

Macroevolution of plant defense strategies

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Theories of plant defense expression are typically based on the concepts of tradeoffs among traits and of phylogenetic conservatism within clades. Here, I review recent developments in phylogenetic approaches to understanding the evolution of plant defense strategies and plant–herbivore coevolutionary interactions. I focus particularly on multivariate defense against insect herbivores, which is the simultaneous deployment of multiple traits, often arranged as convergently evolved defense syndromes. Answering many of the outstanding questions in the biology of plant defense will require generating broad hypotheses that can be explicitly tested by using comparative approaches and interpreting phylogenetic patterns. The comparative approach has wide-spread potential to reinvigorate tests of classic hypotheses about the evolution of interspecific interactions.

Plant–herbivore interactions and the evolution of defense

In most food webs, insect herbivores are one of the major conduits of energy flow between autotrophic plants and the rest of the food web. Thus, it is not surprising that herbivory has led to the evolution of a range of effective plant defenses. Natural selection imposed by insect herbivores appears to have resulted in the evolution of sandpapery leaves, digestion inhibitors and toxins that disrupt physiology from oxygen transport to cardiac function [1]. The early conceptual developments in understanding the biology of plant defense include Dethier's proposition that insect host shifts were determined by shared host plant chemistry [2]; Frankel's hypothesis that plant secondary compounds function as adaptive defenses against herbivory [3]; and Ehrlich and Raven's conclusion that defense and counter-defense coevolution resulted in the adaptive radiation of angiosperms and insect herbivores [4] (see Glossary).

These papers were comparative in nature and generated hypotheses by examining differences across plant species; subsequent defense theories were similarly based on differences among species [5–8]. Nonetheless, a lack of phylogenetic methods and rigorous tests of these comparative hypotheses gave way to a microevolutionary paradigm based on quantitative genetics, which has largely dominated our thinking on plant defense evolution for the past 20 years [9–11]. The use of quantitative genetic methods to measure natural selection has provided strong

support for herbivores as agents of natural selection for plant defense [11–13]. Although the microevolutionary approach has been successful in explaining local adaptation and the maintenance of variation in defensive traits, only recently has there been a resurgence of interest in explaining broader macroevolutionary patterns across plant species.

Here, I highlight recent advances in our understanding of the macroevolutionary patterns of plant defense and argue that such a perspective is necessary to answer outstanding questions about the evolution of defense strategies (Box 1, Table 1). I propose two issues that, conceptually, are central to the evolution of plant defense strategies. First, as in most studies of adaptation, tradeoffs are assumed to be universal. Second, phylogenetic conservatism provides a null hypothesis for patterns of plant

Glossary

Coevolution: reciprocal adaptation between interacting species.

Constitutive defense: plant defensive traits that are always expressed (especially in the absence of herbivory). Many such traits are also induced following herbivore attack. For example, the glucosinolate defenses of plants in the Brassicaceae are expressed constitutively but are induced further after attack [43].

Early divergent (basal): a lineage that branches near the root of a phylogenetic tree. An early divergent lineage is considered relative to later divergent (derived) lineages along the same path from the root to a tip of a phylogeny [44]. Early divergent lineages with few species are not ancestral to their more species-rich sister lineages (they share the same common ancestor and time since divergence).

Evolutionarily labile: traits that appear to evolve independently from the effects of evolutionary history (i.e. character states might change at the tips of clades).

Induced defense: plant traits that are produced or increased in terms of their antiherbivore activity following attack. Many traits, including defense chemicals such as glucosinolates [43], physical structures such as trichomes [45], and attractants of carnivores, such as extrafloral nectar, can be induced following herbivory [17].

Phylogenetic conservatism: the hypothesis that closely related species share more traits or ecological associations than do distantly related species.

Phylogenetic control: accounting for the correlated structure (or non-independence) of observations across species that occurs owing to the shared ancestry of species. For example, in examining the ecological correlates of the evolution of induced defenses, phylogenetically controlled analyses were performed to ensure that a comparative analysis across species was not biased by their evolutionary (phylogenetic) non-independence [17,46].

Phylogenetically independent tradeoff: a negative correlation between two traits across several species that is not biased by the potential phylogenetic conservatism across those species. This can be specifically interpreted as evolutionary divergence in one trait that is repeatedly associated with divergence in the other trait.

Syndrome: a suite of multiple co-varying traits associated with a particular ecological interaction. For example, plants inhabiting certain biotic or abiotic environments can convergently evolve a set of particular defense traits [30].

Univariate tradeoff model: the notion that individual defense traits should be negatively associated across species. For example, plant species producing alkaloids should not produce extrafloral nectar to attract ant defenders [21].

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Box 1. A plea for the comparative approach: common mechanisms versus diversity

One of the key benefits of the comparative approach to studying plant defense is that common mechanisms of species interactions can be revealed that would otherwise be impossible to study when using a single model organism. A triumph of this approach has been the identification of jasmonic acid as the hormonal regulator that activates a range of induced plant responses, such as the production of oxidative enzymes, glucosinolates, alkaloids, trichomes, volatiles and extrafloral nectar [17,45,49,50]. Jasmonate regulation thus exhibits deep phylogenetic conservatism across plants.

Likewise, the comparative approach has revealed a remarkable consistency in the bouquet of volatiles that are induced by plants following herbivory. These volatiles, often implicated in the attraction of predators and parasitoids of herbivores to plants, are highly overlapping across diverse plant species [51]. However, it is currently unclear whether this similarity represents phylogenetic conservatism or convergent evolution.

On the flip side, some plant–herbivore interactions appear to be unique, with highly species-specific interactions even among close relatives (e.g. Refs [17,23,46,52,53]). For example, across plant species, plants that are attacked by one herbivore species release volatiles that attract its legitimate parasitoid, whereas the plants attacked by a congeneric herbivore release a different bouquet of volatiles that do not attract that same parasitoid [53].

It is this challenge, finding the similarities and differences among species and their interaction, which is the spice of evolutionary ecology. Combining a phylogenetic approach with our ability to interpret traits as conserved or convergent (and adaptive) will help us to generally understand the function of widely varying traits.

defense and host use by insects. Although these two concepts should define the backbone of comparative analyses of plant defense evolution, I suggest a broadening of,

and a cautioning in, their application (Box 2). I focus mainly on defense strategies that act directly on insect herbivores, although recent data and conceptual developments additionally call for an evolutionary perspective on multitrophic interactions (e.g. involving carnivores, pollinators, etc.) [14–18]. The macroevolutionary program proposed herein should also apply to such multitrophic interactions. Indeed, the phylogenetic approach to evolutionary ecology of interspecific interactions more generally has broad potential to address classic questions and totally new avenues of research.

History and status of the univariate tradeoff concept

The univariate tradeoff concept for the evolution of plant defense is based on two points. First, if one defense is sufficient, then in species with high levels of that defense, there should be no selection for other redundant defenses (e.g. alkaloids that poison herbivores versus extrafloral nectaries that attract enemies of herbivores); and second, the pattern of a tradeoff should be reinforced by the allocation costs associated with producing a given defense (e.g. limiting nutrients could be used for reproduction in place of a redundant defense).

Early support for this model (i.e. a negative association between two traits) came from studies of *Acacia* species with and without associations with ants. Across several species, non-ant-acacias were found to be rich in hydrogen cyanides, whereas acacias with ants (myrmecophytes) were not [19]. However, this result, although from several species within a genus, did not control for whether the species within a group (ant versus non-ant acacias) were

Table 1. Phylogenetic hypotheses for the evolution of plant defense

Hypothesis	Reasoning	Example	Refs
Univariate tradeoffs	Plants defend with primarily one type of strategy; thus, there should be a tradeoff between defense strategies across species that is generated by defense costs and the lack of a need for redundancy	The abundance of toxic leaf glands trades off with the density of trichomes across 31 species of wild cotton (<i>Gossypium</i> spp.)	[19,21, 22]
Resistance–regrowth tradeoff	Coexisting plant species will have alternative (convergent) defense strategies against herbivores: resist attack versus have a high ability to regrow following defoliation	In Dutch coastal sand dunes <i>Verbascum thapsus</i> is heavily attacked but has strong regrowth ability, whereas <i>Senecio jacobaea</i> is largely resistant (not attacked) but has very poor ability to regrow when damaged	[8]
Apparency	Plants that are apparent to their herbivores (i.e. easy to find) will defend with quantitatively acting traits (e.g. anti-nutritive), whereas less apparent plants will defend with qualitative traits (e.g. toxins); patterns of defense will be convergent	Oak trees which dominate many forests defend with tannins and other phenolics, while ephemeral herbaceous mustards defend with glucosinolates	[6,27, 28]
Convergent syndromes	Plant species growing in similar environments (biotic or abiotic) will converge on suites of co-varying defensive traits	Tropical trees specialized to white sand habitats are slow growing and heavily defended, while species adapted to richer clay soils are fast growing, tolerant of herbivores, and poorly defended (Fig. 2)	[23,26, 29,30]
Escape and radiate evolution	The evolution of a novel defensive trait will lead to diversification in plant lineages because escape from herbivory allows for larger population sizes and reduced probability of extinction	Plant lineages that produce latex or resin canals are more species rich than sister clades that do not produce such defenses	[4,41]
Macroevolutionary escalation of defense	As species evolve from common ancestors, more derived species have more complex or potent forms of defense compared with early divergent species	Derived <i>Dalechampia</i> spp. vines contain more classes of defenses (e.g., resins and sharp trichomes) compared to basal taxa	[4,14, 36,37]
Phylogenetic conservatism (monophyletic origin of defense synthesis)	Biosynthetic machinery needed to produce classes of plant defense compounds are highly conserved and have a single origin. The diversity of specific compounds within a class has occurred via biochemical modification during the speciation process within clades; thus major classes should have a monophyletic origin	The presence of an enzyme (<i>S</i>)-noroclaurine synthase (which is required for the production of defensive benzyloquinoline alkaloids) has a monophyletic origin in the angiosperms. Although the production of benzyloquinoline alkaloids is restricted to a few groups, the biosynthetic machinery needed to produce them have a basal origin	[4,38– 40]

Box 2. Cautionary suggestions for applying phylogenetic approaches to the evolution of plant defense

- Avoid comparing only two species. Many comparative studies in plant–herbivore interactions compare a single pair of species (e.g. two plant species, one with and one without trichomes). Few conclusions are drawn about tradeoffs or patterns of trait association when comparing two species, even if they are closely related. Any association between two traits can only be generalized by studying multiple species.
- Do not expect a tradeoff for arbitrarily selected traits. Although tradeoffs are central to our belief in the limits to adaptation, historically, many researchers have expected tradeoffs between two selected plant defensive traits (because they are assumed to be costly and/or redundant) [21,54]. If plant defenses, similar to most adaptations, comprise multiple traits, they could be organized into suites of co-varying traits or syndromes. Many defensive traits will thus co-vary positively.
- Do not confuse early divergent with ancestral [44]. The extant species in an early diverged or basal lineage of a clade need not have the traits of the hypothesized ancestor; early diverged lineages have been evolving independently for as long as other lineages derived from that common ancestor.
- Do not assume adaptation or a particular agent of natural selection. Here, I have used ‘defense’ loosely to refer to traits thought to provide a fitness advantage (only in the presence of herbivores) compared with plants without that trait. Comparative biologists use the repeated, evolutionarily independent association between plant traits and some ecological aspect to implicate adaptation. For example, the repeated loss of extrafloral nectaries in plant species located in communities without ants (compared with closely related species in communities with ants) would be reasonable evidence to suggest that nectary–ant associations are adaptive. The agents of natural selection can only be identified by manipulating their presence and measuring the resulting natural selection.
- Do not blindly correct for phylogenetic history. The assessment of phylogenetic relatedness (or independence) in generating a pattern can be more or less important depending on the question. For example, in the analysis of quantitatively variable plant traits and their impacts on herbivores, phylogenetic correction might not be necessary to implicate particular traits in resistance [7,55]. However, to infer adaptation, repeated independent associations among traits and ecological attributes are the most convincing.

phylogenetically independent. This phylogenetic control is important from the perspective of statistical independence as well as for assessing whether the ant–plant associations repeatedly evolved (and are therefore likely to be adaptive), or whether associations have a single evolutionary origin. In subsequent analyses across three genera of plants, each with and without associations with ants, there appears to be little support for a univariate tradeoff between plant association with ants and particular defense compounds [20].

Similarly, in a different group of plants, a phylogenetically uncontrolled analysis found no evidence for a tradeoff when comparing 19 species of morning glory *Ipomoea* for the presence of extrafloral nectaries, alkaloids and leaf trichomes [21]. In a more recent phylogenetically controlled analysis of 31 cotton *Gossypium* species, Rudgers *et al.* [22] again found no evidence for a tradeoff between extrafloral nectaries and toxic leaf glands or trichomes, although there was a tradeoff between toxic glands and trichomes. Finally, a similar analysis with seven defensive traits among 24 milkweed *Asclepias*

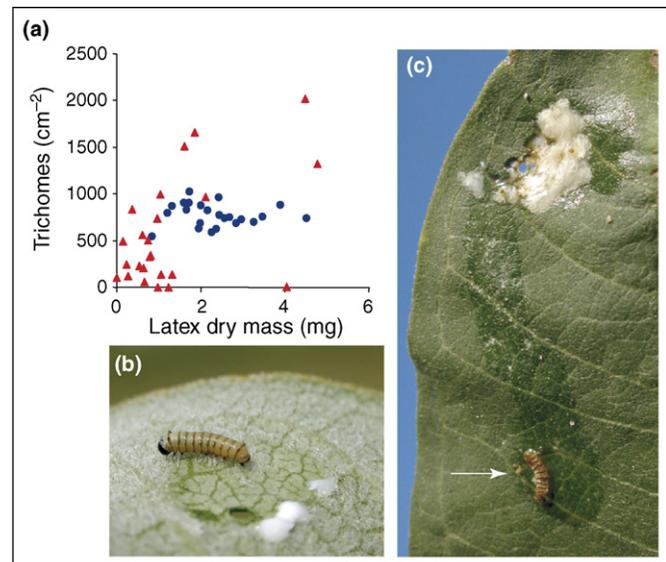


Figure 1. Correlations among plant defense traits across and within species: the evolution of latex and trichomes in milkweeds. Contrary to the univariate tradeoff hypothesis (Table 1, main text), many plant defensive traits are either positively correlated or not correlated at all. In (a), the correlation between latex production and trichome density (two defensive traits of milkweeds) is shown across 24 milkweed *Asclepias* species (red triangles; $P < 0.01$ in both phylogenetically corrected and uncorrected analyses, data from Ref. [23]). Genetic variation is also shown across 23 full-sibling genetic families of *A. syriaca* grown in a common garden (blue circles, $P = 0.75$, data from Ref. [47]). Both data sets are from field experiments at the same site, from same year, and with at least five replicates per species or family. Armbruster *et al.* [48] have argued that such correlations across but not within species are suggestive of adaptation; whereas the opposite is often interpreted as evidence of constraint. Despite the lack of a correlation across genetic families, variation in latex and trichomes was negatively correlated with herbivore damage for *A. syriaca* [47]. These two traits might have a synergistic impact on reducing herbivory. For example, when eggs of the monarch butterfly *Danaus plexippus* hatch, young caterpillars often graze a bed of trichomes before puncturing the leaf surface (b). Once the caterpillars puncture the leaves, they encounter pressurized latex (c). A large fraction of monarch caterpillars die trapped in latex (arrow).

species yielded no evidence for a tradeoff; only five of the 21 possible correlations between defensive traits were statistically significant, and they were all positive (Figure 1) [23]. Thus, despite the *a priori* expectation that there would be tradeoffs between defense traits, evidence across several systems is limited; indeed, tradeoffs at the univariate level are perhaps rare.

Given the lack of univariate tradeoffs in plant defense traits, three general explanations have emerged for why univariate approaches might have limited use. First, it is known that most traits have multiple functions (e.g. trichomes also function as a barrier against evapotranspiration and reflect UV light), and that two traits involved in defense cannot be assumed to be redundant when the full array of selective forces, both biotic and abiotic, are considered. Second, given the many different herbivores that attack plants, and the general lack of strong cross-resistance against herbivorous species, even within a guild [24], several defensive traits are probably needed to defend against the range of possible herbivores. Finally, and perhaps most generally, defense is rarely, if ever, effective as a single trait. Defense traits can act synergistically, that is they can be most effective when acting together [25]. Thus, there should not be an *a priori* expectation that two defensive traits should be negatively correlated.

Convergent evolution of plant defense syndromes

The rejection of the univariate tradeoff model does not mean that tradeoffs are not important. Indeed, they will occur among certain traits, and must occur at some higher level of organization. Here, sets of traits, together forming a strategy or syndrome, are likely to tradeoff against each other. For example, van der Meijden *et al.* [8] proposed that, as a group, resistance traits (i.e. traits that reduce herbivory such as toxins and physical barriers) will trade-off with tolerance traits (i.e. traits that improve regrowth capacity following herbivory). At the extremes, plant species with a resistance strategy will be subject to little selection for tolerance (because they do not receive much damage); tolerant species will not experience strong selection for resistance because herbivory does not reduce their fitness. It is implicit here that resistance and tolerance are strategies, each comprising many traits. This multivariate view of plant defense has led to the development and maturation of theories for the evolution of convergent syndromes [23,26].

Apparency theory

The concept of plant defense syndromes is not new. During the 1970s, Paul Feeny proposed an apparency theory [6], suggesting that plants that are obvious to herbivores (i.e. those that are large, long lived, or dominant) will convergently evolve a suite of defenses that are effective against most herbivores, and will function by quantitatively reducing the edibility or nutritional quality of the plant (e.g. via low levels of nitrogen and water, and high toughness and tannins). Conversely, less apparent plants, such as successional ephemeral herbs, were proposed to contain qualitative barriers to feeding (e.g. alkaloids, cyanides, cardenolide, etc.), although they would be highly nutritious. Although this hypothesis has withstood the test of time and some phylogenetic analysis (e.g. plant woodiness is correlated with abundance of tannins, and tannins and alkaloids show a negative correlation [27,28]), it is less clear how to apply the concept of apparency to most plant species that fall between the extreme ends of the apparency continuum.

Resource availability

In 1974, Janzen [5] argued for a different type of plant defense syndrome. Tropical plants inhabiting resource-poor environments were hypothesized to grow slowly, have low tolerance to herbivory (because the value of each leaf to the plant is high), and invest heavily in sundry chemical defenses (e.g. various phenolics). Conversely, plants growing in resource-rich soils were predicted to outgrow (or tolerate) herbivory and to invest less in defense. Recent experimental work on 20 phylogenetically paired species from white sand and clay soil habitats of Peru support this hypothesis [29,30] (Figure 2). Species grew best on their home soil type in the presence of herbivores, but clay specialists, adapted to resource-rich environments, exhibited faster growth in both habitats when protected from herbivory. In phylogenetically paired analyses, these clay specialists invested less in defense traits (a multivariate summary of four classes of compounds) and more in growth, compared with white-sand specialists.

Coley and colleagues have similarly hypothesized that plant species colonizing high-resource light gaps should have divergent syndromes compared with species living in the more resource-poor understories in tropical forests [7,26]. The core of this resource availability hypothesis is that abiotic resources are the driving factor behind plant evolutionary strategies, and that this sets the template for the types of defense that can evolve. In this case, tradeoffs are invoked at the multivariate level. Recent work has supported the notion that tropical trees fall along an 'escape'-'defense' continuum: extreme 'escape' species (growing in high light) are predicted to have few chemical defenses, but rapid synchronous leaf expansion and low leaf nutritional quality during expansion; extreme defense species (growing in low light) have high levels of various chemical defenses, low nutritional quality and asynchronous leaf expansion [26,31].

An additional hypothesis associated with plant defense evolution in environments varying in resources predicts that plant species will show lower levels of phenotypic plasticity when evolving in resource-poor conditions [32]. Van Zandt [33] recently showed that nine temperate species growing on resource-poor glade sites tended to have higher constitutive defense and lower inducibility compared with congeners growing in resource-rich grassland sites. This study is an excellent example of using the comparative method to address questions associated with the evolution of phenotypic plasticity (i.e. induced defense), and its relationship with selection on traits that alter plant growth rates and habitat suitability.

The above hypotheses and examples illustrate: (i) early predictions of patterns in the types of defense that plants use; and (ii) more recent findings that unrelated plant species have converged evolutionarily on suites of similar strategies [26,29,31]. These suites of strategies apparently maximize fitness given a particular set of ecological conditions or interactions, although more work is needed to identify the relationships between environment and defense syndromes.

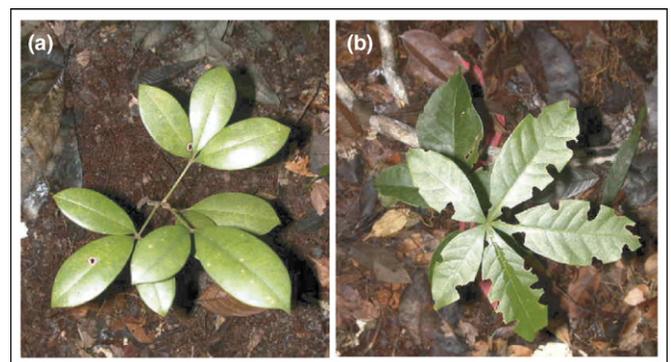


Figure 2. Resource availability and the evolution of plant defense strategies. *Pachira brevipes* (a) and *P. insignis* (b) (Malvaceae) from nutrient-poor white-sand and nutrient-rich clay soils, respectively (near Iquitos, Peru). Each species is adapted to, and occurs primarily on, its respective soil type, in part mediated by interactions between resource availability and herbivory. Across several such phylogenetic pairs of plants, species on nutrient-poor soils exhibit slow plant growth and a high cost of leaf loss, which is associated with heavy investment in defense; on nutrient-rich soils, plant species invest less in defense and are relatively more tolerant of herbivory. These predictions were supported by a phylogenetically controlled reciprocal transplant experiments with 20 Amazonian tree species [29,30]. Reproduced with permission from Paul Fine.

Defense evolution within a lineage

A complementary approach for studying plant defense syndromes involves describing the patterns of multivariate trait associations within lineages. In work on 38 species of *Bursera* trees, Becerra reported convincing evidence for convergent evolution for the combination of terpenoid defenses and the force with which they are released upon attack [34,35]. Species that produce simple chemical mixtures (one to a few monoterpenes) release the compounds with a forceful squirt upon damage, whereas species that use complex mixtures of mono- and diterpenes (up to 12 compounds) do not [34,35]. The ecological drivers of this differentiation in defense combinations have yet to be uncovered.

A preliminary analysis of seven different groups of plant defenses in 24 species of *Asclepias* has revealed multivariate trait associations that also show convergent evolution [23]. Three syndromes have been distinguished in which *Asclepias* species are grouped in the following clusters: (i) high physical defenses (low levels of chemical defense and moderate nutritional quality); (ii) low nutritional quality (with few physical or chemical defenses); and (iii) high chemical defense (low levels of physical defense and moderate nutritional quality). In addition, the quantitative clustering of plant species by defense traits was not found to be congruent with a molecular phylogeny, indicating that the defense characteristics of *Asclepias* are evolutionarily labile, and do not track phylogenetic history. Given this apparent convergence, the question then becomes whether there are consistent ecological correlates that match these different syndromes.

In addition to the abiotic hypotheses discussed earlier, distinct defense syndromes can evolve if different plant species are primarily attacked by particular types of herbivore (e.g. vertebrate versus invertebrate, chewing versus sucking herbivores, etc.) [23]. Thinking about plant defense syndromes forces us to grapple with multivariate defense, assessing whether there is a limited set of adaptive strategies that repeatedly evolve, and whether trade-offs do occur, but at some higher level of trait organization.

Phylogenetic history of plant defense

Several hypotheses for the evolution of antiherbivore defense have suggested that broad patterns will emerge in plant phylogenies that might not be evident from studying a few co-occurring species within a community or a focal lineage (Table 1): (i) the evolution of novel defensive traits will be associated with adaptive radiation [4]; (ii) evolutionary transitions in defense potency or toxicity should increase as taxa become more derived [4,36,37]; and (iii) chemical defense traits will exhibit phylogenetic conservatism owing to constraints on biosynthetic origins [38–40]. I outline these patterns here to demonstrate the ways in which an understanding of broader phylogenetic relationships can aid our understanding of the evolution of plant defenses.

Adaptive radiation

Perhaps the best case of novel defense leading to radiation is that of plant latex and resin canals, which are effective physical barriers to insect feeding (Figure 1) and highly convergent in angiosperms (occurring in ~10% of families

and 10% of species overall). In a landmark study, Farrell *et al.* showed that latex-bearing plant clades were significantly more species rich than were sister clades lacking latex (13 of 16 pairs showed this pattern) [41]. Thus, although there is some evidence that evolution of particular defenses is coincident with adaptive radiation, there is still a gap in our knowledge of such 'escape and radiate' owing to a lack of additional data.

Escalation of defense potency

In Ehrlich and Raven's original coevolutionary hypothesis [4], novel defensive adaptations that free plants from herbivory lead to adaptive radiations. Once these phylogenetically conserved defenses are overcome by herbivores with counter-adaptations (e.g. physiological detoxification systems), it was proposed that only the evolution of an additional novel, more potent defense would enable that plant lineage to continue to diverge and speciate. Thus, evolutionary transitions from simple to complex defense or less potent to more potent defense should occur only among broad phylogenetic classes of plants (e.g. clades), because it was the initial escape from herbivory that enabled the subsequent radiation of each clade.

Arguments for increasing defense potency have been made for plants in the genus *Asclepias* [36,42] and in the carrot family (Apiaceae) [37]; however, none of these analyses were phylogenetically rigorous. Perhaps the best evidence comes from Armbruster's work on *Dalechampia* vines [14], which shows additional defensive strategies in the derived taxa compared with those that are basal. Ultimately, addressing this hypothesis and the scale at which escalation occurs is an empirical question that awaits resolved phylogenies and information about multivariate defense.

Phylogenetic conservatism

Although phylogenetic conservatism is a general null hypothesis for the evolution of traits, it has a special meaning for patterns of plant defense evolution because these are evolutionarily derived from a limited number of biosynthetic pathways. The hypothesis of phylogenetic conservatism in defense posits that the biochemical pathways required to produce various defenses are complex enough that they probably evolve only once or a few times, followed by subsequent modification within a clade (although not necessarily becoming more complex). The domination of particular defense chemical classes in certain plant families is a striking example of conservatism: for example, cardenolides in the dogbane family (Apocynaceae) or glucosinolates among mustards (Brassicaceae).

Recent advances in the study of alkaloids across several plant families with known phylogenies have revealed striking examples of phylogenetic conservatism [38–40]. There are also deviations from this pattern. For example, within the nightshades (Solanaceae), tropane alkaloids represent a well-known group of toxic chemical defenses. Among some tribes within the Solanaceae, such as the Datureae, these alkaloids are highly conserved and occur in most taxa [39]; yet, in other somewhat distantly related tribes (e.g. Physaleae), tropane alkaloids occur sporadically [39].

Box 3. Coevolution, co-speciation and host shifts: including herbivore macroevolution

The original coevolutionary hypothesis [4] was about how reciprocal selection can lead to the escape of plants from herbivory via a novel defense, and the resulting adaptive radiation of those plants. It was then predicted that herbivores, once able to overcome the novel defense, would also radiate onto the diversified host plants. This hypothesis predicts that clades of plants and herbivores will mirror each other in their patterning of speciation events (e.g. topology), but that the divergence time of speciation events for herbivores should lag after the diversification of plants. Few data have been collected so far to address this coevolutionary hypothesis rigorously [56].

Co-speciation suggests a pattern whereby early diverging hosts were associated with early diverging herbivores, and that as one group speciated, so did the other. Several mechanisms, most simply joint allopatry of the two interacting partners owing to some vicariance event, could generate similar phylogenetic patterns, and it is still unclear how to implicate specific processes that generate such patterns. Speciation events that appear synchronous among plants and herbivores (e.g. Ref. [57]) lend further support to co-speciation owing to joint allopatry. Non-synchronous co-speciation can indicate the movement of herbivores onto already diversified host plants, or other types of host shift (e.g. Ref. [58]).

Host shifts and herbivore diversification patterns might thus be crucial to unraveling coevolution. In an explicitly phylogenetic framework, several studies demonstrate that host usage is conserved; that is, closely related herbivores feed on closely related plants [34,56,58,59]. Host shifts occasionally occur on more distantly related plants, and only two studies have identified some of the key chemical and ecological attributes of such distant host shifts.

For example, Becerra [34] demonstrated how shared host plant defensive chemistry can be more important than phylogenetic association in host shifts of leaf beetles on *Bursera* species. In other words, host shifts by the beetles onto distantly related plant species were coincident with a shared chemistry with the former host plant species. Murphy has shown that a host shift in swallowtail butterflies *Papilio machaon* onto a distantly related plant species is associated with shared host chemistry and enemy-free space [16,60]. The current interesting questions for host shifts thus lie at the interface of phylogeny and plant defensive biology; although it is clear that host usage is typically conserved, the exceptions to this rule will provide the key to understanding the key selective factors involved in herbivore diversification.

We do not yet have quantitative metrics to describe the extent of phylogenetic conservatism (e.g. the percentage phylogenetic conservatism that alkaloid content shows in the Solanaceae), but these are needed. The exceptions to conservatism are important in that they can be remarkable examples of convergence (e.g. tropane alkaloids have been reported from a few species in distantly related plant families such as the Brassicaceae and Proteaceae [39]) or losses (i.e. the lack of cardenolides and latex in some *Asclepias* species [23]). In some cases, distantly related taxa that show similar defense mechanisms are examples of true convergent evolution. In other cases, however, the biosynthetic pathways for producing particular defenses will be basal and have a monophyletic origin; here the hypothesis for the sporadic presence of particular defenses rests on differential gene expression (i.e. the lack of expression in many taxa) as opposed to the occasional gain of those genes and pathways [38,39].

Conclusion

The availability of phylogenies among broad plant families and novel statistical approaches are finally enabling the

rigorous testing of early comparative theories of plant defense. Reciprocally, novel theories and patterns will be developed by examining plant defense strategies in a phylogenetic context. Ideas such as the univariate tradeoff model are no longer valid. Others, such as the importance of biotic versus abiotic agents (or their interaction) in shaping patterns of plant defense expression are now waiting to be addressed in a phylogenetic context. Here, I have proposed three alternative strategies to examine the evolution of plant defense syndromes. First, by comparing plant species within a community, either by focusing on congeners or by building a phylogeny of species within a community, we can address the importance of convergence on particular strategies in co-occurring taxa. Second, by focusing on a particular clade, evolutionary transitions between syndromes can be addressed more precisely. Finally, by examining patterns across clades, the signatures of processes such as 'escape and radiate' selection, escalation of defense, and the extent of phylogenetic conservatism in defensive traits can be addressed. A similar set of approaches are needed to study the evolution of exploitation in herbivores (Box 3).

The fact that there was once controversy about whether herbivores impose natural selection for plant defenses seems like a distant memory. Although there is still much to be learned about the context dependence of such selection, I have argued here that filling the key gaps now requires researchers to address some of the classic questions and theories about plant defense expression across taxa.

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