Flora 211 (2015) 1–10

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Limited pollen flow and high selfing rates toward geographic range limit in an Atlantic forest bromeliad

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ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form 5 December 2014
Accepted 4 January 2015
Edited by Bohumil Mandák.
Available online 7 January 2015

Keywords:
Bromeliaceae
Cryptic self-incompatibility
Microsatellites
Mixed mating system
Outcrossing rates

ABSTRACT

Bromeliaceae is a Neotropical family that evolved ecological key innovations in association with extensive adaptive radiation. Its species present a variety of different mating system strategies varying within and among species, within genera and subfamilies. Also, species with a wide geographical range can display large variation in mating system, reproductive success and genetic diversity. Here we combined data from hand pollinations and genetic analysis to assess outcomes of contemporary gene flow and mating system variation at the range edge of *Vriesea gigantea*. Results from pollen germination rates showed that this species is cryptically self-incompatible. Hand-pollination experiments and genetic analysis of progeny arrays revealed that *V. gigantea* has a mixed mating system, with high selfing rates (s = 0.612), and high inbreeding coefficient (F = 0.372). Inbreeding in *V. gigantea* at southern edge of its distribution range was caused by high levels of selfing rather than by mating among relatives. Moreover, strong pollen pool genetic structure was observed (Φ'_{FT} = 0.671), with an increase from north to south. The parameters observed help us to understand historical and ecological conditions under which *V. gigantea* has experienced moderate to high levels of selfing in the face of reduced pollen flow from central to peripheral populations due to recent southward range expansion.

Introduction

Neotropics comprise the most species-rich region on Earth (Antonelli and Sanmartín, 2011). Nevertheless, relatively fewer genetic studies have examined Neotropical plant species, for example those species from the Atlantic Forest (AF), compared to temperate species (Hewitt, 2004; Jaramillo et al., 2006; Turchetto-Zolet et al., 2013). Bromeliaceae is an example of a Neotropical group that has evolved “key innovations” in association with its extensive adaptive radiation, like tank habit and crassulacean acid metabolism (CAM) (Givnish, 2010; Silvestro et al., 2014; Givnish et al., 2014). Moreover, it comprises subfamilies with different and relatively recent evolutionary histories. In particular, tank bromeliads from the AF have the highest diversification rate among bromeliad species, which can be associated with ecological trends such as evolution of differences in growth form, floral morphology, and mating systems (Benzing, 2000; Wendt et al., 2008; Givnish, 2010; Matallana et al., 2010; Givnish et al., 2011; Silvestro et al., 2014; Givnish et al., 2014). Studies on variation of reproductive traits within species can provide important insights in evolution and radiation of recent groups from Neotropics, especially in widespread species adapted to different ecological circumstances (Barrett, 2008 and references therein; Levin, 2012), and should be an important aspect for populations occurring at the species' distribution margins, where adaptations to specific conditions are decisive for their persistence (Darling et al., 2008; Geber, 2008; Sexton et al., 2009; Moeller et al., 2012).

Factors which determine species' range limits include abiotic and biotic factors, which are fundamentally related to the ecological niche of species (Sexton et al., 2009). A recent evolutionary

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http://dx.doi.org/10.1016/j.flora.2015.01.001
0367-2530/

model includes the notion that a fundamental parameter of mating system evolution, the inbreeding depression, may shape the association of traits along geographic distributions and create stable limits of edge dispersion if inbreeding depression decreases from center to periphery. This parameter is thus worth considering when analyzing empirical data (Pujol and Pannell, 2008; Cheptou and Massol, 2009; Pujol et al., 2009; Sun and Cheptou, 2012). Moreover, according to Baker's law, plants colonizing populations located at the margins of a distribution area tend to be autogamous as a consequence of pollinator or mate scarcity (Baker, 1955, 1959; Sun and Cheptou, 2012). Indeed, Kalisz et al. (2004), based on inbreeding depression and marker-based estimates of selfing for wild populations of *Collinsia verna* (Scrophulariaceae), demonstrated that when pollinator visits decrease, populations shift toward intermediate selfing rates through an increase in the proportion of autonomously selfed seeds. As widely known, selfing may allow reproduction when circumstances reduce opportunities for a union of gametes produced by different individuals, a phenomenon known as reproductive assurance (Holsinger, 2000).

Previous studies on reproductive biology of bromeliads showed mating strategies vary among species within genera and subfamilies (Wendt et al., 2001, 2002; Cascante-Marín et al., 2005; Barbará et al., 2007, 2008, 2009; Wendt et al., 2008; Matallana et al., 2010). Notwithstanding, amount of intraspecific variation in mating strategy and selfing capacity has only been assessed for a few species (Cascante-Marín et al., 2006; Paggi et al., 2007; Hmeljevski et al., 2011; Schmid et al., 2011; Zanella et al., 2011; Hmeljevski, 2013; Paggi et al., 2013). Yet such variation may have important consequences during colonization, as the establishment of selfing genotypes that may be favored after long-distance dispersal (the idea in Baker's law).

A species' mating system can be determined through hand-pollination experiments, progeny arrays and pollen pool genetic structure estimates. Also, biological characteristics and genetic patterns can help distinguish between potential mating (biological characteristics) and real mating (genetic patterns), assessing possible outcomes of gene flow at margins of a species' range (Holt and Keitt, 2005; Cheptou, 2012). In this study, the mating system and contemporary gene flow were reported for *Vriesea gigantea* Gaudich., a member of Tillandsioideae subfamily which exhibits high diversity in pollination modes and mating systems (Sazima et al., 1999; Benzing, 2000; Kessler and Krömer, 2000; Krömer et al., 2008; Wendt et al., 2008; Matallana et al., 2010). Mating systems of some Tillandsioideae species have been previously examined using non-molecular approaches, which have shown a particularly high frequency of selfing and mixed systems in *Alcantarea*, *Guzmania*, *Racinea*, *Tillandsia*, *Vriesea*, and *Werauhia* (Lasso and Ackerman, 2004; Ramírez-Morillo et al., 2004; Cascante-Marín et al., 2005; Paggi et al., 2007; Wendt et al., 2008; Ramírez-Morillo et al., 2009; Mattalana et al., 2010; Schmid et al., 2011; Paggi et al., 2013).

Furthermore, some previous reports on mating systems of bromeliads have been based on inferences from population genetic data (Wright's *F*-statistics) for a single plant generation. They have indicated mixed system for different species, *Alcantarea imperialis* (Carrière) Harms, *A. geniculata* (Wawra) J.R. Grant (Barbará et al., 2007, 2009), *A. glaziouana* (Lemaire) Leme and *A. regina* (Vell.) Harms (Barbará et al., 2008, 2009), *V. gigantea* (Palma-Silva et al., 2009), *V. minarum* (Baker) L.B.Sm. (Lavor et al., 2014) and outcrossing system for *Bromelia antiacantha* Bertol. (Zanella et al., 2011 see also Zanella et al., 2012 for a review), *Encholirium horridum* L.B.Sm. (Hmeljevski, 2013), and *Pitcairnia encholirioides* L.B.Sm., which is a self-incompatible species (Hmeljevski et al., 2014). Determination of mating system through a more comprehensive approach, using combinations of direct experiments and genetic analysis (e.g., progeny arrays), has been reported only for three bromeliad species, *Guzmania monostachia* (L.) Rusby ex Mez. and *Tillandsia fasciculata* Sw., both species showed high selfing rates (Cascante-Marín et al., 2006), and *Dyckia ibiramensis* Reitz, which presented mixed mating system (Hmeljevski et al., 2011).

In plants, mating systems have a profound effect on the genetic composition of natural populations (Hamrick, 1982). Likewise, variation in gene flow via pollen transfer and seed dispersal also contributes to the shape of genetic diversity within populations (Charlesworth, 2003; Duminil et al., 2007). Disruption of gene flow via pollen transfer and seed dispersal also contributes to loss of genetic diversity within populations isolated by habitat fragmentation (Hamrick, 1982), which may contribute to increase inbreeding coefficient. In a previous study, populations throughout entire *V. gigantea* (Bromeliaceae) distribution range showed latitudinal trends of decreasing genetic diversity and gene flow from north to south, which is consistent with historical forest expansion from northern half of the present distribution range (Palma-Silva et al., 2009). Further expansion toward the south appears to be inhibited by a lack of gene flow at edges of the current range (Palma-Silva et al., 2009; Paggi et al., 2010). Accordantly, Palma-Silva et al. (2009) found variation in the inbreeding coefficient (F_{IS}) among populations, with higher values occurring in *V. gigantea* populations from southern edge of range. In addition, Sampaio et al. (2012) also found geographic variation in inbreeding depression estimates, with a moderate value ($\delta = 0.31$), for the same central and southern populations of *V. gigantea*; those populations are the focus of the present study (Table 1). Herein, we investigate the mating system and contemporary gene flow of *Vriesea gigantea* at southern edge of the species range in Brazil, by combining data from hand-pollination experiments and genetic analysis of nuclear molecular markers. Specifically, we aimed to: a) characterize the mating system of *V. gigantea* at its southern range edge by estimating different parameters using progeny array and pollen pool genetic structure approaches, and by hand pollination experiments; and b) identify the role of two components, population subdivision or high selfing rate, that may be responsible for the high inbreeding coefficient (F_{IS}) observed previously. Results will help us to understand the relative roles of pollen flow and selfing in shaping southern range limit in *V. gigantea*.

Materials and methods

Study species

Vriesea gigantea (Tillandsioideae) presents an inflorescence with a central axis and several branches on each side, and flowers on each side of the lateral axis (Reitz, 1983; Fig. 1A). The flowers have three petals and their yellowish color and tube-shaped corolla are in accordance with the chiropterophilous syndrome (Vogel, 1969; Krömer et al., 2008). Besides, *V. gigantea* flowers lasted just one night (c.a. 12 h), and petals, stamens, and the style abscised during the following days. Also, the flowers present protogyny, in which style is receptive before anther dehiscence, in the beginning of anthesis (Paggi, 2009). The studied species is a tank bromeliad, frequently epiphytic or rupicolous, occurring in AF, across a wide geographic distribution, from Espírito Santo to Rio Grande do Sul states, Brazil (20°07′S, 40°30′W to 31°56′S, 52°25′W) (Fig. 1C). *Vriesea gigantea* is the only *Vriesea* species occurring in the southernmost part of AF (Smith and Downs, 1977; Reitz, 1983; Zimmermann et al., 2007; Martinelli et al., 2008; Palma-Silva et al., 2009; Fig. 1C).

Study sites

This study was conducted in three populations of *V. gigantea* (Maquiné, Itapuã and Taim; Table 1), located in AF of Rio Grande do