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Why do plants produces so many terpenoid compounds?

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Summary:

All plants synthesize a suite of several hundred terpenoid compounds with roles that include phytohormones, protein modification reagents, anti-oxidants, and more. Different plant lineages also synthesize hundreds of distinct, terpenoids, with the total number of such specialized plant terpenoids estimated in the scores of thousands. Phylogenetically restricted terpenoids are implicated in defense or in attracting beneficial organisms. A popular hypothesis is that the plant's ability to synthesize new compounds arose incrementally by selection when, due to gradual changes in their biotic partners and enemies, the 'old' plant compounds were no longer effective, a process dubbed 'coevolutionary arms race'. Another hypothesis posits that often the sheer diversity of such compounds provides benefits that a single compound cannot. In this review we review the unique features of the biosynthetic apparatus of terpenes in plants that facilitate production of large numbers of distinct terpenoids in each species and how facile genetic and biochemical changes can lead to further diversification of terpenoids. We then discuss evidence relating to the hypotheses that given ecological functions could be enhanced by the presence of mixtures of terpenes and that the acquisition of new functions by terpenoids could favor their retention once the original functions are lost.

Key words: biochemistry, evolution, plant defense, secondary metabolism, specialized metabolites, terpenes.

Introduction

Terpenoids constitute a class of chemical compounds present in all living organisms. However, green plants, and particularly flowering plants exhibit an unusually high number of terpenoids, both per species and in the aggregate, compared with other living organisms. Various publications have estimated that the number of distinct terpenoid compounds (an inclusive term used to describe both terpenes and compounds with terpene moieties linked to other moieties derived from different pathways) in plants could be in the scores of thousands (Chen *et al.*, 2011). And while there are perhaps a few hundred terpenoids that are found in all or almost all plants – therefore they are defined as primary metabolites – the vast majority of terpenoids are restricted to a given lineage or even a single species and are therefore called specialized terpenoids.

The terpenoids of primary metabolism have roles that include hormones, components of electron transfer systems, protein modification agents, membrane fluidity determinants, anti-oxidants, and more, and these diverse roles must evolved early in the history of green plants (some even predate the origin of plants). The lineage-specific terpenoids, which have arisen throughout the evolution of green plants, have generally been postulated to play a role in the ecological interactions of the plant with biotic and abiotic aspects of its environment. Such roles have included defense against herbivores and pathogens, and signals and rewards to beneficial organisms such as pollinators and mycorrhiza (e.g., Heiling *et al*., 2010).

The continuing evolution of new specialized terpenoid structures in plant lineages has of course been caused by the evolution of new genes that encode new enzymes capable of making such new metabolites. The question naturally arises as to what advantages, if any, the plant derives from the ability to synthesize a diverse array of terpenoids. In this review, we begin by describing the contribution of plant terpenoids to human ecology. It was this contribution that led to terpenoids being one of the first classes of plant compounds to be investigated in detail, and our contemporary interest in commercially important terpenes (e.g., taxol, artemisinin, rubber) is still influencing research directions. We next describe the diversity of known terpenoid structures and functions, both in primary metabolism and in specialized metabolism and the possible molecular mechanisms by which such diversity arose during evolution of green plants. We conclude by critically examining the possible reasons to the adaptive value to any given species of having a large diversity of terpenoids, and to the continued evolution of more terpenoid diversity throughout the plant kingdom.

Terpenes in nature and their use by humans

The term ‘terpene’ is derived from τερέβινθος, the Greek name for the terebinth tree, *Pistachia terebinthus*, whose terpene-rich resin was used by people all over the Middle East to treat a variety of ailments. Indeed, terpenes and terpenoids have been important to many facets of human life and culture for millennia. Humans carved ornaments and religious artefacts from amber, the polymerized and fossilized terpene exudate of plants, as long as 10,000 yr ago. Terpene scents such as myrrh and frankincense are mentioned in old religious texts, and are still used in the rituals of many major religions (Langenheim, 2003). Pitch is another example of a terpene mixture of long term importance. This sticky substance – which is the residue of the terpene exudate of diverse trees that is left after the volatile terpenes evaporate from it – was used to waterproof boats, ships, and other structures (Langenheim, 2003). Rubber, a natural terpene polymer, is an essential component in mechanized transport systems as well as many other technological devices.

By far the most intensive use of terpenes by humans is for internal consumption. Some terpenoid compounds are used by humans to produce essential compounds – for example, our bodies synthesize vitamin A from β-carotene, an abundant plant terpene. More generally, terpenes in foods have a major effect on our eating experience. Terpenoid pigments such as bixin, lycopene, and astaxanthin are heavily used in the food industry. Volatile terpenoid compounds impart specific flavors to foods via their detection by the olfactory system, which they reach by the retronasal pathway after they are released from the masticated food. Thus, ginger flavor is due to zingiberene, and nootkatone imparts grapefruit flavor. Many herbs (fresh plant material such as lemon grass) and spices (dried plant material such as saffron) contain volatile terpenoids as major flavor components, and alcoholic drinks, and wine in particular, owe much of their flavor to the presence of terpenes (Stewart, 2013).

Terpenoids in herbs and spices were often used, and are still used today, to preserve food, due to the microbicidal and insecticidal properties that many of them possess (Tassou *et al*., 2012). This property of general toxicity, as well as their ability to dissolve oily and grimy material, could have led to their initial use in ancient human medicine and their continuing use in mouth washes, cough medicines, disinfectants, and insect repellents. However, more complex terpenoids such as taxol and vinblastine have some specific activities on components of human cells that have led to their use in treating specific diseases, mostly cancer (Julsing *et al*., 2006). Others are mimics of animal hormones. Taking advantage of this property, diosgenin, a sterol present in high concentration in the tubers of Mexican yam (*Dioscorea mexicana)* and related species, has been used for the semi-synthetic synthesis of progesterone for birth control pills and other medicinal steroids (Djerassi, 1990).

Many terpenoids exert their effect on the nervous system. Cardenolides such as digitoxigenin can stop a human heart from beating if ingested at high dosage, but are also useful in treatment of heart disease (Agrawal *et al*., 2012). Other terpenoids have psychoactive properties, such as tetrahydrocannabinol (THC), the active compound in *Cannabis* (marijuana), and salvinorin A from *Salvia divinorum*, and such properties were known in antiquity as was beguilingly described by Herodotus in his 5th Century BC book, The Histories.

Terpenes are highly combustible, and the large amounts of terpenoids stored in trees such as conifers in the Northern hemisphere and eucalypts in Australia exacerbate forest fires and help them spread more easily. The combustibility of terpenoids has led to the suggestion, as well as some initial work, that certain terpenes, particularly those that are liquid in ambient temperature, could be produced in biological systems for fuel (Wang *et al*., 2015). Some progress has also been achieved towards genetically engineering microorganisms to produce a variety of terpenoid drugs, such as taxol, vinblastine and artemisinin (for malaria prevention), as well as a variety of fragrances and flavor compounds such as valencene and patchoulol (Schwab *et al*., 2013).

Synthesis of primary and specialized plant terpenes

In the 1920's, it became clear that the basic building unit of the terpenes was the isoprene unit composed of five carbons, and the 'isoprene rule' was formulated, in which a 'regular' terpene was one in which isoprene units were condensed in a 'head to tail' fashion while an irregular terpene was formed in 'head to head' condensation (Fig. 1). It is important to note, though, that the enzymatically catalyzed condensation reactions in the cell involve prenyldiphosphates rather than free prenyls (Fig. 2). Today, both the head to tail and the head to head condensations are considered 'regular' terpene biosynthetic reactions, while the term 'irregular' is applied to a 'head to middle' linkage of isoprene units that is part of the biosynthetic pathway of such compounds as lavandulol and chrysanthemic acid (Demissie *et al*., 2013).

Several extensive reviews on the biochemistry and genetics of plant terpenes have appeared in the last decade (e.g. Chen *et al*., 2011; Zi *et al*., 2014), so only the general facts will be summarized here. The product of the condensation of two isoprene units is called a monoterpene (with a 10-carbon skeleton), of three isoprene units – a sesquiterpene (C15), and four isoprene units – a diterpene (C20). The actual condensation of these isoprene units in the plant occurs with the diphosphate-activated forms, called prenyl diphosphates, and, for head to tail condensations, can happen in two ways, leading to *trans*-prenyl diphosphates and *cis*-prenyl diphosphates (Fig. 2). Most plant triterpenes (i.e., sterols, C30) are produced via the head to head condensation of two *trans*-sesquiterpenyl diphosphates, while most plant tetraterpenes (i.e., carotenoids, C40) are produced via the head to head condensation of two *trans*-diterpenyl diphosphates (Fig. 2).

The diterpenoid hormones called gibberellins (Fig. 3) are common to all vascular plants (Zi *et al*., 2014). As in animals, sterols are abundant membrane compounds in plants, with stigmasterol, sitosterol, and campesterol being the major sterol constituents, and brassinosteroids constitute a class of hormones found in all plants (Fig. 3) (Vriet *et al*., 2013). Carotenoids such α-carotene and β-carotene are major constituents of photosynthetic tissues, and the hormones abscisic acid and strigolactones are derived from degradation of carotenoids (Fig. 3) (Al-Babili & Bouwmeester, 2015).

Some primary metabolites simply have a prenyl group attached to the carbon skeleton. The hormone cytokinin has a single isoprene unit, while phyloquinone, tocopherols, and chlorophylls have a diterpene attached with all, or all but one, of the double bonds reduced (Fig. 4). Polyprenols (with ≥5 isoprene units) constitute another group of *trans* terpene compounds that are found throughout the plant kingdom. Some are free and contain 5–25 isoprene units (Fig. 3), and some are attached to other moieties, as in ubiquinones and plastoquinone (Fig. 4). It is believed that there are no pure *cis*-polyprenols, but dolichols are polyprenols that have a few isoprene units in the *trans* configuration followed by multiple isoprene units linked in *cis* (Brasher *et al*., 2015). When the number of isoprene residues added to a *trans* polyprenyl starter reaches several hundred, the compound is referred to as rubber (Qu *et al*., 2015).

In addition to the terpene compounds listed above that are part of primary metabolism, different plant lineages have evolved the ability to make additional, ‘specialized’ metabolites that enhance the fitness of the plant in its particular ecological niche (discussed later). The number of structurally determined specialized plant terpenes is already in the tens of thousands, and the total number of such plant terpenes is likely to be much higher. Examples include monoterpenes, sequiterpenes, diterpenes and triterpenes found in floral and vegetative parts (e.g, menthol, artemisinin, taxol), triterpenoids and carotenoids (e.g., steroidal alkaloids, cardenolides, and bixin) (Fig. 3). Other compounds are partially derived from a terpene starter, such as monoterpenoid alkaloids (e.g. strychnine, vinblastine) that are synthesized in part from secologanin (Fig. 3), a member of the wide-spread class of iridoid monoterpenes (Geu-Flores *et al*., 2012). A variety of other specialized metabolites exist to which a prenyl unit is added, and then sometimes further modified and even degraded, such as the polyketides humulone and tetrahydrocannabinol, and the coumarin psoralen (Fig. 4).

Generation of structural diversity

There are several causes for terpene diversity that are unique to this class of chemicals and contribute to the huge number of terpenoids found in plants. The first is that the reactions catalyzed by the enzymes known as terpene synthases (TPSs), which use prenyl diphosphates as the precursors to create the basic terpene skeleton, often produce multiple products from a single substrate (a function of stochastic charge migrations in the carbocation intermediate formed in the active site of the enzyme) (summarized in Chen *et al*., 2011). Second, even a single amino acid change in a TPS often leads to drastic changes in the mixture of terpenes produced. Thus, multiple new products can be generated by a single mutation at the beginning of the pathway. Furthermore, TPS genes are typically present as a gene family of 30-100 genes per genome, providing a large platform for the evolution of new terpenes via mutation and selection. Finally, the terpene skeletons are susceptible to a slew of modification reactions by oxidative enzymes, methyltransferases, acyltransferases, prenyltransferases, and a few other types of enzymes that exhibit relaxed substrate and regiospecificity, thus amplifying the number of total compounds produced in the manner of combinatorial biochemistry.

Functions of terpenes of primary metabolism

Terpenes in general are highly hydrophobic. Indeed, in the absence of fatty acid-containing lipids in the archaebacteria, short terpenes are the major constituents of their membranes (in ether linkages to glycerol). Primary and specialized terpenoid metabolites, and prenylated proteins tend to be highly hydrophobic and therefore to be present inside the membrane or tethered to the membrane by virtue of the prenyl group. Examples include ubiquinone and plastoquinone, whose functions in electron transport chains depend upon prenyl chain-mediated membrane association. The presence of double bonds is another important feature of primary metabolite terpenes, allowing the absorption of high-energy radiation or free radicals. Carotenoids in the photosynthetic apparatus and isoprene and structurally diverse monoterpenes are examples of terpenes that protect the plant from such abiotic stress (Velikova *et al*., 2015). As in animals, the specific concentration of triterpene sterols in the membranes has a pronounced effect on the fluidity of plant membranes (Zhou *et al*., 2015).

The potential for a high diversity of structures appears to have resulted in the recruitment of various terpenes as hormones that are recognized by specific receptors. These include the gibberellins, cytokinins, auxins, brassinolides, and the strigolactones (Figs 2, 3). It is noteworthy that multiple forms of each type of hormone exist within a single plant, derived by various modification reactions such as oxidation, reduction, methylation, esterification and other reactions to which most terpenes are susceptible, as described above. Such modification reactions often modulate the strength of the signal given by the hormone molecule as perceived by its receptor, thus making the response finely tunable.

Functions of specialized terpenes

Defense against biological enemies is the best established function for plant terpenoids – whether directly, through targeting of herbivores as toxins or repellants, or indirectly, through the attraction of predators or parasitoid enemies of such herbivores (Kessler & Heil, 2011). However, assigning specific roles to specific terpenes has been difficult because typically multiple, and often very similar, terpenes are produced even within a single plant organ, and additional terpene diversity exists within the entire plant. Given the huge number of specialized terpenes produced by various plant species, it is not surprising that the primary ecological functions of most of them have not yet been elucidated. Attempts to identify specific roles for specific terpenes may often be misguided. Berenbaum & Zangerl (2008) addressed the issue of compound diversification broadly by stating that while work in the late 20th century clearly established that specialized metabolites function in defense (Fraenkel, 1959; Hartmann, 2007), understanding why there are so many different kinds of specialized metabolites remains a key goal.

One explanation for the diversity of specialized metabolism in general was the ‘arms race’ scenario outlined by Ehrlich & Raven (1964), in which specialized metabolites are expected to diversify as a consequence of escalating defense and counter defense between plants and specialized herbivores. In this hypothesis, while biosynthetic capabilities for novel compounds arise throughout evolutionary time, it is not necessarily the diversity of compounds that is adaptive, but rather the most recently evolved compounds, which are now capable of defending the plant against the natural enemy that has not yet evolved a resistance to it. Typically, this arms race occurs on the plant's side, either by adding a new, more complex compound (see Fig. 5a) or by modifying an existing molecule that was originally toxic to the herbivore species (or its ancestor) by adding functional groups to it, such as methyl and acyl groups (using newly evolved methyl and acyl transferases) or by adding oxygen (by oxidative enzymes such as cytochrome P450s; see Berenbaum 1983 for a classic example). This scenario is likely to account for the diversity of benzoxazinoids in maize (Osbourn *et al*., 2003) and glucosinolates in mustards (Angerbirke & Osbourn, 2012). However, note that maize and mustard plants continue to make substantial amounts of several types of the respective class of specialized metabolites and not just one.

While examples of diverse terpenoid compounds have been found in some model organisms, studying their evolution requires comparison with closely related species that occupy different ecological niches, and this is often difficult with model plants because they tend to be cultivated species that have undergone strong artificial selection. Some of the best available studies of terpene diversification have focused on the Burseraceae, a pan-tropical family of trees that produce the terpenoid resins of frankincense (*Boswellia*) and myrrh (*Commiphora*) of the Middle East and copal (*Protium*) of Mesoamerica. *Protium* trees dominate many Amazonian forest sites in abundance and species diversity, with up to 35 species coexisting in some sites, many of them exhibiting complex patterns of constitutive monoterpene and sesquiterpene essential oil composition (Fine *et al*., 2006). The *Protium* TPS-b gene subfamily includes a lineage (C1) of highly duplicated TPS loci common to the Burseraceae and the related citrus family (Rutaceae) (Zapata & Fine, 2013). Patterns of volatile terpene diversification in the Burseraceae have been extensively studied in the 85 species of *Bursera* trees found in tropical Mexico, many of which emit mono- and sesquiterpenes from pressurized resin canals, a so-called 'squirt gun defense', when tissues are wounded by herbivores (Becerra, 1997). *Bursera* trees have specialized herbivores in the flea beetle genus *Blepharida*, whose host-plant utilization patterns, combined with molecular clock analyses of both plant and beetle diversification rates suggest synchronous counter-adaptations by beetles to the evolution of specific classes of defensive terpenoids produced in *Bursera* foliage (Becerra, 2003).

This coevolutionary 'arms-race' process appears to have escalated the complexity and diversity of chemical defenses in the more derived species of *Bursera* (Becerra, *et al*., 2009).

Like their Amazonian relatives in the genus *Protium*, several species of *Bursera* often grow in sympatry, and sympatric species clusters are more likely (than chance) to differ from each other in terpene chemistry, suggesting that their continuous coexistence as distinct species, through avoidance of beetle host shifts, is facilitated by chemical dissimilarity (Becerra, 2007). Two evolutionary paths could lead to the differences in terpene patterns among sympatric species. One would involve the repeated evolution of diverse terpene skeletons in each lineage that colonizes a novel habitat, comparable to the way that *Anolis* lizards repeatedly evolve different trophic niches on different Caribbean islands (Harmon *et al*., 2005; see Fig. 5b). The relative ease in which mutations can lead to the production of new terpenes, or terpenes that are new to this lineage but whose biosynthesis has evolved independently in other species (Pichersky *et al*., 2006; Pichersky & Lewinsohn 2011) suggests that this is a common evolutionary pathway. An alternative, more parsimonious scenario is modeled in Fig. 5(c). Here, selective forces in different environments sort out lineages with preexisting terpene profiles so that sibling species with the same terpene profiles end up in allopatry, while each coexist in geographically separated populations with less closely related congeners that show divergent defense chemistry.

The ancient coevolutionary relationship between *Bursera* tree species and *Blepharida* beetles, mediated by diversification of volatile and non-volatile terpenes in the plant and subsequent behavioral or physiological circumvention of these defenses by the beetles, is not unique, and many of its complex aspects have evolved in many other lineages as well. However, the degree of phylogenetic escalation (i.e., increased diversity of chemicals in some species) varies in plant lineages. In the legume genus *Inga*, another diverse and ecologically dominant group of Neotropical trees, co-occurring species also tend to differ from one another in chemical composition or defense strategy, but show no evidence for phylogenetic escalation of saponins (triterpenes), and only weak diversification of non-terpene phenolic compounds (Kursar *et al*., 2009). Parallel studies reveal that the phylogenetic diversification of North American milkweeds (*Asclepias*) (Fig. 6a) is weakly associated with escalation in phenolic compounds, with a reduction, rather than an escalation, in the amount and complexity of toxic cardenolides (Agrawal *et al*., 2009).

Research on milkweeds highlights the combined chemical and physical defensive functions played by different classes of terpenes that constitute latex. Latex has evolved independently throughout Angiosperm lineages, as it is currently found in over 20,000 plant species from more than 40 families (Agrawal & Konno, 2009). Depending upon the specific lineage, latexes may contain the terpenoid polymer rubber (*cis*-1,4-polyisoprene), sesquiterpene lactones, diterpenes, triterpenes (e.g. cucurbitacins) or cardenolides (Fig. 6) as well as non-terpenoid compounds. Latex is thought to represent a key innovation, such that its presence as a derived trait is correlated with significant increases in species richness and diversification in 13 out of 16 lineages studied (Farrell *et al*., 1991). However, species-rich clades of milkweeds tend to show reduced amounts of latex production, suggesting that counter-defenses by specialized herbivores select for alternative defensive strategies (Agrawal *et al*., 2008). Such counter-defenses include vein cutting or trenching – in which an herbivore clips leaf veins allowing latex to drain from tissues before safely consuming them (Agrawal *et al*., 2009; see Fig. 6). Taken together, the available evidence from *Inga* and *Asclepias* indicates that specialized herbivores are as likely to select for alternative defensive strategies as to trigger the escalation of chemical complexity within a biochemical pathway. Alternative strategies may include physical defenses or even tolerance, in which chemical defense is reduced and plants combat herbivory either with low nutritional quality of leaves or with induced compensatory regrowth (Agrawal & Fishbein, 2006).

Given the mixed current support for the ‘arms-race’ hypothesis as the sole, or even the main, evolutionary driver of phytochemical diversification, what other processes might explain the current diversity of specialized terpenes? Various researchers have proposed alternative hypotheses that can be generally summarized to posit that chemical diversity (constitutive or induced) may be *ab initio* selectively advantageous against a suite of diverse natural enemies (e.g. microbial pathogens as well as vertebrate herbivores). Such an effect might be due to the potential for synergistic interactions between compounds and the decreased chance of simultaneous evolution of resistance to multiple chemicals in the pathogen or herbivore (Firn & Jones, 2003; Richards *et al*., 2015). The hypothesis that diversity is better *per se* is consistent with patterns of evolution in the terpenoid pathway, where, as previously noted, the generation of diversity of terpenoids is augmented by the tendency of TPS enzymes to generate multiple products, up to 50 in some cases, from a single precursor prenyl diphosphate (Steel *et al*., 1998), and the multitude of enzymes with broad substrate specificity that modify the basic skeletons produced by such TPSs (Ginglinger *et al*., 2013; Zi *et al*., 2014). Richards *et al*. (2015) found support for this general hypothesis through the use of multiple regression and path models, revealing that greater functional chemical diversity (albeit in this case amides and phenolic compounds, rather than terpenes) was associated with reduced overall herbivory and increased phototoxicity in *Piper*, another diverse and ecologically dominant genus of tropical trees and shrubs. However, it remains difficult to evaluate this hypothesis due to the difficulty of measuring the costs (metabolic or ecological) and functions associated with specialized metabolites in general, as well as specifically with specialized terpenes.

Regarding the search for function, recent studies illustrate two important caveats: (1) that specialized metabolites can have several, often unexpected, functions depending upon level of organization and ecological context, and (2) that an apparent absence of function may indicate that an appropriate bioassay has not yet been performed. Indeed, multi-functionality of specialized metabolites has become the rule, rather than the exception, as the field of chemical ecology expands from the study of dyadic interactions between organisms (predators and prey, plants and herbivores, flowers and pollinators) to more ecologically realistic networks of community interactions, including microbial symbionts (Hay, 2009; Raguso *et al*., 2015), and the diversity of specialized terpenes best exemplifies this observation, as recounted below.

Multiple functions connect defense with mutualism

It is worth considering cases in which specialized terpenes with known defensive functions in certain contexts come to play different ecological roles. The volatile monoterpenoid 1,8-cineole shows broad-band antimicrobial and insecticidal properties (Lee *et al*., 2004; Hendry *et al*., 2009) and is a dominant leaf essential oil constituent across two highly diverse, ecologically and economically important plant genera with extensive geographic distributions, *Eucalyptus* (> 700 spp.) and *Salvia* (*c*. 1000 spp.) (Perry *et al*., 2003; Batish *et al*., 2008). However, at least one group of insects, the orchid bees (Euglossinae), can physiologically tolerate undiluted quantities of 1,8-cineole. Male orchid bees collect 1,8-cineole in liquid form from flowers of several Neotropical orchid genera, for which they are the exclusive pollinators, to be used in complex courtship behaviors directed at female bees (Zimmermann *et al*., 2006; Fig. 7a). In parallel, female orchid bees collect triterpene resins (mixtures of β-amyrin, β-amyrone, dammadienol and dammadienone) from flowers of some *Dalechampia* species as critical resources for nest construction, and pollinate these flowers in the process (Armbuster, 1993). Phylogenetically informed bioassays reveal that all *Dalechampia* species produce these resins in foliage, where they have been shown to reduce or inhibit damage by generalist and specialist herbivores (Armbuster *et al*., 1997), suggesting that the defensive functions of these resins preceded their co-option as floral rewards in species with specialized pollination (Armbuster *et al*., 2009). In the same Neotropical region, *Asclepias curassavica* and related milkweed plants produce cardenolides which, through their potent inhibition of Na^+/K^+ ATPase pumps, are broadly toxic to many organisms (Agrawal *et al*., 2008; see above). However, specialized milkweed herbivores, including danaiine butterflies and lygaeid bugs, have independently evolved cardenolide-resistant ATPases and the ability to sequester host plant cardenolides as components of defense against their own predators, which, coupled with bright aposematic coloration, provides the foundation for insect mimicry (Petschenka *et al*., 2013; Bramer *et al*., 2015).

In each of these examples, the compounds of interest originated as toxins against a broad spectrum of enemies, subsequently became utilized as attractants or rewards by organisms that had evolved resistance to them, and eventually were co-opted as signals or resources that enhance the survival or reproductive success of such organisms. Zimmer & Ferrer (2007) have outlined a model for the evolutionary modification of such deadly toxins as tetrodotoxin into ecological signals (in courtship) and cues (in predator avoidance) that structure community interactions among organisms with evolved tolerance. Similar transitions play major roles in the evolution of multifunctional plant terpenoids, particularly in the evolution of bark beetle monoterpene pheromone communication through the detoxification of pine oleoresins (Raffa, 2014), and the co-option of terpenoid and aromatic floral defense compounds as pollinator attractants (Schiestl, 2010). It is instructive that not only are some volatile terpenes (e.g. linalool) repellent to facultative flower visitors and attractive to obligate visitors (Junker & Blüthgen, 2010), but also that similar compounds (e.g. β-myrcene) can be attractive or repellent to the same obligate visitor at different concentrations (Terry *et al*., 2007; Fig. 7b). Although plant volatile terpenes likely originated as defense compounds (Schiestl, 2010), they now function as host selection cues in nearly every obligate pollination mutualism that has been described thus far, from specific blends of chiral monoterpene and sesquiterpene hydrocarbons in fig–fig wasp interactions (Chen & Song, 2008), to lilac aldehydes in *Silene*–*Hadena* moth interactions (Dötterl *et al*., 2006), to oxygenated C11 homoterpenes derived from nerolidol in yucca–yucca moth interactions (Svensson *et al*., 2005) (Fig. 7c). A fuller spectrum of bioassays may reveal that these compounds, while selectively attractive to the plants' obligate pollinators, may be broadly repellent to other organisms, ranging from ineffectual pollinators and florivores to microbial pathogens (Junker *et al*., 2011; Junker & Tholl, 2013).

Conclusions and future directions

In closing, examination of the published record regarding the biosynthesis and function of plant terpenoids reveals several emerging patterns:

- The presence in the genome of each plant species of large number of genes already involved in terpene biosynthesis underlies the present ability of plant lineages to make large number of terpenoids. It also provides a large platform for the evolution of new terpenes via mutation and selection.

- New terpenoids keep arising in specific plant lineages, potentially as an outcome of coevolution with natural enemies. However, specialized terpenoids tend to occur as cocktails of multiple, related compounds in both radiating species as well as less quickly evolving species, suggesting that terpenoid diversity provides an advantage *per se*.

- These suites of terpenoid defense compounds do not increase in number indefinitely for several reasons such as excessive cost or the evolution of alternative (non-terpenoid-based) defense pathways.

- Due to the multifaceted chemical and physical properties of terpenoids, some also have non-defensive functions, including signaling to mutualists such as pollinators or symbionts.

However, analysis of these patterns does not yet provide definitive answers as to how terpenoids became the largest class of compounds produced in plants, and often the largest class of specialized compounds that each plant species produces. To understand the evolutionary and ecological processes that gave rise to these patterns, we need to expand our understanding of the actual costs and benefits conferred by specialized terpenes to the organisms that synthesize them, by combining experimental approaches that manipulate chemical diversity with unbiased or untargeted assays in natural or semi-natural community settings. One step forward in this regard, at least for model plants and those amenable to gene silencing approaches, has been to silence genes responsible for a constitutive or inducible metabolite or pathway, and then to measure changes in performance in growth, defense and reproductive success. Baldwin and colleagues (Kessler *et al.*, 2004, 2008, 2013) have used such an approach to identify unexpected community links (e.g. normally deterred enemies) and measure ecological costs and fitness impacts of specialized metabolites. These elegant field studies highlight the importance of unanticipated targets or recipients of specialized terpenes and similar metabolites. More often than not, these recipients include the full spectrum of microbial partners, from pathogens to mutualists, along with other, multicellular organisms in the rhizosphere, where community interactions are necessarily mediated by chemical signals and cues (Turlings *et al.*, 2012; van Dam & Bouwmeester, 2016).

Identifying the molecular targets of plant terpenoids is an exciting challenge. One recently developed method that could be used is activity-based protein profiling (ABPP). Although still in its infancy, this approach uses tagged chemical probes to identify protein receptors, secondary messengers and transcriptional regulators that respond to specific metabolites (e.g. antibiotics; Sadler & Wright, 2015). Conceivably, proteomics-based approach might also be applied more broadly to soil community bacteria exposed to the root exudates of a model plant. Finally, the targets of specialized metabolites might actually be plant cells, either through processes that affect pollen tube growth or seed germination (Khan *et al.*, 2008), or through feed-back transcriptional control on their own biosynthesis (Burow *et al.*, 2015). There is ample scope for experimental approaches that consider the full spectrum of interactions mediated by specialized terpenes, from a plant's own metabolic regulation to its community ecology.

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Fig. 1 The isoprene unit and the head-to-tail and head-to-head forms of condensing isoprene units.

Fig. 2 The classes of terpenes and their origin from the isoprene building blocks, DMAPP (dimethylallyl diphosphate) and IPP (isopentenyl diphosphate), and the *trans* prenyl diphosphate GPP (geranyl diphosphate), *e,e*-FPP (farnesyl diphosphate) and GGPP (geranylgeranyl diphosphate) and the *cis*-prenyl diphosphate NPP (neryl diphosphate), *z,z*-FPP, and NNPP (nerylneryl diphosphate). In plants, two pathways independently contribute to the production of IPP and/or DMAPP, and plants also have isomerases that interconvert both. The acronym of one pathway is MEP, it operates in the plastids, and it starts with pyruvate and glyceraldehyde-4-phosphate. The acronym of the second pathway is MEV, it operates in the cytosol (and possibly partially in the peroxisomes), and it starts with acetyl-CoA.

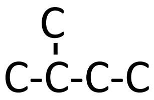
Fig. 3 Examples of plant terpenoids. Monoterpenoids and sesquiterpenoids are generally volatile when not conjugated to polar or large (>200 d) moieties. Higher-order terpenoids are generally not volatile.

Fig. 4 Prenylated compounds. In parentheses, the number of carbons of the terpenoid moiety is indicated. For clarity, in some cases the terpenoid carbons are also shown in red.

Fig. 5 Contrasting models of the diversification of defensive terpenes in plant lineages. (a) This depicts a 'chemical arms race' in which new terpene skeletons evolve in specific lineages, presumably in response to escalating selective pressures by herbivores. Chemical escalation is indicated by the addition of a new skeleton at each node of the tree. In this example, the ancestral lineage made limonene, and α-pinene, α -farnesene, and α-cadinene were successively added to the repertoire of specific lineages. (b, c) Contrasting models for generation of terpenoid diversity in different lineages in relationship to geographic distribution. In (b), terpene diversification occurs repeatedly as the ancestral taxon in each lineage colonizes a new geographic region (i, ii, iii), comparable to repeated trophic niche evolution in *Anolis* lizards that colonize different Caribbean islands (Harmon *et al*., 2005). The chemical dissimilarity between each cluster of related species is thought to promote their co-existence by reducing the probability that specialist herbivores can move between them as host plants. In (c), terpene diversification is more parsimonious, as the biosynthetic capacity to produce each terpenoid set evolves only once and defines each lineage as a synapomorphy. Here, members of each chemically-defined lineage colonize different geographic regions (i, ii, iii), in which, through ecological filtering, they co-exist with species with divergent defense chemistry.

Fig. 6 Convergent evolution of terpenoid latex production in plants and behaviors allowing insects to circumvent latex defenses. (a) A monarch butterfly larva trenching a milkweed leaf, thereby avoiding ingesting latex with cardenolides (photo courtesy of Stan Rehm, with permission). (b) A chrysomelid beetle trenching a leaf of a wild cucurbit plant, whose latex contains cucurbitacins (photo courtesy of David E. Dussourd, with permission). (c) A katydid clipping the midrib of a dogbane leaf, whose latex contains cardenolides (photo courtesy of David E. Dussourd, with permission). Note the terpene-laced droplets of latex at the wounds in all three species.

Fig. 7 Plant–pollinator mutualisms mediated by floral terpenes. (a) A male *Eulaema* orchid bee collecting volatile terpenes from a *Catasetum* orchid (photo courtesy of Ian Morton, with permission); females of the same species collect triterpene resins from other flowers (b) Thrips emerging from a male cycad cone, to which they are attracted (or from which they are repelled) by different concentrations of volatile terpenes (photo courtesy of Irene Terry, with permission). (c) A Yucca moth resting within a flower of *Yucca glauca*, to which it is attracted by volatile terpenes (photo courtesy of R. A. Raguso).



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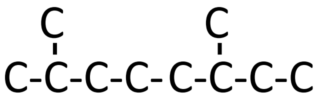
Isoprene



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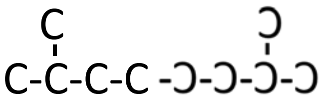
head to tail



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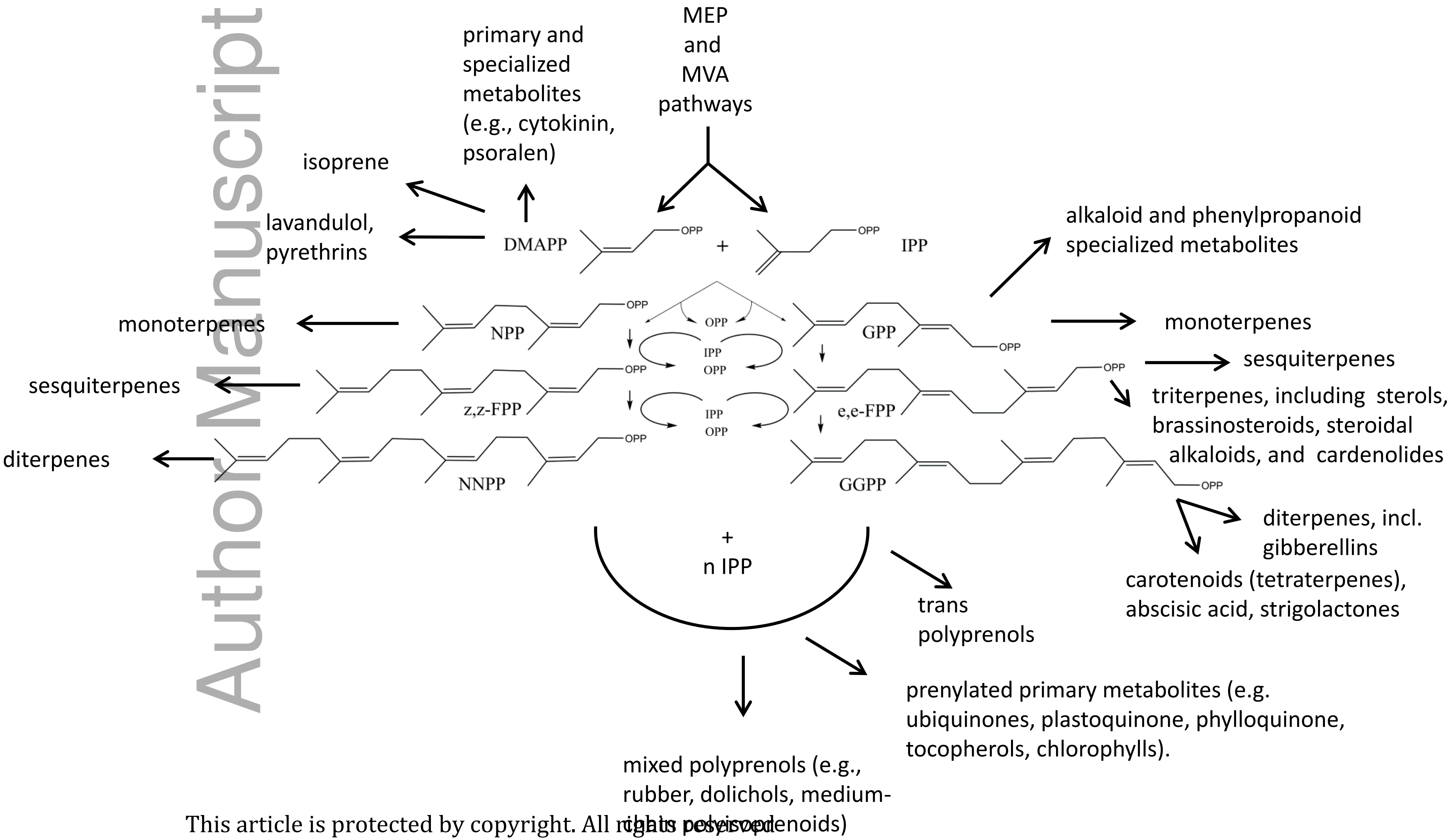
head to head


MEP
and
MVA
pathways
primary and
specialized
metabolites
(e.g., cytokinin,
psoralen)
isoprene
lavandulol,
pyrethrins
DMAPP
OPP
+
OPP
IPP
alkaloid and phenylpropanoid
specialized metabolites
OPP
NPP
monoterpenes
OPP
GPP
OPP
monoterpenes
IPP
OPP
sesquiterpenes
OPP
z,z-FPP
OPP
e,e-FPP
sesquiterpenes
triterpenes, including sterols,
brassinosteroids, steroidal
alkaloids, and cardenolides
IPP
OPP
diterpenes
OPP
NNPP
GGPP
OPP
diterpenes, incl.
gibberellins
carotenoids (tetraterpenes),
abscisic acid, strigolactones
+
n IPP
trans
polyprenols
prenylated primary metabolites (e.g.
ubiquinones, plastoquinone, phylloquinone,
tocopherols, chlorophylls).
mixed polyprenols (e.g.,
rubber, dolichols, medium-
chain polyisoprenoids)

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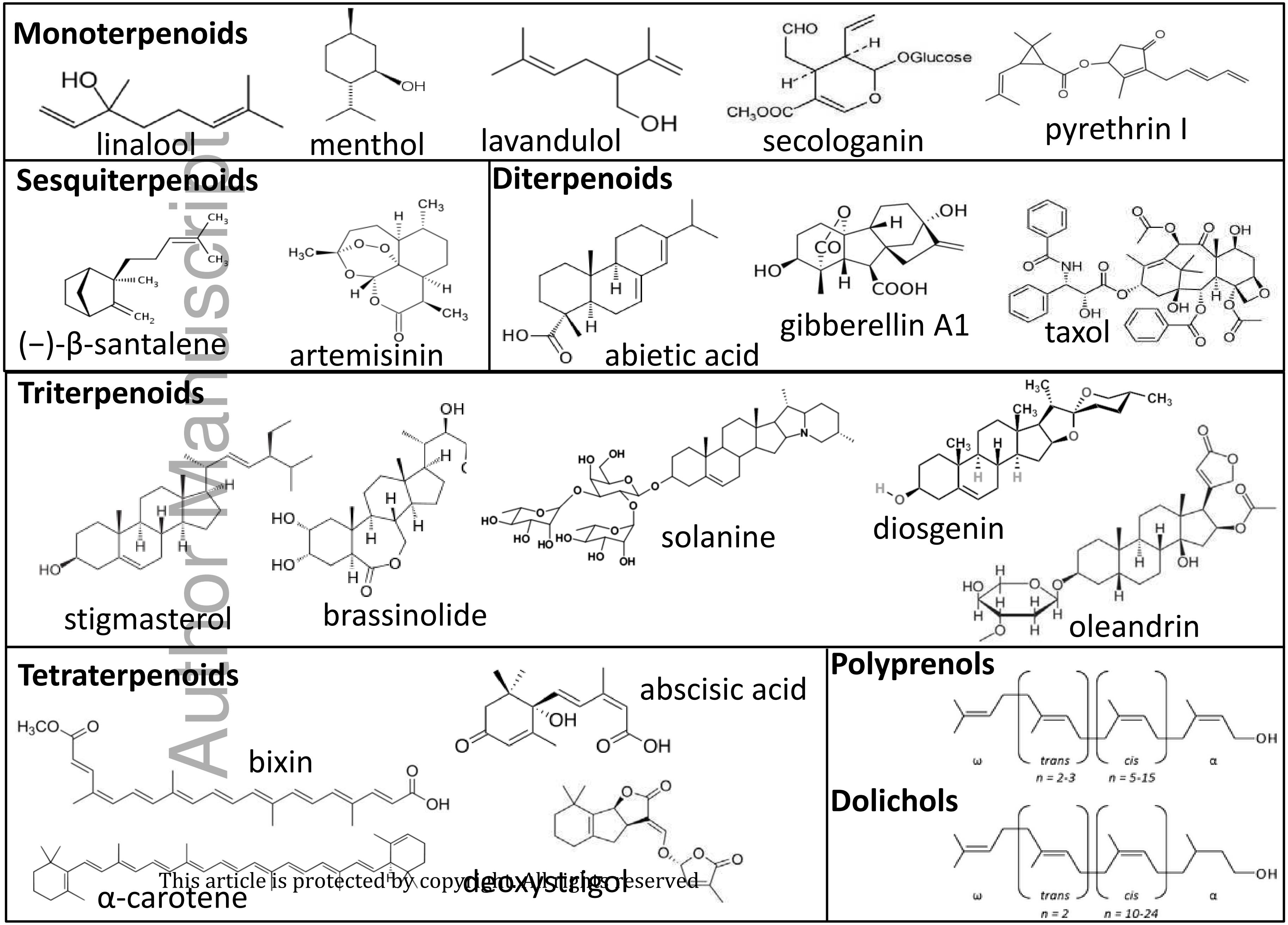

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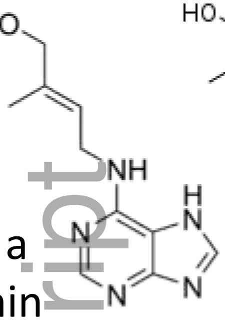
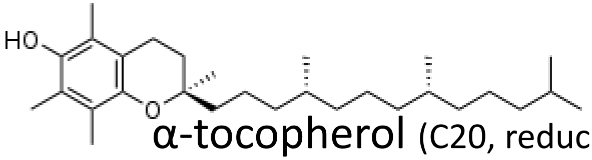
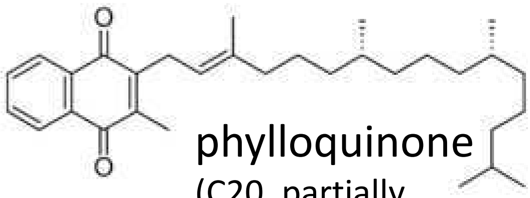


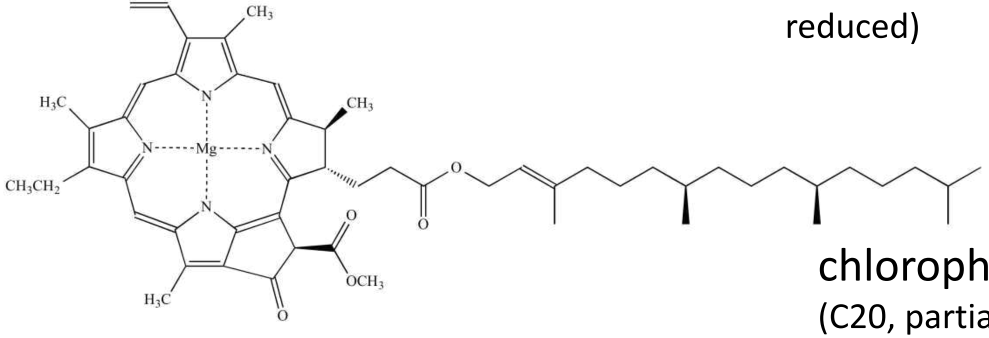
zeatin, a cytokinin (C5)



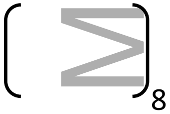
α-tocopherol (C20, reduced)



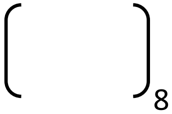
phylloquinone (C20, partially reduced)



chlorophyll *a* (C20, partially reduced)



plastoquinone (C45)

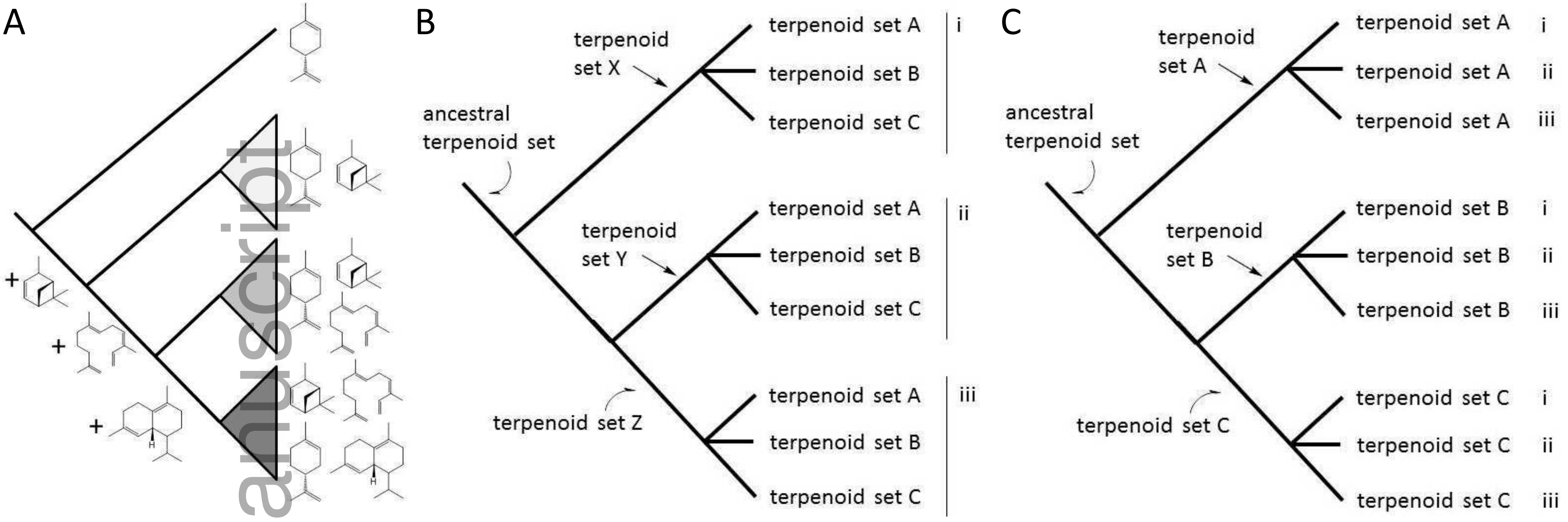


ubiquinone-9 (C45)

tetrahydrocannabinol (C10)

psoralen (C5, partially degraded)

humulone (C5, C5)


A
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i
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terpenoid set B
terpenoid set C
ii
terpenoid set Z
terpenoid set A
terpenoid set B
terpenoid set C
iii
C
terpenoid set A
ancestral terpenoid set
terpenoid set A i
terpenoid set A ii
terpenoid set A iii
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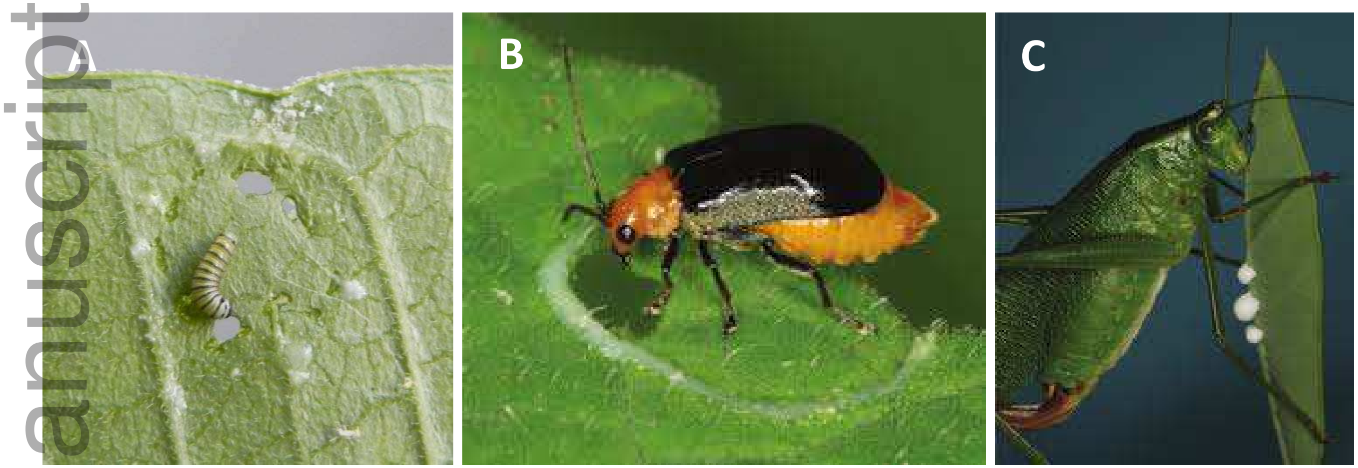
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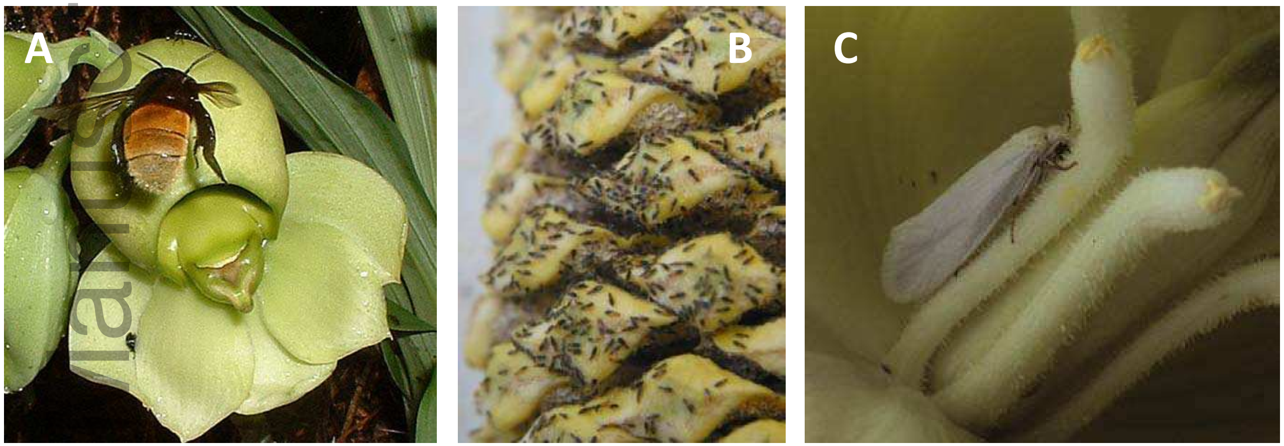
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