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Breeding systems of enantiostylous Cassiinae species (Fabaceae, Caesalpinioideae)

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ABSTRACT

The floral morphology of enantiostylous species favours pollen flow between floral morphs because it relegates pollen deposition and capture to opposite sides of a pollinator's body. This functionality of floral morphology acts as a barrier to spontaneous self-pollination. Although numerous enantiostylous species exhibit this pattern, there are a number of variations, which might play important roles in the evolution of reproductive systems of atypical species. This study aimed to investigate the self-incompatibility and intramorph incompatibility mechanisms of monomorphic enantiostylous species, considering six species and one variety of Cassiinae. Data on floral morphology, pollinator behaviour, patterns of pollen deposition and capture, and breeding system were collected. All species exhibit heteranthery that results in distinct functions of stamens. Six bee species pollinated the flowers when grasping onto the anthers and generated vibratory movements that resulted in pollen release. Pollen deposition and capture occurred on opposite sides of a pollinator's body for the majority of Cassiinae species. All of the evaluated species were self- and intramorph-compatible, except Senna martiana, that seemed to exhibit a case of late-acting self-incompatibility. The absence of physiological self-incompatibility mechanisms in most of the studied species reinforces the relevance of floral morphology and the interaction with pollinators for the reproductive success of monomorphic enantiostylous species.

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1. Introduction

Enantiostyly involves the occurrence of reciprocal floral morphs with stigma and stamens positioned on opposite sides of the axis of bilaterally symmetrical (mono-symmetrical) flowers (Barrett, 2002). The reproductive success of enantiostylous species depends on the occurrence of pollination between floral morphs (intermorph pollination; Barrett, 2002). The floral morphology of the reciprocal floral morphs of these plants determines the locations of pollen deposition (by the anthers) and pollen capture (by the stigma) on opposite sides of a pollinator's body (Barrett, 2002). Thus, the transference of pollen between floral morphs is performed by the pollinators (Delgado and Souza, 1997;

Gottsberger and Silberbauer-Gottsberger, 1988; Westerkamp, 2004; de Almeida et al., 2013). This pattern of pollen flow based on floral morphology has been recorded for enantiostylous species of the subtribe Cassiinae of Fabaceae (Westerkamp, 2004; Laporta, 2005; de Almeida et al., 2013) and also recorded for species with other types of reciprocal herkogamy, such as heterostyly, flexistyly and inversostyly (Jesson and Barrett, 2002; Pauw, 2005), which may reduce selfing rates (Barrett, 2010).

The majority of enantiostylous species are self-fertile. There are no records of physiological self-incompatibility barriers in intramorph crossings in monomorphic enantiostylous species (i.e. that exhibit both floral morphs on the same individual; de Almeida et al., 2013). In contrast, this type of incompatibility is considered a trait of heterostylous species (Ganders, 1979; Web and Lloyd, 1986; Barrett, 2002, 2003). Therefore, morphological mechanisms of barriers to selfing in enantiostylous species are essential to the realization of legitimate matings (between different morphs),

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enhancing xenogamy rates and reducing geitonogamy (Barrett, 2002; de Almeida et al., 2013).

Aside from the existence of a pattern of pollen flow and breeding system in enantiostylous species, a number of variations have been recorded, such as self-incompatibility in *Senna* Mill. (Carvalho and Oliveira, 2003; Leite and Machado, 2010; Wolowski and Freitas, 2010) and *Cassia* L. (Bahadur et al., 1979). In the Caesalpinioideae, self-incompatibility is more commonly recorded in tree species with flowers that are characterized by a humid stigma (Arroyo, 1981; Owens and Lewis, 1989), and it is not known if this incompatibility is related to the occurrence of enantiostyly (Bahadur et al., 1979; Carvalho and Oliveira, 2003; Leite and Machado, 2010).

Enantiostylous species commonly exhibit heteranthery, i.e. the presence of anthers with different forms and functions in the same flower. Heteranthery is another important component for the functioning of enantiostyly because it results in pollen deposition on specific areas of a pollinator's body (Carvalho and Oliveira, 2003; Westerkamp, 2004; Arceo-Gómez et al., 2011; Almeida et al., 2013; de Almeida et al., 2013). Moreover, the different functions of the two groups of stamens (food source and pollination) minimize the risks of pollen limitation and wastage (Vogel, 1978; Barrett, 2002; Vallejo-Marín et al., 2009).

Besides the heteranthery, the indirect pollen deposition at the pollinator body is another trait recognized as a strategy that contibutes to the functioning of enantiostyly because it allows intermorph pollination (Westerkamp, 2004; de Almeida et al., 2013; Almeida et al., 2015). This type of deposition is performed by one or more petals, against which the pollen collides after the vibratory movements made by the bees (ricochet; Westerkamp, 2004); in some cases the pollen grains run through these petals (looping; de Almeida et al., 2013) until they reach the body of the pollinator. The actuation of petals in pollen deposition hinders pollen to be collected by the pollinator, because pollen is mostly deposited by the petals at the dorsum of the pollinator's body, from which it cannot be removed (Westerkamp, 2004; de Almeida et al., 2013; Almeida et al., 2015).

This study aims to investigate the self-incompatibility mechanisms of monomorphic enantiostylous species using species of the subtribe Cassiinae as a model. The following questions are addressed: (1) Which self-incompatibility mechanisms are present in species of the subtribe Cassiinae, and what are their relationships to the functionality of enantiostyly? (2) Do morphological selfing barriers enhance xenogamy rates?

2. Materials and methods

2.1. Study area

The study was conducted in two areas of NE Brazil, over two different time periods. Between May and July 2011, data were collected from an area of coastal sand vegetation (restinga) at the Reserva Particular do Patrimônio Natural (RPPN) Nossa Senhora do Oiteiro de Maracaípe, Ipojuca, Pernambuco State (08°31′48″S and 35°01′05″W). The size of RPPN is 76.2 ha, of which 60.96 ha are occupied by restinga vegetation (Almeida et al., 2009). The climate is warm and wet ('As' *sensu* Köppen 1948), with a mean annual rainfall of 2000 mm (concentrated between May and July) and a mean annual temperature of 23 °C; there is a dry season between October and December (SUDENE, 1990; INMET, 2014).

Between October 2011 and April 2013 data were collected from an area of 350 ha of estepic arborescent savanna (Caatinga; Teixeira, 2001) located in the municipality of Petrolina, Pernambuco State (09°19′36″S, 40°32′51.2″W). The climate is semi-arid and tropical ('Bswh' *sensu* Köppen 1948; Nascimento et al., 2003), with a mean annual temperature and mean annual precipitation of 26.3 °C

and 431 mm, respectively. There is a dry season between May and October and a rainy period between November and April (CPRM, 2005).

2.2. Studied species

Six species and a variety of monomorphic enantiostylous species of the subtribe Cassiinae were examined, including four herbaceous *Chamaecrista* Moench. species [*Chamaecrista amiciella* (H.S. Irwin and Barneby) H.S. Irwin and Barneby, *Chamaecrista calycioides* (DC. ex Collad.) Greene, *Coprosma repens* (Vogel) H.S. Irwin and Barneby, *Chamaecrista rotundifolia* (Pers.) Greene], two species and one variety of trees of *Senna* Mill. [*Senna macranthera* (DC. ex Collad.) H.S. Irwin and Barneby, *Senna macranthera* var. *pudibunda* (Benth.) H.S. Irwin and Barneby, *Stanhopea martiana* (Benth.) H.S. Irwin and Barneby]. *C. rotundifolia* was observed in the restinga vegetation, and the other species were studied in the caatinga.

All of the species exhibit diurnal anthesis and are characterized by a yellow, dialipetalous corolla with one petal that is differentiated from the others (falcate in *C. calycioides*, *C. rotundifolia*, *S. macranthera*, and *S. macranthera* var. *pudibunda*; curved in *S. martiana*; tube-formed in *C. amiciella*; and a landing platform in *C. repens*). All species have two types of stamens (heteranthery; Vogel, 1978; Vallejo-Marin et al., 2009), with pollen released from poricidal anthers by means of vibrational movements of the pollinators. Details of the heteranthery of each species are presented in the Section 3.

2.3. Floral visitors

The behaviours of floral visitors were determined by focal observations conducted throughout the day (approximately 160 h). Floral visitors were classified as pollinators (when areas of pollen capture and deposition at the body were reciprocal) or robbers (when areas of pollen capture and deposition at the body were not reciprocal). The pollen collection strategy was recorded and classified in buzzers (may result in pollination) and biters (results in pollen robbing, can damage the floral structure; Wille, 1963). Floral visitors were collected and identified by specialists.

2.4. Morphological barriers to selfing

The morphological barriers to selfing considered here relate to the effect of floral morphology upon pollen flow because the floral morphology of enantiostylous species conducts pollen for deposition to specific areas of pollinator's body that are isolated from contact with stigma to prevent intramorph pollination (Barrett, 2002; de Almeida et al., 2013).

To evaluate the areas of pollen deposition on a pollinator's body in both floral morphs, pollinators were collected immediately after visiting a flower during the first hours of anthesis and then observed under a stereomicroscope to look for areas with pollen. To identify the areas of a pollinator's body that contacted with the stigma of both floral morphs, the stigmas were covered with grease before the visits; floral visitors were then collected immediately after a visit and observed under a stereomicroscope to identify areas with grease deposition (adapted from de Almeida et al., 2013). The bees were killed using a killing jar containing ethyl acetate; this method is rapid and pollen and grease loss from their body is expected to be minimal.

For each experiment (pollen deposition and capture), a minimum of ten bees of the most frequent species were collected (20 bees for each plant species). For each plant species, the following numbers of individuals (or groups of individuals) were evaluated during these experiments: *C. amiciella*–3, *C. calycioides*–2,

Table 1

Bee species that visited the flowers of six species and a variety of enantiostylous Cassiinae plants. C = *Centris* Fabricius, 1804; FE = *Florilegus* (*Euflorilegus*) Ogloblin, A. A. (1955); XC = *Xylocopa* (*Neoxylocopa*) *cearensis* Ducke, 1910; XG = *Xylocopa* (*Neoxylocopa*) *grisescens* Lepeletier, 1841; XN = *Xylocopa* (*Neoxylocopa*) Latreille, 1802.

Species/Visitors	C sp1	C sp2	FE	XC	XG	XN
C. amiciella	X	X				
C. calycioides	X	X				
C. repens	X	X				
C. rotundifolia			X			
S. macranthera	X			X	X	X
S. macranthera var. *pudibunda*	X			X	X	X
S. martiana	X			X	X	X

C. rotundifolia–2, *C. repens*–5, *S. martiana*–5, *S. macranthera*–3 and *S. macranthera* var. *pudipunda*–3.

2.5. Mechanisms of physiological self-incompatibility (breeding system)

The following treatments were applied to characterize the breeding system (sample sizes are described in Table 2): (a) manual self-pollination (right–R and left–L flowers received pollen from their own anthers); (b) spontaneous self-pollination (R and L flowers were both bagged); (c) cross-pollination (pollination between flowers of different individuals or groups of individuals located at a distance of at least 30 metres from the receptors, as follows: R × R, L × L, R × L and L × R; the first letter is the pollen donor and the second letter is the pollen receptor). To evaluate fruit set after natural pollination, R and L flowers were left untreated and allowed to receive visits (control).

Considering that a low fruit set had previously been recorded after flower manipulation in *S. martiana* (Leite and Machado, 2010) and that the vibration of the stigma favoured receptivity and fruit set in *Chamaecrista chamaecristoides* (Arceo-Gómez et al., 2011), two supplemental manipulations were applied prior to the treatments of manual self-pollination and cross-pollination (intramorph and intermorph compatibility) in *S. martiana*, consisting of either (a) low vibration (the stigma was vibrated with a diapason at a frequency of 400 Hz) or (b) high vibration (the stigma was vibrated with a handheld electric shaver at a frequency higher than 2000 Hz; Arceo-Gómez et al., 2011). Some authors state that the vibrations made by bees allow the exposure of stigma cavity (that is covered by trichomes) and contribute to receptivity (Harder and Barrett, 1995; Buchman, 1996; Arceo-Gómez et al., 2011).

The treatments were performed in a minimum of ten and a maximum of 31 individuals and 1–2 flowers/treatment/individual.

After the manual self- and cross-pollinations, the flowers were bagged until senescence. Fruit set was recorded for all of the treatment and control plants. Fruit set comparisons between R and L flowers and between all pollination treatments were conducted by performing pairwise tests using 2 × 2 tables. The statistical analyses were performed using the software BioEstat 5.0 (Ayres et al., 2007).

To evaluate pollen tube growth, the pistils subjected to intramorph and intermorph pollination treatments were maintained in Gerbox with a 2% solution of agar for 12–24 h after the treatments (*n* = 5 flowers/time interval/treatment), fixed in a 70% FAA solution, and observed by fluorescence microscopy (adapted from Martin, 1959).

3. Results

3.1. Floral visitors

Six bee species were observed visiting the flowers of the studied species (Table 1). All six bee species were identified as pollinators and exhibited similar visiting behaviours: the bees grasped onto the anthers and generated vibratory movements that resulted in pollen release. On *C. repens*, the bees *Centris* sp. 1 and *Centris* sp. 2 used the different petals as landing platforms prior to these movements.

The visits to *Senna* and *Chamaecrista* species were observed, approximately, between 05:00 h and 17:00 h and between 05:00 h and 14:00 h, respectively.

3.2. Dynamics of pollen transfer

Pollen deposition and capture followed a similar pattern for the majority of species, i.e. on opposite sides (left and right) of a pollinator's body (Fig. 1A, C and D). The exceptions were *S. martiana* and *C. repens*, for which the arrangement of the stamens on both sides of the floral axis resulted in the simultaneous deposition and capture of pollen on the left and the right sides, respectively, of a pollinator (details below; Fig. 1B and E).

The conduction and posterior deposition of pollen grains of *C. rotundifolia* was accomplished with four petals (three similar and one falcate) that changed flower architecture and formed an arc during the visit, and thus, the deposition mode was indirect. The flowers are characterized by two stamen sizes and distinct pore directions. The stamens with pores turned to the centre of the falcate petal, deposited pollen at the abdomen and were thus considered feeding stamens (FS), whilst those with pores turned opposite to the stigma, deposited pollen at the dorsum (near the head) and acted as pollination stamens (PS; Fig. 1D). The areas of the pollinators' body that were covered with grease coincided with the areas of pollen deposition of PS (Fig. 1D).

Table 2

Results (fruits/flowers) of breeding system experiments for six species and a variety of enantiostylous Cassiinae plants. CA = *C. amiciella*; CC = *C. calycioides*; CR = *C. repens*; CRO = *C. rotundifolia;* SM = *S. macranthera*; SMp = *S. macranthera* var. *pudibunda*; SMt = *S. martiana;* SMt# = *S. martiana* after vibration; SMt* = *S. martiana* after vibration with electric shaver; NP = natural pollination (control); SP = manual self-pollination; SSP = spontaneous self-pollination; CP INTRA = cross-pollination intramorph; CP INTER: cross-pollination intermorph; R = right flowers; L = left flowers.

	NP		SP		SSP		CP INTRA		CP INTER	
	R	L	R	L	R	L	R	L	R	L
CA	7/23[a]	7/17[a]	4/27[a]	5/22[a]	0/22	0/28	11/21[a]	8/22[a]	12/25[a]	8/17[a]
CR	15/37[a]	23/43[a]	15/35[a]	11/38[a]	0/20	0/22	10/28[a]	9/28[a]	6/25[a]	8/27[a]
CRO	18/47[a]	16/43[a]	8/35[a]	7/32[a]	0/32	0/36	10/23[a]	10/26[a]	7/21[a]	8/23[a]
CC	33/41[a]	36/40[a]	13/35[b]	6/19[b]	0/35	0/22	5/20[b]	6/18[b]	11/21[b]	9/22[b]
SM	10/32[a]	12/30[a]	3/28[b]	3/27[b]	0/24	0/27	7/18[a]	9/22[a]	8/20[a]	7/21[a]
SMp	5/58[a]	7/64[a]	0/27	0/25	0/33	0/32	18/22[b]	14/25[b]	9/18[b]	7/20[b]
SMt	2/20	2/17	1/43	0/35	0/45	0/45	4/40	1/42	2/43	2/37
SMt#	–	–	0/12	0/15	–	–	1/10	0/10	0/15	1/17
SMt*	–	–	0/16	0/18	–	–	1/12	1/13	2/14	1/15

Means followed by the same letter in a row are statistically similar (Chi square test).
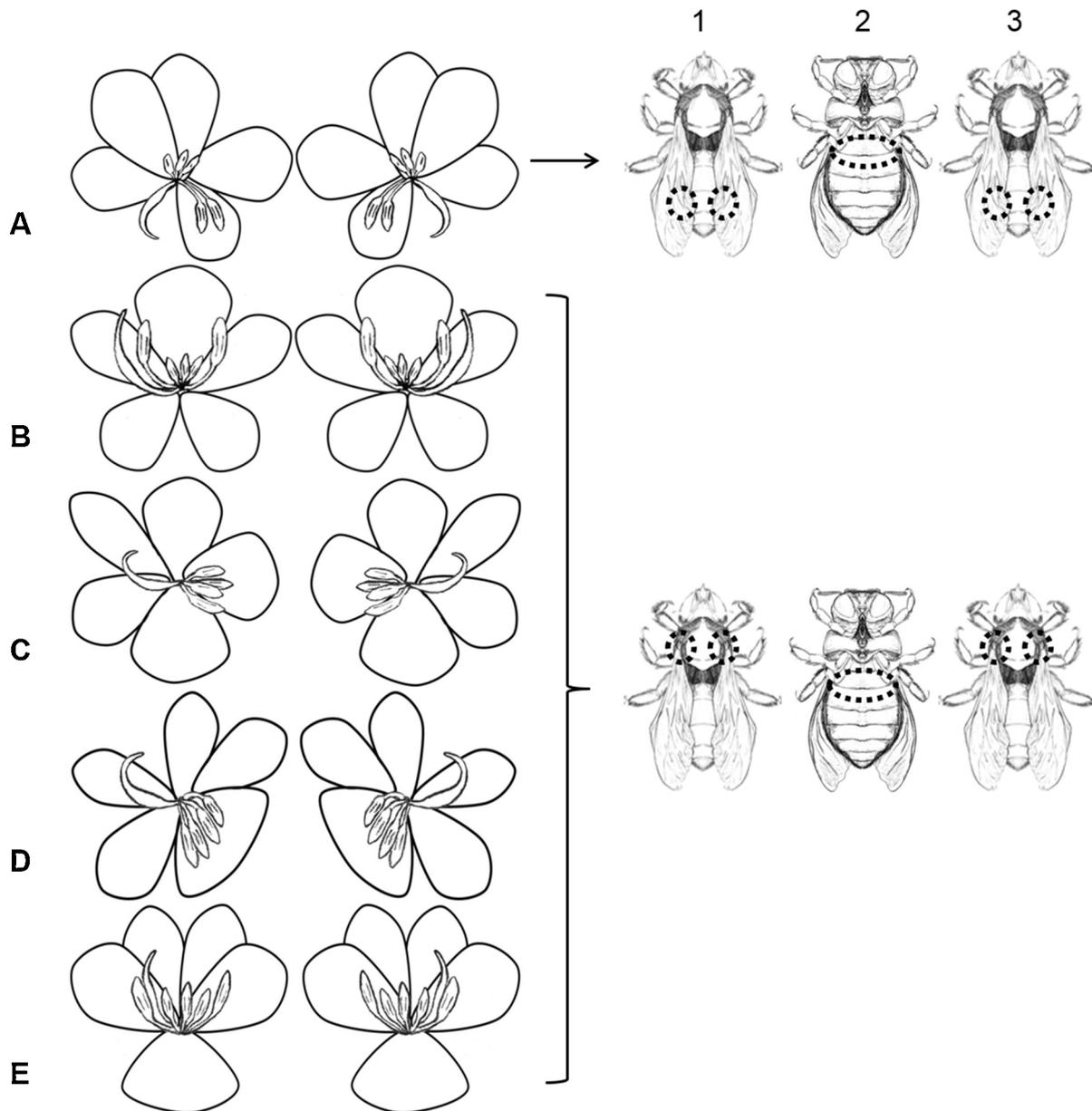
Fig. 1. Areas of pollen deposition by pollination (1) and feed (2) anthers of left (first column) and right (second column) flowers and areas of pollen capture by the stigma (3) of enantiostylous Cassiinae species in NE Brazil. (1) and (3) show the dorsal portion of the bees, and (2) the ventral portion. (A) *S. macranthera* and *S. macranthera* var. *pudibunda;* (B) *S. martiana;* (C) *C. amiciella;* (D) *C. rotundifolia* and *C. calycioides;* (E) *C. repens.* Schemes of bees and flowers adapted from de Almeida et al. (2013) and Almeida et al. (2015), respectively.

The corolla of *C. calycioides* is characterized by a curved arrangement of petals that allow for the conduction of the pollen grains from the PS to the dorsal portion of the thorax (indirect deposition). The anther morphology is similar to that described for *C. rotundifolia*; the FS releases pollen at the ventral portion of a bee (Fig. 1D). There was no modification of the floral architecture during the pollinator visits, and grease vestiges were observed on the pollinator's dorsum in the areas opposite to those of pollen deposition.

C. repens also exhibited a curved petal arrangement that conducted the pollen grains to the *allinotum* (region of contact between thorax and abdomen) of the pollinator's dorsum; thus, the pollen deposition was also indirect (Fig. 1E). One group of seven smaller stamens deposits pollen at the pollinator's venter (FS). The other stamens have three large anthers, two located on the side oppo-

site of the stigma and one following the style deflection and depositing pollen at a bee's dorsum (PS). Grease vestiges were observed in the areas of pollen deposition on the pollinator's dorsum (Fig. 1E).

In *C. amiciella*, a differentiated petal forms a tube that plays a function similar to that described for the falcate petal of the other species and that deposits pollen at a pollinator's dorsum (PS) and venter (FS, Fig. 1C). Pollen capture occurs at the side opposite to that of the pollen deposition of PS (confirmed by the observation of grease vestiges on the pollinator's body; Fig. 1C).

The stamens located at the flower centre of *S. macranthera* and *S. macranthera* var. *pudibunda* deposit pollen at a pollinator's venter (FS), whereas the pollen produced by the stamens positioned opposite to the stigma is deposited directly on the dorsal portion of the abdomen (PS). The styles of the L flowers and R flowers left

grease vestiges on the left side and right side, respectively, of the pollinators (Fig. 1A).

During the pollinator visits to *S. martiana*, the anthers touch the petals in the exact location that creates a small depression that conducts the pollen grains to the dorsum of the pollinator, thus characterizing the indirect deposition of pollen. The smaller stamens that are arranged in the flower centre deposit pollen on the venters of floral visitors (FS), and two larger stamens deposit pollen indirectly on both sides of the dorsum of a pollinator, near the head (PS; Fig. 1B). The larger stamens deposit pollen on both sides of a pollinator's body and may pollinate R and L flowers without morphological barriers. The L and R flowers capture pollen on the left side and right side, respectively, of a pollinator, coinciding with the sites of deposition (Fig. 1B).

3.3. Breeding system

C. amiciella, *C. calycioides*, *C. repens*, *C. rotundifolia*, *Senna macranthera* and R flowers of *S. martiana* set fruit under all of the treatment and natural pollination conditions, except for spontaneous self-pollination (Table 2).

S. macranthera var. *pudibunda* and L flowers of *S. martiana* set no fruit after manual self-pollination. In general, fruit set was low in all treatments and in the control because most fruits were aborted, even after the vibration treatments. There were no significant differences in fruit set between floral morphs or between intramorph and intermorph pollination experiments for any species. However, differences in the fruit set between control pollination and the treatments were observed in *S. macranthera* var. *pudibunda* and *C. calycioides*; in *S. martiana* differences occurred manual self-pollination and the other treatments (Table 2).

The fruits set of *S. martiana* could not be compared statistically because of their low numbers. Numerous pollen tubes had penetrated the ovules of all species by 12 h after both intramorph and intermorph pollinations.

4. Discussion

The six species corroborated the pattern described for the subtribe Cassiinae in relation to the pollination system. The flowers contain poricidal anthers and depend on bees that are able to vibrate the stamens to release the pollen (Buchman, 1983; Gottsberger and Silberbauer-Gottsberger, 1988). Similarly to this study, in other studies with Cassiinae species, the pollinators of the genera *Xylocopa, Centris* and *Bombus* are frequently large bees (Carvalho and Oliveira, 2003; Westerkamp, 2004; Laporta, 2005; Nascimento and Del-Claro, 2007; Almeida, 2012; Almeida et al., 2013; de Almeida et al., 2013).

The small number of floral visitors observed for all species in this study, and also previously reported for *S. corymbosa* (Laporta, 2005), might be explained by the high level of specialization that bees require to collect pollen from poricidal anthers (Buchman, 1983; Frankie et al., 1983; Gottsberger and Silberbauer-Gottsberger, 1988; Forni-Martins et al., 1998; Schlindwein, 2000) and the small number of bees having these specialized traits. A relatively high richness of bee species visiting flowers of the subtribe Cassiinae has been recorded in a number of other studies (Carvalho and Oliveira, 2003; Nascimento and Del-Claro, 2007; Almeida, 2012; Almeida et al., 2013; de Almeida et al., 2013), but, in contrast to this study, the insects reported in those studies were non-specialized pollen robbers.

The heteranthery recorded in the studied species allowed for the deposition of pollen at distinct areas of a pollinator's body (Vallejo-Marín et al., 2009; de Almeida et al., 2013), as documented for other species of the subtribe Cassiinae (Vogel, 1978; Buchman, 1983; Gottsberger and Silberbauer-Gottsberger, 1988; Westerkamp, 2004; Laporta, 2005; Almeida, 2012; de Almeida et al., 2013). The distinct functions of the stamens in pollen flowers are generally accepted to have arisen as a result of selective pressures favouring pollen collection by specialized bees, which are relatively more efficient at pollen collection than non-specialized bees (Vogel, 1978; Carvalho and Oliveira, 2003).

The similar pattern of pollen capture and deposition by floral morphs on opposite sides of a pollinator's body has been observed in *C. amiciella*, *C. calycioides*, *C. rotundifolia*, *Senna macranthera* and *S. macranthera* var. *pudibunda*, corroborating the expectations for enantiostylous species (Bahadur et al., 1990; Barrett, 2002). Enantiostylous species of *Senna, Cassia* and *Chamaecrista* also follow this pattern (Westerkamp, 2004; Laporta, 2005; Almeida, 2012; de Almeida et al., 2013). This feature seems to maximize xenogamy and minimize geitonogamy (Bahadur et al., 1990; Barrett, 1992; Kohn et al., 1996; Barrett, 2002; Westerkamp, 2004; Laporta, 2005; de Almeida et al., 2013). The occurrence of this pattern supports the existence of morphological barriers to selfing because the characteristic floral morphology does not allow for the occurrence of intramorph pollination.

S. martiana and *C. repens* did not follow the above-described pattern because pollen deposition occurred simultaneously on both sides of a pollinator's body. Thus, these species may be considered as atypical enantiostylous species (Almeida et al., 2013; Almeida et al., 2015). In a recent study, when Almeida et al. (2015) organized species of Cassiinae by type of enantiostyly, *S. martiana* and *C. repens* were designated as martiana and repens types, respectively, which were considered atypical conditions of enantiostyly. The first report of an atypical enantiostyly (Almeida et al., 2013) was for *C. flexuosa*, that exhibits a third floral morph (central) that was not recorded in the present study; this condition maximizes the area of pollen capture at the pollinator body. Thus, deviations in the pattern of pollen deposition at the pollinator body may be adaptive features of enantiostylous species (Almeida et al., 2015).

The indirect pollen deposition performed by the petals of *Chamaecrista* species is similar to the pattern of deposition, termed ricochet deposition, observed for *Cassia* and *Chamaecrista* (Westerkamp, 2004; Costa et al., 2007; Dutra et al., 2009); in which pollen grains strike punctually against the petals (Westerkamp, 2004). In *Chamaecrista ramosa*, the pathway of pollen grains is similar to a looping pathway because the pollen grains are transported to the adaxial side of the petals before they are deposited on the dorsum of a pollinator (de Almeida et al., 2013). All of the *Chamaecrista* species examined for this study are characterized by a looping pathway for pollen deposition. Studies of *Senna* species have observed the opposite: pollen is deposited directly by the anthers (Carvalho and Oliveira, 2003; Laporta, 2005; Almeida, 2012), similarly to what was observed for *S. macranthera* and *S. macranthera* var. *pudibunda* in this study. *S. martiana* represents an exception because it exhibits an indirect pollen deposition.

The atypical, low fruit set observed for *S. martiana* is interpreted as a result of a late-acting self-incompatibility mechanism reported in a previous study (Leite and Machado, 2010). This assumption is reinforced by the large number of fruits aborted early during fruit development observed for all of the treatments evaluated in this study. Cases of late self-incompatibility were recorded in Fabaceae species and interpreted as the main cause of fruit abortion (Gibbs and Sassaki, 1998; Rodriguez-Riaño et al., 1999; Freitas and Oliveira, 2002; Leite and Machado, 2010; Wolowski and Freitas, 2010). The effects of this type of incompatibility may be confounded with those of inbreeding depression; the main difference between these two mechanisms is that fruit abscission occurs synchronically in the first, and gradually in the latter (Seavey and Bawa, 1986).

The *Chamaecrista* species considered in this study are self-compatible herbs, whereas *S. macranthera* var. *pudibunda* and *S.*

martiana are self-incompatible trees. This finding confirms the suggestion that the incompatibility mechanisms present in the sub-family Caesalpinioideae (which includes the subtribe Cassiinae) have been lost from several annual, herbaceous groups and are more commonly observed in woody species (Arroyo, 1981).

The absence of physiological mechanisms of intramorph incom-patibility in the studied species was confirmed by the arrival of pollen tubes at the ovules and by the occurrence of fruit set after intramorph crosses. This result corroborates the findings of other studies related to enantiostylous Cassiinae species (Almeida et al., 2013; de Almeida et al., 2013) and differs from those for het-erostylous species (Ganders, 1979; Webb and Lloyd, 1986; Barrett, 2002, 2003). The morphological barriers to selfing for the stud-ied species are essential for the functionality of enantiostyly. We suggest that in enantiostylous species of the subtribe Cassiinae, the floral morphology is the main mechanism that guarantees reproductive success because the characteristic floral morphology maximizes cross-pollination and reduces autogamy.

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