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Focused Topic

Emission Rates of Species-Specific Volatiles Vary across Communities of *Clarkia* Species: Evidence for Multimodal Character Displacement*

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abstract: A current frontier of character displacement research is to determine whether displacement occurs via multiple phenotypic pathways and varies across communities with different species compositions. Here, we conducted the first test for context-dependent character displacement in multimodal floral signals by analyzing variation in floral scent in a system that exhibits character displacement in flower size and that has multiple types of sympatric communities. In a greenhouse common garden experiment, we measured quantitative variation in volatile emission rates of the progeny of two species of *Clarkia* from replicated parental communities that contain one, two, or four *Clarkia* species. The first two axes of a constrained correspondence analysis, which explained 24% of the total variation in floral scent, separated the species and community types. Of the 23 compounds that were significantly correlated with these axes, nine showed patterns consistent with character displacement. Two compounds produced primarily by *C. unguiculata* and two compounds produced primarily by *C. cylindrica* were emitted in higher amounts in sympatry. Character displacement in some volatiles varied across sympatric parental communities and occurred in parallel with displacement in flower size, demonstrating that this evolutionary process can be context dependent and may occur through multiple pathways.

Keywords: allopatry, sympatry, floral traits, context-dependent selection, floral scent.

Introduction

Interspecific interactions have long been hypothesized to have significant effects on patterns of biodiversity (Darwin 1859; Lack 1945; Schluter 2000; Grant and Grant 2008). One such evolutionary process is character displacement, in which interspecific interactions where species co-occur in sympatric communities lead to a pattern of differences in a given species' trait values in sympatric communities relative to allopatric communities (Brown and Wilson 1956; Germain et al. 2017; fig. 1). We stress that character displacement refers to trait change within a species across communities and that displacement of a given species' trait value in sympatry can result in greater differences between two species (e.g., divergence, the classic pattern associated with character displacement) or greater similarity between two species (e.g., convergence; see Grant 1972; Abrams 1986; Germain et al. 2017; see fig. 1). While character displacement has been studied and debated for more than 60 years (Stuart and Losos 2013), there are two key gaps in our understanding of this process. First, outside of a small number of classic systems (e.g., anoles, sticklebacks, Darwin's finches), few studies have examined the potential for character displacement in more than one type of trait (reviewed in Stuart and Losos 2013). Examining variation in multiple types of traits increases our ability to detect nonrepeatable character displacement, which may occur through different phenotypic pathways (e.g., change in different single traits or in combinations of traits) across communities (Losos 2011; Germain et al. 2017), and determine when species interactions lead to shifts in correlated or independently evolving traits. Second, while most studies of character displacement have focused on pairwise interactions (but see Lemmon and Lemmon 2010; T. E. Miller et al. 2014; Grant 2017; Roth-Monzon et al. 2020), many species

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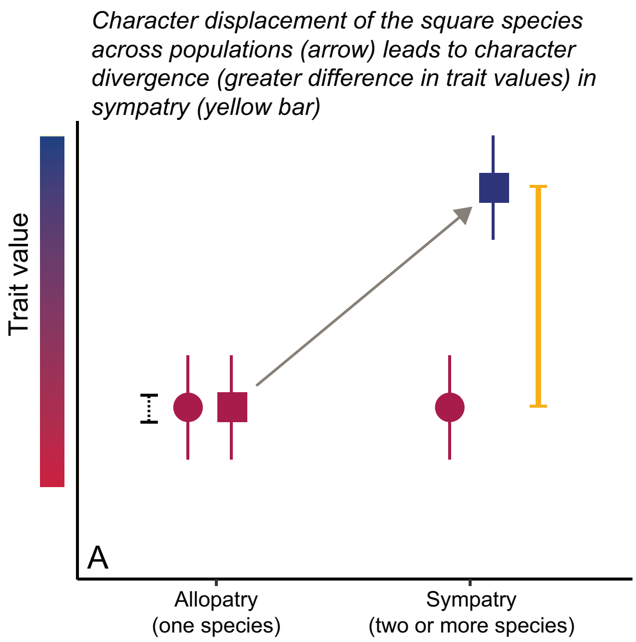

Figure 1: Schematic representation of character displacement, which can lead to character divergence (*A*) or character convergence (*B*). Each panel depicts the trait values of two species (depicted as circles and squares) in two types of communities: allopatry, where each species occurs alone, and sympatry, where the two species co-occur and where additional species may also occur. Character displacement refers to the evolution of trait differences within a species across populations. In both panels, the square species exhibits character displacement, with a red trait value in allopatry and a blue trait value in sympatry (*A*) or the reverse (*B*). This is indicated with a gray arrow in both panels. This character displacement of the square species can result in character divergence if the difference between the two species is greater in sympatry (yellow difference bar in sympatry in *A*, in contrast to the black dotted bar in allopatry, which indicates a difference that is equivalent to zero). Alternatively, character displacement can result in character convergence if the difference between the two species is smaller in sympatry (blue dotted difference bar, denoting a difference that is equivalent to zero, in sympatry in *B*, in contrast to the black bar in allopatry). These schematics depict situations where only one species is displaced, but displacement may occur in one or both species depending on the underlying species interactions in sympatry. The same aesthetics (arrows and colored bars) are used to indicate character displacement and convergence or divergence in figures 2 and 3.

exist in complex ecological communities, where interactions with multiple species could include indirect and higher-order interactions (Mayfield and Stouffer 2017; TerHorst et al. 2018; Roth-Monzon et al. 2020). Testing for character displacement across sympatric communities that vary in species composition or richness (Eisen and Geber 2018; Roth-Monzon et al. 2020) can advance our understanding of the evolutionary consequences of direct and indirect interactions (Walsh 2013; TerHorst et al. 2015). Specifically, communities with more than two species provide an opportunity to test whether additional interspecific interactions affect patterns of character displacement on two focal species.

Among co-occurring flowering plants, pollinators often represent a shared resource that is critical for reproduction (Waser et al. 1996; Ollerton et al. 2011), and there is a growing body of evidence for character displacement in plants mediated by interactions between co-occurring species that share pollinators (reviewed in Beans 2014; Eisen and Geber 2018). Presently, there are two critical gaps in our understanding of this process. First, studies to date have examined character displacement in floral morphology, color, and phenology (reviewed in Beans 2014; Eisen and Geber 2018), which reflects a general bias toward visual traits in pollination (Raguso 2008*a*). Nonetheless, olfactory and reward traits are critical to successful pollination in many systems (Schiestl 2010, 2015; Raguso 2014) but have yet to be integrated into the study of character displacement. Second, character displacement in floral traits is likely to occur via multiple phenotypic pathways or changes in trait combinations across communities (Losos 2011; Germain et al. 2017). Nonetheless, most studies to date have addressed character displacement in one type of floral trait (e.g., color or morphology), not the multimodal bouquet flowers typically present (Leonard et al. 2011).

The emission of floral scent—volatile organic compounds including monoterpenes, sesquiterpenes, and aromatic compounds (Knudsen et al. 2006)—is a complex trait in that individual plants can exhibit qualitative variation in the blend of volatiles and quantitative variation in their emission rates (Raguso 2008*b*). Because scent can be produced not only from petals but also from reproductive floral structures, scent may be correlated with or unrelated to variation in flower size (Effmert et al. 2006; Valdivia and Niemeyer 2006; Burdon et al. 2015; Martin et al. 2017), which could lead to multimodal character displacement in some systems. In addition, species may vary in common volatiles that are produced by a small number of biosynthetic pathways (Dudareva and Pichersky 2006) and in species-specific volatiles that provide "private channels" for communication with specialist pollinators (Raguso 2008*b*; Soler et al. 2010) or promote pollinator specificity in a multispecies community (Wright and Schiestl 2009; Larue et al. 2016). Insights from three areas of floral scent research suggest that floral scent could undergo character displacement. First, floral scent exhibits substantial intraspecific variation across populations in multiple systems, including cacti (Schlumpberger and Raguso 2008), cycads (Suinyuy et al. 2012), saxifrages (Friberg et al. 2019), and orchids (Gross et al. 2016; Chapurlat et al. 2018). These patterns suggest that floral scent may be relatively evolutionarily labile. As a result, scent could evolve in response to geographic variation in selection (Gross et al. 2016), which could lead to variation in character displacement across different communities (Germain et al. 2017; Eisen and Geber 2018). Second, floral scent can be a target of pollinator-mediated selection (Parachnowitsch et al. 2012; Chapurlat et al. 2019), which indicates that floral scent could evolve in response to interactions between co-occurring plant species that share pollinators. Third, differences in floral scent can mediate reproductive isolation between co-occurring species (Waelti et al. 2008; Bischoff et al. 2014; Peakall and Whitehead 2014) and explain variation in the structure of plant-pollinator networks (Junker et al. 2010; Larue et al. 2016; Kantsa et al. 2018, 2019). As such, floral scent may determine how pollinators are partitioned among co-occurring plant species (Wright and Schiestl 2009).

In this study, we test for variation in multimodal character displacement across sympatric communities that contain different numbers of co-occurring species. Specifically, we assess the potential for character displacement in the floral scent of co-occurring species of California native annuals in the genus *Clarkia* (Onagraceae) in the southern foothills of the Sierra Nevada (Kern County, CA). Across the sympatric and allopatric communities in this region, we detected no differences in a number of abiotic factors, including soil properties (nutrient content, moisture content, particle size), environmental properties (slope, aspect), and climate (temperature and precipitation, via data from local weather stations; K. E. Eisen and E. M. Goud, unpublished data). The lack of discernible abiotic environmental differences across communities suggests that spatial variation in traits does not result from confounding abiotic environmental variation. The four outcrossing *Clarkia* species in this region flower concurrently (see "Study System"), have similar floral diameters (Eisen and Geber 2018), and share pollinators, which are primarily solitary bees (Lewis 1953; MacSwain et al. 1973; Singh 2014). These species also exhibit patterns of trait variation that are consistent with character displacement. Where they co-occur, *C. unguiculata* and *C. cylindrica* have converged in flowering time and diverged in flower size (Eisen and Geber 2018). Similarly, *C. speciosa* and *C. unguiculata* have also diverged in flower size, while *C. speciosa* and *C. xantiana* have converged in flowering time in some sympatric communities (Eisen and Geber 2018). These results point to the potential for multimodal character displacement in these species.

While all four *Clarkia* species that occur in the region are nearly unscented to the human nose, scents that are barely perceptible to humans can affect pollinator attraction (Ashman et al. 2005; Cook et al. 2005; Parachnowitsch et al. 2012). A pilot study of these *Clarkia* species (detailed in app. 1; apps. 1–3 are available online) found that all four species emit volatiles that were detectable by gas chromatography mass spectrometry (GC-MS). Specifically, species profiles were composed of common monoterpenes and sesquiterpenes but were somewhat separated by nonmetric multidimensional scaling. These results suggest that floral scent could play a role in pollinator attraction in *Clarkia*, although the functions of the entire floral scent blend and specific compounds are as yet unknown. Given that *Clarkia* communities are often quite dense, volatiles that are emitted by multiple species—the majority of the monoterpenes and sesquiterpenes that comprise *Clarkia* scent blends—could generate a "scent cloud" that could contribute to joint pollinator attraction, while species-specific volatiles may increase the constancy or specificity of foraging by pollinators (Waelti et al. 2008; Wright and Schiestl 2009). As such, we expect a stronger signal of character displacement in species-specific floral scent (Wright and Schiestl 2009), which may have more direct effects on pollinator behavior and thus plant reproductive success relative to shared volatiles. In addition, we also expect a stronger signal of character displacement in floral scent in sympatric communities with fewer species, as character displacement in (Eisen and Geber 2018) and selection on (Eisen et al. 2020) other traits has been relatively stronger in these communities.

To determine whether floral scent may undergo character displacement in *Clarkia*, we conducted a greenhouse common garden experiment to measure quantitative variation in volatile emission rates of the progeny of plants from natural parental communities that contain one, two, or four *Clarkia* species. Specifically, we focused our study on *C. unguiculata* and *C. cylindrica*, as they co-occur more frequently than expected by chance in the region (Eisen and Geber 2018). By eliminating variable abiotic environmental effects on trait values, the common garden enabled us to compare phenotypes across different, replicated parental community types. These data were used to test for significant interactions between species and parental community types on floral volatile emission rates (character convergence or divergence between species within a parental community type) and for significant differences between a species' trait values at different parental community types (evolved trait differences within a species; character displacement). These analyses were used to address three questions regarding the potential for and nature of character displacement in floral scent. First, is variation in volatile emissions across species and parental community types consistent with character displacement? Second, do patterns of character displacement vary across types of sympatric parental communities (e.g., two-species vs. four-species communities)? And third, do multimodal signals (e.g., floral scent and flower size) jointly undergo character displacement?

Methods

Study System

Species in the genus *Clarkia* (Onagraceae) often co-occur and share pollinators, which are primarily solitary bee pollinators that specialize on the genus (Lewis 1953; MacSwain et al. 1973; Singh 2014). Across the genus, species exhibit intra- and interspecific variation in multiple types of floral traits, including flowering time (Lewis 1961; Jonas and Geber 1999; Moeller 2004; Gould et al. 2014; Singh 2014), floral orientation (Lewis 1961), petal coloration (Lewis and Lewis 1955), flower size (Eisen and Geber 2018), and floral scent (T. J. Miller et al. 2014).

In the southern Sierra Nevada (Kern River Canyon, Kern County, CA), communities contain up to four outcrossing *Clarkia* species: *C. unguiculata* Lindley, *C. cylindrica* ssp. *clavicarpa* W. Davis, *C. xantiana* ssp. *xantiana* A. Gray, and *C. speciosa* ssp. *polyantha* Harlan Lewis and M. Lewis. These species are primarily outcrossing because flowers are protandrous and herkogamous, and while they share pollinators (Singh 2014), they are not known to hybridize in the field (MacSwain et al. 1973). All four species of *Clarkia* are among the last plant species to flower in the region (Lewis and Lewis 1955; MacSwain et al. 1973; Moeller 2004), such that intraspecific interactions or interspecific interactions with congeners are likely to have significant effects on the selective environment (Eisen et al. 2020). These interactions may include facilitation for pollination via joint pollinator attraction (Moeller 2004), and they may vary across the parental community types (Eisen et al. 2020). *Clarkia unguiculata* and *C. cylindrica* have largely overlapping flowering times that occur earlier in the season (early to mid-May) relative to *C. xantiana* and *C. speciosa*, which flower in early to mid-June (Moeller 2004; K. E. Eisen and M. A. Geber, personal observation). Significant overlap in the primary pollinators of the earlier and later flowering species (Moeller 2005; Singh 2014; A. R. M. James, unpublished data) creates the potential for pollinator sharing through time (e.g., sequential mutualism; Waser and Real 1979).

In this study, we focus on *C. unguiculata* and *C. cylindrica*, which co-occur more frequently than would be expected by chance (Eisen and Geber 2018). The petal area of *C. cylindrica* exhibits divergent character displacement (an increase in petal area) relative to *C. unguiculata* in communities that contain two and four species of *Clarkia* (Eisen and Geber 2018).

Common Garden Source Parental Community Selection

Our common garden contained three replicates of each of four unique types of parental source communities: *C. cylindrica* alone, *C. unguiculata* alone, *C. cylindrica* and *C. unguiculata* together, and these two species with the two other outcrossing *Clarkia* species (*C. speciosa* and *C. xantiana*) that occur in the Kern River Canyon (for parental community locations, see table S1; tables S1–S6 are available online). In other words, individuals of each species (*C. cylindrica* and *C. unguiculata*) were grown from seeds sourced from three single-species parental communities, three two-species parental communities, and three four-species parental communities. Parental community types thus vary in how many species are present in the community.

Seeds from both species were collected at all parental communities in 2017. Three or more fruits per plant were collected from 50 haphazardly chosen plants of each species at each parental community. The seeds from one fruit from each of 20 plants per parental community and species were combined to ensure that plants in the common garden represented a sufficient range of any possible plant-level variation at each parental community. Because a *Clarkia* fruit generally contains between 30 and 50 seeds (K. E. Eisen and M. A. Geber, personal observation), this pool of seeds from 20 fruits provided sufficient seeds for all five cohorts of plants grown in the study (described below). These field-collected seeds were used in the study (see below), which creates the potential for maternal effects to affect the observed results. Previous work comparing population-level variation in morphology and life history of *C. xantiana* (one of the two additional *Clarkia* species in the region), using both field-collected and greenhouse-crossed seed sources, found similar levels of character variation among populations for both types of seed used (Gould et al. 2014). The results of this study suggest the absence of strong maternal effects in morphology and life history traits in *Clarkia*, although these findings may not be generalizable to floral scent, as maternal effects are often highly trait and environment specific. However, studies of intraspecific floral scent variation have not tested for the potential for maternal effects (e.g., Majetic et al. 2009; Friberg et al. 2017), likely because of serious limitations on high data throughput and analysis.

Plant Germination and Growth

Because of the large number of parental community × species combinations present in the common garden, seeds were started in five batches in September–November 2017. Five replicate individuals from each parental community × species combination were included in each batch of plants. To break dormancy, seeds were placed on moist filter paper in a petri dish, wrapped in parafilm, and stratified at 5°C for 5–7 days and then held at 23°C for 5–7 days before planting. Germinants were transplanted into 656-mL Cone-tainers (Stuewe & Sons, Tangent, Oregon) filled with Lambert soil mix. The pots' positions on the greenhouse bench were randomized. Plants were exposed to supplemental light (16-h days) and maintained at 23°C–25°C during the day and 19°C–21°C at night. Plants were watered twice a week on average and received on average 30–40 mL of water per week in weeks 1–3 after transplanting, 70–80 mL per week in weeks 4–6, and 100 mL per week in weeks 7–10. This watering regime was designed to minimize the possibility of overwatering newly transplanted germinants and underwatering maturing and mature plants. Each pot initially contained two germinants; pots were thinned after 4 weeks to contain one plant. At this time, six prills of Osmocote Smart-Release Plant Food Flower and Vegetable 14-14-14 fertilizer (Scotts Miracle-Gro Company, Marysville, OH) were applied to the soil surface in each pot.

Qualitative Scent Analysis

To inform our quantitative sampling protocols, we conducted two types of qualitative analyses using solid-phase microextraction (SPME) fibers (Supelco [Sigma-Aldrich], Bellefonte, PA; app. 2). First, we determined whether the presence of additional flowers changed the composition of the volatile profile (i.e., due to threshold dosage effects). Past studies of other *Clarkia* and species in other genera have demonstrated that methodological parameters (Raguso and Pellmyr 1998) as well as the amount of floral tissue sampled (Raguso and Pichersky 1995; Ashman et al. 2005) can affect the strength and detectability of floral volatiles by GC-MS. To this end, we compared the profiles of samples that contained three versus six flowers. Specifically, nine total flowers were cut from the same plant and divided between two vials, one with three flowers and the other with six, for static headspace sampling (see full description of methods in app. 2). We chose these numbers of flowers because our greenhouse-grown *Clarkia* tended to have a maximum of 6–10 flowers open at a time. As such, we compared a conservative maximum profile per plant to the profile of half as many flowers. We recovered significantly more monoterpenoid and sesquiterpenoid compounds in samples with six flowers (app. 2). Given this result, we adjusted our quantitative headspace sampling protocol to include a minimum of six open, attached flowers per plant (see below). Second, to determine where volatiles are produced in these flowers, we compared the volatile profiles of dissected petals from six flowers versus those of the remaining tissues of the same six flowers. We found that petals generally contained fewer volatiles than the nonpetal floral tissues (app. 2), which may influence the relationship between flower size and floral scent (see "Discussion").

Quantitative Scent Analysis

Floral volatile samples were collected using the dynamic headspace adsorption technique between November 13, 2017, and February 5, 2018. All collections were made under natural lighting conditions in a well-aerated glassed-in corridor adjacent to the greenhouse where the plants were grown. We used an AIRCARE hygrometer (Essick Air Products, Little Rock, AR) to record the minimum and maximum temperature and percent humidity during sampling; the average minima were 17°C and 23% humidity, while the average maxima were 25°C and 41% humidity. Floral samples were obtained from 15 plants per parental community per species ($N_{\mathrm{total}} = 270$), and one vegetative control sample was collected per parental community per species ($N_{\mathrm{total}} = 18$).

We used 16-ounce PET water bottles to enclose stems for headspace sampling. Water bottles were washed with odorless soap, dried, and baked in a clean drying oven for 15–20 min at 80°C each morning before sampling began. Samples were collected using PAS-500 Micro Air Sampler pumps (Spectrex, Redwood City, CA) connected to traps that contained 0.0100 g of Tenax 80/100 adsorbent (Alltech Associates [W. H. Grace], Deerfield, IL). The flow rate of the pump was set to 200 mL/min. Scent was collected for 6 h, from 900 to 1500 hours, as this corresponds to the period of greatest pollinator activity in natural communities. For floral samples, 6–13 flowers were enclosed per plant (average: 7.4 flowers), and the number of flowers enclosed was recorded for each plant sample. Vegetative controls were obtained from plants that had not begun to flower but had formed buds. During each sampling day, one ambient control sample was collected in an empty PET bottle.

Immediately following the end of the headspace collection period, the traps were removed from the pumps and eluted with 300 μL of GC-MS-quality hexane (Burdick & Jackson GC2; Honeywell International, Charlotte, NC). Samples were then concentrated to 50 μL with a flow of gaseous N_2 and spiked with 23 ng of toluene (5 μL of a 0.03% solution in hexane) as an internal standard in preparation for analysis with GC-MS (see below). Samples were stored at −20°C and labeled with a parental community-neutral identifier code based on the date of sampling (e.g., December 15-1, December 15-2, etc.) to facilitate analysis that would be blind to the species and parental community type (see Becklin et al. 2011).

Scent Analysis via GC-MS

Both solvent-eluted and solvent-free (SPME) volatile samples were analyzed using a GC17A gas chromatograph coupled with a QP5000 quadrupole mass spectrometer (Shimadzu Scientific Instruments, Kyoto, Japan). One-microliter aliquots of the solvent-eluted samples were injected (splitless mode) at 240°C onto a polar GC column (EC Wax, 30 m long, 0.25-mm internal diameter, 0.25-μ film thickness; BGB Analytik, Böckten, Switzerland). The GC oven program (40°C to 240°C, increasing at 20°C per minute, with a 2-min hold at the maximum temperature) was optimized to minimize run length (for more than 300 samples) while allowing for peak resolution to baseline. Electrical ionization mass spectra were generated under 70-eV conditions (scanning range: 40–350 *m*/*z*), and resulting mass spectra were compared with those of MS libraries (Wiley, National Institute of Standards and Technology [NIST], Adams) using Shimadzu GCMSolutions software. Kovats retention indices (KI) were prepared for each compound by running a blend of *n*-alkanes (C7–C30) under the same chromatographic conditions and optimized method. Volatile compounds were identified via (1) direct comparison of retention time and mass spectra with those of authentic standards, (2) comparison with the KI of the best MS library fit for the unknown with published KI values from the plant volatile literature (NIST WebBook; https://webbook.nist.gov/), or (3) in the absence of standards or published KI values, the mass spectral data (ion fragment table) were listed for unknown compounds in reverse order of abundance, starting with the base peak (set to 100%).

Extraction and Processing of Quantitative Data

Peak areas were integrated manually using Shimadzu GCMSolutions software. After excluding compounds that were present in one or two samples out of 270, our quantitative data set contained 54 compounds (table S2). To exclude experimental artifacts from individual plants' profiles, we compared the profile of each sample to the profile of the ambient control that was collected on the same day. If a compound appeared in both a floral sample and the relevant ambient control, we retained this peak in the floral sample only if the floral sample peak area was at minimum five times larger than the peak area of the ambient control. This value was selected to be highly conservative in terms of the compounds that we retained in samples where those compounds were also present in the control. Similarly, to exclude compounds emitted by vegetation, we compared each floral sample to the vegetative control collected from the sample population and applied the same threshold (five times larger than the peak area of the ambient control) to any overlapping compounds. As such, some compounds were retained in the data set but excluded from particular samples where their emission rates were similar to the relevant ambient or vegetative controls.

Emission rates were normalized by dividing total ion current peak areas by that of the internal standard (Svensson et al. 2005), and they then were calculated algebraically using response factors generated with external standard dose-response curves generated from log- and semilog dilutions of the primary floral volatiles identified in these analyses ((*E*)-β-ocimene, α-pinene, β-caryophyllene, benzyl alcohol, and methyl salicylate).

To relate emission rates to floral masses, we measured the fresh and dry masses of 20 flowers (10 male-phase and 10 female-phase flowers) per parental community and species (table S1). Flowers were selected haphazardly from between four and eight plants per parental community and species. Each plant contributed a maximum of five flowers to the 20 total flowers per parental community and species. Fresh masses were recorded immediately after removing the flower from the plant. Flowers were dried for 24 h at 50°C before dry masses were recorded. We present analyses of floral scent emission rates that were standardized by the number of open flowers that contributed to a sample multiplied by the average fresh mass of a flower from that parental community and species, which gives the micrograms of scent per gram of fresh floral mass per hour. Analyzing the data using emission rates that were standardized by the number of open flowers that contributed to a sample (μg scent/flower/h) yielded highly similar results (results not shown).

Additional Common Garden to Test for Wounding Artifacts

We observed differences across species and parental community types in compounds that are generally considered green leafy volatiles (GLVs) and are generally associated with plant wounding (Visser and Ave 1978; Scala et al. 2013) but can be floral volatiles that facilitate pollinator attraction (Brodmann et al. 2008; Brodmann et al. 2012;

see "Results"). To determine whether these patterns resulted from artifacts of the experimental sampling process, we conducted an additional common garden experiment to test for differences in the emission rates of GLVs between wounded and nonwounded plants (see app. 3). Wounding elevated the emission rates of GLVs (e.g., (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate), but the emission rates of these compounds during the 2018 main experiment were more similar to the 2019 nonwounded control plants than to the 2019 wounded plants (see app. 3). Given these results and our use of vegetative controls to exclude compounds with similar emission rates in floral and vegetative samples (described above), observed emission rates of these compounds are unlikely to be an experimental artifact, and we retain the GLVs as floral compounds in our analysis.

Multivariate Statistical Analyses and Dimensionality Reduction

We analyzed emission rates of floral scent that were standardized by floral mass (μg scent/g fresh floral mass/h) to address our questions about the potential for character displacement in floral scent. We used multivariate and univariate methods to first test for significant interactions between species and parental community type, which test for differences in species' emission rates of volatiles across communities (character convergence or divergence). We then used univariate analyses to test for trait change within a species across parental community types (character displacement). All analyses were performed in R (R Core Team 2018). To partition the observed variance in the emission rate of all compounds across the species and parental community types, we performed a permutational multivariate analysis of variance (PERMANOVA) with a Bray-Curtis distance matrix using the adonis function from the vegan package (Oksanen et al. 2019). We specified our replicate parental communities nested within parental community type as strata in the function, which is equivalent to a random effect. To find the compounds that distinguish the parental community types and species, we performed a canonical analysis of principal coordinates (CAP) with a Bray-Curtis distance dissimilarity index using the capscale function from the vegan package (Oksanen et al. 2019).

Univariate Statistical Analyses

We analyzed variation in compound classes and in specific compounds using general linear mixed effects models, which were performed using the lme4 package (Bates et al. 2015). Models were visually assessed to ensure normally distributed residuals with homogenous variance. These models all contained parental community type, species, and their interaction as fixed effects, and parental community nested within parental community type was included as a random effect. The significance of fixed effects in linear mixed models was assessed using the ANOVA function in the lmerTest package (ver. 2.0-29; Kuznetsova et al. 2017) to perform type III *F*-tests using the Kenward-Roger approximation for the denominator degrees of freedom. When ANOVAs returned significant *F* values, we used the Tukey's honest significant difference test to determine which group means were significantly different using the emmeans function with the pairwise option in the emmeans package (Lenth 2019). These tests were performed with the "type = 'response'" option such that intervals were back transformed from the log and square-root scales. Contrasts for models with log-transformed response variables are presented on the log-odds scale, such that ratios greater than 1 indicate larger emission rates in *C. cylindrica* and ratios less than 1 indicate larger emission rates in *C. unguiculata*.

We performed two types of univariate analyses. First, we tested for differences in total scent emission and the emission of certain types of compounds across the species and parental community types using linear mixed effects models as described above. The compound classes we analyzed were monoterpenoids, sesquiterpenoids, GLVs, and aromatics (table S2). To ensure that our models had normally distributed residuals with homogenous variance, total scent, monoterpenoid, and aromatic emission rates were square-root transformed, and GLVs and sesquiterpenoid emission rates were log transformed.

Second, we performed univariate analyses on compounds that were correlated with one or both of the first two CAP axes. Specifically, 23 compounds were correlated with one or both of the first two CAP axes (table S3). These compounds had significant Pearson correlation coefficients with one or both axes at $P < .01$ after applying a false discovery rate correction for 54 tests; in all significant correlations, $|r| > 0.15$. Most compounds were either square-root or log transformed to improve the normality of model residuals (table S3). To test for a significant interaction between species and parental community type, we ran ANOVAs on these models as described above. We applied a false discovery rate correction for 23 tests on the *P* values associated with these species by parental community type interactions. We then tested for differences within each species across the three parental community types using the emmeans function as described above. All data sets and analysis scripts are available on GitHub (https://github.com/kate-eisen/clarkia-scent) and Zenodo (https://doi.org/10.5281/zenodo.4623613). All data and code have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.3tx95x6fx; Eisen et al. 2021).

Results

Diversity of Volatile Organic Compounds

There were 54 volatile organic compounds present in four or more samples: 22 monoterpenoids, 18 sesquiterpenes and C_{15} derivatives, five GLVs, and nine aromatic or nitrogenous compounds (table S2). Thirty-eight of the 54 compounds were found in more than five samples of both species. Of the remaining 16 compounds, 11 compounds were completely or nearly unique to *Clarkia unguiculata* (present in five or fewer *Clarkia cylindrica* samples), and five compounds were completely or nearly unique to *C. cylindrica* (present in five or fewer *C. unguiculata* samples; for exact counts of occurrences of each compound, see table S2). The average number of compounds detected in a sample (mean ± 1 SE) was 13.8 ± 0.4 for *C. cylindrica* and 14.0 ± 0.5 for *C. unguiculata*.

Multivariate Analyses

The PERMANOVA on the scent compounds revealed main effects of parental community type ($R^2 = 0.03$, $P < .001$), species ($R^2 = 0.22$, $P < .001$), and an interaction between the two ($R^2 = 0.04$, $P < .001$).

The CAP indicated that a subset of all compounds helped to define variation among the two focal species × three parental community types per species in our study. The constrained portion of the variance was 24% of the total variance; that is, our independent variables explained 24% of the total variation in the data. CAP axis 1 explained 20% of the total variation in scent and 77% of the constrained variance. In general, *C. cylindrica* individuals had negative values on CAP axis 1, while *C. unguiculata* individuals had positive values (fig. S1, available online; table S4). CAP axis 1 was strongly positively correlated with two monoterpenoids and an aromatic compound and was strongly negatively correlated with sesquiterpenoids (tables 1, S3). CAP axis 2 primarily separated *C. unguiculata* individuals from the three different parental community types (table S4). This axis explained 4% of the total variation in scent and 14% of the constrained variance. It was strongly positively correlated with two monoterpenoids and an aromatic compound and was strongly negatively correlated with two GLVs and a monoterpenoid (tables 1, S3).

Univariate Analyses of Total Scent, Compound Classes, and Single Compounds

Patterns of variation in three compound classes were consistent with character displacement: sesquiterpenes ($F_{2,117.84} = 8.749$, $P = .0003$), GLVs ($F_{2,78.42} = 17.330$, $P < .0001$), and aromatics ($F_{2,89.51} = 4.720$, $P = .0113$). The interaction in sesquiterpene emissions was driven by a significantly larger difference between the species in one-species parental communities relative to two-species parental communities (character convergence in sympatry driven by an increase in the emission rates of *C. unguiculata* in two-species parental communities; fig. 2*B*; table S5). For the GLVs, *C. unguiculata* produced more than *C. cylindrica* in one-species parental communities and two-species parental communities; this character convergence in four-species parental communities was driven by a decrease in the emission rates of *C. unguiculata* (fig. 2*C*; table S5). The interaction in aromatics emissions was driven by greater

Table 1: The six compounds with the strongest positive and negative correlations with the first two canonical analysis of principal coordinates (CAP) axes (see table S3 for all compound correlations with the CAP axes)

Axis, compound	*r*	*P*
CAP axis 1:		
(*Z*)-*β*-ocimene	.528	1.51×10^{-19}
(*E*)-*β*-ocimene	.526	1.70×10^{-19}
2-amino phenyl ethanone	.481	4.60×10^{-16}
Intermedeol	−.646	1.73×10^{-31}
β-cadinene	−.641	2.87×10^{-31}
Unknown $C_{15}H_{24}$ no. 3	−.516	1.04×10^{-18}
CAP axis 2:		
(*E*)-*β*-ocimene	.457	1.26×10^{-13}
(*Z*)-*β*-ocimene	.444	5.16×10^{-13}
(*E*)-2-6-dimethyl-1,3,5,7-octatetraene	.329	4.12×10^{-7}
(*Z*)-3-hexenyl acetate	−.349	6.44×10^{-8}
(*Z*)-3-hexen-1-ol	−.275	3.52×10^{-5}
Sabinene hydrate	−.275	3.52×10^{-5}

Note: For CAP axis 1, in addition to the six compounds in this table, there were 14 compounds that were strongly correlated with this axis ($|r| > 0.15$; table S3). For CAP axis 2, in addition to the six compounds in this table, there were six compounds that were strongly correlated with this axis ($|r| > 0.15$; table S3). A false discovery rate correction for conducting 54 tests was applied to all *P* values (see “Methods”).

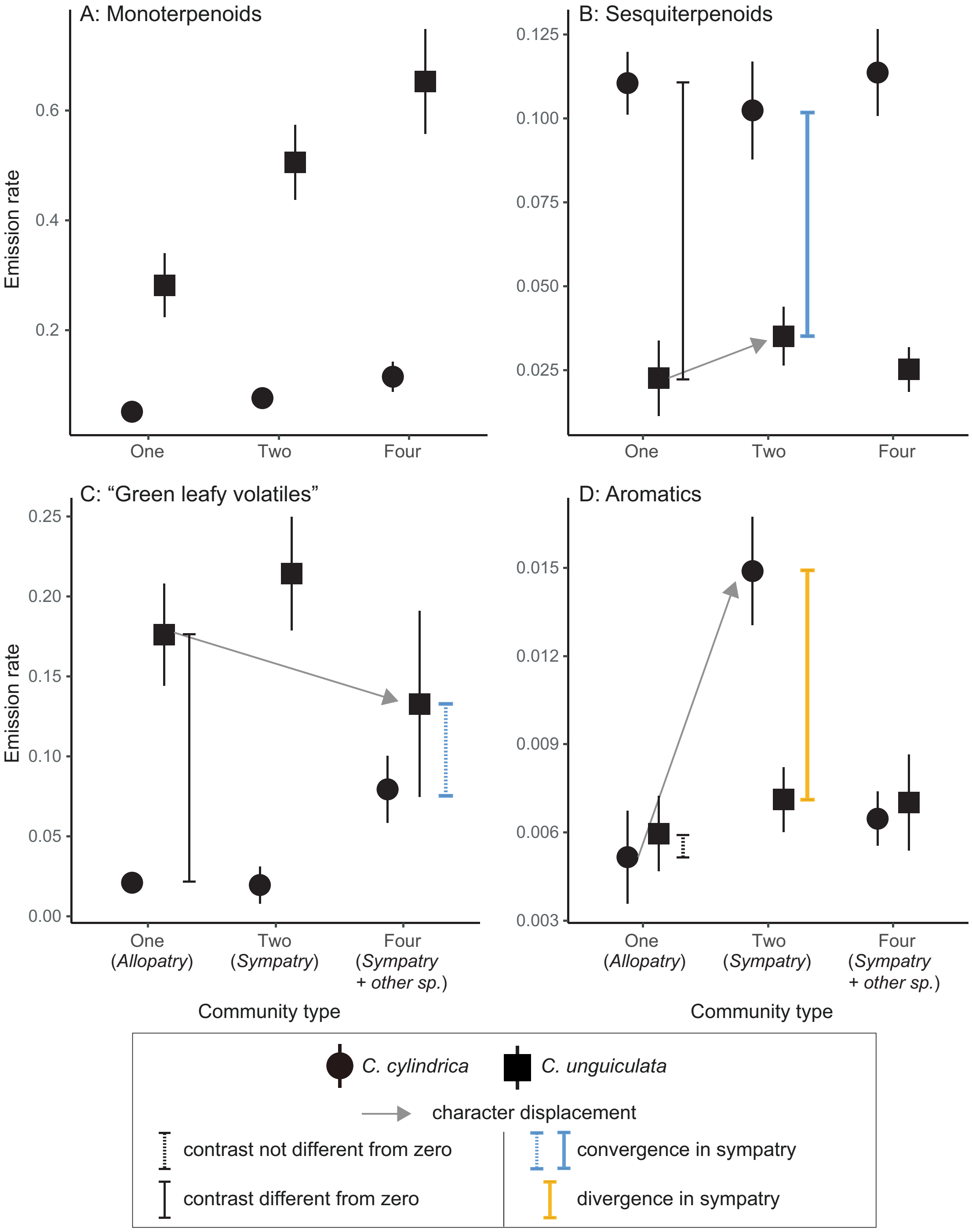

Figure 2: Emission rates (raw values; μg scent/g fresh floral mass/h) of monoterpenoids (*A*), sesquiterpenoids (*B*), green leafy volatiles (*C*), and aromatic compounds (*D*) by *Clarkia cylindrica* (circles) and *Clarkia unguiculata* (squares). Plot aesthetics (use of arrows and colored bars) are consistent with their usage in figure 1. Contrasts within species and between the differences between the species across parental community types were tested using the emmeans function from the emmeans R package (see details in “Methods”). Emission rates of monoterpenoids were higher in *C. unguiculata*, but there was no character displacement (and thus no convergence or divergence) across parental community types (*A*). *Clarkia cylindrica* produced more sesquiterpenes than *C. unguiculata* in all parental community types (*B*), but *C. unguiculata* produced more sesquiterpenes in two-species parental communities relative to one-species parental communities (gray arrow), such that the difference between the species was smaller in two-species parental communities (blue contrast bar). *Clarkia unguiculata* produced more green leafy volatiles than *C. cylindrica* in one-species parental communities, but emission rates in four-species parental communities were equivalent (*C*). This decrease in the emission rates of *C. unguiculata* (gray arrow) generated convergence in four-species parental communities (blue dotted contrast bar). *Clarkia cylindrica* had substantially higher emission rates of aromatic compounds in two-species parental communities (*D*, gray arrow), which generated divergence in two-species parental communities (yellow contrast bar). Note the differences in scale for the *y*-axes across all panels.

production by *C. cylindrica* in two-species parental communities (character displacement in *C. cylindrica*; fig. 2*D*; table S5), such that the difference between the species in two-species parental communities was significantly larger than the differences between the species in one- and four-species parental communities (character divergence in sympatry; fig. 2*D*; table S5). In particular, this pattern was driven by the emission of large amounts of benzyl alcohol by *C. cylindrica* in two-species parental communities (results not shown).

We used the results of the CAP to determine the compounds that we analyzed individually. Of the 23 compounds that were correlated with one or both of the first two CAP axes (see "Methods"), nine compounds had significant parental community type × species interactions in univariate models (table S6). Two of these compounds, 2-amino phenyl ethanone and methyl nicotinate, are primarily produced by *C. unguiculata*—these compounds were present in one and two *C. cylindrica* samples, respectively—and emission rates were higher in both types of sympatric parental communities (fig. 3.1, 3.2; table S5). Two additional compounds, (*E*)-cinnamic aldehyde and veratrole, are primarily or exclusively produced by *C. cylindrica*—these compounds were present in two and zero *C. unguiculata* samples, respectively—and emission rates were higher in both types of sympatric parental communities (fig. 3.7, 3.8; table S5). The remaining five compounds with significant interactions—α-pinene, β-pinene, sabinene hydrate, γ-terpinene, and (*Z*)-3-hexenyl acetate—are more commonly produced by *C. unguiculata*. Specifically, these compounds were present in between 1 and 24 of 135 total *C. cylindrica* samples and had lower emission rates in *C. unguiculata* samples in four-species parental communities relative to one- and two-species parental communities (fig. 3; table S5; exact occurrence counts are given in table S2).

Discussion

By measuring floral scent variation across parental communities that contain different numbers of species in a system that exhibits character displacement in flower size, we conducted the first test for context-dependent multimodal character displacement in floral traits. These species exhibit pronounced differences in their floral scent profiles, with more subtle but significant differences across the parental community types. In an analysis of all of the volatile organic compounds emitted by the two species, the significant interaction between species and parental community type was driven by compounds that were primarily or exclusively emitted by only one species—two aromatic compounds and four monoterpenoids emitted by *Clarkia unguiculata*, and two aromatic compounds emitted by *Clarkia cylindrica*. These patterns were consistent across sympatric parental communities for *C. cylindrica* but not for *C. unguiculata*. In addition, our investigation of the potential for multimodal character displacement revealed that changes in floral scent were associated with changes in flower size in *C. cylindrica* but not in *C. unguiculata*.

Character Displacement Driven by Changes in Species-Specific Volatiles

Because floral scent is a complex trait (see Raguso 2008*b*), character displacement could occur through several pathways, including both qualitative and quantitative changes in compounds that are either shared across the species or unique to one species. In this study, we observed patterns consistent with character displacement in compounds that were generally emitted by only one of the focal species. While monoterpenes in general dominate the floral profiles of these species of *Clarkia* (see results of pilot study in app. 1), the compounds that exhibited character displacement were largely species-specific monoterpenes or aromatic compounds. Changes in these compounds in particular could be linked to increases in plant-pollinator specialization mediated by evolved differences in floral scent in multispecies communities (Wright and Schiestl 2009). Specifically, an increase in species-specific volatile emissions may increase a pollinator's ability to differentiate between two co-occurring plant species, which could increase pollinator constancy and decrease heterospecific pollen transfer among species that share pollinators (Waser 1986; Sargent and Ackerly 2008). Divergence in flower color has been demonstrated to reduce inconstant foraging in multiple systems (Levin 1985; Hopkins and Rausher 2012; Muchhala et al. 2014; Norton et al. 2015). The compounds that exhibited patterns consistent with character displacement in *Clarkia* were benzenoid aromatics (both species) and monoterpenoids (*C. unguiculata*). Among plants that are pollinated by food-seeking bees, scent profiles are commonly dominated by benzenoids, terpenoids, or a mixture of the two types of compounds (Dobson 2006). In both observational and experimental studies, benzenoids have been associated with visitation from apid and halictid bees (Theis 2006; Andrews et al. 2007; Kantsa et al. 2019), such that the increases in benzenoid emission rates in *Clarkia* could result in greater attraction of these pollinator species. In particular, because only *C. unguiculata* is commonly visited by apid bees (*Apis mellifera*, *Xylocopa tabaniformis*, *Bombus* sp.; Singh 2014), the increases in benzenoid emissions could reflect greater pollinator specialization in sympatric communities.

Context Dependency of Character Displacement

Because indirect interactions can modify evolutionary trajectories (Benkman 2013; Walsh 2013; TerHorst et al.

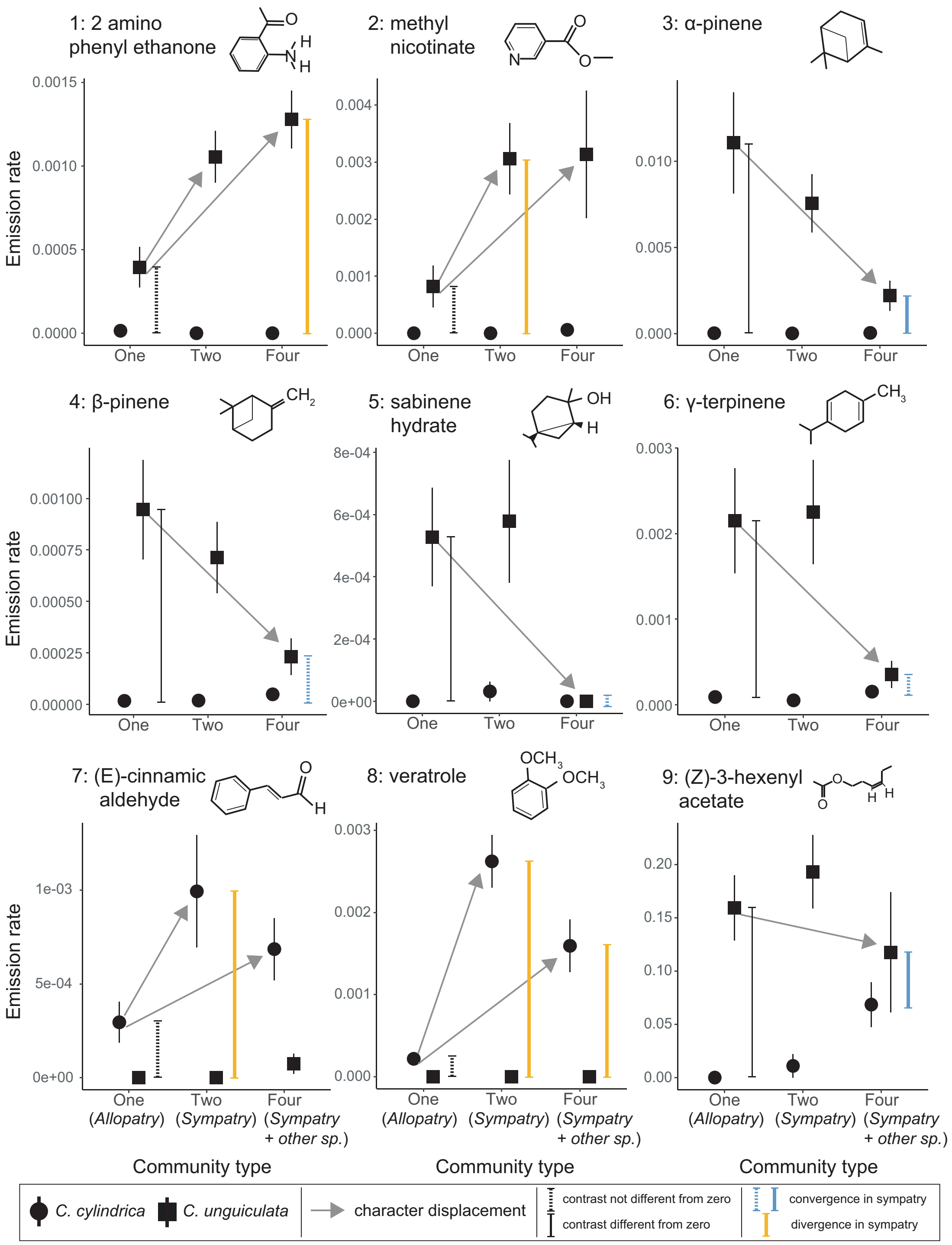

Figure 3: Emission rates (raw values; μg scent/g fresh floral mass/h) by *Clarkia cylindrica* (circles) and *Clarkia unguiculata* (squares) of the nine compounds that were significantly correlated with one or both of the first two CAP axes and also had a significant species × parental community type interaction. Plot aesthetics (use of arrows and colored bars) are consistent with their usage in figure 1. Contrasts within species and between the differences between the species across parental community types were tested using the emmeans function from the emmeans R package (see details in "Methods"). 2-amino-phenyl ethanone (1) and methyl nicotinate (2) were emitted at higher rates by *C. unguiculata* in two- and four-species parental communities (gray arrows; character displacement), leading to character divergence in four-species parental communities for 2-amino-phenyl ethanone and in two-species parental communities for methyl nicotinate (yellow contrast bars). α-pinene (3), β-pinene (4), sabinene hydrate (5), and γ-terpinene (6) were also emitted at lower rates by *C. unguiculata* in four-species parental communities (gray arrows; character displacement), leading to character convergence between the two species in four-species parental communities (solid or dotted contrast blue bars). (E)-cinnamic aldehyde (7) and veratrole (8) were emitted

2015), we tested for variation in character displacement in two types of parental sympatric communities: two-species parental communities that contain the focal species of this study and four-species parental communities that contain the focal species plus two additional congeners that flower later in the summer (Moeller 2004). We found that *C. cylindrica* exhibited similar patterns across both types of parental sympatric communities, while patterns for *C. unguiculata* across the parental community types varied by compound class (monoterpenoids and aromatics). In general, this variation in the patterns observed for our two focal species points to the potential for character displacement to be context dependent (Eisen and Geber 2018; Roth-Monzon et al. 2020) and to occur via different phenotypic pathways across communities (Germain et al. 2017). In particular, our results suggest that changes in the volatile profile of *C. cylindrica* may result primarily from interactions with *C. unguiculata*, which occurs in both types of sympatric communities. For *C. cylindrica*, indirect interactions with the later-flowering *Clarkia* species in the four-species communities may not affect the evolution of floral scent. However, indirect interactions have significant effects on pollinator visitation (e.g., sequential mutualism; Ogilvie and Thomson 2016) and patterns of selection on morphological traits (Irwin 2006) in other systems.

In contrast, *C. unguiculata* had greater emission rates of two aromatic compounds at both types of sympatric parental communities but lower emission rates of four monoterpenoids only in the four-species sympatric parental communities. Similar patterns of intermediate or less displacement were observed across different multispecies communities of freshwater fish (Roth-Monzon et al. 2020), which suggests that evolution in these communities likely occurs in response to multiple species interactions. Because *C. unguiculata* is the earliest *Clarkia* species to flower in the region (Moeller 2004; Singh 2014), its higher total scent emission (see fig. 2) may serve to attract scarce pollinators at the beginning of the flowering season (Filella et al. 2013; but see Burkle and Runyon 2019). However, the observed decrease in monoterpenoid emissions in the four-species parental communities suggests that *C. unguiculata* may invest less in pollinator attraction if, like other species of *Clarkia* (Moeller 2004), it experiences facilitation for pollination via increased pollinator attraction in these communities. While more complex communities may generate facilitation for *Clarkia* species, leading to weaker selection (Eisen et al. 2020) and character displacement in other traits (Eisen and Geber 2018), competition for pollination is generally expected to increase with community complexity (reviewed in Vamosi et al. 2006). These dynamics could generate selection for the emission of more unique or distinctive blends of floral volatiles to increase pollinator specificity in more complex communities, and patterns consistent with this expectation were observed in a community of more than 50 forbs and 75 bees in the northern Rocky Mountains (Burkle and Runyon 2019).

Multimodal Character Displacement: Synergy of Changes in Floral Size and Scent

Because pollinators often exhibit responses to combinations of visual and olfactory traits (Leonard et al. 2011), we conducted the first test for character displacement in multimodal floral signals (fig. 4). Using estimates of volatile emission rates that were standardized by floral fresh mass, we found that changes in the floral scent of *C. unguiculata* were not associated with changes in flower size, while increases in the emission of floral scent of *C. cylindrica* were related to increases in flower size. We hypothesize that this pattern results from differences in the floral parts that produce these compounds (Effmert et al. 2006). Because we used SPME to measure the scent profiles of a relatively small number of floral dissections (six per species), a more thorough investigation of potential differences in emissions across floral parts is needed to more thoroughly address this hypothesis. In a number of other systems, including other *Clarkia* species (Pichersky et al. 1994), *Lithophragma* (Friberg et al. 2013), *Penstemon* (Burdon et al. 2015), and *Aristolochia* (Martin et al. 2017), the complexity of floral scent extends beyond the quantitative and qualitative composition of a scent bouquet to include spatial variation in the emission of volatiles across tissue types.

These differences in the floral sources of the volatiles that may change across the parental community types may signify differences in their functions. For *C. cylindrica*, the increases in both size and volatile emissions in both petals and reproductive parts may serve to increase overall pollinator attraction in sympatry. Increases in flower size or scent emission have been linked to increased pollinator attraction and plant reproductive success in multiple insect-pollinated systems (Conner and Rush 1996; Miyake and Yafuso 2003; Majetic et al. 2009; Sandring and Ågren 2009; Parachnowitsch et al. 2012), although most studies have not tested

at higher rates by *C. cylindrica* in two- and four-species parental communities (gray arrows; character displacement), leading to character divergence in two-species parental communities for (*E*)-cinnamic aldehyde and in two- and four-species parental communities for veratrole (yellow contrast bars). (*Z*)-3-hexenyl acetate (9) was emitted a lower rate by *C. unguiculata* in four-species parental communities relative to one-species parental communities; this character displacement in *C. unguiculata* (gray arrow) generated character convergence in four-species parental communities (blue contrast bar). Note the differences in scale for the *y*-axes across all panels.

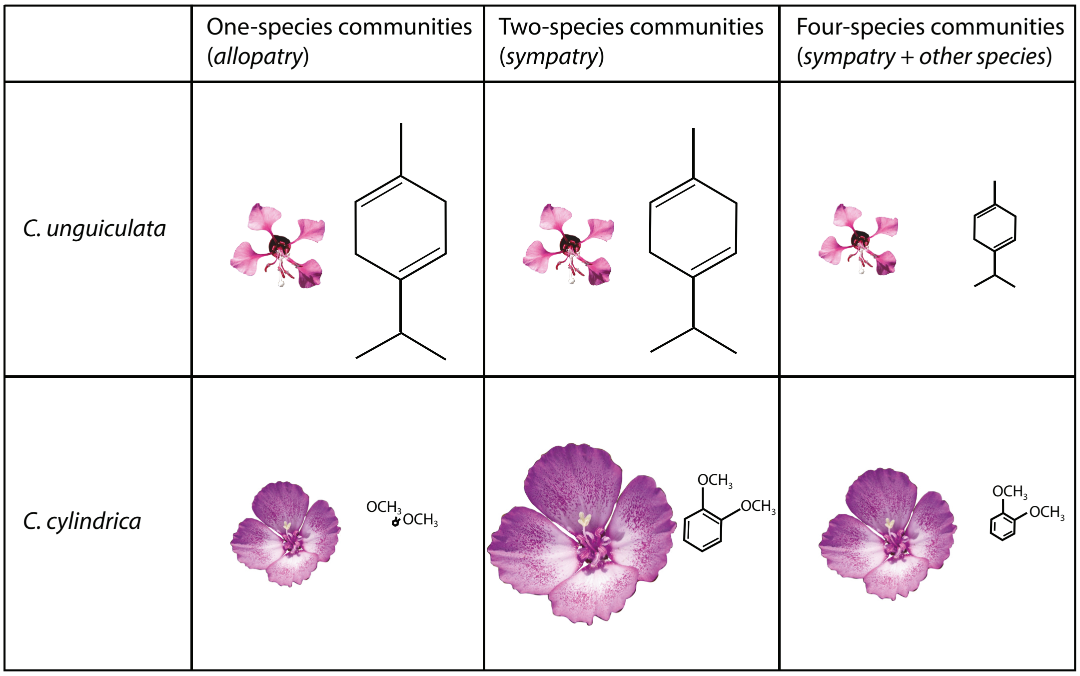

Figure 4: Schematic showing the relative changes in flower size (based on measurements of petal area in Eisen and Geber 2018) and the species-specific floral scent compounds that showed patterns consistent with character displacement (*Clarkia unguiculata*: 2-amino phenyl ethanone, α-pinene, β-pinene, sabinene hydrate, γ-terpinene, and methyl nicotinate; *Clarkia cylindrica*: (*E*)-cinnamic aldehyde and veratrole). We stress that character displacement refers to trait changes within a species across community types, such that the first row of the schematic represents the character displacement that occurs in *C. unguiculata* and the second row represents the displacement that occurs in *C. cylindrica*. Drawings of flowers and chemical compounds (molecules that are representative of the suites of compounds that responded in each species) are scaled proportionally both between the species and across the parental community types. Flower size of *C. unguiculata* is similar across parental community types. Floral scent emission rates of *C. unguiculata* are similar in one- and two-species parental communities, and emission rates in four-species parental communities are about 0.45 times emission rates in one-species parental communities (a decrease in scent emission in four-species parental communities). Flower size of *C. cylindrica* in two-species parental communities is 1.7 times larger than flower size in one-species parental communities, and flower size in four-species parental communities is 1.25 times larger than in one-species parental communities. Floral scent emission rates of *C. cylindrica* are 7.2 times larger in two-species parental communities relative to one-species parental communities and are 4.6 times larger in four-species parental communities relative to one-species parental communities.

for concurrent changes in both traits (but see Parachnowitsch et al. 2012). For *C. unguiculata*, scent emission in the reproductive tissues may serve to cue pollinators to the precise location of the reproductive parts (Dötterl and Jürgens 2005; Burdon et al. 2015). Because the solitary bees that specialize on *Clarkia* forage for pollen (MacSwain et al. 1973), volatiles emitted in the reproductive tissues also indicate the location of the primary rewards for this species. After becoming attracted to a flower, bees can use pollen odors, which are often a distinct subset of the floral bouquet (Jürgens and Dötterl 2004; Effmert et al. 2006), to orient more specifically to the source of pollen (Dobson et al. 1996, 1999). Here, the decrease in floral volatiles that are putatively produced in the reproductive organs in four-species parental communities suggests that *C. unguiculata* may invest less not only in pollinator attraction, as described above, but more specifically in provisioning pollinators with pollen where the community of congeners may facilitate joint pollinator attraction or maintenance (Moeller 2004). This hypothesis could be tested via additional analysis of the pollen volatiles in *C. unguiculata* and with pollinator behavior assays (see below).

Future Directions

This study revealed a pattern of trait variation that is consistent with character displacement, but additional work is needed to rule out alternative hypotheses (Schluter and McPhail 1992). Quantitative genetic approaches, such as an artificial selection experiment, could be used to rule out maternal effects as a potential source of phenotypic variation, to quantify heritability, and to examine how scent responds to selection; these types of studies remain rare in floral volatiles research (Zu et al. 2016). In addition, it is particularly critical to determine whether this variation in scent has functional consequences for pollinator behavior, as it does in other systems with volatile emission rates that are barely perceptible to the human nose (Ashman et al. 2005; Cook et al. 2005; Parachnowitsch et al. 2012; Milet-Pinheiro et al. 2013). Given that the volatiles that mediate pollinator behavior are often a subset of all volatiles emitted by a plant (reviewed in Junker and Blüthgen 2010; Dötterl and Vereecken 2010), pollinators may not respond to the specific changes observed in floral scent profiles across parental community types. Experimental assays of pollinator behavior can be used to determine whether these shifts in volatiles affect pollinator attraction or constancy or whether they are nonfunctional. The potential effects of variation in *C. unguiculata* volatiles on honey bees and bumblebees could be tested in a controlled environment (e.g., Burger et al. 2012; Peter and Johnson 2014). However, a comprehensive assessment of the functionality of floral scent variation in *Clarkia* would need to be field based, as laboratory experiments with the solitary bees that pollinate both species are not tractable.

More broadly, the results of this study highlight the need to continue to integrate chemical phenotypes into the study of floral trait evolution (Leonard et al. 2011; Junker and Parachnowitsch 2015). In combination with visual traits, floral scent can affect species interactions at multiple scales, from specifying highly specialized interactions (e.g., Peakall and Whitehead 2014; Whitehead et al. 2015) to contributing to the structure of complex plant-pollinator interaction networks (Kantsa et al. 2018, 2019). In this study, by testing for character displacement in a complex trait across parental communities that contain different numbers of interacting species, we have generated new insights into the context dependency of character displacement, which may occur through multiple pathways. Moving forward, systems that exhibit variation in both floral scent and species interactions across communities (e.g., Friberg et al. 2019) provide opportunities to study the interplay between complex trait evolution and species interactions, which can generate insight into the repeatability of evolutionary change across variable ecological communities.

Acknowledgments

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Statement of Authorship

K.E.E., M.A.G., and R.A.R. conceived of the study and developed the experimental design. R.A.R. and K.E.E. developed the methods. K.E.E. led the data collection, analysis, and visualization, with support from R.A.R. and M.A.G. R.A.R. provided resources for the collection, identification, and analysis of volatile compounds. K.E.E. wrote the original draft of the manuscript, with input from M.A.G. and R.A.R. All authors contributed to editing the manuscript.

Data and Code Availability

All data and code are available on GitHub (https://github.com/kate-eisen/clarkia-scent) and Zenodo (https://doi.org/10.5281/zenodo.4623613). All data are also available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.3tx95x6fx).

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