasible.

Precision not same as Accuracy

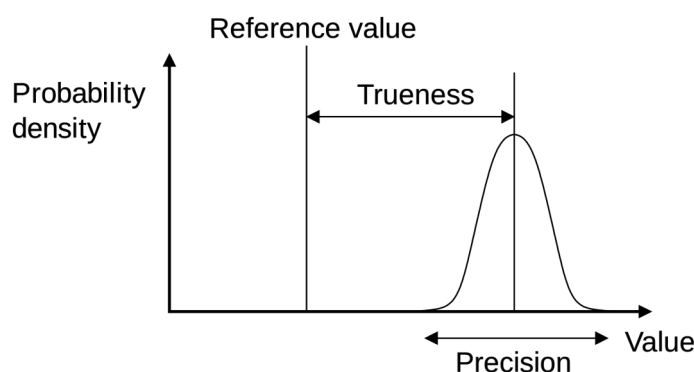
Variation in repeated measurements of a **single sample** indicates the precision of the measurement instrument or procedure but not necessarily its accuracy

A result can be precise (repeated measurements similar) but not accurate (deviate from true value) showing a very **similar result**.

Precision vs Accuracy

Precision is more important in terms of reliability than accuracy .

If results are imprecise the variation in results will be larger and will not allow reliable conclusions to be drawn. Variations in accuracy are **less important** as they tend to **deviate the results in the same direction** away from the true value each time and thus a **causation trend** can still be ascertained.



Reliable Results & Independent Replication

Random errors

and

systematic errors



Neither accurate nor precise



Precise, not accurate



Accurate, not precise



Accurate and precise

Variables

Independent Variable

The variable being altered with a series of values in the experiment that are chosen by the researcher to investigate causation of changing the independent variable on the dependent variable.

Dependent Variable

Variable being measured in the investigation. Often an indirect technique is being used but all conclusions/aim should refer back directly to the actual dependent variable.

Dependent variable	In direct measurement
Rate of photosynthesis	number of bubbles per minutes/colorimetry

Experimental Designs

Simple One Factorial Designs

Also called **In Vitro Studies**

Simple lab experiments allow variables to be controlled easily and follow the pattern below

- A) the manipulation of the independent variable by the investigator
- B) all other confounding variables are kept constant.
- C) The results are compared with positive and negative controls where possible.

This allows a **valid conclusion** on the **causation of the independent variable** on the dependent variable.

Disadvantage

Its findings may **not be applicable to a wider setting** where multiple variables come into play.

Variables

Multi factorial Designs

More than one independent variable/combination of independent variables being investigated as variables are interconnected.

More complicated but more realistic to real life setting.

Example—Field work/Field Trials

Does not permit control of all confounding variables unlike laboratory conditions

Use of randomised block design to help eliminate bias.

Example light intensity/pH/moisture all may affect plant distribution in quadrats OR field trial results

In Vivo vs In Vitro Studies

In Vitro Studies

Experiments on part of an organism/tissue or cell in the laboratory with a simple one factorial design.

In Vivo Studies

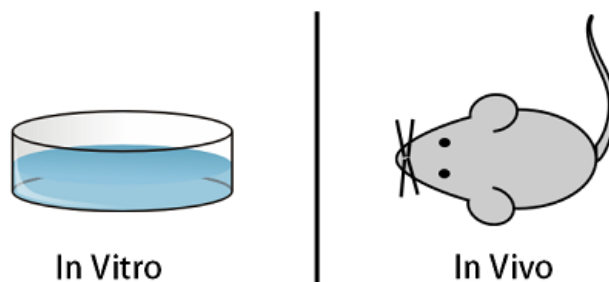
The effects of an independent variable on whole, living organisms through animal testing/clinical trials.

More complex as often confounding variables cannot be controlled & **randomised block design** required.

Examples

Drug to lower blood pressure. Other factors such as exercise, stress and diet may affect this.

Randomised block design is required to reduce effect of confounding variables on result validity.



Correlation vs Causation

Causation studies

Whether In Vitro (simple) or In Vivo multi factorial design there is an independent variable being held constant with confounding variables held constant or randomly distributed through random block design to prove causation of independent variable on dependent variable

Correlation Observational Studies

Investigators use groups that already exist, so there is no truly independent variable.

Both variables are simply being measured (not deliberately altered) and the connection between these two variables examined.

The researchers are not in control of the values of the variables. Simply by making lots of measurements all values of the two variables should be able to be measured and relationship derived.

These 'observational' studies are good at detecting correlation but, as they do not directly test the model by changing one variable & holding other variables constant while measuring the other variable they are not able to determine causation.

Examples

Light intensity Number of daisies

Heart rate blood pressure

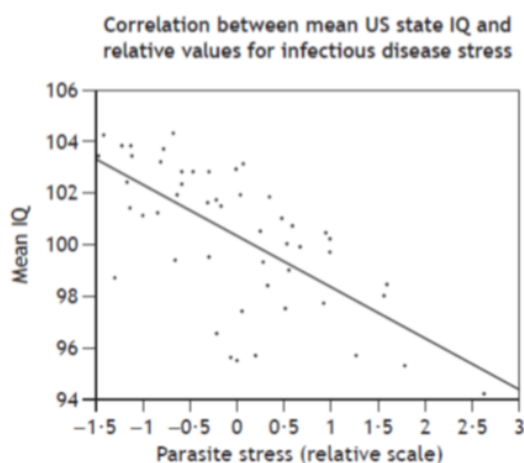
Scatter plot

A scatter plot is used to measure correlation.

It does not matter which variable goes on the X axis

The further the scatter points from the line of best fit the weaker the correlation and suggests some

Other factor is affecting the IQ if correlation weak



Pilot Studies

The use of a pilot study to allows evaluation and modification of experimental design

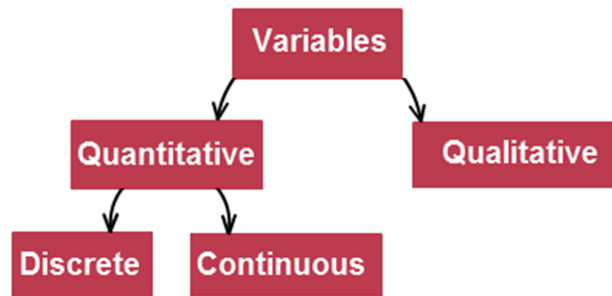
- a) **develop or practice protocols** to become proficient in using an established protocol
- b) check **effectiveness of techniques**,
- c) find a suitable range of **values for the independent variable** to avoid results for the dependent variable ending up 'off the scale'.
E.g. more than 50ml in titration
- d) identify and control **confounding variables**
- e) identifying suitable **numbers of samples** in each replicate
- f) establish the **number of repeat measurements** required to give a true value for each independent datum point
- g) A pilot study can also be used to check whether results can be produced in a suitable time frame

Types of Variables— suitable analysis

Types of Variables

Consequences for type of graph & statistical test to work out p value.

1. Qualitative (not numerical e.g. words)
2. Quantative (measured on a linear numerical scale)



Types of Quantative Data

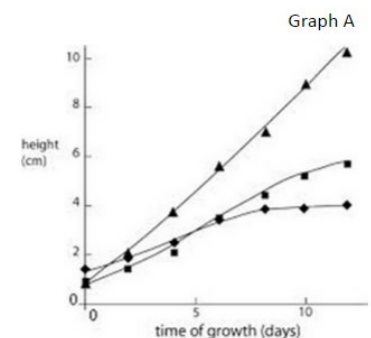
1. Continuous Variables (infinite)

Can take any value on the scale

Continuous variables require a **measuring device**

Example—distance (m), mass, temperature

Types of graph—histogram/line graph



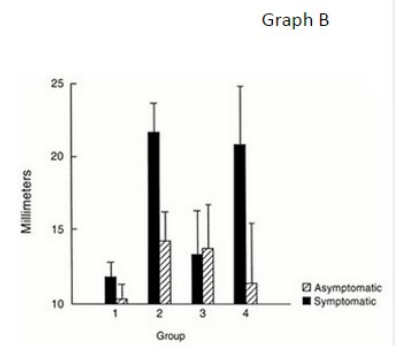
2. Discrete Variables (finite)

Can only take certain values e.g. whole numbers

Discrete variables are **counted**.

Although discrete variables are measured in whole numbers it is acceptable to express derived data, such as the mean as a fraction

Examples number of heart-beats per minute, number of eggs laid



Mean vs Mode Discrete Variables

For derived discrete data the mode (most frequently occurring value) might be a more useful statistic than the mean

E.g. 2.4 children is the mean per family but mode of 2 children per family more useful.

Types of Variables

2. Qualitative Data

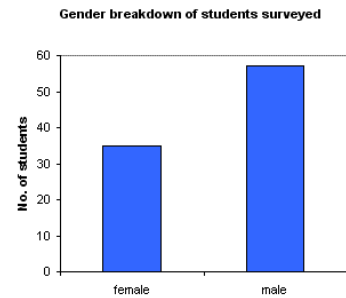
A) Counts

Measured as counts in separate categories

Examples the numbers of each fish species in a pond

the numbers of males and females in a group

the numbers of pink and white flowers in a sample of plants



Care should be taken to ensure that categories are mutually exclusive so that any one individual can only be allocated to one category.

Such categorical data can be processed to produce frequencies, ratios or percentages to compare the counts in each category.

Graph—bar graph

B) Ranked Variables

Include measurements on a scale where the categories are ranked in order of magnitude,

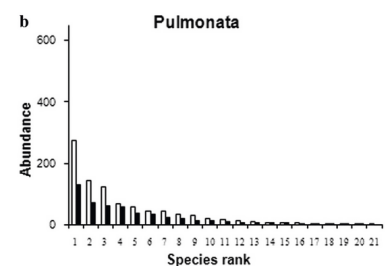
The points on the scale are not at even intervals, therefore it is not a numerical scale and so the data has to be treated as categorical data.

Graph—bar graph

Example

five point scale to describe the abundance of an organism

(1 = rare, 2 = occasional, 3 = frequent, 4 = common, 5 = abundant).



A species scored as 4 is not necessarily twice as common as another species that is scored as 2— not numerical

To make this clear, the values "1" to "5" in the scale above are arbitrary; we could have coded them as "0" to "4" or even "A" to "E".

Scientific Ethics

The principles of integrity and honesty are of key importance in scientific research and are achieved via

- A) unbiased presentation of results
- B) citing and providing references
- C) avoiding plagiarism.
- D) replication of experiments by others (independent replication)
Reduces the opportunity for dishonesty /deliberate misuse of science.

Factors affecting Scientific research

1. **legislation/policy by government**

Legislation **limits** the potential for the **misuse of studies** and data.

2. **Regulation of research e.g. licence required**

Many areas of scientific research are **highly regulated** and **licensed** by government due to risks involved to individuals, subjects and environment,

To conduct experiments researchers must prove that the risks have been considered and

Justified in terms of scientific knowledge outweighs any **risks** to subjects, investigators and the environment

3. **Funding available**

Some areas of research are more popular and therefore receive more charitable donations

E.g. cancer research compared to parasitology in developing countries

Example—stem cell research

Legislation/policy

definition of an embryo in UK

Regulation

Tight regulation in UK of stem cell research

Funding

By government/charities'

Scientific Ethics

Animal Studies

3R's used to **avoid, reduce or minimise** the harm to animal

- A) **Replace** the use of animals with **alternative techniques**
- B) **Reduce** the number of animals to a minimum
- C) **Refine** the way experiments are carried out to **minimise animal suffering** e.g. improve animal welfare

Human Studies

Informed consent must be obtained by each subject understanding risks of experiment

There is also the right of subjects to have their **data withdrawn** from the study

Requirement of confidentiality in human studies through double blind studies where neither researcher nor patients know which drug treatment they are on. re-

Scientific Method

Scientific Method

1. Construction of a testable hypothesis

The null hypothesis can be used in the design of experiments to investigate a possible effect which suggests no link between the independent and dependent variable.

2. Experimental design, gathering, recording, analysis of data

Validity/reliability/accuracy & precision all ensured in experimental design & recording of data

3. Evaluation of result & conclusions

Failure to find an effect i.e. accept the null hypothesis is a valid finding, as long as an experiment is well designed.

Conflicting data or conclusions can be resolved through careful evaluation

4. New hypotheses may be formulated where necessary (refinement of ideas)

In science, refinement of ideas is the norm, and scientific knowledge can be thought of as the current best explanation which may then be updated after evaluation of further experimental evidence

Sharing Scientific Findings

1. Seminars
2. Conference talks
3. Posters
4. Publishing in academic journals.

Peer Review

One off Results

One-off results are treated with caution.

It is very importance that **methods, data and analysis are published alongside conclusions** so that others are able to **repeat an experiment**.

Scientific ideas only become accepted once they have been **checked independently** through **peer review**.

Peer Review

Used in scientific journals.

Specialists with expertise in the relevant field assess the scientific quality of a submitted manuscript a

Make recommendations regarding its suitability for publication in terms of critical evaluation of experimental design,

Data analysis and conclusions in terms of validity & reliability.

Review Articles

These review article summarise previously published material and the current knowledge rather than reporting new facts or analysis in a particular field.

Often more useful as provide many sources simultaneously and can be more reliable as contain multiple sources which confirm same position.

Critical evaluation of science coverage in the wider media.

Increasing the public understanding of science and the issue of misrepresentation of science in the media.

Evaluation of Experimental Design ?

Validity of Experimental Design

1. The experiment should be designed to test the intended aim or hypothesis for it to be valid.

Example

*To investigate the effect of **volume of paracetamol** on catalase activity*

Experimental design—4 values of independent variable of paracetamol

20ml of paracetamol solution,

15 of paracetamol & 5ml of deionised water

10 ml of paracetamol solution & 10ml of deionised water

5ml of paracetamol solution & 15ml of deionised watre

This experiment is designed to alter concentration NOT volume and thus results making conclusions about volume are invalid.

2. Treatment effects should be **compared to controls** (positive/negative). Failure to include appropriate positive & negative controls makes the experimental design invalid.
3. The validity of an experiment may be compromised where **confounding variables** are not sufficiently controlled and may influence the value of the dependent variable.

Reliability of Experimental design

4. **Selection bias** may have prevented a representative sample being selected affecting the reliability of the results.

This can be overcome by random (name generator) /systematic or stratified sampling

5. **Sample size** may not be sufficient to decide without bias whether the modification to the independent variable has caused an effect in the dependent variable.

e.g. sample not representative as wide variations in results leads to conclusion sample size too small

Evaluation of Data analysis?

1. Data are explored through the appropriate use of simple statistical procedures such as

- a) Appropriate **Graphs**

Line graphs for quantitative variables with at least three numerical values on independent variable

Bar graphs for qualitative data or less than 3 numerical values of independent variable

Scatter plots for measuring two variables to ascertain a correlation by the line of best fit

- b) SEM **Error bars/confidence intervals**

Statistical tests used to indicate the variability of data around a mean via confidence intervals/SEM.

Larger error bars mean more variation in data and thus a large sample size should have been used

Smaller error bars mean less variation in data and thus a smaller sample size could have been

Selected

- c) **Measures of Central tendency**

Mean (average), Median (middle number), Mode (most common number), range

(difference between largest & smallest value) & interquartile range (middle 50% of data)

2. Statistical tests are used to determine whether the results are likely or unlikely to have occurred by chance

A statistically significant result is one that is unlikely to be due to chance alone.

The null hypothesis is rejected & alternative hypothesis accepted.

This can be seen if the treatment mean differs from the control sufficiently for their confidence intervals/error bars not to overlap.

3. Consideration should be given to the **validity of outliers and anomalous** results.

Often a confounding variable has not been sufficiently controlled leading to an outlier.

Evaluation of Conclusions

- a) Conclusions should **restate the aim/hypothesis** in light of the experimental results & statistical tests
- b) The **validity and reliability of the experimental design** should be taken into account when deciding on whether conclusions are correct.

Conclusion can not be acceptable if there are issues with validity/reliability of experimental design

Problems with validity of data e.g. lack of controls OR failure to control confounding variables

Problems with reliability of data e.g. sample size not sufficient (large error bars) or selection bias

- c) Consideration should be given as to whether the results can be attributed to **correlation or causation**.

Causation studies

Experimental design: An independent variable is altered and confounding variables held constant in a lab whilst suitable controls are put in place to prove that changing the independent variable **CAUSES** the effect on the dependent variable.

Correlation Studies

Investigators use groups that already exist, so there is **no truly independent variable**.

These 'observational' studies are good at detecting correlation but, as one variable is not deliberately changed whilst other confounding variables are held constant, other **confounding variables may be causing** the result and this **suggests correlation not causation**.