

# Reciprocal intercompatibility between *Byrsonima* Rich. ex Kunth species (Malpighiaceae), in an urban ecotonal fragment of Semi-Deciduous Seasonal Forest and Cerrado

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## Resumen

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*Intercompatibilidad recíproca entre especies de Byrsonima Rich. ex Kunth (Malpighiaceae), en un fragmento ecotonal urbano de Bosque Estacional Semi-Caducifolio y Cerrado*

Las especies de *Byrsonima* presentan monomorfismo floral y comparten gremio de visitantes/polinizadores. Estos rasgos sugieren la evaluación de hipótesis de interincompatibilidad recíproca entre dos especies sintópicas de *Byrsonima*: *B. pachyphylla* y *B. crassifolia*. Se observó el crecimiento del tubo polínico con comportamiento similar en la superficie estigmática, canal del pistilo, ovario y canal micropilar de ambas especies. Además, se describió la autoincompatibilidad parcial en la autopolinización con mayor fructificación en la autogamia. La polinización cruzada y la autopolinización coexisten y se produce una intercompatibilidad recíproca. Los mecanismos de aislamiento precigóticos son improbables por la ausencia de tubos polínicos anormales, producción de frutos y ausencia de híbridos en la área de estudio.

**Palabras clave:** Epifluorescencia; Sistema de apareamiento; Abejas recolectoras de aceite; Crecimiento del tubo polínico; Autocompatibilidad.

## Abstract

*Byrsonima* species present floral monomorphism and sharing visitor/pollinator guild. These traits suggest the hypothesis evaluation of reciprocal inter-incompatibility between two syntopic *Byrsonima* species: *B. pachyphylla* and *B. crassifolia*. Pollen tube growth with similar behavior was observed in the stigmatic surface, pistil canal, ovary and micropylar channel to both species. In addition, partial self-incompatibility in self-pollination with greater fruiting in autogamy was observed. Cross-pollination and self-pollination coexist, and reciprocal intercompatibility occurs. Prezygotic isolation mechanisms are unlikely by the absence of abnormal pollen tubes, higher fruiting production and absence of hybrids in the study site.

**Key words:** Epifluorescence; Mating system; Oil-collecting bees; Pollen tube growth; Self-compatibility.



## Introduction

Malpighiaceae is a monophyletic taxon of tropical and subtropical eudicotyledons, phylogenetically related to self-incompatible families, presenting ancestral homomorphic self-incompatibility system and subsequent variation and diversification of lineages with different mating systems (Igic *et al.* 2008, Davis & Anderson 2010, Barret 2013). Such evidence supports mating systems variation within and among recognized genera in Malpighiaceae (Anderson 1980, Barros 1992, Sigrist & Sazima 2004, Benezar & Pessoni 2006, Costa *et al.* 2006, Bezerra *et al.* 2009, Sazan *et al.* 2014). The floral morphology of the Malpighiaceae is homogeneous, with preferentially co-sexual flowers, gathered in paniculate inflorescences, diurnal anthesis and calyx glands or elaiophores – traits indicative of mellitophily (Vogel 1974, Anderson 1979).

Oil plants and oil-collecting bees are important animal-plant interaction from Neotropical dry forests and savanna (e.g., Cerrado vegetation from Brazil) (Buchmann 1987, Aguiar *et al.* 2020). Three restricted tribes of female oil-collecting bees (i.e., Centridini, Tapinotaspidini, and Tetrapediini), represented by *Centris*, *Epicharis*, and *Monoeca*, are pollinators of Malpighiaceae (Neff & Simpson 1981, Sigrist & Sazima 2004). These bees collected oil for larval provisioning and nest construction (Vogel 1988). *Byrsonima*, the largest genus of Malpighiaceae in the Neotropic (Cronquist 1981, Davis & Anderson 2010, Flora do Brasil 2020), exhibit floral monomorphism and is dependent of oil-collecting bees to pollination (e.g., Gottsberger 1986, Barros 1992, Teixeira & Machado 2000, Benezar & Pessoni 2006, Costa *et al.* 2006, Amorim & de Marco 2011). In addition, *Byrsonima* species also exhibit a complex nature of self-recognition systems, with self-incompatibility and self-compatibility observed among species and populations. For example, self-incompatibility has been observed in six species (e.g., *Byrsonima sericea* DC., Teixeira & Machado 2000 and Matallana *et al.* 2016, *B. microphylla* A.Juss., Costa *et al.* 2006, *B. gardneriana* A.Juss., Machado *et al.* 2006 and Bezerra *et al.* 2009, *B. pachyphylla* A.Juss., Vilas-Boas *et al.* 2013, *B. umbellate* Mart. ex A.Juss., Mendes *et al.* 2011, and *B. lucida* (Mill.) DC., Downing & Liu 2013). On the other hand, self-compatibility has also been commonly observed (e.g., *Byrsonima crassi-*

*folia* (L.) Kunth, Bawa 1974, *B. coccolobifolia* Kunth, Benezar & Pessoni 2006, *B. intermedia* A.Juss., Oliveira *et al.* 2007, *B. crassa* Nied., Peixoto *et al.* 2011, *B. guilleminiana* A.Juss., *B. laxiflora* Griseb., *B. subterranean* Brade & Markgr., *B. umbellate* Mart. ex A.Juss., Barros 1992, and *B. basiloba* A.Juss., Balestra *et al.* 2014), and different self-recognition systems have been reported to *B. intermedia* populations (Vilas-Boas *et al.* 2013, Balestra *et al.* 2014).

The labiality of the self-recognition system suggests the possibility of reciprocal intercompatibility or introgression among *Byrsonima* species. Hitherto, studies evaluating the reciprocal intercompatibility (cross-compatibility) between *Byrsonima* species are absent, but this phenomenon had already been recognized for different plant species, for example, in *Chamaecrista desvauxii* (Collad.) Killip. (Costa *et al.* 2007), *Melocactus* species (Khan *et al.* 2020), and commercial fruits (avocado and olive). *Byrsonima* show floral monomorphism, overlapping of the flowering period, and share pollinators promoting intra- and interspecific pollen flow. These attributes suggest the evaluation of the inter-incompatibility hypothesis between two syntopic populations of *Byrsonima pachyphylla* and *B. crassifolia*. In addition, a comprehensive dataset of mating systems was building and analyzed to investigate the extent of self-incompatibility mechanisms among *Byrsonima* species in Neotropics.

## Materials and methods

### Study sites and species

This experimental study was carried out in two syntopic species of *Byrsonima* in the Ecological Station of the Universidade Federal de Minas Gerais, located in the Belo Horizonte municipality (19°52'S, 43°58'W), with well-defined dry and wet seasons (Alvares *et al.* 2013). The urban ecological fragment has 102 hectares of area, with vegetation formations of forests of gallery, cerrado and secondary forest. *Byrsonima pachyphylla* and *B. crassifolia* are small trees and shrubs, respectively (Bawa 1974, Pereira & Freitas 2002, Vilas-Boas *et al.* 2013, Melo *et al.* 2014). The flower traits of two species are very congruent to the floral display of Malpighiaceae (Vogel 1974, Anderson 1990, Possobom & Machado 2018): terminal and racemose inflorescences, zygomorphic and

hermaphroditic flowers, with five pairs of oil glands (elaiophores). All flowers begin yellow, but flowers of *B. pachyphylla* turn red after first day of flowering (Fig. 1). Mating system treatments were carried out on individuals found in situ over flowering/fruiting season (October 2010 - January 2011, personal observation). Based in the comprehensive dataset building here, the flower season was similar to another population of *B. crassifolia* (Pereira & Freitas 2002). However, *B. pachyphylla* present distinct flowering periods in other populations (e.g., April - July, Vilas-Boas *et al.* 2013, July and October, Melo *et al.* 2014). *Byrsonima* species are visited by oil-collecting bees, and flesh-fruits are globose drupe dispersed by animals (e.g., Amorim & De Marco 2011, Sazan *et al.* 2014).

### Flowers counting and floral visitors

The number of flowers per inflorescence was estimate by direct counting of flower buds in 10 inflorescences per species. The change of floral color was verified daily in flowers marked from pollination treatment. Focal observations of floral visitors were performed continuously during one day (07:00 to 17:00 hs) before pollination treatments (November 27, 2010), and not included nocturnal observations. Floral visitors were classified according to Inouye (1980): legitimate (pollinators) or illegitimate (pollen or oil thieves).

### Intra- and inter-taxa experimental pollinations

Experimental pollination treatments were used to determine the mating systems and reciprocal intercompatibility between *B. crassifolia* and *B. pachyphylla*. A two-fold approach was followed based on using *in vitro* (tube pollen growth) and *in vivo* (fruit:flower set) experimental hand-pollination treatments. Only open flowers of the first day were used, and the number of flowers determined the number of crosses among treatments (981 experimental crosses in 10 individuals of both species, see Table 1 to flowers per treatment and species). Seven pollination treatments on selected flowers in the field were carried out for each approach: (1) pollinator exclusion (inflorescences bagged to exclude pollinators, and flowers not emasculated), (2) self-pollination (flowers bagged and hand-pollinated with pollen from the same flower), (3) “geitonogamy”-pollination (flowers bagged and hand-pollinated with pollen

from a different flower on the same plant), (4) cross-pollination (flowers bagged and hand-pollinated with pollen from a flower of a different plant), (5) bidirectional interspecific cross-pollination (flowers bagged and hand-pollinated with pollen from a flower of a different species), (6) apomixis (inflorescences bagged to exclude pollinators), and (7) control (flowers unbagged/open pollination after visitation of bees were marked). To avoid intra-floral interference on cross-pollinated flowers, in other words, the partial contribution of self-pollen grains on tube formation and fruit-set, all flowers of “geitonogamy”-pollination, cross-pollination, bidirectional interspecific cross-pollination and apomixes treatments were emasculated.

To *in vitro* experiments, flowers were collected 24, 48 and 72 hours after each cross (i.e., six pistils from each treatment, corresponding to 252 flowers in the two species), fixed in FAA-50% (24 hours) and later transferred to ethanol (70%). The pistils were placed in 10N NaOH solution (60 °C for 8 min.), clarified with NaClO (1 hour), washed in distilled water (3 times) and stained with aniline blue (0.2%), squashed on slides to examine pollen tube growth and ovule penetration under epifluorescence microscopy (Martin 1959). Anomalous and normal pollen tubes can be differentiated by the irregular grew on the stylar canal and callose pattern deposition, and/or erratic behavior into the ovary – ‘ovarian incompatibility’ (Gibbs 2014). In addition, the time-lag of the selfed and crossed pistils (i.e., pollen tubes arrived in the ovary) were investigated in all flowers.

To *in vivo* experiments, fruit:flower ratio of seven pollination treatments (see above) was quantified after 20 days (immature fruits) of performed experiments (a total of 729 flowers, 52 mean  $\pm$ 10 SD by treatments). Abortion of immature fruits used to occur between the 5th and 20th day after pollination, and the maturation time took approximately 120 days. However, fruit was not falling after the 30th day of fruit development, as observed in *B. crassifolia* (Pereira & Freitas 2002). In this case, abortion beyond the time slice in this present study was insignificant. Our rationale behind the experimentation of pollinator-excluded conditions was to assess autonomous/spontaneous pollination/pollinator independence or apomixis (Sazan *et al.* 2014). Three indexes were calculated *sensu* Ruiz-Zapata & Arroyo (1978) and Lloyd & Schoen (1992): (i) Self-

incompatibility index (ISI, % artificial self-pollination fruits/% artificial cross-pollination fruits); (ii) Autogamy index (AI, % natural self-pollination fruits/% natural pollination fruits); and (iii) Reproductive efficacy (RE, % artificial cross-pollination fruits / % natural pollination fruits). In detail, the ISI index suggests three classes to species scores: self-compatible ( $ISI \geq 0.8$ ), partially ( $0.8 < ISI < 0.2$ ), and self-incompatible ( $ISI \leq 0.2$ ). In general, ISI and AI values above 0.2 indicated self-compatibility and autogamy (*sensu* Ruiz-Zapata & Arroyo 1978, Lloyd & Schoen 1992). Reproductive efficacy was employed in estimating the performance of the whole pollinator guild (i.e., the pollinators' efficiency to pollen transfer).

The association between responses of fruit set to pollination treatments was calculated using the Chi-square tests for contingency tables (e.g., Bartoš *et al.* 2020). Chi-square test has low statistical power when used to reduce numbers of expected frequencies between treatments (i.e., probability of rejecting the null hypothesis when indeed this hypothesis is false, Roscoe & Byars 1971). For circumventing this problem, Fisher's exact test was also conducted because it is not strongest affected by sample size. Similar results were observed between Chi-square and Fisher's test, and hereafter using the Chi-square test ( $p < 0.05$ ). In the same way, differences between species in the reproductive efficacy and Self-Incompatibility index were evaluated in R Program ver. 3.16.1.

## Results

The flowers production during 15 days was simultaneous to *B. pachyphylla* and *B. crassifolia* in the study site (Fig. 1). On one hand, flowers of *B. crassifolia* are arranged in dense inflorescences gathering 27-34 flowers per inflorescence, which begin yellow and maintain such color until the senescence. On the other hand, *B. pachyphylla* produced around 8-12 flowers per inflorescence, which are yellow soon after the flower opening process (during the first day), and turning red (in the second day; Fig. 1a). In both species the flowers were visited by *Paratrigona* Schwarz, 1938 bees, and by the oil-collecting bees *Epicharis* (*Epicharana*) *flava* Friese, 1900 and *Centris* (*Centris*) *spilopoda* Moure, 1969. The body size and foraging behaviors of oil-collecting bees allowed pollen deposition on the ventral portion of the thorax, performing legitimate visits (Fig. 1c,

d). *Paratrigona* sp. did not contact with the stigma of the flowers and performed illegitimate visits (Fig. 1e). *Ectatomma* cf. *tuberculatum* (Olivier, 1792) ants were observed on the inflorescences, supposedly collecting small arthropods (Fig. 1f). The efficacy of oil-collecting bees in the pollen grains transference was confirmed to both species ( $RE \approx 0.94$  for both species, Table 1).

Fruit set differed between all pollination treatments (Table 1). There was no formation of fruits through apomixis in the *B. pachyphylla* and *B. crassifolia*, but ca. 35.5% of fruits were formed in pollinator exclusion treatment, and ca. 37% in artificial self-pollination (Table 1). Geitonogamy treatment in *B. crassifolia* produced more fruits (58.3%) than *B. pachyphylla* (45%). The intraspecific cross-pollination and open pollination produced more fruits in both species when compared with selfing treatments ( $\approx 71.75\%$  fruit set, Table 1). Similar fruiting was formed in inter-taxa pollination in both species ( $> 62\%$ , Table 1). Fruit set in the pollinator exclusion and selfed treatment was significantly different between species ( $\chi^2 = 6.5$ ,  $p = 0.018$ ), but no difference was observed to crossed treatments (i.e., intra- and inter-specific) and control ( $p > 0.05$ ).

Partial self-incompatibility was observed to *B. pachyphylla* ( $ISI = 0.653$ ) and *B. crassifolia* ( $ISI = 0.405$ ), with greater fruiting in autogamy (AI *B. pachyphylla* = 0.528 and AI *B. crassifolia* = 0.491). No significant difference between species in the Reproductive efficacy and Self-Incompatibility index was detected ( $P = 0.864$ ). Both species were categorized with autogamous and partially selfing, with mixed pollination system (Table 1). In addition, bidirectional interspecific cross treatment supported the reciprocal intercompatibility. Analyses of pollen tube growth (epifluorescence microscopy) corroborate the compatibility system intra- and inter-taxa (Fig. 2). In general, the pollen tube has normal morphology and similar behaviour in the stigmatic surface, in the stylar canal, ovary and micropylar canal (i.e., no callose deposition). The penetration of the micropyle canal occurs in the time-lag of 24 hours in *B. crassifolia* and 48 hours in *B. pachyphylla*, including inter-taxa pollination (Fig. 2).

Based on dataset of mating systems to Malpighiaceae species (Table 2), variation of self-incompatibility among species and populations has been detected in *Byrsonima*.

In detail, ten *Byrsonima* species expressed



**Figura 1.** Detalles de la inflorescencia de (a) *Byrsonima pachyphylla*; (b) *Byrsonima crassifolia* con *Epicharis (Epicharana) flava* recolectando aceite en la flor y (c) yema floral; (d) inflorescencia de *B. crassifolia* con *Centris (Centris) spilopoda* recolectando aceite en yema floral; (e) inflorescencia de *B. crassifolia* con *Paratrigona* sp. colectando granos de polen; (f) *Ectatomma* cf. *tuberculatum* con una gota de agua en la mandíbula, y supuestamente recolectando pequeños artrópodos.

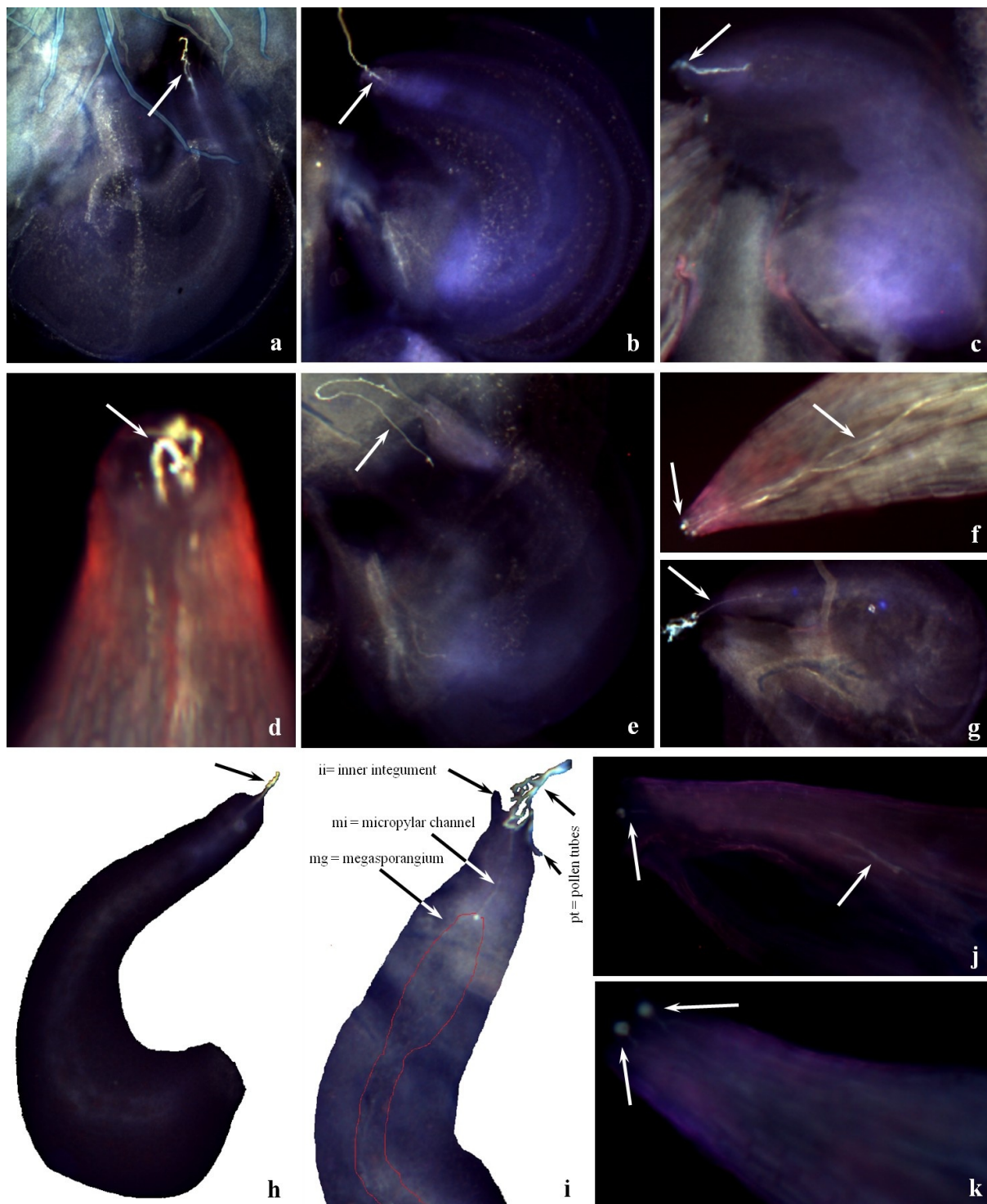
**Figure 1.** Inflorescence details of (a) *Byrsonima pachyphylla*; (b) *Byrsonima crassifolia* with *Epicharis (Epicharana) flava* collecting oil on the flower and (c) floral bud; (d) inflorescence of *B. crassifolia* with *Centris (Centris) spilopoda* gathering oil in floral bud; (e) inflorescence of *B. crassifolia* with *Paratrigona* sp. collecting pollen grains; (f) *Ectatomma* cf. *tuberculatum* with water droplet on the jaw, and supposedly collecting small arthropods.

Treatments	<i>Byrsonima pachyphylla</i>		<i>Byrsonima crassifolia</i>	
	Fruits/Flowers (N)	Fruit set (%)	Fruits/Flowers (N)	Fruit set (%)
Apomixis	0/43	0	0/47	0
Autonomous (or spontaneous) self pollinator	26/69	37.7	21/63	33.3
Hand self-pollination	21/45	46.7	11/40	27.5
“Geitonogamy” pollination	18/40	45.0	28/48	58.3
Intraspecific cross-pollination	35/49	71.4	38/56	67.9
Interspecific cross-pollination	35/46	76.1	33/53	62.3
Open pollination (control not emasculation)	50/68	73.5	46/62	74.2
RE (Reproductive efficacy)		0.971		0.915
ISI (Self-incompatibility index)		0.653		0.405
AI (Autogamy index)		0.528		0.491

**Tabla 1.** Formación de frutos en diferentes tratamientos experimentales de polinización realizados en flores de *Byrsonima pachyphylla* y *Byrsonima crassifolia* (Malpighiaceae). Se presentan el número de frutos / flores y la proporción de frutos (%). Se muestran la eficiencia reproductiva (RE *sensu* Ruiz-Zapata & Arroyo 1978), el índice de autoincompatibilidad (ISI) y el índice de autogamia AI (*sensu* Lloyd & Schoen 1992). N = número.

**Table 1.** Fruit set in different experimental pollination treatments carried out in flowers of *Byrsonima pachyphylla* and *Byrsonima crassifolia* (Malpighiaceae). The number of fruit/flower and fruiting ratio (%) are presented. Reproductive efficiency (RE *sensu* Ruiz-Zapata & Arroyo 1978), index of self-incompatibility (ISI) and autogamy index AI (*sensu* Lloyd & Schoen 1992) are shown. N = number.





**Figura 2.** Tubos polínicos normales (flecha), en el ovario y el micropilo de (a) *B. pachyphylla* (polinización abierta); (b) *B. crassifolia* (autopolinización manual); (c) *B. crassifolia* (polinización cruzada intrapoblacional); (e) *B. crassifolia* (polinización cruzada interespecífica con *B. pachyphylla* el donante de polen); (h, i) Óvulos de *B. pachyphylla* (polinización cruzada interespecífica con *B. crassifolia* el donante de polen); y (g) *B. pachyphylla* (geitonogamia). Tubos polínicos normales en la superficie estigmática y el canal estigmático de: (d) *B. crassifolia* (polinización abierta); (f) polinización cruzada intrapoblacional; (j) *B. pachyphylla* (polinización cruzada interespecífica con donante de polen de *B. crassifolia*); y (k) *B. crassifolia* (polinización cruzada interespecífica con *B. pachyphylla* donante de polen).

**Figure 2.** Normal pollen tubes (arrow), in the ovary and micropyle of (a) *B. pachyphylla* (open pollination); (b) *B. crassifolia* (hand self-pollination); (c) *B. crassifolia* (intrapopulation cross-pollination); (e) *B. crassifolia* (interspecific cross-pollination with *B. pachyphylla* pollen donor); (h, i) Ovules of *B. pachyphylla* (interspecific cross-pollination with *B. crassifolia* pollen donor); and (g) *B. pachyphylla* (geitonogamy). Normal pollen tubes in stigmatic surface and stylar canal of: (d) *B. crassifolia* (open pollination); (f) intrapopulation cross-pollination; (j) *B. pachyphylla* (interspecific cross-pollination with *B. crassifolia* pollen donor); and (k) *B. crassifolia* (interspecific cross-pollination with *B. pachyphylla* pollen donor).

Author	Year	Species	RS	Apo		Geito		Cr_pol		Ha_self		Auto		Nat_pol		ISI	RE
				Fr	Flo	Fr	Flo	Fr	Flo	Fr	Flo	Fr	Flo	Fr	Flo		
Sigrist et al.	2004	<i>Banisteriopsis adenopoda</i>	SC	0	63			25	150	42	171	4	159	20	225	1.47	0.53
Sigrist et al.	2004	<i>B. lutea</i>	SC							2	36	0	87	1	54	3.00	
Sigrist et al.	2004	<i>B. muricata</i>	SC					16	42	2	117	0	66	3	108	0.62	0.07
Sigrist et al.	2004	<i>Diplopterys pubipetala</i>	AG	51	120									26	144		
Sigrist et al.	2004	<i>Dicella bracteosa</i>	SI					2	38	0	45	0	42	0	41	0.00	0.00
Sigrist et al.	2004	<i>Alicia anisopetala</i>	SC					6	183	2	261	2	117	8	198	0.23	1.23
Sigrist et al.	2004	<i>M. cordifolia</i>	SI					18	42	0	36	0	36	0	57	0.00	0.00
Sigrist et al.	2004	<i>M. sepium</i>	SI							0	9	0	9	6	51	0.00	
Sigrist et al.	2004	<i>Stigmaphyllon lalandianum</i>	SI					8	105	0	117	0	117	8	162	0.00	0.65
Bawa	1974	<i>Malpighia glabra</i>	SC					16	31	25	44			12	80	1.10	0.29
Machado et al.	2006	<i>S. paralias</i>	SI					26	30	0	30	0	30	38	45	0.00	0.97
Costa et al.	2006	<i>S. paralias</i>	SC	0	15	12	18	4	18	10	21	0	20	11	37	2.14	1.34
Costa et al.	2006	<i>Heteropterys alternifolia</i>	SI	0	17	0	25	28	35	0	16	0	33	4	30	0.00	0.17
Sigrist et al.	2004	<i>Tetrapterys guilleminiana</i>	SC							3	57	3	57	4	120	1.58	
Sigrist et al.	2004	<i>T. phlomoides</i>	SC							17	132	17	132	96	102	0.14	
Mendes et al.	2011	<i>Byrsonima umbellata</i>	SI*	0	30	10	30	14	30	4	30	0	30	11	30	0.29	0.79
Mendes et al.	2011	<i>B. rotunda</i>	SC	18	30	27	30	20	30	15	30	7	30	18	30	0.75	0.90
Peixoto et al.	2011	<i>B. crassa</i>	SC	1	18			6	18	2	16	6	18	8	14	0.38	1.71
Downing et al.	2013	<i>B. lucida</i>	SI					103	314	5	314	0	736	161	370	0.05	1.33
Vilas-Boas et al.	2013	<i>B. intermedia07</i>	SI					26	35	0	35	0	35	28	35	0.00	1.08
Boas et al.	2013	<i>B. intermedia08</i>	SI*					28	39	6	35	0	35	21	37	0.24	0.79
Boas et al.	2013	<i>B. pachyphylla</i>	SI					15	25	0	25	0	22	11	25	0.00	0.73
Sazan et al.	2014	<i>B. cydoniifolia</i>	SI	3	112	3	102	78	430	5	156	14	286	872	2901	0.18	1.66
Balestra et al.	2014	<i>B. basiloba</i>	SC	0	165	1	115	4	119	5	118	5	159	42	145	1.26	8.62
Balestra et al.	2014	<i>B. intermedia</i>	SI	0	113	5	101	32	87	7	118	0	264	35	254	0.16	0.37
Machado et al.	2006	<i>B. gardneriana</i>	SI					28	30	0	30	0	30	68	129	0	0.56
Benezar et al.	2006	<i>B. coccolobifolia</i>	SC			40	100	64	100			16	116	100	243		0.64
Benezar et al.	2006	<i>B. coccolobifolia</i>	SC	0	90	33	105	59	90	55	168	66	150	76	165	0.50	0.70
Costa et al.	2006	<i>B. gardnerana</i>		0	15							0	41	3	25		
Costa et al.	2006	<i>B. microphylla</i>	SI	0	16	4	20	20	27	2	16	0	31	18	32	0.17	0.76
Costa et al.	2006	<i>B. sericea</i>	SI	0	28	1	24	13	30	0	17	0	28	20	78	0.00	0.59
Benezar	2006	<i>B. crassifolia</i>	SC			8	20	7	20	4	20	1	20	17	20	0.57	2.43
Bawa	1974	<i>B. crassifolia</i>	SC					58	80	48	149			102	406	0.44	0.35
Teixeira et al.	2000	<i>B. sericea</i>	SI	0	13	0	14	14	28	0	12	0	16	33	275	0	0.24
Oliveira et al.	2007	<i>B. intermedia</i>	SC	0	41			14	54	12	45	0	45	19	43	1.03	1.70
Amorim et al.	2011	<i>B. coccolobifolia</i>						0.72				0.54					
Barros	1992	<i>B. coccolobifolia</i>						0.337				0.105					
Barros	1992	<i>B. crassa</i>		80	100												
Barros	1992	<i>B. laxiflora</i>		57.6	72												
Pereira et al.	2002	<i>B. crassifolia</i>	SC					32	40	9	40	0	40	30	40	0.28	0.94
Bezerra et al.	2009	<i>B. gardneriana</i>	SI	0	30			28	30	0	30	0	30	68	129	0.00	0.56
Ribeiro	2007	<i>B. chrysophylla</i>	SC	0	30			9	30	4	30	3	30	11	30	0.44	1.22
Matallana et al.	2016	<i>B. sericea</i>	SI					epifluorescence microscopy									

**Tabla 2.** Resumen de los sistemas reproductivos en los géneros Malpighiaceae en el Neotrópico. Apo: apomixis; Geito: polinización “geitonogamia”; Cr\_pol: polinización cruzada intraespecífica; Ha\_self: autopolinización manual; Auto: autopolinización autónoma (o espontánea); Nat: polinización natural o abierta. ISI= índice de autoincompatibilidad; RE= eficacia reproductiva (Zapata y Arroyo 1978). RS: sistema reproductivo; SC= autocompatible; SI= autoincompatible; AG= agamospermia; \*= facultativo.

**Table 2.** Summary of the reproductive systems in Malpighiaceae genera in the Neotropics. Apo: Apomixis; Geito: “Geitonogamy” pollination; Cr\_pol: Intraspecific cross-pollination; Ha\_self; Hand self-pollination; Auto: Autonomous (or spontaneous) self pollination; Nat: Natural or open pollination. ISI = Self-incompatibility index and RE = Reproductive efficacy (from Zapata and Arroyo, 1978). RS: Reproductive system; SC= self-compatible, SI= self-incompatible, AG= agamospermous, \*=Facultative.

self-incompatibility, and seven species were self-compatible. In addition, agamospermy was recognized only for *Diplopterys pubipetala* (A.Juss.) W.R.Anderson & C.Davis among all 30 Malpighiaceae species. Large spectrum to Self-incompatibility (ISI) and Reproductive efficacy (RE) index was observed to Malpighiaceae (maximum and minimum; average ± Standard Deviation: ISI, 0.00 and 3.00; 0.49±0.67 and RE, 0.00 and 8.62; 1.06±1.40).

## Discussion

The aggregation of hermaphrodite flowers in *Byrsonima* spp. inflorescences might favour self-pollination mechanisms in both senses: intra-floral interference and geitonogamy (Gibbs 2014). In this case, pre-zygotic mechanisms to prevent selfing have been commonly found in Malpighiaceae (e.g., herkogamy, protogyny, stigmatic cuticle, and distinct types of self-incompatibility mecha-

nisms) (Sigrist & Sazima 2004, Downing & Liu 2013, Balestra *et al.* 2014). On the other hand, some species of Malpighiaceae show fruit formation by spontaneous treatment, as for example in *Alicia anisopetala* (A.Juss.) W.R.Anderson (2%), *Tetrapterys guilleminiana* A.Juss. (5%), *Tetrapterys phlomoides* (Spreng.) Nied. (13%) (Sigrist & Sazima 2004), and *Byrsonima* species (44% in *B. coccolobifolia*, 5% in *B. crassifolia*, 23% in *B. rotunda*, 5% in *B. cydoniifolia*) (Benezar & Pessoni 2006, Benezar 2006, Mendes *et al.* 2011, Sazan *et al.* 2014). In addition, values of fruiting by hand self-pollination, observed here to *Byrsonima pachyphylla* (38%) and *B. crassifolia* (33%), corroborated the recurrence of selfing on the Malpighiaceae and *Byrsonima*. This event could be attributed to delayed movement presented by the style towards the anthers (e.g., Benezar & Pessoni 2006) or homogamy in pre-anthesis, that is, presence of open anthers and receptive stigmas in the pre-anthesis bud, when anthers and stigmas are close (see Barros 1992), a possible strategy to facilitate the process of self-pollination even in the absence of pollinators. In addition, in some *Byrsonima* species, the stigmatic cuticle prevents water loss in xeric systems and adhesion of pollen grains to the stigma (i.e., mechanical system to prevent selfing), reinforcing the importance of pollinators in the rupture of the stigmatic cuticle (Sigrist 2004, Downing & Liu 2013).

Delayed selfing strategies are understood as a mechanism to maintain the floral traits promoting outcrossing (Lepers *et al.* 2014). On the other hand, the aggregation of hermaphrodite flowers in inflorescences of *Byrsonima* increases the “geitonogamy” pollination (de Jong *et al.* 1993, Gibbs 2014). *Byrsonima pachyphylla* featured similar fruit:flower ratio between “geitonogamy” and hand self-pollination treatments, differently to *B. crassifolia*, with more 47% of geitonogamous fruits. These discrepancies may be linked to higher plant attractiveness of *B. crassifolia* due to mass flowering ensured by inflorescence architecture with numerous flowers. On the other hand, *B. pachyphylla* is a tree, with inflorescences more distant from each other, modeling the bionomy of floral visitors / pollinators to spend more energy on intra-plant flights/foraging, discouraging the “geitonogamy” pollination in this species. In general, self-compatible species with limited ability of selfing (e.g., stigmatic cuticle, delayed movement of stylet towards the anthers) could maintain

a balance between selfing and outcrossing rates in pollinator-limited environments or seasonally floating (e.g., Karrenberg & Jensen 2012, Voillemont & Pannell 2017, Bartoš *et al.* 2020).

Distinct fruit production from selfing and crossing treatments in *Byrsonima* and other Malpighiaceae genera (*Alicia* W.R.Anderson, *Banisteriopsis* C.B.Rob., *Diplopterys* A.Juss., *Galphimia* Cav., *Heteropterys* Kunth, *Malpighia* L. *Mascagnia* (Bertero ex DC.) Colla, *Peixotoa* A.Juss., and *Tetrapterys*Cav.) (Bawa 1974, Barros 1992, Sigrist & Sazima 2004) is not totally supported by a theoretical base of subsequent events of pistil rejection (i.e., late-acting self-incompatibility systems with early acting inbreeding depression due to deleterious recessive alleles; see Gibbs 2014). On the other hand, homomorphic self-incompatibility systems are widespread on Malpighiaceae, with diversification of reproductive systems within and among genera of the family (Anderson 1980, Barros 1992, Sigrist & Sazima 2004, Benezar & Pessoni 2006, Costa *et al.* 2006, Bezerra *et al.* 2009, Sazan *et al.* 2014). For example, *Byrsonima* presents a wide range of mating systems: self-incompatible (e.g., *B. sericea*) (Costa *et al.* 2006), partially self-incompatible (e.g., *B. umbellata*) (Mendes *et al.* 2011), self-compatible (e.g., *B. intermedia*) (Oliveira *et al.* 2007), and rarely apomixis (e.g., *Diplopterys pubipetala*, *B. cydoniifolia*) (Sigrist & Sazima 2004, Sazan *et al.* 2014). Empirical data support the recurrence of non-obligatory xenogamous or mixed reproductive system and levels of self-incompatibility in the *Byrsonima*. In addition, the study area is an urban forest fragment, and self-compatible phenotypes tend to be selected when there is a shortage of pollinators, in disturbed environments or subject to anthropization.

Different levels of incompatibility has been reported to *B. intermedia* populations (e.g., ISI= 1.0, Oliveira *et al.* 2007; ISI= 0.16, Balestra *et al.* 2014). For *B. pachyphylla* (ISI= 0.65) and *B. crassifolia* (ISI= 0.45) populations in the present study could be considered self-compatible or with partial self-incompatibility, without pollen tube reactions in all levels evaluated (i.e., stigmatic surface, pistil canal, ovary and micropyle channel), but with higher fruit:flower ratio in xenogamy. Similarly, two populations of *B. crassifolia* (ISI= 0.44, Bawa 1974; ISI= 0.57 Benezar 2006) can be considered self-compatible, and with partial self-incompatibility (ISI= 0.28, Pereira &



Freitas 2002). Conversely, *B. pachyphylla* was also considered self-incompatible (ISI= 0, Vilas-Boas *et al.* 2013). This population variability in the levels of incompatibility can be attributed to numerous factors (e.g., differential rates in pollinator-limited environments, variation in the herkogamy or dichogamy levels, and efficiency of stigmatic cuticle in unpredictable environments). Unfortunately, I do not know of the existence of studies evaluating population variability in these traits.

Our hypothesis of inter-taxa incompatibility between two sympatric populations of *B. pachyphylla* and *B. crassifolia* needs more information to corroborate. Fruiting in the manual cross-pollination and pollen tubes grew analysis by fluorescence microscopy techniques reinforces the evidence for the absence of pre-zygotic barriers and reciprocal intercompatibility, although individuals with intermediate morphology were not identified *in situ*. In Malpighiaceae, the similarities in flower morphology, pollinator-dependency and oil-collecting bees guild (e.g., Centridini, Tapinotaspidini tribes) have been identified as the main factors to fixation of a homomorphic self-incompatibility system (Sigrist & Sazima 2004). However, high levels of compatibility observed in the populations studied here, indicate that pre-zygotic barriers between *B. pachyphylla* and *B. crassifolia* are weak or absent, making hybridization likely to occur under natural conditions. In spite of the apparent success of fruit formation from inter-taxa pollinations, hybrids are not distinguished. Similar approaches in *Chamaecrista desvauxii* (Collad.) Killip. (Caesapinioideae) varieties demonstrated that death of the embryo or the endosperm in the seed level is the principal post-zygotic isolation mechanism (Costa *et al.* 2007). Unfortunately, seed germination was not evaluated in present research. Additionally, I do not know any other study reporting the possibility of reciprocal intercompatibility for *Byrsonima* species.

All attributes of *B. pachyphylla* and *B. crassifolia* (i.e., syntopic, sharing oil-collecting bees, floral monomorphism, selfing and inter-taxa pollination) reinforce the failure of pre-zygotic mechanisms. Late-acting self-incompatibility (i.e., level of ovary and microphillar canal) and post-zygotic rejections (i.e., abscission of selfed pistils or fruits) are unlikely by the absence of abnormal pollen tubes in the ovaries and higher fruiting taxa (> 70% fruit:flower ratio in intra- and inter-taxa

pollination). In this scenario, I assume that *B.* and *B. crassifolia* are non-obligatory xenogamous but with high efficacy of oil-collecting bees to pollen transfer. Finally, the absence of morphological variants or hybrids in natural populations supports the hypothesis of inbreeding depression (i.e., embryo or endosperm death in the seed level). Much of this evidence is circumstantial, and at this point I suggest employing seed germination experiments and molecular approach in the offspring to investigate inbreeding depression, hybridization and introgression events in synchronopatric *Byrsonima* species.

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