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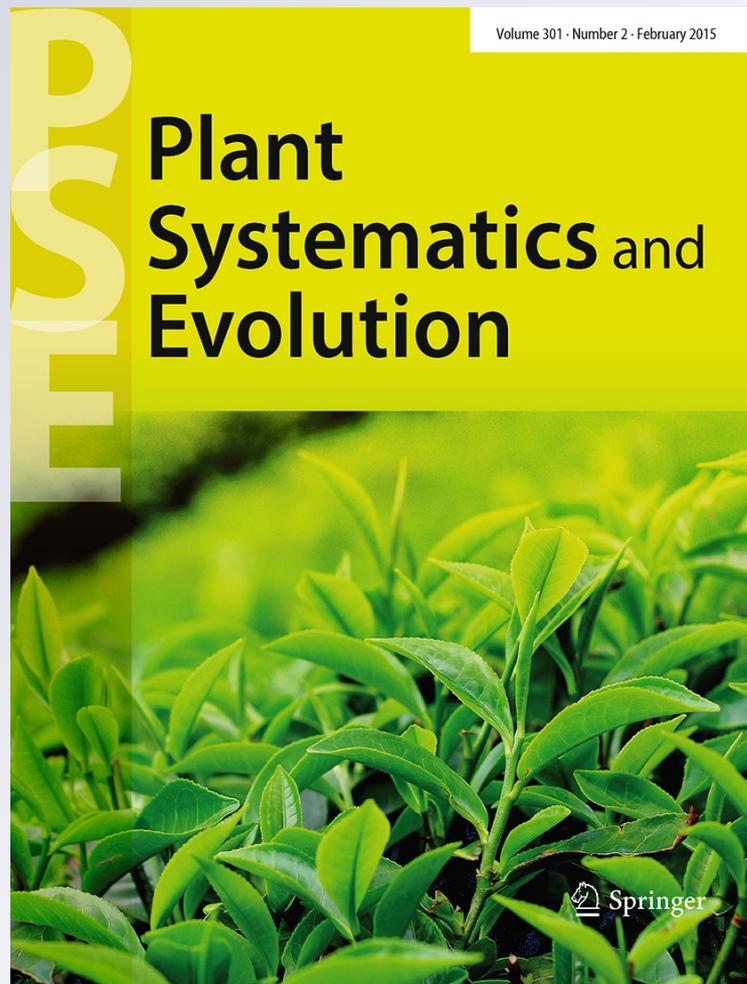
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Reproductive biology of *Vasconcellea quercifolia* A. St.-Hil. (Caricaceae), a moth-pollinated ‘highland papaya’

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Abstract *Vasconcellea* species, often referred to as highland papayas, are wild relatives of common papaya (*Carica papaya*) widely distributed in tropical America, with a preference for cooler climates. The genus deserves special attention as its species show potential as raw materials in the tropical fruit industry, papain source or as genetic resources in breeding programs of *C. papaya*. However, studies related to breeding system and pollination biology of genus are still unexplored. We characterize the reproductive biology of dioecious *Vasconcellea quercifolia*, one of the southernmost Caricaceae. According to floral shape and presence of nectar, we expected that this species had a specialized pollination system, with moths as the primary pollinators. To test this, we studied floral biology, floral visitors, pollinators, and breeding system of this species. Controlled pollination experiments show that *V. quercifolia* is a xenogamous species that depends on its pollinators to set fruits (18 and 79 % fruit and seed set under open-pollination, respectively). Anemophily and apomixis occur very seldom. The treatments of exclusion of floral visitors and pollen load analysis showed

that the main pollinators are moths belonging to Arctiidae, Noctuidae, and Pyralidae. The only reward for pollinators is the nectar produced by male flowers. Female flowers are pollinated by deceit because they do not produce floral rewards.

Keywords Caricaceae · Deceit pollination · Dioecy · Moth pollination

Introduction

Vasconcellea St.-Hil. is the largest genus of Caricaceae, with 21 of the 35 taxa described for this family (Badillo 2000, 2001; Kyndt et al. 2005), exclusively composed of wild relatives of common papaya (*Carica papaya* L.) often referred to as ‘highland papayas’ (National Research Council 1989). They occur exclusively in Central and South America, and spread mainly at high altitudes over the region stretching from south of Mexico to north of Chile, Argentina and south of Brazil, with their center of diversity located in the Andes of South American (Scheldeman et al. 2007). *Vasconcellea* species show interesting potential and scope for domestication in different regions. They could be used as raw material in the industry of tropical fruit, as a source of proteases and other biologically active components of pharmaceutical interest, and also as a genetic resource in programs for the improvement of *C. papaya* (d’Eeckenbrugge et al. 2014). *Vasconcellea quercifolia* A. St.-Hil. is one of the few species of the genus that apart from inhabiting high altitudes it also grows in lowlands and reaches the highest latitudes of distribution (Scheldeman et al. 2007). This species has great attractiveness for human beings: it is resistant to PRSV-P *C. papaya* ringspot virus, it is capable

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of producing vigorous inter-generic hybrids with this species, and is a source of proteases with a proteolytic activity higher of genus (Drew et al. 2006; Siar et al. 2011; Torres et al. 2010), although it is not cultivated nowadays.

As occurs in Caricaceae, *Vasconcellea* species are mostly dioecious, as monoecious and polygamous occur less frequently. Monoecy has been observed only in *Vasconcellea monoica* (Desf.) A. DC., and polygamy has been proposed for *Vasconcellea cundinamarcensis* Badillo and *Vasconcellea pubescens* A. DC. (Badillo 1971, 2000). It is stated that *V. cundinamarcensis* presents rare cases of andromonoecy, a trait shared only with *C. papaya* (Scheldeman et al. 2011). Flowers of studied species of Caricaceae are sexually dimorphic for their size and number for inflorescence, nectar production, and phenology (Baker 1976; Bawa 1980; Bullock and Bawa 1981; Aguirre et al. 2009). Sexual dimorphism is not always absolute as it is possible that individuals with a defined sexual morph produce floral structures of the opposite sex. Variation in sexual expression was observed in cultivated individuals of *C. papaya* and species of *Vasconcellea* and *Jacaratia*, mainly occurring in males (Badillo 1971, 2000; Ronse De Craene and Smets 1999; Aguirre et al. 2009).

Studies on the pollination biology of Caricaceae report sphingid moths as the main pollinators of *C. papaya* (Baker 1976; Garrett 1995), and species of *Jacaratia* (Bullock and Bawa 1981; Piratelli et al. 1998; Bullock 2002). Indeed, it is suggested that a system of deceit pollination occurs due to the absence of reward in female flowers (Baker 1976; Bawa 1980). Floral shape and quantities of floral rewards in studied species of Caricaceae reflect morphology and metabolisms of mentioned pollinators. Sphingid moths are crepuscular or nocturnal visitors with high metabolic rate and long proboscis that visit long-tubular flowers of heavy sweet fragrance and copious nectar as reward (Haber and Frankie 1989; Bawa 1990; Proctor et al. 1996). In deceit pollination systems they are considered effective pollinators because they may not recognize and remember unrewarding morphs (Renner and Feil 1993).

Members of *Vasconcellea* share floral traits related to pollination by nocturnal lepidopterans with the rest of Caricaceae. However, *Vasconcellea* flowers are smaller and conform the classic phalaenophilous syndrome (Faegri and van der Pijl 1979): short narrow corolla tube, greenish flowers, and small amounts of nectar. Previous record on pollination biology of *Vasconcellea* suggests the presence of anemophily for *V. quercifolia* (Colombo et al. 1989). However, according to floral morphology, we hypothesized that this species has a specialized pollination system and is effectively pollinated by moths. In order to provide novel information on reproductive biology and to clarify the pollination system of *V. quercifolia*, we described the

reproductive system, floral traits and flowering strategy related to the attraction of pollinators, and characterized floral visitors and pollinators.

Materials and methods

Study area

Trees were sampled from a natural population located at 'Estancia El Matrero II' (30°51'08.63"S, 60°15'56.37"W), Colonia La Brava, Santa Fe Province, Argentina. The study site is within woods dominated by species of *Prosopis* L., together with *Geoffroea decorticans* (Gillies ex Hook. & Arn.) Burkart, *Phytolacca dioica* L., *Acacia caven* (Molina) Molina, *Erythrina crista-galli* L. and *V. quercifolia*. This forest is an ecotonal community between Chaco and Espinal regions (Pensiero et al. 2005). These lands, where herbaceous communities with fodder value develop, are low and prone to floods during the rainy season (Pensiero et al. 2005). Bovine cattle breeding on a rotational basis has been the main activity in the area for 60 years. The average annual rainfall is about 1,200 mm and the average temperatures range from 16 to 24 °C in the autumn–winter and spring–summer seasons, respectively (INTA 2013). The study site was visited monthly between September and February over the periods 2010–2011 and 2011–2012.

Studied species

Vasconcellea quercifolia is a tree growing up to 7 m tall (Biloni 1990), with a trunk reaching up to 1 m in diameter at breast height. It is markedly adaptable to seasonal temperatures, and it is found in areas of average rainfall which do not show extreme seasonality (Scheldeman et al. 2007). It is distributed throughout the south of the American continent and it comprises a wide region including southern Ecuador, southwestern Bolivia, southern Paraguay, southeastern Brazil, and northern Argentina (Scheldeman et al. 2007). It was described as a dioecious species, with staminate (male) and pistillate (female) flowers produced in separate individuals (Biloni 1990).

Reproductive phenology

The flowering phenology at the population level was described based on the number of scars of fallen flowers, buds, open flowers, and fruits present in three branches (segments of 25 cm long with 3 inflorescences) per female ($n = 15$) and male ($n = 15$) individuals. Sampling dates were evenly spread over each reproductive season (2010–2011 and 2011–2012). In order to describe anthesis

and the phenological stages, 30 female and male floral buds were tagged on 3 individuals of each sex, and observed until flower senescence, at regular intervals. Stigmatic receptivity was evaluated with a 0.1 M guaiacol and 0.1 M hydrogen peroxide solution, considering orange-brown color turn on stigma as indicator of receptive tissue (Tel-Zur and Schneider 2009). Pollen availability was qualitatively determined by touching the anthers with a brush and observing the presence of pollen grains on it. To test pollen viability pollen grains were placed in different germination media (5, 10, 20 and 30 % sucrose solution) and percentage of pollen germination was evaluated using an Olympus CH30 microscope. Stigmatic receptivity, pollen availability and viability were evaluated on ten flowers throughout the flowering cycle.

Floral biology

To study floral morphology, 100 male and female flowers were collected ($n = 5$ flowers from 10 male and female individuals) and preserved in FAA (formalin:acetic acid:alcohol, 1:1:3). The following morphological measurements were recorded for each flower: total length of calyx and corolla, number of lobes of calyx and corolla, ovary length, style length, number and length of stigmatic lobes, stigma diameter, number of ovules, loculae number, number of lower and upper stamens, length of upper and lower anthers, and pistilloid length.

The number of pollen grains per flower was calculated by counting the grains of pollen in flowers in a pre-anthesis stage ($n = 10$, one flower per individual). One theca per flower was taken and opened under a magnifying glass. It was successively rubbed over three small blocks of gelatin–glycerine stained with safranin (Dafni 1992) until no pollen grains were left. The total number of pollen grains found on the blocks was counted under a microscope (Olympus CH30), and it was multiplied by the total number of thecas in each flower. The presence of starch and lipids (pollenkitt) in pollen grains was evaluated by staining with lugol for the former and with Sudan IV for the later two. This is because starchy pollen grains and presence of lipids are expected to be found in moth-pollinated species (Dafni 1992).

Volume and concentration of nectar were measured in male and female flowers using a Hamilton syringe (1701LT, 10 μ l) and manual refractometer ([®]Arcano REFI08 0–80 % Brix), respectively. The measurements were performed after 48 and 76 h from the floral dehiscence.

Floral visitors

Nocturnal observations were made between 2030 and 2400 hours, and diurnal observations between 0900 and

1600 hours. Observations were made during 15 min each hour, on four and six non-consecutive nights and days in 2010 and 2011, respectively, on three individuals on each sex.

Foraging behavior and duration of visits were studied during these observations. Floral visitors were captured using entomological nets when visiting flowers, prepared as per conventional techniques, and identified in the laboratory with the help of a specialist (see Acknowledgments). Insect determination was carried out at the lowest possible taxonomic level (i.e. species, genus or family). Individuals that could not be identified at the species level were assigned to morph-species. Insects are kept in the Entomological Collection of the Department of Agricultural Botany, Facultad de Agronomía, Universidad de Buenos Aires.

Moth visits to female flowers were evaluated by determining the proportion of stigmas with moth scales (Ortega-Baes et al. 2010). Stigmas exposed to free pollination ($n = 65$ stigmas from 13 individuals) were collected in November 2010 and 2011. To avoid contamination and loss of scales, stigmas were isolated in Eppendorf tubes. At the laboratory they were softened in NaOH 8 N 24 h and then colored with safranin. The presence/absence of scales was evaluated under microscope.

To study pollinic load transported by pollinators, under a binocular microscope, a cube of gelatin–glycerine with safranin was passed over the proboscis and the head of the captured moths to extract pollen grains adhering to the bodies. A temporary pollen preparation on a glass slide was made with this jelly cube and the pollen grains were counted under the microscope at 200 \times magnification. Pollen grains observed were classified as co-specific (*V. quercifolia*) and hetero-specific grains (other species). The length of proboscis of captured moths was measured.

Breeding system

Manipulative experiments to determine the breeding system were made during October–November 2010 and 2011. A known number of buds was subjected to the following treatments: (a) *Open pollination* (control): not manipulated flowers exposed to free pollination; (b) *Hand crosspollination (xenogamy)*: flowers bagged with voile bags and subsequently hand-pollinated using a mix of pollen from several individuals; (c) *Anemophily*: flowers bagged with 1 mm mesh, excluding floral visitors and allowing the passage of airborne pollen; and (d) *Apomixis*: flowers bagged with voile bags. The response variables used to compare the treatments were: proportion of fruits and seeds formed, and weight of seeds per fruit. Load of pollen used for crosspollination was made with pollen from randomly selected individuals (at least six), and pollinations were

made between 2400 and 4800 hours following floral dehiscence (see Fig. 3a).

Statistical analysis

Differences in number of open flowers along flowering in male and female individuals, morphological variables of male/female flowers, nectar volume and concentration, and number of co-specific and hetero-specific pollen grains obtained from the stigmatic surface were evaluated applying a *t* test to independent samples (normality and homogeneity of variances were previously tested). Spearman correlation coefficient was calculated for 'corolla tube length' of male flowers and 'length of moth proboscis'. All the analyses were performed with InfoStat software 2011 Version (Di Rienzo et al. 2011). Differences in fruit formation and seed production (number and weight of seeds per fruit) among the pollination treatments were analyzed by the adjustment of general lineal models with the lme function of the nlme package (Pinheiro et al. 2011) of the R statistical language (R Development Core Team 2011), using the interface provided by InfoStat (Di Rienzo et al. 2011).

Results

Reproductive phenology

Flowering of *V. quercifolia* occurred between September and November with the peaks of flowering between mid-October and late November 2010–2011 (Fig. 1a). In both periods, most flowers were available already at the beginning of the observation period (Fig. 1b). Open male flowers outnumbered females throughout the flowering period

($t = 3.74$, $p = 0.0002$ for 2010–2011; $t = 2.53$, $p = 0.023$ for 2011–2012). The onset of fruiting overlapped with the end of flowering. Fruiting started in October and extended until March, being at its peak in November. Fruits ripened 'by pulse' within each individual, from January to March.

Morphology and floral longevity

Both male and female flowers of *V. quercifolia* release a sweet smell, and have green-colored perianths, but show sexual dimorphism in morphology and nectar production (Fig. 2). Male flowers are significantly smaller than females (Table 1), and have a tubular corolla and produce nectar as reward. Conversely, female flowers have free petals along almost all their extension, and do not produce nectar or any other floral reward. These exhibit a 5-locular ovary with 28 ovules, followed by a short style ending in a 5-lobular stigma.

Male flowers have ten stamens. The introrse anthers are inserted in the throat of the corolla tube, forming a staminal column which surrounds the entrance to the tube. There is a pistiloid (4.8 ± 0.13 mm long, $n = 50$) in the internal and basal part of the corolla tube. The pollen grains keep starch as a reserve substance, and there is presence of lipids as pollenkit. The mean number (\pm SD) of pollen grains per flower is 20.246 ± 2.619 ($n = 10$). Nectar is exposed as a continuous liquid layer over the pistiloid surface and it is produced throughout the anthesis period. The volume and concentration of nectar did not differ between floral stages evaluated ($t = 1.76$, $p = 0.09$ for volume; $t = 1.67$, $p = 0.11$ for nectar). Nectar volume and concentration were 1.25 ± 0.36 μ l (mean \pm SD, $n = 20$) and 25.39 ± 4.05 % ($n = 20$), respectively.

Female inflorescences bear 33.62 ± 13.30 flowers ($n = 40$), whereas male ones 48.87 ± 15.36 ($n = 40$)

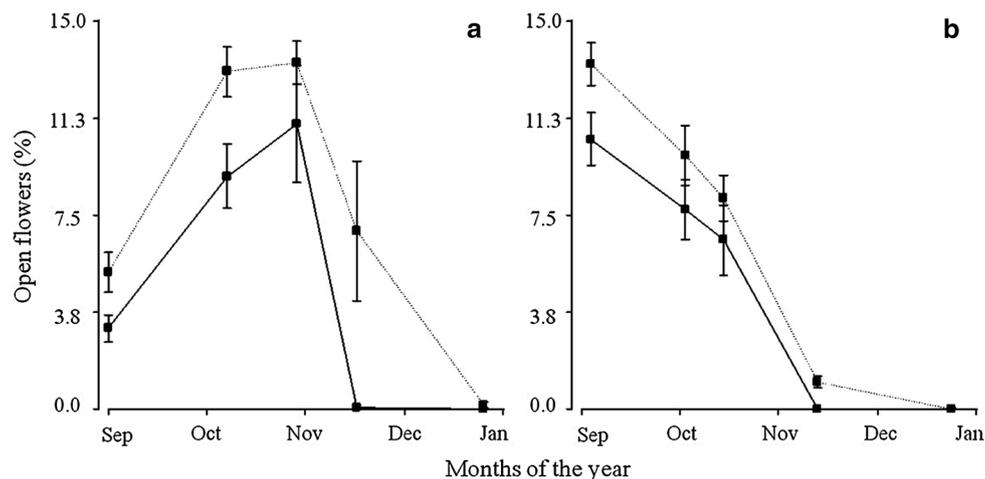


Fig. 1 Flowering rate of male and female trees of *Vasconcellea quercifolia* in two consecutive reproductive seasons. **a** Period 2010–2011, **b** period 2011–2012. Solid line female trees, dotted line male trees

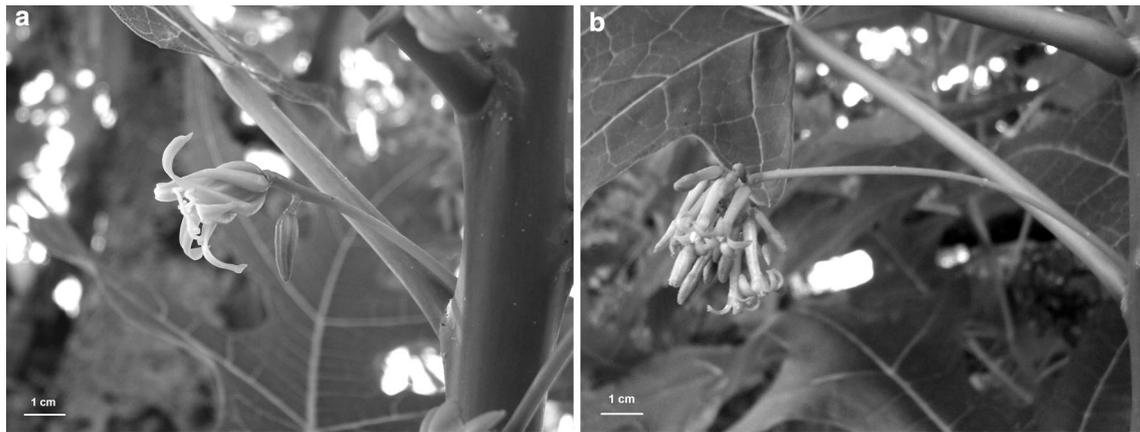


Fig. 2 *Vasconcellea quercifolia* female (a) and male (b) flowers and inflorescences

Table 1 Morphometrics of male and female floral organs of *Vasconcellea quercifolia* (mean \pm SD)

	Variable	Female flower	Male flower
Calyx	Total length (mm)	3.2 \pm 1.1 ^b	1.54 \pm 0.41 ^a
	Number of lobules	5.21 \pm 0.42 ^a	5.02 \pm 0.14 ^a
Corolla	Total length (mm)	17.54 \pm 2.04 ^b	14.19 \pm 2.57 ^a
	Length of the tube (mm)	–	8.77 \pm 1.56
	Number of lobules	5.24 \pm 0.44 ^a	5.01 \pm 0.12 ^a
	Length of lobules (mm)	16.45 \pm 0.47 ^b	5.53 \pm 0.17 ^a
Androecium	Number of lower stamens	–	5.04 \pm 0.27
	Number of upper stamens	–	5.13 \pm 0.57
	Length of lower anthers (mm)	–	1.28 \pm 0.32
	Length of upper anthers (mm)	–	0.96 \pm 0.14
	Pistilloid length (mm)	–	4.80 \pm 1.06
Gynoecium	Length of ovary (mm)	10.21 \pm 2.52	–
	Style length (mm)	1.51 \pm 0.79	–
	Number of stigmatic lobules	5.42 \pm 0.99	–
	Length of stigmatic lobules (mm)	5.68 \pm 0.92	–
	Stigma diameter (mm)	6.69 \pm 1.44	–
	Number of ovules	28.32 \pm 7.73	–
	Number of locules	5.13 \pm 0.43	–

Different letters indicate significant differences (Tukey, $p < 0.05$). Measuring unit: millimeter

flowers. Generally, each individual of *V. quercifolia* exhibits either male or female flowers only. However, one fruit with seeds was found in a male tree.

Flowers of both sexes open at dusk (between 1800 and 1900 hours). Female flowers last 72 h (Fig. 3a) and male flowers 78 h (Fig. 3b). Stigma was receptive and anthers release pollen throughout the floral longevity, being pollen viability of 31.3 ± 5.0 %. The presence of nectar in male flowers was observed throughout floral cycle.

Floral visitors

During the nocturnal observations, we caught individuals belonging to Arctiidae, Noctuidae, Nymphalidae, and Pyralidae families (Table 2). The number of individuals

collected per species was low; and the families Noctuidae and Pyralidae were the most abundant. The total number of visits recorded was 93 (9 in 2010–2011 and 84 in 2011–2012). Visits were observed on flowers of both sexes, but they were more frequent on male trees (73 % of total visits). Three hawkmoths (Sphingidae) were observed (2 on female and 1 on male flowers) in hovering flight, but they were not captured. Therefore, the presence of co-specific pollen on them could not be evaluated. Greater activity of moths occurred between 2100 and 2300 hours.

Moths arrived at male flowers quickly, landing on them, extending the proboscis and inserting it into the corolla tube in search of nectar. They visited several flowers of the same inflorescence. During foraging, the visitors contacted the anthers with their heads and the base of their proboscis.

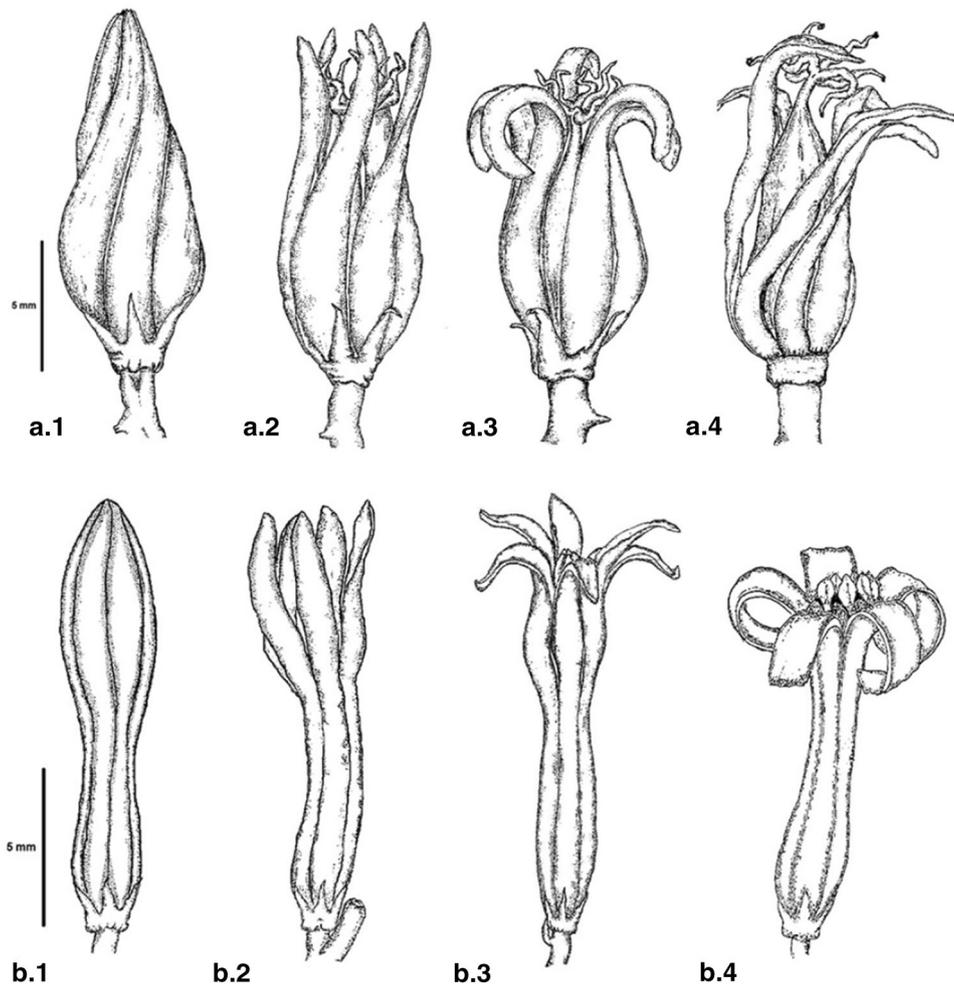


Fig. 3 Floral stages of female (a) and male (b) flowers of *Vasconcellea quercifolia*. **a1** Ripe bud (0 h); **a2** open flower with corolla lobules parallel to main axis (24 h); **a3** open flower with corolla lobules perpendicular to main axis or retrorse and receptive stigma with extended stigmatic lobules (48 h); **a4** ripe flower with brown

stigmatic lobules and corolla withered. **b1** Mature bud (0 h); **b2** open flower with the corolla lobules parallel to the main axis and dehiscent anthers (2 h); **b3** open flower with corolla lobules perpendicular to the main axis (48 h); **b4** mature flower with retrorse corolla lobules (28 h)

In female flowers, the moths arrived and left the flowers quickly.

All moths captured visiting male and female flowers ($n = 26$) carried pollen grains of *V. quercifolia* (Table 2; Fig. 4). Most individuals (81 %, $n = 22$) carried more than the mean number of ovules (28 pollen grains), and only 23 % of the pollinators carried a hetero-specific pollen load (1–33 pollen grains). The average length of proboscis (\pm SD) was 10.0 ± 2.8 mm (range 5–11 mm). Proboscis length was strongly correlated with tube length ($r = 0.8$, $p < 0.002$).

During the diurnal observations, only three individuals of *Apis mellifera* (Apidae) and one individual of hummingbird *Chlorostilbon aureoventris* (Trochilidae) were seen on November 2011. Bees were seen occasionally harvesting pollen on several male flowers but did not visit female flowers, while hummingbirds performed very fast

visits only on the male flowers without contacting the anthers. Bees and hummingbirds seemed to act almost exclusively as pollen and nectar thieves, respectively. Therefore, we only considered nocturnal lepidopterans to be effective pollinators.

Stigmatic pollen loads

Co-specific pollen load was observed in 88 % of the 65 stigmas studied, and it was dominant in 74 % of stigmas ($t = 3.19$, $p < 0.0023$). Only 11 % of the 57 stigmas with co-specific pollen load carried a number of co-specific pollen grains that exceeded the number of ovules. The remaining stigmas (89 %) contained between 1 and 26 co-specific pollen grains. Stigmas with lepidopteran scales climbed to 75 %.

Table 2 Moths captured on flowers of *Vasconcellea quercifolia*

Family	Species/morpho-species	T	PVq	PHS	PrL (mm)
Arctiidae	<i>Euclera rubricincta</i>	1	28	33	10.0
Noctuidae	<i>Autoplusia egea</i>	3	599 ± 391	3 ± 6	11.2 ± 6.0
	<i>Leucania</i> sp.	2	38 ± 46	0 ± 0	–
	<i>Megalographa bonaerensis</i>	2	400 ± 212	0 ± 0	17.5
	<i>Rachiplusia nu</i>	1	21	2	7.5
	Noctuidae sp.1	2	168 ± 124	4 ± 6	–
	Noctuidae sp.2	1	300	0	7.5
Nymphalidae	Nymphalidae sp.1	1	1000	0	10.0
	<i>Ortilia ithra</i>	1	5	0	11.5
Pyralidae	Pyralidae sp.1	3	448 ± 166	0 ± 0	10.5 ± 1.8
	Pyralidae sp.2	2	195 ± 92	0 ± 0	8.7 ± 2.5
	Pyralidae sp.3	1	2	0	9.5
	Pyralidae sp.4	1	800	0	11.5
	Pyralidae sp.5	1	600	0	7.0
	Pyralidae sp.6	1	200	0	–
	Pyralidae sp.7	3	26 ± 21	3 ± 4	7.3 ± 0.4

T number of individuals captured, PVq number of pollen grains of *V. quercifolia* (mean ± SD), PHS number of hetero-specific pollen grains (mean ± SD), PrL length of the proboscis (mean ± SD)



Fig. 4 Pollen grains of *Vasconcellea quercifolia* found on moths. Scale in µm

Table 3 Fruit set, seed set, and weight (g) of seeds per fruit (mean ± SD) in different treatments used to study the breeding system of *Vasconcellea quercifolia*

	Fruit set	Seed set	Weight of ripe seeds
Open-pollination	0.18 ± 0.05 ^b (500)	0.79 ± 0.27 ^a (200)	0.24 ± 0.02 ^a (200)
Hand-pollination	0.86 ± 0.06 ^a (124)	0.77 ± 0.23 ^a (35)	0.21 ± 0.02 ^a (35)
Anemophily	0.04 ± 0.01 ^c (130)	0.39 ± 0.33 ^b (4)	0.09 ± 0.04 ^b (4)
Apomixis	0.02 ± 0.01 ^c (115)	0	0

Different letters represent significant differences between treatments by Fisher test LSD ($p < 0.05$). Numbers between brackets indicate number of treated flower to determine fruit set, and number of used fruits to determine seed set and weight of seeds

Breeding system

Fruit set ($F = 84.33$, $p < 0.0001$), seed set ($F = 7.95$, $p = 0.0005$), and seed weight ($F = 6.55$, $p = 0.0022$) varied among treatments (Table 3). The natural establishment of fruits and seeds (‘open pollination’) was 18 and 79 %, respectively. Cross pollinated flowers produced significantly more fruits than open-pollinated flowers (68 % more fruit set under hand-pollination). However, seed set and weight of seeds did not differ among both treatments.

The seed set and weight of seeds per fruit under ‘anemophily’ treatment were significantly lower than those obtained by ‘open pollination’ and ‘hand-pollination’. We

have not included ‘apomixis’ in this comparison due to the lack of ripe fruits and seeds formed under such treatment.

Discussion

Our results show that *Vasconcellea quercifolia* is a xenogamous species that requires pollinators for the formation of fruits and seeds. Dioecy in this species is morphological as well as functional, a common reproductive characteristic in Caricaceae (Badillo 1971, 2000). The observation that one male plant produced a single fruit with seeds is not enough to reach the conclusion that sexual variation, as reported for some species of *Vasconcellea*

(*V. sprucei*, *V. aprica*, *V. microcarpa*, *V. stipulata*; Badillo 1971, 2000), is a common event in *V. quercifolia*. However, it is possible that the presence of fruiting males is higher in other populations of the species. The frequency of fruiting males in some populations of *Jacaratia mexicana* was above 45 %, while in others they were completely absent (Aguirre et al. 2007).

In *V. quercifolia* sexual dimorphism was pronounced. Male individuals produce larger floral displays than females and nectar as reward. Same flowering traits have been observed in studied species of Caricaceae (Bawa 1980; Bullock 2002), and are consistent with male-biased selection for increased attractiveness hypothesis (Willson 1979; Queller 1997). According to this view, floral traits that increase attractiveness to pollinators have been predicted to evolve through selection on male function rather than female function, due to males usually competing for mating opportunities, whereas female function (seed production) is usually limited by resources other than pollen (Willson 1979; Delph and Ashman 2006). In *V. quercifolia*, components of floral display such as flower number and size and length of flowering phenology are greater in males, with flowering episodes earlier and longer than females and exhibit a larger number of flowers. Furthermore, insect pollinators responded to increased male attractiveness by visiting males more frequently than females. Our results coincide with studies in sexually dimorphic species that have shown allocation to attractive structures benefiting male function more than female function (Conner et al. 1996; Vaughton and Ramsey 1998; Munguia-Rosas et al. 2011).

According to floral morphology, *V. quercifolia* has relatively smaller flowers compared to other studied Caricaceae species (Badillo 1971; Bullock 2002; Aguirre et al. 2007), and its flowers are of similar size to other moth-pollinated species (Oliveira et al. 2004; Sugiura and Yamazaki 2005; Johnson and Anderson 2010). Floral tube length of *V. quercifolia* allows visits by moths with proboscis of similar length (moths) and greater than the corolla length (sphingids), and excludes shorter tongue moths. We propose to medium sized moths as pollinators of *V. quercifolia*. This is supported by a greater fruit set in control than visitors exclusion treatments, by the presence of pollen grains on moth bodies and of moth scales on stigmas, and due to these were seen contacted reproductive organs of male and female flowers. Similarly, a functional fit between pollinators and morphological traits was observed in Caricaceae species hawkmoth-pollinated with relatively larger flowers (Bawa 1980; Haber and Frankie 1989; Bullock 2002). Sphingid visits on flowers shorter than the length of its tongue—non sphingophilous flowers—are usual in their foraging behavior (Haber and Frankie 1989; Agosta and Janzen 2005). On the other hand,

Alexandersson and Johnson (2002) have proposed that individuals with tongues relatively long compared with the length of flower tube would not pick up pollen efficiently. However, considering flower width rather than flower length, it has been demonstrated that sphingid can effectively pollinate relatively short flowers (Moré et al. 2007). In this sense, sphingid could effectively carry pollen between male and female flowers of *V. quercifolia*, but future experiments are necessary to evaluate their role as pollinators of this species.

Over two reproductive seasons, *V. quercifolia* exhibited low natural female reproductive success. Low stigmatic pollen load and infrequent pollinator activity on non-rewarding female flowers led us to suggest pollinator limitation due to insufficient number of visits that ensure minimum amount of pollen grains necessary for fruit development. Low reproductive success is characteristic of non-rewarding species, and is often attributed to pollinator limitation rather than pollen availability (Ferdy et al. 1998; Castillo et al. 2002; Smithson and Gigord 2003; Anderson and Johnson 2006; Sun et al. 2009). Pollinators can distinguish between non-rewarding and rewarding flowers, avoiding the former to optimize foraging efficiency (Smithson and MacNair 1997). Moreover, different studies showed that the probability of visit to empty flowers is reduced with an increase in the frequency of these flowers (Smithson and Gigord 2003; Anderson and Johnson 2006). On this basis it was proposed low density (Jersáková et al. 2006) and wide flower longevity (Proctor and Harder 1995) as a strategy to increase reproductive success of non-rewarding flowers. Results of this study are according with these expectations. Therefore, further studies are needed to clarify if, as occurs in other deceptive species (Alexandersson and Ågren 1996; Sabat and Ackerman 1996; Ferdy et al. 1999), reproductive success in *V. quercifolia* is pollinator-induced density.

In summary, the present study demonstrates for the first time the reproductive system of *Vasconcellea* species and their pollinator guild. In accordance with that predicted by floral syndrome (phalaenophily), moths are the primary pollinators of *V. quercifolia*. Like other Caricaceae, *V. quercifolia* is dimorphic in flower size and present nectar as reward in male flowers only; but in contrast, it has a smaller floral size. *Vasconcellea* species could be evolved toward moth pollination. Additional studies should be conducted to determine whether the findings of this study could be extended to other species of this genus.

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References

- Agosta SJ, Janzen DH (2005) Body size distributions of large Costa Rican dry forest moths and the underlying relationship between plant and pollinator morphology. *Oikos* 108:183–193. doi:10.1111/j.0030-1299.2005.13504.x
- Aguirre A, Vallejo-Marín M, Salazar-Gorozieta L, Arias DM, Dirzo R (2007) Variation in sexual expression in *Jacaratia mexicana* (Caricaceae) in southern Mexico: frequency and relative seed performance of fruit-producing males. *Biotropica* 39:79–86. doi:10.1111/j.1744-7429.2006.00230.x
- Aguirre A, Vallejo-Marín M, Piedra-Malagón EM, Cruz-Ortega R, Dirzo R (2009) Morphological variation in the flowers of *Jacaratia mexicana* A. DC. (Caricaceae), a subdioecious tree. *Plant Biol* 11:417–424. doi:10.1111/j.1438-8677.2008.00154.x
- Alexandersson R, Ågren J (1996) Population size, pollinator visitation and fruit production in the deceptive orchid *Calypso bulbosa*. *Oecologia* 107:533–540. doi:10.1007/BF00333945
- Alexandersson R, Johnson SD (2002) Pollinator-mediated selection on flower-tube length in a hawkmoth-pollinated *Gladiolus* (Iridaceae). *Proc R Soc Lond B* 269:631–636. doi:10.1098/rspb.2001.1928
- Anderson B, Johnson SD (2006) The effects of floral mimics and models on each others' fitness. *Proc R Soc B* 273:969–974. doi:10.1098/rspb.2005.3401
- Badillo VM (1971) Monografía de la familia Caricaceae. Asociación de profesores, Maracay, Venezuela
- Badillo VM (2000) *Carica* L. vs. *Vasconcellea* St. Hil. (Caricaceae): con la rehabilitación de este último. *Ernstia* 10:74–79
- Badillo VM (2001) Nota correctiva *Vasconcellea* St. Hill. y no *Vasconcellea* (Caricaceae). *Ernstia* 11:75–76
- Baker HG (1976) "Mistake" pollination as a reproductive system with special reference to the Caricaceae. In: Burley J, Styles BT (eds) *Tropical trees: variation, breeding and conservation*. Academic Press, London, pp 161–169
- Bawa KS (1980) Mimicry of male by female flowers and intrasexual competition for pollinators in *Jacaratia dolichaula* (D. Smith) Woodson (Caricaceae). *Evolution* 34:467–474. <http://www.jstor.org/stable/2408216>
- Bawa KS (1990) Plant–pollinator interactions in tropical rain forests. *Annu Rev Ecol Syst* 21:399–422. <http://links.jstor.org/sici?sici=0066-4162%281990%2921%3C3399%3APIITRF%3E2.0.-CO%3B2-0>
- Biloni JS (1990) Árboles autóctonos argentinos de las selvas, bosques y montes de la Argentina. Tipográfica Editora Argentina, Buenos Aires
- Bullock SH (2002) *Jacaratia mexicana* A. DC (Caricaceae). In: Noguera FA, Vega-Rivera JH, García-Alderete AN, Quesada M (eds) *Historia Natural de Chamela*. Instituto de Biología, UNAM, México DF, pp 155–157
- Bullock SH, Bawa KS (1981) Sexual dimorphism and the annual flowering pattern in *Jacaratia dolichaula* (D. Smith) Woodson (Caricaceae) in a Costa Rican rain forest. *Ecology* 62:1494–1504. <http://www.jstor.org/stable/1941506>
- Castillo RA, Cordero C, Domínguez CA (2002) Are reward polymorphisms subject to frequency- and density-dependent selection? Evidence from a monoecious species pollinated by deceit. *J Evol Biol* 15:544–552. doi:10.1046/j.1420-9101.2002.00425.x
- Colombo P, Melati MR, Scialabba A, Trapani S, Sortino M (1989) The ecomorphology of *Carica quercifolia* Solms-Laub. in a Mediterranean climate. *Agric Ecosyst Environ* 27:397–409
- Conner JK, Rush S, Kercher S, Jenetten P (1996) Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. Selection through lifetime male and total fitness. *Evolution* 50:1137–1146. doi:10.2307/2410654
- d'Eeckenbrugge, Drew R, Kyndt T, Scheldeman X (2014) *Vasconcellea* for papaya improvement. In: Ming R, Moore PH (eds) *Genetics and genomics of papaya, plant genetics and genomics: crops and models*. Springer Science and Business Media, New York, pp 47–79. doi:10.1007/978-1-4614-8087-7_4
- Dafni A (1992) *Pollination ecology: a practical approach*. Oxford University Press, New York
- Delph LF, Ashman T-L (2006) Trait selection in flowering plants: how does sexual selection contribute? *Integr Comp Biol* 46:465–472. doi:10.1093/icb/icj038
- Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2011) InfoStat, versión 2011. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. <http://www.infostat.com.ar>
- Drew RA, Siar SV, O'Brien CM, Magdalita PM, Sajise AGC (2006) Breeding for papaya ringspot virus resistance in *Carica papaya* via hybridisation with *Vasconcellea quercifolia*. *Austral J Exp Agric* 46:413–418. doi:10.1071/EA04247
- Faegri K, van der Pijl L (1979) *The principles of pollination ecology*. Sinauer Associates, London
- Ferdy J-B, Gouyon P-H, Moret J, Godelle B (1998) Pollinator behavior and deceptive pollination: learning process and floral evolution. *Am Nat* 152:696–705. doi:10.1086/286200
- Ferdy J-B, Austerlitz F, Moret J, Gouyon P-H, Godelle B (1999) Pollinator-induced density dependence in deceptive species. *Oikos* 87:549–560
- Garrett A (1995) *The pollination biology of papaw (Carica papaya L.) in Central Queensland*. Thesis, Central Queensland University, Rockhampton. <http://hdl.cqu.edu.au/10018/25774>
- Haber WA, Frankie GW (1989) A tropical hawkmoth community: Costa Rican dry forest Sphingidae. *Biotropica* 21:155–172. <http://www.jstor.org/stable/2388706>
- INTA. Instituto Nacional de Tecnología Agropecuaria [online]. Observatorio Agrometeorológico E.E.A. Reconquista N° 437 (s.m.n). <http://inta.gob.ar/documentos/estacion-meteorologica-reconquista>. Accessed 15 July 2013
- Jersáková J, Johnson SD, Kindlmann P (2006) Mechanisms and evolution of deceptive pollination in orchids. *Biol Rev* 81:219–235. doi:10.1017/S1464793105006986
- Johnson SD, Anderson B (2010) Coevolution between food-rewarding flowers and their pollinators. *Evo Edu Outreach* 3:32–39. doi:10.1007/s12052-009-0192-6
- Kyndt T, Van Droogenbroeck B, Romeijn-Peters E et al (2005) Molecular phylogeny and evolution of Caricaceae based on rDNA internal transcribed spacers and chloroplast sequence data. *Mol Phylogenet Evol* 37:442–459. doi:10.1016/j.ympev.2005.06.017
- Moré M, Séršic AN, Cocucci AA (2007) Restriction of pollinator assemblage through flower length and width in three long-tongued hawkmoth-pollinated species of *Mandevilla* (Apocynaceae, Apocynoideae). *Ann Mo Bot Gard* 94:485–504
- Munguia-Rosas MA, Ollerton J, Parra-Tabla V (2011) Phenotypic selection on flowering phenology and size in two dioecious plant species with different pollen vectors. *Plant Species Biol* 26:205–212. doi:10.1111/j.1442-1984.2011.00320.x
- National Research Council (1989) *Lost crops of the Incas: little-known plants of the Andes with promise for worldwide cultivation*. National Academy Press, Washington

- Oliveira PE, Gibbs PE, Barbosa AA (2004) Moth pollination of woody species in the Cerrados of Central Brazil: a case of so much owed to so few? *Plant Syst Evol* 245:41–54. doi:10.1007/s00606-003-0120-0
- Ortega-Baes P, Saravia M, Sührling S, Godínez-Alvarez H, Zamar M (2010) Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. *Plant Biol* 13:33–40. doi:10.1111/j.1438-8677.2010.00332.x
- Pensiero JF, Gutiérrez HF, Luchetti AM, Exner E, Kern V, Brnich E, Oakley L, Prado D, Lewis JP (2005) Flora vascular de la provincia de Santa Fe. Claves para el reconocimiento de las familias y géneros. Catálogo sistemático de las especies. Universidad Nacional del Litoral, Santa Fe
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2011) nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-101
- Piratelli AJ, Piña-Rodrigues FCM, Gandara FB, Santos EMG, Costa LGS (1998) Biologia da polinização de *Jacaratia spinosa* (Aubl) Adc. (Caricaceae) em mata residual do sudeste Brasileiro. *Rev Brasil Biol* 58:671–679. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0034-71081998000400015
- Proctor HC, Harder LD (1995) Effect of pollination success on floral longevity in the orchid *Calypto bulbosa* (Orchidaceae). *Am J Bot* 82:1131–1136. <http://www.jstor.org/stable/2446066>
- Proctor M, Yeo P, Lack A (1996) The natural history of pollination. Timber Press, Portland
- Queller DC (1997) Pollen removal, paternity, and the male function of flowers. *Am Nat* 149:585–595. <http://www.jstor.org/stable/2463385>
- R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>
- Renner SS, Feil JP (1993) Pollinators of tropical dioecious angiosperms. *Am J Bot* 80:1100–1107. <http://www.jstor.org/stable/2445757>
- Ronse De Craene LP, Smets EF (1999) The floral development and anatomy of *Carica papaya* L. (Caricaceae). *Can J Bot* 77:582–598. doi:10.1139/b99-026
- Sabat AM, Ackerman JD (1996) Fruit set in a deceptive orchid: the effect of flowering phenology, display size, and local floral abundance. *Am J Bot* 83:1181–1186. <http://www.jstor.org/stable/2446202>
- Scheldeman X, Willems L, Coppens d'Eeckenbrugge G et al (2007) Distribution, diversity and environmental adaptation of highland papayas (*Vasconcellea* spp.) in tropical and subtropical America. *Biodivers Conserv* 16:1867–1884. doi:10.1007/s10531-006-9086-x
- Scheldeman X, Kyndt T, d'Eeckenbrugge GC, Ming R et al (2011) *Vasconcellea*. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, tropical and subtropical fruits. Springer, Berlin, pp 213–249. doi:10.1007/978-3-642-20447-0_11
- Siar SV, Beligan GA, Sajise AJC, Villegas VN, Drew RA (2011) Papaya ringspot virus resistance in *Carica papaya* via introgression from *Vasconcellea quercifolia*. *Euphytica* 181:159–168. doi:10.1007/s10681-011-0388-z
- Smithson A, Gigord LD (2003) The evolution of empty flowers revisited. *Am Nat* 161:537–552. doi:10.1086/368347
- Smithson A, MacNair MR (1997) Negative frequency-dependent selection by pollinators on artificial flowers without rewards. *Evolution* 51:715–723. doi:10.2307/2411148
- Sugiura S, Yamazaki K (2005) Moth pollination of *Metaplexis japonica* (Apocynaceae): pollinaria transfer on the tip of the proboscis. *J Plant Res* 118:257–262. doi:10.1007/s10265-005-0216-4
- Sun H-Q, Cheng J, Zhang F-M, Luo Y-B, Ge S (2009) Reproductive success of non-rewarding *Cypripedium japonicum* benefits from low spatial dispersion pattern and asynchronous flowering. *Ann Bot* 103:1227–1237. doi:10.1093/aob/mcp066
- Tel-Zur N, Schneider B (2009) Floral biology of *Ziziphus mauritiana* (Rhamnaceae). *Sex Plant Reprod* 22:73–85. doi:10.1007/s00497-009-0093-4
- Torres MJ, Trejo SA, Martin MI et al (2010) Purification and characterization of a cysteine endopeptidase from *Vasconcellea quercifolia* A. St.-Hil. latex displaying high substrate specificity. *J Agric Food Chem* 58:11027–11035. doi:10.1021/jf904295x
- Vaughton G, Ramsey M (1998) Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia* 115:93–101. doi:10.1007/s004420050495
- Willson MF (1979) Sexual selection in plants. *Am Nat* 113:777–790. <http://www.jstor.org/stable/i320672>