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Reproductive biology of eight sympatric Myrtaceae from Central Brazil

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SUMMARY

Eight species of Brazilian Myrtaceae were studied for their flowering phenology, pollination biology, breeding system and fruiting success: *Eugenia dysenterica*, *Siphoneugena densiflora*, *Blepharocalyx salicifolius*, *Campomanesia pubescens*, *C. velutina*, *Myrcia linearifolia*, *M. rhodosepala* and *Psidium firmum*. Flowering strategies were mass-flowering and steady-state types, with two species showing a pulsed flowering. All species were bee-pollinated with pollen as the floral reward; flowers opened early in the morning and lasted one day; buzz-pollination was registered in three species. Pollinator-sharing of *Bombus* spp. bees occurred in the four savanna shrubs, pre-dawn pollination by *Ptiloglossa* sp. (Colletidae) was found in arboreal *S. densiflora* and the remaining two trees had open pollination systems. The three forest species were markedly self-incompatible, and two of the savanna species were effectively so, whilst three savanna species showed self-compatibility.

Key words: Myrtaceae, Brazil, cerrados, breeding systems, pollination biology, buzz-pollination.

INTRODUCTION

Although Myrtaceae is the dominant plant family in many different neotropical communities (Keel & Prance, 1979; Mori *et al.*, 1983; Rodrigues *et al.*, 1989; Peixoto & Gentry, 1990), the reproductive biology of the group has been little studied. The breeding systems of a few species of Myrtaceae have been reported in various community surveys (Ruiz & Arroyo, 1978; Frankie *et al.*, 1983; Bullock, 1985) but no studies are available of the broader aspects of reproductive biology, including pollination biology and fruiting success, for any group of neotropical myrtaceous species. Here we report such a study for eight species, representing six genera, which occur in the central Brazilian savanna type *cerrado* vegetation or associated gallery forest.

Fieldwork was carried out over a period of six months in the natural reserve area of the Brasília Botanical Garden, located some 20 km SE of Brasília, between 47° 51–52′ W and 15° 52′–56′ S. The regional climate is Köppen's seasonal Aw (Köppen, 1931); precipitation is between 1400–1500 mm a year and falls mostly between September and March. The soils are deep, nutrient-poor, highly acidic, yellow-red latosols with some shallower cambisols in the areas cut by rivers.

The vegetation consists mainly of a species-rich form of savanna woodland (locally called *cerrado*) traversed by gallery forests and marshes which border the water courses. Where the soil is very poor or shallow the trees thin out and scattered shrubs and grasses predominate; the local term for this open form of cerrado is *campo sujo*. The vegetation of the Federal District has been well described physiognomically by Eiten (1984) and floristically by Filgueiras & Pereira (1990).

MATERIALS AND METHODS

Flowering phenology

During the year 1988 individuals of the eight species (Table 1) were monitored on a weekly basis until flowering was imminent. At this time, two to ten plants of each species were selected and sufficient young inflorescences per plant tagged to yield at least 100 buds (except for *Campomanesia pubescens*). The numbers of flowers present on each were subsequently censused on a daily basis until the day that the last flower opened. In the case of *Myrcia*

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Table 1. *Species investigated with habitat and life-form*

Subtribe/species	Habitat/life form
Eugeniinae	
Eugenia dysenterica Mart. ex DC.	Dense cerrado: tree
Siphoneugena densiflora Berg	Dense cerrado: tree
Myrciinae	
Myrcia linearifolia Camb.	Campo sujo: shrub
M. rhodosepala Kiaersk.	Campo sujo: shrub
Myrtiinae	
Blepharocalyx salicifolius (H.B.K.) Berg	Dense cerrado: tree
Psidium firmum Berg	Campo sujo: shrub
Campomanesia pubescens (DC.) Berg	Campo sujo: shrub
C. velutina (Camb.) Berg	Forest/cerrado: tree

rhodosepala, *M. linearifolia* and *Psidium firmum*, all inflorescences on the plants were marked so that the series of observations of daily flower production represent the whole flowering episode for these shrubs. For the trees *Eugenia dysenterica*, *Siphoneugena densiflora*, *Blepharocalyx salicifolius* and *Campomanesia velutina* only a selection of flowering branches was tagged, so that the actual flowering episode for the tree sometimes lasted slightly longer than that recorded. Likewise, with *C. pubescens*, a shrub which produces aerial shoots of various ages flowering at different periods, it was not always feasible to monitor an individual's whole flowering episode but rather that of each individual shoot. Voucher herbarium specimens of these species are located in the herbarium of the University of the Ecological Reserve of the Instituto Brasileiro de Geografia e Estatística (IBGE).

Floral biology and pollination

Each species was observed for 1–3 observation periods lasting from 15 min to 3 h to establish the identity of the probable pollinator(s). The earliest observation period started at 04.00 h and the latest ended at 11.00 h. Except for *Campomanesia pubescens* and *Blepharocalyx salicifolius* in which the onset of anthesis was not observed, at least one of the observation periods started at anthesis.

Breeding systems

For each species, several inflorescences were marked and enclosed within nylon bags with a 2-mm mesh. Bagged inflorescences were monitored until the morning on which the first sequence of flowers opened, when the remaining unopened buds were removed, the number of open flowers counted, and one of the following applied: (1) automatic self-pollination (i.e. left with no further treatment), (2) self-pollination, (3) cross-pollination. Because of the large numbers of stamens, and the potential damage to the flower in removing them, flowers were not emasculated. Hand pollinations were effected by brushing a dehisced anther against the stigma and were always carried out in the morning. For self-pollinations, an anther was taken from the same flower. In cross-pollinations, an anther was removed from a recently collected flower belonging to a conspecific plant growing at least 50 m away, except for two of the three *Campomanesia velutina* trees which were *c.* 10 m apart.

Most hand-pollinated flowers were left to set fruit and monitored weekly until the first fruit matured when a census was taken. The same procedure was followed for tagged flowers on inflorescences left for natural pollinators. Two of the three trees of *Blepharocalyx salicifolius* were blown down in a storm so that results are limited for this species.

Subsamples of hand-pollinated flowers were collected *c.* 24, 48 and 72 h after pollination and fixed in 70% ethanol. Fixed flowers were subsequently used to check pollen grain germination and pollen tube growth by means of aniline blue staining under fluorescence microscopy (Martin, 1959). The hypanthium and staminal disk were cut away prior to softening for 10–20 min with 8 M NaOH at 60 °C and stained with 0·1% leuco-aniline blue in 0·1 N K_3PO_4 with traces of calcofluor white as a fluorescence enhancer (Jeffries & Belcher, 1974). Preparations were observed with a Leitz Laborlux Microscope fitted with Ploemopak vertical illuminator and appropriate ultra-violet filter. The number of pollen tubes in the styles of selfed- versus crossed-pistils was counted or estimated, and the numbers of penetrated ovules were also counted and compared statistically using Student's *t* test.

For some species, pistils were embedded in wax following standard tertiary-butanol dehydration and infiltration (Johansen, 1940), sectioned at 14 μm with a Reichert Ultracut Microtome and the sections stained with leuco-aniline blue with a trace of calcofluor white, and viewed with a fluorescence microscope.

RESULTS

Flowering strategies

Three flowering strategies were encountered which can be referred to the types recognized in central American Bignoniaceae by Gentry (1974).

(a) 'Big-bang' flowering strategy occurs in two of the tree species, *Eugenia dysenterica* (Fig. 1 *a*) and *Campomanesia velutina* (Fig. 1 *h*), with synchronized mass flowering by individual plants which lasted about 1 wk. Flowering conspicuousness was probably enhanced in *E. dysenterica* by the accompanying flush of new, pinkish-coloured leaflets on these trees. A slight modification of the big-bang flowering strategy which we refer to as 'pulsed-bang' flowering

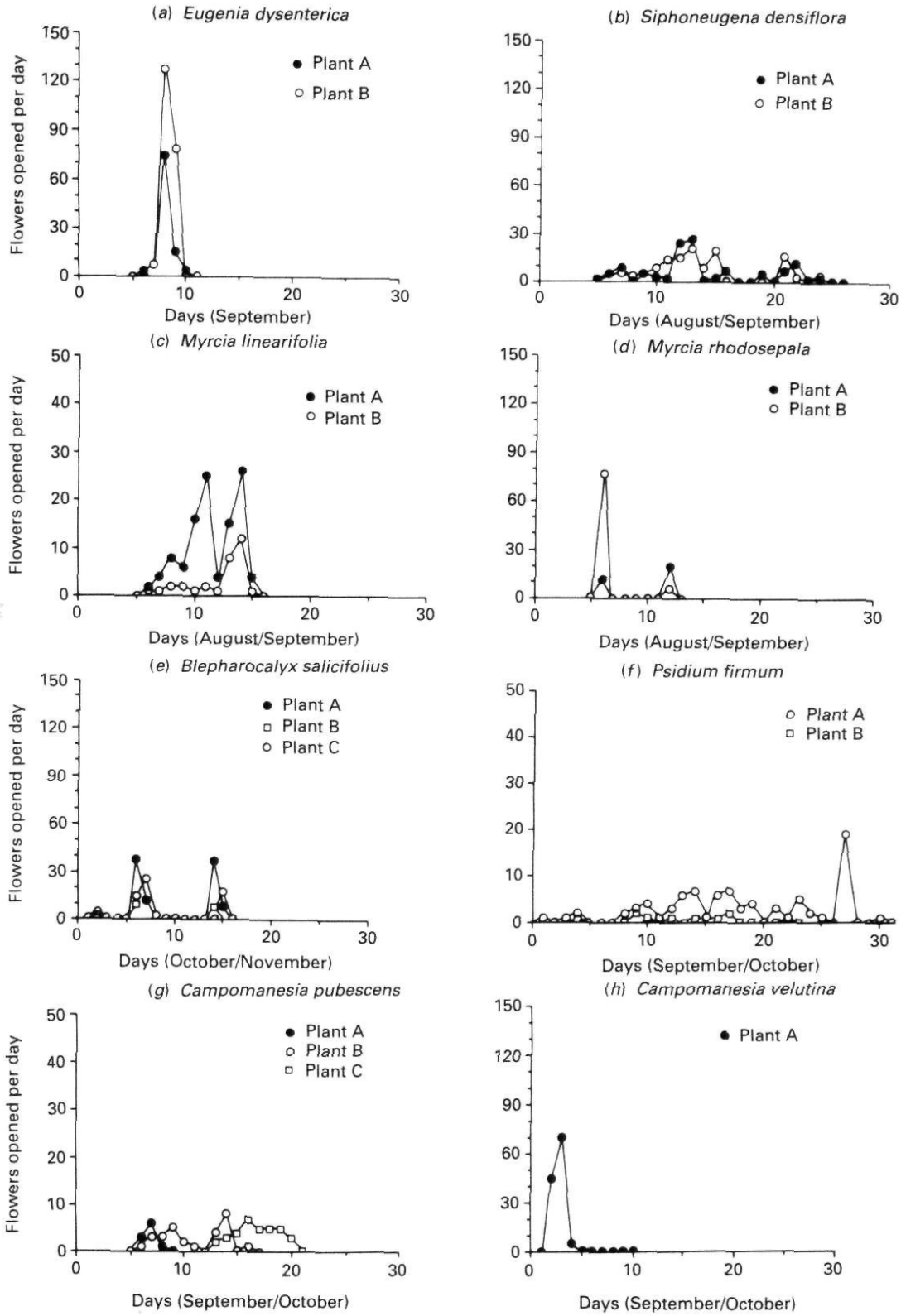


Figure 1. Flowering phenologies in eight species of Myrtaceae in Central Brazil.

was found in *Myrcia rhodosepala* (Fig. 1*d*) and *Blepharocalyx salicifolius* (Fig. 1*e*). This strategy differs from the previous in that although the total number of days on which flowers are open may be similar, 'pulsed-bang' flowering is discontinuous and there may be an interval of several days when no flowers open. However, synchronization of actual flowering days is exact. In *Eugenia parnahybensis*, a locally common *campo sujo* shrub not included in this study, a pulsed-bang type strategy was perfected to such a degree that the whole population was in flower on only two separate days when the shrubs in this area were very conspicuous with their massed, white flowers. This 'pulsed-bang' strategy should

Table 2. *Floral characteristics of eight species of Myrtaceae in Central Brazil*

Species	Flower diameter (mm)	Stamen number	Ovule number	Time of anthesis	*Type of anthesis*
Blepharocalyx salicifolius	8	130–180	6–12	before 07.00 h	*Myrcia*
Campomanesia pubescens	15	90–130	25–80	06.30–07.00 h	*Psidium*
C. velutina	13	70–110	10–30	06.00–06.30 h	*Psidium*
Eugenia dysenterica	15	60–70	4–8	06.30 h	*Psidium*
Myrcia linearifolia	7	80–150	4–6	06.00–06.30 h	*Myrcia*
M. rhodosepala	7	50–150	4–6	06.00–06.30 h	*Myrcia*
Psidium firmum	20	200–300	150–300	06.00–06.30 h	*Psidium*
Siphoneugena densiflora	7	65–110	6–14	03.00–04.00 h	*Myrcia*

Table 3. *Insect visitors to eight species of Myrtaceae in Central Brazil*

Blepharocalyx salicifolius (60 min)	*Bombus atratus* (Swederes)	s.c.[1]
	Centris violacea (Lepeletier)	s.c.
	Ceratilictus theius (Shrottky)	s.c.
	Melipona quinquefasciata Lep.	s.c.
	Partamona cupira Smith	no s.c.
	Scaptotrigona postica (Latr.)	no s.c.
	Trigona banneri Cockerell	no s.c.
	T. spinipes (F.)	no s.c.
	?*Xylocopa frontalis* (Olivier)	s.c.
Campomanesia pubescens (45 min)	*Bombus* spp.	s.c.
C. velutina (45 min)	*Bombus atratus* (Swederes)	s.c.
	B. morio (Franklin	s.c.
Eugenia dysenterica (45 min)	*Bombus* spp.	s.c.
	?*Ceratina* sp.	s.c.
	?*Melipona* sp.	occasional s.c.
	Trigona spinipes	no s.c.
	Trigona sp.	occasional s.c.
Myrcia linearifolia (180 min)	*Bombus atratus*	
	B. morio	
	Trigona spp.	occasional s.c.
M. rhodosepala (120 min)	*Bombus morio*	s.c.
	Bombus sp.	s.c.
	Trigona spp.	occasional s.c.
	Xylocopa ciliata Burmeister	s.c.
Psidium firmum (180 min)	*Bombus* spp.	s.c.
	Ceratina gossypii Schrottky	
Siphoneugena densiflora (590 *min*)	*Ptiloglossa* sp.	s.c.
	Wasps	no s.c.
	Beetles	no s.c.

[1] s.c. = stigmatic contact observed.

not be confused with the 'multiple bang' type (Gentry, 1974) in which several small-scale flowering episodes occur throughout the year, each involving an intermediate sterile phase and the formation of a new inflorescence.

(b) A 'cornucopia' flowering strategy, i.e. with many flowers being produced per plant for over a month or so, is exhibited by *Siphoneugena densiflora* (Fig. 1*b*).

(c) A 'steady-state' flowering strategy, i.e. with relatively few flowers produced each day over a long period of time, is exhibited by two shrubs, *Psidium firmum* (Fig. 1*f*) and *Campomanesia pubescens* (Fig. 1*g*).

(d) A somewhat intermediate pattern which started with a few flowers, peaked and then fell off abruptly was found in *Myrcia linearifolia* (Fig. 1*c*).

Floral biology and pollination

Anthesis always occurred in the early morning, but two different patterns were encountered (Table 2). In the first, '*Psidium* type', the style was either bent at the tip or already erect and the staminal filaments were irregularly crumpled as the petals began to open, and anthers in various stages of dehiscence became visible as the style and filaments expanded and the flower assumed its open appearance. In the

Table 4. *Fruit-set following hand self- or cross-pollinations in eight species of Myrtaceae in Central Brazil*

Species/ treatment	Plants	Flowers	Fruit-set	Fruit-set (%)	*t* test	ISI (+)
Eugenia dysenterica ($t = 0{\cdot}05$; $v = 30$)						
Selfed[1]	1	18	3	16·7	ns	0·83
Crossed[2]	2	15	3	20	—	—
Siphoneugena densiflora						
Selfed	2	17	0	0	—	0
Crossed	3	36	21	58·3	—	—
Myrcia linearifolia ($t = 3{\cdot}53$; $v = 120$)						
Selfed	4	87	2	2·3	*	0·12
Crossed	2	35	7	20	—	—
M. rhodosepala ($t = 0{\cdot}65$; $v = 31$)						
Selfed	2	21	13	61·9	ns	1·24
Crossed	2	35	6	50	—	—
Blepharocalyx salicifolius						
Selfed	3	42	0	0	—	0
Crossed	1	6	2	33·3	—	—
Campomanesia pubescens ($t = 5{\cdot}31$; $v = 33$)						
Selfed	1	15	1	6·7	*	0·09
Crossed[3]	?	20	15	75	—	—
C. velutina						
Selfed	3	33	1	0	—	0
Crossed	2	26	7	26·9	—	—
Psidium firmum ($t = 1{\cdot}16$; $v = 37$)						
Selfed	4	28	23	82·1	ns	1·29
Crossed	3	11	7	63·6	—	—

[1] Bagged, self-pollinated flowers.
[2] Bagged, cross-pollinated flowers.
[3] Cross-pollinations by K. del Claro in different cerrado area.
(+) ISI, index of self-incompatibility (ratio of fruits set from selfed *vs.* crossed flowers).
* $P < 0{\cdot}05$.
Note in those cases of strict self-incompatibility no *t* test was made.

Table 5. *Natural fruit-set in tagged flowers in eight species of Myrtaceae in Central Brazil*

Species	Plants	Flowers	Fruits	Fruit-set (%)	PERS*
Eugenia dysenterica	2	325	22	6·8	0·6–4·6
Siphoneugena densiflora	2	244	31	12·7	0·8–4·7
Myrcia linearifolia	1	125	17	13·6	2·3–6·8
M. rhodosepala	5	212	31	14·6	2·5–7·3
Blepharocalyx salicifolius	2	126	9	7·1	0·7–7·1
Campomanesia pubescens	6	81	15	18·5	4·7
C. velutina	1	122	10	8·2	0·3–4·1
Psidium firmum	10	152	67	44·1	4·6–12·7

* PERS, pre-emergent reproductive success (product of fruit/flower ratio and seed/ovule ratio, cf. Wiens, 1984; Wiens *et al.*, 1987).

second, '*Myrcia* type', the staminal filaments were all incurved so that no anthers were visible as the petals opened, whilst the style, also initially folded, was usually the first to straighten and protrude above the mass of filaments which then began to uncurl, starting with the outermost whorls.

Open flowers of all the species investigated shared the following characteristics: four or five white or whitish petals, many stamens, a sweet scent, no nectar and a dry stigma raised by the style 1–2 mm above the innermost whorls of stamens, which were always slightly shorter than the outermost whorls, and an inferior ovary (Table 2). In *Myrcia linarifolia* the filaments and petals have a pink flush, and in *Psidium firmum* stigmas at the same level as the anthers were found to occur sporadically in the flowers of some of the individuals. Between pollination and ovary enlargement, *Siphoneugena densiflora* and *Blepharocalyx salicifolius* abscissed, respectively, hypanthial and calycinal remnants above the ovary, and all species abscissed styles a few days after pollination.

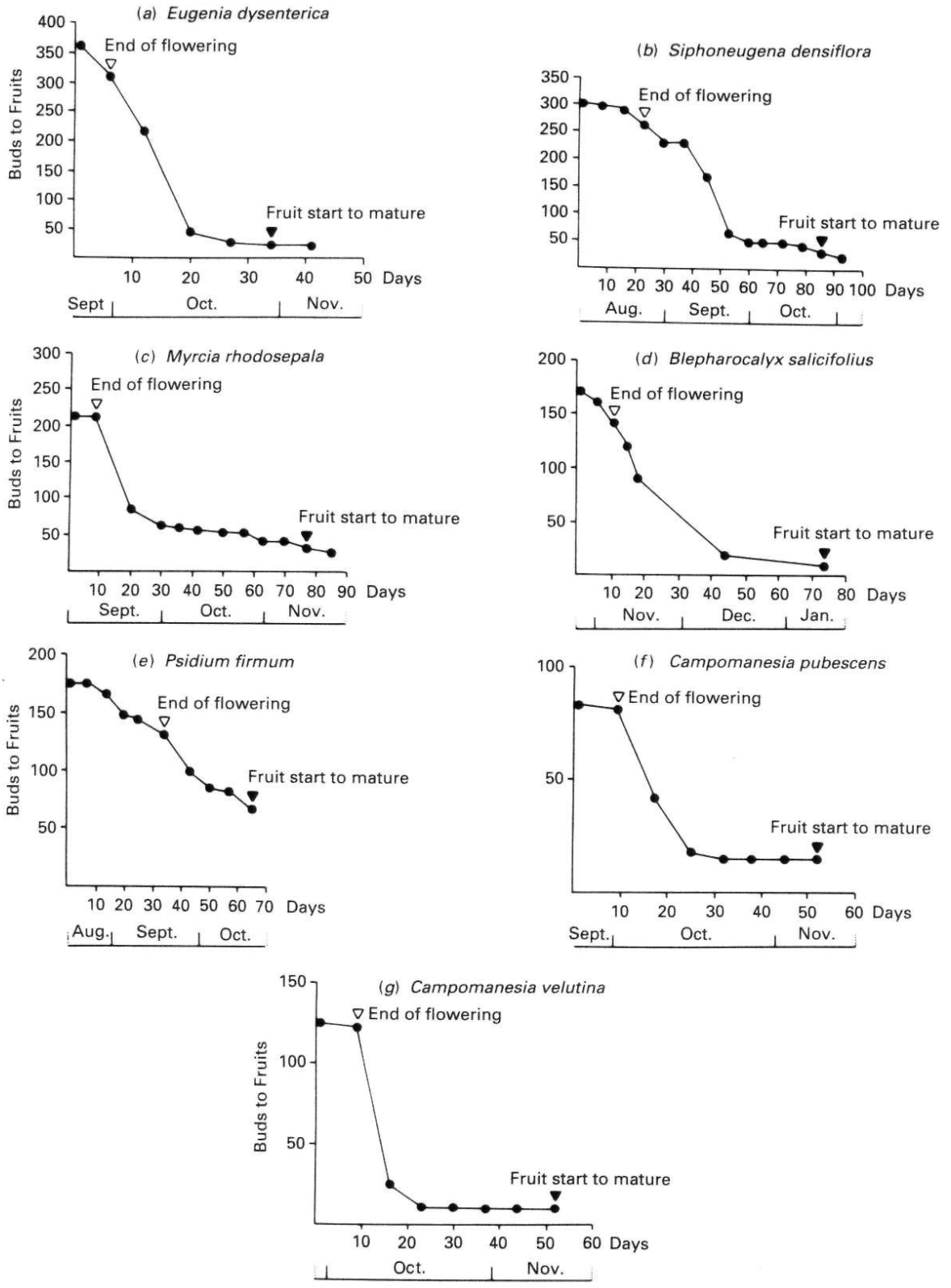


Figure 2. Fruiting success curves for eight species of Myrtaceae in Central Brazil.

All species are bee-pollinated and offer pollen as a reward, but visitation patterns are varied. *Siphoneugena densiflora* was visited almost exclusively by a *Ptiloglossa* sp. (Colletidae) between 06.00 h (shortly before dawn) and 06.45 h. These bees alighted on an open flower, clasped groups of stamens with their legs, buzzed, and flew off to the next inflorescence up the branch. Since inflorescences averaged 1·84 open flowers ($n = 137$ inflorescences) this visitation pattern probably makes for an efficient foraging strategy. Visits to this species during the rest of the morning were mostly by introduced *Apis mellifera* or

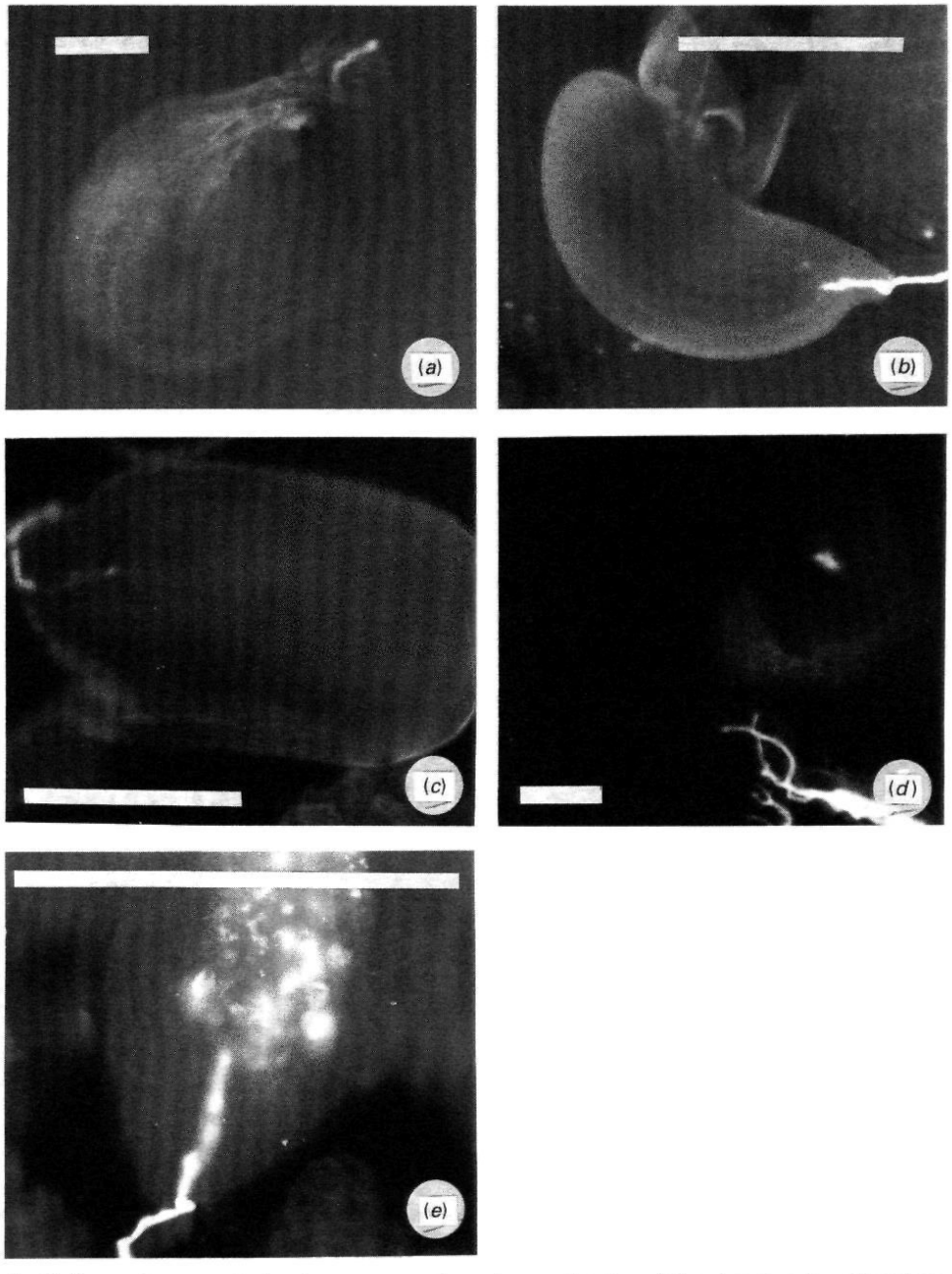


Figure 3. Pollen tube growth in the ovary and ovule penetration following hand pollinations in some myrtaceous species. Leuco-aniline-blue staining, observed under fluorescence microscopy. (*a*) & (*b*), *Campomanesia velutina*: ovules penetrated by pollen tubes 24 h after self-pollination. (*c*) & (*d*), *Psidium firmum*: (*c*), penetrated ovule at 24 h after cross-pollination; (*d*), self-pollen tubes close to the ovules, 72 h after pollination. (*e*), *Siphoneugena densiflora*: penetrated ovule at 72 h after self-pollination. [Scale bar = 100 μm.]

wasps which do not buzz, and do not contact the stigma.

Although observation times were too short to ascertain the identity of all potential pollinators for the other species, two bees of the genus *Bombus* (*B. atratus* and *B. morio*) seem to play an important role since they were observed to visit all of the species studied except *S. densiflora* and made good stigmatic contact in each case. However, two of these *Bombus*-pollinated species, *E. dysenterica* and *B. salicifolius* (both mass-flowering trees in dense *cerrado*) were also intensely visited all morning by a wide range of insects with different kinds of behaviour, many of which did not contact the stigma, but some of which were possible pollinators (Table 3). The remaining species were visited predominantly by *Bombus* spp.

Breeding systems

The results of fruit-set from self- versus cross-pollinations indicate that the species differ in their breeding systems (Table 4). Three species, *Blepharocalyx salicifolius*, *Campomanesia velutina* and *Siphoneugena densiflora* were strictly self-incompatible, and set no fruit when selfed. Two species, *Myrcia linearifolia* and *Campomanesia pubescens*,

Table 6(a–d). *Timing of self- vs. cross-pollen tube growth to the ovary, and ovule penetrations, in four species of Myrtaceae in Central Brazil*

Treatment	Time after treatment (h) 24	48	72
(*a*) *Eugenia dysenterica*			
ASP	6/1	0/0	?
	8/1	2/0	
SP	0/0	0/0	±5/2
	±5/0	0/0	?*/1
		5/1	
		6/1	
		6/1	
		7/1	
		5/3	
CP	1/0	5/0	±5/0
		±15/0	6/1
(*b*) *Siphoneugena densiflora*			
ASP	1/0	3/0	> 11/0
	> 11/0	6–10/0	> 11/0
SP	0/0	3–5/0	> 11/0
	6–10/0	6–10/0	> 11/0
	6–10/0	6–10/0	> 11/0
	6–10/0	> 11/0	> 11/1
	> 11/0		
CP	0/0	2/0	6–10/0
	3–5/0	6–10/0	6–10/0
	6–10/0	6–10/0	6–10/1
	> 11/0	6–10/0	> 11/0
		6–10/1	> 11/0
(*c*) *Myrcia linearifolia*			
ASP	0/0		
	0/0	?	?
	0/0		
	1/0		
SP	3/0	3/0	4/1
	3/0	10/0	20/2
	7/0		
	9/0		
	17/0		
	3/1		
CP	0/0	0/0	0/0
	2/0	0/0	3/0
	5/0	0/0	4/0
	7/0	1/0	12/0
	6/1	2/0	3/2
		2/0	
(*d*) *Myrcia rhodosepala*			
ASP	0/0	0/0	
	0/0	1/0	?
	0/0	2/0	
	3/0		
SP	1/0	0/0	4/0
	1/0	5/0	1/1
	2/0	4/1	2/1
	3/0	4/1	2/1
	6/0	7/1‡	3/1
	8/0	9/2	5/1‡
	9/0		7/1
			10/1
			10/1‡
			6/3
CP	1/0	1/0	2/0
	6/0	7/0†	4/0
	7/0	6/1	6/1
	7/0	12/1	
	8/0	3/2	

Key: ASP, Automatic self-pollination; SP, self-pollination; CP, cross-pollination.

Note that figures prior to the diagonal refer to in style, those after the diagonal refer to in ovule.

* Style abscissed.

† Ovarian tissue covering ovules, not used in means.

‡ Penetration abnormal, not used in means.

Table 6(e–h). *Timing of self- vs. cross-pollen tube growth to the ovary, and ovule penetrations, in four species of Myrtaceae in Central Brazil*

Treatment	Time after treatment (h) 24	48	72
(*e*) *Blepharocalyx salicifolius*			
ASP	0/0	0/0	0/0
	3/0	2/1	±12/4
SP	3/0	2/0	±10/0
	±20/0	7/4	±15/0
	±20/0	±15/5	±20/0
	±30/0	±30/7	±15/3
	±10/1	±30/9	±15/4
CP	4/0	±20/4	9/3
	6/0	±20/6	±10/4
	6/0	±35/8	±20/5
	±12/0		
(*f*) *Psidium firmum*			
ASP	0/0	5/0	2/0
	±30/0		7/0
HSP	0/0	0/0	±90/0
	3/0	3/0	±120/0
HCP	±40/0	±50/2	±60/2
		±100/2	±100/6
(*g*) *Campomanesia pubescens*			
ASP	2/0	0/0	0/0
	±10/0	±10/0	±20/1
			±10/2
SP	0/0	0/0	±7/2
	3/0	3/0	?/3
	?/6	?/3	?/3–6*
(*h*) *Campomanesia velutina*			
ASP	0/0	?	0/0
	0/0		4/0
SP	0/0	±15/0	4/0
	1/0	±50/3	±30/1
	2/0	±30/4	±40/2
	4/0		
	±20/0		
	8/2		
CP	0/0	0/0	0/0
	1/0	0/0	0/0
		2/0	1/0
		±10/0	2/0
		±10/2	±10/2

Key: ASP, Automatic self-pollination; SP, self-pollination; HSP, hand self-pollination; CP, cross-pollination; HCP, hand cross-pollination.

Note that figures prior to the diagonal refer to in style, those after the diagonal refer to in ovule.

* 3 used to calculate mean for *t* test.

were capable of some selfing, but fruit-set was statistically superior (increasing by a factor of *c.* 10) after cross pollinations. The index of self-incompatibility (ISI) values calculated for these two species (i.e. the ratio of fruits set for selfed vs. crossed flowers, (cf. Bullock, 1985) are 0·12 and 0·09, respectively (Table 4), so that these species are less than the maximum value of ISI = 0·2 arbitrarily set for self-incompatible species (Bawa, 1974; Sobrevila & Arroyo, 1982). *M. linearifolia* and *C. pubescens* can therefore be regarded as self-incompatible species. Three species, *Eugenia dysenterica*, *Myrcia rhodosepala* and *Psidium firmum*, were completely self-compatible, setting statistically equal numbers of fruits after cross- and self-pollination.

Natural fruiting success also varied considerably (Table 5), as did fruit-set curves and length of the reproductive episode, from anthesis to fruit maturity (Fig. 2). Estimates of the percentage of ovules which

Table 7. *Comparison of fruit-set following automatic- vs. hand self-pollination in five species of Myrtaceae in Central Brazil*

Species/treatment	Plants	Flowers	Fruits	Fruit-set (%)	Significance
Eugenia dysenterica ($t = 0.835$; $v = 30$)					
Automatic (1)	1	14	1	7·1	ns
Hand (2)	1	18	3	16·7	—
Myrcia linearifolia ($t = 1·85$; $v = 102$)					
Automatic	2	17	2	11·8	ns
Hand	4	87	2	2·3	—
M. rhodosepala ($t = 1·18$; $v = 44$)					
Automatic	1	25	11	44	ns
Hand	2	21	13	61·9	—
Campomanesia pubescens ($t = 0·2$; $v = 32$)					
Automatic	2	19	1	5·3	ns
Hand	1	15	1	6·7	—
Psidium firmum ($t = 2·56$; $v = 34$)					
Automatic	3	8	3	37·5	*
Hand	4	28	23	82·1	—

[1] Bagged flowers left without treatment.
[2] Bagged, hand self-pollinated flowers.
* $P < 0·05$.

form seeds (i.e. pre-emergent reproductive success (PERS) values) of all species were well below 22% and so within the range found by Wiens (1984) for outbreeding species. This would indicate that the eight species investigated, including those lacking genetic self-incompatibility, are likely to be outbreeding.

In the three markedly self-incompatible species self-pollen tubes were observed to penetrate ovules with a similar frequency to those of cross-pollinated pistils (Fig. 3). We applied *t* tests to the mean number of penetrated ovules per flower between self- versus cross-pollinations in these species and were unable to detect any differences at 24, 48 or 72 h after pollinations (Table 6). In *Campomanesia velutina* and *Blepharocalyx salicifolius* rejection of selfed pistils occurred within 1 wk of pollination, but in *Siphoneugena densiflora* abscission took a month to occur.

In the species which set at least some selfed fruits, fruit-set following automatic selfing was compared with that obtained from hand self-pollination (Table 7). In three species, *Eugenia dysenterica*. *Myrcia rhodosepala* and *Campomanesia pubescens*, the differences in fruit-set were not statistically significant; in *Psidium firmum*, hand selfing produced significantly higher fruit-set, but in *Myrcia linearifolia* higher fruit-set was obtained from the automatic selfing, although this was only significant at the 10% level.

DISCUSSION

Flowering strategies and floral biology

It is likely that in the species with a 'big-bang' flowering strategy, in which synchronization is very precise and the flowering period is short, the onset of flowering is environmentally cued by changes in humidity at the threshold of the rainy season.

Abrupt increases in humidity are relatively common at the beginning of the dry season but tend to diminish towards its end. In July 1988, there were eight days with humidity increases above 5%, four in August, and two in September before the end of the third week when the rains started (Pereira Neto, 1988). After the rains began, the three abrupt increases in humidity observed coincided with flowering in the two big-bang species studied (Fig. 4). *Eugenia dysenterica* commenced flowering the day after an increase of 12% had occurred from the previous day (which was the driest day of the year); *Campomanesia velutina* commenced flowering when an increase of 8% from the previous day occurred (which was also two days after the largest daily increase of the year, of 42%, was registered).

The synchronization of pulsed-bang species may be more complex. In *Myrcia rhodosepala*, several flowering days showed no discernible correlation with humidity changes. However, *Blepharocalyx salicifolius* commenced flowering the day after the largest daily increase (42%) in relative humidity of the year had been registered and thereafter opened flowers on all days in which humidity was high (*c.* 80% or higher), until all buds had opened. The onset of flowering of 'cornucopia' and 'steady-state' species showed no precise correlation with daily changes in humidity or other immediate environmental conditions such as temperature, rainfall, etc.

The dry/rainy season transition is the period of the year when the most marked fluctuations in humidity occur over the shortest time intervals, and it is probable that there is greater scope for humidity-linked synchronization of flowering events at this time. Furthermore, flowering at this time may have

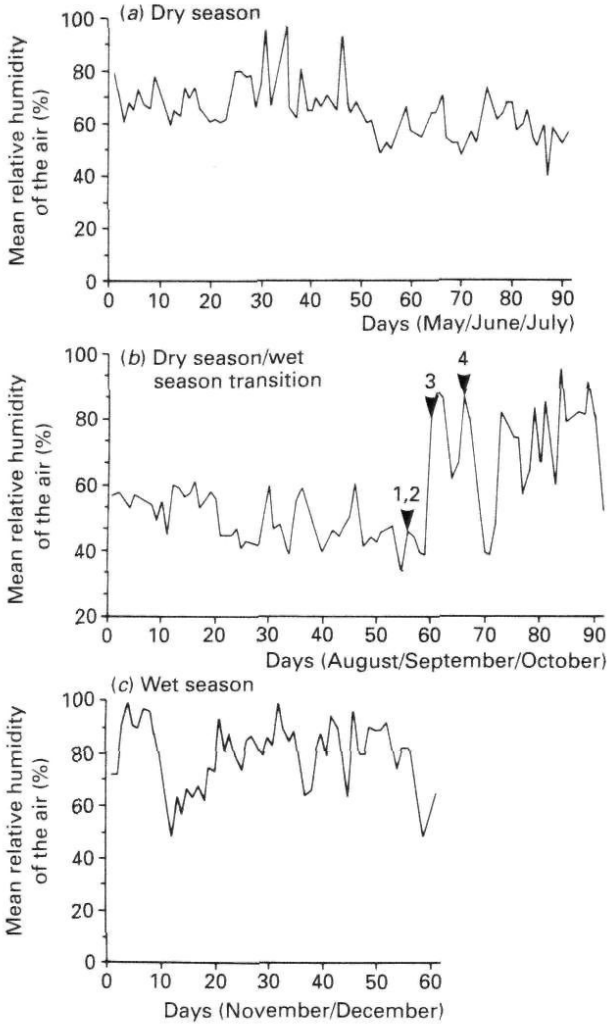


Figure 4. Mean daily humidity of the air near the study site during 1988. Arrows indicate initiation of flowering of 1, *Eugenia dysenterica* (big bang), 2, *Campomanesia pubescens* (steady state), 3, *C. velutina* (big bang) and 4, *Blepharocalyx salicifolius* (pulsed bang).

advantages in terms of good (dry) conditions for pollination, but with the mature seeds subsequently dispersed as the rains arrive, and so facilitate germination and establishment before the next dry cycle. Flowering at the dry/rainy season transition is a favoured strategy within the family and occurred in seven out of 10 species investigated in other South-American forest communities (Frankie *et al.*, 1983; Ferreira & Merona, 1987; Morellato *et al.*, 1989). In the present study, six out of the eight species investigated commenced flowering at the dry/rainy season transition.

Pollination

Bombus species are likely to be the principal pollinators of seven of the eight species in this study, although more detailed observations are needed. The principal species, *B. morio* and *B. atratus* also exhibited a preference for the myrtaceous *Psidium guajava* in a study carried out in secondary vegetation in SE Brazil (Camillo & Garofalo, 1989). Other bee species may also be effective pollinators, especially in mass-flowering species. It is notable that buzz-pollination by *Bombus* spp. was observed in the two species *Myrcia rhodosepala* and *Blepharocalyx salicifolius* and by *Ptiloglossa* bees in *Siphoneugena densiflora*, although none of these species possesses the poricidal anthers usually associated with this behaviour (Buchmann, 1983). The implications of this interesting phenomenon have been discussed elsewhere (Proença, 1992).

Pollination of *Siphoneugena densiflora* by *Ptiloglossa* sp. suggests that the Myrtaceae–Colletidae association which is so striking in Australia (Michener, 1965) probably exists to some extent in the Neotropics; another reported example is *Eugenia salamensis* (Frankie *et al.*, 1983).

Breeding systems

The three strictly self-incompatible (SI) species were all trees growing in dense, predominantly arboreal *cerrado*, which also occur in gallery forests (*Blepharocalyx salicifolius* and *Siphoneugena densiflora*) or mesotrophic forests (*Campomanesia velutina*) in the region. Pollen tube penetration of the ovules in selfed flowers, as observed in these SI species, has been reported in other genera of Myrtaceae, e.g. *Eucalyptus* (Sedgley *et al.*, 1989; Sedgley & Smith, 1989) and self-pollen tube growth as far as the ovary, but without penetration of the micropyle, occurs in some *Melaleuca* species (Barlow & Forrester, 1984).

It is not known whether ovule penetration by self-pollen tubes is followed by fertilization in these Brazilian myrtaceous species, as certainly occurs in some other taxa with late-acting self-incompatibility (Oliveira *et al.*, 1992; Gibbs & Bianchi, 1993). It is perhaps significant that in the self-incompatible species studied here no ovary enlargement occurred in either crossed or selfed pistils, up to the time of abscission of the latter. Moreover, rejection of selfed pistils was synchronous; this suggests late-acting self-incompatibility (Seavey & Bawa, 1986), rather than embryo abortion due to recessive deleterious genes, in which abortion would be expected to occur over the various stages of embryo development (Wiens, 1984).

Self-compatibility and outbreeding

If a low PERS is indicative of outbreeding (cf. Wiens *et al.*, 1987) then all species studied here, self-compatible as well as self-incompatible, are likely to be outbreeding. A certain degree of outcrossing in

self-compatible species may be attained by a steady-state flowering strategy (*Psidium firmum*) or opportunistic behaviour of the pollinators in species with mass-flowering strategies such as *Eugenia dysenterica* and *Myrcia rhodosepala* (Janzen, 1971; Gentry, 1974).

Another possible explanation for outbreeding in self-compatible species (which needs to be verified by *ad hoc* experimentation) is slow growth of self pollen tubes as compared with that of cross pollen tubes, at least in *P. firmum*. Some form of 'preferential self-exclusion' may be operating in this species, similar to that proposed by Bowman (1987) for *Clarkia unguiculata* (Onagraceae, Myrtales). *C. unguiculata* is a self-compatible species in which the use of a genetic marker (flower colour) revealed that 58–100 % of the progeny of mixed self- and cross-pollen were outcrossed; Bowman argued that this was because faster-growing cross-pollen tubes were responsible for most fertilizations.

In *P. firmum* (Table 6*f*) no penetrated ovules were observed in pistils fixed at 48 or 72 h after self-pollination, in contrast to crossed pistils which had penetrated ovules at both of these time intervals. This suggests that cross pollen tubes may grow faster than self pollen tubes although the samples (both $n = 2$) were insufficient for statistical inference. In 72-h selfed pistils, several pollen tube tips were observed quite close to the ovules (Fig. 4*c*) but either had not had enough time to penetrate the micropyles or else were somehow arrested at this point while cross-pollen tubes of the same age had already reached the ovules (Fig. 4*d*). Self-fertilizations must have occurred subsequently, however, since fruit-set following selfing was as successful as that from crossing.

These eight myrtaceous species from the Brazilian cerrados and adjoining gallery forest conform to the general pattern that has emerged for neotropical woody species (Bawa, 1974; Bawa, Perry & Beach, 1985) in that five are self-incompatible taxa It is of interest that in all of these species, representing four genera, a late-acting type mechanism seems to operate, since selfed flowers are rejected after ovule penetration has occurred. In the case of *Siphoneugena densiflora* the delayed abscission of selfed pistils (after 4 w or more), and the equally slow initial development of crossed fruits, is notable.

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REFERENCES

Barlow BA, Forrester J. 1984. Pollen tube interactions in *Melaleuca*. In: Williams, EG, Knox, RB, eds. *Pollination '84*. Symposium proceedings. Melbourne: University of Melbourne.

Bawa KS. 1974. Breeding systems of tree species of a lowland tropical community. *Evolution* **28**: 85–92.

Bawa KS, Perry DR, Beach JH. 1985. Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. *American Journal of Botany* **72**: 331–345.

Bowman RN. 1987. Cryptic self-incompatibility and the breeding system of *Clarkia unguiculata* (Onagraceae). *American Journal of Botany* **74**: 471–476.

Buchmann SL. 1983. Buzz pollination in Angiosperms. In: Jones CE, Little RJ, eds. *Handbook of experimental pollination biology*. New York: Van Nostrand Reinhold Company, Inc.

Bullock SH. 1985. Breeding systems in the flora of a tropical deciduous forest. *Biotropica* **17**: 287–301.

Camillo E, Garofalo CA. 1989. Analysis of the niche of two sympatric species of *Bombus* (Hymenoptera, Apidae) in south-eastern Brazil. *Journal of Tropical Ecology* **5**: 81–92.

Eiten G. 1984. Vegetation of Brasília. *Phytocoenologia* **12**: 271–292.

Ferreira LV, Merona JR. 1987. *Aspectos sobre a ecologia da família Myrtaceae em uma floresta de terra firme na Amazônia Central*. São Paulo: Proceedings 38th Congresso Nacional de Botânica.

Filgueiras TS, Pereira B. 1990. A flora do distrito federal. Brasília. In: Novais Pinto M, ed. *Cerrado: caracterização, ocupação e perspectivas*. Brasília: Editora Universidade de Brasília.

Frankie GW, Haber WA, Opler PA, Bawa KS. 1983. Characteristics and organization of the large bee pollination system in the Costa Rican dry forest. In: Jones CE, Little RJ, eds. *Handbook of experimental pollination biology*. New York: Van Nostrand Reinhold Company Inc.

Gentry A. 1974. Coevolutionary patterns in Central American Bignoniaceae. *Annals of the Missouri Botanical Garden* **61**: 728–769.

Gibbs PE, Bianchi M. 1993. Post-pollination events in species of *Chorisia* (Bombacaceae) and *Tabebuia* (Bignoniaceae) with late-acting self-incompatibility. *Botanica Acta* **106**: 64–71.

Janzen DH. 1971. Euglossine bees as long distance pollinators. *Science* **171**: 203–205.

Jeffries CJ, Belcher AR. 1974. A fluorescent brightener used for pollen tube identification *in vivo*. *Stain Technology* **49**: 199–202.

Johansen DA. 1940. *Plant microtechnique* New York: McGraw Hill Book Co.

Keel SH, Prance GT. 1979. Studies of vegetation of a white sand, black water igapó (Negro River, Brazil). *Acta Amazonica* **9**: 645–655.

Köppen W. 1931. *Grundriss der Klimakunde*. 2nd edn. Berlin: W. de Gruyter.

Martin FW. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology* **34**: 125–128.

Michener CD. 1965. A classification of the bees of the Australian and Pacific Regions. *Bulletin of the American Museum of Natural History* **30**: 1–362.

Morellato LP, Rodgrigues RR, Leitão Filho HF, Joly CA. 1989. Estudo comparativo da fenologia de espécies arbóreas de floresta de altitude e floresta mesófila semidecídua na Serra do Japi. *Revista Brasieira de Botânica* **12**: 85–98.

Mori S, Boom B, Carvalho AT, Santos T. 1983. Ecological importance of Myrtaceae in an Eastern Brazilian wet forest. *Biotropica* **15**: 68–70.

Oliveira PE, Gibbs PE, Barbosa AA, Talavera S. 1992. Contrasting breeding systems in two *Eriotheca* (Bombacaceae) species of the Brazilian cerrados. *Plant Systematics and Evolution* **179**: 207–219.

Peixoto AL, Gentry A, 1990. Diversidade e composição florística da mata de tabuleiro na Reserva florestal de Linhares (Espírito Santo, Brasil). *Revista Brasileira de Botânica* **13**: 19–25.

Pereira Neto M. 1988. *Dados climáticos período 1988*. Annual Report. Brasília: Instituto Brasileiro de Geografia e Estatística, Divisão de Estudos Ambientais.

Proença C. 1992. Buzz pollination – older and more widespread than we think? *Journal of Tropical Ecology* **8**: 115–120.

Rodrigues RR, Morelatto LP, Joly CA, Leitão Filho HF. 1989. Estudo florístico e fitossociológico em um gradiente altitudinal de mata estacional mesófila semi-decídua na Serra do Japi, Jundiaí, SP. *Revista Brasileira de Botânica* **12**: 71–84.

Ruiz TZ, Arroyo MTK. 19978. Reproductive ecology of a secondary deciduous forest. *Biotropica* **10**: 221–230.

Seavey SR, Bawa KS. 1986. Late-acting self-incompatibility in Angiosperms. *Botanical Review (Lancaster)* **52**: 195–212.

Sedgley M, Hand FC, Smith RM, Griffin AR. 1989. Pollen tube growth and early seed development in *Eucalyptus regnans* F. Muell. (Myrtaceae) in relation to ovule structure and preferential outcrossing. *Australian Journal of Botany* **37**: 397–411.

Sedgley M, Smith RM. 1989. Pistil receptivity and pollen tube growth in relation to the breeding systems of *Eucalyptus woodwardii* (*Symphiomyrtus*). *Annals of Botany (London)* **64**: 21–31.

Sobrevila C, Arroyo MTK. 1982. Breeding systems in a montane tropical cloud forest in Venezuela. *Plant Systematics and Evolution* **140**: 19–37.

Wiens D. 1984. Ovule survivorship, brood size, life history, breeding systems and reproductive success in plants. *Oecologia* **64**: 47–53.

Wiens D, Calvin CL, Wilson CA, Davern CI, Frank D, Seavey SR. 1987. Reproductive success, spontaneous embryo abortion and genetic load in flowering plants. *Oecologia* **71**: 501–509.

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