

MODIFICATION OF PHENOTYPIC AND FUNCTIONAL GENDER IN THE MONOECIOUS *ARUM ITALICUM* (ARACEAE)¹

MARCOS MÉNDEZ²

Departamento de Biología de Organismos y Sistemas (Unidad de Ecología), Universidad de Oviedo, E-33071 Oviedo, España

Other than studies on sex-labile *Arisaema* species, studies of gender patterns in Araceae are scarce. The modification of phenotypic and functional gender was investigated in three populations of the monoecious *Arum italicum* Miller. The probability of reproduction and the number of inflorescences produced increased with plant size, and flower number (total, male, staminodes, female, pistillodes) increased with both plant and inflorescence sizes. However, plant and inflorescence sizes were poor predictors of floral sex ratio (female to male flower ratio). In contrast, change in floral sex ratio towards increasing femaleness was found among inflorescences sequentially produced by a plant. This change could not be explained by either a decrease in inflorescence size or a change in the mating environment. Differences in functional gender did not appear to be related to plant size or stage in the flowering period. Instead, different patterns of functional gender were found between plants with different number of inflorescences. Multi-inflorescence plants showed a functional gender around 0.5, while plants with one inflorescence showed a more extreme functional gender (either male, female, or functionally sterile). Sex of flowers in this species did not seem to exhibit a phenotypic trade-off.

Key words: Araceae; *Arum italicum*; floral sex ratio; functional gender; mating environment; size advantage model; trade-off.

Although most angiosperms are cosexual (Charnov, 1982), substantial variation in intraspecific sexual expression has been found (Bertin, 1982; Bawa and Webb, 1983; Ross, 1990). Such variation in resource partitioning to each gender (phenotypic gender) could be related to different reproductive success through each gender (functional gender). Thus, the study of phenotypic gender patterns, their underlying mechanisms, and their consequences for functional gender is basic to understanding the evolution of sexual patterns observed. Lloyd and Bawa (1984) reviewed the terminology, theory, and evidence for intraspecific gender modification in plants. Two causes, not necessarily exclusive, have been proposed for an adaptive modification of gender.

Firstly, an internal cause is the “size advantage” model of Ghiselin (1969, quoted in Lloyd and Bawa, 1984). According to this hypothesis, an increase in plant size, age, or condition involves differences in fitness gain curves for each gender (Lloyd and Bawa, 1984). These differences could be produced by several mechanisms: increasing geitonogamy because of a larger floral display (Klinkhamer, de Jong, and Metz, 1994); increasing ability to mature seeds with plant size (Stephenson, 1981); interference between male and female functions (Lloyd,

1988); or different abilities for gamete dispersal (Bickel and Freeman, 1993). Predictions of an increasing bias toward male or female gender with size are dependent on the specific cause underlying the change in fitness gain curves (Bickel and Freeman, 1993; de Jong and Klinkhamer, 1994).

Secondly, external conditions such as density (Smith, 1981; Lundholm and Aarssen, 1994) or “quality of the environment” (Freeman et al., 1981) could influence the sexual expression. In general, shaded, dry, or nutrient-poor (micro)habitats are related to a bias toward the “cheaper” male gender at individual and population levels (Freeman et al., 1981). Another external factor, a changing mating environment, has been recently found to influence the optimal within-plant seasonal gender modification (Brunet and Charlesworth, 1995). For the protandrous *Aquilegia caerulea*, a prevalence of male phase flowers earlier in the season was followed by a higher maleness in flowers produced later in the season. The inverse change was predicted for protogynous species (Brunet and Charlesworth, 1995).

Intraspecific gender variation studies in the Araceae are scarce. Both the size advantage model (Policansky, 1981; Clay, 1993) and environmental effects (Lovett-Doust and Cavers, 1982) have been discussed in the sex-labile *Arisaema* species. However, the sexual expression in other aroid species remains mostly unexplored. For example, while discussing the role of disabled reproductive organs in plants, Lloyd (1992) proposed a trade-off among fertile and sterile flowers in *Arum*. This proposal has not been tested yet. This paper studied the patterns and variability of gender in *Arum italicum* Miller (Araceae), a monoecious, entomophilous plant. Specific questions addressed were: (1) How are flower number and phenotypic gender related to plant and inflorescence size? (2) Are there any

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² Current address: Department of Ecological Botany, University of Uppsala, Villavägen 14, S-752 36 Uppsala, Sweden.

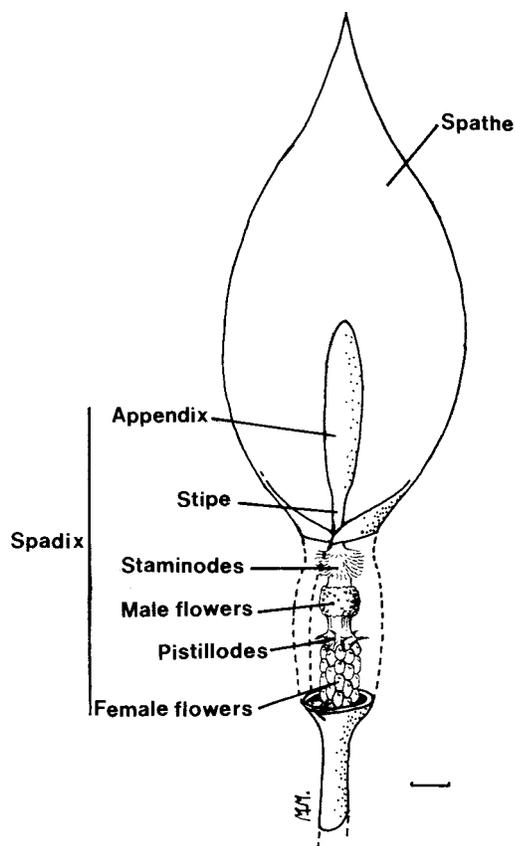


Fig. 1. Inflorescence of *Arum italicum*. The lower portion of the spathe has been removed to show the arrangement of the different types of flowers on the spadix. Bar = 1 cm.

modifications in flower number and phenotypic gender among inflorescences sequentially produced by a plant? (3) Is flower number linked by trade-offs between sexes? (4) How is the functional gender related to phenotypic gender patterns?

MATERIALS AND METHODS

Plant species—*Arum italicum* is an herbaceous, perennial geophyte mainly distributed in southern Europe, although reaching the British islands and the Black Sea (Boyce, 1993). In the study area (province of Asturias, northern Spain), this species is common in riparian forests, hedgerows, roadsides and other wet, shaded, disturbed, lowland habitats. Details about the seasonal growth cycle, perennation organ, and vegetative propagation of this species are given in Méndez and Obeso (1993).

Unisexual flowers in this species are very simple and arranged on a linear axis called a spadix (Fig. 1). Aside from unisexual whorls of fertile flowers, two groups of bristle-like sterile flowers are present: pistillodes (sterile female flowers) and staminodes (sterile male flowers). A full description of both flowers and spadices in the genus *Arum* is given by Boyce (1993). A big, green bract (the spathe) surrounds the inflorescence axis. Usually, 1–3 inflorescences are sequentially produced by fertile plants, separated by a lapse of 2–6 wk. Inflorescences are not preformed the previous year but, if multi-inflorescence plants are dissected at the beginning of the flowering period, inflorescences in different developmental stages can be seen.

In this study, the inflorescence was considered as the unit of analysis because it is a highly integrated structure, functionally analogous to a

protogynous flower (Grayum, 1990). It acts as a trap, attracting and deceiving small Diptera (Dafni, 1984) in two phases. On the first day of anthesis (D day) female flowers are receptive and Diptera are attracted and trapped. Sterile flowers play a role at this moment, limiting the trapping to insects of the right size (Boyce, 1993) and hindering the escape of insects trapped (Proctor and Yeo, 1973; Faegri and van der Pijl, 1979; Boyce, 1993). On the second day of anthesis (D+1 day) male flowers become functional and Diptera leave the inflorescence after being covered with pollen. *Arum italicum* does not self-fertilize (personal observation). Pollination in this and other few more *Arum* species has been reviewed by Proctor and Yeo (1973), Faegri and van der Pijl (1979), Lack and Díaz (1991), and Boyce (1993).

Study sites and variables measured—Three sites were studied in 1994: (1) 122 plants were tagged at Ablaneda (43°30'N, 5°54'W; 130 m above sea level [a.s.l.]), a roadside colonized by ruderal forbs and grasses, *Rubus* sp., and *Salix atrocinerea*; (2) 67 plants were planted in an experimental population at Avilés (43°31'N, 5°54'W; 40 m a.s.l.) with rich soil, to use them in several experiments (for details about the provenance of plants of this population see Méndez, 1997); and (3) 39 plants were tagged at Tamón (43°31'N, 5°52'W; 45 m a.s.l.), a riparian forest with *Alnus glutinosa*, *Corylus avellana*, and few understory plants (*Dryopteris* sp., *Mercurialis perennis*, and *Hedera helix*). Until and after the flowering period, populations were visited every week (Avilés) or every other week (Ablaneda and Tamón). During flowering, Ablaneda and Avilés plants were visited daily and Tamón plants were visited once a week. Inflorescences infected by an unidentified pathogen were excluded from analyses. The definitive sample sizes are shown in the Results section.

Plant size was estimated as the dry mass of leaves produced. Leaves of each plant were collected when abscised. A few leaves in Avilés were missed before collection. In such cases, the missing value was substituted by the mean mass of remaining leaves. This correction was not used when the number of leaves missed exceeded two. Inflorescence size was estimated as dry mass of the spathe. This measurement was highly related to the inflorescence mass ($r = 0.86$, $df = 1, 18$, $P < 0.0001$) in a separate sample. Spathes were harvested after anthesis (D+2 day). Removal of this structure could impair the resorption of nutrients or carbon (Ashman, 1994). However, the spathe decays soon after blooming and is usually grazed by slugs and snails (personal observation). Thus, it had to be collected immediately in order to avoid biomass losses. Leaves and spathes were oven-dried at 60° C for a week and weighed to the nearest 0.01 g.

During the flowering period (February–June), inflorescences produced by each plant were noted, and male and female flowers, staminodes, and pistillodes were counted. Sometimes, herbivores ate male flowers, staminodes, or pistillodes before they were counted. In such cases, the proportion of flowers eaten was estimated at sight and used together with the number of flowers remaining to estimate total flower number. This procedure was too inexact and thus avoided when flowers lost exceeded three-fifths of the total. Inflorescence herbivory affected mostly the Ablaneda population.

Phenotypic and functional gender—Phenotypic gender was calculated as the female/male flower ratio for each inflorescence (equivalent to floral sex ratio of other authors, e.g., Sutherland, 1986). Functional gender should be ideally obtained by considering paternity of seeds produced (Snow and Lewis, 1993). In this study, an indirect estimate of functional gender, G , was calculated for the inflorescences of Avilés, following the formulae given by Lloyd (1980b) and Devlin and Stephenson (1987). Details known on the pollination dynamics in this population were included in order to give as much realism as possible to this estimate. The formula used was $G = F_i/[F_i + F_s(M/M_{ov})]$ where F_i is the number of fruits initiated by the focal inflorescence, F_s is the number of fruits potentially sired by the focal inflorescence during its male phase (i.e., number of fruits initiated by remaining inflorescences

TABLE 1. Relationships between plant size (grams of dry mass of leaves produced) and inflorescence or flower production in three populations of *Arum italicum*: (A) mean \pm 1 SD (sample size) plant size for plants with 0–4 inflorescences (within columns, means followed by different letters were significantly different in the SNK test), (B) R^2 (all relationships were positive), sample size (N), and significance (P) of regressions between plant size and size (spathe mass) of first inflorescence produced, flower production, and floral sex ratio.^a

Variable	Ablaneda			Avilés ^b			Tamón ^b		
A)									
Zero inflorescences	nd			nd			0.91 \pm 0.60 (6) A		
One inflorescence	3.25 \pm 2.04 (5) A			2.93 \pm 0.79 (15) A			1.96 \pm 1.17 (32) B		
Two inflorescences	5.30 \pm 3.41 (10) AB			6.02 \pm 2.47 (17) B			nd		
Three inflorescences	9.09 \pm 4.84 (6) B			6.35 \pm 1.37 (3)			nd		
Four inflorescences	33.24 \pm 0.00 (1)			8.81 \pm 0.00 (1)			nd		
ANOVA results	$F_{2,18} = 3.764^*$			$F_{1,30} = 27.071^{****}$			$F_{1,37} = 12.476^{**}$		
	R^2	N	P	R^2	N	P	R^2	N	P
B)									
Spathe mass	0.81	22	****	0.47	36	****	0.88	30	****
Total flower no.	0.18	13	ns	0.38	33	***	0.23	29	**
Total staminode no.	0.08	14	ns	0.32	34	***	0.00	29	ns
Total female flower no.	0.47	18	**	0.38	35	***	0.21	31	**
Total pistillode no.	0.17	13	ns	0.30	34	***	0.29	31	**
Floral sex ratio	0.10	15	ns	0.00	34	ns	0.04	31	ns

^a ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; nd no data.
^b Log-transformed data to analysis in part A.

in the population during the male phase of the focal inflorescence), M is the number of male flowers of the focal inflorescence, and M_{tot} is the total number of functional male flowers present during the male phase of the focal inflorescence (obtained from data on anthesis date of each inflorescence; range: 0–6 anthesis per day). This index ranges from 0 (exclusively male) to 1 (exclusively female). Inflorescences failing to both initiate and potentially sire fruits were considered functionally sterile.

The functional gender of plants was calculated according to Devlin and Stephenson (1987) as the sum, across all inflorescences produced, of fruits initiated and potentially sired:

$$G = \Sigma(F_i) / \Sigma(F_i) + \Sigma[F_s(M/M_{tot})].$$

Number of fruits initiated was chosen as a measure of reproductive success because in Avilés, an experiment was performed that affected infructescence maturation (Méndez, 1996) and thus a measure based on fruits or seeds matured would be biased. In this site, fruit and infructescence abortion was low in control plants, making the use of fruits initiated appropriate as surrogate of reproductive success. Estimates based on gametes (ovules, pollen) were not used because it was not possible to calculate them with certainty.

Two measures of functional gender, G , differing in the length of male phase (and thus, in the values of F_s , and M_{tot}), were used. G_1 considered only the D+1 day as the functional male period of inflorescences. This is the logical expectation based on the pollination system of this species

TABLE 2. R^2 (bold for negative relationships), sample size (N), and significance (P) of regressions of inflorescence size (spathe mass) vs. flower production or floral sex ratio in three populations of *A. italicum*, as a function of number of inflorescences produced and their order of production.^a

Population and variable	Inflorescence number																				
	Overall			One			Two (first)			Two (second)			Three (first)			Three (second)			Three (third)		
	R^2	N	P	R^2	N	P	R^2	N	P	R^2	N	P	R^2	N	P	R^2	N	P	R^2	N	P
Ablaneda																					
Male	0.12	146	****	0.33	33	***	0.01	38	ns	0.01	34	ns	0.07	15	ns	0.04	11	ns	0.04	11	ns
Staminodes	0.00	155	ns	0.07	38	ns	0.07	39	ns	0.19	36	**	0.00	15	ns	0.00	13	ns	0.05	13	ns
Female	0.25	161	****	0.42	39	****	0.18	40	**	0.09	36	ns	0.06	15	ns	0.00	14	ns	0.00	13	ns
Pistillodes	0.09	147	***	0.06	36	ns	0.07	39	ns	0.03	33	ns	0.00	14	ns	0.08	11	ns	0.23	11	ns
Sex ratio	0.04	146	*	0.04	33	ns	0.08	38	ns	0.20	34	**	0.00	15	ns	0.06	11	ns	0.03	11	ns
Avilés																					
Male	0.13	64	**	0.15	16	ns	0.25	18	*	0.00	17	ns	—	—	—	—	—	—	—	—	—
Staminodes	0.00	64	ns	0.02	16	ns	0.00	18	ns	0.03	17	ns	—	—	—	—	—	—	—	—	—
Female	0.08	65	*	0.35	17	*	0.20	18	ns	0.00	17	ns	—	—	—	—	—	—	—	—	—
Pistillodes	0.04	64	ns	0.15	17	ns	0.02	18	ns	0.04	17	ns	—	—	—	—	—	—	—	—	—
Sex ratio	0.00	64	ns	0.17	16	ns	0.00	18	ns	0.00	17	ns	—	—	—	—	—	—	—	—	—
Tamón																					
Male	0.30	30	**	0.30	30	**	nd	—	—	nd	—	—	nd	—	—	nd	—	—	nd	—	—
Staminodes	0.03	29	ns	0.03	29	ns	nd	—	—	nd	—	—	nd	—	—	nd	—	—	nd	—	—
Female	0.23	30	**	0.23	30	**	nd	—	—	nd	—	—	nd	—	—	nd	—	—	nd	—	—
Pistillodes	0.28	30	**	0.28	30	**	nd	—	—	nd	—	—	nd	—	—	nd	—	—	nd	—	—
Sex ratio	0.02	30	ns	0.02	30	ns	nd	—	—	nd	—	—	nd	—	—	nd	—	—	nd	—	—

^a ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; — not computed; nd no data.

TABLE 3. Mean \pm 1 SD (sample size) value of inflorescence traits in one-inflorescence plants of *A. italicum*, in three populations. For each variable, the second row shows the ANOVA results of the interpopulation comparison. Means followed by different letters were significantly different in the SNK test.^a

Variable	Ablaneda	Avilés	Tamón
Spathe mass (g) ^b	0.57 \pm 0.35 (39) A	0.58 \pm 0.20 (17) B $F_{2,83} = 20.131^{****}$	0.29 \pm 0.17 (30) C
Male flower no.	143 \pm 32 (33) A	118 \pm 25 (16) B $F_{2,77} = 6.236^{**}$	123 \pm 24 (31) B
Staminode no.	65 \pm 18 (38) A	64 \pm 17 (16) A $F_{2,80} = 1.480$ ns	58 \pm 13 (29) A
Female flower no. ^b	64 \pm 15 (39) A	48 \pm 10 (17) B $F_{2,84} = 6.600^{**}$	63 \pm 18 (31) C
Pistillode no.	17 \pm 5 (36) A	16 \pm 7 (17) A $F_{2,81} = 0.286$ ns	16 \pm 6 (31) A
Floral sex ratio	0.45 \pm 0.10 (33) AB	0.41 \pm 0.08 (16) A $F_{2,77} = 5.014^{**}$	0.51 \pm 0.13 (31) BC

^a ns $P > 0.05$; ** $P < 0.01$; **** $P < 0.0001$.

^b Analysis carried out on log-transformed data.

as described above. In addition, G_3 considered an inflorescence exporting pollen from D+1 to D+3 d (3 d). G_3 was used because of the observation of inflorescences being pollinated on days without pollen donor inflorescences in Avilés (Méndez, 1996). This could indicate pollen carry-over in the population (other alternatives are considered in the Discussion).

Statistical analyses—The relationships of number of flowers and floral sex ratio with plant and inflorescence sizes were studied using regression analysis. Trade-offs among types of flowers were studied by means of correlation analysis.

Differences in size, flower number, or floral sex ratio between populations or between plants within populations were tested using ANOVAs and a posteriori Student-Newman-Keuls (SNK) tests. If required, data were log transformed to achieve normality and homogeneity of variances. Differences in size, flower number, or floral sex ratio between inflorescences sequentially produced by a plant were tested using a t test for paired samples, after testing normality of the difference between each pair of values (Zar, 1984).

To remove the effect of size, two methods were used. ANCOVAs using spathe mass as the covariate were used in comparisons between populations or plants. The interaction between covariate and treatment was tested using the MANOVA procedure in SPSS (Hull and Nie, 1981). Residuals of a regression between flower number or floral sex ratio and the spathe mass were used in t tests for paired samples for removing the effect of the inflorescence size in within-plant analyses. Except where otherwise indicated, data are showed as means \pm 1 SD.

RESULTS

Size and phenotypic gender—*Relationships with plant size*—The number of inflorescences produced increased with plant size in all populations studied (Table 1A). The size of the first inflorescence produced, measured as its spathe mass, also increased with plant size in all populations studied (Table 1B).

In general, the number of flowers produced per plant also increased with plant size in all populations (Table 1B), although the percentage of variance explained was not very high ($\leq 48\%$). The relationships between plant size and number of sterile flowers produced were weaker or nonsignificant (Table 1B), excepting pistillodes in Tamón.

In Avilés, all significant relationships between plant size and flower number became nonsignificant when regressions were performed taking separately plants with

1–2 inflorescences (all $P > 0.346$). Thus, the flower number increased in discrete steps with the inflorescence number in this population. No analyses were possible in Ablaneda because of the small sample size. In Tamón, all reproductive plants produced one inflorescence.

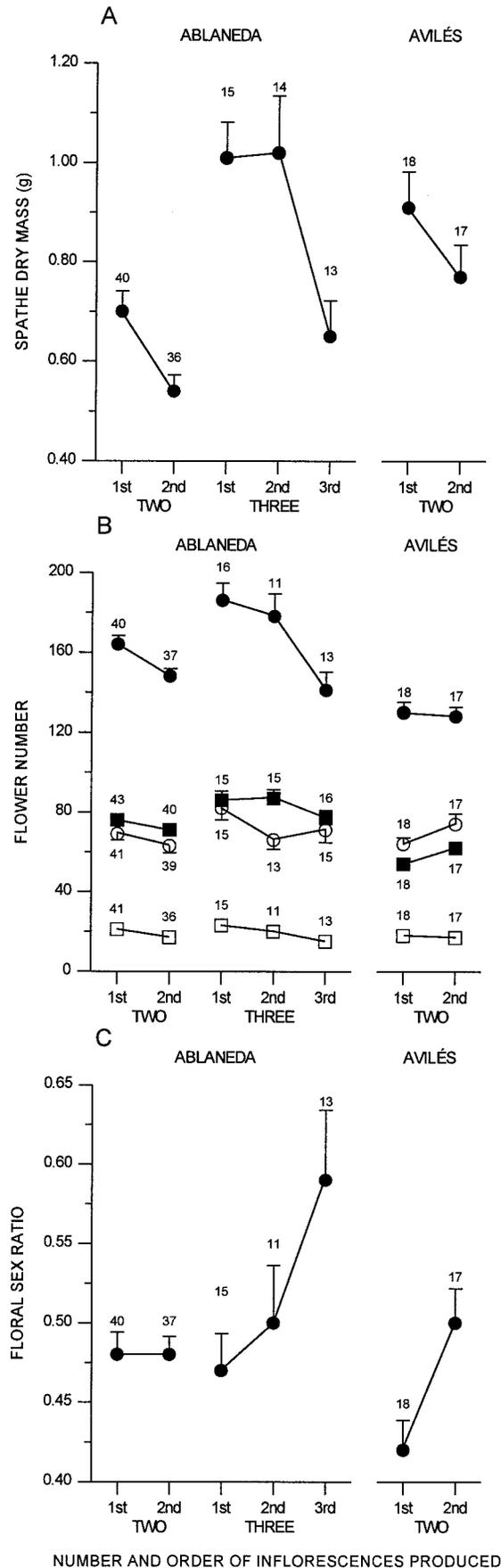
Floral sex ratio was not related to plant size in any population studied (Table 1B). In addition, differences in overall floral sex ratio between plants with different number of inflorescences were nonsignificant both in Avilés (one vs. two inflorescences: 0.41 ± 0.08 , $N = 16$ and 0.46 ± 0.08 , $N = 17$; $F_{1,31} = 2.574$, $P = 0.119$) and in Ablaneda (one vs. two vs. three inflorescences: 0.45 ± 0.10 , $N = 33$, 0.47 ± 0.07 , $N = 29$ and 0.52 ± 0.07 , $N = 6$; $F_{2,65} = 1.561$, $P = 0.218$).

Relationships with inflorescence size—Overall, inflorescence size was positively related to the number of flowers (Table 2). Again, the percentage of variance explained was low ($\leq 30\%$). Relationships of inflorescence size with sterile flowers were nonsignificant (Table 2), except for that of pistillodes in Ablaneda and Tamón.

For Ablaneda and Avilés, most significant relationships between inflorescence size and flower number became nonsignificant when data were analyzed by considering inflorescences in their order of production (Table 2). However, some exceptions to this trend were found. In one-inflorescence plants, an increase in the variance explained was found for female flowers (in both sites) and male flowers (Ablaneda). A significant negative relationship was found for staminodes in the second inflorescence of two-inflorescence plants of the Ablaneda population (Table 2).

ANCOVAs showed no significant difference (all $P > 0.329$) in slope between regressions of male and female flowers with inflorescence size for Avilés and Tamón. However, in Ablaneda these differences were almost significant for the overall data and for the first inflorescence of two-inflorescence plants ($P > 0.052$ and $P > 0.073$, respectively).

The relationships between floral sex ratio and inflorescence size were nonsignificant in Avilés and Tamón (Table 2). This relationship was positive for the overall data set and for the second inflorescence of two-inflorescence plants in Ablaneda (Table 2).



Differences between populations—Between-population comparisons were limited to plants with one inflorescence. Significant differences between populations were found in mean spathe mass (Table 3). Spathes were biggest in Avilés and smallest in Tamón (Table 3). Male and female flower numbers were highest in Ablaneda (Table 3). All comparisons between populations involving sterile flowers were nonsignificant (Table 3). Floral sex ratio decreased in the order Tamón > Ablaneda > Avilés (Table 3).

Similar results were obtained when the effect of inflorescence size was removed using ANCOVA (results not shown). The effect of the spathe mass covariate was significant ($P < 0.001$) in all cases. The covariate X population interaction was significant ($P < 0.05$) only for pistillode number.

Temporal trends in flower production and floral sex ratio—Inflorescences sequentially produced by a plant showed a decrease in spathe mass (Fig. 2A). In Ablaneda, this decrease was significant for the last inflorescence in both plants with two and three inflorescences (Table 4). However, in Avilés this decrease was nonsignificant in two-inflorescence plants (Table 4). Plants with three inflorescences were too scarce to perform any analysis.

In the case of the two-inflorescence plants of Ablaneda, all flower types decreased in number from the first to the last inflorescence produced (Fig. 2B, Table 4). Plants with three inflorescences showed the same general trend, but it was only significant for male flower number and pistillodes (Fig. 2B, Table 4). In Avilés, the mean number of staminodes and female flowers were higher in the second inflorescence produced (Fig. 2B, Table 4). Remaining types of flowers did not significantly vary in number among successive inflorescences (Fig. 2B, Table 4).

For plants with two inflorescences, similar results were obtained after removing the effect of the inflorescence size, excepting female flowers in Ablaneda (Table 4). However, all tests became nonsignificant for three-inflorescence plants (Table 4).

In Ablaneda, the floral sex ratio significantly increased in the second inflorescence produced by two-inflorescence plants (Fig. 2C, Table 4). The same trend was found for three-inflorescence plants, but the increase was nonsignificant (Table 4). In Avilés, the floral sex ratio significantly increased from the first to the second inflorescence produced (Fig. 2C, Table 4). In all cases, similar results were obtained when the effect of the inflorescence size was removed (Table 4).

Trade-offs between flower types—At the three sites, significant correlations among flower types were positive (Table 5). In addition, the correlations for number of flowers of the same type among inflorescences sequen-

Fig. 2. Modification of inflorescence traits in inflorescences sequentially produced by plants of *A. italicum* in two populations: (A) spathe dry mass, (B) flower number (closed circles: male; open circles: staminodes; closed squares: female; open squares: pistillodes), and (C) floral sex ratio. Bars above or under each symbol indicate SE. Numbers above or below bars indicate the sample size.

TABLE 4. Results of paired *t* tests for differences in reproductive traits between the first and the last inflorescence produced by two- and three-inflorescence plants in two populations of *A. italicum*. For each flower type, data in the first row refer to original analyses and data in the second row to analyses after removing the effect of the inflorescence size.^a

Variables	Ablaneda						Avilés		
	Two inflorescences			Three inflorescences			Two inflorescences		
	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>
Spathe mass	29	5.46	***	8	5.79	***	16	2.09	ns
Male flower no.	29	4.84	***	9	4.46	**	16	0.18	ns
	29	4.05	***	8	0.47	ns	15	-0.29	ns
Staminode no.	30	2.52	*	9	0.69	ns	16	-3.34	**
	29	6.17	***	8	-0.59	ns	15	-2.43	*
Female flower no.	33	3.41	**	11	1.79	ns	16	-3.32	**
	28	0.82	ns	7	0.36	ns	15	-3.45	**
Pistillode no.	29	5.79	***	8	3.47	**	16	0.50	ns
	25	4.30	***	5	1.42	ns	15	0.37	ns
Floral sex ratio	28	-2.58	*	8	-2.24	ns	16	-4.83	***
	25	-4.62	***	5	-0.67	ns	15	-4.43	***

^a ns *P* > 0.05; * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001; **** *P* < 0.0001.

tially produced by a plant were either positive or nonsignificant (Table 5).

Functional gender—For inflorescences, G_1 showed a bimodal distribution, with a peak for functionally sterile inflorescences and another peak in exclusively female inflorescences (Fig. 3A). G_3 showed a smoother distribution, with a third central peak for inflorescences contributing roughly equally male and female genders (Fig. 3A). Functionally sterile inflorescences predominated at the beginning and the end of the flowering period (Fig. 4). There was no significant relationship of G_1 or G_3 with the floral sex ratio ($R_s = 0.149$, $N = 41$, $P = 0.352$ for G_1 ; $R_s = 0.030$, $N = 48$, $P = 0.579$ for G_3) or with the spathe mass ($R_s = 0.080$, $N = 41$, $P = 0.619$ for G_1 ; $R_s = 0.008$, $N = 48$, $P = 0.957$ for G_3).

Plants showed different patterns of functional gender as a function of the number of inflorescences produced. Using G_1 , plants with one inflorescence were either functionally sterile or mostly female; plants with ≥ 2 inflorescences showed a more spread gender (Fig. 3B). Using G_3 , one-inflorescence plants were either male or near female; plants with several inflorescences showed a functional gender around 0.5 (Fig. 3B). No significant relationship was found between either G_1 or G_3 and plant size ($R_s = -0.292$, $N = 18$, $P = 0.240$ for G_1 ; $R_s = 0.206$, $N = 22$, $P = 0.357$ for G_3). For two-inflorescence plants, no significant difference was found in G_1 or G_3 between the first and the second inflorescence produced (0.60 ± 0.39 , $N = 13$ vs. 0.73 ± 0.39 , $N = 11$, $F_{1,22} = 0.524$, $P = 0.477$ for G_1 ; 0.43 ± 0.35 , $N = 15$ vs. 0.56 ± 0.38 , $N = 12$, $F_{1,25} = 0.821$, $P = 0.374$ for G_3).

TABLE 5. Correlations between the number of flowers of each type in three populations of *A. italicum*. For each population, correlations within an inflorescence are shown in the lower hemimatrix and correlations between flowers of the first and last inflorescence produced by two- and three-inflorescence plants are shown in the diagonal. *r* values (sample size) and significance are given.^a

Population and variable	Fertile	Sterile	Male	Staminodes	Female	Pistillodes
Ablaneda						
Fertile	0.62 (42)***					
Sterile	0.50 (54)***	0.79 (39)***				
Male	—	—	0.58 (39)***			
Staminodes	—	—	0.38 (147)***	0.82 (41)***		
Female	—	—	0.54 (152)***	0.26 (161)**	0.69 (46)***	
Pistillodes	—	—	0.45 (145)***	0.18 (149)*	0.38 (153)***	0.48 (39)**
Avilés						
Fertile	0.37 (17) ns					
Sterile	0.21 (18) ns	0.78 (17)***				
Male	—	—	0.48 (17) ns			
Staminodes	—	—	0.03 (64) ns	0.82 (17)***		
Female	—	—	0.47 (64)***	0.30 (64)*	0.37 (17) ns	
Pistillodes	—	—	0.48 (63)***	0.03 (63) ns	0.36 (64)**	0.40 (17) ns
Tamón						
Fertile	nd					
Sterile	0.60 (29)**	nd				
Male	—	—	nd			
Staminodes	—	—	0.44 (29)*	nd		
Female	—	—	0.56 (31)**	0.26 (29) ns	nd	
Pistillodes	—	—	0.65 (31)***	0.27 (29) ns	0.56 (31)**	nd

^a ns *P* > 0.05; * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001; — not computed; nd no data.

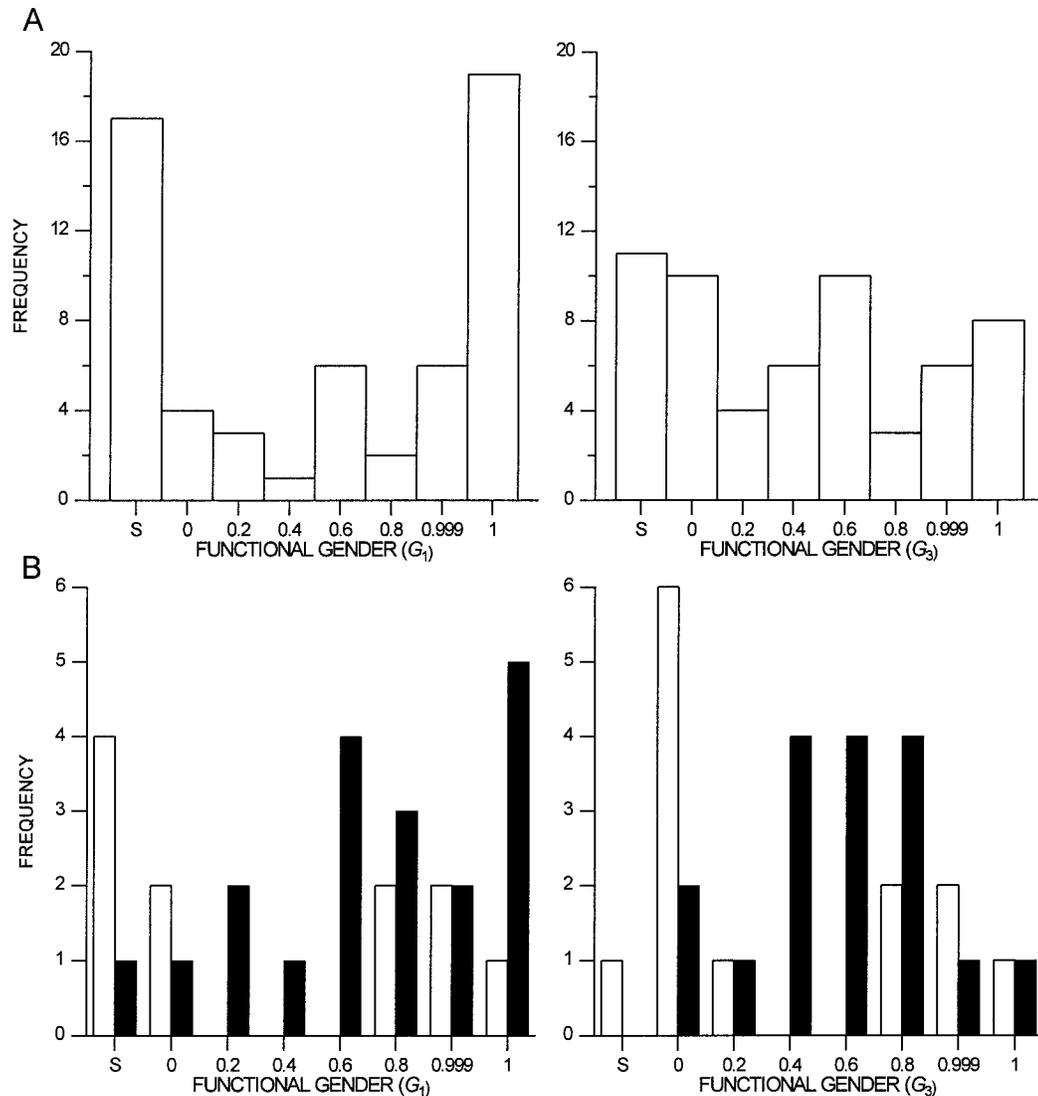


Fig. 3. Functional gender of: (A) inflorescences and (B) plants in the Avilés population (open bars: one-inflorescence plants; closed bars: multi-inflorescence plants). Two gender indexes are shown, G_1 and G_3 (see Materials and Methods for further explanation). Excepting 0, 1, and S, marks on the x-axis refer to the upper value of the intervals. S: functionally sterile.

DISCUSSION

Plant size, reproduction, and phenotypic gender—

Plant size is involved in several phases of reproduction. First, it can determine the probabilities of reproduction (Lacey, 1986). Secondly, it may influence the number of inflorescences or flowers produced (Chaplin and Walker, 1982) and the proportion of resources allocated to reproduction (Samson and Werk, 1986). Finally, it can influence the gender (de Jong and Klinkhamer, 1994). Both the probability of reproduction and the inflorescence and flower number were size dependent in *Arum italicum* (Méndez and Obeso, 1993; present study). However, the increase in flower number seemed to be mainly related to the inflorescence production and its relationship with plant size was not very strong. The same situation seems to occur in other *Arum* species. In *Arum maculatum*, no relationship was found between the number of mature, sagittate leaves and female flower number per inflores-

cence (Jeff Ollerton, Nene College, UK, personal communication).

Size-dependent changes in phenotypic gender have been found in several monoecious species (Burd and Allen, 1988; Fox, 1993). For anemophilous plants, maleness is positively related to plant size; for entomophilous plants, femaleness is related to higher plant size (Bickel and Freeman, 1993). In *Arisaema dracontium* and *A. triphyllum*, femaleness increased with plant size (Policansky, 1981; Lovett-Doust and Cavers, 1982; Clay, 1993). Clay (1993, Fig. 2) proposed four mechanisms for this increase based on different variation of male and female flower number with increases in plant size. *Arisaema dracontium* showed a decrease in male flower number with plant size and an increase in female flower number (Clay, 1993). In *Arum italicum*, both male and female flower number increased with plant and inflorescence size. Although in Ablaneda phenotypic femaleness was allometrically related to inflorescence size, in general both

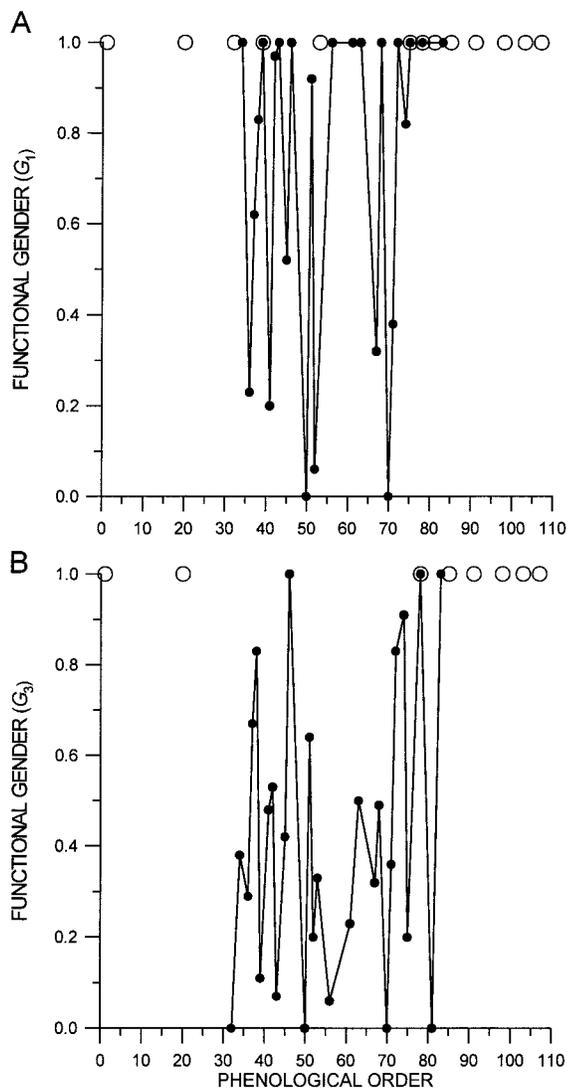


Fig. 4. Functional gender of inflorescences of *A. italicum* in the Avilés population as a function of their phenological order: (A) G_1 , (B) G_3 . Open circles indicate functionally sterile inflorescences.

plant and inflorescence sizes were poorly related to gender modification in the populations studied.

Modification of phenotypic gender in *Arum italicum*—Nevertheless, gender variation was found in *A. italicum*. First, there were differences among populations in the floral sex ratio. These differences were independent of plant or inflorescence size. No environmental variable was measured in this work, so further analysis of these patterns was not possible.

Second, a modification of gender was found among successive inflorescences within a plant. This modification showed certain plasticity, involving either an increase or a decrease in flower number in different populations. Within-plant gender variation has been found for several hermaphroditic species, either toward a higher maleness (Bawa and Webb, 1983) or toward a higher femaleness (Mossop, Macnair, and Robertson, 1994). The monoecious *Zizania palustris* showed a trend toward a

higher femaleness (Goldman, 1991). An allometric relationship between inflorescence size and flower number did not fully explain this modification in *Arum italicum*.

Two hypotheses could explain the modification in phenotypic gender of successive inflorescences in a plant. First, the decrease in inflorescence size found here and in other species (Goldman, 1991; Ashman and Baker, 1992; Mossop, Macnair, and Robertson, 1994) could be associated with the sequential adjustment of reproductive allocation caused by a progressive depletion of resources (Lloyd, 1980a). If earlier inflorescences limit the resources available for later ones, a modification in gender toward increased maleness could be adaptive. However, the trend toward a higher femaleness seems paradoxical because the female function is usually considered more expensive (Freeman et al., 1981). The second hypothesis is that a variation in mating environment could alter the prospects of success by means of one or another gender (Brunet and Charlesworth, 1995). The modification of gender in *A. italicum* agreed with predictions for protogynous species. However, no correlation was found between functional gender and phenology. Such a correlation could occur in other populations or years, but at present no data are available on this subject. Thus, generalization is not possible.

Functional gender—Theory usually predicts that smaller plants should be functionally male (Charnov, 1982; Pickering and Ash, 1993; see, however, Ackerly and Jasienski, 1990). This is due to a lower availability of resources for seed production (Stephenson, 1981) or, in species that produce few flowers, to the unpredictability of pollination (Pickering and Ash, 1993). In *A. italicum*, no modification of functional gender was found with plant size. The relationship with plant size was only indirect, mediated by the number of inflorescences produced. One-inflorescence plants showed a specialization in one gender, while multi-inflorescence plants showed, on average, an even representation by both genders. This favors the hypothesis of a higher unpredictability of pollination for plants with only one inflorescence. In addition, this unpredictability could influence the lack of relationship among phenotypic and functional gender (Devlin and Stephenson, 1987). The use of an indirect estimate of functional gender could also influence the lack of correlation.

Differences between G_1 and G_3 as functional gender indexes were apparent. They are useful as a conceptual model to examine the consequences of pollen carry-over. The main effect of calculating G_3 was an increase of male success and a decrease of functionally sterile inflorescences, and it seems to indicate a benefit of a higher synchronization of flowering at the population level. Pollen carry-over could be achieved by means of a higher longevity of pollen or by means of a gradual escape of pollinators. However, trinucleate pollen of genus *Arum* (Grayum, 1990) is associated with a fast loss of viability (Dafni, 1992). In Avilés, a gradual escape of pollinators was not possible because the spathe was removed on the D+2 day. Although the isolation of the population of Avilés was high, the possibility of arrival of pollen from outside of the population cannot be ruled out as an al-

ternative to explain the inflorescence pollination in days without pollen donors.

Trade-offs among flower types—Lloyd (1992) proposed the existence of a trade-off among fertile and sterile flowers. Also, the theory of sex allocation predicts the existence of a trade-off among allocation to each gender (Charnov, 1982). No such trade-off was found at the phenotypic level analyzed in this paper. This absence of a trade-off also appears to occur in other species (Goldman, 1991; Damgaard and Loeschcke, 1994; Mossop, Macnair, and Robertson, 1994). Competition between male and female functions assumes that these functions share the same resources (Burd and Head, 1992). However, each gender could be limited by different resources (Ashman and Baker, 1992). Also, Worley and Harder (1996) proposed that traits derived from a common process, as in the formation of an inflorescence, are more likely to be positively related. Nevertheless, the existence of a between-year trade-off cannot be discarded and warrants further study.

Although sterile flowers have a specific function during pollination, their exact number may not be very important for the proper functioning of the trap. Their relative variability could explain the lack of relationship with inflorescence and plant sizes.

In conclusion, variability in phenotypic gender in *A. italicum* does not seem to follow the size advantage model. Also, the lack of correlation between phenotypic and functional gender during the flowering period does not give (tentatively) support to the prediction of Brunet and Charlesworth (1995) of an increasing female gender for protogynous species in response to a changing mating environment. Sex of flowers in this species did not seem to exhibit a phenotypic trade-off.

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