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Sexual Dimorphism and Gynoecium Size Variation in the Andromonoecious Shrub *Caesalpinia gilliesii*

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Abstract: **The degree of sexual dimorphism in flowers and inflorescences can be evaluated early in flower development through the study of floral organ size co-variation. In the present work, the gynoecium–androecium size relationship was studied to assess the degree of sexual expression in flowers and inflorescences of the andromonoecious shrub *Caesalpinia gilliesii.* The co-variation pattern of floral organ sizes was compared between small and large inflorescences, under the hypothesis that inflorescence size reflected differential resource availability. Also, staminate and perfect flowers were collected from three populations and compared on the basis of gynoecium, ovule length, filament length, pollen size and number. The obtained results indicated that staminate and perfect flowers differed only in the gynoecium and ovule length, whereas filament length, pollen size, and number varied across populations. The gynoecium size was smaller and its variability was much more higher in staminate than in perfect flowers, as explained by a recent hypothesis about pollinator-mediated gynoecium size selection acting upon perfect flowers. The analysis of the gynoecium–androecium size relationship during flower development, revealed a dissociation of gynoecium growth relative to other floral structures in some buds. Lower gynoecium–androecium regression slopes and smaller gynoecia length characterized smaller inflorescences, thus reflecting the fact that sexual expression was more male-biased. This trend is in agreement with a differential resource-related response at the inflorescence level, however, post-mating resource allocation and the inclusion of other modular levels may also help us to understand the variation in sexual dimorphism in this species.**

Key words: **Gynoecium–androecium size relationship, inflorescence size, sexual expression, floral dimorphism.**

Introduction

Spatial and/or temporal variation in the size of floral organs has diverse consequences on reproduction, and it is of particular interest to understand the reproductive system of a species (Diggle, 1992; Eckhart, 1999). Despite the occurrence of continuous quantitative size variation in the floral organs of several species, others exhibit a substantial change in gynoecium–androecium relative growth that results in a smaller atrophied organ and the lack of one reproductive function (e.g., Diggle, 1991a; Tucker, 1992).

In species with sexual dimorphic systems, the dissociation in gynoecium–androecium growth is a common pattern that reflects sexual expression during floral development (Diggle, 1991a, 1992; Tucker, 1992; Han and Liu, 1999; Zhou et al., 2002; Caporali et al., 2003). Such intraspecific variability in gynoecium growth, appear linked to the resource status of the plant/inflorescence in some species. In a gynodioecious species, the regression slopes for the gynoecium–flower length relationship were steeper for female-biased trees and dependent on the fruiting status of the plant (Gibson and Diggle, 1998). In other species with dimorphic systems, the allocation to reproductive structures, or more precisely, the allometric relationship between the gynoecium and androecium sizes, was a function of inflorescence size (Koelewijn and Hunscheid, 2000; Méndez, 2001). Larger inflorescences were found, however, to be either female- or male-biased (Koelewijn and Hunscheid, 2000; Méndez, 2001).

In andromonoecious species, staminate flowers are also hermaphroditic at the beginning of development and become female-sterile by dissociation of gynoecium growth relative to the androecium (Diggle, 1992). Because of such dissociation, the regression slopes for the gynoecium–flower lengths are lower in staminate than in perfect flowers that show a stronger size correlation among floral structures and less variable gynoecium size (Diggle, 1991 a, b; Ushimaru et al., 2003 a, b). The production of staminate flowers in andromonoecious plants was, in general, related to resource depletion, with a weaker gynoecium–flower length relationship in fruiting inflorescences (Diggle, 1991 a, b). The link between low resource levels and staminate flower production was also evinced when the proportion of perfect flowers instead of the gynoecium size variation was taken into account. Thus, the presence of fruits, as well as other factors like florivory, plant, and inflorescence size, was found to be associated with the proportion of staminate flowers in several andromonoecious species (Emms, 1996; Diggle, 1997; Krupnick and Weis, 1998; Gibbs et al., 1999). In these examples, the obtained pattern also suggests that staminate flower production responds to lower resource

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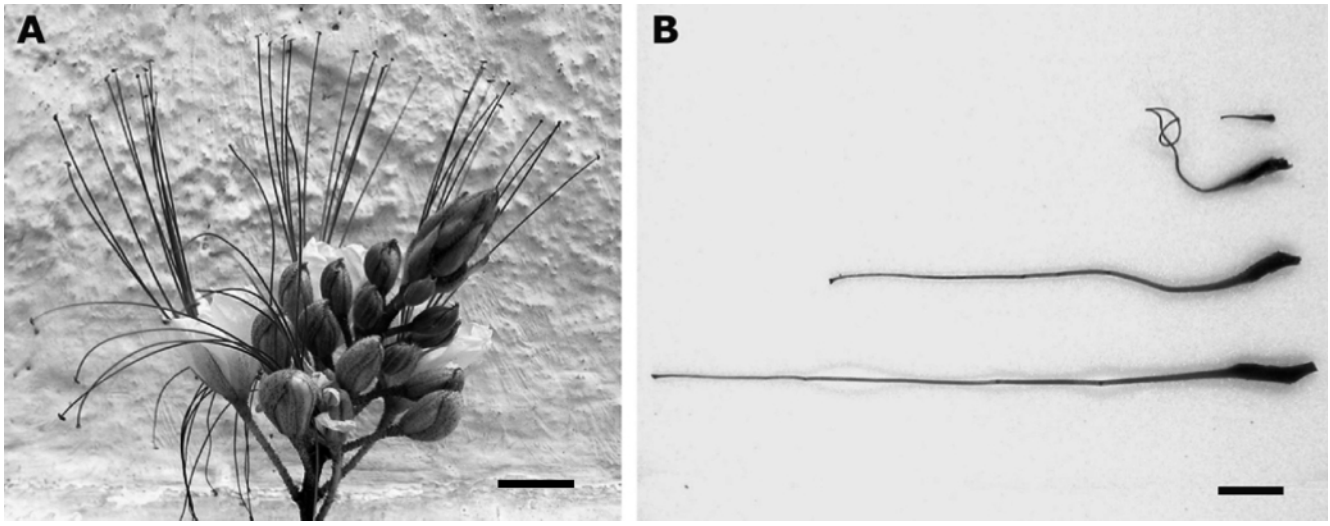

Fig. 1 (**A**) *Caesalpinia gilliesii* inflorescence showing the sequential blooming pattern, bar = 2.4 cm. (**B**) Isolated gynoecia from different flower morphs, from bottom to top two gynoecia of perfect flowers and two of female-sterile flowers. Observe the size variation among and within flower types, and the curved style in one of the sterile gynoecia, bar = 1 cm.

levels. However, earlier changes in the sexual expression cannot be detected if only the final proportion of flower morphs is considered. This may be of special relevance if the regulation of maternal investment is made before anthesis, as in several andromonoecious species (Lloyd, 1980). Moreover, the form and function of a given flower has been observed to depend on spatial and temporal context during its ontogeny (Diggle, 1997). Then, the study of sexual expression through analysis of gynoecium size variation in both flowers and buds may contribute to elucidate if the same factors, for instance the resource level, are operating at the time of sex determination during floral development.

Caesalpinia gilliesii is an andromonoecious shrub that shows variability in the production of staminate and perfect flowers at the plant and inflorescence levels (Cocucci et al., 1992; Jausoro and Galetto, 2001). An inflorescence produces 25 flowers on average which may be all staminate, perfect, or more commonly a mixed array of both flower types (Jausoro and Galetto, 2001). Inflorescences begin blooming from the base and continue upwards (Fig. **1 A**). The perfect flower of *C. gilliesii* has a long flexible style that usually surpasses the stamens (Cocucci et al., 1992; Jausoro and Galetto, 2000). Staminate flowers generally have an atrophied gynoecium (Fig. **1 B**) or it is reduced in size, with a more or less curved style (Cocucci et al., 1992; Jausoro and Galetto, 2000). The different flower types were previously studied in relation to nectary structure and no substantial differences were found (Jausoro and Galetto, 2000), however, other floral features related to sexual dimorphism of the flower (i.e., pollen production) have not been investigated.

Here, the floral dimorphism of *C. gilliesii* is studied with respect to the size of reproductive structures, and to the gynoecium–androecium co-variation size during floral development. We also explored these relationships between different sized inflorescences, as a first step to address factors that may account for the sexual dimorphism in this species. The aim of this work is to address the following questions: 1) What are the main floral features related to sexual dimorphism in *C. gilliesii* flowers? 2) Does the change in the gynoecium–androecium size during floral development reflect sexual dimorphism? 3) Does the pattern of the gynoecium–androecium size relationship observed vary independently of inflorescence size? We postulate that the expression of female-sterile flowers in *C. gilliesii* may respond to differential resource availability, mainly generated by the size of the inflorescence. In the extent to which staminate flower production is reflected by weaker gynoecium–androecium size relationships, smaller inflorescences with a potentially lower resource availability would also exhibit weaker gynoecium–androecium size co-variation and then male-biased sexual expression in comparison with larger inflorescences. As staminate flowers have a reduced gynoecium size, a lower mean gynoecium size is also expected for smaller inflorescences.

Materials and Methods

Sexual dimorphism in Caesalpinia gilliesii *flowers*

To compare staminate and perfect flowers with regard to sexual dimorphism, 20 to 28 flowers per morph per population were collected between October–November of 2000 from three populations: San Nicolás, La Quebrada, and Alta Gracia (SN, LQ, and AG hereafter) in Córdoba province, Argentina. To avoid any difference in the size of the floral organs due to flower position within the inflorescence or to post-anthesis size changes, only newly opened flowers from the three first basal positions were collected. In general, staminate flowers can be easily recognized by a rudimentary gynoecium. If there was some doubt about the sex of the flower, dissection of the ovary and observation of the ovules allowed us to distinguish between staminate and perfect flowers. Ovules of the staminate flowers are smaller and papery in comparison with those of perfect flowers. Gynoecium and filament lengths were measured in all the flowers collected (n = 75 staminate and 93 perfect), and a subsample of 90 flowers (n = 49 staminate and 41 perfect) was used to measure and count the number of ovules and pollen grains per flower. In these flowers, the anther of the

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outer stamen facing the keel was dissected and all the pollen grains were counted under a stereoscopic microscope at 25 ×. Pollen volume was calculated as $\pi PE^2/6$ (Harder, 1998), where P and E are the polar and equatorial diameter, respectively. Measurements of pollen grain diameters were made with an ocular micrometer on 10 pollen grains per flower at 40 ×. The ovule size was estimated as the ovule length from the calazal to the micropilar end at 40 ×. Ovule length should be log-transformed to obtain normality. Comparisons were made with a two-way ANOVA, with population and flower type as fixed factors. Type III sum of squares was used because of unbalanced data (Shaw and Mitchell-Olds, 1993). Because of non-normality, the number of ovules per flower was compared with non-parametric tests. The variability in the size/number of the floral structures was expressed as a percentage of the coefficient of variation (CV = standard deviation/mean ■ 100).

Gynoecium–androecium size relationships

To analyze changes in the gynoecium–androecium size during flower development, whole inflorescences were collected from 47 plants, and fixed in ethylic alcohol : acetic acid (3 : 1) in October 2002 from SN and LQ populations. Only non-fruiting inflorescences were used. The filaments and style continued elongating inside the buds, so that flowers buds were previously dissected in order to measure complete organ length. Because of the differences in length between outer and inner filaments at earlier stages, the inner filament was also measured in flower buds. Again, the filament of the stamen facing the keel was used. The bud, gynoecium, and filament lengths were measured with digital calipers at 0.01 mm (n = 1021 flower buds, 47 inflorescences).

Regression slopes were addressed as a useful tool to examine between and within species variability in the size relationship between a pair of floral organs (e.g., Gibson and Diggle, 1998; Ushimaru and Nakata, 2001, 2002). Following these authors, the regression slopes for the log-transformed data were used here to analyze the intra-specific variability of the gynoecium–androecium size relationships with regard to inflorescence size.

Differences in slopes were tested for three bud size categories that correspond to different stages in floral development (Table **1**, Fig. **2**). Stage I corresponds to the smallest sized buds, where floral organ parts are differentiated and the gynoecium is typically longer than the stamens. At stage II, the shape of the organs is defined and they start to enlarge; the stamens had reached the gynoecium length, and sexual differentiation of the staminate flower had occurred (Carrizo García and Calviño, in prep.■). Stage III corresponds to a period of major organ enlargement, particularly filaments and style, and quantitatively size differences may then be found. At this time, some flower buds are clearly female-sterile.

Inflorescence size characterization

Length and stem diameter have previously been observed to influence sexual allocation, both within and between inflorescences, in a way that longer and/or thicker inflorescences accounted for a higher allocation to female organs (e.g., Vaughton, 1993; Machon et al., 1995). Here, stem width and length were used to characterize each inflorescence with respect to their size. The stem diameter of the inflorescence and the length from the first flower bud to the top of the inflorescence were measured with digital calipers. Inflorescences ranged from 6.30 to 11.83 cm in length, with a mean length of 8.37 ± 1.33 cm (mean ± 1 s.d.). Stem diameter ranged from 2.26 to 4.00 mm in width (3.28 ± 0.10). The Pearson correlation coefficient between inflorescence length and diameter was r = 0.58 ($p = 0.001$), suggesting that larger inflorescences were associated with greater stem diameter. The mean length was used to group inflorescences into two classes. One size class corresponded to inflorescences with length values equal to or higher than the mean ("large" inflorescences), and other class with lengths lower than mean ("small" inflorescences). The two

Table 1 Mean length ± standard deviation of floral structures measured in three bud size categories used in the analysis. I = small, II = intermediate, and III = large. All measurements are expressed in mm

	Bud size categories		
	I	II	III
Gynoecium	5.04 ± 2.21	13.63 ± 7.02	32.84 ± 20.11
Outer filament	3.20 ± 0.76	10.84 ± 8.16	38.31 ± 19.42
Inner filament	1.50 ± 0.53	9.59 ± 9.28	40.40 ± 21.01
Bud	7.29 ± 1.72	13.20 ± 1.57	18.37 ± 2.01
n	90	231	173

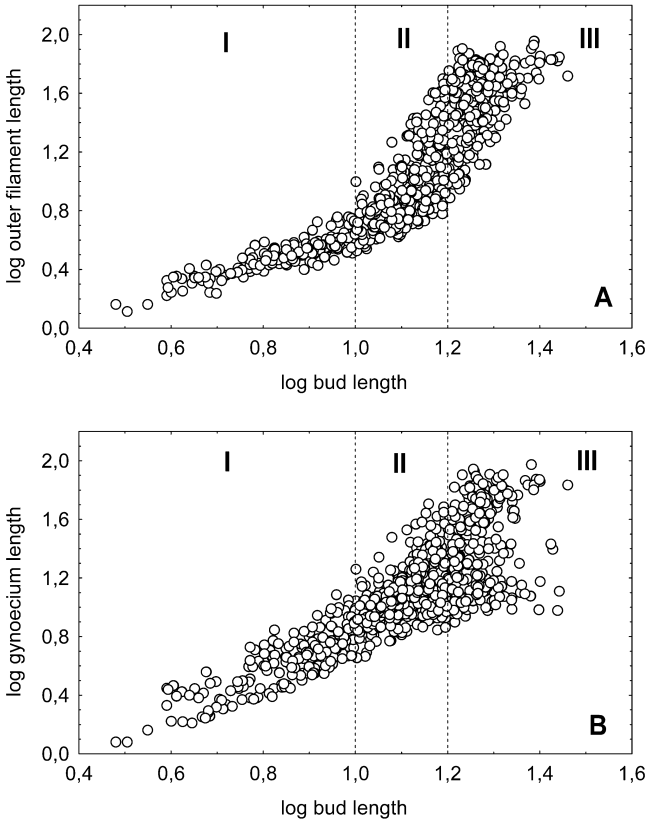

Fig. 2 Log-log plots of the outer filament (**A**) and gynoecium (**B**) lengths against bud length in *Caesalpinia gilliesii* flower buds. Roman numbers indicate bud size categories used in the analysis. I = small, II = intermediate, III = large.

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Table 2 Two way ANOVA for the floral characters measured. The *F* values for each source of variation are shown. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$

Sources of variation	Gynoecium length	Ovule length	Filament length	Pollen number	Pollen volume
Flower type	**183.10******	**37.90******	0.02	0.12	2.67
Population	0.51	**3.95***	**7.68*****	**5.65****	**5.91****
Flower type × Population	2.67	**6.97****	**4.94****	2.95	0.28

Table 3 Floral characters and measurements of *C. gilliesii* flowers. Means and CV (%) from perfect and staminate flowers of three populations are shown. AG= Alta Gracia, SN= San Nicolás, LQ= La Quebrada

Flower type	Gynoecium length (mm)	Ovule length (mm)	Ovule number	Filament length (mm)	Pollen number	Pollen volume ($\times 10^5\mu^3$)
AG						
Perfect	80.23 (22.6)	0.77 (20.7)	8.27 (11.6)	75.38 (10.8)	11888.75 (15.2)	2.34 (9.8)
Staminate	33.11 (59.8)	0.54 (16.6)	8.63 (8.57)	67.11 (18.1)	14115.56 (21.4)	2.28 (4.8)
SN						
Perfect	73.33 (33.8)	0.86 (10.5)	7.71 (19.4)	74.36 (11.7)	11020.00 (19.9)	2.53 (18.5)
Staminate	29.79 (52.3)	0.35 (37.1)	7.57 (14.9)	81.24 (8.7)	10020.20 (33.2)	2.72 (15.8)
LQ						
Perfect	84.06 (28.9)	0.58 (32.7)	8.54 (9.13)	78.53 (11.9)	12872.31 (14.7)	2.20 (8.6)
Staminate	23.50 (87.2)	0.42 (40.5)	8.44 (15.7)	79.11 (11.0)	12260.00 (18.6)	2.35 (13.2)

groups defined in this way had obviously significantly different mean lengths (7.43 ± 0.64; 10.05 ± 2.01 cm in length for small and large inflorescences, respectively; t-value – 4.48, $p<0.0001$), but also differed in their basal stem diameter (3.02 ± 0.40; 3.73 ± 0.13 mm in width, respectively; t-value – 4.83, $p<0.0001$). All inflorescences from the SN population fell into the "large" category and were not considered for further analysis. Then, and because some of the inflorescences collected differed in developmental stage, only young inflorescences (those with no more than 4 open flowers) from the LQ population were used to compare gynoecium–androecium regression slopes between small (S) and large (L) inflorescences (n = 20 inflorescences; 12 short and 8 long).

Within each bud size category, the average bud length did not differ between S and L inflorescences (t-values 0.23, $p=0.820$; – 0.50, $p=0.619$; and – 0.96, $p=0.336$ for small, intermediate, and large flower buds, respectively). The heterogeneity between slopes for S and L inflorescences was compared with ANCOVA (Sokal and Rohlf, 1995). For L inflorescences only, gynoecium–androecium slopes were also compared between SN and LQ populations for the intermediate bud size.

Results

Sexual dimorphism in C. gilliesii *flowers*

Staminate flowers had significantly smaller gynoecia and ovules (Table **2**). Ovule length differed significantly between both floral morphs and populations and, together with gynoecium length, was the highest variable character, especially in staminate flowers (Table **3**, overall CV: 57.6% and 32.8% for gynoecium and ovule lengths, respectively). The difference in ovule length between staminate and perfect flowers was less conspicuous in the LQ population (Table **3**), which may account for the significant flower type per population term in the ANOVA (Table **2**). The number of ovules did not differ between flower types (Mann-Whitney U = 676.0, $p=0.680$), nor across populations (Kruskall-Wallis H = 4.68, $p=0.09$). The size relationship between the gynoecium and filament length was stronger for perfect flowers, as expressed by a steeper regression slope ($\beta=0.62$ and 0.25 for perfect and staminate flowers, respectively; the regression slope for staminate flowers was not significantly different from zero). Despite this, the gynoecium–androecium size variation of perfect flowers resulted in a considerable stigma–anther separation that ranged from – 17 to 25 mm.

The size and number of the pollen grains differed among populations (Table **2**). The filament length showed differences across populations but also a significant flower type × population interaction (Table **2**). Pollen size and number were less variable than gynoecium and ovule lengths (overall CV: 9% and 16.2% for pollen volume and number, respectively).

Gynoecium–androecium size relationships in buds

The filament–bud length relationship showed an initial close co-variation with a rapid increase in size from stage II (Fig. **2A**). The gynoecium–bud length relationship showed a different trend, particularly at stage III (Fig. **2B**). At this time, and for the same bud length, two groups of buds appear: some with short (*ca.* 10 mm) and others with long gynoecia (*ca.* 63 mm) (Fig. **2B**). This trend is clearer when gynoecium is plotted against filament length. The initial co-variation pattern of gynoecium–filament length observed at stage I was followed by divergence growth at a filament length of *ca.* 7 mm, that is, at an intermediate bud size (Fig. **3**, Table **1**). However, there was

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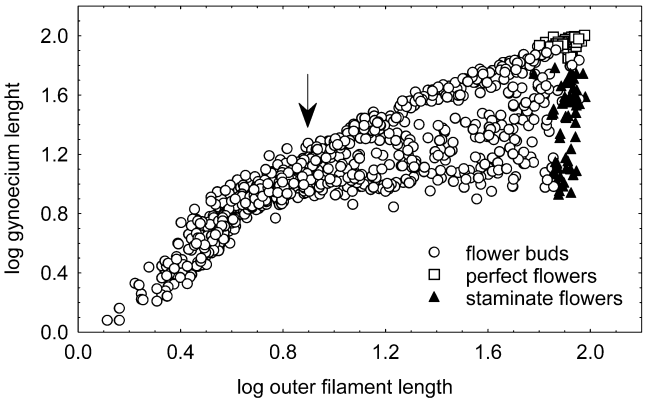

Fig. 3 Log-log plot of the gynoecium and outer filament lengths in *Caesalpinia gilliesii.* The arrow indicates a filament length of ca. 7 mm.

enormous variability in the dissociation timing. Whereas some buds do not surpass the final gynoecium length or reached an intermediate-sized gynoecium of staminate flowers, others grew to the average gynoecium size of perfect flowers (Fig. **3**). An increasing CV in the mean gynoecium length from stages I to III of development reinforces the variability in dissociation timing (CV = 44%, 51%, and 61% for the stages I, II, and III, respectively).

The effect of the inflorescence size

There were no significant differences between regression slopes of S and L inflorescences for the smaller bud size category (Table **4**, Fig. **4A**). All slopes between the floral organs measured were lower for S than for L inflorescences at stage II, and significantly different for gynoecium vs. filament or bud lengths, and for outer–inner filament lengths (Table **4**). The dissociation of the gynoecium was evinced by the lower slopes between the gynoecium–filament at stage II compared with stage I (Table **4**, Fig. **4B**). No difference was found at this stage between SN and LQ populations for the gynoecium–outer filament slopes ($F_{[1,160]} = 0.03$, $p = 0.855$ for slope differences).

The pattern of dissociation was maintained at stage III where L inflorescences also had statistically steeper slopes for the gynoecium–filaments relationships (Table **4**, Fig. **4C**). Furthermore, at stage III, L inflorescences had a significantly higher mean gynoecium length (31.25 ± 1.56 mm and 49.01 ± 3.76 mm for S and L inflorescences, respectively; t-value 4.33, $p < 0.0001$). Mean outer filament length, however, did not differ between S and L inflorescences at stage III (46.04 ± 1.96 mm and 43.10 ± 3.06 mm for S and L inflorescences, respectively; t-value – 0.30, $p = 0.764$).

The androecium growth accelerated at stage II, with steeper slopes for filament–bud lengths compared with that observed at a previous stage (Table **4**). The particularly high slope for the inner filament–bud length relationship at stage II may explain the final equal length of inner and outer filaments observed in mature flowers.

Discussion

Gynoecium–androecium size variation and sexual dimorphism

Because allocation to male structures did not differ between floral morphs, sexual dimorphism of *C. gilliesii* flowers relies on the gynoecium and ovule size differences. An equal allocation to male structures of both flower morphs is common in several andromonoecious species, addressing the fact that staminate are less costly than perfect flowers (Baksh and Iqbal, 1978; Solomon, 1986; Anderson and Simon■, 1989; Diggle, 1991a, b; but see Huang, 2003). Male floral structures were, in comparison, less variable than female structures and mainly different across populations. Staminate and perfect flowers of *C. gilliesii* also had the same number of ovules, indicating that at certain developmental stages all flowers were potentially perfect. The strong size co-variation of floral structures in small buds reinforces this fact. Furthermore, there are no histological differences between staminate and perfect flowers at the beginning of development (Carrizo García and Calviño, in prep.■). From an intermediate bud size, however, the lower regression slopes for gynoecium growth showed that this organ dissociates its growth from the other floral structures in some buds, leading to staminate flowers with a small gynoecium. Nevertheless, there was considerable variability in the timing of dissociation, resulting in a diversity of gynoecia sizes at the final stage of development. The gynoecium size variability was, nevertheless, much higher in staminate than in perfect flowers.

Intermediate floral forms between the short-styled staminate and perfect flowers have often been found in andromonoecious species (Dulberger et al., 1981; Whalen and Costich,

Table 4 Slopes of the log-log regression for gynoecium, androecium, and bud lengths of the three categories of flower bud sizes. The *F* values of the ANCOVA for differences between slopes of short (S) and long (L) inflorescences are shown. All slopes were significantly different from zero. I = small, II = intermediate, III = large flower buds. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Bud size categories	I			II			III		
Regressions	S	L	$F_{(1,86)}$	S	L	$F_{(1,225)}$	S	L	$F_{(1,171)}$
Gynoecium, outer filament	1.66	1.57	0.22	**0.40**	**0.76**	18.96****	**0.51**	**0.87**	3.90*
Gynoecium, inner filament	0.64	0.82	0.11	**0.39**	**0.69**	10.33***	**0.59**	**1.14**	5.95*
Gynoecium, bud	0.67	0.77	0.58	**1.42**	**2.60**	7.96**	1.67	2.90	1.10
Outer filament, bud	0.83	0.77	0.33	2.72	3.36	1.31	2.75	2.68	0.01
Inner filament, bud	1.08	1.03	0.10	3.97	4.86	0.95	2.47	1.91	0.19
Outer filament, inner filament	1.27	1.21	0.16	**1.17**	**1.27**	4.40*	1.02	0.96	3.41

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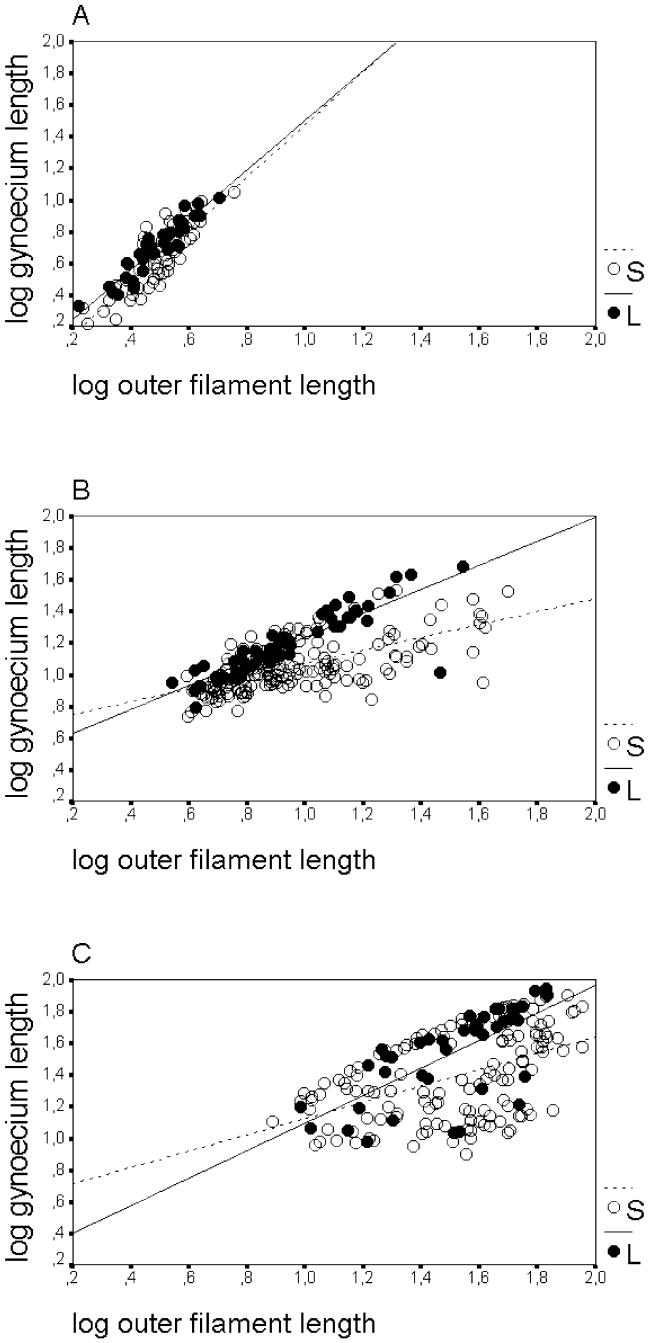

Fig. 4 Linear regressions of gynoecium length on outer filament length for short (S) and long (L) inflorescences of *Caesalpinia gilliesii* for small (**A**), intermediate (**B**), and large (**C**) bud size categories. Differences in slopes are summarized in Table **4**.

1986; Anderson and Symon, 1989; Emms, 1993; Ito and Kikuzawa, 2000; Manicacci and Després, 2001), including several Caesalpinoid andromonoecious taxa where long pistil morphs were always functionally hermaphrodite (Ramírez et al., 1984; Bullock, 1985; Lewis and Gibbs, 1999). For andromonoecious species, functionless gynoecia of staminate flowers are not subject to pollinator-mediated selection of gynoecium size that operates in perfect flowers, so that a higher variability in gynoecium size is to be expected in staminate rather than in perfect flowers (Ushimaru et al., 2003 a). Perfect flowers of *C. gilliesii* showed, however, considerable size variation in their gynoecium size and stigma–anther separation. When gynoecium size variability is associated with a higher stigma–anther separation, such differences can be maintained to favour outcrossing over self-pollination (Thomson and Stratton, 1985; Murcia, 1990; Motten and Stone, 2000). In the studied populations, *C. gilliesii* produces more seeds by cross- than by self-pollination (Calviño, unpublished data). The stigma–anther separation observed in perfect flowers of *C. gilliesii* could also be explained as a self-pollination avoiding mechanism. This is supported by the smaller gynoecium length variability of perfect compared with staminate flowers, suggesting that selection pressure could be acting upon the gynoecium–androecium size relationship in these flowers. Nevertheless, a great variability in the expression of gynoecium size was also observed in perfect flowers when andromonoecy has been induced, and it has been argued that different genes and/or mechanisms may be involved in maintaining such variability in the individual plant (Janoušek et al., 1996).

Sexual dimorphism and inflorescence size

Interestingly, regression slopes for the gynoecium–androecium size relationship were dependent on inflorescence size. For intermediate and large bud sizes, L inflorescences showed steeper slopes for gynoecium size relationships. Despite the considerable scatter of the data, the gynoecium–androecium regression slopes for L inflorescences approximated that observed in perfect flowers. Steeper slopes for gynoecium growth rates were also observed in perfect compared with male flowers of andromonoecious species (Diggle, 1991 a) and in female-biased plants of a gynodioecious tree (Gibson and Diggle, 1998), supporting the idea that femaleness is related to closer co-variation of the gynoecium with other floral structures. Then, steeper slopes of L inflorescences observed for *C. gilliesii* could reflect a higher femaleness for larger inflorescences and, in fact, large flower buds of L inflorescences had a significantly higher mean gynoecium length, a typical feature of perfect flowers.

According to the proposed hypothesis, dependence of sexual expression on inflorescence size would respond to differential resource availability such that higher resource availability would account for female-biased sexual expression. Then, the higher femaleness of larger *C. gilliesii* inflorescences should also be related to higher resource availability. In some andromonoecious species, lowered resource availability generated by the presence of developing fruits was addressed as the triggering factor for staminate flower production (Diggle, 1991 b; Gibbs et al., 1999). In non-andromonoecious species, the length and thickness of inflorescences were associated with higher resource levels and accounted for a higher female allocation (Vaughton, 1993; Machon et al., 1995). As the diameter of the vasculature declines with stem width, such architectural differences may influence the resource supply of the inflorescence (Diggle, 1997). Given these facts, it is likely that different sized inflorescences of *C. gilliesii* may indirectly impose an inequality in the available resources, so that sexual expression would result in larger inflorescences that are female-biased.

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Nevertheless, contrary to other andromonoecious species, the presence of mature fruits is not associated with a higher proportion of staminate flowers in *C. gilliesii* inflorescences (Jausoro and Galetto, 2001). This finding, however, does not contradict the trend observed in the present work for female-biased larger inflorescences. This is because, first, in species with a sequential blooming pattern, sex allocation depends not only on pre-mating factors such as morphology, but also on post-mating factors like the female success of earlier flowers (Emms, 1993; García, 2003). This agrees with the hypothesis of serial adjustment of maternal investment (Lloyd, 1980), where resource allocation to developing ovaries is supposed to be controlled at different times – and with different reproductive units – during one reproductive period (Lloyd, 1980). Then, differential resource availability acting through inflorescence size may be an influential factor in the pre- but not in the post-mating sexual expression of *C. gilliesii* flowers, addressing the fact that floral form and function is ontogenetically contingent (Diggle, 1997). Moreover, many other factors such as the maternal genome and/or mechanical effects may contribute to sexual expression of a flower (Diggle, 1992; Janoušek et al., 1996; Spielman et al., 2001). In fact, L inflorescences of *C. gilliesii* also showed a general trend to have greater slopes than S inflorescences for androecium growth, suggesting that all floral organs could be subject to inflorescence size-dependent variation. Second, as Cox (1988) has pointed out, plants are capable of varying their sexual expression at different modular levels of morphological complexity (i.e., flower, inflorescence, plant), and the same may be true for the pattern of female vs. male investment with higher resource availability (e.g., Emms, 1993). Moreover, it has been pointed out that independence of gynoecial and androecial development may be variable at different levels of plant organization (Diggle, 1992). In *C. gilliesii,* mean inflorescence size is correlated with plant height (Calviño, pers. obs.■), so that the extent to which the observed trend for the gynoecium growth pattern of this species will be translated into differences in the final sex ratio would also depend on morphological level considered.

In summary, sexual dimorphism in *C. gilliesii* flowers was characterized by differences in gynoecium and ovule sizes, highly variable female structures, and equal allocation to male floral organs. The expression of female-sterile flowers was dependent on size of inflorescence, where smaller inflorescences had weaker gynoecium–androecium regression slopes and reduced gynoecia. Considering these features as indicative of male-biased sexual allocation, this work addresses the fact that size of the inflorescence, an indirectly resource-related response, may account for sex determination of the flower during floral development in this species. It also illustrates that study of the size of reproductive organs in buds and flowers, and, moreover, its co-variation pattern, may help to identify if inflorescence size may be an influential factor at the time of sex determination of the flowers. More interestingly, it is also shown that among-inflorescence size variability may act on floral organ development, in analogy to the more common observation of floral organ size changes at the within-inflorescence level (e.g., Diggle, 1997). This may be especially true for those plants where attributes of the entire organism (e.g., plant height) impose constraints on size of the general shoot system (e.g., Machon et al., 1995).

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