An Overview of Simple Pseudoreplication

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STAT 811

February 4, 2020

Overview

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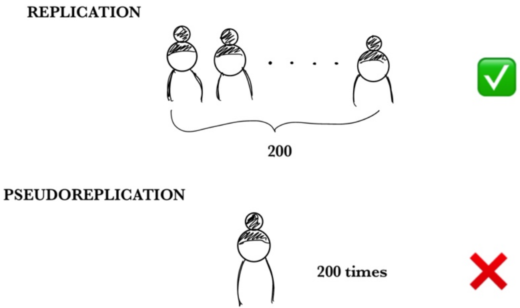
Purposes of Replication

Replication

- an important component of experimental design
- More than one experimental (or observational) unit with the same treatment. Each unit with the same treatment is called a replicate
- controls random or stochastic error
- Increases the precision of a test
- Increases the generalizability of the test
  - eg: If you test across many sites, you can generalize to many others

Motivating Example

Motivating Example



- To determine the amount of human chorionic gonadotropin (hCG), the pregnancy hormone, in the blood of women one month into their pregnancy
- Want to have 200 replicates for your study
- True replication → Persuade 200 different pregnant women to donate blood
- Pseudo replication → Measure the blood levels in the same person 200 times

Definitions

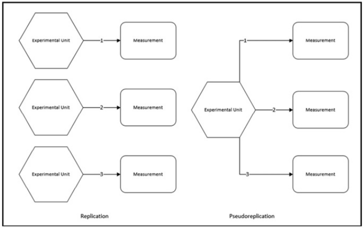


Figure: Replication vs Pseudoreplication

- *Experimental unit* = the smallest entity to which a treatment is applied
- *Sampling unit* = unit that has been sampled from a statistical population
- *True replicate* = sample
- *Pseudo replicate* = subsample

Simple Pseudoreplication

What is Pseudoreplication?

PSEUDOREPLICATION AND THE DESIGN OF ECOLOGICAL FIELD EXPERIMENTS[1]

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Abstract. Pseudoreplication is defined as the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or replicates are not statistically independent. In ANOVA terminology, it is the testing for treatment

- Process of artificially inflating the number of samples or replicates
- Results in replicates not being independent
- Cannot test for treatment effects
  - Confounded with other effects .
- Inferential statistical tests performed on the data are rendered invalid
  - ANOVA assumption violated

Simple Pseudoreplication

- Broadly speaking, pseudoreplication has 3 different forms
  - One of them is known as *Simple Pseudoreplication*
- Occurs when
  - There is only a single experimental unit (= replicate) per treatment, but multiple measurements are made on each experimental unit.
- Most common in observational studies - especially multiple group studies.

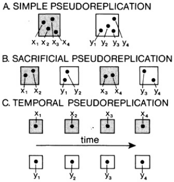

Figure: Various Forms of Pseudoreplication

Simple Pseudoreplication Contd. (1)

- Example:
  - Suppose we are interested in comparing the effectiveness of 2 drugs.
  - You have 2 experimental units, patient 1 and patient 2.
  - Administer drug A to patient 1 and drug B to patient 2.
  - MISTAKE: Taking multiple samples from patient 1 and patient 2 to compare the drug performance
  - WHY?
    - Drugs typically have different effects in different people
    - Can validly be used to increase *sensitivity*, but not to examine the *effect of drug*
    - The treatment factor is completely confounded with the patient
    - Do not know whether the differences arise from some other factor which differs between the two patients
    - No information about generalizing to people other than the two involved.

Drawbacks of Simple Pseudoreplication

Illustration

- We have beetle population distributed over a large field
- Test for short-term effect of herbicide on beetle density
  - Assume that there is actually no effect
- Design A: Divide the field into 2 subfields and apply herbicide to one
- Design B: Divide the field into grids of 4x4 m plots and apply herbicide to n of them
- **Underlying assumption:** experimental units are identical at the time of treatment implementation and remain identical during the duration
- As the number of samples covers the entire unit, the probability that there is a difference between the units approaches 1

Illustration Contd. (1)

- Null hypothesis assumed: No herbicide effect on beetles
    - Null hypothesis tested: No difference between subfields
- Probability of a type 1 error increases significantly as the number of replicates increases

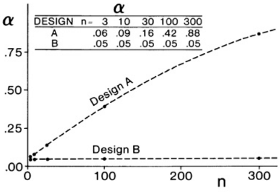

Figure: Design A has pseudoreplication whereas Design B does not

Empirical Demonstration

Data Set

Plot Number	Beetle Density	Treatment
1	25.3133	sprayed
1	13.91215	sprayed
2	58.61689	unsprayed
2	67.59083	unsprayed
3	40.20168	sprayed
3	46.21766	sprayed
4	44.86198	unsprayed
4	62.79626	unsprayed

Figure: Beetle density data set overview

- We have 4 plots and 2 samples of "beetle" density from each plot. Each of the plots (the experimental unit) is either *unsprayed* (control) or *sprayed* (treatment)

Data Analysis

- Run ANOVA to test the herbicide effects without considering the dependent nature of the observations to get the following summary:

```
            Df Sum Sq Mean Sq F value Pr(>F)
factors1     1   1464  1464.0   9.475 0.0217 *
Residuals    6    927   154.5
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure: One-way ANOVA

- Significant effect of herbicide effects
  - Error degrees of freedom is 6 (due to the incorrect count of replicates)
  - allows for easier significance result

Data Analysis Contd. (1)

- Repeat the same process by averaging samples from each experimental unit to be the single replicate.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt1	1	732.0	732.0	4.555	0.166
Residuals	2	321.4	160.7		

Figure: One-way ANOVA

- No Significant effect of herbicide effects
  - Next Steps: Treatment effects might be confounded with plot effects.

Data Analysis Contd. (2)

- Run ANOVA for plot effects

```
          Df Sum Sq Mean Sq F value Pr(>F)
plots1     3 2106.8   702.3   9.885 0.0254 *
Residuals  4  284.2    71.0
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure: ANOVA for plot effects

- Significant difference of beetle density with respect to plots
  - Underlying assumption violated → beetle densities were not the same on the different plots
  - Significant result was still achieved under the assumption violation

Key Takeaways

- Multiple samples per experimental unit do not increase the number of degrees of freedom
- Number of replicates was less which lowered the residual degrees of freedom. This increased the value of the denominator, which resulted in a lower F-test value and thus, the non-significant result.

Remedies

Avoiding Simple Pseudoreplication

- Avoid at all by taking more *true* replicates
  - However, due to real-world constraints, this may often be impossible or undesirable
  - example, not suitable for large scale systems like whole lakes, rivers
- Most simple solution to avoid this kind of pseudoreplication is:
  - Use only a single datum (mean of the samples) for each experimental unit
  - In our drug example, average all the measurements taken from a single patient
- Account for the random factor

Avoiding Simple Pseudoreplication Contd.(1)

- Maximize the independence of your samples if a certain number of replicates is required
- Perform the correct analysis by changing the objectives of the study
  - Treat as a preliminary one rather than the final result.
- Present both results clearly outlining the assumptions violated for the one involving pseudoreplication.

Summary

- **Common mistake** during the analysis phase of an experiment is to treat your replicates as independent when they aren't - *Pseudoreplication*
  - In fact, Hurlbert found that 48% of studies that he looked at committed this error
- One such kind is known as **Simple Pseudoreplication**
  - This happens when you take *multiple measurements per experimental unit*
- Results in inflated type-I error result that demonstrates the dangers of Simple Pseudoreplication with respect to inferential statistics

References

- **Pseudoreplication and the Design of Ecological Field Experiments**, Stuart H. Hurlber. Ecological Monographs, Vol. 54, No. 2. (Jun., 1984), pp. 187-211
- **Influential Points**
  http://influentialpoints.com/Training/Pseudoreplication-useand-misuse.htm
- **Texas A&M**
  https://web.ma.utexas.edu/users/mks/statmistakes/pseudorep.html