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REVIEWS

Biogenic Volatile Isoprenoid Emission and Levels of Natural Selection

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Abstract | Biogenic volatile isoprenoid emission as a biological process has many worthwhile yet unanswered questions of fundamental scientific and ecological merit. Foremost among them is to understand and quantify the long-term feedback effects of volatile emission on climate and climate-driven macro-evolutionary changes. Moreover, we are now at a stage where our understanding of biogenic isoprenoid emission at the molecular and ecophysiological levels holds the key to the doors of next generation breakthroughs in isoprenoid-dependent applications in synthetic chemistry, human bio-therapeutics and agro-food industries. Like any other living trait/process, biogenic volatile isoprenoid emission has several levels of complex organization. We summarise biophysical, chemical and ecological functions of biogenic volatile isoprenoid emission highlighting aspects of evolution at different levels of natural selection.

Keywords: *ecological fitness, evolution, isoprene, isoprenoid biosynthesis, natural selection, photosynthesis, volatile organic compounds*

1 Introduction

Constitutive volatile isoprenoid emission by phototrophic living organisms is a process whose biological costs are not trivial while evidence of the (possibly multiple) benefits are still circumstantial, or purely elusive. More than 1000 Tg carbon per year is emitted in the form of volatile isoprenoids, mainly isoprene (C_5H_8) and monoterpenes ($C_{10}H_{16}$), mainly by terrestrial plants[1] and, as far as we currently know in much lower amount (~10 TgC/yr) by marine phytoplankton.[2,3] To put this in perspective, this is comparable to the carbon loss caused by global deforestation[4] (~1200 TgC/yr). The emitted isoprenoids have a prolonged post-emission impact on the climate, especially through oxidation chemistry of ozone in the troposphere, and formation of secondary organic aerosol and precipitation.[5–7]

Isoprenoids (also called terpenoids) are a large class of versatile macromolecules with great structural diversity despite being all constructed by catenation of five carbon (C5) monomers that are derivatives of isopentenyl pyrophosphate (IPP) and dimethyallyl pyrophosphate (DMAPP). Volatile isoprenoids are small terpenoids whose conjugated double bounds (dienes) readily react with any atom/molecule with unpaired valence electrons. These are made by one (isoprene), two (monoterpenes) or three (sesquiterpenes) C5 units. Drawing from recent research developments, in this review we examine the phenomenon of biogenic volatile isoprenoid emission from an evolutionary standpoint, at different levels of organization spanning a single living prokaryotic cell to populations of forest trees (Fig. 1, Box 1).

2 A Flexible Structure to Function Relation Among Isoprenoids has Accommodated Long Periods of Neutral Drift in Molecular Evolution

The diversity in isoprenoid emission capacity is a product of interactions between many genes, enzymes and metabolites both within and across interacting pathways. The enzymes involved in isoprenoid biosynthesis belong to a family of closely related terpene synthases (TPSs).[8] Since there is no significant homology between plant TPS sequences and the known bacterial genomic equivalents, it is inferred that bacterial and plant TPSs do not share a common ancestry[9] although

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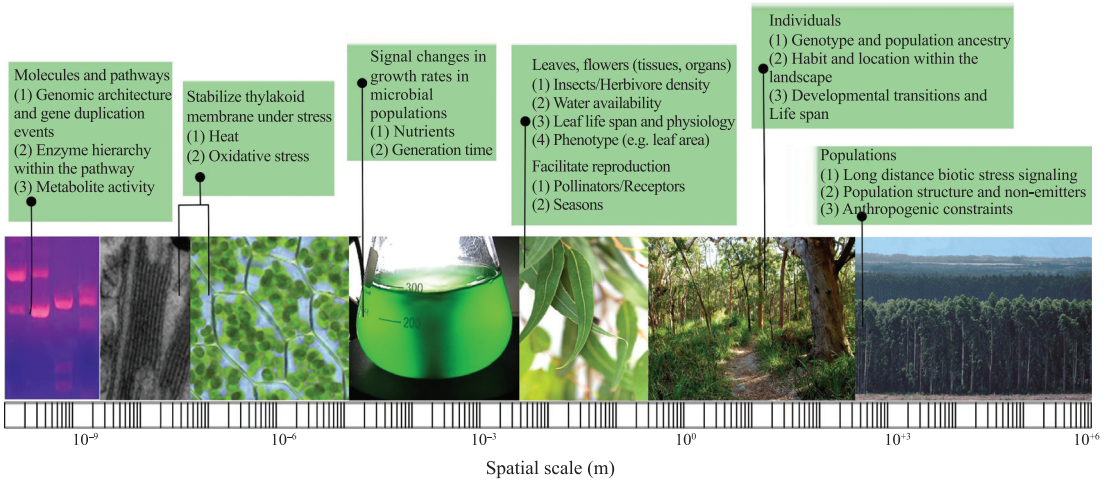

Figure 1: Function of volatile isoprenoids (isoprene and monoterpenes) and means of natural selection.

Box 1 Levels of Natural Selection and the Isoprenoid Emission Trait

Underneath any biological phenomenon, there lies a hierarchy of organization and levels of natural selection that shape and reshape its evolution at different levels from molecules to tissues and from organisms to populations/ecosystems. The debates about the actual unit of natural selection, from the unit being a single gene to a group of organisms, have continued to generate fascinating evolutionary enquiries and polarised disagreements.[96–98] If there is one thing that is becoming clear then it is the modular nature of the unit of selection. By modularity we mean either a hierarchical or egalitarian network of interacting elements.[99,100] A module could be a group of genes (elements) co-expressed/co-regulated by similar environmental stimuli. A module could also be a higher order organization that defines tissues and/or organisms. As life organizes itself into intricate interactions, it is of great benefit to strip any trait/behaviour/process of interest into its fundamental components and dissect discrete facets of natural selection at each level of modular organization.

Level 1: Genes (and enzymes) are seen as the site of natural selection in continuous action since genes are the simplest self-replicating and perpetual entities unlike their carrier organisms which undergo life and death[101,102] (see section 2, Tables 1 and 2).

Level 2: The cell provides a physical framework that facilitates self-correction and selective regulation of a large self-replicating and error-prone chemical regulatory system. Natural selection finds its way through a complex set of cell organelles that share space and resources. The cell membrane concentrates the medium of life within a cell and allows biochemical reactions that are 10 times faster than those in cell-free systems[103] (see sections 3 and 4).

Level 3: Phenotypic characters such as phyllotaxis, leaf shape and area, canopy architecture, etc., are all products of life history evolution and 'canalization', a process in which phenotypes are locked by genotypes and become potentially insensitive to environmental stimuli.[104] Deviation from such set norms constitutes phenotypic plasticity, which is a raw material for natural selection (with or without heritability) especially in long-lived plants[105,106] (see section 5).

Level 4: When phenotypic variation within a population, with regard to a specific trait or a group of traits, is heritable and its occurrence follows the spatial genetic structure of the population, then such traits of one individual will have differential fitness effects on neighbouring individuals[107] (see section 6).

some enzymes are proposed to be monophyletic at least within major plant clades.[8,10] Deduced amino acid sequences of large TPS gene families in some gymnosperms and comparative analyses of angiosperm TPSs suggest that modern TPSs could have evolved from an ancestral diterpene synthase in a eukaryotic ancestor of higher plants, much before specialization of TPS functions and divergence of angiosperms and gymnosperms could take place.[11,12]

Divergence: acquisition of dissimilar characters or traits by related organisms.

Despite an apparently simple chemical structure, a very large number of volatile isoprenoids are present in nature. The idea of "molecular parsimony" suggests that large populations of chemical metabolites (e.g. >60000 terpenoids) with minor differences are synthesized by a relatively small group of enzymes (per organism) because (a) the probability of hitting a chemical conformation of significant potency is always small,[13] (b) the cost of gene transcription and RNA translation could influence organismal fitness,[14] and (c) promiscuity is the norm in enzyme evolution irrespective of enzyme–substrate specificity.[15] TPSs are known to be promiscuous in that they can act on different versions of related substrates and thus may have been the main contributors to the vast diversity of isoprenoids.[16] Moreover, significant single nucleotide polymorphisms (SNPs) in genes encoding for TPSs are associated with the qualitative and quantitative variation in isoprenoid emission profiles.[17]

Minor changes in chemical structures involved in physical/structural function (e.g. membrane lipids, accessory pigments) do not necessarily compromise their functions, and thus are rarely constrained by stringent natural selection.[18] As a result, the same metabolite (e.g. ascorbate[19]), with or without minor changes, could acquire novel biochemical functions under different circumstances across divergent clades. The idea of 'superior biomolecular activity'[20] is proposed to explain structural conservatism and functional divergence in chemical molecules. Often, natural selection promotes 'a chemical blend' (of various stored aromatics and monoterpenes) rather than a specific structural configuration of a single volatile isoprenoid and such chemical mixtures are more potent than a single molecule in terms of 'biomolecular activity'.[21] In this way certain monoterpene blends increase beneficial biological interactions and in some cases are stringently selected to suit interactants in co-evolved biological systems involving flower–pollinator, host–pathogen and plant–herbivore interactions[22] (also see section 5).

3 Modes, Mechanisms and Energy Budget of Carbon Reduction Could be the Major Factors Determining Emission Potential of Isoprenoids

Independent prokaryotic origins of plant cell organelles have resulted in multiple biosynthetic pathways with duplicated functions (*sensu lato*) taking place simultaneously within different organelles.[23,24] Plant isoprenoid biosynthesis occurs through one of the two spatially separated pathways within a plant cell (Fig. 2). The cyanobacterial pathway takes place in the plastid, and is also referred to as the methyl erythritol phosphate (MEP) pathway; while the archaeal pathway, operating in the cytoplasm, is also referred to as the MVA (mevalonic acid) pathway.[25–27] The MVA pathway proceeds further into multiple terminal pathways including steroid and hormone biosynthesis. The MEP pathway proceeds further to synthesise stable and structural terpenoids such as carotenoids.

Several models have been proposed to explain the evolutionary events that caused the divergence of archaea and bacteria; however, there is no consensus.[28] Sequence similarity and pathway reconstruction analyses show that at least three separate horizontal 'whole pathway' transfer events between bacteria and archaea could have taken place[29] but the nature of either the original eukaryotic ancestor or the origin of isoprenoid-dependent membrane architecture remains unexplained.[30] Both MEP and MVA pathways are almost mutually exclusive among prokaryotes with the exception of *Streptomyces* species, which possess both pathways and it is hypothesized that maintaining both pathways could be beneficial for the bacterium since it allows selective use of resources and specialized functions.[31,32] It is not clear which pathway appeared first.

Mutual exclusivity of the two pathways also holds among some eukaryotes such as fungi and animals that possess only the MVA pathway.[33] But, the simultaneous occurrence of the two pathways in plants, despite organellar (spatial) separation, creates a complex scenario for natural selection to act on the functionality of this system.[24] In fact, (a) all the genomic controls on both the pathways are in the nucleus (the MEP pathway enzymes are nuclear encoded and plastid targeted), and (b) the substrate level cross talk between the two pathways takes place through a selective chloroplast membrane interface through selective transport of IPP from plastid to cytosol.[34] The transport of glyceraldehyde-3-phosphate (GAP) from plastid to cytosol, and of phospho-enol-pyruvate (PEP) from cytosol to plastid, suggests 'an umbilical link' between the two pathways, as first postulated[35] and later confirmed through labelling experiments.[36] It was demonstrated that the genetic blocking of either the MVA pathway or the MEP pathway in null mutants, or the complete inhibition of single pathway enzymes in wild-type plants treated with specific inhibitors resulted in a developmental block and a seedling-lethal phenotype. This indicates that the loss of one of the two pathways cannot be compensated by the remaining pathway.[37]

Parsimony: the minimum number of evolutionary changes to infer phylogenetic relationships between closely related taxa. Parsimony also implies that the simplest hypothesis (among many alternatives) that is sufficient to explain an observation is to be preferred and is most likely to be closest to the reality.

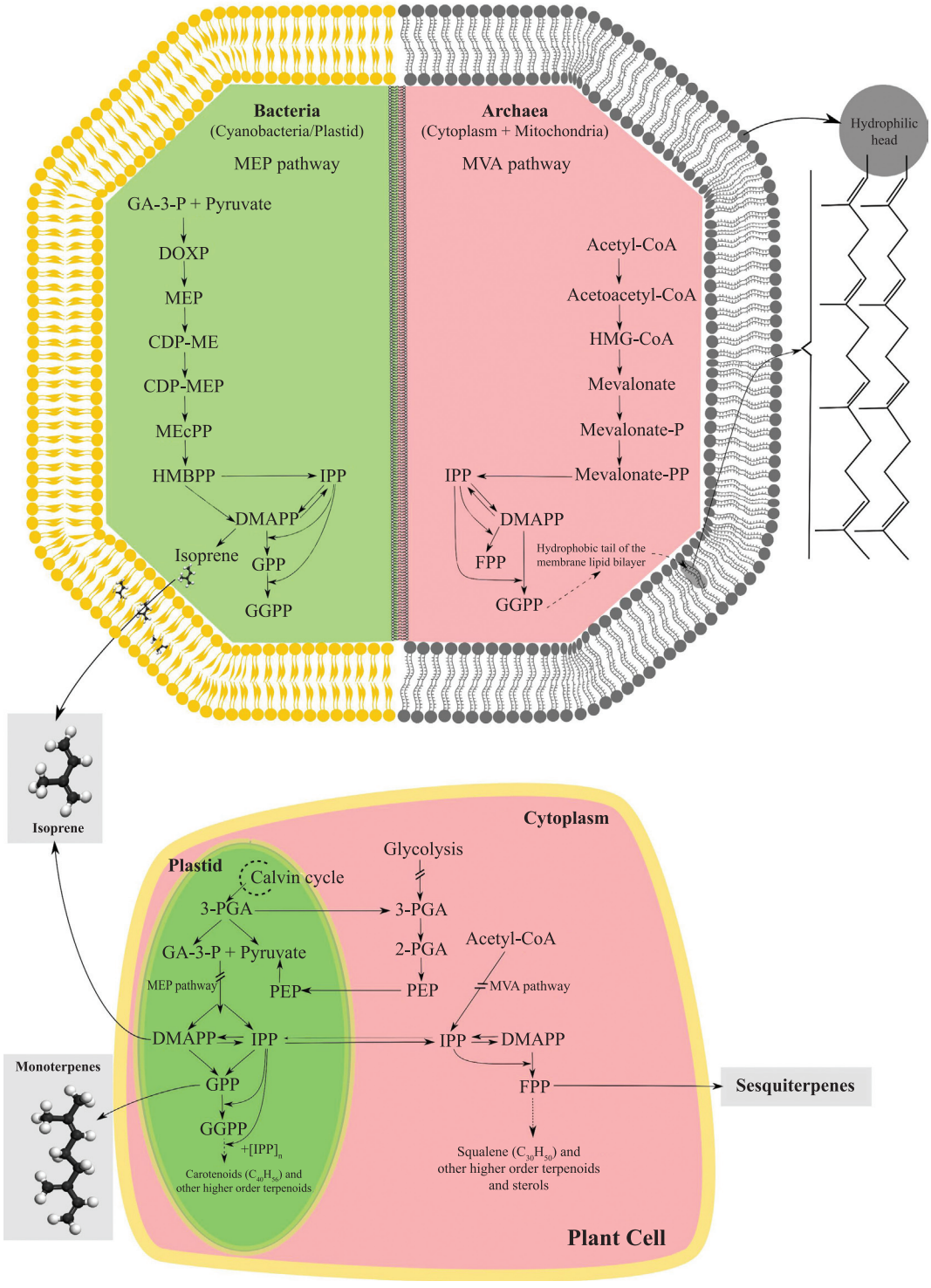

Figure 2: Isoprenoid biosynthetic pathways in bacteria and archaea (top) and in plants (bottom).

Although the MEP pathway contributes to volatile isoprenoid emission in eubacteria as well as in higher plants, surprisingly the MVA pathway outperforms the MEP pathway in *E. coli* engineered for large scale production of isoprenoids.[38] Metabolic flux through the MEP pathway has several bottlenecks and involves allosteric feedback mechanisms.[39,40] The MEP pathway gene expression is regulated by heat, light and circadian transcriptional factors,[41,42] which appear to follow hierarchical and modular organization.[43] The difficulty of working with the MEP pathway is

due to our lack of understanding of the pathway's evolutionary history. Knowing the two pathways and the selection pressures acting on them (e.g. with reference to gene specific subtleties in codon usage, and oxidative stress sensitivity) will provide important leads in solving problems, especially for large scale microbial production of industrially relevant isoprenoids.

The reasons for differences in productivity and efficiency of these pathways in heterologous expression systems potentially lie in distinct and ancient evolutionary histories of bacteria and archaea, and their ways of acquiring and reducing carbon. While photosynthesis by photoautotrophs (starting with cyanobacteria) is the most influential biological phenomenon in the history of the Earth, it is neither the most ancient nor the most efficient way through which carbon could be reduced for storage and transport. Alternative autotrophic carbon reduction pathways had evolved much before photosynthesis among early prokaryotes, mostly extremophiles.[43] Among those, the chemoautotrophic reduction of carbon to acetyl-CoA is a prominent process that supplies carbon to the MVA pathway in archaea and some bacteria. Comparison between energy demands of alternative autotrophic carbon reduction pathways among extant prokaryotes clearly shows large differences in costs per fixed carbon.[43,44] The Rubisco-based aerobic CO_2 fixing system is the most expensive among all known autotrophic CO_2-fixing mechanisms. Correspondingly, photoautotrophs have a large energy and reducing power capacity that not only sustains expensive high turn-over of enzymes and their maintenance but also supports constitutive volatile isoprenoid emission via the MEP pathway which also involves several steps of chemical reduction.[45] It is known

Box 2 Codon Bias and Amino Acid Usage: Contrasting MEP and MVA Pathways in Angiosperms

Table 1: Comparing amino acid frequency in enzymes of two spatially separated isoprenoid biosynthetic pathways in plants.

Amino acid	Frequency of amino acid per 1000 residues/enzyme‡ MEP pathway (*N* = 7 enzymes)* mean ± 1 SE	Frequency of amino acid per 1000 residues/enzyme‡ MVA pathway (*N* = 6 enzymes)** mean ± 1 SE	*P* value ($\alpha = 0.05$) *t* test
Ala	76 ± 6.9	92 ± 8.8	0.165
Cys	**15 ± 0.6**	**21 ± 0.7**	**0.000**
Asp	59 ± 2.0	46 ± 6.1	0.094
Glu	60 ± 5.4	57 ± 3.4	0.687
Phe	39 ± 4.2	35 ± 2.9	0.514
Gly	72 ± 4.7	77 ± 8.1	0.601
His	25 ± 4.5	20 ± 2.2	0.377
Ile	60 ± 2.5	55 ± 2.8	0.197
Lys	65 ± 3.7	58 ± 3.8	0.195
Leu	95 ± 6.2	100 ± 9.7	0.677
Met	20 ± 2.9	26 ± 2.3	0.159
Asn	35 ± 3.7	42 ± 2.0	0.143
Pro	**55 ± 5.0**	**43 ± 2.9**	**0.059**
Gln	31 ± 3.1	33 ± 2.0	0.691
Arg	46 ± 4.0	40 ± 3.1	0.296
Ser	85 ± 11.0	88 ± 3.6	0.821
Thr	51 ± 2.2	50 ± 3.0	0.811
Val	73 ± 4.2	74 ± 4.8	0.912
Trp	9 ± 1.9	11 ± 2.7	0.533
Tyr	26 ± 2.7	26 ± 6.4	0.949
TOTAL†	998	995	

**DXS, DXR, MCT, CMK, MDS, HDS, and HDR; **ACCT, HMGS, HMGR, MVK, PMVK, and PMVDC*; †Total excludes stop codons (For enzyme names, see Table 2); ‡the enzymes responsible for reactions up to the formation of IPP/DMAPP.

Box 2 Continued

Codon bias: the propensity to use a particular triplet codon to specify a particular amino acid.

Codon bias towards optimal/common codons positively correlates with gene expression levels. Abundance of tRNAs influences translational efficiency.[108–110] Codons coding for rare and structurally critical amino acids possess the least abundant tRNAs, viz. cysteine, tyrosine and tryptophan, which curiously also have the highest probability of mutating into stop codons given the way triplet codons have evolved. Mutations that replace less abundant and structurally important amino acids are always minimised and as a result premature terminal mutations are also minimised by natural selection. Codon usage frequency, calculated for each enzyme in both MVA and MEP pathway using a set of at least three representative sequences from angiosperms of which one sequence within each set was from *Arabidopsis thaliana*, showed no significant difference in overall codon frequency (relative to genome wide codon usage in *Arabidopsis*) between all the genes between and within both pathways with minor exceptions. However, the differences became significant when the pathways were divided into two sections with the top section resulting in IPP/DMAPP biosynthesis and the bottom section involving terpenoid synthases and prenyltransferases. The top section of the MVA pathway comprised significantly more cysteine than the MEP pathway (Table 1). Given adverse factors such as (a) limited availability of $tRNA^{Cys}$, which is among the least abundant tRNAs in plants[111] and (b) oxygen sensitivity of the thiol group that puts negative selection pressure on cysteines in cytosolic proteins,[112] cysteine richness of MVA pathway enzymes in plants must have ancient phylogenetic constraints. Cysteine richness in the MVA pathway enzymes is perhaps consistent with the pathway's archaeal evolutionary ancestry given that enzymes in thermophilic archaea were rich in disulfide bonds and were selected to remain stable under extreme temperatures.[113] It is also not a coincidence that energetically less expensive (relative to the Calvin cycle) chemoautotrophic CO_2 fixing mechanisms are restricted to anaerobic habitats (e.g. sulphur bacteria and methanogenic archaea; also see section 3), where cysteine richness in MVA pathway was not under negative selection.

Table 2: Genes and enzymes of the MEP and MVA isoprenoid biosynthetic pathways in plants.

Gene	Corresponding enzyme of the MEP pathway	Gene	Corresponding enzyme of the MVA pathway
Top section of the two pathways (reactions leading to the formation of IPP/DMAPP)			
DXS	1-deoxy-D-xylulose 5-phosphate synthase	*ACCT*	Acetyl-CoA *C*-acetyltransferase
DXR	1-deoxy-D-xylulose-5-phosphate reductoisomerase (aka: CM synthase)	*HMGS*	3-Hydroxy-3-methylglutaryl-CoA synthase
MCT	2-C-methyl-D-erythritol 4-phosphate cytidylyl transferase	*HMGR*	3-Hydroxy-3-methylglutaryl-CoA reductase
CMK	4-diphosphocytidyl-2-C-methylerythritol kinase	*MVK*	Mevalonic acid kinase
MDS	2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	*PMVK*	Phospho-MVA kinase
HDS	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase	*PMVDC*	Diphospho-MVA decarboxylase
HDR	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase		
Bottom section of the two pathways (reactions leading to the formation of isoprenoids)			
IPI	Isopentenyl diphosphate isomerase		
ISPS	Isoprene synthase	NA	NA
GPS	Geranyl pyrophosphate synthase		
MTS	Monoterpene synthase	NA	NA
GPPS	Geranyl geranyl pyrophosphate synthase		
PS	Phytoene synthase	NA	NA

that both isoprene and monoterpene biosynthesis through the MEP pathway principally utilises carbon fixed *de novo* during photosynthesis.[46–48] The carbon cost of volatile biosynthesis in plants is around 2% of photosynthesis under stress-free conditions, and increases under abiotic stress, often exceeding 10% of fixed carbon[49] (both *de novo* and imported from cytosol) with a corresponding increase in energy costs.[50–52]

Atmospheric CO_2 concentration is a key regulator of photosynthesis and it is also shown to influence volatile isoprenoid emission. Increasing CO_2 concentration increases photosynthesis and decreases isoprene emission, at least during short-term acclimation,[53–54] but also in plants exposed to life-long high CO_2.[55] Many have argued a case for CO_2-driven emission changes through geological history.[56] The high cost on a plant's carbon budget due to emission especially during low CO_2 eras (glaciations), in turn likely constraining photosynthesis, may have exerted a significant negative selection pressure on emission capacity.[10] The same logic does not apply to archaea and the MVA pathway, since none of the known autotrophic archaea employs the Calvin cycle to fix carbon. However, the fact that low CO_2 causes increased emission in plants at least over short-term acclimation contradicts the notion of negative selection on emission during glacial periods. The suggestion is confounded by the complex interactive effects of heat and CO_2 on emission, which do not fit any existing mechanistic theories of emission behaviour.[57] There is increasing experimental evidence in favour of a hypothesis proposed in the 1990s that availability of energy (ATPs) controls isoprene synthesis.[58] ATP and reducing power (NADPHs and equivalents) unused by carbon reduction may explain isoprenoid emission behaviour under varied CO_2 and drought scenarios.[52,59–61] As put forth earlier, these findings further support the idea that the mechanisms and energy budget of carbon reduction could have been the primary factors through which natural selection determined isoprenoid emission capacity and behaviour in prokaryotes and later in plants (also see Box 2).

4 Isoprene Emission is Sensitive to Abiotic Stress Operating at the Level of a Single Cell and Population of Cells

Isoprene emission has distinct and testable physical functions at the level of cells (prokaryotes and unicellular eukaryotes) and multicellular tissues. Hypotheses concerning isoprene-mediated scavenging of free radicals[62,63] along with possible membrane stabilization under transient heat stress in higher plants[64] have strong empirical evidence, and now also some mechanistic understanding.[65–67] In thermophilic archaea, isoprenoid-linked phospholipids form their cell membranes (Fig. 2), while in bacteria emitted isoprene could simply physically interact with bacterial membrane as proposed in eukaryotes. However, the function of spurts in isoprene emission at different growth phases among some bacteria is still unknown.[9] The function of isoprene emission among marine phytoplankton such as dinoflagellates and diatoms is also unclear.

Natural selection influences the inter- and intra-specific variation in any heritable characteristic, and the extent of genetic differentiation in certain loci in the genome may reveal the nature of selection.[68] As mentioned earlier, isoprenoid synthases are promiscuous enzymes with flexible substrate affinities and often evolve through gene duplication events. The newly acquired functions of a duplicated enzyme are normally not efficient and it is hypothesized that hyper-transcription could overcome enzymatic inefficiencies in secondary metabolism.[69] A gene duplication event could often be followed by selectively neutral mutations in the duplicate gene; thus neofunctionalization is rare and gene redundancy is common.[70] Therefore, the random emergence of isoprene synthase only in certain limited number of plant phylogenetic clades with altered specificity/efficiency remains unexplained. Two alternative theories have been proposed. One of them posits that the occurrence of isoprene emission capacity in unrelated land plant lineages may be explained by specific environmental conditions that increase fitness of emitting species.[71] In an experiment involving isoprene emitting and non-emitting (RNA*i* mutant) plants acclimated to glacial CO_2 levels (190 ppm), it was found that photosynthesis recovered faster in isoprene emitters than non-emitters after a heat or sun-fleck stress treatment[72] suggesting that low CO_2 periods during the Quarternary could have positively selected for isoprene emission capacity resulting in high emitting plants. However, in most cases, the evolutionary advantage to the isoprene emitting genera is unclear because the trait is often randomly lost. Even if we assume that isoprene emission confers additional fitness to the emitting genus,[73] the fact that isoprene synthases have remained very inefficient ($K_m > 2$ mM) compared to other enzymes of the MEP pathway suggests weak selection.[74] To explain random disappearance and reappearance of isoprene emission in certain limited number of plant lineages we should

Calvin cycle: also known as the photosynthetic carbon reduction cycle. A cyclic pathway used for the fixation of carbon dioxide by photoautotrophic organisms.

Mechanistic theory and modelling: when natural processes are mechanistically determined and the laws of physics and chemistry are sufficient to explain biological phenomena. Mechanistic modelling involves deduction of mathematical relationships between (often) biological variables and it emphasizes the physical, chemical and biochemical principles that explain the observed relationship between variables.

Natural selection: the non-random and differential reproduction of different genotypes acting to preserve favourable variants and to eliminate less favourable variants.

Fitness: the relative competitive ability of a given genotype conferred by adaptive morphological, physiological and behavioural characters. Fitness is often quantified as the average number of surviving progeny of one genotype relative to that of competing genotypes.

Quarternary: period of geological time that covers approximately the last 1.8 million years. The Quarternary is noted for numerous major glacial-interglacial cycles (advancement and receding of polar ice sheets) characterized by significant shifts in global temperature and atmospheric CO_2 concentration.

also account for the roles played by fast growing physiology and long life span in isoprene emitting trees in accumulation of pre-meiotic mutations and tolerance for such mutations in the corresponding loci of their genome.[75]

5 Monoterpenes and Other Aromatics are Under Stringent Selection in Co-Evolved Biological Systems

Plant behaviour, especially in annuals, does not evolve during the course of a plant's lifetime because plants do not have memory in the sense of experience storage within neuronal connections as in animals. Plants "rote-learn" through evolution by natural selection, a very slow process usually operating over many generations at the level of genes, genomes, epigenomes and possibly phenotypic plasticity (which may or may not be determined by genotypes). As a result, several traits may persist in plant populations long after they have lost adaptive relevance. While this might arguably be the case for isoprene emission in long-lived trees (but see[64]), other volatile isoprenoids have well-defined roles in plant defence. For example, induced volatile isoprenoids could be employed effectively to prime intraplant antiherbivory responses.[76–78] Many metabolites derived from the MVA pathway, primarily sesqui-, di-, and saponin tri-terpenoids, have potent antifungal, antimicrobial, and repellent properties or serve to attract predators or parasitoids.[79]

Rote-learning: learning or remembering by repetition rather than through developing an association between phenomena.

Less volatile monoterpenes can be stored under most conditions and their induced emission has an important role in maintaining organismal fitness. Constitutive emission of monoterpenes has been shown to defend plant parts that are at high risk of intense herbivore attack, preventing the loss of those parts that might result in substantial fitness costs.[21] The fact that foliar volatile storing is virtually absent in deciduous trees, which likely are vulnerable to insect attack, suggests a link between monoterpene emission and structural costs of plant parts (also see section 6).

Intraspecific volatile communication mostly involving aromatics and monoterpenes could increase population fitness by benefiting closely related individuals within a large population[80] (also see Box 1). There is evidence to suggest that interspecific signaling and recognition both in aquatic and terrestrial ecosystems involve volatile isoprenoids.[81,82] However, high-cost resource mediated communication strategies involving tertiary trophic interactions (e.g. bodyguard insects such as parasitoid wasps or ants) appear to be under more stringent selection than low-cost constitutive or induced information transmission between plant populations.[83] It remains to be seen whether untargeted, constitutive isoprenoid emission of a species is a function of ecosystem heterogeneity.[84]

Plant functional type (PFT): a collection of plant species (vegetation) with similar suits of co-occuring functional traits that exhibit similar responses to external stimuli and have similar effects on ecosystem function. The equivalent of a PFT in the animal kingdom is a '*guild*'.

6 Reconciling Phenotypic, Genetic Diversity and the Sensitivity of Emission Responses in Individuals or Populations of Trees to Environmental Stimuli

Stringent developmental constraints on isoprenoid emission levels (see review[85]) point towards natural selection acting on the whole plant phenotype. Leaf economics and leaf life span have a significant influence on isoprenoid emission profiles.[53,61] Physical defense strategies involve niche-specific phenotypic adaptations and phenotype constrains chemical defence. For example storing of volatiles is determined by packing efficiency of monoterpenoid storage glands in leaves, which in turn depends on specific leaf area, distribution and thickness of palisade parenchyma. In addition, natural selection appears not to have favoured a trade-off between chemical and physical defences in most plants,[86] which could ultimately mean that isoprenoid emission may not show any relationship with the broad trends in plant phenotypic strategies. On the whole, reconciling phenomic variability with genetic diversity and accounting for their cumulative impact on isoprenoid emission profile has not been possible despite significant progress on both fronts. The contradictions between field trials that ignore genetic variation and species-specific controlled experiments that aim to minimize environmental variation have made the challenge more complicated than it ought to be.[87]

The importance of knowing how natural selection is acting on emission behaviour of plant populations is exemplified by the challenges faced by models trying to forecast global isoprenoid emissions given the significant impact of emissions on regional climate and carbon cycles. Most isoprenoid emission models are parameterized based on leaf level isoprenoid emission measurements and there is a large uncertainty in emission variation within plant functional types (PFTs).[88] There are many local emission discrepancies that are hard to explain,[1] and generalizations are not helped by the fact that emission capacity does not follow consistent phylogenetic patterns.[72,75,89,90] Vegetation models assume presence or absence of a defined PFT, which ignores the significant impact of fine-scale genetic variations on ecosystem dynamics and their impact on spatial dimensions of emission

signature.[91] Ignoring intra-population (fine-scale) variations might work in case of homogeneous clonal plantation emitters, some of which are indeed the most dominant isoprene emitting angiosperms.[89,92] However, many monoterpene emitters in boreal forests (pines, firs, spruces and oaks) exhibit low domestication and live in large, open-pollinated, native populations. Genetic diversity, gene flow, population heterogeneity and structure (relationship between individuals belonging to a single species) is shown to affect VOC emission profiles[93] and such effects are likely magnified in species spread over large geographic areas.[94] Reassessment of recent literature on plant emission response to changing climate has led to the suggestion that carbon input in the form of isoprenoids into the climate system will increase in future.[58] Anthropic pressure to select suitable agricultural traits might also have contributed to diversify emission in cultivated plants, as found in cork oaks over their cultivation range.[94]

7 Going Forward

Simple chemical derivation (reduction), highly reactive hydrocarbon chemistry, a wide range of ecological benefits, and an unlimited scope for diverse structural configurations have contributed to repeated emergence and functional diversification of biogenic volatile isoprenoid emission in evolutionarily distant and unrelated living systems. It is helpful to remind ourselves that each step during the development of a living organism in some ways represents a cusp of one of the major transitions in the evolution of living complexity.[95] We are still aiming at discovering metabolic and biophysical aspects of isoprenoid emissions in eubacteria and protists (diatoms and dinoflagellates), and such information is likely needed to further decode the complexity of volatile emissions in higher plants. With every new finding about some aspect of metabolic regulation of isoprenoid emission, it is becoming clearer that emission from living organisms provides a template to investigate other complex biological phenomena with often unclear function and uncertain origins. At the other end of the spatial scale, the consequences of land-use (vegetation) changes and increased temperatures due to unprecedented anthropogenic interference will not only have an impact on air quality and human lifestyle in a rapidly urbanising world, but is also likely to change the isoprenoid emission profiles of emitting and non-emitting living systems.

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References

1. Guenther, A., Karl, T., Harley, P., Wiedinmyer, C., Palmer, P.I. & Geron, C. Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature. *Atmospheric Chemistry and Physics* **6**, 3181–3210 (2006).
2. Shaw, S.L., Chisholm, S.W. & Prinn, R.G. Isoprene production by *Prochlorococcus*, a marine cyanobacterium and other phytoplanktons. *Marine Chemistry* **80**, 227–245 (2003).
3. Yassaa, N., Peeken, I., Zöllner, E., Bluhm, K., Arnold, S., Spracklen, D., & Williams, J. Evidence for marine production of monoterpenes. *Environmental Chemistry* **5**, 391–401 (2008).
4. van der Werf, G.R., Morton, D.C., Defries, R.S., Olivier, J.G.J., Kasibhatla, P.S., Jackson, R.B., Collatz, G.J. & Randerson, J.T. CO_2 emissions from forest loss. *Nature Geoscience* **2**, 737–738 (2009).
5. Trainer, M., Williams, E.J., Parrish, D.D., Buhr, M.P., Allwine, E.J., Westberg, H.H., Fehsenfeld, F.C. & Liu, S.C. Models and observations of the impact of natural hydrocarbons on rural ozone. *Nature* **329**, 705–707 (1987).
6. Claeys, M., Graham, B., Vas, G., Wang, W., Vermeylen, R., Pashynska, V., Cafmeyer, J., Guyon, P., Andreae, M.O., Artaxo, P. *et al*. Formation of secondary organic aerosols through photo oxidation of isoprene. *Science* **303**, 1173–1176 (2004).
7. Kroll, J.H., Ng, N.L., Murphy, S.M., Flagan, R.C. & Seinfeld, J.H. Secondary organic aerosol formation from isoprene photooxidation under high-NO_x conditions. *Geophysical Research Letters* **32**, L18808 (2005).
8. Sharkey, T. D. Is it useful to ask why plants emit isoprene? *Plant, Cell & Environment* **36**, 517–520 (2013).
9. Sivy, T.L., Shirk, M.C. & Fall, R. Isoprene synthase activity parallels fluctuations of isoprene release during growth of *Bacillus subtilis*. *Biochemical and Biophysical Research Communications* **294**, 71–75 (2002).
10. Sharkey, T.D., Gray, D.W., Pell, H.K., Breneman, S.R. & Topper, L. Isoprene synthase genes form a monophyletic clade of acyclic terpene synthases in the TPS-B terpene synthase family. *Evolution* **67**, 1026–1040 (2012).
11. Tholl, D. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology* **9**, 297–304 (2006).
12. Chen, F., Tholl, D., Bohlmann, J. & Pichersky, E. The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *The Plant Journal* **66**, 212–229 (2011).
13. Jones, C.G., Firn, R.D. & Malcolm, S.B. On the evolution of plant secondary chemical diversity. *Philosophical Transactions of the Royal Society Series B: Biological Sciences* **333**, 273–280 (1991).
14. Stoebel, D.M., Dean, A.M. & Dykhuizen, D.E. The cost of expression of *Escherichia coli* lac operon proteins is in the process, not in the products. *Genetics* **178**, 1653–1660 (2008).

15. Khersonsky, O., & Tawfik, D.S. Enzyme promiscuity, a mechanistic and evolutionary perspective. *Annual Review of Biochemistry* **79**, 471–505 (2010).
16. Pichersky, E., Noel, J.P. & Dudareva, N. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science* **311**, 808–811 (2006).
17. Kulheim, C., Yeoh, S.H., Wallis, I.R., Laffan, S., Moran, G.F. & Foley, W.J. The molecular basis of quantitative variation in foliar secondary metabolites in *Eucalyptus globulus*. *New Phytologist* **190**, 1041–1053 (2011).
18. Firn, R.D. & Jones C.G. A Darwinian view of metabolism, Molecular properties determine fitness. *Journal of Experimental Botany* **60**, 719–726 (2009).
19. Gest, N., Gautier, H. and Stevens, R. Ascorbate as seen through plant evolution, the rise of a successful molecule? *Journal of Experimental Bot*any **64**, 33–53 (2013).
20. Firn, R.D & Jones C.G. The evolution of secondary metabolism—a unifying model. *Molecular Microbiology* **37**, 989–994 (2000).
21. Wittstock, U. & Gershenzon, J. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology* **5**, 300–307 (2002).
22. Schiestl, F.P. The evolution of floral scent and insect chemical communication. *Ecology Letters* **13**, 643–656 (2010).
23. Dyall, S.D., Brown, M.T. & Johnson, P.J. Ancient invasions, from endosymbionts to organelles. *Science* **304**, 253–257 (2004).
24. Hemmerlin, A., Harwood, J.L. & Bach, T.J. A *raison d'être* for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Progress in Lipid Research* **51**, 95–148 (2012).
25. Lichtenthaler, H.K., Rohmer, M. & Schwender, J. Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. *Physiologia Plantarum* **101**, 643–652 (1997).
26. Lange, B.M., Rujan. T., Martin, W. & Croteau, R. Isoprenoid biosynthesis, The evolution of two ancient and distinct pathways across genomes. *Proceedings of the National Academy of Sciences USA* **97**, 13172–13177 (2000).
27. Smit, A. & Mushegian, A. Biosynthesis of isoprenoids via mevalonate in Archaea: the lost pathway. *Genome Research* **10**, 1468–1484 (2000).
28. Matsumi, R., Atomi H., Driessen A.J.M. & van der Oost J. Isoprenoid biosynthesis in Archaea: Biochemical and evolutionary implications. *Research in Microbiology* **162**, 39–52 (2011).
29. Lombard, J. & Moreira, D. Origins and early evolution of the mevalonate pathway of isoprenoid biosynthesis in the three domains of life. *Molecular Biology and Evolution* **28**, 87–99 (2011).
30. Lombard, J., Lopez-Garcia, P. & Moreira, D. The early evolution of lipid membrances and the three domains of life. *Nature Reviews Microbiology* **10**, 507–515 (2012).
31. Gophna, U., Ron, E.Z. & Graur, D. Bacterial type III secretion systems are ancient and evolved by multiple horizontal-transfer events. *Gene* **312**, 151–163 (2003).
32. Gophna, U., Thompson, J.R., Boucher, Y. & Doolittle, W.F. Complex histories of genes encoding 3-hydroxy-3-methylglutaryl-CoenzymeA reductase. *Molecular Biology and Evolution* **23**, 168–178 (2006).
33. Kramer, R. & Abraham, W-R. Volatile sesquiterpenes from fungi, what are they good for? *Phytochemical Reviews* **11**, 15–37 (2012).
34. Lichtenthaler, H.K. Biosynthesis, accumulation and emission of carotenoids, tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. *Photosynthesis Research* **92**, 163–179 (2007).
35. Laule, O., Fürholz, A., Chang, H.S., Zhu, T., Wang, X., Heifetz, P.B., Gruissem, W. & Lange, M. Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences USA* **100**, 6866–6871 (2003).
36. Loreto, F., Pinelli, P., Brancaleoni, E. & Ciccioli, P. ^{13}C labeling reveals chloroplastic and extrachloroplastic pools of dimethylallyl pyrophosphate and their contribution to isoprene formation. *Plant Physiology* **135**, 1903–1907 (2004).
37. Pulido, P., Perello, C. & Rodriguez-Concepcion, M. New insights into plant isoprenoid metabolism. *Molecular Plant* **5**, 964–967 (2012).
38. Vickers, C.E., Bongers, M., Liu, Q., Delatte, T. & Bouwmeester, H. Metabolic engineering of volatile isoprenoids in plants and microbes. *Plant, Cell & Environment* 37, 1753–1775 (2014).
39. Li, Z. & Sharkey, T.D. Metabolic profiling of the methyl-erythritol phosphate pathway reveals the source of post-illumination isoprene burst from leaves. *Plant, Cell & Environment* **36**, 429–437 (2013).
40. Banerjee, A., Wu, Y., Banerjee, R., Li, Y., Yan, H. & Sharkey, T.D. Feedback inhibition of deoxy-D-xylulose-5-phosphate synthase regulates the methyl erythritol 4-phosphate pathway. *Journal of Biological Chemistry* **288**, 16926–16936 (2013).
41. Cordoba, E., Salmi, M. & León, P. Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. *Journal of Experimental Botany* **60**, 2933–2943 (2009).
42. Wiberley, A.E., Donohue, A.R., Westphal, M.M. & Sharkey, T.D. Regulation of isoprene emission from poplar leaves throughout a day. *Plant, Cell & Environment* **32**, 939–947 (2009).
43. Berg, I.A., Kockelkorn, D., Ramos-Vera W.H., Say, R.F., Zarzycki, J., Hugler, M., Alber, B.E. & Fuchs, G. Autotrophic carbon fixation in archaea. *Nature Reviews Microbiology* **8**, 447–460 (2010).
44. Berg, I.A. Ecological aspects of the distribution of different autotrophic CO_2 fixation pathways. *Applied Environmental Microbiology* **77**, 1925–1936 (2011).

45. Sharkey, T.D., Wiberley, A.E. & Donohue, A.R. Isoprene emission from plants: why and how. *Annals of Botany* **101**, 5–18 (2008).
46. Delwiche, C.F. & Sharkey, T.D. Rapid appearance of ^{13}C in biogenic isoprene when $^{13}CO_2$ is fed to intact leaves. *Plant, Cell & Environment* **16**, 587–591 (1993).
47. Loreto, F., Ciccioli, P., Brancaleoni, E., Cecinato, A., Frattoni, M. & Sharkey, T.D. Different sources of reduced carbon contribute to form three classes of terpenoid emitted by *Quercus ilex* L. leaves. *Proceedings of the National Academy of Sciences USA*. **93**, 9966–9969 (1996).
48. Schnitzler, J-P, Graus, M., Kreuzwieser, J., Heizmann, U., Rennenberg, H., Wisthaler, A. & Hansel, A. Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. *Plant Physiology* **135**, 152–160 (2004).
49. Brilli, F., Barta, C., Fortunati, A., Lerdau, M., Loreto, F. & Centritto, M. Response of isoprene emission and carbon metabolism to drought in white poplar (*Populus alba*) saplings. *New Phytologist* **175**, 244–254 (2007).
50. Sharkey, T.D. & Loreto, F. Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia* **95**, 328–333 (1993).
51. Loreto, F. & Schnitzler, J-P. Abiotic stresses and induced BVOCs. *Trends in Plant Science* **15**, 1360–1385 (2010).
52. Dani, K.G.S., Jamie, I.M., Prentice, I.C. & Atwell, B.J. Increased ratio of electron transport to net assimilation rate supports elevated isoprenoid emission rate in eucalypts under drought. *Plant Physiology* **166**, 1–14. (2014).
53. Loreto, F. & Sharkey, T.D. A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. *Planta* **182**, 523–531 (1990).
54. Rosenstiel, T.N., Potosnak, M.J., Griffin, K.L., Fall, R. & Monson, R.K. Increased CO_2 uncouples growth from isoprene emission in an agroforest ecosystem. *Nature* **421**, 256–259 (2003).
55. Scholefield, P.A., Doick, K.J., Herbert, B.M.J., Hewitt, C.S., Schnitzler, J.P., Pinelli, P. & Loreto, F. Impact of rising CO_2 on emissions of volatile organic compounds: isoprene emission from *Phragmites australis* growing at elevated CO_2 in a natural carbon dioxide spring. *Plant, Cell & Environment* **27**, 393–401 (2004).
56. Possell, M. & Hewitt, C.N. Isoprene emissions from plants are mediated by atmospheric CO_2 concentrations. *Global Change Biology* **17**, 1595–1610 (2011).
57. Sharkey, T.D. & Monson, R.K. The future of isoprene emission from leaves, canopies and landscapes. *Plant, Cell & Environment* **37**, 1727–1740 (2014).
58. Loreto, F. & Sharkey, T.D. On the relationship between isoprene emission and photosynthetic metabolites under different environmental conditions. *Planta* **189**, 420–424 (1993).
59. Niinemets, U., Tenhunen, J.D., Harley, P.C. & Steinbrecher, R. A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. *Plant, Cell & Environ*ment **22**, 1319–1335 (1999).
60. Harrison, S.P., Morfopoulos, C., Dani, K.G.S., Prentice, I.C., Arneth, A., Atwell, B.J., Barkeley, M., Leishman, M.R., Loreto, F., Medlyn, B.E. *et al*. Volatile isoprenoid emissions from plastid to planet. *New Phytologist* **197**, 49–57 (2013).
61. Morfopoulos, C., Sperlich, D., Peñuelas, J., Filella, I., Llusià, J., Medlyn, B.E., Niinemets, U., Possell, M., Sun, Z. & Prentice, I.C. A model of plant isoprene emission based on available reducing power captures responses to atmospheric CO_2. *New Phytologist* **203**, 125–139 (2014).
62. Loreto, F. & Velikova, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* **127**, 1781–1787 (2001).
63. Vickers, C.E., Gershenzon, J., Lerdau, M.T. & Loreto, F. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biol*ogy **5**, 283–291 (2009).
64. Singsaas, E.L., Lerdau, M., Winter, K. & Sharkey, T.D. Isoprene increases thermotolerance of isoprene emitting species. *Plant Physiology* **115**, 1413–1420 (1997).
65. Velikova, V., Várkonyi, Z., Szabó, M., Maslenkova, L., Nogues, I., Kovacs, L., Peeva, V., Busheva, M., Garab, G., Sharkey, T.D. & Loreto F. Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. *Plant Physiology* **157**, 905–916 (2011).
66. Velikova, V., Sharkey, T.D. & Loreto, F. Stabilization of thylakoid membranes in isoprene-emitting plants reduces formation of reactive oxygen species. *Plant Signaling & Behavior* **7**, 1–3 (2012).
67. Pollastri, S., Tsonev, T., & Loreto, F. Isoprene improves photochemical efficiency and enhances heat dissipation in plants at physiological temperatures. *Journal of Experimental Botany* **65**, 1565–1570 (2014).
68. Neilsen, R. Molecular signatures of natural selection. *Annual Review of Genetics* **39**, 197–218 (2005).
69. Pichersky, E. & Gang, D.R. Genetics and biochemistry of secondary metabolites in plants, an evolutionary perspective. *Trends in Plant Science* **5**, 439–445 (2000).
70. Hughes, A.L. The evolution of functionally novel proteins after gene duplication. *Proceedings of the Royal Society Series B: Biological Sciences* **256**, 119–124 (1994).
71. Monson, R.K., Jones, R.T., Rosenstiel T.D. & Schnitzler, J-P. Why only some plants emit isoprene? *Plant, Cell & Environment* **36**, 503–516 (2013).
72. Way, D.A., Schnitzler, J-P., Monson, R.K. & Jackson, R.B. Enhanced isoprene-related tolerance of heat- and light-stressed photosynthesis at low, but not high, CO_2 concentrations. *Oecologia* **166**, 273–282 (2011).
73. Lerdau, M. A positive feedback with negative consequences. *Science* **316**, 212–213 (2007).

74. Cornish-Bowden, A. The effect of natural selection on enzymic catalysis. *Journal of Molecular Biology* **101**, 1–9. (1976).
75. Dani, K.G.S., Jamie, I.M., Prentice, I.C. & Atwell, B.J. Evolution of isoprene emission capacity in plants. *Trends in Plant Science* **19**, 439–446 (2014).
76. Frost, C.J., Appel, H.M., Carlson, J.E., De Moraes C.M., Mescher, C.M. & Schultz, J.C. Within-plant signaling via volatiles overcomes vascular constraints on systemic signaling and primes responses against herbivores. *Ecology Letters* **10**, 490–498 (2007).
77. Brilli, F., Ciccioli, P., Frattoni, M., Prestininzi, M., Spanedda, A. & Loreto, F. Constitutive and herbivore-induced monoterpenes emitted by *Populus* × *euroamericana* leaves are key volatiles that orient *Chrysomela populi* beetles. *Plant, Cell & Environment* **32**, 542–552 (2009).
78. Fineschi, S. & Loreto, F. Leaf volatile isoprenoids, an important defensive armament in forest tree species. *iForest* **5**, 13–17 (2012).
79. Vranová, E., Coman, D. & Gruissem, W. Structure and dynamics of the isoprenoid pathway network. *Molecular Plant* **5**, 318–333 (2012).
80. Heil, M. & Karban, R. Explaining evolution of plant communication by airborne signals. *Trends in Plant Science* **25**, 137–144 (2010).
81. Runyons, J.B., Mescher, M.C. & De Moraes, M. Volatile chemical cues guide host location and host selection by parasitic plants. *Science* **313**, 1964–1967 (2006).
82. Rasher, D.B., Stout, E.P., Engel, S., Kubanek, J. & Hay, M.E. Macroalgal terpenes function as allelopathic agents against reef corals. *Proceeding of the National Academy of Sciences USA* **108**, 17726–17731 (2011).
83. Kessler, A. & Heil, M. The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology* **25**, 348–357 (2011).
84. Kigathi, R.N., Weisser, W.W., Veit, D., Gershenzon, J. & Unsicker, S.B. Plants suppress their emissions of volatiles when growing with conspecifics. *Journal of Chemical Biology* **39**, 537–545 (2013).
85. Niinemets, U., Arneth, A., Kuhn, U., Monson, R.K., Penuelas, J. & Staudt, M. The emission factor of volatile isoprenoids, stress, acclimation, and developmental responses. *Biogeosciences Discussions* **7**, 1529–1574 (2010).
86. Moles, A., Peco, B., Wallis, I.R., Foley, W.J., Poore, A.G., Seabloom, E.W., Vesk, P.A., Bisiqato, A.J., Cella-Pizzarro, L. Clark, C.J *et al*. Correlations between physical and chemical defences in plants, tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytologist* **198**, 252–263 (2013).
87. Brown, T.B., Cheng, R., Sirault, X.R., Rungrat, T., Murray, K.D., Trtilek, M., Furbank, R.T., Badger, M., Pogson, B. & Borevitz, J.O. TraitCapture, genomic and environment modelling of plant phenomic data. *Current Opinion in Plant Biology* **18**, 73–79 (2014).
88. Arneth, A., Monson, R.K., Schurgers, G., Niinemets, U. & Palmer, P.I. Why are estimates of global isoprene emissions so similar (and why is this not so for monoterpenes)? *Atmospheric Chemistry and Physics Discussions* **8**, 7017–7050 (2008).
89. Fineschi, S., Loreto, F., Staudt, M. & Peñuelas, J. Diversification of volatile isoprenoid emissions from trees, evolutionary and ecological perspectives. In *Biology, controls and models of tree volatile organic compound emissions* (eds. U. Niinemets & R.K. Monson) pp. 1–20. Netherlands, Springer (2013).
90. Loreto, F., Bagnoli, F., Calfapietra, C., Cafasso, D., De Lillis, M., Filibeck, G., Fineschi, S., Guidolotti, G., Sramko, G., Tokolyi, J. *et al*. Isoprenoid emission in hygrophyte and xerophyte European woody flora, ecological and evolutionary implications. *Global Ecology and Biogeography* **23**, 334–345 (2014).
91. Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M., Wimp, G.M., Fischer, D.G., Bailey, J.K., Lindroth, R.K. *et al.* Community and ecosystem genetics, A consequence of the extended phenotype. *Ecology* **84**, 559–573 (2003).
92. Guidolotti, G., Calfapietra, C. & Loreto, F. The relationship between isoprene emission, CO_2 assimilation and water use efficiency across a range of poplar genotypes. *Physiologia Plantarum* **142**, 297–304 (2011).
93. Welter, S., Bracho-Nunez, A., Mir, C., Zimmer, I., Kesserlmeier, J., Lumaret, R., Schnitzler, J-P. & Staudt, M. The diversification of terpene emissions in Mediterranean oaks: lessons from a study of *Quercus suber, Quercus camariensis* and its hybrid *Quercus afares*. *Tree Physiology* **32**, 1082–1091 (2012).
94. Loreto, F., Bagnoli, F. & Fineschi, S. One species, many terpenes, matching chemical and biological diversity. *Trends in Plant Science* **14**, 416–420 (2009).
95. Szathmáry, E. & Smith J.M. The major evolutionary transitions. *Nature* **374**, 227–232 (1995).
96. Williams, G.C. *Natural selection: Domains, levels and challenges*. Oxford University Press. New York. pp. 208 (1992).
97. Mayr, E. The objects of selection. *Proceedings of the National Academy of Sciences USA* **94**, 2091–2094 (1997).
98. Reeve, H.K. & Keller, L. Levels of selection, Burying the units-of-selection debate and unearthing the crucial new issues. In *Levels of selection in evolution.* (ed. L. Keller), pp. 3–15. New Jersey, USA, Princeton University Press (1999).
99. Schlosser, G. & Wagner, G.P. The modularity concept in developmental and evolutionary biology. In *Modularity in development and evolution* (eds. G. Schlosser & G.P. Wagner), pp. 1–11. Chicago, USA, University of Chicago Press (2004).
100. Burian, R. Selection does not operate primarily on genes. In *Contemporary debates in philosophy of biology*. (eds. F. Ayala & R. Arp). Oxford, Wiley-Blackwell (2008).

101. Williams, G.C. 1966. *Adaptation and natural selection: A critique of some current evolutionary thought.* New Jersey, USA, Princeton University Press, pp. 307 (2009).
102. Dawkins, R. *The extended phenotype*. Oxford, Oxford University Press (1982).
103. Underwood, K.A., Swartz, J.R. & Puglisi, J.D. Quantitative polysome analysis identifies limitations in bacterial cell-free protein synthesis. *Biotechnology and Bioengineering* **91**, 425–435 (2005).
104. Stearns, S. The evolutionary significance of phenotypic plasticity. *BioScience* **39**, 436–445 (1989).
105. Borges, R.M. Plasticity comparisons between plants and animals: concepts and mechanisms. *Plant Signaling & Behavior* **3**, 367–375 (2008).
106. Borges, R.M. Phenotypic plasticity and longevity in plants and animals: cause and effect? *Journal of Biosciences* **34**, 605–611 (2009).
107. Dudley, S.A. & File, A.L. Kin recognition in an annual plant. *Biology Letters* **3**, 435–438 (2007).
108. Gouy, M. & Gautier, C. Codon usage in bacteria, correlation with gene expressivity. *Nucleic Acids Research* **10**, 7055–7074 (1982).
109. Ikemura, T. Codon usage and tRNA content in unicellular and multicellular organisms. *Molecular Biology and Evolution* **2**, 13–34 (1985).
110. Wright, S.I., Yau, C.B.K., Looseley, M. & Meyers, B.C. Effects of gene expression on molecular evolution in *Arabidopsis thaliana* and *Arabidopsis lyrata*. *Molecular Biology and Evolution* **21**, 1719–1729 (2004).
111. Michaud, M., Cognat, V., Duchene A-M. & Marechal-Drouard, L. A global picture of tRNA genes in plant genomes. *Plant Journal* **66**, 80–93 (2011).
112. Fahey, R.C., Hunt, J.S. & Windham, G.C. On the cysteine and cystine content of proteins: differences between intracellular and extracellular proteins. *Journal of Molecular Evolution* **10**, 155–160 (1977).
113. Beeby M., O'Conner B.D., Ryttergaard, C., Boutz, D.R., Perry L.J. & Yeates, T.O. The genomics of disulfide bonding and protein stabilization in thermophiles. *PLOS Biology* **3**, 1549–1558 (2011).

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