

Opinion

Evolution of plant specialized metabolites: beyond ecological drivers

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Plants produce a highly diverse array of specialized metabolites. Traditionally, the evolution of these metabolites has been studied primarily through the lens of plants' ecological interactions with herbivores, pathogens, and pollinators, as many of them exhibit defense and/or attraction functions. However, increasing evidence suggests that many specialized metabolites, along with their precursors, also act as cellular signals that regulate cell growth and differentiation. We propose that these intrinsic functions are at least equally important factors in shaping the evolution of plant chemical defenses. We further discuss how future research that combines modern single-cell techniques and evolutionary genomics will provide novel insights into the evolutionary process of specialized metabolism diversification.

A historical perspective

The diversity of plant specialized metabolites (PSMs) is vast and multifaceted. Unlike primary metabolites, which originate from conserved metabolic pathways, PSMs are often distributed in a taxonomically restricted manner [1,2]. For example, many PSMs are unique to specific plant families or genera. Another dimension of metabolic diversity arises from both quantitative and qualitative variations within a plant, manifesting across different organs and tissue types and through developmental and phenological stages. These spatial and temporal heterogeneities in PSM distribution are further shaped by natural polymorphisms in signaling and responses to biotic stresses, particularly interactions with herbivorous insects. These many dimensions of PSM diversity, thoroughly reviewed by Wetzel and Whitehead [3], have become more accessible due to recent advances in metabolomics technologies. Researchers now frequently employ functional diversity indices, adapted from ecological studies on species biodiversity, to decode these complex chemical landscapes. Several hypotheses, such as the interaction diversity hypothesis [4], have been proposed to explain how these different organismal scales of PSM diversity function and evolve. These frameworks have in common that they posit that the many dimensions of PSM diversity form the foundation of a complex, adaptable chemical phenotype that shapes plant–environment interactions [5].

Interest in PSMs dates back centuries, prompting a historical exploration of evolving perspectives. In 1837, plant physiologist Julius Sachs proposed that many compounds now recognized as PSMs were mere by-products of fundamental energy-related metabolic pathways that are not used in the formation of new cells [6]. This physiology-centered view gained popularity for several decades, which led to the definition of 'primary' and 'secondary' metabolites. Around 50 years later, ecologist Ernst Stahl connected results from different ecological experiments and concluded that many PSMs mediate plant–animal interactions [7]. This ecological perspective on PSM functions gradually gained recognition. With the progressive developments in analytical chemistry, scientists continued uncovering novel ecological functions of PSMs. In a seminal

Highlights

Plants' adaptation to their environment is intimately linked to and reflected in their fascinating capacity to produce a tremendous number of structurally diverse metabolites.

In addition to external ecological functions, such as defense against herbivores and attracting pollinators, intrinsic cellular functions regulating cell growth and differentiation also represent an important selection pressure shaping the evolution of plant specialized metabolites.

Integrating single-cell multi-omics and evolutionary genomics will advance our understanding of how plant specialized metabolisms evolve under both external and internal selection pressures.

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paper published in 1959, Gottfried S. Fraenkel proposed that PSM evolved as a means of 'protecting plants from insects and now guide insects to food' [8]. Since then, the ecology-centered view on the evolution of PSM has been widely accepted, and a large body of studies has demonstrated fitness benefits associated with PSM production in the context of plants' biotic interactions [9,10].

However, recent discoveries using genetic and molecular tools have blurred the boundaries between the ecological and physiological functions of PSMs. For example, growing evidence shows that genetically manipulating the biosynthesis or chemical decoration of PSMs not only changes a plant's defensive status but also affects its growth and development [11–14]. In addition, while PSMs are often assumed to benefit plants, several studies demonstrate that herbivores and pathogens can rapidly evolve strategies to counter these defenses and that some of them even take direct advantage of PSM production, for instance, for the localization of their host plant, sequestering toxins for protecting themselves from predators or initiating infections [15]. This challenges the traditional view that herbivores and pathogens act as the sole driving force behind PSM evolution. Here, we first summarize the experimental evidence supporting both ecological pressures and cellular functions as key driving forces for the evolution of PSMs. We then propose an integrated research framework that unifies these perspectives to advance our understanding of PSM diversification.

Ecological factors driving the evolution of PSMs

Since many PSMs have defensive functions against herbivores and pathogens and/or attract pollinators, it is often hypothesized that their evolution is a consequence of adaptation to various biotic stresses (Box 1). One way to test this hypothesis is to perform experimental evolution studies, in which the evolutionary changes in PSMs are quantified under different herbivory or pollination scenarios. For simplicity, most of these studies manipulate the presence or absence of herbivores and/or pollinators. By combining historical data from natural populations of *Arabidopsis thaliana* and its natural herbivores with real-time experimental evolution experiments, Züst *et al.* demonstrated that variations in the profiles of glucosinolates in *A. thaliana* can be driven by natural enemies [16]. Similarly, indoor experiments manipulating the presence and/or absence of bumblebee pollinators and leaf herbivores (*Pieris brassicae*) on *Brassica rapa* populations also led to rapid evolutionary changes in leaf glucosinolate abundance and floral volatiles [17]. In another study, Agrawal *et al.* found that a 5-year manipulation of insect abundance in replicated field populations of a native plant, *Oenothera biennis*, altered flowering time and lowered defensive ellagitannins in fruits [18]. Using outdoor mesocosms, Malacrinò *et al.* showed that herbivory-induced changes in morphology and/or putative defensive metabolites in the giant duckweed contributed to rapid adaptive evolution in response to snails [19]. Although many PSMs are crucial for defending plants against pathogens and shaping soil microbial communities, no experimental evolution studies have yet been conducted to determine whether microorganisms influence the evolution of PSMs [20].

While experimental evolution can provide direct and mechanistic insights into whether certain ecological factors can drive the evolution of plant chemical defenses, these studies are often conducted under unnatural conditions. In nature, plants are subjected to multiple biotic and abiotic selection pressures simultaneously, and the direction of selection can be highly dynamic [21–23]. Recent experimental evolution studies suggest that the interactions among different kinds of selection pressures, such as pollinators and herbivores, can significantly alter the evolutionary trajectory of plant traits [17]. Furthermore, plant pathogens and tri-trophic interactions can also significantly influence both the direction and intensity of selection imposed by herbivores [20]. Moreover, studies that manipulate herbivores within natural communities indicate that plant–

Box 1. Hypotheses explaining the evolution of novel PSMs

The hypotheses are presented for a given PSM group, with a focus on recently evolved novel metabolites. For each hypothesis, several testable predictions are provided. To avoid confounding factors, predictions are specific for the focal PSM.

Neutral evolution of novel PSMs hypothesis

Novel PSMs arise and persist without being strongly shaped by natural selection, at least initially. Random mutations and genetic drift may lead to the accumulation of new compounds that neither confer a major advantage nor impose a significant cost. Over time, such 'neutral' PSMs may become fixed in certain populations or lineages purely by chance. Key predictions are:

- No direct fitness effect: genetically silencing or knocking out the gene(s) responsible for recent PSM innovations does not affect plant fitness in a straightforward manner; any observed phenotypes are due mainly to pleiotropy rather than a direct function of the PSM.
- Random organ emergence: there is no consistent pattern regarding which organ(s) first evolve or express these novel PSMs.
- Neutral molecular evolution: genes associated with the most recent PSM innovations do not show clear signatures of selection.
- Neutral genetic correlation: variations in PSM among related species correlate more closely with overall neutral genetic distance rather than with ecological or developmental factors.

Ecological factors drive novel PSM evolution hypothesis

Novel PSMs primarily arise and diversify due to ecological pressures. These pressures can include herbivory, pathogen attack, competition, or abiotic stresses (e.g., drought, extreme temperatures). The underlying assumption is that the production of certain PSMs confers a fitness advantage in particular environments, thereby shaping the trajectory of PSM evolution through natural selection. Key predictions are:

- Stress resistance: genetically manipulating a key gene responsible for recent PSM innovations alters resistance to biotic or abiotic stresses.
- Positive selection: this gene shows a molecular signature of divergent or positive selection, for example, an elevated ratio of non-synonymous versus synonymous mutation rates (dN/dS).
- Organ-specific emergence: novel PSMs first emerge in organs (either produced in or transported into) directly exposed to the relevant stress.
- Ecological correlation: variations in PSMs among related species correlate with ecological differences rather than with neutral genetic distance.

Cellular/developmental functions drive novel PSM evolution hypothesis

Novel PSMs are shaped primarily by the plant's internal developmental and physiological processes. Rather than arising just for defense, these compounds may play key roles in growth, reproduction, or cell signaling. Over time, some may acquire defensive or other ecological functions, but their initial driver is fundamentally tied to developmental processes. Key predictions are:

- Growth/development impact: genetically manipulating a key gene responsible for recent PSM innovations disrupts plant growth or development.
- Precursors dependency: removing direct biosynthetic precursors of the novel PSM abolishes normal growth or developmental phenotypes.
- Reproductive organ expression: the gene(s) underlying recent PSM innovations are predominantly expressed in reproductive organs or cells.
- Reproductive origin: novel PSMs first appear in (either produced in or transported into) reproductive tissues among closely related species.
- Developmental correlation: variations in PSMs among related species correlate with differences in developmental or growth patterns, rather than just ecological niche differences.

plant competition, often overlooked in most indoor experimental evolutionary studies, plays a critical role in shaping community structures [24,25] and the evolutionary trajectories of plants [26] (see [Outstanding questions](#)).

Another approach to testing whether herbivores drive the evolution of plant chemical defenses is through phylogenetic analysis, which compares species that diverged millions of years ago and captures the average evolutionary forces that shaped the diversity of phenotypic traits. According

to the herbivore-driven chemical defense evolution hypothesis, the macroevolutionary predictions are: (i) chemical defenses should exhibit a strong phylogenetic signal, (ii) coevolution between plant chemical defenses and herbivores should be detectable, as herbivores can also evolve counter-defense strategies, and (iii) following the ‘escape and radiate’ hypothesis of Ehrlich and Raven [27], coevolution between plant chemical defenses and herbivores should lead to increased diversification rates, with chemical diversity in plants escalating over time. However, recent studies have shown that many chemical defenses exhibit weak or no phylogenetic signal [28–34]. Although few studies have systematically investigated the coevolutionary arms race, a general pattern is that herbivore assemblage matches host chemical defenses better than phylogeny, suggesting that insect herbivores are more likely chasing host plant defenses rather than coevolving with them [35]. Evidence for escalation of chemical diversity is mixed with some trends pointing towards de-escalation rather than a continuous increase in chemical diversity [30,36–39].

Overall, these results suggest that while ecological factors, such as herbivores and pollinators, can drive the evolution of PSMs, they are unlikely to be the sole drivers.

Beyond their role in defense against biotic stresses, many PSMs may also play a role in resisting abiotic stresses, such as UV radiation, drought, and cold [2]. However, current evidence supporting abiotic stresses as evolutionary drivers remains limited to comparative analyses and indirect inferences.

Cellular functions shaping the evolution of PSMs

The production of many defensive metabolites is often linked to core cellular functions [40,41]. While fundamental processes such as respiration and photosynthesis form the basis of cell metabolism, the production of PSMs often incurs metabolic costs. These costs impact energy availability and primary metabolic processes essential for growth and reproduction, a phenomenon commonly referred to as resource allocation costs [42–44]. As a result, the growth-defense trade-offs in plants are frequently seen as a driving force shaping the evolution of induced chemical defenses [45–47]. Direct evidence supporting this hypothesis comes from observed fitness costs in terms of reproductive units when experimentally changing inducible defenses into constitutive ones by supplying jasmonic acid, as for instance demonstrated for the wild tobacco (*Nicotiana attenuata*) [48]. However, the jasmonic acid signaling pathway not only mediates various stress responses but also regulates flower development and fertility [49]. Many defensive metabolites are also produced in flowers, yet the extent to which the pleiotropy of genes involved in jasmonic acid signaling contributes to the evolution of induced defenses remains unclear. While the fitness costs of herbivores preferentially attacking plants with lowered jasmonic acid induced defensive PSM production are well documented, the energetic and metabolic costs of this chemical defense production are not always as evident [50,51]. The fitness costs of these defenses are likely most significant in resource-limited or competitive environments [41,52].

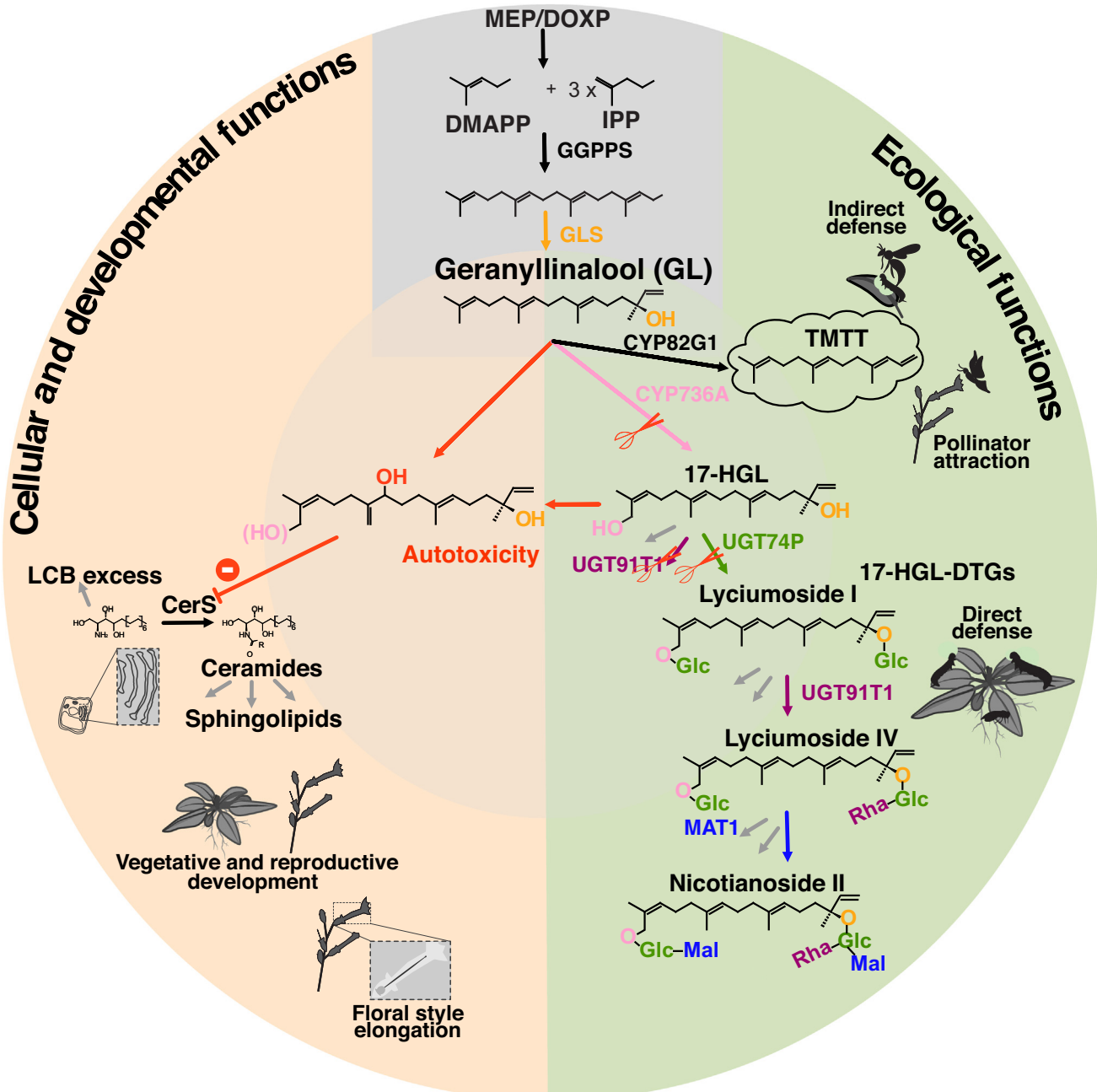
Detoxification processes are closely linked to chemical defense production, as detoxification helps prevent the uncontrolled accumulation of bioactive and toxic signaling molecules (e.g., phytotoxicity) [46,53]. Recent studies have highlighted this connection by genetically modifying the biosynthetic pathways of various defensive metabolites. For example, when the biosynthesis of steroidal glycoalkaloids (SGAs) in tomato and potato was disrupted, the plants exhibited significant growth retardation and severe morphological abnormalities [12]. Common detoxification processes that help prevent the accumulation of toxic intermediates include structural strategies, such as sequestration of PSMs into vacuoles [54,55], trichomes [56,57], or laticifers [58]. Additionally, PSMs can undergo chemical modifications, such as glycosylation, which increases water solubility, facilitates

transport, and reduces reactivity or amino acid conjugation, which reduces toxicity and enhances metabolite stability. Despite these observations, the precise mechanisms linking these biochemical disruptions to growth and developmental changes remain largely unknown.

One of the best mechanistically understood examples of how PSMs mediate cellular functions comes from recent research on 17-hydroxygeranylinalool diterpene glycosides (17-HGL-DTG) in *Nicotiana* species (Figure 1). 17-HGL-DTG biosynthesis involves the hydroxylation of geranylinalool by two cytochrome P450s (CYP736A), the resulting product being diversely decorated by a uridine diphosphate glucosyltransferase (UGT74P) and rhamnosyltransferase (UGT91T1) [11,59]. Subsequently, an acyltransferase (malonyltransferase, MaT) transfers malonyl groups to specific glucose moieties [60,61]. Interrupting the glycosylation or hydroxylation steps in this pathway results in the over-accumulation of geranylinalool and 17-HGL, which is highly prone to unspecific hydroxylation. The unspecific hydroxylation products can then inhibit the biosynthesis of sphingolipids, a highly conserved cellular signaling pathway, which in turn causes phytotoxicity [11,61]. Interestingly, plants such as *Arabidopsis thaliana* and tomato, which do not produce 17-HGL-DTG, produce C(16)-homoterpene 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), an oxidized form of geranylinalool. Silencing the gene coding for the oxidizing enzyme CYP82G1 in *A. thaliana* also led to phytotoxicity and altered plant development [13], likely due to the same mechanisms. While it remains to be determined whether additional detoxification mechanisms exist in other plants, a plausible explanation for the evolution of TMTT and 17-HGL-DTG is that they represent two alternative mechanisms to precisely regulate the abundance of geranylinalool in cells, which is required during certain developmental stages or cell differentiation processes. Whether plants evolve TMTT or 17-HGL-DTG biosynthesis as an autotoxicity-avoidance strategy is dependent on pre-existing genetic makeups that can be recruited into either oxidation or specific hydroxylation and glycosylation processes. Subsequent ecological factors, such as interactions with pollinators or herbivores, may have further influenced and stabilized the production of these metabolites in plants.

This perspective on geranylinalool metabolism evolution underscores the critical importance of biochemical innovations in maintaining the homeostasis of bioactive geranylinalool as well as in fostering the metabolic diversity upon which novel ecological functions can evolve. In this scenario, precursors or early biosynthetic intermediates of the PSM may possess bioactivities that influence plant growth and development. The primary drivers of PSM structural diversification are in this scenario, the biochemical mechanisms that stabilize and regulate the distribution and abundance of these bioactive metabolites, which, if accumulated in excessive amounts or in inappropriate cells, could become phytotoxic. Natural selection likely acts most intensively against the uncontrolled accumulation of potentially harmful intermediates, thereby shaping the evolutionary trajectories of plant chemical defenses (Box 1). Supporting this view, many plants store chemical defenses in less toxic forms, such as glycosides, with variations in glycoside units (in terms of type, position, and number) significantly contributing to the diversity of PSMs [62,63]. Additionally, plants often sequester these compounds in specific tissues or organelles to minimize their exposure to sensitive cellular processes [64,65].

The importance of adopting an evolutionary perspective considering the diversity of internal functions fulfilled by PSMs is further underscored by the expanding universe of cellular regulatory functions – ranging from damage-self signals up to hormonal-like functions – that have been identified in recent years for various PSM classes [66–68]. For instance, a recent study identified a perception mechanism for a *Petunia* floral volatile synchronizing reproductive organ development [69]. Additionally, strigolactones, traditionally considered PSMs, are now recognized as phytohormones. Beyond their direct effects, PSMs or their precursors can also indirectly



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Figure 1. Biosynthesis and duality of cellular and ecological functions of geranylinalool (GL) derivatives for autotoxicity and anti-herbivore protection. In wild tobacco, GL is specifically hydroxylated on carbon 17 by the P450 enzyme CYP736A and leads to the production of 17-hydroxygeranylinalool diterpene glycosides (17-HGL-DTGs) that act as a direct anti-herbivore defense (ecological function). The defense function of 17-HGL-DTGs is mediated by the inhibitory effects of the unspecifically hydroxylated aglycone on the ceramide synthase inside insect herbivores. In *Arabidopsis thaliana* and the tomato, geranylinalool is oxidized by the P450 enzyme CYP82G1 to produce 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), which is released as a volatile acting as an indirect defense by attracting the predators of herbivores (ecological functions). Without CYP736A or CYP82G1, geranylinalool is unspecifically hydroxylated, which inhibits ceramide synthase and leads to autotoxicity (cellular functions). Thus, TMTT and 17-HGL-DTG production protects from phytotoxicity via specific modifications of geranylinalool, the precursor of bioactive cell signaling molecules (unspecifically hydroxylated 17-HGLs). Other abbreviations: CerS, ceramide synthase; CYP, cytochrome P450; DMAPP, dimethylallyl pyrophosphate; DOXP, 1-deoxy-D-xylulose 5-phosphate; Glc, glucose; GGPPS, geranylgeranyl diphosphate synthase; GLS, geranylinalool synthase; IPP, isopentenyl pyrophosphate; LCB, long chain base (sphingolipid building block); Mal, malonyl; MAT, malonyltransferase; MEP, 2-C-methyl-D-erythritol 4-phosphate; Rha, rhamnose; UGT, UDP-glycosyltransferase. Scissor symbols denote gene silencing and knockout in the 17-HGL-DTG pathway.

influence plant growth and development by modulating phytohormonal balances. These new insights into the developmental roles of many PSMs emphasize the importance of a mechanistic and holistic understanding of the functions of PSMs and their precursors. Despite these advances, direct evidence supporting the idea that cellular functions are key drivers of the evolution of plant PSM remains lacking.

Embracing a genetics-guided multi-omics research framework for future studies

Since all heritable changes in PSMs are rooted in genetics, we argue that understanding the evolution of PSM requires a genetics-guided research framework (Figure 2). However, several challenges remain in adopting this framework, including (i) elucidating the underlying biosynthetic pathways and reconstructing their sequential evolution, (ii) understanding the evolutionary

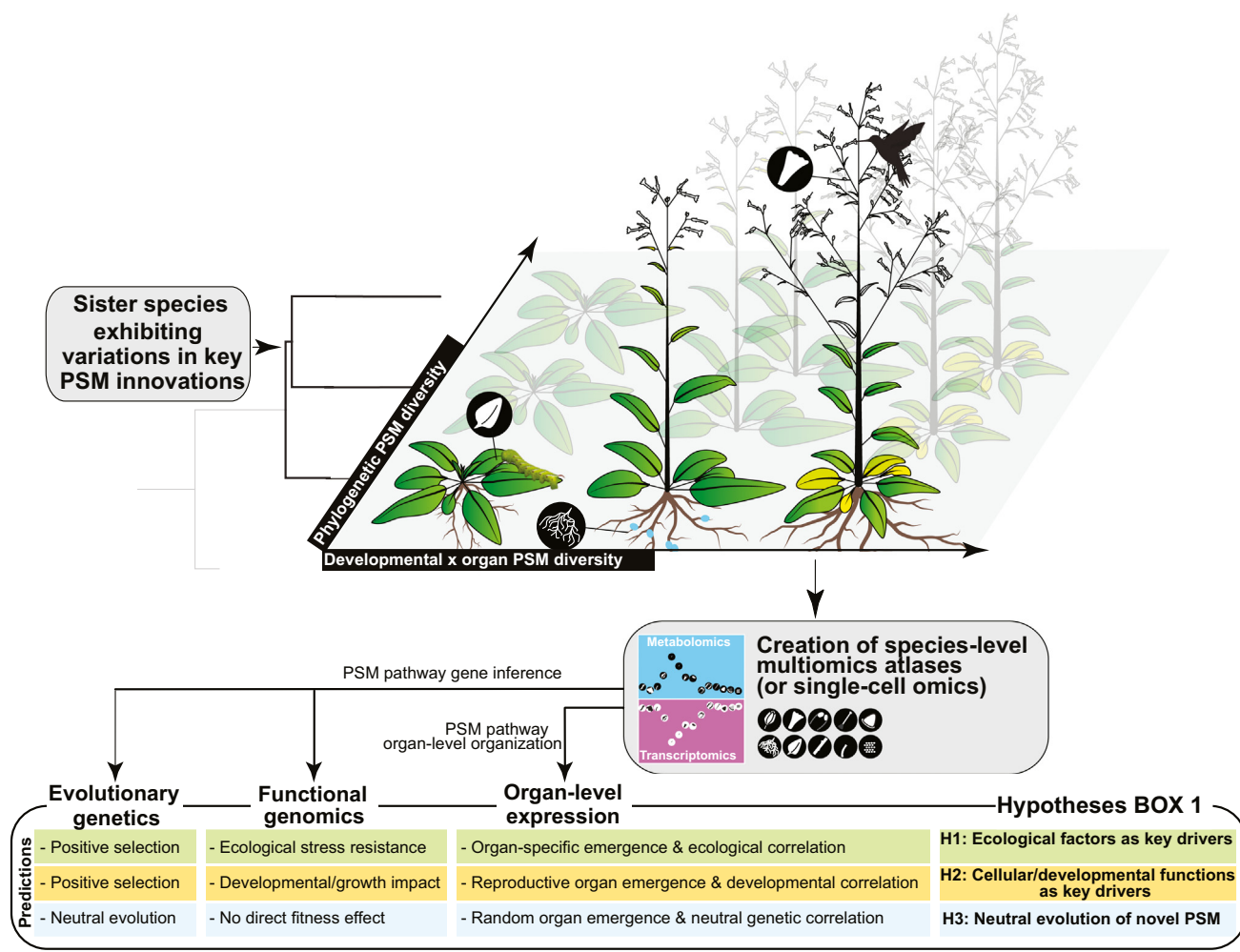


Figure 2. Embracing a genetics-guided multi-omics research framework to study the evolution of plant specialized metabolites (PSMs). This framework consists of three levels of analysis. The biosynthetic pathway of a given PSM is elucidated by integrating multi-omics atlas data with molecular biology tools. Multi-omics atlases of closely related species are further compared to identify the genetic basis of key innovations in PSMs, as well as the organs or cell types where these innovations have evolved rapidly (organ-level expression analysis). Population genetics and molecular evolution analyses are used to detect selection signatures in the underlying genetic mechanisms (evolutionary genetics analysis). Finally, both intrinsic factors (e.g., autotoxicity avoidance) and external factors (e.g., defense strategies) are examined for their roles in shaping PSM evolution, using a combination of genetic manipulation (functional genomics analyses), single-cell multi-omics, and ecological bioassays. Predictions for each of these levels of analysis referred to those presented in Box 1 for the three main hypotheses explaining the evolution of novel PSMs.

patterns of PSMs under neutral conditions and divergent selection, and (iii) disentangling cellular and ecological factors that shape the evolution of PSMs. We go on to discuss how recent developments in genomics and metabolomics, as well as their integration, can help address these challenges.

To understand the evolutionary patterns and drivers of any PSM, a critical step is to identify their biosynthetic pathways, regulations, and transport mechanisms. In model organisms, such as *Arabidopsis*, maize, and rice, metabolite-based genome-wide association study (mGWAS) is a powerful tool to dissect the genetic basis of PSM production. However, mGWAS requires the sequencing and metabolomics profiling of large natural populations, which is often not feasible for many non-model organisms. To overcome this hurdle, gene co-expression analysis can be used. Previous studies showed that genes within a PSM biosynthetic pathway often cluster into tight co-expression modules across different organs, tissues, and cell types [70–73]. This method often relies on expression atlases constructed from various organs and tissues. To achieve this, a cost-effective approach involves combining long-read – for example, full-length complementary DNA (cDNA) sequencing to create reference transcriptomes – and short-read – to quantify transcript abundance – sequencing technologies. Reproductive organs, particularly pollen grains, exhibit unique PSM profiles [74], making them highly valuable in the creation of organ-level atlases aimed at detecting gene–metabolite associations related to organ-specific metabolic innovations. In addition, a phylogenomics study indicated that pollen may serve as an ‘innovation incubator’ for the birth of *de novo* genes [75]. This concept is notably exemplified by the emergence of a novel branch of phenolic compounds unique to the pollen coat of members of the Brassicales, which arose through the divergent evolution of a retrogene expressed in male reproductive tissues [76,77]. Recent advancements in single-cell sequencing offer significant benefits, enhancing the statistical power to identify co-expressed gene modules by providing expression profiles from thousands of distinct cell types [78]. Integrating transcriptome data with metabolomic information at the single-cell level would further improve the resolution and understanding of gene–metabolite relationships, offering a more powerful approach to unraveling the complexities of PSM biosynthesis and regulation [78].

In addition to adaptive evolution, factors such as genetic drift, pleiotropy, and genetic linkage can also lead to the emergence of novel PSMs. To determine whether a new heritable PSM has evolved through adaptive evolution, it is crucial to identify its genetic basis and to explore the evolutionary and diversity patterns associated with it. One approach is to perform a comparative analysis among closely related species that have evolved different PSM profiles. By combining gene–metabolite co-expression analysis and *in vitro* functional validations, one can identify the genetic mechanisms that contribute to recently evolved PSM differences among the focal closely related species. Following this, population genetic techniques – such as genotype-by-sequencing or exon-capture sequencing – can be employed to detect signatures of selection in these genetic underpinnings [79]. However, it is important to note that the absence of a signature of selection does not necessarily mean neutral evolution, as the weak contemporary selective pressures (driven by either cellular or ecological functions) that acted in the past can be overwritten by recent demographic changes.

If the genetic basis of a novel chemical defense shows the highest signature of positive selection among all biosynthetic and regulatory genes, further investigation is needed to determine whether the selection is driven by intrinsic cellular functions and ecological functions as two non-exclusive factors. To investigate intrinsic cellular functions, genetic manipulation of the candidate genes responsible for the novel metabolite is essential. If this is true, silencing the candidate biosynthetic gene will alter the development and growth of plant organs in an environment-independent manner. Since many biosynthetic genes may have pleiotropic functions, to establish the specific role of the metabolites it is necessary to perform double silencing of both the candidate biosynthetic

gene and the genes involved in precursor biosynthesis. Expression atlases, both at organ and developmental levels, would provide critical insights into the underlying mechanisms by revealing when and where the functional metabolites are produced. Since some of the changes in organ development may be too subtle to be detected with the naked eye (e.g., in pollen and ovule development) yet are critical for plant fitness, a single-cell atlas will provide guidance on which organ to examine more closely. To investigate the ecological drivers, manipulating the genetic basis underlying the novel PSM and quantifying the multigenerational fitness consequences in natural communities are essential. However, this approach is often challenging for most plants. A more practical method involves measuring the most relevant ecological functions of the PSM in a controlled environment. To achieve this, gene expression and metabolite atlases across different plant organs or cell types can offer insights into which ecological functions and plant organs are most pertinent.

Concluding remarks and future perspectives

It is commonly assumed that the evolution of PSMs is driven primarily by their ecological functions. However, recent molecular studies have revealed that many PSMs also play crucial roles in different cellular and developmental functions, particularly by regulating the abundance of bioactive molecules produced as part of PSM intermediate biosynthetic steps (Box 1). This phytohormone-like function of PSMs challenges the traditional view of their roles, which was shaped largely by arbitrary classifications of plant metabolites established over 180 years ago. Future studies that integrate both the ecological and cellular functions of PSMs – such as using individual-based models [80] and experimentally estimated key parameters – will help embrace this functional complexity, offering new insights into the evolutionary processes that have shaped plant metabolic diversity in nature (see Outstanding questions).

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Declaration of interests

The authors declare no conflicts of interest.

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Outstanding questions

What is the genetic basis underlying metabolic novelty?

In which organ or cell type do PSMs evolve fastest?

Can cellular function drive the evolution of novel PSMs?

How do PSMs evolve in multitrophic communities?

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