Chapter 7

Going Back to Basics: How to Master the Art of Making Scientifically Sound Questions

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Abstract

Inspired by the famous quote of Leonardo da Vinci, this chapter builds upon the idea that practice without theory is blind and unpredictable. Indeed, theory without practice can be idle. Accordingly, progress in science is made through approaches that integrate hypothesis testing and falsifiability or that investigate weight of evidence for multiple hypothesis, such as the hypothetico-deductive method (HDM) and Bayesian techniques. Here, we provided a straightforward way to combine the HDM with statistical thinking to create a diagram that links variables by causal links, which can improve the scientific method and statistical literacy.

Key words Hypothetico-deductive method, Scientific flowchart, Prediction, *P* value

1 Introduction

> *He who loves practice without theory is like the sailor who boards ship without a rudder and compass and never knows where he may cast.*—Leonardo da Vinci

Tell me what your question is. Perhaps this is the phrase that most young researchers listen to when they begin their scientific activities. Apparently simple, answering this question becomes one of the biggest challenges in scientific training. Whether in qualitative or quantitative research, the entire knowledge-seeking process starts from a question/problem formulated by the researcher at the beginning of the process. This question will guide the researcher in all stages of the research. In the specific case of quantitative research, the question will begin one of the most powerful ways of thinking scientifically: the hypothetico-deductive method (HDM) championed by Karl Popper [1].

This chapter proposes a way of thinking about scientific hypotheses (generated within the HDM) to improve statistical

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thinking by using a flowchart that relate variables by causal links. In addition, we argue that you can easily use flowcharts to (1) tease apart relevant variables and how they affect each other; (2) improve (when necessary) experimental/observational design; (3) facilitate the selection of statistical analyses; and (4) boost data interpretation and communication.

2 Questions Must Precede Statistical Analyses

2.1 A Bestiary of Hypothesis Testing (Are You Asking the Right Question?)

Most students and professors in the biological sciences have an aversion to the word "statistics". Not surprisingly, whereas most academic disciplines in STEM (Science, Technology, Engineering, and Mathematics) have a strong statistical background in their undergraduate level, courses in the biological sciences have poor curricula in how to integrate statistical thinking within a biological context [2]. These courses have been frequently taught without any practical baseline to integrate students in a problem-solving platform [3]. Unfortunately, ethnobiology, ecology, and conservation (hereafter EEC) are not exceptions.

More importantly, a major concern during the statistical training of EEC students is the need of working with complex, multidimensional problems, which demand analytical solutions even more complicated to a public without a background in statistics and mathematics. Hence, some researchers consider statistics as the most problematic part of their scientific research. We argue that the difficulty of using statistics in EEC is associated with the absence of a problem-solving platform stating clear hypotheses derived from a theory. However, we agree that there is a great challenge in ethnobiology to integrate this hypothesis-driven approach, because it was introduced only recently (see [4–6]).

In view of the lack of a problem-solving platform, we frequently notice that students/researchers in EEC usually have a hard time answering basic questions for a scientific research, such as:

1. What is the main theory or logical reasoning of your study?
2. What is the main question of your study?
3. What is your hypothesis? What are your predictions?
4. What is the sampling unit, independent and dependent variables of your work? Is there any covariate?
5. What is the control group?

How can one select any statistical test without answering those five questions? The frequentist statistical framework provides a way to go by progressively supporting or falsifying a hypothesis [1, 7]. The decision to reject a null hypothesis is made using a probability value (usually $P < 0.05$) calculated by comparing observed events with repeated observations that generate a null distribution.

Now, let us teach by example and introduce a "guide to statistical thinking," which connects some essential elements needed before running any multivariate (or univariate) analysis (Fig. 1, see also Fig. 1.3 in [8], and Fig. 1 in [9]). First, imagine you observed the following phenomena in nature: (1) "local people selecting some plants for medical purposes," and (2) "monodominant patches of the tree *Prosopis juliflora*, an invasive species in several regions." On the ethnobiology side, to understand how and why traditional knowledge is constructed, there is a theory or hypothesis (e.g., apparency hypothesis: Gonçalves et al. [10]) explaining the main processes dictating plant selection (Fig. 1a). Then, you can ask one or more questions related to those observed phenomena (Fig. 1b). For example, how does urbanization affect people knowledge about medicinal plant use in different biomes? On the ecological/conservation side, to understand why introduced species affect local native species, you need to understand the ecological niche and evolutionary theories [11, 12]. You can ask, for instance, how does exotic plants affect native plant community structure?

Complex or vague questions difficult the construction of the research flowchart (see description below) and the selection of statistical tests. Instead, a useful question should indicate the relevant variables of your study, such as independent and dependent variables, covariate, sampling unit, and the spatial scale of interest (Fig. 1b). In the provided ethnobiological example, urbanization and people knowledge are the independent and dependent variables, respectively. Also, this study has a broad scale, as it compares different biomes. The next step is constructing the biological hypothesis (Fig. 1c), which will dictate the association between independent and dependent variables. In the ethnobiological example, the hypothesis is that (1) "urbanization affects people knowledge about medicinal plant use," while the ecological hypothesis is that (2) "exotic species affect native community structure." Note this is very similar to the main question. But you can have multiple hypotheses [13] derived from one theory.

After stating the biological (or scientific) hypothesis, it is time to think about the corollary (logic derivation) of the hypothesis, which is called prediction (Fig. 1d). The predicted patterns are a very important step, because after defining it you can operationalize your variables and visualize your data. For example, the theoretical variable "Urbanization" can be measured as "urbanization grade along urban, peri-urban, and rural areas," and "people knowledge" as "the number and type of useful plant species used for different diseases." Thus, the prediction is that urbanization grade decreases the number and type of known plant species utilized for medicinal purposes." In the ecological example, "Exotic species" can be

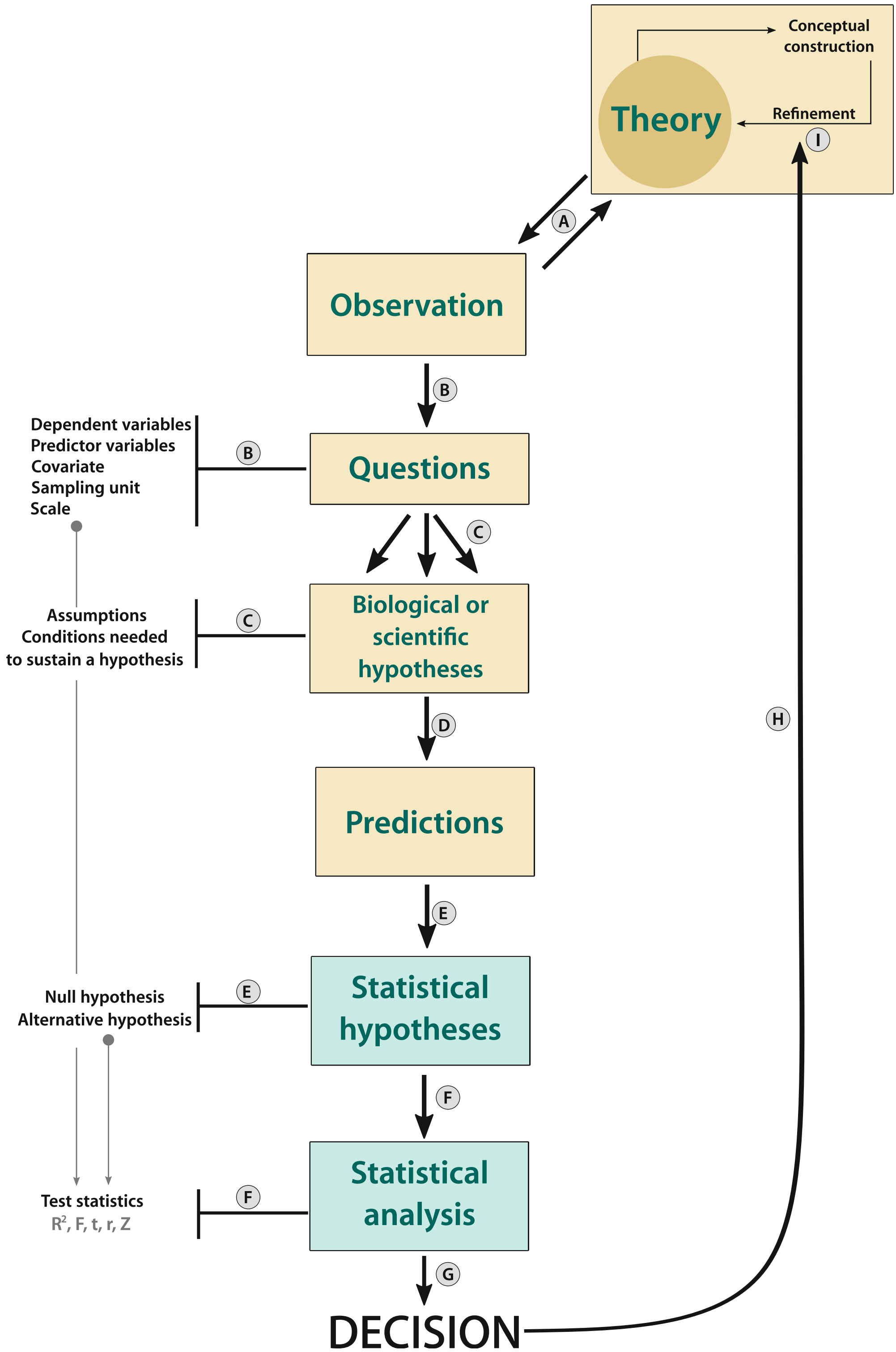

Fig. 1 A guide to statistical thinking combining the hypothetico-deductive method (**a**–**d**, **i**) and frequentist statistics (**e**–**i**). See also Fig. 1 in Underwood [9], Fig. 1 in Ford [15], and Fig. 1.3 in Legendre and Legendre [8]

measured as "the density of the exotic plant *Prosopis juliflora*" and "Community structure" as "native species richness and composition". After you have operationalized your work in the light of the hypothetico-deductive method (HDM), the next step is "thinking statistically" about the formulated biological hypothesis (see Figs. 1e, f).

Then, you need to define the null (H_0) and the alternative (H_1) statistical hypotheses. Two different "statistical hypotheses" can be derived from a biological hypothesis (Fig. 1e). Therefore, we are using the term "statistical hypothesis" in quotation marks, because the so-called statistical hypotheses are predictions sensu stricto, and often confound young students. The null statistical hypothesis represents an absence of relationship between the independent and dependent variables. After defining the null statistical hypothesis, you can derive one or multiple alternative statistical hypotheses, which demonstrate the expected association(s) between your variables (Fig. 1e). In our example, the null hypothesis is that "urbanization grade does not affect the number of useful plant species known for local people." In turn, the alternative hypothesis is that "urbanization grade does affect the number of useful plant species known for local people."

After you operationalized your variables and defined the null and alternative hypotheses, it is time to plot the expected result (Fig. 2, Box 1) and choose an adequate statistical method. For example, if you want to compare the difference in the composition of useful plants between urban, peri-urban, and rural areas, you can run a PERMANOVA (Chap. 8, in this book) that uses the pseudo-F test statistics. Then, you have to choose the probability threshold (the P value) of the statistical test to decide whether the null hypothesis should or should not be rejected [14]. If you find a $P < 0.05$, you should reject the null statistical hypothesis (urbanization does not affect the number and composition of plants). Conversely, a $P > 0.05$ indicates that you cannot reject the null statistical hypothesis. Thus, the test statistics and the P value represents the last part of the statistical hypothesis testing, which is the decision and appropriate conclusions that will be used to feedback to the main theory (Figs. 1g–i). By generalizing your results and falsifying (or not) your hypotheses, the study is seeking to refine the conceptual construction of the theory, which is constantly changed (Fig. 1i, [15]). However, there is a critical point in this last statement, because statistical significance does not necessarily mean biological relevance (see discussion in Gotelli and Ellison [14], Martínez-Abraín [16]). In the words of Ford [15]: "statistics is used to illuminate the problem, and not to support a position." Also, the hypothesis-testing procedure has some uncertainty, which can influence "false-positive" (type 1 error) and "false-negative" (type 2 error) results [17].

Box 1 Type of Variables and Data Visualization:
As described in Sect. 3, the flowchart is essential for connecting relevant variables for the research. To take advantage of this approach, you can draw your own graphical predictions to help you think about different analytical possibilities. Here, we provide a full description of types of variables that you must know before running any statistical analysis and plotting results. Also, we show a brief gallery (Fig. 2) with examples of good practices in data visualization (Fig. 3b, see also Fig. 8.1 in Chap. 8 in this book). Besides connecting different variables in the flowchart, you should distinguish the type of variable. First, you must identify the independent (also known as explanatory or predictor) and dependent (also known as response) variables. The independent variable is the one (or more) that predicts or affects the response variable (e.g., soil fertility is the independent variable that affects the abundance of a focal plant species, the dependent variable). Additionally, a covariate is a continuous variable that can affect the response or the independent variables, or both, but usually is not of interest of the researcher. After defining those variables and connecting them in the flowchart, it is time to differentiate their type: (1) quantitative or continuous, and (2) categorical or qualitative (Fig. 2a, Box 1). The type of variable will define what kind of plot you may select. For example, if you are comparing two continuous variables or one continuous and a binary variable, the best way to visualize them (Fig. 2b) is a scatterplot (Fig. 2c, d). The line represents the values predicted by the statistical model used (e.g., linear, logistic). If you are interested in comparing the range of different traits (or the description of any numerical variable) between categorical variables (e.g., species or local populations), a dumbbell plot is good option (Fig. 2e). Histograms can be also used to show the distribution of two continuous variables of two groups or factors (Fig. 2f). However, if you want to test the effect of an independent categorical variables (like an ANOVA's design) on a numerical variable, boxplots (Fig. 2g) or violin plots can summarize them elegantly. Multivariate datasets, in turn, can be visualized with ordination (Fig. 2h) or cluster plots (not shown). There is a comprehensive website presenting several ways for visualizing data called datavizproject.com.

For the sake of simplicity, we will not further discuss the pros and cons of frequentist statistics, alternative methods (e.g., Bayesian and Maximum Likelihood), and philosophical issues regarding the "p value" (for a discussion on these topics see the forum in Ellison et al. [18]).

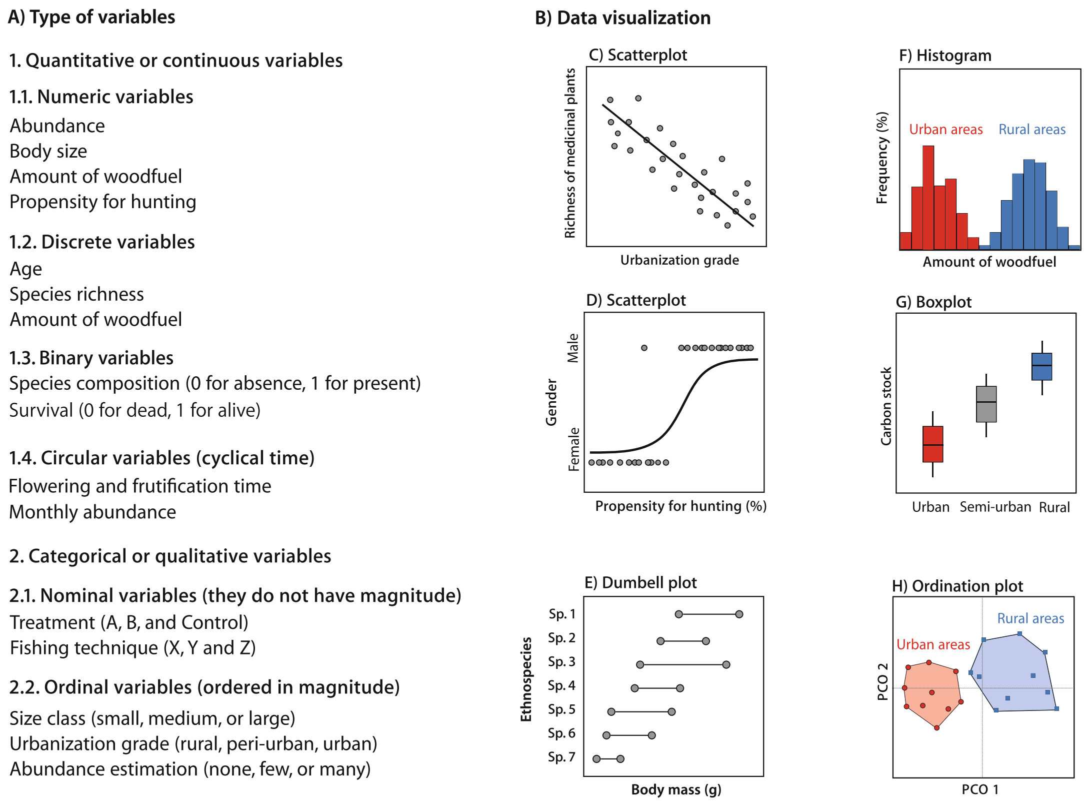

Fig. 2 Type of variables (**a**) and data visualization (**b**) to represent the expected relationship between dependent and independent variables, or covariates

3 Flowchart: Connecting Variables to Improve Experimental Design and Statistical Analysis

McIntosh and Pontius [19] stated that statistical thinking (represented in Fig. 1) includes four important steps: (1) what questions you would investigate (Sect. 4), (2) how and where to collect the data [20], (3) what factors should be considered and how they affect your variables of interest (and how they affect each other), and (4) what statistical analysis you should use and how to interpret and communicate results (Section 4). However, step (3) must be done before collecting the data. For example, if you are interested in investigating the benefits of riparian forests to native fish species, what variables should be included in the study? If you choose rivers with and without riparian forest as the single predictor variable, your sampling design will omit other confounding variables, such as river order and upstream soil organic carbon. Vellend [21] named this issue the "three-box problem" (see also [20]), which is the problem in inferring that X (independent variable) causes Y (dependent variable) when other variables create or magnify the

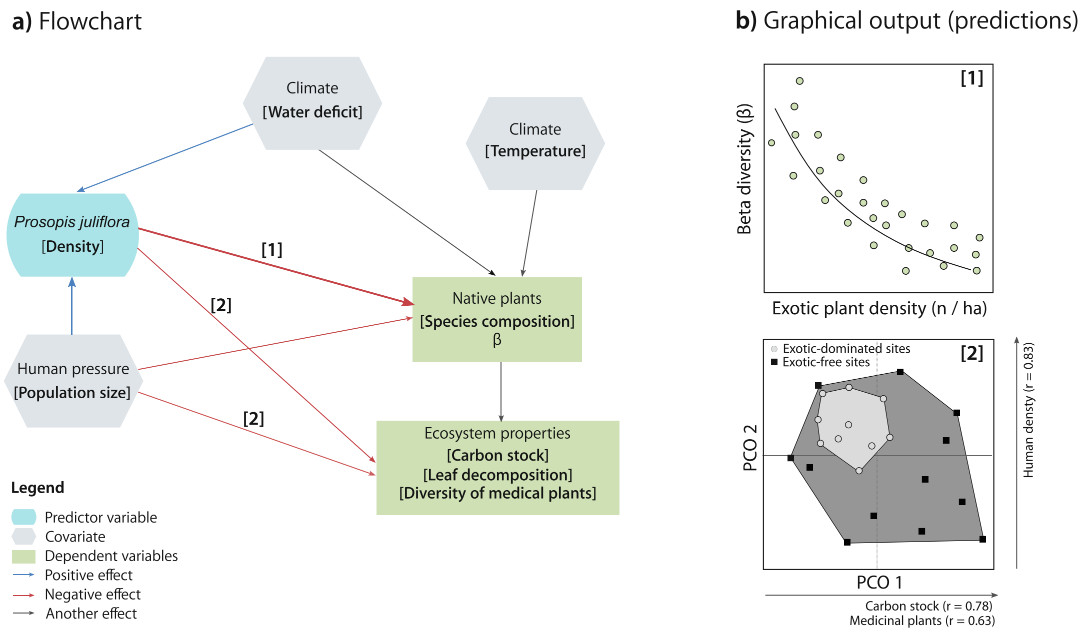

Fig. 3 Example of how to use a flowchart to improve the understanding of the studied system. The theoretical question "What is the impact of invasion on native community and ecosystem properties? can generate two predictions: (1) the exotic plant *Prosopis juliflora* reduces beta diversity of native plant communities, and (2) *Prosopis juliflora* modifies plant composition, and reduces carbon stock and decomposition rates. After stating your predictions, you can construct a flowchart connecting the relevant variables and the expected associations between them (**a**). Also, you can use the information in Box 1 to identify which type of variable you will collect and what plot(s) can be used (**b**)

correlation between X and Y (see Fig. 2 in [20]). A useful tool for understanding the relationship among every relevant variable for your study is a flowchart.

In the "research flowchart" (see also [22]) proposed here, dependent (also known as response) and independent (or predictor) variables, as well as covariates are depicted as boxes (with distinct shapes: Fig. 3). In addition, you can use an arrow to represent a (possible) causal pathway indicating strength and sign (positive or negative) of the predictor variable on the dependent variable (Fig. 3). By doing so, you could improve the experimental or observational sampling design including or controlling for confounding variables, which help you tease apart the contribution of different predictor variables in your system. Importantly, making connections among variables improve your ability to visualize the "big picture" of your research, which in turn affect your experiment, statistical analysis, and literature review. In fact, Arlidge et al. [23] argued that flowcharts facilitate the construction of scientific narratives by improving: (1) the definition of multiple working hypotheses, (2) gathering, interpreting, and spreading data, and (3) the communication of the study content. You can also refer to

Magnusson et al. [22] for how to use flowcharts in statistical analysis.

Furthermore, Ford [15] recommended the use of an analytical framework to foster research development. More importantly, the research flowchart can be used as a strong tool to accomplish Ford's advices, which were: (1) define the asked question, (2) define the theory to be used, (3) define the technique of investigation (e.g., experiment, field observation), (4) define the measurements, (5) define how to make inference, and (6) interpret, generalize, and synthesize from data, which feedback to refine theory and modify (when necessary) future questions (Fig. 1).

4 Fundamental Questions in Ethnobiology, Ecology, and Conservation

> *Theories are generalizations. Theories contain questions. For some theories the question(s) are explicit and represent what the theory is designed to explain. For others the questions are implicit and relate to the amount and type of generalization, given the particular choice of methods and examples used by researchers in constructing the theory. Theories continually change, as exceptions are found to their generalizations and as implicit questions about method and study options are exposed.*—E. David Ford [15]

As we argued before, a relevant, testable question precedes statistical analysis. Thus, we present below 12 questions that can stimulate future research in EEC. Note, however, that we do not mean they are the only relevant questions to be tested in EEC (see, e.g., Sutherland et al. [24] for a full evaluation of cutting edge research avenues in Ecology; and Box 6.1 in Pickett et al. [25]). Specifically, these questions are very broad and can be further developed into narrower questions, hypotheses, and predictions. After each theoretical question, we presented an example of a study testing it and the relevant variables that can stimulate future studies.

(a) How does land use affect biodiversity maintenance and spatial distribution of species at different spatial scales?

    Example: Several studies on different ecosystems and scales investigated how land use affects biodiversity. However, we highlight a study comparing global effects of land use (e.g., human population density, landscape to human uses, time since forest conversion) on terrestrial species (e.g., net change in local richness, average compositional dissimilarity) [26].

(b) What is the impact of biotic invasion on native communities and ecosystem properties?

    Example: Investigating how the establishment of exotic species affects species richness of the recipient, native communities, as well as how it impacts the delivery of ecosystem services. Previous studies have controlled the presence of invasive species or compared historical records (observational study) of these species and how they impact biodiversity. In addition, there is some effort in understanding the predictors of invasibility (e.g., gross domestic product of regions, human population density, coastal mainland and islands) [27].

(c) How does top predator decline affect the delivery of ecosystem services?

Example: Investigating how the removal of large carnivores affects the delivery of ecosystem services, such as carbon sequestration, disease, and crop damage control. Previous studies have investigated this question by controlling the presence of top predators or comparing historical records (observational study) of species and several predictors (e.g., habitat loss and fragmentation, conflict with humans—hunting, utilization for traditional medicine, and depletion of prey) [28].

(d) How does ocean acidification affect primary productivity and food webs in marine ecosystems?

Example: Recent studies tested the individual and interactive effects of ocean acidification and warming on trophic linkages across a food web. Acidification and warming have been manipulated by changing levels of CO_2 and temperature, respectively. Previous studies demonstrated that elevated CO_2 and temperature boosted primary productivity and affected the strength of top down control [29].

(e) How do we reconcile societal needs for natural resources with nature conservation?

Example: There is a growing literature using landscape approaches to improve land management to reconcile conservation and economic development. The studies have mixed objectives, but in general they used stakeholder engagement, institutional support, effective structures of governance as predictor variables, and environmental (e.g., soil and water conservation, vegetation cover) and social-economic (income, social capital, public health, employment) improvements as dependent variables [30].

(f) What is the role of protected areas (PAs) for the maintenance of biodiversity and ecosystem services?

Example: There has been considerable work in the last decade comparing the effectiveness of PAs for biodiversity conservation. Although this question is not completely separated from question E, the design of studies is relatively distinct. In general, researchers contrast the number of species and the delivery of ecosystem services (e.g., water and soil retention, carbon sequestration) between protected and unprotected areas [31].

(g) How to integrate scientific and local people knowledge to mitigate the negative impacts of climate change and land use on biodiversity?

Example: Extreme climatic events can have strong impact on agricultural yield and food production. Recent authors have argued that this effect can be stronger for small farmers. Future studies can investigate how rainfall and temperature affect agricultural yield and how traditional farmers or indigenous people deal with this negative impact. Traditional farming systems have lower soil erosion, and N_2O/CO_2 emissions than monocultures, and thus can be seen as a viable mitigating activity in a changing world [32, 33].

(h) How does climate change affect the resilience and adaptive strategies in social-ecological systems?

Example: The changing climate alters both fisheries and agriculture worldwide, which in turn enforces humans to change how they grow crops. Recent studies have argued that agriculture in some countries will face risks under climate change. These studies compare different production systems, from conventional agriculture to other types made by local people. For example, there is a strong connection between (1) threatened and overfished species, (2) human development index (HDI) and average dependence on fisheries and aquaculture. Also, there is evidence that biodiversity may buffer climate change impacts by increasing land resilience [32, 34]. Also, an interesting approach is to investigate how local people deal with these challenges in terms of their perceptions and behavior.

(i) How does biological invasion affect spatially and temporally the structure and functionality of social-ecological systems?

Example: Many studies demonstrated that invasive species have negative biological, economic, and societal consequences. Here, similarly to question B, researchers controlled the presence of invasive species or compared historical record. However, recent works quantify not only native species richness and composition, but also animal/plant traits that directly affect the delivery of ecosystem services, such as provisioning (food, water), regulating (climate, flood control), supporting (nutrient cycling, soil formation), and cultural (ecotourism, cultural heritage) services [35]. But, invasive species can provoke positive effects on social-ecological systems by incrementing the availability of natural resources, impacting how people manage and use local biodiversity.

(j) What is the relationship between phylogenetic and taxonomic diversity with biocultural diversity?

Example: Recent studies have shown that there is a phylogenetic and taxonomic pattern in the resources that people incorporate into their social-ecological systems, especially medicinal plants. There is a tendency for people, in different parts of the world, to use phylogenetically related plants for the same purposes. Here, researchers can test how much this affects the diversity of practices in a social-ecological system, considering the environment, as well as its structure and functions [36, 37].

(k) What environmental and social-political variables change the structure and functionality of tropical social-ecological systems?

Example: Testing the influence of human-driven environmental changes (e.g., fire, logging, warming) on keystone species and, consequently, how this effect cascade down to other species and ecosystem services (e.g., carbon storage, water cycle, and fire dynamics) [38].

(l) Do species traits influence how local people distinguish useful from useless plant or animal species?

Example: Investigating whether local people have a nonrandom preference when selecting animal or plant species. You can evaluate whether different groups (e.g., tourists) or local populations (e.g., fishermen) select species based on similar traits. Recent studies have shown a potential link between plant (e.g., color, leaf, flowering) and bird (e.g., color, vocalization) traits and some cultural ecosystem services, such as aesthetic, recreational, and spiritual/religious [39].

As you have noticed, the questions were more theoretical and, consequently, you can derive testable predictions (using operational variables) from them (Figs. 1 and 3). For example, from the question "*How does land use affect biodiversity maintenance and spatial distribution of species at different scales?*" we can derive two different predictions: (1) population density (land use operational variable) changes species composition and reduces species richness at the landscape scale (prediction derived from the biotic homogenization hypothesis [40]); (2) the composition of plant traits is different in forest remnants with different matrices (sugarcane, cattle, city, etc.).

5 Final Considerations

Tell me your secrets
And ask me your questions
Oh let's go back to the start
Running in circles, coming up tails
Heads on a science apart

Nobody said it was easy
(...) Pulling the puzzles apart
Questions of science, science and progress
—The Scientist, Coldplay

This is an excerpt of a song by the British rock band Coldplay from their 2002 album "A Rush of Blood to the Head." The lyrics are an amazing comparison between science and the ups and downs of a broken relationship. The band made an astonishingly clear statement that, as a scientist, we (should) frequently ask questions, go back to the start after discovering they were wrong (or not) and run in circles trying to improve our knowledge. The band described in such an accurate way how cyclical (but not repetitive) is the scientific method.

As the song goes: it is not easy, but learning how to ask good questions is an essential step toward knowledge consolidation. By including hypothesis testing in EEC we can be more precise. Definitely, this does not mean descriptive science is useless at all. On the contrary, the development of EEC, and mainly ethnobiology, was built upon a descriptive frontline, which means it was valuable to the foundation of Ethnobiology as a consolidated discipline [41, 42]. However, recent studies advocate that ethnobiology should dialogue with disciplines with stronger theoretical background, such as ecology and evolutionary biology to improve research on biodiversity [43]. In turn, incorporating local knowledge into ecology and evolution will certainly refine their own development, which ultimately benefits biological conservation [44]. In addition, (ii) there is an urgent need for training young researchers in the philosophy and methodology of science, as well as scientific communication and production [45].

As a concluding remark, we believe that the training of students in EEC needs a reappraisal that necessarily goes back to basic concepts and methods. Accordingly, researchers can combine the hypothetico-deductive method with statistical thinking using a research flowchart to go beyond description.

Glossary[1]

Assumption	Conditions needed to sustain a hypothesis or build the theory.
Hypothesis	Testable statement derived from or representing various components of a theory.
Mechanism	Direct interaction of a causal relationship that results in a phenomenon.

[1] After Pickett et al. [25].

Pattern	Repeated events, recurring entities or replicated relationships observed in time or space.
Phenomenon	An observable event, entity or relationship.
Prediction	A statement of expectation deduced from the logical structure or derived from the causal structure of a theory.
Process	A subset of phenomena in which events follow one another in time or space, which may or may not be causally connected. It is cause, mechanism or constraint explaining a pattern.

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