## Research review

# Why do plants produce so many terpenoid compounds?

Author for correspondence:
*Eran Pichersky*
*Tel: +1 734 936 3522*
*Email: lelx@umich.edu*

Received: *19 April 2016*
Accepted: *2 August 2016*

### Eran Pichersky[1] and Robert A. Raguso[2]

[1]Department of Molecular, Cellular and Developmental Biology, University of Michigan, Michigan, MI 48109, USA; [2]Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

## 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 the attraction of beneficial organisms. A popular hypothesis is that the ability of plants to synthesize new compounds arose incrementally by selection when, as a result of gradual changes in their biotic partners and enemies, the 'old' plant compounds were no longer effective, a process dubbed the 'coevolutionary arms race'. Another hypothesis posits that often the sheer diversity of such compounds provides benefits that a single compound cannot. In this article, we review the unique features of the biosynthetic apparatus of terpenes in plants that facilitate the production of large numbers of distinct terpenoids in each species and how facile genetic and biochemical changes can lead to the further diversification of terpenoids. We then discuss evidence relating to the hypotheses that given ecological functions may be enhanced by the presence of mixtures of terpenes and that the acquisition of new functions by terpenoids may favor their retention once the original functions are lost.

## 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 total, 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). Although 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 have 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 plants with biotic and abiotic aspects of their 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 been facilitated 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 compound 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

See also the Editorial by Kessler, **220**: 655–658.

diversity of known terpenoid structures and functions, in both primary metabolism and specialized metabolism, and the possible molecular mechanisms by which such diversity arose during the evolution of green plants. We conclude by critically examining the possible reasons for the adaptive value to any given species of having a large diversity of terpenoids, and for the continued evolution of further 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 has been 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 have evaporated from it – has been 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 masticated food. Thus, ginger flavor is caused by zingiberene, and nootkatone imparts a 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, as a result of their microbicidal and insecticidal properties (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 the treatment of 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 concentrations 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 the 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 to 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 at ambient temperature, could be produced in biological systems for fuel (Wang *et al.*, 2015). Some progress has also been achieved towards the genetic engineering of 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 1920s, 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, whereas an irregular terpene was formed in a 'head to head' condensation (Fig. 1). It is important to note, however, 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 as 'regular' terpene biosynthetic reactions, whereas the term 'irregular' is applied to a 'head to middle' linkage of isoprene units that is part of the biosynthetic pathway of compounds such 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), and so only the general facts are 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 of four isoprene units a diterpene (C20). The actual condensation of these isoprene units in the plant occurs with the diphosphate-activated forms, called prenyl
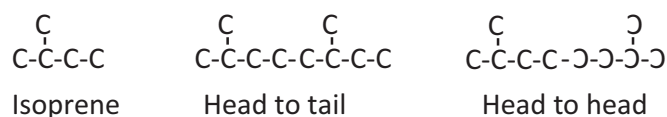


**Fig. 1** The isoprene unit and the head to tail and head to head forms of condensing isoprene units.

primary and specialized metabolites (e.g. cytokinin, psoralen)

MEP and MVA pathways

isoprene

lavandulol, pyrethrins

DMAPP   OPP   +   OPP   IPP

alkaloid and phenylpropanoid specialized metabolites

monoterpenes   NPP   OPP   OPP   GPP   OPP   monoterpenes

IPP OPP

sesquiterpenes   OPP   z,z-FPP   IPP OPP   e,e-FPP   OPP   sesquiterpenes

triterpenes, including sterols, brassinosteroids, steroidal alkaloids, and cardenolides

diterpenes   NNPP   OPP   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)
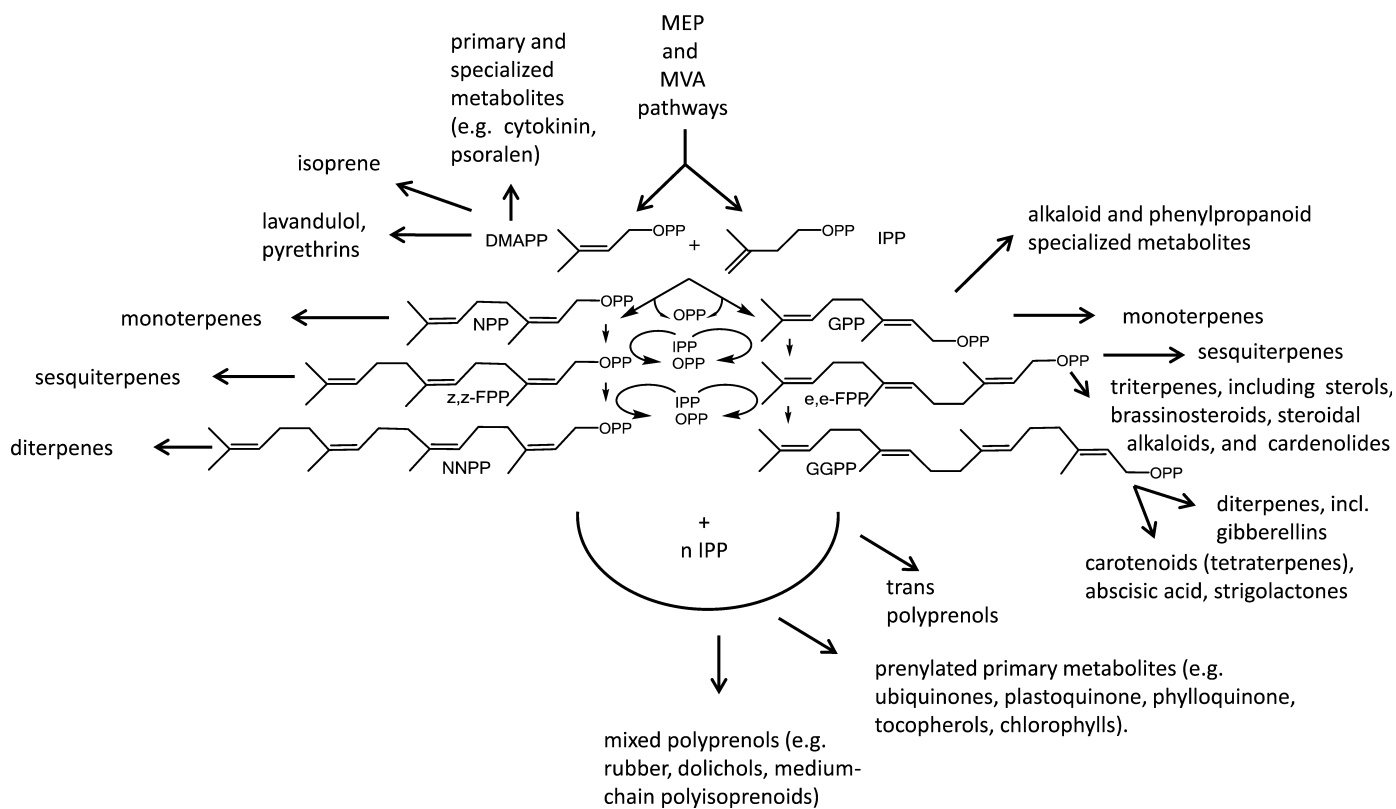
**Fig. 2** The classes of terpenes and their origin from the isoprene building blocks DMAPP (dimethylallyl diphosphate) and IPP (isopentenyl diphosphate), the *trans*-prenyl diphosphate GPP (geranyl diphosphate), *e,e*-FPP (farnesyl diphosphate) and GGPP (geranylgeranyl diphosphate) and the *cis*-prenyl 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 starts with pyruvate and glyceraldehyde-4-phosphate. The acronym of the second pathway is MVA; it operates in the cytosol (and possibly partially in the peroxisomes) and starts with acetyl-CoA.

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, whereas 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 as α-carotene and β-carotene, are major constituents of photosynthetic tissues, and the hormones abscisic acid and strigolactones are derived from the 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, whereas 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, which 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), which are synthesized in part from secologanin (Fig. 3), a member of the widespread 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
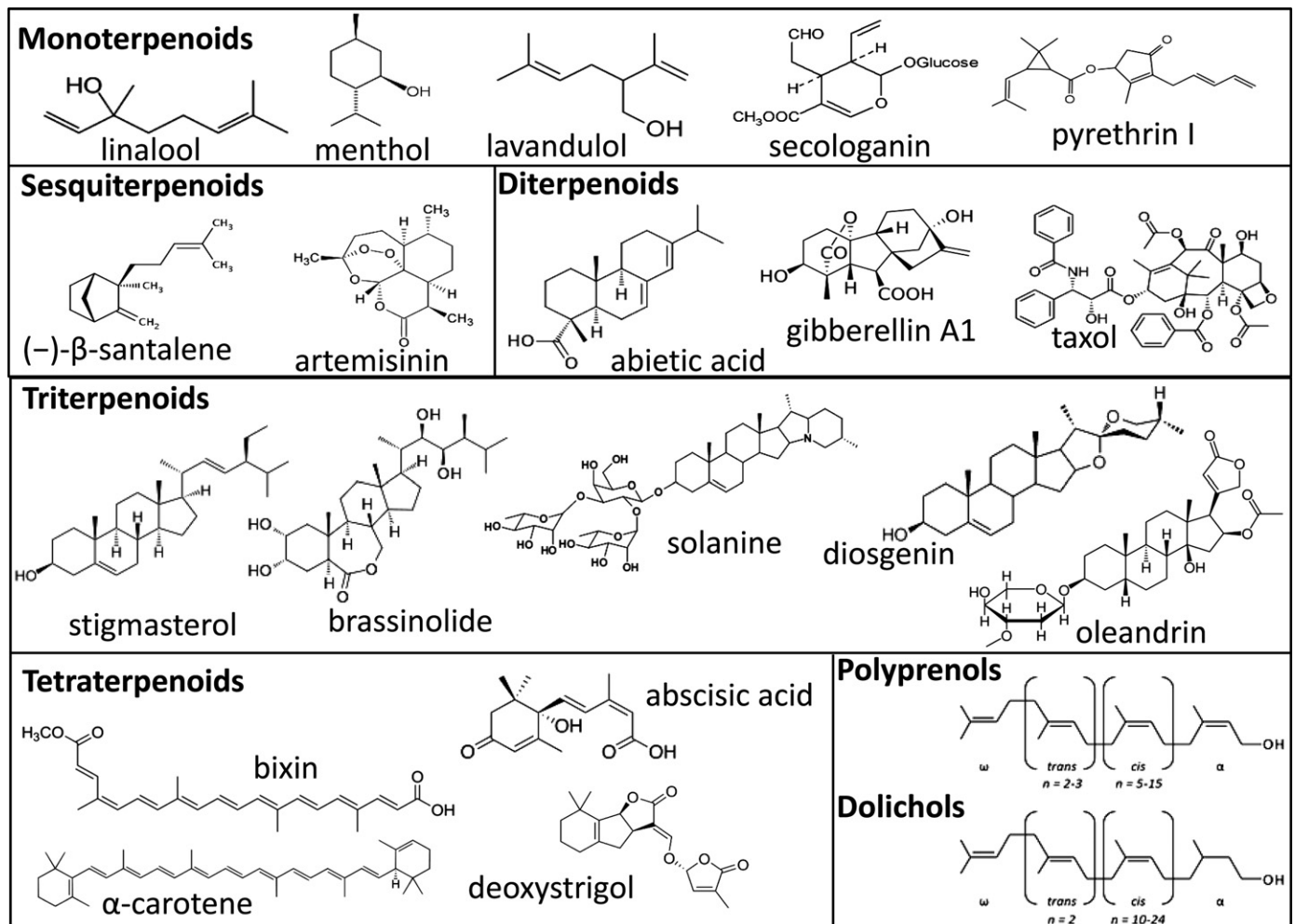
**Fig. 3** Examples of plant terpenoids. Monoterpenoids and sesquiterpenoids are generally volatile when not conjugated to polar or large (> 200 Da) moieties. Higher order terpenoids are generally not volatile.

modified and even degraded, such as the polyketides humulone and THC, and the coumarin psoralen (Fig. 4).

## Generation of structural diversity

There are several causes of 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 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 enzyme 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 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 on 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
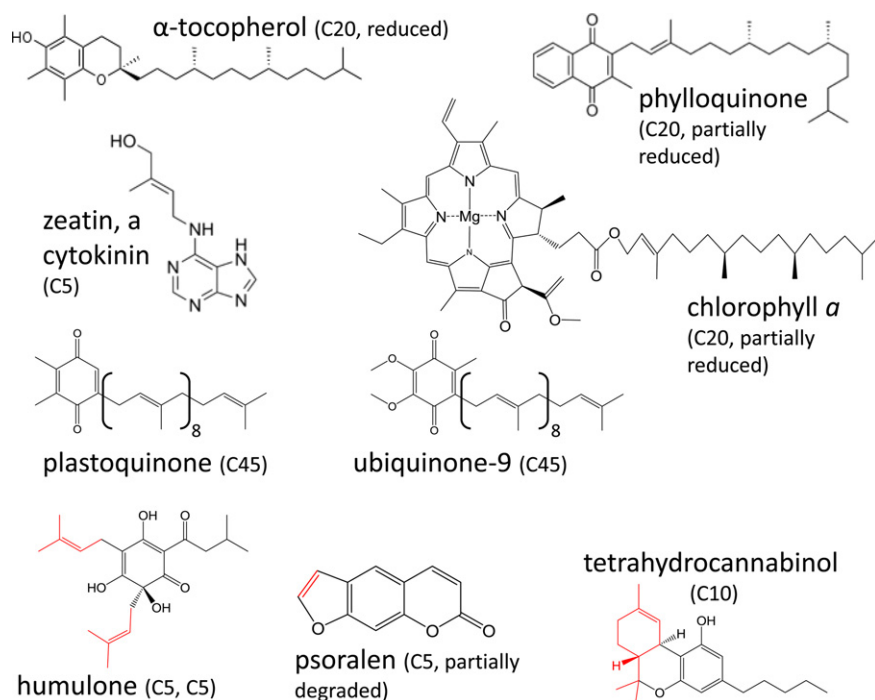
**Fig. 4** Prenylated compounds. The number of carbons of the terpenoid moiety is indicated in parentheses. For clarity, in some cases, the terpenoid carbons are also shown in red.

(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 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 the targeting of herbivores as toxins or repellents, or indirectly through the attraction of predators or parasitoid enemies of such herbivores (Kessler & Heil, 2011). However, the assignment of 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 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, although 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, although 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 them. 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 (Agerbirk & Olsen, 2012). However, it should be noted that maize and mustard plants continue to make substantial amounts of several types of the respective class of specialized metabolite and not just one.

Although examples of diverse terpenoid compounds have been found in some model organisms, the study of 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
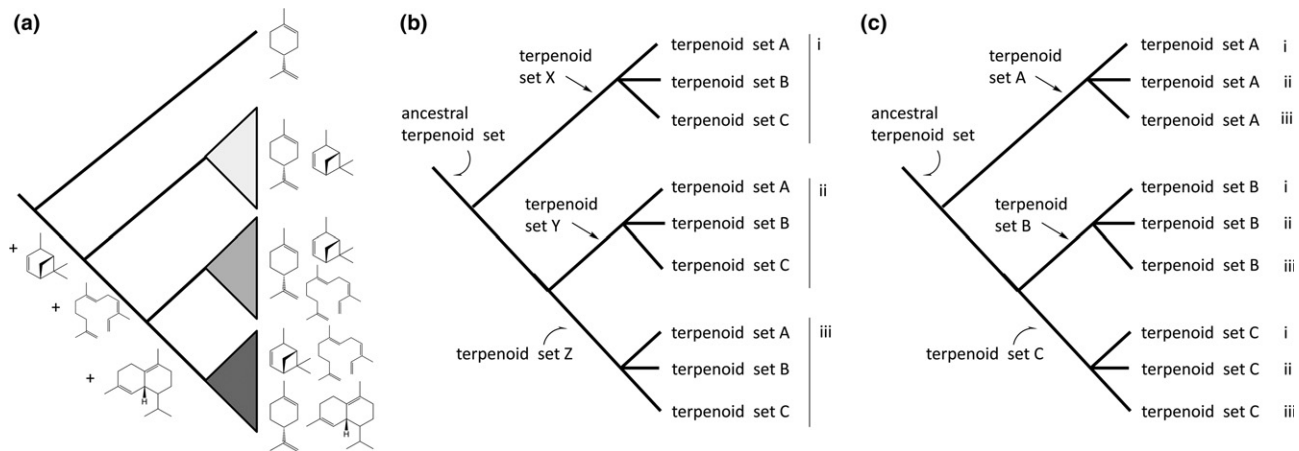
**Fig. 5** Contrasting models of the diversification of defensive terpenes in plant lineages. (a) This depicts a 'chemical arms race', in which new terpene skeletons evolve in specific lineages, presumably in response to escalating selective pressures by herbivores. Chemical escalation is indicated by 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 the generation of terpenoid diversity in different lineages in relation 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 with 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.

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 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 the 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 with the way in which *Anolis* lizards repeatedly evolve different trophic niches on different Caribbean islands (Harmon *et al.*, 2005; see Fig. 5b). The relative ease with 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 pre-existing terpene profiles, so that sibling species with the same terpene profiles end up in allopatry, whilst each coexists 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 the 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 have revealed that the phylogenetic diversification of North American milkweeds (*Asclepias*) (Fig. 6a) is weakly associated with escalation in phenolic compounds, and with a reduction, rather than an escalation, in the amount and complexity of toxic cardenolides (Agrawal *et al.*, 2009).

**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 the ingestion of latex with cardenolides (photograph courtesy of Stan Rehm, with permission). (b) A chrysomelid beetle trenching a leaf of a wild cucurbit plant, whose latex contains cucurbitacins (photograph courtesy of David E. Dussourd, with permission). (c) A katydid clipping the midrib of a dogbane leaf, whose latex contains cardenolides (photograph courtesy of David E. Dussourd, with permission). Note the terpene-laced droplets of latex at the wounds in all three species.

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 > 40 families (Agrawal & Konno, 2009). Depending on 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 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 a 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 a result of 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 (Steele *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 because of the difficulty of measuring the costs (metabolic or ecological) and functions associated with specialized metabolites in general, as well as specifically with specialized terpenes.

With regard to the search for function, recent studies have illustrated two important caveats: (1) that specialized metabolites can have several, often unexpected, functions depending on the 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, multifunctionality of specialized metabolites has become the rule, rather than the exception, as the field of chemical ecology has expanded 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 (Armbruster, 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 (Armbruster *et al.*, 1997), suggesting that the defensive functions of these resins preceded their co-option as floral rewards in species with specialized pollination (Armbruster *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). 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 enhanced 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 probably 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, although 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).
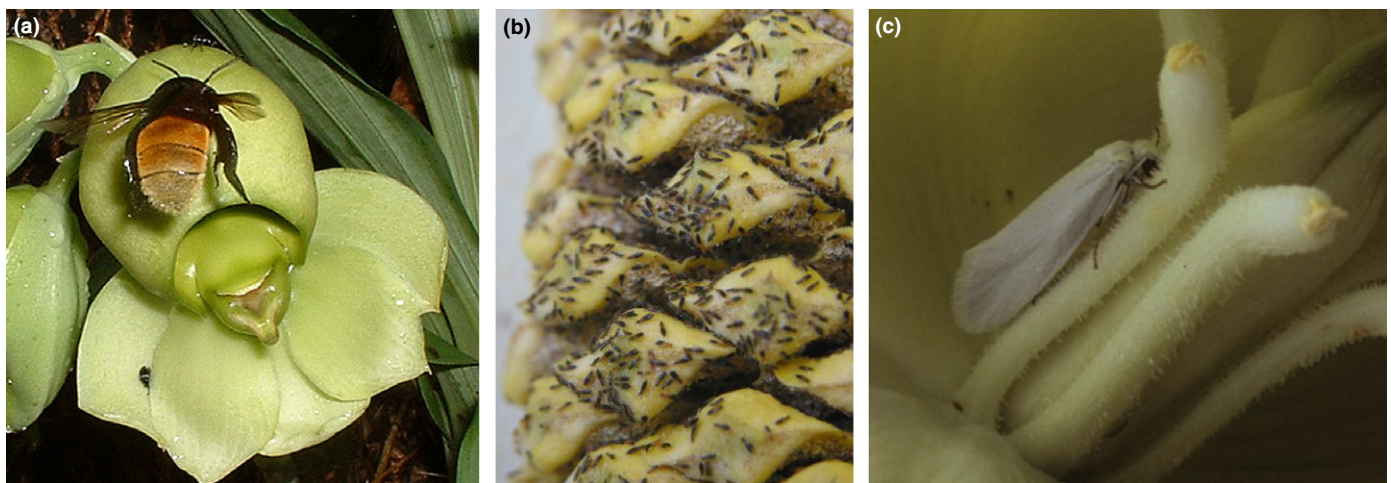


**Fig. 7** Plant–pollinator mutualisms mediated by floral terpenes. (a) A male *Eulaema* orchid bee collecting volatile terpenes from a *Catasetum* orchid (photograph courtesy of Ian Morton, with permission); females of the same species collect triterpene resins from other flowers. (b) Thrips emerging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

## Conclusions and future directions

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

• The presence in the genome of each plant species of a large number of genes already involved in terpene biosynthesis underlies the present ability of plant lineages to make large numbers 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.

• As a result of 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 has not yet provided 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 to measure ecological costs and fitness impacts of specialized metabolites. These elegant field studies have highlighted 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, together 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).

The identification of 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 approaches 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 feedback 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.

## References

Agrawal AA, Fishbein M. 2006. Plant defense syndromes. *Ecology* 87: S132–S149.

Agrawal AA, Fishbein M, Halitschke R, Hastings AP, Rabosky DL, Rasmann S. 2009. Evidence for adaptive radiation from a phylogenetic study of plant defenses. *Proceedings of the National Academy of Sciences, USA* 106: 18067–18072.

Agrawal AA, Konno K. 2009. Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annual Review of Ecology and Evolutionary Systematics* 40: 311–331.

Agrawal AA, Lajeunesse MJ, Fishbein M. 2008. Evolution of latex and its constituent defensive chemistry in milkweeds (*Asclepias*): a phylogenetic test of plant defense escalation. *Entomologia Experimentalis et Applicata* 128: 126–138.

Agrawal AA, Petschenka G, Bingham RA, Weber MG, Rasmann S. 2012. Toxic cardenolides: chemical ecology and coevolution of specialized plant–herbivore interactions. *New Phytologist* 194: 28–45.

Al-Babili A, Bouwmeester HJ. 2015. Strigolactones, a novel carotenoid-derived plant hormone. *Annual Review of Plant Biology* 66: 161–186.

Agerbirk N, Olsen CE. 2012. Glucosinolate structures in evolution. *Phytochemistry* 77: 16–45.

Armbruster WS. 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution* 47: 1480–1505.

Armbruster WS, Howard JJ, Clausen TP, Debevec EM, Loquvam JC, Matsuki M, Cerendolo B, Andel F. 1997. Do biochemical exaptations link evolution of plant defense and pollination systems? Historical hypotheses and experimental tests with *Dalechampia* vines. *American Naturalist* 149: 461–484.

Armbruster WS, Lee J, Baldwin BG. 2009. Macroevolutionary patterns of defense and pollination in *Dalechampia* vines: adaptation, exaptation, and evolutionary novelty. *Proceedings of the National Academy of Sciences, USA* 106: 18085–18090.

Batish DR, Singh HP, Kohli RK, Kaur S. 2008. Eucalyptus essential oil as a natural pesticide. *Forest Ecology & Management* 256: 2166–2174.

Becerra JX. 1997. Insects on plants: macroevolutionary chemical trends in host use. *Science* 276: 253–256.

Becerra JX. 2003. Synchronous coadaptation in an ancient case of herbivory. *Proceedings of the National Academy of Sciences, USA* 100: 12804–12807.

Becerra JX. 2007. The impact of herbivore–plant coevolution on plant community structure. *Proceedings of the National Academy of Sciences, USA* 104: 7483–7488.

Becerra JX, Noge K, Venable DL. 2009. Macroevolutionary chemical escalation in an ancient plant–herbivore arms race. *Proceedings of the National Academy of Sciences, USA* 106: 18062–18066.

Berenbaum M. 1983. Coumarins and caterpillars – a case for coevolution. *Evolution* 37: 163–179.

Berenbaum MR, Zangerl AR. 2008. Facing the future of plant–insect interaction research: le retour à la "raison d'être". *Plant Physiology* 146: 804–811.

Bramer C, Dobler S, Deckert J, Stemmer M, Petschenka G. 2015. Na⁺/K⁺-ATPase resistance and cardenolide sequestration: basal adaptations to host plant toxins in the milkweed bugs (Hemiptera: Lygaeidae: *Lygaeinae*). *Proceedings of the Royal Society B: Biological Sciences* 282: 20142346.

Brasher MI, Surmacz L, Leong B, Pitcher J, Swiezewska E, Pichersky E, Akhtar TA. 2015. A two-component enzyme complex is required for dolichol biosynthesis in tomato. *Plant Journal* 82: 903–914.

Burow M, Atwell S, Francisco M, Kerwin RE, Halkier BA, Kliebenstein DJ. 2015. The glucosinolate biosynthetic gene AOP2 mediates feed-back regulation of jasmonic acid signaling in *Arabidopsis*. *Molecular Plant* 8: 1201–1212.

Chen C, Song Q. 2008. Responses of the pollinating wasp *Ceratosolen solmsi marchali* to odor variation between two floral stages of *Ficus hispida*. *Journal of Chemical Ecology* 34: 1536–1544.

Chen F, Ro D-K, Petri J, Gershenzon J, Bohlmann J, Pichersky E, Tholl D. 2004. Characterization of a root-specific *Arabidopsis* terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. *Plant Physiology* 135: 1956–1966.

van Dam NM, Bouwmeester HJ. 2016. Metabolomics in the rhizosphere: tapping into belowground chemical communication. *Trends in Plant Science* 21: 256–265.

Demissie ZA, Erland LA, Rheault MR, Mahmoud SS. 2013. The biosynthetic origin of irregular monoterpenes in *Lavandula*. *Journal of Biological Chemistry* 288: 6333–6341.

Djerassi C. 1990b. Steroids made it possible, series title. In: Seeman JI, series ed. *Profiles, pathways, and dreams*. Washington, DC, USA: American Chemical Society.

Dötterl S, Jürgens A, Seifert K, Laube T, Weissbecker B, Schütz S. 2006. Nursery pollination by a moth in *Silene latifolia*: the role of odours in eliciting antennal and behavioural responses. *New Phytologist* 169: 707–718.

Ehrlich PR, Raven PH. 1964. Butterflies and plants – a study of coevolution. *Evolution* 18: 586–608.

Farrell BD, Dussourd DE, Mitter C. 1991. Escalation of plant defense: do latex and resin canals spur plant diversification? *American Naturalist* 138: 881–900.

Fine PV, Miller ZJ, Mesones I, Irazuzta S, Appel HM, Stevens MHH, Sääksjärvi I, Schultz JC, Coley PD. 2006. The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87: S150–S162.

Firn RD, Jones CG. 2003. Natural products – a simple model to explain chemical diversity. *Natural Product Reports* 20: 382–391.

Fraenkel GS. 1959. The raison d'être of secondary plant substances. *Science* 129: 1466–1470.

Geu-Flores F, Sherden NH, Courdavault V, Burlat V, Glenn WS, Wu C, Nims E, Cui Y, O'Connor SE. 2012. An alternative route to cyclic terpenes by reductive cyclization in iridoid biosynthesis. *Nature* 492: 138–142.

Ginglinger JF, Boachon B, Höfer R, Paetz C, Köllner TG, Miesch L, Lugan R, Baltenweck R, Mutterer J, Ullmann P *et al.* 2013. Gene coexpression analysis reveals complex metabolism of the monoterpene alcohol linalool in Arabidopsis flowers. *Plant Cell* 25: 4640–4657.

Harmon LJ, Kolbe JJ, Cheverud JM, Losos JB. 2005. Convergence and multidimensional niche. *Evolution* 59: 409–421.

Hartmann T. 2007. From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry* 68: 2831–2846.

Hay ME. 2009. Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. *Annual Review of Marine Science* 1: 193–212.

Heiling S, Schuman MC, Schoettner M, Mukerjee P, Berger B, Schneider B, Jassbi AR, Baldwin IT. 2010. Jasmonate and ppHsystemin regulate key malonylation steps in the biosynthesis of 17-hydroxygeranyllinalool diterpene glycosides, an abundant and effective direct defense against herbivores in *Nicotiana attenuata*. *Plant Cell* 22: 273–292.

Hendry ER, Worthington T, Conway BR, Lambert PA. 2009. Antimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. *Journal of Antimicrobial Chemotherapy* 64: 1219–1225.

Julsing MK, Koulman A, Woerdenbag HJ, Quax WJ, Kayser O. 2006. Combinatorial biosynthesis of medicinal plant secondary metabolites. *Biomolecular Engineering* 23: 265–279.

Junker RR, Blüthgen N. 2010. Floral scents repel facultative flower visitors, but attract obligate ones. *Annals of Botany* 105: 777–782.

Junker RR, Gershenzon J, Unsicker SB. 2011. Floral odor bouquet loses its ant repellent properties after inhibition of terpene biosynthesis. *Journal of Chemical Ecology* 37: 1323–1331.

Junker RR, Tholl D. 2013. Volatile organic compound mediated interactions at the plant–microbe interface. *Journal of Chemical Ecology* 39: 810–825.

Kessler A, Halitschke R, Baldwin IT. 2004. Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science* 305: 665–668.

Kessler A, Heil M. 2011. The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology* 25: 348–357.

Kessler D, Diezel C, Clark DG, Colquhoun TA, Baldwin IT. 2013. Petunia flowers solve the defence/apparency dilemma of pollinator attraction by deploying complex floral blends. *Ecology Letters* 16: 299–306.

Kessler D, Gase K, Baldwin IT. 2008. Field experiments with transformed plants reveal the sense of floral scents. *Science* 321: 1200–1202.

Khan ZR, Pickett JA, Hassanali A, Hooper AM, Midega CAO. 2008. *Desmodium* species and associated biochemical traits for controlling *Striga* species: present and future prospects. *Weed Research* 48: 302–306.

Kursar TA, Dexter KG, Lokvam J, Pennington RT, Richardson JE, Weber MG, Murakami ET, Drake C, McGregor R, Coley PD. 2009. The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proceedings of the National Academy of Sciences, USA* 106: 18073–18078.

Langenheim JH. 2003. *Plant resins: chemistry, evolution, ecology, and ethnobotany*. Portland, OR, USA: Timber Press.

Lee BH, Annis PC, Choi WS. 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *Journal of Stored Products Research* 40: 553–564.

Osbourn AE, Qi X, Townsend BT, Qin B. 2003. Dissecting plant secondary metabolism – constitutive chemical defences in cereals. *New Phytologist* 159: 101–108.

Perry NS, Bollen C, Perry EK, Ballard C. 2003. Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. *Pharmacology Biochemistry & Behavior* 75: 651–659.

Petschenka G, Fandrich S, Sander N, Wagschal V, Boppré M, Dobler S. 2013. Stepwise evolution of resistance to toxic cardenolides via genetic substitution in the Na⁺/K⁺-ATPases of milkweed butterflies (Lepidoptera: Danaini). *Evolution* 67: 2753–2761.

Pichersky E, Noel JP, Dudareva N. 2006. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science* 311: 808–811.

Pichersky E, Lewinsohn E. 2011. Convergent evolution in plant specialized metabolism. *Annual Review of Plant Biology* 62: 549–566.

Qu Y, Chakrabaty R, Iran HT, Kwon EJG, Kwon M, Nguyen TD, Ro DK. 2015. Lettuce (*Lactuca sativa*) homolog of human Nogo-B receptor interacts with cis-prenyltransferase and is necessary for natural rubber biosynthesis. *Journal of Biological Chemistry* 290: 1898–1914.

Raffa KF. 2014. Terpenes tell different tales at different scales: glimpses into the chemical ecology of conifer-bark beetle–microbial interactions. *Journal of Chemical Ecology* 40: 1–20.

Raguso RA, Agrawal AA, Douglas AE, Kessler A, Poveda K, Thaler JS. 2015. The raison d'être of chemical ecology. *Ecology* 96: 617–630.

Richards LA, Dyer LA, Forister ML, Smilanich AM, Dodson CD, Leonard MD, Jeffrey CS. 2015. Phytochemical diversity drives plant–insect community diversity. *Proceedings of the National Academy of Sciences, USA* 112: 10973–10978.

Sadler NC, Wright AT. 2015. Activity-based protein profiling of microbes. *Current Opinion in Chemical Biology* 24: 139–144.

Schiestl FP. 2010. The evolution of floral scent and insect chemical communication. *Ecology Letters* 13: 643–656.

Schwab W, Fuchs C, Huang FC. 2013. Transformation of terpenes into fine chemicals. *European Journal of Lipid Science* 115: 3–8.

Steele CL, Crock J, Bohlmann J, Croteau R. 1998. Sesquiterpene synthases from grang fir (Abies grandis) – comparison of constitutive and wound-induced activities, and CDNA isolation, characterization and bacterial expresson of delta-selinene synthase and gamma-humulene synthase. *Journal of Biological Chemistry* 273: 2078–2089.

Stewart A. 2013. *The drunken botanist: the plants that create the World's great drinks*. Chapel Hill, NC, USA: Algonquin Books of Chapel Hill.

**Svensson GP, Hickman MO Jr, Bartram S, Boland W, Pellmyr O, Raguso RA. 2005.** Chemistry and geographic variation of floral scent in *Yucca filamentosa* (Agavaceae). *American Journal of Botany* **92**: 1624–1631.

**Tassou CC, Chorianopoulos NG, Skandamis PN, Nychas GJE. 2012.** Herbs, spices and their active components as natural antimicrobials in foods. In: KV Peter, ed. *Handbook of herbs and spices, vol. 2. Issue 228.* Cambridge, UK: Woodhead Publishing, 17–50.

**Terry I, Walter GH, Moore C, Roemer R, Hull C. 2007.** Odor-mediated push–pull pollination in cycads. *Science* **318**: 70.

**Turlings TC, Hiltpold I, Rasmann S. 2012.** The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. *Plant and Soil* **358**: 51–60.

**Velikova V, Muller C, Ghirardo A, Rock TM, Aichler M, Walch A, Schmitt-Kopplin P, Schnitzler JP. 2015.** Knocking down of isoprene emission modifies the lipid matrix of thylakoid membranes and influences the chloroplast ultrastructure in poplar. *Plant Physiology* **168**: 859–870.

**Vriet C, Russinova E, Reuzeau C. 2013.** From squalene to brassinolide: the steroid metabolic and signaling pathway across the plant kingdom. *Molecular Plant* **6**: 1738–1757.

**Wang X, Ort DR, Yuan JS. 2015.** Photosynthetic terpene hydrocarbon production for fuels and chemicals. *Plant Biotechnology Journal* **13**: 137–146.

**Zapata F, Fine PV. 2013.** Diversification of the monoterpene synthase gene family (TPSb) in Protium, a highly diverse genus of tropical trees. *Molecular Phylogenetics & Evolution* **68**: 432–442.

**Zhou YP, Stuart-Williams H, Grice K, Kayler ZE, Zavadlav S, Vogts A, Rommerskirchen F, Farquhar GD, Gessler A. 2015.** Allocate carbon for a reason: priorities are reflected in the $^{13}C/^{12}C$ ratios of plant lipids synthesized via three independent biosynthetic pathways. *Phytochemistry* **111**: 14–20.

**Zi JC, Mafu S, Peters RJ. 2014.** To gibberellins and beyond! Surveying the evolution of (di)terpenoid metabolism. *Annual Review of Plant Biology* **65**: 259–286.

**Zimmer RK, Ferrer RP. 2007.** Neuroecology, chemical defense and the keystone species concept. *Biological Bulletin* **213**: 208–225.

**Zimmermann Y, Roubik DW, Eltz T. 2006.** Species-specific attraction to pheromonal analogues in orchid bees. *Behavioral Ecology and Sociobiology* **60**: 833–843.

## About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.

- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.

- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)

- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**