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Fruits, frugivores, and the evolution of phytochemical diversity

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Plants produce an enormous diversity of secondary metabolites, but the evolutionary mechanisms that maintain this diversity are still unclear. The interaction diversity hypothesis suggests that complex chemical phenotypes are maintained because different metabolites benefit plants in different pairwise interactions with a diversity of other organisms. In this synthesis, we extend the interaction diversity hypothesis to consider that fruits, as potential hotspots of interactions with both antagonists and mutualists, are likely important incubators of phytochemical diversity. We provide a case study focused on the Neotropical shrub *Piper reticulatum* that demonstrates: 1) secondary metabolites in fruits have complex and cascading effects for shaping the outcome of both mutualistic and antagonistic fruit–frugivore interactions, and; 2) fruits can harbor substantially higher levels of phytochemical diversity than leaves, even though leaves have been the primary focus of plant chemical ecology research for decades. We then suggest a number of research priorities for integrating chemical ecology with fruit–frugivore interaction research and make specific, testable predictions for patterns that should emerge if fruit interaction diversity has helped shape phytochemical diversity. Testing these predictions in a range of systems will provide new insight into the mechanisms driving frugivory and seed dispersal and shape an improved, whole-plant perspective on plant chemical trait evolution.

Keywords: chemical ecology, fruit defense, phytochemical diversity, Piper, secondary metabolites, seed defense, seed dispersal, specialized metabolites, toxic fruit

Introduction

One of the most intriguing features of plants is their production of complex mixtures of secondary metabolites; hundreds or even thousands can sometimes be detected in a single tissue sample (Wiggins et al. 2016, Sedio et al. 2018). Most of the structures and functions of these metabolites are undescribed, but there are numerous well-studied examples in which secondary metabolites play a key role in shaping plant interactions, and, ultimately, the evolution of both plants and consumers (Hartmann 2007, Futuyma and Agrawal 2009). Still, it is unclear why plants produce so many secondary metabolites simultaneously, and many competing evolutionary hypotheses have

been proposed (Box 1). Perhaps the most well-supported is the interaction diversity hypothesis, which suggests that chemical diversity is an emergent consequence of diverse selective pressures from the many different antagonists and mutualists with whom the plant interacts (Whitehead et al. 2021). Comparing support for this and other hypotheses in natural systems remains challenging, in large part due to the multidimensional nature of phytochemical diversity and the challenges associated with summarizing large multifarious datasets with meaningful metrics (Wetzel and Whitehead 2020). Furthermore, there is often a disconnect between the occurrence patterns of secondary metabolites and measured ecological variables, such as herbivory (Carmona et al. 2011, Whitehead et al. 2017, Salazar et al. 2018). Here, we argue that much of the uncertainty surrounding the origins and maintenance of plant chemical diversity may be due to a historically narrow view of the interactions that shape plant chemical traits, and, in particular, a lack of focus on interactions surrounding a key plant organ – the fruit.

Most theory and empirical research in chemical ecology has focused on leaves and leaf herbivores (Iason et al. 2012), and there is convincing evidence that interactions with leaf herbivores have helped shape secondary metabolite evolution (Kursar et al. 2009, Richards et al. 2015, Salazar et al. 2018). However, plant-associated communities include diverse vertebrates, invertebrates, and microbes that interact with leaves, roots, stems, flowers, and fruits. Fruits, in particular, are highly nutritious resources and face complex and highly variable selection pressures from different consumers over time. As they develop on the parent plant, they must defend against complex communities of antagonistic herbivores, seed predators, and pathogens (Andersen 1988, Kolb et al. 2007, Tiansawat et al. 2017). Once mature, animal-dispersed fruits must attract mutualists that will effectively disperse their seeds away from the parent plant (Howe and Smallwood 1982, Schupp et al. 2010, Ruxton et al. 2014). Finally, post-dispersal, seeds face an entirely new environment (i.e. the 'seedscape'; Beckman and Rogers 2013), with

Box 1. Key hypotheses that explain the evolution of phytochemical diversity.

Several hypotheses have been proposed to explain the evolution of phytochemical diversity. Under any of these hypotheses, diversity can be initially generated through various genetic and biochemical mechanisms (Gershenzon et al. 2012), but the hypotheses differ in their explanation for how that diversity is maintained by natural selection. Most are united by the assumption that the majority of secondary metabolites (other than perhaps some essential precursors or evolutionarily transient metabolites) have an adaptive role. An exception is the screening hypothesis, which provides a null alternative to that assumption.

Most secondary metabolites are adaptive

- *Interaction diversity hypothesis.* Different metabolites provide different advantages in the diversity of ecological interactions that plants face, including those with herbivores, pathogens, pollinators, seed dispersers and microbial mutualists (Berenbaum and Zangerl 1996, Iason et al. 2011, Whitehead et al. 2021).
- *Synergy hypothesis.* Diverse mixtures are more biologically active because of greater than additive effects of secondary metabolites when they occur in mixtures. Evidence suggests that synergies among compounds are common (reviewed by Richards et al. 2016); however, it is still unclear whether the probability of beneficial synergies increases with the diversity of a mixture, providing a selective advantage for phytochemically diverse plants (Whitehead et al. 2021).
- *Slowed adaptation hypothesis.* Diverse mixtures provide a more effective defense because they slow down the evolution of counter-adaptations by herbivores or other enemies. This hypothesis has been little examined in the context of secondary metabolite diversity (Palmer-Young et al. 2017), but similar principles have been supported in the context of pesticide mixtures used in agriculture to slow the evolution of resistance in pests (Cloyd 2010) or multi-drug therapies used in medicine to slow the evolution of antibiotic resistance (Baym et al. 2016).
- *Specific communication hypothesis.* Diverse mixtures enhance communication with mutualists by encoding more information about the specific state or quality of the plant (Gershenzon et al. 2012, Kessler 2015, Nevo et al. 2018).

Most secondary metabolites are non-adaptive

- *Screening hypothesis.* Diversity is maintained, even though most metabolites have no adaptive function, because selection acts on enzymes and other biosynthetic mechanisms that generate diversity (Jones and Firn 1991, Firn and Jones 2003). This hypothesis rests on the assumption that biological activity is a rare property for a metabolite to possess, and therefore plants must make and 'screen' a large number of metabolites in order to gain an advantage. A key requisite of this hypothesis is that the fitness benefit a plant gains from producing one or a few rare bioactive metabolites must outweigh the metabolic costs of producing numerous biologically-inactive metabolites. Although it has been the subject of debate for decades (Berenbaum and Zangerl 1996, Firn and Jones 2006, Owen and Peñuelas 2006, Pichersky et al. 2006), the screening hypothesis and its assumptions remain largely untested.

novel competitors, seed predators/pathogens, and mutualists, such as fungal symbionts (Loiselle 1990, Dalling et al. 2011, 2020, Abdel-Lateif et al. 2012). No other plant organ travels through such variable environments and interacts with such complex communities of mutualists and antagonists over its development and lifespan. Furthermore, considering the relatively direct consequences of seed survival, dispersal probability, and seedling recruitment for reproductive fitness, selection pressure may be especially strong for fruit chemical traits that can mediate fruit interactions. Overall, if interaction diversity shapes phytochemical diversity, fruit interactions are likely a critical, but historically underappreciated, force in plant chemical trait evolution.

The goal of this synthesis is to bring together theory from chemical ecology and seed dispersal ecology to consider the hypothesis that the diverse interactions surrounding fruits are key drivers of phytochemical diversification (hereafter the 'fruit-driven diversity hypothesis'). We consider this hypothesis to be one extreme of a likely continuum of different possible mechanisms of phytochemical diversification across plant lineages and compound classes, and it is not mutually exclusive with other drivers (such as selective pressures in other plant parts), that shape phytochemical phenotypes. We focus here on fruits because, despite their central importance for plant fitness, they have been largely left out of theory on plant secondary metabolite evolution. Furthermore, research on fruit chemical ecology has largely focused on the effects of single isolated metabolites or total amounts in a particular class (e.g. total phenolics), with less attention given to how the complexity and variability in fruit chemical composition shapes fruit–frugivore interactions. Below, we first provide background information on our current understanding of the evolution of phytochemical diversity and the functional roles of secondary metabolites in fruits. Next, we use a case study of secondary metabolites in the tropical shrub *Piper reticulatum* as an example of a system where current evidence supports the fruit-driven diversity hypothesis as a key mechanism maintaining phytochemical diversity. Finally, we provide a roadmap for future research that could test the generality of these patterns across systems and transform our understanding of both the origins of phytochemical diversity and the mechanisms shaping variation in the outcome of frugivory and seed dispersal.

Background

Historical context and recent advances in the study of phytochemical diversity

Biologists and chemists have pondered the origins and distribution of plant secondary metabolites for more than a century (Abbott 1887, Stahl 1888, Hartmann 2008). Most early scholars assumed these diverse compounds were largely waste products of metabolism with no biological function. It was the mid-20th century before pioneering work by Fraenkel (1959) and Ehrlich and Raven (1964) provided a convincing argument that secondary metabolites evolved primarily for their role in defense against insect herbivores, helping to ignite the field of plant chemical ecology and inspiring decades of work. As the field grew in the following decades, the prevailing assumption was that all (or at least most) secondary metabolites had an adaptive role waiting to be elucidated. Then, in the 1990s, the idea that the majority of compounds were 'redundant' and had no biological function re-surfaced under the tenets of the screening hypothesis (Jones and Firn 1991, Box 1). This hypothesis posits that most secondary metabolites are inactive, but selection acts on biochemical mechanisms that increase diversity because plants that make and 'screen' a large number of metabolites will benefit from the rare metabolites that have potent biological activity. The idea that most metabolites are non-adaptive sparked much debate on the origins of phytochemical diversity, and opponents of the screening hypothesis provided a number of clear alternatives (Box 1) that illustrated the potential adaptive function of diverse mixtures of secondary metabolites (Romeo et al. 1996). However, the debate was not settled, perhaps in part due to the limitations of methods in chemical ecology at that time to assess the scope of phytochemical diversity and its relationship to ecological and evolutionary variables.

The last decade has ushered in a new era of discovery. This is largely due to the advent of the field of metabolomics and rapid advances in mass spectrometry (MS), nuclear magnetic resonance (NMR), and associated bioinformatic tools that have enabled relatively rapid, non-targeted approaches to characterizing complex chemical mixtures (Sedio 2017, Dyer et al. 2018, Aron et al. 2020). Simultaneously with the data revolution brought on by metabolomics, chemical ecologists made an important theoretical advance by starting to apply metrics and concepts developed for the study of species diversity to the study phytochemical diversity (Wetzel and Whitehead 2020). There is an increasing recognition of the inherent multi-dimensional nature of phytochemical diversity, which can refer broadly to the complexity of chemical composition and its variation in space and/or time (Box 2).

Recent evidence has supported the view that a main ecological benefit of secondary metabolite diversity is that different metabolites can serve different functions, allowing plants to adaptively respond to a wide range of species interactions (i.e. the interaction diversity hypothesis; Whitehead et al. 2021). Perhaps due to the historical context of the field (Fraenkel 1959, Ehrlich and Raven 1964), the dominant view in chemical ecology is still that defense against herbivores is the main driver of phytochemical diversification, and thus far the interaction diversity hypothesis has been mainly considered in the context of plants defending themselves against diverse enemies (Gershenzon et al. 2012, Whitehead et al. 2021). However, we know that secondary metabolites can function in many types of biotic interactions, such as attracting the natural enemies of herbivores (Kessler and Heil 2011), attracting or manipulating pollinators or seed dispersers (Cipollini and Levey 1997b, Adler 2000, Raguso 2008, Borges 2015), and shaping soil microbial communities

Box 2. Dimensions of phytochemical diversity.

Secondary metabolite diversity can refer broadly to the complexity of chemical composition and its variation in space and/or time, and, like species diversity, cannot be perfectly summarized with any single metric (Wetzel and Whitehead 2020). The long history of research on species diversity provides a rich theoretical and mathematical starting point for exploring phytochemical diversity (Magurran and McGill 2011). However, much work remains to determine how best to apply existing diversity concepts to describe patterns of phytochemical diversity (Wetzel and Whitehead 2020). Below we define several diversity metrics and concepts that can provide a starting point for studies that aim to link phytochemical diversity to ecological and evolutionary variables.

- *Richness.* The number of unique metabolites in a mixture.
- *Evenness.* The extent to which metabolites in a mixture have equal relative abundances.
- *Structural complexity.* The extent of dissimilarity among molecular features (e.g. functional groups) within and across metabolites in a mixture. Often used as a proxy for functional diversity, though the link between the two is indirect and variable.
- *Alpha diversity.* The average diversity detected per sampling unit. Alpha diversity, along with beta and gamma, are concepts of scale that can be applied to multiple other metrics (e.g. average richness, average structural complexity).
- *Gamma diversity.* The total diversity detected across all sampling units.
- *Beta diversity.* The variability or turnover in composition detected across sampling units. Can be used to describe the amount of spatial and/or temporal variability at multiple scales depending on the sampling units used (e.g. sub-individual, inter-individual, inter-species). There are numerous metrics for assessing beta-diversity that can be applied to phytochemical diversity (Anderson et al. 2011, Wetzel and Whitehead 2020).
- *Autocorrelation.* Describes the spatial or temporal pattern of chemical variation across samples. High autocorrelation indicates neighboring samples are more similar in composition than expected, and low autocorrelation indicates variability is distributed across the sample set (Wetzel and Whitehead 2020).

(Abdel-Lateif et al. 2012, Tian et al. 2020). The fruit-driven diversity hypothesis is an extension of the logic of the interaction diversity hypothesis to include mutualistic and antagonistic interactions surrounding fruits.

Occurrence and functions of secondary metabolites in fruits

Fruit secondary metabolites may play a variety of adaptive roles, and we refer readers to a number of key reviews for a more thorough overview of this topic (Cipollini and Levey 1997b, Levey et al. 2007, Rodríguez et al. 2013, Borges 2015, Dalling et al. 2020, Nevo and Ayasse 2020). Here, we provide a brief overview of the major categories of hypotheses that have been proposed to explain the occurrence of fruit secondary metabolites, with specific named hypotheses from the literature listed in Box 3. We focus on the roles of secondary metabolites in biotic interactions, though many metabolites may also provide protection against abiotic stress, act as regulators of metabolism, or function as 'primary' metabolites (Erb and Kliebenstein 2020). Considering the diversity of potential functions of secondary metabolites, it is critical to emphasize that no single hypothesis can explain the occurrence of all compounds, and any given compound can have multiple functional consequences (Izhaki 2002), leading to complex tradeoffs and constraints in fruit secondary metabolite evolution.

First, a major function of secondary metabolites in both abiotically and biotically-dispersed fruits is defense against antagonistic consumers. Antagonists include vertebrate and invertebrate seed predators as well as pathogens, all of which can have large impacts on plant fitness and population dynamics (Hulme 1998, Kolb et al. 2007, Tewksbury et al. 2008b, Beckman and Muller-Landau 2011, Mordecai 2011, Zalamea et al. 2021). Secondary metabolites that defend against these diverse antagonists are likely ubiquitous in fruits (including seeds and surrounding appendages). For example, polyphenols (e.g. tannins), which are known to contribute to plant defense, were detected in > 80% of seeds from 196 species studied in a woody plant community (Gripenberg et al. 2018). The relative importance of these chemical defenses over physical defenses is variable across species and may be linked to functional seed traits such as dormancy type (Zalamea et al. 2018, Dalling et al. 2020). In addition to seeds, secondary metabolites also commonly occur in the pulp of fleshy fruits, a tissue that functions primarily to attract animal seed dispersers (Herrera 1982, Cipollini and Levey 1997b). In many cases, the diversity or abundance of pulp secondary metabolites decreases during fruit ripening (Schaefer et al. 2003, Whitehead and Bowers 2013, Maynard et al. 2020). However, even ripe pulp often contains compounds that are deterrent or even highly toxic to some consumers, a pattern commonly explained by the defense tradeoff hypothesis, in which toxic secondary metabolites deter dispersers, but these costs are offset by the benefits incurred by protecting seeds against antagonists (Box 3, Cipollini and Levey 1997b). The strength of these potential tradeoffs will depend on the ecological context as well as

Box 3. Key hypotheses that explain the evolution of secondary metabolites in fruits.

Many hypotheses have been proposed to explain the various ways in which individual secondary metabolites could provide an adaptive advantage in fruit interactions. Alternatively, some secondary metabolites in fruits could be non-adaptive. Here, we list several of these key hypotheses with a non-exhaustive list of examples of studies that provide supporting evidence for each. The adaptive hypotheses described here were defined and/or reviewed in detail by Cipollini and Levey (1997b) and Cipollini (2000), and we direct readers to those papers for a more comprehensive overview. These hypotheses are not mutually-exclusive; no single hypothesis will explain the occurrence of all metabolites, and many metabolites have multiple functions.

Secondary metabolites are adaptive and driven by fruit antagonists

- *Defense tradeoff hypothesis.* Secondary metabolites deter invertebrate and microbial pests, but this comes at the cost of also deterring dispersers (Herrera 1982, Cipollini and Stiles 1993, Cipollini and Levey 1997a, b, Schaefer et al. 2003, Cipollini et al. 2004, Cazetta et al. 2008, Whitehead et al. 2016, Maynard et al. 2020).
- *Directed deterrence hypothesis.* Secondary metabolites deter vertebrate seed predators but have no effect on dispersers (Cipollini and Levey 1997b, Tewksbury and Nabhan 2001, Filardi and Tewksbury 2005, Levey et al. 2006).

Secondary metabolites are adaptive and driven by dispersers

- *Attraction/association hypothesis.* Secondary metabolites provide reliable foraging cues that dispersers can associate with rewards (Cipollini and Levey 1997b, Nevo et al. 2018, 2020a).
- *Attraction/repulsion hypothesis.* Primary nutrients attract dispersers, and secondary metabolites induce them to leave early in a foraging bout to prevent excessive removal or increase dispersal distance (Barnea et al. 1993, Cipollini and Levey 1997b).
- *Protein assimilation hypothesis.* Secondary metabolites interfere with protein metabolism, thereby inducing dispersers to leave early in a foraging bout to seek other food sources (Izhaki and Safriel 1989, Cipollini and Levey 1997b).
- *Gut retention time hypothesis.* Secondary metabolites influence gut retention time of dispersers, thereby affecting dispersal distance, deposition site, and the impacts of gut conditions on seeds (Murray et al. 1994, Cipollini and Levey 1997b, Wahaj et al. 1998, Traveset et al. 2007, Tewksbury et al. 2008a, Baldwin and Whitehead 2015, Baldwin et al. 2020).
- *Direct nutritional benefits hypothesis.* Secondary metabolites provide direct dietary benefits to seed dispersers, for example by acting as antioxidants or reducing parasite loads (Cipollini 2000, Schaefer et al. 2008, Alan et al. 2013, Neco et al. 2019).

Secondary metabolites are non-adaptive and driven by plant physiological constraints

- *Physiological constraints hypothesis.* Secondary metabolites are non-adaptive in the context of seed dispersal and are present as a consequence of selection for defense of other plant parts (Ehrlén and Eriksson 1993, Cipollini and Levey 1998, Eriksson and Ehrlén 1998, Cipollini et al. 2002, Whitehead and Poveda 2011, Whitehead and Bowers 2013, Nevo et al. 2020b).

the extent to which the mode of action of a given chemical defense is consumer-specific (Box 3).

In addition to defensive metabolites, the ripe pulp of animal-dispersed fruits can contain a variety of metabolites that function to alter disperser foraging behavior or physiology. Compounds that influence foraging behavior can include volatiles used as olfactory cues (Rodríguez et al. 2013, Borges 2015, Nevo et al. 2018, 2019) or pigments used as visual cues (Schaefer et al. 2008, Cazetta et al. 2012), either of which could serve to enhance associative learning or act as honest signals of nutritional rewards (Cipollini and Levey 1997b, Nevo et al. 2019, 2020a). Beyond the cues that initially drive fruit selection, other metabolites can alter foraging and feeding behavior in complex ways, for example by preventing excess removal by any single animal through dose-dependent toxicity (Barnea et al. 1993, Cipollini and Levey 1997b) or reducing the probability of frugivores destroying seeds through mastication (Samuni-Blank et al. 2012). In other cases, certain metabolites may have important direct benefits for animal health and physiology and be part of the reward system (Cipollini 2000). For example, antioxidants could serve nutritive functions (Schaefer et al. 2008, Alan et al. 2013) and antimicrobial or insecticidal compounds could reduce animal parasite loads (Baker 1996, Raman and Kandula 2008, Neco et al. 2019). Finally, many compounds may influence frugivore physiology in ways that affect the gut retention time of seeds, with consequences for seed viability and dispersal distance (Box 3, Murray et al. 1994).

Finally, though still poorly-understood, fruit secondary metabolites likely play a critical role in mediating beneficial

microbial interactions. In general, fruits and seeds provide habitat for diverse communities of microorganisms with complex potential consequences for fruit physiology and development, pathogen resistance, seed germination, and seedling success (Shade et al. 2017, Droby and Wisniewski 2018, Nelson 2018, Verma and White 2019). Secondary metabolites are known to shape microbial community dynamics (Aizenberg-Gershtein et al. 2015, Pusztahelyi et al. 2015), providing a means through which plants may manipulate fruit epiphytic or endophytic communities to their advantage. Once dispersed, successful establishment in competitive environments often depends on associations with soil microbiota, including mycorrhizal fungi and N-fixing bacterial symbionts, such as *Rhizobia* (Wang and Qiu 2006, Poole et al. 2018). Certain secondary metabolites that occur in seeds, such as flavonoids, are thought to be one of the primary ways that plants may selectively attract beneficial microbiota from the soil (Abdel-Lateif et al. 2012, Hassan and Mathesius 2012, van Dam and Bouwmeester 2016). As our ability to characterize both microbial communities and secondary metabolites is rapidly expanding, future work in this area will likely lead to many new insights into the mechanisms through which metabolites in fruits can help shape the microbial environment and influence seed survival and success.

Although there is growing support for a diversity of functional roles for secondary metabolites in both fruit flesh and seeds, we also emphasize that, from an evolutionary perspective, it is still unclear to what extent selective pressures imposed by fruit interactions (versus physiological or phylogenetic constraints) have shaped fruit chemistry. A key alternative to the adaptive hypotheses described above is that fruit secondary metabolites may be present largely due to physiological constraints (Box 2, Cipollini and Levey 1998, Eriksson and Ehrlén 1998). For example, some toxic secondary metabolites in ripe fruit flesh could be present as a consequence of strong selection for the defense of leaves or unripe fruit, combined with constraints that limit the exclusion of secondary metabolites from ripe fruit (Eriksson and Ehrlén 1998, Whitehead and Poveda 2011, Nevo et al. 2020b). Past work in a handful of systems has supported the idea that fruit secondary metabolites serve an adaptive role and cannot be explained solely as a consequence of leaf defense (Cipollini et al. 2002, Beckman 2013, Whitehead and Bowers 2013). Nonetheless, additional research is needed to test the generality of the multiple potential adaptive functions of secondary metabolites in fruits across diverse plant taxa. Furthermore, we need more information about the extent to which the expression of chemical traits is constrained across plant parts (e.g. fruits, flowers, roots and leaves). Certainly, in some cases, fruit secondary metabolites may occur largely as a consequence of defense of vegetative plant parts, but in other cases the opposite may be true – secondary metabolites in vegetative parts may occur largely as a consequence of selection on fruits.

Case study

In this section, we review past work on the tropical shrub *Piper reticulatum* (Piperaceae) that, taken together, provides evidence that the fruit-driven diversity hypothesis explains much of the phytochemical diversity in this system. A key underlying assumption of this hypothesis is that secondary metabolites have an adaptive role in interactions between fruits and diverse communities of frugivores, including vertebrates, invertebrates or microbes. Thus, we first review evidence demonstrating the multiple roles of amides, a diverse class of N-containing secondary metabolites, in *P. reticulatum* fruit. Next, we review what is known about the composition of *P. reticulatum* fruits relative to that of other plant parts, focusing in particular on a major prediction of our hypothesis: fruits (including seeds and surrounding appendages) will contain higher secondary metabolite diversity than other plant parts.

This case study focuses on a single species, and a more general test of the fruit-driven diversity hypothesis will also require a broader examination across taxa. However, we consider case studies such as this one, which target particular classes of compounds and systems in which a strong link between secondary metabolite diversity and function can be established, to also be critical for understanding the role of fruit-frugivore interactions in phytochemical diversification. The major evolutionary mechanisms that maintain phytochemical diversity will undoubtedly vary enormously across plant taxa and classes of compounds. Thus, we advocate for broad-scale metabolomic surveys alongside deeper investigation of model systems such as this one that provide targeted analyses of within-class chemical diversity and tests of mechanisms linking that diversity to the outcome of species interactions.

Study system background

Piper is a diverse (1000 + species) pantropical genus with fruits borne on distinctive spike-shaped infructescences (Dyer and Palmer 2004). In the neotropics, they are dispersed primarily by short-tailed fruit bats in the genus *Carollia*. Across different *Piper* species, bats consume 50–95% or more of the fruits (Thies and Kalko 2004, Maynard et al. 2020). In turn, *Piper* fruits are a dietary staple for the bats, comprising 50–98% of the diet across different *Carollia* species (Fleming 1991, Maynard et al. 2019).

Piper reticulatum provides an ideal case study because it is, to our knowledge, the only plant species that has been a model for both non-targeted phytochemical studies that compare secondary metabolite diversity across organs (Whitehead et al. 2013, Schneider et al. 2021) and ecological studies that have explored how fruit secondary metabolites function in fruit defense and seed dispersal (Whitehead and Bowers 2014, Baldwin and Whitehead 2015,

Whitehead et al. 2016, Baldwin et al. 2020). Ecological studies have focused on one particular class of compounds, the amides, which are particularly diverse and abundant in this species (Dyer et al. 2004). Amides are a group of nitrogen-containing compounds that occur in a number of different *Piper* species, including *P. nigrum* (black pepper), where the amide piperine occurs in high concentrations in the seed and gives black pepper its spice (Semler and Gross 1988). Toxic effects of *Piper* amides have been reported for a wide range of leaf herbivores, both specialist and generalist (Dyer et al. 2003). Amides often occur in complex mixtures, and their bioactivities can be non-additive, with examples of both synergistic and antagonistic interactions among compounds (Scott et al. 2002, Dyer et al. 2003, Richards et al. 2010, Whitehead and Bowers 2014).

Amides in *Piper reticulatum* fruit shape mutualistic and antagonistic fruit interactions

There are multiple lines of evidence that amides in fruit pulp or seeds can affect the outcome of interactions with both fruit mutualists and antagonists. In laboratory bioassays, Whitehead and Bowers (2014) found that individual amides and amide-rich extracts have strong dose-dependent negative effects on three different fungal strains isolated from rotting fruits. The effects of amide mixtures on fungi were non-additive and included synergistic or antagonistic effects that varied across fungal taxa. The specific composition of amides in unripe fruits was more potent than that of ripe fruits – up to 12 times more bioactive at the same total amide concentration – suggesting plants may 'fine-tune' chemistry for different developmental stages (Whitehead and Bowers 2014, Fig. 1A). In addition to the effects on fungi, amides had weak negative effects on the feeding preferences of an insect seed predator (*Sibaria englemani*, Hemiptera: Pentatomidae, a *Piper* specialist), though these effects were limited to certain compounds (Whitehead and Bowers 2014). Beyond the general evidence of amide bioactivity, there is also evidence that plants benefit from producing complex amide mixtures: in natural populations, higher amide richness in seeds is associated with lower levels of seed damage (Whitehead et al. 2013). Overall, amides appear to serve a key function in both fruit pulp and seeds by acting as a defense against fruit pests.

However, the benefits of amides as a defense may come with a cost in terms of seed dispersal and seedling success. Whitehead et al. (2016) found that amides alter the preferences of three different species of *Carollia* bats (Fig. 1B), reducing both the number of infructescences bats remove and the proportion they consume from an infructescence once they begin feeding. As expected based on the interaction diversity hypothesis (Box 1), the strength of these effects varied across compounds and three *Carollia* species (Whitehead et al. 2016). Fruits containing high doses of amides were often removed but then dropped below a feeding roost, sometimes with as little as 10% of the fruit consumed. *Piper* seeds inside intact fruits have very low germination rates (Sides and Whitehead unpubl.), and there is likely high competition below bat feeding roosts, which could, in some contexts, mean these seeds have little chance of survival. However, ripe *Piper* fruits on the forest floor are often rapidly discovered by ants and carried away piecemeal (Clemente and Whitehead 2020). The fate of these secondarily-dispersed seeds is still being addressed in our ongoing work, and these results will help shape understanding of the ultimate fitness consequences of amide-mediated changes in bat behavior.

In addition to the effects on bat foraging behavior, amides also have important effects on bat physiology that could affect both plant and bat fitness. Baldwin and Whitehead (2015) found that amides from *Piper reticulatum* reduced the

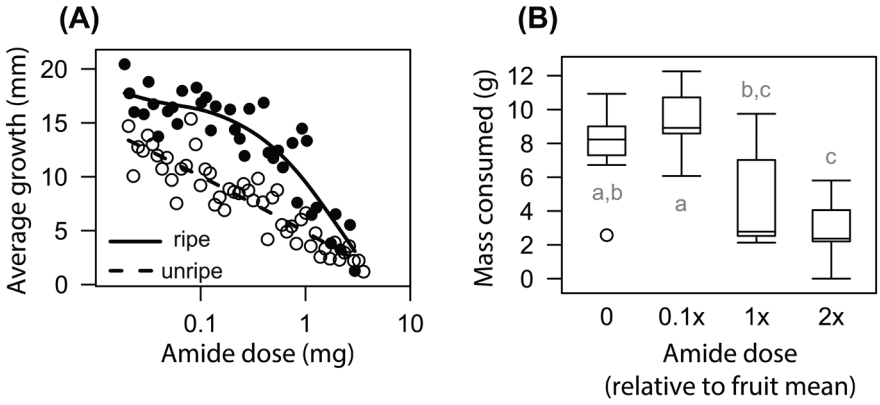

Figure 1. Amides likely represent a defense tradeoff in fruit, defending against fungi (A) but reducing the preferences of seed-dispersing bats (B). To test the effects of amides on fungal growth (A), amides were extracted from unripe and ripe fruits, added to agar media and inoculated with unidentified fungal strains isolated from rotting *P. reticulatum* fruits. The composition of amides from unripe fruits is significantly more potent (at $\alpha = 0.05$) than that from ripe fruit at the same concentration. To test the effects of amides on bat preference (B), amides were extracted from ripe fruits and fed to bats at different concentrations relative to the average extracted from one fruit (1x). Note 2x is still within the range of concentrations bats experience in ripe fruits (Fig. 3). Data are from Whitehead and Bowers (2014) (A) and Whitehead et al. (2016) (B); refer to those studies for methodological details and additional interpretation.

retention time of seeds in bat guts (Fig. 2). At the high end of the range of natural variation in amides, seed passage could be as fast as three minutes, which may severely limit nutrient assimilation by bats. Although there was no evidence that gut passage or variation in retention time predicted germination success in lab trials (Baldwin and Whitehead 2015), altered seed treatment in bat guts due to rapid passage could impact the quality of dispersal in myriad ways, including effects on dispersal distances, the number of seeds deposited per defecation, seed chemistry, or the seed microbiome. Baldwin et al. (2020) modeled how amides may affect dispersal distances based on their effects on both gut retention and fruit removal times in bats, and estimated that the amide content typical of ripe *P. reticulatum* fruits could reduce dispersal distance by an average of 43.9% (Baldwin et al. 2020).

Fruits of *Piper reticulatum* contain a higher diversity of secondary metabolites than leaves or other organs

Whitehead et al. (2013) compared amide richness and abundance in unripe and ripe fruit pulp, seeds, flowers, leaves, and roots of *P. reticulatum* (Fig. 3). Thirty total amides were detected across all tissue types, and structures were elucidated for 13 of these compounds. In general, amide richness was higher in reproductive tissues, especially seeds, compared to leaves and roots (Fig. 3). Seeds also had the highest total amide concentration. Ripe fruit pulp had lower concentrations than other parts but still high chemical richness that exceeded that of leaves (Fig. 3).

More recent evidence suggests that the pattern of higher chemical diversity in *Piper reticulatum* fruits relative to leaves is not limited to amides. Using a non-targeted LC-MS-MS based metabolomics approach, Schneider et al. (2021) compared chemical composition across leaves, unripe fruit pulp, ripe fruit pulp, and seeds for 12 species of *Piper*. Patterns were variable across species, but *Piper reticulatum* contained an estimated 588 total secondary metabolites across all tissue types. Many of these compounds (256 total) were shared across tissues, but at least 272 compounds spread across several classes were unique to fruits (found in fruit pulp or seeds but not in leaves), compared to only 60 that were unique to leaves (Schneider et al. 2021, Fig. 4). On average, samples of unripe fruit pulp, ripe fruit pulp, or seeds contained 23–34% higher chemical richness than leaves (Schneider et al. 2021, Fig. 5). Interestingly, the four tissue types did not differ in the average structural complexity of composition (Schneider et al. 2021), suggesting the increased richness in *P. reticulatum* fruits relative to leaves is likely due to additional numbers of structurally-related compounds within chemical classes.

In sum, the existing data from *P. reticulatum* shows that fruits contain a higher diversity of secondary metabolites than leaves, and are particularly rich in amides (Whitehead et al. 2013, Schneider et al. 2021). Amides have multiple functional roles in fruits, affecting defense against fungal pathogens and insect seed predators (Whitehead and Bowers 2014) and the behavior and physiology of seed-dispersing bats (Baldwin and Whitehead 2015, Whitehead et al. 2016). These effects can have cascading consequences in the seed dispersal process, affecting, for example, secondary seed dispersal and dispersal distance (Baldwin et al. 2020, Clemente and Whitehead 2020). Together, these studies suggest that amides may have evolved largely for their ecological roles in protecting seeds and shaping the quality of seed dispersal. This may also be true for the many other compounds that are unique to fruits or occur in both fruits and leaves (Fig. 4). Overall, our case study is consistent with the fruit-driven diversity hypothesis, but does not exclude other mechanisms that contribute to phytochemical diversity. Additional data will help us more fully understand the fitness consequences of amides and the broad generality of these results across systems.

Integrating frugivory and chemical ecology: a roadmap

To better understand the extent to which fruit–frugivore interactions have shaped phytochemical diversification, we suggest three broad approaches: 1) comparative chemistry across plant organs that includes fruits (both seeds and surrounding appendages); 2) ecological studies that address how intraspecific variation in fruit phytochemical composition and diversity affect fruit–frugivore interactions and plant fitness, and; 3) macroevolutionary studies that examine whether patterns of phytochemical composition and diversity at the whole-plant level reflect a signature of selection from fruit–frugivore interactions. Below we further expand on these approaches and make specific predictions for expected patterns based on the fruit-driven diversity hypothesis.

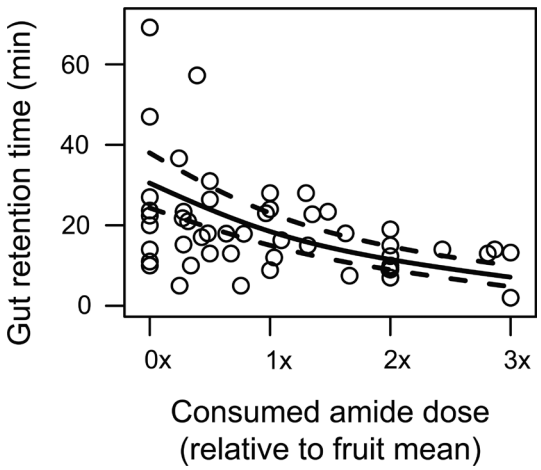

Figure 2. Amides can reduce the gut retention time of seeds in bat guts. Amides were extracted from ripe fruits and fed to bats at different concentrations relative to the average extracted from one fruit (1x). Note 3x is still within the range of concentrations bats experience in ripe fruits (Fig. 3). Data are from Baldwin and Whitehead (2015); refer to that study for methodological details and additional interpretation.

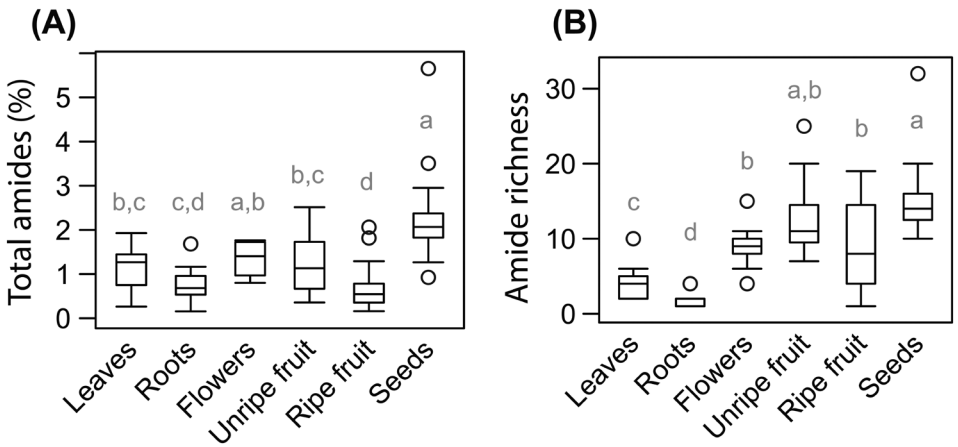

Figure 3. Average amide concentration (A) and richness (B) in different tissues of *P. reticulatum.* Richness represents the number of detected amides in GC-MS analysis. Boxes show the median, 25th and 75th percentile, and whiskers show the maximum and minimum across n = 16 plants. Different letters represent statistically supported differences at $\alpha = 0.05$ in a Tukey's HSD post-hoc comparison following a linear mixed model. Data are from Whitehead et al. (2013); refer to that study for further methodological details and interpretation.

Comparative chemistry across plant organs

Comparative chemistry across plant tissues or organs can provide key baseline data for understanding metabolite function and how selective pressures in different parts may have shaped plant chemical traits. Yet, there are still only a limited number of studies that have provided these comparisons in an ecological context for wild plants (Cipollini et al. 2002, Beckman 2013, Whitehead et al. 2013, Maynard et al. 2020, Schneider et al. 2021). To understand phytochemical diversity, we especially need studies that document all detectable (known and unknown) compounds. This may include non-targeted metabolomics, which seeks to document all detectable compounds in a sample, or more targeted approaches that focus on particular compound classes, but still report all detected (known and unknown) compounds in that group (e.g. all detectable amides, or all detectable flavonoids). Both are highly valuable and complementary, as non-targeted approaches can provide the best broad view of diversity, but focusing on particular classes with known functions can provide a more clear view of how diversity links to ecological and evolutionary processes.

If interactions involving fruits have driven phytochemical diversification in a particular group of plants or class of compounds, then fruits (relative to leaves or other plant parts) should be highly diverse in terms of the numbers of compounds (richness) and the structural complexity of compound mixtures. In the case study above, we demonstrate support for this prediction for one species, *Piper reticulatum.* To our knowledge, the first test of this hypothesis using a metabolomics approach across multiple species is provided by Schneider et al. (2021). In that study, data from 12 species of neotropical *Piper* provide further overall support for the hypothesis, but also emphasize that patterns will vary across species. Across all 12 species, fruits had higher total numbers of estimated compounds (higher gamma diversity, Box 2) and more unique compounds (92 compounds found only in fruits and never in leaves, compared to only 4 compounds found only in leaves but never in fruits). However, different species varied in terms of whether fruits or leaves had a higher average compound richness – three species had higher richness in fruits and two species had higher richness in leaves. This emphasizes that no single hypothesis is likely to explain patterns of phytochemical diversification in all species (even a closely related group with similar dispersal ecology) and that we need further studies in a variety of systems to better understand the generality of the patterns in our case study.

Beyond improving our understanding of relative levels of diversity across tissues, comparative chemistry can provide a critical starting point for understanding metabolite function. Fruits may often contain many unique compounds that are not found in leaves or other plant parts, and, furthermore, different specific tissues within the fruit pericarp (e.g. exocarp, mesocarp and endocarp) or seed (e.g. radical, hypocotyl, cotyledons, endosperm and seed coat) may be distinct from one another. In cases where compounds are highly tissue-specific, it is possible to greatly narrow down the unique selective pressures that may drive their production. For example, a volatile compound that occurs primarily in ripe fruit pulp (but not unripe pulp or seeds) is a good candidate compound to explore for its role in the attraction of animal seed dispersers. In cases where compounds are shared across tissues, further research can seek to determine whether this is due to adaptive multifunctionality (Izhaki 2002, Neilson et al. 2013) or physiological constraints (Adler et al. 2006, Kessler and Halitschke 2009, Keith and Mitchell-Olds 2019).

In addition to documenting differences in mean composition, studies focused on comparative chemistry should also seek to understand how variability in composition (i.e. beta diversity, Box 2) may differ across tissues. Spatial variation in chemical traits could occur at multiple scales: across repeating organs on a plant, across individual plants in a population, and across species in communities. Temporal variation could occur during development and dispersal or in response

to biotic interactions (e.g. induced defenses). Numerous metrics have been described in the species diversity literature that quantify beta diversity as either the overall multivariate variability in a dataset or the turnover along a specified spatial or temporal gradient (Anderson et al. 2011). Researchers are just starting to apply these concepts to phytochemical diversity (Wetzel and Whitehead 2020), and our understanding of links between plant interactions and phytochemical beta-diversity is still in its infancy. Initial evidence suggests that, at least in some cases, variability in chemistry may be even more important than average trait values in shaping the outcome of species interactions (Moore et al. 2014, Shimada et al. 2015, Wetzel et al. 2016, Pearse et al. 2018). We further discuss how this variability may shape fruit–frugivore interactions in the next section. Here, we emphasize that we need further studies that compare the extent and nature of variability across different organs to better understand both the evolutionary causes and ecological consequences of phytochemical variability. Initial evidence suggests that beta-diversity across individual plants and species may be even higher for fruit chemical traits than leaves (Schneider et al. 2021), and further studies comparing beta-diversity across organs may provide key insights into the major selective pressures that shape variation in chemical traits.

Effects of fruit phytochemical composition and diversity on fruit–frugivore interactions

Although a growing number of studies are starting to explore the chemical ecology of frugivory and seed dispersal, we still only have a limited number of examples where researchers have shown how intraspecific variation in fruit chemical traits correlates with the outcome of biotic interactions, such as seed predation or removal rates, that may affect fitness. An underlying assumption of the fruit-driven diversity hypothesis is that secondary metabolites have adaptive roles in fruits, which has been supported in several systems (Whitehead and Bowers 2013), but could also be debated (Cipollini and Levey 1998, Eriksson and Ehrlén 1998). Thus, we need further investigation of how different aspects of secondary metabolite composition (e.g. quantities of specific compounds, quantities of compound classes or shifts along multivariate axes) that vary within species are correlated with the outcome of fruit ecological interactions across a wide range

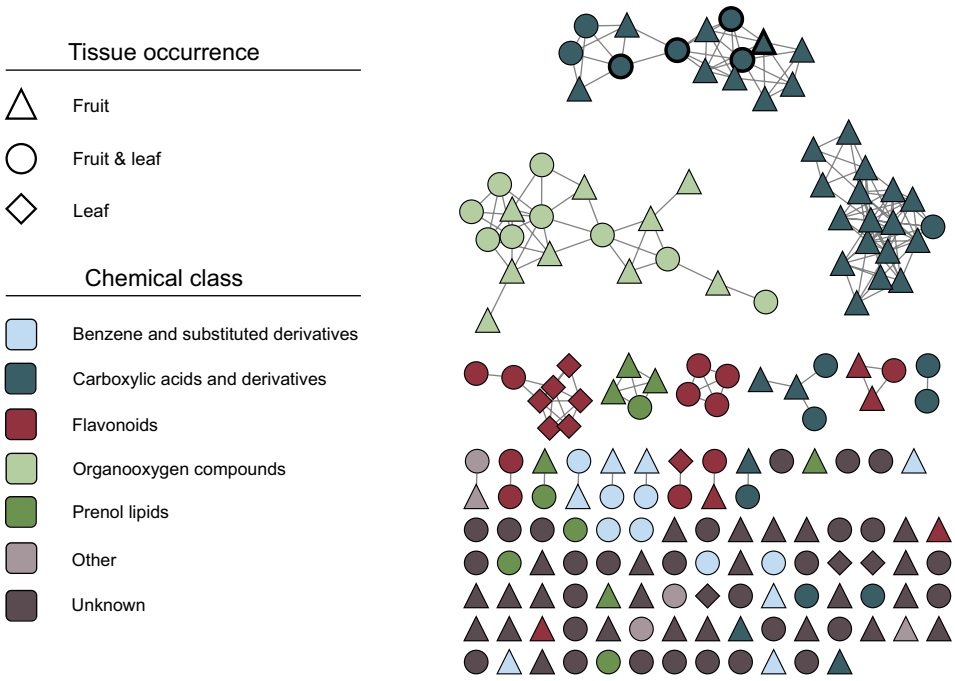

Figure 4. Molecular network of 171 features from *Piper reticulatum* leaves, unripe pulp, ripe pulp, and seeds (n = 3 samples per tissue) generated with GNPS (Wang et al. 2016). Each node represents a molecular feature (i.e. a putative individual secondary metabolite), and the connectedness of nodes by edges represents chemical similarity between features. Features in the network represent the subset of all detected metabolites (588 total) that were characterized in tandem MS analyses. Symbols indicate whether the feature was detected in fruit (unripe pulp, ripe pulp and/or seed), leaves, or both. Colors indicate chemical class according to the standardized ClassyFire hierarchical chemical classification (Djoumbou Feunang et al. 2016). Five features had library or neighborhood matches in ClassyFire as amides (grouped in ClassyFire as 'carboxylic acid amides', a sub-class of carboxylic acid derivatives) and are highlighted in bold in the top cluster; other nodes in this cluster had no matches at this level and may be amides or related compounds. Note that most clusters are composed of compounds unique to fruits or shared between fruits and leaves, suggesting an evolutionary origin in fruits for several classes of compounds. Only one group of flavonoids show the opposite pattern, suggesting an evolutionary origin in leaves. Data are from Schneider et al. (2021); refer to that study for further methodological details and interpretation.

of study systems. Many compounds have multiple ecological effects (Izhaki 2002), and long-term studies in model systems will be essential to truly understand the fitness consequences of particular fruit metabolites.

Beyond demonstrating that particular compounds or multivariate compound compositions can have adaptive value, a next step is to specifically examine whether fruit chemical diversity per se can provide a selective advantage to plants. The interaction diversity hypothesis (Box 1) predicts that individual plants with higher chemical richness or structural diversity will have higher fitness because different compounds will provide different advantages in different pairwise interactions. This is a different mechanism than, for example, a diverse mixture providing a more effective defense against any single antagonist due to compound synergies (the synergy hypothesis, Box 1), or providing a more specific odor blend that can attract a specific mutualist (the specific communication hypothesis, Box 1), and leads to different predictions. Individual plants that have more unique defensive compounds in fruits may not be better defended against any single seed predator, but they will be better defended against a complex community of seed predators, pathogens, and other antagonists and ultimately have higher fitness than neighboring plants with low fruit chemical diversity (Whitehead et al. 2021). For classes of compounds that function to increase the effectiveness of mutualistic interactions (e.g. attractants, behavioral modifiers), higher chemical richness may be associated with an increased diversity of mutualistic consumers, potentially leading to higher total seed dispersal effectiveness across the complete landscape of interactions (Schupp et al. 2010). To test these and other predictions, we need studies that characterize both chemical diversity and ecological or fitness correlates across a large number of individuals in a population.

Furthermore, studies seeking to relate chemical diversity to ecological interactions should focus not only on the complexity of phytochemical composition (e.g. richness, structural diversity), but also the role of variability in composition (i.e. beta diversity, Box 2) at multiple spatial and temporal scales. In general, consumers experience negative consequences of variation in plant traits due to the increased costs associated with foraging, predator avoidance, nutrient assimilation, and detoxification on variable diets (Adler and Karban 1994, Wetzel et al. 2016, Pearse et al. 2018). Thus, selection by antagonists is expected to increase plant trait variability at multiple scales (Herrera 2009, Sobral et al. 2014, Salazar et al. 2016, Massad et al. 2017). In contrast, selection by mutualists could be in the opposite direction, whereby mutualists exert stabilizing selection on chemical traits (Herrera 2009, Sobral et al. 2010). For example, Shimada et al. (2015) found that rodents removed fewer seeds from oak trees that had higher sub-individual variance and skew in the distribution of tannin content across individual acorns. In contrast, individual differences in average tannin content had no effect, confirming the important, but underappreciated role that variability may play in shaping the outcome of interactions. However, even if individual dispersers select for low variability, diverse mutualistic guilds (e.g. seed disperser assemblages that include many species of birds, bats, and other mammals) may ultimately select for high variability (Herrera 2009).

In addition to overall variability in composition across fruits, various metrics of beta diversity can also describe autocorrelation or directional turnover in composition along a specified gradient (Box 2, Anderson et al. 2011, Wetzel and Whitehead 2020). Considering the dramatic shifts that fruits experience in their abiotic and biotic environment during pre-dispersal, dispersal, and post-dispersal processes, we predict high directional turnover in composition through time. For example, past work on volatiles in animal-dispersed fruits has suggested clear shifts in chemical composition during ripening that are likely essential for providing olfactory signals of ripeness and nutritional rewards to dispersers (Nevo et al. 2019). Comparisons across 98 species showed that these shifts are physiologically constrained such that compositional shifts occur mostly within a biochemical pathway, but that the specific individual compounds and relative abundances may shift dramatically (Nevo et al. 2020b). Similarly, the specific composition of individual compounds within a class of defensive compounds should shift predictably during ripening to still allow some level of protection while avoiding negative impacts on dispersers (*Piper reticulatum* case study, above). Overall, these patterns suggest that the changing temporal landscape of fruit selection pressures may be particularly important in explaining within-class chemical diversity (Nevo et al. 2020b). Future work should seek to explain how the extent and pattern of compositional shifts through pre-dispersal, dispersal, and post-dispersal processes shape overall plant fitness by allowing fruits to adapt to the unique

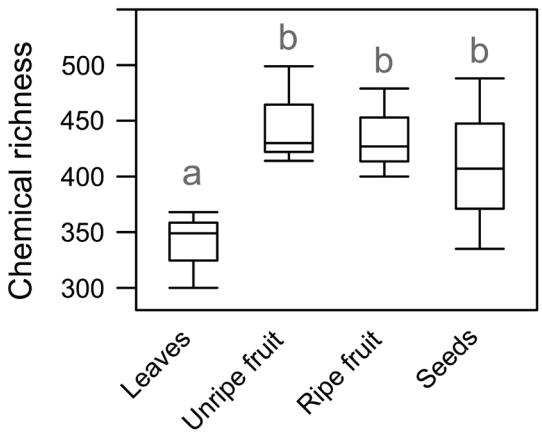

Figure 5. Average chemical richness is higher in fruit tissues (unripe fruit pulp, ripe fruit pulp, and seeds) compared to leaves in *P. reticulatum.* Richness represents the number of detected metabolites in n = 3 samples per tissue using a non-targeted LC-MS-MS based approach. Boxes show the median, 25th and 75th percentile, and whiskers show the maximum and minimum. Different letters represent statistically supported differences at $\alpha = 0.05$ in a Tukey's HSD post hoc comparison following a linear mixed model. Data are from Schneider et al. (2021); refer to that study for further methodological details and interpretation.

selective pressures they experience at these different stages despite developmental constraints and ecological tradeoffs imposed by varying interactions.

Macroevolutionary patterns of phytochemical composition and diversity across tissues

Only a few studies have examined fruit secondary metabolite evolution at the macroevolutionary scale (Lomáscolo et al. 2010, Gripenberg et al. 2018, Nevo et al. 2020b). Our ongoing work is using phylogenetic-comparative methods across a large number of tropical trees and shrubs that vary in dispersal mode (including bird-, bat- and non-animal dispersed species) to help fill this gap, but much more work is needed at this level to understand how fruit interactions may have influenced the evolution of plant chemical traits. If fruit consumers have shaped the evolution of fruit chemical traits, we expect that differences in the evolutionary history across species will be reflected in different phytochemical compositions. For example, we expect convergence in chemical traits across lineages that share disperser assemblages (e.g. syndromes associated with mammal versus avian-dispersed species; Cipollini et al. 2002, Lomáscolo et al. 2010) or share similar pressures from antagonists (e.g. syndromes associated with increased fungal defenses in seeds that must survive and germinate in very wet soils).

More specifically to the fruit-driven diversity hypothesis, we expect plants that interact with a larger diversity of fruit consumers to have higher phytochemical diversity. Compared to abiotically or autochorously-dispersed species, animal-dispersed plants likely interact with a large number of consumers, including mutualists and a large number of antagonists due to their high nutritional content. Antagonist and mutualist communities are likely to exert conflicting selection pressures, and the relative importance of selection from different groups will shift over time and throughout development. In general, conflicting selection pressures are likely to increase chemical trait diversity (Siepielski and Benkman 2010). Thus, we expect that secondary metabolite richness, structural complexity, and intraspecific variability should be highest in fruit of animal-dispersed compared to non-animal dispersed species. Additionally, we expect that species that experience stronger pressure from diverse communities of antagonists (e.g. species that occur in tropical vs temperate zones; Salazar and Marquis 2012, Hargreaves et al. 2019) should have higher richness, structural diversity, and intraspecific variability than species that experience more stable selective pressure from a smaller guild of antagonists. Because many other factors may also contribute to patterns in phytochemical diversity across lineages and communities, these comparisons are best made while controlling for phylogeny and large differences in abiotic conditions. Furthermore, it is important to note that the macroevolutionary patterns observed across species could be different from the patterns observed across individuals within a species (Agrawal and Hastings 2019). For example, within a species, we expect that fruits producing more diverse mixtures of defensive metabolites will experience lower levels of damage from seed predators. However, across species, we might expect that species that have historically experienced the highest level of seed predator pressure will have higher chemical diversity because of a long evolutionary history that has led to phytochemical diversification over time.

Conclusion

Theoretical and empirical work in chemical ecology has largely focused on leaves and the role of leaf herbivores in driving the evolution of secondary metabolites. Fruits, in contrast, are often assumed to be chemically benign or contain secondary metabolites largely as a result of strong selection for the defense of leaves. Here, we suggest that, at least in some plant lineages, selection pressures in fruits may be the primary driver of phytochemical diversification. The fruit-driven diversity hypothesis is not mutually exclusive with other mechanisms that generate phytochemical diversity and may not be important in all systems, but considering the diversity of interactions surrounding fruits and their impacts on fitness, we argue that frugivores are likely an underappreciated selective force shaping overall plant chemical composition and diversity. Evidence from *Piper* and from other systems (e.g. capsaicin in chilies, emodin in buckthorn, and glycoalkaloids in *Solanum*; Cipollini et al. 2002, Levey et al. 2007) has clearly demonstrated that fruit secondary chemistry can be adaptive and have complex and cascading consequences for both mutualistic and antagonistic fruit interactions at the pre- and post-dispersal stage. Our case study further illustrates that, at least in some systems, the chemical diversity of fruits can far exceed that of leaves. Understanding the generality of these patterns will require much additional data in a variety of plant lineages and ecosystems. These studies promise to provide a more whole-plant context for chemical ecology and greatly expand our understanding of the evolutionary origins of phytochemical diversity. Simultaneously, exploring the role of secondary metabolite diversity in fruits will rapidly advance our understanding of the mechanisms driving seed dispersal, a critical ecological process that shapes plant regeneration and the structure of entire ecological communities.

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Susan R. Whitehead: Conceptualization (equal); Data curation (lead); Funding acquisition (equal); Visualization (supporting); Writing – original draft (lead); Writing – review and editing (lead). **Gerald F. Schneider**: Data curation (supporting); Visualization (supporting); Writing-original draft (supporting); Writing – review and editing (supporting). **Ray Dybzinski**: Funding acquisition (equal); Visualization (lead); Writing – original draft (supporting); Writing – review and editing (supporting). **Annika S. Nelson**: Writing – original draft (supporting); Writing – review and editing (supporting). **Mariana Gelambi**: Writing – original draft (supporting); Writing – review and editing (supporting). **Elsa Jos**: Writing – original draft (supporting); Writing – review and editing (Supporting). **Noelle G. Beckman**: Conceptualization (equal); Funding acquisition (equal); Writing – original draft (supporting); Writing – review and editing (supporting).

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