Evolution of chemodiversity – From verbal to quantitative models

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Abstract

Plants harbour an astonishing amount of *chemodiversity*, i.e., diversity of specialized metabolites, at different scales. For instance, individual plants can produce a large number of different specialized metabolites and individuals in a population can differ in their metabolite composition. Given the ecological and economic importance of plant chemodiversity, it is important to understand how it arises and is maintained over evolutionary time. For other types of biodiversity, i.e., species diversity and genetic diversity, quantitative models, that is, mathematical models and computer simulations, have long played an important role in addressing such questions. Here we review models and hypotheses for the evolution of plant chemodiversity and, in particular, explore what quantitative models have been proposed so far and what gaps there are in quantitative modeling of chemodiversity. For each model or hypothesis we review its ingredients, i.e., the biological processes that are assumed to shape chemodiversity, the scales at which the model explains or claims to explain chemodiversity, and the extent to which the model has been formalized as a mathematical or simulation model. From this review, a mixed picture emerges. We identified a small number of quantitative models for the evolutionary dynamics of plant chemodiversity. In addition we found a number of models that use equations to derive an optimal defense, but are not dynamic. Many influential models, however, have remained verbal so far. Furthermore, we identify some quantitative models used for genetic variation that have not been used for chemodiversity so far, but could be easily extended to do so. We end by outlining our vision for future model building for the evolution of plant chemodiversity.

Keywords: chemodiversity, phytochemical diversity, secondary metabolites, mathematical model, simulation

1 Introduction

Chemodiversity is the diversity of specialized metabolites (SMs) at different levels of organisation – from single tissues to entire communities (see Wetzel & Whitehead, 2020, for a review). SMs are compounds produced by an organism that are not directly involved in its most basic survival and reproduction mechanisms, but may be important for the interaction with herbivores, pollinators, and conspecifics, or protect from abiotic stresses (Wetzel & Whitehead, 2020). SMs are also often called 'secondary metabolites' (Stone & Williams, 1992; Cipollini & Levey, 1997; Hamberger & Bak, 2013; Moore *et al.*, 2014; Dyer *et al.*, 2018; Rokas *et al.*, 2020), 'phytochemicals' (in plants, Allstadt *et al.*, 2012; Richards *et al.*, 2016; Dyer *et al.*, 2018; Defossez *et al.*, 2021), or 'natural products' (Firn & Jones, 2003). Examples of SMs are alkaloid or terpenoid defense chemicals, flavonoid pigments, and numerous other compounds and compound families with known or unknown properties. The set of SMs that an organism produces is called its chemotype.

Chemodiversity can be quantified in various ways (Wetzel & Whitehead, 2020): it can focus on particular compound families or not, and it can be measured within as well as between units of scale. For example, both the number of SMs per individual and the differences in SMs between individuals in a population are aspects of chemodiversity.

The existence of widespread chemodiversity is puzzling from an evolutionary perspective. SMs are often synthesized in complex metabolic pathways that involve multiple enzymes modifying a precursor metabolite into the SM over several steps (Jones & Firn, 1991; Firn & Jones, 2003, 2009). Given the inherent costs of these pathways, one would expect evolution towards a small number of the most beneficial metabolites (Jones & Firn, 1991). Despite this, a high diversity of SMs has been found within and between plant populations and to some extent also in fungal and bacterial populations (Calf *et al.*, 2018; Rokas *et al.*, 2020; Li *et al.*, 2020; Defossez *et al.*, 2021). Evidently, there are mechanisms for the maintenance of this chemodiversity. These mechanisms are what models around chemodiversity attempt to elucidate.

For both species diversity and genetic diversity, quantitative models – both mathematical and simulation models – have long been an important part of scientific inquiry (see e.g. Wright, 1937; Kimura, 1983; Hubbell, 2001). They are for instance used as proof-of-concept models to test the validity of verbal models (Servedio *et al.*, 2014). Other functions are to generate predictions and hypotheses that can be tested empirically (Servedio *et al.*, 2014) and to estimate parameters from data. Running *in silico* experiments with quantitative models can give clues to what aspects of a system are the most relevant, and thus which measurements should be taken in an experiment. In that way they can make empirical studies more efficient, and sometimes provide a statistical model that can be fit to data. Additionally, a good quantitative model can unify several studies that differ in methodology so they form a coherent narrative (Otto & Rosales, 2019). Because of these various contributions of quantitative models to scientific inquiry, we argue that chemodiversity too should be investigated in this way.

A better understanding of the evolution of chemodiversity that might be conferred by such models also has an applied relevance. For instance, models that predict local adaptation and geographic structure of chemodiversity (Calf *et al.*, 2018; Defossez *et al.*, 2021), could be taken into account in conservation planning, the way genetic diversity already is (see e.g. Frankham *et al.*, 2002, Ch. 16). Moreover, models for the evolution of chemodiversity might help predict why some introductions of plants or herbivores to new places succeed while others do not, which will be discussed further in Box 3.

In this article, we review the work that has been done so far on developing verbal and, in particular, quantitative models for chemodiversity. For each model, we discuss which biological processes it focuses on and at which scale it addresses chemodiversity. We are not the first to review possible explanations for chemodiversity. There are for instance in the excellent reviews by Stamp (2003), Moore *et al.* (2014), Dyer *et al.* (2018), and Wetzel & Whitehead (2020); therefore we do not want to dwell on reviewing the empirical support for the different explanations for chemodiversity. Instead, we focus on how the verbal models described in those reviews have been tested through quantitative models, and discuss how quantitative models for other types of diversity could be adapted to modeling chemodiversity. We also discuss some of the empirical work that has been done on them to elucidate how quantitative models can connect verbal models and empirical studies. We end by outlining important avenues for future research.

2 Chemodiversity models

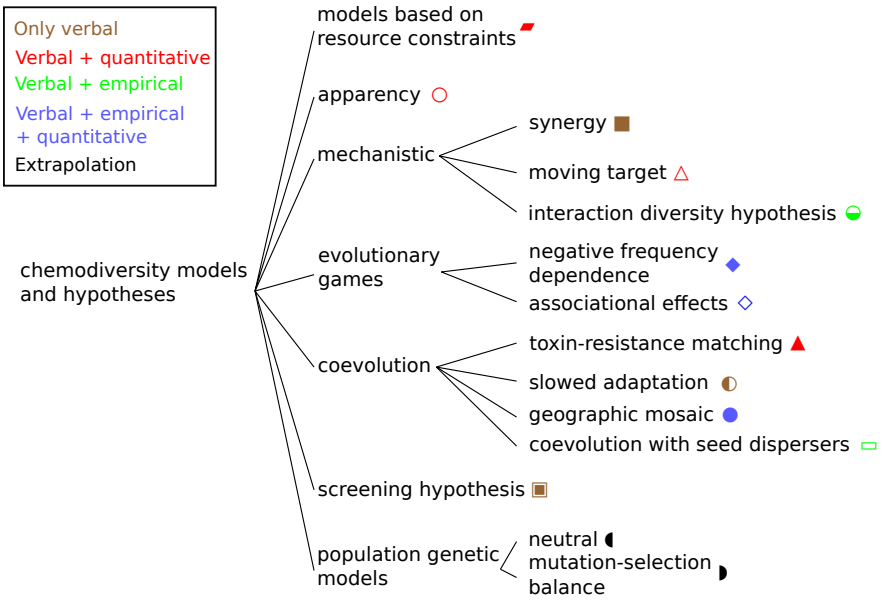

Figure 1: Tree showing a rough conceptual grouping of chemodiversity models and hypotheses. Each model or hypothesis has a unique symbol, which is then used in Fig. 2 to indicate which ingredients are part of the model and in Fig. 3 to indicate at what scales the model can explain chemodiversity. Note that similarity in shape between symbols here does not indicate any relationship between models. Models with brown symbols have only a verbal model, green symbols additionally have empirical evidence, red a quantitative model, and blue both a quantitative model and empirical evidence. Black symbols stand for models that have, to our knowledge, not been previously used in the context of chemodiversity but that we argue can be extended to modeling chemodiversity (i.e. they are our extrapolation).

There is currently no single unifying theory that can explain chemodiversity at all scales and within as well as between units at each scale. However, there are numerous hypotheses and verbal models (Fig. 1), each of which focuses on a different set of "ingredients", i.e., biological players and processes that can contribute to shaping chemodiversity (Fig. 2, Box 1). Moreover, the different hypotheses and models cover different scales of organization (Fig. 3, Box 2). Although there are connections and overlaps between many hypotheses and they are certainly not all mutually exclusive, to provide some structure for this review, we have placed all models into a hierarchy and grouped similar models together (Fig. 1). Note that sometimes different terms are used in different publications to describe the same or similar models (for instance in the excellent reviews of Stamp, 2003; Dyer *et al.*, 2018; Wetzel & Whitehead, 2020).

Although these various hypotheses on the development and maintenance of chemodiversity are commonly used to generate hypotheses for empirical research (Li *et al.*, 2020; Whitehead *et al.*, 2021), there are fewer

quantitative models of these hypotheses. Here, we will review extant quantitative models of chemodiversity, as well as research that could serve as a starting point for the creation of models to fill the numerous gaps in the state of the art.

Box 1: Ingredients of chemodiversity models

Here we explain the biological processes that act as key ingredients (Fig. 2) in current models and hypotheses for chemodiversity and, without aspiring to completeness, give some exemplary empirical studies supporting their role in shaping chemodiversity. How the ingredients are used in the various theoretical models is explained in section 3.

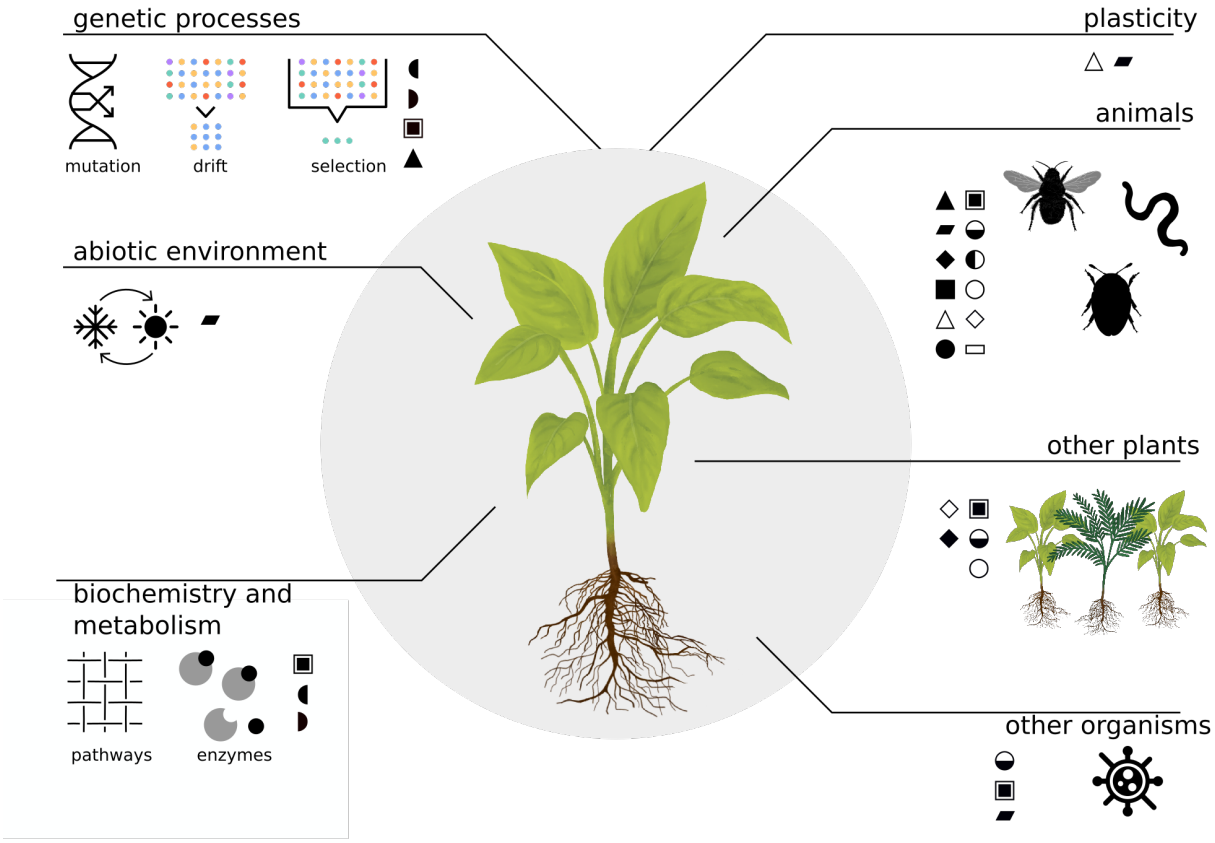

Figure 2: Overview of the key ingredients of chemodiversity models. To see which hypothesis/model a symbol stands for, please refer to Fig. 1. The ingredients listed in the figure are further explained in Box 1. Models and hypotheses are listed as connected to particular ingredients when these ingredients are key components of the model as described below in section 3.

- **Genetic processes**: SMs are produced by enzymes, which are coded for in genes, which in turn have regulatory genes. Therefore, genetic processes can give rise to chemodiversity. Genes for enzymes in the same metabolic pathway are often organized as operons, i.e. located closely together in the genome (Hamberger & Bak, 2013). Within these operons, gene duplication and subsequent divergence is common, which facilitates a flexible gene network that can undergo rapid evolution (Hamberger & Bak, 2013).

  Mutations may change the function of the enzyme coded for by a gene, so it has a different substrate profile or catalyzes different reactions and therefore produces different SMs than its ancestor. Meanwhile changes in gene regulation may change the abundance of SMs, and duplication and deletion may open new pathways of SM synthesis or close them off. Random mutations thus bring about genetic and consequently chemical diversity.

  Genetic drift is the phenomenon of changing allele frequencies through chance effects on reproduction and survival rather than natural selection based on fitness. A gene might become fixed or die out randomly within one population but not another, resulting in chemodiversity.

  Natural selection occurs when SMs which confer fitness benefits become more common, and SMs that confer fitness costs become more rare. Similarly, if there is a fitness benefit to lack of specificity in the production of SMs, this trait may be selected for.

- **The abiotic environment**, such as water and nutrient availability, temperature, or elevation, can determine how much a plant can invest in growth and defense (Bryant *et al.*, 1983; Coley *et al.*, 1985; Herms & Mattson, 1992; Hamilton *et al.*, 2008; Smakowska *et al.*, 2016; Monson *et al.*, 2021). Moreover, changes in the abiotic environment may cause stress responses that lead to changes in the concentrations of certain SMs or induce biosynthesis of new SMs (Agati & Tattini, 2010).

- **Biochemistry and metabolism**: Metabolic pathways for the production of SMs can involve multiple enzyme-catalyzed reactions and can be branched. Which new SMs can be produced by changes in these pathways is constrained by the extant pathways. Many of the enzymes involved with SM synthesis are "promiscuous", meaning that they accept multiple substrates and thus can produce multiple different SMs at low cost (Aharoni *et al.*, 2005). Thus, small modifications to the enzyme suite can have a profound impact on the metabolic pathway (Moghe & Last, 2015; Shoji, 2019).

- **Phenotypic plasticity**: Phenotypic plasticity can explain chemodiversity even among genetically identical individuals in a population. For example, an individual which experiences a more nutrient-poor micro-environment may invest these resources differently from an individual in the same population that experiences a more nutrient-rich micro-environment, leading to a variation in chemical composition (Stamp, 2003; Defossez *et al.*, 2021).

- **Interactions with animals** play a role in the majority of hypotheses and models for the evolution of chemodiversity and their importance has broad empirical support (Hambäck *et al.*, 2014; Calf *et al.*, 2018; Li *et al.*, 2020; Whitehead *et al.*, 2021). For example, Calf *et al.* (2018) found that there were differences between the composition and total amounts of glycoalkaloids produced by the bittersweet nightshade *Solanum dulcamara* sampled from different locations, and that slugs *Deroceras reticulatum* consistently showed preference for leaves from populations which produced fewer glycoalkaloids, which corresponded to populations where few slugs were present, hinting at local adaptation of plant populations. Moreover, plants produce various SMs that serve as visual, olfactory and/or gustatory signals to lure pollinators (Borghi *et al.*, 2021) and attract seed dispersers (Cipollini & Levey, 1997; Nevo *et al.*, 2018; Baldwin *et al.*, 2020). Furthermore, toxicity from SMs may influence frugivore behaviour and gut retention time of seeds, and in that way influence the spread of seeds (Cipollini & Levey, 1997; Baldwin *et al.*, 2020).

- **(Indirect) interactions with other plants:** Plants can interact directly with each other, for example through SMs that hinder the access to resources for other plants, e.g. heterospecific competitors. For example in *Brassica nigra*, the benefit of these SMs for the plants was higher when their strategy was rare (Lankau & Strauss, 2007, 2008). Interactions with other plants can also be indirect, often through interaction with other species in the environment (associational effects, Hambäck *et al.*, 2014). In *Piper* plants, more chemodiverse communities were found to have lower plant mortality and local species extinction than less chemodiverse communities (Salazar & Marquis, 2022).

- **Interactions with other organisms**: Separately from animal mutualists, a plethora of microbial mutualists are guided by plant SMs to their host, and the formation of symbiotic structures is induced by SMs (De la Peña & Loyola-Vargas, 2014), for example rhizobia (Cooper, 2004) and the hyphal branching of arbuscular mycorrhizal fungi (AMF) (Akiyama *et al.*, 2005). Differences in root exudates among populations within species have been found to alter the rhizosphere soil composition, leading to distinct soil chemical communities (Mueller *et al.*, 2020). Mutualists can in turn also affect patterns of chemodiversity. For example, endophytes and a symbiosis with AMF resulting in functional arbuscular mycorrhiza can modify the chemical composition of different plant tissues (Schweiger & Müller, 2015; Yadav *et al.*, 2022).

Box 2: Scales of chemodiversity

Chemodiversity can be quantified at various scales of organization (Wetzel & Whitehead, 2020). Terms such as richness and evenness, alpha, beta and gamma diversity that are familiar from species diversity are frequently used. However, different authors use different definitions for these terms (see e.g. discussion in

Kessler & Kalske, 2018; Li *et al.*, 2020), depending on the scales their work focuses on. Meanwhile Wetzel & Whitehead (2020) use alpha, beta and gamma diversity to describe general relationships between scales without either term referring to a specific scale. We argue that using the same terms to speak about different scales can be confusing, especially when comparing studies, and when there are more than two scales that may be of interest.

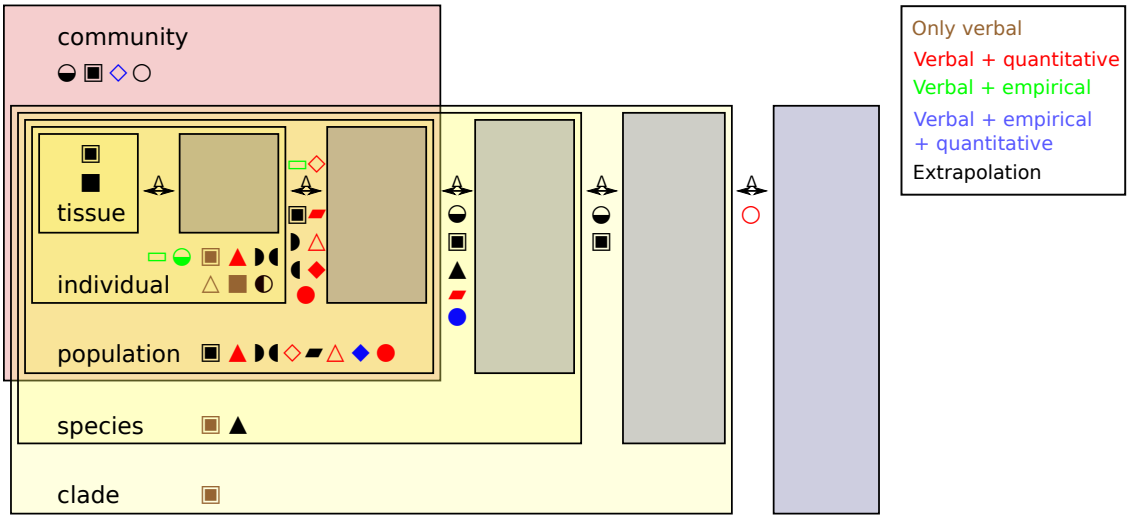

Figure 3: Schematic overview of the scales at which the different models and hypotheses (potentially) explain the evolution of chemodiversity. Please refer to Fig. 1 for the models each symbol refers to. Models with brown symbols have only a verbal model, green symbols additionally have empirical evidence, red a quantitative model, and blue both a quantitative model and empirical evidence. Black symbols stand for models that have, to our knowledge, not been previously used in the context of chemodiversity but that we argue can be extended to modeling chemodiversity (i.e. they are our extrapolation). For example, the filled red diamond next to the label "population" indicates that there is a quantitative model suggesting that negative frequency dependence can explain the maintenance of chemodiversity within a population and the same symbol between the two "individual" boxes indicates that it can maintain differences between individuals. For some symbols, we used different color in different places in the figure to indicate a different type of support for chemodiversity at the different scales. The community level is orthogonal to the species level because populations can be considered nested both in their species and in a multi-species community.

We argue that instead of using alpha, beta, and gamma diversity, it is more helpful to explicitly state the scales under consideration (tissue, individual, population, species, clade, or community) and additionally specify whether diversity within units at those scales or differences between units at those scales are considered (Fig. 3). For example one can quantify chemodiversity within individuals as the average number of SMs produced per individual, and between-population chemodiversity as number of SMs that are pro-

duced only by one of the populations. Note that between-unit chemodiversity is not the same as within-unit chemodiversity at the next higher scale. For example, the average number of metabolites that are not shared between randomly picked individuals in the population is not the same as the total number of metabolites in the whole population. At each scale, chemodiversity could be quantified using different metrics, e.g. metrics focusing on richness or evenness. In Fig. 3, we place all hypotheses at the scale or scales at which they have been shown to explain, claim to explain, or can be logically inferred to explain chemodiversity.

3 Model descriptions

3.1 Models based on resource constraints

The SMs an organism produces are limited by the resources the organism has access to. Furthermore, the organism may optimize SM production subject to constraints based on available resources or other abiotic or biotic factors (Fig. 2). These ideas are the basis for a group of models that aim to explain variation in quantitative defense level depending on abiotic and biotic conditions. For instance, according to the **carbon nutrient balance hypothesis**, the relative availability of carbon and nitrogen determines which types of compounds are preferentially produced (Bryant *et al.*, 1983). For instance, in low-carbon (i.e. shady) habitats, likely more nitrogen-based toxins are produced. While the hypothesis at times correctly predicts the chemicals which are found in empirical studies, it has come under criticism for frequently failing to do so (Hamilton *et al.*, 2008).

Many of the hypotheses in this group are based on the idea that a plant's investment in defense competes with investment into growth, reproduction, and storage. According to the **resource availability hypothesis** (Coley *et al.*, 1985; Hahn & Maron, 2016), plants in environments with low resource availability are selected to grow slowly and be well defended, whereas plants grown at high resource availability are selected to grow fast and invest less in defense. The **growth-differentiation hypothesis** (Herms & Mattson, 1992) and the **coordinated resource allocation hypothesis** (Monson *et al.*, 2021) expand on the resource availability hypothesis by modeling the maximum possible growth rate in more detail and by adding reserve safety margins, i.e., storage pools that allow plants to plastically respond to biotic and abiotic challenges (Fig. 2). Coley *et al.* (1985) also propose an equation (which is then extended by Herms & Mattson, 1992; Monson *et al.*, 2021) modeling how plant growth rate depends on investment in defense. In these models (at least with the selected parameter values), plant growth rate is optimized at intermediate defense levels and the optimal strategy depends on the maximum possible growth rate in the absence of herbivores, i.e. resource availability. Thus, if the microenvironments of individuals in a population differ in resource availability, these hypotheses thus offer a quantitative prediction for differences in defense levels between individuals within a population (Fig. 3). Analogously, differences between different populations of the same species are predicted if the habitats of the populations differ in resource availability. Similarly, in a verbal model ,Vannette &

Hunter (2011) assumes that the expression of plant defenses has a nonlinear relationship with the density of arbuscular mycorrhizal fungi (AMF), such that variation in plant defense between populations in different habitats could be explained by variation in AMF density.

Differences in defense levels between individuals or populations in response to environmental conditions are often based on phenotypic plasticity. Many SMs are not produced continuously, but induced when their effects are beneficial. For example, exposure to higher ultraviolet-B radiation induces the production of flavonoids, which are important SMs in photoprotection (Agati & Tattini, 2010). Using a simple mathematical framework called **error-management theory**, Orrock *et al.* (2015) computed the optimal herbivore cue strength at which a plant should express its induced defense. This threshold represents a balance between the costs of a false alarm and the costs of being attacked unprepared. Since these costs but also the exposure to herbivores might differ between different plant individuals and populations (Fig. 2), this model could explain chemodiversity in expressed defense between individuals and between populations (Fig. 3).

Clearly, plasticity cannot explain all differences in defense expression though because there are often also strong genetically-based differences in a controlled environment (see e.g. Garrido *et al.*, 2012). Populations and species as a whole can have the SMs they produce be selected to the typical resources in their location, consequently affecting their chemodiversity (Stamp, 2003; Hahn & Maron, 2016; Defossez *et al.*, 2021). However, at the intraspecific level patterns appear to be more complex, in part because locations with high resource availability also tend to be locations with high herbivore pressure, such that it is still unclear how much of intraspecific defense variation can be explained by the resource-availability hypothesis and other models based on constraints (Hahn & Maron, 2016).

3.2 Apparency

Feeny (1976) developed the **plant apparency hypothesis** as a verbal model where plants in a community produce different SMs depending on how easy it is for herbivores to find them relative to other plants in the community (Fig. 2): highly apparent plants (e.g. oak trees) produce high concentration, so called quantitative compounds that affect most herbivores; less apparent species (e.g. small annual plants) produce low concentration, highly toxic, so called qualitative compounds that some herbivores are immune to. The apparency hypothesis addresses chemodiversity at the community level as well as between clades (see Fig. 3).

Partial support that the verbal apparency model can work in principle, comes from a quantitative model by Yamamura & Tsuji (1995). They used an optimal control theory approach to find the optimal investment in growth vs. defense over the life time of the plant. With apparency understood as herbivory pressure, their model results supported the apparency hypothesis. They found that more apparent plants produced quantitative defenses whereas less apparent ones did not, while for those that did, the investment in de-

fense increased with apparency. With apparency understood as length of the growth period, only the first prediction was true and only under some parameter settings.

The plant apparency hypothesis has been criticized for being difficult to test in practice. SMs are not easily divided into quantitative and qualitative compounds, but often have properties of both. This makes it different to correlate apparency to the presence of quantitative and qualitative compounds (Stamp, 2003). However, which SMs are beneficial to particular plants in a population still might differ depending on their apparency.

3.3 Mechanistic

Hypotheses in this group focus on why having multiple defense SMs is beneficial to plants, but they do not explicitly consider evolution on the herbivore side (as opposed to the hypotheses in the next section).

The **synergy hypothesis** proposes that the effect of a mixture of SMs is more than the sum of the effects of each SM in isolation. These nonlinear effects of mixtures of SMs then explain why organisms produce multiple SMs. On the molecular level, synergistic effects are explained by molecular interactions between SMs, for example by one SM facilitating the movement of another SM across cell membranes (Richards *et al.*, 2016). The synergy hypothesis addresses chemodiversity within tissues and within individuals (see Fig. 3, and Wetzel & Whitehead, 2020). In empirical studies, it was found that mixtures of SMs affect generalist herbivores more than specialists (Richards *et al.*, 2016). Synergistic effects are difficult to study in the lab in part because it is difficult to isolate large enough amounts of the SMs involved (Dyer *et al.*, 2018). Here, quantitative modeling can be a useful tool to formulate hypotheses and guide experiments. So far, to our knowledge, such models do not exist.

The **moving target hypothesis** is a term proposed by Adler & Karban (1994) and used by Li *et al.* (2020) to refer to an inducible defense where a plant in a population randomly changes its phenotype in response to an attack by herbivores (Fig. 2). This is closely related to the 'novel weapons hypothesis' that is proposed in biological invasions (Box 3). Herbivores then cannot adjust effectively to this defense as it is unpredictable. Wetzel & Whitehead (2020) uses the term more broadly to cover hypotheses that describe changes in SMs within individuals over time, functioning like a moving target that herbivores have a hard time adjusting to, even if it does not follow the precise mechanisms described in Adler & Karban (1994). Relatedly, variation between plant individuals in a population can suppress herbivores because they need to adjust their detoxification apparatus when moving between plants with different defense metabolites, which costs energy and is not instantaneous (Pearse *et al.*, 2018). However, the conditions under which this phenomenon would give an advantage to individuals with rare metabolites and promote the evolution of chemodiversity between individuals are not well understood yet and would probably depend on the details of herbivore behavior.

Adler & Karban (1994) analysed a quantitative, stochastic, general plant-herbivore model. In this model, plants could have three defense strategies: a strategy that does not adapt to herbivore damage (constitutive defense), one where plants adapt to herbivore damage by randomly changing their defense (moving target), or one where all plants switch to whichever defense results in the most optimal growth rate in the current situation (optimal inducible defense). They found that in an environment with a fluctuating number of herbivores, the optimal inducible defense is superior when there is just one herbivore species. When there are at least two herbivores with different resistances to defense strategies, the moving target strategy is superior as long as the cost of defense is not too high. When the cost of defense is high, constitutive low levels of defense are superior.

Eagle-eyed readers will have noticed that this model only included chemodiversity on the within population and between-individual level, not on the within-individual level, although this is part of the verbal model (Fig. 3). It is arguable that the defense strategy is analogous to a chemotype. To make this an explicit chemodiversity model capable of investigating chemodiversity on the individual level, a chemical model would need to be added that simulates the actual metabolites.

The **interaction diversity hypothesis** (Kessler & Kalske, 2018; Whitehead *et al.*, 2021) suggests that plants possess a variety of SMs because they interact with a large number of other organisms (both antagonists and mutualists, as well as interspecific or intraspecific competitors, see Fig. 2) and different compounds are active in interaction with the different organisms. Independent selection on each compound, potentially in coevolution with the respective interacting species, then leads to the emergence of chemodiversity. Although the name is rather new, the idea is relatively old and has also been called the "common-sense scenario" (Berenbaum & Zangerl, 1996). Although the interaction diversity hypothesis mostly seeks to explain the diversity of SMs within an individual (Fig. 3, with empirical support by Whitehead *et al.*, 2021), the reasoning can be extended to explain chemodiversity at other scales. For example, if two populations or species differ in the set of interacting animals, we would expect differences in their metabolite composition. Although the ideas of multiple interaction partners driving chemodiversity is very prominent and well-supported, there do not currently appear to be any quantitative models based on this idea.

3.4 Evolutionary games

Models and hypotheses explaining plant chemodiversity or variation in defenses via evolutionary games focus on the competition of plants "playing" different defense strategies. That is, they have interactions with other plants as important ingredient (Fig. 2).

One way in which variation in defense traits can be maintained between individuals in a population and within populations (see Fig. 3) is **negative frequency dependence** where the respective rare phenotype or genotype has an advantage. Sato *et al.* (2017) and Augner *et al.* (1991) created quantitative models of

evolutionary change in plant defense in populations over time. Sato *et al.* (2017) found a pattern of negative frequency-dependent selection that could lead to stable coexistence of undefended plants and defended plants which paid a cost for their defense. The underlying mechanism is that when defended plants become too common, optimally foraging herbivores start to forage also on defended plants despite lower profitability because it is too costly to search for the few undefended plants. This matched up well with their data on a field site of *Arabidopsis halleri* that comprised hairy and glabrous morphs. Similarly, Augner *et al.* (1991), showed that, in a game-theoretical model of a grazed population with two morphs, both could coexist if the fitness benefit a defended player has if an undefended opponent is grazed is higher than the profit a non-defended player makes if another non-defended opponent is grazed. These models show that diversity, including chemodiversity, can exist when there is a benefit to being the rare morph in a mixed population.

A coexistence mechanism closely related to negative frequency dependence is a rock-paper-scissors game. This appears to explain the coexistence of high-sinigrin *Brassica nigra* genotypes, low-sinigrin *B. nigra* genotypes, and other competing species (Lankau & Strauss, 2007). Here the high-sinigrin type has an advantage in the competition with other plant species and thus has an advantage when *B. nigra* is rare. As *B. nigra* is then becoming more common and intraspecific competition becomes stronger, it no longer pays to produce so much sinigrin which is not effective against conspecifics, and the low-sinigrin type has an advantage. These dynamics were also captured in an individual-based simulation model by Lankau (2009).

Associational effects are the effects that plants have on each other without direct interaction with each other but through interaction with the other species, in particular herbivores (Hambäck *et al.*, 2014) (Fig. 2). These exist under many names. One kind of associational effect is social heterosis, which can explain chemodiversity at the within-population scale. Social heterosis refers to a scenario wherein individuals have different traits that are beneficial both to themselves and others in their neighbourhood but cannot all exist in one organism at the same time. In this case, individuals in a diverse group have higher fitness than those in a monoculture, as demonstrated in a quantitative model by Nonacs & Kapheim (2007). In chemodiversity, this could take the form of a population of plants where different plants have different chemical profiles which repel different insect herbivores, both from the individual plant itself and its neighbours. If the production of SMs is costly, it may be detrimental to an individual to produce too many SMs, but beneficial to be in an environment where other individuals produce different SMs from oneself. In this way, social heterosis generates negative frequency-dependent selection on a single or community scale (Fig. 3) as rare types are more likely to find themselves in mixed neighbourhoods with other types. This type of associational effect is also called associational resistance (increased resistance through associational effects).

Another example of associational effects of chemodiversity is the model by Hambäck *et al.* (2014), which modeled associational effects in a community of plants with different traits involved with both visual and olfactory detection by herbivores. Volatile SMs form odor plumes that can be detected by herbivores. The

model included different plant trait values for the rate of detection of these plumes from a distance, the relative herbivore attraction to different types of plants within the patch when SMs act as attractors, and the rate of movement away from a plant to a different plant in the patch, and the rate of movement out of the patch of the herbivores when SMs act as repellants. Their model found both scenarios where mixed-trait plots had associational resistance to herbivory, and scenarios where mixed-trait plots had associational susceptibility, and scenarios where one trait displayed associational susceptibility and the other associational resistance, depending on the nature of the trait of the plant and whether mixed-trait plots had the same number of plants as the monocultures they were compared to. Hambäck *et al.* (2014) did not model evolutionary changes, nor were SMs explicitly modeled in the model. However, their model could be modified in the future to include these aspects, and in a multi-chemotype system these kinds of associational effects should certainly be taken into account.

3.5 Coevolution

Coevolution is a phenomenon where the evolution of two or more interacting species is influenced by the evolution of the respective other species in the interaction (Ehrlich & Raven, 1964). In the case of the evolution of chemodiversity, chemodiversity can be conceptualized in different ways in the co-evolutionary framework. In some studies, the richness and/or evenness of SMs is used as a trait of individuals (e.g. Calf *et al.*, 2018), in others, the individual SMs are treated as separate traits (Speed *et al.*, 2015). Most coevolutionary models and hypotheses for chemodiversity focus on coevolution with herbivores and envision plants and herbivores to be in an "arms race". There is evidence from field, lab and phylogenetic studies for the coevolutionary arms race hypothesis of chemodiversity on a population, species and lineage level (Thorsteinson, 1953; Becerra *et al.*, 2009; Jander, 2014; Richards *et al.*, 2016).

Many quantitative models of **coevolution with herbivores** can be found in the literature (e.g. Gilman *et al.*, 2012; Speed *et al.*, 2015; Ashby & Boots, 2017; Sandoval-Castellanos & Núñez-Farfán, 2023). However, the one that most explicitly addresses chemodiversity is by Speed *et al.* (2015). This model deals with chemodiversity within individual plants and within populations (see Fig. 3). In this individual-based model, the traits that make plants produce toxins have direct resistance counterparts in insect herbivores, so that an insect that feeds on a plant with trait A has to have the corresponding resistance A to the same degree as the plant to be able to reproduce. We have dubbed this style of model a **toxin-resistance matching model**. When plants and insects were allowed to evolve toxins and resistances through random mutation and selection (Fig. 2), an arms race developed. Selection favoured plants which produced whatever toxin the insects were least resistant to at a time, while other toxins became less prevalent until the insect resistances evolved in turn and a different toxin would be the toxin they were least resistant to. This caused cycles in which multiple toxins were present in the plants at any time in different concentrations, giving one possible

explanation of chemodiversity within individuals, within populations, and in populations through time (Fig. 3). Which chemicals dominate in the population at any given time is independent of the chemicals which dominate in separate populations which are going through their own coevolutionary processes, thus causing chemodiversity between populations and within the species.

Other quantitative coevolutionary models do not directly address chemodiversity, but contain elements which could be used in chemodiversity models. Gilman *et al.* (2012) developed a model similar to the one by Speed *et al.* (2015) and also show that coevolving with multiple traits can give the 'victim' species an edge, although their model is not explicitely about chemodiversity. Ashby & Boots (2017) developed a host-parasite model which uses a toxin-resistance matching model to model host defenses and parasite resistance traits. This model displayed dynamics of both stable equilibrium where either all traits or none were present, and infinite cyclical evolution. In the case of infinite cycles, there were two types of cycle occurring in the same simulation: fast-cycling of the possible resistances at the same number of resistances in a plant and slow cycling of number of resistances that existed in a plant. This was possible through a dynamic where immediate benefits or detriments to the exact resistances an individual possessed caused rapid changes in the resistances present, while subtle fitness costs of maintaining resistances created a slow dwindling of the number of resistances until a threshold was reached where having many was beneficial again. Both types of cycles displayed negative-frequency-dependent-selection and are thus linked to the game theory models discussed above. These dual cycles are a feature not found in the model of Speed *et al.* (2015) or in the model by Gilman *et al.* (2012) which is very similar to the model by Ashby & Boots (2017) but only found stable equilibria. While the models of Ashby & Boots (2017) and Gilman *et al.* (2012) are not specific to chemodiversity, these and other matched-trait models of coevolution could easily be translated to a chemodiversity context by making the resistance traits correspond to metabolites.

Some authors have proposed that chemodiversity can come about when individual plants produce multiple toxins to make it more difficult for herbivores to adapt to counter every single toxin of the set a plant produces (Speed *et al.*, 2012; Wetzel & Whitehead, 2020). This **slowed adaptation hypothesis** is essentially one verbal description of an evolutionary process for which toxin-resistance matching is the mathematical description. The slowed adaptation hypothesis may play a role in biological invasions (Box 3). However, this is not the only possible verbal explanation for a mechanism of toxin-resistance matching. In the model by Speed *et al.* (2015), the observed chemodiversity is the result of constant, moving-target like, innovation and the remains of innovations past, rather than of slowed adaptation of herbivores to a standing set of toxins. Additionally, while all models described in this review that have an explicit or implicit description of individual metabolites use a toxin-resistance model, this is not the only possible way to model interactions with and between metabolites. Quantitative models which explore synergy between metabolites, for example, would require the effects of metabolites to differ depending on which other metabolites are present.

Another coevolutionary hypothesis which is based on spatial structure is the **geographic mosaic of coevolution hypothesis**. In separated populations, different concentrations in SMs may derive from opposite selection pressures of specialists, which use certain SMs for host plant finding, versus generalists that are repelled or deterred by the same SMs (van der Meijden, 1996; Enge *et al.*, 2012). Geographic differences in herbivore abundance may thus result in a selection pressure mosaic (Thompson, 1999; Zangerl & Berenbaum, 2003). In a general mathematical model for the geographic mosaic of coevolution that could also be applied to toxin-resistance matching, Gomulkiewicz *et al.* (2000) showed that a spatial setup with coevolutionary hot spots (mutual selection) and cold spots (only one species exerts selection on the other, but not vice versa) could under some conditions allow the maintenance of polymorphism in both species and differences in allele frequency between populations. For a plant toxin coevolving with a herbivore, this would mean that the model could explain coexistence of toxic and nontoxic plant individuals in the same patch as well as differences in the frequency of toxic plants between patches (see Fig. 3).

A recent coevolutionary model that does not fit clearly into any of the previously discussed categories investigates the circumstances under which non-linearity of costs and benefits of herbivore resistance can lead to a mix of herbivory-resistant and herbivory-tolerating plants in a population (Sandoval-Castellanos & Núñez-Farfán, 2023). The model is an individual-based model that compares additive and multiplicative versions of a fitness function that includes the fitness costs and benefits of resistance and tolerance as well as the fitness cost of inbreeding depression. It concludes that the nonlinear fitness function needs to be concave, the allocation of costs and benefits multiplicative, selfing non-heritable, and tolerance costly to promote a mix of strategies. The 'resistance' in this model does not necessarily refer to SMs, but but the model could be easily modified to include different SMs.

To really make the models specific to chemodiversity, introducing ingredients like the branching pathways through which chemicals are created would be useful. Additionally, while toxin-resistance matching has been used in most quantitative coevolutionary models, empirical studies frequently test linear additive or nonlinear synergistic effects of SMs (Richards *et al.*, 2016; Dyer *et al.*, 2018). Therefore, developing models that use these additive or synergistic effects could really bring the empirical and quantitative modeling research in this field together.

Coevolution with seed dispersers is another way in which coevolution can potentially promote chemodiversity. For example, in the genus *Piper*, fruit toxicity affects dispersal by coevolved frugivores. This was statistically modeled by Baldwin *et al.* (2020) based on empirical data collected in a system where *Carollia* fruit bats consume *Piper* fruits. They found that higher levels of defensive amides correlate with shorter gut retention times and lower dispersal distances, while lower concentrations of amides correlate with higher dispersal distances as well as optimal ripeness of the fruit and maximum attractiveness to the fruit bats. In this way, the ripe *Piper* seeds are dispersed as far as possible, while unripe fruits are less likely to

be eaten in the first place. Models like this can be used to make quantitative predictions for future empirical studies. In its current form, such a model could explain differences in amide concentration between fruits within an individual or between individuals when fruits differ in ripeness (Fig. 3). For purposes of studying chemodiversity, it would be interesting to not only include total amide concentrations as a predicting variable, but also diversity of amides. It may be possible to shine a light on how coevolution of seed dispersal influences chemodiversity on the within-plant and between-population scale. Moreover, gut retention time in frugivores is not the only way chemodiversity may play a role in the coevolution of fruit-bearing plants and their seed dispersers. For example, SMs also may act as olfactory cues to frugivores, inhibit seed germination, and act as toxins Cipollini & Levey (1997). So far, we did not find any quantitative models addressing the potentially important consequences of coevolution with seed dispersers for the evolution of plant chemodiversity.

3.6 Screening hypothesis

The models reviewed in the previous sections generally assume that every metabolite has a specific biological activity in the interaction with one or more species. Metabolites without a specific strongly advantageous activity would be expected to be selected away because of their inherent costs. The screening hypothesis first proposed by Jones & Firn (1991) (see also Firn & Jones, 2003; Firn, 2009) challenges this view. Based on the observation that many metabolites produced by plants and fungi have no known function, they argue that strong specific biological activity is in fact a rare phenomenon. Thus, in order to defend themselves against a variety of herbivores, plants need to "screen" a large number of "candidate metabolites". They keep a diversity of metabolites, even many without a function, because this diversity and the underlying multitude of enzymes allows them via mutation to rapidly generate new metabolites and thus increase their chances to find at least some that have strong activity. Since maintaining a diversity of enzymes and metabolites still has costs, the screening hypothesis predicts that the metabolic pathways would be selected such that they can produce a high number of metabolites with as little enzymatic machinery as possible, mostly via promiscuous enzymes that can take multiple substrates, leading to grid-like metabolic pathways. Keeping a diversity of metabolites around can then also allow plants to rapidly respond to new herbivores or herbivores that have evolved a counter-defense (Jones & Firn, 1991).

In summary, the screening hypothesis is based on the ingredients of random mutation and natural selection, metabolic pathways, and interactions with other organisms (Fig. 2). Though the focus in the original formulation is on herbivores, by the same logic, a diversity of metabolites can help plants to develop metabolites that are beneficial in the interaction with other plants or with mutualists and soil communities. The screening hypothesis has been formulated as a verbal model and to our knowledge, it has not been formulated as a quantitative model. Nor are the ideas of grid-like metabolic pathways, promiscuous enzymes, and rare metabolic activity incorporated into other quantitative models.

The screening hypothesis mostly addresses chemodiversity within units of scale, i.e. it attempts to explain why a plant individual, or a plant population, species or clade produces so many different metabolites (Fig. 3). Although this has been less discussed by the authors of the screening hypothesis, if a complex metabolic network allows plants to rapidly generate new metabolites, this could also help explain differences between individuals, populations, and species. This appears to be supported by the observation that many enzymes involved in generating chemodiversity are less conserved than those that are involved in more primary metabolism (Weng *et al.*, 2012).

The screening hypothesis has been criticized because evolutionary foresight appears to be required for evolution to create a complex network of pathways just based on the chance that some of its products might eventually prove useful (Pichersky *et al.*, 2006). Proponents of the hypothesis counter that no evolutionary foresight is required if the rapid adaptation made possible through the network is sufficiently beneficial to be selected for (Firn & Jones, 2006). A formal mathematical description or computer model could demonstrate to what extent these criticisms are warranted. Such models have, to our knowledge, not yet been developed.

3.7 Population genetics models

Chemodiversity is, at least in part, underpinned by genetic variation. Thus, it would seem natural to extend classical population genetic models to study chemodiversity.

In biodiversity research (Hubbell, 2001) and in population genetics (Kimura, 1983), **neutral models** play an important role as null models for more interesting ecological and evolutionary processes though there is also much debate over how useful they are (see e.g. Clark, 2009; Kern & Hahn, 2018). While neutral biodiversity models assume that species within a guild are equivalent in terms of their birth, death, and migration rates, neutral models in population genetics assume that allele frequencies change just through mutation and drift without selection. We argue that similar neutral models should also be created for chemodiversity. That is, diversity in such models would come about and be maintained through random mutation and genetic drift without selection (Box 1). A neutral model for chemodiversity should additionally include pathways and enzymes that are encoded by the modeled genes and that thus also evolve neutrally (Fig. 2). A neutral model could in principle be formulated at any scale of organization, though a neutral model derived from neutral models in population genetics would most straightforwardly address chemodiversity within populations, within and between individuals (Fig. 3). This model would serve as a null model for the origin and maintenance of chemodiversity. Any explanations of the origin and maintenance of chemodiversity which propose that there is a selective advantage to producing a diversity of SMs can then be compared to it. We have not found any sources where a model of chemodiversity is compared to such a null model, but we think that such an approach would be extremely useful.

A slightly more complex explanation for chemodiversity is **mutation-selection-drift balance** (see e.g.

Wright, 1937). Mutation-selection-drift models in population genetics generally assume that mutations are either unconditionally beneficial or deleterious. There are no complex selection patterns like balancing selection or negative frequency dependent selection. Thus variation under this model is observed due to mutations that are transiently segregating in the mutation before they are getting either lost or fixed. In a chemodiversity extension of such models, new SMs would come about through random mutation in existing metabolic pathways. Here it is assumed that at least some SMs improve the organisms' fitness and are under positive selection, while others may be detrimental or are not worth the cost of their production and are thus under negative selection. The probability that a mutation is fixed or lost and the expected time to do so then depends on the strength of selection relative to genetic drift. During their time to loss or fixation, mutations and their effects on the chemical phenotype would then transiently contribute to chemodiversity. Like neutral models, mutation-selection-drift models could explain chemodiversity within populations, and within and between individuals (Fig. 3), but potentially also at all other scales. For example if different SMs randomly become fixed in different populations and clades this could explain differences between these units. So far, there do not seem to be any mutation-selection-drift models of chemodiversity in the literature.

Box 3: Biological invasions as testing ground for chemodiversity models

Connecting models for the evolution of chemodiversity to empirical data can be challenging because in natural population we can usually only observe a snapshot of the evolutionary process at the current time, but the long evolutionary history remains hidden. Biological invasions of plants or interacting animals offer exciting opportunities for testing these theories because here the interaction partners often experience sudden large changes in selection regime, sometimes resulting in rapid evolution on ecological time scales (Cox, 2004).

Invasive species may also benefit from high intraspecific variation in chemical composition, i.e. chemodiversity on a population level. For example, individuals of the plant *Tanacetum vulgare* (Asteraceae) are highly diverse in their terpenoid profiles, with different terpenoids occurring in different concentrations (Wolf *et al.*, 2011). Terpenoids have various ecological functions (Cheng *et al.*, 2007), and effects on antagonists are highly species-specific. Therefore, a high chemodiversity within invasive populations may impede the adaptation of herbivores and microorganisms native to the habitat (Wolf *et al.*, 2011; Tewes *et al.*, 2018), as predicted by the slowed adaptation hypothesis. Furthermore, the simple unfamiliarity of the novel SMs is thought to provide a competitive advantage. This is called the 'novel weapons hypothesis' (Callaway & Ridenour, 2004) in the context of biological invasions and closely related to the moving target hypotheses described above.

Furthermore, multiple introductions from different source populations to North America may have led to hybridisation of individuals of different chemotypes, producing novel mixtures of terpenoid profiles and thus

novel chemotypes. Indeed, few chemotypes were only found in individuals in North America, where *T. vulgare* is invasive (Wolf *et al.*, 2011). Moreover, potential inbreeding in invasive populations in interaction with the environment may lead to rapid evolutionary changes (Schrieber & Lachmuth, 2016), potentially also resulting in novel chemotypic variation. Overall, changes in chemodiversity and the functions in species-interactions in non-native populations have been largely neglected (Tewes *et al.*, 2018), although chemical composition can highly vary between native and non-native populations (Tewes *et al.*, 2018; Pankoke *et al.*, 2019) and due to hybridization (Piola *et al.*, 2013), offering great opportunities to develop theoretical models and test them with empirical studies. The novel-weapons hypothesis has already been mathematically modeled (Allstadt *et al.*, 2012), albeit not as a chemodiversity model, and is ripe for expanding upon.

4 Vision

It would be easy to fall into the trap of assuming that the different frameworks and hypotheses described here must be in competition, that through modeling and empirical work, we can find one correct and all others incorrect. Instead, we agree with Dyer *et al.* (2018) that the hypotheses do not have to contradict each other. In fact, many of the hypotheses and selection mechanisms described here may be at play at once within the same plant species. While the members of particular set of SMs cannot all have both synergistic and toxin-resistance matching fitness effects at the same time, this set of SMs might have synergistic effects on herbivory at the plant level, and also be under negative frequency-dependent selection on the population level. This set can then simultaneously be in a co-evolutionary arms race with herbivores on the lineage level, where new SMs come about within a pathway through the process well-described by the screening hypothesis. Another set of SMs in the same plant may only exist transiently as random mutations in a pathway under mutation-selection-drift balance. For this reason, it is useful to think of the different hypotheses and selection methods as different possible factors in the evolution and maintenance of chemodiversity, and not as mutually exclusive. At the same time, some of these hypotheses may well be invalid, or some might contribute less to explaining chemodiversity than others. Quantitative models can serve to investigate the validity and explanatory power of these hypotheses. Combining multiple hypotheses in a single quantitative model would enable us to compare the relative explanatory power of different models.

This does not mean that every quantitative model of chemodiversity has to be able to test all possible mechanisms involved with the evolution and maintenance of chemodiversity. A model should include no more complexity than it needs to (Servedio *et al.*, 2014), and there is also the risk of overfitting in overly complex models. Nevertheless, one should be mindful of alternative explanations when making a model that investigates a particular set of hypotheses. A model can show a verbal hypothesis to be flawed if the stated assumptions cannot be made to produce the suggested result, but it cannot prove a hypothesis to be definitely correct (Otto & Rosales, 2019).

When we make a model to test a verbal hypothesis, we need to incorporate enough detail to describe what that hypothesis is about. That means that a test of the coevolutionary arms race needs to incorporate a focal species and an interacting co-evolving species. For the screening hypothesis it is necessary that a molecular model be included that works as described by Jones & Firn (1991); Firn & Jones (2003), but it is not fundamentally necessary that any other species is involved. When modeling a very specific system, it is useful to ensure that all aspects of that system are carefully considered, and all relevant details are included, and all assumptions are justified (Servedio *et al.*, 2014). For example, if one were to model a biological invasion as described in Box 3, it would be prudent to include elements of the slowed adaptation and novel weapons/moving target hypotheses.

We have shown here that there is a great body of work on the description of current chemodiversity and its evolutionary history, and there are good evolutionary frameworks for the evolution of chemodiversity that are compatible with various mechanisms for the maintenance of chemodiversity. For many of the hypotheses, we also found a small number of quantitative modeling studies. However, quantitative models that explicitly address chemodiversity where individuals produce a diverse set of metabolites are few and far between. Such models will often be sufficiently complicated that it might not be possible to capture them in a system of equations that can then be solved analytically. Thus, numerical solutions and individual-based models as in Speed *et al.* (2015) appear to be the most productive ways to model the evolution of chemodiversity in these cases.

5 Conclusion

In conclusion, while there has been much verbal theory-crafting on chemodiversity and there is a small number of interesting quantitative models for many of the verbal hypotheses, there is much space for the development of mathematical models. This can be done by expanding existing models of chemodiversity, adapting existing models of diversity, sometimes by combining different submodels to create coherent models of chemodiversity. In this way, the existing theory can be tested for validity, the explanatory power of different hypotheses can be explored, and the results can be used to interpret empirical data to better understand the dynamics underlying extant chemodiversity.

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