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Broad diversity in monoterpene–sesquiterpene balance across wild sunflowers: Implications of leaf and floral volatiles for biotic interactions

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Abstract

Premise: As plant lineages diversify across environmental gradients, species are predicted to encounter divergent biotic pressures. This study investigated the evolution of volatile secondary metabolism across species of *Helianthus*.

Methods: Leaves and petals of 40 species of wild *Helianthus* were analyzed via gas chromatography–mass spectrometry to determine volatile secondary metabolite profiles.

Results: Across all species, 500 compounds were identified; 40% were sesquiterpenes, 18% monoterpenes, 3% diterpenes, 4% fatty acid derivatives, and 35% other compounds such as phenolics and small organic molecules. Qualitatively, annuals and species from more arid western climates had leaf compositions with a higher proportion of total monoterpenes, while erect perennials and species from more mesic eastern habitats contained a higher proportion of total sesquiterpenes. Among species, mass-based leaf monoterpene and sesquiterpene abundance were identified as largely orthogonal axes of variation by principal component analysis. Profiles for leaves were not strongly correlated with those of petals.

Conclusions: Volatile metabolites were highly diverse among wild *Helianthus*, indicating the value of this genus as a model system and rich genetic resource. The independence of leaf and petal volatile profiles indicates a low level of phenotypic integration between vegetative and reproductive structures, implying vegetative defense and reproductive defense or pollinator attraction functions mediated by terpene profiles in these two organs can evolve without major trade-offs. The major biosynthetic pathways for the major terpenes in wild *Helianthus* are already well described, providing a road map to deeper inquiry into the drivers of this diversity.

KEYWORDS

Asteraceae, GC-MS, *Helianthus*, monoterpene, phenotypic integration, sesquiterpene

Plant secondary metabolites consist of diverse classes of volatile and nonvolatile compounds (based on a threshold molecular weight of approximately 300 D) that contribute to a wide range of interactions with abiotic and biotic aspects of the environment (Dudareva et al., 2004; Boncan et al., 2020). These metabolites have been shown to contribute to plant speciation and coevolution (Ehrlich and Raven, 1964). Many of these metabolites contribute color, flavor, aroma, and medicinal bioactivity to plant products and serve as industrial raw materials (Balandrin et al., 1985; Chadwick et al., 2013).

Terpenoids (hereafter "terpenes") include more than 30,000 compounds and are among the largest and most-diverse classes of biological compounds in living organisms on Earth (Breitmaier, 2006). Much of the diversity of terpenes (>25,000 unique compounds) can be found across the plant kingdom, while each plant species produces a small fraction of this diversity. The number, type, quantity, and relative ratio of terpenes varies widely among plant species, which in turn creates unique chemical features, fragrance, taste, and pigmentation in plants (Gershenzon and Dudareva, 2007; Chen et al., 2011; Boncan et al., 2020).

The majority of observed plant terpene diversity is associated with plant diversification, and many secondary metabolites within this compound class are lineage-specific (Pichersky and Raguso, 2018). However, a few hundred terpenes evolved early in the evolutionary history of the plant kingdom, and these primary terpenes exist in almost all plants (Chen et al., 2011; Pichersky and Raguso, 2018; Zhou and Pichersky, 2020). Due to the high structural similarity of terpenes and the multiple context-dependent observed functions of individual compounds, assigning a specific role to each terpene is difficult, leaving many terpenes with no clearly identifiable role in plant physiology or ecology (Pichersky and Raguso, 2018). The terpenes essential for primary metabolism are required for plant survival through their roles as phytohormones, protein modifiers, transporters, antioxidants, and photosynthetic pigments (Chen et al., 2011; Pichersky and Raguso, 2018; Zhou and Pichersky, 2020). The terpenes involved in secondary metabolism have an enormously diverse assemblage of functions mediating plant–environment interactions through sensory, signaling, or toxicological attributes. These compounds may attract mutualists like pollinators, seed dispersers, microbial symbionts, or enemies of enemies like parasitoids (Dudareva and Pichersky, 2006; Zeng et al., 2016; Pichersky and Raguso, 2018; Boncan et al., 2020), repel or inhibit pathogens and herbivores (Burnett et al., 1974; Rogers et al., 1987; Charlet et al., 2008; Chadwick et al., 2013; Prasifka et al., 2015; Silva et al., 2018), decrease competition with neighboring plants through allelopathy (Macias et al., 2002), or increase tolerance to abiotic stressors like drought or salinity (Kleiber et al., 2017; Yadav et al., 2017).

Depending on the plant species, between 18 and 152 genes from the terpene synthase (TPS) family are involved in terpene biosynthesis (Chen et al., 2011; Pichersky and Raguso, 2018). Many of these genes have evolved through the duplication, subfunctionalization, and neofunctionalization of an ancestral gene likely encoding a bifunctional kaurene synthase in plants, though some terpenes long predate the origin of land plants (Pichersky and Raguso, 2018; Boncan et al., 2020). These TPS genes encode enzymes such as isoprene synthases, monoterpene synthases, sesquiterpene synthases, and diterpene synthases that are present in plastids and the cytosol and produce different terpene backbones from isoprene building blocks (Chen et al., 2011; Vranova et al., 2013; Zhou and Pichersky, 2020). In addition to the TPS family of enzymes, terminal enzymes such as short-chain dehydrogenases/reductases (SDRs) are also involved in the biosynthesis of terpenes (Ringer et al., 2005; Moummou et al., 2012). After synthesis, terpenes (along with many other secondary metabolites) are often stored in specialized structures such as resin ducts, resin blisters, leaf storage cavities, laticifers, or glandular trichomes to avoid autotoxic effects (Gopfert et al., 2009; Vranova et al., 2013). Based on the arrangement and number of isoprene units, terpenes are classified into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), homoterpenes (C11,16), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40), or polyterpenes ($C > 40$). Most secondary terpenes including hemi-, mono-, homo-, sesqui-, and diterpenes are volatile and thus readily detectable by gas chromatography (Chen et al., 2011; Pichersky and Raguso, 2018; Liu et al., 2020; Zhou and Pichersky, 2020). Degree of genetic variation for underlying enzymes, functional redundancy, and enzyme promiscuity in the underlying metabolic pathways that manufacture a given class of secondary metabolites may strongly influence the relative capacity for individual metabolites to evolve independently of one another in nature or through artificial selection or bioengineering (Harborne, 1990; Junker et al., 2018; Kuken and Nikoloski, 2019; Dowell and Mason, 2020).

Like many other phytochemicals, the biosynthesis of terpenes is resource consuming and requires energetic investment. Terpene production is therefore predicted to be subject to the same types of resource allocation trade-offs typical of most plant secondary metabolites (Stamp, 2003; Agrawal, 2007, 2011). While there have been many efforts to develop generalized models for expected energetic trade-offs in secondary metabolite production in plants (resource availability hypothesis, growth–differentiation balance hypothesis, univariate trade-off model, etc.), it has become apparent that potential trade-offs in defense investment are highly lineage-specific and difficult to generalize without the holistic context of lineage physiology and habitat ecology (Coley et al., 1985; Stamp, 2003; Agrawal, 2007, 2011; Zust and Agrawal, 2017; Hahn et al., 2019). In the most extreme cases, concentrations of individual compounds may evolve in near-lockstep due to constraints on the flow of shared precursors. In this case, changes in the regulation of shared precursor production may have downstream effects on multiple compounds at once. The degree to which plant terpene production is subject to these forms of constraint is likely highly lineage-specific, given that a particular lineage of plants may express detectable concentrations of perhaps a few dozen to a few hundred terpenes from among the tens of thousands known, representing a small subset of sprawling underlying metabolic pathways.

For determining the degree of constraint versus lability in secondary metabolite evolution within a given lineage, phylogenetic comparative studies of diverse yet closely related groups of species can provide strong insights (Johnson et al., 2014; Mason et al., 2016; Lichman et al., 2020). In particular, these kinds of lineage-focused approaches can permit inquiry into potential drivers of secondary metabolite variation, including ploidy, growth form and life history, or native habitat environmental conditions. Here we examined the evolution of volatile terpene metabolism in leaves and petals across the wild sunflowers (*Helianthus*, Asteraceae). Of the approximately 50 described species, the genus occupies diverse habitats across North America and possesses wide diversity in plant morphology (Heiser et al., 1969; Onemli and Gucer, 2010), ecophysiology (Mason et al., 2016), phenology (Henry et al., 2014; Mason et al., 2017a), and resistance to biotic and abiotic stresses (Chandler and Jan, 1984; Rogers et al., 1987; Seiler and Jan, 2014), and phytochemistry (Seiler, 1985; Mason et al., 2016, Harun, 2019), With respect to terpene chemistry, previous studies have reported that leaves and flowers of cultivated and wild *Helianthus annuus* are rich in terpenes (Marechal and

Rigal, 1999; Ceccarini et al., 2004; Ukiya et al., 2007; Prasifka et al., 2015; Adams et al., 2017; Lawson et al., 2019). Despite this, and the known utility of wild *Helianthus* species as a genetic resource for crop improvement (Seiler, 1992; Kane et al., 2013; Kantar et al., 2015), terpene profiles across the genus are largely unknown. The limited information available is derived from chemosystematic studies (the presence–absence of several sesquiterpene lactones; Spring and Schilling, 1989, 1990, 1991) or more applied entomological inquiries focused on a small number of sesquiterpenes (Rossiter et al., 1986; Rogers et al., 1987). In this study, we leverage analytical chemistry and phylogenetic comparative approaches to investigate the breadth and depth of volatile terpene diversity across the genus *Helianthus*, addressing for the first time the phenotypic integration of terpene production in vegetative versus reproductive organs and assessing evidence for underlying constraints on the evolution of terpene metabolism.

MATERIALS AND METHODS

Plant germplasm and growth

For this study, a common garden approach was employed, with diverse species grown under uniform high-resource conditions to minimize environmentally induced trait variation (Mason and Donovan, 2015). Seed accessions of 48 species of wild *Helianthus* were obtained from the USDA National Plant Germplasm System (Appendix S1). One accession was included per named species, with the exception of species for which multiple ploidy levels are known (*H. decapetalus* and *H. divaricatus*). For these species, one accession per ploidy level was included based on flow cytometry data in recent studies (Bock et al., 2014; Qiu et al., 2019), for a grand total of 48 target accessions.

In the spring of 2018, seeds were scarified and germinated on filter paper in petri dishes. Once root hairs were present, seedlings were placed into seedling trays filled with moist sand under growth lights with a 12-h photoperiod. Each tray was initially supplemented with 10 g of pelleted slow-release fertilizer (Osmocote Plus 15-9-12; Scotts, Marysville, OH, USA). Once true leaves were present, the five healthiest seedlings were moved to the greenhouse and transplanted into 10-inch-diameter azalea pots filled with a homogenous mixture of 70% sand and 30% potting soil. Pots were randomized spatially within the greenhouse to minimize spatial effects on traits. Twenty grams of the pelleted slow-release fertilizer was added to each pot to ensure high nutrient availability. Plants were hand-watered to field capacity daily until they were well established, then with an automatic drip watering system to ensure daily watering to field capacity. For pest control, plants were preventatively treated with imidicloprid (0.22% systemic granules, Bonide, Oriskany, NY, USA), and then all plants were uniformly treated with permethrin if any piercing/sucking insects were identified (0.25% liquid, Bonide). For eight accessions, none of the five replicates survived to maturity and were excluded from the study. Each of the major clades within the genus was represented well by the surviving 40 accessions (Stephens et al., 2015).

Leaf and petal sampling

Given the large variation in growth rate and phenology among *Helianthus* species, leaf sampling was standardized by ontogenetic stage to ensure valid comparison (Mason and Donovan, 2015; Mason et al., 2016), with the period of first flowering (anthesis) chosen for sampling. Plants were checked at least once per week for flowering. Once each plant had open flowers, the most recently fully expanded leaf pair was selected for sampling. The two leaves were removed from the plant using sharp shears where the petiole meets the stem. In the same flowering stage, fresh, unwilted petals from open flowers were sampled in bulk. Fresh leaf and petal samples were placed into sealed plastic bags in a cooler on ice during sampling, then transported to the laboratory and placed in microcentrifuge tubes and frozen at −80°C until analytical chemical analysis. Only undamaged fresh leaves and petals were sampled, avoiding any plant parts with visible insect damage or any plants that appeared unhealthy or with more than very minor insect damage. This reduction in the number of biological replicates ensured data quality. Additionally, because of the ephemeral nature of wild *Helianthus* flowers, and the low reproductive output of many perennial species in their first year, it was not always possible to obtain fresh, unwilted petals, resulting in fewer samples for petals than for leaves. For leaf tissue, 112 replicate plants of 37 species were able to be sampled, or $N = 3.02$ replicates on average. For petal tissue, 54 replicate plants of 24 species were able to be sampled, or $N = 2.25$ replicates on average.

Analytical chemistry analysis

Plant volatile profiles were determined by headspace solid-phase microextraction gas chromatography-mass spectrometry (SPME GC-MS; Adam et al., 2005; Deng et al., 2006; Zhu et al., 2013; Burzynski-Chang et al., 2018; Tholl et al., 2021) using a single quadrupole QP2020 (Shimadzu, Kyoto, Japan). For this purpose, leaf and petal samples were ground to homogeneity in liquid nitrogen, and ~50 mg of tissue was added to 10-mL glass headspace vials with the total sample mass recorded. The vials were incubated at 90°C for 20 min with agitation at 250 rpm. Then, a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVS/CAR/PDMS) SPME fiber was inserted into the vial for 10 min to extract volatiles from the headspace. The fiber was then desorbed in the inlet of the GC-MS for 3 min at 225°C. Between samples, the fiber was conditioned at 270°C for 10 min. Column flow was 1.5 mL/min with a split ratio of 25:1 using a purge flow of 3.0 mL/min after 4 min sampling time. Initial temperature in the GC-MS was 30°C for 1 min, which was then increased to 150°C at

12°C min^{-1}, held for 1 min, then increased to 225°C at 9°C min^{-1}, then increased to 250°C at 50°C min^{-1}, and held for 5 min. The source and interface temperatures of the mass spectrometer were kept at 200°C and 250°C, respectively.

Mass spectra of peaks were compared against the National Institutes of Standards and Technology standards database (Lemmon et al., 2017), and peak identities with minimum similarity of 70% were selected as naming conventions. Using retention time and mass spectra similarity hits for each peak, the raw data was manually processed to avoid any possible mislabeling. The peak area for each compound within each sample was normalized by dividing the area by the initial sample mass placed into the glass headspace vial. Isomers can be notoriously tricky to differentiate by mass spectral data alone; we therefore report the top NIST library hit for each peak regardless of isomer identity (three versions of NIST library: NIST17-1, NIST17-2, and NIST17-S). Our data set therefore contains multiple instances of some metabolites, which are likely isomers of the same compound where our analysis could not fully differentiate identity.

Statistical analyses

In addition to the mass-normalized peak areas generated for individual compounds, we calculated additional summary statistics for each sample. For each subclass of compounds identified, we summed the normalized peak areas to calculate estimates of total monoterpenes, total sesquiterpenes, total diterpenes, total terpenes, total fatty acid derivatives, and an additional category of "total other" for all other miscellaneous nonterpene compounds (including ketones, epoxides, benzaldehydes, triazenes, alkanes, alkenes, and alcohols). Further, summing all normalized peak areas gave an estimate of total volatile compounds for each sample. It is important to note that summing mass-normalized peak areas gives a semiquantitative relative estimate of compound abundance, as the slope of the relationship between tissue concentration and mass-normalized peak area will differ somewhat among compounds due to a range of factors including matrix effects (Burzynski-Chang et al., 2018). For research applications needing more precise quantification for a small number of target compounds (e.g., fine-scale intraspecific or intraindividual variation), compound-specific calibration curves should be used (Verzera et al., 2011; Cincotta et al., 2015; Burzynski-Chang et al., 2018; Tholl et al., 2021). This approach is not feasible for exploratory work describing compound variation among hundreds of compounds, and the semiquantitative results obtained here should be robust when orders-of-magnitude variation in compound abundance is present.

For leaf and petal samples, separately, proportions (percentages) of total monoterpenes, total sesquiterpenes, total diterpenes, total fatty acid derivatives, and total other were calculated by dividing values of these traits by total volatile compounds. The number of observed compounds within each sample was also recorded as an estimate of volatile compound diversity. A subset of "major" compounds, defined as having a mean proportion greater than 0.5% across all species, was identified separately for leaves and petals. To investigate the distribution of species in phytochemical trait space, we performed a descriptive principal component analysis (PCA) of the major compounds in leaves and petals (39 and 44 compounds in 37 and 24 species, respectively) using the prcomp function in R version 1.4.1717 (R Core Team, 2020).

Phylogenetic comparative methods were conducted using the Stephens et al. (2015) 37-species phylogeny of the diploid backbone of the genus and carried out in R using phylolm (Ho and Ane, 2014), Rphylopars (Goolsby et al., 2017), and MCMCglmm (Hadfield, 2010). Because this phylogeny does not contain polyploids, hybrids, or several difficult-to-grow diploid species, these analyses included the subset of species in our data set that were present in the phylogeny (25 species for leaves and 15 for petals). Ancestral state reconstruction was performed using species means for all traits assuming a Brownian motion model of evolution.

To investigate potential trade-offs or constraints in metabolite evolution, pairwise correlations (R^2) among the compounds in both leaf and petal tissues were estimated using generalized linear mixed models (GLMMs) and phylogenetic generalized linear mixed models (PGLMMs) in the MCMCglmm package (Hadfield, 2010; Nakagawa and Schielzeth, 2013; Villemereuil and Nakagawa, 2014), with leaf or petal metabolite concentrations as a fixed effect and species as a random effect to account for intraspecific variation. For PGLMM models, an additional species-level random effects term was specified to account for phylogenetic structure using the inverse of the phylogenetic covariance matrix. PGLMMs were performed on the subset of observations from the species represented in the phylogeny tree), and GLMMs (i.e., not accounting for phylogeny) were run on the full data set. To calculate the correlation of compounds between leaves and petals, we used species means because leaf and petal tissue was collected from different individuals, so ordinary least squares (OLS) and phylogenetic generalized least squares (PGLS) regressions were run instead of GLMMs. PGLS regression was run using the phylolm function in the phylolm package (Ho and Ane, 2014), and OLS regression was performed using the lm function. Pagel's lambda was estimated for all PGLS regressions, and PGLMM models fit using MCMCglmm are equivalent to a Pagel's lambda model (Pagel, 1999; Villemereuil and Nakagawa, 2014).

RESULTS

Volatile compounds

The GC-MS analysis across all 166 samples (40 species and two tissue types) identified a total of 500 compounds, comprising 40.2% sesquiterpenes, 17.8% monoterpenes, 3.4%

diterpenes, 4% fatty acid derivatives, and 34.6% other nonterpene compounds including primarily alkanes, alkenes, aldehydes, ketones, and epoxides. A total of 467 compounds were detected in leaves, while 210 compounds were detected in petals. Species varied from 17 to 78 detected compounds in leaves (*H. heterophyllus* versus *H. argophyllus*, respectively) and 7 to 55 detected compounds in petals (*H. tuberosus* and *H. maximiliani* versus *H. winteri*, respectively), while total compound abundance as quantified by mass-normalized peak area varied over 100-fold in leaves and 300-fold in petals (Appendices S2–S4). On average, leaves contained more compounds than petals did, as well as a higher total compound abundance. While leaves and petal volatile profiles contained a similar proportion of monoterpenes, leaves contained a higher proportion of sesquiterpenes and therefore a higher proportion of total terpenes, while petals contained a higher proportion of other nonterpene compounds (Table 1).

In leaves, major variation was observed among species in the balance of monoterpenes and sesquiterpenes, spanning from 9–91% monoterpenes and 3–90% sesquiterpenes (Figure 1A). The highest leaf proportions of total monoterpenes were found in species mostly native to desert, dry grassland, and Mediterranean habitats including annuals such as *H. annuus*, *H. exilis*, *H. niveus*, *H. neglectus*, and erect perennials such as *H. winteri*, *H. arizonensis*, *H. californicus*, and *H. laciniatus* that do not fall into either the large perennial or the southeastern perennial major clades within the genus (Stephens et al., 2015). The highest leaf proportions of total sesquiterpenes belonged to erect perennial species within the large perennial clade (Stephens et al., 2015) that are mostly native to mesic woodland habitats (*H. glaucophyllus*, *H. microcephalus*, *H. resinosus*, *H. divaricatus*, *H. giganteus*, *H. smithii*, *H. tuberosus*, *H. decapetalus)*. Other taxa within the genus fall somewhere between the two ends of this spectrum of high-monoterpene, arid-habitat annuals and erect perennials versus high-sesquiterpene, mesic-habitat, erect perennials.

Among species, petals demonstrated a similar degree of variation in volatile profile composition, with species varying from 3–92% monoterpenes and 0–90% sesquiterpenes (Figure 1D). However, petals contained a much larger share of "other" compounds. In leaves, such compounds made up 1–15% of volatile profiles, with the exception of the outlier *H. heterophyllus*, which was composed of 61% "other" compounds—overwhelmingly driven by the abundance of 1-pentadecene. For petals, nine species contained over 30% "other" compounds, up to 85% in both *H. heterophyllus* and *H. tuberosus*—driven by compounds such as 1-pentadecene, 1-undecene, benzaldehyde, and gentisaldehyde.

Among closely related species of varying ploidy levels, there were no consistent patterns of increasing or decreasing terpene levels or directional change in monoterpene–sesquiterpene balance. Likewise, within the taxa of variable ploidy level (*H. decapetalus* and *H. divaricatus/H. hirsutus*), there was no consistent directional effect on terpene levels or monoterpene–sesquiterpene balance.

Across all detected compounds, 39 compounds in leaves and 44 compounds in petals were selected as major compounds comprising more than 0.5% of mass-normalized peak area on average across all species. These compounds together made up >63% of the total mass-normalized peak area of all volatile compounds across species in both leaves and petals, and 28 compounds were shared between leaves and petals (Appendices S5–S12). The 39 compounds present in leaves included 10 monoterpenes (borneol, bornyl acetate, camphene, 3-carene, D-limonene, β-myrcene, two isomers of α-pinene, β-pinene, sabinene, and γ-terpinene), 15 cadinene-type sesquiterpenes (α-amorphene, δ-amorphene, α-muurolene, γ-muurolene, τ-muurolol, α-cadinene, β-cadinene, γ-cadinene, cadina-1,4,-diene, cadina-3,5-diene, τ-cadinol, α-calacorene, *trans*-calamenene, epizonarene, β-ylangene), 10 other sesquiterpenes (two isomers of α-bergamotene, γ-bisabolene, *epi*-bicyclosesquiphellandrene, caryophyllene, α-cedrene, copaene, germacrene D, humulene, longipinene, β-selinene), and two nonterpenes (gentisaldehyde, 1-pentadecene). Compounds present in petals but not in leaves include six additional monoterpenes (acoradiene, *o*-cymene, pseudo-limonene, dehydro-sabinene, α-terpinene, terpinen-4-ol), three additional cadinene-type sesquiterpenes (*Z*-α-*trans*-bergamotol, cadalene, an additional isomer of cadinene), and several assorted nonterpenes (benzaldehyde, benzeneacetaldehyde, oxirane-5-hexenyl, 9-oxabicyclo-6,1,0-nonane, 1-undecene, 1,2-cyclo-octanediol). Complete mass-normalized peak area data for individual leaf and petal samples, species means, and major compound data sets and summary statistics are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.8gtht76sn (Mason et al., 2022).

TABLE 1 Summary of volatile profiles from leaves and petals of wild species of *Helianthus* (mean ± SD).

Tissue	Number of compounds	Total volatile compounds[a]	Percentage of total					
			Monoterpenes	Sesquiterpenes	Diterpenes	Terpenes	Fatty acid derivatives	Others
Leaves (37 species)	52.7 ± 11.7	354,907.2 ± 382,914.7	39.7 ± 25	53.3 ± 24.7	0.7 ± 0.9	93.8 ± 11.5	0.5 ± 1.5	5.6 ± 10
Petals (24 species)	29.2 ± 15.4	159,990.8 ± 194,308.4	42.8 ± 26.8	30.9 ± 23	0.2 ± 0.4	73.9 ± 30.6	1.4 ± 3	24.6 ± 28.6

[a]Mass-normalized peak

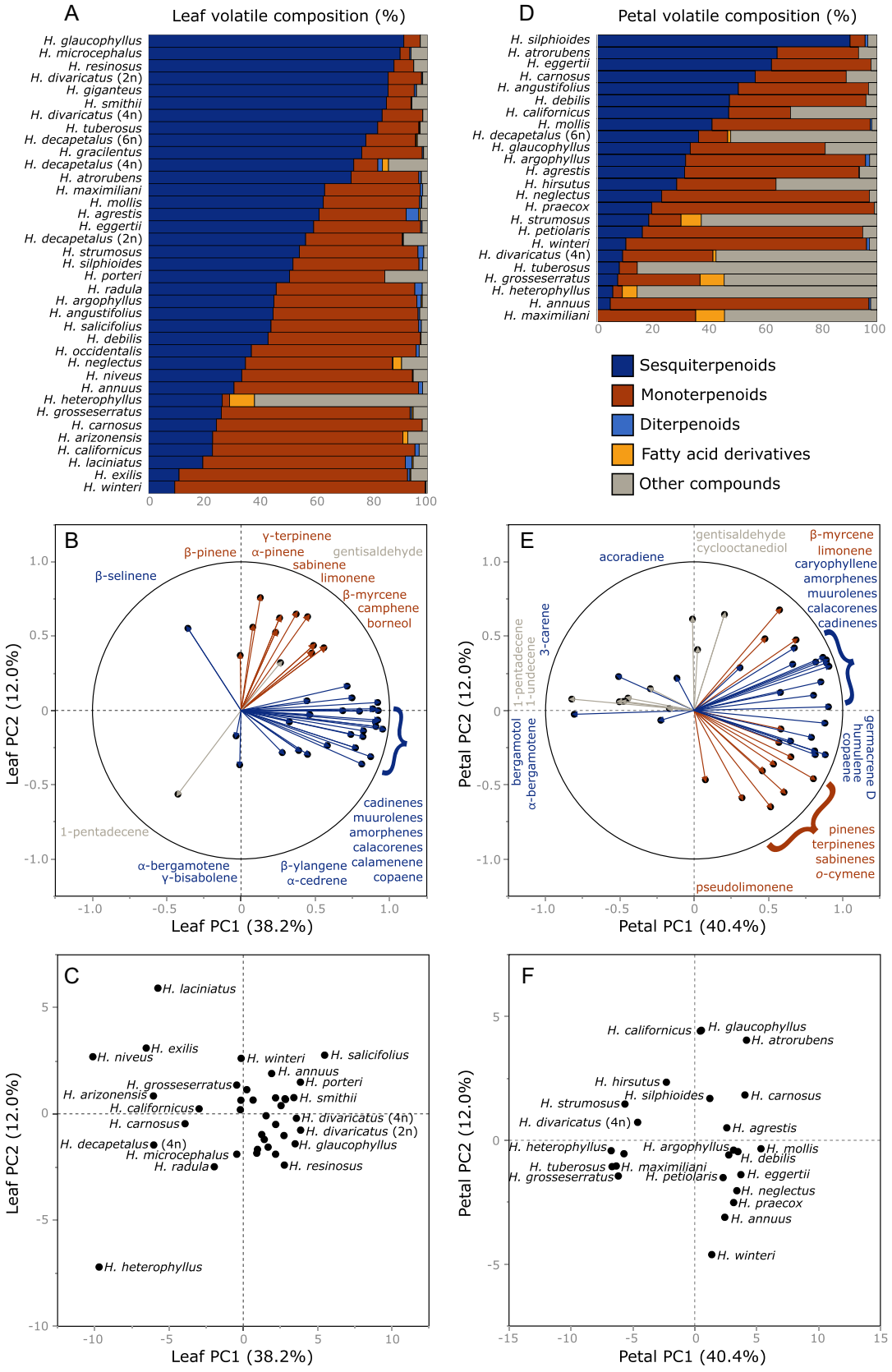

FIGURE 1 (See caption on next page)

Principal component analysis

Principal component analysis was performed using the 39 major compounds detected in leaves (Figure 1B, C) and 44 compounds detected in petals (Figure 1E, F). For both tissue types, the first two principal components captured over half of variation in the major compound datasets. In leaves, the first principal component axis (PC1) explained 39% of variation and loaded positively with most sesquiterpenes, while PC2 explained 12% of variation and loaded positively with most monoterpenes (Figure 1B). In petals, PC1 explained 40% of the variation and loaded positively with most sesquiterpenes and some monoterpenes and loaded negatively with several nonterpene "other" compounds and bergamotene-related sesquiterpenes (Figure 1E). When species separation across the first two PC axes for both petals and leaves was examined, closely related species tended to cluster together (Figure 1C, F).

Heatmap clustering and ancestral state reconstruction

In the heatmap for the 39 major compounds in leaves, α-pinene was the most abundant monoterpene present in leaves overall, and germacrene D was the most abundant sesquiterpene, though these compounds were not necessarily highly abundant in all individual species (Figure 2). In addition to these major compounds, the clustering algorithm grouped together the most abundant monoterpenes (camphene, D-limonene, γ-terpinene, sabinene, β-pinene, β-myrcene), and the abundant sesquiterpenes were grouped into two clusters (caryophyllene and δ-amorphene as one cluster; humulene, epizonarene, α-cadinene, α-muurolene, γ-muurolene, and γ-cadinene as another). Other sesquiterpenes of lower proportional abundance were clustered together. Generally, closely related species had more similar total terpene abundances and similar proportional profiles (Figure 2). Most notably, ancestral state reconstruction revealed that the common ancestor of the large perennial clade likely had high total terpene levels and that species within this clade had on average the highest total terpene levels found in *Helianthus* (Figure 2). Most members of the large perennial clade also have the highest proportional abundances of individual sesquiterpenes. By contrast, the annual and the southeastern perennial clades had lower total terpene levels and a higher proportional abundance of individual monoterpenes (Figure 2).

Correlation analysis

As described above, phylogenetic (PGLS or PGLMM) and nonphylogenetic (OLS or GLMM) correlation analyses were conducted for summary metrics and the major compounds in leaves and petals. Statistically significant correlations $R^2 \geq 0.75$ were considered strong, correlations between 0.50 to 0.75 moderate, and those ≤0.50 weak, following recommendations of Poorter et al. (2014) for trait–trait correlations (Appendices S13–S18).

Most interestingly, metrics of volatile profile composition were largely uncorrelated between leaves and petals. There were no significant correlations between leaves and petals for total volatile compounds, total terpenes, total monoterpenes, total sesquiterpenes, total diterpenes, total fatty acid derivatives, or the number of compounds detected. Only a few individual compounds showed any significant correlation between leaves and petals: the monoterpene β-myrcene (weakly positive, OLS $R^2 = 0.24$, PGLS $R^2 = 0.32$), the sesquiterpene α-muurolene (weakly positive, OLS $R^2 = 0.23$, PGLS $R^2 = 0.24$), and the nonterpene 1-pentadecene (moderately positive, OLS $R^2 = 0.69$, no data for PGLS), likely driven by the distinctive abundance of this compound in *H. heterophyllus*. Overall, these results indicate that leaf and petal volatile profiles are not highly correlated, and we interpret this low covariance among organs as low phenotypic integration (sensu Pigliucci and Preston, 2004) in volatile secondary metabolism between vegetative and reproductive structures across *Helianthus*.

Within leaves, there were moderate to strongly positive correlations among most metrics, including between total volatile compounds and total terpenes (strongly positive, GLMM $R^2 = 0.91$, PGLMM $R^2 = 0.88$), between total terpenes and total monoterpenes (moderately positive, GLMM $R^2 = 0.52$, PGLMM $R^2 = 0.59$), and between total terpenes and total sesquiterpenes (strongly positive, GLMM $R^2 = 0.74$, PGLMM $R^2 = 0.75$). Correlations between total monoterpenes and total sesquiterpenes were much weaker (positive, GLMM $R^2 = 0.18$, PGLMM $R^2 = 0.28$), and there were no substantial correlations between diterpenes and either monoterpenes or sesquiterpenes. The number of compounds detected in leaves was correlated with total terpenes (weakly positive, GLMM $R^2 = 0.24$, PGLMM $R^2 = 0.19$) and also weakly positively correlated with total monoterpenes, total sesquiterpenes, and total diterpenes.

Within petals, correlations were again moderate to strong positive among most metrics, including between total volatile compounds and total terpenes (strongly positive, $R^2 = 0.82$, petals PGLMM $R^2 = 0.87$), between total terpenes and total monoterpenes (moderately to strongly positive, GLMM

FIGURE 1 Interspecific variation in the composition of volatile secondary metabolites in leaves and petals from wild species of *Helianthus* (37 and 24 species, respectively). Proportional composition of all detected volatile compounds in leaves (A) and petals (B), allocated into major classes of monoterpenes, sesquiterpenes, diterpenes, lipids, and all other nonterpene compounds (primarily alcohols, aldehydes, ketones, and other small organics). Metabolite loadings derived from PCA performed on species means for the 39 most abundant compounds in leaves (C) and 44 most abundant compounds in petals (D), with individual metabolites color-coded by major class and a subset of focal compounds listed near the corresponding eigenvector. Species separation in principal component chemospace for leaves (E) and petals (F), with the most disparate species listed. Clumped species names are omitted for readability.

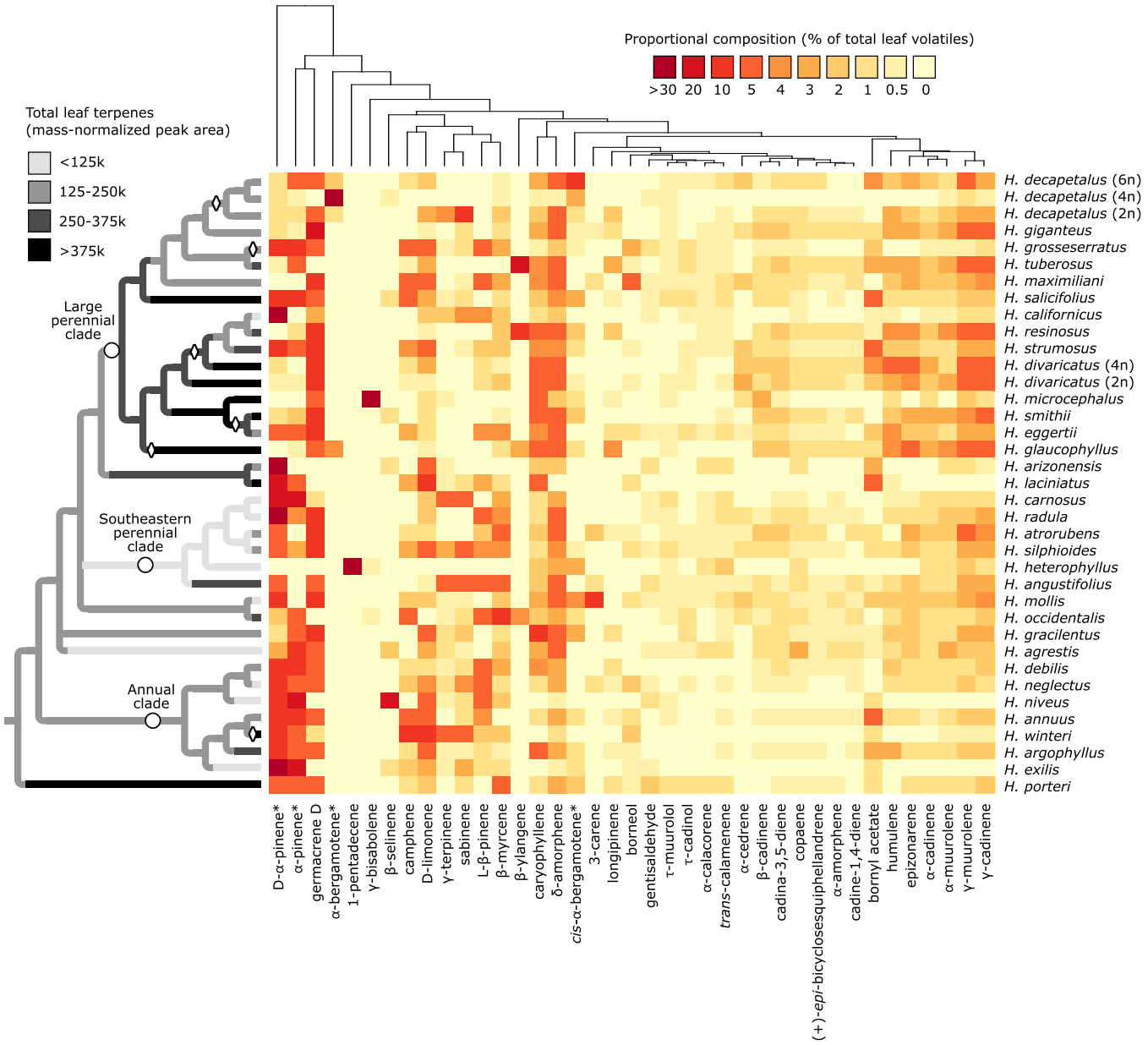

FIGURE 2 Heatmap of proportional composition of the 39 most abundant leaf volatile secondary metabolites across wild species of *Helianthus*. Heatmap scale reflects proportional mass-normalized peak area of each compound expressed as a percentage of the total volatile profile for that species. Rows are sorted based on phylogenetic relationships among species, with species relationships derived from the well-resolved phylogeny of the diploid backbone of the genus (Stephens et al., 2015), with polyploids and other missing taxa grafted onto the backbone based on available taxonomic and genetic evidence (Bock et al., 2014; Baute et al., 2016; Qiu et al., 2019). ◊, grafted lineages. Branches of the phylogeny reflect the ancestral state reconstruction of total leaf terpene content, expressed as mass-normalized peak area across all terpene compounds identified by GC-MS (see Materials and Methods). Columns represent individual compounds, sorted using the hclust function in R (Perry, 2021; hierarchical farthest neighbor clustering based on dissimilarity using the Ward method) to generate a dendrogram of similarity in compound variation. Asterisks represent likely isomers of the same compound detected with distinct retention times (see Materials and Methods).

$R^2 = 0.65$, PGLMM $R^2 = 0.86$), and between total terpenes and total sesquiterpenes (moderately positive, GLMM $R^2 = 0.73$, PGLMM $R^2 = 0.72$). Correlations between total monoterpenes and total sesquiterpenes were somewhat stronger than in leaves (weakly positive, GLMM $R^2 = 0.32$, PGLMM $R^2 = 0.44$), likely reflecting the larger role of nonterpenes in petal volatile profiles and the more substantial co-occurrence of these two classes of terpenes in opposition to compounds like 1-pentadecene, 1-undecene, and gentisaldehyde. As in leaves, again there were no significant correlations between diterpenes and either monoterpenes or sesquiterpenes. The number of compounds detected in petals was correlated with total terpenes (moderately positive, GLMM $R^2 = 0.51$, PGLMM $R^2 = 0.56$) and weakly positively correlated with total monoterpenes, total sesquiterpenes, and total diterpenes. This positive relationship between the

number of compounds detected and the overall abundance of terpenes and terpene subclasses indicates that in both leaves and petals *Helianthus* species that synthesize many different kinds of terpenes can do so without sacrificing the overall quantity of terpenes produced. Rather, it appears that increasing the production of additional compounds, or the production of higher levels of a given subclass, results in higher terpene levels overall rather than a compensatory reduction in other compounds or subclasses. This positive relationship between number of compounds and overall abundance of terpenes indicates that there are no apparent biosynthetic trade-offs within or among subclasses of terpenes in regard to production of terpenes per unit tissue mass. However, this lack of trade-off should be interpreted with caution because a positive relationship between the number of compounds (richness) and total abundance across compounds can be at least partially driven by technical aspects underlying analytical chemistry approaches, where samples with higher total compound abundance have higher observed compound richness due to detection thresholds (Wetzel and Whitehead, 2020).

In both leaves and petals, there were moderate to strong positive correlations among most sesquiterpenes, especially among germacrene D, copaene, caryophyllene, humulene, longipinene, and the various cadinene-type sesquiterpenes (e.g., α-cadinene, β-cadinene, γ-cadinene, cadina-3,5-diene, α-muurolene, γ-muurolene, τ-muurolol, δ-amorphene, epizonarene, α-calacorene, and *trans*-calamenene). For example, γ-cadinene and δ-amorphene are strongly positively correlated in leaves (GLMM $R^2 = 0.88$, PGLMM $R^2 = 0.83$) and moderately positively in petals (GLMM $R^2 = 0.69$, PGLMM $R^2 = 0.61$). Likewise, α-cadinene and γ-muurolene are moderately positively correlated in leaves (GLMM $R^2 = 0.67$, PGLMM $R^2 = 0.72$) and strongly positively in petals (GLMM $R^2 = 0.94$, PGLMM $R^2 = 0.98$). Conversely, these consistent correlations were not observed among monoterpenes, where strength and direction of correlations were variable among compounds and with differing patterns in leaves versus petals. For example, sabinene and γ-terpinene are weakly positively correlated in leaves (GLMM $R^2 = 0.50$, PGLMM $R^2 = 0.33$) and petals (GLMM $R^2 = 0.38$, PGLMM $R^2 = 0.29$), while D-α-pinene and 3-carene were uncorrelated in leaves and weakly negatively correlated in petals (GLMM $R^2 = 0.30$, PGLMM $R^2 = 0.37$). It therefore seems as though across *Helianthus* sesquiterpenes are more tightly up- or downregulated as a unit as lineages have diversified, while monoterpenes have more loosely evolved in relation to one another.

Total monoterpenes were negatively correlated with multiple nonterpene compounds, for example 1-pentadecene in leaves (moderately negative, GLMM $R^2 = 0.45$, PGLMM $R^2 = 0.50$) and 9-oxabicyclo-6,1,0-nonane in petals (moderately negative, GLMM $R^2 = 0.51$, PGLMM $R^2 = 0.46$). In both leaves and petals, nonterpene compounds were negatively correlated with most terpene compounds. In petals, total fatty acid derivatives were weakly to moderately negatively correlated with both total monoterpenes (GLMM $R^2 = 0.41$, PGLMM $R^2 = 0.58$) and total sesquiterpenes (GLMM $R^2 = 0.26$, PGLMM $R^2 = 0.40$). This pattern may suggest a weak trade-off in biosynthesis between terpenes and fatty acid derivatives in petals, which also contain proportionally more fatty acid derivatives and less terpenes than do leaves (Figure 1).

DISCUSSION

Terpene biosynthesis in wild *Helianthus*

In this study, a substantial diversity of volatile compounds was observed across the 40 species of *Helianthus* considered. The dominant compounds detected align with the few previous studies of *Helianthus* leaf and floral volatiles, mostly in wild and cultivated *Helianthus annuus* (Schuh et al., 1997; Ceccarini et al., 2004; Ogunwande et al., 2010; Bertoli et al., 2011; Adams et al., 2017; Lawson et al., 2019). Monoterpenes and sesquiterpenes formed the two largest classes of compounds in both leaves and petals, though with an abundance of nonterpene compounds present in petals of a portion of species. The highest proportions of monoterpenes were observed in annuals and erect perennials from arid habitats, while the highest proportions of sesquiterpenes were in erect perennials from mesic habitats. Other species were intermediate, for example, species from the southeastern perennial clade (erect perennials such as *H. atrorubens*, *H. silphioides*, and *H. angustifolius* and basal rosette perennials such as *H. carnosus* and *H. radula*) span a range of intermediate compositions. Interestingly, those taxa from the large perennial clade with higher proportions of leaf monoterpenes occupy on average drier habitats than most other clade members (e.g., *H. grosseserratus*, *H. salicifolius*, *H. maximiliani*). However, given the large overlapping ranges of many *Helianthus* species, the attribution of leaf monoterpene–sesquiterpene balance to climate and life history predictors should be considered preliminary and requires a higher degree of intraspecific sampling to verify.

Within both leaves and petals, the evolution of increased volatile production was dominated by the production of terpenes, with just under 90% of variation in total volatiles attributable to variation in total terpenes among species (PGLS). In leaves, monoterpene and sesquiterpene production appears to be mostly independent, loading heavily with orthogonal PC axes and only weakly positively correlated. In petals, the production of these two subclasses is more correlated given the larger importance of nonterpene compounds to petal volatile profiles.

As terpenoids, the sesquiterpenes and monoterpene partially share the same biosynthetic pathway, branching off from the mevalonate (MVA) and methylerythritol phosphate (MEP) backbone pathways that produce dimethylallyl pyrophosphate (DMAPP), isopentyl-PP, geranyl-PP, and farnesyl-PP (Figure 3; Bick and Lange, 2003). Geranyl-PP is the substrate for a range of terpene synthases that produce monoterpenes and their derivatives, while farnesyl-PP is the equivalent substrate for terpene synthases producing sesquiterpenes and their derivatives (Figure 3). A lack of negative

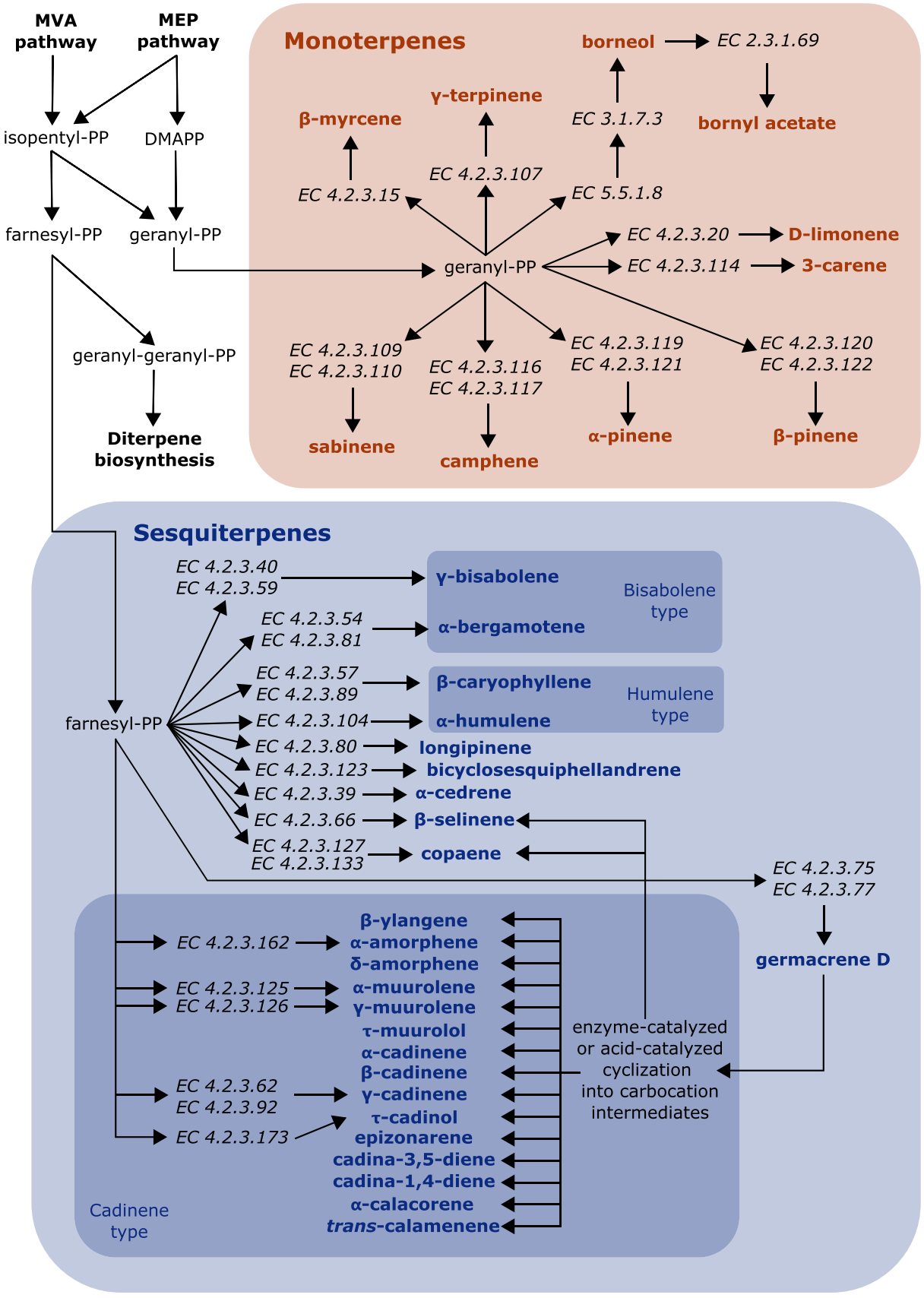

FIGURE 3 (See caption on next page)

correlations among species on an absolute basis (concentrations per mass of tissue) indicate that among diverse *Helianthus*, the terpenoid backbone pathways do not constrain the production of these two subclasses, such that an increase in monoterpenes is associated with a mild increase in sesquiterpenes and vice-versa. When viewed in terms of the proportional composition of volatile profiles, there is wide variation among species in the relative balance of monoterpenes and sesquiterpenes, but this variation is not independent of total terpene production (Figures 1 and 2), such that among species, higher overall terpene production is associated with the production of proportionally more sesquiterpenes.

Given the structure of the terpenoid biosynthetic pathways, where diverse mono- and sesquiterpenes can be generated from the same two precursors, the relative production of individual compounds by *Helianthus* species are likely more related to cross-talk between the MVA and MEP pathways that generate geranyl-PP and farnesyl-PP (Bick and Lange, 2003) and to the effects of different ratios of the diverse terpene synthases that act upon them (Iijima et al., 2004) and the terminal enzymes that contribute to functional group diversity (Ringer et al., 2005; Moummou et al., 2012).

Within *Helianthus*, the strongest pairwise relationships among individual terpenes are among the cadinene-type sesquiterpenes, which is unsurprising given the abundance of germacrene D in most high-sesquiterpene species and its major role as a precursor to a wide range of cadinene-type sesquiterpenes through either enzyme- or acid-catalyzed cyclization into carbocation intermediates (Setzer, 2008; Wedler et al., 2015). The covariation within *Helianthus* volatile profiles indicates that the "cadinyl" carbocation likely is a major route to cadinene-type sesquiterpene production in *Helianthus*, alongside direct enzymatic synthesis from farnesyl-PP. From a resource allocation perspective, the De Jong model predicts that major divergences in allocation tend to lead to negative covariation among classes of phytochemicals, whereas subsequent minor resource allocations usually lead to lead to positive correlations (De Jong, 1993; Zust and Agrawal, 2017). Under this perspective, it would appear that, based on covariation observed within terpene subclasses across *Helianthus*, investment in monoterpenes versus sesquiterpenes represents a minor resource allocation subsequent to a larger upstream resource allocation between terpenes and other energetic investments. Further inquiry into broader secondary metabolism is needed to examine where such upstream resource allocation splits may occur, whether investment in other aspects of secondary metabolism or investments in growth and reproduction.

Previous work on nonvolatile leaf secondary metabolism may shed partial light on resource allocation to other secondary metabolites. Species in the southeastern perennial clade have been documented to have higher leaf phenylpropanoid concentrations and higher leaf tannin activity than species in the large perennial or annual clades (Mason et al., 2016). When coupled with the results of the present study, this pattern suggests a potential divergence in investment between phenylpropanoids and terpenes across the genus; the large perennial clade invests most heavily in terpenes, the southeastern perennial clade most heavily in phenylpropanoids, and the annual clade relatively less in both major classes of compounds. However, given the known plasticity in the expression of plant secondary metabolism (Wetzel and Whitehead, 2020) and likely intraspecific diversity of *Helianthus* species across ranges (Adams et al., 2017), this potential divergence in investment represents a hypothesis that cannot be confirmed without simultaneous phenotyping of volatile and nonvolatile secondary metabolism with ontogenetic, environmental, and genotype standardization.

Phenotypic integration between leaves and petals under diversification

The composition of volatile compounds in leaves and petals were substantially different. Leaves had on average more sesquiterpenes, while petals had on average more monoterpenes and nonterpene compounds. This difference may be related to the known roles of sesquiterpenes and their derivatives in herbivore defense (Chadwick et al., 2013; Prasifka et al., 2015) and the known role of many monoterpenes in pollinator attraction (Dudareva and Pichersky, 2006; Zeng et al., 2016). Within *Helianthus*, multiple studies have linked nonvolatile or low-volatility sesquiterpene lactones to herbivore defense (especially florivores: Rossiter et al., 1986; Rogers et al., 1987; Prasifka et al., 2015), though there has been little to no work published on the role of floral volatiles in relation to pollinator visitation within crop or wild *Helianthus* (Pham-Delegue et al., 1990; Bertoli et al., 2011; Mallinger and Prasifka, 2017). While in the present work, leaves from wild *Helianthus* had higher numbers of compounds and a greater abundance of total terpenes, petals had a wider diversity of

FIGURE 3 Biosynthesis of major terpenes found in leaves and petals of wild species of *Helianthus*. Pathways are derived from the Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa and Goto, 2000). Enzyme-catalyzed reactions are represented by Enzyme Commission (EC) numbers, most of which represent the activity of terpene synthases (a class of phosphate lyases). Many cadinene-type sesquiterpenes are derived from enzyme- or acid-catalyzed reactions with carbocation intermediates derived from germacrene D (Setzer, 2008; Wedler et al., 2015). Note that in addition to the canonical pathways described here, many metabolites may be produced via non-enzymatic reactions or via enzyme promiscuity (Zulak and Bohlmann, 2010; Khersonsky and Tawfik, 2010; Moore et al., 2014; Junker et al., 2018). *Abbreviations*: mevalonate (MVA), mevalonate-independent pathway (MEP), dimethylallyl-pyrophosphate (DMAPP), pyrophosphate (PP).

compound classes (e.g., terpenes, fatty acid derivatives, alkanes, alkenes, ketones). Flowers from wild *Helianthus* are known to exhibit a high degree of diversity in size, color, and morphology (Mason et al., 2017b), and thus the finding of high diversity in floral volatile profiles indicates complementary chemical diversity that may impact the processes of pollination, florivory, and floral pathogen resistance.

The lack of phenotypic integration between leaf and floral volatile profiles indicates a lack of constraint on the evolution of differential production of secondary metabolites in different organs. In many plant systems, the production of higher concentrations of metabolites in leaves can be associated with increased concentrations in other organs like roots, stems, and flowers (Bezemer and van Dam, 2005; Adler et al., 2006; De Deyn et al., 2009; Kessler and Halitsche, 2009; Parker et al., 2012; Schiestl, 2014; Sampaio et al., 2016; Jacobsen and Raguso, 2018), which may constrain the ability of plants to optimize chemical profiles in different organs in support of divergent functions such as root microbial symbiosis, shoot herbivore defense, and flower pollinator attraction. This constraint may operate at the level of plasticity within individuals (i.e., herbivore-induced defenses), among genotypes or populations within a species, or across lineages of species diversifying under natural selection. The results here for *Helianthus* suggest that this form of constraint is not present with respect to interspecific variation in constitutive secondary metabolism, which can permit the independent optimization of leaf and floral volatile metabolism by natural selection. Whether this is the case requires further inquiry. If this lack of constraint also holds at smaller scales, it would be useful information for breeding cultivated *Helianthus*.

Terpene profile evolution and utility to crop improvement

Across the genus, the three major clades of *Helianthus* have diverged in average leaf terpene levels and relative balance of monoterpenes and sesquiterpenes. Within clades, variation in relative balance appears qualitatively related to factors such as aridity, in parallel to the divergence among the three major clades into warm arid, warm mesic, and cool mesic regions of North America (Mason and Donovan 2015). While ploidy level has been suggested to drive variation in plant secondary metabolism including terpenes (Parsons et al., 2019; Iannicelli et al., 2020), we do not observe consistent differences among closely related species of differing ploidy levels, nor among cytotypes of *H. decapetalus* or *H. divaricatus*. The high evolutionary lability of both total volatile abundance and the relative composition of volatile profiles in both leaves and petals indicates substantial divergence during *Helianthus* diversification, yet the specific drivers of this variation are not yet understood. Future work should examine impacts of volatile metabolism diversity on folivores, florivores, pathogens, and pollinators to determine whether this diversity likely arrives from natural selection from mutualists or natural enemies, or else from a neutral process like genetic drift. Sequencing the many terpene synthase genes across *Helianthus* species is one avenue to identify potential biosynthetic mechanisms underlying the impressive terpene diversity seen here.

The domestication of cultivated sunflower (*Helianthus annuus*) took place approximately five millennia ago in eastern North America (Crites, 1993; Blackman et al., 2011). During domestication and improvement, sunflower underwent significant recurrent population bottlenecks, an increase in seed and oil yield, decrease in overall fitness under stress, and a substantial reduction in genetic diversity (Mandel et al., 2011; Baute et al., 2015; Palmgren et al., 2015; Badouin et al., 2017; Park and Burke, 2020). Given limited research to date, whether diversity in volatile profiles has been reduced during these bottlenecks is unknown. Previous work suggests substantial diversity in leaf terpene profiles in wild populations of *Helianthus annuus* across its native range (Adams et al., 2017), and our findings indicate yet further diversity in the genus-wide tertiary germplasm pool (Kantar et al., 2015). The importance of nonvolatile terpenes in mediating sunflower florivory has been established (e.g., Prasifka et al., 2015), but few studies have explored the role of sunflower volatile terpenes in insect pest resistance (but see Roseland et al., 1992; Wong et al., 2021), or for other biotic interactions like pollinator attraction. Given strong evidence of the importance of volatile profiles in other plant systems (Pichersky and Raguso, 2018; Boncan et al., 2020), an obvious next step is to relate the variation observed across wild *Helianthus* to specific agriculturally important biotic interactions. Should variation in wild *Helianthus* volatile profiles be found to drive more favorable interactions, these crop wild relatives could be leveraged in support of crop improvement beyond the diversity currently present in the cultivated sunflower germplasm. Hundreds of millions of dollars in sunflower annual crop value is attributable to traits already derived from crop wild relatives including disease resistance (Seiler et al., 2017), though insect pest resistance has historically been less straightforward to identify and introgress than pathogen resistance given typically more complex polygenic basis within the genetic background of wild species that is less directly transferrable to *H. annuus*. One of two avenues could overcome this barrier—first, a focus on identifying how underlying volatile chemistry mediates target biotic interactions, and second, a focus on understanding how sequence and expression variation in terpene synthases drive variation in observed volatile profiles. Together, these may be a more fruitful avenue to leveraging crop wild relatives for the improvement of target biotic interactions, permitting a focus on increasing or decreasing the abundance of individual compounds while working wholly within the *H. annuus* genetic background. Identifying the genetic architecture of variation in the expression of individual terpene compounds within cultivated sunflower is achievable with currently available association-mapping resources for sunflower (Masalia et al., 2018; Stahlhut et al., 2021;

Todesco et al., 2022) and thus may permit more straightforward up- or downregulation through either traditional breeding or emerging gene editing methods (Wang et al., 2020; Pan et al., 2022).

CONCLUSIONS

We here demonstrate that the genus *Helianthus* is a useful model system for studying the evolution of plant volatile chemistry, amenable to examination of the evolution of volatile-mediated biotic interactions across habitats and environmental gradients. A low level of phenotypic integration between leaves and petals indicates that vegetative and reproductive organ volatile chemistry has evolved largely independently without major trade-offs or constraints. Weak positive covariation between monoterpene and sesquiterpene abundance indicates that differential biosynthesis of terpene subclasses likely represents a minor resource allocation tradeoff, with overall investment in terpene production likely related to other unmeasured traits. Given the genetic resources available for sunflower and knowledge of the major terpenoid biosynthetic pathways, determining the underlying genetic drivers of volatile profile variation is likely achievable within this system.

AUTHOR CONTRIBUTIONS

C.M.M. and A.R. designed the study. A.R. performed the greenhouse experiment and sampled tissue. S.M., A.L., and J.A.D. prepared samples for analysis and conducted GC-MS. K.B. quantified metabolomic data from raw GC-MS output. K.B., E.W.G., and C.M.M. performed data analysis and created figures. K.B. and C.M.M. wrote the manuscript with input from A.R., A.L., S.M., J.A.D., and E.W.G.

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DATA AVAILABILITY STATEMENT

Data used for this study are included in the supporting information (Appendices S19–S22) as well as the Dryad Digital Repository: https://doi.org/10.5061/dryad.8gtht76sn (Mason et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Wild *Helianthus* accessions used in this study.

Appendix S2. Proportional composition of summary statistics for volatile compounds in leaves and petals of each species.

Appendix S3. Species means for proportional composition of volatile compounds and summary statistics in leaves.

Appendix S4. Species means for proportional composition of volatile compounds and summary statistics in petals.

Appendix S5. Mass-normalized peak areas for 39 major volatile compounds and summary statistics in all individual leaf samples.

Appendix S6. Mass-normalized peak areas for 44 major volatile compounds and summary statistics in all individual petal samples.

Appendix S7. Proportional composition for 39 major volatile compounds and summary statistics in all individual leaf samples.

Appendix S8. Proportional composition for 44 major volatile compounds and summary statistics in all individual petal samples.

Appendix S9. Species mean mass-normalized peak areas for 39 major volatile compounds and summary statistics for leaves.

Appendix S10. Species mean mass-normalized peak areas for 44 major volatile compounds and summary statistics for petals.

Appendix S11. Species mean proportional composition (percentages) for 39 major volatile compounds and summary statistics in leaves.

Appendix S12. Species mean proportional composition for 44 major volatile compounds and summary statistics in petals.

Appendix S13. GLMM correlations among compounds and summary statistics in leaves.

Appendix S14. PGLMM correlations among compounds and summary statistics in leaves.

Appendix S15. GLMM correlation among compounds and summary statistics in petals.

Appendix S16. PGLMM correlation among compounds and summary statistics in petals.

Appendix S17. OLS correlations between leaf and petal mass-normalized peak areas for individual compounds and summary statistics.

Appendix S18. PGLS correlations between leaf and petal mass-normalized peak areas for individual compounds and summary statistics.

Appendix S19. Mass-normalized peak area for volatile compounds and summary statistics in leaves and petals for all individual samples.

Appendix S20. Proportional composition of volatile compounds and summary statistics in leaves and petals of all individual samples.

Appendix S21. Species means for mass-normalized peak area for volatile compounds and summary statistics in leaves and petals.

Appendix S22. Species means for proportional composition of volatile compounds and summary statistics in leaves and petals.

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