

# Pseudoreplication: a *sine qua non* for regional ecology

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Keywords: statistics, sampling, experimentation, experimental design, landscape ecology

## Abstract

We question whether classical experimentation is adequate for real progress in landscape or regional ecology. One cannot do classical experimentation unless one can replicate the treatment. There is conflict between the need to replicate and the need to study processes at appropriately large scales.

Because of the difficulties in doing controlled field experiments at regional scales, we propose that landscape ecologists take greater advantage of natural field experiments. Natural experiments must be coordinated, standardized, and synchronized over space and through time, and will require the cooperation of multiple investigators. Distributed computer networks can help provide the automated region-wide monitoring which will supply natural experiments with pre-treatment data.

Regions or landscapes need not be 'replicated', and indeed, cannot be. One can achieve a relational understanding between a system's response and environmental characteristics. This understanding is not definitive, but allows for the development of testable hypotheses, in the classical sense. The confounding of space, time, and/or other environmental factors in pseudoreplicated natural experiments only allows for the development of hypotheses – 'how-possibly' explanations. Discrimination among competing hypotheses can be done at smaller scales and used to infer processes occurring at larger scales. Use of natural and controlled field experiments in complementary roles is a more promising approach than views of one or the other as methodologically inferior.

## Introduction

Hurlbert (1984) defined pseudoreplication as "... a consequence of the actual physical space over which samples are taken or measurements made being smaller or more restricted than the inference space implicit in the hypothesis being tested (p. 190)." "Assuring that the replicate samples or measurements are dispersed in space (or time) in a manner appropriate to the specific hypothesis being

tested is the most critical aspect of the design of a mensurative experiment" (Hurlbert 1984, p. 189-190). Temporal pseudoreplication is committed if measurements taken through time are used as replicates. Hurlbert found pseudoreplication in more than a quarter of the ecological field experiments he reviewed.

Pseudoreplication does not describe a particular type of experimental design, but rather a particular category of misinterpretations or misanalyses. An

experiment lacking treatment replication is not 'pseudoreplicated,' it is only weakly designed. Pseudoreplication is not inevitable, but only occurs if the investigator misleads the reader by applying inappropriate statistical analyses or misstating the strength of the evidence obtained. The essence of pseudoreplication is not design but deception, intentional or otherwise.

Hurlbert (1984) himself recognized valid scientific contributions where replication was impossible, particularly in impact assessment studies. He felt that in such cases ecological but not statistical inferences could be drawn even though treatments were not replicated. Despite these allowances, Hurlbert's paper has created a preoccupation with statistical significance and classical experimentation in ecology.

Hawkins (1986) urged caution in responding to what he saw as a harmful 'backlash' to the pseudoreplication problem. From firsthand experience, he feared that 'pseudo-understanding' reviewers might judge all nonreplicated studies as **pseudo**-replicated and scientifically inadequate. Hawkins used circumstantial ecological (rather than statistical) evidence to infer that significant differences in population density between two experimental streams were in fact due to his nonreplicated **labor**-intensive removal treatment. Despite criticism for pseudoreplication, Hawkins maintained that **nonreplicated** studies might nevertheless produce strong ecological evidence.

Stewart-Oaten *et al.* (1986) outlined a statistical procedure which they felt would allow statistical comparison of impact assessment without pseudoreplication. Sampling through time before and after discharge at a control and an impact site will permit statistical detection of the effect of a discharge. They cautioned that the control site must be chosen in accordance with stringent assumptions for their cross-site comparison to satisfy Hurlbert's objections. We suspect that Hurlbert would still define such a procedure as pseudoreplication.

Pseudoreplication has a greater impact on experimental design as scale and duration increase. The 'inference space' must be blanketed with sites to avoid pseudoreplication. If substantial time is required to measure all sites, the region may change

during measurement, and temporal **pseudoreplication** will occur. Sampling must be repeated often enough to permit adequate temporal resolution, and must be continued long enough to detect regional changes. Regional experimenters need frequent instantaneous 'snapshots' of entire regions over long periods. The logistic problems of conducting regional experiments without **pseudoreplication** are clearly immense, and may be insurmountable.

Local ecological investigations, usually assume homogeneity and equilibrium. These initial assumptions conceptually eliminate that which regional ecologists attempt to detect – spatial and temporal interactions across heterogeneous areas. One goal of landscape ecology is to quantify the constant redistribution of organisms, materials or energy among landscape components which are not at equilibrium (Risser *et al.* 1984).

While local experiments are interpreted as if all other things were equal, experimental conditions at different locations are rarely the same. Indeed, if spatial heterogeneity is what one is trying to understand, how can results be compared spatially without pseudoreplicating?

The classical 'scientific method', in which all variables save one are held constant while that variable is manipulated, is not a practical approach for landscape and regional ecology, and sacrifices the economies of a multivariate approach. In a multifactorial experiment, effects of several variables can be investigated simultaneously. Factorial designs also yield data on interaction impossible with a classical design.

For fear of pseudoreplicating, landscape ecology is becoming more reductionist when the object is to become more holistic. The sanctioned conceptual framework and experimental techniques available to regional ecologists are inconsistent with the nature of regional ecology. This deep conflict has resulted in stagnation.

### Problems impeding progress in regional ecology

Much of landscape ecology to date has been based on description and simulation. Initial description

has been limited to patch patterns (Neef 1982; Wiens *et al.* 1985; Risser *et al.* 1984), e.g. determination of fractal dimensions of patch borders (Bradbury *et al.* 1984; Krummel *et al.* 1987; Turner 1987; Milne 1988). Description has recently extended to patch dynamics, the spread of disturbance, and processes occurring across the boundaries of heterogeneous landscape units (Roughgarden *et al.* 1988; Turner *et al.* 1989). The development of any new discipline commences with description, but should progress to manipulation and experimentation. Landscape ecology has failed to make this transition.

Space or time is the only 'experimental' variable or 'treatment' in such observational ('mensurative' *sensu* Hurlbert 1984) studies. 'Treatments' in an observational experiment are isolated from each other in space and time, while treatments in a classical experiment always must be interspersed with each other in space and time (Hurlbert 1984). Purely observational studies only demonstrate correlations, and alone give no basis for selecting among multiple explanatory models of underlying processes.

Classical experiments, in contrast to observational studies, are exclusively capable of unambiguously demonstrating causality (in a practical sense) with statistical significance levels. Four stringent criteria distinguish classical experimentation: controls, manipulation, randomization, and independent replication. Different experimental units must receive different treatments, and the assignment of treatments to experimental units must be randomized (Hurlbert 1984). Classical experiments are theoretically neutral, and allow strong inference (Platt 1964).

Classical experiments are not well-suited to regional ecology. It is difficult to manipulate or replicate over large spatial scales and to maintain a manipulation for long periods. Manipulation is always disturbance, and may propagate undesired effects through other system components. Replication implies that the experiment be repeatable, i.e., to some degree independent of time and space.

Replication permits estimates of error and variability, but is more difficult as spatial and temporal scales increases. At larger scales, rare events be-

come ordinary. Random events may be definable in large systems, but are not predictable (Gleick 1989). More replication is necessary with capriciousness and heterogeneity, but replication at large scales is expensive. Longer experiments are increasingly susceptible to stochastic or 'nondemonic' intrusions (Hurlbert 1984).

What constitutes reasonable or adequate replication? The answer to this question is not absolute, but is subject to interpretation of the factors believed important with regard to the measurements being made. This interpretation may change in light of new information about underlying factors. Replicates are not strictly valid or invalid, but can assume a spectrum of validity. Indeed, particular replicates might be better (*viz.* more similar) in some regards than others.

The logistics of classical experimentation over wide areas often require some separation of sampling in time. The use of samples separated in time as replicated treatments, however, is temporal pseudoreplication (Hurlbert 1984). Restricting the experiment to a few sites which can be sampled within a short period, on the other hand, is spatial pseudoreplication, since the inference space **will be** larger than the area covered by replicates (Hurlbert 1984). Insufficient or inadequate replication may be worse than no replication at all if the experimenter (or reader) fails to consider the power of the statistical tests when interpreting the results.

Classical experimentation is most practical for a reductionist approach. Discrepancies in design and method among sites complicate 'bottom-up' techniques (Caraco and Lovett 1989). Varying levels of precision, resolution, duration, and timing make integration of results obtained with classical experiments difficult (Wimsatt 1980; Berkowitz *et al.* 1989). A potpourri of classical study outcomes may not have homogeneous measurement scales or statistical independence (Wachter 1988). More than **a collection** of point results is required to detect spatio-temporal patterns in the landscape.

Ecologists can barely manage experimental manipulations at the scale of ecosystems (much less landscapes), and then only by manipulating some specialized ecosystem, such as a watershed or lake (Schindler 1990). These systems lend themselves to

manipulation because their boundaries are defined by the flow of water or nutrients. Sadly, few ecosystems (and even fewer landscapes) have such well-defined borders. A classical experimental approach with more amorphous systems will be difficult if not impossible.

The apparent incongruence between population/community-level experimental approaches and ecosystem/landscape approaches is widely recognized (Fenchel 1987; May and Seger 1986; Vitousek 1990; see Carney 1989 and comments by Fenchel 1989 and Allen and Hoekstra 1989). There has been a lack of satisfaction with progress using higher-level approaches, even among proponents (e.g. Franklin 1989; Pomeroy *et al.* 1989; Simberloff 1980). The International Biome Programme, an early attempt to employ higher-level approaches, is often regarded as disappointing (Macfadyen 1975; Mitchell *et al.* 1976). Despite all efforts, large-scale ecology does not seem to have developed a powerful central thrust comparable to that found in, say, molecular biology. We seem no closer to elucidation of unifying principles of regional ecology than when we started.

Because of the difficulty of experimental manipulation at higher levels, few researchers have tried to determine the relative importance of exogenous variables, such as the weather or the surrounding landscape, on populations. Andrewartha and Birch (1954) underscored the importance of weather in controlling population outbreaks. Far from settling the question of key factors, however, their work was sparked a 30-year debate on the relative importance of abiotic vs. biotic factors in controlling insect outbreaks. Population-level ecologists typically focus on observable site-specific factors, such as resource availability or the impact of higher trophic levels in attempting to understand population-level phenomena. This local approach may be insufficient to resolve overwhelming exogenous factors interceding from higher-order processes.

Roughgarden *et al.* (1988) were unable to explain the structure of intertidal communities before considering events in offshore waters. Small-scale field manipulation, by itself, was misleading when larger scales were considered. They entreat study of the

'mesoscale'; that which lies between the local study site and the biogeographic and ecosystem scale. Progress at the mesoscale, they suggested, requires fusing the 'reconstructural logic' commonly used in the earth sciences with standard field experimentation. Reconstructural logic is inference based on pseudoreplication.

Vitousek (1990) suggested that cases of biological invasion of ecosystems by exotic species which alter ecosystem properties and/or processes could be used to integrate population biology and ecosystem ecology. He considers changes in an Hawaiian ecosystem after invasion by a nitrogen-fixing *Myrica* species which quadruples inputs of otherwise-limiting nitrogen. Unless deliberate introduction is used as a treatment, such 'experiments' will be natural, and comparisons will be pseudoreplicates.

O'Hara (1988), considering cladistic systematics, suggests retreat to a pragmatic level of explanation, which he calls 'how-possibly' explanations. A how-possibly explanation merely removes objections that a questioner has to the possibility of an explanation. Rather than classical experiments, how-possibly experiments can be done to suggest how something may have taken place. How-possibly experiments, however, only provide plausible answers, not deductions based on laws. There may be a larger number of acceptable how-possibly explanations for any given question.

Scriven (1959) recognized statements generated by the collective outcomes of repeated experiments as normic statements: statements of what usually or normally happens. They are not universal, and are not probability-based, but represent a generalization with exceptions. A normic statement describes conditions that are necessary, but not sufficient, for an event to occur. Although not predictive, the information content of normic statements is high in terms of explanatory power. Induction-based pseudoreplicated experiments will result in normic statements.

Between ecosystem and landscape levels there may be an important transition in viewpoint and method as wide as that between community- and ecosystem-levels. Progress in regional ecology will require adoption of a conceptual perspective that admits heterogeneity and disturbance (Pomeroy *et*

*al.* 1989; Tilman 1989). Classical experimentation cannot manage the complexity of increasing scale and duration.

How should ecologists collect data relevant to landscapes? Methodology is by definition time- and space-dependent. Dependence on scale severely restricts the utility of classical experimental approaches for regional ecology.

Classical experimentation in practice is neither objective nor theoretically neutral (Kuhn 1962; Kiefer 1977; Berger and Berry 1988; Warren 1986). Classical experiments, *sensu stricto*, are idiosyncratic. Generalizations are not permitted; results are true only for particular cases. Regional ecology must infer what is true rather than falsify what is not.

Some ecologists feel that ecology will evolve to be a 'hard' objective science, and that ecologists should work toward making ecology as rigorous as physics or chemistry. Egler (1986) calls this 'physics envy,' and suggests that the small-scale and short-duration experiments of chemistry and physics are poor models for ecology. Ecology is complex (particularly at landscape and regional scales) and ecological data are anything but 'hard facts' (Fagerström 1988). Zonneveld (1982) characterized landscape ecology not as science but rather as a 'state of mind.'

### A prescription for progress in regional ecology

Other disciplines not permitting classical experimentation appear to make progress. Astronomy, like regional ecology, is prevented from direct experimentation by remoteness in space. Geology, systematics, and biogeography cannot experiment in the classical sense because of remoteness in time. Medicine and psychology must pseudoreplicate because of ethical considerations. How do these necessarily pseudoreplicating disciplines progress?

One technique common to pseudoreplicating disciplines is space-for-time substitution (SFT), the use of presumed chronosequences (Pickett 1989). The observer infers a temporal trend from study of different-aged sites. SFT assumes that spatial and temporal variation are equivalent and that impor-

tant processes are independent of space and time.

SFT is analogous to age-cohort versus serial-cohort life-table studies. When it is not practical (or possible) to follow a cohort until the demise of the last individual, one can simply count the number of individuals alive, born, and dying in each cohort during an interval of time. SFT is a vertical rather than a horizontal approach.

SFT is useful for structural and compositional dynamics (i.e. description), but provides limited discrimination of functional processes, since observations are of short duration. Study of **chronosequences** is sensitive to rare events – nondemonic intrusions affecting some but not all members of the cohort.

We suggest that much ecological work is inferential and inductive. This results from the 'softness' of ecology rather than poor quality research. Because regional ecology is a conceptual extension of ecosystem and landscape ecology in time and space, it is generally impossible to experiment in the classical sense at regional scales and durations. Careful pseudoreplication leading to induced conclusions will be necessary. Regional ecologists cannot afford to eschew induction and pseudoreplication as inferior; indeed, we must embrace these as primary investigative tools.

We suggest quasi-experiments as a compromise between classical experimentation and descriptive techniques. Quasi-experiments assume that there would have been no changes in a region if no 'treatment' had been 'applied' or if the 'treatment' had no effect. Before/after experiments and impact studies are practical examples of quasi-experiments. Quasi-experimentation requires *a priori* serendipitous pre-'treatment' measurements, since the 'treatment' is not controllable, randomizable, or predictable. Observational studies lay groundwork for quasi-experimentation.

Quasi-experiments may take advantage of natural phenomena or catastrophes which alter or manipulate wide areas. El Niño, the Gulf Stream, and the San Andreas fault represent natural forces with the potential to affect much larger regions than ecologists ever will. Island biogeography, arguably the developmental basis for landscape ecology, emerged from a series of 'natural experi-

ments.' Accidental manipulations may also be treated as quasi-experiments.

Quasi-experimenters will always pseudoreplicate spatially or temporally, and must make intelligent and informed comparisons to insure legitimate results. Quasi-experimentation is a form of SFT. Like SFT, the success of this technique rests on the ability of ecologists to perceive and identify conditions which are important with regard to the hypothesis of interest to ensure valid pseudoreplication.

Quasi-experiments are not a *panacea*. There is danger of spurious correlation; other variables can confound or obscure relationships. Conclusions cannot be made about processes from examination of correlative patterns revealed during quasi-experimentation, since any number of processes can result in the same pattern. Stochastic processes (nondemonic intrusions) have greater impact on quasi-experiments than on other designs.

Distributed networks can supply 'ground truth' for remote observations, and provide 'before treatment' characterization for quasi-experiments across regions (Pickering *et al.* 1990). Computerized geographic information systems (GIS) can relate mesoscale processes to local and regional scales (Burroughs 1986; Johnson 1990; Kuchler and Zonneveld 1988).

Quasi-experiments occurring in a natural laboratory will not unambiguously establish causal links, but may identify a few driving variables based on circumstantial evidence. Quasi-experiments cannot choose among the infinity of hypotheses which explain a particular set of observations. Smaller-scale classical experiments, however, can be designed which will resolve among competing hypotheses consistent with the outcomes of regional quasi-experiments.

Discrimination among competing hypotheses can be done at smaller scales and used to infer processes occurring at larger scales. Using careful process research and modelling, we can narrow the range of credible hypotheses for observed patterns. Regional ecologists can use these how-possibly explanations as tools to study aspects of larger situations in detail.

External validity from a single 'perfect' experi-

ment, particularly at landscape scales, is a dangerously restrictive concept. Experiments at scales larger than lab benches cannot be replicated exactly, only repeated approximately. Difficulty in replicating large-scale manipulations makes quantifying cause-effect relationships difficult, but this loss of statistical inference to pseudoreplication may be offset by carefully developing ecological inferences from an investigation. Most hypotheses are not tested in the isolation of one finely-controlled definitive experiment, but in the wider context of a series of experiments or observations. Repetition of an 'experiment' adds weight to an inference (Hawkins 1986). A congruent group of non-replicated quasi-experiments will outline an evolving procedure for conducting research in landscape ecology. Shared prior beliefs may make studies by separate research teams less than independent, but so does the current scientific paradigm (Kuhn 1962; Greene 1981). We may learn more by repeating than we would have by replicating the original experiment.

Quasi-experimental comparisons can be evaluated in the same ways that ecologists presently evaluate the validity of statistical assumptions in classical experimental situations. Comparison and interpretation of results from experiments on the same phenomenon conducted under different conditions is at the heart of traditional research synthesis. The position of the research in relation to the edifice which is emerging from the collective weight of existing work provides grounds for evaluation.

A controversial statistical technique known as meta-analysis represents an extreme of purposeful pseudoreplication. Meta-analysis uses formal statistical techniques to sum up a body of separate but similar experiments (Wachter 1988; Mann 1990). Meta-analysis produces a unified result from diverse contradictory studies, but the meta-analysis itself is not an experiment. Meta-analysis is increasingly used to evaluate pseudoreplications in psychology, medicine, education, agronomy, and sociology (Mann 1990). Progress in pseudoreplicating disciplines is made in small steps. Meta-analysis is gaining acceptance among the soft sciences as a way of resolving the direction of these small steps (Mann 1990).

Debate about meta-analysis is essentially debate as to whether careful pseudoreplication can be valid. Meta-analysts use disparate (albeit carefully selected) pseudoreplicated studies as data. Opponents say there is danger in comparing studies that may differ in important yet unrecognized regards. The *a priori* selection and application of criteria used for inclusion of studies is the subjective yet critical crux of this controversy.

Regional ecologists must relax the classical statistical requirements of publication to a level achievable at regional scales to prevent valuable but pseudoreplicated regional quasi-experiments from being discarded. Classical experiments cannot be done over wide areas without confounding some information. Inductive conclusions must be permitted in the literature by editors and reviewers without penalty if underlying assumptions are clearly stated. Normic statements (with requisite qualifiers) must be encouraged and allowable as science.

This prescription will succeed only if investigators, granting agencies, reviewers, and editors can successfully discriminate important similarities and differences among regional situations. Ecologists may be unable to do this adequately. This is tantamount to admitting that we are not yet capable of regional ecology.

While genuine replication is a powerful tool that should be used when possible, the scale of ecological research should not be dictated by statistical constraints. We maintain that one cannot reasonably hope to do regional ecology without pseudoreplication. Astronomers cannot directly manipulate stars and systematics cannot repeat evolutionary events. A parallel situation exists for regional ecologists.

Evidence at regional scales will be anecdotal, circumstantial, and accidental as often as it is experimental. The difference in support given an hypothesis by a classical experiment, a quasi-experiment, a simulation, or an analytical description is one of degree, not kind. Through multiple cross-site or before-after comparisons, we can study the relative importance of landscape-level factors on particular populations that are not feasible to study by manipulative experimentation.

Hurlbert (1984) acknowledges that before/after experiments can decidedly imply 'treatment' effects. He simply and correctly points out that it is philosophically inappropriate to apply statistical tests to these results. To do so would imply that the difference is attributable to the 'treatment' alone, an implication which natural experiments cannot guarantee. Regional ecologists must be willing nevertheless, in light of enough circumstantial evidence, to accept that it is.

### Acknowledgements

Ideas presented here represent an amalgam of concepts and opinions from many sources, several of whom do not agree with us. Some were generated by discussions with the following persons: J. Chamberlin, D.C. Coleman, D.A. Crossley, Jr., B. Ekbom, F.B. Golley, B. Haines, E.C. Hargrove, A. Huryn, L.R. Pomeroy, K.B. Weinrich, and J.W. Wenzel.

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