Size Variations of Flowering Characters in *Arum maculatum* (Araceae)

Marion Chartier and Marc Gibernau*
Université Paul Sabatier
Laboratoire d'Evolution & Diversité Biologique (UMR 5174)
Bât. 4R3-B2
31062 Toulouse cedex 9, France

ABSTRACT

For entomophilous species, the size-advantage model predicts a bias to the female function with resource increase. This model has been shown to be expressed on *Arum italicum* Mill. (Araceae), whose bigger or latest inflorescences are female-biased. Here, we characterise the reproductive-trait variations of *Arum maculatum* L., a closely related species that is smaller and produces a single inflorescence per year.

By measuring the different floral parts and counting the flower number of differently-sized inflorescences, we showed that (1) for *A. maculatum*, 34% of spadix size is allocated to the appendix, 12% for female flowers and 7% for male flowers. Due to its size range, *A. maculatum* could in a manner be considered as a "small" *A. italicum*. (2) As for *A. italicum*, the appendix size is the longest part of the inflorescence, and increases proportionally more than the male and female parts size with plant vigour. This is not surprising, as the appendix is an essential attracting organ for pollinators. (3) There is no correlation in *A. maculatum* between the flowers number and the other floral characters, and no bias for female part size in bigger inflorescences. Thus, contrary to *A. italicum*, there is no expression of the size-advantage model for *A. maculatum*, whose range of sizes might be too small. Another explanation is selection for a high number of male gametes that may counterbalance the potential bias for female flowers.

* e-mail: gibernau@cict.fr

INTRODUCTION

Proposed by Ghiselin (1969, quoted in Lloyd & Bawa, 1984), the size-advantage model postulates a sex change when an increase in body size is related to differential abilities to produce or sire offspring. For entomophilous plant species, such as Aroids, the size-advantage model predicts a female-biased gender expression with size increase (Klinkhamer *et al.*, 1997). This is expressed by a proportionally bigger increase of the female function than the male function in bigger individuals. This topic has been mainly studied in Araceae on *Arisaema* species presenting an extreme reproductive variation with a gender modification according to size (Gusman & Gusman, 2003). Apart from *Arisaema*, this question has been mainly studied on *Arum italicum*. It has been shown in this species that there is a disproportionate allocation to the appendix versus the floral parts, with a length representing 44% of the spadix size (Méndez, 1998; Gibernau & Albre, 2008), and a mass representing 29% of the total inflorescence weight (Méndez, 2001). Such results are not surprising as the appendix is the inflorescence's attractive organ, producing heat and volatile organic compounds (Bermadinger-Stabentheiner & Stabentheiner, 1995; Kite *et al.*, 1998; Diaz & Kite, 2002). In general, fertile parts and appendix weights increase with plant vigour, which is representative of an increase of the reproductive effort when

resources become more abundant (Méndez, 1998; Gibernau & Albre, 2008). In Spanish *A. italicum*, Méndez (2001) showed that the mass allocated to male flowers increases proportionally more than for female flowers with increase in the inflorescence's weight, which is in contradiction with the size-advantage model prediction. However, the same author (1998) found no significant difference between the proportional increase of male and female flower number with spadix size increase. On the contrary, in the South of France, Gibernau & Albre (2008) found that the female flower number increased proportionally more than male flowers with spadix size increase in *A. italicum*, showing that a bias to female parts can exist, which is in accordance to the size-advantage model. Thus, *A. italicum* floral sex ratio, which is male-biased (mean = .74), decreases with spadix size increase (Gibernau & Albre, 2008). Furthermore, the sex ratio from the last inflorescences (which are smaller) tends to be female-biased (Méndez, 1998) which is also in accordance with the sized-advantage model, as the more vigorous plants are the ones able to produce up to three inflorescences per year. Then, when considering the floral parts size and mass variations, the resources allocation in *A. italicum* seems to be different for female parts, for which an increase in size would rather be due to an increase in flower size, than it is for male parts, for which an increase in size may likely be due to an increase of flower number (Méndez, 2001; Gibernau & Albre, 2008).

Here, we study the floral variations and the expression of the size-advantage model of *Arum maculatum* (L.). Its flowering cycle is protogynous; female flowers being receptive before stamens release the pollen. *Arum maculatum* is pollinated by deceived diptera attracted by the floral odour mimicking the odour of their ovipositing site (sapromyophily) (Gibernau *et al.*, 2004). The deceiving odour is produced by a sterile organ situated at the apex of the inflorescence called the appendix. *Arum maculatum* is mainly pollinated by

one diptera species, the midge *Psychoda phalaenoides* whereas *A. italicum* is pollinated by several diptera species (Lack & Diaz, 1991; Diaz & Kite, 2002; Albre *et al.*, 2003). In a way, we can consider from a pollination point of view that *A. maculatum* may be a "specialist" and *A. italicum* rather an "opportunist" species. Another difference is that mature plants of *Arum maculatum* generally produce only one inflorescence whereas mature *Arum italicum* produce an average of two inflorescences (Albre & Gibernau, 2008).

The aim of this paper is to document the reproductive characters of *Arum maculatum* in three different French populations, and to characterize its allocation strategy. Does *A. maculatum*, which produces a single inflorescence per year, express the size-advantage model for big inflorescences? Does its higher pollinator-specificity result in a different resource allocation in terms of the relative appendix size or the numbers of fertile flowers?

MATERIALS AND METHODS

34 inflorescences of *Arum maculatum* L. were collected during the spring of 2008 in 3 populations situated in Bagnères-de-Bigorre (Midi-Pyrénées, France), La Loubatière (Languedoc-Roussillon, France) and Smarves (Poitou-Charentes, France). The inflorescences were collected before the release of pollen and preserved in 70% alcohol.

The lengths of the different parts of the spadix, i.e. fertile male and female zones and appendix, were measured with a digital calliper. Moreover, male and female flowers per inflorescence were counted on 13 inflorescences.

Data analyses consisting of linear regressions, Shapiro tests, Bartlett tests, ANCOVA (for differences between regression slopes), as well as a Kruskal-Wallis test followed by a multiple comparison test (for inter populations differences of floral part sizes) were performed using R 2.5.1 (2007) software. Codes are available from M. Chartier.

RESULTS

Morphology

The mean sizes of the different parts of the inflorescences are shown in Table 1. The appendix appears to be the most important part of the inflorescence, representing 33.8 ± 5.4% of the total spadix length. The fertile parts are shorter than the appendix representing 11.9 ± 1.9% of the total spadix length for the female zone and 7.2 ± .9% for the male zone (Table 1). Moreover, the female zone appears to be 1.6 times longer than the male zone. The inflorescences from the population of Bagnères-de-Bigorre appear to be longer than in the two other populations (Table 1). Zone lengths are significantly different between populations only for the male part ($X^2_{2,34}$= 7.07, df = 2, P = .03), Bagnère-de-Bigorre having significantly longer male zone length than Smarves (P < .05).

The mean numbers of male and female flowers are shown in Table 2. Here again, in the population of Bagnères-de-Bigorre, inflorescences seems to have more flowers than in the two other populations. But inflorescence numbers per population are too low to test for any difference between populations.

Intra-plant relationships

The three measured floral zones sizes were positively correlated with the spadix size, which is logical due to the autocorrelation of these traits (Fig. 1). The appendix length increases proportionally more (slope = .5) than the male (slope = .06) and female zone lengths (slope= .07). Surprisingly, male and female zone size slopes are not significantly different ($F_{1,32}$ = .11, P= .75) i.e. both parts seem to increase in the same proportions with the spadix size (Fig. 1).

The numbers of male and female flowers are not significantly correlated with spadix length (Fig. 2) but are significantly different, with a male-biased sex ratio (mean sex ratio = .75).

Table 1. Reproductive morphological measures in millimetres (mean ± SD) and percentages (mean ± SD) against total spadix length of *Arum maculatum* inflorescences collected in 3 French populations at La Loubatière, Bagnères-de-Bigorre (BB) and Smarves. The sum of the lengths and percentages of the appendix, the female zone and the male zone are not equal to the spadix length and percentage, as the appendix stipe and the two sterile zones were not measured. N = number of measured inflorescences.

Zone	La Loubatière (N=9) Length (mm)	%	BB (N=19) Length (mm)	%	Smarves (N=6) Length (mm)	%
Spadix	68.6 ± 7.8	100	75.7 ± 11.0	100	67.3 ± 14.6	100
Appendix	21.1 ± 5.7	30.4 ± 5.2	27.2 ± 6.2	35.8 ± 5.1	22.4 ± 7.3	32.7 ± 4.1
Female zone	8.2 ± 1.5	12.0 ± 2.0	8.6 ± 1.4	11.4 ± 1.7	8.6 ± 1.8	13.0 ± 2.3
Male zone	4.9 ± .4	7.2 ± .9	5.5 ± 1.1	7.3 ± .8	4.6 ± .4	7.0 ± 1.1

Table 2. Mean numbers of male and female flowers of *Arum maculatum* inflorescences at La Loubatière, Bagnères-de-Bigorre (BB) and Smarves. N = number of inflorescences measured.

	Loubatière (N=5)	BB (N=5)	Smarves (N=3)
Female flower number	26.8 ± 5.8	37.6 ± 13.1	27.3 ± 5.5
Male flower number	88.0 ± 19.3	128.6 ± 65.5	74.7 ± 19.1

DISCUSSION

An important part of the spadix size of *A. maculatum* (34%) was found to be allocated to the appendix, against 11.9% for female part and 7.2% for male part. Moreover, the appendix size increases proportionally more than fertile zones lengths (Fig. 1). This pattern is similar for *A. italicum*, whose appendix represents 44% of the spadix size, against 16.5% for the female part and 6% for the male part (Gibernau & Albre, 2008). However, a
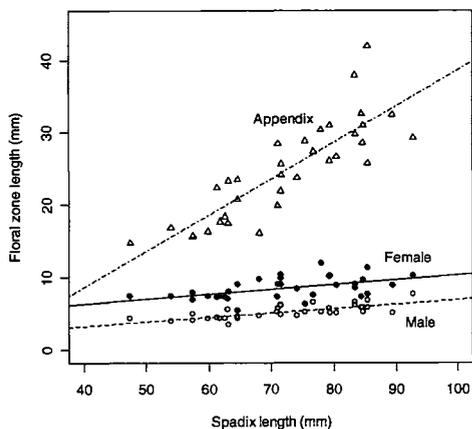

Figure 1.   Relationships between the lengths of the spadix and the appendix, the male flower zone, and the female flower zone. Each of the measured parts increases with spadix length (appendix: R^2 = .72, $F_{1,32}$ = 79.99, P < 10^{-9}; female zone: R^2 = .24, $F_{1,32}$ = 11.45, P < 10^{-2}; male zone: R^2 = .53, $F_{1,32}$ = 37.81, P < 10^{-6}). Female and male zone increase in the same proportions (slopes are not significantly different: $F_{1,32}$ = .11, P =.75). The slope of the appendix regression is higher than for the other zones ($F_{2,96}$ = 18.5, P < 10^{-5}).

mean of 53% of spadix size is allocated to appendix and fertile zones for *A. maculatum*, against 67% for *A. italicum*. This difference can be explained by the fact that *A. maculatum* is on average smaller than *A. italicum*, with a mean *A. maculatum* spadix size of 64 mm (range 47–75) against 102 mm (range 52–181) in *A. italicum* (Gibernau & Albre, 2008). Then, the size range of *A. maculatum* seems to correspond to the smallest size range of *A. italicum*, and *A. maculatum* could be considered as a "small" *A. italicum*. Another explanation is that there may be a morphological constraint on the sterile flowers (as part of the floral trap) and stipe (as inflorescence sustaining organ) for their minimal sizes. Thus, there might be a threshold below which the stipe and the sterile flower can't be decreased without affecting the reproductive success.
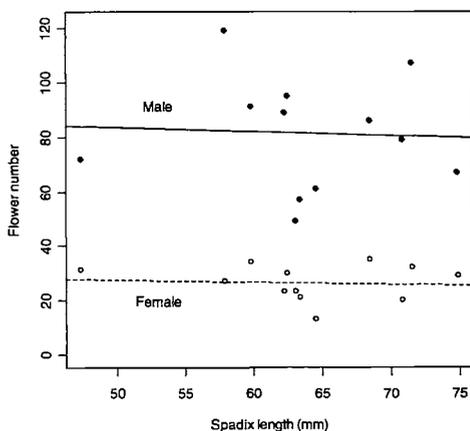

Figure 2.   Relationship between the length of the spadix and the male and female flower number. No significant correlation was found (male: $F_{1,11}$ = .03, P = .86; female: $F_{1,11}$ = .07, P = .80).

For both species, the appendix constitutes the biggest spadix part. The appendix is crucial for pollination achievement as it attracts insects by heating and producing volatiles organic compounds (Bermadinger-Stabentheiner & Stabentheiner, 1995; Kite *et al.*, 1998; Diaz & Kite, 2002; Albre & Gibernau, 2003), especially for these *Arum* species, which are obligatory allogamous (Lack & Diaz, 1991; Albre *et al.*, 2003). Thus, the more important investment made by the plant when more resources are available concerns the pollinator attraction function. In *A. italicum*, it has been shown that appendix length was positively correlated with the number of insects caught by the inflorescence (Méndez & Obeso, 1992), which is likely also to occur in *A. maculatum*. For *A. maculatum*, one single *Psychoda phalaenoides* is supposed to be sufficient to ensure fructification, but more pollinators might be necessary for *A. italicum*, that bears 2.4 times more flowers and produces 4.6 times more seeds (Lack & Diaz, 1991; Albre & Gibernau, 2008). In both cases, it seems that attracting a great number of pollinators increases the chances (1) to ensure efficient pollen dispersal, (2) to attract at least one pollinator bearing fresh allogamous pollen (Lack & Diaz, 1991; Albre *et al.*, 2003).

Male and female parts sizes increase proportionally with spadix size for *A. maculatum*, traducing a reproductive effort when more resources are available, even when pooling different populations. However, their male and female flower number is not significantly correlated to spadix size. Thus, an increase of these parts may be due to an increase of flowers sizes for both genders, which is different than for *A. italicum*, whose male part size increase is more due to an increase of the flower number (Gibernau & Albre, 2008). Considering the small size range of *A. maculatum*, an increase of the flower number with spadix increase might be more costly than an increase of the size and number of gametes. On the contrary, reducing too much the flower number may compromise the reproductive success. This may explain the absence of correlation between the flower number and the spadix size. Another explanation is that we masked any relationship by pooling inflorescences from several populations. This has to be tested by measuring the flowers size and gamete number against the spadix size in *A. maculatum* and *A. italicum* from a broader sampling in the same populations.

The expression of the size-advantage model in *A. italicum* results in a proportionally bigger increase of the female part vs. male part sizes with the spadix length increase (Gibernau & Albre, 2008) and in a bias to female part in the last inflorescence (Méndez, 1998). On the contrary, *A. maculatum*, which produces a single inflorescence per year, doesn't show any expression of the size-advantage model. We found no difference between the increase of the female and male part size against the spadix size increase, and no correlation between the floral sex-ratio and any other floral character. This can be explained by the combination of different factors. (1) The range of spadix sizes of this species may be too small to see the expression of the model. It has been shown on *A. italicum* that plant size, and *a fortiori* spadix sizes are dependent on bulb size (Méndez & Obeso, 1993). The sizes of *A. maculatum* represent the lower range of size distribution of *A. italicum*, and its mean sex-ratio (.74) corresponds to *A. italicum* smallest inflorescences sex-ratio (.75) (Gibernau & Albre, 2008). In fact, *A. maculatum* inflorescences appear to be equivalent (in terms of floral number and sex-ratio) to small *A. italicum*. The resources the bulbs of *A. maculatum* can accumulate might be limited, leading to a constraint on the maximal inflorescence size the plant can produce without affecting its seed survival and/or production capacity. (2) In the studied populations, *A. maculatum* attracted 2 to 10 times more pollinators than *A. italicum* (data non published), so pollinators might not be a limiting factor. A greater pollen dispersion and selection for a big number of male gametes (i.e. male flowers) may counterbalance the potential bias for female flowers.

Further studies are needed to assess whether there is a difference of pollination efficiency between these two species due to their different pollinator specificity that could explain their different resource allocation strategies. A study of the gender variations at the gametes level, by the evaluation of ovules and pollen grain numbers variations would help understanding more precisely whether these plants adjust their sex ratio in relation to their vigour (e.g. available resources).

ACKNOWLEDGMENTS

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