Carrion and Dung Mimicry in Plants

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Mimicking Livor Mortis: a Well-Known but Unsubstantiated Color Profile in Sapromyiophily

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Abstract By emitting strong scents resembling rotting organic materials suitable for oviposition and/or foraging of flies, sapromyiophilous flowers mimic the substrates that attract flies as pollinators. It has been suggested that the wide range of volatile organic compounds emitted by this deceptive pollination system reflects the trophic preferences of flies to different types of substrate, including herbivore and carnivore feces, carrion, and fruiting bodies of fungi. Previous studies suggest that floral scents play a particularly important role in sapromyiophily. However, few studies on the relative importance of floral color or synergy between visual and olfactory cues in sapromyiophily have been substantiated. In this study, we analyzed fetid floral odor, floral pigment composition, and reflectance of an *Amorphophallus konjac* C. Koch inflorescence, and we conducted bioassays with different visual and/or olfactory cues to explore an unsubstantiated color profile in sapromyiophily: mimicking livor mortis. Our analysis showed *A. konjac* can emit oligosulphide-dominated volatile blends similar to those emitted by carrion. Necrophagous flies cannot discriminate between the color of an inflorescence, livor mortis, and floral pigments. We concluded that mimicking livor mortis may represent a common tactic of pollinator attraction in "carrion flower" systems within angiosperms.

Keywords *Amorphophallus* · Deceit pollination · Livor mortis · Pigments · Sapromyophily · Spectral reflectance

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Introduction

Deceit pollination is a common natural phenomenon in angiosperms, which is a textbook case to study the evolutionary relationship between flowers and pollinators (Vereecken and McNeil 2010). There are about 7500 deceptive plant species in different angiosperm families, and these plants are divided into several deceptive pollination categories, such as: food deception, sexual imitation, and oviposition site mimicry (Renner 2006; Vereecken and McNeil 2010). In these strategies, oviposition site mimicry (OSM) is a well-known phenomenon reported from at least 20 unrelated families, such as the Araceae, Rafflesiaceae, and Aristolochiaceae (Jüergens et al. 2013; Urru et al. 2011). In general, OSM flowers usually attract saprophagous, coprophagous, and necrophagous flies and/or beetles for pollination through deceptive tactics (Jüergens et al. 2013; Vereecken and McNeil 2010). In

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sapromyiophily, flowers often emit a strong and unpleasant scent reminiscent of decaying organic matter (Jüergens et al. 2013). The floral scents from these deceptive pollination systems involving dimethyl disulphide, dimethyl trisulphide, phenol, *p*-cresol, skatole, indole, various carboxylic acids, *etc.* often exploit a wide range of trophic and oviposition preferences in flies or beetles (Jüergens et al. 2006, 2013; Stensmyr et al. 2002; Urru et al. 2011). Additionally, OSM also is likely to involve visual signals because many sapromyiophilious arums, stapeliads, orchids, and birthworts often show similarities in flower/inflorescence colors with colors that are red or purple reminiscent of "livor mortis" (Jüergens et al. 2013; Kelly and Gaskett 2014; Raguso 2004; Urru et al. 2011; Fig. 1).

In general, the formation of livor mortis is a dynamic process according to forensic studies (Goff 2009). Dull red livor mortis is observed after a period of about 2–4 h following death in a carcass because of postmortem hypostasis. This is a physical process of blood (protoheme) beginning to settle to the lowest portions of the body by gravity (Nashelksy and McFelley 2003). The situation continues for 9–12 h following death, at which time the color of livor mortis will not change. As time goes on, greenish discoloration begins to form in a carcass due to the reaction of hydrogen sulfide with the hemoglobin in blood to form sulfhemoglobin (sulfheme) (Clark et al. 1997). This pigment is greenish and may be seen in areas where livor mortis has formed. At last, greenish discoloration will become brown or even black (Goff 2009). To most botanists, the first impression of sapromyiophilous flowers is that they look like livor mortis or the greenish discoloration of decomposing flesh (Figs. 1, 2)In this study, we analyzed fetid floral odor, floral pigment composition, and the reflectance spectrum of a famous sapromyiophilious taro, *Amorphophallus konjac* C. Koch, and we conducted bioassays to decouple the effects of scent and color as attractants for saprophilous insects. We also wanted to explore whether mimicking livor mortis is a color profile of *A. konjac* inflorescences.

Methods and Materials

Amorphophallus konjac (Araceae) is a traditional food and medical plant in China, distributed in southwest China, Japan, and India (Li and Hetterscheid 2010). The inflorescence of *A. konjac* consists of a long, dull red spathe and an approximately 60 cm spadix (Fig. 1). We noticed a strong and unpleasant carrion-like odor from *A. konjac* during anthesis. To the human observer, the brownish to red colors of the inflorescence and pedicel look similar to the stage of livor mortis in a dead animal (Fig. 2). Previous studies indicated that bees (Trigone), beetles (Cetoniidae, Nitidulidae, Scarabaeidae, *etc.*), and flies (Calliphoridae and Platystomatidae) were pollinators and/or visitors of *Amorphophallus* spp. (Gibernau 2003). However, we observed various saprophagous flies and nocturnal clown beetles, rove beetles, earwigs, and nitidulids visiting the inflorescence of *A. konjac* (Fig. 2) at the Kunming Botanical Garden, Kunming Institute of Botany, the Chinese Academy of Sciences (KBG: 25.127 N and 102.743 E, 1788 m.a.s.l.). Nowadays, it is difficult to identify natural populations of *A. konjac* in the field because of wide introduction and cultivation in Southeast Asian countries.

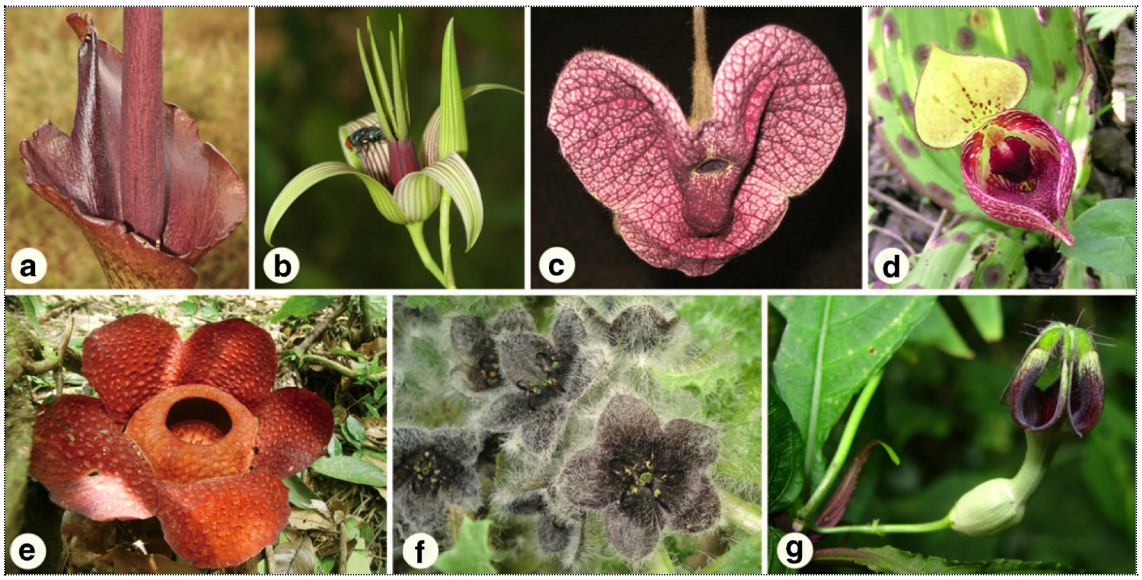

Fig. 1 Representative sapromyiophilous flowers in angiosperms. **a**, *Amorphophallus konjac* (K. Koch); **b**, *Stemona tuberosa* L.; **c**, *Aristolochia griffithii* J. D. Hooker; **d**, *Cypripedium sichuanense* Perner (photograph by Gui-Ling Zheng); **e**, *Rafflesia tuan-mudae* Becc. (photograph by Yi-Feng Huang); **f**, *Jaborosa rotacea* (Lillo) Hunz. & Barboza (photograph by Marcela Moré); **g**, *Ceropegia christenseniana* Hand. (photograph by Shao-Le Huang)

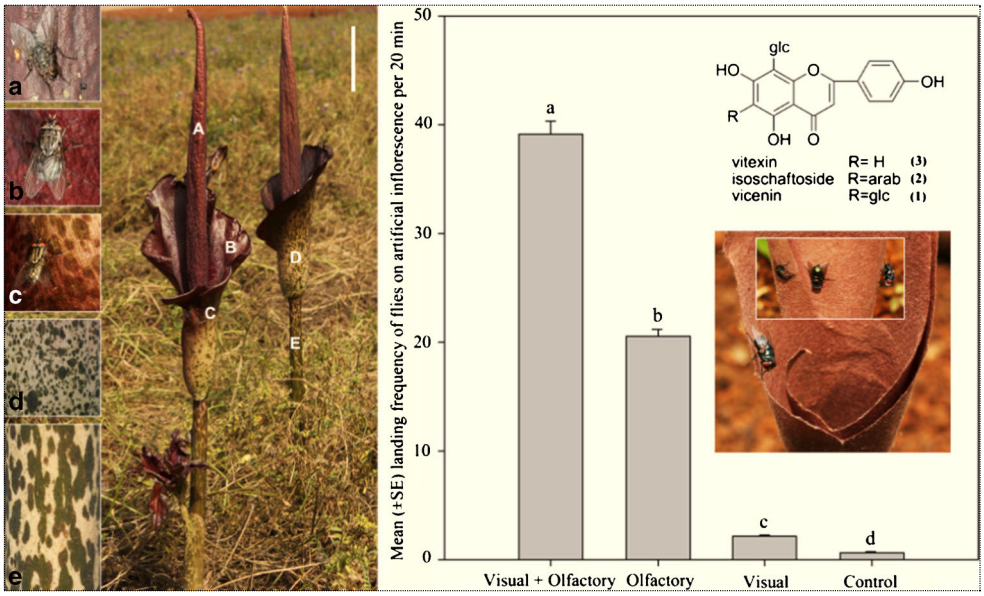

Fig. 2 The inflorescence of *Amorphophallus konjac* and the attractiveness of real and artificial inflorescences to fly visitors. *Left figure*: Inflorescences look like corpses in livor mortis (*dull red*). Appendix (A) and spathe (B & C) attract different flies; the base of the spathe (D) and the stem (E) of *A. konjac* exhibit greenish coloration; Scale bar, 9 cm. *Right figure*: artifical inflorescences with different odor and color treatments (visual+olfactory, olfactory, visual, and control) were offered to flies in a patrolling area for 20 min (N=20). Means (± SE) number of flies landing on the inflorescences is plotted. *Lowercase on top of error bars* indicates significant differences between the different treatments at significance levels of $P < 0.05$. *Inset*: structure of an artificial inflorescence dyed with extracted pigments from *A. konjac*

Extraction and Analysis of Color Pigments of *A. konjac* The spathe and appendix of the spadix of an *A. konjac* inflorescence (1.0 kg) were extracted with 35 % aqueous methyl alcohol (1 L× 3) at room temperature. The solvent was evaporated *in vacuo*, and the residue was partitioned with pure ethyl acetate and H_2O to create an aqueous solution. The solution (20 g) was subjected to column chromatography over PR18 silica gel eluting with 3–10 % aqueous methanol to generate color fractions (500 mg). The fractions were separated by preparative reversed-phase using a C_{18} HPLC SunFire™ column (Waters Corporation) with a gradient flow from 3 to 10 % aqueous methanol. Three compounds were separated and their fractions (fraction 1=15 mg; fraction 2=15 mg; fraction 3=105 mg) were used for later analysis by ^{1}H NMR and ^{13}C NMR.

NMR Spectroscopy ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 400.13 and 150.9 MHz, respectively, equipped with a 5 mm probe, in deuterated water (D_2O), with all shifts referred to internal tetramethylsilane (TMS). Spectra were recorded with the following parameters:

^{1}H NMR: pulse width (PW), 10 μs (flip angle 90C°); PL1, −3.0 dB; acquisition time, 5.11 s for 64 K data table with a spectral width (SW) of 6410.256 Hz (16 ppm); CPD mode decoupling, waltz16; digital resolution 0.0978 Hz/pt. The number of accumulated scans was 2 for each sample (2–3 mg in 0.5 mL of H_2O).

^{13}C NMR: pulse width (PW), 11.8 μs (flip angle 90C°); PL1, −0.6 dB; acquisition time, 0.86 s for 64 K data table with a spectral width (SW) of 37,878 Hz (250 ppm); CPD mode decoupling, waltz16; digital resolution 0.578 Hz/pt. The number of accumulated scans was 800 for each sample (2–3 mg in 0.5 mL of H_2O).

Reflectance Spectra Analysis of Flower, Livor Mortis Model, and Pigments The spectral reflectance of *A. konjac* inflorescences (N=40; four different positions on the appendix of 10 inflorescences), the surface of dead cattle in a stage of livor mortis (N=18, three positions per six individuals), pigments from flower spathe and appendix (N=17; pigment extracts were evenly daubed on artificial inflorescences), bovine protoheme (N=19; evenly daubed on a filter paper; Sigma-Aldrich, USA), the dark purple spot region on the stem of *A. konjac* (N=20), bovine sulfheme (N=22; evenly daubed on filter paper), the non-spot region on the stem of *A. konjac* (N=20; two different positions on the appendix of 10 inflorescences) were measured using an Ocean Optics USB4000-UV–vis spectrometer (Ocean Optics Inc., Dunedin, FL, USA). For this measurement, a fiber optic and reflection probe (R200-7-UV–VIS 200 μm) was held with a RPH-1 probe holder at a 45° angle to the object surface. An Ocean Optics DH-2000 Deuterium Tungsten Halogen light source was used to produce light, and a WS-1 diffuse reflectance white standard was used to calibrate the reflectance.

Color similarity of flower, livor mortis, sulfheme, protoheme, and floral pigments as perceived by floral visitors was determined by plotting the spectral reflectance as loci in the blowfly *Lucilia* color vision model suggested by Troje (1993). There are five types of photoreceptors in blowfly, of which four [two p-type (R7p and R8p) and two y-type (R7y and R8y)] are used to construct a fly color space by considering the relative spectral sensitivities of different photoreceptors (Arnold et al. 2009; Troje 1993). To plot the reflectance as loci in the fly vision model, the relative radiation absorbed by each of the four photoreceptors was calculated by integrating the product of photoreceptor absorption [*i.e.*, the receptor sensitivities of fly *Lucilia* sp. from Hardie and Kirschfeld (1983)], the standard D65 illumination (Wyszecki and Stiles 1982), and the spectral reflectance of tested objects (flower, livor mortis, sulfheme, protoheme, and floral pigments) from 300 to 700 nm (at 4 nm steps). The spectral reflectance of typical green foliage was used as background (Chittka et al. 1994). The conversion of photoreceptor absorption (P) into receptor excitations (E) was calculated by the equation E=P/(P+1) from Naka and Rushton (1966). The color locus of each tested object was structured by difference in relative excitations between two p-type (R7p and R8p) and two y-type (R7y and R8y) photoreceptors of paired stimulus to form four separate quadrants p+y+(UV), p-y+(blue), p-y- (green), and p+y- (purple) (Fig. 3). The blowfly vision model is suitable for modelling visual discrimination of floral visitors of *A. konjac* because most of the visitors were *Lucilia* spp. (Table S1). Based on the blowfly vision model, the spectral stimuli are not discriminated within a quadrant, but discriminated among quadrants by flies.

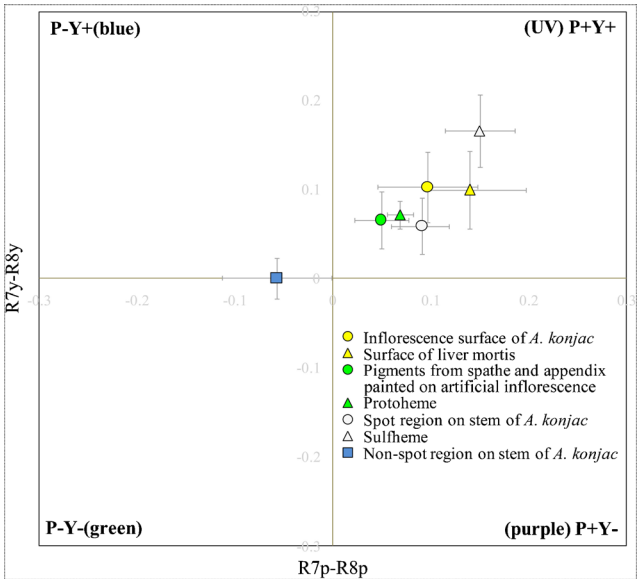

Fig. 3 Color loci of Reflectance spectra of inflorescence, livor mortis, sulfheme, protoheme, spot region, and non-spot region on stem of *Amorphophallus konjac*, and floral pigments (pigment extracts were evenly daubed on artificial inflorescence) as perceived by blowflies according to the model suggested by Troje (1993). Loci of inflorescence surface of *A. konjac*, pigments from spathe and appendix, spot region on stem of *A. konjac*, surface of livor mortis, and protoheme and sulfheme are located at the same UV quadrant. Loci of non-spot region on a stem of *A. konjac* are located at the border of blue and green quadrants. Flies exhibit a categorical color vision system, and spectral stimuli with loci in the same quadrant would not be discriminated by them (Troje 1993; Arnold et al. 2009)

Collection and Identification of Inflorescence Scent Species in Araceae typically have protogynous inflorescences. A previous study indicated that *A. konjac* has the same reproductive strategy (Lamprecht and Seymour 2010). Based on field observations, the chamber of the spathe often imprisons the fly and/or beetle pollinators on the first day, and releases them on the second day. Therefore, we analyzed the floral scent from first day flowers. Scent samples of five *A. konjac* inflorescences were collected between 10:30 and 20:30 at the KBG locality, which coincided with the time of floral scent emission. Floral scent was collected using dynamic headspace adsorption by enclosing a single newly opened inflorescence with a Tedlar bag (Dupont, USA). The scent was drawn from the bag into a tube containing the adsorbent Porapak Q (150 mg, mesh 60/80, Waters Associates, Inc.) using a pump with an inlet flow rate of 300 ml min^{-1}. Trapped VOCs were eluted with 300 μl dichloromethane (99.5 %) and concentrated to one-fifth of the original volume by a gentle stream of nitrogen (200 ml min^{-1}). Next, *n*-nonane was added as an internal standard (3000 ng) to each sample for quantification. Volatiles were also collected from ambient atmosphere as control to identify any background compounds in floral scent samples.

The extracts were analyzed using an Agilent Technologies HP 6890 gas chromatograph, equipped with an HP-5MS column, and linked to an HP 5973 mass spectrometer. Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}. Split inlet and FID were held at 250 °C. The column temperature was programmed to rise from 40 °C (5-min hold) to 250 °C (20-min hold) at 3 °C/min. Compounds were tentatively identified by comparing their MS spectra and relative retention times with that provided in the Wiley 7n.1 mass spectral library. For some compounds, identification was confirmed by comparing their GC retention time and mass spectrum with those of standard compounds purchased from Sigma-Aldrich, USA (Table 1). The mean amounts of fetid scents from *A. konjac* were compared according to One-Sample *T* Test with SPSS 13.0 software (SPSS Inc., Armonk, NY, USA).

Behavioral Bioassays To evaluate the relative importance of visual and olfactory cues of *A. konjac* to saprophagous insects, we used an array of four artificial inflorescences to attract insects at the KBG location without a natural population of *A. konjac* present in early April, 2013. Four isometrical artificial inflorescences were constructed according to *A. konjac*

Table 1 Average relative amounts (%) of fetid scents from inflorescences of A*morphophallus konjac* (*N*=5)

No.	Compound	RI	CAS #	Relative amount±S.E. (%)
1	2-Pentanone	665	107-87-9	1.3±0.7
2	3-Hydroxy-2-butanone*	721	513-86-0	0.1±0.1
3	Isoamyl alcohol	732	30899-19-5	5.8±1.0
4	2-Methyl-1-butanol	736	137-32-6	3.2±1.1
5	Dimethyl disulphide*	748	614-92-0	26.3±13.4
6	3-Methyl-2-pentanone	754	565-69-5	2.3±0.8
7	Hexanal*	802	66-25-1	0.2±0.2
8	Butyl acetate	812	123-86-4	0.3±0.3
9	4-Hydroxy-4-methyl- 2-pentanone	832	123-42-2	2.1±0.9
10	3-Methyl-1-pentanol	846	589-35-5	1.5±0.6
11	Butyl ether	884	142-96-1	1.6±1.4
12	Butyl 2-propenoate	902	1214-39-7	1.0±1.0
13	Butyl propionate	910	137-40-6	0.5±0.5
14	Dimethyl trisulphide*	972	3658-80-8	42.9±7.0
15	Benzyl alcohol*	1031	100-51-6	0.1±0.1
16	Linalool*	1098	78-70-6	0.4±0.4
17	n-Undecane*	1100	17301-28-9	0.8±0.2
18	n-Nonaldehyde	1102	124-19-6	1.9±0.5
19	Decanal	1195	112-31-2	0.8±0.3
20	n-Dodecane*	1200	112-40-3	1.3±0.4
21	Dimethyl tetrasulphide	1215	5756-24-1	1.7±0.4
22	n-Tridecane*	1300	629-50-5	1.1±0.3
23	β-Caryophyllene	1436	87-44-5	2.5±1.1
24	Humulene	1458	6753-98-6	0.3±0.2

Tentatively identified compounds based on mass spectral data and KOVATS retention indices according to Wiley 7n.1 mass spectral library. Compounds marked with an asterisk were identified based on GC retention times and mass spectra of standard compounds

inflorescence size and were placed on four corners of a square area. The distance between neighboring artificial inflorescences was 2 m. The four inflorescences presented visual+ olfactory cues (foul odor+dull red filter paper dyed by pigments from *A. konjac*), olfactory cue (foul odor+white filter paper), visual cue (dull red filter paper), and control (white filter paper), respectively (Fig. 2). In each artificial inflorescence, an odorless cotton wool ball containing either 10 μl dimethyl disulfide, 15 μl dimethyl trisulfide (Sigma-Aldrich, purity 95–99 %) (two main compounds in *A. konjac*; Table 1) and 75 μl dichloromethane or 100 μl of dichloromethane (control) were added to a 2 ml GC vial that was inserted into the base of the inflorescence. The color of the artificial inflorescences was dyed with pigments extracted from three inflorescences. All bioassays were conducted between 15:00–19:00 p.m., when the inflorescence of *A. konjac* emits a putrid odor. To avoid any location effect, the position of each artificial inflorescence was changed every 5 min. Each trial lasted 20 min and was replicated 20 times. Each scented or control cotton wool ball was used only once after the dichloromethane had evaporated. We recorded the number of insect landings on the artificial inflorescence arrays during each trial. Representative individuals were captured when landing on the artificial inflorescences. In this study, One-way ANOVA was used to compare the mean landing frequency of flies on each artificial inflorescence subjected to four experimental treatments in the field, followed by *post hoc* analysis (Tukey test). Data were log 10-transformed to achieve variance homogeneity.

Results

Composition of *A. konjac* Flower Color Pigments Three compounds were separated using HPLC, including vicenin (**1**, 15 mg), isoschaftoside (**2**, 15 mg), and vitexin (**3**, 105 mg). The UV spectrum (base) of all three isolates showed the characteristic absorption bands of flavone at 277, 328, and 395 nm. Compound **3** was found to possess a molecular formula of $C_{21}H_{20}O_{10}$ based on the negative high resolution electron spray ionization mass spectrometry (ESI-MS) spectrum (*m/z*=431 $[M\text{-}H]^-$). Its ^1H NMR spectrum (D_2O)

indicated the presence of apigenin [δ_H 7.62 (2H, d, J=8.4 Hz, H-2′ 6′), 6.53 (2H, d, J=8.4 Hz, H-3′ 5′), 6.25 (2H, s, H-3, 6), 4.60 (1H, H-1), 3.7–3.40 (4H, H-2″, 3″, 4″, 5″)]. The ^{13}C NMR and DEPT (D_2O) spectrum of compound **3** displayed C-glucoside signals [δ_C 80.3 (d, C-5″), 78.3 (d, C-3″), 74.1 (C-1″), 69.8 (C-2″, 4″), 60.7 (C-6″). The Heteronuclear Multiple Bond Coherence correlations of compound **3** together with literature (Hu et al. 2006) classified it as vitexin (8-C-glucosyl-apigenin). The NMR patterns of compounds **1** and **2** were similar to vitexin with the exception for additional sugar signals. Additionally, the negative ESI-MS spectrum of compounds **1** and **2** (m/z=563 and 593 $[M\text{-}H]^-$, respectively) in combination with the literature showed that compounds **1** and **2** were vicenin (6,8-di-C-glucosyl-apigenin) and isoschaftoside (6-C-arabinosyl-8-C-glucosyl-apigenin), respectively. The ratio of vitexin: isoschaftoside: vicenin was approximately 7:1:1.

Color Similarity Among Flower, Livor Mortis, and Floral Pigments Reflectance spectra of the color of *A. konjac* inflorescences are similar to the color of tissue that has been discolored by livor mortis and their corresponding protoheme and sulfheme extracted from livor mortis. The spectral curve of sulfheme is slightly higher than that of a spot region on the stem of *A. konjac* (Fig. S1). But all of these reflectance spectra are quite different from that of a non-spot region on the stem of *A. konjac*, which is raised apparently along 400–650 nm. Color space analysis revealed that color loci for all materials fall in the UV quadrant (p+y+) of blowfly color space except the non-spot region on a stem of *A. konjac*. Flies exhibit a color vision with four separate quadrants (p+y+, p+y-, p-y-, and p-y+), which correspond to the difference in relative excitation of the two paired opponent photoreceptor types (R7p and R8p, R7y, and R8y). Since flies are unable to discriminate stimuli chromatically within each quadrant (Arnold et al. 2009; Shuttleworth and Johnson 2010; Troje 1993), our data suggest that flies are unable to distinguish the inflorescence from livor mortis and their corresponding pigments. However, according to this color vision model, flies should be able to discriminate livor mortis and the red floral coloring from a non-spot region on the stem of *A. konjac* (Fig. 3).

Flower Scent Composition We identified 24 compounds from five individuals (Table 1). The floral scent samples of *A. konjac* were dominated by dimethyl disulphide (26.3 ±13.4 %) and dimethyl trisulphide (42.9±7.0 %). However, it certainly is not limited to just these. The presence of dimethyl tetrasulfide is unusual in this or previous studies. Additionally, lots of aliphatic esters, alcohols, and ketones were found from fetid inflorescences of *A. konjac*. The average total scent emission per inflorescence was 4988±384.32 ng h^{-1} per individual (N=5).

Behavioral Bioassays In this study, flies were the only floral visitors of the artificial *A. konjac* inflorescence. In total, we recorded 1251 fly visits. Two hundred and sixty-one flies were caught and identified according to specimens deposited in KBG, of which, 81.2 % flies were female (Table S1). The flies represented 12 genera and three families, Calliphoridae (227), Sarcophagidae (19), and Muscidae (15). Main visitors were *Lucilia* spp. (93), *Chrysomya spp.* (74), *Achoetandrus rufifacies* (31), and *Aldrichina grahami* (29). Only 14 flies landed on the control artificial inflorescence in the observed periods. Bioassay experiments indicated that the combination of floral pigments and synthetic scent consisting of dimethyl disulphide and dimethyl trisulphide can manipulate fly preference behavior. Visitation frequency of flies to the artificial inflorescence with visual+olfactory cues, olfactory cue only, visual cue only, and control is shown in Fig. 2. The results showed that there were significant differences in the landing frequency of flies on the artificial inflorescences with the four different treatments. The landing frequencies of flies on the artificial inflorescences depended significantly on the presence of the foul-scented and/or colored attractants. Either olfactory or visual signals resulted in a significant higher landing frequency than the control, and the combination of olfactory and visual signals did significantly better than any signal alone (Fig. 2).

Discussion

The pollination syndrome of sapromyophily has evolved several times independently in unrelated angiosperm families (Jürgens et al. 2006, 2013; Vereecken and McNeil 2010). The flowers of these plants mimic carrion, animal excrement, or the fruiting bodies of fungi by emitting awful smells that saprophagous insects can not distinguish from odors of their brood substrates (Jürgens et al. 2013; Vereecken and McNeil 2010). Previous studies have indicated that floral odor plays a particularly important role in the sapromyiophilous mimicry system, and the most common volatiles are oligosulfides (Jürgens et al. 2013; Urru et al. 2011). The potential function of these compounds to floral visitors has been supported previously by some behavior bioassays (Moré et al. 2013; Shuttleworth and Johnson 2010; Stensmyr et al. 2002). In this study, oligosulfides (Table 1) occurred in the floral scent of *A. konjac* at a rate of 70.9 %, which substantiated the unique floral odor profile in carrion-mimicking flowers.

Sapromyiophilous flowers, however may present dull red color, large size, and even unusual thermal signals to their potential pollinators (Angioy et al. 2004; Davis et al. 2008; Raguso 2004; Stensmyr et al. 2002, Seymour et al. 2003). Previous studies have demonstrated that flies can perceive different colors (Aak and Knudsen 2011; Fukushi 1989), and flower colors may be of importance as an orientation

cue for pollinators (Beaman et al. 1988; Jersáková et al. 2012). For example, visual cues are important in carrion mimicry and probably work in concert with olfactory stimuli (Bänziger 1991). Odor cues can enhance the induction of landing by female *Lucilia sericata* Meigen, and visual cues are important when selecting a final landing site (Wall and Fisher 2001). Gomes et al. (2007) indicated that odor cues may enhance the induction of landing on a resource for the blowfly *Chrysomya megacephala* Fabricius, while visual cues are important when selecting a final landing site in a natural condition. Floral visual cues play an important role in floral mimicry systems (Jersáková et al. 2012). Of course, the combined effect of heat with visual and olfactory stimuli in attracting the necrophagous flies in Araceae should be taken into account as has been noted previously (Angioy et al. 2004; Seymour et al. 2003).

Interestingly, dull red is a common color for fly-pollinated brood site deceptive flowers (Jüergens et al. 2013; Raguso 2004; Urru et al. 2011). The anecdotal color profile of mimicking livor mortis has never been tested in sapromyiophily, by comparing the floral color to that of flesh in livor mortis. In this study, our analysis confirmed that flies may not discriminate floral color of *A. konjac* from that of flesh in livor mortis (Fig. 3). In fact, forensic studies indicate that the color formation and change is a dynamic process as carcass decays (Goff 2009). Dull red livor mortis is observed after several hours following death in a carcass (Nashelksy and McFelley 2003). After about 12 h, greenish discoloration begins to form in a carcass (Clark et al. 1997). Finally, greenish discoloration will become brownish or black (Goff 2009). Here, we found that the color of livor mortis and greenish discoloration occurred on the surface of the inflorescence of *A. konjac* (Fig. 2). Therefore, we hypothesize that the inflorescence of *A. konjac* mimics different decay phases along with the use of a larger body carcass to attract a wider range of saprophagous insects. Of course, the link between the color of *A. konjac* and different stages of decay was not investigated in this study, and needs additional experimentation.

Our results indicated that 82.2 % of collected specimens on the artificial inflorescences were female flies (N=261; Table S1). Some other carrion mimicking plants also attract more female flies (Pape and Bänziger 2000; Stensmyr et al. 2002). Therefore, a reasonable explanation is that female flies have more sensory pits than males (Sukontason et al. 2004) or that the putrid odor/dull red color may represent a preferred substrate for an ovipositing fly (Davis et al. 2008; Raguso 2004). As for why a huge inflorescence has evolved in *A. konjac*, we hypothesize that the large size of *A. konjac* inflorescence may mimic a large animal carcass and thereby enhance the plant's attractiveness towards potential pollinators. Given the combined effect of heat with visual and olfactory cues in attracting flies in Araceae (Angioy et al. 2004; Stensmyr et al. 2003), thermogenesis may be also invoked to explain the large inflorescences in *A. konjac*. In this study, although several types of flies visited the artificial inflorescence of *A. konjac*, previous studies indicated that beetles were the most effective pollinators in *Amorphophallus* (Beath 1996; Gibernau 2003; Punekar and Kumaran 2010). Therefore, to identify whether both flies and beetles are pollinators of *A. konjac*, more field observations on the pollinators of natural *A. konjac* populations are required. Our preliminary observations indicate that fly pollinators could promote natural fruit sets of *A. konjac* at the KBG location, however, clown beetles, rove beetles, earwigs, and nitidulids play the most important role in natural fruit sets and seed sets of *A. konjac* (unpublished data). Nonetheless, we think flies, at least to a small extent, affect reproductive success of *A. konjac* plants in natural populations. Additionally, it is not yet known whether the fly responses to floral color that were observed in this study are innate or learned. Our results were obtained with experienced flies at the KBG location. Establishing whether inexperienced flies have innate responses to livor mortis color would require further behavioral studies with naïve insects in a controlled environment.

In summary, given that thermal cue (Lamprecht and Seymour 2010), floral color mimicking livor mortis, and floral odor mimicking rotting carrion are deployed by inflorescences of *A. konjac*, we suggest that this plant is an outstanding example of evolutionary tactics that exploit insects for pollination purposes. The tactic may be a consequence of convergent evolution with other oviposition site mimicry plants in angiosperms.

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