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Theory of plant defensive level: example of process and pitfalls in development of ecological theory

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Several hypotheses appear regularly in the literature as explanations for the level of plant defense, i.e. why some plants are so well defended and others are not. These hypotheses include optimal defense, carbon: nutrient balance, growth rate and growth-differentiation balance. However, there is considerable dissatisfaction with the progress with the plant defense hypotheses. At least part of the dissatisfaction with the hypotheses and research framed by them arises from the nature of the development of theory. Progress toward a mature stage requires attention to how theory develops (e.g. clearly distinguishing between the theoretical domain and the subset that can be tested, establishing criteria of robustness, properly accounting for discrepancies). In addition, part of the dissatisfaction with the development of the theory stems from inadequate approaches, such as failure to identify and test assumptions in experimental designs, confusing the hypotheses and their predictions, choosing a subsystem (e.g. plant age or part) that may be inappropriate for the test, and defaulting to a less precise hypothesis for explanation of the results.

Investigation of why some plants are well defended and others are not has led to development of numerous hypotheses. For the last 25 years, four of these hypotheses have been particularly important in guiding ecological and evolutionary research on this topic. The optimal defense (OD) hypothesis addresses how the defensive requirements of plants generate the evolution of secondary metabolites, with a cost of defense to maximize fitness (McKey 1974, 1979, Rhoades 1979). The carbon: nutrient balance (CNB) hypothesis explains the phenotypic variation in secondary metabolites in terms of the carbon: nutrient ratio in plants (Bryant et al. 1983, Tuomi et al. 1988). The growth rate (GR) hypothesis focuses on genotypic variation in plant defenses, with resources shaping inherent growth rate and, therefore, constraints on defense (Coley et al. 1985). The growth-differentiation balance (GDB) hypothesis predicts how plants balance allocation between growth and differentiation (which includes secondary metabolites and mechanical defenses, Loomis 1932, 1953) and was expanded to account for the evolutionary consequences of the opposing selective forces of competition and herbivory (Herms and Mattson 1992). Routinely in recent research publications, these four hypotheses are referred to as the foundation for the research described on plant defense against herbivores.

Despite all of the research represented by and relating to these hypotheses, there is dissatisfaction with what has been accomplished in this area of research and with these hypotheses. There are three sources of dissatisfaction: 1) Some of the dissatisfaction may derive from full theory development being unrealistic due to the complexity of it all (Berenbaum 1995). 2) Some, and perhaps even much, of the dissatisfaction is a consequence of the nature of theory and how theory develops. 3) But some of the dissatisfaction may reflect an inadequate approach to the issue. The degree to which the first explanation (# 1) accounts for the majority of the dissatisfaction can only be ascertained by examining the process (# 2) and pitfalls (# 3) of theory development.

The purpose here is to outline the process and pitfalls of theory development on plant defensive levels, which serves as an example for theory development in ecology. A detailed comparison and contrast of the plant defense hypotheses is provided elsewhere (Stamp 2003).

Process of theory development

To evaluate any theory, it is first necessary to understand how theory develops in general. ''Theory'' refers to a generally accepted view of the pattern and explanation of a major phenomenon. The development of a theory goes through stages: early, immature and mature.

In the early stage of development of a theory, the theory is imprecise and not defined well operationally (Loehle 1987, 1988). For example, many studies have

used the OD hypothesis, which was developed in the 1970s, for a theoretical framework. Basically this hypothesis states that: 1) any observed defensive pattern is possible if it is adaptive and 2) defense has a cost to fitness (Rhoades 1979). It is difficult to construct a test for which it would be possible to reject the OD hypothesis, especially for part 1 (Rhoades 1979, Fagerström et al. 1987, Hamilton et al. 2001). That is, because it is a verbal and somewhat vague "model" and so, in contrast to the other plant defense hypotheses which have graphical/mathematical models, results can always be explained, in some way (and are) by the OD hypothesis. Although Fagerström et al. (1987) tried to formalize the OD hypothesis, their discussion indicated several issues that needed to be resolved (and remain unresolved). Imprecision in the early stage is the very nature of theory development; seldom are there short cuts.

Subsequent work on a theory increases the empirical content and testability of hypotheses. "Hypothesis" refers to an explanation that accounts for a category of observations related to the phenomenon. Hypotheses usually carry with them assumptions. An assumption is a premise that is accepted as true, without proof or thorough examination. A hypothesis is only useful to us if it makes predictions that we can test. "Prediction" refers to statements of expectation that are based on the hypothesis, and "test" refers to collecting observations or conducting an experiment to examine a prediction. The most useful predictions are "if-then" statements, with the "if" part setting up the conditions for the test and the "then" part stating what is expected.

But at the immature stage of theory development, the predictions are vague and, consequently, the tests are inconclusive (Loehle 1987, 1988). During the immature stage, there are likely to be many competing hypotheses and conflicting and unassimilated facts. For example, the OD, CNB, GR and GDB hypotheses are often regarded as competing hypotheses (e.g. as discussed in Karban and Baldwin 1997), and much data are difficult to interpret in terms of these hypotheses.

Only at a later, more mature stage, when more empirical content has been obtained and more distinct predictions are possible, can hypothesis testing be more efficient. However, this process is complicated by the difficulty in elaborating concepts in ecology (Loehle 1996). When elaboration of a concept is limited, testing hypotheses about mechanisms of operation can be more constructive (Loehle 1996). For instance, the success of the gene-for-gene recognition (between plant and pathogen) hypothesis (Flor 1971, reviewed by Staskawicz et al. 1995, Crute 1998), which explains plant defensive response, resides in its providing mechanistic predictions at the molecular level that can be tested explicitly.

In practice, testing a model, or more specifically these hypotheses, is not so much trying to validate the model (or prove it wrong) as it is to determine the limits of application, by identifying when the model does not work (Dunbar 1995). The determination of such boundary conditions occurs through falsification of predictions. But that is then often followed by development of ad hoc auxiliary hypotheses due to reluctance to reject a major hypothesis. This eventually leads to a new major hypothesis (and revision or elaboration of the theory), when the auxiliary hypotheses do not hold up. This process works well provided that the predictions and tests logically follow from the hypotheses. But when theory is not rigorously tested and unassimilated exceptions accumulate, stagnation occurs.

To approach a mature stage of theory development requires defining the domain of application, testing the assumptions, converting the verbal statement to a mathematical structure, determining how to measure the variables, developing new techniques, and determining the criterion of robustness (Loehle 1987). But no study or collection of studies on plant defense has accomplished that. For example, although mathematical statements (or graphical models) exist for the GR hypothesis (Coley et al. 1985) and the GDB hypothesis (Herms and Mattson 1992), these have not been tested. "The lack of maturity of much theory in ecology explains much of the inconclusive wrangling that takes place…" (Loehle 1987, p. 404).

Why is theory development so difficult?

Theory does not develop through a precise match with empirical data because such a match will not occur. "The concrete experience that scientists label an experiment cannot itself be connected to a theory in any complete sense. That experience must be put through a conceptual grinder that in many cases is extremely coarse." (Suppes 1967). The variation inherent in populations and ecosystems exacerbates this problem. In the formal terms of philosophy of science, this means that to assess the fit of the "model of the experiment" to the "model of the theory", especially complex theory, which most biological theory is, is quite difficult. The "model of the experiment" refers to an observed, physical entity (i.e. data gathered in a structured format). Strictly speaking, the "model of the theory" refers to an abstract, mathematical (or graphical) entity. There are several reasons why assessment of fit is difficult.

First, whether by scientists or science philosophers, discussion of theory, even about complex theory, is usually in relatively simple and often informal language rather than the highly abstract, non-linguistic terms required for a model of theory (Suppes 1967). Although that facilitates communication, it is likely to mention only a few of the items that would have to be specified

to form a complete explanation of complex phenomena. Specifically in terms of the theory of plant defense, reviews (Karban and Baldwin 1997, Hamilton et al. 2001) tend to simplify the more formalized plant defense hypotheses and thus predictions, to the exclusion of important features of the verbal, graphical and mathematical models and so at the risk of misinterpretation (Stamp 2003). For example, Karban and Baldwin (1997, p. 77) so reduced the description of the GDB hypothesis that they said that according to the GDB hypothesis "plants do not regulate the production of secondary metabolites to any extent". The relatively complex verbal, graphical and mathematical models of the GDB hypothesis (Herms and Mattson 1992) contradict that statement. The point is that incorporation of complexity into the hypothesis was lost in the "translation". This problem is common in the plant defense literature and leads to misinterpretation of whether experimental design and results fit the hypothesis in question (Stamp 2003).

Second, the model of the experiment, which is typically discrete and bounded, is a different logical type than the model of the theory, which usually contains continuous functions or infinite sequences (Suppes 1967). Tests of plant defense hypotheses are system specific and, consequently, quite discrete and bounded. Assessment requires isomorphism between the model of the experiment and the model of the theory. Isomorphism means there is a one-to-one mapping between the models, or the behavior of the "phenomenal" system (e.g. an experiment) is the same as specified for the abstract system of the theory (Thompson 1989). One-to-one correspondence between a model of the experiment and a model of the theory, such as for the CNB hypothesis or even for the gene-for-gene theoretical model of plant-pathogen recognition, now seems unrealistic (Herms and Mattson 1992, Crute 1998).

Third, the process inserts another body of theory, theory of experimentation, between the theory in question and its associated empirical experience (Suppes 1967). The theory of experimentation actually is comprised of other bodies of theory, such as statistics, probability and experimental design, resulting in the insertion of an elaborate hierarchy of theories. Inserted theories are not formally recognized in a way that allows thoughtful evaluation of fit between a model of an experiment and a model of a focal theory. For instance, meta-analysis is a statistical procedure that uses experiments as the sampling units. It has been applied to issues of plant defense (Koricheva et al. 1998, Koricheva 2002). But its utility is compromised when hypotheses have been misapplied and assumptions of predictions have not been tested, which has often been the case in plant defense research, due to the immature stage of the theory.

Fourth, some additional insertions may be needed to explain observed discrepancies from the theory in question. The discrepancies that can be accounted for by an inserted theory can be presented as "hedged statements" (Beckner 1967). For example, "this applies, except for…". Then the exceptions are explained with a "bypassing statement". For instance, in population-genetics theory, Mendel's laws of independent assortment and segregation and the Hardy-Weinberg equilibrium apply, except when crossing-over, linkage, inversion and other cytological and genetic aspects accounted for by cytological theory and molecular genetics occur (Thompson 1989). The deductive component of theory is modified here because the phenomenal system behaves differently than the abstract system specified by population-genetics theory. That is, there are exceptions to the generalizations, but they can be explained by drawing on other theory. Insertions that might be needed for evaluation of plant defense theory include the theories of: biochemical evolution in plants, signaling mechanisms, gene-for-gene recognition, hypersensitive response, systemic acquired resistance, tolerance to herbivory, soil nutrient cycling, allelopathy, microbial-plant symbioses, growth and differentiation, and plant allometry.

The question is how do we distinguish between legitimate discrepancies (i.e. accounted for by the hedge-bypass process) and falsehoods (or empirical refutation) of theory (or hypothesis). For example, it has been difficult to demonstrate a cost of defense to fitness, which is a central issue in all four plant defense hypotheses. Sometimes a cost is detected, but often not and then usually studies provide some rationale for lack of detection of cost (reviewed by Simms 1992, Bergelson and Purrington 1996, Karban and Baldwin 1997). Based on a meta-analysis, it was suggested that failure to detect costs may reflect exclusion from the experimental design of interactions between plants and other important factors, such as competitors, pollinators, array of herbivores and so forth (Koricheva 2002). This is an example of creating an auxiliary hypothesis that maintains the original hypothesis about cost of defense. As logical as that auxiliary hypothesis may be, we should be careful about defaulting to a less precise hypothesis (e.g. part 1 of the OD hypothesis: any observed defensive pattern is possible if it is adaptive) when we get stuck in our explanations of the results.

Typical pitfalls in testing the plant defense hypotheses

Central to theory development is testing hypotheses. However, it can be difficult to derive appropriate predictions from hypotheses and, thus, easy to confound the issues inadvertently. An example will illustrate this and also show why it is important to understand the differences among these hypotheses and carefully apply

what we collectively have learned so far about plant defenses.

This example is quite typical of many recent research articles. (And I do apologize for having to pick on anyone, and I readily admit that I have been as confused as anyone!) This particular example comes from a research paper published in the journal Ecology and so presumably reflects the collective view of the authors, reviewers and editors on the theory of plant defense. Although the work itself is well done and can serve as a model of attention to detail in many ways, it falls prey to the considerable confusion about the theory of plant defense.

The purpose of the paper (Siemens and Mitchell-Olds 1998) is to address the evolution of constitutive and inducible defenses, which is certainly a worthy goal. This article refers to the four major hypotheses of plant defense (OD, CNB, GR and GDB) for its framework, via specifically citing the hypotheses or the main papers developing the hypotheses. The authors make the prediction that there should be a negative genetic correlation between constitutive and inducible defenses, i.e. a genotype with a high constitutive defensive level should have a low induced defensive level, and vice versa.

The authors point out that this prediction is grounded in the OD hypothesis. That is true in that Rhoades (1979, p. 13) states, "defenses are costly: therefore, commitment to defense is decreased when enemies are absent and increased when organisms are subject to attack", and then discusses expected patterns of induced defenses for different kinds of plants. However, it is the GR hypothesis (which extends the OD hypothesis, Herms and Mattson 1992) that specifically spells out the predicted trade-off between constitutive and induced defenses. The GR hypothesis predicts that given an evolutionary history of relatively poor resources and limited ability to replace lost tissue, a plant species will have high constitutive and low inducible defenses, whereas given an evolutionary history of a competitive environment with better resources and so greater ability to replace lost tissue, another species will have low constitutive and high inducible defenses (Coley et al. 1985, Coley 1987). For example, a plant species that thrives in low-light environments should have high constitutive and low inducible levels of defense, whereas a plant species that thrives in full sunlight should exhibit low constitutive and high inducible levels of defense.

Siemens and Mitchell-Olds (1998) used artificial selection on a herbaceous annual (*Brassica rapa*) to develop genotypes (= subpopulations) with relatively high levels of a preformed defensive compound (glucosinolate), or its activating enzyme (myrosinase), and correspondingly genotypes with low levels. They evaluated defensive concentrations by examining the cotyledons of seedlings. In the experiment, the authors tried to induce resistance in half of the seedlings of each defensive type, using either a pathogen or insect herbivore. They found a neutral or positive genetic correlation between constitutive and inducible defenses. That result did not fit their prediction. Siemens and Mitchell-Olds (1998) concluded that constitutive and inducible levels of secondary metabolites are not alternative defensive strategies.

But there are problems with their interpretation. These problems are rooted in our collective misunderstanding of what these hypotheses of plant defense do and do not predict and what we have learned to date about plant defense.

First, as implied by both the OD and GR hypotheses, which refer to interspecific patterns, the prediction of Siemens and Mitchell-Olds (1998) requires a comparison of genotypes from a pool that exhibits sufficient range in expression of defenses. Although some species exhibit considerable genetic variation in level of defenses (Dirzo and Harper 1982, Zangerl and Berenbaum 1990, Vrieling et al. 1993, van Dam and Vrieling 1994, Mauricio 1998), other species seem to have fixed levels (Muzika et al. 1989, Holopainen et al. 1995). The assumption about variation should be identified clearly and examined at the outset. It could be supported by sufficient information in the literature or testing directly.

For their project, Siemens and Mitchell-Olds (1998) chose a herbaceous annual species. Presumably their choice reflects important practical considerations, such as a system for which major defenses are easily quantifiable and sufficient sample size is obtainable within budgetary and spatial constraints. Obviously, we have to choose systems that are economical and feasible. But this species would be classified as a ruderal (Grime 1979) and so, according to the habitat template-plant defense model (which incorporates the GR hypothesis; Coley 1987), is expected to have relatively low constitutive defense and low inducible defense, compared to other species. If that is indeed the case, the range of expression of the defenses of this species may not allow a good test. According to the habitat template-plant defense model (Coley 1987), a good candidate would be a species that naturally grows well in both the competitive environment (therefore, predicted to have low constitutive and high inducible defenses) and the stress-tolerant environment (depicted to have high constitutive and low inducible defenses), and so presumably would display the necessary variation in defense. The point is that the assumption about defensive variability should be examined.

Second, although the short-term ("two cycle") selection process yielded subpopulations that differed in levels of one or the other defensive chemical, the selective regime did not mimic the two most important selective pressures in shaping plant defenses (herbivory and competition or resource availability). The most precise published prediction of an evolutionary trade-

off between constitutive and inducible defenses comes from the GR hypothesis, which is about interspecific variation in levels of plant defense due to evolution under different regimes of herbivory and resource availability. Specifically, it predicts that species with an intrinsically slow growth rate, reflecting evolution in their resource-poor environment, would have high constitutive defense and low inducible defense, and species with an intrinsically fast growth rate, reflecting their resource-rich environment, would have low constitutive defense and high inducible defense (Coley et al. 1985, Coley 1987). It does not predict a marked trade-off between genotypes that have not evolved under the opposing selective regimes of resource availability and herbivory. Concentration of a secondary metabolite is a function of the selective agent or regime (Harborne 1990). Simply selecting for a low level of a particular defensive compound may yield a different genotype than competition would; and selecting for plants with a high level of a particular compound may result in quite a different genotype than herbivory would.

In the Siemens and Mitchell-Olds' (1998) study, the test genotypes differed in one preformed compound or its activating enzyme, but we do not know how well these genotypes fit the rest of the proposed GR-hypothesis scenario, e.g. in terms of growth rate, total constitutive defense, and total induced defense. It seems inappropriate to expect that short-term selection for a particular defensive compound would provide suitable genotypes for a test of the constitutive-induced defense prediction of the GR hypothesis.

Again, assumptions are made that should be tested. Is there a good correlation between concentration of a single defensive chemical and total constitutive defense? Data from other studies suggest that we should not make that assumption (Zangerl and Berenbaum 1987). Does selection for a high level of one chemical yield a facsimile of a genotype subjected to herbivory? Presumably herbivory would select for high levels of both the preformed compound and its activating enzyme, plus some other defensive traits. In another study (cited in Siemens and Mitchell-Olds 1998), they used a selection regime for high versus low levels of both the preformed compound and its activating enzyme, which yields more realistic genotypes. The high defense plants had a slower growth rate than the low defense plants, which suggests this other set of derived genotypes was closer to fitting the test situation, but the constitutive-versus-inducible-defenses trade-off was not examined.

Third, another question is whether plants of the age used were likely to exhibit the variation in expression of defenses necessary to test the prediction. This study used seedlings. Presumably seedlings were used because it was more feasible and economical than using older plants. The ontogeny of plants affects levels of constitutive and induced defenses (Nowacki et al. 1976, Ohnmeiss and Baldwin 1994, Stout et al. 1996b). Seedlings may or may not have much constitutive defenses. Plants that have little or no reserves in their root and stem systems are predicted to show, with leaf damage, a decline or no change in overall defensive concentrations, irrespective of pre-damage constitutive level (Tuomi et al. 1988). This means that the developmental stage chosen for this experiment might not be as responsive to the selective regime and inducing agents as would be desired for a good test of the prediction about a trade-off between constitutive and inducible defenses. Preliminary tests could have resolved this issue.

Fourth, defensive concentrations were determined by assessing one plant part, the cotyledon, a part that is very important but has a quite limited life-span. Again, the choice reflects feasibility (Siemens and Mitchell-Olds 1998). But it raises another question of whether the cotyledon's defensive chemistry is representative of the plant's. Leaf age affects levels of constitutive and induced defenses, with patterns differing among defensive chemicals (Stout et al. 1996a). In Siemens and Mitchell-Olds' (1998) study, the defensive differences in the cotyledons generated by the selection regime did not affect the resistance of true leaves to insect herbivores. Furthermore, increases in only one of the chemicals reduced seed production, reflecting the weak correlation between cotyledon and true leaves in levels of the other chemical. These results suggest that defensive chemistry of cotyledons was not representative of whole plants, yet the OD and GR hypotheses are about whole plant patterns (i.e. a trade-off within plants between constitutive and induced defenses reflecting cost of defense to fitness).

This example illustrates the state of the "art" of testing predictions of the plant defense hypotheses. As clever as some of the tests are, we too often fail to address the assumptions of the hypotheses and predictions directly. We do not exhibit a clear understanding of how what we have learned about defensive chemical differences among plant ages or leaf ages plays a role in our tests of predictions of the hypotheses. Because of the lack of understanding about what particular hypotheses do and do not predict and how hypotheses are subsumed by others (Stamp 2003), we tend to set up predictions and tests that represent a mixture of the hypotheses. Consequently, we do not have clear tests of the hypotheses.

Conclusion

At the outset of hypothesis testing, there should be a detailed written evaluation of the hypothesis to be tested, preferably by those who propose the hypothesis, but if not, then at least by those who attempt to test the hypothesis. The hypothesis should be methodically pulled apart and analyzed in detail. An effort should be

made to define the domain (or limits) of application clearly. It helps to distinguish formally between the theoretical domain and the "realized" domain (the subset that can actually be tested). Analysis should include an assessment of the isomorphism between the model of the hypothesis (i.e. the abstraction) and the model of the experiment (i.e. a test). Testing should include explicit examination of assumptions of both the hypothesis and predictions. An effort should be made to determine the criterion of robustness. Anticipating the need for "hedged" and "bypassing" statements and then plans to take those into account in the experimental design will help with that. To do less than such an evaluation will result in more empirical data in the category of "unassimilated exceptions" and contribute to more "inconclusive wrangling". To the degree that maturation of theory is possible, it will depend on this more formal approach.

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