

## MATING SYSTEM VARIATION AND ASSORTATIVE MATING OF SYMPATRIC BROMELIADS (*PITCAIRNIA* SPP.) ENDEMIC TO NEOTROPICAL INSELBERGS<sup>1</sup>

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- *Premise of the study:* The mating system is an important component of the complex set of reproductive isolation barriers causing plant speciation. However, empirical evidence showing that the mating system may promote reproductive isolation in co-occurring species is limited. The mechanisms by which the mating system can act as a reproductive isolation barrier are also largely unknown.
- *Methods:* Here we studied progeny arrays genotyped with microsatellites and patterns of stigma–anther separation (herkogamy) to understand the role of mating system shifts in promoting reproductive isolation between two hybridizing taxa with porous genomes, *Pitcairnia albiflos* and *P. staminea* (Bromeliaceae).
- *Key results:* In *P. staminea*, we detected increased selfing and reduced herkogamy in one sympatric relative to two allopatric populations, consistent with mating system shifts in sympatry acting to maintain the species integrity of *P. staminea* when in contact with *P. albiflos*.
- *Conclusions:* Mating system variation is a result of several factors acting simultaneously in these populations. We report mating system shifts as one possible reproductive barrier between these species, acting in addition to numerous other prezygotic (i.e., flower phenology and pollination syndromes) and postzygotic barriers (Bateson–Dobzhansky–Muller genetic incompatibilities).

**Key words:** asymmetric introgression; breeding system; Bromeliaceae; evolution of selfing; herkogamy; hybridization; progeny array; reinforcement; reproductive isolation; reproductive biology; speciation.

The shift from outcrossing to selfing has puzzled evolutionary biologists for over a century (e.g., Darwin, 1876; Stebbins, 1957; Jain, 1976; Lande and Schemske, 1985; Barrett, 2003, 2010, among others). Sympatric, closely related species that vary in their mating systems provide an opportunity to examine

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the mechanisms responsible for the early stages of transitions in mating system (Foxe et al., 2010; Ness et al., 2010; Willi and Määttänen, 2010; Busch et al., 2011; Pettengill and Moeller, 2012). Despite the large variation in mating system frequently observed in plants and in some animals (i.e., Jarne and Charlesworth, 1993), selfing is rarely studied as a prezygotic reproductive barrier to gene flow (but see Levin, 1985; Martin and Willis, 2007; Briscoe Runquist et al., 2014; Brys et al., 2014).

Selfing may contribute to reproductive isolation in several ways. Selfing reduces the likelihood of pollen transfer between species (e.g., Fishman and Wyatt, 1999; Martin and Willis, 2007) and often leads to increased genetic incompatibilities between species (Brandvain and Haig, 2005). Consistent with selfing acting as a reproductive barrier, increased divergence in traits associated with selfing has been observed in sympatric relative to allopatric populations of several species: flower color (e.g., *Phlox*: Levin, 1985; Hopkins and Rauscher, 2012), flower size (e.g., *Solanum*: Whalen, 1978; *Arenaria*: Fishman and Wyatt, 1999; *Mimulus*: Grossenbacher and Whittall, 2011), and reduced herkogamy: physical separation between anthers

and stigmas (e.g., *Ipomea*: Ennos, 1981; Smith and Rausher, 2007; *Mimulus*: Grossenbacher and Whittall, 2011). In contrast, when closely related species occur in allopatry (i.e., their geographic ranges do not overlap), divergence in floral traits is not always needed to ensure reproductive isolation (Mayr, 1942).

In this study, we focus on populations of the hybridizing species *Pitcairnia albiflos* Herb. and *P. staminea* Lodd. (Bromeliaceae). They are sister taxa (for morphological phylogeny, see Saraiva, 2013; molecular phylogeny, Schubert et al., 2013) and are exclusively saxicolous (rock-dwelling) species adapted to isolated inselbergs (dome-shaped granitic or gneissic rock outcrops), which represent patchy habitats similar to terrestrial islands (Porembski and Barthlott, 2000; Barbará et al., 2007). These rare bromeliad species are endemic to a narrow geographic area in southeastern Brazil (Rio de Janeiro and Niteroi cities) and are known from only a few populations. The two species occur in sympatry in only two localities within the city of Rio de Janeiro: a large area on the Pão de Açúcar Mountain and the other a smaller population on a mountain with a difficult and dangerous access (Palma-Silva et al., 2011). Both species are self-compatible. Whereas *P. albiflos* is pollinator-dependent, *P. staminea* is capable of higher levels of autonomous seed set in the absence of pollinators (Wendt et al., 2001).

The two bromeliad species are well differentiated morphologically (Wendt et al., 2000, 2001) and genetically, based on nuclear microsatellite markers ( $\theta_{ST} = 0.22$  and  $G'_{ST} = 0.65$ , both  $P < 0.0001$ , Palma-Silva et al., 2011). Previous studies based on nuclear and plastid microsatellite markers have demonstrated that barriers to reproductive isolation between these bromeliads are permeable with asymmetric introgression toward *P. albiflos* (Palma-Silva et al., 2011), thus attesting to the porous nature of the genome and indicating that divergence with gene flow is possible in this system. Species integrity was hypothesized to be maintained by the simultaneous action of multiple post- and prezygotic barriers (Wendt et al., 2001). Possible prezygotic barriers consist of divergence in flowering phenology, pollination syndromes, and mating systems. Possible postzygotic barriers include hybrid inviability, lower germination rates (Wendt et al., 2001), Bateson–Dobzhansky–Muller incompatibilities (i.e., incompatibility because of cytonuclear or nuclear–nuclear interactions, Palma-Silva et al., 2011), as well as partial male sterility (reduced pollen viability, Wendt et al., 2001).

Here, we expand our previous work (Wendt et al., 2001, 2002; Palma-Silva et al., 2011) by evaluating mating system variation in allopatry and sympatry using progeny array analysis. Progeny-array-based methods are robust, informative, and sufficiently powerful to estimate outcrossing and selfing rates of individuals (Wang et al., 2012). We aim to understand the potential contribution of one particular floral trait, herkogamy,

and intra- and interspecific variation of the mating system, which may increase reproductive isolation between these two bromeliad species, leading to assortative mating and protection of *P. staminea* genome from introgression from *P. albiflos*. First, we examined the extent of variation in outcrossing and selfing rates, based on the genotyped progeny arrays, among one sympatric population of *P. albiflos* and *P. staminea* and two allopatric populations of *P. staminea*. Second, we aim to understand the potential contribution of herkogamy and intra- and interspecific variation in mating system to assortative mating in these two bromeliad species and to the protection of the *P. staminea* genome from introgression from *P. albiflos*. Specifically, we found that, at the population level, *P. staminea* had higher selfing rates and increased herkogamy in sympatric relative to allopatric populations, which might help to protect *P. staminea* from introgression with *P. albiflos*. We discuss mating system variation as one of the reproductive barriers between these species acting in addition to multiple other prezygotic (i.e., flower phenology, pollination syndromes) and postzygotic barriers (i.e., Bateson–Dobzhansky–Muller genetic incompatibilities).

## MATERIALS AND METHODS

**Biological samples and DNA extraction**—We analyzed progeny arrays based on fruits collected from open-pollinated maternal families (groups of offspring from a known mother plant) in the largest and best-studied hybrid zone of *P. albiflos* and *P. staminea* located in Pão de Açúcar (PAO) in the city of Rio de Janeiro. In previous studies, the spontaneously self-pollinated *P. staminea* exhibited a higher inbreeding coefficient ( $F_{IS} = 0.24$ , Palma-Silva et al., 2011) than the pollinator-dependent *P. albiflos* ( $F_{IS} = 0.11$ , Palma-Silva et al., 2011). Moreover, these same authors reported lower inbreeding coefficients in allopatric populations of *P. staminea* (from Andorinha and Itacoatiara rock outcrops, Niteroi city, Rio de Janeiro) than in sympatric populations of *P. staminea*. The Pão de Açúcar sympatric population is approximately 13 and 11 km, respectively, from the allopatric populations of *P. staminea* in Itacoatiara (ITA) and Andorinha (ANDO). The allopatric population of the pollinator-dependent *P. albiflos*, previously examined by Palma-Silva et al. (2011), had a lower and nonsignificant level of inbreeding ( $F_{IS} < 0.010$ ), thus indicating high levels of outcrossing in this population. For this reason, we did not include the allopatric population of *P. albiflos* in the present study. Moreover, *P. albiflos* was previously reported to present herkogamy (Wendt et al., 2002), corroborating the pollinator dependence reported by Wendt et al. (2001) for this species.

We randomly collected up to 10 maternal families from each population (Table 1). Pure and hybrid mother plants (MP) were previously classified using Bayesian admixture coefficients from the STRUCTURE v2.3.4. program, following Palma-Silva et al. (2011). Ten seeds per mother plant were collected and germinated in Petri dishes with culture medium according to Paggi et al. (2007). After this period, seedlings were stored in 2% CTAB buffer at  $-20^{\circ}\text{C}$  until DNA extraction. In total, 48 MP and 464 progenies were assayed. Total genomic DNA was extracted using either Invisorb Spin Plant Mini Kit (Strattec Molecular, Berlin, Germany) or a modified approach based on the method of Palma-Silva et al. (2007). DNA was quantified using electrophoresis in a 1.5% agarose gel

TABLE 1. Population names, localities, geographical coordinates, and sample sizes of *Pitcairnia albiflos*, *P. staminea*, and their hybrids in the gneissic-granitic inselbergs of the Atlantic Rainforest in southeastern Brazil. Pure and hybrid individuals were classified using Bayesian admixture coefficients from STRUCTURE based on data of Palma-Silva et al. (2011).

Species (abbreviation)	Population name	GPS position	Altitude (m a.s.l.)	Sample size	
				Families	Progenies
<i>P. albiflos</i> (alb-PAO)	Pão de açúcar, Rio de Janeiro city	22 57.178S, 43 09.532W	30	10	99
Hybrids (hyb-PAO)				8	71
<i>P. staminea</i> (sta-PAO)				10	99
<i>P. staminea</i> (sta-ANDO)	Andorinha, Niteroi city	22 58.441S, 43 01.586W	112	10	97
<i>P. staminea</i> (sta-ITA)	Itacoatiara, Niteroi city	22 58.392S, 43 02.477W	120	10	97

and stained with Gelred (Biotium, Hayward, CA, USA) or by Eppendorf BioPhotometer.

**Microsatellite marker assay**—All progeny arrays were genotyped using six of the most informative nuclear microsatellite markers: PaA05, PaA10, PaC05, PaD07, PaZ01 (Paggi et al., 2008), and VgA04 (Palma-Silva et al., 2007), which had previously been used for genotyping adult plants from allopatric and sympatric populations of *P. albiflos* and *P. staminea* (Palma-Silva et al., 2011). All polymerase chain reactions (PCR) were performed in an Applied Biosystems Veriti thermocycler (Applied Biosystems) following previously described methods (Palma-Silva et al., 2007). The nuclear microsatellite alleles were resolved on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems). Molecular sizes in base pairs were precisely sized against the LIZ (500) molecular size standard (Applied Biosystems) using GENEMAPPER version 4.1 software (Applied Biosystems). Additionally, all microsatellite alleles were visually verified and scored.

**Determination of outcrossing and selfing rates**—Outcrossing and selfing rates were estimated per population and per individual family using likelihood methods in the MLTR v.3.4 software (Ritland, 2002). The presence of null alleles was checked with the Microchecker software (van Oosterhout et al., 2004). For three loci (PaA10, PaA05, PaZ01), we could not rule out the presence of null alleles. Thus, we ran the MLTR software with the option Null Alleles selected, which allowed us to estimate all mating system parameters considering the possibility of null alleles. At the population level, we used the Newton–Raphson method (Ritland, 2002). For family-level estimates, we applied the method of moment procedure, designed for small sample sizes (Ritland, 2002). The maximum likelihood model estimates pollen and ovule frequencies, the genotype of each MP, and the proportion of progeny produced through either cross-fertilization ( $t$ ) or self-fertilization ( $s = 1 - t$ , Ritland, 1990) within families and within populations. These procedures calculate: (1) multilocus outcrossing rate ( $t_m$ ), (2) average maternal plant inbreeding coefficient based on progeny genotypes ( $F_p$ ), (3) multilocus correlated paternity [ $r_p(m)$ ], which is the proportion of full sibs among outcrossed progeny (Ritland, 2002), and (4) correlation of selfing among families ( $r_s$ ). A lack of correlation of selfing ( $r_s = 0$ ) is present when selfing rates do not vary among families. Standard errors of the mating system parameters were estimated based on 1000 bootstrap replicates among families within populations. Inbreeding depression for each population ( $\delta$ ) was calculated using the method of Goodwillie et al. (2005), as:

$$\delta = 1 - 2[(1 - s)F/s(1 - F)],$$

where  $s$  is the selfing rate (estimated as above) and  $F$  is the inbreeding coefficient of the mother plants. Analysis of variance (ANOVA) tests were followed by post hoc Tukey tests to verify whether the outcrossing rates differed among populations and species, using individual families as repetitions. This statistical analysis was performed using SPSS 11.0 (IBM, Armonk, New York, USA).

**Measurement of anther–stigma distances**—For estimating herkogamy of sympatric *P. albiflos*, *P. staminea*, and their hybrids, and allopatric populations of *P. staminea*, we measured the minimum distance between anther and stigma of one flower per plant for up to 31 plants per population (Fig. 1B). A total of 94 flowers were measured. Measurements were taken on the first day of flower opening between 8:00 and 12:00. The variation in herkogamy among populations and species was analyzed using a Kruskal–Wallis multiple-comparisons test. The statistical analyses were performed using the SPSS 11.0 software.

**Genetic assignments of progenies and early-acting barriers**—Progeny array analyses allow us to detect differences between the alleles of the maternal plant and its offspring. These differences will reveal both the selfing rates and, when outcrossing, the kind of pollen that effectively fertilized the ovules (Rieseberg et al., 1998). To explore the possibility of early-acting barriers (i.e., assortative mating, postmating prezygotic barriers or early-acting postzygotic barriers) and to search for evidence of ongoing hybridization in the progeny arrays, we used adult individuals of *P. albiflos* ( $n = 47$ ) and *P. staminea* ( $n = 40$ ) as references for assigning the ancestries of progenies to one or the other species. Mother plants and adult individuals were previously genotyped at 15 microsatellite loci by Palma-Silva et al. (2011). The relative contribution by each parental species to the progeny was estimated using the model-based clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000). The analyses were carried out under the admixture model, assuming independent allele frequencies, and a burn-in period of 250 000 steps followed by run lengths

of 1 000 000. We used  $K = 2$  for this set of analyses based on the presence of two species' gene pools in the data (Palma-Silva et al., 2011). We assigned progeny using the conservative threshold  $Q > 0.9$  as *P. albiflos*,  $Q < 0.1$  as *P. staminea* and  $0.1 < Q < 0.9$  as hybrids as done by Palma-Silva et al. (2011).

## RESULTS

**Mating system**—The results indicated mixed mating with low to high frequencies of outcrossing rates (ranging from 0.287 to 0.808) with significant variation within and between species ( $F_{4,43} = 6.249$ ,  $P < 0.0001$ ; Fig. 1A). At the intraspecific level, allopatric populations of *P. staminea* displayed comparatively higher outcrossing rate estimates ( $t_m = 0.670$  and  $0.589$ ) than the sympatric population ( $t_m = 0.287$ ) (Tables 2 and 3; Fig. 1A). The highest outcrossing rate estimates were observed for *P. albiflos*

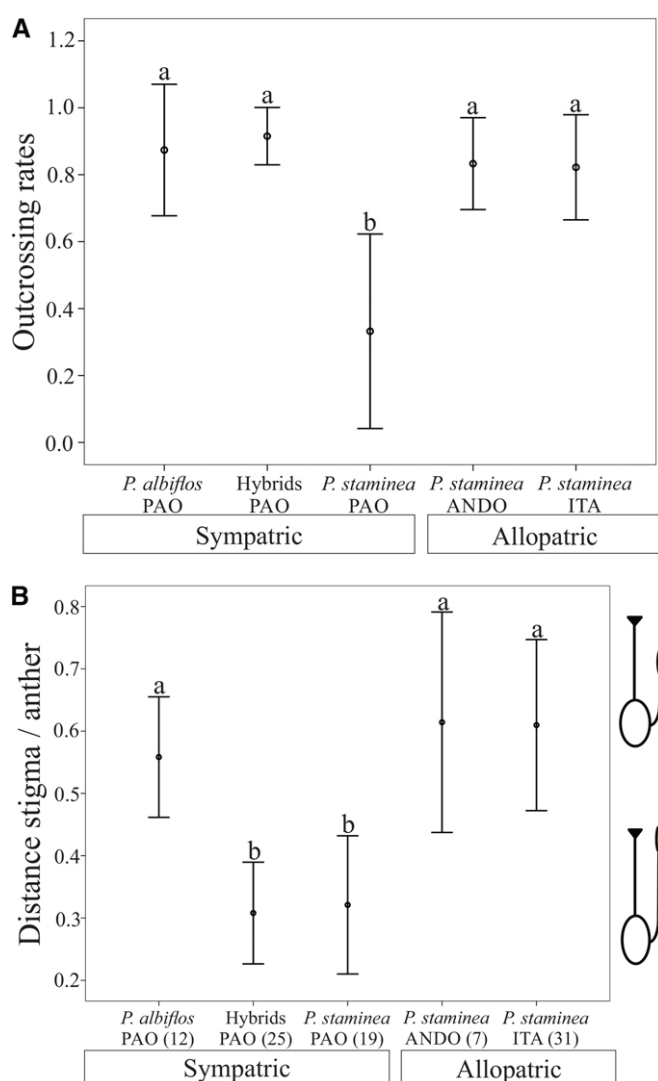


Fig. 1. Comparison between the means ( $\pm$ SE) of (A) individual progeny outcrossing rates, (ANOVA  $F_{4,43} = 6.249$   $P < 0.0001$ ) and (B) herkogamy (Kruskal–Wallis  $\chi^2 = 20.87$ ,  $df = 4$ ,  $p < 0.001$ ) in sympatric (*Pitcairnia albiflos*, *P. staminea*, and hybrids) and allopatric populations of *P. staminea*. Sample sizes per population are in parentheses. The drawings illustrate distances between stigma and anthers. Bars with the same letter indicate that the means are not significantly different.

TABLE 2. Mating system parameters at the population level for *Pitcairnia albiflos*, *Pitcairnia staminea*, and their hybrids. Bootstrap-based standard deviations are in parentheses.

Species/Population	Type	$F$	$t_m$	$r_s$	$r_p(m)$	$s$	$\delta$
<i>P. albiflos</i> -PAO	sympatric	0.063 (0.095)	<b>0.808 (0.095)</b>	0.474 (0.383)	0.068 (0.043)	<b>0.192</b>	0.434
Hybrids-PAO	sympatric	0.064 (0.670)	<b>0.796 (0.073)</b>	0.506 (0.186)	0.314 (0.114)	<b>0.204</b>	0.466
<i>P. staminea</i> -PAO	sympatric	0.521 (0.119)	<b>0.287 (0.067)</b>	0.870 (0.128)	0.170 (0.180)	<b>0.713</b>	0.124
<i>P. staminea</i> -ANDO	allopatric	0.037 (0.114)	<b>0.670 (0.083)</b>	0.557 (0.237)	0.183 (0.152)	<b>0.330</b>	0.844
<i>P. staminea</i> -ITA	allopatric	0.022 (0.085)	<b>0.589 (0.110)</b>	0.830 (0.107)	0.107 (0.103)	<b>0.411</b>	0.935

Notes:  $F$ , maternal plant inbreeding coefficients;  $t_m$ , multilocus outcrossing rate;  $r_s$ , multilocus correlation of selfing;  $r_p(m)$ , multilocus correlation of paternity;  $s$  ( $1 - t_m$ ), selfing rate;  $\delta$ , inbreeding depression.

and hybrids ( $t_m = 0.808$  and  $0.796$ , respectively, Table 2). Accordingly, maternal inbreeding coefficients were high for the sympatric *P. staminea* ( $F_p = 0.521$ ), and low for all other populations (ranging from 0.022 to 0.064) (Table 2). The correlation of selfing  $r_s$  ranged from 0.474 (*P. albiflos*, Table 2) to 0.870 (*P. staminea* sympatric population, Table 2), indicating that selfing rates varied among families in all populations (Table 2; Appendix S1, see Supplemental Data with online version of this article).

A low correlation of paternity [ $r_p(m) = 0.068$ ] was only seen in *P. albiflos*, indicating that most individuals within the progeny array were half sibs rather than full sibs, in accordance with the high outcrossing rates estimated for this species. The highest correlation of paternity was observed for the hybrids [ $r_p(m) = 0.314$ ], suggesting that despite the high outcrossing rate, multiple mating events (pollination visits) are likely to be rare in hybrid mother plants, possibly due to them being less attractive to pollinators. Moderate levels of correlation of paternity were detected for *P. staminea* populations, ranging from 0.107 to 0.183 (Table 2). Moderate inbreeding depression was found for the highly outcrossed populations of *P. albiflos* and hybrids ( $\delta = 0.434$  and  $0.466$ , respectively). Inbreeding depression values were higher for the two *P. staminea* allopatric populations (St-ANDO  $\delta = 0.844$ , and Sta-ITA  $\delta = 0.935$ ) and much lower in the sympatric population of *P. staminea* ( $\delta = 0.124$ ), suggesting that inbreeding depression is not severely affecting progeny fitness in the sympatric population of *P. staminea*.

**Herkogamy and outcrossing rates**—Herkogamy differed significantly among populations (Kruskal–Wallis  $\chi^2 = 20.87$ ,  $df = 4$ ,  $p < 0.001$ ). Lower average stigma–anther distances were detected in flowers from the sympatric population and hybrids of *P. staminea* ( $0.321 \pm SE 0.055$  and  $0.308 \pm SE 0.041$  mm, respectively) than in the allopatric *P. staminea* (ANDO:  $0.614 \pm SE 0.088$  and ITA:  $0.610 \pm SE 0.069$  mm) and sympatric populations of *P. albiflos* ( $0.558 \pm SE 0.048$  mm). As expected, at the population level, the variation in stigma–anther distances corresponded to the variation in outcrossing rates in both

TABLE 3. Outcrossing rates ( $t_m$ ) at the individual family level for *Pitcairnia albiflos*, *Pitcairnia staminea*, and their hybrids.

Species/Population	Type	N (families)	Outcrossing		
			$t_m > 0.8$	$0.8 < t_m > 0.2$	Selfing $t_m < 0.2$
<i>P. albiflos</i> -PAO	sympatric	10	8	2	0
Hybrids-PAO	sympatric	8	6	2	0
<i>P. staminea</i> -PAO	sympatric	10	2	5	3
<i>P. staminea</i> -ANDO	allopatric	10	6	4	0
<i>P. staminea</i> -ITA	allopatric	10	7	3	0

*P. albiflos* and *P. staminea* (Fig. 1A, 1B). The *P. staminea* allopatric populations and *P. albiflos* sympatric population displayed high outcrossing rates and high herkogamy, while the *P. staminea* sympatric population had the lowest outcrossing rate and low herkogamy (Fig. 1A, B). This association was not detected in hybrids, in which outcrossing rates were high and herkogamy was low.

#### Genetic assignments of progenies and early-acting barriers—

Figure 2 clearly shows that genetic assignment of progenies to either species is affected by early acting barriers, such as assortative mating, postmating prezygotic barriers or early-acting postzygotic barriers between *P. albiflos* and *P. staminea*, demonstrating that mother plants tend to be fertilized by pollen from the same species (Fig. 2). Most progenies from the *P. albiflos* MP had  $Q$  values  $> 0.9$ , and only 7 of 99 progenies (~7%) from *P. albiflos* mother plants were identified as hybrids: alb-pao238 (1 hybrid seedling), alb-pao227(4 hybrid seedlings), and alb-pao225 (2 hybrid seedlings). Most of progenies from the *P. staminea* MP had  $Q$  values  $< 0.1$ , with only 2 of 97 progenies (~2%) recognized as hybrids, one hybrid seedling in sta-pao242 and another in sta-pao249. Finally, most progenies from the hybrid mother plants had intermediate  $Q$  values ( $0.1 < Q < 0.9$ ). Of a total of 71 progenies, 12 were identified as pure *P. albiflos* ( $Q > 0.9$ ) and only five as pure *P. staminea* ( $Q < 0.1$ ).

## DISCUSSION

In this study, we investigated divergence in the mating systems and stigma–anther clustering in one sympatric population of *P. staminea* and *P. albiflos* and two allopatric populations of *P. staminea*. Our progeny array results revealed two important patterns regarding outcrossing rates: (1) variation among populations of the same species: there was a higher selfing rate in the *P. staminea* sympatric population than relative to two allopatric populations, despite the small geographic distance (11–13 km) separating the sympatric and allopatric populations, and (2) variation between closely related species: in sympatry, *P. staminea* showed higher selfing rates than *P. albiflos*. These results agree with differences in the reproductive biology of these species (Wendt et al., 2001).

The higher hybrid frequencies in the progeny arrays observed in *P. albiflos* agrees with the asymmetric introgression from *P. staminea* into *P. albiflos* observed in our previous work (Palma-Silva et al., 2011). Furthermore, there was an association between the direction of reproductive isolation (asymmetric introgression toward *P. albiflos*) and the increased selfing rates in the *P. staminea* sympatric population. In agreement with our results, other studies have confirmed asymmetric introgression from selfers to outcrossers (*Pitcairnia*: Wendt et al., 2002;

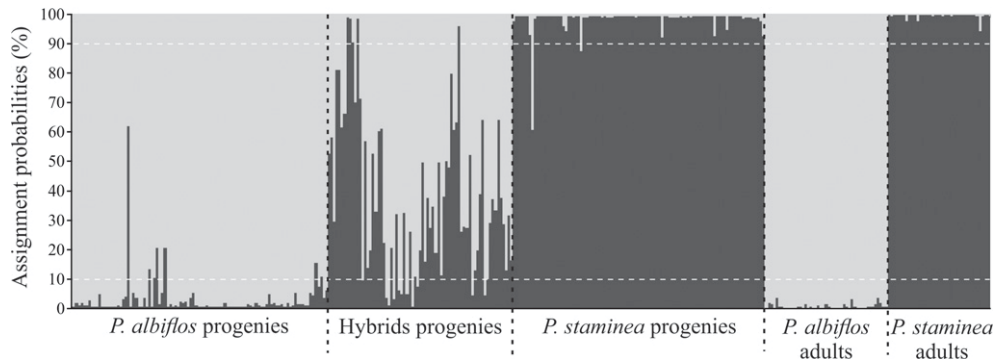


Fig. 2. Bayesian admixture proportions ( $Q$ ) of *P. albiflos* and *P. staminea* individuals estimated by STRUCTURE, assuming  $K = 2$  in the sympatric population PAO. Adult individuals of the sympatric population PAO based on data of Palma-Silva et al. (2011) were used as references for STRUCTURE analysis. Individuals were assigned using the threshold  $Q > 0.9$  as *P. albiflos*,  $Q < 0.1$  as *P. staminea*, and  $0.1 < Q < 0.9$  as hybrids (dashed white lines).

*Mimulus*: Sweigart and Willis, 2003; Martin and Willis, 2007; Pettengill and Moeller, 2012; *Centaurium*: Brys et al., 2014), or increased self-pollination in sympatric populations of closely related congeners (*Phlox*: Levin, 1985; *Solanum*: Whalen, 1978; *Helianthus*: Rieseberg et al., 1998; *Arenaria*: Fishman and Wyatt, 1999). Likewise, several studies suggest that differences in mating system, caused by divergent selfing rates, may be an important reproductive barrier in plants (i.e., Jain, 1976; Lowry et al., 2008, and studies therein), including bromeliads (Wendt et al., 2002; Matallana et al., 2010).

We observed that higher outcrossing rates in each species were associated with higher degrees of herkogamy in these populations. For example, the *P. albiflos* sympatric population and the *P. staminea* allopatric populations, and low outcrossing rates in the *P. staminea* sympatric population were concurrent with lower stigma and anthers clustering. An exception was observed in hybrids, likely due to their lower pollen viability (<65% of viability—Wendt et al., 2001), which may preclude self-pollination, even in the presence of the observed lower herkogamy. Similarly, in *Helianthus annuus* L. and *H. petiolaris* Nutt. hybrid zones, Rieseberg et al. (1998) observed that in semisterile hybrids, backcrossing with the fertile parental species was more likely than selfing.

It is broadly accepted that a reduced distance separating the anthers and stigma increases the chance for autogamous pollination in self-compatible species (Webb and Lloyd, 1986; Barrett, 2003; Levin, 2010; de Vos et al., 2012, among others). A negative correlation between herkogamy and selfing rates has been reported in a range of plant species: *Ipomea hederacea* Jacq. (Ennos, 1981); *Mimulus guttatus* DC. (Ritland and Ritland, 1989; van Kleunen and Ritland, 2004) *Turnera ulmifolia* L. (Belaoussoff and Shore, 1995); *Mimulus ringens* L. (Karron et al., 1997); *Aquilegia caerulea* E. James (Brunet and Eckert, 1998); *Datura wrightii* Regel (Elle and Hare, 2002). Nevertheless, other studies have found this not to be the case (Baker et al., 2000; Medrano et al., 2005).

Besides herkogamy, mating systems in plant populations can be affected by multiple factors, such as population density and size, the spatial distribution of individuals, phenology patterns, pollinator behavior, and modes of pollination (see review by Barrett, 2010; Wright et al., 2013, among others). Several factors may thus act simultaneously to shape levels of selfing in populations of *P. staminea*. For instance, *P. albiflos*, the species with the highest outcrossing rates, is nocturnal and pollinated by bats and hawkmoths, while in the same locality *P. staminea* are diurnally visited by hummingbirds and butterflies.

The differences in pollinator behavior may also influence differences in outcrossing rates between these two bromeliad species. Unfortunately, no information is currently available on the precise identity of pollinator species in allopatric populations of *P. staminea*. However, because of the spatial proximity of populations and the similarity in environment conditions, we suspect both allopatric and sympatric populations of *P. staminea* to have similar pollinator spectra. However, we cannot exclude the possibility that the observed difference in outcrossing rates would be influenced by different pollinator community members or different pollinator abundances in these different sites.

Herkogamy and selfing rates might determine, in combination with other factors, the hybridization and introgression dynamics between *P. albiflos* and *P. staminea*. The interaction between co-occurring related species has been found to be a stimulus for the evolution of herkogamy variation and reduction in selfing in other plant species (Ennos, 1981; Smith and Rauscher, 2007; Grossenbacher and Whittall, 2011). The proximity of stigma and anthers indicates that lower herkogamy in *P. staminea* may enhance prezygotic isolation from *P. albiflos*, possibly through mechanical protection against costly heterospecific pollen flow by increased selfing. Our results showed that differences in herkogamy and the variation in mating systems between these closely related species are more pronounced in sympatry than in allopatry, a pattern compatible with that expected under the reinforcement hypothesis (Abbott et al., 2013).

Although our results are consistent with the hypothesis that lower herkogamy and higher selfing rates might increase prezygotic isolation, they do not imply that anther–stigma clustering and mating system variation are the only barriers between these species. In fact, the simultaneous action of multiple prezygotic barriers was previously inferred to be important in maintaining species integrity in this system (i.e., pollination syndromes and flowering phenology: Wendt et al., 2001), in conjunction with postzygotic barriers (Bateson–Dobzhansky–Muller incompatibilities: Palma-Silva et al., 2011). Nevertheless, our results suggest herkogamy as one potentially important floral trait for the enhancement of selfing in the presence of heterospecific pollen flow. Thus, our results offer an incentive for further investigation of the consequences of variation in floral traits on the evolution of reproductive isolation in this and other systems.

Possible alternative explanations for the variation in mating system in this inhospitable and heterogeneous inselberg landscape include neutral processes such as drift, transmission advantage of selfing genes, reduced inbreeding depression,

reproductive assurance, or combinations of those factors (e.g., Darwin, 1876; Stebbins, 1957; Jain, 1976). Reproductive assurance is the most widely accepted explanation for the evolution of selfing (Herlihy and Eckert, 2002). It allows autonomous selfing plants to ensure seed set when pollinators and/or potential mates are scarce (Baker, 1955). Additionally, reproductive assurance may be of adaptive value where populations have restricted gene exchange (Grossenbacher and Whittall, 2011), a pattern commonly observed for inselberg bromeliads (Sarhou et al., 2001; Barbará et al., 2007, 2009; Boisselier-Dubayle et al., 2010; Palma-Silva et al., 2011).

The progeny array results presented here suggests a potential link between the evolution of the mating system and the evolution of prezygotic reproductive isolation. Further analyses of heterospecific pollen deposition and the examination of relationships between herkogamy, selfing, and the production of hybrid seeds will facilitate tests of the hypothesis raised here that hybridization has favored increased selfing in sympatry (Fishman and Wyatt, 1999; Martin and Willis, 2007; Smith and Rausher, 2007). In the future, it will be important to determine the frequency of independent transitions between mating systems across the Bromeliaceae family by using phylogenies of groups for which data on reproductive isolation are available, to infer the role of mating system variation in divergence with gene flow in this species-rich, neotropical radiation.

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