#### Teaching the principles of design of experiments



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# Writing a protocol

The protocol for the experiment should include at least the following headings, many of which are interrelated.

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- What is the purpose of the experiment?
- What are the treatments?

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- Proposed statistical analysis

Litter 1 1 1 1 2 2 2 2 3 3 3 3 Pig 1 2 3 4 5 6 7 8 9 10 11 12 Feed 3 1 1 2 3 1 3 2 1 2 3 2

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Look at pig 5: is its weight-gain  $y_5$  or  $y_{21}$  or  $y_{231}$ ? Calling it  $y_{231}$ 

- ignores the pigs;
- encourages non-blindness;
- encourages operation by treatment instead of by litter.

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What is the purpose of the experiment? To find out how three different feeds affect the weight of piglets after 8 weeks of life

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• What are the observational units? 12 piglets  $\times$  8 weeks

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- What are the observational units? 12 piglets  $\times$  8 weeks
- What measurements are to be recorded? The weight of each piglet in kilograms, in the early afternoon of the Tuesday closest to its *n*-th week birthday, for n = 1, ..., 8.

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- Plan See above
- Proposed statistical analysis For each week, do an analysis of variance incorporating litters and feeds, giving means and standard errors of differences for the feeds.

# What is the purpose of the experiment?

If the answer is something vague like 'to investigate new varieties of sunflower' or 'to find out about the nutritional effects of margarine', there may be a perfectly worthwhile exploratory experiment to be done but a statistician is unlikely to be able to help you. If the answer is something vague like 'to investigate new varieties of sunflower' or 'to find out about the nutritional effects of margarine', there may be a perfectly worthwhile exploratory experiment to be done but a statistician is unlikely to be able to help you.

Usually it is better to state a specific question that is to be answered, such as

- to estimate how much better drug A is than drug B in reducing inflammation (of course, the two drugs would need to named more precisely)
- to test the hypothesis that there is no effective difference between organic and inorganic nitrogen as a source of fertilizer
- to fit a model for how much sucrose is absorbed at different distances from the surface of the liver.

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5mg of ciprofloxacin 4 hours after contact.

Give each treatment a simple code like A, B, C for reference later.

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In an experiement to compare two new pesticides, we should ask the scientist if he just wants to compare the two new pesticides with each other or if he wants to compare them both with the effect of doing nothing. If the latter then there is a third treatment, 'do nothing', which is often called control. You should always ask if a control is needed.
### What is the structure on treatments?

Examples of treatment structure include:

unstructured This means that there is no structure to the treatments at all.

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several new treatments plus control The control may be 'do nothing' or it may be the current standard.

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all combinations of two factors For example, three cultivars of rye-grass combined with four quantities of fertilizer.

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increasing doses of a quantitative factor However, factors such as the amount of fertilizer are indeed quantitative, but not really on a continuous scale, because the farmer will use a whole number of bags of fertilizer.

# Factorial treatments

Twelve treatments are all	fact
combinations of:	Cul

factor	levels
Cultivar $(C)$	Cropper, Melle, Melba
Fertilizer $(F)$	0, 80, 160, 240 kg/ha

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$$E(Y_{\omega}) = \tau_{C(\omega),F(\omega)}$$

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$$E(Y_{\omega}) = \lambda_{C(\omega)} + \mu_{F(\omega)}$$

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$$E(Y_{\omega}) = \kappa$$

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$$E(Y_{\omega}) = \tau_{C(\omega),F(\omega)}$$

$$\downarrow \qquad \leftarrow \text{Interaction}$$

$$E(Y_{\omega}) = \lambda_{C(\omega)} + \mu_{F(\omega)}$$

$$E(Y_{\omega}) = \lambda_{C(\omega)} \qquad E(Y_{\omega}) = \mu_{F(\omega)}$$

$$\downarrow$$

$$E(Y_{\omega}) = \kappa$$

$$\downarrow$$

$$E(Y_{\omega}) = 0$$

Treatments are all	factor	levels
combinations of:	Timing $(T)$	early, late
	Fertilizer (F)	0, 80, 160, 240 kg/ha

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How many treatments are there?

Treatments are all<br/>combinations of:factorlevelsTiming (T)<br/>Fertilizer (F)early, lateFertilizer (F)0, 80, 160, 240 kg/ha

How many treatments are there?

Timing	Fertilizer			
	0	80	160	240
None				
Early				
Late				

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This is where you describe exactly how you apply the treatments to the experimental units, and what you do from then on until all measurements have been taken.

There should be sufficient detail for other scientists to replicate your work.

The experimental units are what you apply the treatments to. Describe them carefully. It is helpful to note how many there are. For example (the dots denote missing technical details)

- 20 male Wistar rats ...
- ► 3 parts of the orchard (which parts? how separated? what area?)
- 12 student volunteers each used every day for 5 days (this is 60 experimental units if treatments can be changed daily)

► 4 subplots in each of 3 strips in each of 2 fields.

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How should the experimental units be chosen? Should they be as alike as possible or be representative? If they are unalike then any variation between the experimental units may swamp any differences between the treatments that you are trying to find, especially in a small experiment. If they are not representative, you cannot extrapolate your conclusions. The observational units are what you take measurements on. In many experiments they are the same as the experimental units. Then you can simply say so. The observational units are what you take measurements on. In many experiments they are the same as the experimental units. Then you can simply say so.

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If a fruit grower sprays parts of his orchard but counts the number of apples on each tree then the observational units are the trees.

Of course, you need to know what you are going to measure before you can say what objects you are going to measure, so fill in the 'What measurements are to be recorded?' box at the same time as this one.

On the data sheet, there is usually one row for each observational unit.

It is helpful to state the number of observational units, especially if they are not the same as the experimental units.

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# Irrigation channels in a rice experiment



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### What measurements are to be recorded?

Write down everything to be recorded, for example

- weight in kg at 15 days old
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Record everything at the proper time; do not go back and make it up later. For example, if you are supposed to record temperature then do so; don't simply look back at someone else's temperature records later and copy the figure.

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Do not make 'neat' copies: you are likely to introduce errors.

# The danger of makeing intermediate calculations

In an experiment on leafstripe disease on barley, one measurement was the percentage of disease on each plot. The agronomist took a random sample of ten quadrats in each plot, inspected 100 tillers (sideshoots) in each quadrat to see how many were infected, and averaged the ten numbers. Only the average was recorded in the 'official' data. I saw a collection of pieces of paper like this.

Plot 8	6	Plot 23	0
	0		0
	7		0
	3		0
	6		0
	0		0
	4		0
	5		28
	6		0
	4		0
Average	4.1	Average	28

In a simple experiment this can be very straightforward. For example, four feeds are each allocated to two pens.

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In really complicated cases (such as incomplete-block designs, or confounded factorial designs), you need to write out the complete systematic design.

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• Watch out for false replication.

### Replication for power



Solid curve defines the interval [-a, a] used for the hypothesis test; dashed curve gives the probability density function of the test statistic  $\Delta/\sqrt{\nu\Gamma}$  if the real difference is  $\delta$
A field was divided into three areas and one pesticide applied to each area. Ladybirds were counted on three samples from each area.

- Treatments = ?
- Experimental units = ?
- Obersvational units = ?
  - Replication = ?

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A field was divided into three areas and one pesticide applied to each area. Ladybirds were counted on three samples from each area.

Treatments	=	3 pesticides
Experimental units	=	3 areas
Obersvational units	=	9 samples
Replication	=	?

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Treatments	=	3 pesticides
Experimental units	=	3 areas
Obersvational units	=	9 samples
Replication	=	1





- Blocking
- Constraints on applying or changing treatments: for example, some cannot be applied to small areas.

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Costs

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Choice of systematic design

#### Randomization used

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- systematic bias (for example, doing all the tests on treatment A in January then all the tests on treatment B in March)
- selection bias (for example, choosing the most healthy patients for the treatment that you are trying to prove is best)
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- cheating by the experimenter.

How do we randomize? Write down a systematic plan. Then choose a random permutation (from a computer, or shuffle a pack of cards) and apply it to the systematic plan.

Explain how this was done.

This gives the exact details of which coded treatment is allocated to which experimental unit.

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