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The evolution of floral signals in relation to range overlap in a clade of California Jewelflowers (*Streptanthus* s.l.)

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Because of their function as reproductive signals in plants, floral traits experience distinct selective pressures related to their role in speciation, reinforcement, and prolonged coexistence with close relatives. However, few studies have investigated whether population-level processes translate into detectable signatures at the macroevolutionary scale. Here, we ask whether patterns of floral trait evolution and range overlap across a clade of California Jewelflowers reflect processes hypothesized to shape floral signal differentiation at the population level. We found a pattern of divergence in floral scent composition across the clade such that close relatives had highly disparate floral scents given their age. Accounting for range overlap with close relatives explained additional variation in floral scent over time, with sympatric species pairs having diverged more than allopatric species pairs given their age. However, three other floral traits (flower size, scent complexity and flower color) did not fit these patterns, failing to deviate from a null Brownian motion model of evolution. Together, our results suggest that selection for divergence among close relatives in the composition of floral scents may play a key, sustained role in mediating speciation and coexistence dynamics across this group, and that signatures of these dynamics may persist at the macroevolutionary scale.

KEY WORDS: **Coexistence, contemporary and historical approaches, floral scent, heterospecific pollen transfer, phylogenetic ecology, reproductive character displacement, reproductive interference.**

Angiosperms employ a remarkably diverse array of floral signals to attract or repel pollinators and enemies. The study of floral traits has a long history in evolutionary biology, with many decades of research revealing complex patterns of selection and trait diversity in floral characters (e.g., Darwin 1886; Thompson 2005; Harder and Johnson 2009; Schiestl 2010). Floral traits can induce strong prezygotic isolating barriers between species, and shifts in scent composition (e.g., Galen and Cuba 2001; Okamoto et al. 2015), flower color (e.g., Bradshaw and Schemske 2003; Muchhala et al. 2014), size (Koski and Ashman 2016), and floral shape (e.g., Fulton and Hodges 1999; Galen and Cuba 2001) have all been implicated in promoting reproductive isolation and speciation. Despite the recognition that floral trait evolution has important causes and consequences for speciation and coexistence with close relatives, broad-scale macroevolutionary patterns of floral trait evolution over deep time and sympatry across whole clades remain much less explored. Here, we ask whether macroevolutionary patterns in floral trait evolution and contemporary patterns of species coexistence are consistent with population-level processes hypothesized to shape the tempo and mode of differentiation in floral signals among recently diverged lineages. We integrate contemporary and historical perspectives to investigate

macroevolutionary trajectories of floral shape, color, and scent evolution and patterns of contemporary range overlap with clademates in the clade *Streptanthus* (s.l.), California Jewelflowers (Brassicaceae).

There are several major hypotheses predicting how floral traits may evolve on a macroevolutionary scale. On the one hand, floral phenotypes are hypothesized to evolve rapidly when populations are isolated or encounter new suites of pollinators (Parachnowitsch et al. 2012; Schiestl and Johnson 2013). This mechanism can promote the evolution of prezygotic reproductive isolation, and, if persistent over time, should result in a macroevolutionary pattern in which floral divergence occurs concomitantly with speciation events. Similarly, disruptive or directional selection is expected to drive sexual signal differentiation between closely related lineages when they occur in sympatry, either via character displacement to reduce competition intensity (Dayan and Simberloff 2005), hybridization reduction (via reinforcement) (Emerson and Kolm 2005), or heterospecific pollen transfer (Muchhala et al. 2014; Arceo-Gómez and Ashman 2016; Weber and Strauss 2016). In theory, this process should produce a macroevolutionary signature of increased levels or rates of phenotypic divergence among close relatives that coexist. On the other hand, floral trait convergence (rather than divergence) may be predicted if similar traits in a community positively impact the selective benefit of having those traits (Geber and Moeller 2006; Koski and Ashman 2016). For example, if convergent species share the same pollinators and either facilitate each other in attracting a critical mass of pollinators, are deceptive mimics of rewarding species, or reduce the use of flowers by enemies (e.g., Moeller 2004; Lev-Yadun and Ne'eman 2012). Yet, another hypothesis is macroevolutionary stasis, in which species' floral traits remain relatively stable over large areas and over long periods of time. For example, pollinator fidelity and selection against hybridization may lead to stabilizing selection, which over sustained long periods would be predicted to result in macroevolutionary stasis (Cresswell 1998). Finally, it is important to note that these hypotheses are not mutually exclusive: all of the above processes may operate simultaneously to shape the evolution of different floral traits, at different points in the history of a lineage, or across a variable landscape, calling into question whether a single pattern should be expected to prevail in a detectable manner at the macroevolutionary scale. For example, local ecological conditions, including the presence or absence of close relatives in a community, resource availability, or pollinator variation, may impose selection on floral traits across populations, over time, or over space (e.g., Schemske and Bierzychudek 2001; Thompson 2005; Parachnowitsch et al. 2012).

In light of these hypotheses, we evaluate the macroevolutionary trajectories of floral traits in the Jewelflower Streptanthoid clade, a group of ~54 species that rank among the most diverse in floral signals within Brassicaceae (Al-Shehbaz 2012) and exhibit varying degrees of range overlap in the western United States. Specifically, we ask: (1) What are the major patterns of floral trait variation across Streptanthoids? (2) Do closely related species share similar floral traits (as predicted by their recent common ancestry), or do recently diverged species differ dramatically in floral traits (as predicted by divergence of floral traits at or near speciation)? And, (3) is the degree to which floral traits have diverged from a common ancestor associated with range overlap of close relatives?

We investigated these questions by integrating GC-MS volatile phenotyping, geo-spatial range reconstruction, spectrometer colormetrics, and comparative phylogenetic analyses across 22 species of Streptanthoids spanning the phylogeny. Our results provide evidence for divergent evolution among close relatives and enhanced rates of evolution associated with range overlap for the similarity of floral scent blends, but not for floral size, color, or scent complexity (the number of floral scent compounds). These results suggest that divergent selection on floral scent similarity may play an important role in speciation and coexistence dynamics across this group. Beyond this, our results suggest that different floral traits can support strikingly different hypotheses at the macroevolutionary scale.

Methods

STUDY SYSTEM

The Streptanthoid complex (Brassicaceae) includes mostly annual or short-lived perennial plants in the genus *Streptanthus* and closely related *Guillenia* and *Caulanthus* (Cacho et al. 2014). Streptanthoids typically occupy bare habitats, such as rocky outcrops and dry rocky slopes, with a distribution centered in western North America, and with a large number of species endemic to the California Floristic Province (JMII). Field observations of at least six *Streptanthus* species indicate that they are visited by a variety of pollinators, with bees being some of the most abundant. Daytime observations of sympatric *S. tortuosus/S. diversifolius* (Table Mtn, Butte Co.) populations revealed the two species shared at least two dominant pollinator species: *Bombus vosnesenskii* and *Apis mellifera* (S. Y. Strauss, pers. obs.). Pollinator communities also differed between these species in sympatry: only *S. diversifolius* was visited by a diurnal moth, *Schinia sueta*. Observations of sympatric populations of *S. breweri* and *S. hesperidis* (in Napa Co., and in Knoxville, Napa/Lake Co.) revealed similar patterns: mostly overlapping pollinator species, with some divergence between the two *Streptanthus* species (S. Y. Strauss, unpubl. data). Thus, to date, we understand these species to share many, but not all, pollinator species in sympatry.

TRAIT MEASUREMENTS

We examined flower size, flower color, floral scent complexity (compound richness in the floral blend), and floral scent composition (compound presence, absence, and abundance in the floral scent blend) across 22 species of Streptanthoids that we grew from field-collected seeds in a greenhouse common garden. Seeds were collected from sites across California (Table S1), planted in October of 2014, and grown in normal potting soil amended with sand. The number of replicate populations per species ranged from 1 to 3 (average = 1.5 ± 0.72), with 1–13 individuals per population (average = 5.1 ± 3.04). Floral traits were measured on common garden plants as they emerged in spring and summer of 2015.

FLORAL SCENT

To quantify floral scent, we collected headspace floral scent profiles from all populations grown in a greenhouse common garden using dynamic headspace sampling via a closed-loop striping system over a 24-h period. To achieve sufficient scent concentrations, inflorescences from multiple plants from the same population were grouped together into bouquets (grouping inflorescences of multiple intact potted plants from the same population under a single glass collection beaker). Scents were sampled over a continuous 24-h sample period to capture the full profile of diurnal and nocturnal emissions using Super Q traps (Supelco; Sigma-Aldrich, St. Louis, MO). We sampled the scent of each population an average of 2.9 ± 1.33 times for a total of 97 samples. During each collection period, control samples were collected simultaneously from glass beakers in the same manner as plant samples, but with ambient air in place of plants. Volatiles were eluted from traps using 400 μL of hexane and samples were stored at –20°C until analysis.

Gas chromatography/mass spectrometry (GC/MS) was conducted at the University of California, Davis, using a 7890B Agilent GC fitted with a 30 m × 0.25 mm × 0.25 lm HP-5 Ultra Inert column coupled to an Agilent 5977A mass spectrometer (Agilent Technologies). We injected 1 μL into the gas chromatograph using a splitless mode and an autosampler. Oven temperature was held at 60°C for 3 min and then increased by 10°C/min until it reached 300°C, after which it was kept at 315°C for 1 min. Both injector and transfer line temperatures were kept constant at 250°C. Helium served as the carrier gas with a constant flow rate set to 1.2 mL/min. Electron impact mass spectra were obtained by scanning between 30 and 550 *m/z*.

GC-MS data were processed using MassHunter GC/MS Acquisition software version B.07.00 (Agilent) and MSD ChemStation Enhanced Data Analysis Software version F.01.00 (Agilent) to register chromatogram peaks. Automatic peak integration was conducted using the RTE integrator in the software Chemstation version E.02.00 (Agilent Technologies) set to the automatic minimum-area detection threshold. Peak alignment was based on retention times. Corresponding spectra of individual peaks were saved in a user-built mass-spectral library. Although our downstream analyses were agnostic to external compound identification, we assigned tentative identifications to compounds by comparing spectra and retention indices against published databases (Adams 2007; NIST 2011 and Wiley 275). In several cases, peaks displayed strong matches to common green leaf volatiles or to synthetic compounds that did not appear in controls. To be conservative, we report results with these compounds removed. However, removal had no qualitative bearing on any analysis. We further identified contaminant compounds as those present in ambient air controls, which were run simultaneously with normal samples using identical procedures but no plants. Contaminant compounds were removed from all samples and analyses, regardless of which run they were found in.

After removing contaminants, peak areas (representing integrated MS ion currents) were standardized to relative peak contributions to overall composition (percentages). We compared within- and between-species Bray-Curtis dissimilarity in scent profiles based on the square root transformed peak areas using a mantel test in the R (R Development Core Team 2017) package "vegan" (Oksanen et al. 2017) using 1000 permutations. There was considerable variation in scent profiles across populations, as might be predicted from differences in abiotic and biotic conditions among populations of a given species, but overall intraspecific scent distance was significantly lower than interspecific comparisons (Mantel $r = 0.057$, $P = 0.011$, mean intra-specific Bray–Curtis distance of 0.52, interspecific mean of 0.67). We created a species-level dataset by averaging relative peak areas (integrated MS ion currents) for each compound across multiple samples of each species. We analyzed scent in two ways: (1) complexity, the total number of compounds in each species' scent profile, also known as scent richness, and (2) composition, the square root transformed species averages of standardized peak areas (a measure of the relative amount and identity of the compounds present). For distance-based analyses, we calculated a scent dissimilarity matrix using the Bray–Curtis index of dissimilarity between each species pair using floral scent composition data. For scent complexity, we calculated a Euclidean distance matrix of the absolute difference in the total number of compounds in each species' scent.

FLOWER COLOR

Because flower color is an important signal mediating pollinator choice, and because bees are dominant pollinators of Streptanthoid species, we used a spectrometer approach to quantify sepal coloration (one of the most conspicuous organs of the Streptanthoid flower) in *Apis melifera* visual color space. We sampled the color of each species an average of 5.5 (min 3–max 11) times

for a total of 99 samples. We lacked color samples from four species in our dataset (Table S3), which were thus omitted from all analyses involving color. We measured sepal reflectance spectra from 300 (UV) to 750 (IR) nm. Scans were conducted using an Ocean Optics USB2000 spectrometer and a deuterium/tungsten halogen light source (Ocean Optics, Dunedin, FL) with a 3-s integration time and boxcar of 12 and averaged three independent scans per sample. True black and true white control references were scanned before each sample. Color data were processed using SpectraSuite (Optic 2009, Ocean Optics, Dunedin, USA) software. Spectra were normalized to their minimum values from 300 to 700 nm using the "procspec" function in the R package "pavo" (Maia et al. 2013). We compared within- and between-species distance in Maxwell chromatacity color space according to the *Apis mellifera* visual system using the "maxwell" function in the R package "colsci" (Maia et al. 2013; White 2017) and a mantel test in the package "vegan" (Oksanen et al. 2017) using 1000 permutations. Population-level variation in color profiles was present in our samples, as predicted from differences across abiotic and biotic communities, but overall intraspecific color distance was significantly lower than interspecific comparisons (Mantel $r = 0.105$, $P = 0.001$). Individual spectra were then averaged to achieve a species-level dataset using the "pavo" function "aggspec," and species pairwise distances in Maxwell chromatacity color space were calculated according to the *Apis mellifera* visual system as above.

FLOWER SIZE

Flower size can be an important trait influencing pollinator visitation (Harder and Johnson 2009). We measured floral size on three fully expanded flowers haphazardly chosen from the fully mature flowers at mid-height of mature inflorescences. Floral size was estimated as the mean sepal length (measured as the distance between the sepal base to the tips of the sepals) multiplied by mean floral width (measured as the petal length from the lateral view of the flower) of these three flowers. We measured flower size on an average of 5.94 ($\pm$4.05) individuals per species (Table S3). We lacked floral size measurements on several species for which we had scent data (Table S3). However, because our measurements of flower size were meaningfully correlated with those reported in the Jepson Manual ($F_{1,10} = 10.24$, $r = 0.71$, $P = 0.009$), we supplemented flower size data from the Jepson manual for those species. For all distance-based analyses using flower size, we calculated squared Euclidean pairwise distance in flower size for each species pair.

RANGE OVERLAP

We calculated species ranges from geo-referenced occurrence data compiled from the Global Biodiversity Information Facility (GBIF; http://data.gbif.org). We compiled data using the function "gbif" in the dismo R library and the California Consortium of Herbarium, using the "getConsortium" function in the R library "Jepson." Record curating consisted of removing duplicate coordinate records, coordinates that lacked subdegree resolution, and extreme outliers. Records with dubious distributions were double checked against herbarium specimens or confirmed by collectors and expert opinion. Coordinates were mapped separately for each species and checked by hand for any obviously erroneous records using the packages "sp" (Bivand et al. 2008) and "rgdal" (Bivand et al. 2015). This resulted in a total of 5970 records, with the mean number of records per species being 106.6 (median 49, maximum 928). We estimated the geographic range for each of our 22 focal species represented by at least five collection records. Ranges were calculated by placing a 10 km buffer around each collection location and then merging all overlapping areas to construct a range. The buffer method is more conservative than hull methods, which by enclosing all collection records in a polygon, can include large amounts of habitat that are potentially never used by the species. Buffers were placed and merged using the "gBuffer" and "joinPolys" functions in the "rgeos" and "PBSmapping" libraries, respectively. Range overlap was calculated for each species pair, and species pairs were classified as either sympatric (area occupied by both species > 0) or allopatric (area occupied by both species $= 0$).

PHYLOGENETIC COMPARATIVE ANALYSES

For all phylogenetic analyses, we used phylogenetic hypotheses from Cacho et al. (2014). Analyses were run on 1000 trees randomly drawn from the posterior distribution, or in cases where a single tree was required, a majority-rule consensus tree that was constructed using the full postburnin distribution and consensus branch lengths calculated using the least squares method via the "consensus.edges" function in the package "phytools" (Revell 2012).

For each of the four floral traits (floral size, color, scent composition, and scent richness), we asked whether trait disparity between species pairs was predicted by the phylogenetic relatedness of those species using a disparity through time (DTT) framework (Harmon et al. 2008). For each trait, the expected Brownian Motion DTT was simulated using 1000 simulations on each of 1000 trees in the posterior distribution. We assessed significant deviations from the Brownian expectation using the morphological diversity index (MDI) statistic (Harmon et al. 2003), a measure of the area between the mean observed and simulated DTT across the 1000 trees with the "dtt" function in the R package "geiger" (Harmon et al. 2008). A positive MDI reflects a pattern where closely related species are more divergent than the Brownian Motion expectation, and a negative MDI reflects a pattern where closely related species are more similar than expected. Significance of the MDI (interpreted as a significant deviation from

the Brownian expectation) was assessed according to the 95% confidence interval of the 1000 simulations run on each of the 1000 trees for each trait. Next, we tested whether species pairs with range overlap had a higher net rate of floral trait evolution (pairwise trait dissimilarity / phylogenetic distance) than allopatric ones. We assessed significance using a simulation-based approach: for each trait, we compared the observed difference in the net rate of trait evolution between sympatric and allopatric pairs to a distribution of differences generated from 1000 Brownian motion simulations of that trait on each of 1000 trees in the posterior distribution. This approach has the benefit of holding the tree and biogeographic distribution constant while generating a null distribution of trait differences under a Brownian Motion expectation.

Finally, because signal complexity is predicted to impact signal specificity (Schaefer and Ruxton 2015), we also asked whether scent complexity (the number of compounds in a species' floral blend) correlated with external factors hypothesized to impact the evolution of specific signals. We used a phylogenetic generalized least squares (PGLS) framework to evaluate whether the evolution of floral scent complexity is correlated with flower size, range size, or the number of overlapping clade-mates in a given species' range, using the phylo.pls function in the package "geomorph."

Results

For floral scent, we found a total of 53 compounds across our 22 focal species (Fig. 1, Table S2). These compounds represented a diversity of classes including terpenes, benzenoids, alcohols, and esters. Most of the compounds had high database match scores to common floral compounds (Table S2). Scent richness, or the number of distinct compounds found in the floral scents of a given Streptanthoid species (scent complexity trait) ranged from 0 to 13 detectable compounds (mean 4.41 $\pm$ 3.78). Flower size ranged from 0.2 to 1.5 cm^2 (mean 0.63 $\pm$ 0.31, Fig. 1). Flower color occupied a substantial portion of bee visual space, ranging from greens to purples in RGB human visual space (Figs. 1 and S1).

TEMPO AND MODE OF FLORAL TRAIT EVOLUTION

DTT analyses revealed a pattern where the evolution of floral size, flower color, and scent complexity (number of compounds in a species' blend) was consistent with a Brownian motion model of trait evolution over the evolutionary history of Streptanthoids (Fig. 2A–C). In contrast to these traits, scent dissimilarity deviated significantly from a Brownian motion null. Scent similarity displayed significantly higher DTT than Brownian motion evolution, reflecting a pattern where closely related species tended to have dissimilar scent blends, and only distantly related clade-mates displayed high scent similarity (Fig. 2D). The MDI statistics also reflected these results: DTT did not deviate significantly from the Brownian expectation for flower size (MDI = 0.23 $\pm$ 0.06, $P = 0.12 \pm 0.06$, Fig. 2A), scent complexity (MDI = 0.22 $\pm$ 0.10, $P = 0.14 \pm 0.07$, Fig. 2B), or flower color (MDI = 0.29 $\pm$ 0.07, $P = 0.1 \pm 0.11$ Fig. 2C). But DTT for floral scent composition was significantly higher than the Brownian expectation throughout the evolutionary history of the clade (MDI = 0.41 $\pm$ 0.05, $P < 0.001 \pm <0.001$, Fig. 2D).

Accounting for contemporary range overlap with clade-mates explained additional variation for floral scent composition, but not for flower size, scent complexity, or flower color (Fig. 2E). Sympatric and allopatric species pairs experienced similar amounts of divergence per unit time for flower size ($\mu_{sym\text{-}allo} = 0.04 \pm 0.006$, $P = 0.24 \pm 0.03$), scent complexity ($\mu_{sym\text{-}allo} = 0.5 \pm 0.12$, $P = 0.33 \pm 0.05$), and flower color ($\mu_{sym\text{-}allo} = 0.024 \pm 0.005$, $P = 0.25 \pm 0.03$), but sympatric species pairs had more divergent scent profiles per unit time compared with allopatric species for scent composition ($\mu_{sym\text{-}allo} = 0.14 \pm 0.02$, $P < 0.001 \pm <0.001$). No additional variation in floral scent complexity, a measure hypothesized to impact signal specificity (Schaefer and Ruxton 2015), was explained by the additional factors tested (Fig. S2). PGLS analyses revealed no relationship between scent complexity and any of the factors we examined: flower size (across 1000 trees: mean $F = 0.72 \pm 0.31$, $P = 0.42 \pm 0.11$, Fig. S2A), range size ($F = 0.11 \pm 0.07$, $P = 0.74 \pm 0.08$, Fig. S2B), or the number clade-mates a species overlaps with in range (0.19 ± 0.08, $P = 0.67 \pm 0.06$, Fig. S2C).

Discussion

A major challenge in biology is translating the complexity and variability of interspecific interactions at local scales (e.g., Thompson 2005) to an understanding of when and how biotic interactions shape macroevolutionary patterns of trait evolution across whole clades (Jablonski 2008). Here, we asked whether processes hypothesized to shape floral trait evolution at the population-level correspond to detectable signatures at the macroevolutionary scale across the California Jewelflowers (*Streptanthoids*). For floral scent composition (one out of the four floral traits we examined), we found patterns consistent with both high trait disparity early in the divergence of lineages and enhanced divergence associated with contemporary range overlap. However, this was not the case for all floral characters: floral size, scent complexity, and flower color did not deviate significantly from a null Brownian motion model of evolution. Together, our results are consistent with scent divergence having been an important factor mediating speciation and coexistence dynamics across this group, leaving a signature on macroevolutionary patterns of scent diversity across the clade. More generally, our data illustrate that different floral traits can display strikingly different evolutionary trajectories across a clade.

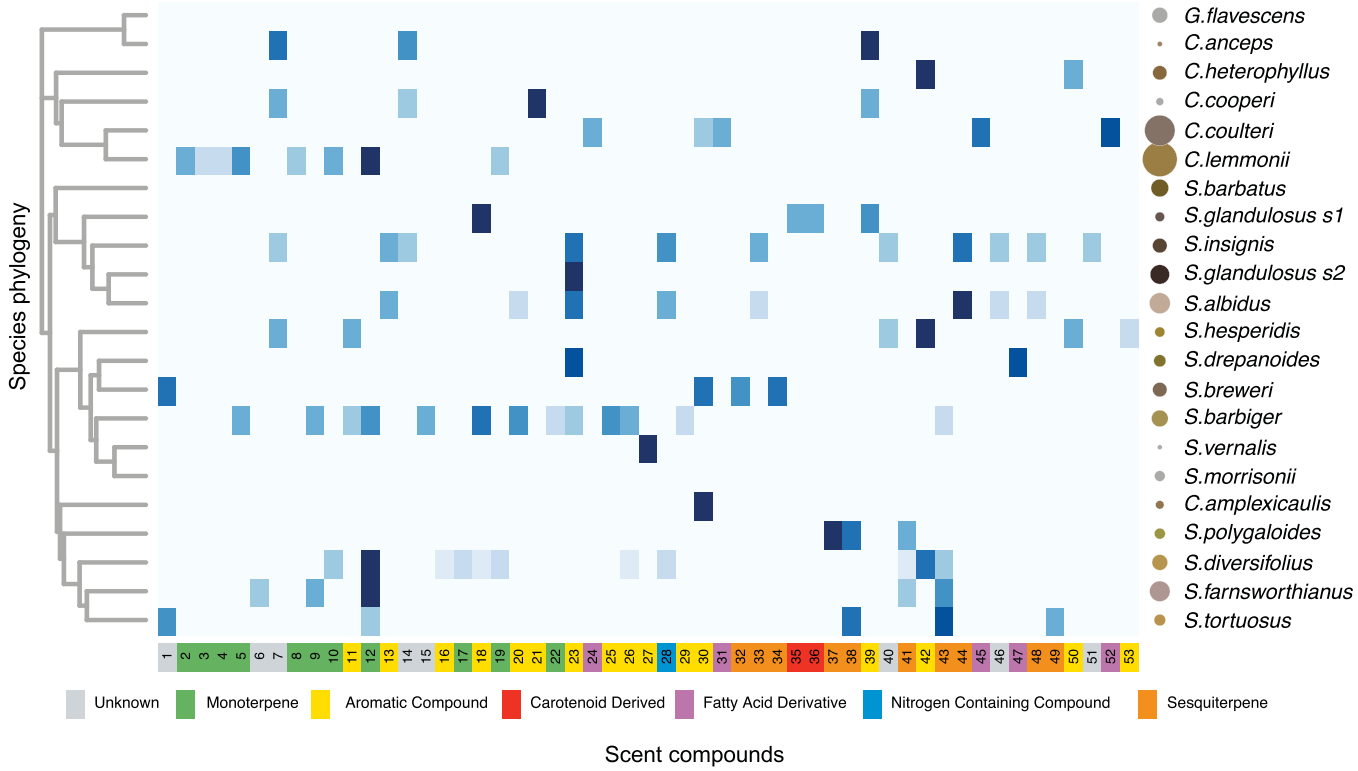

Figure 1. **Floral data and phylogenetic relationships of Streptanthoid species. Phylogeny depicted to the left represents the pruned majority rule consensus tree of the posterior distribution from Cacho et al. (2014). Scent compounds are sorted by retention time (increasing from left to right, see Table S2 for more detailed compound information). Shadings of internal blue squares represent the square root relative abundance of a given compound in the blend. The size of the circles to the right of the heatmap is proportional to flower size. The color of the circles (except for species depicted in gray, for which we lacked color spectrometer data) represents the RGB representation of the species-averaged flower color spectra created using the "spec2rgb" function in the "pavo" R package.**

Decades of work have shown that floral scent is a complex trait that can have direct repercussions on species interactions and reproductive isolation (reviewed in Raguso 2008). Research across scales is now accumulating supporting this ecologically important phenotype as a trait with striking capacity for rapid evolution. Within species, floral scent can diverge markedly over very short spatial and temporal scales with repercussions for pollinators and reproductive isolation of plants (e.g., Parachnowitsch et al. 2012; Gottsberger et al. 2013; Friberg et al. 2014; Whitehead and Peakall 2014). Recent experimental evolution work has shown that divergent pollinator selection can cause evolutionary divergence in floral scent phenotypes in just a few short generations in the laboratory (Gervasi and Schiestl 2017). Our finding that floral scent composition is highly disparate among recently diverged species compared to other floral traits at a macroevolutionary scale suggests that these microdynamics may translate to patterns of high-scent diversity within and across clades. In particular, our results support the hypothesis that scent composition is either less constrained than flower shape and color (e.g., due to relative amounts of heritable variation), exposed to stronger selection (e.g., Gross et al. 2016), or both. Perhaps due in part to the technical aspects of its quantification, the evolution floral scent has traditionally received less attention in a phylogenetic framework compared to other floral traits, such as floral color and shape (e.g., Gómez et al. 2006; Harder and Johnson 2009). However, other studies that have investigated the evolution of scent profiles using phylogenies have generally found scent to be a complex and highly variable trait across closely related taxa (e.g., Azuma et al. 1999; Prieto-Benítez et al. 2016), consistent with our results.

Although results from this and other scent-based research paint a picture of scent as phenotypes with high disparity among close relatives, the underlying pathways that produce these phenotypes may be more constrained. Interestingly, while we found high disparity in compound blends, compound richness adhered to a Brownian motion pattern of evolution. This finding (that close relatives tended to have similar numbers of compounds, but different compound identities) could reflect a scenario where relatively small changes in pathways result in turnover in the identity, rather than number, of compounds produced. Scent dissimilarity in this study was measured without taking into account similarity in the structure of nonshared compounds (as is the common practice in the field), but recent innovation in the development of methods

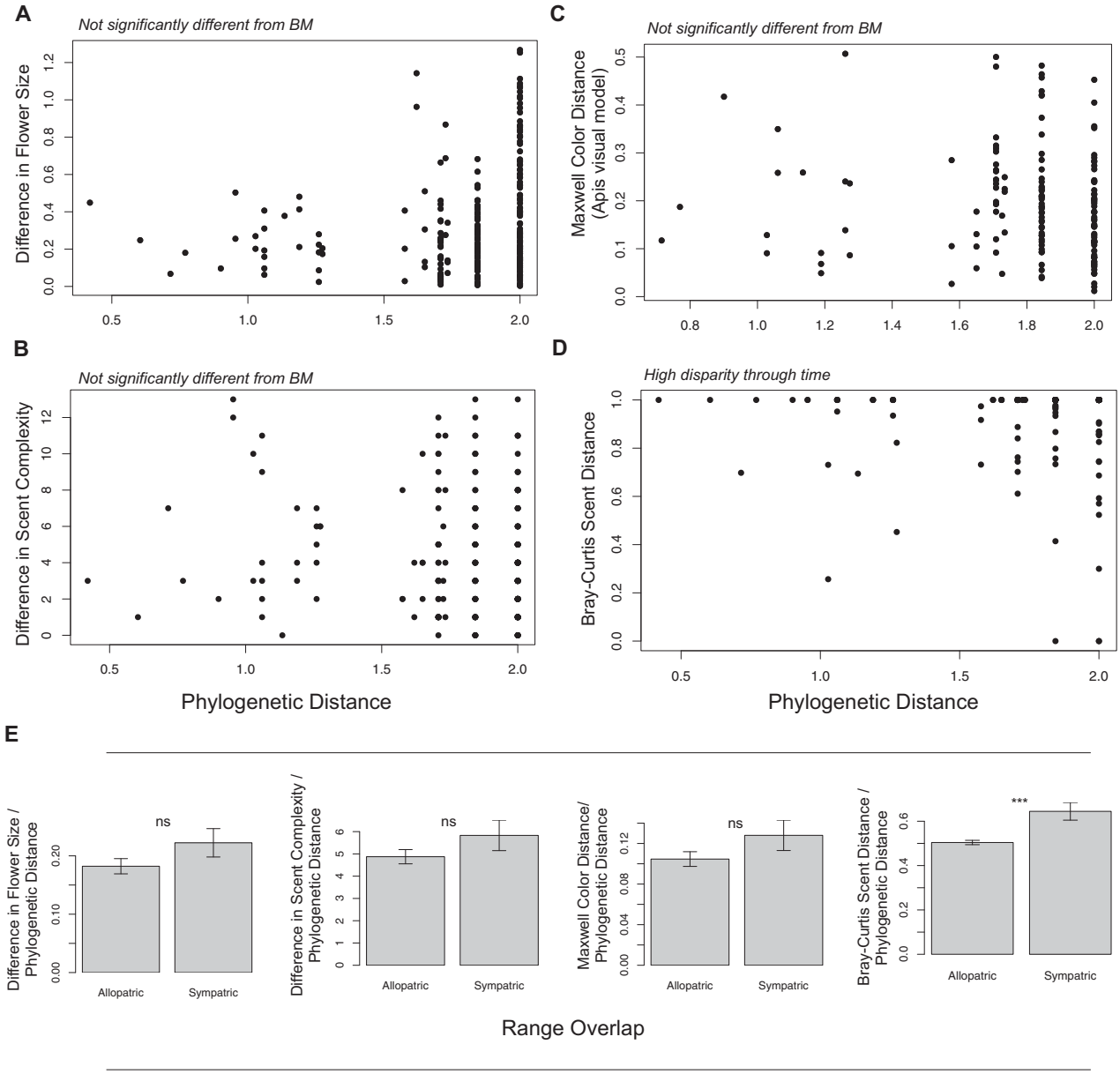

Figure 2. **The relationship between floral trait similarity, phylogenetic distance (based on the consensus phylogeny), and sympatry across streptanthoids for floral traits in this study. (A–D) Pairwise trait distance plotted against phylogenetic distance, each dot represents a pairwise comparison of two species for flower size (A), flower scent complexity (B), flower color (C), and flower scent composition (D). Significant deviations from patterns expected under Brownian motion were determined through DTT simulation tests. Flower size, numbers of scent compounds, and flower color did not deviate from the Brownian expectation, but close relatives displayed higher than expected disparity in floral scent composition. (E) The relationship between sympatry and floral trait evolution. Bars represent the means and SEs for the amount of trait divergence per unit branch length for allopatric and sympatric species pairs. Sympatric pairs were more divergent than expected given their age than allopatric pairs for floral scent composition, but not for flower size, floral scent complexity, or flower color.**

to quantify chemical structural similarity across species (Sedio 2017; Sedio et al. 2017) present exciting opportunities to tackle these questions in future work. The development of multivariate models of trait evolution for dealing with chemical (or chemical-like) data is also needed if we are to accurately decipher nuanced patterns.

To our knowledge, this study is the first to test for a relationship between contemporary coexistence among close relatives and the divergence rate of floral scent profiles across a phylogeny. We found that closely related species with range overlap had more divergent scents than expected given their age compared to species pairs with nonoverlapping ranges. Numerous studies

have documented divergence among sympatric close relatives in floral characteristics. For example, sympatric species of *Mitella* diverge strongly in scent and have no overlap in their specialized gnat pollinators (Okamoto et al. 2015); a study of hummingbird pollinated plants found that flower color is selected to diverge in sympatry, owing to competition for hummingbird pollinators (Muchhala et al. 2014); and across 41 sister-species pairs in the Cape flora region of South Africa, range overlap was associated with pollination system and edaphic shifts (van der Niet et al. 2006).

Although less frequently investigated, co-occurrence has also been hypothesized to drive convergence, rather than divergence, in floral traits (e.g., Moeller 2004; Koski and Ashman 2016). However, we found no evidence of convergence of floral traits in our study, as close relatives with range overlap had more dissimilar (rather than more similar) scent profiles than expected given their age.

Scent phenotypes could drive divergence and coexistence among close relatives if pollinators display scent fidelity in Streptanthoids (see Oyama et al. 2010, e.g., with floral color in sister species of *Antirrhinum*). Pollinator observations have revealed that sympatric *Streptanthus* species can share some, but not all, pollinator species (see methods); moreover scent differences among species could lead to pollinator constancy within foraging bouts. Behavioral studies of pollinators using scent arrays are one way forward in experimentally testing these ideas. Finally, it is important to remember that while we found a significant relationship between scent divergence and contemporary range overlap, the causal directionality of this relationship remains unknown, as both divergent evolution of floral traits in situ and environmental filtering based on local community composition could produce this pattern. In other words, additional work is needed to distinguish between a scenario in which coexistence of close relatives drives floral scent divergence (i.e., character displacement or reinforcement), and a scenario in which close relatives that have diverged more than expected in their floral scent for their age more commonly coexist when they come together upon secondary contact.

A natural follow-up to this research would be to compare the patterns of divergence we documented across species to detailed quantifications of divergence within species in relation to sympatry (micro-to-macro approach). Although we had some replication within and across populations for the 22 species included in our study, replication was relatively low. Thus, the design of our study did not allow for the robust quantification of within species variation in scent profiles (a common caveat of macroevolutionary work). However, our limited sampling did reveal that, while scent divergence between species was greater than that within species, there was still substantive variation across populations of the same species. Work in other systems at the population scale demonstrates that scent can vary considerably intraspecifically and can be shaped by selection from very local and distinct pollinator and enemy communities or abiotic environmental conditions at very local scales (e.g., Moeller 2004; Soler et al. 2011; Delle-Vedove et al. 2017). Yet, species often have characteristic blends that can be detected even in the face of sources of substantive intraspecific variation (Gross et al. 2016). For example, recent studies in well-sampled species of *Zamia* and *Macrozamia* showed high species specificity of scents despite variation among populations (Suinyuy et al. 2013). This pattern also holds among famously divergent *Lithophragma* populations and species (Soler et al. 2011; Suinyuy et al. 2013; Friberg et al. 2014). Additional work at the population and sister-species scales is needed to understand the degree of intraspecific variation in scent profiles, and the processes that shape inter- and intraspecific patterns of scent diversity.

Flower color is another trait that has received attention as important for species divergence (e.g., Schemske and Bierzychudek 2001; Bradshaw and Schemske 2003; Hopkins and Rausher 2012; Grossenbacher and Stanton 2014; Muchhala et al. 2014). Intraspecific variation in floral color is also present in Streptanthoids, with many species having cream/green yellow versus lavender/purple polymorphisms across populations, but monomorphic sepal color within populations. These polymorphisms might reflect selection by pollinators, but evidence also suggests a potential role for abiotic factors as well. In *Streptanthus polygaloides*, color morph variation has been linked to elevation and soil chemistry (Pope et al. 2014), although it is unclear whether soil or elevation are also linked to pollinator community shifts. However, purple morphs are also more common on serpentine soils in *Collinsia sparsiflora* (Scrophulariaceae) (Wright and Stanton 2011), and in *Leptosiphon androsaceus* and *L. parviflorus* (Polemoniaceae) (Kay et al. 2011; O'Dell and Rajakaruna 2011). Thus, local-scale selection may have a strong influence on the evolution of floral trait variation in Streptanthoids as well. Analyses at the population level will help our understanding of local dynamics in flower color and their relation to intraspecific patterns of divergence.

Generally speaking, reconciling patterns of species-specificity, macroevolutionary trends, and trait divergence among populations in signaling traits remain a major challenge in the study of reproductive signaling. Future studies evaluating how and when variation across populations shapes evolutionary patterns across species would build on the work presented here, and inform our greater understanding of links between local microevolutionary processes and broad-scale macroevolutionary patterns.

AUTHOR CONTRIBUTIONS

MGW, SYS, NIC, and SR conceived of the project; MGW wrote the first draft of the manuscript; MGW and NIC analyzed the data, with input

from SYS; MGW, MIQP, SRR, and CP collected the data. SYS, NIC commented on draft.

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DATA ARCHIVING

Data for this paper have been archived at Dryad under https://doi.org/10.5061/dryad.9mc8j51.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Species plotted according to the first two vectors of a principal coordinates analysis (PCoA) of a Bray Curtis dissimilarity matrix of scent chemistry.

Figure S2. Species location in Maxwell chromatacity color space according to the *Apis mellifera* visual system.

Figure S3. We found no significant relationship between scent complexity (the number of compounds in a species' floral blend) and three external factors hypothesized to impact the evolution of specific signals: flower size, range size, or the number of overlapping clade-mates in a given species' range.

Table S1. Specimen collection information for seeds used in this study.

Table S2. Compounds present in floral scent extracts.

Table S3. Presence/absence character matrix for each type of data for each species in the dataset.