New Phytologist

Review

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Explaining intraspecific diversity in plant secondary metabolites in an ecological context

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Received: *23 June 2013*
Accepted: *22 August 2013*

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New Phytologist (2013)
doi: 10.1111/nph.12526

Key words: biosynthesis, evolvability, herbivore, heritability, plant defence, transgenic, variance.

Summary

Plant secondary metabolites (PSMs) are ubiquitous in plants and play many ecological roles. Each compound can vary in presence and/or quantity, and the composition of the mixture of chemicals can vary, such that chemodiversity can be partitioned within and among individuals. Plant ontogeny and environmental and genetic variation are recognized as sources of chemical variation, but recent advances in understanding the molecular basis of variation may allow the future deployment of isogenic mutants to test the specific adaptive function of variation in PSMs. An important consequence of high intraspecific variation is the capacity to evolve rapidly. It is becoming increasingly clear that trait variance linked to both macro- and micro-environmental variation can also evolve and may respond more strongly to selection than mean trait values. This research, which is in its infancy in plants, highlights what could be a missing piece of the picture of PSM evolution. PSM polymorphisms are probably maintained by multiple selective forces acting across many spatial and temporal scales, but convincing examples that recognize the diversity of plant population structures are rare. We describe how diversity can be inherently beneficial for plants and suggest fruitful avenues for future research to untangle the causes and consequences of intraspecific variation.

I. Introduction

Plant secondary metabolites (PSMs) are ubiquitous in the plant kingdom, and play myriad ecological roles in defence against herbivores and pathogens past and present, mediating interactions with pollinators and mycorrhizal fungi, attracting predators of herbivores, plant–plant signalling and protection against abiotic stressors such as UV-B radiation, frost and drought (Dixon & Paiva, 1995). Yet, because of their sheer number and diversity, modes of action remain to be discovered and adaptive

advantages still await rigorous demonstration for most PSMs, presenting an ongoing challenge for ecologists. Like most traits of organisms, PSMs are variable at all scales including among species, populations, individuals, and different plant parts (Suomela *et al.*, 1995; Holeski *et al.*, 2012a), and it is this variation and its causes and consequences that are the subject of this review.

A plant population can exhibit presence–absence polymorphisms for individual PSMs, but more common, and often overlaid, is quantitative variation in PSM concentrations. The great diversity of PSMs also allows for variation in the number and evenness of concentrations (i.e. PSM richness and α-chemodiversity) of PSMs in individual plants as well as qualitative variation between individuals and groups (β-chemodiversity). When discussing qualitative variation, phenotypes with different PSM profiles are referred to as chemotypes. These perspectives on chemical variability and diversity are elaborated upon in Supporting Information Notes S1.

Particularly against specialists, the effectiveness of defence is rarely determined by summing concentrations across broad PSM classes and more often linked to specific individual compounds which sometimes account for small fractions of total defence allocation (Adler *et al.*, 1995), emphasizing the importance of qualitative PSM diversity. The effectiveness of PSMs as herbivore deterrents can also be influenced by plant nutritional quality, as herbivores titrate nutritional reward against the cost of ingesting PSMs (Au *et al.*, 2013).

Diversity in the specificity of biochemical interactions between plants and their enemies parallels PSM diversity. For example, digoxin is highly specific, targeting the Na^+/K^+ ATPases (Katz *et al.*, 2010), whereas tannins bind many diverse proteins. Terpenes act as direct defences through generalized toxicity and as receptor-specific cues to attract parasitoids (indirect defence), so both quantitative and qualitative diversity influence the ecological interactions of plants possessing these compounds. Small structural variations can also have vastly different effects. For example, specific ellagitannin identity influences redox conditions in caterpillar guts (Appel, 1993; Salminen & Karonen, 2011). These observations emphasize that the use of cost–benefit analyses to explain quantitative PSM variation will probably fail if the group of PSMs considered is too inclusive.

All chemical phenotypic variation is attributable to the effects of genes, environment and the interaction of these, and this extends to phenotypic variation with plant ontogeny and phenology. Here, we first review how PSMs respond in time and space to plants' biotic and abiotic interactions with their environment. We then address the genetic and molecular basis of PSM qualitative diversity, with an emphasis on the insights to be gained into PSM diversity from manipulating the genes that are ultimately responsible. Having described the phenomenon of PSM variation, we turn to how quantitative genetic variation contributes to PSM quantitative variation, discuss the evolvability of trait means and variances and finally consider the ecological context and consequences of PSM variation, which we argue can be a valuable species trait in itself. Finally, we provide a synthesis and suggest some future research priorities.

II. PSM variation in time: ontogeny, phenology and induced defences

Much of the qualitative and quantitative PSM variation in a species occurs among tissue types and ontogenetic stages, and across circadian and annual cycles (Kim *et al.*, 2011; Holeski *et al.*, 2012a). Optimal defence theory suggests that different plant tissues are defended in accordance with their value to the plant, the costs of defence and the risk of herbivore or pathogen attack (Rhoades & Cates, 1976). These factors change throughout plants' ontogeny and, accordingly, so too do PSM concentrations (Barton & Koricheva, 2010). Plant age and size can also influence PSM concentrations, either as a life history strategy or as a consequence of changing resource availability. Consequently, population age structure can also contribute to chemical variation (Moore & Foley, 2005; Andrew *et al.*, 2007).

1. Herbivore-induced changes in PSMs

Biotic interactions often change PSM profiles and net production by inducing defensive responses. Induction can occur locally at the site of attack or infection, or can be systemic (Kessler & Baldwin, 2002). A growing list of examples also demonstrate persistent, transgenerational induction of PSMs by herbivory on maternal plants, mediated by epigenetic mechanisms including DNA methylation, histone modification, smRNAs and enhanced homologous recombination (Holeski *et al.*, 2012b). Inducible chemical defence may allow plants to avoid any costs associated with defence when enemies are absent, while constitutively defended plants must pay these costs whether or not defences are required (Karban & Baldwin, 1997; Mithofer & Boland, 2012); however, maintaining the capacity to induce defences may also be costly.

Changes to PSM synthesis can also be induced remotely by volatile emissions from con- or heterospecific neighbours that are under herbivore attack (Baldwin *et al.*, 2006). Despite extensive efforts, few of the signalling molecules have been identified (Jander & Clay, 2011) beyond salicylates and jasmonates (Erb *et al.*, 2012), and reactive oxygen molecules such as hydrogen peroxide (Orozco-Cardenas *et al.*, 2001). Jasmonate signalling is widespread and has been well characterized in plants including *Arabadopsis thaliana*, tomato (*Lycopersicon esculentum*) and *Nicotiana attenuata* (Turner *et al.*, 2002). Wild tobacco (*N. attenuata*) plants treated with methyl jasmonate increase production of nicotine and are attacked less often by herbivores, although induction exacts a cost to seed production if herbivores are absent (Baldwin, 1998). Attack signals are propagated and amplified by signalling molecule cascades that travel between cells, through the phloem of plants and through the atmosphere to neighbouring plants and to natural enemies of the plants' attackers (Degenhardt *et al.*, 2003). Previously attacked plants and their neighbours may be 'primed' to a higher alert level than naïve plants by the detection of a variety of volatile organic compounds (VOCs), including green leafy volatiles, which comprise six-carbon aldehydes, alcohols and esters (Engelberth *et al.*, 2004). Detection of different attackers and the propagation and reception of signalling molecules are all under genetic control and subject to variation, although few VOC

receptors have been identified (Baldwin *et al.*, 2006). The consequences of volatile-induced defences extend beyond individual plants to influence both the mean and variance of defence and thus susceptibility to herbivores and pathogens in plant populations and communities (Baldwin, 1998; Meldau *et al.*, 2012).

Soon after their discovery, it was suggested that the variability produced by induced defences could reduce herbivory, thus improving plant fitness (Schultz *et al.*, 1982; Karban *et al.*, 1997).

III. PSM variation through space: the role of environment

Both the type and amounts of many PSMs differ strongly across physical and biotic environments as a result of local adaptation or genotypic sorting and selection across habitats. However, much of this variation is attributable to phenotypic plasticity of genotypes in response to variations in resources for growth such as nutrients, light and water, and the presence or absence of enemies, competitors or mutualists. Such changes to concentrations of PSMs in plant tissue may be a result of specific up- or down-regulation of their biosynthesis, or may occur more passively as dilution and concentration effects (Koricheva, 1999), driven by changed allocation to quantitatively dominant fractions such as cellulose.

Patterns of plant defence along gradients of latitude, climate, resource availability and herbivore pressure have attracted enormous empirical and theoretical attention, with much of the latter work subsequently found wanting (Hamilton *et al.*, 2001). In particular, plant defence patterns are rarely predicted by the carbon-nutrient balance hypothesis (except, perhaps, for phenolics; Bryant *et al.*, 1983). This and other plant defence theories have been comprehensively reviewed elsewhere (Stamp, 2003) and while it is clear that PSMs are often influenced by their environment, this does not always occur in predictable ways (Endara & Coley, 2011).

IV. Genes and biosynthetic pathways underlying PSM variation

Plant secondary metabolites were long considered to be waste products, 'flotsam and jetsam on the metabolic beach' (Haslam, 1986), until Fraenkel (1959) coherently advocated a defensive role against herbivores. Since then, the role of PSMs has been expanded to include the amelioration of so many biotic and abiotic stresses that a tendency exists to treat all PSMs as functional and adaptively valuable. When interpreting chemodiversity and quantitative variation, there is a risk of applying adaptive defensive explanations uncritically. However, in the past decade molecular tools have arisen with which to manipulate specific genes and pathways to experimentally test the roles and functions of individual PSMs.

Natural mutants can allow powerful tests of the adaptive function of PSMs. For example, the CORONATINE INSENSITIVE1 (COI1) mutant in *A. thaliana* (Feys *et al.*, 1994) suggested an important role for jasmonates in mediating plant defense. This was later confirmed through gene-silencing techniques in other plants (e.g. rice (*Oryza sativa*); Ye *et al.*, 2012). Other natural mutations in the jasmonate pathway have helped dissect the steps of wound-induced and indirect defence.

Reintroduction of genes into natural mutants is a powerful complementary method of demonstrating adaptive roles. Terpenes produced by maize (*Zea mays*) roots in European lines indirectly defend against root herbivores by attracting entomopathogenic nematodes (Rasmann *et al.*, 2005). When a gene for caryophyllene synthase was reintroduced into borer-susceptible North American maize lines (Kollner *et al.*, 2008), nematode attraction increased and borer damage declined (Degenhardt *et al.*, 2009).

Extraordinary insights into how chemodiversity influences ecological interactions have been gained by testing the fitness of isogenic mutants in which key genes have been silenced. For example, silencing the putrescine N-methyl transferase gene in *N. attenuata* led to a 95% reduction in constitutive and induced nicotine concentrations (Steppuhn *et al.*, 2004), leading to greater herbivore damage and confirming nicotine as a direct resistance trait. Key to the success of this approach in Baldwin's group has been an efficient transformation system, the ability to establish transgenic plants in natural habitats and a comprehensive screening process addressing all identified biotic and abiotic stresses. Silencing of other genes involved in the biosynthesis of floral attractants and repellents has demonstrated the complex interrelationships with pollinators that lead to changes in fitness (Kessler *et al.*, 2008). Just as importantly, silencing multiple genes in the jasmonate pathway has revealed the complexity of signalling but also revealed how herbivore attack reconfigures primary metabolism (Schwachtje & Baldwin, 2008).

However, the gene-silencing approach to demonstrating adaptive function has been criticized (Agrawal, 2011). Specifically, (1) it is expensive, (2) expression and transformation systems are not universally available, (3) it is less well-suited to long-lived plants such as trees, (4) legal restrictions preclude the release of genetically modified organisms in many places, and (5) adaptive value of some traits/genes may only be apparent under unusual environmental conditions, which may not be observed during field experiments which are limited in spatial extent and duration. Nonetheless, with advances in molecular genetics, these approaches will be used more widely and, provided that they are coupled with a strong ecological understanding, they can provide specific tests of the adaptive value of chemical variance.

V. Mechanisms for diversification of PSMs

Most PSMs originate from a small group of precursor compounds, which eventually become modified into diverse end-products. For example, all 40 000+ isoprenoid compounds (Buckingham, 1994) originate from pyruvate and D-glyceraldehyde 3-phosphate entering the methylerythritol phosphate pathway in the chloroplast or from acetyl-CoA entering the mevalonate pathway (Fig. 1, Dudareva *et al.*, 2004; Külheim *et al.*, 2011). A second group of > 10 000 PSMs (Wink, 2008), including alkaloids and glucosinolates, are amino acid derivatives. Here we will outline some mechanisms by which these pathways evolve to increase PSM diversity.

1. Gene duplication and neofunctionalization

Genes encoding secondary metabolism have often evolved from genes of primary metabolism. For example, *BRANCHED-CHAIN*

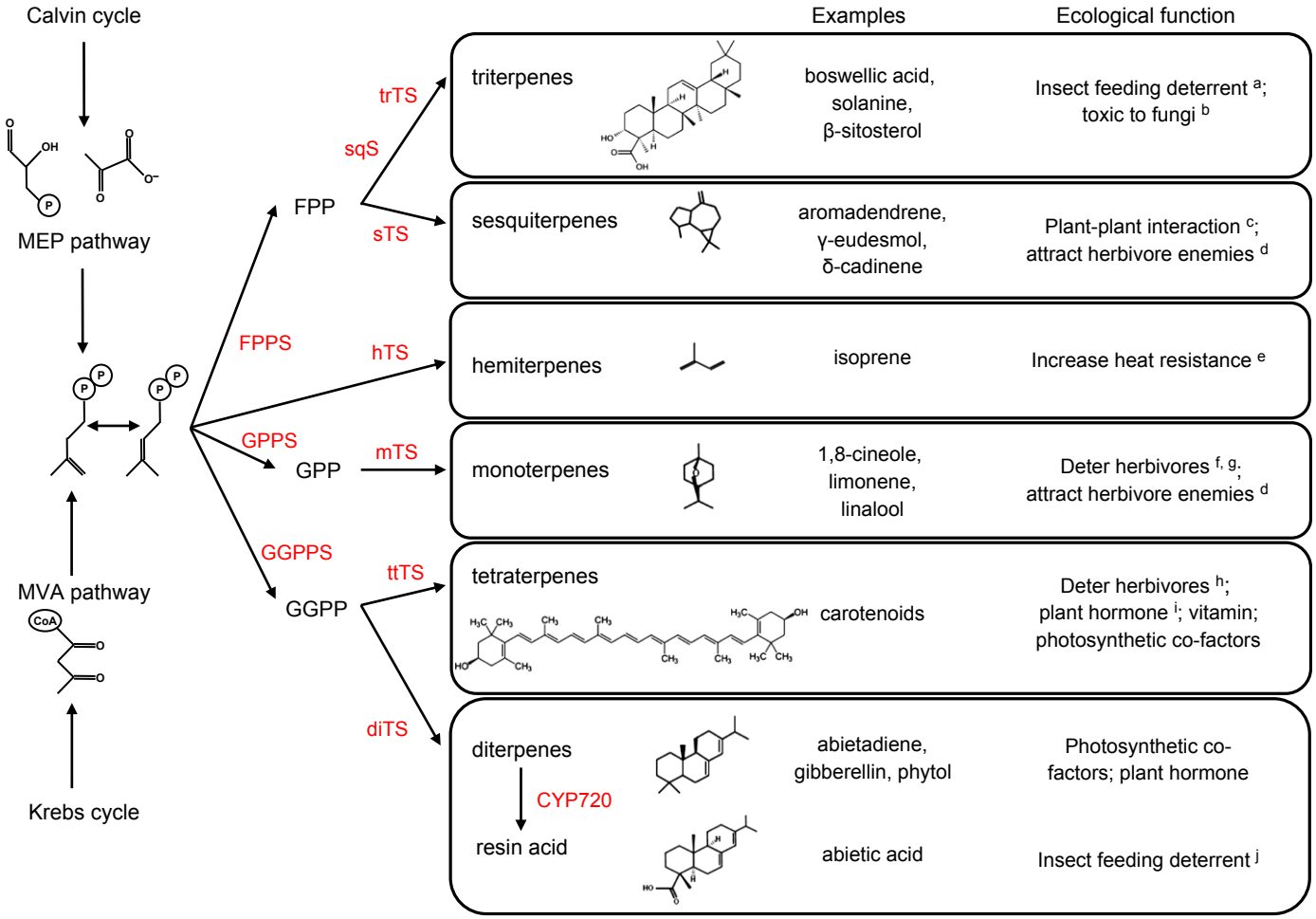

Fig. 1 Schematic overview of basic terpenoid biosynthesis with examples for different terpene subgroups shown with some ecological functions. Enzymes are abbreviated in red: MEP pathway, methylerythritol phosphate pathway; MVA pathway, mevalonate pathway; FPPS, farnesyl pyrophosphate synthase; GPPS, geranyl pyrophosphate synthase; GGPPS, geranyl geranyl pyrophosphate synthase; trTS, triterpene synthase; sqS, squalene synthase; sTS, sesquiterpene synthase; hTS, hemiterpene synthase; mTS, monoterpene synthase; diTS, diterpene synthase; ttTS, tetraterpene synthase). Compound abbreviations are shown in black: FPP, farnesyl pyrophosphate; GPP, geranyl pyrophosphate; GGPP, geranyl geranyl pyrophosphate. References: [a]Shinoda *et al.* (2002); [b]Osbourn (1996); [c]Baldwin *et al.* (2006); [d]Degenhardt *et al.* (2003); [e]Sasaki *et al.*(2007); [f]Boyle & McLean (2004); [g]Lawler *et al.* (1999); [h]Phillips & Croteau (1999); [i]Chrispeels & Varner (1967); [j]Keeling & Bohlmann (2006).

AMINOTRANSFERASE4 is involved in glucosinolate biosynthesis, but shares a common ancestor with genes involved in leucine biosynthesis (Schuster *et al.*, 2006). The most important mechanism in diversifying secondary metabolism is whole-genome and local-gene duplication (Kliebenstein *et al.*, 2001a,b; Kliebenstein, 2008; Kroymann, 2011). Whole-genome duplication occurs rarely, with three and four occasions known from *A. thaliana* (Bowers *et al.*, 2003) and *Populus trichocarpa* (Tuskan *et al.*, 2006), respectively. Local duplications probably create more biosynthetic gene diversity and can arise from unequal crossing-over during meiosis, which produces chromosome regions containing tandem repeats (Ober, 2010).

Unequal crossing-over favours genomic regions that already contain tandem repeats, creating large regions of duplicated genes. *Populus trichocarpa* contains up to 24 tandem-repeated S-locus-specific glycoprotein genes (Tuskan *et al.*, 2006) and *Vitis vinifera* contains a cluster of 20 putative terpene synthase genes and 25 pseudo-genes from the same family (Martin *et al.*, 2010). Here, gene copies are liberated from functional constraints and accumulate mutations, leading to neofunctionalization, including the synthesis of novel PSMs or, more commonly, loss of function resulting in a pseudogene (Zhang, 2003). Hartl *et al.* (2010) found evidence for neofunctionalized serine protease inhibitors in *Solanum nigrum* with overlapping or synergistic functions against herbivores. Another outcome of gene duplication is subfunctionalization (Lynch & Force, 2000; Ober, 2010), where the functions of gene duplicates diverge to perform complementary parts of the original function. Subsequent mutations in the regulatory elements of one copy might then alter temporal or local expression patterns (Hinman & Davidson, 2007).

Genome duplication and increases in ploidy are a major source of duplicate genes. Allopolyploidy, where divergent genomes are combined, can lead to particularly significant gene rearrangements affecting PSMs. The natural allotetraploid species *Nicotiana quadrivalvis* and *Nicotiana clevelandii* have retained biosynthetic genes from one parent and regulatory genes from the other (Wu

et al., 2006). The result is more diverse responses to herbivore attack via induction of trypsin proteinease inhibitors than in the parent species (Anssour & Baldwin, 2010).

Diversification of PSMs can also arise from point mutations of structural biosynthetic genes. Subtly different terpene synthases often produce completely different products from the same substrate. A single-amino-acid substitution in *Salvia fruticosa* 1,8-cineole synthase, from Asn to Ile at position 338, changes the product profile from mostly (73%) 1,8-cineole with some α-terpineol, β-pinene, α-pinene, sabinene and myrcene to a mixture of sabinene (48%) and limonene (37%) (Kampranis *et al.*, 2007).

2. Transcriptional regulation

Small RNAs can regulate many aspects of metabolism. Silencing several dicer-like proteins (dicer is an endoribonuclease that fragments double-stranded RNAs to produce guide RNAs, which silence cognate genes at the transcriptional and post-transcriptional levels; dicer-like proteins play a similar role) in *N. attenuata* changed the variability of nicotine concentrations in flowers which influences pollination by birds and hence outcrossing rates (Kessler *et al.*, 2012). Small RNAs also coordinate transcriptional changes after herbivore attack, so their role in mediating PSM variation will probably become a major new research focus.

3. Enzyme promiscuity and modification of PSMs

Much chemodiversity may arise not from genetic diversity, but rather from promiscuous or 'sloppy' enzymes. Mutations leading to neofunctionalization may increase enzyme promiscuity with only minor consequences for original enzyme functions (Aharoni *et al.*, 2005). Indeed, Zulak & Bohlmann (2010) have suggested that terpenoid diversity may be largely the result of sloppy enzymes.

Enzymes that modify metabolites, such as cytochrome P450 monooxygenases (CYP450) and *O*-methyltransferases, also diversify PSMs in many biosynthetic pathways (Mizutani & Ohta, 2010). CYP720B4 from *Picea sitchensis* can transform at least 24 diterpene substrates from eight different diterpene skeletons to a diverse array of end-products via consecutive oxidation steps (Hamberger *et al.*, 2011). The absorption spectrum of anthocyanidins changes dramatically upon methylation of B-ring hydroxyl groups, leading to flower colour changes when these compounds accumulate in petals. CrOMT2 (*Catharanthus roseus O*-methyl transferase 2) catalyses 3′ and 5′ *O*-methylation of the B-ring of substrates including myrecitin and dihydromyrecitin in *Catharanthus roseus* (Cacace *et al.*, 2003).

VI. Examples of diversity from specific biosynthetic pathways

The examples in this section illustrate the diversity of PSM biosynthetic pathways and hence the molecular origins of both qualitative and quantitative PSM diversity. Cyanogenic glycoside variability in *Lotus* spp. is a classic, widely studied presence/absence polymorphism with a recently elucidated molecular basis. Phenylpropanoids are quantitatively important and play diverse roles. Glucosinolates and terpenoids were chosen to provide thoroughly studied examples where gene duplication and neofunctionalization have led to significant structural, functional and ecological variation. We have necessarily been selective in choosing these examples, and it is evident that numerous other well-studied examples are also available in the literature.

1. Cyanogenesis

Cyanogenesis, the liberation of hydrogen cyanide from the hydrolysis of cyanogenic glycosides in response to tissue disruption, occurs in 5% of angiosperms (Lamont, 1993). The presence/absence phenotype can result from the presence/absence of one or as many as five (Neilson *et al.*, 2006) cyanogenic glycosides (cyanogens), β-glycosidases that cleave the glucose from the cyanogen, or both (Gleadow *et al.*, 2003). Cyanogenic glycosides are synthesized from hydrophobic amino acids such as valine, leucine, tyrosine and isoleucine (Fig. 2). The first committed step is catalysed by a membrane-bound CYP450 from the CYP79 subfamily and then a CYP71, followed by glucosylation by a UDP-glucosyltransferase (Halkier & Möller, 1989; Jones *et al.*, 1999). It was recently confirmed in *Trifolium repens* that the first committed step is the target of selection for cyanogenesis. The polymorphism is controlled by two unlinked loci which segregate as present/absent in the genome. The *Li*/*li* locus encodes the β-glucosidase linamarase (Hughes, 1991; Olsen *et al.*, 2007) and the *Ac*/*ac* locus encodes a CYP79D15 that catalyses the first

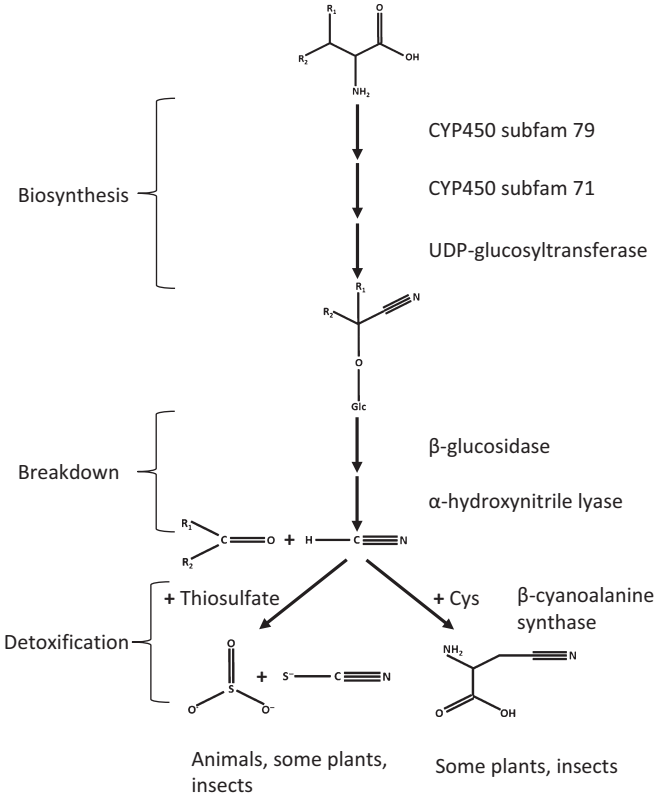

Fig. 2 Schematic overview of the biosynthesis, breakdown and detoxification of cyanogenic glucosides. For each step the active enzyme is named on the right. subfam, subfamily; Cys, cysteine.

biosynthetic step for both linamarin and lotaustralin (Olsen *et al.*, 2008). Both genes have undergone multiple gene deletions, suggesting that repeated macromutation, rather than the persistence of alleles through long-term balancing selection, is the cause of this widespread polymorphism (Olsen *et al.*, 2013).

Many nonsynonymous polymorphisms and indels in exons in *CYP79D15* exist among *T. repens* populations. No statistical association has been detected between these polymorphisms and quantitative cyanogen variation, but few individuals have been studied. However, quantitative variation could equally arise from sequence variation in the promoter region or regulatory elements (Olsen *et al.*, 2008).

2. Isoprenoids

The isoprenoids are a particularly diverse class of PSMs with numerous biological activities and include volatile mono- and sesquiterpenes, diterpene resin acids and precursors of steroids and saponins. Isoprenoid synthesis arises out of the chloroplast methylerythritol phosphate (MEP) and the cytosolic mevalonate (MVA) pathways (Fig. 1), which both produce isopentyl pyrophosphate (IPP), which can be converted to dimethylallyl pyrophosphate by isopentyl pyrophosphate isomerase. Prenyl synthases then produce substrates for the multi-product terpene synthases (TPSs) (Bohlmann *et al.*, 1998; Lichtenthaler, 1999; Dudareva *et al.*, 2004), the major agents of product diversification.

More than 100 TPS genes have been functionally characterized and plant genomes are known to code for between two (*Physcomitrella patens*) and 113 (*Eucalyptus grandis*) putatively functional TPS genes (Chen *et al.*, 2011). Typical TPS gene copy numbers for higher plants range from 24–33 for plants that do not utilize terpenes as pollinator attractors or defences to 58–113 for plants that do (C. Külheim *et al.*, unpublished data).

As well as sequence changes, differential expression of duplicated genes can produce novel terpene chemotypes. In *Picea sitchensis*, up to three (+)-3-carene synthases are present (*PsTPS-3car1–3*), resulting in variable concentrations of (+)-3-carene, which defends against spruce weevil (*Pissodes strobi*). While all chemotypes express *PsTPS-3car1*, *PsTPS-3car2* and *PsTPS-3car3* are only expressed in the resistant and susceptible chemotypes, respectively. *PsTPS-3car3* may have been deleted or not duplicated in susceptible trees (Hall *et al.*, 2011).

Terpene chemotypes have been well described for many species. In oregano (*Origanum vulgare*; Crocoll *et al.*, 2010), chamomile (*Matricaria chamomilla*; Irmisch *et al.*, 2012) and tea tree (*Melaleuca alternifolia*; A. Keszei & W. J. Foley, unpublished data), this chemotypic variation is mostly a result of transcription-level control of multiple terpene synthases. In thyme (*Thymus vulgaris*), Vernet *et al.* (1986) discovered an epistatic relationship between five loci, each with two alleles, by crossing the six chemotypes. Those five loci probably encode either activators promoting action along one possible biosynthetic pathway or repressors decreasing action along another.

3. Glucosinolates

Glucosinolates (Fig. 3) are sulphur-rich anionic compounds and important plant defences (Halkier & Gershenzon, 2006) occurring near-universally in the Brassicaceae, Capparidaceae and Caricaceae. Over 130 glucosinolates are described, including nearly 40 from *A. thaliana* (Kliebenstein *et al.*, 2001a,b). They are synthesized from amino acids, mostly methionine, tryptophan and phenylalanine, but also alanine, leucine, isoleucine and valine. Glucosinolate diversity arises from elongation of the carbon side-chain of the amino acid and subsequent transformations including hydroxylation, *O*-methylation, desaturation, glycosylation and acylation (Halkier & Gershenzon, 2006).

Methionine can undergo multiple carbon chain elongation cycles, greatly diversifying the glucosinolate end-products. These aliphatic glucosinolates comprise the most abundant class in *A. thaliana* and are important for herbivore defence (Mewis *et al.*, 2006). Carbon chain elongation starts with transamination, followed by condensation catalysed by methylthioalkylmalate synthase (MAM), followed by isomerization and oxidative decarboxylation (Benderoth *et al.*, 2009). *Arabidopsis thaliana* contains three copies of *MAM*, each acting on different carbon chain lengths. Individual glucosinolate profiles are determined epistatically: *MAM1*→*MAM2*→*MAM3* (Kroymann *et al.*, 2003; Kroymann & Mitchell-Olds, 2005). *MAM1* and *MAM2* arose by duplication and neofunctionalization of an ancestral *MAMa* gene. *MAM1* strongly influences qualitative glucosinolate variation and probably evolved under positive selection (Benderoth *et al.*, 2006) from specialist herbivores (Mewis *et al.*, 2006). Contrastingly, *MAM2* is not involved in qualitative glucosinolate variation and evolved under balancing selection, probably from generalists (Kroymann *et al.*, 2003).

Further modifications to glucosinolates also occur, such as benzoic acid esterification in *A. thaliana* seeds (Reichelt *et al.*, 2002). Additionally, upon mechanical damage by herbivores, glucosinolates are exposed to endogenous thioglucosidases (myrosinases) and hydrolysed; the resultant aglycones are rearranged to form toxic compounds such as isothiocyanates and nitriles (Halkier & Gershenzon, 2006). Product profiles from hydrolysis vary among *A. thaliana* ecotypes: 'Columbia' produces isothiocyanates while 'Landsberg erecta' produces nitriles. These differences map to a locus encoding an epithiospecifier modifier (Lambrix *et al.*, 2001; Zhang *et al.*, 2006).

4. Phenylpropanoids

One out of every five photosynthetically fixed carbon atoms is estimated to enter the phenylpropanoid pathway (PPP; Fig. 4), a major reservoir of PSM diversity (Colquhoun, 2012). Phenylpropanoids are derived from the amino acid phenylalanine (Winkel-Shirley, 2001) and include > 3000 compounds (Wink, 2008) among which are simple phenolics, polyphenolics including tannins and lignin, coumarins, flavonoids, phloroglucinols and stilbenes, flower and leaf pigments (e.g. anthocyanins such as cyanidin) and volatiles that mediate plant–pollinator interactions (e.g. eugenol and derivates).

Phenylpropanoid diversity is created at the first committed step of the PPP, by a chalcone synthase that produces a variable number of chalcones. The removal or addition of various functional groups from end-products further expands this diversity (Holton & Cornish, 1995; Winkel-Shirley, 2001; Tanner *et al.*, 2003). Studies

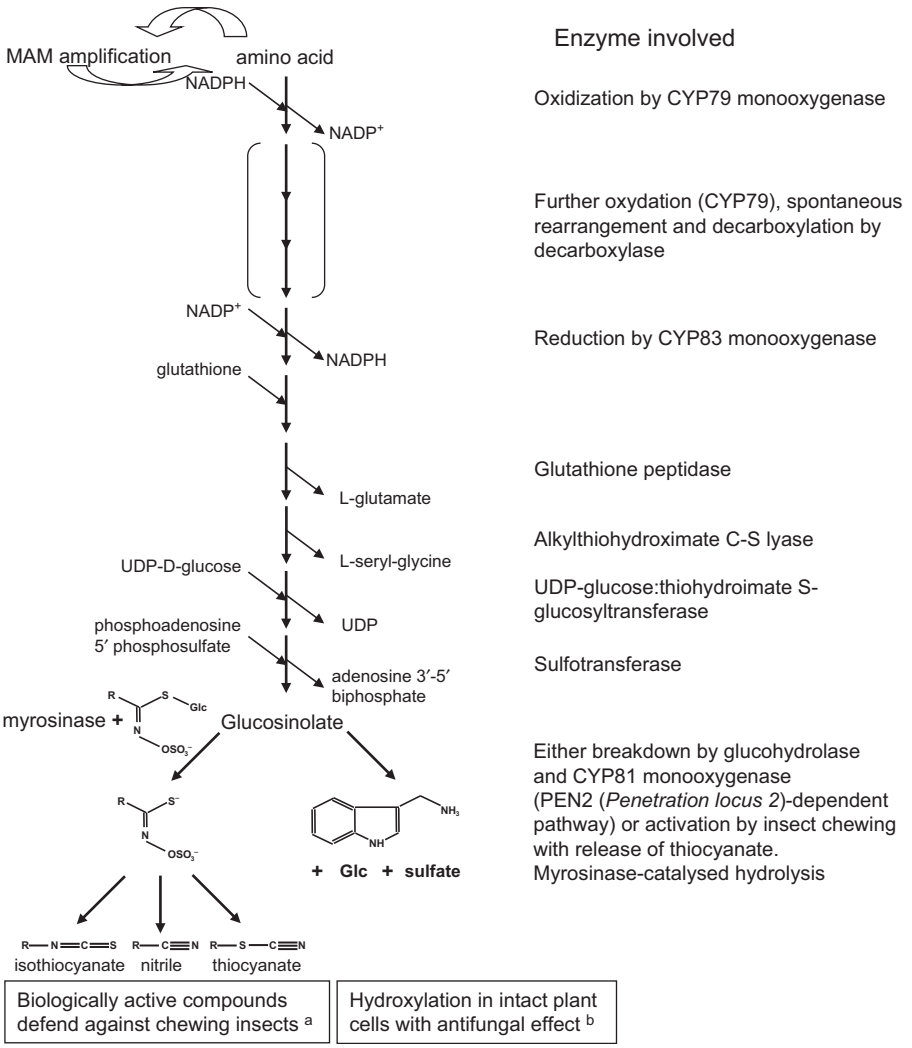

Fig. 3 Schematic overview of glucosinolate biosynthesis and breakdown. For each step of glucosinolate biosynthesis the active enzyme is mentioned; methylthioalkylmalate (MAM) amplification contributing to glucosinolate diversity is indicated at the top. References: [a]Halkier & Gershenzon (2006); [b]Bednarek *et al.* (2009). MAM, methylthioalkylmalate synthase.

in *A. thaliana* have revealed many enzymes and intermediates in the PPP and have demonstrated its broader involvement in plant metabolism (Fraser & Chapple, 2011). However, in species where phenolic compounds are more concentrated and diverse and where gene families are expanded, (e.g. conifers), alternative pathways and mechanisms for phenylpropanoid synthesis may exist (Porth *et al.*, 2011).

Many phenylpropanoids affect plant ecology, but tannins have received the most attention historically. These confer herbivore resistance through protein-binding effects in vertebrates and as pro-oxidants in insects (Barbehenn & Constabel, 2011). Recent analytical advances are revealing strong structure–function relationships in ellagitannins (Salminen *et al.*, 2010). Despite this, genes for gallic acid biosynthesis have only recently been identified (Feller & Martens, 2012) and biosynthetic details for other ecologically important tannins remain unknown.

Structural differences can arise among condensed tannins from closely related plants as a result of differing PPP precursors (Scioneaux *et al.*, 2011). For example, poplars have very different proanthocyanidin profiles, with *Populus angustifolia* dominated by procyanidin/prodelphinidin and *Populus fremontii* dominated by procyanidin dimers (Scioneaux *et al.*, 2011). Early predictions of additive inheritance of quantitative phenylpropanoid variation (Driebe & Whitham, 2000) have recently been revised in light of the suggestion by Scioneaux *et al.* (2011) that composition and chain length are controlled by dominance of specific PPP and proanthocyanidin-polymerization genes. Thus, quantitative and qualitative variation of the same PSM class may be controlled by distinct genetic mechanisms.

VII. How and why is chemical diversity maintained?

Jones & Firn (1991) proposed a simple model to explain the high degree of chemical complexity seen in some PSM classes, such as terpenoids. They suggested that, because any new compounds arising as a result of mutation have a low probability of being biologically active, plants are more likely to evolve active compounds if they produce a diverse and rapidly mutating complex of compounds. Many PSMs found in plants have no *known* biological activity but these are retained because they increase the probability of producing new active compounds by acting as precursors. This is known as the screening hypothesis, because plants produce a large number of metabolites that are subsequently 'screened' for biological activity.

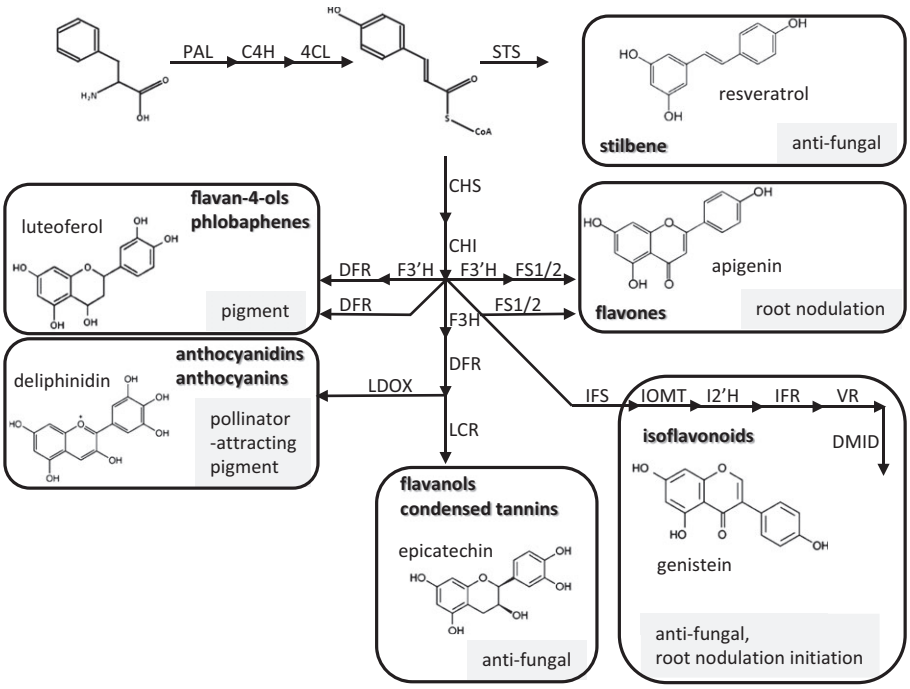

Fig. 4 Schematic overview of phenylpropanoid biosynthesis with examples for different classes with a variety of ecological functions (shaded text). Enzyme abbreviations: PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; STS, stilbene synthase; CHS, chalcone synthase; CHI, chalcone isomerase; F3′H, flavonoid 3′-hydroxylase; DFR, dihydroflavonol reductase; FS1/2, flavone synthase 1/2; F3H, flavonoid 3-hydroxylase; LDOX, leucoanthocyanidin dioxygenase; LCR, leucoanthocyanidin reductase; IFS, isoflavone synthase; IOMT, isoflavone *O*-methyl transferase; I2′H, isoflavone 2′-hydroxylase; IFR, isoflavone reductase; VR, vestitone reductase; DMID, 7,2′-dihydroxy 4′methoxyisoflavanol dehydratase.

Consistent with, but not necessarily confirming, the predictions of the screening hypothesis, Becerra *et al.* (2009) have shown that, in a large plant radiation, the diversity of PSMs and of pathways used to produce these PSMs has increased over evolutionary time; by contrast, Pelser *et al.* (2005) found that, despite the presence of substantial inter- and intraspecific variation in pyrrolizidine alkaloids in 24 species of *Senecio*, individual compounds had been frequently lost and gained through evolution and were distributed incidentally throughout the clade. Similarly, Kursar *et al.* (2009) detected little phylogenetic signal in anti-herbivore defences in a radiation of *Inga* species, suggesting that local abiotic and biotic selection pressures are the main drivers of chemodiversity and that inactive precursor molecules are often not maintained through evolution.

Effective defences can clearly arise without qualitative diversity, so chemodiversity is not always as great as the screening hypothesis would suggest. *Plantago lanceolata* is chemically variable within and between populations, but this is based upon only two iridoid glycosides (aucubin and catalpol) and two phenylpropanoid glycosides (verbascoside and plantamajoside; Adler *et al.*, 1995). Conspecific individuals and closely related species of *Eucalyptus* also show great quantitative variation in concentrations of defensive formylated phloroglucinol compounds, but PSM richness ranges from one to dozens of compounds (typically macrocarpals, euglobals and sideroxylonals; Moore *et al.*, 2004). These observations suggest two interpretations that contradict assumptions of the screening hypothesis. First, the cost of inactive compounds may sometimes have caused them to be lost and, secondly, strong directional selection may cause the accumulation of effective bioactive compounds at the expense of inactive ones. In the second scenario, we might ask not only whether that situation represents only a temporary victory in the arms race between plants and their enemies but also how rapidly lost diversity can be restored by evolution once these defences are breached.

VIII. Evolvability of PSM variation

Quantitative variation and β-chemodiversity are maintained in plant populations despite frequently high estimates of heritability and strong selection on PSM traits, evidenced by numerous demonstrations of the role of PSMs in allowing plant survival in natural environments. The adaptive value of PSMs is often viewed as a product of coevolution with plants' enemies; however, given simple pairwise co-evolutionary arms races, the often extreme variability of PSMs is hard to explain. Strong and consistent directional selection should erode quantitative variation. However, many selective forces act on PSMs, including multiple herbivores (Lankau, 2007; Iason *et al.*, 2011), pollinators (Irwin *et al.*, 2004), pathogens, mutualists, competitors (Kliebenstein *et al.*, 2005; Lankau & Strauss, 2008), and abiotic stresses (Burchard *et al.*, 2000), and the most thorough tests of the adaptive value of traits are those that test their effects against the entire gamut of natural plant enemies (e.g. *N. attenuata* in Utah; Schuman & Baldwin, 2012). All of these vary spatiotemporally, thus reducing the average strength of selection or generating balancing selection. Plant defence can impose direct (metabolic and physiological) and ecological costs, which are context-dependent (Strauss *et al.*, 2002). Chemical defence tradeoffs with growth, competitive ability and other forms of defence, such as mechanical defence or tolerance, may sometimes be important (Irwin *et al.*, 2004), but increasingly the joint evolution of multiple defence strategies is seen to be the norm (Núñez-Farfán *et al.*, 2007). In the case of induced defences, this is possibly as a result of shared regulatory pathways such as jasmonic acid signalling (Woldemariam *et al.*, 2011).

Summarizing the evolutionary ecology of plant defence, Agrawal (2011) highlighted the difficulty of obtaining ecologically relevant measures of costs and advocated measuring costs of defence traits in a multivariate context considering covariation in traits.

Recent evidence shows rapid qualitative and quantitative evolution of PSMs in response to changed herbivore pressure. Züst *et al.* (2012) showed that existing geographical patterns in a polymorphic glucosinolate locus of wild *A. thaliana* are correlated with the historical distributions of two specialist aphids and further demonstrated experimentally that differential selection could recreate these patterns within only five generations. PSMs may be among the most evolvable traits in the broad sense because they are relatively unconstrained by costs and tradeoffs. Unlike with primary metabolic processes, plants can survive under favourable ecological circumstances when PSM synthesis is switched off entirely. Yet when increased synthesis is required, examples abound of the ability of plants to up-regulate resource acquisition via increased photosynthetic capacity or nutrient uptake and thus minimize or avoid entirely any measureable tradeoff between PSM synthesis and growth (Mitra & Baldwin, 2008). Although, ultimately, there must be an upper limit to the concentrations of PSMs achievable in plant tissue, and costs of PSM synthesis can be demonstrated (Rausher, 2001), the quantitative PSM variation that we observe necessarily occurs below that level, and the key to understanding this variation lies with understanding the genetic and biochemical processes responsible.

On average, plant life history, growth and morphology have a more negative effect on susceptibility to herbivores than do PSMs, and so PSMs may be well suited to evolve defensive roles because they are less constrained by pleiotropy of the genes controlling them (Carmona *et al.*, 2011). Another recent demonstration of the rapid evolution of chemical defence traits come from Agrawal *et al.* (2012). They excluded herbivores from *Oenothera biennis* plots and documented reduced allocation to a specific chemical defense (ellagitannins of fruits) and increased competitive ability (earlier flowering) within 4 yr. There is now a need for similar studies in plants with a broader range of mating systems, including outcrossing, selfing and vegetative reproduction, in addition to the unusual, functionally asexual, mode of seed production in *O. biennis*.

Introductions of plants into new habitats can also provide evidence for rapid chemical evolution in response to environmental and demographic change (Bossdorf *et al.*, 2005). Numerous studies have looked for reduced defence in invasive plants, as predicted by the 'evolution of increased competitive ability hypothesis', and for the qualitatively different defences demanded by new, naïve herbivores as predicted by the 'shifting defence hypothesis', with mixed results (Alba *et al.*, 2011; Doorduin & Vrieling, 2011). One good example is that of *Melaleuca quinquenervia* introduced into Florida, which displays a different terpene composition from populations in its native range (Franks *et al.*, 2012). In addition, changes in the genetic variance/covariance matrix (G-matrix) have occurred, suggesting that the propensity for further evolutionary change has been altered.

Heritability is often interpreted as a measure of the ability of a population to respond to natural selection. It can be compared among traits by virtue of its being scale-free; however, it can be misleading as a predictor of evolutionary response, because of its dependence on environmental variance (Houle, 1992). The coefficient of genetic variance (CV_a) has been proposed as a better measure of a trait's evolvability, as it predicts the proportional change under a unit strength of selection (Houle, 1992). Across taxa and traits in general, and for growth, life history and morphology specifically, Hansen *et al.* (2011) found that evolvability was uncorrelated with heritability. However, they did report a moderate positive correlation ($r = 0.47$) for physiological traits, including PSMs, which is borne out by studies focusing on chemical traits within species. Heritability and CV_a were positively correlated ($r = 0.61$) across 18 PSMs in *Betula pubescens* spp. *czerepanovii* (Haviola *et al.*, 2012) and in *O. biennis* they were positively correlated ($r = 0.74$) across 15 chemical traits, although no significant correlations occurred among another ten foliar, life history and fitness traits in that study (Johnson *et al.*, 2009). These results suggest that the high heritability of chemical traits (Geber & Griffen, 2003; Rowe *et al.*, 2008) probably does reflect high evolvability.

1. The evolvability of evolvability

The possibility for new mutations to enhance genetic variation and facilitate the evolution of novelty is an alternative perspective on evolvability ('the evolvability of evolvability'; Wagner & Altenberg, 1996; Pigliucci, 2008). This depends on how genotype determines phenotype, that is, the genetic architecture of a trait and the constraints imposed by relationships with other traits. Importantly, the effect of new mutations must be considered, rather than just standing variation, which heritability and CV_a express. PSMs may be particularly evolvable in this sense for several reasons. First, as described earlier, mutation of a single nucleotide in a biosynthetic gene can completely alter its product profile. Mutations with such large consequences offer exceptional opportunities for chemical novelty to evolve. Secondly, a lack of pleiotropic effects of the genes underlying PSM variation may leave these biochemical systems relatively free to evolve (Debat & David, 2001; Carmona *et al.*, 2011). Thirdly, by increasing the dimensionality of the adaptive landscape, expanded biosynthetic gene families and their products enhance the scope for phenotypes to evolve as populations can skirt fitness troughs more easily (Gavrilets, 1999).

Evolvability can also be promoted by 'capacitors' of variation, such as the heat-shock proteins. In particular, heat-shock protein 90 (Hsp90) is involved in phenotypic plasticity (Sangster *et al.*, 2007) and can buffer genetic variation (Rutherford & Lindquist, 1998; Queitsch *et al.*, 2002) such that it is only expressed under certain environmental conditions. Thus, previously silent phenotypic variation can be released under novel conditions (Sangster *et al.*, 2007). Although Hsp90 is essential for R-protein-mediated defence, it is not yet known whether it buffers natural PSM variation. However, it does plays a role in regulating induced defences in *A. thaliana* (Sangster *et al.*, 2007). Additional buffering mechanisms exist in *A. thaliana*, including the signalling protein encoded by the *ERECTA* gene (Hall *et al.*, 2007). If it is widespread, such hidden genetic variation in PSM plasticity may be particularly important in the context of rapid climate change.

2. Evolution of genetically controlled responses to environmental variation

As a source of phenotypic variation, environmental variation is often divided, somewhat artificially, into macroenvironmental variation, which can be measured and controlled, and microenvironmental variation, which occurs at the scale of the individual plant. Phenotypic variance resulting from microenvironmental variation (microenvironmental variance) is often indistinguishable from stochastic biosynthetic 'noise', especially when multiple independent phenotypic measurements cannot be conducted on each genotype. In plants, which are highly modular, the influences of stochastic and/or microenvironmental variance may often be apparent within individuals, for instance among leaves or branches.

The responses of organisms to variation in abiotic or biotic environments are often under genetic control. The result is a genotype × environment (G × E) interaction, which can be viewed as genetic variation in reaction norms, and can sometimes cause genetic variance to differ among environments (Orians *et al.*, 2003). For example, genetic variation in the induction of PSMs by herbivores is only visible in the presence of herbivores (Agrawal *et al.*, 2002). Underlying genetic mechanisms have been identified for some induced chemical defences (Rowe *et al.*, 2008; Schuman *et al.*, 2009), but those mediating G × E effects on constitutive PSMs are likely to be highly polygenic and have proven elusive. As global change alters both the biotic and abiotic environments of plants, interactions between genes and the environment could determine the availability of the genetic variance needed to adapt to new environments.

The residual component of variance, which is typically attributed to microenvironmental variation, has traditionally been assumed to be constant, but recent theory has explored the potential for genetic effects on environmental variance and its implications for both natural and artificial selection. Recognition of the importance of these factors has been driven by the animal-breeding community, where a failure to consider them can mean that genotypes favouring high stochastic variation are inadvertently selected for by common breeding methods (e.g. truncation selection; Hill & Mulder, 2010). In the plant literature, genetic analysis of stochastic variation has been limited to model systems (maize and *A. thaliana*), although quantitative trait locus (QTL) studies have started to identify the molecular mechanisms (Ordas *et al.*, 2008; Shen *et al.*, 2012). In general, genetic effects on environmental variance are widespread, but small (genetic coefficients of variation up to 0.23; Ordas *et al.*, 2008), yet they can contribute > 20% of the total phenotypic variance (Shen *et al.*, 2012). Jiménez-Gómez *et al.* (2011) identified QTLs affecting stochastic noise in glucosinolate production and associated transcript abundance in *A. thaliana*. Known QTLs for mean phenotype levels also affected stochastic noise, but several novel associations were found in that study. Interestingly, polymorphisms controlling both glucosinolate-related and global transcriptomic stochastic noise occur in nature, indicating that such genetic effects may influence the evolution of PSMs and other traits. Although it sets a baseline for quantitative chemical variation and influences the apparent strength of selection, the extent and evolvability of residual variation are rarely discussed explicitly in empirical studies of PSM evolution.

The evolution of genes affecting variance has received considerable theoretical attention (Hill & Zhang, 2004; Mulder *et al.*, 2007). In two-gene systems, selection affects variance-controlling genes more strongly than the effector loci (Pettersson *et al.*, 2012). Stabilizing selection in stable environments (e.g. with predictable herbivore pressure and resource availability) favours low-variance alleles, as they enhance the efficacy of selection on a chemical phenotype. By contrast, the high-variance allele may be favoured in new environments or under directional selection, although the associated adaptive phenotype might not be passed on via the breeding value. This is one way that variance can increase under directional selection (Hill & Zhang, 2004), while simultaneously reducing the efficacy of selection. In highly variable environments (e.g. herbivory or pathogens are patchy or unpredictable) or under disruptive selection, high-variance alleles for PSM production may also be favoured. It is unknown how costly the required molecular machinery may be, and specific chemical traits may differ in this respect. Where QTLs affect both mean and variance (Jiménez-Gómez *et al.*, 2011), costs may be small, but indirect costs are difficult to predict.

IX. Evolutionary strategies and diversity in plant chemical defence

Perhaps the most powerful and obvious explanation for quantitative chemical variation and β-chemodiversity is the fact that environments are inherently variable, in space and time and across many scales, and variability, regardless of its origin, improves the likelihood of a plant's phenotype matching its environment. This is the lottery principle, originally proposed by Williams as an explanation for sex (1975). Spatial heterogeneity in herbivory is most likely to occur for species that are not ecosystem dominants, that disperse propagules widely and that are attacked by specialist herbivores. However, because competition also favours phenotypic variability (Bell, 1982), whether the predictions of the lottery principle are borne out may depend upon the relative strengths and heterogeneity of herbivore and pathogen pressure, versus ecological tradeoffs between defence and competitive ability.

Bet-hedging describes a suite of strategies by which plants adjust the mean and variance of a trait among their offspring so as to reduce temporal (usually year-to-year) variation in their fitness (Simons, 2009). A particular pattern of offspring defence may produce high arithmetic mean fitness across years, but if that pattern fails to ensure a reasonable proportion of survivors in years of high herbivore pressure, geometric mean fitness will be low, and the long-term survival of the genotype poor. Bet-hedging can be conservative, when among-sibling variation is reduced around a mean PSM type or level that ensures survival under most herbivory conditions, or diversifying, when propagule variation is increased to maximize the chances of some offspring surviving in any given year. Bet-hedging of plant chemical defence is plausible; however, because spatial (between-sibling) variation in the intensity of herbivory encountered reduces its likelihood, while between-cohort variation increases it (Starrfelt & Kokko, 2012), it might be

more likely to occur in locally common annual plants where herbivore densities vary from year to year.

The occurrence of undefended chemotypes in populations of largely defended individuals can sometimes be described as automimicry (Speed *et al.*, 2012). In rare cases where herbivores choose food items on a plant species rather than a plant individual basis, automimics are parasites on the public good (i.e. the benefit accrued to the population as a whole from the presence of defended individuals), and their fitness should decline with their frequency in the population. Mathematical models can demonstrate evolutionarily stable strategies that allow automimicry, both within and between plants (Till-Bottraud & Gouyon, 1992), but empirical tests are rare. Lawler *et al.* (1999) described an ecological conditioned flavour aversion, whereby herbivores used concentrations of readily tolerated terpenes in *Eucalyptus* as a reliable olfactory cue to assess the concentration of antifeedant formylated phloroglucinol compounds (FPCs). This situation seems to present an opportunity for more sophisticated mimicry, where high terpene concentrations could mimic defence by FPCs without their cost. Because successful mimics must be difficult to identify, mimicry might be more successful against generalist than specialist herbivores, but will fail if pre-ingestive cues such as volatile compounds or pigments allow herbivores to reliably assess defence. Hamilton & Brown (2001) suggested that overwintering aphids use the highly variable PSMs responsible for autumn leaf colour as an honest signal of defence.

Frequency-dependent selection (FDS) and local adaptation by herbivores are key to other explanations of β-chemodiversity. Because herbivores and pathogens become locally adapted to common plant chemotypes, the fitness of plant chemotypes should decrease as their frequency in the population increases and overall parasitism (herbivory and infection by pathogens) in a population should decrease with chemotypic diversity. Over time, the frequency of rare phenotypes increases as a consequence, resulting in time-lagged oscillations between host and parasite genotypes. Mixed evidence for FDS is available from a range of predator–prey interactions; for example, Pasteels & Gregoire (1984) showed that predatory insects avoided insect prey with rare defensive secretions, but Janz *et al.* (2005) found no evidence that preferred host-plant frequency affected rates of oviposition by a polyphagous butterfly. However, studies addressing FDS on plant defence are uncommon (Núñez-Farfán *et al.*, 2007). Siemens & Roy (2005) demonstrated FDS for resistance to a rust fungus in *Arabis holboellii* but not for herbivore resistance mediated by glucosinolates in the same species. By contrast, many examples demonstrate local adaptation by plant enemies (Kaltz & Shykoff, 1998; Hereford, 2009), even though it can be prevented by high levels of herbivore immigration (Tack & Roslin, 2010).

1. Diversity can be inherently beneficial for plant individuals, families, populations and communities

Intraspecific variation in PSMs was a formative example in developing the concepts of community and ecosystem genetics. Variation in condensed tannins in cottonwood (*Populus*) influences the trees that beavers select, which in turn affects tree fitness and stand composition. Tannins negatively affect nitrogen mineralization but are positively correlated with fine-root production (Whitham *et al.*, 2006). Although this idea is an attractive way to link intraspecific variation in PSMs to community- and ecosystem-wide consequences, understanding the genes that underlie the trait has proved elusive despite the early availability of the *Populus trichocarpa* genome (Wang *et al.*, 2013).

Biodiversity can boost community primary productivity and other ecosystem functions via well-studied facilitation, additive and dominance effects (Tilman *et al.*, 2001), and recently interest has grown in the relationship between these functions and intraspecific genetic diversity (Sangster *et al.*, 2008). The magnitude of genetic diversity effects on ecosystem function can match those of species diversity (Crutsinger *et al.*, 2006). For example, genetic diversity can often reduce herbivory and damage by pathogens in agricultural systems (Cantelo & Sanford, 1984; Smithson & Lenne, 1996) and Hughes & Stachowicz (2004) showed that genetic diversity increased the resistance of a seagrass (*Zostera marina*) population to grazing geese. Nevertheless, increased diversity does not always reduce herbivore populations (Johnson *et al.*, 2006; Kotowska *et al.*, 2010). Utsumi *et al.* (2011) showed that aphid population size increased in more genotypically diverse plots, and proposed two explanations: source–sink effects where susceptible plants provide a source of aphids for defended neighbours, and reduced mortality of natural enemies of aphids. Although PSMs are likely to contribute to these effects, studies that have explicitly considered genetically-based diversity in secondary chemistry in this context are rare and less encouraging. Two studies (Poelman *et al.*, 2009; Tack *et al.*, 2012) observed that qualitative glucosinolate diversity in *Brassica oleracea* cultivars was associated with insect diversity, but that most insects responded similarly to the various compounds. Furthermore, Macel *et al.* (2002) found that qualitative diversity in pyrrolizidine alkaloids in eight *Senecio* species appeared to be selectively neutral in the context of herbivory by a specialist herbivore.

The approach used in the kinds of studies mentioned above may not be without problems. To maximize the chance of detecting effects, diversity is commonly sourced from provenances across large geographical areas, so the range of phenotypic diversity (i.e. the magnitude of quantitative diversity or the chemotypic diversity among families or clones) created in experimental populations often deliberately exceeds that seen in natural populations, yet the experimental spatial scale usually remains small (Tack *et al.*, 2012). Consequently, the importance of the ecological effects of genetic and phenotypic diversity may be overestimated and Tack & Roslin (2011) suggest that they may be generally small. At the same time, the number of genetic families or clones in common gardens will sometimes be less than that that seen in natural populations, and this might accelerate the process of herbivore specialization on hosts, for example.

Quantitative diversity in chemical defence can directly reduce herbivory on individuals and populations via Jensen's inequality (Jensen, 1906). If the relationship between PSM concentration and the benefit gained by a herbivore is concave, then the net benefit to a herbivore (and hence the damage experienced by an individual exhibiting heterogeneity among its parts or a heterogenous population) will decrease with increasing heterogeneity given the

same plant or population trait mean. The ability of induced defences to produce such heterogeneity among plant parts or individuals may offer a significant advantage over constitutive defences (Karban *et al.*, 1997; Karban, 2011). Shelton (2004) produced a number of dynamic programming models parameterized with data from a generalist herbivore of *Raphanus sativus* defended by glucosinolates, showing that such quantitative variation, at a range of scales, should reduce herbivore fitness.

Another mechanism has been demonstrated by which qualitative PSM diversity among individuals may also reduce herbivory experienced by plant individuals in some, probably rare, cases. Just as generalist herbivores include many plant species in their diet to spread the detoxification load across enzymes and pathways (Marsh *et al.*, 2006), and balance their nutrition (Felton *et al.*, 2009), intraspecific PSM variation might promote plant switching by specialists. Mody *et al.* (2007) showed that *Chrysopsyche imparilis* caterpillars switched regularly between individual plants of *Combretum fragrans*, thus shortening feeding bouts on individual plants with the result that the foliage was never markedly reduced.

The sessile nature of plants allows some scope for group selection when animals make feeding decisions at the level of the patch instead of, or in addition to, the level of the individual plant, and PSM diversity can contribute to a herbivore's decision to feed in or depart from a patch. If increased diversity is beneficial to the offspring seedlings clustered around a maternal plant, then selection may act upon these sources of variation. Stabilizing selection might also act more strongly to ensure the ideal proportion of automimics among the offspring of a maternal plant where most or all automimics and automodels are siblings because the opportunity for unrelated plants to exploit the common good is reduced. Within-family automimicry allows some offspring to avoid the cost of defence but benefit from defence by its siblings, which is now a family rather than a common good. This situation is analogous to that described by Hare & Eisner (1993) where insects produce clutches of eggs where only a proportion are defended, but the group as a whole benefits.

The production of chemically variable offspring may allow a maternal plant to ensure that some offspring can circumvent the attack by locally adapted herbivores predicted by the Janzen–Connell hypothesis (Janzen, 1970; Connell, 1971) to occur within the high-seedfall zone surrounding the parent (Langenheim & Stubblebein, 1983). A meta-analysis (Hyatt *et al.*, 2003) suggested that seedlings were probably more affected by proximity to parent trees in tropical than in temperate forests, and so predictions about chemodiversity should differ accordingly.

Finally, an alternative perspective is to view undefended seedlings as acting to dilute the impact of herbivores on their better-defended siblings, rather than as beneficiaries of within-family diversity. These family- or group-selection mechanisms all depend upon an individual's likelihood of herbivory being influenced by the density and quality of its neighbours. There are examples of herbivores that make feeding decisions only at the level of the patch, only at the level of the individual plant or plant part and at both levels (Hjalten *et al.*, 1993; Bergvall & Leimar, 2005; Moore *et al.*, 2010; Emerson *et al.*, 2012). Many of these group-selection mechanisms have been proposed previously (Denno & McClure, 1983), but whether they are valid and how they may favour chemodiversity will depend upon many circumstances including habitat complexity, seed dispersal and herbivore identity. Their importance can only be addressed by the accumulation of empirical data matching the dynamic dispersal and movement of herbivores to data about plant population structures, such as detailed spatial mapping of plant chemical defence genotypes and phenotypes.

2. PSM variation may be ecologically essential

The roles of both quanitative variation and chemodiversity in ameliorating biotic and abiotic stress may play critical roles in creating the ecological niche differentiation among individual plants that is essential to reduce competition and allow coexistence. Variation in responses to the environment among individual plants has been shown to allow species coexistence in a variety of plant communities (Clark, 2010; Jung *et al.*, 2010). If diversity is a prerequisite for finding niche space, this demand might be met by genetic diversity or by plasticity. Few studies to date have specifically looked at the role of PSM variability and diversity in these processes, but Mraja *et al.* (2011) showed that the species richness and composition of plant communities can alter the concentration and composition of PSMs in *P. lanceolata* in experimental grasslands. It is likely that the species diversity of ecological communities and the phenotypic diversity of plant populations are mutually reinforcing, as plant defensive traits structure communities just as the broader community context shapes defence phenotypes and selection upon them (Lankau & Strauss, 2007; Poelman *et al.*, 2008). For example, quantitative chemical variation and chemodiversity can promote biodiversity by allowing species to invade and persist in dynamic systems, whether that diversity occurs among (β-chemodiversity; Lankau & Strauss, 2007, 2008) or even within (α-chemodiversity) individuals (Iason *et al.*, 2005).

X. Conclusions and future directions

Although the variance of PSM traits is often measured, especially in studies of selection, as a source of error around population mean trait values, little attention is paid to variation *per se*, particularly for quantitative variation. Genetic and environmental contributions to mean phenotypes are often measured together, but usually in common gardens and with limited numbers of genotypes and environments; marker-based techniques are available to estimate heritability and genetic correlations in the wild but are not widely used (Andrew *et al.*, 2007). Appropriate statistics to describe the chemotypic diversity of wild plant populations from large sample sizes are rarely reported; in many cases only standard errors are reported for trait means and sample sizes are omitted or obscure. In order to advance understanding of how and why phenotypic variation in PSM traits exists, we encourage researchers to publish summary statistics describing phenotypic, environmental and genetic variance, as well as heritability (h^2) and evolvability (CV_a) whenever these are available. The publication of CV_a for secondary chemistry is becoming more common, but Garcia-Gonzalez *et al.*

(2012) caution that this is often calculated inappropriately. Although interest in the ecological importance of intraspecific genetic diversity is booming (Dall *et al.*, 2012), only a few studies (Johnson *et al.*, 2006; Carmona *et al.*, 2011) have yet considered variation of PSM phenotypes explicitly.

In this paper we have summarized many explanations for, and potential advantages associated with, quantitative variation and qualitative diversity in PSMs. Efforts should now be made to understand the relative contributions of additive and nonadditive genetic, environmental, microenvironmental and stochastic variation to phenotypic variation across plant taxa and environments. Of particular interest is the extent to which variation in each of these compartments is heritable and what role they play as reservoirs and generators of quantitative variation and chemotypic diversity, as well as how they respond to a variety of selection pressures. Under what circumstances is chemical variability likely to be beneficial and how does selection act upon the molecular structures and processes underlying it?

The explosion in understanding the molecular basis of chemical variation should soon allow development of tools to directly test the adaptive value of variance in natural environments. For example, we envisage the creation of lines and the assembly of populations that express specific levels of variance in one or several PSMs being used to facilitate comparisons with the same lines in which the drivers of variance have been silenced. This could include both constitutive and induced PSMs. Exposing these lines to selection by herbivores and pathogens may allow explicit tests of many of the hypotheses that we have discussed.

As data accumulate, it should prove rewarding to search for patterns in the partitioning of chemical phenotypic variance across plant life history and functional traits. What circumstances favour the maintenance of environmental over genetic variance and vice versa? Does environmental variance expand when genetic diversity is lost, and can the loss of environmental variance lead to evolutionary dead ends? Is stochastic noise actively selected for in order to maintain phenotypic variation where it is beneficial or are stochastic fluctuations deliberately dampened (Masel & Siegal, 2009)? Many axes of variation might influence the degree and partitioning of chemical phenotypic variability, particularly those that influence the spatial and temporal variability of plants' environments. We hope that this review will stimulate the development of theory and predictions along these lines.

Because it seems clear that highly variable environments and diverse selection pressures maintain variability and prevent strong directional selection on PSMs, examples of rapid PSM trait evolution and associated changes in chemical variability should be sought where plants have invaded new habitats and where climate or other changes have altered the biotic and abiotic landscape. Also of interest in a world subject to unprecedented rates of anthropogenic change is the extent to which the genetic variation underlying quantitative PSM variation and chemodiversity has been lost from many plants of economic importance as a result of breeding, harvesting and the reduced diversity of biotic interactions characteristic of monocultures.

When Geber & Griffen (2003) reviewed heritability and selection differentials on a variety of plant traits, they concluded that PSMs were indeed particularly highly heritable but also that their heritability was negatively correlated with directional selection, seemingly because direct selection on these traits was opposed by indirect selection (i.e. selection on correlated traits and against costs of chemical defence). This, they suggested, would constrain PSM evolution. Carmona *et al.* (2011) arrived at almost the opposite conclusion, proposing that the evolutionary and ecological importance of PSMs in herbivore defence may be a consequence not of their effect on herbivores (which they found to be weaker than that of many other traits), but of the constraints on their evolution, particularly pleiotropy, being the weakest. The merit of these opposing conclusions can only be addressed with microevolutionary studies of more chemical defence traits over multiple years and environments. Artificial selection experiments can illuminate these issues perhaps more clearly than measures of selection in the wild (Agrawal *et al.*, 2012), but extending both of these approaches to long-lived plants remains a challenge. We expect that explicit consideration of the causes and consequences of variation in plant secondary chemicals will enrich our understanding of the ecology and evolution of plant defence more generally.

Acknowledgements

Thanks to Heather Rowe, Ian Baldwin and two anonymous reviewers for useful comments which improved the manuscript. Support for W.J.F. and C.K. was from the Australian Research Council Discovery and Linkage Schemes.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Notes S1 Definitions and discussion of the types of chemical diversity discussed in this review and appropriate metrics for quantifying and partitioning diversity.

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