Recent Advances Towards an Evolutionary Comprehension of Araceae Pollination

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Abstract—A correspondence between floral traits and pollinator types is found in Araceae. Hence different complexes of floral traits are associated with bee-, fly- and beetle-pollinated aroids. Using the method of non-metric multidimensional scaling (NMDS) bee-pollinated species appear to have very different floral traits from fly- and beetle-pollinated taxa, these two groups showing some overlapping. This imperfect discrimination between fly- and beetle-pollinated aroids may have several explanations which are discussed. Interestingly the species belonging to the Lasioideae and Orontioideae subfamilies are grouped together not in relation to fly- and bee- pollinated groups and may represent a generalist pollination system. Finally, floral traits of fly deceptive species appear to be characteristic of the fly-pollinated taxa and not clearly different from fly-mutualistic species.

Keywords—bee, beetle, co-adaptation, floral traits, fly, pollination syndrome.

In all pollination systems, pollinators visit flowers looking for a resource, which can be alimentary (stigmatic fluid, pollen, floral parts, etc.) or reproductive (mating and/or laying site). Flowers in return have developed adaptations to transform this pollinator behaviour (e.g., resource seeking) into a 'pollinating act', and thus ensure their reproduction.

In insect-pollinated taxa, it is assumed that floral traits have evolved in relation to the interaction between the flower and the 'most efficient' pollinator in order to increase the frequency of this interaction and thus of plant

reproductive success (Stebbins 1970; Cruden 2000; Fenster et al. 2004). The 'most efficient' pollinator will be the agent among the different flower visitors whose visits ensure a higher seed production due to its particular behaviour or its high frequency/abundance. In consequence, pollinators become selective agents of floral traits associated with attraction, but also of fertile/sexual parts of the flowers leading to adaptations that increase visits by efficient pollinators (Fenster et al. 2004).

Thus, in specialized pollination systems, we expect to find some kind of correlation or association between the most efficient pollinator and the floral traits associated with it. Consequently, the selective pressure of the different types of pollinators has led to pollination syndromes (reviewed in Fenster et al. 2004): adaptive floral character complexes resulting in different types of floral architecture adapted to particular groups of pollinators. On the contrary, in non-specialized pollination systems, no such association or correlation will be found as different pollinators are likely to exert different even opposite selective pressures on floral traits (Waser et al. 1996).

Araceae is a monocotyledon family mainly pollinated by insects and this type of interaction may lead to a process of specialization/adaptation of aroid inflorescences (Mayo et al. 1997; Gibernau 2003). Grayum (1986) was the first author to suggest the existence of such adaptive traits in relation to the pollinator in his palynological study. He grouped pollen grains into 5 groups according to their exine sculpturing: psilate, striate, verrucate(+tuberculate), foveolate (+reticulate), and spinose. He found that "*psilate pollen is intimately and almost exclusively associated with beetle pollination and spinose pollen is equally closely associated with fly pollination...Verrucate and tuberculate pollen in Araceae also seems to be fairly well correlated with beetle pollination....Striate and foveolate exines...being less extreme, might be adaptable to a wider range of vectors. Sculpturing, not size, is thus the overriding factor*". This first study showed clearly that some floral characters, here the exine sculpturing of pollen grains, may be adapted to the type of pollinator. In fact, most of the Araceae seem to have quite a specific or specialized pollination system which can be divided into three types: bee, beetle, and fly (Gibernau 2003).

The first type occurs in the Neotropics within the genera *Anthurium* Schott and *Spathiphyllum* Schott (Montalvo and Ackerman 1986; Schwerdtfeger et al. 2002), which are pollinated by male euglossine bees in a way very similar to orchids (Dressler 1982). The male euglossines visit the inflorescences to collect resin and/or odoriferous substances used in the building of their nests. In the neotropical genus *Monstera* Adanson, typically considered a bee-pollinated genus, the data are contradictory. Several species are described as pollinated by stingless bees *Trigona* Jurine, which collect stigmatic and gums (Madison 1977; Ramirez and Gomez 1978; Ramirez 1980) whereas *M. obliqua* has been found to be beetle-pollinated (Chouteau et al. 2007).

The second type of specialized system is beetle-pollination. Even if the different genera and families of beetles are implicated (review in Gibernau 2003),

the interaction is functionally the same. Pollinating beetles are attracted to the receptive inflorescences in which they mate and eat various floral parts (sterile flowers or tissue, pollen grains). This pollination system is widespread within the Araceae and present in five subfamilies: Orontiodieae, Pothoideae, Lasioideae, Philodendroideae, and Aroideae (Gibernau 2003).

The third type of specialized system is fly-pollination. This interaction is mainly represented by one Asian fly genus, *Colocasiomyia* de Meijere (Drosophilidae), which pollinates Asian genera from the subfamilies Monsteroideae, Schismatoglottideae, Philodendroideae, and Aroideae (Sultana et al. 2006). The notable exceptions are a chloropid fly which pollinates *Peltandra virginica* Rafinesque in North-America (Patt et al. 1995) and unidentified drosophilids in the African genus *Culcasia* Palisot de Beauvois (Knecht 1983). Here again, the interactions follow the same functional schema. Flies visit the receptive inflorescences in order to mate and females oviposit their eggs on the inflorescences surface. The adults feed on the stigmatic secretions and on pollen whereas larvae eat decomposing matter and rotting flowers such as the stamens after pollen release. The inflorescence becomes part of the reproductive cycle of the pollinator. Insects visit Araceae inflorescences not only for food rewards (nectar, pollen, or floral tissue) but also to meet sexual congeners, achieve copulations, and sometimes lay their eggs.

In some genera of Araceae; depending on the genus, generalist or deceit systems can be found. Generalist pollination systems are rare and only documented in two species, *Lysichiton camtschatcense* Schott (Tanaka 2004) and *Symplocarpus renifolius* Schott (Uemera et al. 1993), both belonging to the basal Orontioideae subfamily. In these cases, various insects, a few of them documented as efficient pollinators, can be found in low frequencies. One interesting point is that not a single aroid species is known to offer nectar as a reward (Schwerdtfeger et al. 2002). In the deceit pollination system, the inflorescence dupes the pollinators by mimicking its laying sites (faeces, mushrooms, dead animal, etc.). Hence, the insects visit the inflorescence in order to complete their reproductive cycle. Through this deceptive attraction, the insects perform pollination but without actually receiving any reward (e.g., *Arum* L., *Helicodiceros* K. Koch, *Amorphophallus* Decaisne). This pollination system has only been documented in the Araceae family for some genera of the Aroideae subfamily.

In a previous study of the Araceae family, it has been shown that some specific floral traits were associated with pollination by bees, beetles, and flies. However, the discrimination between the three types of pollination was not perfect (Chouteau et al. 2008). In order to further understand the relationship between aroid floral traits and the type of pollinator, the floral characters of new species were measured and a new discriminant analysis was performed. For example, we added *Monstera obliqua* Miquel, a beetle-pollinated species belonging to a bee-pollinated genus, to our analysis. Moreover, we investigated whether deceit pollination systems were characterized by unique floral traits.

More precisely, the purposes of this study were: 1) to see if the different types of pollination in the Araceae family are correlated with specific floral traits and 2) to see if the boundaries between different types of pollination are clearly delimited.

Material and Methods

This study was conducted on 22 species belonging to 19 genera of *Araceae* sampled from the living collections of the Botanical Garden of the Montet (Nancy, France) as well as from the field in Corsica, French Guiana, Japan, and Sarawak (Borneo, Malay) (Table 1). Data from 46 species belonging to 27 genera taken from Chouteau et al. (2008) were added to our new data set. Note that five genera are common to both sets of data. Overall, the new analysis included 68 species belonging to 41 genera.

The same floral traits which have proven to discriminate among the types of pollinators in Araceae were measured (Chouteau et al. 2008). In order to count the pollen grains, inflorescences were collected during the first day of their flowering cycle, before the pollen is released (e.g., lost) in order to be able to calculate the pollen/ovule ratio. Floral ratio can vary according to the pollinator, thus for each inflorescence, the total numbers of flowers (female and male) were counted. In some cases, the number of male flowers was estimated by counting the number of male flowers on a 5 mm slice cut in the middle of the male zone. The total number of stamens was obtained by multiplying the number of stamens on the slice by the total length of the male zone and dividing by 5 (for details see Chouteau et al. 2008). The mean number of stamens per flower was counted on 10 flowers from at least three separate inflorescences. Different types of pollinator have different level of pollination efficiency which may affect female flowers. The number of ovules per flower was estimated by counting the number of locules on 10 flowers and the number of ovules per locule on these 10 flowers for each inflorescence collected. Ovule number per inflorescence was obtained by multiplying the mean number of ovules per flower by the mean number of flowers per inflorescence bearing ovules.

To estimate the number of pollen grains per inflorescence, three stamens were dissolved individually in 300 µl of 95% sulphuric acid, for 3-5 days at 24° C. The solution was homogenized every day. The last day, 1 µl was collected and carefully placed on a microscope slide. The number of pollen grains was counted for three independent replicates of 1 µl. The number of pollen grains per stamen was obtained by multiplying the mean of the triplicate by 300 (for details see Chouteau et al. 2008). For two species *Aridarum nicolsonii* Bogner and *Piptospatha elongata* (Engl.) N.E.Br., no pollen grains were observed after the acid digestion, thus pollen grains per flower were directly counted from three fresh stamens squashed between microscope slides and cover glasses. Pollen grains per inflorescence were obtained by multiplying the mean number of pollen

Species	Location of collection	Sample number	Date of collection
Symplocarpus renifolius Schott	Katashina-mura Tone-gun, Japan	3	April 2007
Lysichiton camtchatcense Schott	Katashina-mura Tone-gun, Japan	3	April 2007
Monstera oblique Miquel	Petit Saut Dam (French Guiana)	8	June 2007
Philodendron acutatum Schott	Petit Saut Dam (French Guiana)	3	June 2007
Aglaonema commutatum Schott	Nancy Botanical Garden(France)	3	August 2007
Callopsis volkensis Engl.	Nancy Botanical Garden(France)	3	August 2007
Chlorospatha longipoda (K. Krause) Madison	Nancy Botanical Garden(France)	3	August 2007
Dracontioides descicens (Schott) Engl.	Nancy Botanical Garden(France)	3	August 2007
Nephthytis hallaei (Bogner) Bogner	Nancy Botanical Garden(France)	3	August 2007
Schismatoglottis neo-guineensis (Linden ex André) N. E. Br.	Nancy Botanical Garden(France)	3	August 2007
Scindapsus hederaceus Schott	Nancy Botanical Garden(France)	3	August 2007
Spathicarpa hastifolia Hook.	Nancy Botanical Garden(France)	3	August 2007
Ulearum sagittatum Engl.	Nancy Botanical Garden(France)	3	August 2007
Aridarum nicolsonii Bogner	Sarawak (Borneo, Malay)	9	December 2004
Dieffenbachia seguine Schott	Nouragues (French Guiana)	7	July 2006
Dieffenbachia paludicola N.E. Br. ex Gleason	Nouragues (French Guiana)	7	July 2006
Helicodiceros muscivorus (L.f.) Engl.	Corsica (France)	12	April 2004
Arum concinnatum Schott	Crete (Greece)	9	April 2007
Homalomena hostiifolia Engl.	Sarawak (Borneo, Malay)	3	December 2004
Homalomena sp.	Sarawak (Borneo, Malay)	3	December 2004
Piptospatha elongata (Engl.) N.E.Br.	Sarawak (Borneo, Malay)	3	December 2004
Piptospatha grabowski (Engl.) Engl.	Sarawak (Borneo, Malay)	3	December 2004

Table 1. List of species, locations and dates of collection, and sample numbers studied.

grains per flower by the mean number of flowers bearing pollen. In the same way, the pollen grain volume per inflorescence was obtained by multiplying the mean number of pollen grains per inflorescence by the mean pollen volume of the species concerned (see below).

The size of elongate pollen grains was estimated by measuring the diameter of the polar and equatorial axes of the grains. The volume of a single pollen grain was estimated using the formula $\pi PE^2/6$ (Harder 1998), where P is the polar axis and E the equatorial axis diameter. For globose pollen, the diameter D was measured and the volume calculated with the formula $(4/3)\pi(D/2)^3$. Generally, 10 pollen grains per inflorescence were measured from three independent inflorescences (generally $N = 30$).

The pollen-ovule ratio was calculated for the inflorescence by dividing the mean number of pollen grains per inflorescence by the mean number of ovules per inflorescence.

For each inflorescence studied, the stigma area (estimated as a circle) of 10 flowers was calculated using the diameter (0.01mm resolution) of the stigmas measured at 20× magnification under a dissecting microscope equipped with an ocular micrometer and using the formula $\pi D^2/4$ where D is the measured diameter. To obtain the total stigmatic area of the inflorescences, the mean stigma area was multiplied by the mean number of flowers bearing stigmas for each species. Life form, growth mode, and climatic region were obtained using Mayo et al. (1997) and from personal observations (for details see Chouteau et al. 2008).

The discriminant analysis was conducted for three types of pollinating insects (grouping variable) – bee, beetle and fly – according to the data available in the literature (see Gibernau 2003 for a review). Twenty-three species were coded as beetle-pollinated, 21 as fly-pollinated, seven as bee-pollinated, and 17 as unknown. The 17 floral traits (variables) available for all species were selected in order to test any discrimination among the three pollinator groups: stigma area per flower and per inflorescence, mean volume of a pollen grain, pollen volume per flower and per inflorescence, pollen number per flower and per inflorescence, number of locules per female flower, number of ovules per locule, per flower and per inflorescence, pollen-ovule ratio, number of male and female flowers, sexual type of the flower, growth mode, and life form. A preliminary step consists in performing a stepwise backward discriminant analysis which allowed us to reduce the number of discriminant floral traits (Systat 8.0). The results of the discriminant analysis are not totally reliable because both categorical and continuous data were used. Consequently, differences among the six floral traits (e.g., resulting from the discriminant analysis) were represented with the method of non-metric multidimensional scaling (NMDS) using the Gower similarity measure which allows using both categorical and continuous data (PAST 1.74). This non parametric method represents the studied species in a two-dimensional coordinate system preserving the ranked differences between the species. The more two points are separated in the score plot, the more dis-

tinct the floral traits are. The stress value gives the percentage of difference not optimally represented by the analysis. In order to visualize the type of pollinator, each species has been coded by a coloured symbol representing the different types of pollinator. Differences in floral traits between the different types of pollinators were tested using ANOVA (Systat 8.0).

Results

Table 2 summarizes the floral traits, life form, growth mode, and pollinator type for the 22 newly studied aroid species.

The stepwise backward discriminant analysis of the 68 species held back six variables, even if some other variables showed significant differences between pollinator types (Table 3): pollen volume per inflorescence, pollen number per inflorescence, number of female and male flowers, sexual type of the flower, and growth mode. The NMDS plotting distinguishes the three pollinator groups although some overlapping occurs (see Fig. 1) and some species were misclassified (see below). The bee-pollinated group is characterized by species with bisexual flowers, an evergreen life form type, and a high number of male and female flowers (Table 3). Beetle-pollinated species are characterized by a high pollen volume per inflorescence, a medium number of male and female flowers, and almost always bearing unisexual flowers (see Table 3). Fly pollination is associated with species with low numbers of male and female flowers and a relatively low number of pollen grains per inflorescence (Table 3). The P/O was much higher in beetle- (mean: 44,004) and fly-pollinated species (mean: 32,511) than in bee-pollinated species (mean: 10,605), but these differences were not significant (Table 3). Pollen grain volume in relation to pollinator class displayed the same kind of difference, with pollen volume of beetle-pollinated species being significantly larger (mean: 419,828 μm³) than related fly- (mean: 28,903 μm³) and bee-pollinated (mean: 15,145 μm³) species (Table 3). In the same way, the flower stigma surface was significantly larger in beetle-pollinated species (mean: 3.85 mm²) than in fly- (mean: 0.90 mm²) or bee-pollinated (mean: 0.64 mm²) taxa (Table 3).

Now we shall consider species classification according to pollinator type since some species had been misclassified – in fact, 12 out of 51. Four beetle-pollinated species were classified among fly-pollinated species, namely: *Anubias barteri* Engl. and *A. heterophylla* Engl., *Caladium bicolor* (Ait.) Vent. and *Xanthosoma conspurcatum* Schott, and two among bee-pollinated taxa, *Monstera obliqua* and *Rhaphidiphora schottii* Hook. f. (Fig. 1). Inversely, *Alocasia amazonica* Andre., *A. portei* Becc. & Engl., *A. macrorrhizos* (L.) G.Don, *Aglaonema commutatum* Schott, *Schismatoglottis neo-guineensis* (Linden ex André) N. E. Br., and *Homalomena* sp. fly-pollinated species were classified among beetle-pollinated species (Fig. 1).

The fly-pollinated *Dracontium polyphyllum* L. appears close to *Anaphyllopsis americana* (Engl.) A. Hay and *Dracontioides descicens* (Schott) Engl., two 'unknown taxa'. These species are clearly not related to fly- and bee-pollinated groups

Species	Life form	Growth mode	Pollinator	Stigma area (mm²)	Pollen grains volume (μm³)	Number of male flowers per inflorescence	Number of female flowers per inflorescence	Number of pollen grains per flower	Number of pollen grains per inflorescence	Number of ovules per flower	Number of ovules per inflorescence	Ratio pollen / ovule per inflorescence
Symplocarpus renifolius	S	T	fly	0.8 ± 0.1	75,584 ± 17,155	145 ± 18	145 ± 18	108,667 ± 78,630	14,620,667 ± 8,594,082	1.0 ± 0.0	145 ± 18	108,667 ± 78,630
Lysichiton camtschatcense	S	H	fly	0.06 ± 0.01	161,540 ± 8,759	236 ± 118	236 ± 118	23,156 ± 8,365	4,798,578 ± 1,319,381	2.0 ± 0.0	471 ± 237	11,578 ± 4,483
Monstera obliqua	E	HE	beetle	0.3 ± 0.2	177,594 ± 73,014	56 ± 10	56 ± 10	26,850 ± 7,522	1,528,067 ± 536,866	4.0 ± 0.0	226 ± 38	6,713 ± 1,881
Philodendron acutatum	E	HE	beetle	1.4 ± 0.4	319,477 ± 67,866	2,982 ± 92	746 ± 4	8,910 ± 7,623	25,166,460 ± 23,404,527	62.8 ± 3.6	46,851 ± 2,456	525 ± 472
Aglaonema commutatum	E	T	fly	0.03 ± 0.01	91,774 ± 7,492	181 ± 15	13 ± 7	31,175 ± 13,965	5,895,750 ± 3,159,736	1.0 ± 0.0	13 ± 7	430,252 ± 349,413
Callopsis volkensis	E	G		0.13 ± 0.02	34,306 ± 8,431	121 ± 1	11 ± 1	1,356 ± 317	164,136 ± 39,191	1.0 ± 0.0	11 ± 1	15,222 ± 4,968
Chlorospatha longipoda	E	T	beetle	0.002 ± 0.001	12,287 ± 3,186	64 ± 4	33 ± 3	2,600 ± 521	165,557 ± 28,892	19.5 ± 3.5	632 ± 81	269 ± 80
Dracontioides desciens	S	H		0.16 ± 0.04	4,797 ± 2,307	141 ± 4	141 ± 4	153,500 ± 13,763	21,620,766 ± 2,468,006	1.9 ± 0.1	272 ± 9	79,583 ± 11,912
Nephthytis hallaei	E	G		0.19 ± 0.03	30,346 ± 10,570	58 ± 12	9 ± 3	1,089 ± 504	58,903 ± 13,916	1.0 ± 0.0	9 ± 3	6,963 ± 2,591
Schismatoglottis neoguineensis	E	T	fly	0.07 ± 0.02	2,212 ± 1,029	198 ± 3	277 ± 48	59,490 ± 9,666	11,808,060 ± 2,104,416	21.6 ± 1.0	5,942 ± 775	2,041 ± 640
Scindapsus hederaceus	E	HE		0.004 ± 0.001	121,876 ± 8,295	76 ± 3	76 ± 3	54,400 ± 15,546	4,132,233 ± 1,105,668	1.0 ± 0.0	76 ± 3	54,400 ± 15,546
Spathicarpa hastifolia	S	G		0.07 ± 0.01	12,286 ± 3,185	12 ± 3	17 ± 2	4,793 ± 2,387	58,593 ± 37,755	1.0 ± 0.0	17 ± 2	3,610 ± 2,644
Ulearum sagittatum	E	G		0.28 ± 0.09	17,121 ± 2,824	48 ± 5	22 ± 1	480 ± 480	19,583 ± 5,916	1.0 ± 0.0	22 ± 1	450 ± 163
Dieffenbachia seguine	E	H	beetle	4.47 ± 0.4	2,509,040 ± 418,007	235 ± 26	46 ± 8	6,760 ± 2,056	1,531,040 ± 558,747	1.97 ± 0.08	90 ± 18	18,444 ± 5,938
Dieffenbachia paludicola	E	H	beetle	21.01 ± 6.02	4,462,440 ± 341,046	169 ± 18	10 ± 1	2,703 ± 1,787	497,366 ± 341,956	7.17 ± 1.63	69 ± 18	6,223 ± 3,532
Helicodiceros muscivorus	S	G	fly	0.02 ± 0.01	33,459 ± 14,424	261 ± 46	158 ± 28	10,446 ± 1,899	2,700,942 ± 559,726	4.5 ± 0.7	726 ± 212	3,944 ± 1,066
Aridarum nicolsonii	E	R	fly	0.99 ± 0.29	4,849*	353 ± 113	98 ± 17	20 ± 10	6,724 ± 3,003	14.5 ± 2.7	1,385 ± 372	5 ± 2
Arum concinnatum	S	G	fly	0.21 ± 0.06	17,157*	114 ± 26	57 ± 14	2,588 ± 2,835	302,870 ± 359,358	7.4 ± 3.3	437 ± 237	755 ± 831
Homalomena hostifolia	E	T	beetle	2.43 ± 0.30	3,054*	210 ± 29	145 ± 27	7,850 ± 4,621	1,716,310 ± 1,111,200	255.6 ± 50.9	37,942 ± 14,934	43 ± 24
Homalomena sp	E	T	fly	3.21 ± 0.38	3,054*	289 ± 45	169 ± 19	37,461 ± 17,081	10,474,250 ± 4,154,627	207.7 ± 34.6	35,327 ± 9,359	300 ± 117
Piptospatha elongata	E	R	fly	1.11 ± 0.05	8,181*	233 ± 53	129 ± 20	58 ± 24	13,471 ± 7,153	19.4 ± 3.0	2,555 ± 744	5 ± 2
Piptospatha grabowski	E	R	fly	2.42 ± 0.43	8,181*	317 ± 97	110 ± 25	15,778 ± 14,879	4,071,111 ± 2,747,889	44.8 ± 3.3	4,969 ± 1,388	970 ± 925

Table 2. Life form, growth mode, pollinator, and floral traits measured for 22 aroid species in 19 genera. Life form was coded: E = Evergreen, S = Seasonally dormant. Growth mode was coded: T = Terrestrial, H = Helophyte, G = Geophyte, E = epiphyte, HE = Hemiepiphyte, FF = Free floating.

(Fig. 1). Interestingly, these three species belong to the Lasioideae subfamily and are grouped with *Lysichiton camtschatcense* and *Symplocarpus renifolius* two examples of a generalist pollination system. The other species with unknown pollinators were tentatively classified as follows: *Stenospermation sessile* Engl., *S. longipetiolatum* Engl., and *Scindapsus hederaceus* Schott may be bee-pollinated. *Homalomena philippinensis* Engl. and *H. rubescens* Kunth may be among the beetle-pollinated species. *Pistia stratiotes* L. is rather fly-pollinated, but its outstanding position may suggest an original unknown pollination system (Fig. 1). All the other taxa with unknown pollinators, *Synandrospadix vermitoxicus* Engl., *Pseudodracontium fallax* Serebr., *Gonatopus boivinii* (Decne) Engl., *G. angustus* N.E. Br., *Zamioculcas zamiifolia* (Loddiges) Engl., *Callopsis volkensis* Engl., *Nephthytis hallaei* (Bogner) Bogner, *Spathicarpa hastifolia* Hook., and *Ulearum sagittatum* Engl. were

Floral character	Beetle pollination (N = 23)	Fly pollination (N = 21)	Bee pollination (N = 7)	Statistic values $F_{2,48}$ –
Flower sexual type [1]	1.91 ± 0.29	1.85 ± 0.36	1 ± 0	26.08***
Growth mode [2]	3.04 ± 1.06	1.86 ± 0.85	4 ± 1	15.52***
Pollen volume per inflorescence	$14.6 ± 20.6 \times 10^{11}$	$1.84 ± 2.95 \times 10^{11}$	$2.22 ± 2.73 \times 10^{11}$	5.06*
Pollen number per inflorescence	$11.6 ± 21.2 \times 10^{6}$	$5.60 ± 5.55 \times 10^{6}$	$23.97 ± 29.84 \times 10^{6}$	2.72
Male flower number	496.5 ± 650.5	210.5 ± 123.6	710.6 ± 881.2	2.75
Female flower number	254.6 ± 333.8	119.2 ± 69.5	853.4 ± 849.7	9.97***
Life form [3]	1.09 ± 0.29	1.57 ± 0.51	1 ± 0	10.98***
Flower stigma area	3.85 ± 5.55	0.90 ± 1.01	0.64 ± 0.23	3.95*
Mean pollen grain volume	$420 ± 1,022 \times 10^{3}$	$28.9 ± 38.4 \times 10^{3}$	$15.1 ± 17.1 \times 10^{3}$	2.04
Ovule number per flower	22.81 ± 53.11	22.83 ± 45.3	5.28 ± 3.54	0.43
Nb of locules per flower	2.96 ± 2.75	1.61 ± 1.06	2.43 ± 0.53	2.51
Nb of ovules per locule	7.39 ± 17.61	13.87 ± 19.79	2 ± 1	1.46
Pollen-ovule ratio	44,004 ± 90,220	32,511 ± 94,616	10,605 ± 7,131	0.41

1 The flower sexual type was coded: 1 = bisexual, 2 = unisexual.

2 The growth mode was coded: 1 = geophyte, 2 = helophyte, 3 = ground, 4 = hemiepiphyte, 5 = epiphyte.

3 The life form was coded: 1 = evergreen, 2 = seasonally dormant.

Table 3. Group means (± standard deviation) used in the discriminant analysis for the different floral characters according to type of pollinator in 51 species of Araceae. The first six variables were selected by the discriminant analysis. Note that some variables showing statistically significant differences were not included in the analysis. The level of significance of the ANOVA results is coded as follows: * P < 0.05, ** P < 0.01, *** P < 0.001.
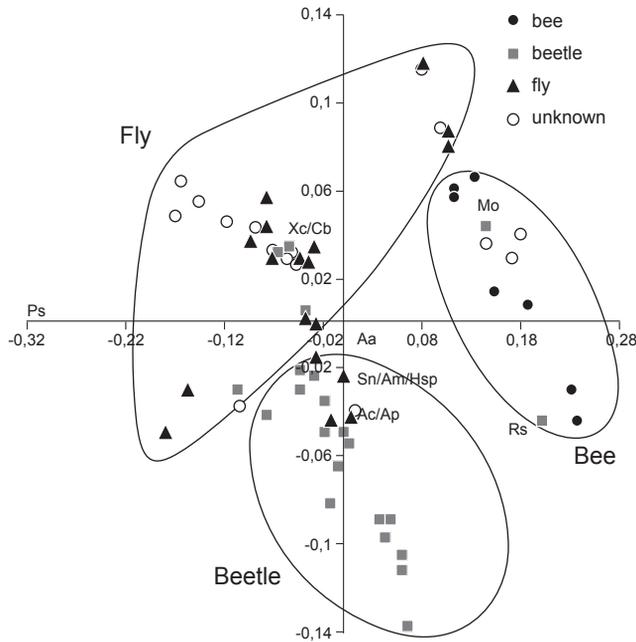
Fig. 1. Non-metric multidimensional scaling (NMDS) representation of the six selected floral traits (see results section) comprising overall data (48 genera, 68 species). Stress value = 0.12. The Letter Codes are 'misclassified' species which floral characters do not correspond to their type of pollinator. Among fly-pollinated species Xc: *Xanthosoma conspurcatum*, Cb: *Caladium bicolor*, Ab: *Anubias barteri* and Ah: *Anubias heterophylla*. Among bee-pollinated species Mo: *Monstera oblique* and Rs: *Rhaphidophora schottii*. Among beetle-pollinate species: Aa: *Alocasia amazonica*, Sn: *Schismatoglottis neo-guineensis*, Am: *Alocasia macrrorhizos*, Hsp: *Homalomena* sp., Ac: *Aglaonema commutatum* and Ap: *Alocasia portei*. Outstanding species on the left Ps: *Pistia stratiotes*.

clearly classified as fly-pollinated (Fig. 1). Note that the classifications of the unknown species must be considered as hypotheses to validate in the field.

Finally, the deceit pollination systems is not clearly defined in this analysis since fly-deceptive taxa such as *Arum italicum* Mill. or *Helicodiceros muscivorus* (L.f.) Engl. are close to two mutualistic fly-pollinated species like *Colocasia esculenta* (L.) Schott or *C. fallax* Schott and the two beetle-pollinated species *Caladium* Ventenat and *Xanthosoma* Schott within the fly-pollinated 'cloud' (Fig. 1).

Discussion

We confirm a generally good correspondence between floral traits and pollinator types. Hence, different complexes of floral traits are associated with bee-, fly-, and beetle-pollinated aroids (Fenster et al. 2004; Chouteau et al. 2008). It appears that bee-pollinated taxa have very different floral traits from fly- and beetle-pollinated ones, these two latter groups showing some overlapping. This
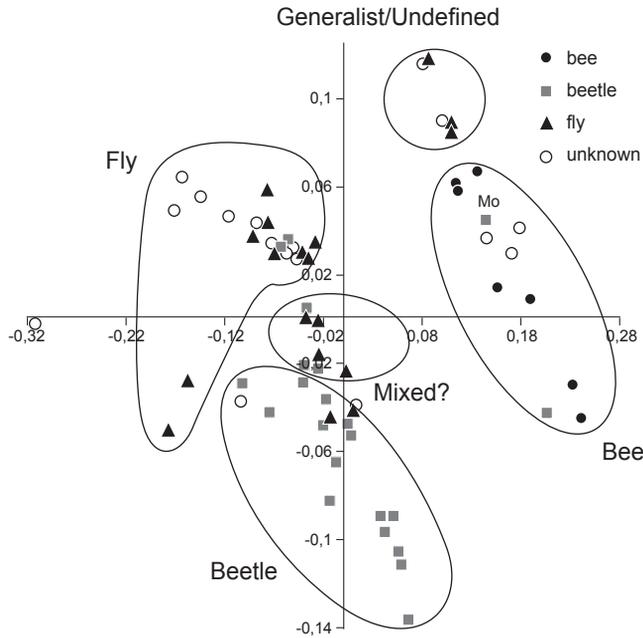
Fig. 2. Interpretation of the NMDS representation represented in figure 1 with respect to the floral traits and pollination types already known in the Araceae family.

imperfect discrimination between fly- and beetle-pollinated aroids may have several explanations. The first is that some of the species misclassified by the discriminant analysis may have been wrongly labeled as beetle- or fly-pollinated due to incomplete observations. Thus, field validations are needed for these 'intermediate'/misclassified species. Second, fly- and beetle-pollination may represent partially similar selective pressure on flowers leading to a convergence of some floral traits and thus an incomplete discrimination. In such a case, the shift from one type of pollination to another might be due to a change in a single character that is not included in our analysis, for example the odour or the colour of the spathe. Third, some of the misclassified species may indeed be pollinated by both flies and beetles. Consequently, floral traits are under a double selective pressure leading to the evolution of floral traits with characteristics intermediate between fly and beetle-pollinated floral traits. These intermediate species may represent a mixed pollinated system (Fig. 2), which needs to be validated by further field studies.

Interestingly the three species belonging to the Lasioideae subfamily are grouped with *Lysichiton camtschatcense* and *Symplocarpus renifolius,* two examples of a generalist pollination system in an intermediate position between fly- and bee-pollinated groups. We hypothesize that this group in an intermediate position between fly- and bee-pollination systems may represent a generalist pol-

lination system (Fig. 2). This hypothesis needs to be validated in the field and through study of other potentially generalist species, such as *Calla palustris* L..

Floral traits of fly-deceptive species appear to be characteristic of the fly-pollinated taxa with no clear discrimination. However, more data are needed in order to increase the sample size of deceptive species (N = 6). Moreover it could be interesting to include some beetle-deceptive pollinated species such as *Amorphophallus* species to test whether they will be grouped close to beetle-pollinated species or not.

Pistia stratiotes, an aquatic species, appears to be related to fly-pollination even if its pollinators are still unknown. But its outstanding position on the NMDS representation may suggest an original pollination system may be linked to its aquatic habit and the extreme reduction of its inflorescence which is function-ally one male and one female flower. Further studies on this very common aquatic tropical species are needed.

Surprisingly, *Monstera obliqua* a species pollinated by small nitidulid scarabs (Chouteau et al. 2007), appears within the bee-pollinated group. We proposed several explanations. First, *M. obliqua*, belonging to the Monsteroideae sub-family, may be phylogenetically constrained against any drastic floral changes from bee-pollination characteristics which are present in other genera of this subfamily. Second, the pollination of *M. obliqua* by nitidulid scarabs may be recent from an evolutionary point of view and the selective pressure of the scarabs on the floral trait characteristics still be ongoing. Third, pollination by nitidulid beetles may not require different floral traits, and those associated with bee-pollination may also be adapted to nitidulid beetle pollination leading to a reduced selective pressure on floral traits by beetles. Fourth, *M. obliqua* may be pollinated by bees and the conclusions of Chouteau et al. (2007) could be erroneous because nitidulid scarabs may not be efficient pollinators. Further studies of pollination in the genus *Monstera* are needed to explain the pattern resulting from the discriminant analysis.

In order to fully understand the evolution of floral traits in relation to pol-linators in Araceae further work is needed. In particular, more species should be studied in order to document all the different aroid taxonomical groups, but also to increase the sample sizes of the different pollination systems, particularly the newly proposed ones (generalist, mixed) and deceit pollination. More floral traits could be added, most obviously, the types of exine sculpturing of pollen grains, since large data sets are available (Thanikaimoni 1969; Grayum 1992; Hesse 2006). However, in some cases, the exine sculpturing of pollen grains var-ies within a genus, as in *Syngonium* Schott (Grayum 1986), and thus one must be carefully assign a type of pollen exine to a given genus particularly when the same species has not been studied. Finally, the recent molecular phylogeny of the aroid genera will permit the mapping of discriminant floral traits on the phylogenetic tree in order to study their changes and (co-)evolution in relation to phylogeny and pollinator type (Cabrera et al. 2008).

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