Rationale. We conducted two types of additional analyses using Solid Phase Micro Extraction (SPME) fibers (Supelco, Inc., (Sigma-Aldrich), Bellefonte, PA). To determine if the presence of additional flowers changed the composition of the volatile profile (i.e. due to threshold effects), we compared the profiles of samples with three versus six cut flowers from the same plant. To determine if volatiles are produced in the petals and/or reproductive parts of these flowers, we compared the volatile profiles of dissected petals from six flowers versus those of the remaining tissues of the same six flowers.

Methods. We used the 65 μ "Stabilflex" field-assembly fibers (with both divinylbenzene and polydimethylsiloxane in the adsorbent matrix) because of their proven versatility in trapping different biosynthetic classes of volatile compounds (Goodrich and Raguso 2009).Both types of collections were performed on plants of both species from one one-species community (*C. cylindrica*: MHG; *C. unguiculata*: CB323) and from one two-species community (SC for both species). Three replicates of each comparison were sampled for each analysis (e.g. 3 samples with 3 flowers and 3 samples with 6 flowers from each site for each species).

Samples were collected in 20 mL glass scintillation vials sealed with Nalophan (PTE) film (Toppits ®, Cofresco Frischhalteprodukte, Minden, Germany). One ambient control vial was sampled during each sampling period. All samples were equilibrated for 60 minutes, and exposed to a SPME fiber for 30 minutes. After the exposure period, fibers were inserted into the GC injection port for thermal desorption. Analysis via GC-MS followed the same method used for the solvent-eluted samples.

Peak areas were integrated manually using Shimadzu GCMSolutions software. We observed 45 compounds across all samples (Table S2), and compounds were identified using the same protocols as the solvent-eluted samples (see Methods). To exclude experimental artefacts,

each sample was compared to the concurrently collected ambient control. No ambient control samples contained the compounds detected in the floral samples.

Statistical analysis. To determine if the presence of additional flowers changed the composition of the volatile profile (i.e. due to threshold effects), we compared the number of compounds observed in the three flower samples versus the six flower samples. Specifically, we calculated the total number of compounds in each sample, and the number of monoterpenoids, sesquiterpenoids, aromatics, and "green leafy volatiles" (see Table S2). To test for differences in these count data, we ran paired Wilcoxon signed rank tests for each compound class.

To determine if volatiles are produced in the petals and/or reproductive parts of these flowers, we compared the presences and absences of all compounds across petal and non-petal samples. We performed a Permutational Multivariate Analysis of Variance (PERMANOVA) using a jaccard distance matrix on the jaccard dissimilarity values between samples using the adonis function from the vegan package in R (Oksanen 2018). We visualized the differences between the petal and non-petal samples using the multivariate dispersion, which shows the average distance to the group centroid.

Results. Samples with six flowers contained significantly more compounds than samples with three flowers (Z = -2.4382, P = 0.015). Specifically, the samples with six flowers contained more monoterpenoids (Z = -2.4945, P = 0.013), and sesquiterpenoids (Z = -2.1264, P = 0.033). In the PERMANOVA analysis of samples from petals and non-petals, there was a significant effect of sample type (R_2 = 0.1555, P = 0.002). Petal and non-petal samples formed two distinct clusters based on their multivariate dispersion (Figure 1). Non-petal samples contained significantly more compounds than petal samples (Z = 3.0618, P = 0.0022).

Conclusions. The significant increase in monoterpenoids and sesquiterpenoids in the samples with six flowers suggests that increasing the floral tissue in a sample can increase the probability of detecting a fuller complement of compounds, more representative of a blooming inflorescence. As such, we collected quantitative samples from plants with six or more open flowers in our study. The dissected flower tissues were separated in multivariate space, which suggests that scent is differentially produced across types of floral tissue in both species. In particular, the non-petal samples contained more compounds than the petal samples, which suggests that increases in volatile production may not be strongly correlated with increases in petal size in these species.

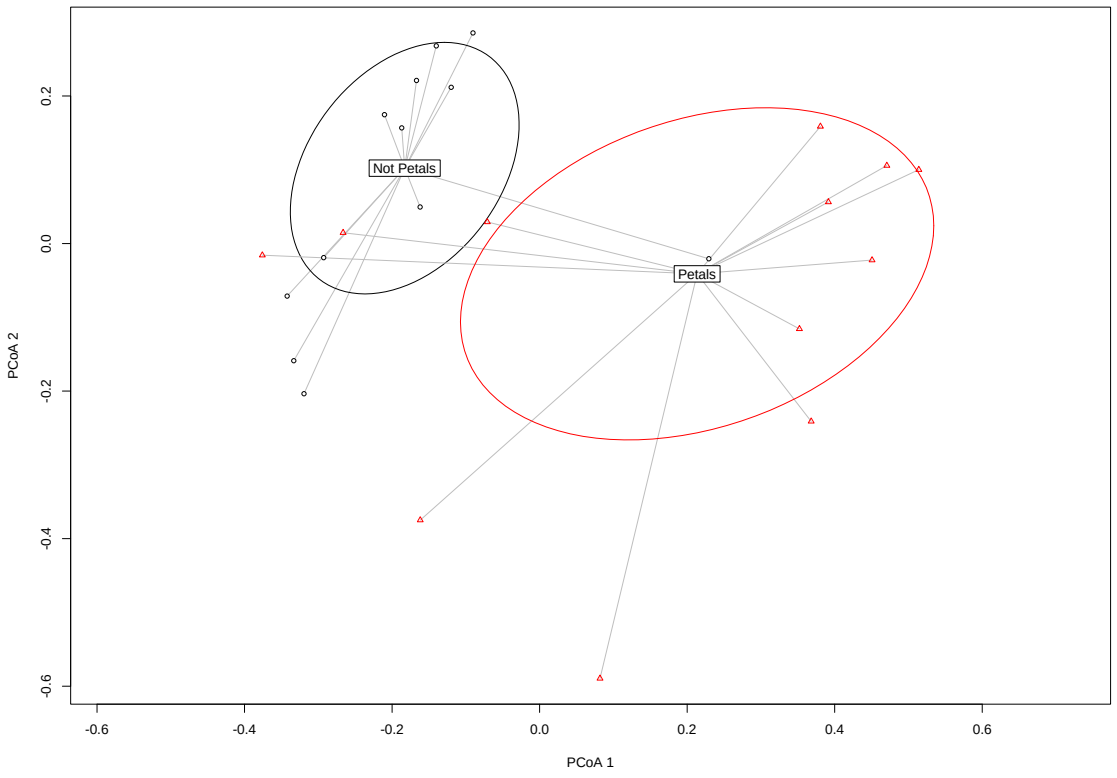

Figure 1. Multivariate dispersion of the petal and non-petal samples, performed using jaccard distances. The ellipses show the group standard deviations, and lines represent the average distance to the group centroid.