Convergent Evolution in Plant Specialized Metabolism

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Keywords

Abstract

Plants synthesize a multitude of compounds that contribute to adaptation to their ecological niches. Such compounds serve as attractants of other living organisms beneficial to the plants or as defense against other biotic as well as abiotic agents. Selection for increased fitness, a never-ending process, has resulted in each plant lineage synthesizing a distinct set of specialized metabolites appropriate for its environment. The total number of specialized metabolites found in the plant kingdom far exceeds the capacity of any one plant genome to encode the necessary enzymes, and just as a plant lineage acquires the ability to make new specialized compounds during evolution, it also loses the ability to make others. Although the ability of plants to make novel, specialized metabolites continues to evolve, there are also many examples in which different plants have independently evolved the ability to make compounds already present in other plant lineages or to make different compounds that fulfill the same role—both are examples of convergent evolution. Here, we discuss many examples of convergent evolution in specialized metabolism. There are many genetic and biochemical mechanisms that can give rise to convergent evolution, and we conclude that, overall, convergent evolution in plant specialized metabolism is surprisingly common.

Contents

INTRODUCTION

There are several hundred thousand recognized plant species, and the true number may be in the millions. Estimates of the number of chemical compounds synthesized by an individual plant

PRIMARY METABOLITES IN PLANTS

The number of primary metabolites in plants can be extrapolated from work done in yeast, where 16% of the genes in the yeast genome were found to be responsible for 584 metabolites (18, 72). The Arabidopsis genome, the smallest plant genome so far elucidated in full, contains roughly 4.5 times the number of genes found in yeast (26,500 versus 6,000). If we estimate that no more than 50% of the genes in the *Arabidopsis* and yeast genomes are devoted to primary metabolism (including biosynthesis and modification of macromolecules), then we derive the number of ∼8,000 metabolites (including intermediates) for *Arabidopsis*.

are even more difficult to arrive at. The number of primary metabolites, which is defined here as the type of compounds synthesized by all or most plant species, is probably under 10,000, and it likely exceeds the number of compounds found in other eukaryotes because plants are true autotrophs (see sidebar Primary Metabolites in Plants). It should also be borne in mind that because plants are drastically different organisms than animals, some primary metabolites found in all (or almost all) plants may not be present in other eukaryotes, and vice versa.

Plants also produce a plethora of compounds that some biologists refer to, rather illogically, as "natural compounds" or as "phytochemicals" (illogical to plant biologists because all compounds made in plant cells fit this definition; these terms come from the field of pharmacology, where they do make sense as a way of distinguishing them from synthetic medicines) (14, 87). These compounds have also been misleadingly called "secondary metabolites," an anachronistic term that was coined when scientists did not know the biological functions of such compounds and suspected that they had no function at all but were waste products (19, 26). Regardless of the imprecision and inconsistencies in these old terms, their general intent was to define compounds that are present in some plant species and not in others, and therefore could not logically be involved in the basic, primary metabolism operating in all plants. As more evidence concerning their functions accumulates, it has become clear that the ability to synthesize such compounds evolved in different plant lineages, and these compounds represent adaptations to specific ecological situations, for example, attraction of specific pollinators or defense against specific herbivores. For this reason, these compounds have recently been termed "specialized metabolites" (53, 64, 87), and we refer to them as such here.

The number of specialized metabolites made, in the aggregate, by plant species has been estimated at roughly 200,000 (14). Recent data from metabolic profiling investigations show a large number of metabolites detected in each species examined and great

diversity among species (87), suggesting that 200,000 is probably a gross underestimation, particularly given the fact that so few plant species have ever been investigated for their content of specialized metabolites. Furthermore, many specialized metabolites are intermediates in complex pathways and are not typically found in high concentrations in the plant tissues being examined, thus avoiding detection. Issues concerning efficacy of extraction and stability of compounds also limit the number of compounds detected in these studies (the above arguments apply to primary metabolites as well). Conversely, extraction artifacts and chemical instability may generate novel structures that are essentially absent *in planta*.

For the same reasons, it is difficult to estimate the number of specialized metabolites that each plant species can synthesize. At best, estimates have been given on the basis of genes with known function, with or without the caveat that the exact activity of the proteins encoded by the majority of genes in any genome is not presently known (see, e.g., 18). However, assuming conservatively that in plants 10–20% of the genes in the genome encode enzymes for specialized metabolism (52, 71), an average of ≤1.5 proteins per enzyme (18), and a roughly 1:1 ratio between the number of enzymes and the number of compounds produced in the cell (some enzymes are redundant and use the same substrate to give the same product, and some enzymes can use multiple substrates to give multiple products, but a general rule of one enzyme, one product is reasonable), the plant model *Arabidopsis thaliana*, with roughly 26,500 genes, can be estimated to make 1,750–3,500 specialized metabolites (including intermediates). Other plants such as rice and poplar are estimated to have 35,000 genes, so the number of specialized metabolites they make is somewhat higher. However, it is clear that each plant species can synthesize only a small fraction of the total number of specialized metabolites found throughout the plant kingdom.

On the molecular level (i.e., genes and proteins), what mechanisms enabled plants to evolve the ability to make so many different specialized metabolites? The process of gene duplication (45, 48, 51), followed by random mutations that can occasionally give rise to a new enzyme (although at least one gene copy retains the original function), has been amply documented and discussed. However, orthologous genes encoding enzymes of specialized metabolism can diverge in different species even without a prior duplication (52, 65). Whether following gene duplication or not, genetic variations arising from random mutations leading to variability in fitness are the material on which natural selection operates (48), and genes required for specialized metabolism are no different (53). Although the selective advantage of particular specialized compounds has often been difficult to document, the widespread presence of a gene (or allele) for a given specialized compound in individuals of the same species is likely in most cases to be an indication that it presently confers a selective advantage to the organism (and this is why this gene, or allele, has spread in the population). However, it is also likely that at any given period in the plant's history a small proportion of the specialized metabolites that the plant makes no longer confer a selective advantage (54).

The acquisition of new enzymes with new functions in specialized metabolism is generally viewed conceptually as divergent evolution, which is undoubtedly appropriate in the majority of cases (52). Convergent evolution, traditionally viewed as less common than divergent evolution, has received less attention. Here, we argue that the concept of convergent evolution not only explains, in some cases, the presence of the same specialized metabolites in polyphyletic plant lineages but also explains how selection to achieve similar physiological functions in different lineages can lead, paradoxically, to greater chemical diversity in the plant kingdom.

DEFINITION OF CONVERGENT EVOLUTION, WITH EMPHASIS ON FUNCTION

The essence of convergent evolution is that the same biological function evolves independently more than once. The evolution of eyes (for sight) of vertebrates and insects is an often cited example. On any level—anatomical, cellular, molecular—there may or may not be resemblance, but the function is judged to be the same. The evolution of wings (for flying) of birds and bats is also an example of convergent evolution. In this case, both lineages evolved wings from the forelimbs of their common ancestor, but clearly did so independently because phylogenetic information indicates that intervening taxa did not and do not possess wings.

In this review, we divide the discussion on convergent evolution within plants into two main categories. The first includes examples of different lineages independently evolving the ability to synthesize compounds that fulfill the same or very similar function, even though they come from different biochemical pathways and are structurally different. In the second category are those cases where different lineages independently evolved the ability to synthesize identical chemicals. We also discuss examples where convergent evolution has occurred in the ability to make chemically identical compounds or functionally identical compounds in plants and other organisms.

THEORETICAL AND PRACTICAL LIMITS ON VERIFICATION OF CONVERGENT EVOLUTION

Some specialized metabolites found in plants (or in some plant lineages) are also found in other taxa, such as (some) bacteria or fungi, and some specialized metabolites found in one plant lineage are found in other, but not closely related, plant lineages, whereas intervening plant lineages do not seem to synthesize such compounds. In determining whether such cases indicate convergent evolution, a common concern is to verify that, indeed, intervening lineages do not make such compounds. This is not an easy task. When we state that a given compound is not found in a given species, this statement cannot be absolute (85) because as the adage goes, absence of evidence is not evidence of absence. The amount of the compound may be below the detection level of the instrument, or perhaps the right tissue or developmental stage or time was not chosen for examination. Indeed, most plant species have not been investigated at all for the presence of specialized compounds.

Thus, it is possible that some compounds presently considered to be limited to some lineages are indeed universally found in plants. Moreover, it has been hypothesized that some specialized metabolites detected in certain plants were actually synthesized by microorganisms living in association with these plants (85). However, considering that hundreds of thousands, and possibly millions, of specialized metabolites have been found in plants and that each plant genome is theoretically capable of producing probably fewer than 5,000 such compounds, it follows that the syntheses of the majority of these compounds indeed occur in restricted plant lineages. Moreover, because there is no reason to assume that the genomes of plants were ever much larger than what they are today, it follows that the likely explanation for the restricted distribution of a given compound is in most cases a gain in synthetic ability in the given plant lineage, not a loss of synthetic ability in the lineages in which this compound is not found or other alternative explanations.

In practice, the more we know about how a compound is synthesized, the better position we are in to determine whether convergent evolution might have occurred. For example, if a given compound is synthesized by two unrelated enzymes in different lineages, that would be a clear indication of convergent evolution. Unfortunately, for the majority of specialized metabolites, we know little or nothing about their synthesis.

DIFFERENT CHEMICALS, SAME FUNCTION

There are many examples of different plant lineages utilizing different compounds for the same physiological or ecological roles. Classical examples are found in the use of different pigments. For example, anthocyanins are important flavonoid pigments, widely distributed in the plant kingdom, imparting blue, pink, red, and magenta hues in flowers, as well as the red and purple color of strawberries and blueberries (75). Plants within the order Caryophyllales (except for one family within the order: the Caryophyllaceae) apparently lack anthocyanin pigments but instead produce betacyanins, a group of pigments belonging to the betalain class that mimic the color range of anthocyanins and which are also present in some fungi (73) (**Figure 1a**). Interestingly, members of the Caryophyllaceae lack betacyanins but contain anthocyanins (it appears that no known plant species accumulates both betacyanin and anthocyanin pigments). It seems that an ancestral Caryophyllaceae member evolved a different pigmentation system that ultimately replaced anthocyanins in its descendants (75). It has been hypothesized that this dramatic pigmentation change was due to a loss of dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS) activities in betacyanin-containing plants, based on the fact that some betacyanin-accumulating plants do accumulate dihydroflavonols (67). However, recent findings have indicated that at least some members of the betacyanin-accumulating Caryophyllales possess operational DFR and ANS genes (when heterologously expressed in *Escherichia coli*), but these genes are expressed only in the seed coats (67, 68), where proanthocyanindins, but not anthocyanins, accumulate (67). Therefore, an explanation for the mutual exclusivity of the two pathways in the same tissue is still not evident.

Another example of chemical equivalency can be found among the yellow pigments (**Figure 1b**). Xanthophylls are important yellow carotenoid pigments in flowers of tomato and other plants, whereas other plants rely on chalcones and aurones, both of which are phenolic, water-soluble, vacuolar pigments, to impart yellow coloration to their flowers. Cacti, portulacas, amaranths, and other members of the order Caryophyllales, on the other hand, utilize betaxanthins, also members of the betalain class, for the same purpose (73, 75).

Different volatiles, too, can have similar olfactory properties. Flowers that are pollinated by a particular class of insects, such as (diurnal) bees or (nocturnal) moths, have been described as possessing a specific pollination syndrome, a constellation of morphological and biochemical (color, scent) characters held in common. However, even though moth-pollinated flowers are typically white and scented and have long floral tubes, there is no one volatile chemical present in all of them, even for flowers from several species that are visited by the same species of moth (15). Among the most common volatiles emitted in moth-pollinated flowers is linalool, an acyclic monoterpene alcohol, yet many moth-pollinated flowers do not emit linalool but instead emit other terpenes and/or phenylpropanoids such as eugenol or methylbenzoate (55, 66). Some generalist moths visit a range of species that in some cases emit nonoverlapping bouquets (58). Although such compounds—individually or in combination—attract moths, and thus have the general function of attractants, the moths recognize most of these chemicals as distinct entities and use the information to distinguish between plants (12, 59). However, it is possible that some volatiles are not interpreted as distinct smells by the moth, as it has been established that some volatiles are perceived as causing the same olfactory sensation in humans, particularly if they share a common moiety (84).

SAME CHEMICALS, (SOMETIMES) SAME FUNCTION BUT VIA DIFFERENT REACTIONS OR ENZYMES, OR BOTH

Same Product from the Same Substrate but with Unrelated Enzymes

There are two basic ways in which convergent evolution results in identical chemical

a

Pelargonidin-3-O-glucoside

Betanin

Lycopene

b

Portulacaxanthin II

Aureusin

Neoxanthin

structures: In one, the substrate is the same but the enzymes are unrelated, and in the other, the substrates are different altogether. There are several well-established cases in which the same product is produced from the same substrate, but the enzymes catalyzing their interconversion in at least two different lineages are not orthologous. For example, the flavone apigenin is synthesized in the Apiaceae family by a flavone synthase (FNS) belonging to the oxoglutarate-dependent dioxygenase (OGD) family, whereas in most other plant species the oxidoreductase that catalyzes the same reaction is a member of the cytochrome P450 family (75). As with the switch from anthocyanin pigments to betalains discussed above, the sequence of events that led to this is not clear. Did the Apiaceae lineage lose the ability to make flavones at some point and only later evolve a new enzyme to make flavones again? Did an OGD gene evolve into an FNS in the Apiaceae family while the lineage still possessed a functional cytochrome P450 FNS, and if so, what was the selective pressure driving this evolution? The answers to these recurring questions are important in elucidating the mechanism(s) leading to convergent evolution. In the case of apigenin, the only clear evidence for convergent evolution came to light when the sequences of the two genes (and their encoded proteins) in the different lineages involved in apigenin biosynthesis were obtained and compared, and it was determined that they were not related. Prior to this observation, no obvious discontinuity in the mechanisms by which different plant families make apigenin had been apparent.

Comparisons of the enzyme sequences involved in the biosynthesis of specialized compound in different lineages have now identified many more, if somewhat subtler, examples of convergent evolution. As discussed above, linalool is a compound found in many plant species, so there was no obvious reason to imagine that the ability to synthesize linalool in plants evolved more than once. Analysis of the sequences of linalool synthases from many plant species, including *Clarkia breweri*, mint, and *Arabidopsis*, shows that the linalool synthases in these species all belong to the large family of terpene synthases (TPSs). However, these sequence comparisons also show that in each plant lineage the respective linalool synthase is more similar to other TPSs responsible for the synthesis of other monoterpenes in that lineage than it is to linalool synthases from different lineages (8, 10, 17). The situation is similar with other terpene synthases as well: TPSs with different activities from the same species tend to be more similar to each other than TPSs with the same activity from distantly related species. The conclusion is that within the TPS family there is a fair amount of divergent evolution but also occasionally convergent evolution. Evidently, small changes in primary sequences of TPS can change its function to produce a slightly different terpene from the same or a slightly different substrate (37, 53), so linalool synthase activity, for example, could have arisen multiple times with relative ease.

This type of convergent evolution, whereby a similar function arose not from a completely unrelated sequence but from a homologous,

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Figure 1

Convergent evolution of pigmentation. (*a*) Red fruits that utilize different pigmentation systems. An anthocyanin, pelargonidin-3-*O* glucoside, is a major pigment of the strawberry fruit (*Fragaria ananassa*) (*upper panel*) (28). The main red pigment in pitaya (*Hylocereus polyrhizus*) fruit is betain, a betaxanthin (56). In tomato fruit, red color is due to the carotenoid lycopene (75). Tomato flower image courtesy of N. Galpaz. (*b*) Yellow flowers displaying convergently evolved pigmentation systems. The yellow color of *Portulaca grandiflora* flowers (*upper panel*) is largely due to the presence of portulacaxanthin II, a xanthocyanin (73). The yellow hues of snapdragon (*Antirrhinum mujus*) flowers are due to aureusin an aurone glucoside (1). The yellow pigments in tomato flowers consist of xanthophylls such as neoxanthin (21).

although not orthologous, gene has been termed repeated evolution (11) and may in fact represent the majority of convergent evolution cases in plant specialized metabolism. There are many such examples already identified, and limitation of space precludes us from listing most of them, so a few examples will have to suffice.

The enzymes that convert eugenol to methyleugenol in basil (*Ocimum basilicum*) and *C. breweri* both belong to a large family of *O*-methyl transferases, but the basil enzyme is more closely related to isoflavone methyl transferase, whereas the *C. breweri* enzyme is more similar to caffeic acid methyl transferase (22, 82). *Arabidopsis* and *Perilla frutescens* enzymes that add a malonyl group to the same position in anthocyanin pigment molecules evolved from different branches of a large family of acyltransferases (42). And the enzymes that substitute the 3-hydroxyl and 5-hydroxyl functionalities on the benzene ring of monolignin precursors in angiosperms and *Selaginella* are not orthologous, but all belong to the cytochrome P450 oxidoreductase family (83).

Caffeine and related xanthine alkaloids are present sporadically in the plant kingdom. Caffeine accumulates in the aerial parts of the coffee plant (*Coffea arabica*, Rubiaceae), leaves of tea (*Camelia sinensis*, Theaceae) and mate (*Ilex paraguariensis*, Aquifoliaceae), and seeds of guarana (*Paullinia cupana*, Sapindaceae) and kola (*Cola acuminata*, Sterculiaceae). Additionally, caffeine is present in the flowers of *Citrus* spp. (Rutaceae), almost exclusively in the androecium (2). Various roles for caffeine in plants have been postulated, from defense of vegetative parts to preventing visits of unwanted insects to the flowers, to habituating beneficial pollinators and seed dispersers. In coffee and tea, where caffeine biosynthesis has been examined in greatest detail, the enzymes that methylate the purine intermediates (**Figure 2**) evolved from different branches of the SABATH carboxyl methyl transferase family (2, 88), thus representing another example of repeated evolution. This observation strongly suggests that caffeine biosynthesis, although being widespread in plants, evolved

independently at least twice. The ubiquity of the intermediates, the simplicity of the pathway, and the preexisting diversity of the methyl transferase repertoire of each plant species make this outcome rather unsurprising.

Stilbenes are a group of phenolic compounds associated with defense against fungal diseases. Stilbenes occur in distant taxa such as *Pinus*, *Arachis*, and *Vitis*. The key enzyme in their formation, stilbene synthase (STS), is a polyketide synthase that has a mechanism of action similar to the better studied enzyme chalcone synthase (CHS) (77). CHSs are ubiquitous in the plant kingdom, being a key enzyme in flavonoid biosynthesis. Both CHSs and STSs catalyze the condensation of hydroxylated or non-hydroxylated *p*-cinnamoyl-CoA with three molecules of malonyl-CoA. However, CHS enzymes generate three-ring chalcones, whereas STS enzymes catalyze the formation of two-ring stilbene-type products with the loss of one carbon. Sequence comparisons have indicated that STSs arose independently multiple times in different plant lineages, in each case from CHSs, as these STSs are typically more similar to the CHS from their own lineage than to other STSs from different lineages (77).

A similar type of repeated evolution has occurred in the biosynthesis of pyrrolizidine alkaloids, a group of alkaloids produced by multiple plants as a defense against herbivores (47, 57). Pyrrolizidine alkaloids are found scattered in a few unrelated families such as the Asteraceae, Boraginaceae, Fabaceae, and Orchidaceae, and isolated occurrences have been described in single species of some additional families, such as the Apocynaceae, Celastraceae, Convolvulaceae, and Ranunculaceae (46). Homospermidine synthase (HSS) catalyzes the first specific step in pyrrolizidine alkaloid biosynthesis, transferring the aminobutyl moiety of spermidine to putrescine to form homospermidine. It has been shown that HSS was recruited from the ubiquitous enzyme deoxyhypusine synthase (DHS), which transfers the aminobutyl moiety of spermidine to a Lys residue of its protein substrate, the eukaryotic initiation factor 5A, thus catalyzing the first of two reactions required for
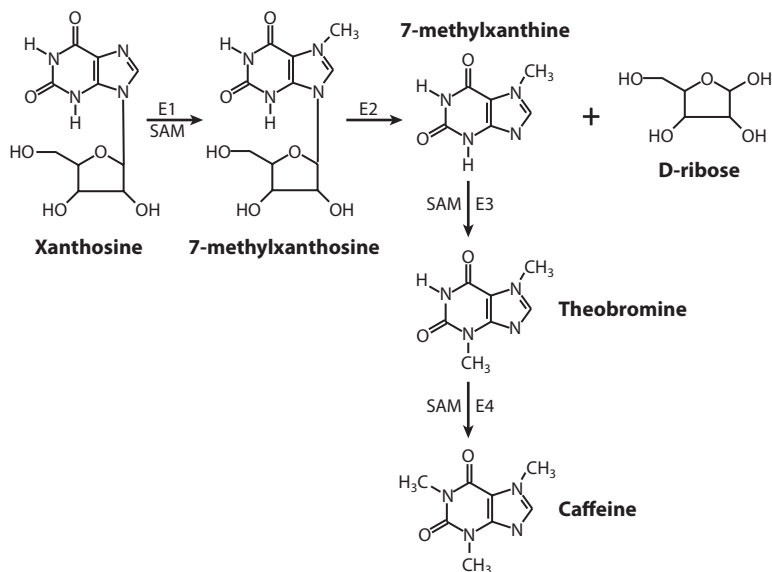
Figure 2

Convergent evolution in purine alkaloid accumulation. Caffeine biosynthesis involves a series of methylation reactions sequentially catalyzed by distinct *N*-methyl transferases (E1, E3, E4) and a 7-methylxanthine nucleosidase (E2). Although plants from several unrelated families accumulate caffeine and other purine alkaloids with fewer methyl groups, and the biosynthetic pathway is similar, the methyltransferase genes in some lineages have evolved independently from different branches of the SABATH carboxyl methyl transferase gene family (2, 88).

the post-translational activation of this factor (46). Despite their completely different reaction products, the two enzymes share common reaction mechanisms. The characterization of cDNAs encoding HSS and DHS from various species has shown that HSS has apparently been independently recruited from DHS at least four times during angiosperm evolution (57).

Finally, the enzyme eugenol synthase (EGS), which catalyzes the formation of eugenol by reduction of coniferyl acetate, presents an interesting conundrum. Basil (*O. basilicum*) EGS and petunia (*Petunia hybrida*) EGS are derived from different branches of a family of reductases involved in the synthesis of Phe-derived specialized metabolites, thus representing another example of repeated evolution (34, 35). Interestingly, however, *C. breweri* has one EGS that is more closely related to basil EGS and another more closely related to petunia EGS, and both are expressed in floral tissue (35). Both enzymes have similar

substrate affinities and turnover rates as well. Why both enzymes have evolved in the same lineage is not clear. Perhaps some subtle differences in the presence of these enzymes in tissues, cells, and subcellular compartments exist that have not yet been elucidated. However, in such a case, it could be reasonably asked why evolution of expression patterns and/or subcellular localization might not have occurred over time to arrive at the same distribution of enzyme activity presently observed. Nevertheless, the abundance of repeated evolution examples suggests that multiple originations of the same enzymatic function via small changes in protein-coding regions may occur as readily as changes in gene expression patterns.

Same Product from Different Substrates

As mentioned above, the other basic route in chemical convergent evolution occurs when

a second pathway evolves so that chemical transformation of different substrates gives rise to the same product. For example, the biosynthesis of methyl anthranilate in grapes is catalyzed by a member of the BAHD acyl transferase family, which replaces the CoA group on anthranilate-CoA with methanol; whereas in maize, methyl anthranilate is synthesized by the transfer of a methyl group from S-adenosyl-L-methione to anthranilic acid, a reaction that is catalyzed by a member of the SABATH family of methyl transferases (36, 81) (**Figure 3**). The monoterpene geraniol is a major constituent of geranium and rose essential oils and is a precursor to geranial, a lemon-scented monoterpene aldehyde present in plants from diverse plant families such as lemongrass, lemon basil, citrus, and eucalyptus. In lemon basil glandular trichomes, the geranial precursor geraniol is formed from gernayldiphosphate by geraniol synthase, a member of the TPS gene family (31) and geranial is subsequently derived from the oxidation of geraniol by dehydrogenases (32). In contrast, in tomato and watermelon, it has been shown genetically that geranial is a degradation product of lycopene and other noncyclic carotenoids (40, 41). Although the genes involved have not yet been isolated, a carotenoid cleavage dioxygenase from rice can perform these reactions (33).

A more complete example is the synthesis of the monoterpene β-phellandrene, reported to be synthesized in leaves of grand fir (*Abies grandis*, a gymnosperm) and in stems and roots of tomato by typical monoterpene synthases that are similar to many other monoterpene synthases from both gymnosperms and angiosperms (6, 79). These tomato and grand fir enzymes use the "universal" monoterpene synthase substrate, geranyl diphosphate (GPP), and as is the case with many monoterpene synthases (5), they actually produce several monoterpenes, but β-phellandrene is the main one. In tomato glandular trichomes, however, β-phellandrene is synthesized from neryl diphosphate (NPP), the *cis*-isomer of GPP (65). This latter β-phellandrene synthase, although still a member of the terpene synthase
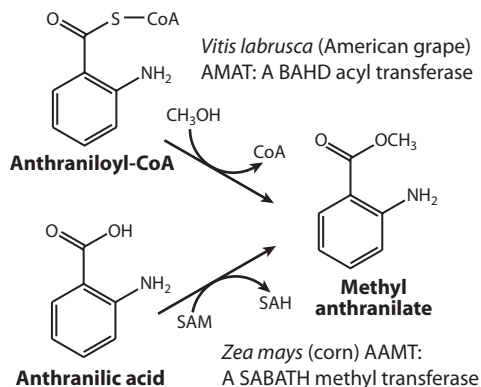
Figure 3

Plants have evolved at least two ways to synthesize methyl anthranilate. In ripening American grape (*Vitis labrusca*) berries, anthranilic acid is first activated by ligating it to CoA, and the resulting anthraniloyl-CoA is used as a substrate by the enzyme anthraniloyl-CoA:methanol transferase (AMAT) to transfer the anthranilic acyl moiety to methanol to form methyl anthranilate, the "foxy" flavor in these grapes. AMAT belongs to the BAHD family of acyl transferases (74, 81). In corn (*Zea mays*) seedlings, methyl anthranilate is emitted from tissue damaged by herbivory, and its synthesis is catalyzed by the enzyme anthranilic acid methyl transferase (AAMT), a methyl transferase of the SABATH that transfers a methyl group from S-adenosyl-L-methione (SAM) to the carboxyl group of anthranilic acid (13, 36).

family, is a member of a monophyletic group of TPS enzymes that consist mostly of diterpene synthases and whose members are larger (by approximately 150 amino acids) than the grand fir and tomato stem and root β-phellandrene synthases (65). Both GPP and NPP are made from the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), but the corresponding enzymes, GPP synthase (GPPS) and NPP synthase, belong to two unrelated gene families, the *trans*-prenyltransferases and the *cis*-prenyltransferase families, respectively (65).

Plants of many families contain cyanogenic glycosides, which consist of an α-hydroxynitrile derived from the sequential oxidation of amino acids by two cytochrome P450 oxidoreductases followed by the addition of one or more sugar units by glycosylases (90). During herbivory

or when the tissue is otherwise damaged, the cyanogenic glycosides come in contact with glycosidases, which remove the sugar units, and the resulting unstable cyanohydrin aglycones are converted by hydroxynitrile lyase (HNL; nitrilase) to hydrogen cyanide (HCN) and a corresponding aldehyde (or ketone) (**Figure 4**). HCN, and often the carbonyl-containing product too, are toxic to the animal causing the damage.

Although they constitute a family of related molecules derived from a variety of amino acids, metabolism of cyanogenic glycosides provides an example of the same final product being obtained from different precursors because HCN is a common final product of cyanogenic glycoside breakdown. Furthermore, phylogenic analysis of the presence of specific cyanogenic glycosides in different lineages suggests that in some cases the ability to synthesize the same cyanogen glucoside, starting with the same amino acid, arose independently more than once, for example, synthesis of cyanogenic glycosides from phenylalanine in *Prunus* (Rosaceae) and *Eucalyptus* (Myrtaceae) (9, 25). But the most remarkable aspect of this system is that enzymes with HNL activity arose independently multiple times. In the Rosaceae family, HNL is a flavin adenine dinucleotide-containing enzyme belonging to the glucose-methanol-choline oxidoreductase family (16, 29). HNLs from other species belong to the α/β-hydrolase superfamily of proteins (29). In addition, the enzyme from *Sorghum bicolor* (a monocot) belongs to the carboxypeptidase branch of the α/β-hydrolase family, whereas the enzymes from cassava (*Manihot esculenta*) and the rubber tree (*Hevea brasiliensis*), both from the dicot family Euphorbiaceae, belong to another branch of the α/β-hydrolase family—another example of repeated evolution (27, 80).

BIOCHEMICAL CONVERGENCE BETWEEN PLANTS AND OTHER TAXA, INCLUDING MIMICRY

Although the main topic of this review is convergent evolution of specialized metabolism
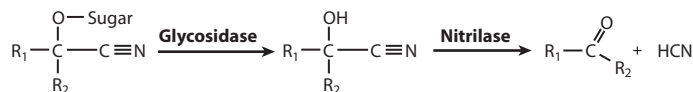
Figure 4

The final step in the breakdown of cyanogenic glycosides is catalyzed by a nitrilase. The reaction results in the production of toxic hydrogen cyanide (HCN) and an aldehyde or a ketone, which are often toxic as well. Plants have independently evolved nitrilases at least three times (29). In cases where R_1 is a phenyl and R_2 is a hydrogen (e.g., in the cyanogenic glycosides prunasin and amygdalin), the second product in addition to HCN is bezaldehyde, a compound that is also produced in plants via cinnamic acid (50, 78).

within the plant kingdom, it is worth noting a few examples of convergent evolution between plants and other organisms. In such cases, it may be instructive to make a distinction as to whether the parallel pathways evolved simply because each lineage has benefitted from making a given compound completely independently of the other or whether mimicry is involved.

The first category includes some cases of terpene biosynthesis. Although plants produce volatile monoterpenes for attracting pollinators and for defense, some insects can synthesize identical compounds. For example, the bark beetle *Ips pini* possesses a bifunctional enzyme that condenses IPP and DMAPP to give GPP, and then converts this intermediate to myrcene, a precursor of the major aggregation pheromone ipsdienol (24). The insect GPPS is only distantly related to plant GPPSs (23), and moreover, in plants myrcene is synthesized from GPP by a typical TPS (4). Another example involves some basic polyketides made in plants for defense that are also synthesized by some bacteria and fungi, possibly for defense as well but against different organisms. However, the plant enzymes and reactions involved are distinct from those in the other taxa (7, 30). Last, moths in the Zygaena family have evolved the ability to make cyanogenic glycosides, used for defense against predators, that are identical to those found in the plants on which they feed. However, as yet, there is no information on the enzymes and genes responsible for the synthesis of these cyanogenic glucosides in the insects, so lateral gene transfer cannot be ruled out (89).

Mimicry is a second category of convergent evolution. In this case, the ability of plants to synthesize a compound appears to have evolved because another organism interacting with these plants also synthesizes an identical compound or a compound with properties in common. For example, female thynnine wasps (Hymenoptera: Tiphiidae) synthesize and emit a set of 2,5-dialkylcyclohexan-1,3-diones that serves as sex pheromones to attract males. Flowers of the Australian orchids in the genus *Chiloglottis* emit the same set of compounds, thus luring male wasps to visit the flowers and inadvertently pollinate them (20, 63). Although nothing is presently known about the synthesis of this compound in either the insects or the orchids, it is likely to be a case of convergent evolution, because there is no reason to assume any association between the orchids and the wasps prior to the evolution in the orchids of the ability to synthesize these compounds and thus attract the insects. A similarly intriguing case is the reported synthesis of the C15 terpene ester methyl farnesoate and its derivatives in plants (3, 76). These compounds serve as juvenile hormone III in insects (69). Presumably, the ability to synthesize these compounds in plants was selected after it arose by chance because the constant exposure of a caterpillar feeding on the plants to these compounds disrupted normal larval development and thus protected the plants. Although little is known about the complete biosynthetic pathway of these compounds in plants, the enzymes that methylate farnesoic acid in insects and plants, a key step in the pathway, are not homologous (69, 86).

Molecular details are available on the synthesis of several defense compounds in plants that mimic neurotransmitters. Approximately 70 cannabinoids have been isolated from the marijuana plant (*Cannabis sativus*) (43), including tetrahydrocannabinol (THC), a well-known psychoactive component that also possesses analgesic, anti-inflammatory, appetite-stimulating, and antiemetic properties. Cannabinoids accumulate in the glandular trichomes of the plant (70).

The pharmacological actions of THC result from its binding to the cannabinoid receptor CB1, located mainly in the central nervous system, and the CB2 receptor, present mainly in cells of the immune system (43). THC acts as a partial agonist on both receptors. The endogenous ligand, anandamide, also known as N-arachidonoylethanolamine, has a completely different chemical structure (**Figure 5**). Nevertheless, both compounds
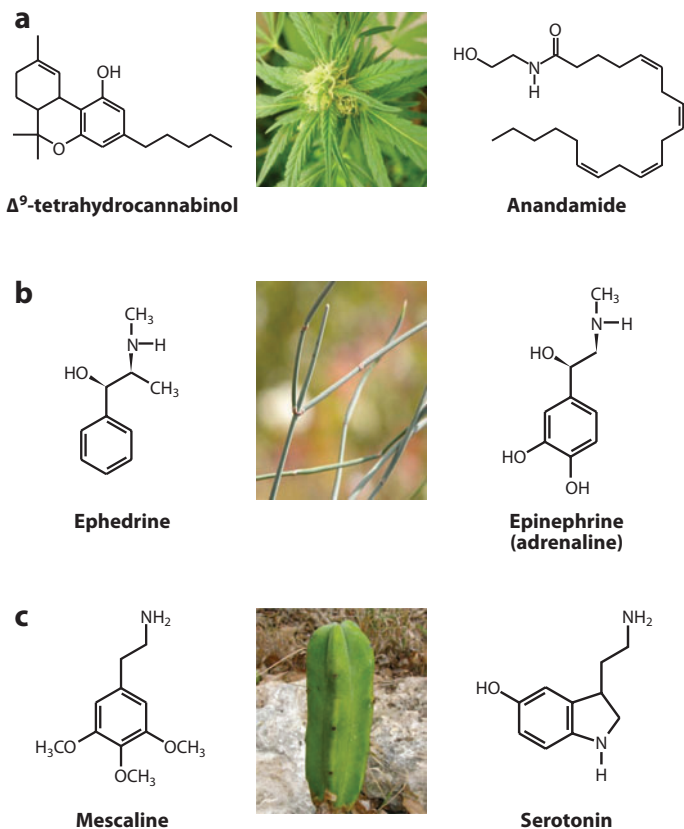
Figure 5

Interkingdom convergent evolution. Many plant specialized metabolites cause pharmacological consequences for animals that consume them. These effects occur through binding to mammalian neuroreceptors. (*a*) Δ^9-Tetrahydrocannabinol (THC), the major neuro-active principle of marijuana (*Cannabis sativus*), binds to the CB1 and CB2 receptors in the brain. Interestingly, the endogenous ligand of these receptors is anandamide (*N*-arachidonoylethanolamine), a molecule whose structure does not appear to be similar to THC (43). (*b*) Ephedrine, present in *Ephedra sinica*, a plant traditionally used in Chinese medicine, binds to the adrogenic receptors, mimicking the natural mammalian ligand adrenaline (61). (*c*) Mescaline, a hallucinogenic phenylpropyl amine alkaloid, is present in some Cactaceae species such as the San Pedro cactus [*Echinopsis* (*Trichocereus*) *pachanoi*] (62). Mescaline binds to and activates the serotonin 5-HT$_{2A}$ receptor (44).

bind to the cannabinoid receptors in the brain and have similar effects on the regulation of feeding behavior and the neural generation of motivation and pleasure.

Phenylpropanolamine alkaloids (ephedrine alkaloids) are produced by several plant species and affect the sympathetic nervous system of animals by a variety of mechanisms that include direct agonist activity at adrenergic receptors (i.e., they compete with epinephrine) and indirect effects via carrier-mediated exchange with norepinephrine (61). For example, the plant *Ephedra sinica* contains varying levels of ephedrine alkaloids, the main compound being ephedrine and its diastereoisomer pseudoephedrine (38) (**Figure 5**). Another plant alkaloid, mescaline (3,4,5-trimethoxyphenethylamine) (**Figure 5**), has a powerful psychedelic effect on humans. Mescaline occurs naturally in various cacti such as peyote (*Lophophora williamsii*) and the San Pedro cactus [*Echinopsis* (*Trichocereus*) *pachanoi*] (62). Mescaline binds to and activates the serotonin 5-HT$_{2A}$ receptor (44).

Indeed, many plants contain neuroactive and endocrinally active compounds, such as dopamine and serotonin in banana, dopamine and norepinephrine in potato, epinephrine (adrenaline) in cacti (39), and melatonin in tomato (49). Examination of the enzymes involved in both animal and plant lineages is likely to find convergent evolution in most cases. The selective advantage for the plants in being able to make these compounds is still not clearly elucidated.

CONCLUSIONS

The study of convergent evolution in plant specialized metabolism is hindered by an incomplete database of the metabolite repertoire of each plant species and by our very rudimentary knowledge of how plants synthesize such compounds and of which genes and enzymes are involved in the synthesis of a given compound in different plant lineages. Nonetheless, as our knowledge in both of these areas expands, more examples of convergent evolution are uncovered. Typically, the evidence that first comes to light is that nonorthologous genes/enzymes are involved in the synthesis of the same compounds in different lineages. This evidence constitutes direct, positive proof that convergent evolution has occurred. Discontinuity in the distribution of a given compound [e.g., the presence of glucosinolates in the derived genus *Drypetes* in the Euphorbiaceae in addition to their presence in 15 other families (60)] is suggestive of convergent evolution but cannot be used as a definitive proof that convergent evolution has occurred, because of the uncertainty in the underlying reasons for the inability to detect the compound in some lineages. Furthermore, until gene and protein sequences are compared, alternative hypotheses for the presence of the same compound in distally related lineages, such as multiple gene losses or lateral gene transfer, cannot be ruled out.

Convergent evolution in plant specialized metabolism is just one facet of the process by which plants rapidly and constantly evolve the ability to synthesize a set of compounds to productively interact with their environment. Given a finite genome size, and the fact that selection constantly favors the ability to synthesize new specialized metabolites as a plant's biotic and abiotic environment changes, and that compounds that once conferred an adoptive advantage to the plant eventually may no longer do so, genomes will evolve new functions without always maintaining the old ones. This leads to the continuing diversification of plant specialized metabolism; inevitably, though, some chemical solutions that arise anew in one plant lineage will be not novel but rather identical to solutions having already arisen one or more times in other plant lineages.

SUMMARY POINTS

1. Different specialized metabolites can have identical ecological function.

2. Many examples exist whereby different plant lineages evolved the ability to make the same specialized metabolite.

3. In many cases, a compound is made in two different lineages from the same substrate but by a nonorthologous protein, although the enzymes still share some family resemblance.

4. Examples of two completely unrelated enzymes making the same product are also found.

5. Plants have evolved the ability to make compounds found in animals, a form of mimicry that serves the plants in attracting useful animals and deterring harmful ones.

6. Humans have taken advantage of these forms of mimicry in developing traditional and modern medicines.

DISCLOSURE STATEMENT

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83. Lignin should be considered a primary metabolite, but variations in lignin are lineage-specific, and Weng et al. show that some of the same variation occurred independently more than once.

Contents