THE EVOLUTION OF FLORAL SCENT AND OLFACTORY PREFERENCES IN POLLINATORS: COEVOLUTION OR PRE-EXISTING BIAS?

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Coevolution is thought to be a major factor in shaping plant–pollinator interactions. Alternatively, plants may have evolved traits that fitted pre-existing preferences or morphologies in the pollinators. Here, we test these two scenarios in the plant family of Araceae and scarab beetles (Coleoptera, Scarabaeidae) as pollinators. We focused on floral volatile organic compounds (VOCs) and production/detection of VOCs by scarab beetles. We found phylogenetic structure in the production/detection of methoxylated aromatics in scarabs, but not plants. Within the plants, most of the compounds showed a well-supported pattern of correlated evolution with scarab-beetle pollination. In contrast, the scarabs showed no correlation between VOC production/detection and visitation to Araceae flowers, with the exception of the VOC skatole. Moreover, many VOCs were found in nonpollinating beetle groups (e.g., Melolonthinae) that are ancestors of pollinating scarabs. Importantly, none of the tested VOCs were found to have originated in pollinating taxa. Our analysis indicates a Jurassic origin of VOC production/detection in scarabs, but a Cretaceous/Paleocene origin of floral VOCs in plants. Therefore, we argue against coevolution, instead supporting the scenario of sequential evolution of floral VOCs in Araceae driven by pre-existing bias of pollinators.

KEY WORDS: Floral evolution, mimicry, pollination, pollination syndromes, sensory bias, sensory trap.

Coevolution defined as "reciprocal evolutionary change between interacting species driven by natural selection" (Thompson 2005) is commonly thought to be a major factor in shaping plant–pollinator interactions (Feinsinger 1983). Because pollinators are generally believed to select for floral traits, and plants can also impose selection on pollinators (Pauw et al. 2009), a co-evolutionary scenario makes sense, at least in specialized pollination. Coevolution of adaptive traits on a macroevolutionary level has, however, rarely been tested rigorously against alternative hypotheses, such as one-sided or sequential evolution (Ramirez et al 2011). Besides floral morphology mediating the fit with the pollinator, floral signals are of key importance for the attraction and (in)direct assessment of a reward by the pollinator (Raguso 2008; Schiestl 2010). Thus, floral signals and the corresponding sensory systems in pollinators may have been shaped by coevolution. Sensory preferences by pollinators have been shown to impact evolution of floral signaling (Vereecken and Schiestl 2008). As a possible consequence, floral signals often show convergent evolutionary trajectories, which are epitomized by the pollination syndromes (Vogel 1954; Fenster et al. 2004; Willmer 2011). Although far from being clearcut, plants pollinated by a similar guild of animals have often evolved similar visual (Fenster et al. 2004) and olfactory (Knudsen and Tollsten 1993; Dobson 2006) signals. While sensory preferences that are common to given taxonomic groups of pollinators are usually implied as drivers of convergent floral signal evolution, it is largely unknown how such preferences evolve in the pollinators themselves. Although this question has been rarely addressed,

Table 1. Occurrence of VOCs and groups of VOCs in Araceae and Scarabaeidae. One typical compound is shown per taxa and VOC group.

	Skatole	Indole	p/m-Cresol	Fatty acid derivative esters	Aliphatic acyloins	Methoxylated aromatics
ARACEAE						
Amorphophallus	x[1]	x[2]		Ethyl acetate[3]		Veratrole[3]
Anthurium		x[4]	x[5]	Ethyl hexanoate[4]	Acetoin[4]	Eugenol[4]
Alocasia				Methyl hexanoate[6]		
Arisaema						
Arum	x[2]	x[2]	x[2]	Methyl butyrate[2]	Acetoin[7]	p-Methylanisole[2]
Caladium				Pent-3-yl acetate[8]		Veratrole[8]
Colocasia						2-Methoxy-6-methylacetophenone[9]
Dracunculus		x[10]				
Helicodiceros						
Homalomena				Methyl-2,4-decadienoate[11]		Veratrole[11]
Montrichardia				Pent-3-yl acetate[12]	2-Hydroxy-5-methylhexan-3-one[12]	1,3,5-Trimethoxybenzene[13]
Peltandra						
Philodendron				(Z)-2-Pentenyl acetate[14]	2-Hydroxy-5-methylhexan-3-one[9]	p-Methoxystyrene[14]
Sauromatum	x[15]	x[15]	x[16]	Methyl butyrate[16]	Acetoin[16]	Anisole[15]
Spathiphyllum		x[17]	x[17]	Propyl/isopropyl tetradecanoate[18]		Eugenol[19]
Taccarum				an Ethyl dodecadienoate[20]	2-Hydroxy-5-methylhexan-3-one[9]	
Zantedeschia				Pentyl hexanoate[9]		Veratrole[9]
SCARABAEIDAE						
Geotrupinae						
Geotrupes	x[21]	x[21]	x[21]			
Aphodiinae						
Aphodius	x[22]					
Scarabaeinae						
Canthon		x[23]	x[23]			2-Methoxy-3-methyl-1,4-benzoquinone[23]
Kheper	x[24]	x[24]				
Ontophagus						2-Methoxy-3-methyl-1,4-benzoquinone[25]
Pachylomerus				Ethyl butyrate[26]		
Melolonthinae						
Holotrichia						Anisole [27]
Liparetrus						Eugenol[28]
Maladera						Eugenol[29]
Phyllophaga			x[30]	Ethyl acetate[31]		Anethole[32]
Rhizotrogus					Acetoin[33]	
Amphiphallon					Acetoin[34]	
Dynastinae						
Cyclocephala			x[8]	Ethyl acetate[31]	2-Hydroxy-5-methylhexan-3-one[8]	1,3,5-Trimethoxybenzene[8]
Erioscelis						p-Methoxystyrene[14]

Continued.

Table 1. Continued

	Skatole	Indole	p/m-cresol	Fatty acid derivative esters	Aliphatic acyloins	Methoxylated aromatics
Oryctes				Ethyl 4-methyloctanoate[35]		
Scapanes					Acetoin[36]	
Strategus				sec-Butyl acetate[36]	Acetoin[36]	
Cyphonistes						2,3-Dimethoxy-1,4-benzoquinone[37]
Rutelinae						
Anomala				(Z)-3-Hexenyl acetate[38]		Anethole[39]
Phyllopertha						Eugenol[40]
Popillia						Eugenol[41]
Parastasia						Veratrole[42]
Cetoniinae						
Dyspilophora						Eugenol[43]
Epicometis						Anethole[44]
Cetonia						Anethole[44]
Euphoria						Eugenol[45]
Oxycetonia						Eugenol[41]
Oxythyrera						Eugenol[43]
Pachnoda				Butyl butyrate[46]	Acetoin[46]	Eugenol[43]
Potosia						Eugenol[44]
Protaetia						Eugenol[41]
Atrichelaphinus						Methyleugenol[47]
Cotinis				Ethyl acetate[31]		

[1]Smith and Meeuse (1966), [2]Kite et al. (1998), [3]Kite and Hetterscheid (1997), [4]Schwerdtfeger et al. (2002), [5]Hentrich et al. (2007), [6]Miyake and Yafuso (2005), [7]Stökl et al. (2010), [8]Maia, Dötterl et al. (unpubl. ms.), [9]Kaiser (2011), [10]Chen and Meeuse (1971), [11]Kumano and Yamaoka (2006), [12]Maia (pers. comm.), [13]Gibernau et al. (2003), [14]Gottsberger, Dötterl et al. (unpubl. ms.), [15]Borg-Karlson et al. (1994), [16]Hadacek and Weber (2002), [17]Roman Kaiser Internal Nascent database, [18]Lewis et al. (1988), [19]Chuah et al. (1996), [20]Maia (2011), [21]Inouchi et al. (1988), [22]Osborne et al. (1975), [23]Francke and Dettner (2005), [24]Burger et al. (2008), [25]Schmitt et al. (2004), [26]Burger et al. (1995), [27]Ward et al. (2002), [28]Allsopp (1992), [29]Benyakir et al. (1995), [30]Zarbin et al. (2007), [31]Camino-Lavín et al. (1996), [32]Crocker et al. (1999), [33]Nojima et al. (2003), [34]Tolasch et al. (2003), [35]Allou et al. (2006), [36]Rochat et al. (2000), [37]Krell et al. (1999), [38]Leal et al. (1994), [39]Cherry et al. (1996), [40]Ruther (2004), [41]Klein and Edwards (1989), [42]Kumano-Nomura and Yamaoka (2009), [43]Donaldson et al. (1990), [44]Tóth et al. (2003), [45]Cherry and Klein (1992), [46]Larsson et al. (2003), [47]Johnson et al. (2007).

it is commonly assumed that signal detection and preferences in pollinator insects are an adaptive outcome of a (diffuse) coevolutionary process, shaping pollinator senses/preferences as well as floral signals (Stowe 1988). Regarding color vision, this adaptationist view on pollinator senses has recently been criticized, and doubt has been shed on its ubiquitous importance (Chittka and Menzel 1992; Briscoe and Chittka 2001; Chittka et al. 2001). Several lines of evidence suggest that flower colors are an adaptation to the pollinators' visual systems but not vice versa (Chittka and Menzel 1992). On a macroevolutionary level, it has been shown that the possession of three-color receptors (ultraviolet [UV], blue, green) is not a derived trait of flower-visiting insects like bees, but ancestral for the insects as a whole, basal clades of which are not anthophilous (Chittka 1996). Also, color receptor sensitivities do not differ significantly between Hymenoptera (many of which regularly visit flowers) and other, nonanthophilous insect orders

(Chittka 1996). However, the selective advantages in preferences for the most rewarding flower color have also been shown, supporting the argument for the adaptive (micro)evolution of sensory preferences in pollinators (Raine and Chittka 2007).

Little is known about floral scent and the corresponding olfactory preferences of pollinators (Raguso 2008). However, a recent analysis has shown that there is widespread overlap in floral scent compounds and insect VOCs, suggesting correlated evolution between floral scent and insect use/preferences for certain VOCs (Schiestl 2010). This pattern was interpreted as the outcome of sequential evolution driven by pre-existing bias, because several insect groups that produce "floral-like" VOCs are considerably older than angiosperms. However, phylogenetic analysis at a lower hierarchical level is required to differentiate specifically between coevolutionary and sequential evolution (Ramirez et al. 2011).

Table 2. Araceae genera with data on floral VOC chemistry pollinated by scarab beetles.

Araceae genera	Scarab subfamily and genera	Type of association	References
Amorphophallus	Scarabaeinae (*Ontophagus*, *Heliocopris*), Rutelinae (*Adoretus*), Dynastinae (*Peltonotus*, *Phaeochorus*), Cetoniinae sp.	Deception	(Bogner 1976; Beath 1996; Kite and Hetterscheid 1997; Grimm 2009; Punekar and Kumaran 2009)
Arum	Aphodiinae (*Aphodius*)	Deception	(Knoll 1926; Kullenberg 1953)
Caladium	Dynastinae (*Cyclocephala*)	Mutualism	(Pellmyr 1985; Maia and Schlindwein 2006)
Homalomena	Rutelinae (*Parastasia*)	Mutualism	(Kumano and Yamaoka 2006; Kumano-Nomura and Yamaoka 2009)
Montrichardia	Dynastinae (*Cyclocephala*)	Mutualism	(Ramirez and Brito 1992; Gibernau et al. 2003)
Philodendron	Dynastinae (*Cyclocephala*, *Erioscelis*)	Mutualism	(Gottsberger and Silberbauer-Gottsberger 1991; Gibernau et al. 2000; Gibernau and Barabe 2002; Maia et al. 2010)
Sauromatum	Scarabaeinae (*Sissiphus*)	Deception	(Dakwale and Bhatnagar 1982)
Taccarum	Dynastinae (*Cyclocephala*)	Mutualism	(Maia 2011)
Zantedeschia	Cetoniinae (*Anoplochilus*, *Leucocelis*)	Mutualism	(Singh et al. 1996)

Among the many different pollination systems that have evolved within Araceae (Gibernau 2003), scarab pollination may be roughly separated into two different mechanisms. The first mechanism is deceptive pollination, whereby plants mimic oviposition substrates, usually dung, and attract dung scarabs as pollinators (usually coprophagous groups in the Aphodiinae and Scarabaeinae; Table 2; Kite et al. 1998; Punekar and Kumaran 2009). Alternatively, some groups have evolved mutualistic associations with phytophagous scarabs (Table 2), offering beetles warm mating sites and food (Gottsberger 1990; Maia 2011). The Scarabaeidae have shown an evolutionary transition from a coprophagous to a phytophagous lifestyle (Grimaldi and Engel 2005). Therefore, several different associations to flowers exist. For example, some of the basal groups of these beetles are primarily associated with dung, and never visit flowers, with the notable exception of deceptive pollination in dung mimics (Sakai and Inoue 1999). In addition, the adults of some basal groups of phytophagous scarabs (Melolonthinae) also do not visit flowers but feed on leaves (Leal 1998; Krell 2006). Hence, flower visitation has evolved within a derived, monophyletic group of scarabs, consisting of Rutelinae, Dynastinae, Cetoniinae (including Valginae), and Trichiinae (Browne and Scholtz 1999; Smith 2006). This diversity in ancestral character states leading to an association to flowers presents an ideal scenario for testing the hypothesis about whether specific chemical signals/preferences have evolved together with anthophily, or whether the use of given VOCs by scarab beetles predates flower visitation.

In this article, we take advantage of the high diversity of pollination systems in the Araceae, and the evolutionary shift from a coprophagous to phytophagous/anthophilous lifestyle in scarabs, which are one of the major pollinator groups. We analyze the available data on floral scent chemistry in Araceae, and VOC production/detection in the beetles, within the dated phylogenetic background for both groups. Specifically, we inquire whether floral VOC production and insect VOC production/detection have evolved through the interaction of the two partners, in a coevolutionary or sequential way. Coevolution suggests the correlated evolution of (1) floral VOCs and pollination by scarabs in plants, and (2) insect VOCs production/detection and anthophily in insects; phylogenetic structure in the evolution of the traits should be apparent either in both partners or in none (in case traits evolve multiple times independently). In addition, the timing in the appearance of VOCs in plants and insects should be congruent. Sequential evolution suggests that evolutionary patterns of floral VOC production and insect VOC production/detection may also be correlated with scarab pollination and anthophily, respectively; in case the traits show no correlated evolution, phylogenetic structure may be apparent in one partner, but not in the other. The evolutionary timing of VOC production/detection should, however, differ in plants and beetles.

Materials and Methods
SELECTION OF VOCs AND TAXA
For our analysis, we used (groups of) VOCs that are produced by both arum lilies and scarab beetles. Such shared VOCs are suitable for analyzing the correlation in signaling/signal detection between plants and insects. In Araceae, the criterion for inclusion in the analysis was the chemical proof of production in flowers. Data on floral VOCs produced by Araceae were searched for online using the ISI Web of Science and Google Scholar. In addition, Knudsen et al. (2006) and the Roman Kaiser Internal Nascent

database were used. All Araceae genera with floral VOC data (17 genera) were included in the phylogenetic analysis. For scarab VOCs, one of three criteria needed to be fulfilled: (1) chemical proof of the occurrence of VOCs in the insect, (2) olfactory detection shown by electrophysiology, or (3) attraction to VOCs shown from bioassays. We used the online database "pherobase" (El-Sayed 2008) to search for appropriate species records. For all records in the pherobase, the original literature was double-checked. In addition, the review papers by Leal (1998) and Francke and Dettner (2005) were searched. Within the Scarabaeidae (including Geotrupinae), 32 genera from seven subfamilies with VOC data were included in the phylogenetic analysis. Following this literature research and tabulation of VOC occurrence, VOC groups were selected for analysis based on their shared occurrence in the experimental plants and insects.

SCARAB POLLINATION

The literature on Araceae pollination by scarab beetles was searched in the Web of Science, Google Scholar, and relevant reviews. The search was limited to the Araceae genera for which VOC data were available, as only these species could be included in the analysis.

PHYLOGENIES OF SCARABAEIDAE AND ARACEAE GENERA

Molecular phylogenies for both Araceae and scarab beetle genera were reconstructed using previously published molecular sequences. In the Araceae, recently published molecular phylogenies based on six chloroplast markers were used (Cabrera et al. 2008; Cusimano et al. 2011). The alignment of the sequence data used in Cusimano et al. (2011) was obtained from Tree-Base (www.treebase.org). All genera that were not included in our study were deleted. A maximum-likelihood tree with branch lengths was obtained through a heuristic search implemented in Paup 4 (Swofford 2003). We chose the GTR+G+I model of sequence evolution; whereby, the proportion of invariable sites (I) and the gamma shape parameter (alpha) was set on "estimate." The starting branch lengths were obtained using the Rogers–Swofford approximation method, branch-length optimization was one-dimensional Newton–Raphson with a pass limit of 20. The starting tree(s) were obtained via a stepwise addition, using a random addition sequence. Ten replicates were calculated in the analysis. One tree was retained, the topology of which was basically identical with the published phylogenies (Fig. 1; Cabrera et al. 2008; Cusimano et al. 2011).

In the scarabs, 18s ribosomal RNA datasets were available from the genebank for 15 of the 32 genera used in our analysis. The sequences were aligned using ClustalX (Thompson et al. 1997). The same analysis parameters as for the Araceae dataset were used to generate a maximum-likelihood tree in Paup 4. One

tree was retained in the analysis, the topology of which was identical to the tree published in Howden (1982; also see Fig. 2). All dung scarabs, phytophagous scarabs, and subfamilies within phytophagous scarabs formed monophyletic clades. Within phytophagous scarabs, Rutelinae and Dynastinae were considered to be sister groups of Melolonthinae, and Cetoniinae was considered to be a sister group to the other three subfamilies. Within the subfamilies, genera without sequence information were added, and all genera were treated as polytomies, with equal branch lengths. However, the topology of this molecular tree differed from other morphological phylogenies (Browne and Scholtz 1998), where Melolonthinae is a sister group to the rest of the phytophagous subfamilies. The latter topology was used for constructing the chronogram at the subfamily level (Fig. 3), as it fitted better with the fossil record (Krell 2006).

ANALYSIS OF PHYLOGENETIC STRUCTURE

To detect phylogenetic structure in the dataset for each compound, the most parsimonious number of gains and losses (parsimony steps) in the corresponding phylogeny was compared to the parsimony steps in a series of randomly modified phylogenetic trees. This method assumes that if the occurrence of compounds in genera is at least partly due to common ancestry, fewer parsimony steps would be expected than when trees are randomly reshuffled. To test this assumption, the number of parsimony steps was calculated for each compound using the molecular phylogenies of Araceae and scarab beetles at the genus level. Subsequently, a distribution of parsimony steps was calculated for 100 simulated trees by randomly reshuffling the terminal taxa of the original phylogeny by using Mesquite 2.7. It was then assessed whether the number of parsimony steps for the original phylogeny was within the confidence intervals (5% level) calculated for the distribution of parsimony steps from the simulated trees. For compounds that showed significantly less parsimony steps, phylogenetic structure was assumed.

CHARACTER EVOLUTION ANALYSIS

VOCs were mapped onto the respective Araceae and scarab phylogeny and the ancestral state of character evolution was assessed by parsimony criteria using Mequite 2.7 software (Maddison and Maddison 2009). In addition, Pagels' tests for correlated character evolution, also implemented in Mesquite (Pagel 1994), were calculated using 10 extra iterations and 1000 replicates for simulations to obtain probability values.

Within the Araceae, correlation between the production of VOCs in flowers and pollination by scarab beetles was calculated, differentiating between deceptive pollination by coprophagous scarabs and mutualistic pollination by phytophagous scarabs. For skatole, indole, and p/m-cresol, which are known as dung constituents (Kite et al. 1998), we used "pollination by coprophagous
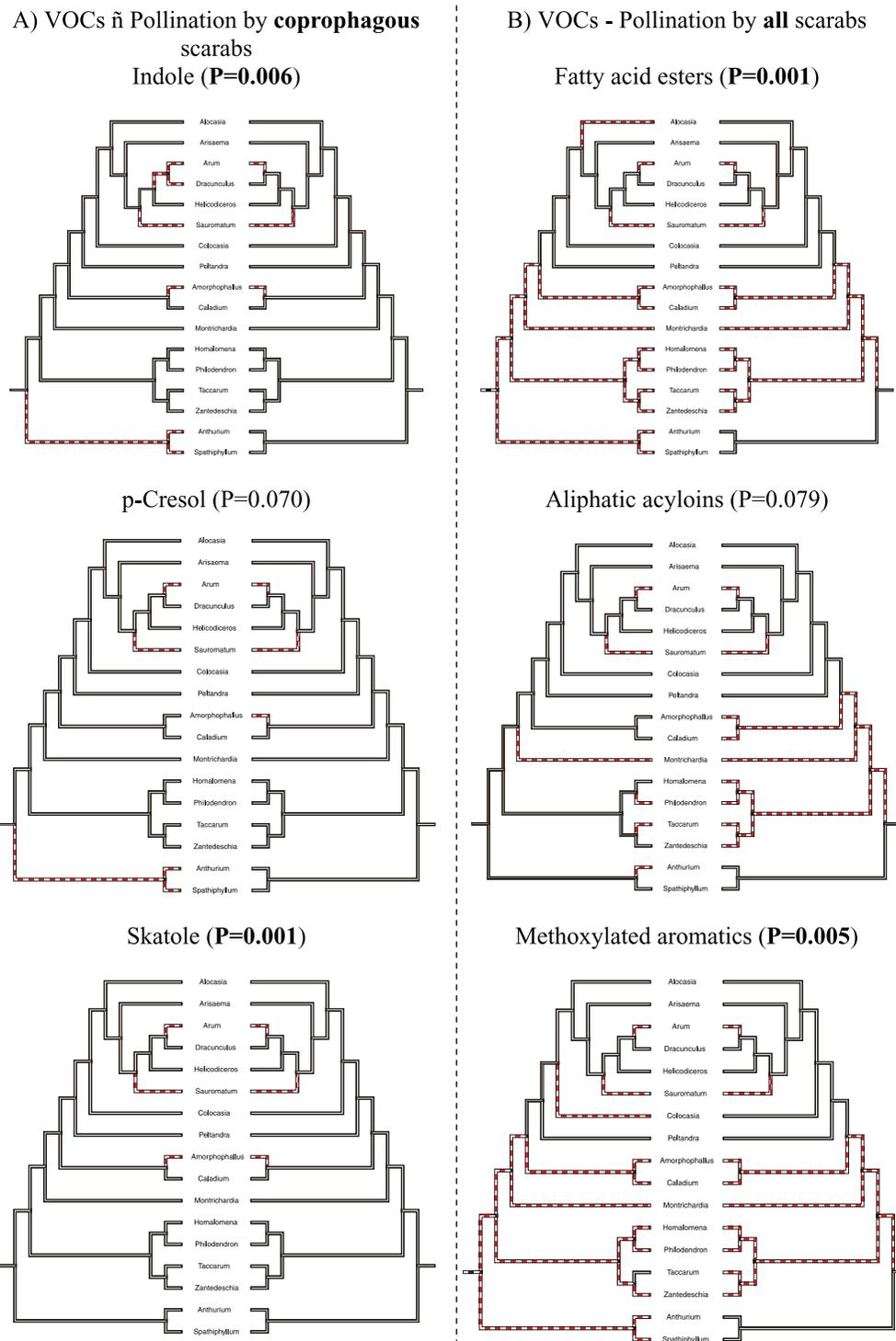
Figure 1. Correlated evolution of floral VOCs and scarab pollination in the Araceae. On each phylogeny mirror image, the left phylogeny depicts the occurrence of a given compound (class), and the right side pollination by dung- or all scarab beetles. Dashed branches correspond to the presence of a trait. Half dashed branches correspond to equivocal presence/absence of a trait in the phylogenetic reconstruction. P values are from Pagels' test of correlated evolution. Significant values are given in bold.
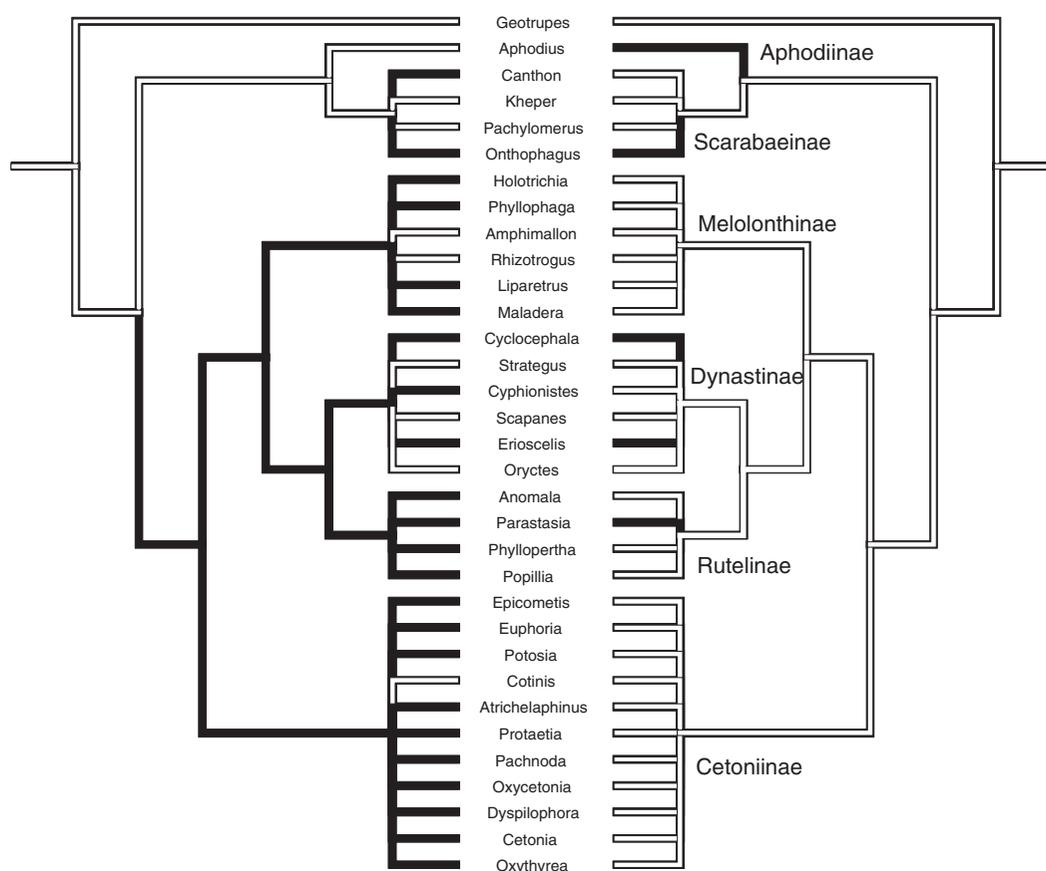
Figure 2. Lack of correlated evolution of methoxylated aromatics (left side) and pollination of Araceae (right side) in the Scarabaeidae. The *P* value from 1000 simulations in Pagels' test of correlated evolution is 0.757. Phylogeny based on 18s ribosomal RNA (see section Methods for details). Note that only scarab genera with VOC data available are included in this analysis.

scarabs" as the corresponding variable. For methoxylated aromatics (MA), aliphatic acyloins (AA), and fatty acid derived esters (FAD) we used "pollination by all scarabs" as the corresponding variable. All scarabs included coprophagous species, as MA and FAE were shown to be used by some dung scarabs. For scarab beetles, the correlation between VOC production/detection and visitation to Araceae flowers was calculated, again discriminating between deceptive and mutualistic pollination. This correlation was calculated at both the genus and subfamily level, because only five genera of scarabs in our analysis were pollinators that had available VOC data. The analysis at the subfamily level assumes the widespread use of similar VOCs within subfamilies, which has been found before in several insect groups (Symonds and Elgar 2008). This analysis is less sensitive to missing data, which is a caveat in our scarab genus dataset, in which only a small sample of the huge scarab beetle diversity is represented.

Results
VOCs IN PLANTS AND INSECTS
A wide range of VOCs was found to occur in both Araceae flowers and scarab beetles. Suitable VOCs for the analysis included

skatole, indole, and p/m-cresol, as they were widespread in both Araceae and scarab beetles (Table 1). Phenol was also widespread, but was excluded from the analysis because its documentation is unreliable, as it can easily contaminate samples. In addition to these VOCs, we included three groups of volatile compounds, specifically, FAE, AA, and MA, which are all widespread and characteristic signaling compounds in both Araceae and scarab beetles (Francke and Dettner 2005; Dobson 2006; Table 1).

POLLINATION
Scarabs of all subfamilies, except Geotrupinae and Melolonthinae, pollinate Araceae flowers (Table 2). Among the Araceae for which floral VOC data are available, nine genera are reported to be, at least partly, visited by scarab beetles, all of which belong to the subfamily Aroideae (Cabrera et al. 2008; Table 2). These scarab beetles belong to 11 genera representing five scarab subfamilies (Table 2). VOC data have been reported for five of these genera in the literature (i.e., *Aphodius, Ontophagus, Cyclocephala, Erioscelis,* and *Parastasia*). Several other Araceae genera (i.e., *Monstera, Rhaphidophora* [Monsteroideae], *Dieffenbachia, Xanthosoma, Syngonium,* and *Anubias* [Aroideae]) are
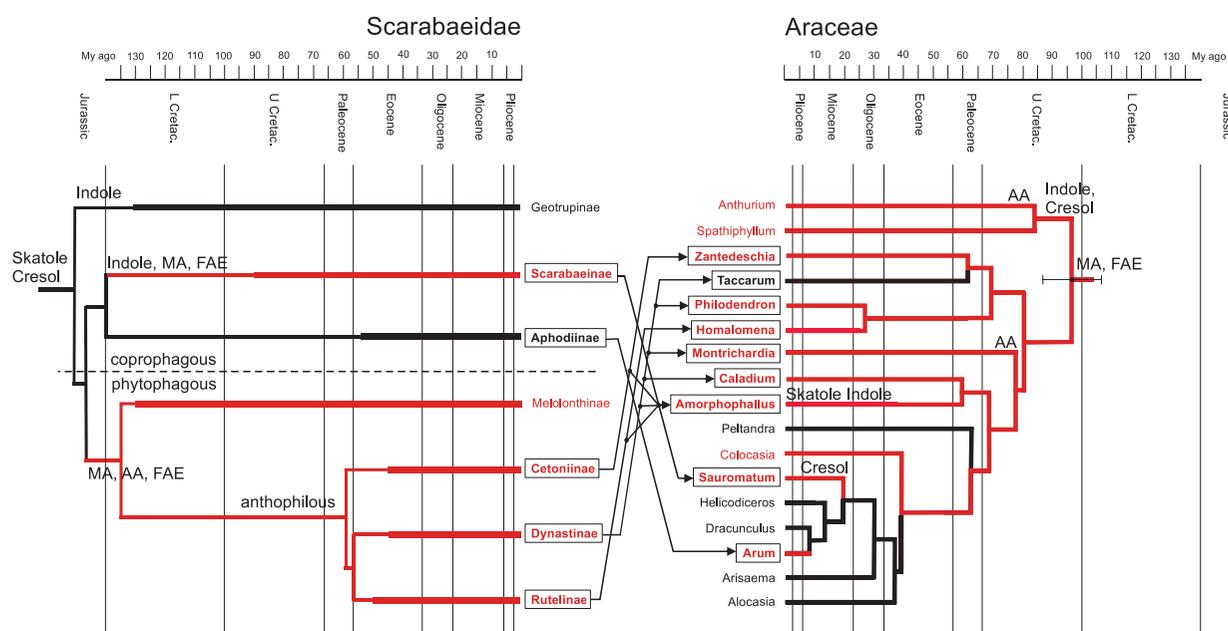
Figure 3. Comparison of dated phylogenies of Scarabaeidae and Araceae (after Krell 2006) (scarabs), Nauheimer, Metzler, and Renner unpubl. ms. (aroids), with the reconstructed evolution of methoxylated aromatics indicated in red. In the aroids, the 95% highest posterior density interval of age estimation is given for the oldest split. The predicted oldest appearances of the other VOCs (classes) are indicated with names or abbreviations in the chronograms (MA = methoxylated aromatics; AA = aliphatic acyloins; FAE = fatty acid esters). Interactions through pollination are indicated with arrows. In the scarabs, bold lines begin with the oldest fossil record of the clade. Note that *Anthurium* and *Spatiphyllum* are partly pollinated by euglossine bees that also show preferences for some of the here analyzed VOCs such as MA.

also pollinated by scarab beetles (Gibernau 2003), but lack floral VOC data.

PHYLOGENETIC STRUCTURE AND HISTORICAL CHARACTERISTICS

Araceae
In the Araceae, phylogenetic structure was not detected for any of the investigated compounds (groups). In other words, the random reshuffling of terminal branches of the tree did not lead to significantly more evolutionary origins than that indicated by the original tree. However, most compound(s) (groups) were found to be widespread across different genera. Such homoplasy is expected in a scenario of nonrelated plants adapting to common groups of pollinators. Fatty acid esters and MA were suggested to be basal VOC classes for all analyzed Araceae from the analysis of historical characteristics (Fig. 1). Indole, cresol, and AA were suggested to be equivocal compounds for ancestral origin and/or multiple independent origins in the clade (Fig. 1). For skatole, three independent origins were suggested. The maximum number of independent origins is shown in Table 3.

Scarabaeidae
In the analysis of scarabs at the genus level, MA showed significant phylogenetic structure (Fig. 2). In other words, the random reshuffling of terminal branches of the tree led to significantly

Table 3. Estimated time of origin of VOCs (based on Fig. 3) and maximum number of independent origins in Araceae and scarab beetles.

VOCs	Oldest estimated appearance (maximum number of independent origins)	
	Araceae	Scarab beetles (subfamily level)
Skatole	Paleocene (3)	Jurassic (1)
Cresol	Upper Cretaceous (4)	Jurassic (2)
Indole	Upper Cretaceous (4)	Jurassic (2)
Methoxylated aromatics	Lower–Upper Cretaceous (4)	Jurassic (2)
Aliphatic acyloins	Upper Cretaceous (6)	Jurassic (1)
Fatty acid esters	Lower–Upper Cretaceous (4)	Jurassic (2)

more evolutionary origins than that indicated by the original tree. The signaling function of this compound group was also suggested to be ancestral for the whole clade of phytophagous scarabs (Figs. 2, 3). The ancestral origin of skatole was ambiguous in the clade of coprophagous scarabs, with multiple independent origins being possible.

For the other compounds (i.e., indole, cresol, fatty acid esters, and AA), multiple independent origins were also suggested. In the analysis at the subfamily level, cresol was suggested as ancestral for dung scarabs plus Melolonthinae. In comparison, the ancestral origin of indole was equivocal for the same group. Fatty acid esters were suggested as ancestral for phytophagous scarabs, but ancestral origin was equivocal for the whole family. In addition, AA were suggested ancestral for phytophagous scarabs. Again, the maximum number of independent origins is shown in Table 3.

CORRELATED EVOLUTION BETWEEN VOCs AND POLLINATION

Within the Araceae, strong associations were detected between the production of most compounds and pollination by either dung or phytophagous scarab beetles. The occurrence of indole and skatole in Araceae was strongly correlated with pollination by dung beetles (Fig. 1; Table 4). Fatty acid esters and MA were correlated with pollination by all scarabs. This result shows that the production of the here analyzed VOCs by the Araceae is linked to pollination by scarab beetles that also use these VOCs in their chemical ecology. Therefore, the observed overlap in floral VOCs probably evolved through the interaction of plants with these pollinators.

However, in the beetles, generally no correlation between VOCs and the pollination of arum lilies (anthophily) was found (Fig. 2; Table 4). The only significant association was found for skatole ($P = 0.02$) at the subfamily level. All other compounds showed no such association, neither for dung beetles visiting dung mimics, nor phytophagous scarabs visiting rewarding arum lilies. For instance, indole is a widespread attractant in coprophagous scarabs; however, only a few genera (i.e., *Onthophagus, Sissiphus, Heliocopris,* and *Aphodius*) are known to pollinate arum lilies. Some beetle subfamilies never seem to visit flowers, such as Geotrupinae (here used synonymously for Geotrupidae). Similarly, MA are used by several genera of Melolonthinae, which generally do not visit flowers. Therefore, the signaling functions of VOCs in scarabs appear to have evolved independently from the visitation to Araceae.

RECONSTRUCTING THE TIMING OF VOC EVOLUTION
Araceae
The oldest split in the Araceae analyzed in this study is between *Anthurium* (Pothoideae), *Spathiphyllum* (Monsteroideae), and the Aroideae (all other analyzed genera), which is estimated to have occurred approximately 95 million years ago (Mya) (Fig. 3; Nauheimer, Metzler, and Renner unpubl. ms.). *Anthurium* and *Spathiphyllum* are not beetle pollinated, but are partly pollinated by flies and euglossine bees. However, the Monsteroideae subfamily does contain two genera pollinated by scarab beetles, *Monstera* and *Rhaphidophora,* which were not included in our analysis

(Gibernau 2003). The Aroideae clade, which contains all the Araceae genera pollinated by beetles in the current study, is suggested to be approximately 80 My old (Nauheimer, Metzler, and Renner unpubl. ms.). Tracing the historical characteristics combined with the dated phylogenies indicates that most Araceae VOCs evolved in the Upper Cretaceous (Table 3; Fig. 3). The only exception is suggested to be skatole, which first originated in *Amorphophallus* during the Paleocene.
Scarabaeidae
The scarab beetle subfamilies of Melolonthinae and Geotrupinae are among the oldest groups, ranging back to the Jurassic (Krell 2006). In general, neither subfamily visits flowers. Pollinating coprophagous scarabs are also estimated to be of similar age; however, Araceae genera pollinated by these beetles (i.e., *Amorphophallus, Sauromatum, Arum*) originated much later, between 60 and 15 Mya (Fig. 3). Among phytophagous scarabs, anthophilous (pollinating) groups are relatively young, with an estimated origin in the Paleocene, around 60 Mya (Fig. 3; Krell 2006). Their origin roughly corresponds to the origin of some of the taxa that they pollinate (i.e., *Zantedeschia, Taccarum*). However, none of the groups of compounds that were analyzed here originated exclusively in this group of primary pollinators. Indeed, all VOCs are suggested to be much older, namely of Jurassic origin (Fig. 3; Table 3).

Discussion

Although it is often assumed that the senses of pollinators and floral signaling by plants reciprocally select for each other leading to coevolution, this hypothesis remains little investigated (Chittka et al. 2001; Ramirez et al. 2011). In this analysis, we examined patterns of VOC evolution in both plants and their pollinators, to assess whether a pattern of coevolution or sequential evolution is supported. We found clear evidence for the sequential evolution of VOCs in insects and plants, whereby the use of given VOCs by pollinators is evolutionarily older than the occurrence of the same groups of VOCs in the flowers that they pollinate. Our findings support a scenario of pre-existing bias, with pre-existing chemical communication in insects leading to their selecting plants that produce specific floral scent compounds (Schiestl 2010; Ramirez et al. 2011). This phenomenon may explain the convergent evolution of floral traits in response to similar pollinator groups, leading to pollination syndromes in floral signaling.

The first notable finding of our analysis is the considerable overlap in VOC chemistry between plant floral scent and beetle chemical communication (Table 1). Such chemical similarity between phylogenetically distinct groups of organisms has been previously recorded, but has only recently been subject to quantitative analysis (Schiestl 2010). Schiestl (2010) demonstrated a broad overlap in VOC production between insects, angiosperms,

Table 4. Correlated evolution of VOC production/preference and attraction to Araceae in scarab beetles as well as VOC production and scarab pollination in Araceae. The table shows statistical values of Pagels' tests of correlated evolution. Significant values are given in bold.

	Log-likelihood four parameter	Log likelihood eight parameter	Difference	*P*
Scarabaeidae subfamily level (genus level)	**Attraction to dung mimics**			
Skatole	5.60 (11.88)	3.40 (14.96)	2.20 (−3.08)	**0.024** (0.963)
Indole	6.26 (11.96)	4.70 (16.01)	1.56 (−4.05)	0.084 (0.942)
Cresol	7.10 (13.74)	6.95 (16.01)	0.15 (−2.27)	0.723 (0.81)
	Attraction to all Araceae			
Methoxylated aromatics	6.374 (32.32)	6.110 (32.73)	0.264 (−0.41)	0.630 (0.757)
Aliphatic acyloins	7.21 (28.22)	7.05 (28.28)	0.17 (−0.07)	0.668 (0.744)
Fatty acid esters	6.37 (30.84)	6.11 (30.89)	0.26 (−0.05)	0.615 (0.754)
Araceae	**Pollination by coprophagous scarabs**			
Skatole	14.46	8.61	5.84	**0.001**
Indole	17.57	13.89	3.68	**0.006**
Cresol	15.81	14.19	1.62	0.070
	Pollination by all scarabs			
Methoxylated aromatics	21.32	17.71	3.62	**0.005**
Aliphatic acyloins	21.34	19.55	1.79	0.079
Fatty acid esters	20.46	15.54	4.91	**0.001**

Values in the table were calculated by using a test for correlated evolution implemented in mesquite 2.7 (Pagel 1994).

and gymnosperms. As discussed in Schiestl (2010), such an overlap in VOC production may be "neutral," and not related to the interactions that plants and insects may engage in. Alternatively, the sharing of VOCs may be an evolutionary consequence of a mutualistic or antagonistic interaction, which may evolve in a sequential or coevolutionary manner. Schiestl (2010) suggested that adaptive evolution causes the observed overlap in VOC production by different groups, which was based on the comparison of patterns of correlation in different groups of plants and insects, as well as different groups of VOCs.

Our analysis strongly supports the adaptive view on the evolution of floral VOCs in plants, in that it shows that the occurrence of floral VOCs shared with scarab beetles is phylogenetically correlated to pollination by scarab beetles. This pattern of correlated evolution was significant in five out of six of the analyzed compound(s) (groups), suggesting that these compounds have particular functions in the scarab pollination system, most likely in attracting the insects to the flowers. Indeed, the significance of an aliphatic acylion, (*S*)-2-hydroxy-5-methyl-3-hexanone, in attracting a dynastine pollinator of *T. ulei* has been recently demonstrated (Maia 2011). Methoxylated aromatics have also been found to be attractive for pollinating dynastine beetles (Gottsberger and Dötterl unpubl. ms.).

Additional lines of evidence come from patterns of convergent evolution in some VOCs of different scarab-pollinated plants. Methoxylated aromatics and/or fatty acid esters, for example, are characteristic constituents of water lilies (Nymphaeaceae), *Magnolia ovata* (Magnoliaceae), and a phytelephantoid palm (Arecaceae), all of which are pollinated/visited by dynastine scarabs (Ervik et al. 1999; Ervik and Knudsen 2003; Kaiser 2006; Gottsberger et al. in press). For the deceptive pollination systems within Araceae, our study provides clear evidence for the attractiveness of plant-produced VOCs to pollinators. Skatole, indole, and cresol are typical constituents of dung (Kite et al. 1998), and their attractiveness to dung beetles has been previously shown in bioassays (Francke and Dettner 2005). In addition, some scarabs have obviously adopted some of these compounds as sex pheromones, such as skatole and indole in *Kheper bonelli* (Scarabaeinae) (Burger et al. 2008). These three compounds frequently occur in flowers of different plant families that are pollinated by dung beetles and flies (Dobson 2006; Jürgens et al. 2006; Urru et al. 2011).

The convergent evolution of floral traits in response to the same pollinator groups has been epitomized in the pollination syndromes that typically include floral signals (Vogel 1954; Faegri and van der Pijl 1979; Fenster et al. 2004; Willmer 2011). Similar patterns of floral scent compounds in unrelated plant lineages have been found in several pollination systems, and are perhaps the

most pronounced in the pollination systems of moths (Knudsen and Tollsten 1993; Dobson 2006), bats (Dobson 2006), and brood-site mimicry systems (Urru et al. 2011). Our analysis supports this concept by showing that particular compound groups evolve upon the interaction with given pollinators. However, the question remains about whether plant signaling evolves in response to insect chemical communication, or vice versa, or synchronously.

An interesting finding, in this respect, is the lack of any correlated evolution between VOC production and preference in scarab beetles and their pollination of arum lilies (Table 3). This observation indicates that, among studied VOCs, scarabs have not evolved any specific VOC preferences in connection with the pollination of arum lilies. This finding is evidence against a coevolutionary scenario, which would require both partners to show adaptive change upon the origin of an interaction (Thompson and Cunningham 2002; Anderson and Johnson 2008). However, this evidence is limited by the low volume of data available on the chemical ecology of scarab–Araceae interactions. The chemical ecology of scarab beetles has clearly focused on economically important species, which are usually not pollinators (Leal 1998).

Additional and strong support against the coevolutionary view comes from comparison in the timing of diversification, and the corresponding reconstruction of VOC evolution. The Araceae is considered an old angiosperm family, with the oldest fossil pollen described being from 125-My-old strata in Portugal (Friis et al. 2004). Indeed, our reconstruction of historical characteristics in connection with dated phylogenies (Nauheimer, Metzler, and Renner unpubl. ms.) suggests the early evolution of most VOCs, during the mid-early Cretaceous. However, the evolution of VOCs in scarabs appears to be considerably older, originating in the Jurassic. A Jurassic origin has been deduced owing mostly to the presence of many VOCs within Geotrupinae and Melolonthinae, with a fossil record that is up to 130 My old (Fig. 3; Krell 2006). The anthophilous scarab groups (i.e., Rutelinae, Dynastinae, and Cetoniinae) are thought to be considerably younger, but none of the VOCs analyzed in this study originated exclusively within these groups.

The clearest evidence against coevolution comes from deceptive plant-pollinator interactions. Here, some 130-My-old beetle groups pollinate relatively young groups of arum lilies (Fig. 3). In these mimetic associations, it is obvious that signals and signal detection have not evolved synchronously. The use of dung-associated VOCs to locate appropriate substrates for oviposition is probably as old as the actual use of dung. Because competition for dung can be fierce, strong immediate responses to these VOCs are vital to successfully compete for a dung resource (Tribe and Burger 2011), allowing plants to interfere with these signals, leading to deceptive mimetic pollination (Mant et al. 2002). In the mutualistically pollinated Araceae, however, the evidence also points against coevolution.

Ancestral, nonpollinating scarabs use methoxylated aromatics, aliphatic acyloins, and fatty acid esters for chemical communication. The derived pollinating groups share these compounds, suggesting phylogenetic conservatism in chemical communication. Phylogenetic conservatism indicates that VOC utilization evolves slowly, in a gradual manner. Indeed, we detected phylogenetic structure in the methoxylated aromatics used by scarabs. Phylogenetic conservatism is often found in the evolution of pheromones in the form of a pattern that is best described as "variation around a theme." While chemical groups of volatile compounds remain unchanged, slight qualitative modifications or changes in quantitative patterns occur at speciation events (Mant et al. 2002; Symonds and Elgar 2008; Symonds et al. 2009). Similarly, color receptors in insect were shown to be highly conserved, with three receptor types (UV, blue, green) as a basal state for the whole insects (Chittka 1996).

In conclusion, our analysis yields strong support for a pre-existing bias explaining patterns of floral VOC evolution in the Araceae. We show that, although specific floral VOC compounds evolve in plants upon scarab beetle pollination, the opposite is not true, because the production of these compounds by beetles clearly predates the evolution of beetle-pollinated arum lilies. Thus, when scarab beetles were recruited as plant pollinators, their pre-existing olfactory detection abilities selected for specific floral scent compounds. This scenario largely matches the suggested one-sided adaptation in floral color to pre-existing sensory preferences for colors in insects (Chittka 1996). Indeed, this evolutionary scenario may be widespread, and explain the pertinent, although not ubiquitous, evolution of pollination syndromes in floral signaling. However, it may be premature to exclude the possibility of (micro) coevolution in highly specialized, mutualistic pollination systems, such as those that have evolved in some Dynastine scarabs and arum lilies (Gottsberger and Silberbauer-Gottsberger 1991; Maia and Schlindwein 2006; Maia et al. 2010). In such close relationships, the floral scents of plants and the olfactory preferences of scarabs may have coevolved, allowing highly specific communication between the plants and their pollinators (Maia 2011). In conclusion, a better understanding of chemical communication between plants and pollinators at the species and individual-compound level may allow us to test this hypothesis in the future.

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