

The Evolutionary Ecology of Plant Chemical Defenses: From Molecules to Communities

María-José Endara,¹ Dale L. Forrister,^{2,3}
and Phyllis D. Coley^{2,3}

¹Grupo de Investigación en Biodiversidad, Medio Ambiente y Salud (BIOMAS), Universidad de las Américas, Quito, Ecuador; email: maria.endara.burbano@udla.edu.ec

²School of Biological Sciences, University of Utah, Salt Lake City, Utah, USA

³Smithsonian Tropical Research Institute, Panamá, República de Panamá

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Keywords

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Abstract

Classic theory relates herbivore pressure to the ecology and evolution of plant defenses. Here, we summarize current trends in the study of plant–herbivore interactions and how they shape the evolution of plant chemical defenses, host choice, and community composition and diversity. Inter- and intraspecific variation in defense investment is driven by resource availability. The evolution of defenses at deeper nodes of plant phylogeny is conserved, yet defenses are highly labile at the tips. On an ecological timescale, while greater specialization of tropical herbivores enhances local diversity by reducing the performance of plants with similar defenses, in temperate ecosystems with more generalist herbivores, rare defense profiles are at a disadvantage. On an evolutionary timescale, host choice by herbivores is largely determined by plant defenses rather than host phylogeny, leading to evolutionary tracking by herbivores rather than cocoladogenesis. The interplay between plants and herbivores shapes both the origin and maintenance of diversity.

1. INTRODUCTION

Because plants and their insect herbivores together account for more than half of the macroscopic diversity on land, their interactions play an extremely important role. These relationships affect many aspects of biodiversity and ecosystem function, influencing processes as diverse as nutrient cycling and carbon sequestration (Díaz et al. 2006), community assembly, and species diversity (Maron et al. 2019). Here, we review recent advances in our understanding of how these defenses shape the ecological and evolutionary interactions in the arms race between plants and herbivores.

Since the early days in the study of plant defenses, much of the research has focused on resolving three main questions: What is the significance of plant defenses, why do defenses vary across species, and what are their ecological and evolutionary consequences for shaping patterns of biodiversity? Over the last decades, significant contributions have been made to resolve some of these questions, such as proving the adaptive value of defensive traits (Dethier 1954, Fraenkel 1959) and determining the selective pressures that have led to the variety of defensive strategies seen across and within species (Stamp 2003). For example, studies have shown that variation in the amount and type of defense responds to the optimal balance between herbivore pressure and access to resources (Coley et al. 1985, Feeny 1976). Over the past 50 years, development of plant defense theory has provided an effective framework for predicting outcomes and developing experiments (Stamp 2003).

Recently, modern ecological and evolutionary theory, coupled with methodological innovations in quantifying phylogenetic relationships and defensive chemistry, has greatly expanded our ability to critically address long-standing research questions about plant–herbivore interactions. Thanks to these innovations, we can refine the testing of classical hypotheses, such as the contribution of plant defenses to local coexistence through negative density-dependent effects of herbivores (Connell 1978, Janzen 1970) and the role of the interactions between plants and herbivores in trait divergence and coevolution (Ehrlich & Raven 1964, Futuyma & Agrawal 2009). The general consensus is that plant chemical defenses play an essential role in community assembly and evolution for both plants and insect herbivores.

In this review, we explore current trends in the study of the ecology and evolution of plant defenses. First, we provide a brief summary of the conceptual frameworks that have aimed to understand variation in plant defenses across and within species, an important research direction since the mid-twentieth century. Then, we address questions that are currently under active investigation but that lack a recent synthesis. For example, how do plant–insect interactions direct the evolution of defenses, how do defenses shape host selection, and how do plant–herbivore interactions influence community assembly and diversity?

Rather than reviewing all classes of plant defenses, we focus on plant defensive chemistry. While all defensive traits negatively affect herbivores, chemical defenses are among the most effective and diverse and have been invoked as a major axis in plant and herbivore interactions (Agrawal et al. 2009, Endara et al. 2017, Futuyma & Agrawal 2009, Thompson 1988). We highlight the use of metabolomics, a recently developed chemical analytical technique that, coupled with phylogenetic comparative methods, has greatly enriched our ability to explore the chemical diversity of plants at unprecedented taxonomic and geographic scales (Defosse et al. 2021, Forrister et al. 2023, Sedio et al. 2017). This has contributed to stronger inferences and more rigorous contributions to long-standing questions in the evolutionary ecology of plant defenses. The integration of metabolomics with genomics and transcriptomics is also providing a mechanistic link between plant physiology (i.e., subcellular processes) and the role it plays in mediating ecological interactions. This broadscale, micro-to-macro perspective has proven useful in our quest to understand the ecology and evolution of plant defenses. Throughout this review, we attempt to take this perspective by synthesizing our understanding of plant defense theory across scales.

2. PLANT DEFENSES

In plants, a variety of defensive traits have evolved, including physical structures (e.g., hairs, thorns), toxic chemical compounds (secondary metabolites, e.g., saponins, nonprotein amino acids), reduced nutritional quality, and phenological escape. These defensive traits are considered direct defenses because they act directly on the herbivore. Plants have also evolved partnerships with species from the third trophic level, which are termed indirect defenses. For example, plants may provide food to predators of insect herbivores (e.g., extrafloral nectar) or produce volatile signals to attract parasitoids of herbivores. All these defensive traits can vary independently from each other (Endara et al. 2017) and contribute to host choice by insect herbivores (Endara et al. 2017, 2018).

Plants are widely considered to be chemical factories, with the estimated size of the metabolome of an individual species ranging between 5,000 and tens of thousands of compounds (Ferne et al. 2004). Across the entire plant kingdom, the total estimated chemical diversity is between 200,000 and 1,000,000 small molecules (Dixon & Strack 2003, Fang et al. 2019). Within the single Neotropical tree genus *Inga* (Leguminosae), we have cataloged more than 9,000 compounds (Endara et al. 2022, Forrister et al. 2023). A small portion of a species' metabolome is involved in primary metabolism, which is broadly conserved across all plant species and underpins all cellular processes. In contrast, the vast majority of chemical diversity consists of specialized plant metabolites, also known as secondary metabolites. These are derived from a small group of precursor compounds used in primary metabolism, which are eventually modified into diverse end products. For example, the >40,000 isoprenoid compounds branch out of the central carbon metabolism (Moore et al. 2014), and another >10,000 metabolites, including alkaloids, glucosinolates, and phenylpropanoids, are amino acid derivatives (**Figure 1**) (Wink 2003).

Plant secondary metabolites mediate interactions between a plant and its external environment (Walker et al. 2022), from deterring pests and pathogens (Lokvam & Kursar 2005, Schneider et al. 2019, Tiku 2020) and attracting pollinators (Sosenski & Parra-Tabla 2019) to allowing a plant to survive harsh abiotic conditions [e.g., UV protection and prevention of desiccation (Schneider et al. 2019, Wang et al. 2019)]. These metabolites belong to many chemical classes, including phenolics, terpenes, alkaloids, glucosinolates, and nonprotein amino acids (Hopkins et al. 2009; Lokvam et al. 2004, 2007; Lokvam & Kursar 2005; Tiku 2020). The chemistry and biology of most major classes of chemical defenses have been extensively reviewed. Therefore, we summarize only the main classes of secondary metabolites in **Figure 1**. For comprehensive reviews, see Harborne (1988), Rosenthal & Berenbaum (1991), Wang et al. (2019), and Wink (2003).

Thus, plants invest in an astonishingly diverse array of secondary metabolites, with the type and number of defensive metabolites varying across species and even within individual plants. For example, plants with different life history traits and habitat preferences show differences in defense investment. Young expanding leaves invest more in secondary metabolites than mature leaves (Wiggins et al. 2016). These patterns have been rationalized by various theories discussed in the next section; these theories in turn have stimulated a multitude of studies and established the basic conceptual framework linking defense and herbivory (Coley et al. 1985, Feeny 1976, Herms & Mattson 1992, Rhoades & Cates 1976).

3. MICROEVOLUTIONARY PATTERNS OF DEFENSE INVESTMENT: PLANT DEFENSE THEORY

For many years, the study of plant–insect interactions focused primarily on the role of secondary metabolites in plants and on the costs and benefits of these defenses. Once it was established that secondary chemicals were not waste products but functioned as defenses against herbivores

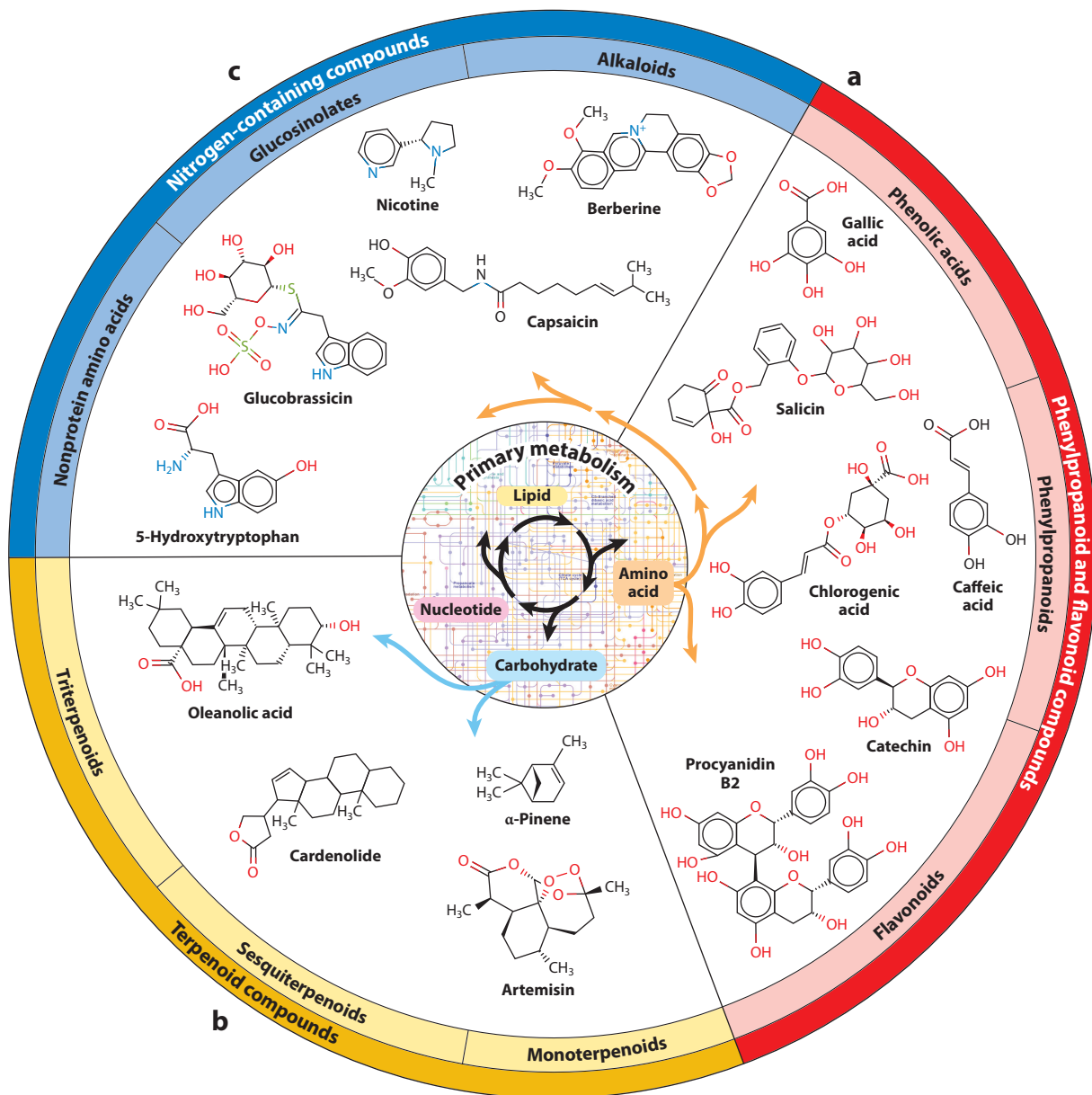


Figure 1

Examples of selected plant specialized metabolite structures arranged by major classes that have been implicated in defense against pests or pathogens. Biosynthetic origins from primary metabolism are depicted with arrows for each of the three compound groups: (a) phenolics, phenylpropanoids, and flavonoids; (b) terpenoid compounds; and (c) nitrogen-containing compounds.

(Dethier 1954, Fraenkel 1959), interest shifted to explaining why the amount and type of defenses differed considerably within and among species. To this end, many conceptual models have been proposed (Stamp 2003), most of them based on the premise that variation in defense expression across species is related to optimal levels of investment, such that the benefits outweigh the cost

(Feeny 1976, McKey 1974, Rhoades & Cates 1976). Here, we focus on theories aimed at explaining differences in defenses among species. Then, we discuss other influential theories that have helped us understand differences in defenses among individuals within species.

3.1. Interspecific Patterns of Defense Investment

Plant apparency theory (Feeny 1976, Rhoades & Cates 1976) and the resource availability hypothesis (Coley et al. 1985) stand out as the most prominent plant defense theories that address interspecific variation in defense expression. Plant apparency theory proposes that differences in defenses between species are related to a species' predictability to herbivores (Feeny 1976, Rhoades & Cates 1976). Thus, it proposes that species that are more apparent or conspicuous to herbivores, such as large, evergreen, and long-lived plants, have evolved defenses that are effective against most herbivores and that reduce damage quantitatively (e.g., defenses that are more effective at higher concentrations, such as tannins). Meanwhile, because of the ephemerality and short lifetimes of unapparent species, such as herbs, selection is proposed to favor qualitative defenses against generalist herbivores (e.g., defenses that are effective at low concentrations, such as alkaloids). Immediately upon its publication, this theory was accepted, and it has profoundly shaped the field. However, some of its assumptions are challenging to test, such as the classification of plant species and defenses along the apparency and quantitative–qualitative continua, respectively.

The resource availability hypothesis (also known as the growth rate hypothesis) (Coley et al. 1985) differs in emphasizing the role of the environment in the evolution of defenses. Based on the observation that species from resource-rich environments exhibit inherently higher growth rates than species from resource-poor environments (Chapin 1980; Coley et al. 1985; Grime 1977, 2006), this hypothesis predicts that species from less productive environments are selected to exhibit higher levels of constitutive defenses and therefore experience lower herbivore consumption than fast-growing species. This prediction relies on the idea that for slow-growing species, the opportunity cost of investing in regrowth after herbivory losses would be high because of their inherently slow growth and the low accessibility of resources in the environment to which they are adapted. Qualitative and quantitative reviews across a wide range of species and latitudes are consistent with the resource availability hypothesis in showing that growth rate shows a negative correlation with defense investment and a positive correlation with herbivory levels across plant species, independent of their apparency (Endara & Coley 2011).

3.2. Intraspecific Patterns of Defense Investment

Other important theories that make predictions about differences in defense investment are the carbon–nutrient balance hypothesis (Bryant et al. 1983) and the growth–differentiation balance hypothesis (Herms & Mattson 1992). It is important to note that these theories pertain to plasticity, as they address intraspecific differences in defense expression.

The carbon–nutrient balance hypothesis predicts intraspecific disparities in defenses across different resource levels. This hypothesis suggests that resources in excess for growth are likely shunted to defense. Thus, higher levels of carbon-based defenses are predicted in individuals growing under high light intensities or low nutrient levels (Massad et al. 2012). While the utility of this theory has occasionally been questioned (Hamilton et al. 2001), it has recently regained prominence in the study of climate change effects on plant defense expression (Fabian et al. 2018, Watanabe et al. 2021).

The growth–differentiation balance hypothesis, in contrast, rationalizes defense allocation within individual plants in response to resource gradients. It follows predictions from optimal

defense theory (Glynn et al. 2007; Herms & Mattson 1992; Stamp 2003, 2004), which predicts that tissues of higher value for plant fitness will be better defended (e.g., flowers and fruits will be better defended than leaves). In general, the growth–differentiation balance hypothesis predicts a negative correlation between growth and differentiation (e.g., investment in defenses) for plants that grow under conditions that range from intermediate to high resource levels. Although it is considered one of the most theoretically mature hypotheses (Stamp 2004), testing its predictions has proven difficult. For instance, in order to assess the curvilinear pattern of defense investment as originally proposed by Herms & Mattson (1992), a minimum of five resource levels are needed (Stamp 2004). In addition, it is difficult to separate allocation costs between growth and some structural differentiation products that can belong to either the defense category (e.g., trichomes) or the growth category (e.g., cell walls). However, it has gained considerable support in the last few years (Ahmed et al. 2014, Chen et al. 2015, Xie et al. 2022).

Together, intraspecific and interspecific hypotheses of defense investment have provided an effective framework for predicting outcomes and developing experiments during the last 50 years. Currently, the field of plant defense theory is moving from organismal-level observation into the cellular scale. Detailed molecular studies (e.g., ones that combine transcriptomic, proteomic, and metabolomic approaches) on a few model plant species have shown that trade-offs in growth and defense are the result of active regulation in coordinated cellular processes (e.g., cascades of molecular signals, phytohormone cross talk) while maintaining stored resource pools for future biotic and abiotic stresses (Monson et al. 2022). The integration of the optimization framework that lies beneath plant defense theory with recent breakthroughs in omics is allowing a deeper understanding of the processes and mechanisms that underlie defense expression not only at this microevolutionary level (Monson et al. 2022, Züst & Agrawal 2017) but also at larger scales.

4. THE EMERGENCE OF PLANT METABOLOMICS AS AN ANALYTICAL TOOL

For many years, the study of the ecology and evolution of plant defensive compounds was limited to examining the role of single secondary compounds [e.g., nicotine (Steppuhn et al. 2004)] or chemical classes [e.g., phenolics (Coley & Barone 1996, Wink 2003)]. Constrained by the chemical tools available between the 1950s and the 2000s, researchers were not able to perform full characterizations of the chemical defensive profiles of individual plants. This substantially limited their capacity to quantify chemical similarity among plant species in order to determine similar functions with respect to antiherbivore activity, as well as to identify similar biosynthetic pathways of chemical evolution or the role of upstream molecules (e.g., genes, RNAs, proteins) in physiological processes and ecological interactions. Furthermore, tests of classical plant–herbivore interaction hypotheses often require exploration of the role of chemical diversity in the evolution of herbivore–host associations (Endara et al. 2022, Richards et al. 2015), the causes and consequences of herbivory variation (reviewed in Wetzel et al. 2023), and the ecological and evolutionary consequences of chemical variation across large taxonomic, phylogenetic, and geographic scales (Defossez et al. 2021, Forrister et al. 2023, Sedio et al. 2017). However, this situation is rapidly changing, as ecologists are partnering with chemists and employing the latest technological advances to explore the largely undiscovered chemical diversity of plants (Castro-Moretti et al. 2020, Dyer et al. 2018) and to characterize the entire suite of defensive compounds expressed in different species. Specifically, untargeted metabolomics methods combine statistical analyses with analytical instrumentation, including liquid chromatography, gas chromatography, mass spectrometry (MS), and nuclear magnetic resonance. This powerful approach simultaneously provides qualitative and

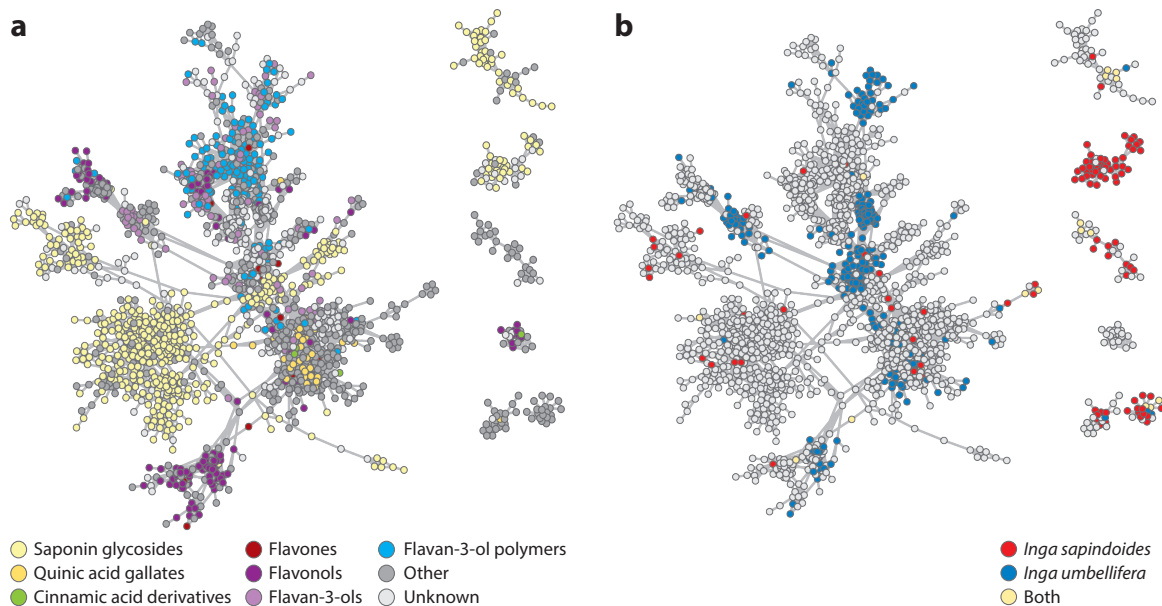


Figure 2

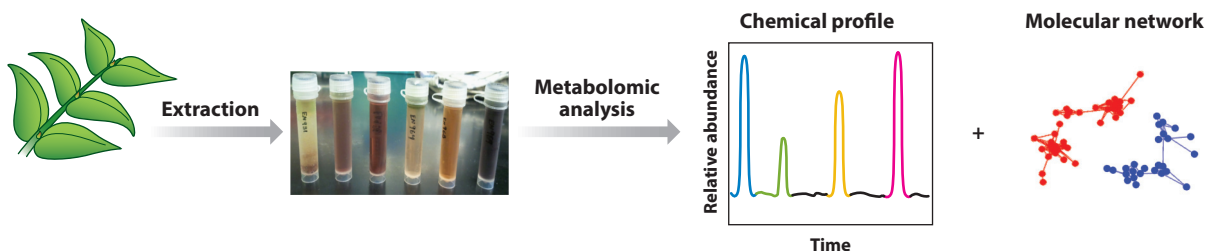
Molecular network of metabolites found in 98 species of tropical trees from the genus *Inga*. Each dot represents a single compound connected and clustered in space by lines (edges) based on their spectral similarity using the Global Natural Product Social Molecular Networking site (GNPS 2023, Wang et al. 2016). (a) Feature-based molecular networking groups related metabolites into molecular networks based on spectral similarity. Molecular networking thus serves as a proxy for structural similarity even when compounds do not receive annotation based on spectral database matches (*light-gray dots*). (b) Molecular networking is used to determine the chemical similarity between species based on the presence or absence of compounds, as well as their structural similarity. Compounds present in *Inga sapindoides* are shown as red dots, those present in *Inga umbellifera* as blue dots, and those present in both as yellow dots. Figure adapted from Forrister et al. (2023).

quantitative data on dozens to hundreds of compounds per species (Defosse et al. 2021, Endara et al. 2022, Sedio et al. 2017).

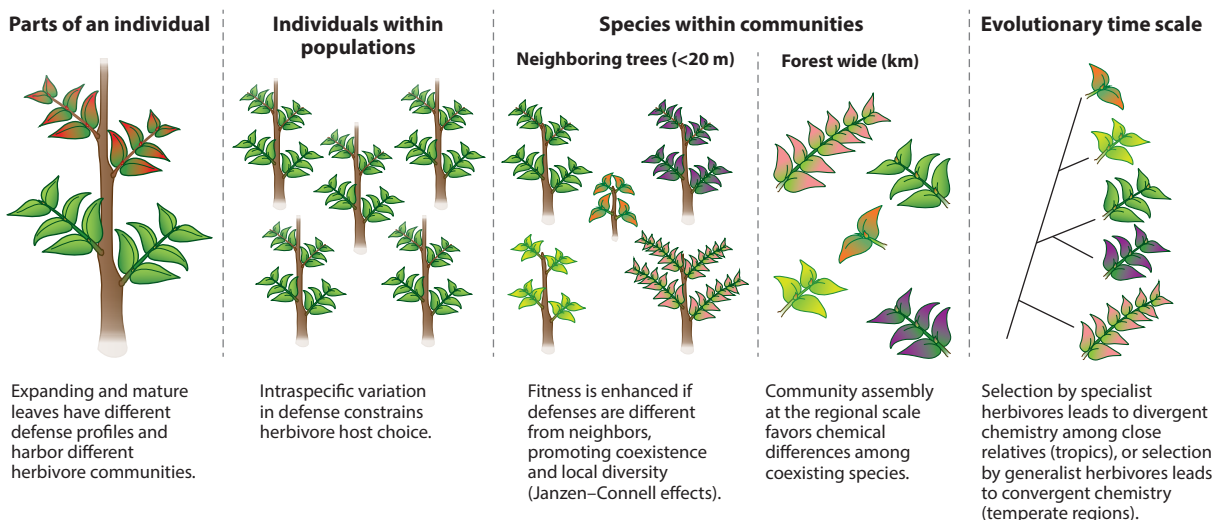
A major advantage of these metabolomic approaches is the ability to determine the chemical similarity between species based on the presence or absence of compounds, as well as their structural similarity (**Figure 2a**). This is particularly important for interspecific comparisons, since most compounds are not shared (**Figure 2b**), and traditional diversity measures, such as Bray–Curtis, do not take into account that most of these unshared compounds are biosynthetically related (Endara et al. 2022, Sedio et al. 2017). Thus, chemical similarity of species can be characterized not only by the minority of compounds that are shared but also by the chemical similarity of the compounds that are not shared (**Figure 2a**). These new metabolomic methods allow chemical similarity using the entire suite of compounds present in a species to be mapped onto phylogenetic trees to assess patterns of defense evolution (Forrister et al. 2023). Additionally, researchers can quantify changes in chemical investment between different plant tissues [e.g., between expanding and mature leaves (Wiggins et al. 2016)] or between light environments (Schneider et al. 2019) and can identify chemotypes within plant populations (Endara et al. 2018).

While metabolomics is extremely promising in terms of its potential to quantify chemical variation across scales (**Figure 3**), as well as for the discovery of new metabolites, the identification and structural elucidation of compounds pose a daunting task for analytical chemists. As the field of metabolomics has matured, many new analytical and computational approaches are better able to

a Metabolomics and the phytochemical landscape



b Chemical variation across increasing temporal and spatial scales



c Ecological consequences of chemical variation

Figure 3

A schematic of (a) the metabolomic analysis, (b) the variation in defense profiles across temporal and spatial scales, and (c) some ecological consequences. Differences in defenses reduce herbivory by specialists and enhance plant fitness within an individual as leaves develop, among individuals within a population, among neighboring individuals of different species (Janzen–Connell effects), among species within a broader community, and among close relatives.

link spectra to structure. For example, high-resolution mass spectrometers are used to isolate and break apart metabolites into fragments, providing an MS/MS spectrum that gives structural information about metabolites. This technique can be used to search global spectral libraries (Treutler et al. 2016, Wang et al. 2016) and to group similar compounds into molecular networks based on their spectral similarity (Figure 3) (Endara et al. 2022; Forrister et al. 2023; Nothias et al. 2020; Sedio et al. 2017, 2021; Wang et al. 2016).

As the capacity to quantify and characterize the structural composition of a metabolite advances, we greatly improve the opportunity to connect an organism's chemical composition (phenotype) to its underlying biosynthetic pathways, regulatory networks, and genetic architecture (genotype). The adoption of untargeted metabolomics of wild plants by ecologists and molecular biologists opens windows into long-standing and new questions related to the underlying

selective forces shaping the micro- and macroevolutionary patterns of chemical defense investment and variation.

5. MACROEVOLUTION OF PLANT CHEMICAL DEFENSES

5.1. Phylogenetic Patterns of Defense Expression

Ehrlich & Raven (1964) incorporated a macroevolutionary framework into the study of plant–insect interactions. In their seminal paper, they considered the reciprocal nature of the adaptive responses between plants and herbivores and introduced the idea that plants and butterflies are coevolving, a concept that has dominated our understanding of the interactions between plants and insects for the last 60 years.

According to Ehrlich and Raven's paradigm, there is taxonomic conservatism in the expression of defenses in plants and in the use of hosts by insect herbivores. They suggested that this results from an ongoing coevolutionary arms race between plants and enemies. Specifically, this theory predicts that after the evolution of a new defense in response to herbivore pressure, a plant species is able to escape most herbivores. Once in a herbivory-free zone, this plant species radiates into a clade in which all its descendants share the new chemical defense. Thus, closely related plants have similar defenses. This process may be repeated with the evolution of new plant defenses. Ehrlich and Raven suggested that this historical process of defense evolution may explain the distribution of plant secondary metabolites we see in the plant kingdom, as well as a substantial fraction of plant species diversity.

Although no specific model for the evolution of chemical defenses was put forth by Ehrlich and Raven, the first evidence supporting the macroevolution of chemicals came from Berenbaum (1983). In the Parsley–Swallowtail system (Apiaceae plants and Pieridae butterflies), Berenbaum proposed a scenario in which plants sequentially evolved several related phenolic compounds (hydroxycoumarins, linear furanocoumarins, and angular furanocoumarins) in increasingly derived taxa as a response to counteradaptations from the adapted lineage of butterflies. This pattern was explicitly defined as an escalation of defense by Vermeij (1994), based on the observation that the production of secondary metabolites in plant lineages is associated with a degree of phylogenetic nesting. In other words, the evolution of novel defenses is proposed to be incremental and sequential, often through the gradual embellishment of core structures into more complex and derived compounds, during the diversification process of a lineage (Agrawal 2007, Berenbaum & Feeny 1981).

In recent decades, our understanding of the deep evolutionary origins of metabolism has grown considerably, providing a framework for understanding the evolution of plant chemical defenses (Moore et al. 2014, Ober 2010, Scossa & Fernie 2020, Wang et al. 2019). To understand how novelty in plant chemicals is generated, in the next section we focus on the evolutionary mechanisms by which defensive chemicals have evolved and how these mechanisms vary at different taxonomic scales.

5.1.1. Evolution of biosynthetic innovations: Novelty at broad phylogenetic scales. Because plant defensive chemicals are derived from a limited number of biochemical pathways (Liscombe et al. 2005), the major mode of evolution for a novel chemical defense is assumed to be gene duplication (Moore et al. 2014, Ober 2010, Scossa & Fernie 2020). Gene duplications are rare, nondeleterious mutational events that allow for the expansion of existing pathways of primary metabolism. Although the majority of gene duplications are lost over evolutionary time, a small portion result in gene paralogs that, under relaxed selection, can acquire potentially adaptive mutations. Once fixed within populations, these genes can result in novel enzymatic functions, termed

neofunctionalization (Scossa & Fernie 2020). Neofunctionalization may result in biosynthetic innovations, often as a result of a shift in substrate specificity, and/or changes in the regulation of metabolic pathways (Moore et al. 2014, Scossa & Fernie 2020). The rare adaptive duplication events that became fixed within populations are thought to have given rise to the major expansion of the plant metabolome that accompanied plants' colonization of terrestrial habitats approximately 500 million years ago (Emiliani et al. 2009, Tohge et al. 2013). Together, this succession of rare evolutionary events suggests that the evolution of biosynthetic innovations through gene duplication happens once or a few times during the evolutionary history of a clade. This process produces novelty only at the level of chemical class [such as the invention of an entirely novel class of structures, e.g., glucosinolates (**Figure 1**)] and at broad phylogenetic scales [e.g., clades, such as the plant family or genus level (Agrawal 2007, Wink 2003, Zhang et al. 2021)].

Comparative studies performed at these broad taxonomic scales have found the dominance of a particular defense class (Wink 2003). At this phylogenetic scale, the expression of lineage-specific metabolite classes has been shown for certain plant families: coumarins for Apiaceae (Berenbaum 1983), cardenolides for the Apocynaceae (Agrawal & Konno 2009), quinolizidine alkaloids and nonprotein amino acids in Fabaceae (Wink 2003), glucosinolates for Brassicaceae (Hopkins et al. 2009), and tropane alkaloids for Solanaceae (Griffin & Lin 2000, Wink 2003), among other examples.

5.1.2. Evolution of novel chemical defenses: Novelty at finer taxonomic scales. With the development of new phylogenetic tools and the ability to characterize the entire metabolome, different patterns of defense evolution have been found at finer taxonomic scales (species level). Examinations of the phylogenetic structure of plant chemical defenses within genera have revealed that close relatives are not similar in their suite of defensive chemicals (Agrawal & Fishbein 2006, Becerra 1997, Becerra et al. 2009, Forrister et al. 2023, Kursar et al. 2009, Sedio 2013). This has resulted in a pattern of poor congruence between their phylogenetic histories and the expression of plant defenses. For example, for the genus *Inga*, we have found that closely related species are highly divergent in the expression of chemical defenses (Forrister et al. 2023, Kursar et al. 2009). Similarly, for the genus *Ficus* in Papua New Guinea, evolution of defenses follows a pattern of divergence toward the tips of the phylogeny (Volf et al. 2017). The fact that closely related species at this phylogenetic scale are dissimilar in defenses suggests that these traits are experiencing rapid evolutionary change. Thus, at finer taxonomic scales, secondary metabolites may evolve by faster and simpler mechanisms than at broad taxonomic scales. Changes to regulatory mechanisms (e.g., differential gene expression) have been suggested as possible mechanisms (Burow et al. 2010, Durbin et al. 2003, Tewari et al. 2003, Windsor et al. 2005). Differential expression of individual or biosynthetically related metabolites can result in the evolution of novel combinations of existing defense compounds. Rapid changes to regulatory mechanisms can shape a species' chemical profile and lead to divergent defense profiles between closely related species at the tips of the phylogenetic tree without the evolution of key biosynthetic innovations (Agrawal & Fishbein 2006, Coley et al. 2018, Courtois et al. 2016, Forrister et al. 2023). Mounting evidence from numerous plant genera [e.g., *Bursera* (Becerra 1997, Becerra et al. 2009), *Mimulus* (Wills et al. 2016), *Piper* (Richards et al. 2015), *Protium* (Salazar et al. 2018), *Psychotria* (Sedio 2013)] has shown that divergence in the profile of plant chemical defenses among close relatives is common. Our work suggests that pests and pathogens might be the drivers selecting for this divergence in defensive traits (see Section 4.1) (Endara et al. 2017, Forrister et al. 2023).

We have found strong evidence for both mechanisms driving defense evolution in the genus *Inga*: biosynthetic innovations at the clade level and divergence at the tips of the phylogeny. The chemical profiles of *Inga* are dominated by phenolics, saponins, and nonprotein amino acids, as well

as the overexpression of L-tyrosine and related depsides. The overexpression of tyrosine evolved once and is restricted to a single clade consisting of 21 species that invest in 5% to 20% leaf dry weight tyrosine (Coley et al. 2019), which is toxic to generalist herbivores at these concentrations (Lokvam et al. 2006). The relatively recent (~4.47 Mya) evolution of this defense is likely the result of a gene duplication event (Coley et al. 2019). Several species within this clade have evolved more derived compounds, including tyrosine and tyramine depsides, providing evidence for the escalation of defense through gradual modifications of core structures. In contrast, the other defense classes found in most *Inga* species show little to no pattern of phylogenetic conservatism, with many individual compounds and compound classes rapidly switching between presence and absence across the phylogeny. This is likely a result of changes in the transcriptional regulation of biosynthetic pathways (Courtois et al. 2016, Forrister et al. 2019, Moore et al. 2014).

While patterns of variation in taxonomic conservatism in the expression of chemical defenses across and within clades are clear, more work is needed to integrate evolutionary theory, macroevolutionary patterns of plant defenses, and our understanding of the evolutionary genomics of plant metabolism in order to understand the evolution of novelty in plant chemical defenses. Metabolomics has the potential to provide the mechanistic link between metabolic profiles and their underlying evolutionary and biosynthetic processes (Dyer et al. 2007, Walker et al. 2022) through integration with other omics approaches such as genomics, transcriptomics, and proteomics.

5.2. Phylogenetic Patterns of Host Use by Herbivores

Another expectation from the arms race hypothesis (Ehrlich & Raven 1964) is that host association should be conserved in phytophagous insects at both ecological and evolutionary timescales. Because plant defenses were assumed to be phylogenetically conserved across all levels of phylogenetic divergence, host shifts in insects were predicted to be rare, and if shifts happened, these were predicted to occur between closely related plants (Agrawal 2007, Futuyma & Agrawal 2009). Thus, at the ecological level, the assemblage of herbivores in a local community was expected to be related to the phylogenies of the assemblages of the host plant species (Lewinsohn et al. 2005, Ødegaard et al. 2005, Weiblen et al. 2006). At an evolutionary level, parallel cladogenesis, or similarity in the sequence of speciation events between both groups, was expected (Mitter & Brooks 1983).

Early studies using natural history observations found results consistent with the main premises of the theory (Benson et al. 1975, Gilbert 1982, Turner 1981). Nevertheless, with the development of new phylogenetic tools and analytical methods that allow host phylogeny and trait information to be combined, tests have found results that are not in full agreement with the original expectations from the arms race hypothesis. Phylogenetic conservatism for herbivore host association changes with the taxonomic scale of the host plant. Ecological studies of herbivore assemblages have found the phylogeny of the hosts at the species level to be a poor predictor of community assembly (Becerra 2007; Endara et al. 2017; Nakadai et al. 2014; Novotny et al. 2002, 2010; Sedio 2013). Instead, similarity in defensive chemicals between plant hosts strongly constrain host selection at the whole-community level (Becerra 2007, Endara et al. 2017, Massad et al. 2017, Richards et al. 2015, Salazar et al. 2018, Volf et al. 2017). Our studies with lepidopteran herbivores associated with the genus *Inga* across the Amazon Basin have shown the key role that host chemistry plays in structuring herbivore community assembly both within (Endara et al. 2017) and across (Endara et al. 2018) sites. Plant species pairs that are more similar in the herbivore communities they host are also more similar in defensive traits, but these plant species are not necessarily closely related. Interestingly, the phylogeny of the host

plant community plays a bigger role in structuring herbivore communities at broader taxonomic levels. For example, in the temperate forests of Japan and the Czech Republic, the phylogenetic relationships between pairs of confamilial host plant species drive the assembly of generalist herbivores, whereas relatedness between congeneric species plays a minor role (Volf et al. 2017).

At the macroevolutionary level, explicit analyses of codiversification between plants and herbivores have found similar patterns to those at the ecological scale. In general, host shifts among closely related herbivores are more strongly correlated with the chemistry of the new hosts than with their phylogenetic relationships (Becerra 1997; Becerra & Venable 1999; Berenbaum 2001; Endara et al. 2017, 2018; Wahlberg 2001). Thus, the phylogenies of insects rarely match those of their hosts (Agosta 2006; Brooks & McLennan 2002; Endara et al. 2017, 2018; Janz 2011). These findings suggest that the most common pattern of evolutionary interaction between plants and their insect herbivores is resource tracking (host trait tracking) (Agosta 2006; Brooks & McLennan 2002; Endara et al. 2017, 2018; Janz 2011), in which herbivores associate with novel hosts that express a resource similar to the ancestral host. Thus, associations between plants and insects are more labile than expected under a model of tight evolutionary processes.

In summary, results from coevolutionary studies between plants and herbivores are consistent with the idea that plant chemical defenses exert strong constraints on herbivore host choice, with most herbivore species being restricted to a few plant hosts with similar defenses. In the next section, we review the evidence suggesting that the interactions between both groups may also enhance ecological coexistence and diversity in plants on an ecological timescale.

6. PLANT DEFENSES SHAPE LOCAL TREE DIVERSITY

A long-standing goal of ecological research is to understand the mechanisms that allow many tree species to coexist in tropical forests, where the diversity of a single hectare [e.g., ~650 species in Yasuní National Park, Ecuador (Valencia et al. 2004)] can surpass that of the United States and Canada combined (~500 species). This high diversity is widely considered to be maintained by negative density dependence (NDD) processes (Chesson 2000, Comita et al. 2014, Johnson et al. 2012, Mangan et al. 2010), in which competitive exclusion is prevented because the densities of each species are kept in check. One possible mechanism hypothesized to drive NDD patterns in forests is resource niche partitioning (Chesson 2000). This mechanism selects for neighbors that have different sets of adaptations to the local abiotic environment. However, resource attributes of habitats have been shown to selectively filter species, leading to neighbors having similar traits for resource acquisition and tolerance to abiotic stress (Cavender-Bares et al. 2009, Fine & Kembel 2011, Hardy et al. 2012, Kraft et al. 2011, Vleminckx et al. 2019). Thus, the potential of abiotic niches alone to explain diversity in species-rich biomes such as tropical rain forests has been questioned (Wills et al. 2016, Wright 2002).

Interactions with herbivores or pathogens may provide an additional explanation for NDD patterns. Recent work suggests that specialist herbivores may play a role in maintaining the high local diversity of rainforests by preventing most plant species from becoming abundant (Comita et al. 2014, Forrister et al. 2019). Specifically, the probability of survival of individual plants significantly decreased near conspecific (Comita et al. 2014) and congeneric neighbors that are similar in chemical defensive traits and shared herbivore species (Forrister et al. 2019). Under this scenario, neighbors with different defense profiles do not share herbivores and can coexist, promoting high local diversity (Coley & Kursar 2014; Comita et al. 2014; Forrister et al. 2019; Salazar et al. 2016a,b). Because plants have many types of defenses that evolve independently from one another (Endara et al. 2017), with chemistry including hundreds or thousands of compounds that could also evolve independently (Coley & Kursar 2014, Sedio et al. 2021), defensive traits may provide a great number of niche dimensions and may be a key axis of ecological divergence.

Studies measuring traits from many plant genera and testing their effects on community assembly in tropical forests have consistently found similar patterns. Neighboring species are more different in defenses than would be expected from a random draw of the forest [e.g., *Bursera* (Becerra 1997, Becerra et al. 2009), *Inga* (Coley & Kursar 2014, Endara et al. 2022, Forrister et al. 2023, Kursar et al. 2009, Sedio et al. 2017), *Mimulus* (Wills et al. 2016), *Piper* (Richards et al. 2015, Salazar et al. 2016a,b), *Psychotria*, *Ocotea* and *Eugenia* (Sedio et al. 2017)]. This pattern holds true whether or not neighboring species are closely related (Endara et al. 2022, Kursar et al. 2009, Richards et al. 2015). In contrast, coexisting species share similar adaptations to the abiotic environment [e.g., traits associated with resource acquisition (Baraloto et al. 2012, Sedio et al. 2012, Wright 2002)]. These results, together with the effects of defensive chemistry and pests on NDD patterns, suggest that herbivores and pathogens may be shaping coexistence in plant communities and playing a critical role in the maintenance of diversity in tropical forests.

For temperate and boreal ecosystems, different mechanisms might be operating in determining coexistence patterns. Contrary to the patterns observed in tropical forests, neighbors in the most northern sites are highly similar in defensive chemistry (Sedio et al. 2021). These results are probably a reflection of the latitudinal gradient in biotic interactions (Schemske et al. 2009).

7. PLANT DEFENSES AND THE LATITUDINAL GRADIENT IN LOCAL TREE DIVERSITY

The latitudinal gradient in species diversity is one of the most prevalent patterns on Earth, but there remains considerable debate regarding its origin and the mechanisms that maintain this gradient (Mittelbach et al. 2007, Schemske et al. 2009). This pattern has tentatively been linked to a gradient in biotic interactions, with higher strength, specialization, and frequency of such interactions occurring toward the equator (Schemske et al. 2009). Our review of research developed in tropical forests and temperate and boreal ecosystems suggests that herbivores may play a very different role in processes regulating local diversity across this gradient. In this section, we briefly discuss how host shifts shape plant community assembly and how this relates to the maintenance of species diversity.

Although herbivore host choice is strongly determined by plant defenses, the extent of diet specialization in herbivores varies between tropical and higher latitude ecosystems (Coley & Barone 1996). Local estimates of diet breadth for leaf-chewing herbivores along a latitudinal gradient that included 13 sites in North, Central, and South America; Papua New Guinea; Japan; and central Europe indicate that host association is correlated with latitude, with a higher frequency of specialized herbivores toward tropical areas (Forrister et al. 2015). Generalism in host association is more prevalent toward the northern forests (Forrister et al. 2015). In addition, feeding trials with herbivores from temperate forests have shown they have a broader diet than tropical ones (Basset 1994), probably reflecting the higher palatability of temperate plants (Coley & Aide 1991).

As we discussed in Section 6, in tropical forests, the general pattern for community assembly is that cooccurring species are more divergent in chemical defenses than expected by chance (Becerra 2007; Endara et al. 2022; Kursar et al. 2009; Salazar et al. 2016a,b; Sedio et al. 2017; Vleminkx et al. 2018), with an average community similarity lower than 25% (Forrister et al. 2023). In these forests, because of the high levels of specialization, herbivores preferentially forage on subsets of species with similar defensive profiles (Becerra 2007; Endara et al. 2017, 2018; Sedio 2013). Thus, not sharing herbivores with neighbors, or being chemically rare, gives a species the advantage of reduced damage or enemy release (Forrister et al. 2019, Yguel et al. 2011). This in turn may promote the coexistence of species that are defensively divergent, providing a high-dimensionality niche space within which a very large number of cooccurring species might sort in ecological

time. Together, these results suggest that in tropical forests, herbivores are selecting for divergent defenses among neighbors, contributing to their high level of coexistence and enhancing diversity.

On the other hand, at higher latitudes such as boreal forests, cooccurring species do not show chemical overdispersion (Sedio et al. 2018). For seven forests in temperate and boreal ecosystems in North America, similarity in chemical expression decreases with latitude, with a mean community similarity ranging between 80% and 95% (Sedio et al. 2021). Boreal forests show the highest chemical similarity between neighbor species, with a similarity of approximately 95% (Sedio et al. 2021). This pattern may be related to the key role that mammalian herbivores play in these ecosystems. Most mammals are generalists because of their high resource requirements and their need to have a diverse diet in order to minimize the overingestion of specific secondary metabolites [e.g., the detoxification limitation hypothesis (Freeland & Janzen 1974, Hoy et al. 2019, Marsh et al. 2006)]. In boreal forests, generalist mammal herbivores show a negative frequency-dependent foraging behavior, selectively exploiting rare resources such as plants with rare defensive profiles (Basey 1999, Edenius et al. 2002, Hoy et al. 2019). In this scenario, and contrary to tropical forests, being rare is not beneficial for a plant species because it increases the probability of being consumed. Taken together, these results are consistent with herbivores exerting selective pressure for convergence in defenses among neighbors in boreal forests. This in turn may minimize the number of available niches, with a consequent reduction in forest diversity.

In summary, host shifts and diet breadth in herbivores play pivotal roles in community assembly. We suggest that community composition and diversity also might be shaped by herbivores exerting disparate selective pressures at different latitudes, resulting in divergence in defenses toward the equator and convergence at the poles. Thus, plant–herbivore interactions may be key to understanding the role that the biotic environment is playing in the latitudinal gradient in species diversity.

8. CONCLUSIONS AND FUTURE DIRECTIONS

The availability of new chemical analytical tools and phylogenetic comparative methods has let us advance long-standing hypotheses on the ecology and evolution of plant defenses. Thanks to the integration of ecological theory and modern chemistry, we have been able to tap into the great diversity of secondary metabolites at unprecedented scales and explore the causes and consequences of this great diversity. However, despite the power of new methodological approaches that can reveal the enormous diversity of compounds within a single leaf, determining the function or mode of action of any single compound remains a formidable challenge. Much more work is needed that explores the function of plant compounds and their adaptive significance. Molecular networks (**Figure 2**) can suggest related functions for compounds with similar structures. Field observations and bioassays with herbivores and pathogens can further shed light on particular interactions of mixtures containing unknown compounds.

Here, we argue that the interactions between plants and their herbivores might be driving defense evolution, as posited by Ehrlich & Raven (1964). However, different mechanisms might be operating at different taxonomic scales. For example, at the tips of the phylogeny, the mode and speed of evolution seem to be different from what has been traditionally accepted. We argue that changes to the regulation of biosynthetic pathways can lead to novel combinations of existing secondary metabolites, which would allow a faster and simpler evolution of defense profiles that could outpace the relatively short generation time of herbivore insects.

With respect to herbivores, our review suggests that host use is more compatible with a pattern of resource tracking rather than with a tight model of coevolution and cladogenesis. We argue that for host selection by herbivores, host defenses, or host resources (Brooks & McLennan

2002), including food availability, are more important than host phylogeny. Hence, in order to test hypotheses about the role of host range in herbivore ecology and evolution, characterizing host traits is essential.

The inclusion of metabolomics in community ecology has allowed us to collect trait data at large scales, permitting the study of the relative role of the biotic environment in community assembly and maintenance of local diversity. These studies have yielded several patterns, such as the significance of being rare in the community and the role of plant–herbivore interactions in coexistence. Nevertheless, these still await additional empirical testing.

Another challenge is understanding the role of plant–herbivore interactions in driving divergent evolution of plants. Specifically, almost 60 years after Ehrlich and Raven’s proposal, we have not yet identified how the evolution of defenses is connected to the emergence of reproductive barriers, assortative mating, or any other mechanism that leads to lineage divergence. We propose one possible scenario for herbivore-driven divergent evolution based on the fact that herbivores can create a moving target for defense adaptations in a way that the abiotic environment does not (Dobzhansky 1950). For plant populations that experience partial barriers to gene flow, selection drives divergence in defensive traits more rapidly than in resource acquisition traits. If the populations become rejoined, the greater divergence in defensive traits might lead to hybrids with lower fitness. If so, this could set in action the initial conditions necessary for the creation of reproductive barriers and ultimately speciation.

The promise of metabolomics, coupled with genomics and transcriptomics, opens many avenues for pursuing the role of plant defenses against herbivores in promoting speciation and the extraordinary diversity of tropical forests.

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