

## RESEARCH PAPER

**Morphological variation in the flowers of *Jacaratia mexicana* A. DC. (Caricaceae), a subdioecious tree**A. Aguirre<sup>1</sup>, M. Vallejo-Marín<sup>2</sup>, E. M. Piedra-Malagón<sup>3</sup>, R. Cruz-Ortega<sup>4</sup> & R. Dirzo<sup>5</sup>

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Mexico; sexual variation.**Correspondence**A. Aguirre, Departamento de Biología  
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**ABSTRACT**

The Caricaceae is a small family of tropical trees and herbs in which most species are dioecious. In the present study, we extend our previous work on dioecy in the Caricaceae, characterising the morphological variation in sexual expression in flowers of the dioecious tree *Jacaratia mexicana*. We found that, in *J. mexicana*, female plants produce only pistillate flowers, while male plants are sexually variable and can bear three different types of flowers: staminate, pistillate and perfect. To characterise the distinct types of flowers, we measured 26 morphological variables. Our results indicate that: (i) pistillate flowers from male trees carry healthy-looking ovules and are morphologically similar, although smaller than, pistillate flowers on female plants; (ii) staminate flowers have a rudimentary, non-functional pistil and are the only flowers capable of producing nectar; and (iii) perfect flowers produce healthy-looking ovules and pollen, but have smaller ovaries than pistillate flowers and fewer anthers than staminate flowers, and do not produce nectar. The restriction of sexual variation to male trees is consistent with the evolutionary path of dioecy from hermaphrodite ancestors through the initial invasion of male-sterile plants and a subsequent gradual reduction in female fertility in cosexual individuals (gynodioecy pathway), but further work is needed to confirm this hypothesis.

**INTRODUCTION**

Most species in the Caricaceae, a small family of tropical trees and herbs, possess separate male and female structures in different individuals, a sexual system known as dioecy. In contrast with the high incidence of dioecy in the Caricaceae, this sexual system is relatively rare among flowering plants, occurring only in approximately 6% of species (Richards 1986). Dioecy is thought to have evolved from cosexual ancestors through the suppression of male or female reproductive structures in different individuals. In some cases, the separation of the sexes is not complete, and individuals of one sex may produce sexual structures of the other sex. Although sexual variation in dioecious taxa is a widespread phenomenon (Darwin 1877; Ross 1982; Ehlers & Bataillon 2007), we know

relatively little about the consequences of this variation for the ecology and evolution of dioecious species.

The analysis of sexual variation in dioecious species can provide information on the ecology and evolution of separate sexes in at least two ways. First, present-day patterns of sexual variation can be used to infer the evolutionary history leading to the evolution to dioecy. For instance, two of the main hypotheses for the evolution of dioecy from cosexuality make different predictions of the type of sexual variation observed in dioecious taxa. The *gynodioecy pathway hypothesis* posits that dioecy evolved from hermaphrodite or monoecious ancestors in a two-step process. A male-sterile mutation first spreads through the population, rendering some of the individuals female, while others remain hermaphrodite (*i.e.* gynodioecy) (Darwin 1877; Charlesworth & Charlesworth 1978a);

then, selection acting on pollen-producing hermaphrodites favours specialisation in male function, and hermaphrodites gradually lose female reproductive functions. Generally, dioecious taxa that evolve through this pathway tend to have female plants that are invariant in sexual expression, and male plants that occasionally set seed (Lloyd 1976; Ross 1982). In contrast, the *cosexual pathway hypothesis* (often referred to as the *monoecy pathway*) suggests that the transition from cosexuality to dioecy occurred through the gradual specialisation towards either male or female functions of a cosexual (monoecious, *i.e.* separate female and male flowers in the same individual) ancestor (Ross 1982). Because in this pathway both male and female individuals are derived from the same cosexual phenotype through gradual specialisation of either sexual function, the theory predicts that sexual variation might occur in both male and female plants (Charlesworth & Charlesworth 1978b; Ross 1982). Analysis of the extent and type of sexual variation in present-day dioecious taxa is thus a useful way to distinguish between some of the different evolutionary scenarios leading to the separation of the sexes.

Second, the type of sexual variation present in extant populations can determine the trajectory of further evolutionary change. For example, the re-invasion of cosexuals into dioecious populations could depend on whether cosexual individuals combine both sexes in the same flower (perfect flowers), or whether sexes are segregated to different flowers on the same individual (separate staminate and pistillate flowers). In species with dimorphic flowers, the production of perfect flowers may result in floral phenotypes that are intermediate between staminate (male) and pistillate (female) flowers. To the extent that intermediate floral phenotypes are unable to optimally perform both male and female functions, this type of sexual interference may act against hermaphrodites. This selective disadvantage against intermediate floral phenotypes could be avoided if cosexuals produce separate staminate and pistillate flowers on the same plant. In contrast, if seed set is limited by the availability of pollinators, producing perfect flowers that can self-fertilise autonomously may be more advantageous than producing separate staminate and perfect flowers that still require the aid of a pollen vector. Therefore, the type of sexual variation (*i.e.* producing perfect or staminate/pistillate flowers or both) has the potential to determine the selective benefits and costs of cosexuality. Studies of sexually variable dioecious species rarely distinguish whether individuals combine sexes within or between flowers, and thus we know little about the potential benefits and costs of cosexuality in these taxa.

In the present study, we characterise sexual variation in flowers of the tropical tree *Jacaratia mexicana* (Caricaceae). We show that female individuals of *J. mexicana* produce only pistillate flowers, and that males may produce up to three different types of flowers – staminate, pistillate and perfect. We describe the different types of flowers using 26 morphological characteristics, and show

that female-fertile flowers produced in male plants are smaller in size and have fewer ovules than pistillate flowers in female individuals. In addition, we show that hermaphrodite flowers have fewer stamens than staminate flowers in male individuals.

## MATERIAL AND METHODS

### Study area

Trees were sampled from natural populations present in the Sierra de Huautla Biosphere Reserve in Central-South Mexico. The reserve is located in the state of Morelos (18°20′–19°07′ N, 98°37′–99°30′ W) (Aguirre *et al.* 2007). The state of Morelos comprises part of the Balsas River basin, and is one of the most important centres of endemism in Mexico (Rzedowski 1991). Seasonally tropical dry forest covers the majority of the state's area (60% or about 2843 km<sup>2</sup>) (Trejo & Dirzo 2000). The climate is warm-subhumid (mean annual temperature 22–26 °C and annual rainfall 800–1000 mm) (García 1988). The landscape is characterised by a series of alternating valleys and mountain ranges (Trejo & Dirzo 2000).

### Studied species

The genus *Jacaratia* (Caricaceae) comprises six dioecious species of trees generally occurring in seasonally dry tropical forests from Mexico to northern Argentina (Badillo 1971; Olson 2002). *Jacaratia mexicana* A. DC. is a tree 5- to 25-m tall, with a trunk reaching up to 1 m in diameter at breast height. It is distributed in Mexico and Central America at elevations up to 1500 m a.s.l. (Badillo 1971). Flowering in *J. mexicana* occurs during the dry season when trees have lost their foliage (Bullock 1992). In Central Mexico, flowering takes place between November and February. This species has been traditionally described as dioecious, with staminate (male) and pistillate (female) flowers produced in separate individuals (Baker 1976; Bullock 1992; Pennington & Sarukhán 1998). As in other species in the Caricaceae (*e.g.* *Carica papaya* and *Jacaratia dolichaula*; Bawa 1980), *J. mexicana* is sexually dimorphic for flower size and number. Pistillate flowers are larger and heavier than staminate flowers [pistillate flowers: floral length = 31.65 ± 0.270 mm (mean ± standard error), mean dry mass = 265 mg; staminate flowers: floral length = 17.31 ± 0.37 mm; mean dry mass = 15 mg; fruit length = 19.5 ± 0.34 cm]. Pistillate flowers are borne solitarily, while staminate flowers form panicles (Bullock 1992). Staminate flowers of *J. mexicana* produce small amounts of nectar (*ca.* 1.9 ml) with a high amino acid content (Bullock 2002). Staminate flowers have a sterile pistil (pistilloid) (Badillo 1971; Pennington & Sarukhán 1998). Pistillate flowers do not produce nectar, although in *J. dolichaula* they secrete a stigmatic exudate that could attract pollinators. As in other species in the genus (*e.g.* *J. dolichaula*), female flowers have similar fragrances to male flowers. Anthesis is nocturnal, and the main visitors

are sphingid moths (Sphingidae), which visit both types of flower (Bullock 2002), and nocturnal bees (*Megalopta* sp., Halictidae), which visit mainly staminate flowers.

Although *J. mexicana* has been traditionally described as dioecious (Baker 1976; Bullock 1992; Pennington & Sarukhán 1998), sexual variation has been reported since its original description (De Candolle 1864; Díaz-Luna & Lomelí-Senci6n 1997). In a previous investigation, we found that in some populations inside the Sierra de Huautla Reserve up to 50% of male trees are sexually variable, producing both staminate and perfect (hermaphrodite) flowers, and are capable of setting viable seeds, concluding that *J. mexicana* is better described as functionally subdioecious (Aguirre *et al.* 2007).

### Flower collection

In December 2005, flowers were collected from different trees in the locality 'Cañada de Ajuchitlán' (18°27'64" N; 99°00'24" W) inside the Sierra de Huautla Biosphere Reserve. Preliminary observations of *J. mexicana* revealed that three types of trees are found within the reserve: female (with only pistillate flowers), male (with only staminate flowers) and hermaphrodite (with staminate, perfect, and occasionally pistillate flowers) (Aguirre *et al.* 2007). Hermaphrodite individuals are hereafter referred to as fruiting males. We randomly sampled staminate, pistillate and perfect flowers from the crown of each tree, trying to include as many individuals as possible. Pistillate flowers were labelled as pistillate solitary (PS) if they were collected from female trees (N = 18 trees) and as pistillate in panicle (PP) if collected from a fruiting male (N = 20 trees). Staminate flowers (SM) were collected from male trees (N = 20 trees), and perfect flowers (HM) were collected from fruiting males (N = 14 trees) (Fig. 1).

### Floral measurements

Floral measurements were done using digital calipers with the aid of a Nikon SMZ-1500 dissecting microscope (Nikon Corp., Kanagawa, Japan). Pictures of representative structures were taken with a Nikon Coolpix 4900

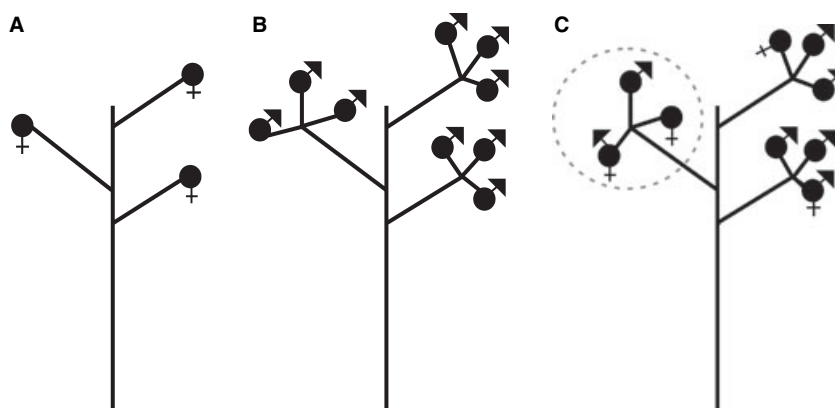
digital camera (Nikon Corp., Chiyodaku, Tokyo, Japan). In total, 26 different morphological measurements were taken in the following manner. Measurements for pistillate flowers (PS and PP) included: (A) sepal length, (B) width of the apical tip of the sepals, (C) width of the base of sepals, (D) width and (E) length of the ovary, (F) width and (G) length of the ovary cavity, and (H) length and (I) width of the stigmas (Fig. 2A). In addition, for HM flowers, measurements also included (J) length and (K) width of the longest anther filament, and (L) length and (M) width of the anthers (Fig. 2B). Measurements for staminate flowers (SM) included the variables A–C, J–M as well as (N) length and (O) width of the pistiloid (rudimentary pistil) and (P) corolla tube length (Fig. 2C). In addition, we recorded sepal number (all floral morphs), loculae and stigma number (PS, PP and HM morphs), and stamen number (HM and SM morphs). Measurements were done in triplicate, when possible, and average values were used in the analyses.

### Flower preparation for scanning electronic microscopy

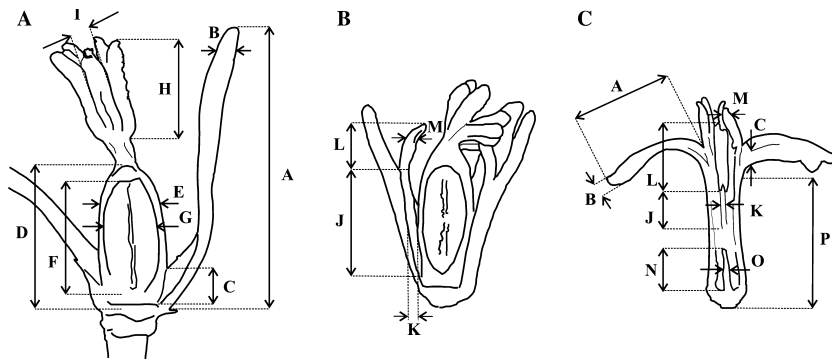
Immediately after collection, flowers were fixed in a solution of 3% glutaraldehyde in 0.15 M potassium buffer (K<sub>2</sub>HPO<sub>4</sub>). The glutaraldehyde was replaced by ethanol through a series of dilutions (10%, 30%, 50% and 70%), to reach a final solution containing 100% ethanol. Samples were then dried to critical point with CO<sub>2</sub> in an EMITECH K850, and then covered using a Sputter coater with gold in an EMITECH K550 (Quorum Technologies Ltd, Sussex, UK). The samples were processed in a scanning electron microscope (SEM, Hitachi S-2460N) operating at Kv15 (Hitachi, Japan) and in a Hitachi tabletop microscope TM1000 (Hitachi High-Technologies Corp., Tokyo, Japan). Photographs were taken with a 35-mm Pentax Z-10 camera.

### Data analysis

Statistical analyses were carried out using Statistica software 7.0 (StatSoft, Inc. 2004). Means are presented with associated standard error (mean ± SE). Correlations



**Fig. 1.** Schematic representation of the occurrence of four floral morphs in *J. mexicana* trees. A: Female tree, with only pistillate flower (PS). B: Male tree with only staminate flowers (SM). C: Male tree with pistillate (PP), staminate (SM) and hermaphrodite flowers (HM). The dashed circle indicates the presence of the three different floral morphs in the same panicle.



**Fig. 2.** Schematic representation of the different structures measured in the four floral morphs. A: Pistillate flower (both PS and PP). B: Hermaphrodite flower (HM). C: Staminate flower (SM).

among traits were calculated using Spearman correlations. Variables that were not significantly correlated were used to compare morphological differences between floral morphs using non-parametric statistics as the criterion of variance homoscedasticity was generally not met. Wilcoxon signed-rank tests were used for comparing structures between two floral morphs, and Kruskal–Wallis tests for analyses of variables with more than two levels (flower type). *Post hoc* multiple comparisons were done using mean rank tests with adjusted *P*-values.

## RESULTS

### Sexual variation among trees

Based on the production of the different flower morphs, we found three types of individuals in the population: female trees producing only pistillate flowers (Fig. 1A), male trees producing only staminate flowers (Fig. 1B), and fruiting male trees (hermaphrodites) producing pistillate and perfect flowers in addition to staminate flowers (Fig. 1C). Within individual fruiting males, the occurrence of the staminate, pistillate and perfect flowers varies: some panicles have exclusively staminate flowers, others have staminate and perfect flowers, and yet others can have the three floral morphs (staminate, perfect and pistillate). However, fruiting males always produce more staminate flowers than either perfect or pistillate flowers.

### Description of structures in the different flower types

Mature flowers of *J. mexicana* display marked sexual dimorphism. Pistillate flowers from female trees (PS) are borne singly at the end of the reproductive branch, and are held by a robust pedicel ( $35.2 \pm 4.27$  mm long) (mean  $\pm$  SE). In contrast, staminate flowers from male trees are borne in panicles containing between seven and 80 flowers (Díaz-Luna & Lomelí-Sención 1997), and are attached to the branch through a much shorter pedicel ( $2.35 \pm 0.43$  mm long). Staminate flowers present a rudimentary pistil or pistiloid with nectarostomes at its base (Fig. 3S and T). Flowers in sexually variable individuals (fruiting males) are borne in panicles that combine staminate (SM), pistillate (PP) and perfect (HM) flowers. Sta-

minate flowers borne in either male or fruiting male trees are morphologically indistinguishable (data not shown). However, the morphology of PP and HM flowers in fruiting males is intermediate between staminate and pistillate flowers.

Pistillate flowers from female trees (PS) have significantly larger sepals and ovaries than any of the other floral types [Kruskal–Wallis test,  $H$  ( $df = 3$ ,  $N = 72$ ) = 60.614,  $P < 0.001$ ; and  $H$  ( $df = 3$ ,  $N = 72$ ) = 57.332,  $P < 0.001$ , respectively] (Table 1). At the bud stage, pistillate flowers from female trees (PS) are significantly longer than all other floral morphs (Table 1). In contrast, the length of flower buds in PP, HP and SM was not statistically different. This similarity among floral buds occurring in panicles makes it very difficult to distinguish between the different sexual morphs at early developmental stages.

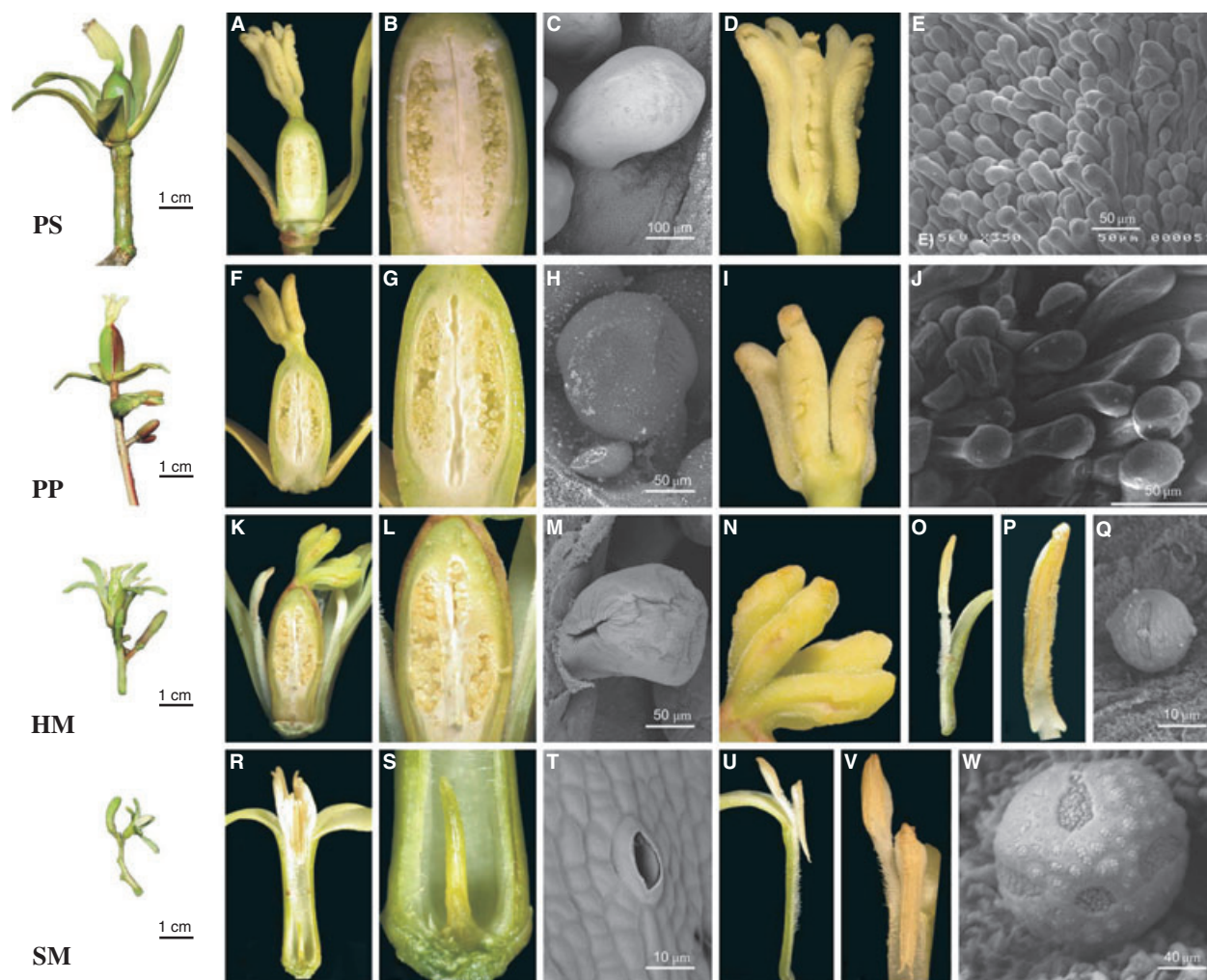
### Differences between pistillate and perfect flowers

Pistillate flowers in female trees (PS) have larger ovaries, longer styles and longer but fewer stigmas than pistillate flowers in fruiting males (PP) (Wilcoxon test,  $Z = 4.15$ ,  $N = 32$ ,  $P < 0.0001$ ; and  $Z = 4.78$ ,  $N = 32$ ,  $P < 0.0001$ , respectively) (Table 2, Fig. 3). However, both perfect (HM) and pistillate flowers from fruiting males (PP) were not statistically different in terms of sepal and ovary length. HM flowers were statistically different in stigma number in relation to PP and PS flowers. HM flowers had a reduced stigma length and ovary size relative to PP flowers. PS, PP and HM flowers produce ovules that are morphologically similar (Fig. 3).

### Differences between perfect and staminate flowers

Perfect flowers (HM) produce significantly fewer stamens than staminate flowers (SM) (differences in median for each morph; Wilcoxon test  $Z = 4.14$ ,  $N = 34$ ,  $P < 0.0001$ ; Table 3). Stamens in HM flowers are free, and attach to the base of the corolla through a single verticil (Fig. 3O and P), while stamens in SM are fused to the corolla through two verticils of different length (Fig. 3U and V). SM flowers have both longer anther's filaments and longer anthers than HM flowers (Wilcoxon test  $Z = 4.88$ ,  $N = 34$ ,  $P < 0.0001$ ; and  $Z = 4.53$ ,  $N = 34$ ,  $P < 0.0001$ ,





**Fig. 3.** Flower types in *Jacaratia mexicana* and their representative structures. Pistillate flower of female trees (PS): (A) longitudinal section of the PS, (B) cavity of ovary showing large quantity of ovules, (C) ovule, (D) stigmas, (E) stigma surface. Pistillate flower of male trees (PP): (F) cross section of the PP, (G) cavity of ovary and ovules, (H) ovule, (I) stigmas, (J) stigma surface. Hermaphrodite flower (HM): (K) longitudinal section of HM, (L) cavity of ovary showing smaller ovules than PP and PS, (M) ovule showing atypical morphology as compared to PS and PP, (N) stigmas, (O) complete stamen with sepal, (P) anther, (Q) pollen grain. Male flower (MC): (R) longitudinal section, (S) reduced ovary (pistilloid), (T) nectarostome located at the base of the pistilloid, (U) entire stamen showing two anthers attached to the sepal, (V) front view of anthers, and (W) pollen grain.

**Table 1.** Morphological differences among four floral morphs of *Jacaratia mexicana*.

structures	PS, N = 53 (mm, mean $\pm$ SE)	PP, N = 60 (mm, mean $\pm$ SE)	HM, N = 42 (mm, mean $\pm$ SE)	SM, N = 60 (mm, mean $\pm$ SE)
Bud length	31.65 $\pm$ 0.270 <sup>a</sup>	16.93 $\pm$ 0.96 <sup>b</sup>	17.07 $\pm$ 0.86 <sup>b</sup>	17.95 $\pm$ 0.48 <sup>b</sup>
Bud width	9.11 $\pm$ 0.13 <sup>a</sup>	6.80 $\pm$ 0.26 <sup>b</sup>	5.04 $\pm$ 0.34 <sup>b</sup>	3.52 $\pm$ 0.17 <sup>c</sup>
Sepal length	39.69 $\pm$ 1.20 <sup>a</sup>	25.17 $\pm$ 1.24 <sup>b</sup>	20.92 $\pm$ 1.39 <sup>b</sup>	11.03 $\pm$ 0.36 <sup>c</sup>
Sepal width, apical tip	4.13 $\pm$ 0.13 <sup>a</sup>	2.97 $\pm$ 0.11 <sup>b</sup>	2.85 $\pm$ 0.07 <sup>b</sup>	1.69 $\pm$ 0.10 <sup>c</sup>
Sepal width, base	7.09 $\pm$ 0.22 <sup>a</sup>	4.18 $\pm$ 0.12 <sup>b</sup>	3.29 $\pm$ 0.12 <sup>b,c</sup>	2.54 $\pm$ 0.09 <sup>c</sup>
Ovary length	20.98 $\pm$ 0.90 <sup>a</sup>	16.32 $\pm$ 0.55 <sup>a,b</sup>	13.00 $\pm$ 0.70 <sup>b</sup>	5.28 $\pm$ 0.24 <sup>c*</sup>
Ovary width	9.76 $\pm$ 0.37 <sup>a</sup>	6.51 $\pm$ 0.19 <sup>b</sup>	4.38 $\pm$ 0.39 <sup>b</sup>	0.58 $\pm$ 0.03 <sup>c*</sup>

The different flower morphs were compared using multiple comparison tests on mean rank values. Statistically significant differences among floral morphs for each trait are indicated with different letters. Floral morphs as in Fig. 1.

\*Measurements correspond to the pistilloid.

**Table 2.** Morphological differences among flowers with female function.

structures	PS, mm (mean ± SE)	PP, mm (mean ± SE)	HM, mm (mean ± SE)
Ovary cavity length	15.07 ± 0.62 <sup>a</sup>	12.13 ± 0.45 <sup>b</sup>	7.33 ± 0.81 <sup>c</sup>
Ovary cavity width	6.39 ± 0.29 <sup>a</sup>	4.40 ± 0.18 <sup>b</sup>	2.37 ± 0.33 <sup>c</sup>
Style length	4.41 ± 0.47 <sup>a</sup>	1.57 ± 0.13 <sup>b</sup>	0.19 ± 0.15 <sup>c</sup>
Style width	2.93 ± 0.13 <sup>a</sup>	2.32 ± 0.08 <sup>b</sup>	0.32 ± 0.21 <sup>c</sup>
No. of stigmas/flower	4.94 ± 0.05 <sup>a</sup>	5.00 ± 0.07 <sup>a</sup>	4.28 ± 0.19 <sup>b</sup>
Stigma length	13.76 ± 0.54 <sup>a</sup>	9.83 ± 0.34 <sup>b</sup>	6.33 ± 0.33 <sup>c</sup>
Stigma width	3.00 ± 0.12 <sup>a</sup>	1.83 ± 0.04 <sup>b</sup>	1.53 ± 0.07 <sup>b</sup>

Sample sizes as in Table 1. The different flower morphs were compared using multiple comparison tests on mean rank values. Statistically significant differences among floral morphs for each trait are indicated with different letters. Floral morphs as in Fig. 1.

**Table 3.** Morphological differences between flowers with male function.

structures	HM, mm (mean ± SE)	SM, mm (mean ± SE)
No. of stamens/flower	4.78 ± 0.29 <sup>a</sup>	8.20 ± 0.42 <sup>b</sup>
Filament length	9.44 ± 0.29 <sup>a</sup>	4.15 ± 0.12 <sup>b</sup>
Filament width	0.94 ± 0.02 <sup>a</sup>	0.57 ± 0.01 <sup>b</sup>
Anther length	5.88 ± 0.16 <sup>a</sup>	4.19 ± 0.09 <sup>b</sup>
Anther width	1.36 ± 0.05 <sup>a</sup>	1.01 ± 0.02 <sup>b</sup>

Sample sizes as in Table 1. The different flower morphs were compared using multiple comparison tests on mean rank values. Statistically significant differences among floral morphs for each trait are indicated with different letters. Floral morphs as in Fig. 1.

respectively) (Table 3). Both types of flowers produce similar-looking pollen with an approximate diameter of 20 µm (Fig. 3Q and W).

## DISCUSSION

In this paper we have shown that male individuals of *J. mexicana* are sexually variable and can produce up to three different floral morphs: staminate, perfect and pistillate. Our results indicate that pistillate flowers in male plants (PP) tend to be smaller than pistillate flowers in female individuals (PS). In contrast to pistillate flowers in female plants, female-fertile flowers in male plants are produced in panicles and can be intermingled with staminate flowers. In addition, we found that perfect flowers in male plants have, on average, half the number of stamens of staminate flowers.

### Floral morphology of sexually variable individuals

Our analysis of floral morphology in *J. mexicana* is one of the few available studies of floral morphology in sexual variants in the Caricaceae, which has been analysed in

detail only in *Carica papaya* L (Ronse Decraene & Smets 1999). *Carica papaya* is known to produce sexually variable individuals in cultivation, although sexual variation is rare in natural populations (Badillo 1971). In *C. papaya*, a single plant may produce staminate, perfect and pistillate flowers. Interestingly, perfect flowers in *C. papaya* can be of two types (Storey 1969). One type, the *elongata*, produces 10 anthers arranged in two whorls, as in regular staminate flowers, and has a functional ovary. The second type, *pentandria*, has a functional ovary but only five anthers in a single whorl. Storey (1969) suggests that during the evolution of unisexuality, the bisexual flower was transformed into a pistillate flower by first suppressing the lower level whorl of anthers that resulted in a perfect flower with only five anthers, resembling the extant *pentandria* floral type. The same author suggests that the *elongata* flower resembles the ancestral bisexual type, while the *pentandria* type is an intermediate stage in the transition towards pistillate flowers. The pistillate flower was subsequently produced by the abortion of the second whorl of stamens. In our survey of sexual variation in *J. mexicana*, we found perfect flowers with five or fewer stamens, the *pentandria* type, but we did not find perfect flowers with 10 stamens as in the *elongata* type of *C. papaya*. Our failure to find *elongata*-type flowers could be due to a number of factors, including a more stringent canalisation of floral development than in *C. papaya*. In any case, in *J. mexicana* the production of carpels in perfect flowers is associated with a reduction in stamen number, and probably with a consequent reduction in pollen production. Further studies are required to determine whether this reduction in stamen number compromises male fitness in sexually variable individuals. From the remaining five species of *Jacaratia*, only *J. digitata* has been reported to produce perfect flowers, although these flowers have a malformed ovary and lack styles and stigmas (Badillo 1971).

The pistillate flowers produced in male plants of *J. mexicana* are morphologically similar but smaller in size to those produced in female plants. The smaller size of pistillate flowers could represent a more limited access to resources in male individuals, where all flowers occur in racemes. Because pistillate flowers in female plants occur singly, they may also suffer from less intense resource competition from adjacent flowers, thus resulting in larger flowers. This resource competition hypothesis could be experimentally tested by reducing the level of resource competition among flowers of male plants by, for example, comparing the size of pistillate flowers where adjacent floral buds are removed *versus* that of flowers when all buds are allowed to develop. The fitness consequences through seed production of differences in size between pistillate flowers in male and female individuals remain to be tested. However, Aguirre *et al.* (2007) showed that fruits developing in male individuals produce fewer, smaller fruits and seeds than those of female plants, and that seeds from male plants are also less likely to germinate and survive the seedling stage.

**Table 4.** Distribution, life form, habitat and sexual system of genera in the family Caricaceae.

genus	no. of species	life form	distribution	habitat	sexual system	sexual variation	variable sex
<i>Cylicomorpha</i> Urban	2	Bottle trees	Equatorial Africa	Humid habitats, montane forests	Dioecious	None recorded	–
<i>Horovitzia</i> Badillo	1	Perennial herb	Mexico	Cloud forest	Dioecious	None recorded	–
<i>Jarilla</i> Rusby	3	Tuberous herbs	South Mexico, Guatemala	Dry tropics	Dioecious	None recorded	–
<i>Jacaratia</i> A. DC.	7	Bottle trees	Mexico to northern Argentina	Mostly dry tropics	Dioecious	Yes	Males (fruiting males) <sup>a</sup>
<i>Vasconcellea</i> Saint-Hilaire	21	Bottle trees, tuberous herbs	South America, Andean valleys in Ecuador	Wet and dry tropics	Dioecious, monoecious <sup>b</sup> , and polygamous <sup>c</sup>	Yes	Males (fruiting males) <sup>d</sup>
<i>Carica</i> L.	1	Bottle tree	Mexico to northern Argentina	Dry and wet tropics	Dioecious (polygamous in cultivation) <sup>e</sup>	Yes	Males, hermaphrodites (fruiting males) <sup>f</sup>

<sup>a</sup> *Jacaratia digitata* (Poep. et Endl.) Solms, *J. spinosa* (Aubl.) A. DC., *J. mexicana* A. DC.

<sup>b</sup> *Vasconcellea monoica* (= *Carica monoica* Desf.) Badillo (1971, 2000).

<sup>c</sup> *Vasconcellea pubescens* (= *Carica pubescens* Lenne et Koch) Badillo (1971, 2000).

<sup>d</sup> *Vasconcellea sprucei* (= *Carica sprucei* Badillo 1971, 2000), *Vasconcellea aprica* (= *Carica aprica* Badillo 1971, 2000), *Vasconcellea microcarpa* ssp. *microcarpa* (= *Carica microcarpa* Jacq. ssp. *microcarpa*), *Vasconcellea stipulata* (= *Carica stipulata* Badillo 1971, 2000).

<sup>e</sup> *Carica papaya* L. Badillo (1971, 2000).

<sup>f</sup> Badillo (1971: 27); Storey (1969).

Taxonomy of the family after Badillo (2000). Data from Kyndt *et al.* (2005), Olson (2002) Badillo (1971, 2000), Van Droogenbroeck *et al.* (2004), Diaz-Luna & Lomeli-Sención (1992, 1997), <http://www.iucnredlist.org>, and this study.

### Future directions: exploring the pathway to dioecy

Our study revealed that sexual variation in *J. mexicana* is restricted to male plants. It has been proposed that determining whether sexual variation is restricted to males or is found in both male and female plants can help to discern the evolutionary history of dioecious species (Charlesworth & Charlesworth 1978a,b; Ross 1982). The fact that female individuals are constant in their sex expression is consistent with the hypothesis that dioecy in this taxon evolved *via* the gynodioecy pathway. Interestingly, when sexual variation occurs in the family Caricaceae (Table 4), it is restricted to male individuals, and that seems to be the case in species of different genera and life forms. Further studies on the patterns of sexual expression among individuals are required to test the hypothesis that dioecy in this group has indeed evolved through the gynodioecy pathway.

The type of sexual variation available in near-dioecious species can influence the trajectory of further evolutionary modification of sexual systems. Our results indicate that *J. mexicana* can produce perfect, staminate and pistillate flowers in sexually variable individuals. This flexibility in sexual expression may allow *J. mexicana* to exploit the hypothesised advantages of sexual variation in near dioecious species. For example, the separation of the sexes in different staminate and pistillate flowers within individu-

als (monoecy), rather than individuals with only perfect flowers (hermaphrodite), could help to reduce within-flower self-fertilisation and interference among sexual organs (Barrett 2002), thus facilitating the invasion of sexually variable plants into dioecious populations. We are not aware of any studies that assess the extent of genetic control on the production of staminate, pistillate and perfect flowers in any sub-dioecious species, and this is an avenue clearly worth investigating.

The widespread occurrence of dioecy in the Caricaceae, together with the ongoing development of molecular tools in this group including the characterisation of sex-determining chromosomes (Liu *et al.* 2004; Ma *et al.* 2004), and an ongoing effort to sequence the genome of *C. papaya* (Hawaii Papaya Genome Project at the University of Hawaii; <http://cgpbr.hawaii.edu/papaya>), makes the Caricaceae a promising model system to study the genetic, ecological and evolutionary consequences of separating sexes in different individuals.

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