

Are deception-pollinated species more variable than those offering a reward?

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Abstract In most pollination systems, animals transfer pollen among plants of a given species. Pollinator visitations do not come without cost, so plants usually offer a reward. However, the flowers of some plant species, mostly orchids, lack rewards and deceive animals into visiting their flowers. Deceptive species are thought to have high levels of variation in traits associated with advertisement and pollinator attraction, which have been attributed to genetic drift, or disruptive selection due to pollinator behavior. Rewarding species are assumed to have less variation due to stabilizing selection. We compared variability in floral morphology and fragrance composition between deceptive and rewarding species. Because both suites of traits are often linked with floral advertisement and pollinator attraction, we expected variation to be greater in species with deceptive pollination systems than in those offering rewards. We obtained floral morphology metrics for 20 deceptive species and 41 rewarding species native or naturalized in Puerto Rico, Venezuela, and Ecuador. Floral fragrances were sampled from eight deceptive species and four rewarding species. We found that the amplitude of variation in floral morphology and fragrance composition covaries significantly. Comparison of coefficients of variation for morphology indicated that, overall, deceptive species show significantly higher variation than rewarding species, and this pattern was also found among just orchids or just nonorchids. There were no

statistical differences in morphological variation between orchids and nonorchids within a functional pollination group. Fragrance variation, measured by Jaccard distance, tended to be greater for deceptive species than for rewarding species. Although overlap in measures of variation occurs between the two groups, the data support the hypothesis that populations of deception-pollinated species are more variable than rewarding species in traits associated with pollinator attraction.

Keywords Orchidaceae · Floral fragrances · Floral traits · Flower advertisement · Morphological variation · Pollinator attraction · Pollination mechanisms

Introduction

Most flowering plants require the services of pollinators for successful reproduction, and these usually visit flowers for food such as nectar, oils, and pollen (Simpson and Neff 1983). The floral displays of plants, with their morphologies, colors, and fragrances, function to attract certain animals that learn to associate these floral signals with the rewards.

Most species of flowering plants offer rewards to their pollinators, but some do not (Little 1983; Dafni 1984; Ackerman 1986; Nilsson 1992; Jersáková et al. 2006a). Flowers without rewards deceive pollinators by exploiting aspects of their behavior. Deceptive pollination systems are most prominent in the Orchidaceae, where they occur in nearly all tribes and account for approximately 10,000 species (van der Pijl and Dodson 1966; Ackerman 1986). Such systems also exist in Apocynaceae, Araceae, Begoniaceae, Bignoniaceae, Caricaceae, Lowiaceae, and Scrophulariaceae, and species of other families (Gentry

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1974; Haber 1984; Sakai and Inoue 1999; Renner 2006). Several ways are known by which plants deceive their pollinators, but the most common form of deception pollination is having the appearance of a potential food resource without mimicry of specific models (generalized food deception; Ackerman 1986; Jersáková et al. 2006a).

After emergence or arrival to a new area, flower visitors often investigate a wide variety of species, but soon form search images for the most rewarding flowers and restrict their foraging to a small subset of available species (Heinrich 1975, 1976; Meléndez-Ackerman et al. 1997). Under these circumstances, there should be stabilizing selection for advertisement traits associated with pollinator attraction (morphology, color, and fragrances; Dafni 1992) to enhance the learning process of pollinators.

On the other hand, food deceptive flowers usually rely on visits from either recently emerged insects or those that monitor food availability in their habitats as the energetic returns diminish from the plants they do visit (Nilsson 1980; Ackerman 1981, 1983). It has been proposed that variation among deceptive flowers may increase the number of flower visits by individual pollinators by interfering with the associative learning process, causing pollinators to visit more flowers before they learn to avoid them (Heinrich 1975; Ackerman 1981; Moya and Ackerman 1993; Ayasse et al. 2000). Such levels of variation may be maintained by negative frequency-dependent selection (Ackerman and Galarza-Pérez 1991; Smithson and Macnair 1997; Gigord et al. 2001), or perhaps through relaxed selection or some other means of genetic drift (Tremblay et al. 2005; Salzmänn et al. 2007a). Considerable variation in floral traits has been reported for several deceptive species (Ackerman and Galarza-Pérez 1991; Moya and Ackerman 1993; Ayasse et al. 2000; Aragón and Ackerman 2004; Salzmänn et al. 2007b; Luo and Chen 2008), but only once have levels of variation been compared between rewarding and deceptive flowers. Salzmänn et al. (2007a) contrasted floral fragrance variation of a deception-pollinated species with that of a rewarding congener and found the variation in the former species to be substantially greater. This result supports the deception variation hypothesis; however, we do not know whether high variation in floral fragrances is indeed a general phenomenon in deception-pollinated species.

In this study we compare variation between deceptive and rewarding species in two of the three components assumed to be involved in advertisement: floral morphology and fragrances. We predict that fragrance and morphological variation will covary, since they are both associated with pollinator attraction (Dafni 1992), and that this variation should be greater in deceptive species than those offering rewards to their pollinators.

Methods

Morphological variation

We compared the degree of variation in flower morphology between deceptive and rewarding species. Our choice of species was largely opportunistic. We specifically tried to avoid autogamous species, and when we were not sure, we excluded pollinators with mesh bags to determine if flowers would set fruit. Data of most species were taken from single populations. We randomly selected one fresh flower per plant, from 8 to 40 individuals per species (Table 1). Field measurements were taken with dial calipers. We collected and pickled samples of small flowered species and then measured them in the laboratory with a dissecting microscope fitted with an ocular micrometer. Voucher specimens are on deposit at the Herbarium of the University of Puerto Rico, Río Piedras Campus (UPRRP).

We included 60 species in the study (Table 1). Data for three species were taken from herbarium specimens, and four species came from the archive data of published studies (Nielsen 2000; Ackerman and Galarza-Pérez 1991; Nassar et al. 1997). Most species were from Puerto Rico ($n = 48$), but some came from the Andes of Ecuador ($n = 10$) and the dry forests of Venezuela ($n = 2$). Of the 60 total species, 41 have rewarding flowers and represent 25 families, while 20 species have deceptive flowers and represent three families. Two of the deceptive species, *Lepanthes rupestris* and *Lepanthes woodburyana*, are presumably pollinated by sexual deception (see Blanco and Barbosa 2005). One naturalized, deceptive species, *Arundina graminifolia* (Orchidaceae), and one naturalized rewarding species, *Scaevola taccada* (Goodeniaceae), are included. Additionally, we obtained data from both male and female flowers of an automimic, monoecious species, *Begonia decandra* (Begoniaceae).

We took measures of morphological traits that appeared to be involved in floral display (advertisement). Because flower morphology differs drastically among taxa and pollination syndromes, it would not have been possible to apply the same set of measurements to all species and capture variation in flower morphology within each species (e.g., orchid versus legume versus tubular flowers). In general, we measured length and width of the total floral display, and when possible and relevant we also measured length and width of each independent structure involved in the display such as the lip of orchids and banners of legumes (Table 1). We avoided structures that appeared to change during anthesis.

A coefficient of variation (CV) was calculated for each trait measured, and an average CV was calculated for each species. We compared average coefficients of variation between four groups of species (deceptive orchids,

Table 1 Morphological variability in rewarding and deceptive species, ordered by rank

Species	Family	Site	Pollination system	<i>N</i> plants	<i>N</i> : Traits	CV average
<i>Bletia patula</i> Graham	Orchidaceae	PR	Deception	25	6: dorsal sepal L & W, petal L & W, lip L & W	23.0
<i>Dilomilis montana</i> (Sw.) Summerh.	Orchidaceae	PR	Deception	26	2: dorsal sepal L, lip mid lobe W	20.1
<i>Psychilis krugii</i> (Bello) Sauleda	Orchidaceae	PR	Deception	31	4: flower W, sepal W, lip lateral lobe W, lip mid lobe W	19.5
<i>Ionopsis utricularioides</i> (Sw.) Lindl.	Orchidaceae	PR	Deception	14	4: dorsal sepal L & W, lip L & W	19.3
<i>Plumeria krugii</i> Urb.	Apocynaceae	PR	Deception	29	3: flower W, petal L & W	18.9
<i>Lepanthes rupestris</i> Stimson	Orchidaceae	PR	Sexual deception	40	6: flower W, petal L & W, W across lateral lobes of lip	18.0
<i>Begonia decandra</i> Pav. female	Begoniaceae	PR	Deception	31	4: flower W, petal W, stigma cluster L & W	17.4
<i>Gonzalagunia spicata</i> (Lam.) Gómez Maza	Rubiaceae	PR	Reward	35	4: flower L & W, petal L & W	17.3
<i>Prosthechea cochleata</i> (L.) W. E. Higgins	Orchidaceae	PR	Deception	21	4: dorsal sepal L & W, lip L & W	17.1
<i>Begonia decandra</i> Pav. male	Begoniaceae	PR	Reward	38	4: flower W, petal W, androecium L & W	16.8
<i>Erithalis fruticosa</i> L.	Rubiaceae	PR	Reward	33	4: flower L & W, petal L & W	16.6
<i>Morinda citrifolia</i> L.	Rubiaceae	PR	Reward	30	2: flower W, petal W	16.6
<i>Epidendrum ibaguense</i> Kunth	Orchidaceae	ECU	Deception	32	3: flower W, lip W, lip + column L	16.5
<i>Plumeria alba</i> L.	Apocynaceae	PR	Deception	21	3: flower W, petal L & W	16.4
<i>Hillia parasitica</i> Jacq.	Rubiaceae	PR	Reward	38	3: flower W, petal L & W	16.4
<i>Epidendrum</i> sp.	Orchidaceae	ECU	Deception	21	3: flower W, lip W, lip + column L	15.2
<i>Rodriguezia lehmannii</i> Rchb. f.	Orchidaceae	ECU	Deception	32	3: flower L & W, lip W	15.1
<i>Pilosocereus lanuginosus</i> (L.) Byles & C. D. Rowley	Cactaceae	VEN	Reward	22	2: flower L, corolla diameter	14.5 ^a
<i>Tolumnia variegata</i> (Sw.) Braem	Orchidaceae	PR	Deception	18	8: dorsal sepal L & W, petal L & W, lip L, lip wing L & W, column wing L	14.2
<i>Wedelia trilobata</i> (L.) A. S. Hitchc.	Asteraceae	PR	Reward	30	4: head L & W, ligulate corolla L & W	13.9
<i>Palicourea riparia</i> Benth.	Rubiaceae	PR	Reward	25	2: flower L, petal W	13.6
<i>Rhynchospora ciliata</i> (G. Mey.) Kük.	Cyperaceae	PR	Reward	29	3: number of white bracts, widest white bract W, longest white mark on bract W	13.4
<i>Sobralia rosea</i> Poepp. & Endl.	Orchidaceae	ECU	Reward	32	3: petal W, lip L & W	13.1
<i>Simarouba tulae</i> Urb.	Simaroubaceae	PR	Reward	34	3: flower W, petal L & W	12.9
<i>Lantana involucrata</i> L.	Verbenaceae	PR	Reward	30	5: flower L & W, petal L & W, lip W	12.8
<i>Xyris jupicai</i> Rich.	Xyridaceae	PR	Reward	30	3: flower W, sepal W, stamen L	12.8
<i>Krameria ixina</i> L.	Krameriaceae	PR	Reward	30	4: flower L & W, lateral petal W, oil petal W	12.4
<i>Vanilla barbellata</i> Rchb.f.	Orchidaceae	PR	Deception		5: petal L & W, lip height, lip L & W	12.4 ^b
<i>Lobelia portoricensis</i> (Varke) Urb.	Lobeliaceae	PR	Reward	34	3: flower L & W, petal L	12.1
<i>Miconia</i> sp.	Melastomataceae	PR	Reward	30	3: flower L & W, petal W	12.0
<i>Andira inermis</i> (W. Wr.) Kunth ex DC.	Fabaceae	PR	Reward	17	4: banner L & W, keel L, wing W	12.0
<i>Arundina graminifolia</i> (D. Don) Hochr.	Orchidaceae	PR	Deception	22	5: flower L, dorsal sepal W, petal W, lip L & W	12.0
<i>Nepsera aquatica</i> (Aubl.) Naudin	Melastomataceae	PR	Reward	30	5: flower W, petal L & W, androecium L & W	11.9
<i>Gesneria cuneifolia</i> (DC.) Fritsch	Gesneriaceae	PR	Reward	30	2: corolla L & W	11.8
<i>Tabebuia heterophylla</i> (DC.) Britt.	Bignoniaceae	PR	Reward	19	4: flower L & W, flower height, gullet width	11.6
<i>Epidendrum cochlidium</i> Lindl.	Orchidaceae	PR	Deception	8	3: flower W, lip W, lip + column L	11.5
<i>Cuphea strigillosa</i> Lindl.	Lythraceae	PR	Reward	30	4: flower L & W, petal L & W	11.3
<i>Lepanthes woodburyana</i> Stimson	Orchidaceae	PR	Sexual deception	26	6: flower W, petal L & W, W across lateral lobes of lip	11.3
<i>Stigmaphyllon emarginatum</i> A. Juss.	Malpighiaceae	PR	Reward	21	4: flower W, lateral petal W, flag petal L & W	11.1

Table 1 continued

Species	Family	Site	Pollination system	<i>N</i> plants	<i>N</i> : Traits	CV average
<i>Avicennia germinans</i> (L.) Stearn	Verbenaceae	PR	Reward	25	2: flower W, lip W	10.7
<i>Hamelia axillaris</i> Sw.	Rubiaceae	PR	Reward	29	2: flower L & W	10.6
<i>Ipomoea pes-caprae</i> (L.) R. Br.	Convolvulaceae	PR	Reward	19	2: flower L & W	10.6
<i>Ornithidium coccineum</i> (Jacq.) Salisb. ex R. Br.	Orchidaceae	PR	Reward	10	5: dorsal sepal L, lateral sepal L & W, flower depth, flower W	10.3
<i>Elleanthus aurantiacus</i> Rehb. f.	Orchidaceae	ECU	Reward	25	2: flower L & W	10.1
<i>Vanilla claviculata</i> Lindl.	Orchidaceae	PR	Deception		5: petal L & W, lip height, lip L & W	10.0 ^b
<i>Calceolaria</i> sp.	Calceolariaceae	ECU	Reward	25	3: hood W, lip W, lip-hood L	9.7
<i>Maxillaria acuminata</i> Lindl.	Orchidaceae	ECU	Reward	31	3: lateral sepal L, lip L, lip mid lobe W	9.7
<i>Oxalis corniculata</i> L.	Oxalidaceae	PR	Reward	29	4: flower L & W, petal L & W	9.4
<i>Lantana camara</i> L.	Verbenaceae	PR	Reward	30	5: inflorescence W, flower W, throat W, lip L & W	9.4
<i>Cestrum diurnum</i> L.	Solanaceae	PR	Reward	16	3: corolla L, corolla apex W, petal W	8.5
<i>Sobralia crocea</i> (Poepp. & Endl.) Rehb. f.	Orchidaceae	ECU	Deception	21	3: dorsal sepal L & W, lip mid lobe W	8.4
<i>Stachytarpheta jamaicensis</i> (L.) Vahl.	Verbenaceae	PR	Reward	30	3: flower W, lip W, white spot W	8.0
<i>Asclepias curassavica</i> L.	Apocynaceae	PR	Reward	30	4: corolla L & W, corona L & W	7.7
<i>Talipariti tiliaceum</i> (L.) Fryxell var. <i>pernambucense</i> (Arruda) Fryxell	Malvaceae	PR	Reward	16	2: androecium diameter, petal W	7.6
<i>Microchilus plantagineus</i> (L.) D. Dietr.	Orchidaceae	PR	Reward	30	4: flower W, lateral sepal L, lip W, lip + spur L	7.3
<i>Malaxis massonii</i> (Ridl.) Kuntze	Orchidaceae	PR	Deception	17	5: lip L & W, lip mid lobe L & W, lip lateral lobe L	7.1
<i>Malvaviscus penduliflorus</i> DC.	Malvaceae	PR	Reward	30	1: flower L	7.0
<i>Scaevola taccada</i> (Gaertn.) Roxb.	Goodeniaceae	PR	Reward	13	3: flower W, corolla L, mid petal W	6.9
<i>Gentiana</i> sp.	Gentianaceae	ECU	Reward	21	3: flower W, petal W, filament L	5.6
<i>Pitcairnia angustifolia</i> Aiton	Bromeliaceae	PR	Reward	30	1: flower L	5.6
<i>Pilosocereus moritzianus</i> (Otto) Byles & R. D. Rowley	Cactaceae	VEN	Reward	23	2: flower L, corolla diameter	5.3 ^a

CV coefficient of variation, *PR* Puerto Rico, *ECU* Ecuador, *VEN* Venezuela, *W* width, *L* length

^a Data recalculated from Nassar et al. (1997) and Rivera-Marchand and Ackerman (2006)

^b Data from Nielsen (2000)

deceptive nonorchids, rewarding orchids, rewarding non-orchids) by a single classification analysis of variance (ANOVA) followed by cross-comparison *t* tests. We also used an ANOVA to test whether the combined average CVs of all deceptive species differed from that of all rewarding species.

Fragrance variation

Variation in floral fragrance composition was assessed for eight deception-pollinated species representing two families and four rewarding species each from a different family (Table 2). Seven of the deceptive species and three of the rewarding species analyzed were also measured for morphological variation. We sampled one flower from each

of 3–15 individuals per species (average 7.7, Table 2) by employing a swab sampling technique with solid-phase microextraction fibers (SPME; Supelco Co., Bellefonte, PA, USA).

The SPME fibers we used consist of 10 mm long, 100 µm diameter silica fibers, coated with 100 µm polydimethylsiloxane (PDMS). New fibers were conditioned for at least 4 h in the gas chromatograph injection port at 250°C. Conditioned fibers were used immediately or kept inside a clean screw cap (Teflon lining) glass test-tube (Kimax) in a glass desiccator at 4°C and protected from contamination by inserting the end of the fiber cover apparatus into a previously punctured gas chromatography (GC) injection port septum (Gorlo et al. 1997). The cap-septum was conditioned by leaving it in contact with the

Table 2 Fragrance variation in rewarding and deceptive species

Species	Family	Pollination system	<i>N</i> plants	Average SCA	Average Jaccard distance
<i>Epidendrum anceps</i> Jacq.	Orchidaceae	Deception	3	6×10^7	0.77
<i>Tolumnia variegata</i> (Sw.) Braem	Orchidaceae	Deception	15	6×10^7	0.68
<i>Bletia patula</i> Graham	Orchidaceae	Deception	4	1×10^9	0.68
<i>Psychilis krugii</i> (Bello) Sauleda	Orchidaceae	Deception	12	2×10^8	0.58
<i>Guaiacum officinale</i> L.	Zygophyllaceae	Reward	8	6×10^7	0.51
<i>Dilomilis montana</i> Summerh.	Orchidaceae	Deception	5	4×10^8	0.51
<i>Plumeria alba</i> L.	Apocynaceae	Deception	10	1×10^8	0.46
<i>Lepanthes rupestris</i> Stimson	Orchidaceae	Sexual deception	10	6×10^8	0.32
<i>Scaevola taccada</i> (Gaertn.) Roxb.	Goodeniaceae	Reward	6	1×10^8	0.31
<i>Ipomoea pes-caprae</i> (L.) R. Br.	Convolvulaceae	Reward	9	3×10^9	0.25
<i>Arundina graminifolia</i> (D. Don) Hochr.	Orchidaceae	Deception	6	1×10^9	0.21
<i>Cestrum diurnum</i> L.	Solanaceae	Reward	4	1×10^8	0.16

SCA sum of corrected area, a measure of fragrance quantity

injector during fiber conditioning and GC/mass spectrometry (MS) analysis. Fibers were reused and cleaned as above before each sampling procedure.

Sampling involved gently swabbing the perianth while positioning the SPME fiber parallel to the surface to be sampled. In the same motion, we manually rotated the fiber to ensure that its entire surface area was exposed to the flower. Sampling time was 3 min unless otherwise specified. All samples were taken in the field or the shade house from flowers still attached to the plant.

For every sampling bout, we had a field blank. We exposed a clean fiber for 3 min while we swab-sampled the flowers. These field blanks were kept 1–3 m distant from the fragrance source. All samples were protected from heat and stored at 4°C whenever samples could not be run immediately. A conditioned blank accompanied each batch of loaded fibers whenever they were placed in storage. With this desorption schedule and monitoring of the blanks, memory effects on the fibers were insignificant.

Each fiber was introduced directly into the injector, initially in the splitless mode, then programmed to open the splitter after 5 min. The optimum desorption time was 5 min for PDMS at 250°C. We used an SPB-5 column (32 nm, 30 m) to separate the compounds in the GC/MS (Hewlett Packard 5090 series 2). Column temperatures were optimized at 40°C for 5 min, first increasing at 25°C/min to 70°C, then increasing at 7°C/min to 190°C, followed by an increase at 10°C/min to 250°C, and finally held for 10 min. The MS scan was set at 45–800 Da to decrease background noise.

Chromatographic peaks were identified tentatively by retention time, mass spectra, elution patterns, and comparison with a mass spectral library (NIST MS 2002). Except for where specified, a reverse match quality of 900

or higher was accepted for positive identification (1,000 being a perfect match).

The RTE Integrator was used to generate an area percent report (Enhanced Chemical Station G1701AA version A.03.00; Hewlett Packard, 1989–1996). All integration parameters were set as default, except to enable smoothing of data points after sampling, and the maximum number of peaks was set to 25.

We took the GC/MS results and constructed a matrix of compounds that accounted for at least 5% of the fragrance captured by each fiber. From this matrix we calculated average Jaccard distances using PC-ORD version 5 after eliminating outliers and relativized by maximum (McCune and Grace 2002; McCune and Meford 2006). This was our measure of fragrance composition variation in a sample.

We first assessed whether or not fragrance variation covaried with morphological variation by testing the correlation between average Jaccard distances for the fragrance data and average coefficients of variation for the morphological data (JMP 5.1.1 statistical package). We then asked whether fragrances of rewarding flowers were more variable than those of deceptive flowers using non-parametric Mann–Whitney *U* test (freeware PAST version 1.97; <http://folk.uio.no/ohammer/past/version.html>).

Results

Morphological variation

The average coefficients of variation for the 60 species sampled showed no discontinuity between deceptive and rewarding species. Some rewarding species were quite variable, and some deceptive species were not (Table 1;

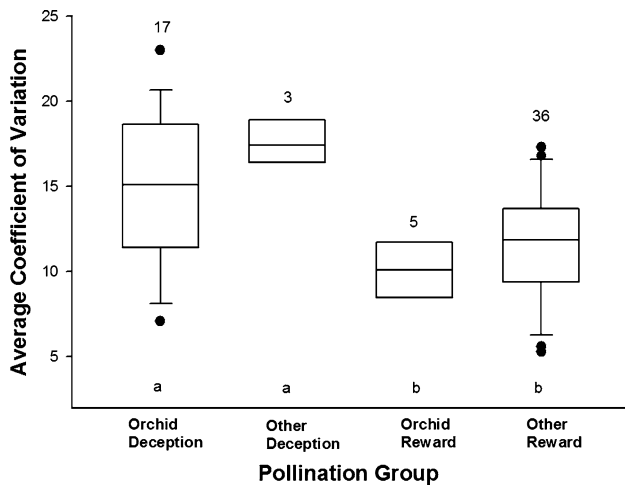


Fig. 1 Average coefficients of variation from morphological traits of flowers. Data segregated as orchids and nonorchids. Groups indicated by a different letter are significantly different. Numbers above the boxes indicate sample sizes

Fig. 1). The average CV for all deceptive species was 15.2%, whereas for all rewarding species it was 11.1%, and these two groups were significantly different (Fig. 1; $F_{1,60} = 18.1$, $P < 0.0001$).

The four groups of species (deceptive orchids, deceptive nonorchids, rewarding orchids, and rewarding nonorchids) differed significantly in their mean CVs for floral morphology (ANOVA: $F_{3,57} = 6.47$, $P < 0.001$). Deceptive orchids had a mean CV of 14.7%, rewarding orchids had a CV of 10.1%, whereas other deceptive species averaged 17.6%, and other rewarding species averaged 11.3%. The cross-comparisons using t tests indicated that the CVs within a family (Orchidaceae) were different for the two functional groups, deceptive and rewarding species. In fact, variations in deceptive species were different from rewarding species regardless of taxonomic grouping (orchids and nonorchids) (Fig. 1).

Variation in deceptive female flowers of the monoecious *B. decandra* was slightly higher than that of male flowers (17.4% and 16.8%, respectively), and in both cases the CVs were higher than the average for deception-pollinated species (Table 1; Fig. 1).

Fragrance variation

We asked whether or not variation in fragrance composition covaried with floral morphology among eight species (including a sexual deceptive species, *L. rupestris*). Spearman's rank correlation coefficient for average Jaccard distances (fragrances) and CVs (floral morphology) was significant ($r_s = 0.74$, $N = 10$, $P = 0.01$).

Fragrance variation produced by rewarding flowers was less than that of deceptive flowers, and the differences were

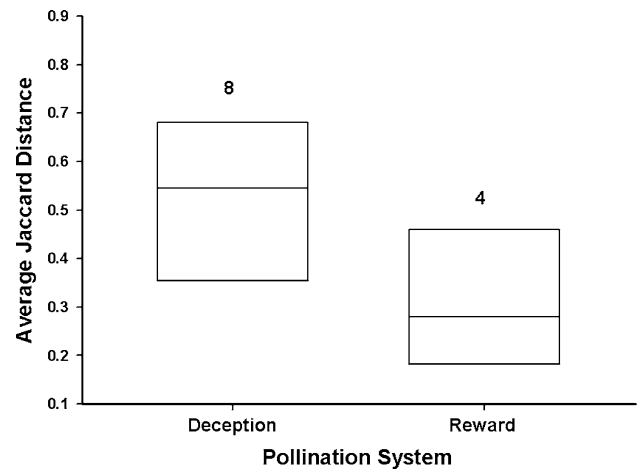


Fig. 2 Average Jaccard distances for floral fragrances of species with food deception-pollination systems and for rewarding pollination systems. The two groups are statistically different. Numbers above the boxes indicate sample sizes

nearly significant (Fig. 2; Mann–Whitney U test, $U = 4$, $P = 0.07$).

Discussion

Our analyses of floral morphologies showed that deceptive species have a higher degree of variation than rewarding species, verifying the assumption made in the literature. Floral fragrances varied similarly, but to a lesser extent, as the differences were not quite statistically significant. Still, it is remarkable that a trend for fragrances was evident despite the relatively small sample of species.

While variation in floral color and morphology is often visibly evident, intrapopulation variation in floral fragrances has rarely been characterized (e.g., Moya and Ackerman 1993; Salzman et al. 2007a, b). In one study, an Italian population of *Dactylorhiza romana* with considerable variation in color also had variable floral fragrances (Salzman and Schiestl 2007). Here, we have shown that floral fragrances can be just as variable as floral morphology within a population and that the amplitude of variation in these traits covaries among species; that is, species with high levels of morphological variation are usually the ones that also have highly variable floral fragrances. If we assume that both suites of traits have the same basic function, advertisement and pollinator attraction, then we expect that the mechanism affecting variation in floral morphology should be the same as for floral fragrances.

The pattern of variation seen in each pollination group is likely due to different processes. In outcrossing rewarding species, we expect pollinators to form strong search images

based on common forms while unusual variants would receive relatively fewer visits. Stabilizing selection would ensue, reducing population variability. There are other possible explanations for lower variability such as founder effects or persistent inbreeding, but founder effects are just as likely or even more so in deception-pollinated species, and we intentionally avoided autogamous species. Thus, for our data set of rewarding species, we expect that stabilizing selection would be the prevalent process in maintaining lower levels of variation.

Selection may also explain high levels of variation in deceptive species, but through some form of disruptive selection. For example, negative frequency-dependent selection has been proposed as the mechanism for generating variable populations in deception-pollinated orchids where rare variants have higher fitness than common forms (Ackerman and Galarza-Pérez 1991). Because pollinators form search images based on their experiences, common variants that offer no reward will likely be avoided more quickly than unusual ones. Rare variants should receive a relatively higher number of visits than common forms and experience higher fitness. There is some experimental evidence that this may occur for some color and fragrance phenotypes in populations of European orchids (Ayasse et al. 2000; Smithson and Mcnair 1997).

Negative frequency-dependent selection is not the only explanation for high variation in deceptive species. Ackerman et al. (1997) did not detect frequency-dependent selection on fragrance production in the deceptive orchid *Tolumnia variegata*, nor was it detected for color variation in *Psychilis monensis*, *Bletia patula* or *L. rupestris* (Aragón and Ackerman 2004; Ackerman and Carronero 2005; Tremblay and Ackerman 2007, respectively). Others have reported the lack of detectable selection on floral characteristics, and even variation patterns presumed to represent strong cases of frequency-dependent selection may actually result from other processes (Schemske and Bierzychudek 2001; Jersáková et al. 2006b). For deceptive species and perhaps orchids in general, genetic drift through repeated founder events and/or small effective population sizes may play as much a role in generating variation as negative frequency-dependent selection (Gentry and Dodson 1987; Zimmerman and Aide 1989; Ackerman and Zimmerman 1994; Tremblay and Ackerman 2001). Deceptive species of orchids have much lower fruit set and higher percentage of plants that fail to fruit than rewarding species (Neiland and Wilcock 1998; Tremblay et al. 2005). If selection from pollinators is weak and visits are often random with respect to floral characteristics, then drift could account for high variation.

Some variation may be a consequence of relaxed selection. When variants have an equal chance at contributing genetically to the next generation, then the chance of

unusual variants being involved is greater and this may be particularly true for many orchids with their small effective populations and biparental seed crops (Zimmerman and Aide 1989; Tremblay et al. 2005). Salzmann et al. (2007a) noted that fragrance variation in a deceptive *Anacamptis* species is quite high, but the quantitative output was likely below the threshold detectable by pollinators. The reduction in fragrance production presumably altered the capacity for flower visitors to learn quickly, since combined signals such as color or fragrance are generally more potent than just a single one (Kunze and Gumbert 2001). Indeed, studies such as these prompted Raguso (2008) to suggest that food deceptive flowers without specific models should be scentless. Loss of a floral fragrance no doubt has occurred in some lineages, but we found that food deceptive plants can have very strong odors, such as that emitted by diurnally pollinated *T. variegata* (Orchidaceae) and nocturnally pollinated *Plumeria alba* (Apocynaceae). Furthermore, the quantity captured from deceptive flowers after 3 min of swabbing a flower with a SPME fiber yielded as much fragrance as that of rewarding flowers (Table 2).

Our sample of deceptive species is strongly biased towards orchids, while rewarding species are broadly represented across a number of families, and this bias reflects the real representation of deception pollination in flowering plants. However, we showed that rewarding orchids and rewarding species of other families were similar in their floral variability, as were deceptive orchids and deceptive nonorchids. Furthermore, we found the deception–reward pattern is upheld within a single family (orchids). Another approach to reduce bias would be to compare closely related taxa of both pollination systems. Salzmann et al. (2007a) did this for one species pair of *Anacamptis*, but broader sampling is possible in genera that have a number of species with each type of pollination system. Phylogenies and knowledge of pollination of a group would be ideal, such as the temperate South African genus *Disa* (Johnson et al. 1998), or for the tropical American *Sobralia* (Dressler 2005; K. Neubig, personal communication; present data). Nevertheless, we feel that our results are a robust assessment of the hypothesis that deceptive species vary in characteristics associated with pollinator attraction more so than pollinator rewarding species (e.g., Heinrich 1975).

Although there is a statistical difference between deceptive and rewarding species in both mean coefficients of variation and Jaccard distances, the overall pattern of variation is continuous. There are some rewarding species that are quite variable and some deceptive species that are not. Perhaps a bimodal pattern may exist, but in a broad survey such as this there are numerous factors that may reduce the resolution of patterns; for example, greater

variation may arise than expected through processes such as introgressive hybridization, directional or disruptive, and even relaxed selection, or annual variation in selection regimes (Anderson and Hubricht 1938; Schemske and Bierzychudek 2001; Rivera-Marchand and Ackerman 2006; Tremblay et al. 2010; Morales et al. 2010).

Less variation than what one may expect may arise through persistent inbreeding or genetic bottlenecks via founder events. In fact, we suspect this may explain the low levels of variation in *A. graminifolia*, an outcrossing, recently naturalized orchid species employing deception pollination. Because naturalized species often suffer genetic bottlenecks, at least until subsequent introductions or hybridization events provide an infusion of additional diversity (Ellstrand and Schierenbeck 2000; Pérez et al. 2006; Lockwood et al. 2007; Wang et al. 2008), the low levels of morphological and fragrance variation in this orchid species are not surprising.

Another potential source of error is that we did not test whether the morphological traits that we measured or floral fragrances that we characterized were actually involved in pollinator attraction. It is reasonable to expect that some of them might not have had any direct relationship with pollination and plant fitness. Indeed, variation in flower color (not tested here) may be more important for pollinator attraction than the fragrance or morphological traits we measured. Such imbalances in trait importance to pollinator attraction may be reflected in the case of *T. variegata*, a deceptive species whose variation in floral morphology was described as extraordinary (Ackerman and Galarza-Pérez 1991), but here we found that its average CV was actually quite near the mean for the entire sample. On the other hand, its floral fragrance was among the most variable that we measured. Given the numerous potential sources of error that may obscure differences between the two functional groups, it is remarkable that most of our results were statistically unequivocal.

Our study supports the hypothesis that flowers of deceptive species have a larger degree of variation in both floral morphology and fragrances than do rewarding species. Various mechanisms responsible for this pattern have been suggested, and it remains to be seen which are prevalent under what conditions.

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