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IDEA AND PERSPECTIVE

The many dimensions of phytochemical diversity: linking theory to practice

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Abstract

Research on the ecological and evolutionary roles of phytochemicals has recently progressed from studying single compounds to examining chemical diversity itself. A key conceptual advance enabling this progression is the use of species diversity metrics for quantifying phytochemical diversity. In this perspective, we extend the theory developed for species diversity to further our understanding of what exactly phytochemical diversity is and how its many dimensions impact ecological and evolutionary processes. First, we discuss the major dimensions of phytochemical diversity – richness, evenness, functional diversity, and alpha, gamma and beta diversity. We describe their potential independent roles in biotic interactions and the practical challenges associated with their analysis. Second, we re-analyse the published and unpublished datasets to reveal that the phytochemical diversity experienced by an organism (or observed by a researcher) depends strongly on the scale of the interaction and the total amount of phytochemicals involved. We argue that we must account for these frames of reference to meaningfully understand diversity. Moving from a general notion of phytochemical diversity as a single measure to a precise definition of its multidimensional and multiscale nature yields overlooked testable predictions that will facilitate novel insights about the evolutionary ecology of plant biotic interactions.

Keywords

Beta diversity, chemical ecology, functional diversity, Phytochemical diversity, plant secondary metabolism, plant–insect interactions, scale, species interactions, trait variability.

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INTRODUCTION

One of the most astounding features of the natural world is the enormous diversity of chemical compounds produced by plants. Over a century ago, biologists noted that different plant families and species produced unique suites of phytochemicals (defined in Box 1) and pondered their ecological and evolutionary roles (Abbott 1887; Stahl 1888; LoPresti & Weber 2016). Since then, researchers have demonstrated the significance of phytochemistry in plant interactions with herbivores, microbes, competitors, pollinators and seed dispersers (Iason *et al*. 2012). What has eluded the field until recently is the significance of chemical diversity itself. At least 200 000 phytochemicals have been described (Kessler & Kalske 2018), and many orders of magnitude may exist. While some plants produce just a few major phytochemicals, many produce thousands of unique compounds, often with apparently redundant functions (Tasin *et al*. 2007). Diversity is further amplified by plasticity; phytochemistry varies through ontogeny and phenology (Wiggins *et al*. 2016; Barton & Boege 2017), in response to abiotic conditions and biotic interactions (Coley *et al*. 1985; Kessler & Baldwin 2002; Dicke & Baldwin 2010), and spatially among branches, organs and even bite-sized pieces of tissue within organs (Shelton 2005; Herrera 2009). All of this diversity means that organisms that interact with plants face astounding chemical complexity – hundreds of compounds in single encounters and potentially tens of thousands of compounds across lifetimes.

A surge of recent research, fuelled by metabolomics and other modern approaches in analytical chemistry (Hartley *et al*. 2012; Sedio 2017; Dyer *et al*. 2018; Richards *et al*. 2018), has finally begun to address the ecological and evolutionary roles of phytochemical diversity itself. This new line of research has been enabled by a key conceptual advance – the use of concepts and metrics from the species diversity literature (Iason *et al*. 2005; Dyer *et al*. 2014; Moore *et al*. 2014; Marion *et al*. 2015). The species diversity literature is replete with theory on the calculation and biological interpretation of metrics that assess the multiple dimensions and scales of diversity, including richness, evenness, diversity indices (e.g. Shannon), functional diversity, and alpha, gamma and beta diversity (Magurran & McGill 2011). By analogy between communities of biological species and mixtures of phytochemicals, these metrics have been used to quantify the variation in phytochemical diversity across plant samples and assess how that variation is linked to key ecological and evolutionary

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variables (e.g. Kursar *et al*. 2009; Richards *et al*. 2015; Bustos-Segura *et al*. 2017; Salazar *et al*. 2018). Complementing this empirical work, several recent reviews have greatly advanced our conceptual understanding of phytochemical diversity by summarising its evolutionary causes, biochemical origins and ecological consequences (Dyer *et al*. 2014; Moore *et al*. 2014; Schuman *et al*. 2016; Dyer *et al*. 2018; Kessler & Kalske 2018; Lämke & Unsicker 2018). However, we still lack a unified definition of what exactly phytochemical diversity is and how its many dimensions can be quantified and related to biological hypotheses.

In this perspective, we argue that our efforts to understand phytochemical diversity will be greatly advanced by more precise links between our ecological and evolutionary hypotheses and the approaches we use to measure phytochemical diversity (Fig. 1). To help establish these links, we first provide a holistic definition of phytochemical diversity as a concept (Box 1), outline its many dimensions (Box 1, Fig. 2) and relate each dimension to major hypotheses. This section also discusses the challenges in the application of metrics from the species diversity literature to phytochemistry. We hope this discussion will help researchers carefully consider which of the many dimensions and scales of phytochemical diversity relate to the particular ecological and evolutionary processes they are addressing. In the second section of the paper, we show the surprising ways that the perception of phytochemical diversity, both by chemical ecologists and organisms interacting with plants, varies depending on the frame of reference from which it is observed or experienced. A fungal endophyte growing inside a plant cell will not experience the same phytochemical diversity as a browsing ungulate because these two organisms interact with very different total amounts of plant material and total abundances of metabolites. We argue that explicit consideration of the frames of reference defined by sampling methods is essential in any study of phytochemical diversity.

Although the complexity and variability of phytochemical traits can be daunting to describe, meeting these challenges can help us to answer some of the most fundamental questions in evolutionary ecology (Box 2). Phytochemistry is essential to shaping plant interactions with other organisms and therefore the structures of entire communities and ecosystems. With new advances in chemistry and bioinformatics, we are poised to embrace phytochemical diversity as a key biological feature that varies across plant individuals, genotypes, species and communities. This variation may help explain ecological and evolutionary processes as diverse as herbivore performance, pollinator attraction, pathogen spread, and adaptation and diversification of plants and their consumers. We hope that the definitions and concepts we outline will help guide the study of phytochemical diversity, from study design through analysis and interpretation, and allow researchers to test long-standing hypotheses about the ecology and evolution of phytochemical diversity.

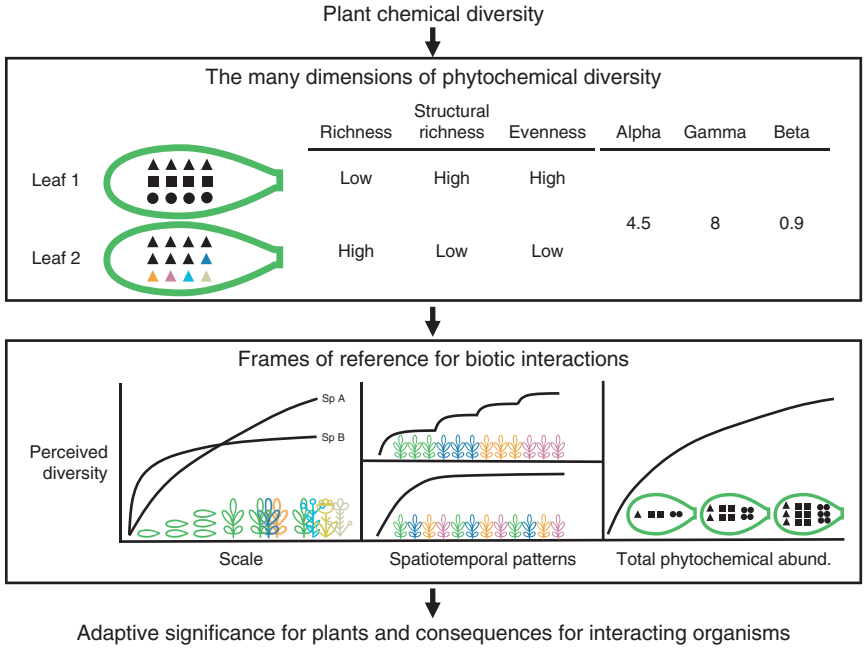

Figure 1 The many dimensions of phytochemical diversity and frames of reference for biotic interactions influence the role of phytochemical diversity in biotic interactions and the adaptive significance for plants. Phytochemical diversity is multidimensional – with dimensions including richness, structural richness, evenness, alpha, gamma and beta diversity, and others – and each of these dimensions can vary independently or in a correlated fashion with independent or interrelated causes and consequences. The phytochemical diversity that is perceived by organisms that interact with plants (and researchers measuring phytochemical diversity) varies with frames of reference, including overall scale, spatiotemporal patterns in how plants are encountered, and the total concentration of phytochemicals experienced. Each of these dimensions and frames of reference shapes the ecology and evolution of phytochemical diversity, and how we study it. Shapes represent compound structural classes (e.g. terpenoids or alkaloids), and their colours represent different compounds within classes. Plants of different colours represent plants with different chemical phenotypes.

Box 1. Defining multiple dimensions of phytochemical diversity

PHYTOCHEMICALS

We use the term phytochemicals to describe plant-derived compounds which are thought to function primarily in interactions with the biotic and/or abiotic environment rather than in basic metabolic processes. These compounds have been referred to as secondary or specialised metabolites, though the primary–secondary dichotomy is ambiguous, and many compounds are not functionally specialised.

PHYTOCHEMICAL DIVERSITY

We broadly define phytochemical diversity as a multidimensional concept that encompasses the complexity of phytochemical composition and the variation in composition across spatial and temporal scales (Fig. 2). Our definition is broader than previous definitions, which have tended to focus primarily on the complexity of composition within a single sample or taxon (Richards *et al.* 2015; Dyer 2018). We argue that variability in phytochemistry is a key facet of diversity that should be discussed together with chemical complexity to fully understand the ecology and evolution of phytochemical diversity. As we discuss in detail in the Frames of Reference section, fully describing diversity requires an understanding of how complexity accumulates with the scale of observation, and any single diversity metric is an abstraction from diversity in its fullest form. Abstractions are useful because they help us understand and summarise variation, but they also obscure biologically important information and require careful interpretation. Each metric we use is a proxy for a unique aspect of diversity; we need to carefully choose these metrics and match them to our research questions.

RICHNESS

Phytochemical richness is the count of unique compounds present in a plant sample or group of samples. Richness can be defined at many scales (e.g. organ, individual, genotype, species or community).

EVENNESS

Phytochemical evenness describes the distribution of total compound production among all the compounds within a sample. A sample where all compounds have equal concentration is perfectly even, whereas a sample composed of one abundant compound and multiple low-concentration compounds has low evenness.

DIVERSITY INDICES

Diversity indices combine the richness and evenness components of diversity into a single metric, weighting the contribution of each compound such that compounds with lower abundances contribute less to the overall estimate of diversity. There are many such indices in the species diversity literature (e.g. Shannon diversity or Simpson diversity).

FUNCTIONAL DIVERSITY

Functional diversity of a phytochemical mixture describes the range of biological activities exhibited by the compounds present.

STRUCTURAL DIVERSITY

Structural diversity describes the complexity of the molecular structures present in a phytochemical mixture. This is often used as a proxy for functional diversity, but does not directly predict function (see main text).

ALPHA, GAMMA AND BETA DIVERSITY

Alpha, gamma and beta diversity are theoretical constructs that describe the hierarchical, multiscale nature of diversity. Phytochemical alpha diversity is the average diversity at the scale of a single sampling unit (i.e. 'local' diversity). Gamma diversity is the diversity at the scale of the statistical population that contains all plant sampling units (i.e. 'regional' diversity). The key difference is that alpha is calculated by averaging diversity across samples, whereas gamma diversity is calculated by pooling samples and counting the total number of unique compounds. Finally, the variation in diversity as we move between the alpha and gamma scales is beta diversity – the compositional turnover among plant units or samples in space and/or time. Chemical beta diversity has been thought of in the context of chemotypic diversity – the discrete number of multivariate chemical phenotypes

Box 1. Continued

in plant population – but beta diversity can also be considered more generally as the continuous change or turnover in chemical diversity and/or composition through space and time. The relationships among alpha, beta and gamma diversity are best understood by plotting the scale–diversity relationship, which shows the cumulative increase in total diversity as a function of the number of samples examined. Previous definitions of alpha, beta and gamma have tied these concepts to particular discrete scales of plant organisation. Our definitions, however, are more general because they are derived from the diversity–scale relationship, and thus can be applied at smaller or larger scales depending on the questions (as they have been in the species diversity literature). For example, alpha may describe average diversity at the scale of a single leaf, and gamma may describe diversity at the whole plant scale. Or alpha may describe the average diversity of a species within a community, and gamma may describe diversity of the entire community. Deciding the scale of alpha and gamma is a flexible biological question that should be based on an understanding of the scale of the biotic interaction or research question of interest.

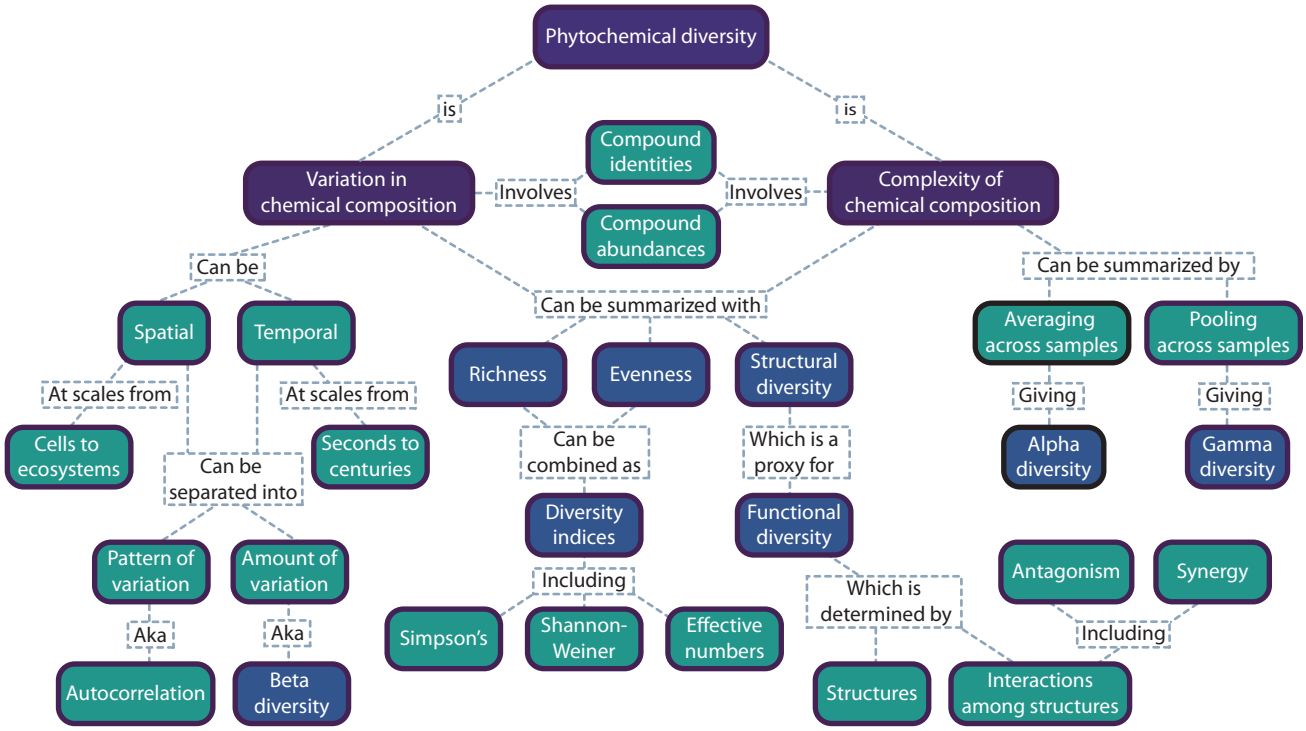

Figure 2 A concept map linking the many dimensions of phytochemical diversity. Our definition of phytochemical diversity (purple boxes) includes the complexity of chemical composition and the variation in composition in space and time. Although no single metric can summarise the complexity of phytochemical diversity, many concepts and metrics from the species diversity literature (blue boxes) can be applied to phytochemical data.

DIVERSITY AS A MULTIDIMENSIONAL CONCEPT

Phytochemical diversity is inherently multidimensional (Box 1, Fig. 2). In this section, we review metrics that describe key dimensions of phytochemical diversity and what is known and unknown about the importance of each in ecology and evolution. We hope that this section will stimulate: (1) careful consideration of which dimensions are relevant for specific biological questions and (2) studies that explore multiple dimensions simultaneously to better understand their potentially independent or interrelated causes and consequences.

Richness

Importance for plant interactions

Richness is the simplest metric of phytochemical diversity, but also one of the most informative diversity. From a plant evolutionary perspective, compound richness summarises the complexity of a plant's biosynthetic pathways. Understanding the complexity of this machinery and how it varies is central for evolutionary hypotheses about the causes of phytochemical diversity, such as the screening hypothesis and the interaction diversity hypothesis (Box 2). From the perspective of consumers, richness, if measured at the appropriate scale (see

Box 2. Key hypotheses about the causes and consequences of phytochemical diversity

Many hypotheses have been proposed to explain the evolutionary causes and ecological consequences of phytochemical diversity. We list several of the major hypotheses here to give readers context for our discussion in the main text of how these hypotheses can be tested using empirical data for specific facets of phytochemical diversity. These hypotheses are not mutually exclusive, and address different phytochemical patterns at different scales and levels of organisation.

COEVOLUTIONARY ARMS RACE HYPOTHESIS

This hypothesis was originally proposed by Ehrlich & Raven (1964) and proposes that plants have accumulated phytochemical diversity in a stepwise evolutionary process. Plants evolve novel defences, followed by an adaptive radiation into enemy-free space. A plant's enemies, in turn, evolve counter-adaptations and radiate in parallel to their host plants. This process is thought to have occurred repeatedly, leading to a diversity of plant defences and species diversification of both plants and their enemies.

SCREENING HYPOTHESIS

The screening hypothesis proposes that plants that develop biosynthetic pathways with more diverse products have a higher probability of producing biologically active, fitness-enhancing compounds. Thus plant enemies, which select for active compounds, indirectly select for diversified biosynthetic pathways with promiscuous biosynthetic machinery. Key to this hypothesis is that fitness-enhancing, biologically active phytochemicals are rare among all possible chemical structures, and most phytochemicals have no direct adaptive benefits (Jones & Firn 1991; Carmona *et al*. 2011).

SYNERGY HYPOTHESIS

This hypothesis (reviewed in Richards *et al*. 2016) proposes that the biological activities of compounds, and their fitness-enhancing benefits, often increase in a non-additive manner with the presence of multiple compounds of the same or different structural classes. This hypothesis could provide an evolutionary explanation for why plants maintain phytochemical diversity rather than producing just a few major compounds.

INTERACTION DIVERSITY HYPOTHESIS

This hypothesis proposes that phytochemical diversity arises evolutionarily not from any single biotic interaction, but instead from the diverse selective pressures imposed by the multitude of biotic interactions among plants and their associated community of herbivores, pathogens, pollinators and other mutualists, each of which may exert only a small selective effect on their plant-partner. Although not named, this hypothesis has been inherent in the literature on plant–herbivore interactions for decades, and was referred to as the 'common sense scenario' by Berenbaum & Zangerl (1996). We use the term 'interaction diversity hypothesis' after the review by Kessler & Kalske (2018) to distinguish this hypothesis from other evolutionary processes generating phytochemical diversity.

SLOWED ADAPTATION HYPOTHESIS

This hypothesis suggests that phytochemical diversity benefits plants by increasing the number of adaptations that herbivores need to surmount defences, thereby slowing overall adaptation. Although the importance of rapid pest adaptation to single toxins has been long recognised in agriculture (e.g. Tabashnik *et al*. 2013), these ideas have been little tested in evolutionary ecology (but see Palmer-Young *et al*. 2017).

MOVING TARGET HYPOTHESES

The original moving target hypothesis, proposed by Adler & Karban (1994), posited that induced plant responses to herbivory were phenotypic change merely for the sake of change, which could be physiologically difficult for herbivores. We use the term 'moving target hypotheses' to refer to a broad grouping of hypotheses united by the idea that sources of within-individual phytochemical beta diversity could decrease herbivore preference, performance or adaptation through a variety of mechanisms (Schultz 1983; Karban *et al*. 1997; Ruel & Ayers 1999; Wetzel & Thaler 2016; Wetzel *et al*. 2016; Pearse *et al*. 2018).

Box 2. Continued

PLANT COMMUNITY VARIABILITY HYPOTHESES

We use the term 'plant community variability hypotheses' to refer to a group of hypotheses that suggest fitness is increased when a plant is chemically divergent from neighbours in a community (i.e. the community has higher beta diversity; Salazar *et al*. 2016b; Massad *et al*. 2017). For example, the semiochemical diversity hypothesis (reviewed in Randlkofer et al. 2010) suggests that herbivore host location is disrupted in complex chemical environments that include host and non-host species. Related ideas include the associational effects hypothesis (Underwood *et al*. 2014), non-additive population dynamics hypothesis (Underwood 2009; Wetzel *et al*. 2016), gut acclimation hypothesis (Wetzel & Thaler 2016) and the classic resource concentration hypothesis (Root 1973).

Frames of Reference), can summarise the number of unique compounds an organism will face in an encounter with a plant.

Practical application and examples

Estimating the richness requires effective chromatographic separation of compounds and/or deconvolution methods to accurately estimate the number of compounds present. Certainly, it can be useful to also apply richness to subsets of compounds (e.g. alkaloid richness). In principle, richness (as well as other metrics) can be applied to describe the diversity of molecular 'features' (i.e. signals representing paired retention times and m/z ratios) in NMR and MS-based metabolomics (Liu & Locasale 2017); however, these do not always reflect unique compound identities. Increasingly, advances in bioinformatics are improving the linkage between features and unique compounds (Olivon et al 2017), which should improve the applicability of richness and other metrics to these types of data. Although estimating the number of compounds present is not without challenges, richness does not rely on the compound identifications, structural descriptors or quantifications, making richness relatively straightforward to assess. Consequently, it is pervasively used by studies of phytochemical diversity. Although there are many unanswered questions regarding the role of phytochemical richness *per se* in ecology and evolution, we know that richness is associated with important ecological and evolutionary variables. For example, higher chemical richness of phytochemicals in the hyper-diverse genus *Protium* is associated with lower herbivore species diversity (Salazar *et al*. 2018).

Key limitations and challenges

There are several important limitations to richness as a summary of phytochemical diversity. First, because our ability to detect compounds depends on their abundances in a sample, richness cannot be understood meaningfully without reference to sampling methods (see Frames of Reference). Second, richness equally weights high-concentration and low-concentration compounds (some of which may be ecologically irrelevant). Third, many low-concentration compounds may be biosynthetic precursors or breakdown products that lack bioactivity (though others can be biologically relevant). If a large number of compounds are not bioactive or present below their bioactivity thresholds, richness would represent a phenotypic axis that is not important in many ecological contexts – even if the evolution of that phenotype is worthy of study. Fourth, richness must be interpreted in relation to the methods of a study. No methods, even untargeted metabolomics, capture all the phytochemistry in a plant because extraction and analytical methods inevitably filter compounds. Richness estimates should therefore not be mistaken for absolute diversity. This observation applies equally to all of the dimensions and metrics described below.

Evenness

Importance for plant interactions

Evenness is a key dimension of diversity, yet virtually nothing has been written about how phytochemical evenness might relate to species interactions. From an evolutionary perspective, patterns of evenness indicate how plants allocate their biosynthetic effort among the compounds they produce, within and among biosynthetic pathways, and can provide perspective on the adaptive significance of phytochemical diversity. For example, if phytochemical diversity were explained primarily by the screening hypothesis (Box 2), then we hypothesise plants should generally have very low-phytochemical evenness, where a few biologically active compounds under selection occur at high concentration and numerous biologically inactive compounds occur at low abundances. Alternatively, if most compounds contribute to plant fitness (e.g. synergy and interaction diversity hypotheses; Box 2), then plants should produce most compounds at biologically significant concentrations, resulting in relatively higher evenness than would be predicted by the screening hypothesis. Levels of evenness may also depend on compound effectiveness, biosynthetic correlations, interaction intensity and other factors. Regardless of the drivers, it is clear that plants vary in evenness at multiple scales—some with one major and many minor compounds and others with many compounds in relatively equal proportions.

From the perspective of an interacting consumer, it is unclear whether evenness *per se* matters. On the one hand, high evenness may negatively impact consumers because it forces simultaneous processing of many compounds, reducing the efficiency of detoxification mechanisms and potentially slowing counter-adaptations. On the other hand, high evenness may benefit herbivores by diluting any single compound,

allowing herbivores to consume more tissue before experiencing negative effects (Freeland & Janzen 1974; Bernays *et al*. 1994; Marsh *et al*. 2006). In many cases, it may not be evenness *per se* that matters to consumers but the identities of the compounds present. A plant with low evenness could be toxic if the most abundant compounds were potent – or it could be palatable if the most abundant compounds were benign.

Practical application and examples
Although evenness is an implicit component of commonly used diversity indices, we know of no studies that have explicitly explored the role of phytochemical evenness. There are many metrics of evenness developed for species diversity (Maurer & McGill 2011). The most common are Shannon and Simpson evenness, which are the common Shannon and Simpson diversity metrics (discussed below) with richness divided out. Researchers should be aware that, contrary to popular belief, these metrics are not mathematically independent of richness, and evenness is best understood as a relative measure within a certain level of richness (Jost 2010).

Key limitations and challenges
Although we believe evenness deserves significant attention, it is challenging to study. First, quantifying evenness requires estimation of individual compound abundances and total phytochemical abundance, which could be estimated gravimetrically or based on peak areas. This is true even if the goal was only to make relative comparisons of evenness within a study because compounds can vary immensely in analytical responses. Without standards with which to correct abundances, we would incorrectly rank samples in their relative evenness. Thus, researchers should not make conclusions based on evenness (or diversity metrics that rely on evenness) without rigorous estimates of individual compound abundances. Currently, this is largely infeasible for metabolomics-scale studies. Furthermore, even where abundances can be calculated, it is unclear what measure of abundance is the most relevant for calculating evenness. Abundances are often calculated on a mass basis, but one alternative is to calculate evenness on a molar basis. Molar calculations would be more appropriate in cases where biological activity is more dependent on the number of molecules than total mass. However, in some cases, higher mass molecules could have multiple functional groups with multiple bioactivities, making mass more indicative of bioactivity. One thing is certain; bioactivity varies considerably among compounds, and even a mixture with perfectly even mass or molar abundances could be highly uneven in terms of bioactivity. Thus, in a model experimental system, a third approach to calculating evenness could be to measure dose–response relationships for each compound in a mixture, calculate standardised measures of bioactivity (e.g. ED50s), and determine the mixture's evenness in terms of relative bioactivities. Considering the uncertainties described above, we argue that experimental approaches in model systems will be critical to disentangling the importance of phytochemical evenness. Evenness is a theoretically and practically challenging subject, but it is such a fundamental dimension of diversity that we need to continue exploring it in order to advance our understanding of phytochemical diversity.

Diversity indices

Importance for plant interactions
Diversity indices combine richness and evenness into a single metric (Box 1). The strength of these indices is that they distil some of the multivariate complexity of diversity into one number that can be used in further analyses. Moreover, diversity indices excel as a representation of how most biologists would subjectively describe diversity.

Practical application and examples
Diversity indices have been used in an increasing number of phytochemical studies and results suggest that they can provide an ecologically relevant measure of phytochemical diversity. For example, higher Simpson's diversity of ^{1}H-NMR features across *Piper* species was associated with higher Simpson's diversity of herbivore species feeding on that host (Richards *et al*. 2015). In another example, the Shannon diversity of monoterpenes in Scots pine tree (*Pinus sylvestris*) was positively associated with increased diversity of ground vegetation beneath individual trees (Iason *et al*. 2005). In choosing a diversity index, researchers should consider that different indices differ in how they weight high- and low-abundance compounds, but the lack of information on the relative importance of phytochemical richness and evenness makes weighting decisions currently arbitrary. An elegant solution to this uncertainty was described by Marion *et al*. (2015), who explored how diversity metrics vary with 'diversity order' (Hill 1973; Jost 2006). Diversity order is a parameter that allows researchers to adjust the sensitivity of diversity metrics to low-concentration compounds. Examining diversity across different orders stems the loss of information associated with combining richness and evenness into one metric and can reveal how diversity in abundant and rare compounds varies among plant species or other factors (Fig. 3, for statistical methods see Appendix S1 in Supporting Information). Increased application of these methods would increase the nuance with which we view diversity and help us link our metrics to what matters for biotic interactions.

Key limitations and challenges
Although we do not want to discourage researchers from using diversity indices, there are three key issues with their use. First, combining richness and evenness into one metric inevitably conceals independent variation in the two (Jost 2007). A lack of a relationship between a biological variable and a diversity metric could mean that neither richness nor evenness affect that variable, or they could both affect it in opposite directions. Second, combining richness and evenness implies that these two different dimensions of diversity are to some extent interchangeable. If, for example, we conclude that plant fitness increases with a diversity index, we imply that plants could achieve higher fitness by increasing richness, evenness or both, which may not be correct. Third, as discussed above, there are considerable challenges associated with quantifying evenness outside the model plant systems. Thus, the use of diversity indices should be complemented with independent examinations of the roles of richness, evenness and their potential interactions.

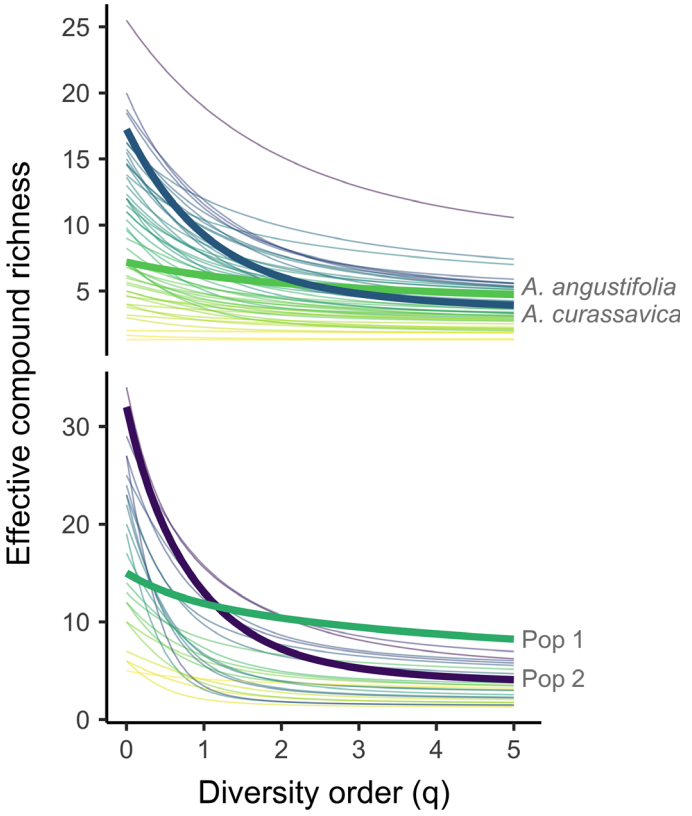

Figure 3 Diversity profile plots showing the effective compound richness for 52 species of milkweed (*Asclepias*) with one curve per species (top) (Rasmann & Agrawal 2011) and for 24 populations of *Lithophragma affine* with one curve per population (bottom) (Friberg et al. 2019). Diversity order (q) is a parameter representing the relative contribution of high- and low-concentration compounds, from equal weighting at q = 0 (which is equivalent to compound richness) to nearly all weight on high-concentration compounds at q = 5 (Marion et al. 2015). Order q = 1 is the exponential of Shannon diversity and q = 2 is inverse Simpson diversity. *A. curassavica* is more diverse than *A. angustifolia* in terms of compound richness (low q) but less diverse in terms of richness of abundant compounds. This is visible in that the lines cross as the weight on low-concentration compounds is decreased (q → 5). The same is true for populations 1 and 2 of *L. affine*. Each species or population is coloured by its diversity at q = 0 (compound richness), with darker colours having higher richness.

Functional and structural diversity

Importance for plant interactions

Functional diversity is what truly matters for ecological processes. In studies of the effects of phytochemical diversity on species interactions, all other metrics (richness, evenness, diversity indices) are essentially proxies for functional diversity. While complete measures of functional diversity are unobtainable – they would require evaluation of all possible biological activities in the full range of plant processes and interactions – functional diversity is often inferred based on the structural diversity of compounds (but see limitations below). The proximate biosynthetic causes of structural diversity are elegantly summarised by Kessler & Kalske (2018) and will not be reviewed in detail here. However, it is critical to consider that the phytochemical phenotype, on which selection acts, is the integrated result of a vast number of genes coding for biosynthetic and modifying enzymes that interact in complex pathways, often serve multiple functions, and may be differentially expressed temporally or across plant tissues to generate functional diversity in ecological interactions.

Practical application and examples

Where structures are known, there are several well-established metrics for assessing the structural dissimilarity among compounds (Cao *et al.* 2008a; Cao *et al.* 2008b; Backman *et al.* 2011). Increasingly, functional properties of known structures can also be inferred using high-throughput bioactivity screening databases (Wang *et al.* 2009) and *in silico* estimates of structure–function relationships (Terstappen & Reggiani 2001; Raies & Bajic 2016). However, the reality is that the vast majority of structures of phytochemicals are undescribed, making these metrics inapplicable. Studies in ecology are just beginning to calculate the structural similarity metrics for complex mixtures of undescribed compounds using either raw MS-MS fragmentation data (Sedio 2017; Sedio *et al.* 2018a) or NMR data (Richards *et al.* 2015; Richards *et al.* 2018). These methods use spectral features to generate measures of structural similarity among compounds, which can then be visualised using molecular networks (e.g. Sedio *et al.* 2017). These approaches are rapidly advancing the field by contributing much-needed information on functional mechanisms that underlie the ecology and evolution of phytochemical diversity. Future work in this area should focus on assessing which functional features of individual phytochemicals or phytochemical mixtures shape ecology and evolution, and aim to integrate across the multiple dimensions of functional diversity, for example, functional richness, functional evenness and functional divergence, as has been developed in the assessment of other phenotypic traits (Villéger *et al.* 2008).

Key limitations and challenges

The main challenge associated with structural diversity is that it is not a direct measure of functional diversity. We caution that there are many examples of small changes in structure that led to enormous differences in function (Geithe & Krautwurst 2015) as well as very different structures with similar functions (Dauplais *et al.* 1997). Furthermore, functional diversity of a mixture depends not only on the bioactivity of each structure, but also on the potential interactions among structures (Richards *et al.* 2016). Thus, uses of structural diversity as a proxy for functional diversity should be interpreted carefully.

Beta diversity

Importance for plant interactions

Beta diversity is an essential component of phytochemical diversity for plant interactions because it represents how organisms experience phytochemistry – as variation in space and time (Wetzel & Thaler 2016; Salazar *et al.* 2016a; Massad *et al.* 2017; Pearse *et al.* 2018) (Fig. 2). It is the dimension of diversity that underlies the moving target hypothesis and the plant community variability hypothesis (Box 2). Indeed,

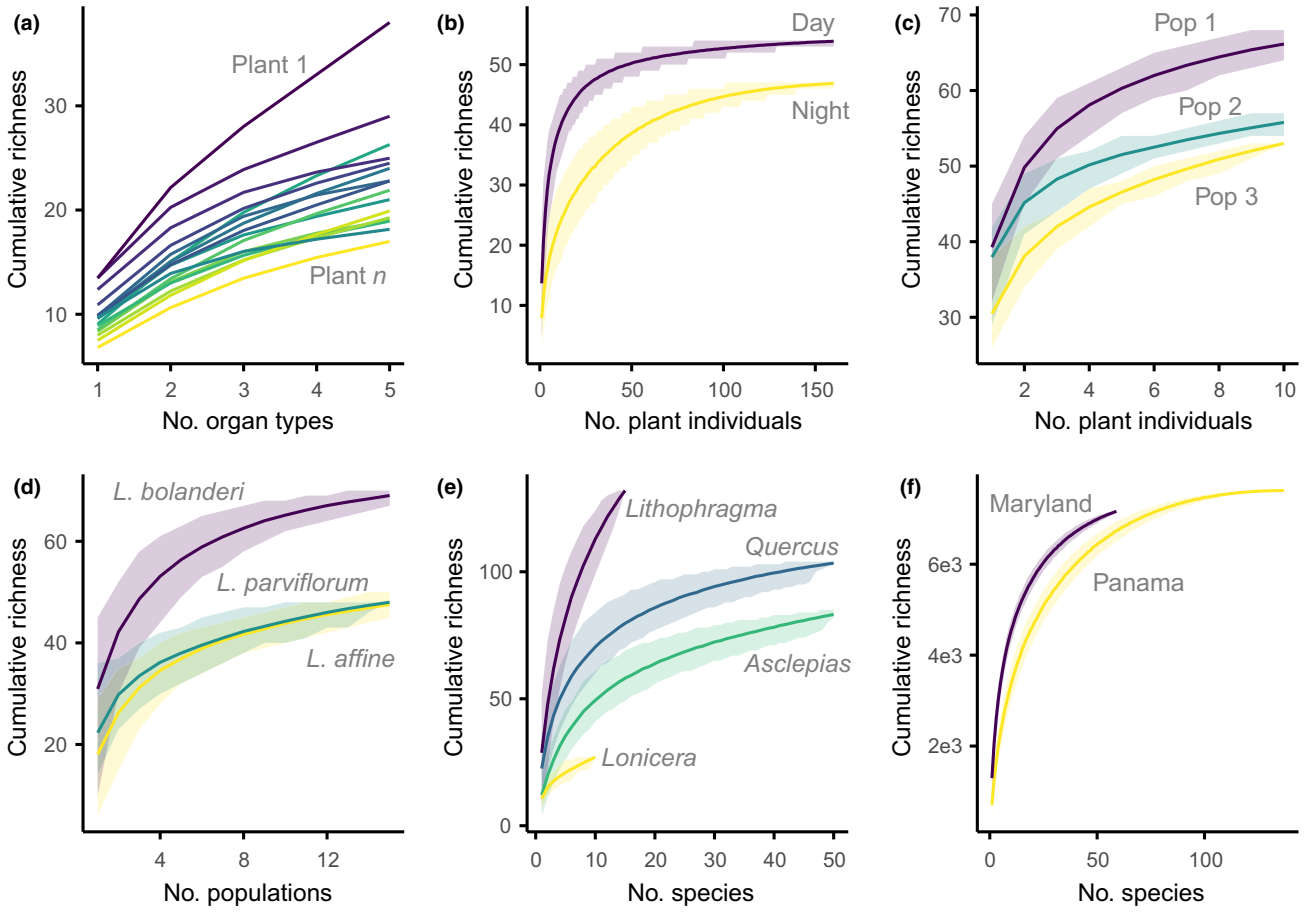

Figure 4 Chemical diversity–scale relationships are positive across multiple levels of biological organisation for diverse groups of plants. Lines are bootstrapped means, and shading indicates 80% probability regions. Darker colours indicate individuals or groups with higher alpha diversity (mean richness in one sample). (a) Cumulative richness of purported defensive amide compounds as a function of number of organ types (e.g. leaves, fruits, roots) for 16 plant individuals of *Piper reticulatum* (Piperaceae) (Whitehead et al. 2013). (b) Cumulative richness of floral volatiles in *Gymnadenia conopsea* (Orchidaceae) as a function of the number of plant individuals sampled during the day or night within one population (Chapurlat et al. 2018). (c) Cumulative richness of leaf volatiles as a function of the number of plant individuals examined in three populations of *Artemisia tridentata* (Asteraceae) (Karban et al. 2016). (d) Cumulative richness of floral volatiles as a function of number of populations examined for three species in *Lithophragma* (Saxifragaceae) (Friberg et al. 2019). (e) Cumulative compound richness as a function of the number of species examined within four plant genera: iridoid glycosides in *Lonicera* (Caprifoliaceae) (Lieurance et al. 2015), cardenolides in *Asclepias* (Apocynaceae) (Rasmann & Agrawal 2011), leaf volatiles in *Quercus* (Fagaceae) (Pearse et al. 2013) and floral volatiles in *Lithophragma* (Saxifragaceae) (Friberg et al. 2019). (f) Cumulative richness of the leaf metabolome as a function of number of tree species examined at Barro Colorado Island, Panama or the Smithsonian Environmental Research Center, Maryland, USA (Sedio et al. 2018b).

evidence is accumulating that spatial and temporal variation in plant chemistry has important ecological effects on biotic interactions (Herrera 2009; Bustos-Segura *et al.* 2017; Pearse *et al.* 2018).

Practical application and examples

There are a litany of metrics and multivariate statistical methods that can be used to summarise beta diversity. We direct readers to excellent sources in the species diversity literature for full discussion of these applications (e.g. Anderson *et al.* 2010; Jost *et al.* 2011; Magurran 2011). Broadly, beta diversity can refer either to directional turnover across a defined spatial, temporal or environmental gradient or to overall variability in composition across samples (Anderson *et al.* 2010). Both turnover and variation could be of great interest in the study of phytochemical diversity, but measures of mean variability across samples have been more commonly employed. These include classic metrics, such as Whittaker's (1960) original measure of beta = gamma/alpha, as well as multivariate methods that are based on similarities or dissimilarities among samples, such as the Jaccard dissimilarity index (*d*), multivariate ordination (e.g. non-metric multidimensional scaling) or Rao's quadratic entropy index. For example, Salazar *et al.* (2016a) used Rao's index to measure the chemical variability (i.e. beta diversity) across *Piper* species in different communities in a diverse tropical forest. Increasing phytochemical beta diversity in a community was strongly associated with decreased total levels of herbivore damage (Salazar *et al.* 2016a). Although we know of no published examples, beta-diversity metrics can also describe temporal variation. For example, we applied Jaccard dissimilarity (*d*) to data from Trowbridge *et al.* (2019) to quantify seasonal variation in

volatile terpenes in piñon pine (*Pinus edulis*). We calculated mean dissimilarity across sequential samples (up to nine per season) for 36 individuals and found that within-individual temporal beta diversity in terpene emission was substantial (mean $d = 0.57$; range $d = 0.41$–0.71), nearly equivalent to beta diversity across individuals within a single period (mean $d = 0.61$; range $d = 0.56$–0.72).

Key limitations and challenges

The complexity of beta diversity is a challenge. Beta diversity has diverse biological causes and consequences, and researchers need to carefully match the process under study with appropriate types and scales of beta diversity. Often, studying beta diversity will require large number of samples collected at multiple scales. For example, the plant variability–herbivore movement hypothesis posits that variability forces herbivores to move more within plants to find suitable patches of plant tissue, and movement is expensive and dangerous (Schultz 1983; Wetzel & Thaler 2016). Testing this hypothesis might require measuring chemistry in numerous samples per plant as well as numerous plants (to examine variation in beta diversity). Measuring temporal beta diversity may be even more difficult because repeated destructive sampling from the same plant could induce phytochemical responses. In addition to these practical challenges, work is needed to determine which metrics of beta diversity, developed over decades in the context of species diversity, will be the most relevant for describing phytochemistry.

FRAMES OF REFERENCE: DIVERSITY NEEDS A DENOMINATOR

In this section, we explore the sweeping implications of a theoretical truth that has been mostly overlooked in the literature on phytochemical diversity: the diversity that an organism encounters (or a researcher observes) will depend strongly on the amount of material that is experienced (or sampled). In two subsections below, we explore how the experience or observation of phytochemical diversity depends on two key phenomena. The first is the scale-dependence of phytochemical diversity, whereby the total diversity encountered increases with the scale of observation. The second is the abundance-dependence of phytochemical diversity, whereby the total diversity encountered increases with the total abundance of phytochemicals. In each subsection, we first illustrate the ubiquity of these relationships using empirical data, then explore how these relationships should inform our sampling designs, and finally show how an understanding of these phenomena leads to novel hypotheses and insight about the role of phytochemical diversity in ecology and evolution.

The scale-dependence of phytochemical diversity

Observed chemical diversity increases with scale

Scale is critically important to consider in studies of phytochemical diversity because biotic interactions involving plants occur across a huge range of scales, from the cellular (e.g. microbes) to the community (e.g. grazing mammals). In the species diversity literature, it is well-appreciated that the more area one searches, the greater species richness one will find on average. Probability theory indicates that the scale-dependence of diversity should also apply to phytochemical diversity – the more plant units (e.g. leaves, plants, populations) one samples, the more phytochemicals one should observe. Importantly, this is also true for organisms that interact with plants – biotic interactions at fine scales should on average involve fewer phytochemicals than those at larger scales. Though the scale-dependence of phytochemical diversity has rarely been explored empirically, the nature of scale–diversity relationships – where they start and level off, their overall height and their initial slope and curvature – contains essential biological information about diversity. This information at least should be used to inform sampling designs and at best can be used to make inferences about the ecology and evolution of plant interactions.

We illustrate the importance of scale by quantifying chemical diversity–scale relationships using published data from seven plant families across six levels of biological organisation (Fig. 4). These relationships start at the average compound richness within one unit (alpha diversity) and increase with scale to the total measured richness (gamma diversity). The path they travel in between depends on how compounds are distributed among units (beta diversity) and indicates how diversity accumulates with scale. We calculated these relationships by averaging cumulative richness across bootstrapped samples at each scale (methods and R code in Appendix S1). This statistical method is analogous to sample-based rarefaction, which is common in the species diversity literature (Gotelli & Colwell 2011). Traditionally, rarefaction is used to compare richness between samples or sites of different sizes; here we demonstrate how rarefaction can be used to explore the scaling patterns explicitly, facilitating key biological insights, such as how the phytochemical diversity experienced might vary with the host breadth of the interacting species, or how phytochemical diversity varies evolutionarily across species within a clade. All of the published datasets we found that were suitable for this type of analysis (non-targeted analyses of all detected compounds within or across classes) were focused on spatial variation, though a similar approach could be applied to temporal variation.

Four key implications from empirical chemical diversity–scale relationships

First, chemical diversity increased with scale at all levels of biological organisation for a diversity of plants, providing strong empirical evidence for the scale-dependence of phytochemical diversity (Fig. 4). The pervasiveness of the relationship suggests that our default assumption should be that biotic interactions that occur at different scales will involve different levels of phytochemical diversity even for the same plant individual, population or species.

Second, the chemical diversity–scale relationships we examined were nonlinear and varied in curvature within and among systems. This means that differences in diversity among plant types can vary with the scale of observations or interactions. For example, sagebrush populations one and two had similar compound richness at fine scales but diverged at larger scales (Fig. 4c). Thus, sedentary organisms that feed

from single host plants, such as a gall-forming flies, would experience similar levels of phytochemical diversity in either population, whereas a mobile organism, such as a grasshopper, would perceive population one to have higher phytochemical diversity. In contrast, sagebrush populations two and three differed in richness at fine scales and converged at larger scales, such that they would seem different to gall-formers but similar to grasshoppers.

Third, variation in the shapes of some chemical diversity–scale relationships was great enough that curves crossed, which means that even relative differences in diversity among samples depend on scale. This is apparent in the variation in amide richness among organs within *P. reticulatum* individuals (Fig. 4a). Which *P. reticulatum* individuals will be more or less diverse to an interacting organism will depend on how the organism interacts with a plant. A key lesson from these observations is that we cannot meaningfully compare phytochemical diversity without reference to scale.

Fourth, our search for data revealed a dearth of phytochemical datasets at the highest and lowest relevant levels of biological organisation. Few studies have characterised variation in phytochemical diversity within individual plants or among species within communities. Such data, particularly on the spatiotemporal distribution of chemistry within plant individuals, would help answer major questions about the evolution of phytochemical diversity.

Determining biologically relevant scales

The importance of scale–diversity relationships in determining how organisms that operate at different scales perceive phytochemical diversity implies that we need to choose our sampling scales carefully to match the scales relevant for our systems, organisms and questions. In Box 1, we advocated for flexible definitions of the key scale-related dimensions of diversity. Here we discuss the ways to define relevant scales biologically.

Defining alpha, beta and gamma

Alpha, beta and gamma phytochemical diversity have previously been defined at discrete plant scales, for example, defining alpha diversity at the scale of plant individuals, gamma diversity at the scale of plant communities, and beta diversity as the differences in chemistry among individuals (e.g. Kessler & Kalske 2018). Because selection acts at the scale of individuals, this is a natural way to assess hypotheses focused on plants, selection on phytochemistry, and evolution of phytochemical diversity within populations and among species (e.g. screening hypothesis, Box 2). However, as they have been in the species diversity literature (Whittaker 1960; Magurran & McGill 2011), these concepts can be flexibly applied to any hierarchically structured set of samples.

The scale at which alpha, beta and gamma are defined should depend on the question of interest. For example, hypotheses focused on the effects of phytochemical diversity on pollinator or herbivore physiology (e.g. Pearse *et al*. 2018) would be best addressed by defining alpha diversity as the compound richness an organism encounters in a single foraging bout, gamma diversity as the total compound richness encountered across an organism's lifetime, and beta diversity as the differences in phytochemistry among foraging bouts. We would examine alpha diversity to test the synergy hypothesis (Box 2) because it is focused on diversity experienced simultaneously by herbivores. In contrast, we would examine spatial and temporal beta diversity to test the moving target hypothesis (Box 2), which is focused on sequential diversity.

Other hypotheses would require these concepts to be defined at higher scales. For example, hypotheses focused on the effects of phytochemical diversity on consumer population dynamics (e.g. Wetzel *et al*. 2016) would be best addressed by defining alpha diversity as the richness consumed by an individual across its lifetime, gamma diversity as the richness consumed across a whole population, and beta diversity as the differences in phytochemical consumption among consumer individuals. In this example, we could examine the effects of phytochemical diversity on the average consumer using alpha diversity, predict the effects of inter-individual differences on population dynamics using beta diversity, and examine the full array of phytochemicals faced by the herbivore population, which may be important for changes in herbivore genotypic frequencies, using gamma diversity. Note that alpha diversity in this example is equivalent to gamma diversity in the previous example, illustrating the important point that there are no universal scales for alpha, beta and gamma diversity.

Spatial and temporal autocorrelation in phytochemistry

How an organism's experience of phytochemical diversity varies with scale depends not only on alpha, beta and gamma diversity, but also on spatial and temporal patterns in phytochemistry, including autocorrelation. Autocorrelation is often overlooked by phytochemical diversity studies because it is not captured by standard diversity metrics, but it can drastically influence how organisms encounter phytochemical diversity and how scientists sample it. If chemically similar plants are spatially aggregated, then it could take longer for a moving organism to experience a plant population's full phytochemical diversity (Fig. 5, Appendix S1). Spatial and temporal patterns also influence our ability to detect phytochemical diversity, with greater sampling breadth required at high aggregation and greater depth required with spatiotemporal evenness. Characterising these patterns for our biological understanding will require spatially and/or temporally explicit sampling, akin to the phytochemical mapping program recommended by Hunter (2016).

Harnessing the chemical diversity–scale relationship to make testable predictions

Our ability to test major hypotheses related to the causes and consequences of phytochemical diversity (Box 2) has likely been slowed because these hypotheses tend to be vague about the spatial and temporal scales of the patterns and processes they explain. We propose that explicitly considering scale allows novel predictions to be derived from classic hypotheses (Box 2). Essentially, we should expect the spatiotemporal distribution of phytochemical diversity to reflect the spatiotemporal scales of the interactions that select for phytochemical diversity. In other words, we should not expect phytochemical diversity to be

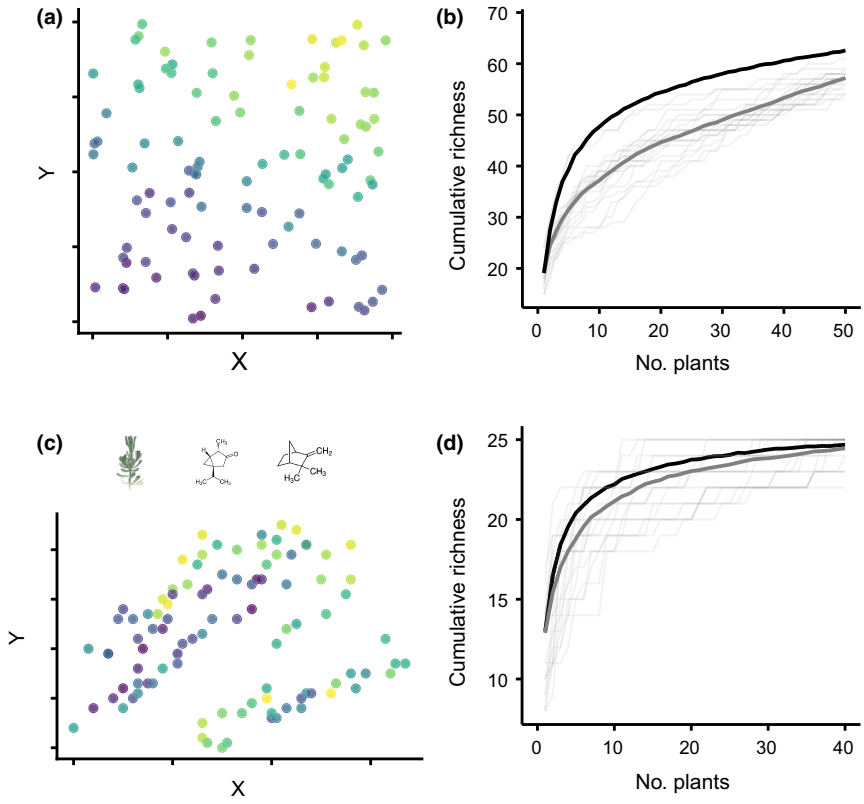

Figure 5 The effect of spatial autocorrelation on perception of plant chemical diversity. (a) A simulated map of 100 plants exhibiting high-spatial autocorrelation in the presence of compounds. Points with more similar colours represent plants with more similar chemistry (closer principal component scores). (b) A spatially explicit examination of how phytochemical richness accumulates with scale. Each thin grey line shows the accumulation of richness starting from a different initial plant and emanating out to the plant's sequence of 1–49 nearest neighbours. The mean of these accumulation curves is a spatially explicit rarefaction (thick grey line), which falls substantially lower than the corresponding non-spatial rarefaction curve (black line), indicating that taking the spatially autocorrelated pattern of chemistry into account, which could be how an organism experiences a plant population, slows the accumulation of chemical richness with scale. (c) A map of 98 sagebrush plants (*Artemisia tridentata* Asteraceae) in one population (Karban et al. 2014) coloured by volatile chemical similarity as in (a). This population exhibits a significant but small degree of positive chemical spatial autocorrelation (Mantel test: $r = 0.07$, $P = 0.024$). (d) Spatially explicit chemical accumulation and rarefaction on sagebrush data, line types as in (b). Detailed statistical methods and R code for this analysis are provided in Appendix S1. Sagebrush drawn by Devyn Orr

universally fitness-enhancing; we should expect it to be fitness-enhancing at the right spatial and temporal scales.

In practice, we are advocating for studies to organise hypotheses and empirical measurements around the scales important for the species interactions under study. For example, if we think selection for phytochemical diversity arises because synergisms among compounds benefit plants in biotic interactions (synergy hypothesis; Box 2), then we should expect compound richness to be high within plant tissues at fine spatial and temporal scales such that interacting organisms cannot avoid it. In contrast, if we think phytochemical diversity is beneficial primarily because plants face a diversity of biotic interactions (interaction diversity hypothesis; Box 2), then we should expect to see high-spatial beta diversity among organs involved in interactions with different species or high-temporal beta diversity corresponding to seasonal variation in biotic interactions.

The diet-breadth phytochemical diversity hypothesis

Phytochemical diversity is unavoidable for organisms that interact with plants. Previous thinking has been that reduced mobility and increased diet specialisation benefit herbivores by reducing the amount of plant diversity they experience, while generalism and increased mobility bring other benefits at the cost of increased exposure to plant diversity (Bernays & Minkenberg 1997; Bernays 2001). Our examination of diversity–scale relationships suggests an additional interpretation – mobility and diet specialisation influence not only the amount of diversity experienced, but also the relative importance of different types and scales of diversity.

Specialists with low mobility have reduced exposure to variability across host plants. This reduction in spatial variability, however, increases the relative importance of temporal variability within individuals, including induced responses and ontogenetic variation. We propose that this means that low-mobility specialists will often select for high-temporal chemical variation in plants. The result, in some systems, will be that temporal variation is a primary defence against low-mobility specialists, whereas in other systems specialists may be well-adapted to within-host temporal variability, depending on whether the plant or the herbivore is 'in charge' of the coevolutionary dynamic (Ali & Agrawal 2012). Mobile

generalist herbivores, in contrast, can avoid temporal variation within individuals by moving among individuals or species, but doing so forces exposure to variability among individuals or species. This suggests both that mobile generalists have adaptations for coping with among-plant variability and that among-plant variability could be a key axis of defence against mobile generalists. By selecting for plants that differ phytochemically from their conspecific or heterospecific neighbours, generalists could be a major selective force in the generation of novel phytochemical defences in plant communities. Testing this hypothesis will require studies at multiple levels; from physiological studies of the effects of phytochemical diversity, to selection experiments that manipulate plant attack by generalists and specialists, and to phylogenetic comparisons of the phytochemical diversity of plants that host different proportions of generalists and specialists.

The abundance-dependence of phytochemical diversity

Observed chemical diversity increases with total chemical abundance

Researchers studying species diversity appreciate that the number of species encountered generally increases not only with area searched, as discussed above, but also with the total abundance of organisms in a community. Here we show that the same is true for phytochemical diversity – the higher the total abundance of phytochemicals in a plant, the greater the diversity of phytochemicals one will, on average, observe. We term this phenomenon the abundance-dependence of phytochemical diversity. In a re-analysis of published data, we found that observed phytochemical richness increased with total phytochemical abundance in each of the datasets we examined across four biological levels: within individuals of *Lonicera bella*; among individuals of *Artemisia tridentata*, *Lonicera bella* and *Piper reticulatum*; among species within *Quercus*, *Asclepias* and *Lonicera*; and among species within communities (Fig. 6). In these examples, total abundance was estimated using the chromatographic peak areas.

There are plausible biological explanations for the chemical diversity–abundance relationship (discussed below). However, at least part of this relationship must be an artefact of analytical detection thresholds. Compounds have minimum concentrations for detection; these depend on many factors including chemical properties, extraction methods, instrument parameters and thresholds for separating peaks from noise. The key consequence is that the number of compounds we detect in a sample declines with overall concentration, a fact appreciated by chemists who have run single plant extract samples at multiple concentrations. For example, we found

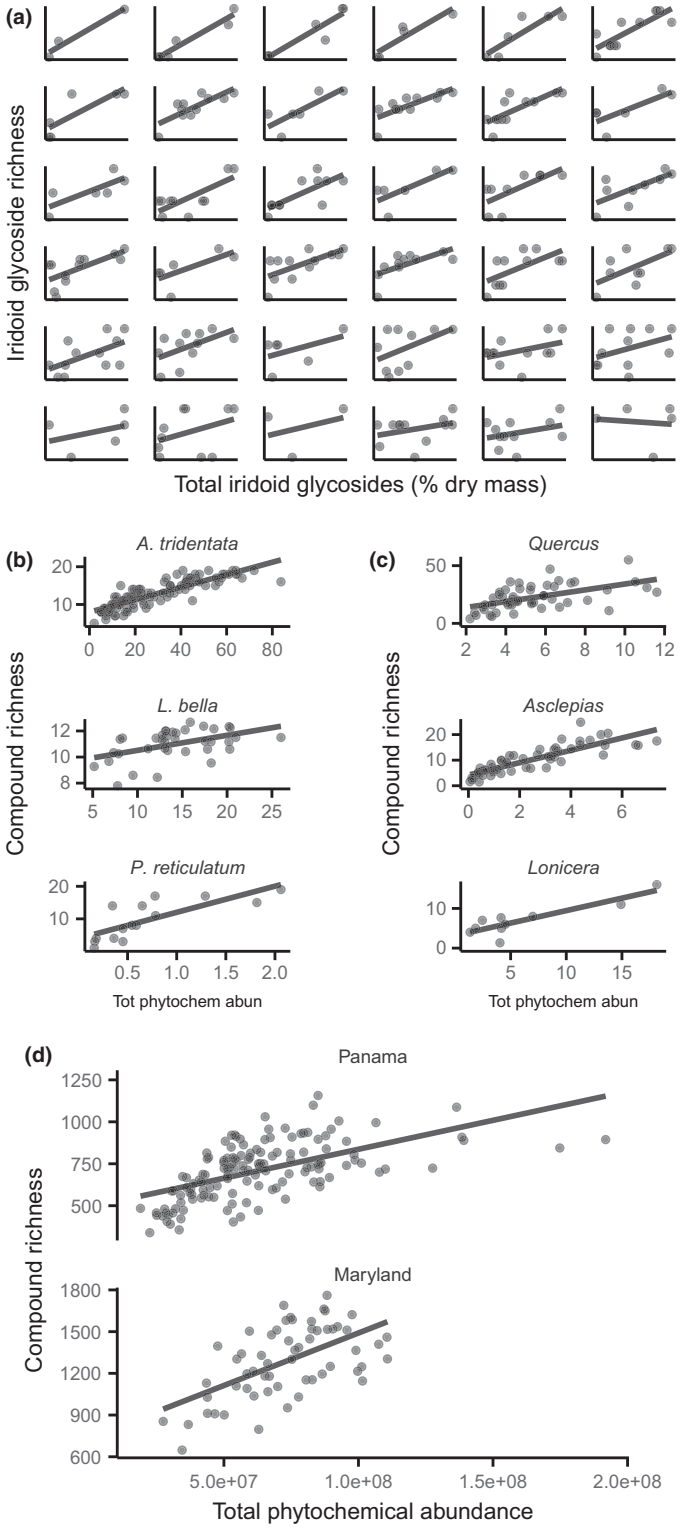

Figure 6 Chemical diversity–abundance relationships are positive across multiple levels of biological organisation. (a) Iridoid glycoside richness and abundance within 36 individuals of *Lonicera bella* (Caprifoliaceae), ordered by steepness of slope (Whitehead & Bowers 2013). (b) Compound richness and total phytochemical abundance across plant individuals in populations of *Artemisia tridentata* (Asteraceae) (Karban et al. 2016), *L. bella* (Whitehead & Bowers 2013) and *Piper reticulatum* (Piperaceae) (Whitehead et al. 2013). Abundance units are peak area, % dry mass and % dry mass. (c) Compound richness and total phytochemical abundance across species within three genera: *Quercus* (Fagaceae) (Pearse et al. 2013), *Asclepias* (Apocynaceae) (Rasmann & Agrawal 2011) and *Lonicera* (Lieurance et al. 2015). Abundance units are peak area, μg mg^{-1} dry mass and % dry mass. (d) Phytochemical richness and abundance (peak area) across tree species within communities at Barro Colorado Island, Panama or the Smithsonian Environmental Research Center, Maryland, USA (Sedio et al. 2018b).

observed phytochemical richness in a single sample of *Physalis angulata* increased continuously from 0 to 135 compounds depending on the concentration at which the sample was analysed (see Fig. S1 in Appendix S1). This phenomenon is a major hurdle to the study of phytochemical diversity because it confounds our ability to assess the effects of phytochemical richness on biological processes independently of the effects of total abundance. Without accounting for differences in abundance across samples, it is uncertain whether any correlation between diversity and an ecological interaction is driven by diversity or abundance or both. More broadly, this phenomenon illustrates one of our major points – diversity cannot be meaningfully understood without accounting for the frame of reference from which it is observed.

Determining a relevant frame of reference: total plant mass or total metabolite abundance

When calculating phytochemical diversity, a critical decision is to whether analyse chemistry and estimate diversity per unit plant biomass or per unit total phytochemical biomass (i.e. abundance). Diversity per unit plant biomass may be most relevant for understanding how consumers interact with phytochemical diversity on a per bite basis. Certainly, the physiological effects of phytochemical diversity may depend on the amount of plant material through which it is distributed. In other contexts, however, calculations of diversity per unit phytochemical biomass may be more informative. For example, insect herbivores that sequester plant defences may have specialised mechanisms in their gut to isolate phytochemicals such that phytochemistry is experienced partially independently from nutritional chemistry (Opitz & Mueller 2009). This perspective is also essential to understanding how plants allocate limited resources among biosynthetic pathways and compounds.

A key recognition is that every diversity estimate implicitly has a denominator. Typical methods involve extracting metabolites with a fixed ratio of solvent to plant biomass and then analysing all samples identically, implicitly measuring diversity per plant mass. Alternatively, some researchers begin with a fixed amount of plant biomass, but then evaporate samples to dryness and re-suspend extracts to a fixed ratio of solvent to extract mass. Assuming the extract is mainly phytochemicals (which will depend on extraction methods), these methods measure diversity per total phytochemical mass. Despite the important difference in frame of reference, studies rarely justify or discuss the implications of their choice between these methods.

We propose that studies, whenever feasible, should use methods that allow diversity to be disentangled from total abundance. A major component of disentangling diversity and abundance will be careful methods development, which should include running samples at multiple concentrations to understand how variation in abundance among samples within a study affects diversity estimates. As mentioned above, diversity could be examined per unit phytochemical biomass by evaporating phytochemical extracts to dryness, obtaining extract masses and diluting samples for analysis at a set ratio of extract mass to solvent. However, these methods are not possible for all sample types (e.g. volatile samples that cannot be evaporated to dryness). Alternatively, researchers could use residuals of the diversity–abundance relationship to understand the relative divergence of different samples from the expected relationship (see Appendix S1 for a brief exploration of these two potential methods, although more work is needed to develop rigorous methods that account for the relationship between diversity and abundance). The bottom line is that when abundances vary among samples within a study, methods that account for the diversity–abundance relationship would help us to avoid confounding the effects of diversity and abundance and enable us to test biological explanations (below) for the abundance-dependence of phytochemical diversity.

Testable hypotheses and predictions

Given the prevalence of diversity–abundance relationships in the datasets we examined (Fig. 6), it is possible the analytical relationship between diversity and abundance (Fig. S1) is only a partial explanation. We propose three non-exclusive biological hypotheses for this phenomenon and discuss their implications for the ecology and evolution of phytochemical diversity.

Biosynthetic probability hypothesis

Phytochemical abundance and diversity may be fundamentally linked by the branching structure of biosynthetic pathways. Much of phytochemical diversity is generated from a limited set of common precursors, often primary metabolites. For example, two diphosphate compounds are modified by terpene synthases to produce 20 000 + terpenes (Tholl 2006). Given that at least some movement of substrate through biosynthetic pathways is random, with higher probabilities for abundant compounds and lower probabilities for rarer compounds, the more precursor that is allocated to phytochemistry, the more diversity will be generated. This hypothesis predicts that factors that increase total allocation to phytochemical biosynthesis will also increase phytochemical diversity. For example, induced responses to herbivores may shift both richness and total abundance concurrently along this relationship's major axis (Fig. 6). If this hypothesis were supported, it would represent a fundamental biosynthetic relationship between phytochemical abundance and diversity, making plant species or genotypes that deviate from the average abundance–diversity relationship (e.g. outliers in Fig. 6) all the more biologically noteworthy.

Minimum functional concentration hypothesis

If most phytochemicals have an adaptive role, then they must also have a minimum functional concentration at which they can benefit the plant. Thus, on average, plants that produce many unique metabolites with adaptive roles will also inherently have a larger sum total of phytochemical abundance all else being equal. Although one might expect trade-offs between different types of defences in different environments, limiting the total abundance and diversity a plant can maintain, this was not supported in a meta-analysis on costs of defence. Koricheva *et al.* (2004) found little evidence for genetic trade-offs between different types of co-occurring chemical defences. Instead, highly defended plants appear to be 'jacks-of-all-trades and masters of all', producing numerous compounds at high concentrations.

Correlated evolution hypothesis

Perhaps the most biologically interesting hypothesis for the abundance–diversity relationship is that factors that select for increased phytochemical diversity may also select for increased phytochemical abundance and vice versa (Rasmann & Agrawal 2011). This requires that diversity *per se* has an adaptive value in specific interactions, as posited by the synergy hypothesis (Box 2), and that plants benefit more from having higher total abundance and higher diversity simultaneously. In other words, phytochemical diversity and abundance may be facets of a general plant defensive syndrome and therefore evolve in concert (Agrawal & Fishbein 2006; Rasmann & Agrawal 2011). This is an intriguing possibility, but it is still unclear how selection may act on diversity and abundance simultaneously. Answers to these and other questions focused on how selection and constraints have shaped the production of phytochemicals would lay the ground rules for understanding the evolution and ecology of phytochemical diversity.

THE FRONTIER OF PHYTOCHEMICAL DIVERSITY

We are at the brink of a new era of understanding in the ecology and evolution of phytochemical diversity. The field has just begun to quantify the diversity of phytochemistry and treat it as a feature of interest. We know phytochemical diversity is pervasive across systems and at all levels of biological organisation, but we do not know why. We know phytochemical diversity is multidimensional and that each dimension can have biological significance, but in general we do not know the role of each dimension, or how dimensions are related evolutionarily or ecologically. We know phytochemical diversity varies substantially with scale within and among systems in ways that can influence the diversity encountered by organisms. However, we do not know which scales are most relevant for interactions, how the ecological consequences of diversity vary with scale or how biotic interactions have shaped the evolution of chemical diversity–scale relationships. Finally, we know that the phytochemical diversity we measure is correlated with phytochemical abundance, but we do not know if this phenomenon is only a measurement artefact or also indicative of biological processes that link phytochemical diversity and abundance within plants at biosynthetic or evolutionary levels. If we embrace the multidimensional, multiscale nature of phytochemical diversity and incorporate that knowledge into our study designs, it will empower us to push through the frontier to develop a deep, nuanced understanding of phytochemical diversity as a keystone feature of plants that mediates interactions and shapes entire communities.

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AUTHORSHIP

Both authors contributed to the ideas, analyses and writing.

DATA AVAILABILITY STATEMENT

Data available from the Figshare Repository: https://doi.org/10.6084/m9.figshare.10006610.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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