Statistical experimental design principles for biological studies

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An apology, and a caveat

- Apology: I am not a genetics/genomics expert!
  - But I DO know a bit about design…
- Caveat: Experimental design is a science! (as is statistical analysis…)
  - Every experiment is different
  - This talk is necessarily simplistic, and *very* incomplete…
  - Intended as a reminder of some concepts, some bits of advice, and a plea…

An experiment – recap:

- Apply treatments (experimental conditions) or treatment combinations to subjects (or experimental units).
- Carefully control/remove other influences as much as possible.
- Observe one or more responses (observed or measured results)

- A way to infer cause => effect.

- Genotype/Species/Variety is a treatment? Yes...

What is statistical experimental design concerned with?

- **Everything! The entire process leading to the final dataset…**
- **Recognising sources of variation (systematic, random) that influence your results and hence controlling for them**
- **How to ensure that we obtain data that provides 'honest' and accurate answers to our questions**

Key point:

- **Good statistics cannot rescue a bad dataset!**
- **And a bad dataset may not be able to answer your questions (honestly, that is)**
- **Do the experiment right => get a good dataset**
- **Consider design from the very beginning of planning the experiment**

Fundamentals

Randomisation

Blocking

Replication

Randomisation

- Allocate treatments to subjects randomly (ideally using a proper random number generator, not your own personal judgement)
- Also randomise:
  - Spatial layout
  - Order of processing
- Avoid systematic patterns (But: see Blocking)
  - Protects against unforeseen biases

Blocking

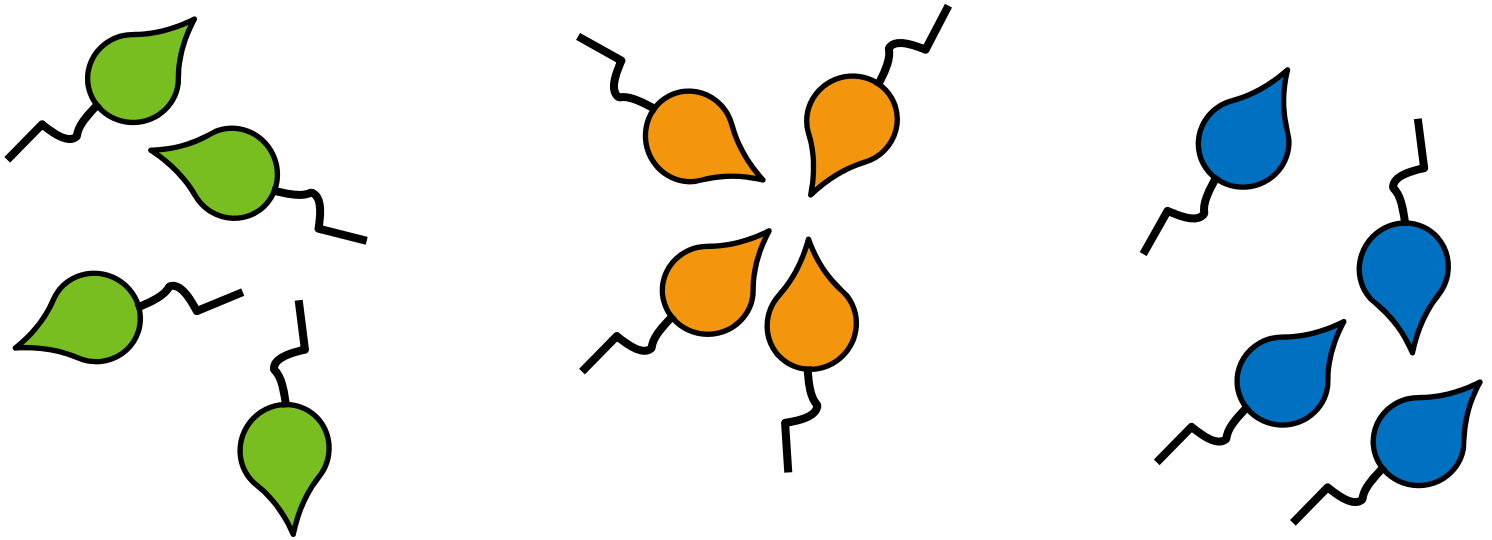
- Identify groupings that may effect your results
  - Choice of operator/technician/machine
  - Cage/Table/shelf/assay plate/batch
- Try to assign (ideally) one of each treatment to each group! (Randomised Block Design or RBD)
  - (Example coming later…)

Replication

- Sample size
  - G*Power (http://www.gpower.hhu.de/en.html)
  - Russ Lenth's applets (http://homepage.stat.uiowa.edu/~rlenth/Power/)
  - Simulation studies in the literature? (Google scholar)

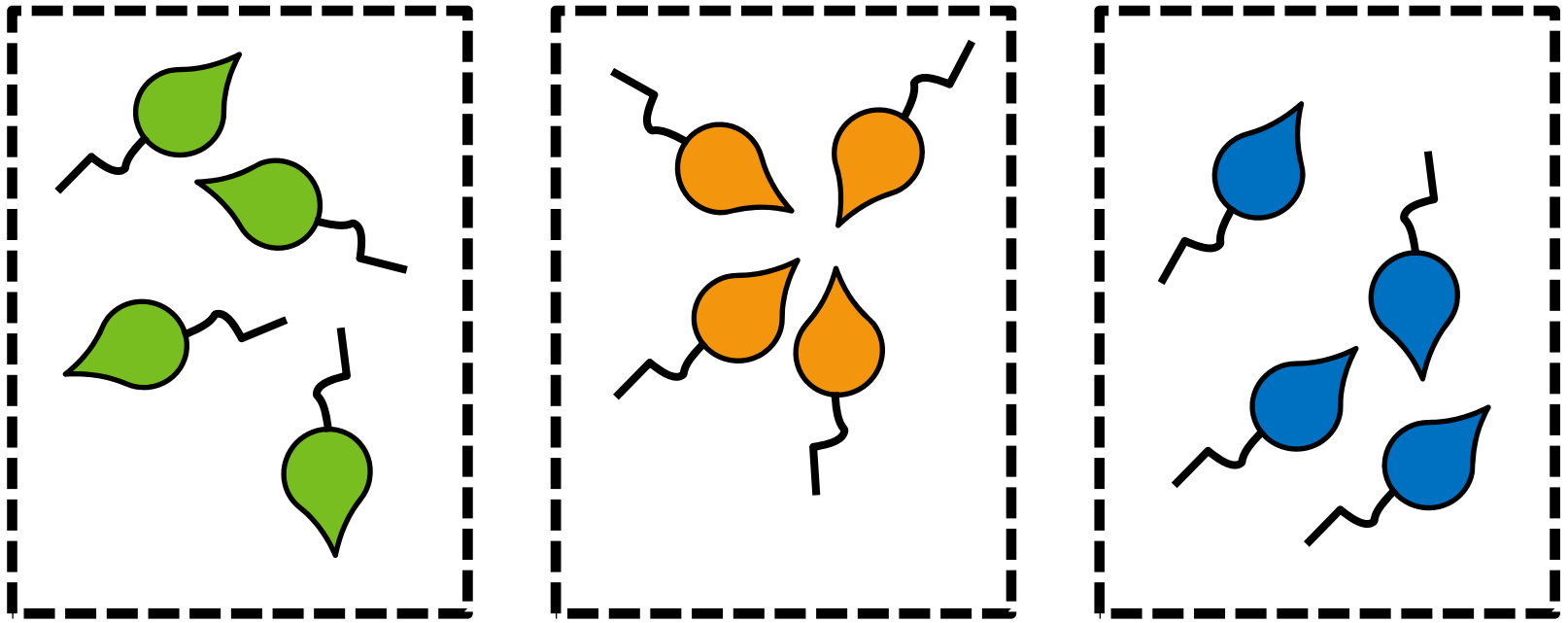
- Biological/internal/technical replicates
  - Don't confuse these – Internal/technical replicates are not a substitute for biological replication.
  - (more later…)

12 mice, three treatments (Colour)



Completely randomised design
Balanced (equal info on each treatment, treatments compared with equal accuracy)

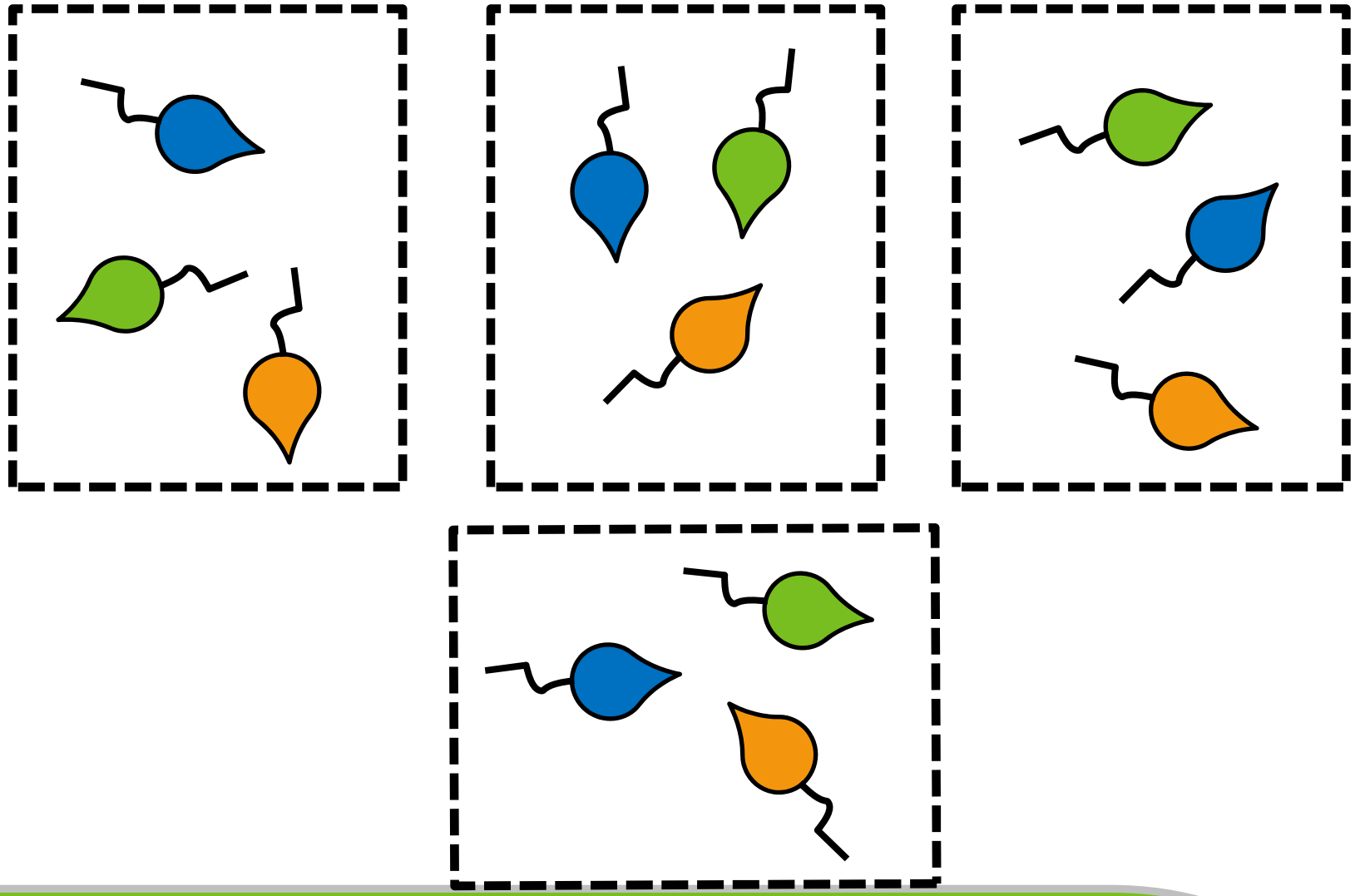
But, the mice must be housed…



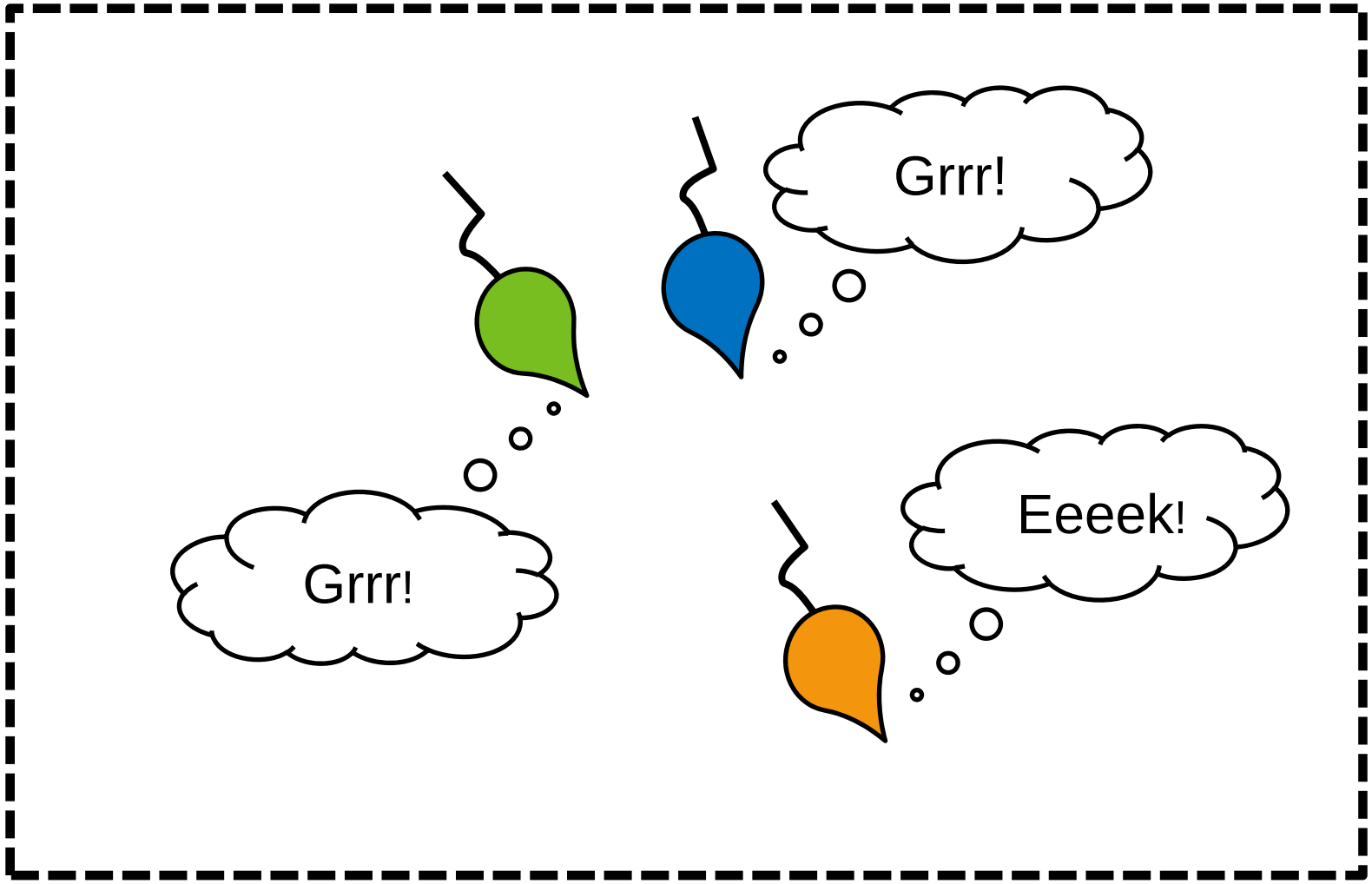
Here, treatments are <u>confounded</u> with cages

Cannot separate Cage effects from Trt effects!

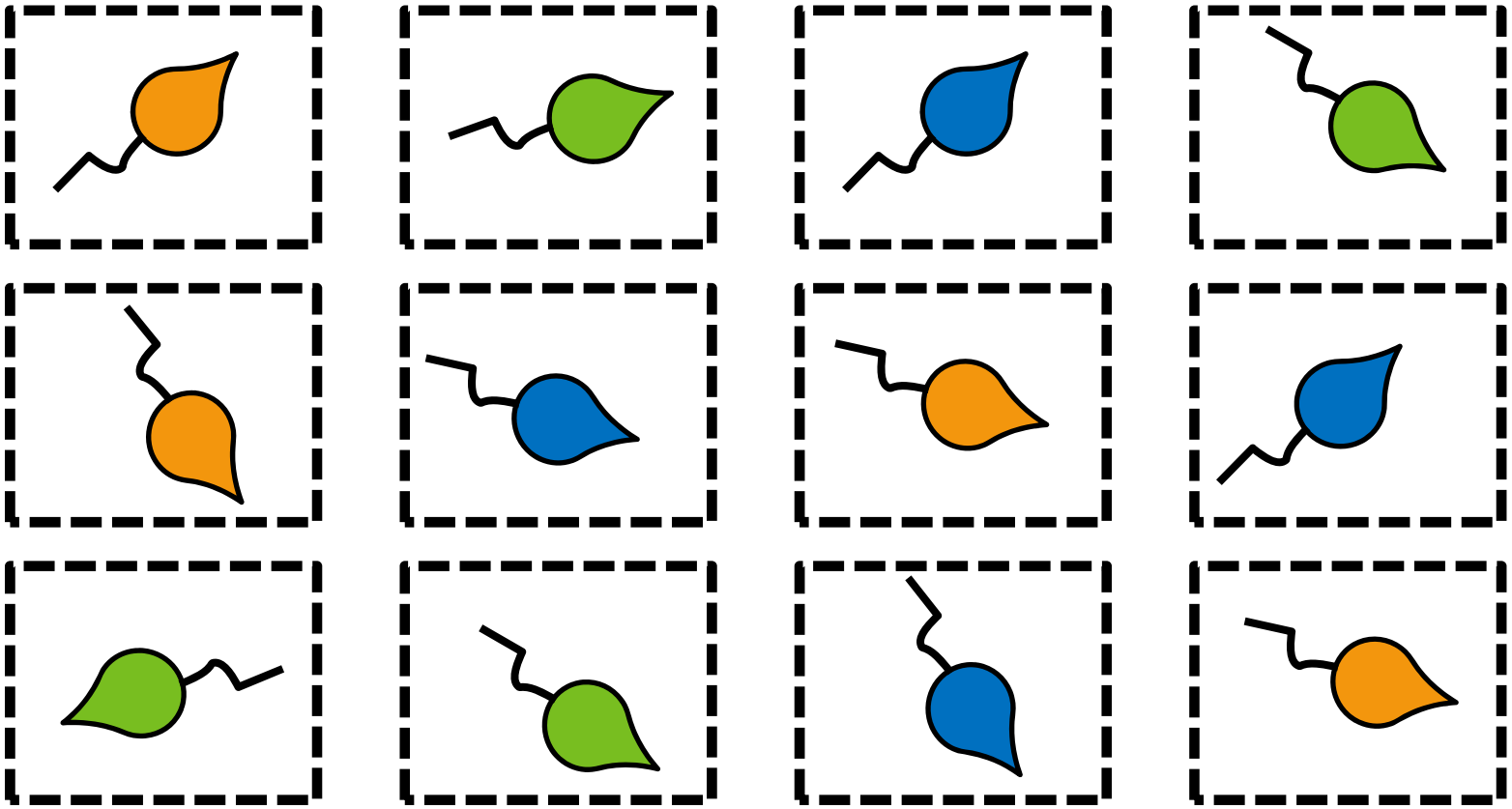
Cages as Blocks



Animals (especially) can pose a problem…

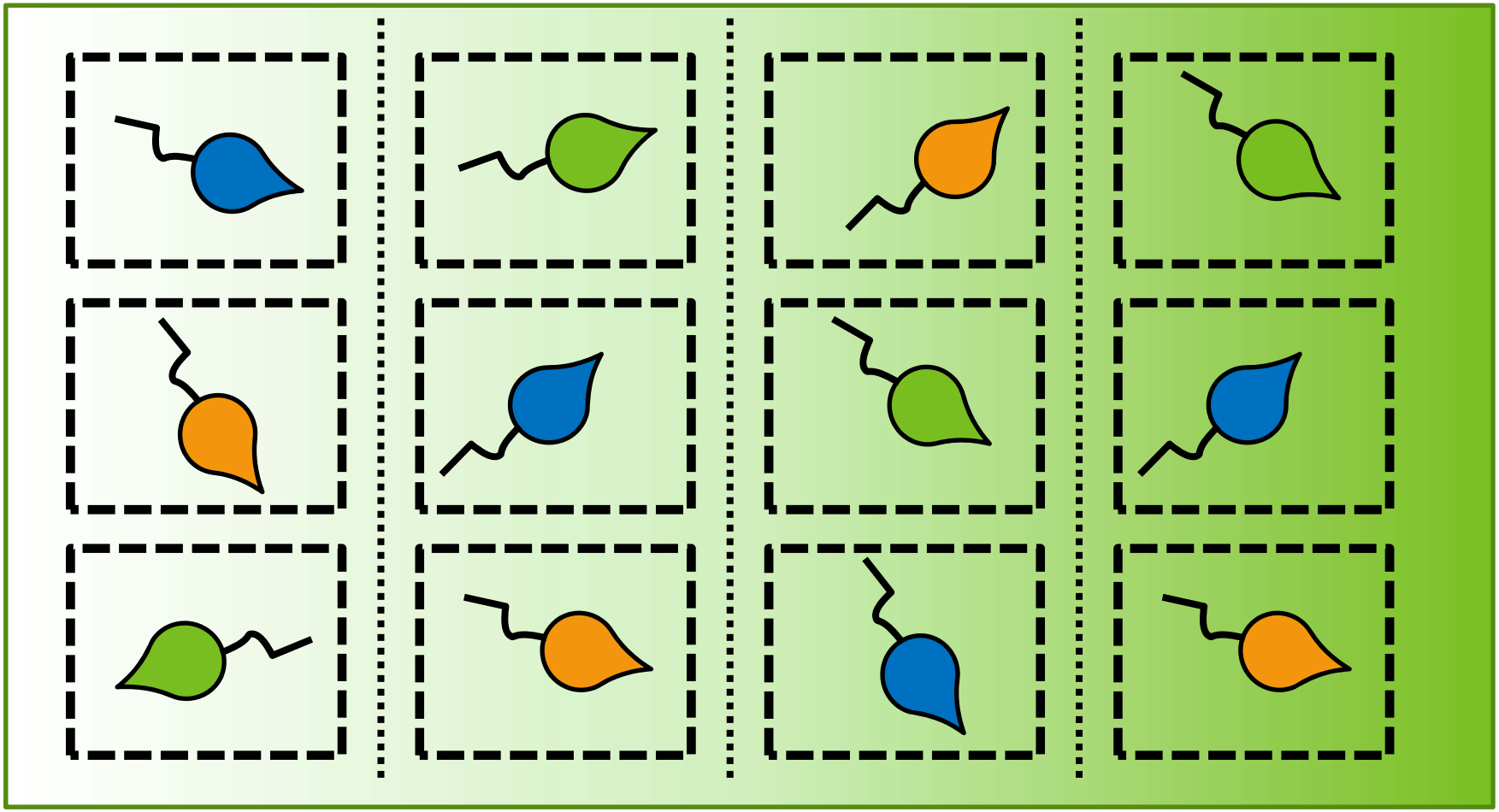


Cages as Experimental Units / Subjects…



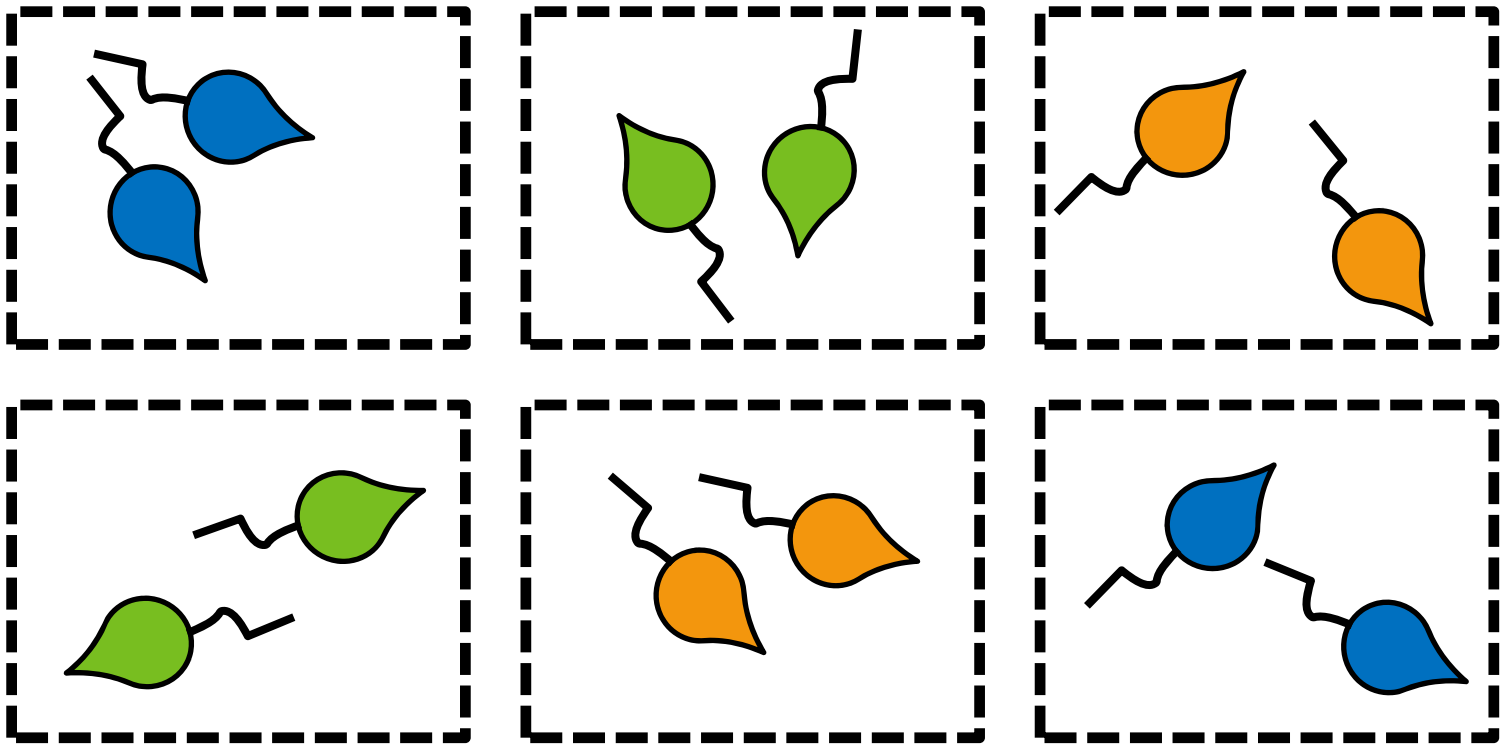
…& Cage = Mouse

(Aside- env. trend across columns of cages?



=> Block by Columns!

Not enough cages? A compromise:



But, Cages are the Experimental units

Only two replicates of each Trt!

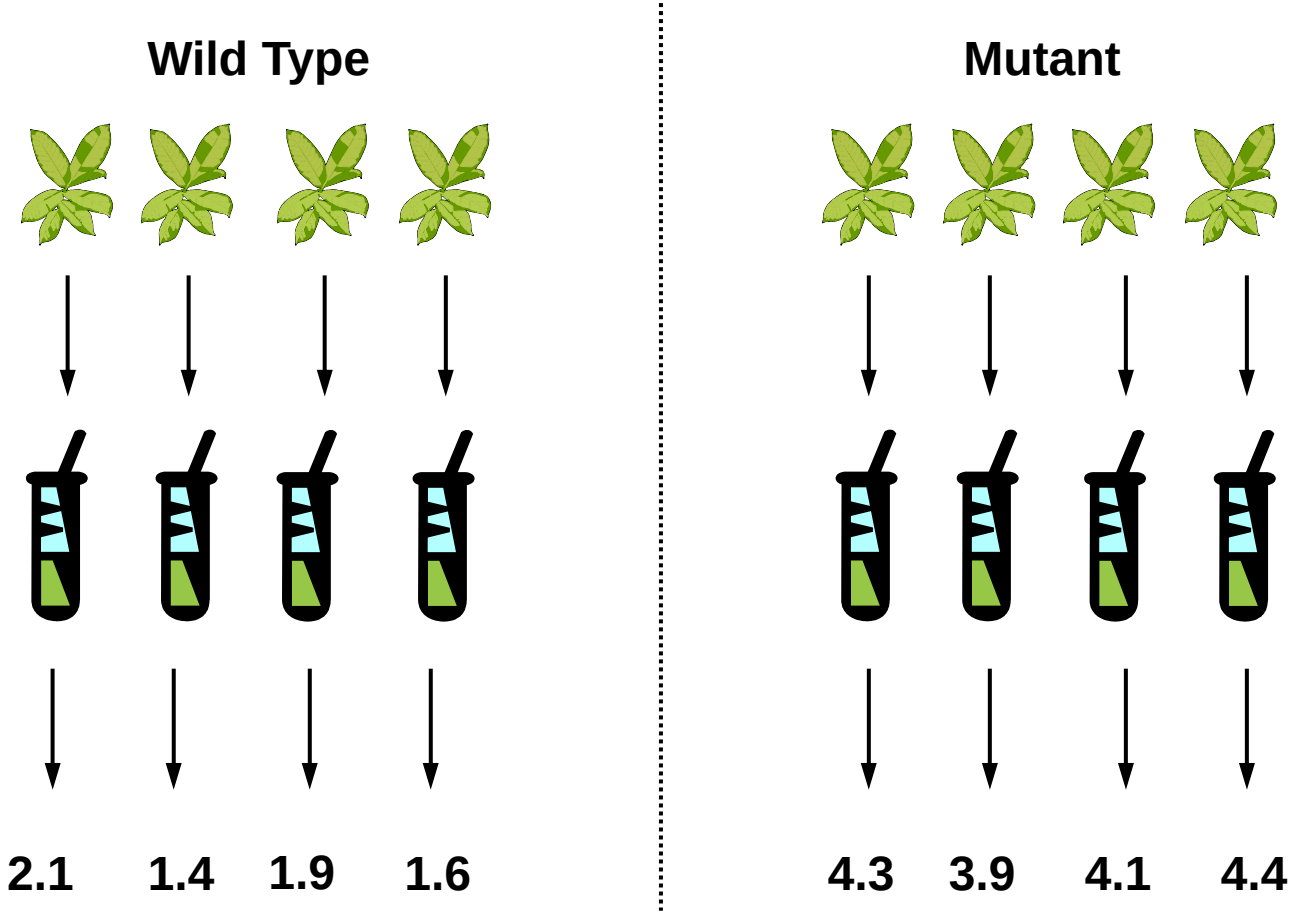
More on replication:

- **The replication used determines the population(s) being sampled...**

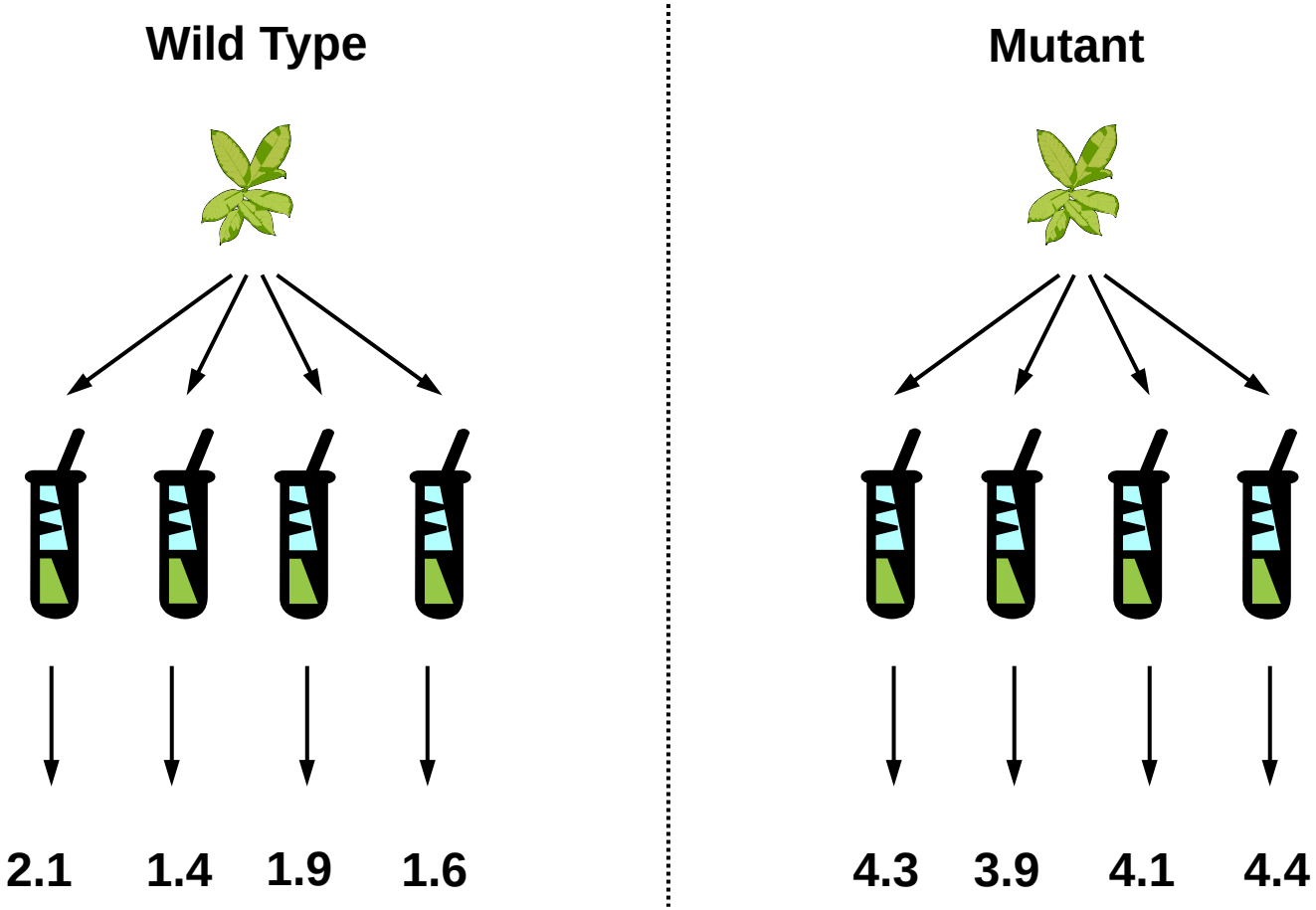
...hence…

- **The replication used determines the conclusions that may be validly drawn from a statistical test**

Comparison between populations of plants



Comparisons between two plants



Recording

- **Details of treatments and associated randomisation, blocking, types of replication should always be recorded**

- **Check what information is required for the analysis, or talk to the statistician about what is required.**

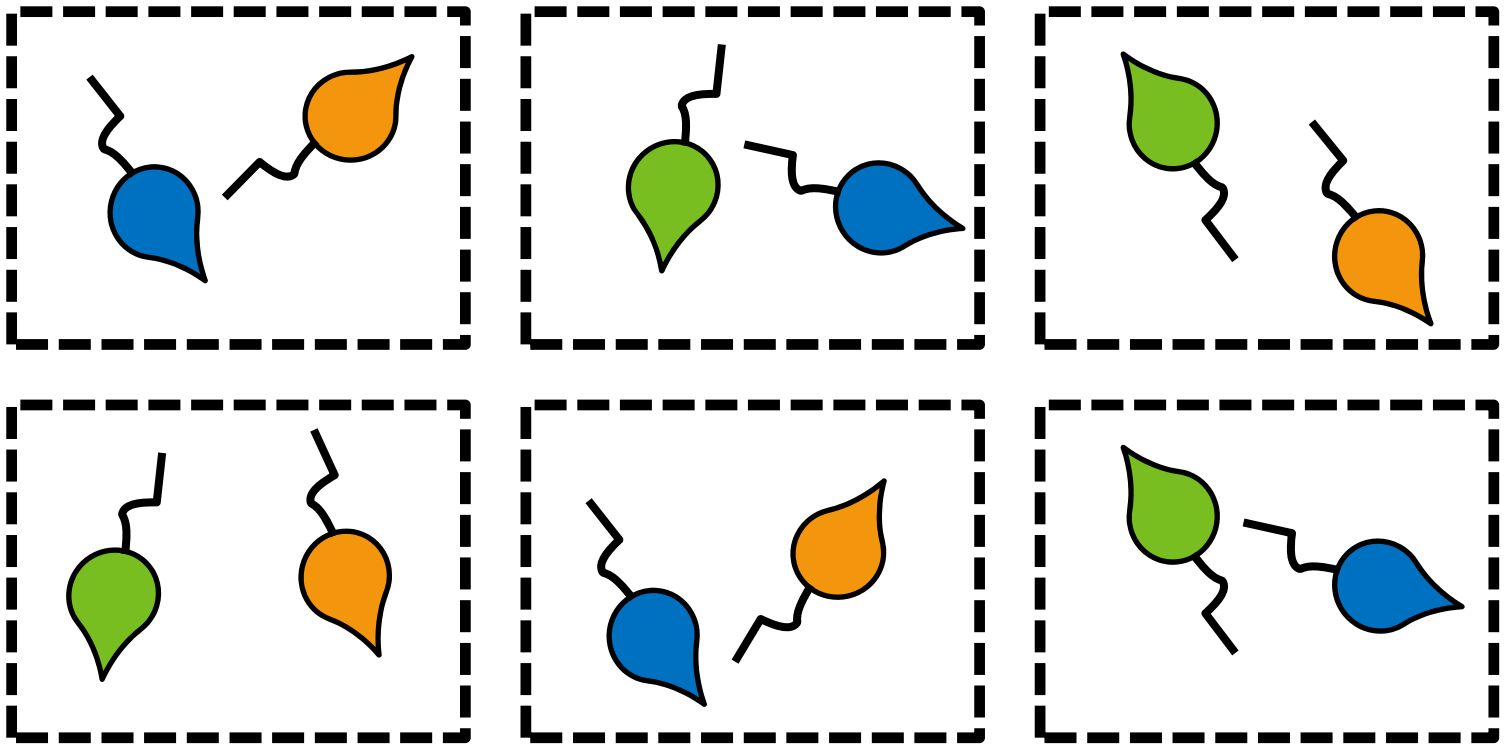
- **This is important meta data when distributing your data…**

More on Blocking…

- ‘One of each treatment per block’ is not always feasible – blocks may be too small
  - ‘Incomplete blocks’

- Think about:
  - Balance – treatments occur together in the same blocks the same number of times.
  - Linkage – common treatments in different blocks provide links between blocks – ensure you have plenty of linkage.

Balanced Incomplete Block Design (BIBD)



- Trts occur together the same number of times
- Plenty of treatment-linkages between blocks

Notes on incomplete blocks

- BIBDs are only availble for certain combinations of parameters.
- However, the general principles are still relevant
  - ‘Unbalanced incomplete blocks’
- Note that teasing apart the treatment effects from block effects in unbalanced situations is dependent on the capabilities of the analysis method
  - Check on said capabilities, consider simpler experiment if necessary…

Treatment structure

- Sir Ronald Fisher => factorial treatment structure

		Environment			
		I	II	II	IV
Genotype	A	5	5	5	5
	B	5	5	5	5
	C	5	5	5	5

(3x4 factorial)

- 5 = Number of replicates of each treatment

Factorial treatment structure

- **Separate main effects and interactions**
- **Can include more factors (in theory)**
- **Unbalanced numbers of treatments usually OK:**

		Environment			
		I	II	II	IV
Genotype	A	5	1	5	2
	B	2	5	3	5
	C	5	5	5	4

But beware missing treatment combinations

- **The more that entire treatments are missing from the factorial, the harder it is to tease apart main effects and interactions and the more confounded different treatment effects become.**

		Environment			
		I	II	II	IV
Genotype	A	5	0	5	0
	B	2	5	3	5
	C	5	5	0	4

Don’t get too ambitious!

- Two-way factorials are much easier to interpret than three-way, four way… etc.
- The higher the factorial structure, the more likely the structure will be incomplete
  - (missing values, treatment combos which don’t make sense, treatment combos you aren’t interested in and don’t include).
- Statisticians and their tools can’t work miracles!
- Simpler is better…
- Talk to a statistician!

Plea:

- If you don't already know how to design and conduct good experiments…

  …then…

- …involve a statistician in the early stages of planning your experiment!

- And note – the statistician may need time…

Sir Ronald Fisher, 'the father of modern statistics'…

"To consult the statistician after an experiment is finished is often merely to ask them to conduct a post mortem examination.

They can perhaps say what the experiment died of."

(R. Fisher)

Thank you