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Title: Sexual selection drives floral scent diversification in carnivorous pitcher plants (Sarraceniaceae)

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ABSTRACT

Plant volatiles mediate vital ecological services, including pollination and herbivory. Empirical studies show that both pollinators and herbivores exert strong selective pressures on plant phenotypes, leading to the expectation that volatiles from floral and vegetative tissues should exhibit the respective signatures of sexual and natural selection. We tested this hypothesis in the North American pitcher plants, which have modified leaves to capture prey and provide an ideal opportunity to understand the evolution of scent compounds across different plant organs. We collected a comprehensive dataset of floral and vegetative volatiles from across the NA Sarraceniaceae, and used multivariate analysis methods to investigate scent evolution in this unique taxon. Our major findings revealed that (i) flowers and traps produced highly distinct scent profiles, consistent with the hypothesis that volatiles alleviate trade-offs due to incidental pollinator-consumption; (ii) across species, floral scent separated into distinct regions of scent space, while traps were showed little evidence of clustering – this may be due to convergence on a generalist strategy for insect capture; and (iii) floral scent evolved much more rapidly than trap scent, showing that even in carnivorous taxa, our framework for phenotypic evolution should incorporate pollinator-mediated sexual selection, and herbivore-mediated natural selection.

Keywords:

Sarraceniaceae, volatiles, carnivorous plant, pollinator-prey, modularity, multidimensional characters

Background

Signal diversity reflects contributions from both sexual selection, which can fuel the evolution of dramatic ornaments and armaments (1-3), and natural selection (4,5). Our knowledge of the processes that contribute to signaling phenotypes comes from empirical studies manipulating selective regimes (e.g. 6-8), in combination with cross-taxon comparisons (e.g. 9-11). Together, these provide insight into the environmental constraints, phylogenetic history and selective pressures that contribute to the evolution of signaling traits. Importantly, such studies have shown that in many signaling modalities, sexual and natural selection operate distinctly: sexual selection often acts on specific components of trait phenotypes, and characters likely to evolve under sexual selection often exhibit a signature of rapid diversification and elevated evolutionary rates (12-15).

Despite an established interest in of the causes and consequences of signaling diversity in the visual and auditory modalities (16,17), surprisingly little is known about how selection affects the evolution of scent constituents (volatiles) that operate as signals, even though there is abundant empirical evidence showing that scent is an important part of many angiosperm phenotypes. For example, volatiles are likely subject to strong sexual selection from pollinators (18-20), as well as natural selection in association with antagonists and herbivores (21,22). Given the importance of volatiles in mediating plant pollinator (23-25) and herbivore interactions (26-28), we should expect to see differential signatures of sexual and natural selection acting on scents emitted by flowers and vegetation. Yet despite this strong expectation that volatile traits should evolve across in different ways between tissues subject to disparate selective regimes, these predictions have not yet been tested within a phylogenetic framework.

The North American (NA) carnivorous pitcher plants (Sarraceniaceae) are especially useful for understanding how volatiles can be shaped by contrasting selective pressures. First, sexual selection is expected to act strongly on flowers, which can be outcrossing limited (29,30). Specialist flowers typically appear briefly in the spring, and emit strong scents that attract bumblebee pollinators (or in smaller species, solitary bees) (30-32). Second, leaf tissues, which persist for months and represent a long-term investment throughout the growing season, can be subject to intense vegetative damage by endemic noctuid moths, a primary herbivore of *Sarracenia spp.* (33,34). Third, leaf tissues are often modified into conical pitchers to trap insects for supplemental nutrition (35). This additional foliar function also allows us to test the additional longstanding hypothesis that scent evolution in carnivorous plants is shaped by pollinator-prey conflict (PPC) – the idea that if pollinators are limited, then volatiles should target "private" sensory channels in the receiver to avoid consuming pollinators. However, the primary prediction of this, that volatiles should be strongly divergent across *Sarracenia* traps and flowers, has never been tested. In at least several species of *Sarracenia*, traps produce detectable levels of volatiles (36) which may function as attractants. Thus, the Sarraceniaceae provide a unique opportunity to disentangle the effects of sexual and natural selection on the dynamics of scent evolution, because while both floral and vegetative tissue function can produce similar attractive scents, *only* floral tissues are directly involved in outcrossing. This study allows us to investigate the underexplored evolution of an important signaling modality (24,38), and is, to our knowledge, the first study to examine how rates of scent evolution can vary across functionally distinct tissues.

In this study, we combined a phylogenetically comprehensive sample of NA Sarraceniaceae flower and trap volatile data with multidimensional data analysis techniques to (i) identify correlated clusters of flower and trap scent diversification, (ii) investigate the lability of scent phenotype and whether there is evidence of phylogenetic signal, (iii) ask whether the tempo of scent evolution differs between flowers and traps, and whether evolutionary rates reflect expected contributions from natural and sexual selection, and finally (iv) examine the hypothesis that volatiles might alleviate PPC. We find that within species, flowers and traps produced highly distinct scent profiles and that floral scent evolves much more rapidly than trap scent,

suggesting that even in carnivorous taxa, scent evolution may depend heavily on pollinator- and herbivore-mediated selection.

Methods

Plant material and volatile sampling

Species sampling included all major recognized species complexes (39,40) of the NA Sarraceniaceae, in addition to one hybrid South American species. Plants were washed, bare-rooted, and vegetation removed prior to potting in a 40:60 mix of pumice and peat moss. Pots were kept outdoors (Seattle, WA 47.606° N, 122.332° W) in an artificial bog and bottom watered using the municipal water supply (unfertilized). Volatiles were collected using established plant headspace collection methods (25,41,42) from flowers during anthesis, and from mature traps covered with pollination bags (1mm mesh) to prevent the incursion of macroscopic insects (NA species: $n_{(flower)}$ = 4-20, $n_{(trap)}$ = 6-22; $n_{(total)}$ = 358 samples). Briefly, plants were enclosed for 24h using Nylon bags (Reynolds; IL, USA), and scented headspace air pulled through cartridges containing 50mg of Porapak Q adsorbent (mesh size 80-100, Waters Corp.; MA, USA). Empty nylon bags were run in parallel with all plant samples and were subtracted to control for ambient environmental contaminants. Headspace samples were run on a gas chromatograph with mass spectrometric detection (GC-MS, 7890A GC paired with a 5975C MS) (Agilent Technologies; Palo Alto, CA, USA). Dynamic headspace methods were supplemented using samples taken with solid-phase microextraction fibers (75µm CAR-PDMS SPME) (Supelco; PA, USA) (see S1-A for details).

Data processing for plant scents

Chromatogram peaks from the MSD output were tentatively identified using the NIST08 mass spectral library. Compounds with a >30% library match were retained and verified using a combination of published Kovats indices and available authentic standards (see S1-A for details). Because plant volatile traits can comprise hundreds of individual compounds, we performed a model reduction to retrieve volatile features most important for explaining variation across species and tissues. The singular value decomposition (SVD) is a statistical procedure that provides a data decomposition separating variables into a set of orthogonal modes that optimally capture linearly uncorrelated, orthogonal axes that extract the maximal variance across a data matrix. The SVD is the underlying algorithm used in principal component analysis (PCA); unlike PCA however, the SVD does not require that the data for each plant have mean-zero and unit variance. The measured intensity levels of individual components can vary over many orders of magnitude (up to 10^9 arbitrary MSD units), with reasonable intensity levels being greater than 10^4. Intensity levels two orders of magnitude below this are considered noise fluctuations in the measurements. We therefore used noise-reduction thresholds in combination with SVD to extract a sparse, but representative matrix of correlated chemical representations of floral and vegetative profiles. Raw data matrices were log transformed so that compounds with the strongest intensity (on the order of 10^9) did not render the remaining, but significant, data irrelevant (e.g. in the range of 10^4 to 10^7). Thus since each plant has only a positive intensity level of only a small subset of the compounds measured, the data is sparse (mostly zeroes) in the space of possible volatiles. Thus it makes little sense to mean subtract (as in PCA) since this would render the majority of volatiles zero and non-negative. Instead, we used the SVD to extract the dominant correlated expression levels of volatile production. The SVD modes, which are like PCA modes, extract the most meaningful complex volatile bouquets derived from the hundreds of individual compounds. Final scent distances across species were calculated as the distance between centroids across the three main axes of scent divergence, SVD modes 2, 3, and 4. Using a similar procedure, we also conducted nested SVDs to examine modules of chemical divergence only across flowers, and only across traps. The first SVD mode is not highly informative since it represents the average chemical profile of the entire dataset. All clustering analyses were performed in Matlab R2016a (see S2-A for analysis workflow).

Constructing a time-calibrated phylogeny

We generated a time-calibrated molecular phylogeny using data from the most recent *Sarracenia* phylogeny (Fig 3) (40). First, we imported Stephens et al.'s (40) 199 nuclear gene alignment into BEAST 2 (v. 2.4.0) (43) and constrained the tree to ensure the resultant tree had the same topology as Stephens et al.'s (2015) species tree. We calibrated the tree following divergence time estimates from a family-level phylogeny using a normal prior distribution (44): the *Sarracenia* crown node was constrained to a mean of 4.18 Ma (offset 2.0, sigma 1.5), the *Sarracenia* stem node was constrained to a mean of 22.76 Ma (offset 14.0, sigma 5.0), and the stem node of *Darlingtonia, Heliamphora* and *Sarracenia* was constrained to a mean of 34.91 (offset 25.0, sigma 5.0). We conducted two runs of 50 million generations, sampling every 5000 generations, and used Tracer v1.6 (45) to verify that both runs reached stationarity and converged on the posterior distributions of trees. Results were combined using LogCombiner and TreeAnnotator with a 10% burn-in. Prior to analyses, we pruned the tree to include the *Sarracenia* species for which we had chemical data, with the exception of the uncommon varietal *S. flava maxima*, as it was not included in the Stephens et al. (40) phylogeny. As the placement of *S. rubra ssp. gulfensis* was unresolved in Stephens et al.'s (40) species tree, we conducted all analyses with *S. rubra ssp. gulfensis* as sister to *S. jonesii*, as sister to *S. alata*, and sister to both *S. jonesii* and *S. alata*. As the results were primarily qualitatively similar, unless mentioned, we here only report the results conducted on the tree where *S. rubra ssp. gulfensis* is sister to both *S. jonesii* and *S. alata*.

Analysis of scent evolution

Recent development of multivariate comparative methods now allow analysis of high-dimensional multivariate phenotypes in a phylogenetic context (e.g. 46,47). While these methods were developed and have typically been used for shape data, we here apply these methods to scent data; another high-dimensional, multivariate trait. We estimated phylogenetic signal in trap and flower scent to test whether phylogenetic relatedness influenced scent using K_{mult} (49), a multivariate generalization of Blomberg's (49) K statistic. We tested whether scent abundance (volatile emissions analysed as a continuous variable) and/or scent composition (as measured by absence/presence of volatiles) exhibit phylogenetic signal. We used 1000 permutations to determine whether phylogenetic signal is significant compared to that expected under a Brownian motion model of evolution.

To test whether trap and flower scent evolved independently in the NA pitcher plants, we first evaluated whether trap and flower scent abundance and/or composition co-varied with one another across the phylogeny, following Adams and Felice (50). To test the significance of the correlation, we permuted the phenotypic data on the tips of the tree 1000 times, each time calculating the correlation scores to which the observed correlation score was compared. Second, we estimated the net evolutionary rate over time for the scent emitted by each organ using σ^2_{mult} (51). As floral volatiles tend to be emitted at higher intensities than traps (see Results), we used proportional data to standardize scent across flowers and traps. To assess significance, the ratio between trap and flower scent was compared to 1000 phylogenetic simulations in which data on the tips are obtained under Brownian motion using a common evolutionary rate for all traits. We further examined the evolution of different types of scents by estimating σ^2_{mult} for flower and trap volatiles that have previously been associated with bee attraction and those that have been associated with herbivory deterrence (S1-B). All analyses of scent evolution were conducted using the geomorph R package (47).

Chemical versus temporal and spatial divergence

To determine whether increased potential for pollinator-prey conflict was related to chemical divergence between flowers and traps, we conducted a linear regression using combined blooming periods and between-organ height differences taken from the Flora of North America (52). To account for shared phylogenetic history among species (53), we computed phylogenetic independent contrasts for an index combining

temporal and spatial data, and for the chemical difference between flowers and traps using the pic function in the ape R package (53). We used the resulting values to conduct a linear regression to test whether the level of chemical divergence between flowers and traps was inversely related to the level of spatial and temporal divergence of these two traits (see S1-C for details).

Results

Scent composition was dominated by terpenoids, benzenoids, and aliphatics

Volatile emissions in the NA Sarraceniaceae were dominated by mono- and sesqui- terpenes (48% of detected compounds), and included major contributions from limonene, α-pinene, and caryophyllene. Aliphatic emissions (e.g. tridecane and pentadecane) were also widespread (34%). Finally, benzenoid emissions, including a number of aromatic esters, comprised 18% of detected volatiles (see S3-A for details). Both volatile sampling techniques (Porapak Q and SPME) identified very similar sets of volatiles in our clustering analysis (S2-C). Species-specific compounds accounted for 31% of emitted compounds, and the total number of compounds detected from each species ranged from 39 volatiles in *S. leucophylla* and *S. psittacina*, to 84 in *S. alabamensis*. Flowers tended to emit greater quantities of scent (ng/mg wet weight/h) and overall, produced a greater number of volatiles ($p=0.01$, $t(38)=2.7$). This difference in volatile number was driven by an increase in the diversity of terpenoids ($p<0.0001$); the number of benzenoids and aliphatics did not differ across tissues ($p>0.1$).

Volatile blends were highly distinctive across traps and flowers

Species (Fig. 1a) and organs (Fig. 1b) in the NA Sarraceniaceae were readily distinguished on the basis of scent (Fig. 1c). Across species, there was marked scent divergence across flowers, while clustering was not well-defined across traps from different species**.** Across-species separation in traps volatiles was very low (Fig. 1c), and the mean spread was more than six times greater in flowers than traps (variance $5.2\text{x}10^{-3}$(fl) vs. $0.84\text{x}10^{-3}$ (tr)). Furthermore, within each species, flowers and traps were highly divergent (Fig. 1c).

Modularity in volatile composition across NA pitcher plants

We ran further nested classifications to examine how volatiles covaried within tissues. In flowers, the SVD revealed that across-species floral composition involves strongly correlated expression of terpenoids, including caryophyllene, sabinene, β-pinene, and β-myrcene. Superimposed on this primary floral mixture, the SVD second mode reveals separation of volatiles along two directions, generating first, a module characterized by correlated production of α-curcumene, (±)-linalool, cis-α-bisabolene, and α-zingiberene, and a second contrasting strategy which flowers emitted combinations of α-ionone, eucalyptol, tetradecanal, sulcatone, and a handful of terpentine derivatives, including terpinolene and α-terpineol (Fig 2).

Nested classification for volatiles within vegetative tissues found that despite low pitcher clustering in the combined organ analysis, chemical divergence across pitchers of different species is best explained by a mixture of caryophyllene, benzoic acid ethyl ester, mesitylene, α-farnesene, and benzaldehyde. The second SVD mode reveals two alternate pitcher strategies, the first involving predominantly terpenoid volatiles, including β-ocimene, humulene, sabinene, and linalool, but also 1-octanol and 3-(*Z*)-hexenol; the second involving predominantly benzenoid compounds, including benzoic acid ethyl ester, benzoic acid methyl ester, benzaldehyde, as well as 2-heptanone (S2-D).

Phylogenetic signal and rates of scent evolution differed between flowers and traps

Our estimation of phylogenetic signal using continuous scent emissions data showed that floral scent did not exhibit significant phylogenetic signal (K_{mult} =1.02, p>0.05) while trap scent emissions did (K_{mult} =1.07, p<0.05). On the other hand, when estimating phylogenetic signal on presence/absence data, we found the inverse: floral scent exhibited moderate to significant phylogenetic signal, depending on the topology of *S. rubra ssp. gulfensis*, *S. jonesii* and *S. alata*, (K_{mult} =0.95-0.96, p=0.03-0.05) while trap scent did not (K_{mult} =0.94, p>0.05). Together, this indicates that the traps of closely related species tend to emit similar intensities of volatiles but the compounds themselves are not similar. Conversely, the flowers of closely related species tend to emit similar compounds, but tend to differ in scent abundance.

We found that flower and trap scent did not significantly co-vary in either volatile emission rates or composition (p>0.05). Further supporting this result, the net evolutionary rate for the entire set of volatiles from flowers and traps differed significantly, whereby floral scent evolved 30% faster than trap scent ($\sigma^2_{mult.flower}/\sigma^2_{mult.trap}$ = 1.3, p<0.01). When estimating the net evolutionary rate of volatiles that have been associated with bee-visitation, we found that these scents evolved 15 times as fast in flower versus traps ($\sigma^2_{mult.flower.bee}/\sigma^2_{mult.trap.bee}$ = 15, p<0.01). Conversely, herbivory-related volatiles evolved greater than two times faster in traps than flowers ($\sigma^2_{mult.flower.herbivory}/\sigma^2_{mult.trap.herbivory}$ = 2.4, p<0.01).

Chemical separation was not predicted by spatial or temporal divergence

We found no significant relationship with the level of chemical divergence between flowers and traps, and the level of temporal and spatial divergence: flowers and traps did not produce more divergent scent bouquets, even when they matured at similar times and heights (S2-E).

Discussion

Distinct scent partitioning across pitchers and flowers

Our study revealed distinct scent divergence between flowers and trapping leaves, consistent with the hypothesis that scent production in these tissues is subject to distinct selective pressures in the NA Sarraceniaceae. This divergence is partially explained by a greater production of scent compounds in flowers, which emit a greater intensity and broader range of terpenoids than traps. Within tissues, scent variance in flowers was more than six times greater than that in traps (Fig 1b). This disparity may result from selection for pollinator constancy amongst flowers, which are specialists and typically recruit one main pollinator. In contrast, the lack of distinct clustering in traps may reflect a more generalist trap strategy to attract a wide variety of insect genera and species. Surveys of unbagged traps in our study plants confirm a range of trapped insects that include dipterans (flies, mosquitoes), lepidopterans, and hymenopterans (honeybees).

Nevertheless, a handful of floral volatiles, including limonene, caryophyllene, α-pinene, and sabinene, were also produced in traps. There are several possibilities for this overlap, which could result from either floral mimicry in traps (e.g 55), or convergence on similar tactics for invertebrate attraction (56). One intriguing possibility is that flower and trap scents are aligned for long distance insect attraction, and it is only at close distances that divergence is necessary to distinguish flowers and traps. This is consistent with a recent study showing that floral scent in *Pinguicula*, a sticky trap carnivore, attracts both pollinators and prey, whereas only prey are attracted to leaf scents (57). This synergistic effect of flower and leaf scent on insect attraction is also observed in other taxa (e.g. 58). Finally, because vegetative and floral tissues often share overlapping biochemical pathways (59,60), another possibility is that the expression levels of these compounds across flowers and traps are not readily decoupled.

Independent regulation of floral and vegetative scent?

Our SVD analysis identified several suites of correlated chemicals produced *within* each tissue (Fig 2, S2-D). These integrated chemical modules identified within flowers and traps have several ramifications. First, it is widely recognized that many traits evolve in a concerted manner (61,62) and have the potential to constrain or facilitate evolution (63,64). This is also the case for floral phenotypes (65,66), and in wild *Brassica*, selection on a single volatile compound can pleiotropically alter the scent of the entire volatile bouquet, typically by increasing emissions of non-target compounds (38). Furthermore, VOCs from similar chemical classes in the *Brassica* study had stronger correlation coefficients than those from different classes (38). This is consistent with our analysis of trap compounds, and the observed benzenoid- or terpenoid- dominated strategies might represent biochemical or genetically constrained modules (S2-D). Nevertheless, our phylogenetic analyses revealed that *across* floral and vegetative tissues, scent did not significantly covary in emission rate or composition. This indicates that although within-tissue emissions may covary as a unit, volatiles in this taxon might be regulated independently across tissues. This concurs with other studies of floral and vegetative traits in which selection for functional independence can occur (67-69).

Phylogenetic signal of scents in the NA Sarraceniaceae

In the NA Sarraceniaceae, closely-related species tended to produce more similar volatile bouquets in flowers, and more similar quantities of scent in traps. Interestingly, the reverse was not true – we found that neither scent abundance in flowers, nor scent composition in traps exhibited phylogenetic signal. This ambiguity may reflect the homoplasious nature of trap volatiles (S2-D), or of scent characters more generally: the presence of phylogenetic signal in volatile composition appears to depend on the taxon (e.g. 70-72), as well as on the compounds sampled (73-75).

Rates of volatile evolution in flowers and traps

In many angiosperm systems, pollinators and herbivores are forceful drivers of floral and vegetative diversity. However, despite the acknowledged importance of scent in mediating these crucial interactions, there is surprising little data on how selective forces influence the evolution of scent diversity across taxa. Here, we found that floral scent evolved 1.3 times faster than scent from trapping leaves, raising the possibility that sexual selection contributes to volatile diversity in the outcrossing NA *Sarracenia*. We investigated this further by examining specific volatiles that were electrophysiologically and behaviorally relevant to *Bombus* and solitary bee pollinators in this clade. This uncovered an even stronger effect in which these volatiles evolved an astonishing 15 times more rapidly in flowers than in traps. Our results reveal pollinator-mediated sexual selection may have an outsized importance on the rates of floral scent evolution in the NA pitcher plants. Furthermore, our finding that sexual selection imposed by pollinators could profoundly influence volatile traits in the NA pitcher plants are augmented by recent studies showing that in dioecious species, pollinators are also associated with the evolution of sexual dimorphism in floral scent (19,76).

Although scent from trapping leaves evolved more slowly than floral scent, we found that compounds associated with herbivory still evolved at more than double the rate in traps than in flowers. In the NA *Sarracenia*, one of the chief herbivores are noctuid moths (genus: *Exyra*). Vegetative damage from these pitcher plant specialists (34) can exert strong selective pressure on pitcher traits, reducing plant size and leaf growth (33). Our data indicate herbivore-associated compounds evolved much more quickly in traps than in flowers, suggesting that herbivory, likely from *Exyra* damage, has played a significant role in the evolutionary history of NA pitcher plants.

Together, these results provide the impetus for integrative studies that will not only link scent production with specific pollinator and herbivore interactions, but which will also explore the functional consequences of these interactions on pitcher plant fitness.

Pollinator-prey conflict (PPC) in the carnivorous plants

In carnivorous plants, insects function as both pollinators and prey. This unusual life history gives rise to the PPC (77), a trade-off which is most apparent in outcrossing, pollen-limited species (37) like the NA Sarraceniaceae (29,30,78). Flower and trap scents were highly distinct, consistent with the hypothesis that traps and flowers might target private sensory channels to alleviate pollinator-prey conflict. Nevertheless, species with a greater potential for conflict (i.e. less physical separation between flowers and traps), did not produce flower and trap scents that were more divergent. Thus, volatiles may act in concert with temporal and spatial separation to alleviate PPC, or alternatively, may not be involved in PPC at all – we emphasize the need for functional data on the sensory systems of different insect guilds (pollinators, prey, herbivores) to distinguish between these possibilities.

Summary and Conclusions

There is now strong evidence that animal mutualists and antagonists can have robust effects on plant scent, but how these forces influence scent evolution and volatile diversity, especially with respect to the sensory ecology of the receivers remains an open question (24,79). This study is, to our knowledge, the first to address how sexual and natural selection might influence rates of scent evolution, and recognizes the outsized influence of sexual selection in floral volatile evolution. We also re-emphasize the importance of physiological studies that specifically target the olfactory sensory biology of Sarraceniaceae mutualists and antagonists, as well as data on how pollinator, herbivore, and prey interactions interact to influence plant fitness. These studies, along with longer-term selection experiments, are crucial for distinguishing whether scent modularity results from biochemical constraints, or from insect-mediated ecological selection. Finally, we suggest that while the traditional emphasis on prey capture in defining carnivorous plant phenotypes is a useful one, our framework should be expanded to include generous roles for herbivore- and pollinator- mediated natural and sexual selection, at least in the context of scent evolution.

Supporting Data

Source material accessions are stored at the University of Washington Herbarium (S3-B). Primary data files and resources for data analyses are provided through the electronic supplementary materials (S1-S3) or upon request.

Competing Interests

We have no competing interests.

Authors' contributions

WH, NK, JR conceived and designed the study; WH collected and analysed chemical data; JN conducted phylogenetic rates analyses; NK generated code for the SVD analysis; WH, NK, JN carried out data and statistical analyses; WH, NK, JN, JR drafted and revised the manuscript. All authors gave final approval for publication.

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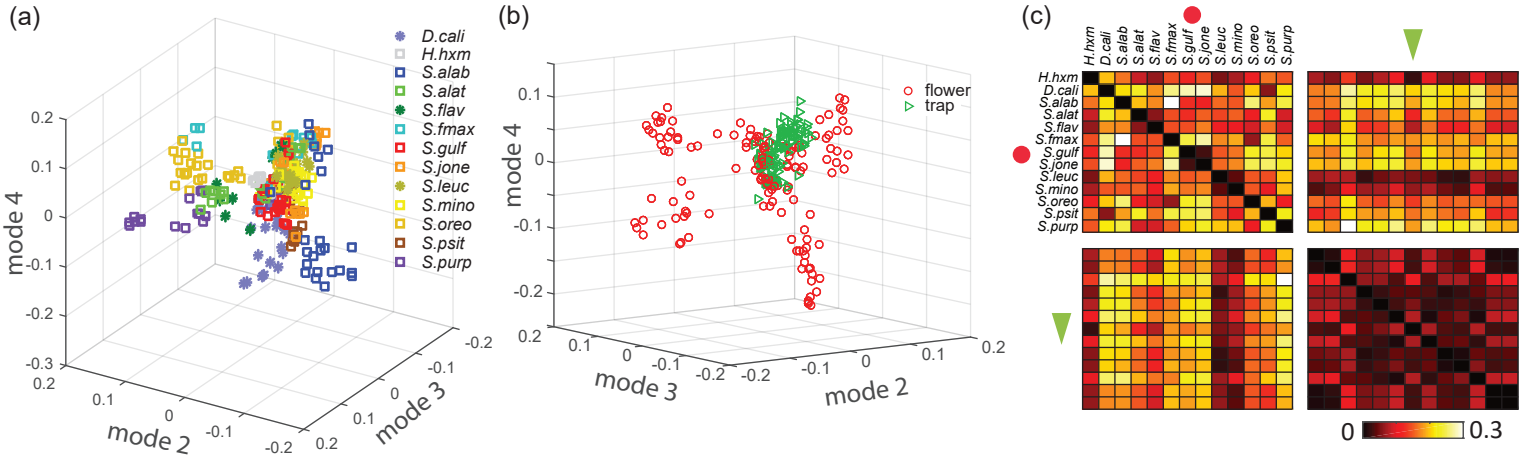
Figure Captions

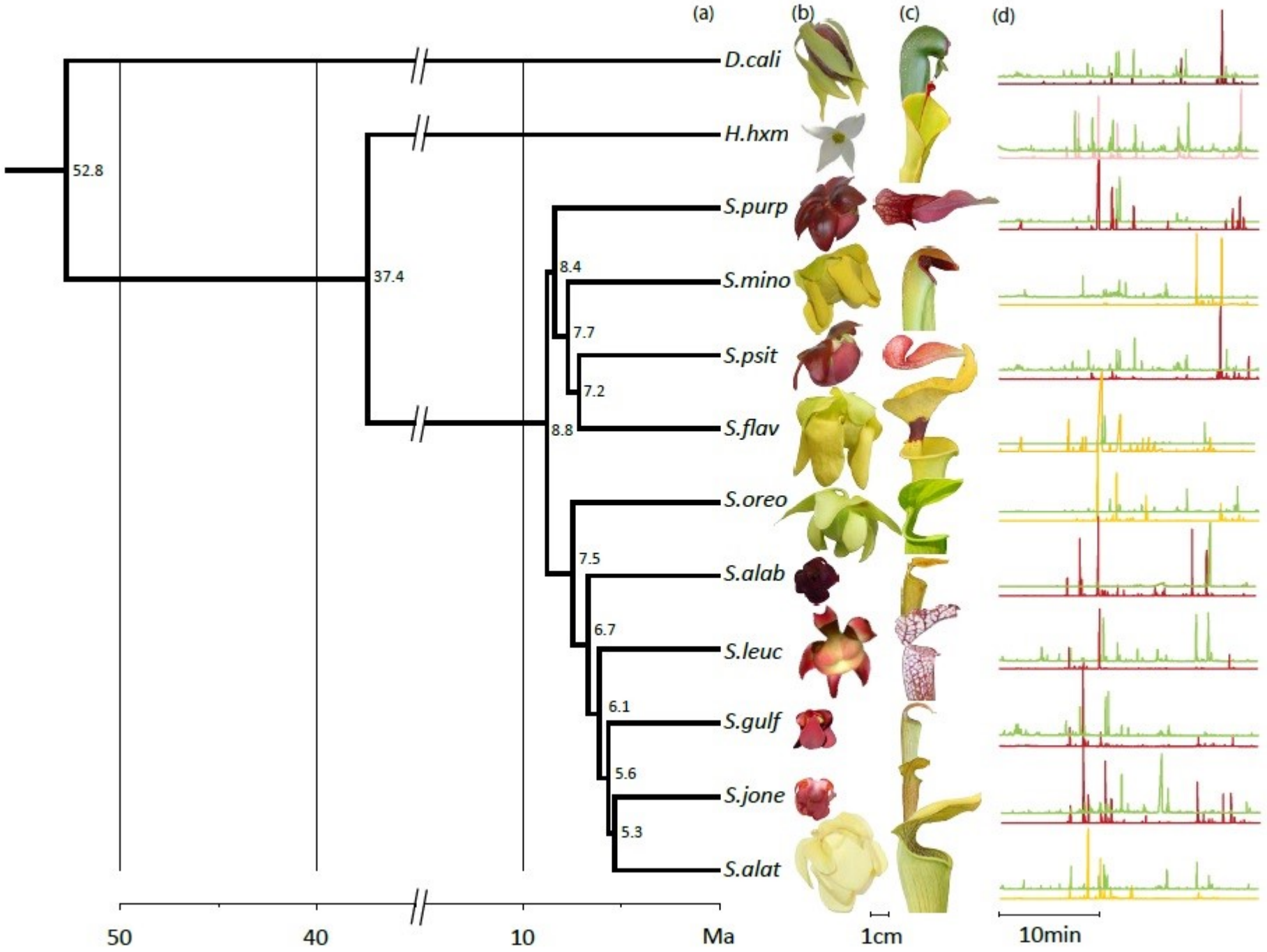
Figure 1. Scent differences shown (a) as scatterplots across species, (b) across tissues, and (c) as a heatmap across tissues and species. Scatterplots are adjusted to show the most important axes of separation. The color bar shows the magnitude of scent divergence: bright colors represent a high degree of separation and dark colors represent low separation. Flowers = red circles; traps = green triangles. Species names are abbreviated as the first generic initial, followed by 3-4 letters from the specific epithet; full names and abbreviations are provided in accession table S3-B.

Figure 2. Volatiles (numbers reference to S3-A) most important for explaining floral diversity extracted from nested singular value decompositions are shown as a heatmap of relative intensity (color bar, left). Top: correlation modes 1-4.

Figure 3. Time-calibrated phylogeny of the (a) Sarraceniaceae with (b) flowers, (c) traps, and (d) scent chromatograms (upper green trace = trap; lower trace = flower); amplitudes are scaled for ease of visualization (*H.hxm* flower photo courtesy of the UW botany greenhouse).



Mode 4
Mode 3
Mode 2
Mode 1
0.2
0
-0.2
0.4
aliphatics
benzenoids
terpenoids
S. purp
S. psit
S. oreo
S. mino
S. leuc
S. jone
S. gulf
S. fmax
S. flav
S. alat
S. alab
D. cali
H. hxm
20
10
0
Acetate, 2-Methyl-1-butyl (1)
Acetic acid, hexyl ester(2)
Allyl isovalerate
Cetene(7)
Decanal(8)
Decane(9)
Dodecanal(12)
Dodecane(13)
Dodecene, 1-(14)
Eicosane(15)
Ethanol, 2-(2-butoxyethoxy)-(17)
Ethylhexanol, 2-(19)
Heneicosane(18)
Heptadecane(19)
Heptanone, 2-(21)
Hexadecanal(22)
Hexadecane(23)
Hexadecanoic acid, ethyl ester(24)
Hexana
Hexanoic acid, ethyl ester(26)
Hexanol, 1-
Hexenal, (E)-2-
Hexenal, 2-(27)
Hexenol acetate, (Z)-3-(28)
Hexenol, (E)-2-
Isoamyl acetate(30)
Mesityl oxide(31)
Nonadecane(33)
Nonanal(35)
Nonanedial, 2,6-(E,Z)-(34)
Nonanoic acid, methyl ester(36)
Nonanone-2
Octadecane(39)
Octadecanoic acid(40)
Octanal(43)
Octanoic acid, methyl ester
Octanol, 1-(46)
Pentadecane(49)
Sulcatone(51)
Tetradecanal(53)
Tetradecane(54)
Tetradecanoic acid, ethyl ester(55)
Tetradecene, 1-(56)
Tricosane
Tridecane(57)
Undecane(58)
Acetic acid, phenylmethyl ester(59)
Acetophenone(60)
Anisole(61)
Benzaldehyde(62)
Benzenemethanol, α-methyl-(65)
Benzoic acid, 2-methylpropyl ester(67)
Benzoic acid, 4-methoxy-, methyl ester(68)
Benzoic acid, ethyl ester(69)
Benzoic acid, methyl ester(70)
Benzyl alcohol(71)
Cymene, p-(73)
Cymenene, p-(74)
Diethyl phthalate(75)
Dimethoxybenzene, 1,4-(76)
Durene(79)
Ethanone, 1-(4-ethylphenyl)-(79)
Ethylbenzaldehyde, 4-(81)
Mesitylene(84)
Methyl salicylate(85)
Phenylethyl alcohol(87)
Styrene(88)
Xylene, o-(89)
Ionone, (E)- β-(160)
Borneol(90)
Camphene(91)
Carene, 3-(92)
Carvone, D-(+)-(93)
Elemol(95)
Eucalyptol(96)
Ipsdienol(98)
Limonene(99)
Limonene, 1,2-oxide
Limonene, iso-(103)
Linalool(103)
Linalool oxide, (E)-(104)
Linalool oxide, (Z)-(105)
Menthatriene, 1,3,8-p-(107)
Myrcene, β-(108)
Monoterpene, unk 1(109)
Ocimene, allo-(111)
Ocimene, β-(112)
Ocimene, γ-(113)
Phellandrene, β-(115)
Phellandrene, α-(116)
Pinene, α-(116)
Pinene, β-(117)
Pinocamphone, Iso-(118)
Sabinene(119)
Terpinen-4-ol(120)
Terpinene, α-(121)
Terpinene, γ-(122)
Terpineol, α-(123)
Thujene, α-(126)
Verbenone(129)
Bergamotene, trans-α-(132)
Bisabolene, cis-α-(133)
Bisabolene, β-(134)
Bisabolol, α-(135)
Cadinene, γ-epi-α-(136)
Cadinene, γ-(137)
Cadinene, δ-(138)
Calacorene, δ-(139)
Caryophyllene(140)
Caryophyllene oxide
Caryophyllene, γ-(141)
Cedrene, 7-epi-α-(142)
Copaene, α-(143)
Cubebene, β-(145)
Cubebene, α-(144)
Curcumene, α-(146)
Elemene, β-(146)
Farnesene, β-(148)
Sesquiterpene, unk 3 (171)
Farnesene, (Z)-β-(149)
Farnesene, α-(150)
Germacrene D(154)
Guaiol(156)
Gurjunene, β-
Humulene, γ-(159)
Muurolene, γ-(164)
Nerolidol, (Z)-(165)
Nerolidol, (E)-(165)
Santalene, (+)-epi-β-(168)
Santalene, (-)-α-(167)
Santalene, β-(169)
Sesquiphellandrene, β-(170)
Tricyclene, α-
Ylangene(172)
Zingiberene, α-(173)


(a)
(b)
(c)
(d)
D.cali
H.hxm
S.purp
S.mino
S.psit
S.flav
S.oreo
S.alab
S.leuc
S.gulf
S.jone
S.alat
52.8
37.4
8.4
7.7
7.2
8.8
7.5
6.7
6.1
5.6
5.3
50
40
10
Ma
1cm
10min