Reproductive biology and pollination of *Aechmea distichantha* Lem. (Bromeliaceae)

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RESUMO

(Biologia reprodutiva e polinização em *Aechmea distichantha* Lem. (Bromeliaceae)). A biologia reprodutiva, incluindo fenologia, biologia floral, polinização e sistemas reprodutivos foram estudados em Floresta com Araucária no Paraná. A fenologia e reprodução de plantas terrícolas foram acompanhadas em outubro 2006 e de maio a outubro de 2007. O pico de floração ocorreu de junho a setembro e a frutificação de junho a outubro. A antese durou um dia. As flores foram polinizadas principalmente por *Stephanoxis lalandi* e a borboleta mais freqüente foi *Lychnuchoides ozias ozias*. A concetração de nectar declinou durante a antese, enquanto que o volume de néctar permaneceu constante. *Aechmea distichantha* é auto-compatível com 30-45% de frutificação nos testes de autopolinização. A luz solar influenciou a reprodução: quando controlados o tamanho das bromélias e das inflorescências, plantas no sol produziram mais sementes por frutos que plantas na sombra. A reprodução também foi associada com tamanho das inflorescências quando controlado o tamanho da bromélia. Inflorescências maiores em plantas de mesmo tamanho produzem mais flores e mais sementes por fruto.

Palavras-chave: beija-flores, biologia floral, borboletas, fenologia floral, sistemas reprodutivo

ABSTRACT

(Reproductive biology and pollination of *Aechmea distichantha* Lem. (Bromeliaceae)). Reproductive biology, including phenology, flower biology, pollination, and the reproductive system in the bromeliad *Aechmea distichantha* were studied in an Araucaria forest in the state of Paraná. Phenology and reproduction in terricolous plants were followed in October 2006 and May - October of 2007. Flowering peaked from June to September and fruiting was from June to October. Flower anthesis lasted one day. Flowers were pollinated the most by the hummingbird *Stephanoxis lalandi* and the most common butterfly visitor was *Lychnuchoides ozias ozias*. Nectar concentration declined during anthesis, while nectar volume was constant. *Aechmea distichantha* is self-compatible with 30-45% fruit formation in self-pollination tests. Sunlight influences reproduction: when controlling for bromeliad and inflorescence size, plants in sunlight produced more seeds per fruit than plants in the shade. Reproduction was also associated with inflorescence size when controlling for bromeliad size. That is, larger inflorescences in similar sized plants produced more flowers and more seeds per fruit.

Key words: breeding systems, floral phenology, floral biology, hummingbirds, butterflies

Introduction

Bromeliaceae is a large family of monocotyledons in which the majority of species are pollinated by hummingbirds (Martinelli 1997; Buzato *et al.* 2000; Varassin & Sazima 2000; Kaehler *et al.* 2005; Machado and Semir 2006; Piacentini & Varassin 2007) but bats may also be important (Sazima *et al.* 1995; Sazima *et al.* 1999). Insects may also pollinate these plants, yet this is seldom demonstrated (Benzing 2000; Varassin & Sazima 2000; Siqueira Filho & Machado 2001; Kaehler *et al.* 2005; Lenzi *et al.* 2006). Also, insect pollination is often in association with other pollinating animals (Benzing 2000; Varassin & Sazima 2000; Siqueira Filho & Machado 2001; Wendt *et al.* 2001; Lenzi *et al.* 2006) and hence may confuse the true source of pollination.

The Bromeliaceae comprise a wide variety of reproductive systems. Selfing has been found in most species and in several genera, including *Aechmea*, *Alcantarea*, *Billbergia*,

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Canistrum, *Pitcairnia* and *Vriesea* (Martinelli 1997; Siqueira Filho & Machado 2001; Wendt *et al.* 2001; Wendt *et al.* 2002; Lenzi *et al.* 2006; Paggi *et al.* 2007), yet incompatibility systems are also found in *Aechmea*, *Billbergia* and *Quesnelia* (Martinelli 1997; Canela & Sazima 2003). Spatial and temporal mechanisms to avoid selfing include dichogamy, dioecy and herkogamy (Martinelli 1997; Siqueira Filho & Machado 2001). However, these mechanisms to avoid selfing are not always effective due to the behavior of the pollinating animals (Siqueira Filho & Machado 2001).

Reproduction may also be influenced by the environment in which the plant is found and in which resource availability may vary (Lechowicz & Bell 1991). Environmental variation may also influence fitness by affecting the number and abundance of pollinating species (Herrera 1995). In the Bromeliaceae, environmental variation can influence reproductive success within and among populations (Paggi *et al.* 2007). For example, light regime can influence individual size and consequently their reproductive success (Lenzi *et al.* 2006). Small scale variation, such as flower numbers and density, can influence visitation by pollinators and hence influence reproductive success (Grindeland *et al.* 2005; Makino *et al.* 2007).

Here we examine reproductive biology of the bromeliad *Aechmea distichantha* Lem. This bromeliad is common in the Atlantic Rain Forest and is considered a generalist or pioneer epiphyte (Borgo & Silva 2003). Specifically, we examine phenology, floral biology and the reproductive system to examine how variation in the local environment may influence reproductive success, in southern Brazil.

Materials and methods

Aechmea distichantha was studied at the Gruta de Bacaetava Municipal Park (GBMP) (25º13'54"S, 49º12'26"W). Created in May 2000, the park comprises an area of 17.4 ha, in Colombo, in the southern Brazilian state of Paraná. The climate is subtropical, with an annual average temperature of 16º C and 1450 mm average rainfall, in an *Araucaria* forest (Prefeitura Municipal de Colombo 1999).

We studied terricolous plants of *Aechmea distichantha*. Plants were visited weekly during two phases. The first, in October 2006 was to observe flower visitors. The second, May – October 2007, included observations of flower visitors and the experimental study. Twenty plants that were available on the ground were studied weekly through the flowering period to record flowering phenology, morphology and biology. The number of flowers in each inflorescence was counted and the duration of time over which the flower was open was registered. The plants occurred in two different light environmental settings: open areas (considered here as a sunny environment) or in forest understory (considered here as a shade environment).

To compare productivity in different light environmental settings and with different pollinators, we must first control for local variation among plants. First, plant size may confound comparisons of seed set if larger plants produce more. Similarly, larger plants may have larger flowers and so on. Thus, we estimated plant size by measuring each plant. The radius (r) of the plant was estimated by measuring the length of the external-most leaf of the rosette from the base to the tip. Plant height (h) was the length of the most internal leaf near the floral scape. Plant size was estimated using those two measurements as radius and height in the formula for the volume of a cylinder ($4/3\ \pi r^3 h$). Similarly, radius and height of the inflorescence were measured and used to calculate volume as an estimate of inflorescence size.

We tested whether light regime influenced reproductive success, as other studies have shown that pollination success is greater under lighter conditions. Since larger plants produce larger inflorescences and larger inflorescences produce more flowers, we must first control for plant size. Using regression analysis, we estimated the relationship between bromeliad size and inflorescence size (Zar 1999). From that regression, we used the residuals to examine how inflorescence size influences productivity, as if we were comparing inflorescence size among similarly-sized bromeliads. Similarly, we also examined whether fruit production was due to the number of flowers, controlling for inflorescence size. That is, if all inflorescence were the same size, do those with more flowers produce more fruits? While this may seem obvious, it may not be the case if fruit abortion is common. The number of flowers and fruits were counted in 20 individual plants. Seeds were counted from five fruits per plant. We tested whether light regime (plant in full sun exposure or plant in forest understory) influenced reproductive success as the number of fruits and seeds produced per plant, controlling for number of flowers per inflorescence by a covariance analysis.

Breeding system experiments comprised manipulations to test how pollination influences fruit production: 1) Open pollination (flowers were not manipulated), 2) apomixis (flowers were emasculated and the stigma removed), 3) autonomous pollination, 4) selfing and 5) crossing (Radford *et al.*, 1974). Reproductive success, measured as fruit set, was compared among treatments by independence tests using contingency tables (Zar 1999).

Plants were observed in the field to attempt to discover which animals were pollinators. Flowers were observed with binoculars during bouts of approximately 4h in the morning. Some flower visitors were photographed. Visits and time duration were noted. Hummingbirds were identified from field guides while invertebrates were collected and identified by specialists. We avoided observing visitors more than once at the same plant (Kaehler *et al.* 2005).

Nectar was collected, with capillary tubes from flowers that were previously covered by small bags to prevent removal by animals, from 12 plants every hour on the hour from 07:00 – 17:00h. Therefore, nectar samples are the cumulative production during the time interval while they were sacked. We registered date, time, temperature, relative humidity, and nectar volume at each collection. Samples were then frozen for later analysis. Volume was measured with a Hamilton 50 µl microsyringe and sugar concentration was measured with an Atago portable refractometer. Nectar production was tested for partial correlations with temperature, humidity and time of day (Zar 1999).

Results

Plant diameter varied from 140 - 280 cm (mean = 193 cm) and height from 50 – 100 cm (mean = 68.5 cm). The inflorescence is a spike, pinkish with bluish petals. Formation of the scape (25 – 45 cm long) takes around 60 days. Fruit (60 – 310 inflorescence^{-1}) formation is complete approximately 40 days after flowering. Inflorescence height varied between 10 – 22 cm and diameter 7 – 10 cm. On average there were 129 flowers inflorescence^{-1} and fruit set of 120 seeds fruit^{-1}. This production results in an average of 15,480 seeds inflorescence^{-1}.

The peak flowering period of *Aechmea distichantha* was June – September 2007, with approximately 10 simultaneous plants flowering month^{-1}. Inflorescences lasted 20 - 30 days and the number of flowers per inflorescence was 70 – 330, with 5 – 10 open flowers inflorescence^{-1} at any given moment. Anthesis began between 05:00 – 06:00h and ended 17:00 – 18:00h on the same day. Fruit were found from June - October.

When reproductive success was calculated after controlling for plant size, we first found that larger inflorescences produced a larger number of flowers (ln number of flowers = 5.0 + 0.65 x residual inflorescence volume, $r^2 = 0.28$, $F = 8.42$, df = 1,18, $p < 0.05$). However, neither the size of the inflorescence nor the number of flowers per inflorescence was influenced by light regime (after controlling for the size of the bromeliad, $t < 1.0$, df = 18, $p > 0.10$). However, the number of fruit produced per plant was much greater in the sun than in the shade, after controlling for number of flowers per inflorescence ($t = 3.66$, df = 18, $p < 0.05$, Fig. 1A) and the number of seeds produced per fruit was greater in the sun than shade ($t = 2.83$, df = 18, $p < 0.05$, Fig. 1B). Therefore, controlling for plant and inflorescence size, we found that more seeds are produced per fruit in the sun.

Aechmea distichantha can be self-compatible, as fruit production in the self-pollination tests was 30 – 45% (Tab. 1). Fruit set in natural conditions was 80% (Tab. 1). Fruit set was lowest in apomixis (10%, $\chi^2 = 24.0$, df = 4, $p < 0.05$) with few seeds per fruit. Fruit set was more or less equal among the autonomous, selfing and crossing treatments ($\chi^2 = 1.82$, df = 2, $p > 0.05$, Tab. 1).

Of the 200 observed visits to flowers during 65 hours of observation, 33% were hummingbirds and 68% were butterflies (Tab. 2). *Stephanoxis lalandi lalandi* (Vieillot, 1818) was the most commonly observed hummingbird, accounting for 57% of the visits, followed by *Thalurania glaucopis* (Gmelin, 1788) (26%, Tab. 2). Of the butterflies, *Lychnuchoides ozias ozias* (Hewiton, 1878) was the most common (50%), followed by *Heliconius ethila narcaea* (Godart, 1819) and *Phoebis neocypris* (Hübner, 1823) (Tab. 2). While hummingbirds visited at any time during flowering, butterflies were most observed during June and July, and seemed to be tied to climatic conditions (clear days, warmer temperatures). The bee *Trigona spinipes* (Fabricius, 1793) was observed as a pollen collector, foraging and damaging the flowers and apparently interfering with the visits by the other species.

Hummingbirds and butterflies both may cause geitonogamy because they visit more than one flower inflorescence^{-1}. Butterflies forage slowly, spending up to 1 min flower^{-1}, while hummingbirds usually visit a flower for less than 10s. Flowers in the open areas were visited more often (both, butterflies and hummingbirds) than plants in the understory condition.

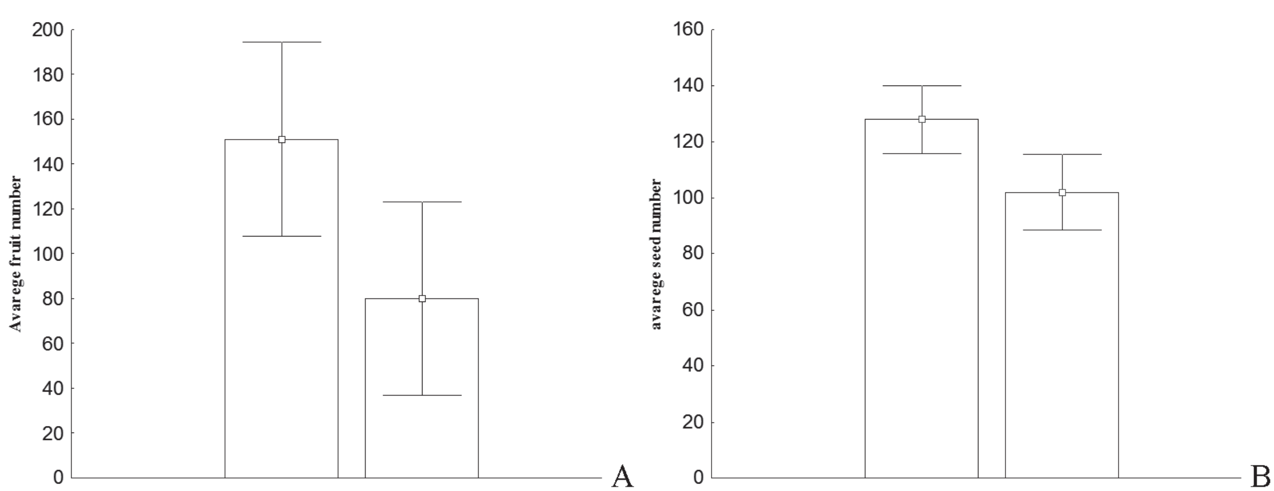

Figure 1. Average (+95% confidence interval for the mean) number of fruits (A) and seeds (B) produced in sunny (left) and shady conditions (right) in *Aechmea distichantha*.

Table 1. Fruit set (%) in the pollination treatments (n = 20 flowers) in *Aechmea distichantha*.

Pollination Treatment	Fruit Set
Autonomous	30
Selfing	45
Crossing	50
Open	80
Apomixis	10

Nectar content on average was 11.6 µl (95% CI = 10.6 – 12.7) and remained constant throughout anthesis (r = 0.20 for correlation with hour of the day, df = 40, $p > 0.05$), indicating that production occurs only at the beginning of anthesis. Temperature and time were correlated (r = 0.60, df = 42, $p < 0.001$) while humidity was independent of both temperature and time (r = -0.12, and r = 0.05 respectively, df = 42, $p > 0.05$), therefore temperature and humidity were tested for a correlation with nectar production and concentration. Nectar volume was independent of both, temperature or humidity (r = -0.30 and 0.16, respectively, df = 40, $p > 0.05$). Nectar concentration, however, was greatest during anthesis early in the morning after which it gradually decreased (r = -0.58, df = 40, $P < 0.001$). Concentration was correlated with temperature (r = 0.40, df = 40, P = 0.0105), but not with humidity (r = 0.17, df = 40, $p > 0.05$).

Discussion

Most plants in GBMP flowered in winter when rainfall was low, in contrast to reported flowering from January to April in Southeastern Brazil (Buzato *et al.* 2000). Plants with flowers pollinated by birds tend to flower during the wet season, with some species flowering throughout the year (Arizmendi & Ornelas 1990; Sazima *et al.* 1995; Araújo *et al.* 2004). The extended flowering season of *Aechmea distichantha* favors visits by hummingbirds and butterflies. A consequence of this prolonged availability may keep pollinators in the area and thereby influence their availability to other species in the local plant community (Machado & Semir 2006). In this study, 68% of flower visits were due to hummingbirds, which emphasizes their importance as pollinators (Benzing 2000, Siqueira & Machado 2001; Lenzi *et al.* 2006). The use of bromeliads by both hummingbirds and butterflies is common and the butterflies seem to favor the genus *Tillandsia* (Varassin & Sazima 2000).

Aechmea distichantha is facultatively selfing, *sensu* (Lima & Vieira 2006), and may benefit from "reproductive assurance," since they may reproduce successfully even in the

Table 2. Visitation rates (flowers $hour^{-1}$) among flower visitors at *Aechmea distichanta* in the GBMP.

Pollinators (flower visitors)	2006	2007				Total	%
	Oct	Mai	Jun	Jul	Aug		
Hummingbirds							
Aphantochroa cirrochloris (♂)				0.06		1	1.5
Chlorostilbon a. aureoventris (♀)	0.06					1	1.5
Leucochloris albicollis (♂)	0.06		0.04			2	3.1
Phaethornis pretrei (♂)		0.07	0.04	0.11	2.0	6	9.2
Ramphodon naevius (♂)		0.07				1	1.5
Stephanoxis lalandi (♀♂)	1.62	0.27	0.29			37	57
Thalurania glaucopis (♀♂)	0.06	0.13	0.58			17	26.1
Monthly hummingbird total	29	8	23	3	2	65	
Butterflies							
Epityches eupompe		0.07				1	0.7
Heliconius beschei		0.13	0.17			6	4.5
Heliconius ethilla narcaea		0.87	0.62			28	20.7
Hesperocharis erota				0.11		2	1.5
Lychnuchoides ozias ozias			2.79	0.06		68	50.4
Phoebis neocypris			0.17	0.83		19	14.1
Vettius artona			0.25			6	4.5
Vettius diversus			0.21			5	3.7
Monthly butterfly total	0	16	101	18	0	135	
Hours of observation	16	15	24	9	1		

absence of pollinators (up to 30%, Zhang & Li 2007). Despite that self-compatibility is common in bromeliads (Martinelli 1997; Siqueira Filho & Machado 2001; Lenzi *et al.* 2006), the importance of selfing has not been previously examined in the genus *Platyaechmea*. Fruit formation in the apomixy treatments indicates that asexual reproduction can occur. Low levels of apomixy were found in the genus *Pitcairnia*, with few and small seeds being produced as a consequence (Wendt *et al.* 2001). Thus, apomixy in that species plays a small role in reproduction, as with *A. distichantha*. Greater reproductive success in natural conditions (80%) as compared to the experimental treatments was also found in *Aechmea beeriana* (98%, Nara & Webber 2002), *Canistrum aurantiacum* (89%, Siqueira Filho & Machado 2001) and in *Vriesea longiscapa* (86%, Martinelli 1997). Greater success in control flowers may be due to lower rates of abortion than in manipulated flowers. It is more likely that the greater reproductive success in control flowers was due to the pollinator carrying pollen from a wide variety of source plants as observed by Kawai & Kudo (2008), with greater genetic variability that may result in greater reproductive success. Such long distance gene flow may be due to pollinating species that travel large distances (Barbará *et al.* 2009).

Light environment directly influenced reproductive success in *Aechmea distichantha*: inflorescences in the sun had 180% more fruit and 120% more seeds than those in the shade. This may be due to resource allocation in response to photosynthetic constrains as evidenced by Cervantes *et al.* (2005). The larger production of fruits and seeds may be also affected by pollinator behavior since bromeliads in sunny places were visited more often. Pollinator foraging may be influenced by the environmental light (Kilkenny & Galloway, 2008) and pollen limitation due to reduced pollinator visits may cause the variation in reproduction among bromeliad populations (Paggi *et al.* 2007). Since hummingbirds are very sensitive to environmental changes in resource supply (Cotton 2007) and avoid unrewarding patches (Sandlin 2000), they may have visited the bromeliads in sunny patches more often due to greater flower availability.

The volume of nectar produced in the morning remained constant throughout the day (when not removed by pollinators), similar to that found in *Aechmea lindenii* (Lenzi *et al.*, 2006), which suggests that *Aechmea distichantha* only produces at the beginning of anthesis. On the other hand, concentration was greatest early, at the beginning of anthesis, and gradually declined. This suggests that the flower reabsorbs the solutes, as has been observed in other hummingbirds pollinated species (Freitas & Sazima 2001; Varassin *et al.* 2001) including Bromeliaceae (Canela & Sazima 2003). While concentration was independent of volume in *A. distichantha*, in *A. pectinata* both varied during anthesis (Canela & Sazima 2003). In both cases, nectar quality (from the pollinator perspective) is greatest in the morning, which appears to be a common trend in the Bromeliaceae (Machado & Semir 2006).

The greater rate of hummingbird visits in the morning is likely to be due to the greater quality of the nectar at that time (Canela & Sazima 2003; Machado & Semir 2006). On the other hand, butterfly visits were always associated with good climatic conditions (higher temperatures, no rain, pers. obs.).

Aechmea distichantha, while self-compatible, has greater reproductive success in natural conditions, perhaps due to pollinator efficiency and consequently greater outcrossing. The greater success in the sun also suggests that it is due to pollinator efficiency, as trap-lining hummingbirds are more likely to find the more visible flowers. Additionally, greater success in the sun may be the result of the combined benefit of greater resource allocation that also influences pollinator visitation.

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