

The pollination biology of *Pseudostiffia kingii* H. Rob. (Asteraceae), a rare endemic Brazilian species with uniflorous capitula

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Abstract (The pollination biology of *Pseudostiffia kingii* H. Rob. (Asteraceae), a rare endemic Brazilian species with uniflorous capitula). *P. kingii* is a monotypic genus within the Asteraceae family that grows under extreme environmental conditions and possesses an unusual single-flowered capitulum. It is endemic to Chapada Diamantina, Bahia, Brazil, a region dominated by a mosaic of rocky, sandy flats and bogs in the upper elevational zone (above 900–1,000 m). To gain a better understanding of the pollination biology of *P. kingii*, we examined its floral development, floral rewards, reproductive strategies, and floral visitors. The flowers exhibit asynchronous diurnal anthesis with three distinct floral stages, each differing with regard to male–female maturation and nectar availability; these floral stages appear to influence the activity of the flower visitors. Although several floral characteristics of *P. kingii* suggest that it is an entomophilous species, observations revealed it as a generalist species for which hummingbirds and bees are the main floral visitors, differing

slightly in the diurnal period of their visits. *P. kingii* can reproduce through xenogamy, geitonogamy, and what appears to be allogamy, but with different sizes of fruits (achenes) produced depending on the reproductive strategy employed. This study revealed some important features of pollination and floral biology within Asteraceae and provides clues for pollen dispersion in a harsh environment.

Keywords Compositae · Floral biology · Nectar · Rocky fields · Visitors

Introduction

Asteraceae is one of the largest angiosperm families, with ~24,000 species and a cosmopolitan distribution (Funk et al. 2009). Despite the large number of species, its pollination biology has been poorly studied in comparison to other groups of plants (Cabrera and Dieringer 1992). Pollinators and pollination mechanisms are diverse within the family (Torres and Galetto 2002, 2008), which includes self-incompatible (Sazima and Machado 1983; Johnson and Midgley 1997; Kirchner et al. 2005; Lafuma and Maurice 2007; Horsburg et al. 2011) and self-compatible species (Andersson 1996; Cerrana 2003; Grombone-Guaratini et al. 2004). Differences in the floral characters in this family, including stigma morphology (Torres and Galetto 2007) and the occurrence of protandry (De Nettancourt 1997; Houlston and Chapman 2004), can also affect pollination and reproduction, though pollinators are usually required to produce viable seeds through sexual reproduction (Torres and Galetto 2008). Furthermore, the environment can also play an important role in determining reproductive strategies (e.g., apomixis, cleistogamy), particularly in extreme climatic regions (Abrahamson 1975; Girão et al. 2007).

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Mani and Saravanan (1999) stated that some Asteraceae flowers, which are usually tubular, yellow, and nectariferous, fit into the entomophilous pollination syndrome, with most of their visitors belonging to the family Lepidoptera. However, recent studies conducted by Torres and Galetto (2002, 2008, 2011) and Chacoff et al. (2006) suggest an evolutionary trend toward a generalist pollination system, with a comparatively short floral phenology in the most derived tribes within the family and multiple visitors for flowers that produce a hexose-rich nectar. In contrast, many species in the more basal tribes present specialized pollination systems, with longer flowering periods and few visitors on flowers that offer sucrose-rich nectar.

Pseudostiffia kingii H.Rob. [tribe Moquinieae, according to Robinson (2009)] belongs to a monotypic genus within Asteraceae and is a sister group of Vernonieae. The plants are distinctive in that they exhibit one flower per capitulum, which is organized in a terminal panicle. *P. kingii* is endemic to Brazil, occurring in rocky fields in the area of Chapada Diamantina, a region known for its extreme environmental conditions and, in particular, its arid winters (Garcia et al. 2007). *P. kingii* has a shrub growth habit and can reach a height of up to 2.5 m, and the leaves are discolored. The capitula (located on a terminal panicle) are discoid, with a single flower, and the multi-serial involucre bracts are chestnut brown (Figs. 1–4). The flowers are actinomorphic and bisexual, present a tubular corolla (12–14 mm in length), and are lilac in color; a mild odor is produced throughout the flower's lifespan. The androecium is formed by five stamens with united anthers; it is epipetalous, purple, and exhibits introrse dehiscence, with secondary pollen presentation. The gynoecium is bicarpellate; the stigma is bifid and the ovary is inferior with a single ovule, forming a cypsela fruit with a whitish

setaceous pappus. Flowering occurs between March and August in Chapada Diamantina (Oliveira 2006).

The purpose of this study was to elucidate the floral biology and pollination system of *P. kingii*, an unusual species in the Asteraceae family which exhibits uniflorous capitula, at the Parque Municipal de Mucugê, Chapada Diamantina, state of Bahia, northeastern Brazil. The specific questions addressed were as follows:

1. What are the main floral features influencing the reproductive biology of *P. kingii*?
2. Do flower rewards vary among the floral stages?
3. Which species comprise the flower visitors of *P. kingii*?
4. What is the reproductive strategy of *P. kingii*?

Materials and methods

Study area

The study was conducted in May 2007, May 2008, and July 2008 at the Parque Municipal de Mucugê, Chapada Diamantina, state of Bahia, northeastern Brazil (41°20'34"W, 12°59'39"S). Three populations of *P. kingii* were selected in the following sampling sites: two populations on the trail to the river Tiburtino (41°20'30"W, 12°59'49"S, 935 m a.s.l. and 41°20'40"W, 13°00'17"S, 924 m a.s.l.) and one population on the trail to Capa Bode (41°19'30"W, 12°59'15"S, 1,090 m a.s.l.).

The landscape in this region is dominated by a type of savanna known as *Campos rupestres* (rocky fields) located at elevations over 900 m a.s.l. Herbaceous and shrubby vegetation grows in the shallow, sandy, acid, and nutrient-



Figs. 1–4 Floral events in *P. kingii* (Pictures Andrew Morse). 1 Beginning of anthesis. 2 End of anthesis (pre-anthesis). 3 Functional male phase. 4 Functional female phase

poor soils associated with rocky outcrops (Harley 1995; Conceição and Pirani 2007).

Sampling procedures

Floral biology

To define the flower stages of *P. kingii*, we marked flowers while they were still enclosed in buds ($n = 17$) and recorded the floral development. Flowers were observed daily from the bud stage until senescence, and the floral stages were defined considering the major changes occurring in the petals, sepals, and reproductive organs.

Stigmatic receptivity was tested at each floral stage with 10 % hydrogen peroxide following the method of Dafni et al. (2005) ($n = 5$ for each floral stage). The pollen grains exposed on the stigma during secondary pollen presentation (functional male stage) ($n = 5$) and the grains found in older flowers (female stage) were tested for viability with 1 % neutral red, according to the protocol of Kearns and Inouye (2003), ($n = 5$).

To determine the role of stigma morphology in the pollination biology of *P. kingii*, we examined the morphology of the style branches during secondary pollen presentation (following Torres and Galetto 2007) and photographed the flowers in the last floral stages under a stereoscopic microscope (NIKON SMZ 1000). Flowers were also examined for the presence and distribution of short hairs on the style.

Floral rewards

To test whether the availability of floral rewards varied among the flowers at different stages, we compared the following at each floral stage (see the “Results” section for details of the floral stages): (a) daily nectar production ($n = 25$ flowers per stage) using microcapillary tubes of 1–5 μL ; (b) sugar concentration ($n = 5$ flowers per plant) using a pocket refractometer (28–62 % BRIX); and (c) total sugar content ($n = 25$ flowers per stage) using volume and concentration data. The average amount of sugar produced per flower was expressed in milligrams (Galetto and Bernardello 2005). A *t* test was performed on the data following Bonferroni’s correction, and the normality of the data was tested by a Kolmogorov–Smirnov test.

Nectar samples were collected to quantify the sugar composition (fructose, glucose, and sucrose); the samples were preserved on Whatman No. 1 filter paper prior to analysis. Gas chromatography was used to identify and quantify the carbohydrates. The samples were processed with trimethylchlorosilane and hexamethyldisilazane in

pyrimidine (Sweeley et al. 1963), and the derivatives were injected into a gas chromatograph (Konik 3000-HRGS) equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector, and an OV 101 column (2 m height), 3 % on Chromosorb G/AW-DMCS (mesh 100–120). Nitrogen was used as the carrier gas (30 mL/min), and the following temperature program was used: initial temperature of 208 °C/2 min, then 1 °C/min up to 215 °C, which was maintained for 1 min, and finally 8 °C/min up to 280 °C, which was maintained for 5 min. Sugar standards (Sigma Chemical C.) were processed using the same protocol.

Reproductive strategies

To determine the compatibility system of *P. kingii*, fruit production was assessed according to the following pollination treatments and using the methods of Dafni et al. (2005): (a) control-non-manipulated flowers were naturally exposed to animal visits ($n = 80$); (b) xenogamy or cross-pollination—pollen grains were transferred manually from one plant to another ($n = 60$); (c) geitonogamy or self-pollination—pollen grains were transferred manually from one flower to another on the same plant ($n = 60$); and (d) spontaneous self-pollination—flowers isolated in voile fabric bags were not manipulated ($n = 70$); for all the treatments, flowers were isolated prior to anthesis in voile fabric bags (mesh 0.05 mm). Fruit formation was assessed after two months. The length and width of fruits were measured with a digital caliper. The data were subjected to analysis of variance (ANOVA) and Tukey–Kramer’s multiple comparison test.

Rapid responses in the style were also analyzed in experiments because some flowers presented a shriveled stigma at the female stage. In this procedure, flowers before anthesis were isolated in voile fabric bags (mesh 0.05 mm), and pollen was transferred manually to achieve geitonogamy ($n = 10$) and xenogamy ($n = 10$) according to the same procedure described above; some flowers were only isolated in a voile bag with no pollen transfer ($n = 10$).

Flower visitors and relationships with regard to *P. kingii* floral biology

To analyze the frequency of flower visitors in relation to flower reward production and stigma receptivity, we observed the visitors of *P. kingii* for two weeks in two periods (May 2007 and July 2008). During the latter period, observations were made for two populations, ~10–12 plants each in full bloom, from the first visit on the plant to the time of day when there was a decrease in visits (between 05:00 and 17:00 h).

Results

Floral biology

The flowers of *P. kingii* exhibit diurnal anthesis, and the petals began to open at ~08:00 h (Fig. 1); however, it was possible to find flowers that began to open as early as 06:00 h. Although the flowers remained closed, at ~1 h before the beginning of anthesis, the lacinia separated, exposing the anthers above the corolla (Fig. 2). The anthers had already dehisced at this stage, which was considered a pre-anthesis phase, even though the flower was not yet sexually functional. Secondary pollen presentation (on the style) occurred at 2.5 h after anthesis (flower opening) when the flowers reached the first functional stage, the male phase (Fig. 3). As the style emerged vertically, the anthers and filaments also extended above the corolla tube (Fig. 3).

During the second day of flowering (i.e., nearly 25 h after the flowers started to open), the style branches began to separate, marking the transition to a new stage. The flowers were functionally female (female phase) thereafter; this third and final floral stage was characterized by the complete separation of style branches (Fig. 4). Flower senescence began at ~29 h after opening, and the corolla faded in color.

The stigma of *P. kingii* flowers was receptive only during the female stage, a stage when the style branches were completely separated. Receptivity decreased during senescence. The pollen grains were viable from the time they were first exposed until the last floral stage. The exposed pollen grains were immediately available to visitors, and we observed anecdotally that the number of pollen grains decreased as the flower stages progressed due to visitor activity. In many cases, pollen grains were totally absent from the flowers at the female stage.

The style branches of *P. kingii* were acute, distinctly swollen, and scabrid, with short sweeping hairs below the bifurcation. When the branches separated, the pollen remained attached behind and below the stigmatic surface, opposite to the receptive region.

Floral rewards

The flowers in the pre-anthesis phase produced nectar in amounts too small to be measured (volume <0.05 μL). The volume of nectar increased during the ensuing functional male and female stages to 0.9 and 2.2 μL , respectively.

The nectar volume and concentration and total content of sugars differed between the flowers in the last two floral stages: The female stage produced more nectar and had higher total content and concentration of sugars than the male stage (Table 1). The sugar composition also differed between stages (Table 1): The female stage had the highest concentrations of fructose and glucose and the lowest concentration of sucrose. However, sucrose was still the dominant sugar in both stages. Amino acids were detected in very low concentrations, ~0.004 mg/1,000 μL .

Reproductive strategies

The flowers in all treatments produced fruits. The fruit length did not vary among the treatments ($P = 0.1147$; $F = 2.00$): spontaneous self-pollination (3.82 mm \pm 0.57, $n = 65$ fruits), flowers open to visits (3.95 mm \pm 0.45, $n = 63$ fruits), geitonogamy (3.96 mm \pm 0.50, $n = 55$ fruits), and xenogamy (3.75 mm \pm 0.74, $n = 57$ fruits). In contrast, there were significant differences in the fruit width between the treatments of spontaneous pollination (0.76 mm \pm 0.15, $n = 65$ fruits) and geitonogamy (0.77 mm \pm 0.18, $n = 55$ fruits) when compared to xenogamy (0.87 mm \pm 0.22, $n = 57$ fruits) and the flowers open to visits (0.96 mm \pm 0.24, $n = 63$ fruits) ($P < 0.001$; $F = 12.640$).

Observations of flower morphology and development (see “Floral biology” above) revealed that the pollen grains in *P. kingii* do not touch the receptive surface of the stigma on the same flower. Thus, it appears most likely that the fruits formed in the “spontaneous self-pollination” treatment were the result of agamospermy; however, additional pollen tube examination should be performed to verify such a conclusion, as emasculation in Asteraceae flowers is very difficult to achieve. The stigmas of the

Table 1 Analysis of nectar characteristics of *P. kingii*: volume, total content of sugar, concentration, and composition of sugars in the functional male and female phenophases

Nectar features	Averages \pm SD		Statistical analyses		
	Functional male	Female	<i>t</i>	<i>P</i>	Degrees of freedom
Volume (μL)	0.9 \pm 0.56	2.2 \pm 0.96	5.7	0.0001	48
Sugar content (mg)	0.43 \pm 0.28	1.40 \pm 0.69	6.5	0.0001	48
Sugar concentration (%)	38.8 \pm 0.6	51.2 \pm 0.5	3.2	0.0059	8
Sugars (%)					
Fructose	2.1 \pm 0.5	25.3 \pm 1.5			
Glucose	6.2 \pm 0.8	12.5 \pm 4.2			
Sucrose	91.7 \pm 0.3	62.2 \pm 5.6			

flowers that received pollen (geitonogamy and xenogamy) in the female phase began to shrivel within the first 5 min, whereas the stigma of the flowers that did not received pollen shriveled after ~5 h.

Flower visitors and relationships with regard to *P. kingii* floral biology

Birds, butterflies, ants, bees, and wasps were all observed visiting the flowers of *P. kingii*. The hummingbirds *Chrysolampis mosquitus* (Linnaeus 1758), *Calliphlox amethystina* (Boddaert 1783), and *Chlorostilbon lucidus* (Shaw 1812) and the bees *Apis mellifera* (Linnaeus 1758), *Centris* sp., and *Bombus* sp. were the most frequent visitors (Fig. 5). The hummingbird species with the highest number of observations was *C. lucidus*, and both male and female individuals were observed visiting flowers. We also recorded the presence of many ants (not identified) searching for nectar on the flower buds and opened flowers and different morphotypes of spiders, which were possibly seeking prey.

Visits to *P. kingii* flowers usually began at 05:00 h, a time when there was nectar, but little pollen in the flowers (i.e., most flowers in the female stage) (Fig. 5). The hummingbirds arrived first, followed by the bees between 06:00 and 07:00 h, with the latter peaking at ~10:00 h. Although there were fluctuations throughout the day and slight differences between morning and afternoon, both groups of

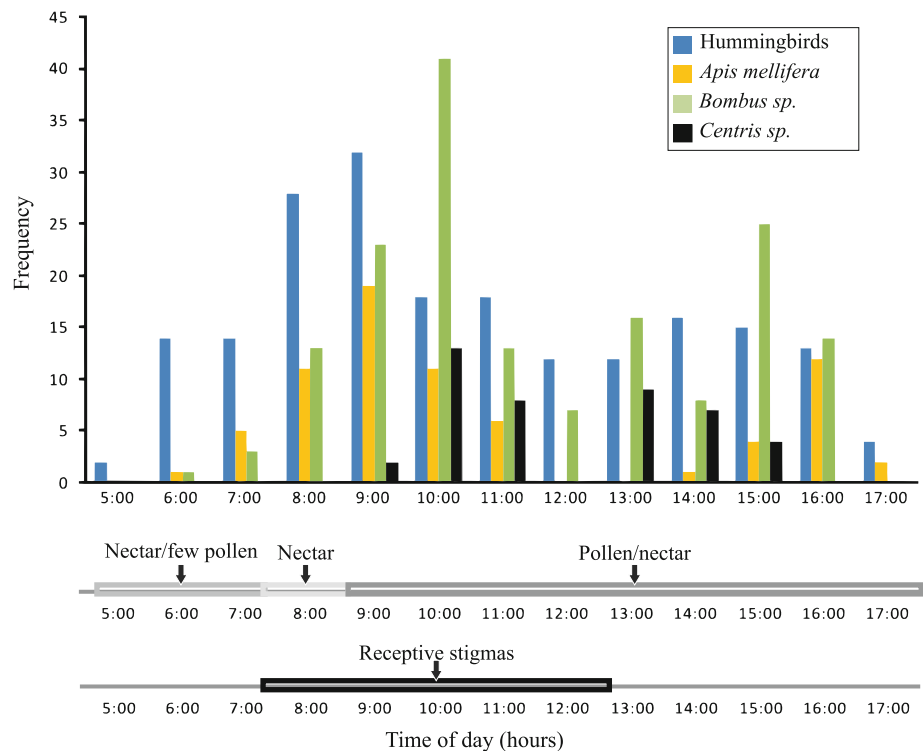
main flower visitors (hummingbirds and bees) could be found on the flowers throughout the day (Fig. 5).

Individual hummingbirds were observed visiting several different flowers, many times on the same plant, spending a few seconds at each flower. The bees exhibited a similar behavior, visiting neighboring flowers on the same plant and frequently on the same capitulescence; however, they spent more time visiting each flower (>10 s).

Discussion

Similar to other plants in the family Asteraceae, such as *Mikania urticifolia* Hook.et Arn. (Cerrana 2003), *Bidens* sp. (Grombone-Guaratini et al. 2004), and *Mikania micrantha* Kunth (Hong et al. 2008), *P. kingii* exhibits protandric flowers. Stigma receptivity occurred at the end of flower development, a stage when the pollen grains were almost completely absent from the flowers. Protandry can be functionally important in avoiding self-pollination, as the receptive surface of the stigma does not contact the pollen grains of the same flower or even of neighboring flowers. Consequently, autogamy is reduced and allogamy is increased (Lloyd and Webb 1986), resulting in a population with decreased homozygosity of unfavorable alleles (Charlesworth and Charlesworth 1987). According to Lloyd and Webb (1986), many plants that have separate phases of pollen exposure and receptivity, as occurs in protandrous species, are self-incompatible. Although there

Fig. 5 Frequency of flower visitors of *P. kingii* (Scheme Juliana Hipólito) and the main floral events that occurred in Chapada Diamantina, Mucugê, in May 2007 and July 2008. The *bottom half* indicates the availability of floral rewards (*first line*) and stigma receptivity (*second line*) over the 2-day flowering period. Here, hummingbirds refer to all species observed: *C. mosquitus* (Linnaeus 1758), *C. amethystina* (Boddaert 1783), and *C. lucidus* (Shaw 1812)



are records of many self-incompatible Asteraceae species (Sazima and Machado 1983; Johnson and Midgley 1997; Kirchner et al. 2005; Lafuma and Maurice 2007), there is a large diversity of reproductive strategies and compatibility systems in the family (e.g., Torres and Galetto 2008). Such diversity is also found in *P. kingii*. In this study, seeds were formed through cross-pollination (xenogamy treatment), pollen from the same plant (geitonogamy treatment), and in the spontaneous self-pollination treatment.

Fruit formation in the absence of pollinator intervention may represent a reproductive strategy in environments that lack pollinators or that limit their movements through such landscapes as fragmented sites (Girão et al. 2007) or rocky fields, which exhibit extreme edaphic and hydrologic conditions (Garcia et al. 2007). Although data that elucidate how pollen is dispersed and the factors that affect pollinator mobility are still lacking (Viana et al. 2012), it is not unexpected that fruits can be formed via different reproductive strategies, including apomixes, because *P. kingii* occurs exclusively in rocky fields. However, the flowers exhibited rapid changes in their stigmas when pollen was transferred, and the seeds originating by the predominance of allogamous pollination were larger and thus can represent better viability or seedling survival.

For seeds dispersed by wind, it is advantageous to be smaller or lighter to cross great distances (Picó et al. 2003); however, larger seeds can have a higher nutrient content (Fenner 2006) and germination rate (Stebbins 1970; Morse and Schmitt 1985). In this study, the seeds that resulted from cross-pollination were larger than the seeds produced by geitonogamy or autogamy. Hence, the difference in size (width) between *P. kingii* fruits suggests that, even though the seeds produced by cross-pollination may remain closer to the original population than those produced by self-pollination or apomixis, they may have more reserves for germination and seedling growth. Further studies are needed to establish the role of seed size in the dispersal and germination success of *P. kingii*.

In *P. kingii*, the female and male phases can overlap on the same plant. Hence, pollination by visitors that move within the same plant may lead to geitonogamy, which appears to be common in flowers that last longer than one day and are protandric (Medan and Ponessa 2003; Davila and Wardle 2006). A decrease in the autogamy of protandric flowers will depend on the reproductive strategy of the plant in addition to the behavior of flower visitors (Jersáková and Johnson 2007).

Pollination in *P. kingii* occurred between 08:30 and 12:30 h, a time when pollen was available and the stigmas were receptive in one-day- and two-day-old flowers, respectively. As a result, the potential pollinators of this species should be active during this period of overlap between the functional male and female stages.

We observed that hummingbirds began to visit *P. kingii* when nectar was the main reward. Bees, mainly from the genus *Bombus*, increased their frequency of flower visits when pollen was released. Both *Bombus* species and hummingbirds were frequent visitors during all sampling periods, but there appeared to be a tendency of the bees to avoid flowers without much reward, whereas the hummingbirds visited the flowers at the pre-anthesis phase looking for reward.

In addition to the environment, at a minor scale, the floral stage and nectar traits can also influence pollinator activity (Veddeler et al. 2006). A large volume of nectar with a high concentration of sugar can lead to a longer period of time spent on the flower because the nectar is viscous and the visitors are consuming extra nectar (Thomson 1986). This activity increases the movement of pollinators among the flowers of different plants and the likelihood of pollen being deposited on conspecific stigmas (Thomson and Plowright 1980).

Bees appear to be the main pollinators of *P. kingii*, particularly those of the genus *Bombus*. Hummingbirds were more frequent visitors, but their ability to access the floral reward without touching the receptive surface of the stigma makes it difficult to confirm hummingbirds as pollinators without precise pollen transfer tests. Among other visitors, bees from the genera *Apis* and *Centris* were observed less frequently on the flowers than hummingbirds and *Bombus* bees, but their body size would permit contact with the reproductive structures while removing nectar, making them potential pollinators.

Several characteristics of *P. kingii*, such as its color, shape, and nectar characteristics, suggest that it is an entomophilous species (Faegri and van der Pijl 1979). Entomophily has been observed in many other Asteraceae species (Stebbins 1970; Mani and Saravanan 1999), and recent empirical studies suggest that there is a high diversity of flower visitors in the family, with a tendency toward generalization in the most derived groups (Torres and Galetto 2002, 2008). *P. kingii* appears to maintain this generalization, sustaining different groups of pollinators, and the nectar composition may be in part responsible for this pattern. Although the nectar of some of the species in the family is composed of hexoses (Torres and Galetto 2002), *P. kingii* nectar is composed mainly of sucrose, which may be nutritionally advantageous to both hummingbirds and long-tongued bees, such as those in the genus *Bombus*, which can digest this disaccharide. Sucrose is also the dominant sugar in the nectar of several species of Vernoniaceae, the sister group of *P. kingii* (Torres and Galetto 2002).

This study of *P. kingii* reveals a number of attributes that influence its pollination biology and some possible explanations for its reproductive strategies. These include the

distinct uniflorous capitula with well-defined (morphologically and physiologically) floral stages, the mixed reproductive system, and, lastly, a group of different flower visitors (vertebrates and invertebrates) that can both collect nectar and use its energy. These results lay the groundwork for complementary studies on the genetic structure and evolutionary relationships of *P. kingii* which will aid in the conservation of this unique and endemic species.

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References

- Abrahamson WG (1975) Reproductive strategies in dewberries. *Ecology* 56:721–726
- Andersson S (1996) Floral reduction in *Crepis tectorum* (Asteraceae): tradeoffs and dominance relationships. *Biol J Linn Soc* 57:59–68
- Cabrera L, Dieringer G (1992) Reproductive biology of a population of *Acourtia runcinata* (Asteraceae: Mutisieae: Mutisieae) at the northeastern limit of its range. *Am Midl Nat* 128:83–88
- Cerrana MM (2003) Flower morphology and pollination in *Mikania* (Asteraceae). *Flora* 199:168–177
- Chacoff V, Aizen M, Galetto L (2006) Nectar concentration and composition of 26 species from temperate forest of South America. *Ann Bot* 97:413–421
- Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Syst* 18:237–268
- Conceição AA, Pirani JR (2007) Diversidade em quatro áreas de campos rupestres na Chapada Diamantina, Bahia, Brasil: espécies distintas, mas riquezas similares. *Rodriguésia* 58:193–206
- Dafni A, Kevan PG, Husband BC (2005) Practical pollination biology. *Enviroquest*, Ontario
- Davila YC, Wardle GM (2006) Reproductive ecology of the Australian herb *Trachymene incisa* subsp. *incisa* (Apiaceae). *Aust J Bot* 50:619–626
- De Nettancourt D (1997) Incompatibility in angiosperms. *Sex Plant Reprod* 10:185–199
- Faegri K, Van der Pijl L (1979) The principles of pollination ecology. Pergamon Press, Inglaterra
- Fenner M (2006) Relationships between seed weight, ash content and seedling growth in twenty-four species of Compositae. *New Phytol* 95:697–706
- Funk VA, Susanna A, Stuessy T, Robinson H (2009) Classification of Compositae. In: Funk VA, Susanna A, Stuessy T, Bayer RJ (eds) Systematics, evolution and biogeography of the compositae. IAPT, Vienna, pp 171–189
- Galetto L, Bernardello G (2005) Rewards in flowers: nectar. In: Dafni A, Kevan PG, Husband BC (eds) Practical Pollination Biology. *Enviroquest*, Ontario, pp 261–313
- Garcia QS, Jacobi CM, Ribeiro BA (2007) Resposta germinativa de duas espécies de Vellozia (Velloziaceae) dos campos rupestres de Minas Gerais, Brasil. *Acta Botânica Brasilica* 21:451–456
- Girão LC, Tabarelli M, Lopes AV, Bruna EM (2007) Changes in tree reproductive traits reduce functional diversity in a fragmented Atlantic forest landscape. *PLoS ONE* 2:908
- Grombone-Guaratini MT, Solferini VN, Semir J (2004) Reproductive biology in species of *Bidens* L. (Asteraceae). *Scientia Agricola* 61:185–189
- Harley RM (1995) Introduction. In: Stannard BL (ed) Flora of the Pico das Almas–Chapada Diamantina, Bahia, Brazil. Royal Botanic Gardens, Kew, pp 43–78
- Hong L, Niu H, Shen H, YE W, CAO H (2008) Development and characterization of microsatellite markers for the invasive weed *Mikania micrantha* (Asteraceae). *Mol Ecol Resour* 8:193–195
- Horsburg M, Semple JC, Kevan PG (2011) Relative pollinator effectiveness of insect floral visitors to two sympatric species of wild aster: *Symphyotrichum lanceolatum* (Willd.) Nesom and *S. lateriflorum* (L.) Löve & Löve (Asteraceae: Astereae). *Rhodora* 113:64–86
- Houliston GJ, Chapman HM (2004) Reproductive strategy and population variability in the facultative apomict *Hieracium pilosella* (Asteraceae). *Am J Bot* 91:37–44
- Jersáková J, Johnson SD (2007) Protandry promotes male pollination success in a month-pollinated orchid. *Funct Ecol* 21:496–504
- Johnson SD, Midgley JJ (1997) Fly pollination of *Gorteria diffusa* (Asteraceae), and a possible mimetic function for dark spots on the capitulum. *Am J Bot* 84:429–436
- Kearns CA, Inouye DW (2003) Techniques for pollination biologists. University of Colorado, Colorado
- Kirchner F, Luijten SH, Imbert E, Riba M, Mayol M, Gonzalez-Martinez SC, Mignot A, Colas B (2005) Effects of local density on insect visitation and fertilization success in the narrow-endemic *Centaurea corymbosa* (Asteraceae). *Oikos* 111:130–142
- Lafuma L, Maurice S (2007) Increase in mate availability without loss of self-incompatibility in the invasive species *Senecio inaequidens* (Asteraceae). *Oikos* 116:201–208
- Lloyd DG, Webb CJ (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. *NZ J Bot* 24:163–178
- Mani MS, Saravanan JM (1999) Pollination ecology and evolution in compositae (Asteraceae). Science Publishers, Oakville
- Medan D, Ponessa G (2003) Movement-assisted dicogamy in *Atamisquea emarginata* (Capparaceae). *Plant Syst Evol* 236:195–205
- Morse DH, Schmitt J (1985) Propagule size, dispersal ability, and seedling performance in *Asclepias syriaca*. *Oecologia* 67:372–379
- Oliveira EC (2006) A família Compositae no Município de Mucugê, Chapada Diamantina, Bahia. Dissertação de mestrado, Universidade Estadual de Feira de Santana, Feira de Santana, Bahia, Brasil
- Picó FX, Ouborg NJ, Van Groenendael JM (2003) Fitness traits and dispersal ability in the herb *Tragopogon pratensis*: decoupling the role between inbreeding depression and maternal effects. *Plant Biol* 5:522–530
- Robinson H (2009) Moquinieae. In: Funk VA, Susanna A, Stuessy T, Bayer RJ (eds) Systematics, Evolution and Biogeography of Compositae. IAPT, Vienna, pp 477–482
- Sazima M, Machado IC (1983) Biologia floral de *Mutisia coccinea* St. Hill (Asteraceae). *Revista Brasileira Botânica* 6:103–108
- Stebbins GL (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annu Rev Ecol Syst* 1:307–326
- Sweeley EC, Bentley R, Makita M, Wells WW (1963) Gas liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J Am Chem Soc* 85:2497–2507
- Thomson JD (1986) Pollen transport and deposition by bumble bees in Erythronium: influences of floral nectar and bee grooming. *J Ecol* 74:329–341
- Thomson JD, Plowright RC (1980) Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. *Oecologia* 46:68–74

- Torres C, Galetto L (2002) Are nectar sugar composition and corolla tube length related to the diversity of insects that visit Asteraceae flowers? *Plant Biol* 4:360–366
- Torres C, Galetto L (2007) Style morphological diversity of some Asteraceae species from Argentina: systematic and functional implications. *J Plant Res* 120:359–364
- Torres C, Galetto L (2008) Importancia de los polinizadores en la reproducción de Asteraceae de Argentina Central. *Acta Botanica Venezuelica* 31:473–494
- Torres C, Galetto L (2011) Flowering phenology of co-occurring Asteraceae: a matter of climate, ecological interactions, plant attributes or of evolutionary relationships among species? *Organ Divers Evol* 11:9–19
- Veddeler D, Klein AM, Tschamtk T (2006) Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos* 112:594–601
- Viana BF, Boscolo D, Mariano-Neto E, Lopes LE, Lopes AV, Ferreira PA, Pigozzo CM, Primo LM (2012) How well do we understand landscape effects on pollinators and pollination services? *J Pollinat Ecol* 7:31–41