doi:10.1111/evo.12318

# THE EVOLUTION OF POLLINATOR–PLANT INTERACTION TYPES IN THE ARACEAE

**Marion Chartier,[1,2] Marc Gibernau,[3] and Susanne S. Renner[4]**

[1]*Department of Structural and Functional Botany, University of Vienna, 1030 Vienna, Austria*

[2]*E-mail: chartier.marion@gmail.com*

[3]*Centre National de Recherche Scientifique, Ecologie des Forêts de Guyane, 97379 Kourou, France*

[4]*Department of Biology, University of Munich, 80638 Munich, Germany*

Received August 6, 2013
Accepted November 17, 2013

**Most plant–pollinator interactions are mutualistic, involving rewards provided by flowers or inflorescences to pollinators. Antagonistic plant–pollinator interactions, in which flowers offer no rewards, are rare and concentrated in a few families including Araceae. In the latter, they involve trapping of pollinators, which are released loaded with pollen but unrewarded. To understand the evolution of such systems, we compiled data on the pollinators and types of interactions, and coded 21 characters, including interaction type, pollinator order, and 19 floral traits. A phylogenetic framework comes from a matrix of plastid and new nuclear DNA sequences for 135 species from 119 genera (5342 nucleotides). The ancestral pollination interaction in Araceae was reconstructed as probably rewarding albeit with low confidence because information is available for only 56 of the 120–130 genera. Bayesian stochastic trait mapping showed that spadix zonation, presence of an appendix, and flower sexuality were correlated with pollination interaction type. In the Araceae, having unisexual flowers appears to have provided the morphological precondition for the evolution of traps. Compared with the frequency of shifts between deceptive and rewarding pollination systems in orchids, our results indicate less lability in the Araceae, probably because of morphologically and sexually more specialized inflorescences.**

**KEY WORDS:** **Ancestral state reconstruction, inflorescence traits, pollination syndromes, phylogeny, trap flowers.**

Interactions between a plant and its pollinators exert great influence on its morphological and physiological adaptations. To the extent that such adaptations are evolutionarily conservative, they can be traced on a phylogeny at least if traits can be unambiguously coded and species are reasonably densely represented in the phylogeny. Studies using historic reconstructions have inferred the evolution of long-tubed flowers adapted to pollinators with long mouthparts (Whittall and Hodges 2007), radial or bisymmetric flowers adapted to different pollinators (Knapp 2010), or perfume-producing flowers adapted to oil-collecting bees (Renner and Schaefer 2010). In orchids, it has also been possible to infer evolutionary shifts between different types of mimicry and deception (Vereecken et al. 2012) and between deceptive and rewarding pollination systems (Johnson et al. 2013). Such shifts between antagonistic and mutualistic pollination interactions are of interest because theory predicts that mutualistic interactions are vulnerable to cheaters (Bronstein 2001). Over evolutionary time, this is expected to lead to the repeated replacement of mutualistic interactions by interactions in which plants deceive their pollinators by attracting them to consistently rewardless flowers. Yet of the 415 families of flowerings plants, only 32 have evolved pollination modes that rely on deceit (Renner 2006). Quantitatively most important among these families are the Orchidaceae, in which thousands of species are thought to rely on deceptive pollination, often involving sexual deception. The first phylogenetic studies addressing evolutionary transitions between deceptive and rewarding pollination in orchids, however, revealed unexpected results. Thus, in the Orchidinae, the ancestral state seems to have been bee-pollinated, food-deceptive flowers, with sexual deception evolving later (Inda et al. 2012),






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but in *Disa* (also in the tribe Orchideae), deception was the ancestral condition from which nectar production, and thus rewarding pollination, evolved at least nine times (Johnson et al. 2013).

Next to orchids, it is the Araceae family that contains the largest number of deceptively pollinated species (Renner 2006), usually involving trapping mechanisms in which pollen is received on the first day and exported on the second (all Araceae are protogynous; Mayo et al. 1997). A recent analysis by Bröderbauer et al. (2012) inferred that trapping inflorescences (some offering liquid sustenance for the trapped insects) evolved at least 10 times, and in 27 of the 120–130 genera of the family. Their much smaller species number compared to orchids and the documented presence of multiple deceptive systems makes the Araceae well suited for studying how mutualistic pollination interactions may give rise to antagonistic ones and whether there is a directional asymmetry in the frequency with which one interaction type replaces the other. This is the main topic we address here, using a new phylogeny that extends recent plastid DNA phylogenies (Cabrera et al. 2008; Cusimano et al. 2011; Nauheimer et al. 2012b) by adding a single-copy nuclear gene.

We build on, but do not duplicate, previous comparative analyses of Araceae pollination. These studies focused on possible correlations between pollen sculpturing and pollinator types (Grayum 1986; Sannier et al. 2009), in the case of Sannier et al. using principal component methods applied to discrete characters on a phylogeny for 56 species from 33 genera for which pollen characters and pollinator orders (beetles, flies, bees, thrips) could be scored. Both studies inferred a strong correlation between verrucate or tuberculate exines and beetle pollination, and a weak association between spinose exines and fly pollination. Two other discriminant analyses compared bee-, beetle-, or fly-pollinated Araceae in terms of flower number per inflorescence, pollen/ovule ratios, stigmatic area, pollen diameter, and stamen number (Chouteau et al. 2008; Gibernau et al. 2010). More recently, trapping structures and pollinator types (beetles, flies, bees, and "generalists") were mapped on a plastid-DNA family phylogeny (Bröderbauer et al. 2012), and floral volatile organic compound and pollination by scarab beetles on a reduced plastid phylogeny with 17 genera (Schiestl and Dötterl 2013). Ours is the first family-wide analysis to focus on antagonism/mutualism shifts.

The specific pollination mutualisms in Araceae involve rewards in the form of liquid food for adult insects, floral perfume for male euglossine bees, or mating-sites and egg-laying sites (references see Materials and Methods). The antagonisms involve pollinator trapping and consequently the reduction of their fitness, if females lose egg-laying and larval development opportunities. In species of *Arisaema*, insects are even killed in female inflorescences from which they cannot escape (Vogel and Martens 2000). Figure 1 illustrates the types of floral morphologies associated with the different types of pollination interactions found in Araceae. To infer the distribution of pollinator interaction types, we compiled data on pollinators, pollination mechanisms, and 21 quantitative and qualitative floral traits. We explored possible correlations among these characters and pollination modes using stochastic trait state reconstructions (Nielsen 2002; Huelsenbeck et al. 2003; Bollback 2006), which permitted simultaneous analyses of qualitative and quantitative characters with up to six states. Bayesian stochastic trait mapping also has the advantage of providing a statistically well-understood measure of confidence for the obtained correlations (predictive sampling; Huelsenbeck et al. 2003). The specific questions we wanted to answer with these data were (i) what is the phylogenetic distribution of mutualistic and deceptive pollination systems in the Araceae? And (ii) are there inflorescence traits that are correlated with interaction types, for example, unisexual flowers, spadix appendages, or constricted spathes?

## Materials and Methods

### MOLECULAR PHYLOGENETICS

For a nuclear phylogeny of the Araceae, we newly generated 42 sequences of the nuclear *Phytochrome C* gene (*PhyC*) and added these to existing Araceae *PhyC* sequences from Cusimano et al. (2010) and Nauheimer et al. (2012a) for a total of 72 sequences. DNA was isolated from leaf material harvested in the living collections of the botanical gardens of Lyon (France), Montet (Nancy, France), and Munich (Germany) in 2008 and 2009. Herbarium vouchers and other details are listed in Table S1, which also shows GenBank accession numbers. Several species per genus were included for *Alocasia* (5 spp.), *Arisaema* (2 spp.), *Arum* (3 spp.), *Biarum* (2 spp.), and *Nephthytis* (2 spp.), and the species *Calla palustris* was sequenced twice (because of its unexpected systematic placement; Ulrich et al. 2013).

Genomic DNA was isolated from silica-dried material, using the QIAquick Gel Extraction Kit and a microcentrifuge (Quiagen, Crawley, West Sussex, UK). *PhyC* was then amplified using three sets of internal and external primers (see Fig. S1 for their position); 750R and 430F were designed by Mathews and Donoghue (1999), AF, AR, and A20F by Cusimano et al. (2010), and 748R-Ara (5′-ACAAGATCCATGACATTAGGTGATT-3′) for this study. Amplification reactions were performed with U-*Taq* DNA Polymerase with ThermoPol Buffer (New England Biolabs, Frankfurt am Main, Germany) and 10–50 ng of template DNA per 25-μL reaction volumes. For recalcitrant material, we used a more reactive polymerase (Phusion® High-Fidelity DNA Polymerase; New England Biolabs, Frankfurt am Main, Germany). PCR products were purified using 0.15 μL of Shrimp Alkaline Phosphatase (SAP) enzyme, 0.015 μL of Exonuclease I enzyme (Fermentas


**Figure 1.** Araceae inflorescences and their associate pollination modes (pollinator order and pollination type); see Table S2 for supporting information. (A) *Lysichiton camtschacensis* (Proto-Araceae), rewarding mutualism, bees, flies; (B) *Anthurium sp.* (Pothoideae), photographed in French Guiana, rewarding mutualism, bees, beetles; (C) *Monstera adansonii* (Monsteroideae), rewarding mutualism, bees, beetles; (D) *Dracontium polyphyllum* (Lasioideae), unknown type of interaction involving flies; (E) *Stylochaeton hypogaeus* (*Stylochaeton* clade), deceptive system involving beetles; (F) *Calla palustris* (Calloideae), suspected rewarding mutualism involving flies and beetles; (G) *Montrichardia linifera* (Calloideae?), mating mutualism, beetles; (H) *Dieffenbachia amoena* (Spathicarpeae), mating mutualism, beetles; (I) *Amorphophallus* (*Pseudodracontium*) *lacouri* (Thomsonieae), deceptive system involving beetles; (J) *Colocasia gigantea* (*Colocasia* clade), oviposition mutualism, flies; (K) *Arum maculatum* (Areae), deceptive system involving flies; (L) *Arisaema fimbriatum* (*Pistia* clade), deceptive system involving flies. Picture (A) F. Muller; pictures (C)–(E) D. Scherberich.

GmbH, St. Leon-Rot, Germany), and 2 μL of water for 5 μL of DNA. Sequencing relied on an ABI 3100 Avant capillary sequencer and assembly and editing on the software Sequencher (Gene Codes, Ann Arbor, MI). Clean contigs were BLASTed in GenBank (http://blast.ncbi.nlm.nih.gov/blas.cgi) to check for possible contamination, and an alignment was then generated with ClustalW (Thompson et al. 1994) in MEGA5 (Tamura et al. 2011) using the default settings, with subsequent checking by eye.

The topology of a maximum likelihood tree from the *PhyC* nuclear matrix did not statistically contradict plastid topologies obtained in earlier studies (Cusimano et al. 2011; Nauheimer et al. 2012b), and we therefore concatenated our nuclear matrix with the most recent plastid alignment of six plastid markers (*rbcL*, *matK*, partial *trnK* intron, partial *tRNA-Leu* gene, *trnL-trnF* spacer, and partial *tRNA-Phe* gene) from Nauheimer et al. (2012b). This resulted in a 5342 nucleotides long combined matrix of 135 species from 119 genera of which 63 were lacking *PhyC*. In 11 genera, the *PhyC* sequence and the plastid sequences came from a different species of the same genus. Trees were rooted on Acoraceae (*Acorus gramineus*), the sister clade to all other monocots, and on Tofieldiaceae (*Pleea tenuifolia* and *Tofieldia calyculata*) as a representative of the Alismatales, an order of 14 families to which the Araceae belong; Araceae and Tofieldiaceae are the two first-branching clades in the Alismatales.

Maximum likelihood (ML) tree searching relied on the GTR + G substitution model, which was the best-fit model found for the combined matrix by JModeltest (Guindon and Gascuel 2003; Darriba et al. 2012) and which happens to also be the model implemented in RAxML 7.0.4 (Stamatakis 2006; Stamatakis et al.

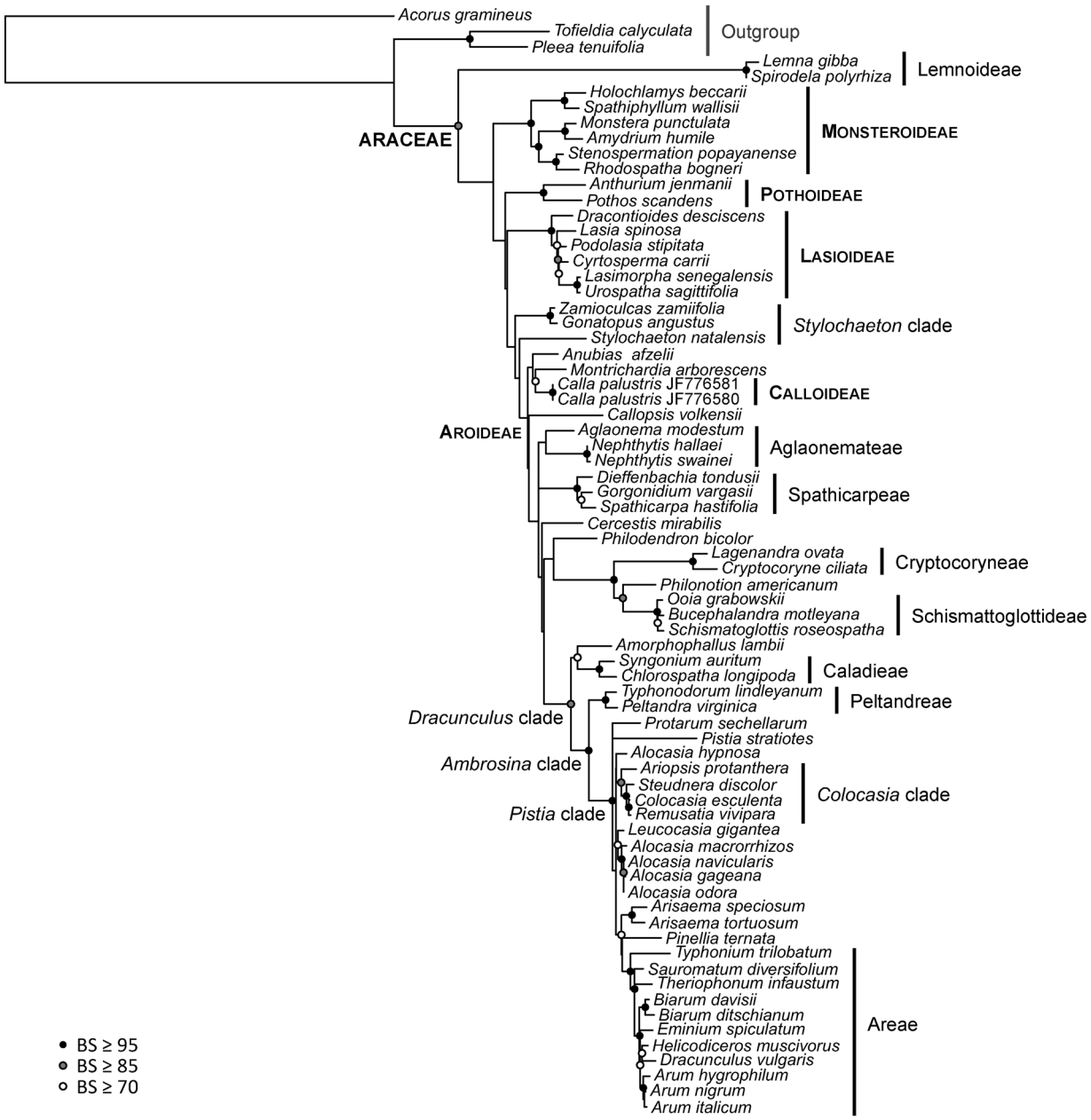


**Figure 2.** **Maximum likelihood phylogeny of the Araceae based on an alignment of the nuclear *PhyC* gene obtained from 72 species; Figure S2 shows a tree for 135 species obtained from a combined alignment of nuclear *PhyC* and six plastid markers; this larger tree was used for the reconstructions in Figure 3. Levels of maximum likelihood bootstrap support (BS) from 1000 replicates are given as explained in the inset. Clade names follow Cusimano et al. (2011).**

2008). Statistical support was assessed with 1000 bootstrap replicates, using the same substitution model.

### CODING OF POLLINATION INTERACTIONS AND FLORAL TRAITS

Information on pollinator orders (Hymenoptera, Coleoptera, and Diptera) and interaction types was compiled from the sources listed in Table S2, which also shows our coding. Rewarding mutualisms in Araceae are pollination interactions that involve a food reward, such as stigmatic exudates, small amounts of nectar (Vogel and Martens 2000; Diaz and Kite 2006), or liquid floral perfume for male euglossine bees (Hentrich et al. 2007, 2010). Mating/oviposition site mutualisms involve flies or beetles that mate or oviposit in the inflorescences (Gibernau et al. 1999; Sakai 2002; Urru et al. 2010; our Table S2). Antagonistic interactions involve the attraction and trapping of pollinators without either a food reward or a suitable site for larval development (Urru et al. 2011). Tofieldiaceae were coded as rewarding based

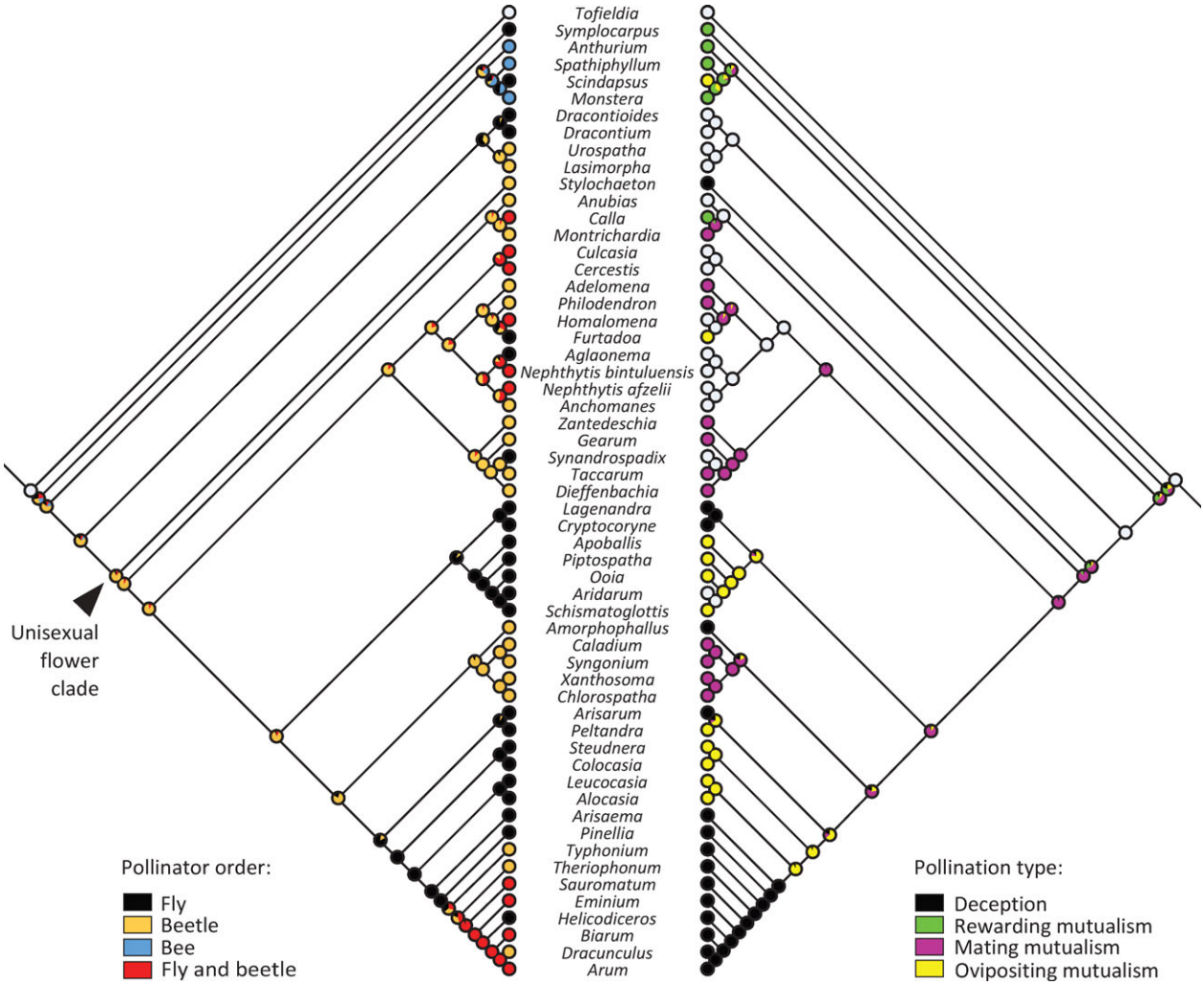


**Figure 3.** **Evolution of pollination modes in the Araceae, with pollinator orders on the left and pollination type on the right, inferred by maximum likelihood ancestral state reconstruction on a reduced phylogeny that includes the 57 of the total genera with pollination information (see Table S2 for supporting references). Branch color coding is explained in the legend. Tips that lack a colored circle, such as *Tofieldia*, have no known (coded) trait state. Figure S3 shows a parsimony ancestral state reconstruction of pollinator order (left) and pollination modes (right) in Araceae.**

on their nectar-producing flowers with few stamens (Azuma and Tobe 2011; S. S. Renner, pers. obs.).

Tables S3 and S4 show the 21 characters coded, namely interaction type, pollinator order, and 19 floral traits. Of the 21 characters, eight were quantitative traits and 13 were qualitative traits. The quantitative traits (male flower number per inflorescence, female flower number per inflorescence, number of ovules per flower, number of locules per flower, number of pollen grains per flower, stigmatic area, pollen–ovule ratio per inflorescence) were coded as ordered characters using simple gap coding (Almeida and Bisby 1984). Simple gap coding consists in plotting the frequency curve of a character and visually determining discontinuities in the distribution by the presence of peaks, dips, or gaps (data were log-transformed for better visualization). The gap-coded characters were originally measured in material from 109 species (shown in Table S5) belonging to 81 genera, built on the matrices of Chouteau et al. (2008) and Gibernau et al. (2010). New data were added for *Calla palustris*, using five inflorescences harvested at three sites in the vicinity of Gerardmer (Retournemer, Belbriette, and Gerardmer, Vosges, France) in 2008 (Table S6). For genera with more than one species, data were averaged before log-transformation. Eight of the remaining characters were taken from Cusimano et al. (2011), namely flower sexuality (unisexual, bisexual), pollen exine surface (smooth, spinose, striate, reticulate/other), pollen size (small, medium, large, very large as defined in Table S3), spathe shape (as defined in Table S3), pollen starch (present, absent), spadix zonation (as defined in Table S3), appendix (present, absent), and five from Mayo et al. (1997),

namely life cycle (seasonally dormant, evergreen), inflorescence position (as defined in Table S3), geographic distribution (temperate zone, tropics), pollen strands (present, absent), sterile flowers type (absent, fleshy, elongated).

### TESTS FOR CORRELATED EVOLUTION

Ancestral state reconstructions and tests for trait-correlated evolution were performed on the ML tree resulting from the combined nuclear and plastid data, but with species lacking relevant data pruned so that we only kept either those 81 of the 135 species for which we had floral traits and/or pollination modes, or the 57 for which we had floral traits and pollination modes. Parsimony and ML ancestral state reconstructions were performed in Mesquite 2.75 (Maddison and Maddison 2011), the latter relying on the Mk model, where the M stands for Markov and k refers to the number of states observed; Lewis 2001). This model assumes that forward and backward changes are equally likely,

We tested for character evolution correlations by running a "Character association (correlation)" analysis in SIMMAP 1.5 (Bollback 2006, http://www.simmap.com). This test is based on numerous stochastic mapping reconstructions on a tree. The strength of the association is determined by comparing the observed associations of the two character states to the expected associations obtained under the hypothesis that the characters are independent, which is here the probability of being in the first state multiplied by the probability of being in the second state. This difference is quantified using a statistic (d for each pairs of states, D for the pairs of characters). The significance of the differences between the observed and expected associations is then assessed by comparison with data randomly simulated so that there is genuinely no correlation between the traits (predictive sampling; Huelsenbeck et al. 2003; Bollback 2006). Before running simulations, posterior distributions of the rates and bias prior parameters for each character were estimated in SIMMAP 1.5, following the Markov chain Monte Carlo (MCMC) approach described in the manual and using the default settings. A thousand samples were performed to obtain the statistic D, and 500 predictive samples to assess its significance.

## *Results*

### NUCLEAR PhyC PHYLOGENY OF THE ARACEAE

The trees from the nuclear sequence data and from the combined nuclear and plastid data yielded well-resolved phylogenies (Figs. 2, S2). The main difference from the most recent plastid-only Araceae phylogeny is that the monotypic genus *Calla* (which has bisexual flowers) is placed with *Montrichardia* and *Anubias*, whereas this genus was embedded among more derived Aroideae (which have unisexual flowers) in the tree of Nauheimer et al. (2012b). Neither placement of *Calla* has statistical support. Another difference was that *Lasimorpha* grouped with *Urospatha* (with a bootstrap support of 100), whereas in the plastid-only tree it was in a polytomy with other Lasioideae.

### RECONSTRUCTION OF POLLINATION MODES ON THE COMBINED NUCLEAR/PLASTID PHYLOGENY

Araceae pollinators and pollination interaction types (Table S2) are distributed on the phylogeny as shown in Fig. 3 (ML reconstruction) and S3 (parsimony reconstruction). Inferences were similar regardless of whether the 81-taxon tree or the 57-taxon tree (see Materials and Methods) were used. With the sparse current knowledge of pollinators and incomplete species sampling near the base of the family phylogeny, an ancestral pollinator cannot be inferred.

The ancestral interaction type was ambiguous in the ML analysis (Fig. 3, right), but was reconstructed as "rewarding" under parsimony reconstruction (Fig. S3, right). The outgroup, Tofieldiaceae, offers nectar in its flowers and based on the small size of its flowers, their white color, and their open shape, is pollinated by bees or flies, although no observations have been published. The early-diverging genus *Symplocarpus* offers (small amounts of) liquid rewards to its pollinators. A reward-based pollination by bees was inferred for the next diverging subfamilies Pothoideae and Monsteroideae (subfamily names can be seen in Fig. 2), with the exception of *Scindapsus*, which is pollinated by ovipositing flies. In the next diverging clade, some species are fly pollinated (*Dracontioides* and *Dracontium*), others beetle pollinated (*Urospatha*, *Lasimorpha*), but the types of interaction (whether rewarding or deceptive) are not known. There appear to have been numerous switches between pollination by beetles and flies (Fig. 3, S3, left). Finally, deception evolved at least five times (Fig. 3 and S3, right), in two cases associated mostly with beetles (*Stylochaeton* and *Amorphophallus*), and in three mostly with flies (Cryptocoryneae, *Arisarum*, Areae).

### INFLORESCENCE TRAITS AND INTERACTION TYPES

Because our correlation analyses were exploratory rather than hypothesis testing, we report all correlations, not just significant ones. Of the 21 studied traits, all but three (pollen strands, ovules per flower, locules per flower) were correlated with each other (either positively or negatively), with pollinators (flies, beetles, bees), or with interaction types (Tables 1, 2; full results see Table S7). The strongest correlations involved spadix zonation, flower sexuality, or pollination type (highest *D* values in Table 1), and 11 of the 33 significant correlations, even if weak, involved flower sex, that is, trait changes were linked to the transition from bisexual to unisexual flowers (Table 1).

**Table 1.** ***D*** **statistics (upper part of the table) and** ***P*** **values (lower part of the table) from a character association test applied to the 21 floral traits listed in Tables S3 and S4.** ***P*****-values above the 0.05 significance threshold are highlighted in gray (lower part) and in bold.** ***D*** **statistics (with highlighting increasing in intensity with the strength of the correlations) from 0.2 to 0.4, 0.4–0.6, and 0.6–0.8.**

| D statistic / p-value | Pollination type | Pollinator order | Life cycle | Inflorescence position | Flower sexuality | Pollen exine surface | Distribution | Spathe shape | Pollen starch | Mean pollen size | Spadix zonation | Pollen strands | Ovules per flower | Locules per flower | # of male flowers | # of females flowers | Pollen per flower | Pollen/ovule ratio | Stigma area | Sterile flower type | Spadix appendix |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pollination type | – | 0.48 | 0.08 | 0.30 | 0.42 | 0.41 | 0.21 | 0.38 | 0.31 | 0.24 | 0.70 | 0.09 | 0.29 | 0.25 | 0.29 | 0.27 | 0.33 | 0.23 | 0.18 | 0.42 | 0.41 |
| Pollinator order | 0.01 | – | 0.08 | 0.26 | 0.29 | 0.33 | 0.19 | 0.30 | 0.20 | 0.21 | 0.56 | 0.08 | 0.25 | 0.26 | 0.25 | 0.24 | 0.26 | 0.19 | 0.17 | 0.27 | 0.28 |
| Life cycle | 0.32 | 0.71 | – | 0.06 | 0.05 | 0.08 | 0.04 | 0.07 | 0.05 | 0.06 | 0.10 | 0.03 | 0.08 | 0.07 | 0.08 | 0.08 | 0.08 | 0.07 | 0.06 | 0.07 | 0.04 |
| Inflorescence position | 0.29 | 0.38 | 0.83 | – | 0.27 | 0.20 | 0.13 | 0.24 | 0.15 | 0.16 | 0.29 | 0.05 | 0.19 | 0.16 | 0.21 | 0.18 | 0.21 | 0.15 | 0.11 | 0.25 | 0.16 |
| Flower sexuality | $<10^{-2}$ | 0.03 | 0.05 | 0.04 | – | 0.29 | 0.18 | 0.57 | 0.55 | 0.23 | 0.72 | 0.05 | 0.17 | 0.21 | 0.24 | 0.20 | 0.33 | 0.17 | 0.11 | 0.45 | 0.30 |
| Pollen exine surface | 0.03 | 0.21 | 0.82 | 0.86 | 0.02 | – | 0.18 | 0.31 | 0.23 | 0.20 | 0.53 | 0.08 | 0.31 | 0.22 | 0.26 | 0.23 | 0.29 | 0.24 | 0.17 | 0.28 | 0.38 |
| Distribution | 0.16 | 0.22 | 0.65 | 0.45 | 0.04 | 0.24 | – | 0.16 | 0.12 | 0.12 | 0.23 | 0.04 | 0.12 | 0.12 | 0.15 | 0.12 | 0.13 | 0.09 | 0.09 | 0.15 | 0.13 |
| Spathe shape | 0.19 | 0.47 | 0.90 | 0.62 | $<10^{-3}$ | 0.35 | 0.40 | – | 0.38 | 0.23 | 0.56 | 0.07 | 0.24 | 0.21 | 0.26 | 0.22 | 0.32 | 0.21 | 0.14 | 0.35 | 0.26 |
| Pollen starch | 0.05 | 0.22 | 0.30 | 0.37 | $<10^{-3}$ | 0.12 | 0.19 | 0.03 | – | 0.20 | 0.49 | 0.05 | 0.15 | 0.17 | 0.20 | 0.16 | 0.28 | 0.14 | 0.10 | 0.41 | 0.26 |
| Mean pollen size | 0.53 | 0.71 | 0.74 | 0.80 | 0.05 | 0.79 | 0.50 | 0.52 | 0.16 | – | 0.30 | 0.06 | 0.20 | 0.20 | 0.21 | 0.19 | 0.20 | 0.14 | 0.12 | 0.18 | 0.17 |
| Spadix zonation | $<10^{-3}$ | $<10^{-2}$ | 0.19 | 0.43 | $<10^{-3}$ | 0.00 | 0.24 | 0.03 | $<10^{-3}$ | 0.38 | – | 0.10 | 0.37 | 0.39 | 0.34 | 0.34 | 0.43 | 0.30 | 0.21 | 0.51 | 0.53 |
| Pollen strands | 0.31 | 0.53 | 0.96 | 0.93 | 0.07 | 0.78 | 0.66 | 0.86 | 0.33 | 0.76 | 0.17 | – | 0.08 | 0.07 | 0.08 | 0.08 | 0.08 | 0.07 | 0.06 | 0.07 | 0.04 |
| Ovules per flower | 0.32 | 0.69 | 0.76 | 0.78 | 0.14 | 0.16 | 0.59 | 0.72 | 0.39 | 0.56 | 0.12 | 0.86 | – | 0.25 | 0.23 | 0.23 | 0.25 | 0.23 | 0.16 | 0.23 | 0.19 |
| Locules per flower | 0.61 | 0.40 | 0.92 | 0.95 | 0.06 | 0.84 | 0.54 | 0.94 | 0.28 | 0.57 | 0.07 | 0.92 | 0.32 | – | 0.21 | 0.20 | 0.22 | 0.16 | 0.14 | 0.19 | 0.19 |
| # of male flowers | 0.42 | 0.73 | 0.82 | 0.71 | 0.04 | 0.65 | 0.40 | 0.66 | 0.18 | 0.51 | 0.26 | 0.85 | 0.79 | 0.86 | – | 0.23 | 0.26 | 0.19 | 0.16 | 0.25 | 0.20 |
| # of females flowers | 0.29 | 0.52 | 0.72 | 0.67 | 0.04 | 0.58 | 0.55 | 0.66 | 0.23 | 0.44 | 0.11 | 0.76 | 0.47 | 0.73 | 0.54 | – | 0.26 | 0.18 | 0.15 | 0.20 | 0.21 |
| Pollen per flower | 0.17 | 0.62 | 0.76 | 0.62 | 0.01 | 0.31 | 0.60 | 0.23 | 0.03 | 0.68 | 0.03 | 0.75 | 0.55 | 0.67 | 0.47 | 0.29 | – | 0.21 | 0.16 | 0.31 | 0.24 |
| Pollen/ovule ratio | 0.12 | 0.44 | 0.57 | 0.49 | 0.01 | 0.08 | 0.52 | 0.22 | 0.08 | 0.49 | 0.03 | 0.52 | 0.09 | 0.69 | 0.44 | 0.38 | 0.16 | – | 0.14 | 0.18 | 0.28 |
| Stigma area | 0.13 | 0.35 | 0.77 | 0.84 | 0.03 | 0.36 | 0.34 | 0.78 | 0.23 | 0.52 | 0.08 | 0.69 | 0.64 | 0.80 | 0.60 | 0.55 | 0.54 | 0.43 | – | 0.15 | 0.18 |
| Sterile flower type | 0.04 | 0.35 | 0.61 | 0.29 | 0.00 | 0.25 | 0.25 | 0.13 | 0.01 | 0.60 | 0.01 | 0.46 | 0.38 | 0.69 | 0.34 | 0.38 | 0.10 | 0.14 | 0.14 | – | 0.25 |
| Spadix appendix | 0.02 | 0.04 | 0.19 | 0.26 | 0.01 | 0.00 | 0.16 | 0.07 | 0.03 | 0.20 | 0.00 | 0.25 | 0.22 | 0.15 | 0.23 | 0.19 | 0.16 | 0.07 | 0.31 | 0.05 | – |

Fly pollination was positively correlated with ovipositing mutualisms ($P = 0.022$; Table 1) and negatively with mating mutualisms ($P = 0.002$), whereas beetle pollination was positively correlated with mating mutualisms ($P = 0.006$) and negatively with ovipositing ($P = 0.028$). Bee pollination is correlated with bisexual flowers ($P = 0.002$) and spadices without a zonation (as illustrated in Fig. 1A–C; $P = 0.016$); fly pollination is correlated with the presence of a spadix appendix (as illustrated in Fig. 1K, L; $P < 10^{-3}$); and beetle pollination with unisexual flowers ($P = 0.018$) arranged in two-zoned spadices ($P = 0.008$). Several qualitative floral traits were significantly correlated with pollination interaction type. Thus, rewarding mutualisms were positively correlated with simple spadices (no zonation, $P = 0.004$; see Fig. S4 and S5 for an illustration of the evolution of spadix zonation and pollination type), absence of a spadix appendix ($P = 0.028$), bisexual flowers ($P < 0.001$), reticulate pollen ($P = 0.038$), and the absence of starch in the pollen ($P = 0.012$). Mating mutualism was positively correlated with unisexual flowers ($P = 0.01$), both types of zoned spadices ($P = 0.002$ and 0.018), absence of an appendix ($P = 0.016$), and fleshy sterile flowers ($P = 0.008$). Oviposition mutualisms were also positively correlated with zoned spadices ($P = 0.022$) and long appendices ($P = 0.042$). Deception was positively correlated with zoned spadices ($P = 0.004$), a spadix appendix ($P = 0.026$), unisexual flowers ($p = 0.054$), spiny pollen ($P = 0.012$), and elongated sterile flowers ($P = 0.034$). There were no correlations between pollen type and pollinator order.

## Discussion

Our main questions concerned the evolutionary distribution of mutualistic and antagonistic (deceptive) pollination interactions and whether inflorescence traits are correlated with these interaction types. We asked these questions because next to Orchidaceae, Araceae are the angiosperm family with the greatest number of antagonistic pollination interactions (*Introduction*). For at least two groups of Orchidaceae, recent work has shown that evolutionary transitions between deceptive and rewarding pollination systems are frequent, although not necessarily in the direction expected from theoretical work on the invasion of mutualisms by cheaters (Bronstein 2001). Thus, in the large orchid genus *Disa*, deception was the ancestral condition from which nectar production evolved at least nine times (Johnson et al. 2013). However, Orchidaceae differ fundamentally from Araceae in relying on single flowers, not inflorescences, as the attraction unit for pollinators, which may make mutualism/antagonism switches easier because less structural change may be required (no spadices and spathes need to be modified). Based on the first nuclear/plastid DNA phylogeny for the Araceae and all available data on pollinators and interaction types (Table S2), a rewarding mutualism may have been ancestral

**Table 2.** Significant correlations (statistic *D* [P-value]) among Araceae pollination types, pollinator orders, and floral traits. Negative values of *D* indicate a negative correlation between trait states; positive values indicate a positive correlation. ns = nonsignificant correlations.

| | | Pollination type | | | |
|---|---|---|---|---|---|
| | | Rewarding mutualism | Mating mutualism | Oviposition mutualism | Deception |
| Pollinator order | Fly | n.s. | −0.064 (0.002) | 0.05 (0.022) | n.s. |
| $D = 0.483$ $P = 0.01$ | Beetle | n.s. | 0.085 (0.006) | −0.036 (0.028) | n.s. |
| | Bee | n.s. | n.s. | n.s. | n.s. |
| | Fly and beetle | n.s. | n.s. | n.s. | n.s. |
| Flower sexuality | Bisexual flowers | 0.09 ($<10^{-3}$) | −0.053 (0.01) | n.s. | n.s. |
| $D = 0.420$ $P = 0.004$ | Unisexual flowers | −0.09 ($<10^{-3}$) | 0.053 (0.01) | n.s. | n.s. |
| Pollen exine surface | Reticulate/other | 0.028 (0.038) | n.s. | n.s. | −0.038 (0.024) |
| $D = 0.409$ $P = 0.03$ | Smooth | n.s. | n.s. | n.s. | n.s. |
| | Spinose | n.s. | −0.045 (0.022) | n.s. | 0.051 (0.012) |
| | Striate | n.s. | n.s. | n.s. | n.s. |
| Pollen starch | Absent | n.s. | n.s. | n.s. | 0.062 (0.012) |
| $D = 0.309$ $P = 0.05$ | Present | n.s. | n.s. | n.s. | −0.062 (0.012) |
| Spadix zonation | No zonation | 0.078 (0.004) | −0.044 (0.02) | n.s. | n.s. |
| $D = 0.704$ $P < 10^{-3}$ | Female/male | n.s. | 0.066 (0.002) | n.s. | −0.026 (0.048) |
| | Female/sterile/male | n.s. | 0.057 (0.018) | n.s. | n.s. |
| | Female/male/sterile | n.s. | n.s. | n.s. | n.s. |
| | Female/sterile/male/sterile | −0.039 (0.016) | −0.072 ($<10^{-3}$) | 0.043 (0.022) | 0.067 (0.004) |
| Sterile flower type | No sterile flower | 0.055 (0.006) | n.s. | n.s. | n.s. |
| $D = 0.423$ $P = 0.036$ | Fleshy sterile flowers | −0.05 (0.01) | 0.057 (0.008) | n.s. | −0.037 (0.042) |
| | Elongated sterile flowers | n.s. | n.s. | n.s. | 0.045 (0.034) |
| Spadix appendix | Absent/inconspicuous | 0.038 (0.028) | 0.064 (0.016) | −0.042 (0.026) | −0.06 (0.026) |
| $D = 0.410$ $P = 0.018$ | Well developed | −0.038 (0.028) | −0.064 (0.016) | 0.042 (0.026) | 0.06 (0.026) |
| | | Pollinator order | | | |
| | | Fly | Beetle | Bee | Fly and beetle |
| Flower sexuality | Bisexual flowers | n.s. | −0.04 (0.018) | 0.054 (0.002) | n.s. |
| $D = 0.288$ $P = 0.026$ | Unisexual flowers | n.s. | 0.04 (0.018) | −0.054 (0.002) | n.s. |
| Spadix zonation | No zonation | n.s. | −0.032 (0,036) | 0.045 (0.016) | n.s. |
| $D = 0.555$ $P = 0.002$ | Female/male | −0.048 ($<10^{-3}$) | 0.041 (0.014) | n.s. | n.s. |
| | Female/sterile/male | −0.027 (0,026) | 0.049 (0.008) | n.s. | n.s. |
| | Female/male/sterile | n.s. | n.s. | n.s. | n.s. |
| | Female/sterile/male/sterile | 0.075 ($<10^{-3}$) | −0.056 (0.004) | n.s. | n.s. |

in the family, although this received little support from ML inference. Mating and ovipositing mutualisms each evolved several times (Fig. 3, right), and changes in Araceae inflorescences were significantly associated with changes in pollination modes. Our analyses of trait correlations were exploratory (as we conducted all pair-wise combinations), rather than hypothesis testing, which is why we decided to report significant and nonsignificant results. In addition, we relied on the Mk model (Lewis 2001), which does not account for the characters themselves having an influence on clade diversification rates (Maddison et al. 2007). Even if all major clades are represented in our reconstruction, information on Araceae pollinators and their behavior on the inflorescences is too sparse to test for the effect of these traits on diversification rates, with information available for perhaps 200 of the family's c. 3300 described species and for 56 of the 120–132 genera (Table S2; Boyce and Croat 2013).

An earlier study inferred that Araceae were ancestrally beetle pollinated and that fly pollination evolved from beetle pollination (Sannier et al. 2009). In our reconstruction, fly pollination appears as early as beetle pollination, a finding probably due to the different and more numerous genera included compared to the study of Sannier et al. These authors (and Grayum 1986) also found a strong correlation between verrucate and tuberculate exines and beetle pollination, which we did not find, again because of our

larger taxon sampling, which appears to have "destroyed" earlier spurious correlations.

Most Araceae species belong in the unisexual flower clade (marked in Fig. 3, left), which comprises 1876 (57%) of the family's c. 3300 species (Boyce and Croat 2013). The evolution of unisexual flowers in turn permitted the separation of sexually specialized flowers along the spadix, the female flowers near its base, and the males toward the apex. This separation often coincides with the surrounding spathe becoming constricted in its middle (Fig. 1G, J, K). The basal part of the spathe then forms a chamber surrounding the spadix's female section (Fig. 1G–L), and sometimes also its male section (Fig. 1K, L), whereas its upper zone expands and forms a landing platform. Such inflorescences are characteristic of Araceae that flower for several hours to several days (Gibernau 2003) and that keep their pollinators within boat-shaped spathes or floral chambers (e.g., closed spathes). Within these floral chambers, active insects and receptive female flowers are close to each other, leading to pollination if insects carry pollen grains. The male flowers are above (or at the top of) the floral chamber, so that during the subsequent male phase, all the inflorescence's anthers release their pollen simultaneously onto the insects, just as flies or beetles are released from the trap's basal chamber due to wilting processes (Vogel and Martens 2000; Mori and Okada 2001; Maia et al. 2010). Where the spathe is constricted, the spadix can bear sterile flowers (Fig. 1G, H) that can prevent insects from leaving a deceptive inflorescence, or lack flowers (e.g., *Sauromatum hirsutum*; color photo in Cusimano et al. 2010). In our sampling, half of the 46 genera with unisexual flowers have a spadix apex consisting of functional male flowers (Fig. 1G, H), a trait associated with mating mutualism and beetle pollination. The remaining genera have elongated sterile spadix tips (Fig. 1I–L) associated with ovipositing mutualisms and deception (Fig. S3). Both types of apices often protrude from the spathe and serve as landing/taking-off rods for beetles and/or flies (Gibernau et al. 2004) or as scent- and warmth-emitting structures attracting and luring insect pollinators (Knoll 1926; Kite 1995; Seymour et al. 2003). Having landed on the appendage or spathe, insects then glide into the basal chamber, where some will deposit pollen on the receptive stigmas (Bröderbauer et al. 2012, 2013 for details on the morphology of trapping mechanisms).

The relative stability of deceptive pollination in Araceae (Fig. 3 and S3) may partly be an artifact of the few data available so far on their pollination (as compared to orchids). Thus, our understanding of transitions from brood site mutualisms involving beetles and flies that are trapped, fed, and released the next day to deceptive systems where insects are unable to oviposit or their larvae unable to develop in the inflorescences requires many more field observations. In the relatively well-studied *Colocasia* clade, *Alocasia*, *Colocasia*, and *Steudnera* are pollinated by species of *Colocasiomyia* (Diptera: Drosophilidae) that lay eggs on the spadices and whose larvae develop in the decomposing spadix tissues, but without damaging young seeds (Miyake and Yafuso 2005). The flies depend on these Araceae for oviposition, larval growth, pupation (in some species), and adult feeding and mating (Sultana et al. 2006). Fieldwork combined with phylogenies for these and other Araceae and their pollinators is required for a fuller understanding of evolutionary switches. This study lays a foundation by clarifying how the evolution of unisexual flowers and spadices covered by specialized spathes relates to pollination modes and shows that shifts between deceptive and food-rewarding pollination systems in Araceae are extremely rare compared to orchids, where nectar has been lost and revolved many times (Johnson et al. 2013). It is also clear from our comparative study that in Araceae the change from bisexual to unisexual flowers, their separation along an elongate spadix, and the spathe constriction were the key morphological changes that facilitated the evolution of trap-based interactions and, in some cases, deception.

## ACKNOWLEDGMENTS

For leaf material, we thank J. Bogner of the Munich Botanical Garden, D. Scherberich of the Lyon Botanical Garden, and G. Ferry and C. Denjean of the Nancy Botanical Garden; S. DeWitt Smith, University of Nebraska-Lincoln, is thanked for statistical advice; M. Silber, University of Munich, for support in the lab; D. Bröderbauer, University of Vienna, for discussing trap pollination; M. W. Pennell, University of Idaho, J. Ollerton, University of Northampton, and two anonymous reviewers for helpful comments on the manuscript; and F. Muller, Guatemala City, and D. Scherberich for photos. Financial support came from a European Synthesys grant to M. Gibernau and a travel grant from Paul Sabatier University of Toulouse (ATUPS) to M. Chartier.

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Associate Editor: L. Jesson

## *Supporting Information*

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Table S1.** Species names, their authors, herbarium vouchers, and GenBank accession numbers.
**Table S2.** The 125 Araceae genera currently recognized, the pollination interactions coded for this study, and supporting references.
**Table S3.** Character states used in the trait reconstruction.
**Table S4.** Twenty-one characters and their states coded for the 57 Araceae used in the trait reconstructions.
**Table S5.** List of the 109 species for which morphological measurements were obtained.
**Table S6.** Morphological measurements for *Calla palustris*.
**Table S7.** List of the complete correlated trait results obtained in SIMMAP.
**Figure S1.** Phytochrome C primer positions.
**Figure S2.** Maximum likelihood phylogeny of the Araceae based on a combined alignment of nuclear *PhyC* and six plastid genes, introns, and spacer regions from Nauheimer et al. (2012b) obtained from 135 taxa and 5342 nucleotides.
**Figure S3.** Parsimony reconstruction of pollinator order (left) and pollination modes (right) in Araceae.
**Figure S4.** Maximum likelihood reconstruction of spadix zonation (left) and pollination modes (right) in Araceae.
**Figure S5.** Parsimony reconstruction of spadix zonation (left) and pollination modes (right) in Araceae.