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An unexpected case of wind-pollination: ambophily in an ornithophilous tropical mountaintop Orobanchaceae

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Short Report

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Abstract

Some plant species have a kind of mixed pollination system – ambophily – which rely both on biotic and abiotic (most commonly wind) as pollen vectors. Ambophily remains poorly addressed in the pollination literature and may have been overlooked because existing studies do not quantify the wind contribution in animal-pollinated species. Moreover, it is expected in species pollinated by different insect groups and generalist floral phenotype. *Esterhazya eitenorum* is a (sub-)shrub endemic to a high-altitude grassland (*campos de altitude*) area in southeastern Brazil. Pollinated mainly by hummingbirds and secondarily by large bees, it presents features generally associated with ornithophily. Bird-pollinated species are not expected to be ambophilous, due to their high resource investment in floral construction and maintenance. However, here we detect ambophily in *E. eitenorum* by testing the potential for pollen export by wind as well as seed set after floral visitor exclusion and spontaneous self-pollination treatments, and comparing these with natural conditions. *Esterhazya eitenorum* has an ambophilous pollination system, with effect size analyses between floral visitor exclusion treatments and natural conditions revealing significant contributions from both abiotic and biotic components. Although its floral phenotype corresponds to the main pollinator, the occurrence of ambophily in *E. eitenorum* underscores the need for an independent approach to the association of floral phenotypes with pollination vectors, in particular for the detection and better understanding of the evolution of mixed pollination systems.

Introduction

Bird pollination is widespread in angiosperms, and hummingbirds the main vertebrate pollinators in the Neotropics (Martín González et al. 2015). Hummingbird-pollinated flowers are quite peculiar, usually reddish in color, tubular in shape, with no noticeable odor and high nectar production (Cronk and Ojeda 2008). These characteristics define ornithophily (Cronk and Ojeda 2008), which is one of the most predictable pollination syndromes (Danieli-Silva et al. 2011). Such high predictability may cause researchers to take for granted that only birds pollinate ornithophilous flowers, hindering research on the contribution of other vectors (e.g., bees; Mayfield et al. 2001).

The classical concept of pollination syndromes implies specialization between the plant and a single functional group of pollinators (Rosas-Guerrero et al. 2014). However, pollination is embedded in a specialization-generalization gradient, and most angiosperm species are pollinated by more than just one visitor group (Ollerton et al. 2009). Furthermore, some species, named ambophilous, use both biotic and abiotic pollination vectors (Culley et al. 2002). This mixed pollination system involving animals and the wind is often considered a transitional system from zoophily (animal pollination) to anemophily (wind pollination) or vice versa (Culley et al. 2002; Costa and Machado 2012).

Ambophily depends on both plant morphology and environmental factors that allow both animal and wind pollen transfer (Costa et al. 2018). Because it has an abiotic component, usually the wind, ambophily is expected to occur in open areas, encompassing flowers with exposed anthers and stigma, which allow pollen to be dispersed and captured by wind (Stelleman 1984; Friedman and Barrett 2009).

Most studies in ambophilous systems report species pollinated by diverse insect groups, with a generalist floral phenotype (Culley et al. 2002; Carvalho 2021). To date, there are no records of ambophily for hummingbird-pollinated species. The only case where ambophily was related to bird-pollination refers to *Syzygium mundagam* (Myrtaceae). Its flowers lack typical ornithophilous features and are pollinated both by wind and Nectariniidae birds (Kuriakose et al. 2018).

Studies based on pollination syndromes may overlook considerations of other existing pollination vectors and their contribution (Ollerton et al. 2009). This is even more pronounced in the case of predicting wind contribution in species considered zoophilous, hence the importance of approaches independent of floral phenotype in pollination studies. In a study in high-altitude grasslands in southeastern Brazil, tests for wind pollination potential were conducted at the community level, including sampling on a species of Orobanchaceae with an ornithophilous floral phenotype and previously shown to be pollinated by hummingbirds and large bees (Bergamo et al. 2021). In this paper, we report an ambophilous pollination system in *Esterhazya eitenorum* and discuss the possible factors leading to ambophily in an ornithophilous species.

Material And Methods

Study system

This study was conducted in a high-altitude grassland (a vegetation type named *campos de altitude*) in Itatiaia National Park, located in the states of Minas Gerais and Rio de Janeiro (~ 2300 m above sea level, 22° 21' S, 44° 40' W). *Campos de altitude* predominantly consists of grasses, herbs, and shrubs growing on rocky outcrops and shallow soil (Safford 1999). *Esterhazya eitenorum* is a shrub/subshrub with known occurrence only for the *campos de altitude* of Itatiaia (Flora and Funga of Brazil 2022). Previous studies in this community have shown that most species present zoophilous generalist pollination systems and a low frequency of animal visitation (Bergamo et al. 2021). The flowers of *E. eitenorum* are tubular (length 38.29 ± 3.72 mm and diameter 9.10 ± 1.21 mm, n = 10 flowers; mean ± SD), pink in color, and last for two days. Its reproductive structures are exserted, with anther height of 38.96 ± 4.03 mm and stigma height of 40.82 ± 3.94 mm (n = 10). They produce nectar with an average sugar concentration of 0.15 mg/µL (n = 10). This species is pollinated primarily by hummingbirds and secondarily by large bees of the genus *Bombus* (Bergamo et al. 2021) and presents features fitting the ornithophilous pollination syndrome (Faegri and van der Pijl 1979; Cronk and Ojeda 2008).

Visitor observation

We conducted one-hour observation sessions on patches of *E. eitenorum* individuals, totaling 29 hours divided into two periods. Bergamo et al. (2021) conducted the first observation period, corresponding to four hours between November 2016 and April 2017 and five hours between October 2017 and March 2018. The remaining twenty hours of observation were performed in January 2021. Visitors were classified according to their behavior in the flower, as: i. pollinator - visitor contacted the stigma and carried pollen grains adhered in an appropriate place on the body; and ii. thief - visitor collected pollen

and/or nectar without touching the reproductive parts of the flower. Visitation frequency was computed per pollinator group dividing number of flower visits by total hours observed.

Spontaneous self-pollination

In order to test for spontaneous self-pollination, flower buds on ten individuals were bagged with polyester fabric (weft < 0.25 mm) preventing pollen carried by both wind and animals (adapted from Dafni 2005). We performed this test to rule out the possibility of self-fertility in the wind-pollination treatments. Flowers at late anthesis were also bagged to estimate seed set (number of seeds / number of treated flowers) under natural conditions (i.e., wind + animal pollination). Fruits and seeds were counted ca. 45 days after the treatments. For comparison between seed set under self-pollination and under wind-pollination, we computed effect sizes by standardized mean differences (Cohen's d, Hedges & Olkin 1985).

Ambophily test

We conducted tests to verify if *E. eitenorum* pollen can be transported by wind. For this, flowers were stimulated with a hand blower (BBQ FAN 1.5V) generating wind flow for 30 seconds. For pollen detection, slides containing solid glycerin were positioned 15 cm away from the flower in the opposite direction to the generated wind flow. The slides were stained with acetocarmine and analyzed under light microscopy to detect the presence of conspecific pollen. In addition, exclusion tests were conducted to verify the contribution of wind and animals to fruit and seed set. Such tests were conducted in two periods, in January 2021 (n = 8 individuals) and the second in February 2022 (n = 5 individuals). Individuals had floral buds bagged with tulle fabric bags (0.9 mm weft) that prevent visitors from accessing the flowers but allow wind-borne pollen to pass through (Dafni et al. 2005, we also tested that blown *E. eitenorum* pollen passed through). The seed set of this treatment were compared with treatments seed set under natural conditions using effect size (see above), to characterize the species as ambophilous.

Results

Esterhazya eitenorum shows contribution of animals (hummingbirds and large bees) and wind in reproductive success (seed set). The frequency of visitation was higher for hummingbirds (0.27 visits/hour) than for large bees (0.17 visits/hour). We observed *Apis mellifera* individuals collecting pollen, as well as beetles and ants collecting nectar without touching all reproductive structures. Since treatments of spontaneous self-pollination did not result in fruit set, the visitor-exclusion treatment (only wind) showed that the species is capable of producing seeds through wind pollination (Table 1, we have not computed effect sizes because there was no seed set in the spontaneous self-pollination treatment). Of the total 13 individuals subjected to the ambophily-test treatments, ten produced fruit in the natural-condition treatments (wind and animals) and five produced fruit in the visitor-exclusion treatments (only wind). In the visitor-exclusion treatments, one out of eight individuals tested in 2021 and four out of five individuals in 2022 produced fruits. The effect size between natural-condition and visitor-exclusion

treatments showed a significant contribution from both components (0.57 [0.15, 0.99 CIs]), confirming ambophily for this species.

Table 1
Seed production in *Esterhazya eitenorum*, after different treatments for testing ambophily, carried out in an area of *high-altitude grasslands* (*campos de* altitude) in Southeastern Brazil.

Treatments	Individuals (with fruit/total)	Fruit set (Fruits/Flowers)	Total seed number
Natural conditions (animal + wind)	10/13	12/58	1276
Visitor exclusion (only wind)	5/13	5/43	613
Spontaneous self-pollination	0/10	0/40	0

Discussion

The floral characteristics of *E. eitenorum*, as well as the observation of hummingbirds as the main pollinators, support the categorization of the species as ornithophilous. However, our study showed that *E. eitenorum* also relies on the wind as a pollen vector and therefore should be considered ambophilous. The occurrence of ambophily in *E. eitenorum* underscores the need for a methodological approach independent to the association of floral phenotypes with pollen vectors, because although its floral phenotype corresponds to the main pollinator, it is also pollinated by large bees, and the wind contributes to the reproductive success of the species.

Species pollinated by hummingbirds are not expected to be ambophilous, due to the high investment of resources in floral construction and maintenance, including large attractive elements and copious nectar production. In addition, hummingbird-pollinated species generally have protective mechanisms for reproductive structures and to avoid nectar robbing (Cronk and Ojeda 2008). *Esterhazia eitenorum* occurs in a pollinator-scarce environment (Bergamo et al. 2021) and despite having a specialized floral phenotype, it is pollinated by two groups of visitors (hummingbirds 75% and large bees 25%) in addition to wind. The low flower visitation in this species may be reflected in its generalist pollination system, since environments where pollination is unpredictable favor the establishment of generalist systems to ensure species reproduction (Waser et al. 1996).

The flowers of *E. eitenorum* differ from other ornithophilous plants in the community in having exserted anthers and stigma and a larger corolla diameter, making resources more accessible to other visitor groups. Exposure of the reproductive structures to wind flow above the corolla is a facilitating feature for wind exportation and reception of pollen. The presence of anther trichomes in *Esterhazya* species has been considered a protective and partitioning structure for pollen release (Hesse et al. 2000). During our field observations, we observed *E. eitenorum* releasing pollen to the wind flow. It seems that the trichomes can dose this release or even provide a form of secondary pollen presentation, leaving it more exposed.

Future studies analyzing in detail the anther structure of this species in relation to pollen transport function are promising for understanding the ecology and evolution of its pollination system.

Wind contribution to pollination of *E. eitenorum* varied between the periods when the exclusion experiments were conducted. In the first year, only one individual out of the eight sampled produced fruits and seeds, and in the second period four out of five individuals. These data reinforce the temporal variation nature of each vector's contribution in ambophilous systems (Lázaro and Traveset 2005; Costa et al. 2018). The variation in wind contribution among individuals can occur either by phenotypic characteristics of the individuals or by local microhabitat characteristics. Spatial position defines the plants' ecological context, as well as the microtopographical conditions and the community to which it is exposed (e.g., competition), generating different interaction outcomes (Chamberlain et al. 2014).

Campos de altitude have structural and climatic characteristics that supposedly facilitate the occurrence of ambophily, either because of the open areas with intense wind flow, or because the plant community is characterized by generalist floral phenotypes and pollinator groups (Bergamo et al. 2021). Two other species of the genus occurring in *campos de altitude*, *E. campestris and E. macrodonta*, also presented low frequency of floral visitation (Sazima et al. 1996; Freitas and Sazima 2001). The results found here differ significantly from *Esterhazya splendida* studied in *cerrado (Brazilian savannah)*, where the visitation frequency was ten times higher and by a greater number of hummingbird species (Ferreira et al. 2016).

Low visitation may be common for species of the genus that occur in *campos de altitude*, as may be the use of wind as a strategy increasing reproductive success. *Esterhazya splendida* also occurs in the area where this study was conducted, but no individuals were found in the sampling area. However, the species can be a model for comparison with other environments and verification of presumed interpopulational variation in the pollination system due to environmental pressures. The occurrence of wind pollination is a derived condition in angiosperms and has evolved several times from animal-pollinated ancestors (Friedman and Barrett 2009). Despite having several independent origins, wind pollination has emerged more frequently in some clades, most likely due to their morphological characters (e.g., Poaceae, Cyperaceae) facilitating wind pollination (Friedman and Barrett 2009). More than the floral morphology, the occurrence sites of *Esterhazya* species, generally related to environments of more open vegetation (Flora and Funga of Brazil 2022) and the low visitation by pollinators may exert environmental pressures that favor the establishment of ambophily in the genus.

The use of the ornithophilous pollination syndrome concept and the occurrence of hummingbirds as the main pollinators can hide the real pollination system of ambophilous species. In this context, the reporting of ambophily in *E. eitenorum* may seem surprising at first glance. However, the low visitation in open environments, in addition to floral characteristics that promote pollen flow by wind such as exserted reproductive structures, demonstrate the plausibility of an ambophilous system. Our results point to the need to investigate ambophily in other *Esterhazya* and in zoophilous species with exserted reproductive structures occurring in open sites.

Declarations

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Amanda Pacheco and Pedro Joaquim Bergamo. The first draft of the manuscript was written by Amanda Pacheco and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

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Figures



Figure 1

Flowers of *Esterhazya eitenorum*, an ambophilous species occurring in the high-altitude grasslands (*campos de altitude*) of Itatiaia National Park, Rio de Janeiro - Brazil. Photos: Nathália Susin Streher