



## SHORT COMMUNICATION

## THE EXCEPTIONAL FLOWERING BEHAVIOUR OF *SPATHIPHYLLUM GRANDIFOLIUM* ENGL. (ARACEAE) – AN INDICATOR FOR SELF-POLLINATION?

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### INTRODUCTION

*Spathiphyllum* is a genus of the aroid subfamily Monsteroideae comprising 50 species of which the majority (47 spp.) are distributed in the Neotropics (Cardona 2004). The remaining species occur in Africa, Asia, and Oceania (Tropicos.org 2019; Zuluaga *et al.* 2015). The herbaceous plants are terrestrial, mainly growing in humid places, and frequently forming populations along the banks of rivers and streams (Bunting 1960). For those species of which we know the flowering time, this is throughout the year with a peak in the late dry and early rainy season or in the early rainy season only (Díaz Jiménez *et al.* 2016; Hentrich *et al.* 2010; Montalvo & Ackerman 1986). The inflorescence consists of an erect leaf-shaped, green or white spathe and a subcylindrical spadix with bisexual flowers (Bunting 1960; Cardona 2004). As in most Araceae, the flowers of *Spathiphyllum* are protogynous, starting with a female phase (receptive stigmas) and proceeding with a male phase (emergence and dehiscence of anthers; Mayo *et al.* 1997). In the cases described so far, all stigmas of an inflorescence become receptive at once and wilt after one to several days (Díaz Jiménez *et al.* 2016; Hentrich *et al.* 2010; Montalvo & Ackerman 1986). Subsequently, the male phase initiates with the emergence of few stamens per day, in an acropetalous way (from base to top) within the flowers along the inflorescence. In all *Spathiphyllum* species studied to date, the two phases are clearly separated temporally, suggesting that the plants depend on pollination vectors for their successful reproduction. Indeed, investigations on their

pollination biology report male euglossine bees (*Euglossini*), honey bees (*Apini*) and stingless bees (*Meliponini*) as pollinators (Díaz Jiménez *et al.* 2016; Hentrich *et al.* 2010; Montalvo & Ackerman 1986). Euglossine bees gather volatile organic compounds (VOCs) that are produced by the flowers in both sexual phases during the morning hours (Hentrich *et al.* 2010; Williams & Dressler 1976), bees of the tribes *Apini* and *Meliponini* collect pollen of the male phase inflorescences and accidentally alight on those in the female phase (Díaz Jiménez *et al.* 2016; Montalvo & Ackerman 1986).

During a field trip to the Hacienda Alejandría (4°51'28"N, 75°53'03"W, 935 m asl), located in the Cauca river valley (La Virginia municipality, Risaralda department, Pereira, Colombia), we observed a flowering pattern in *Spathiphyllum grandifolium* ENGL. (section *Spathiphyllum*; Bunting 1960), which was completely different from that described for other *Spathiphyllum* species. In the course of our short stay, we studied the plants to document their reproductive biology as far as possible. Here, we present our results and discuss them in the context of the occurrence of self-pollination in the genus.

### MATERIALS AND METHODS

The study was conducted between 12-17 July and on 17 September 2017 on two small populations (distance: 200 m) of 28 and seven *Spathiphyllum grandifolium* plants that grew scattered in a secondary forest fragment (ca. 14 ha) of the Hacienda Alejandría. In July, three plants were flow-



ering, two in the smaller and one in the larger population. In September, we returned to examine the mature fruits. A voucher specimen was collected and deposited in the herbarium of the Universidad del Valle, Cali, Colombia (CUVC; P. Díaz J. & J.M. Ruiz-Idarraga 1366). The climate in the region is warm and humid with an annual rainfall of 1882 mm and a mean annual temperature of 24.9 °C (IDEAM, 2018). There are two rainy seasons (April/May, October/November), a minor dry season (January) and a main dry season (June-August).

*Spathiphyllum grandifolium* is distributed in Colombia and Ecuador in different kinds of vegetation at altitudes between 0-1200 m (Cardona 2004; Tropicos.org 2019). It commonly forms large populations (F. Cardona, pers. comm.) and grows on riverbanks and low hills that are very humid or that are temporarily inundated (Cardona 2005; Tropicos.org 2019). The plants are ca. 1.7 m high (Fig. 1A) and their inflorescence has a green, upright spathe with a cylindrical, white, 6-15 cm long spadix that turns slightly cream-yellowish during flowering (Fig. 1B; Cardona 2005). The flowers consist of six tepals and four stamens that surround a conical pistil that is 3-4 mm longer than the tepals and has a round stigma. The ovary is trilocular with 3-9 ovules. The fruits are obovoid and their 3-4 mm long seeds are obovoid-oblong (Cardona 2005). According to the information obtained from herbarium specimens, the species flowers in both rainy seasons (April/May, November/December) and the dry seasons (January, July) in the study area (Tropicos.org 2019).

The flowering behavior and floral visitors of *Spathiphyllum grandifolium* were studied between 12-17 July on one inflorescence of each population. The weather during the observations was completely dry and hot. In the first inflorescence, the male phase had already initiated and was overlapping the female phase. The second inflorescence was in the female phase when we started the observations. The duration of the female phase was determined by testing the stigmas for receptivity by applying hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>; first inflorescence only) and by observing if the stigmas had wilted (color changes from white to brownish). The emergence of the stamens during the male phase was recorded in both inflorescences. For 24 hours on the first day and between 07:00 h and 13:00 h on the three following days, we checked the smell hourly at both inflorescences to determine the presence/absence of floral scent.

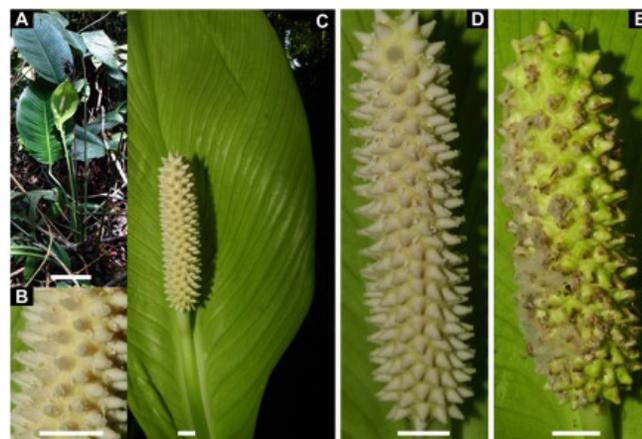
During a 24 hours period (06:00 h-06:00 h), preliminary visitor observations were conducted in both inflorescences to get an overview of the floral visitors and their visiting time (visiting species and visiting time were recorded). Between 06:00 h-19:00 h, observations were continuous; during the night (19:00 h-06:00 h), we only observed the in-

florescences in time blocks of 10 minutes every 50 minutes and used red filters for our headlights. In the subsequent five days, sporadic visitor observations at both inflorescences were carried out between 07:00 h and 14:00 h in time blocks of 10 minutes every two hours. All observations were made at a distance of approximately 2 m.

The ability to self-pollinate autonomously was tested by covering the third inflorescence in pre-anthesis with a cloth (mesh size < 1 mm) until the end of anthesis. Subsequently, fruit set and the seed number of 20 mature fruits (seven at the base, seven at the middle, and six at the top; randomly chosen) was determined manually. The same approach was carried out for five unbagged infructescences of different, randomly selected plants.

## RESULTS

In both inflorescences studied, all stigmas were receptive during the six days of observation (Figs. 1B, C). In the first inflorescence with the overlapping sexual phases, the flowers from the top to the middle of the inflorescence were in the male phase (basipetal emergence) from the first to the last day of observations. All anthers were exposed at once and shed many strands of clotted pollen, which fell down and made contact with the receptive stigmas below.



**Figure 1.** A: Individual plant of *Spathiphyllum grandifolium* ENGL. with inflorescence. B-E: Inflorescences of *Spathiphyllum grandifolium* ENGL. B: First inflorescence with overlapping sexual phases showing pollen masses on the receptive stigmas. C: Overview of the first inflorescence with spathe during overlap of sexual phases. D: Second inflorescence during overlap of sexual phases, first day of emergence of anthers – transition to male phase (basipetal dehiscence of multiple anthers) and receptive stigmas. E: Non-manipulated immature infructescence with remains of anthers and old spider webs (left side).

Photos: (A) J. M. Ruiz-Idarraga; (B-E) P. Díaz Jiménez. Scale bars: A = 30 cm; B-E = 1 cm.

During the entire study period, no progression in flowering was observed, i.e. no new anthers from the same or from other flowers of the inflorescence emerged. In the second, female, phase inflorescence, the male phase initiated on day six (Fig. 1C). Many anthers of different flowers at the distal end of the inflorescence emerged spontaneously (Fig. 1C) and, as with the other inflorescence, shed pollen masses onto the receptive stigmas below. During the entire study period, we could perceive no floral scent.

The observed visitors were one ant (one visit between 08:00 h-09:00 h), one beetle (two visits between 09:00 h-11:00 h), one wasp (one visit between 08:00 h-09:00 h) and four flies (11 visits between 08:00 h-14:00 h). However, all visitors only landed on the spathe but never had contact with the spadix (Fig. 2). The different insects remained on the spathe from one to 60 seconds.

Fruit-set of the bagged inflorescence as well as in the randomly selected infructescences was 100 %. Seed-set of the bagged inflorescence was five seeds/ fruit ( $5 \pm 1.07$ ;  $N = 20$ ), similar to the unbagged ones ( $6 \pm 1.22$ ;  $N = 100$ ).



**Figure 2.** Wasp (top) and fly (bottom) on spathe of *Spathiphyllum grandifolium* ENGL. Photo taken by P. Díaz Jiménez.

## DISCUSSION

The flowering pattern of the *Spathiphyllum grandifolium* plants observed during our study is exceptional not only for the genus *Spathiphyllum*, but also for the family Araceae. The male phase had already started while the stigmas were still receptive (incomplete protogyny) and proceeded in the opposite direction from the usual – from top to bottom (basipetally). Many anthers per flower emerged simultaneously and shed large masses of clotted pollen - a phenomenon generally known for aroid species with unisexual

flowers whose inflorescences form a kettle trap and which are predominantly xenogamous (Gibernau, 2015; Maia *et al.* 2010). In these, the purpose of mass shedding is that the trapped insect visitors are dusted with pollen before leaving the inflorescence (Gibernau, 2003; Maia *et al.* 2010). In the plants studied, the flowering pattern apparently had a different function - it promotes self-pollination by gravity, which permits the pollination of proximal flowers by the pollen of distal ones.

In our only test on autonomous self-pollination and in five unbagged inflorescences, 100 % of the flowers developed fruits and had an average seed set that was almost as high as the average number of ovules. Although, we cannot preclude the possibility that unbagged inflorescences were pollinated by floral visitors during our absence, the flowering phenology, the high reproductive success and the result of our bagging experiment support the possibility of a seed development without pollination vectors.

In *S. humboldtii* fructification in the absence of pollinators has also been observed (Hentrich *et al.* 2010). Unlike the plants we studied, this species has a clear separation of the sexual phases. Seeds are produced by apomixis, without the prior fertilization of the ovule. Due to the specifics of our experiment, we cannot rule out that the seeds in the plants studied were also the outcome of apomixis. Further, we did not determine the viability of the developed seeds.

Previous investigations of the reproductive biology of *Spathiphyllum* demonstrated the importance of floral fragrances for the successful outcrossing of the plants – either by offering VOCs as a reward for male euglossine bees or by deceiving pollen-collecting bees into visiting female phase inflorescences that emit a similar scent to the male phase ones (Hentrich *et al.* 2010; Montalvo & Ackerman 1986). In this context, it is remarkable that the flowers of the *S. grandifolium* plants studied emitted no perceptible floral scent either during the day or at night – at least to our noses. Nevertheless, the emission of minute amounts of VOCs that are large enough to attract specific pollinators is still feasible (compare Hentrich *et al.* in press). It might be, therefore, that pollinators were simply not present during our observations (e.g. due to their population dynamics) or that they visited the flowers during our absence. Further, the importance of visual cues as attractants for pollinators has not yet been studied in this genus. In addition, we actually observed visiting insects, but they were very rare and infrequent and none of them alighted on the spadix during our study. Given that the study area is an isolated secondary forest fragment in an open agricultural landscape, the absence of pollinators may also be related to the loss of suitable habitats for many insect species.

Our investigation is the first to document the reproductive biology of *Spathiphyllum grandifolium*. Due to the

limited number of plants and the short study period, our results still need to be verified by an extended and detailed field assay to confirm their validity for this species. Nevertheless, we feel that our observations are an important piece of the puzzle for understanding the reproductive biology of the genus *Spathiphyllum*. Until now, the pollination biology has been studied in only a few species, most of which present a clear outcrossing strategy (Díaz Jiménez *et al.* 2016; Hentrich *et al.* 2010; Montalvo & Ackerman 1986). *Spathiphyllum humboldtii* seemed to be an exception in being apomictic (Hentrich *et al.* 2010). So far, it is not known how widespread autogamy and self-pollination in the genus is and what their occurrence is related to – e.g. if there is a relation to phylogeny or to the identity of their pollinators. *Spathiphyllum humboldtii* belongs to a completely different section of the genus than does *S. grandifolium* (*Dysspathiphyllum* vs. *Spathiphyllum*; Bunting 1960). If our results prove to be valid, *S. grandifolium* will be the first species in the section *Spathiphyllum* with the ability to self-pollinate. Two closely related species (*S. cochlearispathum*, *S. friedrichsthalii*) have been shown to be clearly outcrossing (Díaz Jiménez *et al.* 2016; Montalvo & Ackerman 1986).

Since the emission of floral fragrances played an important role in the pollination of the species studied so far, the lack of a strong floral scent, as in *S. grandifolium*, could be an indicator for distinct pollinators or self-pollination. Several *Spathiphyllum* species are known, that apparently do not smell to humans and whose pollination biology has not yet been studied (e.g., *S. minor*, *S. pygmaeum*, and *S. tenerum*; Bogner 2011; Cardona 2005; G. Gerlach, pers. comm.). On the other hand, the apomictic *S. humboldtii* emits a distinct and pleasant floral fragrance that attracts pollinators. Apparently, this species has a mixed pollination system, combining apomixis with outcrossing. Also, in the *S. grandifolium* plants studied, outcrossing was not excluded. In the second of our study plants, we demonstrated that in the first days of flowering, there is still a clear female phase, which promotes the idea of a “delayed autonomous self-pollination” (Lloyd & Schoen 1992). This kind of phenology permits cross-pollination and assures reproductive security in the absence or only limited presence of a pollinator (Goodwillie *et al.* 2005; Lloyd & Schoen 1992). The development of delayed autonomous self-pollination systems in the genus *Spathiphyllum* could be an indicator for a weak outcrossing-mechanism. Species that depend on pollen-collecting bees are pollinated when the bees confuse female phase inflorescences with male ones (Díaz Jiménez *et al.* 2016; Montalvo & Ackerman 1986). This pollination system might be fragile since the female flowers do not offer any reward, and the results of other studies suggest that reproductive success depends on the number of plants in the population and the bee species attrac-

ted (Díaz Jiménez *et al.* 2016; Montalvo & Ackerman 1986). Another indicator that supports the idea of a weak outcrossing-mechanism is the finding of nucellar polyembryony in *S. patinii* (Schürhoff & Jüssen 1925). Schürhoff and Jüssen (1925) observed the occurrence of additional embryos not originating from the egg cell but from nucellar tissue in fertilized ovaries, which enables the plant to produce larger quantities of seeds even with a low pollination success. We believe that our findings offer new perspectives for a more complete study of the reproductive biology of *S. grandifolium*, as well as for other species in the genus.

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