

Between-year Changes in Functional Gender Expression of *Palicourea padifolia* (Rubiaceae), a Distylous, Hummingbird-pollinated Shrub

CLEMENTINA GONZÁLEZ, JUAN FRANCISCO ORNELAS* and LEONOR JIMÉNEZ

Departamento de Biología Evolutiva, Instituto de Ecología A.C., Km 2.5 Carretera Antigua a Coatepec No. 351, Congregación El Haya, Xalapa, Veracruz 91070, México

Received: 5 March 2004 Returned for revision: 10 August 2004 Accepted: 16 September 2004 Published electronically: 16 November 2004

• **Background and Aims** Because distylous species have two hermaphroditic style-length floral morphs, they face two sex allocation problems: the equilibrium morph ratio and the optimal allocation to pollen and seed production in each floral morph. Gender specialization is expected among distylous species when floral morphs differ in reproductive output. However, spatio-temporal variability in female reproductive output between morphs needs to be investigated to fully understand patterns of sexual expression and gender specialization in distylous plants. Between-year variation in flower and fruit production of hummingbird-pollinated *Palicourea padifolia* (Rubiaceae) was examined, focusing on functional gender expression of long- and short-styled morphs and comparing their reproductive performance in five consecutive years (1998–2002).

• **Methods** Between-year variation in inflorescence, floral bud and fruit production was monitored and quantified. These traits were then used as parameters to determine functional gender differences between floral morphs through time.

• **Key Results** Inflorescence production varied among years but no significant differences were found between floral morphs. Long-styled plants initiated more floral buds per inflorescence every year than short-styled plants, suggesting higher allocation to pollinator attraction and, potentially, an increase in male fitness through pollen donation. Although fruit production was similar between morphs, their functional gender shifted across years.

• **Conclusions** The gender expression inconsistency across years is surprising because a number of floral characters and attributes that contribute to differently attracting and rewarding effective pollinators in *P. padifolia* suggest gender specialization. The evidence that morphs of distylous species might specialize in functional gender mostly comes from differences among populations in seed production and non-equilibrium morph ratios based on 1-year field population surveys. The results suggest that more sampling through time is needed to detect gender specialization among distylous species with a perennial habit.

© 2004 Annals of Botany Company

Key words: Cloud forest, distyly, gender expression, hummingbirds, *Palicourea padifolia*, Rubiaceae, sex-allocation theory.

INTRODUCTION

Patterns of sexual expression in hermaphroditic plants often depart from strict equisexuality (Bawa and Webb, 1983; Lloyd and Bawa, 1984; Ross, 1990; Pickering and Ash, 1993; Ashman, 1999; Méndez and Traveset, 2003). In heterostylous species, as they have two or three hermaphroditic style-length floral morphs, there are two sex allocation problems: the equilibrium morph ratio and the optimal allocation to pollen and seed production in each floral morph (Casper, 1992). Botanists have classically viewed this polymorphism as an outcrossing mechanism, since in most situations reciprocal morphs can only cross-fertilize. However, the floral morphs of several distylous species specialize in their reproductive effort through male and female function (Lloyd, 1979). Some heterostylous species exhibit such extreme gender specialization that they are functionally dioecious (Opler *et al.*, 1975; but see Muenchow and Grebus, 1989), but less pronounced gender specialization has been commonly reported for naturally pollinated individuals (reviewed by Casper, 1992). Although gender specialization is not a regular feature of heterostyly, reproductive differences between morphs has been reported for most studied distylous species (reviewed

by Casper, 1992). Differences between morphs in compatible pollen loads on stigmas are common in distylous species. In more than half of the studied species such a difference has been reported (14 out of 21 species) and, in almost all of those, pollen reception was greater in long-styled flowers (Casper, 1992, and references therein; see also Bjorkman, 1995). It is noteworthy that, in all other species studied, stigmas of long-styled flowers are larger than those of short-styled flowers (Dulberger, 1992; Dulberger and Ornduff, 2000), a condition opposite to that of morphological distylous *Palicourea* species (Rubiaceae), in which stigmas of short-styled are much larger (Ornelas *et al.*, 2004a). In five Rubiaceae, *Palicourea lasiorrachis* (Feinsinger and Busby, 1987), *P. fendleri* (Lau and Bosque, 2003), *P. padifolia* (Ornelas *et al.*, 2004a), *Psychotria suerrensii* (Stone, 1996) and *Mitchella repens* (Hicks *et al.*, 1985), pollen of long-styled flowers is more efficient in reaching legitimate stigmas, while short-styled morphs are more successful in the reception of pollen. Hence, a generalization about gender specialization is not possible without consideration of all aspects of floral dimorphisms in distylous species, especially in the size of the stigma (Dulberger and Ornduff, 2000; Ornelas *et al.*, 2004a). The evidence that morphs might specialize in functional gender mostly comes from differences among populations

* For correspondence. E-mail ornelasj@ecologia.edu.mx

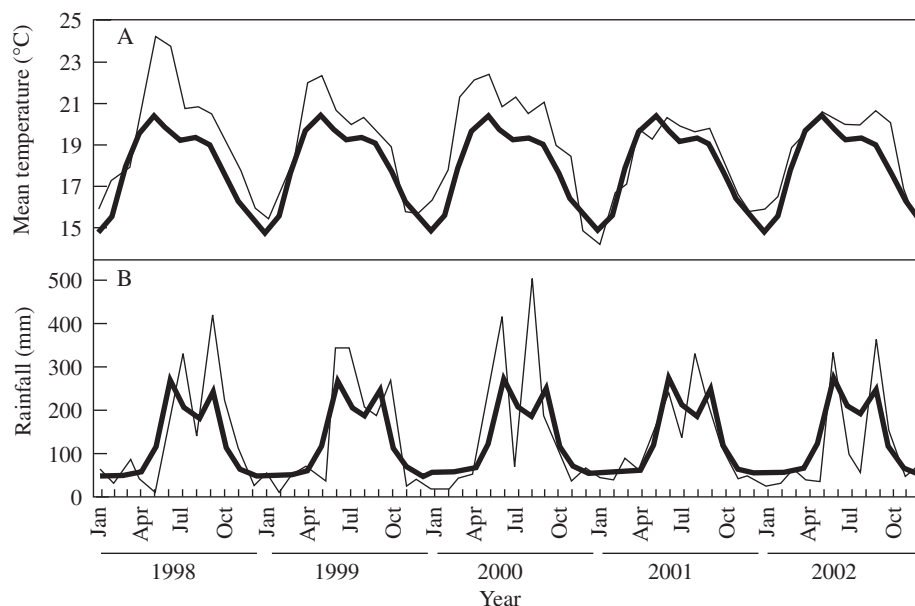


FIG. 1. Monthly mean temperature (°C) and accumulated precipitation (mm) data from the Xalapa meteorological station, 2 km away from the study site. Thick lines indicate the 77-year average.

in seed production and non-equilibrium morph ratios (Casper, 1992). No striking pattern of gender specialization in distylous plants emerges from the data summarized by Casper (1992) and there is no tendency for a particular floral morph to be more biased or specialized in one gender (see also Lau and Bosque, 2003, and references therein). However, year-to-year variation in functional gender between floral morphs needs to be investigated because morphs may shift their sexual expression through time. The first sex allocation problem for heterostylous plants posed by Casper (1992), the equilibrium morph ratio, and the probability of pollen transfer between and within morphs has been addressed in previous papers (Ornelas *et al.*, 2004a, b). Here the year-to-year variation in flower and fruit production of distylous, hummingbird-pollinated *Palicourea padifolia* is examined for five consecutive years (1998–2002). This information was then used to determine gender expression differences between floral morphs. As far as is known, repeated sampling of the same natural population or a combination of field surveys and hand pollination experiments demonstrating differences in fruit or seed production are rarely available, and therefore, little evidence for gender-specific reproductive allocation in heterostylous plants.

MATERIALS AND METHODS

Study site

This study was done from March 1998 to November 2002 in a *P. padifolia* population located in the Parque Ecológico Francisco Xavier Clavijero (19°30'N, 96°57'W; at 1225 m a.s.l.), a 29-ha cloud forest remnant near Xalapa City, Veracruz, Mexico. Floristic details of the area are given by Castillo Campos (1991).

The climate is mild and humid throughout the year, with a dry and cold season from November to March. Mean annual temperature is 17.9 °C and mean annual precipitation is 1500 mm. Extreme episodes of drought occurred during two of the study years. Rainfall within a year varied over the study period. No precipitation occurred in May 1998 and it was very low in 1999 (26.1 mm) and 2002 (28.9). Dry conditions of spring and early summer coincide with the flowering peak of *P. padifolia* (Contreras and Ornelas, 1999). The above-average temperatures in spring and early summer (April–June) 1998 were also more dramatic than 77-year average values (Fig. 1). Mean temperature in May 1998 was four degrees higher (24.2 ± 2.5), and three degrees higher in June (23.7 ± 2.1) than the 77-year average for the area (20.4 ± 1.1). The observed above-average temperatures and below-average precipitation are typical during El Niño events.

Study species

Palicourea padifolia (Roem. & Schult.) C. M. Taylor and Lorence (Rubiaceae) is a long-lived, understorey shrub 2–7 m tall (Ree, 1997; Contreras and Ornelas, 1999; Fig. 2). It occurs in middle-elevation cloud forests from southern Mexico to Panama (Taylor, 1989). The population studied (approx. 500 reproductive individuals) is isoplethric (1:1 morph ratios) and morphologically distylous, with a diallelic incompatibility system that prevents fruit production among cross intramorph pollinations (Contreras and Ornelas, 1999; Ornelas *et al.*, 2004a). Differences between its floral morphs in some floral traits commonly associated with distyly have been reported previously (Contreras and Ornelas, 1999; Ornelas *et al.*, 2004a, b). Individuals of *P. padifolia* bear 30–80 inflorescences, each opening two to four yellow flowers per day and 80–90 floral buds may

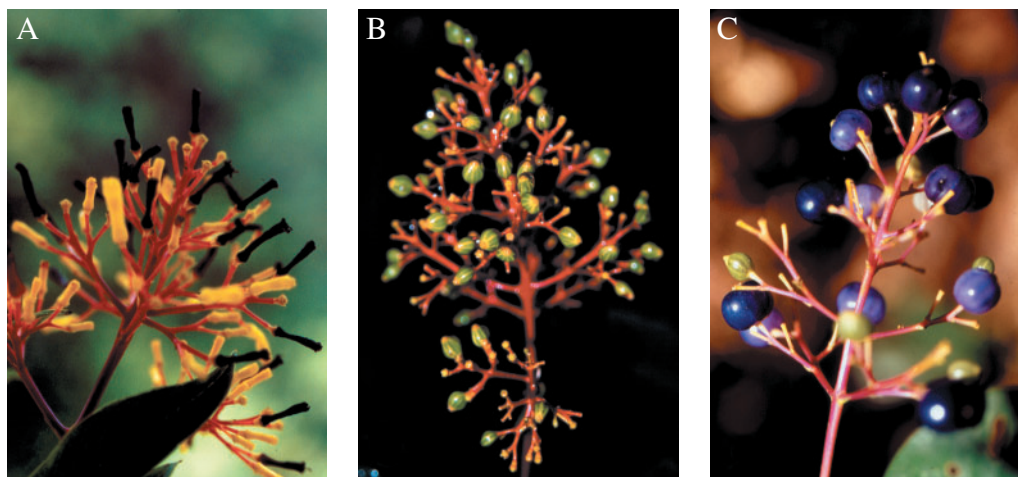


FIG. 2. Flowers and fruits of *Palicourea padifolia*: (A) inflorescence with drought-killed buds; (B) infructescence with initiated fruits; (C) infructescence with developed and ripe fruits.

eventually reach the flower stage during the blooming season, which extends from mid-March to August for the whole population and peaks in May (Contreras and Ornelas, 1999). Flowers of the long-styled (LS) plants have an exerted stigma and stamens are positioned just about half way up the corolla tube. In flowers of short-styled (SS) individuals, the top of the stigma is located just above the midpoint of the corolla tube and stamens are slightly exerted; stigma-anther separation is more pronounced in SS flowers (Contreras and Ornelas, 1999). The fixed number of anthers per flower (four), ovules (two) and carpels (two) suggests that the phenotypic gender is fixed in this species. One-day flowers are about 1.5 cm long with narrow-tubular corollas and almost reciprocal placement of sex organs between morphs (Contreras and Ornelas, 1999; Hernández and Ornelas, 2003). Corolla tubes of LS plants are approx. 2.5 mm shorter than those of SS plants (Contreras and Ornelas, 1999). The distylous polymorphism in *P. padifolia* is also characterized by marked dimorphism in pollen size and number, and stigma size and shape. Anthers of LS flowers have approx. 1.3 times more pollen grains and are 6.5 times smaller than those in SS flowers (Ornelas *et al.*, 2004a). Stigmas of both morphs are bilobed, and stigmatic lobes are approx. 2.5 times longer in SS flowers than those in LS flowers (Ornelas *et al.*, 2004a). In consequence, the surface of the stigma in SS flowers is larger than the surface of the stigma in LS flowers. LS flowers secrete 3 μ l more nectar after repeated removal than SS flowers (Ornelas *et al.*, 2004b). Flowers are visited by small bees, bumblebees, butterflies and hummingbirds (Contreras and Ornelas, 1999). Of the 11 hummingbird species that visit the flowers (82 % of floral visits per plant) of *P. padifolia*, territorial *Amazilia cyanocephala* individuals are the most frequent (30 % of floral visits per plant) and effective pollinators. Insects have been observed collecting pollen but their visitation frequency is much lower (18 %) than hummingbird visitation (Ornelas *et al.*, 2004a). Flower production is similar between style morphs and varies from year to year (Contreras and Ornelas 1999;

Ornelas *et al.*, 2004b), but the probability of an SS floral bud eventually reaching the flower stage is higher than that of LS floral buds (A. Hernández and J. F. Ornelas, unpubl. res.). Fruit development usually starts in May but asynchronous ripening starts in August and extends until October (Ornelas *et al.*, 2004b). Ripe fruits with typically two seeds are eaten and presumably dispersed by birds. Based on manual pollination experiments, it is suggested that LS plants are 'more male' in terms of fruit production in the present study population, whereas SS plants are 'more female' (Ornelas *et al.*, 2004a, b).

Data collection

One hundred and twenty-six reproductive individuals (69 plants of LS morph and 57 of SS morph) from the Clavijero population (1998: LS = 9, SS = 5; 1999: LS = 20, SS = 13; 2000: LS = 15, SS = 15; 2001: LS = 15, SS = 15; 2002: LS = 10, SS = 9) were surveyed. All selected plants were located along the edge of the main trail, where light and microclimatic conditions are similar to the forest interior (Williams-Linera, 2003). Selected individuals (1–3 m tall) were synchronously initiating inflorescence development (same phenological stage). Because some plants failed to flower in the second year of the present study, different plants were used every year. Thus, our results must be taken to represent the typical fate of randomly sampled LS and SS individuals in the population over time. Ten inflorescences per plant were tagged in March of each year, and each was monitored approximately every 15 d from bud initiation (March) until fruit formation (October). On each census, the total number of inflorescences per plant was quantified and, on each tagged inflorescence, the number of initiated buds and the number of initiated fruits (immature) per inflorescence were counted to determine female reproductive output. Results of previous field surveys of *P. padifolia* fruit crops standing over the fruiting season (Contreras and Ornelas, 1999; Ornelas *et al.*, 2004b), and the number of ripe fruits in relation to maximum bud production per

TABLE 1. Results of two-way nested ANOVAs for the production of inflorescences per plant, initiated floral buds, and initiated fruits (immature) in LS and SS morph in five years of study (1998–2002)

Variable	Floral morph			Year			Plant (floral morph)			Year × floral morph			Error	
	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F	d.f.	MS
No. of inflorescences	1	1.74	0.42 NS	4	72.81	17.63***	36	3.74	0.91 NS	4	1.34	0.32 NS	96	4.13
No. of initiated buds	1	76.38	22.51***	4	68.44	20.17***	36	8.45	2.49***	4	4.94	1.46 NS	1306	3.39
No. of initiated fruits	1	1.05	0.24 NS	4	158.45	36.67***	36	20.98	4.85***	4	21.84	5.05***	1306	4.32

*** $P < 0.001$.

infructescence (Ree, 1997), have been used as measures of female reproductive success. A drawback of these estimates is that post-pollination processes can subtract from potential fruit set leading to an underestimation of pollinator efficiency (see also Cane and Schiffhauer, 2003). Fruit initiation is a more accurate measure of female reproductive response to pollination than ripening fruits exposed to fruit consumption, seed predation, disease and physical damage.

Data analysis

To describe the effects of floral morph and year on the maximum number of inflorescences per plant, the maximum number of initiated buds per inflorescence and the maximum number of initiated fruits per infructescence, nested two-way ANOVAs were used. Floral morph and year were treated as fixed factors. Between-plant variation was assessed with floral morph as the main factor and plant nested within floral morph. Along with the main effects, a floral morph × year interaction was included in the model.

To determine gender expression patterns for each floral morph of *P. padifolia*, the standardized functional gender of each plant, as originally defined by Lloyd and Bawa (1984) and according to the re-elaboration of the formula utilized by de Jong and Klinkhamer (1989), was calculated as $G_p = (d_i/l_i)/(E + d_i/l_i)$, where E measures the ratio of maternal (d_i) to paternal (l_i) investment in the whole population ($E = \Sigma d_i/\Sigma l_i$). In the case of *P. padifolia* maternal investment was considered as the maximum number of initiated fruits per infructescence and paternal investment was considered as the maximum number of initiated floral buds. Measures of E were taken as the sum of maternal/paternal investment in the whole population per year. G_p varies between 0 and 1 and indicates to what extent individuals specialize in one sex, relative to the average ratio of expenditures in the whole population. G_p values close to 0 indicate male specialization, whereas those close to 1 indicate female specialization. To determine morph differences on gender expression through time, a two-way ANOVA model was used.

To meet parametric analysis assumptions, floral bud, fruit and inflorescence data were square root or arcsine transformed as required (Zar, 1984), but untransformed data are reported in figures. All statistical analyses were run using general linear modelling with SuperANOVA and StatView (Abacus Concepts, 1989, 1996).

RESULTS

Flower production and reproductive output

Considerable among-year variation in inflorescence number was found, but differences between floral morphs and the year × floral morph interaction were not significant (Table 1). In 1999, inflorescence production was three times as high as the production in 1998 (lowest of the study years) and almost doubled the rest of the years (Fig. 3A). By contrast, significant differences were found between floral morphs for the number of initiated floral buds per inflorescence, and the floral morph × year interaction was not significant (Table 1). LS plants initiated more floral buds per inflorescence than SS plants independently of year, except in 1998 where floral morphs did not differ statistically (Fig. 3B). On average, plants initiated more floral buds per inflorescence in 1998 and 2002 than in the rest of the study years. A major flower loss (floral buds and open flowers with clear signals of tissue killing, see Fig. 2A) occurred during the flowering peak of 1998 (30 % per inflorescence). The observed loss was the result of a prolonged dry season in that year (Fig. 1B). In 1999, plants lost around 5 % of floral buds and open flowers per inflorescence. Unfortunately flower loss was not quantified in 2000 and 2001 but it is likely that <5 % of flower production was lost in those years. In 2002 the relative incidence of flower wilting was again quantified and 34 % of flower loss was detected per inflorescence.

Floral morphs initiated the same number of fruits. However, significant differences were found between years and the floral morph × year interaction was also significant (Table 1). Plants almost doubled fruit initiation per infructescence in 2000, 2001 and 2002 over preceding years (Fig. 3C). The effect of flower loss on the variation of subsequent fruit production for years with detailed data on flower loss was analysed using ANCOVA. In the model, floral morph and year (1998, 1999 and 2002) were treated as fixed factors, flower loss (proportion of floral buds and open flowers with clear signals of tissue killing per inflorescence) was the covariate, and the proportion of initiated fruits was the dependent variable. Variation in fruit initiation was mainly explained by year (year effect, $F_{2,744} = 24.13$, $P = 0.0001$) and the floral morph × year interaction was also significant ($F_{2,744} = 18.84$, $P = 0.0001$). However, the contribution of floral morph to explaining variation in fruit initiation was not significant ($F_{1,744} = 0.08$, $P = 0.78$) when controlling for flower loss ($F_{1,744} = 8.41$, $P = 0.0038$).

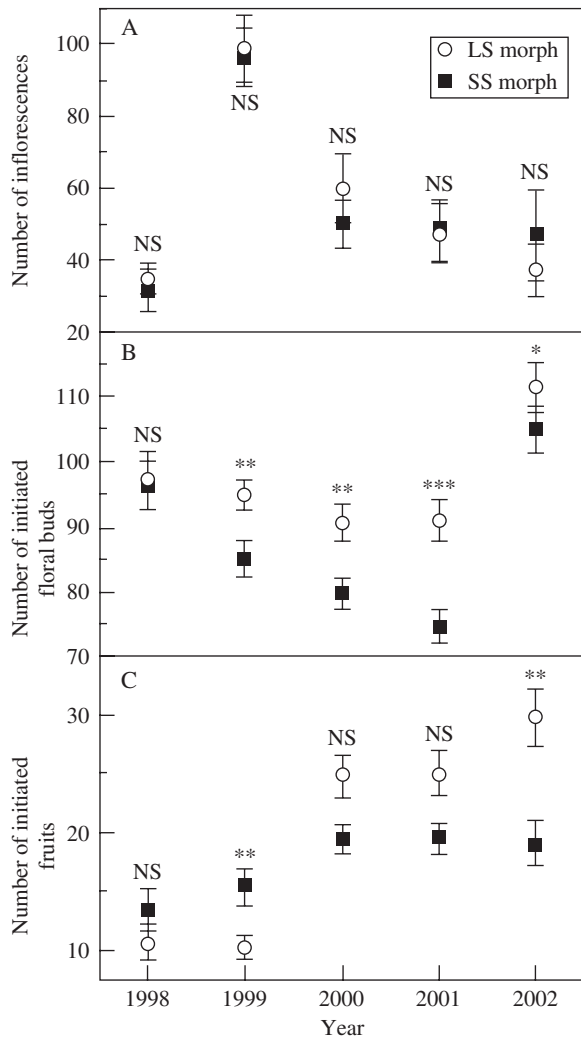


FIG. 3. Effects of year and floral morph on (A) number of inflorescences per plant, (B) number of initiated floral buds per inflorescence, and (C) number of initiated fruits per infructescence of *Palicourea padifolia*. Data indicate means \pm standard error. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

Gender expression

Significant differences for functional gender (G_p) were found between floral morphs and among years, and the floral morph \times year interaction was also significant (two-way ANOVA: floral morph effect, $F_{1,1091} = 14.6$, $P = 0.0001$; year effect, $F_{4,1091} = 4.5$, $P = 0.0013$; floral morph \times year interaction, $F_{4,1091} = 7.1$, $P = 0.0001$). In 1998 and 1999, SS plants had a median G_p value closer to 1 than LS plants, suggesting gender specialization (Fig. 4). Departures from equisexuality were not significantly different between morphs in 2000 and 2001. However, SS morph plants showed a pronounced shift toward maleness in 2002.

Pooling data across years (average values for individual plants, $n = 126$), the number of initiated fruits increased as the number of initiated floral buds increased ($r = 0.34$). The overall relationship explained a low proportion of the variation in the number of initiated fruits for individual plants ($r^2 = 0.07$). The slope of the regression was

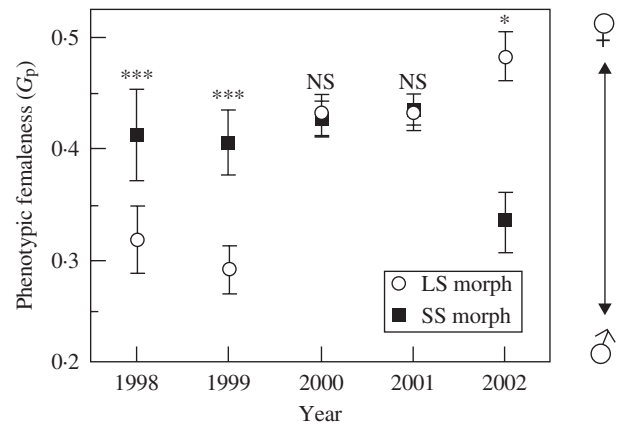


FIG. 4. Effects of year and floral morph on the phenotypic femaleness (G_p) of *Palicourea padifolia*. Values close to 1 indicate female allocation. Data indicate means \pm standard error. *, $P < 0.05$; ***, $P < 0.001$; NS, not significant.

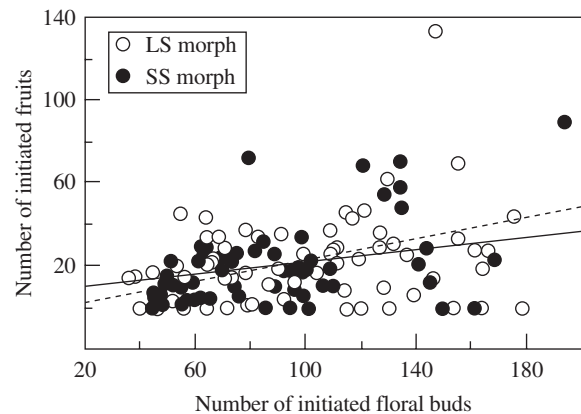


FIG. 5. Relationships between number of initiated floral buds and number of initiated fruits per plant in LS morph (continuous line) and SS morph (dashed line) of *Palicourea padifolia*.

significantly different from 0 (one-tailed, $t = 3.15$, d.f. = 125, $P = 0.0021$). After splitting the present data by floral morph, the overall relationship was only observed for SS plants ($r = 0.43$) with a higher proportion of the variation in number of initiated fruits explained by the number of initiated floral buds ($r^2 = 0.12$). The slope of the regression was significantly different from 0 (one-tailed, $t = 2.72$, d.f. = 56, $P = 0.0087$) (Fig. 5).

DISCUSSION

Flower production and pollinator attraction

Although vegetative and reproductive growth is frequently limited to a short period each year (Wright, 1991), long-term demographic trade-offs between current and future reproduction occur in animal-pollinated plants (Hoglund and Sheldon, 1998). In a long-term study, Inouye *et al.* (2002) found that the number of inflorescences produced by *Delphinium barbeyi* (Ranunculaceae) is determined by

the previous flowering period. It was observed that plants of *P. padifolia* produced three times as many inflorescences in 1999 as those different plants that experienced the extreme drought episode in 1998. The limitation of a 5-year study is acknowledged and more sampling may be needed to detect a temporal pattern (see Inouye *et al.*, 2002). During the dry season, plants can experience water deficits and employ a variety of metabolic and behavioural acclimation responses to drought (Mulkey *et al.*, 1991). Several studies suggest that sex allocation trade-offs are more pronounced under stressful conditions (Costich, 1995; see also Barrett, 1998; Galen, 2000) and that, therefore, should favour the evolution of gender specialization in some taxa. Considerable loss of flowers, particularly during the driest years, was observed. However, flower loss in *P. padifolia* cannot be attributed to hydric stress. Although a month with no rainfall is likely to cause stress in cloud forests, flower loss seemed not to affect subsequent fruit initiation (ANCOVA results).

It was found that LS morph plants initiated more floral buds almost every year, suggesting higher investment to pollinator attraction and potentially an increase in male fitness through pollen donation (see also Ornelas *et al.*, 2004b). By contrast, the significant regression results for the relationship between bud and fruit initiation support the hypothesis that SS plants are investing more resources to female function. Nonetheless, it is necessary to follow the same individual plants through time to examine potential sex allocation trade-offs between flower and fruit production.

Variation in functional gender expression

Surveys of fruit or seed production in heterostylous populations have stimulated some thinking about the functional gender processes responsible for the breakdown of heterostyly. Male and female functions might differ in distylous species when floral morphs produce different quantities of fruits and/or seeds. Because so little is known about fitness through male function in distylous species, the evidence that morphs might specialize in functional gender comes mostly from differences in seed production. The evidence of gender specialization comes from crossing experiments and from naturally pollinated individuals, in which more seeds or fruits are obtained following an intermorph cross in one direction than in the reciprocal (reviewed by Casper, 1992). Although morph specific differences in reproductive output are almost ubiquitous in heterostylous populations (Casper, 1992; Dulberger, 1992), a major limitation to understanding sexual selection in heterostylous plants is a lack of data on variation of functional gender expression through time. With a combination of field surveys and hand-pollination experiments (Contreras and Ornelas, 1999; Ornelas *et al.*, 2004a, b), consistent morph differences in seed production for *P. padifolia*, in which SS morphs behave as more female than LS morphs, have been shown. Year-to-year variation in reproductive output between floral morphs was then investigated because morphs may shift their sexual expression through time, particularly those with a perennial habit. By repeated samplings on the same population, inconsistency

across years in gender expression between *P. padifolia* morphs was found. SS morph individuals initiated proportionally more fruits per infructescence in 1999 than LS individuals, but this result was not consistent over the years. In fact, the opposite pattern was observed in 2002. The observed changes in functional gender expression over the years cannot be explained by deviations from an equal morph ratio because the present *P. padifolia* population has been isoplethic (1:1 floral morphs) for several years (Ornelas *et al.*, 2004b; A. Hernández and J. F. Ornelas, unpubl. res.). A perennial habit might not change the expected morph ratio (isoplethic for most distylous species), but differences between morphs in their ability to clone (Charnov, 1982; Casper, 1992) and stochastic processes such as local plant extinction, due to ongoing fragmentation, might skew ratios and affect the equilibrium of this population.

Gender specialization and distyly

Although gender specialization is not a regular feature of heterostyly, reproductive differences between morphs has been reported for most studied distylous species (reviewed by Casper, 1992), including for *P. padifolia* (Ree, 1997; Contreras and Ornelas, 1999; Ornelas *et al.*, 2004a, b). In 1998 and 1999, it was observed that SS individuals had a higher level of femaleness, whereas the opposite pattern was observed in 2002. A more direct test of a functional gender trade-off for the above observation would be to have followed the same individuals through time. This problem was explored further by pooling individual data across years and then regressing inflorescence number against bud initiation and fruit initiation. Inflorescence number was a poor predictor of bud initiation ($r = -0.003$) and the slope of the regression was not significantly different from 0 (one-tailed, $t = -0.003$, d.f. = 125, $P = 0.94$). By contrast, the relationship between inflorescence number and fruit initiation is negative and stronger ($r = -0.19$) and the slope was significantly different from 0 (one-tailed, $t = -3.36$, d.f. = 125, $P = 0.001$). When data were split by morph, the relationship is significantly stronger but only for the LS morphs ($r = -0.26$) and the slope of the regression was also significantly different from 0 (one-tailed, $t = -3.3$, d.f. = 68, $P = 0.0015$).

Gender specialization in species with floral polymorphisms can be originated by different causes, including changes in the frequency, abundance and composition of the pollinator assemblage, within and between seasons. This could influence plant reproductive success, particularly for distylous, self-incompatible plants because pollinator species differ in their efficiency to transfer outcrossed pollen (Ornelas *et al.*, 2004a). It is known that hummingbird visitation promotes pollen flow from LS to SS morph individuals (Ornelas *et al.*, 2004a), but the role of insects promoting pollen flow in the opposite direction has not been evaluated experimentally for *P. padifolia*. Pollen-collecting insects might be acting as principal pollinators in years where LS individuals are more female, because pollen transfer from SS to LS could be facilitated by the anther position of SS flowers and, therefore, accessibility to pollen grains

(see also Ornduff, 1975; Harder and Barrett, 1993; Ree, 1997; Estrada and Jiggins, 2002). Altered pollination conditions leading to unsatisfactory pollen dispersal may be the critical proximate ecological factor explaining changes in functional gender expression through time. Lastly, an alternative explanation for the inconsistency of morph differences in fruit production is differential herbivory through time. Ornelas *et al.* (2004b) found that fruit production in *P. padifolia* was negatively correlated with the attack of foliar herbivores, and LS plants experienced the strongest negative effect, with a significant reduction in fruit number. Nonetheless, experimental manipulation is needed to explore whether or not attack by herbivores modifies the functional gender expression of either morph.

The inconsistency across years in gender expression is surprising because this species has a number of floral characters and attributes that contribute to differently attracting and rewarding effective pollinators, suggesting gender specialization (Contreras and Ornelas, 1999; Hernández and Ornelas, 2003; Ornelas *et al.*, 2004a, b). SS individuals have significantly longer corollas, larger stigmatic surfaces, larger pollen grains, and receive more legitimate pollen when visited by hummingbirds than LS individuals (Contreras and Ornelas, 1999; Ornelas *et al.*, 2004a). By contrast, LS flowers secrete more dilute nectar and receive proportionally more illegitimate pollen than SS flowers (Ornelas *et al.*, 2004b). Here, it has also been shown that LS morphs develop more floral buds per inflorescence than SS morphs. These dissimilarities between morphs should lead to differences in their abilities to gain reproductive success through male (pollen) versus female (seeds) function. The higher pollen production by LS flowers, its successful delivery on SS stigmas by hummingbirds, and higher deposition of illegitimate pollen on their stigmas (Ornelas *et al.*, 2004a; A. Hernández and J. F. Ornelas, unpubl. res.), support the idea of LS morph having the ability to gain higher reproductive success through male function. However, the range of G_p values for *P. padifolia* morphs corresponds to ranges reported by de Jong and Klinkhamer (1989) for hermaphroditic, monocarpic plants, and the small functional gender differences between floral morphs indicate no strong specialization in one gender.

In conclusion, inconsistency was found across years in functional gender expression between floral morphs in *P. padifolia*. This result challenges the notion that morph differences in fruit and/or seed production based on 1-year field population surveys is sufficient to propose gender specialization among distylous species with a perennial habit. Temporal variation in gender function between floral morphs may depend on the ecological conditions of each population, particularly the spatio-temporal variability and the effectiveness and composition of the pollinator fauna and the frequency and damage intensity of foliar herbivores.

ACKNOWLEDGEMENTS

We thank Lucinda McDade, Marcos Méndez, Angélica Hernández, Rogelio Macías and Fabiana Pezzani for suggestions and constructive criticism; Carlos Lara, Ana Lucía Castillo and Angélica Hernández for help during fieldwork,

and staff from the Xalapa Meteorological Station and Comisión Nacional del Agua for sharing their data. Phenological data for 2001 was kindly shared by A. Hernández. Our work has been partially funded by the Departamento de Ecología y Comportamiento Animal of the Instituto de Ecología, AC (Ref. 902-11-563) and a grant from CONACyT (Ref. 409OP-N9608).

LITERATURE CITED

- Abacus Concepts.** 1996. *Abacus Concepts, StatView Reference*. Berkeley: Abacus Concepts.
- Abacus Concepts.** 1989. *Abacus Concepts, SuperANOVA*. Berkeley: Abacus Concepts.
- Ashman TL.** 1999. Determinants of sex allocation in a gynodioecious wild strawberry: implications for the evolution of dioecy and sexual dimorphism. *Journal of Evolutionary Biology* 12: 648–661.
- Barrett SCH.** 1998. The evolution of mating strategies in flowering plants. *Trends in Plant Science* 3: 335–341.
- Bawa KS, Webb CJ.** 1983. Floral variation and sexual differentiation in *Muntingia calabura* (Elaeocarpaceae), a species with hermaphroditic flowers. *Evolution* 37: 1271–1282.
- Bjorkman T.** 1995. The effectiveness of heterostyly in preventing illegitimate pollination in dish-shaped flowers. *Sexual Plant Reproduction* 8: 143–146.
- Cane JH, Schiffhauer D.** 2003. Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon* [Ericaceae]). *American Journal of Botany* 90: 1425–1432.
- Casper BB.** 1992. The application of sex allocation theory to heterostylous plants. In: Barrett SCH, ed. *Evolution and function of heterostyly*. New York: Springer Verlag, 209–223.
- Castillo Campos G.** 1991. *Vegetación y Flora del Municipio de Xalapa, Veracruz*. Xalapa: Instituto de Ecología, A.C.
- Charnov EL.** 1982. *The theory of sex allocation*. Princeton: Princeton University Press.
- Contreras PS, Ornelas JF.** 1999. Reproductive conflicts of *Palicourea padifolia* (Rubiaceae), a distylous shrub of a tropical cloud forest in Mexico. *Plant Systematics and Evolution* 219: 225–241.
- Costich DE.** 1995. Gender specialisation across a climatic gradient: experimental comparison of monoecious and dioecious *Ecballium*. *Ecology* 76: 1036–1050.
- de Jong TJ, Klinkhamer PGL.** 1989. Size dependency of sex-allocation in hermaphroditic, monocarpic plants. *Functional Ecology* 3: 201–206.
- Dulberger R.** 1992. Floral polymorphisms and their functional significance in the heterostylous syndrome. In: Barrett SCH, ed. *Evolution and function of heterostyly*. New York: Springer Verlag, 40–84.
- Dulberger R, Ornduff R.** 2000. Stigma morphology in distylous and non-heterostylous species of *Villarsia* (Menyanthaceae). *Plant Systematics and Evolution* 225: 171–184.
- Estrada C, Jiggins CD.** 2002. Patterns of pollen feeding and habitat preference among *Heliconius* species. *Ecological Entomology* 27: 448–456.
- Feinsinger P, Busby WH.** 1987. Pollen carry over: experimental comparisons between morphs of *Palicourea lasiorrhachis* (Rubiaceae), a distylous, bird-pollinated, tropical treelet. *Oecologia* 73: 231–235.
- Galen C.** 2000. High and dry: drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *American Naturalist* 156: 72–83.
- Harder LD, Barrett SCH.** 1993. Pollen removal from tristylous *Pontederia cordata*: effects of anther position and pollination specialization. *Ecology* 74: 1059–1072.
- Hernández A, Ornelas JF.** 2003. Correlación morfo-específica en flores de *Palicourea padifolia* (Rubiaceae). *Boletín de la Sociedad Botánica de México* 73: 35–41.
- Hicks DJ, Wyatt TR, Meagher TR.** 1985. Reproductive biology of distylous Partridgeberry *Mitchellia repens*. *American Journal of Botany* 72: 1503–1514.
- Hoglund J, Sheldon BC.** 1998. The cost of reproduction and sexual selection. *Oikos* 83: 478–483.

- Inouye DW, Morales MA, Dodge GJ. 2002. Variation in timing and abundance of flowering by *Delphinium barbeyi* Huth (Ranunculaceae): the roles of snowpack, frost, and La Niña, in the context of climate change. *Oecologia* **130**: 543–550.
- Lau P, Bosque C. 2003. Pollen flow in the distylous *Palicourea fendleri* (Rubiaceae): an experimental test of the disassortative pollen flow hypothesis. *Oecologia* **135**: 593–600.
- Lloyd DG. 1979. Evolution toward dioecy in heterostylous populations. *Plant Systematics and Evolution* **131**: 71–80.
- Lloyd DG, Bawa KS. 1984. Modification of the gender of seed plants in varying conditions. *Evolutionary Biology* **17**: 225–338.
- Méndez M, Traveset A. 2003. Sexual allocation in single-flowered hermaphroditic individuals in relation to plant and flower size. *Oecologia* **137**: 69–75.
- Muenchow GE, Grebus M. 1989. The evolution of dioecy from distyly: reevaluation of the hypothesis of the loss of long-tongued pollinators. *American Naturalist* **133**: 149–156.
- Mulkey SS, Wright SJ, Smith AP. 1991. Drought acclimation of an understory shrub (*Psychotria limonensis*; Rubiaceae) in a seasonally dry tropical forest in Panama. *American Journal of Botany* **78**: 579–587.
- Opler PA, Baker HG, Frankie GW. 1975. Reproductive biology of some Costa Rican *Cordia* species (Boraginaceae). *Biotropica* **7**: 234–247.
- Ornduff R. 1975. Heterostyly and pollen flow in *Hypericum aegypticum* (Guttiferae). *Botanical Journal of the Linnean Society* **71**: 51–57.
- Ornelas JF, Jiménez L, González C, Hernández A. 2004a. Reproductive ecology of distylous *Palicourea padifolia* (Rubiaceae) in a tropical montane forest. I. Hummingbirds' effectiveness as pollen vectors. *American Journal of Botany* **91**: 1052–1060.
- Ornelas JF, González C, Jiménez L, Lara C, Martínez AJ. 2004b. Reproductive ecology of distylous *Palicourea padifolia* (Rubiaceae) in a tropical montane forest. II. Attracting and rewarding mutualistic and antagonistic visitors. *American Journal of Botany* **91**: 1061–1069.
- Pickering CM, Ash JE. 1993. Gender variation in hermaphrodite plants: evidence from five species of alpine *Ranunculus*. *Oikos* **68**: 539–548.
- Ree RH. 1997. Pollen flow, fecundity, and the adaptive significance of heterostyly in *Palicourea padifolia* (Rubiaceae). *Biotropica* **29**: 298–308.
- Ross M. 1990. Sexual asymmetry in hermaphroditic plants. *Trends in Ecology and Evolution* **5**: 43–47.
- Stone JL. 1996. Components of pollination effectiveness in *Psychotria suerrensis*, a tropical distylous shrub. *Oecologia* **107**: 504–512.
- Taylor CM. 1989. Revision of *Palicourea* (Rubiaceae) in Mexico and Central America. *Systematic Botanical Monographs* **26**: 1–102.
- Williams-Linera G. 2003. Temporal and spatial phenological variation of understory shrubs in a tropical montane cloud forest. *Biotropica* **35**: 28–36.
- Wright SJ. 1991. Seasonal drought and the phenology of understory shrubs in a tropical moist forest. *Ecology* **72**: 1643–1657.
- Zar JH. 1984. *Biostatistical analysis*. Englewood Cliffs: Simon & Schuster.