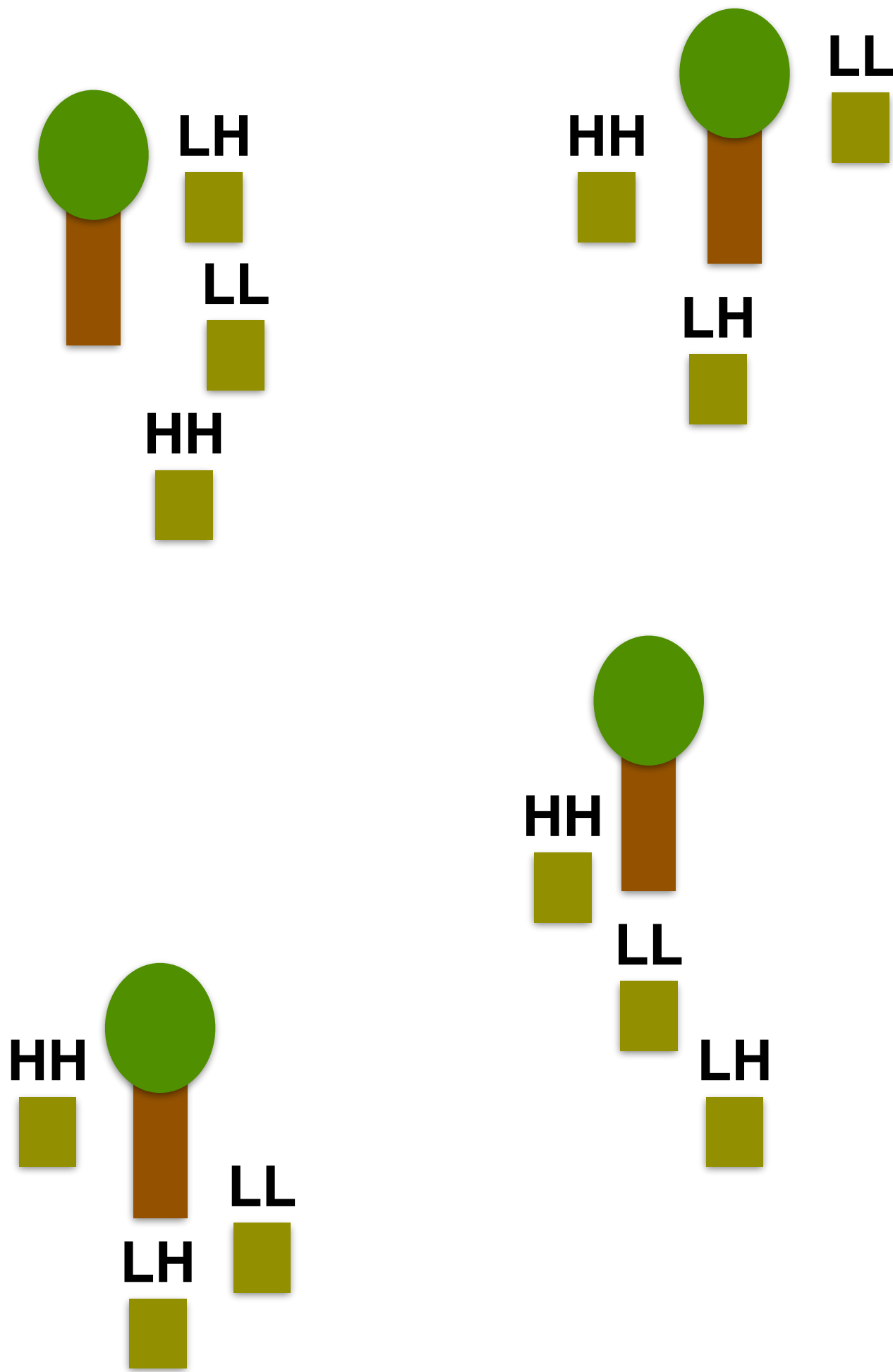
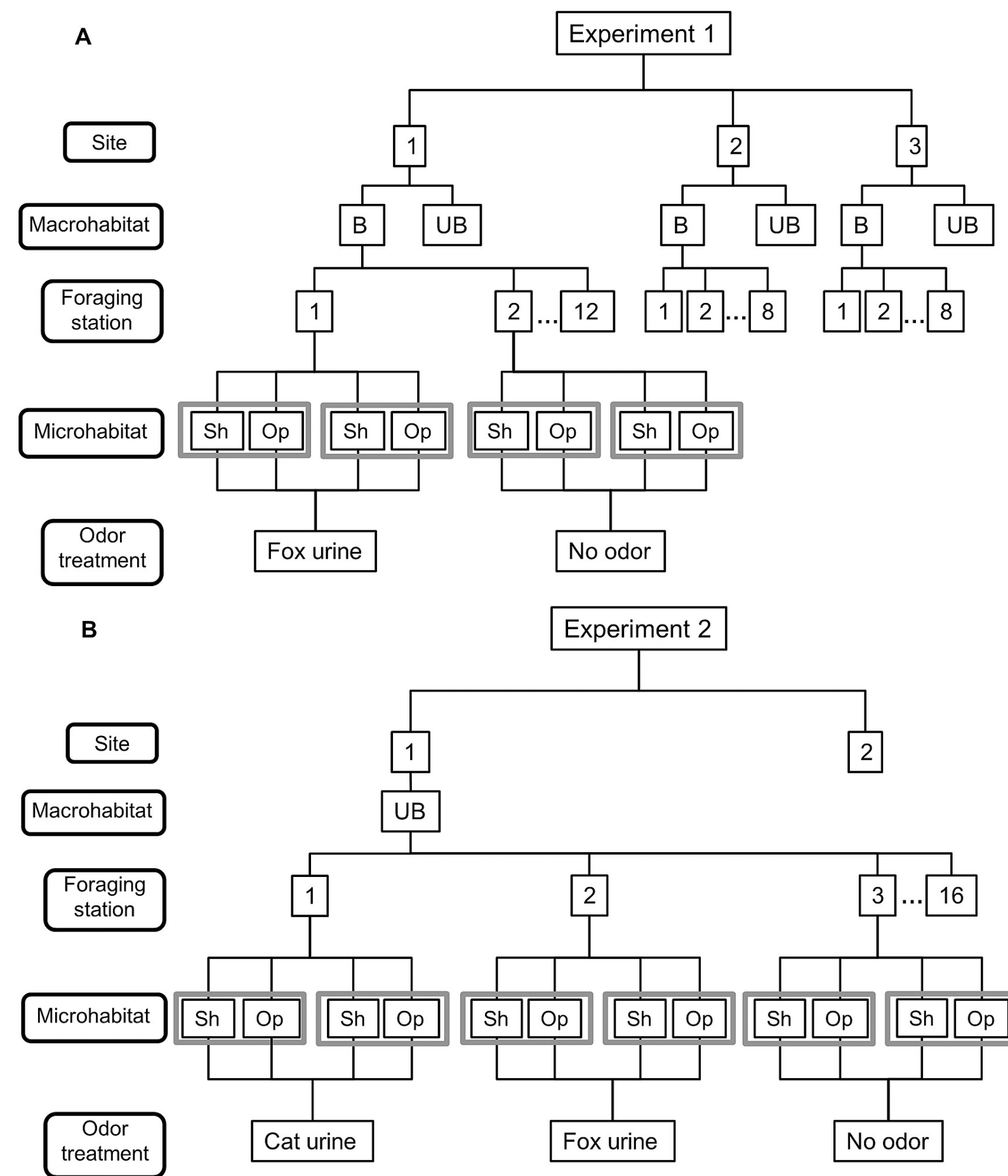


Foundational Statistics

Study / Experimental Design Continued



Key terms I forgot last time!

Variable

A set of measurements of the same type that comprise the sample. The characteristic that differs (varies) from observation to observation

The protein content of koala milk.

Factor: A variable that is categorical (not numeric).
Example: Experimental Diet

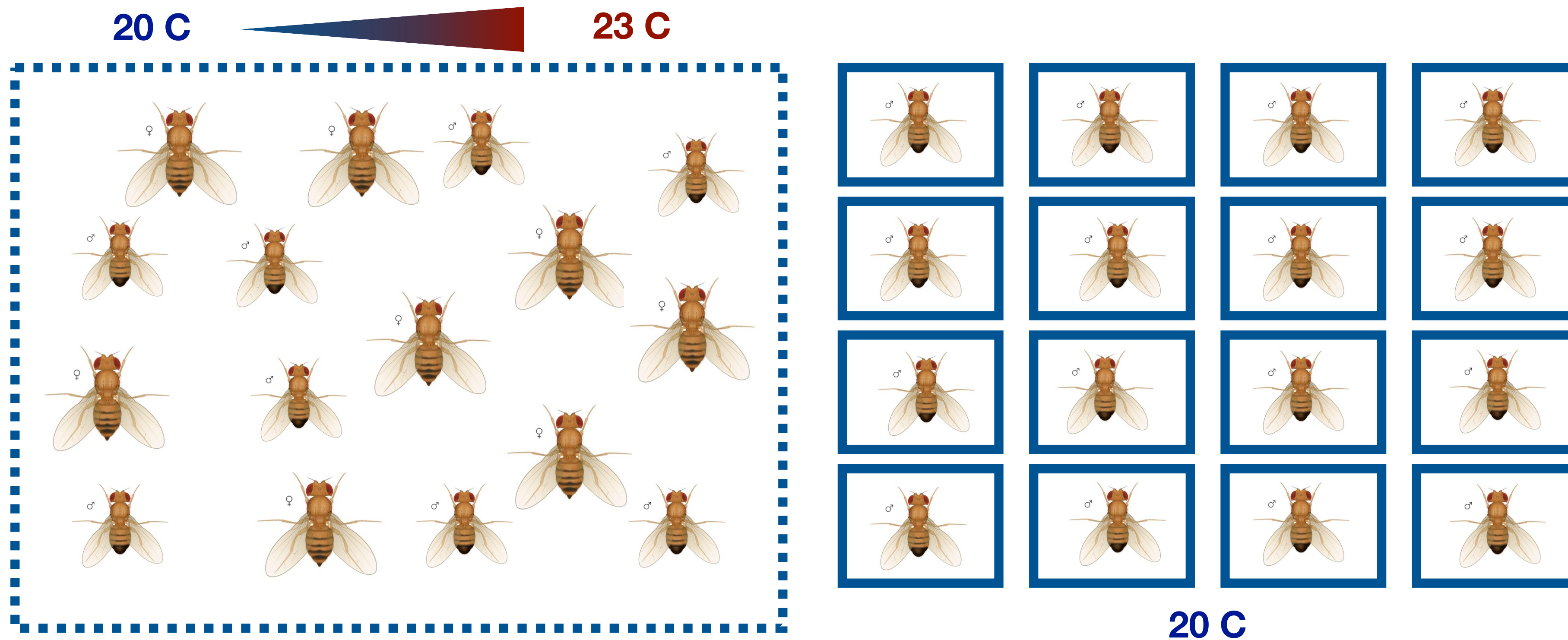
Factor levels: The different states of a factor in a study.
Example: “High-protein” and “Low-protein” Diet

In R, factors are objects that have the specific class of “factor” with attributes “levels”

Minimizing the effects of background variation (especially important with small n)

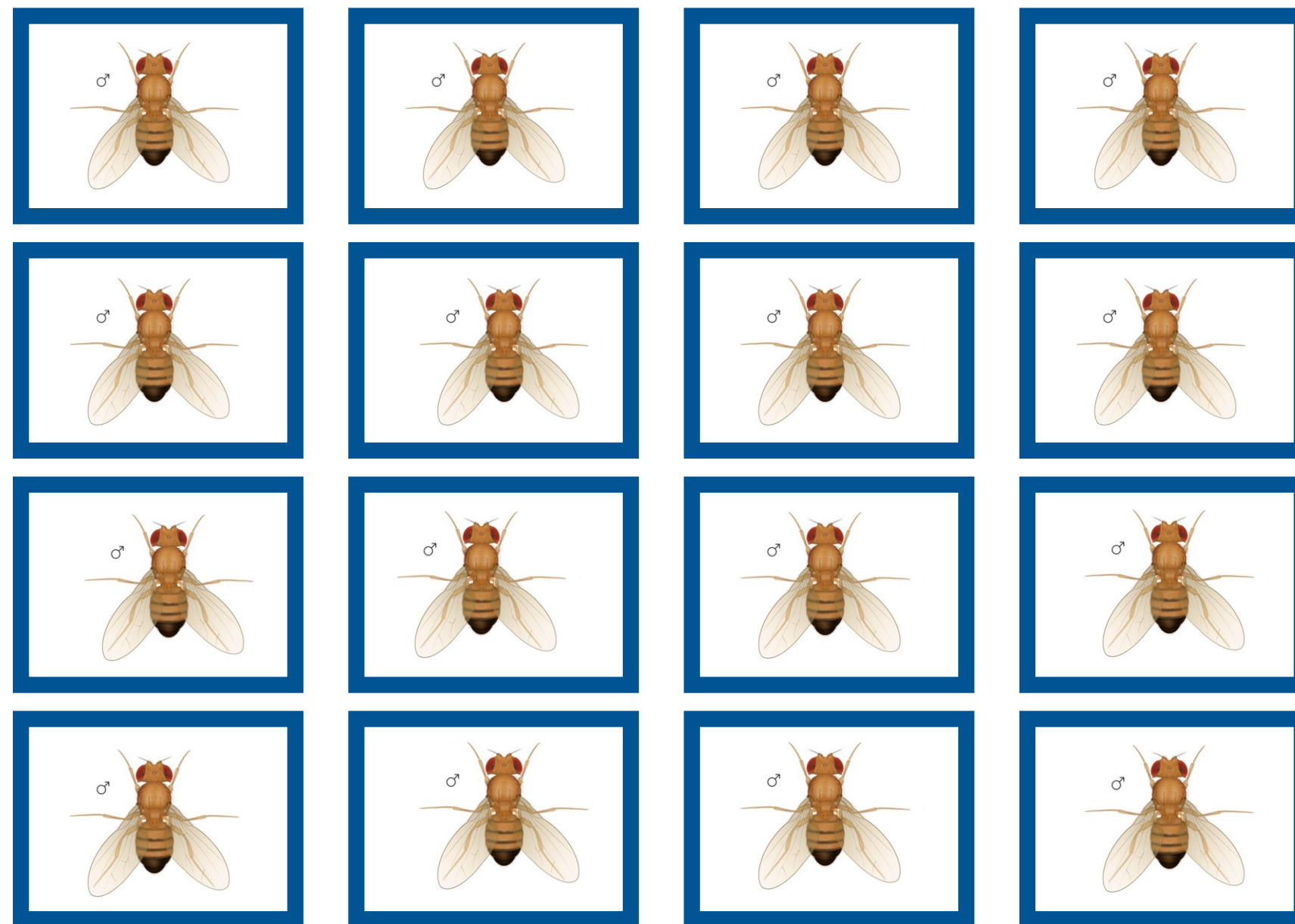
Can reduce “noise” by making the experimental conditions constant

- Fix environmental conditions
- Use only subjects that are the same age, genotype, etc.



Minimizing the effects of background variation (especially important with small n)

Constant conditions might not be desirable, either. By limiting the conditions of an experiment, we also limit the generality of the results—that is, the conclusions might apply only under the conditions tested and not more broadly.



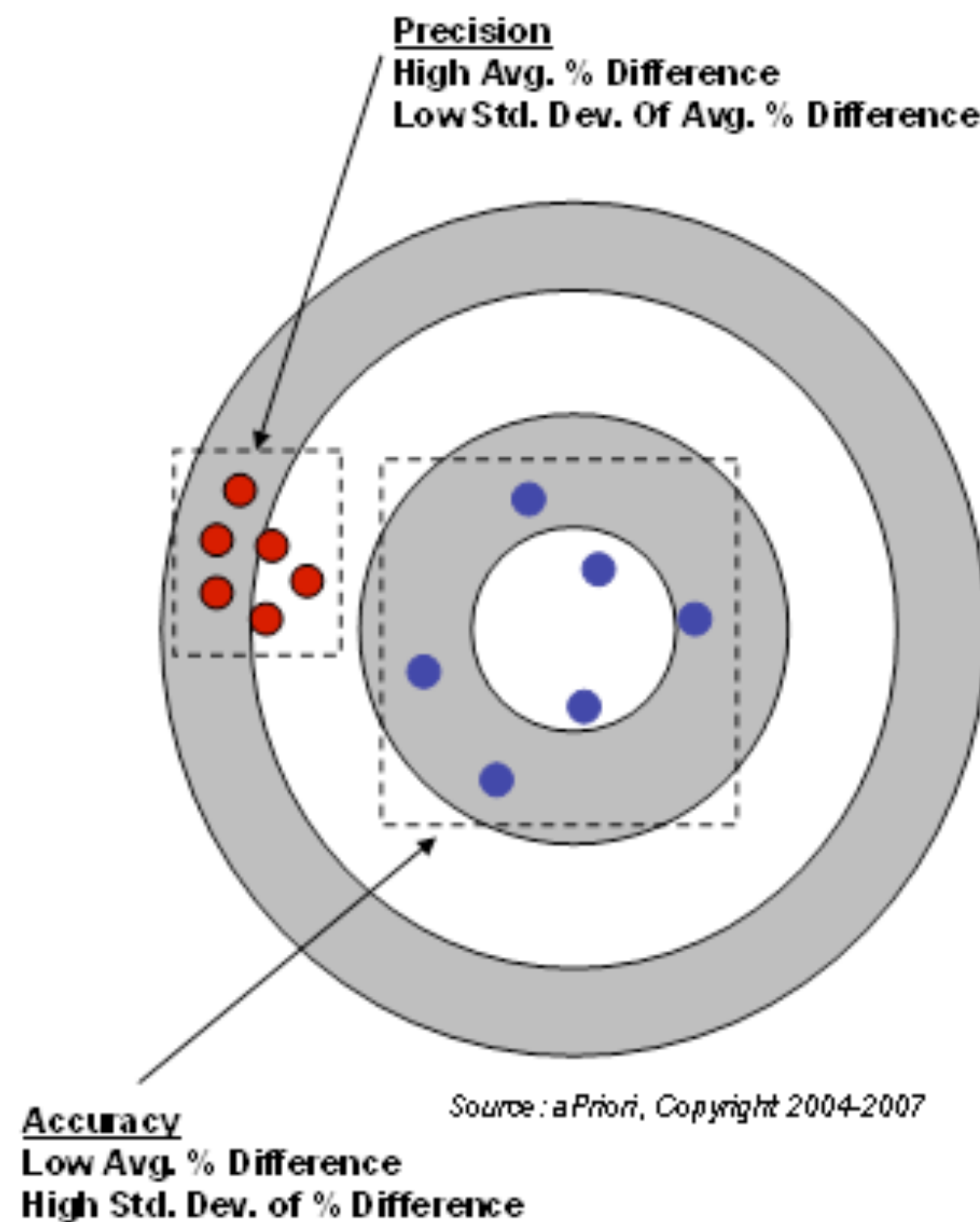
**Scope of inference is restricted
to these specific conditions**

Replication

- Replication is the assignment of each treatment to multiple, independent experimental units.
- Without replication, we would not know whether response differences were due to the treatments or just chance differences between the treatments caused by other factors.
- Studies that use more units (i.e., that have larger sample sizes) will have smaller standard errors and a higher probability of getting the correct answer from a hypothesis test.
- Larger samples mean more information, and more information means **better parameter estimates** and more powerful tests.

Replication

- Replication is especially important when the process of measurement itself is “noisy,” also known as **low precision**.



Precision is the closeness of repeated estimates to one another

Accuracy is the closeness of an estimated value to its true value

Replication and pseudo-replication

- Replication is not about the total number of measurements or individuals, but the number of independent units in the study. An “experimental unit” is the independent unit to which treatments are assigned.

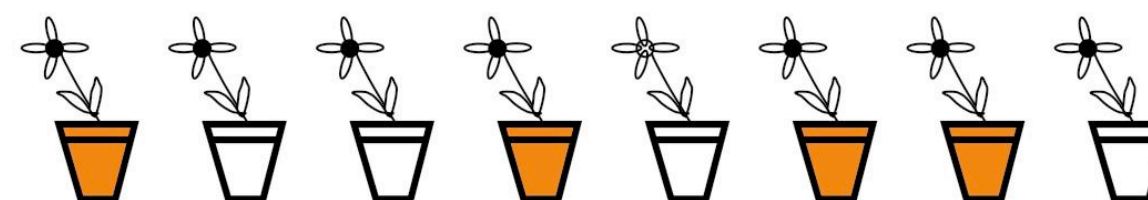
Two pots
 $n = 1$ for each treatment



Two chambers
 $n = 1$ for each treatment



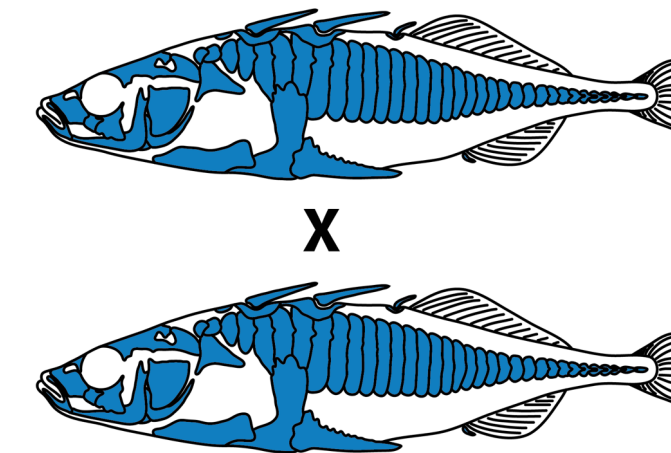
Eight replicates
 $n = 4$ for each treatment



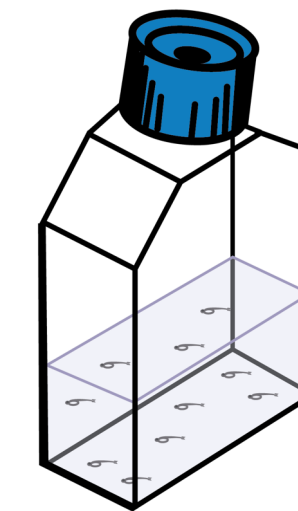
- The figure shows three experimental designs used to compare plant growth under two temperature treatments (indicated by the shading of the pots). The first two designs are un-replicated.

Pseudo-replicates

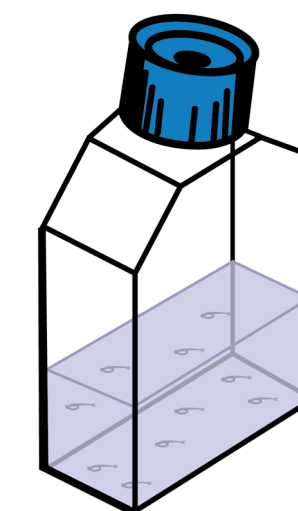
- Replicates that are used for inferences at the **incorrect scale**.
- Note - **pseudoreplication** is therefore context dependent
- **Nested ANOVA** is one proper way to avoid pseudoreplicates, but the model must be set up appropriately
- **Example** - experimental fish in two different tanks, each tank receiving a different treatment. (observations not independent with respect to treatment)



Germ-free



Conventional
Microbiota



Balanced Designs

- A completely balanced design means that all groups being compared have the same sample size.
- Why important?
 - reduce sampling error differences between groups,
 - hypothesis tests more powerful, reliable, and robust

$$s_{\bar{y}_1 - \bar{y}_2} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

(Assuming $s_1 = 1$ and $s_2 = 1$)

For $n_1 = 10$ and $n_2 = 10$

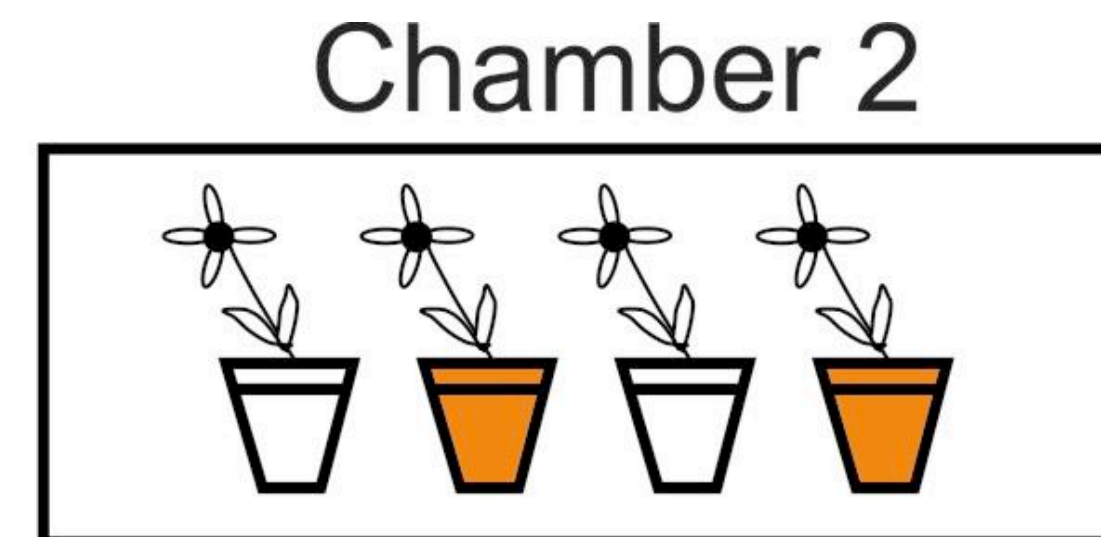
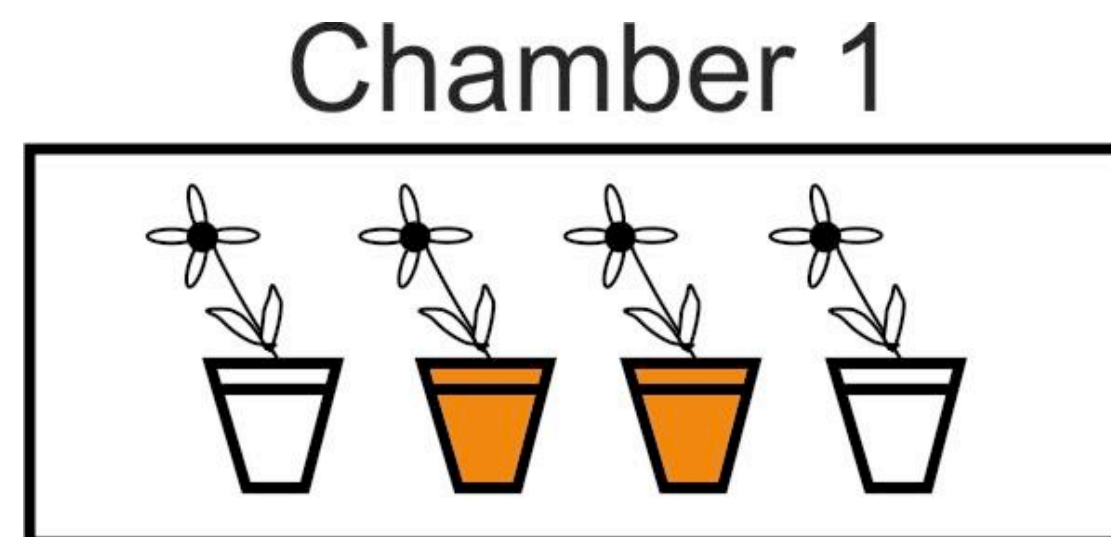
SE = 0.447

For $n_1 = 15$ and $n_2 = 5$

SE = 0.516

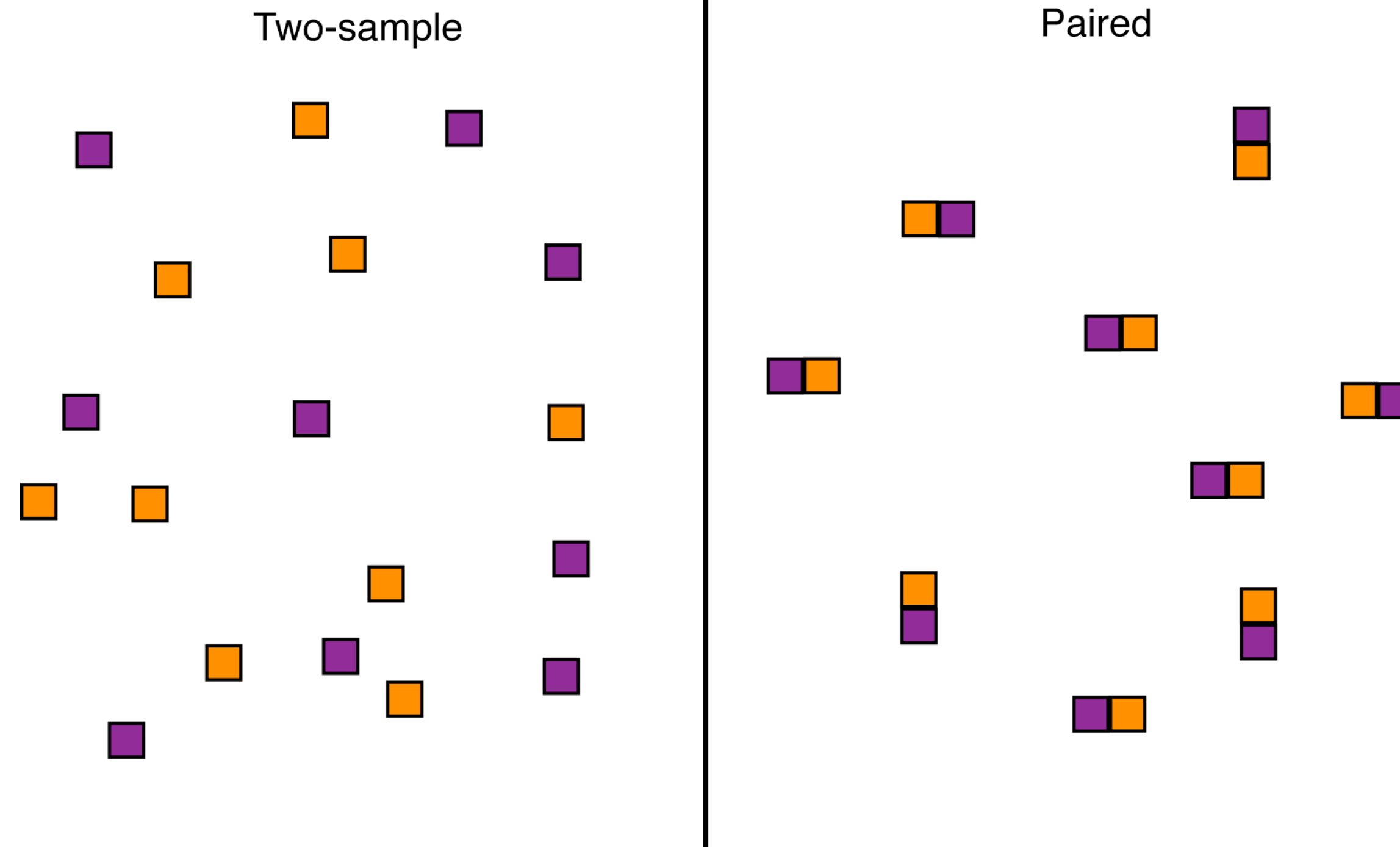
Blocking

- Blocking is the grouping of experimental units that have similar properties. Within each block, treatments are randomly assigned to experimental units.
- Blocking essentially repeats the same, completely randomized experiment multiple times, once for each block.
- Differences between treatments are only **evaluated within blocks**, and in this way the component of variation arising from differences between blocks is discarded.



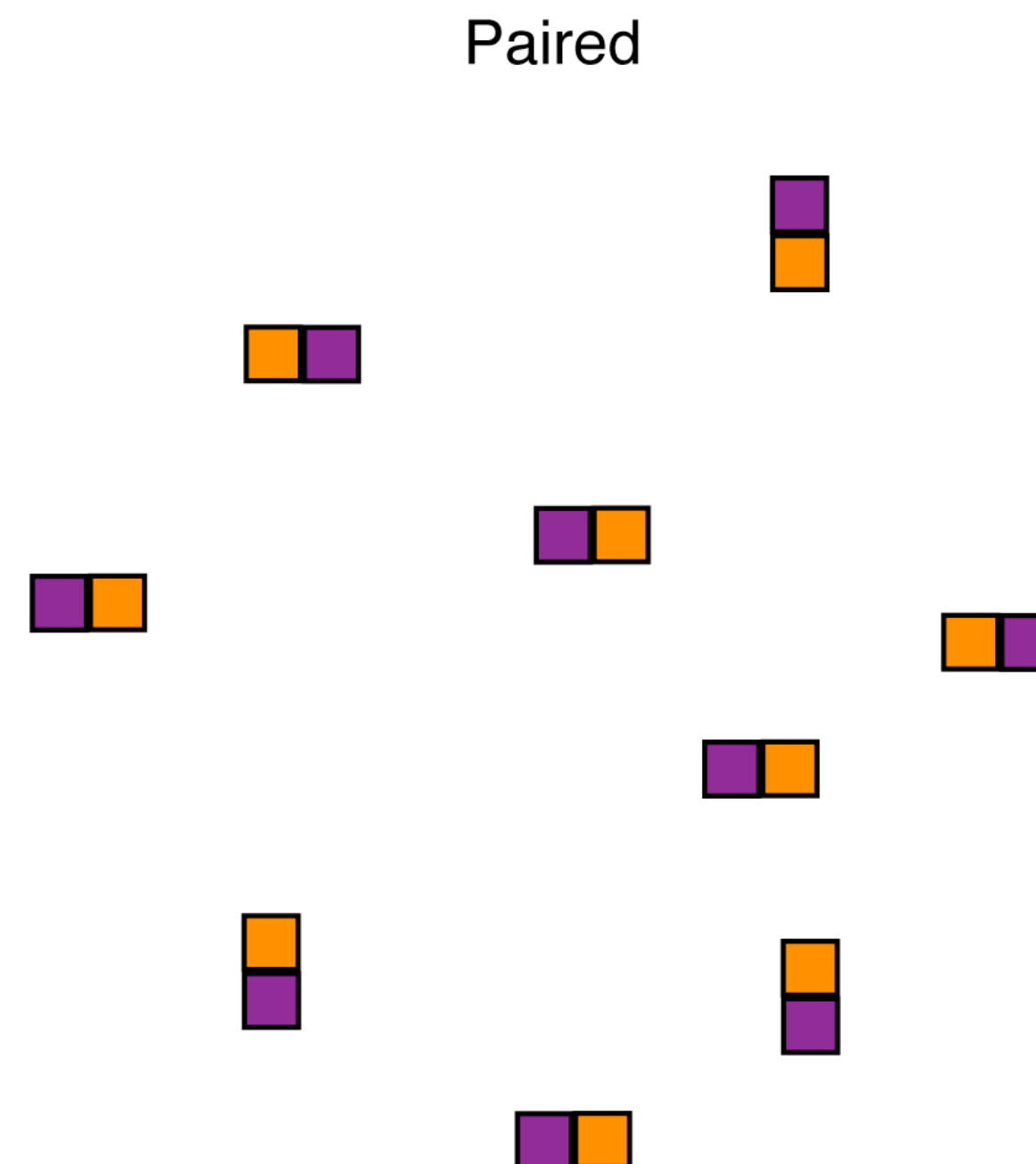
Blocking: paired design

- **Two-sample design without blocking:** randomly assign plots across the landscape to treatments (example: clear-cut vs. not cut forest)
- **Paired blocking design:** take a random sample of forest plots and clear-cut a randomly chosen half of each plot, leaving the other half untouched.



Blocking: paired design

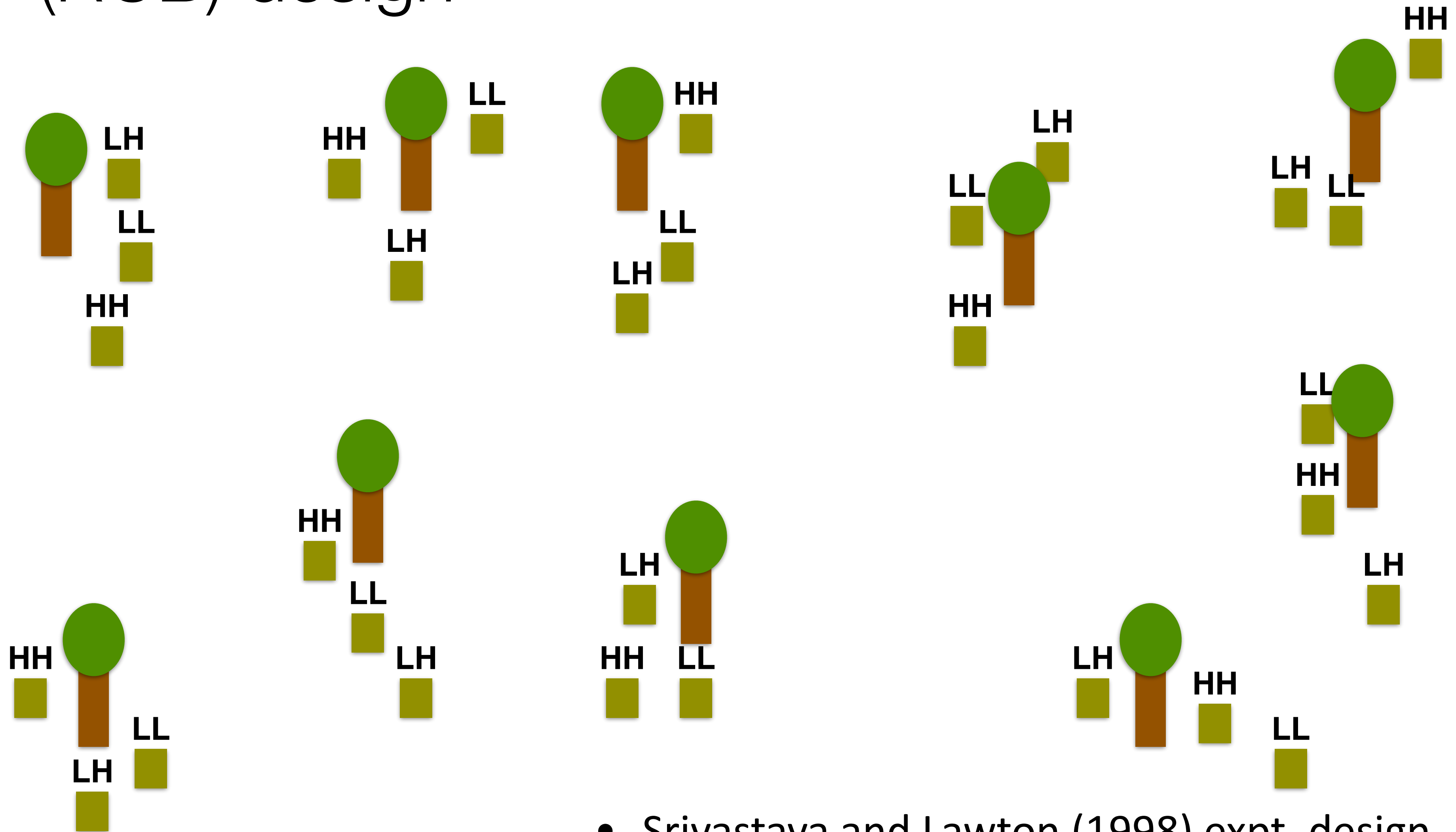
- **Paired blocking design:** The effect size of the treatment is calculated within each block, and the average effect across all blocks is compared to a distribution expected under the null hypothesis.
- **More powerful** than random 2-sample design because it accounts for variation between plots that sometimes obscures the effects we are looking for. For example many other factors over space may contribute to patch salamander density.



Blocking: randomized complete block (RCB) design

- RCB design is analogous to the paired design, but usually has more than two treatments, and each treatment is applied once at random to every block.
- Blocking is worthwhile if units within blocks are relatively homogeneous, apart from treatment effects, and units belonging to different blocks vary because of environmental or other differences.

Blocking: randomized complete block (RCB) design



What if you can't do experiments?

- The best observational studies incorporate the features of good experimental design when applicable: blinding, replication, balance, blocking
- Randomization is out of the question, because in an observational study the researcher does not assign treatments to subjects. Instead, the subjects come as they are.
- Two strategies are used to limit the effects of confounding variables in observational studies

Matching - make comparisons within sample units that are similar e.g. if comparing 2 genotypes, do so within similar body sizes

Record potentially confounding variables and include them as factors in the analysis - record body size and include as a co-variate.

