Annals of Botany **00**: 1–14, 2025
https://doi.org/10.1093/aob/mcaf263, available online at www.academic.oup.com/aob

ANNALS OF BOTANY Nonprofit since 1887

ORIGINAL ARTICLE

From bats to bees: changes in flower anthesis and nectar traits drive a pollination ecotype in dwarf *Caryocar brasiliense* (Caryocaraceae)

Caio S. Ballarin[1,2,*], Pedro A. Lacerda-Barbosa[1,2], Ana Paula Moraes[3], Felipe Girotto Campos[2] and Felipe W. Amorim[1,2,*]

[1]Laboratório de Ecologia da Polinização e Interações – LEPI, Instituto de Biociências, Departamento de Biodiversidade e Bioestatística, Universidade Estadual Paulista 'Júlio de Mesquita Filho' (UNESP), Street Prof. Dr. Antonio Celso Wagner Zanin, Botucatu, São Paulo 18618-689, Brazil, [2]Programa de Pós-Graduação em Biologia Vegetal, Instituto de Biociências, Departamento de Biodiversidade e Bioestatística, Universidade Estadual Paulista 'Júlio de Mesquita Filho' (UNESP), Street Prof. Dr. Antonio Celso Wagner Zanin, Botucatu, São Paulo 18618-689, Brazil and [3]Laboratório de Citogenômica e Evolução de Plantas, Instituto de Biociências, Departamento de Biodiversidade e Bioestatística, Universidade Estadual Paulista 'Júlio de Mesquita Filho' (UNESP), Street Prof. Dr. Antonio Celso Wagner Zanin, Botucatu, São Paulo 18618-689, Brazil
[*]For correspondence. E-mail: csballarin@gmail.com or felipe.amorim@unesp.br

Received: 02 August 2025 Returned for revision: 15 October 2025 Accepted: 20 October 2025

- **Background and Aims** Widespread plants often display different phenotypes that influence pollination. In the Brazilian Cerrado, *Caryocar brasiliense* typically grows as a bat-pollinated tree, but a hemicryptophyte dwarf form occurs in a population near the southern limit of the Cerrado distribution in Brazil, where large-bodied bees are the main visitors. We investigated whether these forms represent distinct pollination ecotypes and assessed the ecological drivers and the potential role of polyploidy underlying their divergence.
- **Methods** We compared floral traits associated with pollinator attraction and reward (flower size, anthesis timing, nectar dynamics and chemical composition) between forms. Pollinator exclusion experiments evaluated the effectiveness of diurnal and nocturnal visitors. Genome size and chromosome number were assessed to explore the role of polyploidy in trait divergence.
- **Key Results** The dwarf form had smaller flowers, later anthesis and nectar secretion peaking in the early morning, with sucrose-enriched, hexose-dominant nectar. Bee visitation rates were over 50 times higher than bat visits and positively associated with nectar sugar content. Diurnal visitors, especially large bees, significantly increased fruit and seed set. Genome size and chromosome number were consistent across ecotypes, suggesting no polyploidy.
- **Conclusions** A shift from bat to bee pollination in the dwarf form of *C. brasiliense* appears to be driven by changes in floral traits, rather than by polyploidy or absence of bat pollinators. This highlights the importance of anthesis timing and nectar chemical composition in shaping pollinator-mediated divergence across heterogeneous landscapes.

Key words: Bee pollination, Cerrado, chiropterophilous, nectar dynamics, neotropical savanna, pollinator shift.

INTRODUCTION

Plant species with broad geographical ranges are subject to different environmental pressures related to the climatic, edaphic and topographical conditions of the regions in which they occur (Etterson and Mazer, 2016; Etterson *et al.*, 2016; Hoffman *et al.*, 2020). Local environmental variation across a plant's distribution can shape its ecological niche and mediate phenotypic changes that affect plant–pollinator interactions (Miner *et al.*, 2005; Etterson and Mazer, 2016). For instance, plants can exhibit pollination ecotypes (i.e. evolutionary adjustments to environmental variation in pollinator assemblages; Armbruster, 1985; Johnson, 2025) when their geographical range extends the distribution of their primary pollinator (Grant and Grant, 1965 ; but see also Moir *et al.*, 2025). In the absence of the most efficient floral visitor, secondary visitors can mediate the selection toward floral traits that match their sensory and morphological preferences (Stebbins, 1970). In this phenomenon, referred to as the 'Grant-Stebbins model' (*sensu* Johnson *et al.*, 2006), shifts in plant pollination niches are determined by the distribution of primary pollinators across a heterogeneous landscape (Johnson, 2010; Van der Niet *et al.*, 2014).

The absence of the primary pollinator is among the best-documented drivers of pollination ecotypes (see

Pérez-Barrales et al., 2007; Newman *et al.*, 2014). Nevertheless, many cases do not conform to the expectations of the 'Grant-Stebbins model' (Van der Niet *et al.*, 2014; but see also Johnson, 2025; Kay and Anderson, 2025). For instance, biotic selective pressures can induce morphological or physiological changes in reproductive organs, affecting pollinator visitation and behaviour (Potts *et al.*, 2003; LoPresti *et al.*, 2018; Pardee *et al.*, 2018; Ballarin *et al.*, 2024*a*; Rodríguez-Sánchez *et al.*, 2024). In such cases, regardless of the occurrence of the primary pollinator, changes in plant phenotypes may lead to the decoupling of the interaction between plants and their main pollinators (Thomson and Wilson, 2008). This occurs when the fitness gain from increased specialization on the primary pollinator does not outweigh the fitness cost of reduced adaptation to secondary, less effective pollinators (Aigner, 2001). Under these conditions, selection may favour intermediate phenotypes that maintain compatibility with both primary and secondary pollinators and maximize their combined fitness contribution, rather than traits exclusively specialized for the primary pollinator (Aigner, 2001). Thus, if secondary pollinators become more frequent than the primary pollinator due to environmental filters, plants may exhibit adaptations to both primary and secondary pollinators.

Additionally, phenotypic variation affecting pollination may arise in response to environmental filters or from genomic changes such as polyploidy, which can simultaneously modify multiple plant traits influencing pollinator attraction, including plant and flower size, phenology, floral colour, and scent (Porturas *et al.*, 2019; Rezende *et al.*, 2020). Such phenotypic variation can also lead to a pollinator shift when primary pollinators reduce or cease legitimate flower visitation (Rezende *et al.*, 2020). By doubling the genome, polyploidy increases genome size and may produce distinct nucleotypic effects (i.e. trait changes linked to total DNA content; see Doyle and Coate, 2019). Such phenotypic variation, whether driven by environmental filters or genomic alterations, can ultimately trigger pollination shifts when the balance of pollinator visitation is altered. However, studies addressing the evolutionary processes beyond the occurrence of a pollinator shift remain scarce and have largely focused on traditional scenarios in which the primary pollinator is absent, thereby overlooking novel or underexplored potential drivers of such shifts (Kay and Anderson, 2025).

The Brazilian Cerrado (the Neotropical savanna of Brazil) is characterized by rich biodiversity and high environmental heterogeneity, providing an ideal context to evaluate whether pollinator shifts follow the 'Grant-Stebbins model' or arise from alternative drivers, such as past adaptation to ancient abiotic conditions or polyploidization events. The Cerrado is a fire-prone ecosystem that spans nearly 2 million km^2 (Klink and Machado, 2005), and exhibits a high elevational variation, ranging from 100 to 1700 m a.s.l (Silva *et al*., 2006). Pleistocene climatic fluctuations, together with frequent fire and frost events at higher elevations, have favoured the occurrence of a highly diverse assemblage of acaulescent, hemicryptophytic, dwarf and xylopodium-bearing species in the Cerrado (Simon *et al.*, 2009; Simon and Pennington, 2012; Cássia-Silva *et al.*, 2022; but see De Toma *et al*., 2022 for examples in other stressful ecosystems). The persistence of such species in this seasonal and fire-prone ecosystem may be explained by preadaptations that allow them to protect their meristems by growing below ground (Simon *et al.*, 2009; Simon and Pennington, 2012; Cássia-Silva *et al.*, 2022). Moreover, the environmental heterogeneity of the Brazilian Cerrado, characterized by variation in altitude, soil types, microclimates and fire regimes, may play a key role in promoting genomic and phenotypic diversification. In such complex landscapes, polyploidization can provide adaptive potential by generating random genetic variation, among which some variants may enhance tolerance to environmental stressors and increase phenotypic plasticity (Van de Peer *et al.*, 2021 ; Turcotte *et al.*, 2024). This mosaic of environmental filters and cytogenetic variation creates a valuable framework for investigating the processes underlying pollinator shift events.

Caryocar brasiliense A.St.-Hil. (Caryocaraceae Voigt) is an endemic species widely distributed throughout the Brazilian Cerrado (Prance and Silva, 1973). Although *C. brasiliense* typically grows as a tree throughout most of its distribution (Fig. 1A), in the southernmost part of the Cerrado range (i.e. near the southern limit of the Cerrado in Brazil), a more recent lineage of *C. brasiliense* exhibits a bushy, dwarf and hemicryptophytic life form, in which the main trunk remains entirely underground (*C. brasiliense* subsp. *intermedium*, Fig. 1B, C). In the core region of the Cerrado (the central and most continuous portion of its range, where the typical ecological, floristic and climatic characteristics of the Cerrado are least affected by transitions to adjacent biomes), *C. brasiliense* produces yellowish-cream, brush-like flowers that secrete abundant, diluted nectar. These flowers open before sunset (around 1700 h) and remain functional for approximately 24 h, attracting a wide array of nocturnal and diurnal floral visitors, including bees, hawkmoths, hummingbirds and nectarivorous bats. However, the Palla's long-tongued bat, *Glossophaga soricina* (Chiroptera: Phyllostomidae), is the primary pollinator of *C. brasiliense* trees in the central Cerrado (Gribel and Hay, 1993).

Nevertheless, despite the broad distribution of *G. soricina* across Brazil, including the southernmost Cerrado (Dias *et al.*, 2017; Amorim *et al.*, 2023), and the floral similarity in shape, colour and scent between the arboreal and the dwarf forms of *C. brasiliense*, the flowers of *C. brasiliense* subsp. *intermedium* are displayed close to the ground due to the plant's shrubby growth form (Fig. 1B, C). This represents an unfavourable trait for bats, as it hinders their flight manoeuvres and increases their exposure to ground predators, but poses no obstacle for bees (Diniz *et al*., 2019; but see Amorim *et al*., 2023). Indeed, our previous observations indicate that bees are the most frequent visitors to the dwarf form of *C. brasiliense*, whereas bats are rarely recorded. This suggests that bees, although considered resource robbers or secondary pollinators (Araujo *et al*., 2020), may contribute significantly to plant fitness due to their high visitation frequency (see Aigner, 2001). Considering the overlapping distribution of bats and the dwarf *C. brasiliense*, the 'Grant-Stebbins model' may not fully explain this potential pollinator shift, offering an opportunity to investigate whether pollination niches can arise from alternative ecological processes (see Johnson, 2025; Kay and Anderson, 2025).

In this sense, we asked which potential alternative forces may have driven the potential pollinator shift observed in *C. brasiliense* subsp. *intermedium*. To address this question, we first investigated whether the core and southernmost populations of *C. brasiliense* represent distinct pollination ecotypes by comparing several floral traits, including flower size, anthesis timing, as well as nectar dynamics and chemical composition across Cerrado populations. Furthermore, we conducted an

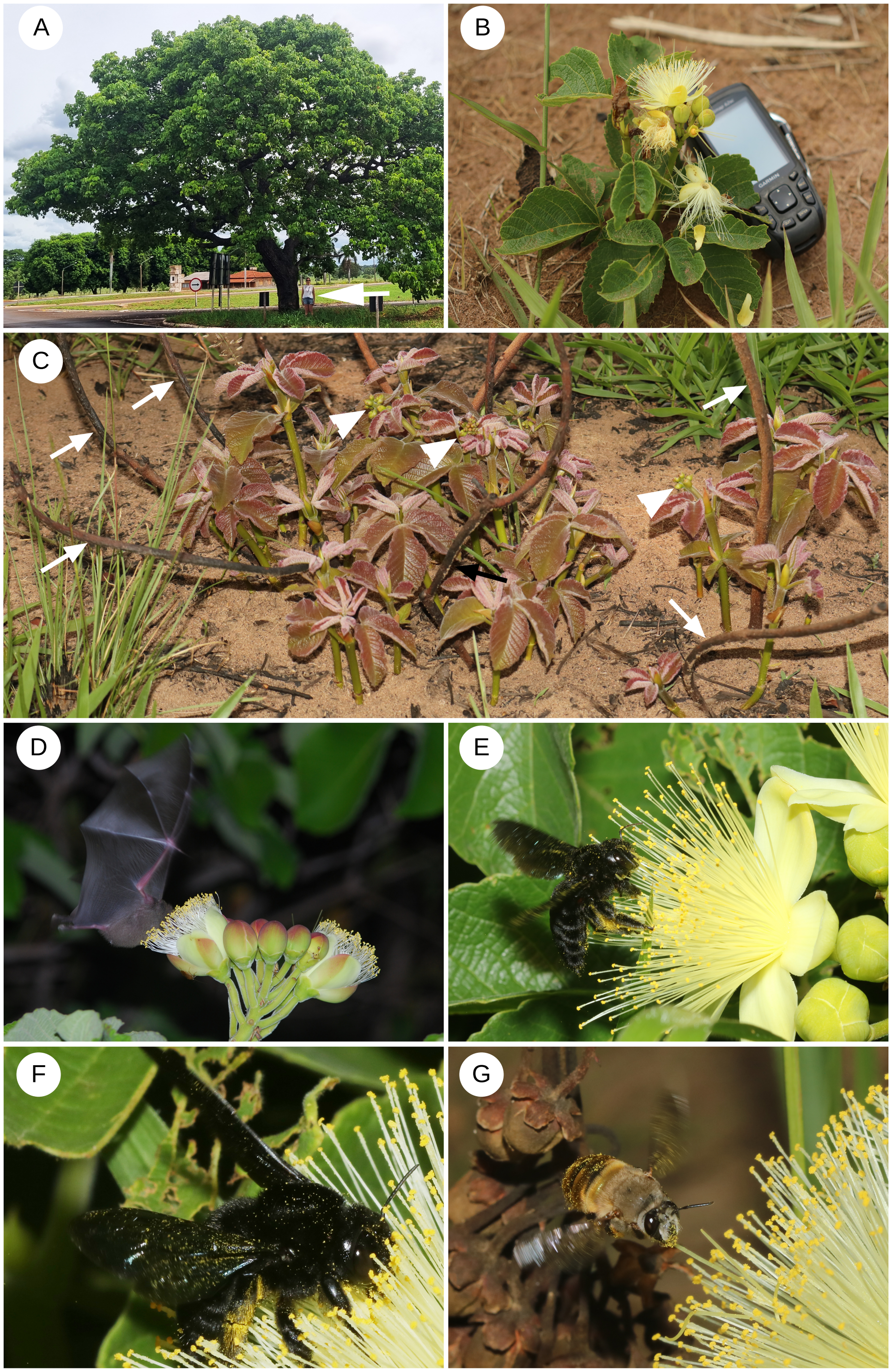

Fig. 1. Plant morphology and main pollinators of the arboreal and dwarf forms of *Caryocar brasiliense*. (A) The typical arboreal (phanerophytic) life form of *C. brasiliense*. This tree is approximately 13 m tall. The white arrow points to a person (1.73 m tall) standing next to the tree for scale. (B) A 30-cm-tall dwarf plant in bloom. Note the 20-cm-long GPS device placed next to the plant. (C) Newly produced sprouts of a dwarf plant a few weeks after a fire event. Arrows indicate old burned branches, and arrowheads point to flower buds at early developmental stages. Note that both the new sprouts and the old burned branches emerge from the ground, revealing the hemicryptophytic life form of this ecotype, in which the main trunk is entirely underground. (D) The Pallas's long-tongued bat (*Glossophaga soricina*) visiting a *C. brasiliense* tree in Central Brazil (photo credit: Christiano Peres Coelho and Paulo E. Oliveira). (E) A large-bodied bee (*Xylocopa* sp.) visiting flowers of the dwarf form of *C. brasiliense* in Botucatu, São Paulo state, in the southernmost region of the Cerrado. (F) A large-bodied bee (*Xylocopa* sp.) on a flower of the dwarf form, covered with pollen while contacting both anthers and pistils. (G) A nocturnal bee (*Ptiloglossa* sp.) covered with *C. brasiliense* pollen approaching a flower of the dwarf form and making contact with the stigma. (A–G) These images collectively illustrate how differences in plant life form between the arboreal and dwarf ecotypes may translate into differences in pollinator frequency and efficiency, potentially leading to pollinator shifts.

exclusion experiment to test whether a pollinator shift had occurred in the dwarf population towards diurnal pollination. Lastly, we analysed the genome size of *C. brasiliense* in both ecotypes and chromosome number on the dwarf ecotype to assess whether one of the observed morphotypes could result from polyploidization, or whether the dwarfism observed in the southernmost population represents a potential adaptive response to harsh Cerrado environments shaped by seasonal fire and frost events. Both scenarios may have contributed to the emergence of distinct pollination niches.

MATERIAL AND METHODS

Study species and sites

Caryocar brasiliense is a fire-prone and keystone species widely distributed in the savanna vegetation within the Brazilian Cerrado. It produces terminal racemose inflorescences arranged above the canopy. Each inflorescence bears conspicuous, actinomorphic, white to yellowish-cream, scented flowers with a brush-like morphology, containing 350–500 stamens (Gribel and Hay, 1993).

Caryocar brasiliense exhibits a nocturnal anthesis, with flowers opening in the late afternoon, just before the onset of bat activity. Early in the evening, bats (frequent visitors of the arboreal form, Fig. 1D) begin flying across the landscape in search of floral resources and to establish their nightly foraging routes (Gribel and Hay, 1993; Bobrowiec and Oliveira, 2012). In addition to glossophagine bats, the sulphur-containing compounds in the floral scent, along with the abundant nectar and pollen, attract a broad range of floral visitors, including non-flying mammals, hummingbirds, hawkmoths, bees, wasps and flies (Paiva *et al*., 2019; Souza *et al*., 2022). Although the brush-like morphology of the flowers allows for mixed pollination systems, as it does not impose morphological constraints preventing legitimate visits by diverse pollinators (Amorim *et al*., 2013; Primo *et al*., 2022; de Matos Costa *et al*., 2025), bats are substantially more efficient pollinators than bees in the arboreal form (see Dias, 2017).

Caryocar brasiliense has populations with a disjunct distribution within the Cerrado, which results from multiple maternal lineages (Collevatti *et al*., 2003). This genetic differentiation may give rise to phenotypic variation among populations occurring in different regions of the species' range. For example, the typical *C. brasiliense* subsp. *brasiliense* is a tree that can reach up to 15 m in height and occurs predominantly in the core region of the Cerrado (Fig. 1A). In contrast, the most recent lineage, *C. brasiliense* subsp. *intermedium*, is a dwarf form found in high-elevation peripheral areas at the southernmost boundaries of the Cerrado. This ecotype grows as a hemicryptophyte shrub, with flowers being produced by plants ranging from just a few centimetres above the ground (Fig. 1B) up to 2.0–3.0 m in height, with its main trunk prostrated and often entirely located below ground (Fig. 1C).

We studied populations of *C. brasiliense* at three sites across its range, encompassing both the arboreal and dwarf forms (Fig. 2; Supplementary Data Table S1; Fig. S1). The dwarf form occurs in the municipality of Botucatu, São Paulo State, south-eastern Brazil, whereas the arboreal populations are located further north. Distances between the two arboreal populations are relatively short compared to those separating them from the dwarf population. All sites share similar main soil types, and vegetation structure varies from '*campo sujo*' (dwarf form) to Cerrado '*sensu stricto*' and Cerrado '*sensu lato*' (arboreal populations). Climatic variables were summarized using historical records since the 1960s, providing an overview of environmental conditions at each site. Although detailed information on historical fire regimes, including extent, frequency and intensity, is unavailable for these specific locations (but see Segura-Garcia *et al*., 2025, for fire regimes within the Cerrado), fire is recognized as an important selective pressure shaping plant–animal interactions in Brazilian ecosystems, albeit often poorly investigated (Ballarin *et al*., 2024*a*). These abiotic factors, while not precisely quantifiable at a long-term evolutionary scale, probably exerted selective pressures that contributed to the differentiation of the dwarf hemicryptophyte life form. Fieldwork was carried out during the flowering peak of *C. brasiliense*, which lasts for 1–2 months between June and December, depending on the locality, from 2019 to 2022.

Floral traits

To evaluate whether the arboreal and the dwarf forms of *C. brasiliense* represent distinct pollination ecotypes, we measured several traits related to plant–pollinator matching (e.g. floral size, and anthesis) and pollinator rewards (nectar production dynamics and chemical composition). Anthesis in the dwarf form was recorded using time-lapse cameras (Supplementary Data Video S1), while anthesis in the arboreal form was monitored through focal observations of individual flowers. Using a digital calliper, we measured: (1) petal height and width (mm); and (2) stamen and carpel length (mm). Petal area in *C. brasiliense* was approximated as elliptical and calculated using the formula: petal area = $\pi \times$ [(petal width/2) $\times$ (petal height/2)]. For these measurements, we randomly selected three to five mature flowers per individual across all studied populations. In the dwarf population, we measured 380 flowers from 121 individuals, while for the arboreal populations, we measured 425 flowers from 108 individuals.

To test whether morphometric floral traits differ between arboreal and dwarf forms, we performed linear models (LMs), considering form (arboreal vs. dwarf) as a fixed factor and the following floral traits as response variables: petal height and width, stamen and carpel length, and petal area. A multiple-testing correction using the Benjamini–Hochberg method was applied to control the false discovery rate. In addition, we conducted a non-metric multidimensional scaling (NMDS) analysis to assess multivariate differences in floral morphology between plant forms, based on petal area, stamen length and anther length. NMDS was performed using Bray–Curtis distances and the 'metaMDS' function from the *vegan* package in R (Dixon, 2003). In this analysis, individuals were categorized by plant form ($n = 2$) and population ($n = 3$). Finally, to assess whether the morphometric traits differed between plant forms and populations, we performed a permutational multivariate analysis of variance (PERMANOVA) using the '*adonis2*' function from the *vegan* package in R, with 10 000 permutations. Plant form, population and their interaction were included as fixed factors.

We evaluated nectar production dynamics only in the dwarf form, as the arboreal form has already been characterized in the literature (see Bobrowiec and Oliveira, 2012). Although the nectar removal intervals are not directly comparable, those

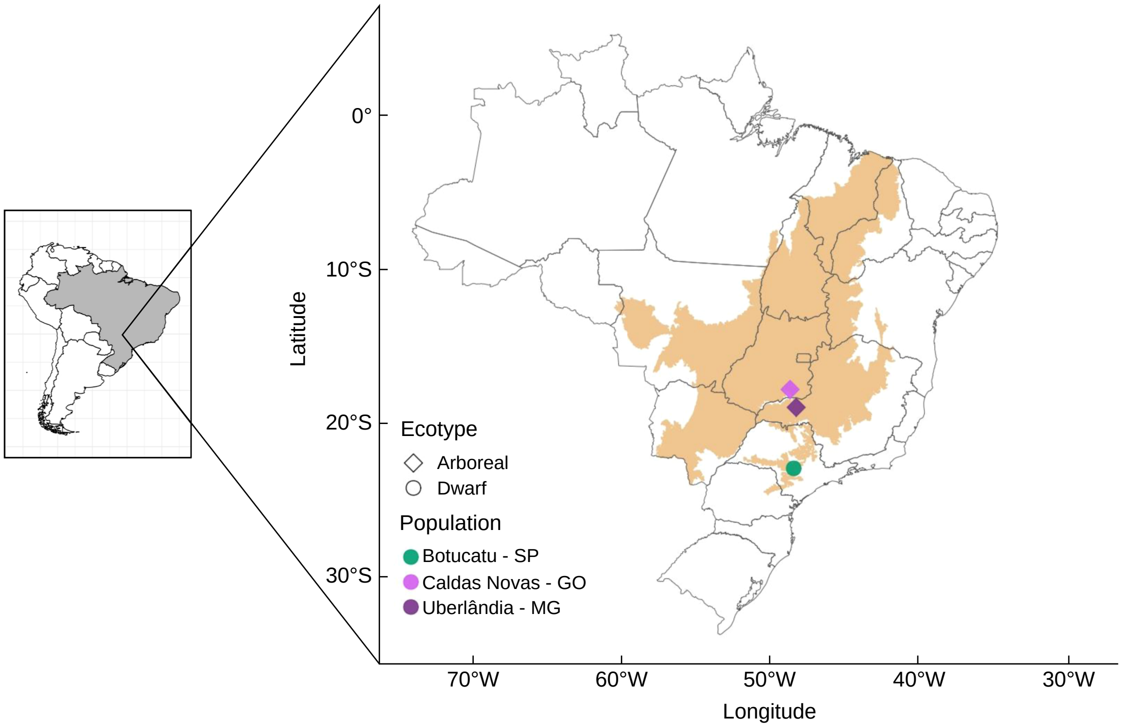

FIG. 2. Geographical locations of the study sites. Botucatu – SP in the southernmost region of the Cerrado, where *Caryocar brasiliense* exhibits the hemicryptophytic and dwarf life form (green circle); Caldas Novas – GO and Uberlândia – MG, areas in the core region of the Brazilian Cerrado, where *C. brasiliense* presents the typical arboreal form (purplish diamonds). The polygon in beige represents the distribution of the Cerrado (the Neotropical savanna of Brazil), where *C. brasiliense* occurs throughout its entire range. Due to the large geographical range of the Cerrado, climatic differences and variation in fire and frost regimes are expected. The municipality of Botucatu, located at higher elevation, frequently experiences both fire and frost events. Lower temperatures with frequent frosts are conditions less common in the core region of the Cerrado, where mean annual temperatures are higher.

data allow for a contextual comparison of nectar production peaks between the two forms. In a dwarf population, we selected 90 flowers (two to three per individual) and isolated them with nylon mesh bags to prevent interference from floral visitors. Over an 18-h period, corresponding to the complete lifespan of flowers in *C. brasiliense* subsp. *intermedium*, we sampled nectar in six sets of 15 flowers each, at 3-h intervals starting as soon as the dwarf flowers had fully opened, from floral opening to senescence. Nectar volume was measured using a microlitre syringe (Hamilton, NV, USA) and sugar concentration (percentage sucrose, mass/mass) was determined with a pocket refractometer (0–90 %; Instrutherm, RT-280) as in Amorim *et al.* (2013). Sugar mass was then calculated following Galetto and Bernardello (2005).

We used the one-way analysis of variance (ANOVA) and a Tukey *post hoc* test to compare nectar traits across time intervals and characterize the pattern of nectar production, including secretion, cessation and possible reabsorption (Ballarin *et al.*, 2022). For this analysis, we considered nectar volume, concentration and amount of sugar as response variables to test for differences among time intervals throughout the flower lifespan.

Finally, we also compared the chemical composition of nectar sugars between the arboreal and dwarf forms of *C. brasiliense*. Nectar was extracted from five flowers per plant form. The soluble sugar profile was analysed using high-performance ion chromatography (Dionex ICS-5000+). Filtered samples and standards (0.22-μm filter) were run through a chromatography system with a quaternary pump, autosampler, DCS-5000 electrochemical detector (Thermo®), P-100 column (Carbopack®) and Ag/AgCl reference electrode. Sugars were identified by comparing retention times to standards. Quantification was performed using standard curves for D-(+)-glucose (≥99.5 %), D-(+)-mannose (≥99.5 %), D-(−)-fructose (≥99 %) and sucrose (≥99.5 %; Sigma-Aldrich®; Suksom *et al.*, 2015; Campos *et al.*, 2023). The sucrose / hexose ratio was calculated as sucrose / (glucose + fructose). Values below 0.1 were classified as hexose-rich nectar, values between 0.1 and 0.499 as hexose-dominant nectar and values above 0.5 as sucrose-dominant nectar (Baker and Baker, 1983).

Pollinator efficiency and reproductive success

We recorded both diurnal and nocturnal floral visitors only in the dwarf form of *C. brasiliense* through focal observations and infrared (IR) motion- and heat-activated cameras (Bushnell Natureview model 119740, Bushnell Corp., Overland Park, KS, USA). We distinguished legitimate from illegitimate visits by classifying as legitimate those in which visitors contacted anthers and stigmas of the flower. To assess the visitation rates by diurnal and nocturnal visitors, we calculated the number of visits per unit of time. The unit of time was defined as the product of the number of flowers observed and the total hours of sampling (*sensu* Muchhala *et al.*, 2009), which accounts for differences in the number of flowers observed across sampling methods and periods of observations. In the dwarf populations, floral visitor observations totalled 174 h during the day and

166 h at night, carried out in 2019 and 2021. We did not conduct focal observations of floral visitors in the arboreal form. However, bat visitation to arboreal *C. brasiliense* is widely recognized as frequent and essential for reproductive success in this typically chiropterophilous species (Gribel and Hay, 1993; Bobrowiec and Oliveira, 2012; Souza *et al*., 2022; Fig. 1D).

We also investigated whether bee visitation in the dwarf form is associated with the nectar production rhythm. Following Ballarin *et al*. (2022), we conducted a generalized linear model (GLM) to analyse the hourly frequency of bee visits in relation to the amount of sugar produced by flowers throughout the day. Predictor variables included the sugar content (mg) of floral nectar and bee size class (i.e. small-, medium- and large-bodied bees), while the response variable was bee visitation frequency. The GLM was fitted using a Poisson distribution for the error structure.

To evaluate the effects of diurnal and nocturnal pollinators on fruit and seed set, we conducted floral visitor exclusion experiments only in the dwarf form of *C. brasiliense*, as chiropterophily in the arboreal form is well established and recognized as essential for reproductive success (Gribel and Hay, 1993; Bobrowiec and Oliveira, 2012). In 2019, we randomly selected 31 individuals of *C. brasiliense* subsp. *intermedium*. For each individual, 5–11 flowers were marked per treatment, totalling 199 flowers for the nocturnal exclusion and 200 for the diurnal exclusion. To exclude diurnal floral visitors, flowers were left exposed to nocturnal visitors from dusk (1800 h) until dawn (0430 h), after they were bagged with nylon mesh to prevent visits during the day. Conversely, to exclude nocturnal visitors, flowers were bagged at dusk (1800 h) and unbagged at dawn (0430 h), thereby allowing access only to diurnal floral visitors. Fruit and seed set were assessed for each individual by calculating the ratio of fruits or seeds to the number of flowers (as in Van der Niet *et al*., 2014). To test whether fruit and seed set were influenced by diurnal and nocturnal floral visitors, we fitted two linear mixed models (LMMs), with fruit and seed set as response variables, exclusion treatment (diurnal or nocturnal) as the fixed factor, and individual identity as a random effect. *Post hoc* multiple comparisons between treatments were performed using Tukey tests with the '*emmeans*' package in R (Lenth, 2016).

Cytogenetic analysis: genome size estimation and chromosome number. Genome size estimation was carried out using approximately 50 mg of fresh young leaf tissue from each sample, co-macerated with the 25 mg of leaf tissue from the internal reference *Solanum lycopersicum* cv. 'Stupicke' (2C = 1.96 pg; Doležel et al., 1992). Both materials were macerated in 0.5 mL of extraction buffer (Ebihara et al., 2005) supplemented with RNase (0.025 μg mL^{-1}). The nuclear suspensions were filtered and stained with 12.5 μL of a 1 mg mL^{-1} propidium iodide solution (Sigma). Leaf samples were collected from two dwarf populations in the southernmost Cerrado (Botucatu, São Paulo State) and one arboreal population from the core region of the Cerrado (Uberlândia, Minas Gerais State), with three individuals per population analysed in triplicate.

A flow cytometry experiment was performed using a FACSCanto II cytometer (Becton Dickinson, San Jose, CA, USA), kindly made available by the Sector of Microbiology and Immunology, Institute of Biosciences (IBB-UNESP). Histograms were generated using FACSDiva software, based on 5000 events per run. Statistical evaluation was performed using FlowJo™ v.10.8 software (BD Life Sciences; https://www.flowjo.com/). Quality control was based on two estimates: the coefficient of variation (CV) of each measurement and the standard deviation (s.d.) among genome size estimates. Both values were required to remain below 5 %, ensuring that observed variation was attributed to technical factors rather than representing intraspecific genomic variation. The genome size for the three analysed populations was estimated following Pellicer and Leitch (2014). Flow cytometry measures the fluorescence emitted by stained nuclei as they pass through a laser beam, assuming a direct relationship between the fluorescence intensity and the amount of DNA in the nuclei. Finally, we used a one-way ANOVA to test whether genome size differed among the three populations.

The chromosome number of dwarf *C. brasiliense* individuals from the southernmost Cerrado was analysed in meiotic cells. Floral buds were collected and fixed in a 3:1 solution of absolute ethanol and glacial acetic acid (v/v) at room temperature for 24 h and subsequently stored at −20 °C in the same solution. For slide preparation, anthers were squashed in a drop of 60 % acetic acid. Coverslips were removed by freezing the slides in liquid nitrogen, followed by air-drying. Slides were subsequently stained with 2 % Giemsa solution. Chromosomes were observed under an Olympus BX51 microscope, and selected metaphases were photographed using a CCD camera and analysed with the software CellSens (Olympus, Inc.). Image contrast and brightness were adjusted for uniformity using Adobe Photoshop CS5 (Adobe Systems, Inc.).

RESULTS

Floral traits

Although flowers of the dwarf form of *C. brasiliense* exhibit similar colour and morphology to those of the arboreal ecotype (Fig. 1), they are significantly smaller (see Supplementary Data Table S2; Fig. 3A). Specifically, the dwarf form has shorter stamens and carpels, reduced petal height and width, and a smaller petal area compared to the arboreal form ($P < 0.001$; Supplementary Data Table S2). Differences between plant forms remained significant after Benjamini–Hochberg correction. NMDS ordination revealed that variation in floral morphology is influenced primarily by plant form and, to a lesser extent, by population (stress: 0.039; $R^2 = 0.99$; Fig. 3B). In addition, the PERMANOVA indicated significant differences in floral morphometric traits across plant forms and populations. Most of the observed variation was explained by the plant form (*Pseudo-F* = 291.06; $R^2 = 0.58$; $P < 0.001$), followed by population (*Pseudo-F* = 19.82; $R^2 = 0.03$; $P < 0.001$). Surprisingly, floral anthesis also differed between the plant forms. While flowers of the arboreal form opened late in the afternoon, around 1700 h, those of the dwarf form began opening later at night, after 0200 h (Video S1).

In the dwarf form of *C. brasiliense*, nectar secretion began concomitantly with floral opening. Newly opened flowers possessed 238.90 ± 57.14 μL of nectar, with a sugar concentration of 11.40 ± 1.71 % yielding a total of 29.04 ± 10.27 mg of sugar (Fig. 4). The highest sugar content was recorded between

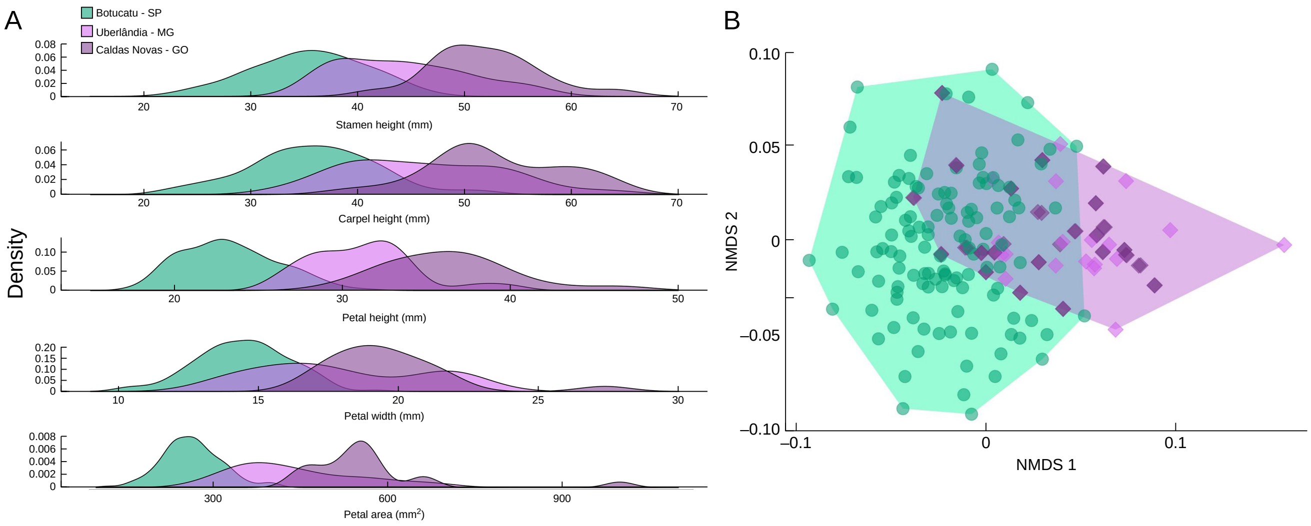

Fig. 3. Floral attributes of the arboreal and dwarf forms of *Caryocar brasiliense*. (A) Floral size of the dwarf (green) and arboreal (purplish) forms. (B) In the non-metric multidimensional scaling (NMDS), green circles represent the dwarf ecotype, located in Botucatu – SP, within the southernmost region of the Cerrado, and purplish diamonds represent the arboreal ecotype, located in Uberlândia – MG and Caldas Novas – GO, within the core region of the Cerrado. The blurred polygons represent the multivariate space occupied by the dwarf and arboreal forms. PERMANOVA was used to test for differences between forms. These floral morphological differences demonstrate the reduction in flower size in the dwarf form, which tends to increase contact of reproductive organs with visiting bees.

0500 h and 0800 h, when flowers produced 43.41 ± 20.17 mg of sugars (Fig. 4A) in 312.10 ± 129.28 μL of nectar (Fig. 4B) with a sugar concentration of 13.10 ± 0.99 % (Fig. 4C).

Nectar production remained active until approximately 1100 h, after which nectar volume declined significantly (148.20 ± 46.00 μL; $F_{5,54} = 23.65$, $P < 0.001$), followed by a decrease in sugar content (39.76 ± 12.20 mg of sugar; $F_{5,54} = 12.61$, $P < 0.001$). At the end of the floral lifespan, nectar volume dropped sharply, while sugar concentration increased significantly ($F_{5,54} = 152.30$, $P < 0.001$), suggesting water evaporation rather than nectar reabsorption.

Nectar sugar composition also differed between *C. brasiliense* forms. The dwarf form produced hexose-dominant nectar with higher sucrose content, whereas the arboreal form produced hexose-rich nectar, with sucrose virtually absent (Table 1).

Pollinator efficiency and reproductive success

Bees were the most frequent floral visitors of the dwarf form of *C. brasiliense*, whereas bat visitation was rare (Supplementary Data Tables S3 and S4). We recorded 926 visits by 28 bee species, corresponding to a visitation rate of 0.0107 visits per flower per hour. In contrast, bats were recorded visiting the flowers of the dwarf form only twice, resulting in a rate of 0.0002 visits per flower per hour, indicating that bees had nearly 53.5-fold higher visitation frequency than bats.

Bee visitation occurred predominantly in the morning, between 0600 h and 1300 h, with a marked peak in large bees during the early morning (Fig. 5A; Supplementary Data Video S1). However, a secondary peak in bee visitation was also observed in the late afternoon, primarily involving medium-sized bees. This increase was largely attributable to *Trigona spinipes*, which frequently landed on flowers to feed on the viscous, sugar-rich nectar solution. The amount of sugar (mg) produced in nectar significantly influenced the visitation rate of bees ($\chi^2 = 4.72$; $R^2 = 0.40$; $P < 0.05$; Fig. 5B). However, bee size class (small, medium or large) did not respond differently to nectar production rhythms ($P > 0.05$; Fig. 5B).

Due to their different sizes and behaviours, bees varied in their pollination efficiency according to our qualitative observations of their contact with both anthers and stigmas. Large-bodied bees belonging to the genera *Xylocopa*, *Ephicaris*, *Bombus*, *Centris*, *Eulaema*, *Euphrisia*, *Ptiloglossa* and *Oxaea* frequently contacted the stigmas of *C. brasiliense* flowers while collecting pollen or nectar. In contrast, medium-bodied (e.g. *Apis mellifera* and *Trigona spinipes*) and small-bodied bees (e.g. *Tetragonisca angustula* and *Paratrigona lineata*) contacted the stigma with intermediate and low frequency, respectively. Fruit and seed set were notably high in the dwarf form of *C. brasiliense*. A total of 86.15 % of flowers exposed to diurnal pollinators developed into fruits, compared with 32.66 % of those exposed to nocturnal pollinators. Regarding seed set, 63.50 % of diurnally pollinated flowers produced one or more seeds, corresponding to 1.24 seeds per flower. In contrast, 18.09 % of nocturnally pollinated flowers produced seeds, with an average of 0.32 seeds per flower. Pollinator exclusion experiments confirmed that diurnal floral visitors were more effective pollinators than nocturnal ones for the reproductive success of the dwarf form. Flowers from which nocturnal visitors were excluded produced more fruits ($F_{1,62} = 132.27$; $R^2 = 0.72$, $P < 0.001$; Fig. 6A) and more seeds ($F_{1,62} = 151.43$, $R^2 = 0.77$, $P < 0.001$; Fig. 6B) than those from which diurnal visitors were excluded.

Genome size and chromosome number

The genome size of *C. brasiliense* was estimated at 1C = 0.50 ± 0.025 pg (489 Mbp), with no difference between the dwarf and arboreal forms collected from the southernmost and core regions of the Cerrado, respectively ($F = 0.2625$, $P = 0.7744$; Fig. 7A–C). This lack of variation suggests that no polyploidy event has occurred between these morphotypes.

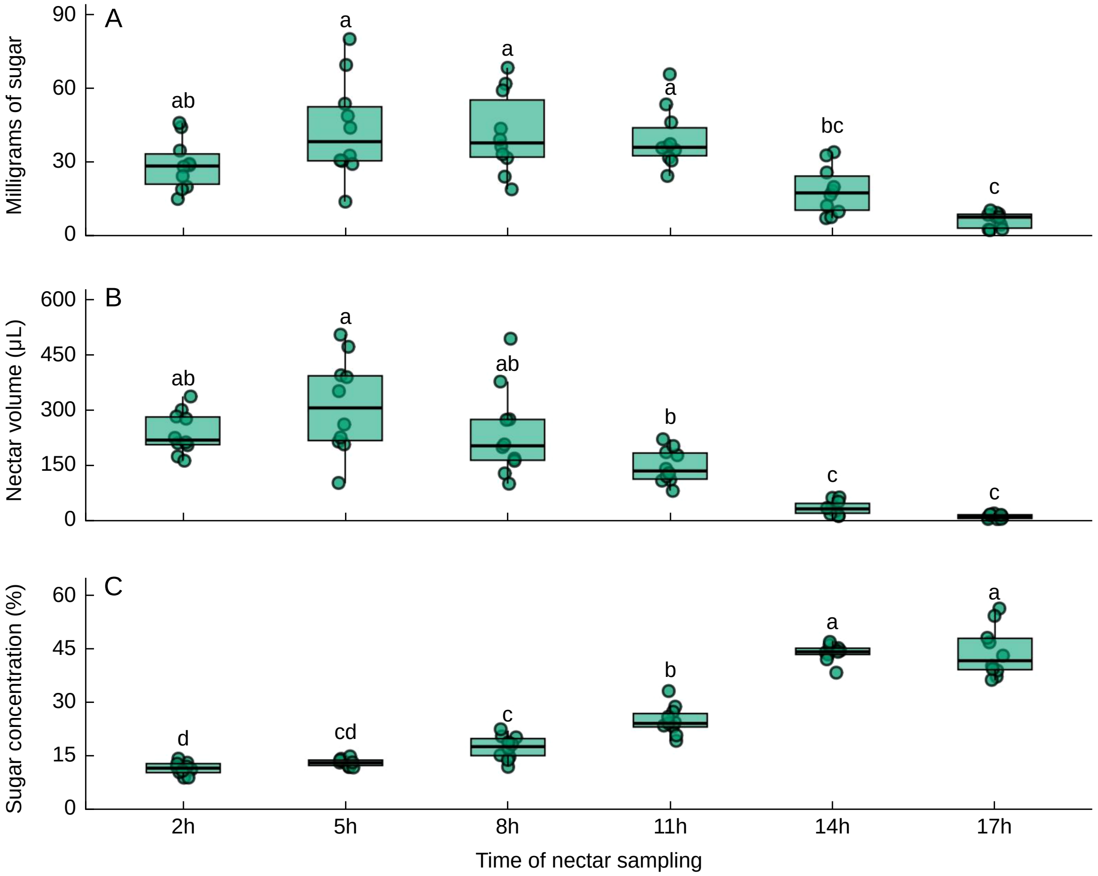

FIG. 4. Nectar secretion pattern of the dwarf ecotype of *Caryocar brasiliense* over flower lifespan in the southernmost region of the Brazilian Cerrado: (A) amount of sugar (mg), (B) nectar volume (mL) and (C) sugar concentration (percentage, w/w). Nectar sampling times on the *x*-axis indicate the actual time of day (e.g. 2h = 0200 h) starting from the moment flowers had fully opened. Different letters above the boxplots represent significant differences ($P < 0.05$) among each period of nectar sampling after the Tukey *post hoc* test for multiple comparisons among pairs of means. The peak in energy content (mg sugar) occurs in the morning, when bees, but not bats, are active.

TABLE 1. Nectar sugar composition of the dwarf and the arboreal forms of *Caryocar brasiliense*.

	Sugar composition (µg)			Sugar total (µg)	Proportion (%)	
Ecotype	Sucrose (S)	Glucose (G)	Frutose (F)	(S + G + F)	S/(F + G)	Type
Arboreal	0.000 ± 0	0.014 ± 0.003	0.046 ± 0.010	0.060 ± 0.013	0	Hexose-rich (HR)
Dwarf	0.005 ± 0.001	0.011 ± 0.004	0.035 ± 0.015	0.051 ± 0.019	0.108	Hexose-dominant (HD)

Nectar from the dwarf and arboreal ecotypes were sampled at Botucatu – São Paulo and Caldas Novas – Goiás, respectively. Classification of nectar type followed Baker and Baker (1983), in which: HD = hexose-dominant nectar; HR = hexose-rich nectar.

Furthermore, a haploid chromosome number of $n = 23$ was observed in meiotic cells of the dwarf form (Fig. 7C, D). The small chromosome size is also consistent with the reduced genome size of this species (Fig. 7D).

DISCUSSION

Our results show the presence of a distinct pollination ecotype in the dwarf form of *C. brasiliense*. Although the species is generally classified as chiropterophilous, dwarf populations in the southernmost Cerrado exhibited a shift in the pollination system from nocturnal bat pollination to primarily diurnal large-bodied bee pollination. This transition appears to be driven by coordinated changes in plant height and floral traits, including reduced flower size, altered anthesis timing, and distinct nectar dynamics and composition that discouraged bat visits while favouring bees. Together, these patterns indicate that ecological filtering and trait divergence may have contributed to the emergence of an alternative pollination strategy in the dwarf form, possibly representing an adaptive response to local selective pressures (Kay and Anderson, 2025; Moir *et al.*, 2025).

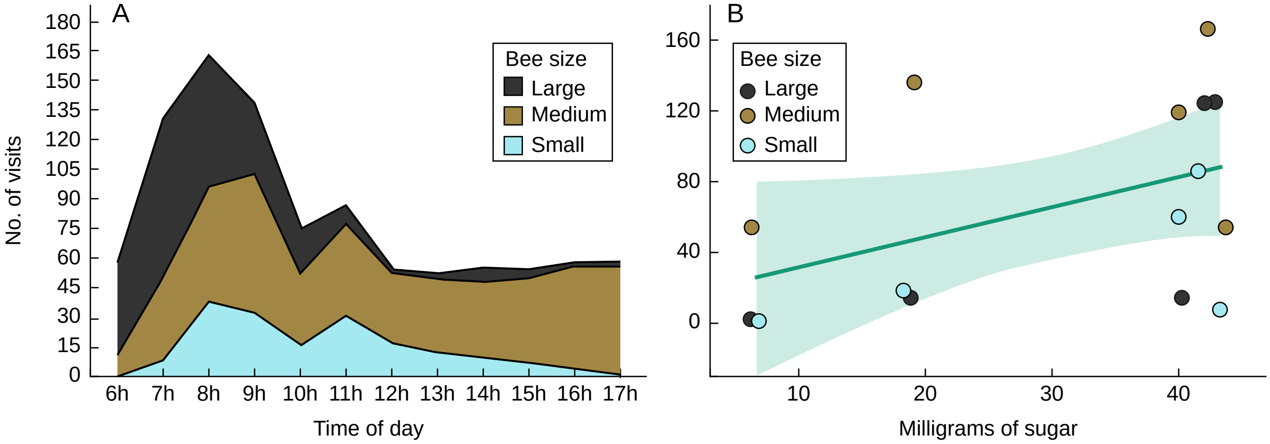

Fig. 5. Bee activity on flowers of the dwarf form of *Caryocar brasiliense* throughout the day. (A) Bee visits per hour. Visits were grouped according to bee sizes: large- (black), medium- (brown) and small-bodied (light blue) bees. (B) Relationship between the daily rhythm of nectar production (measured as mass) in the dwarf form of *C. brasiliense* and the number of bee visits to its flowers. Black, brown and light blue circles represent the visitation frequency of large-, medium- and small-bodied bees, respectively. The highest activity of all bees, particularly the large-bodied and most effective bees, occurs in the early morning, coinciding with the period of highest nectar volume and energy content, demonstrating a positive relationship between bee activity and nectar energy availability.

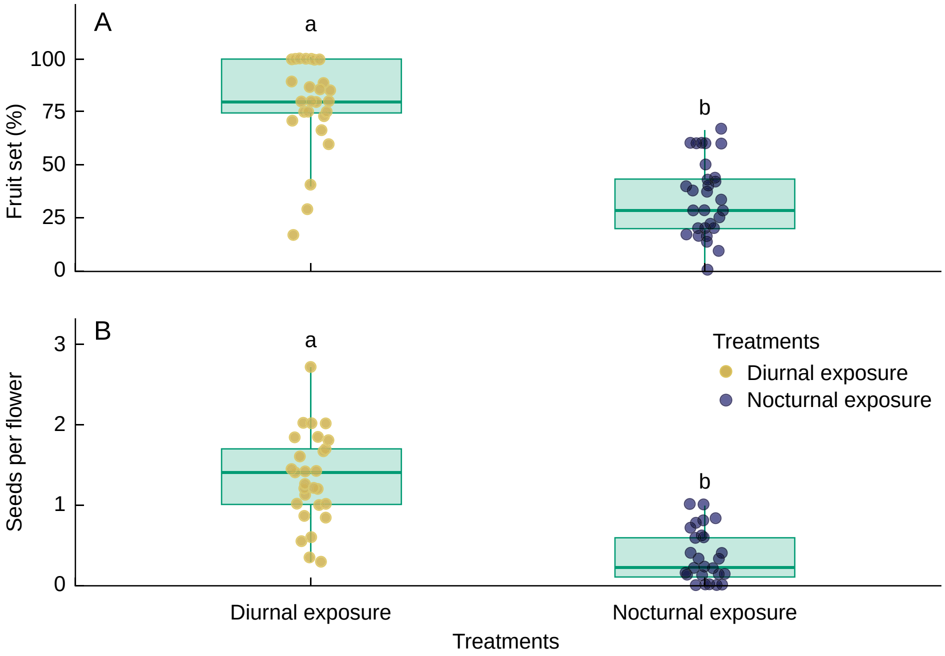

Fig. 6. Reproductive output of the dwarf form of *Caryocar brasiliense* after the exclusion experiment. (A) Fruit set represented as the percentage of flowers converted into fruits. (B) Seed set represented as the number of seeds in each flower. Blue and yellow circles depict nocturnal and daily excluded treatments, respectively. Different letters above the boxplot represent significant differences ($P < 0.05$) among treatments. Visits by diurnal visitors, mainly bees, result in higher reproductive success, indicating a pollinator shift from nocturnal to diurnal pollinators in the dwarf form.

Phylogeographical and palaeo-distribution evidence further reinforce the divergences in pollination ecotypes. Central and northern Cerrado populations of *C. brasiliense* occupied historically large and stable refugia (see Ab'Saber, 2000), whereas peripheral south-eastern populations, including the dwarf form in Botucatu municipality within São Paulo state, probably persisted in smaller refugia during the Quaternary (Collevatti *et al.*, 2003, 2012). These patterns suggest that the dwarf ecotype represents a more recent lineage relative to widespread arboreal populations, consistent with divergence times of ~3.3 Myr for south-eastern lineages (Collevatti *et al.*, 2003, 2012). Therefore, the emergence of dwarfism and associated floral traits favouring bee pollination may have occurred after the establishment of the central–northern lineages, potentially reflecting adaptation to local environmental pressures in these southern refugia.

Although nectar-feeding bats such as *Glossophaga soricina* remain common in the southern Cerrado, including in the study region where the dwarf ecotype occurs (see Amorim *et al.*, 2023), floral traits associated with diurnal pollination, such as

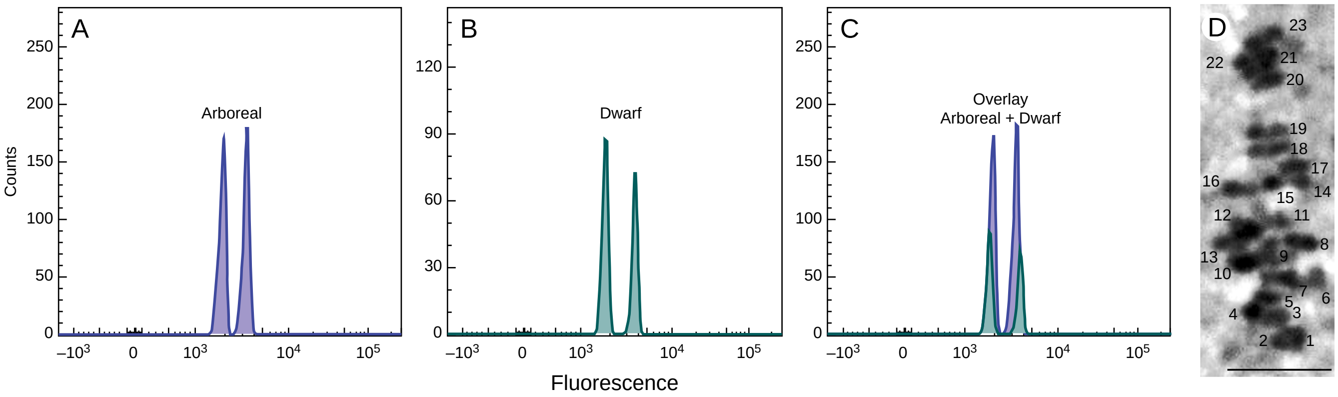

Fig. 7. Genome size and chromosome number of *Caryocar brasiliense.* (A) Representative histogram of an arboreal individual from the core Cerrado. (B) Representative histogram of a dwarf individual from the southernmost Cerrado. (C) Overlay of histograms A (arboreal form) and B (dwarf form). (D) Metaphase I showing the 23 chromosome pairs indicated by numbers. Bar indicates 10 µm. Graphs in A–C show the fluorescence emitted by the samples (*x*-axis) and the number of events detected in each fluorescence (*y*-axis). Although genetic factors may contribute to pollinator shifts, ploidy level and genome size do not differ between arboreal and dwarf forms.

smaller flower size, earlier nectar availability and higher sucrose content, favour bee visitation. The predominance of bees as the most frequent and effective pollinators (i.e. contributing to greater fruit and seed set) suggests a shift in plant–pollinator interactions that may have resulted from past phenotypic changes enhancing compatibility with diurnal visitors. These results align with the view that secondary pollinators can exert meaningful selective pressure when their visitation rates are high and their contribution to reproductive success is additive (Aigner, 2001; Kay and Anderson, 2025).

These patterns are unlikely to be explained by polyploidy, as genome size and chromosome number were similar between the arboreal and the dwarf forms. Chromosome counts have previously been reported for *C. brasiliense* populations from both Cerrado regions, all showing $2n = 46$ (Ehrendorfer *et al.*, 1984; Maglio *et al.*, 1984), although it remains unknown whether any of those individuals belonged to the dwarf morphotype. Altogether, these findings suggest that the morphological differences associated with the pollination shift were not a consequence of polyploidy but may have arisen through ecological filtering and selection. Nonetheless, differences in population genetic structure between arboreal and dwarf populations may also exist. While polyploidy and variation in genome size represent large-scale chromosomal rearrangements, other forms of genomic variation, such as polymorphisms or large-scale chromosomal inversions, were not investigated in this study and may also have contributed to the pollinator shift.

Among the abiotic forces that can influence plant life history, altitude is a particularly interesting geographical factor because substantial environmental variation can occur over short spatial scales, potentially promoting phenotypic differentiation among populations distributed along elevational gradients (Körner, 2007). Indeed, dwarf ecotypes of native plant species have been documented in high-elevation habitats (De Toma *et al.*, 2022). In the southernmost regions of the Brazilian Cerrado, areas located at relatively high altitudes, such as the Botucatu municipality, are subject to both fire and frost, two environmental stressors that may favour individuals capable of protecting their meristems below ground (for fire effects on the Cerrado ecosystem see Hoffmann *et al.*, 2019; Pilon *et al.*, 2022; Ballarin *et al.*, 2024*a*). Altogether, these findings suggest that, in addition to the low soil fertility associated with seasonal drought, which has already been identified as a factor driving dwarfism in Cerrado vegetation (Oliveira-Filho *et al.*, 1989), fire and frost may act as selective pressures that constrain plant size and height (Chiminazzo *et al.*, 2025*a*, 2025*b*).

In addition to the proximity of flowers to the ground resulting from dwarfism in the southernmost populations of *C. brasiliense*, a factor that may discourage bat visitation (Diniz *et al.*, 2019), the shift in flower anthesis seems to be the primary trait limiting bat pollination, as it decouples glossophagine bat foraging behaviour from floral resource availability. It is important to note, however, that nectarivorous bats can effectively access the nectar and pollinate the smaller flowers of the dwarf form, suggesting that changes in floral morphology alone do not constitute a reproductive barrier. Nevertheless, the delayed floral opening after 0200 h probably reduces bat visitation, as these trap-lining species plan their foraging routes in the early evening (Sazima *et al.*, 1999). In contrast, peak nectar availability coincides with the foraging period of bees, which may have driven the synchronization of floral traits with diurnal pollinator activity (Takimoto *et al.*, 2022). Therefore, under reduced visitation by bats, individuals with smaller flowers and diurnally available floral rewards may have achieved greater reproductive success due to more frequent bee visits.

In this context, we outline two possible explanations for the convergence of floral traits in the dwarf form toward bee preferences. First, the reduction in floral size, shifts in anthesis timing and changes in nectar sugar profile could have arisen directly through bee-mediated selection, as bees currently act as the primary pollinators of the dwarf form. Alternatively, dwarfism may have initially reduced plant height and produced smaller flowers, with subsequent modifications in anthesis timing and nectar traits resulting from altered pollinator visitation and an improved mechanical fit to bee size. In this scenario, developmental constraints could also play a role: allometry may have caused smaller flowers as a proportional consequence of reduced plant size (see Wanderley *et al.*, 2016), whereas pleiotropy may have led a single genetic change to simultaneously affect multiple traits, such as plant height and flower size (see

Johnson, 2025). However, given the lack of comparative genetic data and the limited knowledge of the relative evolutionary histories of the two forms, it is not possible to unequivocally favour one scenario over the other.

Besides changes in nectar production dynamics, bees adjusted their activity in response to the amount of nectar produced by the dwarf form of *C. brasiliense*, which is comparatively richer in sucrose and thus offers a higher energetic yield than the sucrose-lacking nectar of the arboreal form. Although nectar production is widespread in flowering plants (Ballarin *et al.*, 2024*b*), differences in sugar chemical composition between plant species are well documented across clades adapted to distinct pollinators, with bee-pollinated plants typically producing sucrose-rich nectar, and bat-pollinated plants favouring hexose-rich nectar (Baker and Baker, 1983; Schmidt-Lebuhn *et al.*, 2007; Kröemer *et al.*, 2008; Amorim *et al.*, 2013; Abrahamczyk *et al.*, 2017).

Fruit and seed set in the dwarf form of *C. brasiliense* were markedly higher than those previously reported for the arboreal form (Gribel and Hay, 1993; Roque *et al.*, 2023). It is possible that the reduced flower size increased the potential for self-pollination in the dwarf ecotype, allowing reproductive assurance in scenarios with limited bat visitation. Nonetheless, nocturnally pollinated flowers produced fewer seeds than those pollinated during the day. It is worth noting, however, that part of the fruit production observed in nocturnally pollinated flowers may have been facilitated by visits from nocturnal bees of the genus *Ptiloglossa*, which were also recorded visiting flowers at night.

Although *C. brasiliense* is a self-compatible species, this outcome was expected given the negligible number of bat visits, which probably resulted in a predominance of self-pollination. Previous studies have suggested that self-pollination in *C. brasiliense* may contribute to seed abortion (Collevatti *et al.*, 2009). Therefore, while self-pollination may provide reproductive assurance under pollinator scarcity, the observed shift from bats to bees probably represents a more reliable evolutionary pathway for maintaining reproductive success in the dwarf form of *C. brasiliense*.

Bat pollination has been regarded as an evolutionary dead end (Tripp and Manos, 2008). Although pollinator shifts involving bats and bees more commonly occur in the opposite direction, i.e. from bee to bat pollination (Knox *et al.*, 2008; Tripp and Manos, 2008; Martén-Rodríguez *et al.*, 2010; Rosas-Guerrero et al., 2014), cases of reversal from bat to bee pollination have been reported, such as in the genus *Cayaponia* (Curcurbitaceae; Duchen and Renner, 2010; Kobal *et al.*, 2024). Shifts from diurnal to nocturnal pollination systems have previously been documented (Luckow and Hopkins, 1995; Perret *et al.*, 2003; Tripp and Manos, 2008; Martén-Rodríguez *et al.*, 2010), but these are typically accompanied by conspicuous changes in floral morphology and attraction. In our study, the reduced floral size alone was not sufficient to explain the reduced bat visitation or increased bee activity. Rather, the shift in floral anthesis and nectar dynamics appears to have played a central role in enhancing compatibility with diurnal pollinators. Although changes in flower lifespan in response to pollination have been documented as an energy-saving strategy in harsh environments (Arroyo *et al.*, 2022), this study represents, to the best of our knowledge, the first report of a pollination-mediated shift in anthesis timing resulting from a change in primary pollinators. Our findings also reinforce the role of generalist floral visitors, such as bees, as effective pollinators under specific ecological contexts. Furthermore, considering that other floral traits such as scent (Peter and Johnson, 2014; Sayers *et al.*, 2021), reward type (Castañeda-Zárate *et al.*, 2021) and colour (Bradshaw and Schemske, 2003) have been implicated in pollinator shifts, we encourage future studies to explore how changes in chemical and visual cues, as well as in resource quality and quantity, might further facilitate bee attraction and reinforce the ongoing transition to diurnal pollination in this system.

CONCLUSION

In this study, we suggest that past adaptations to local environmental pressures have led to a pollinator shift from bats to bees in the dwarf ecotype of the cultural keystone species *C. brasiliense* in the southernmost Brazilian Cerrado. The transition from bat- to bee-mediated pollination illustrates how shifts in different floral traits (i.e. floral anthesis timing) and resource presentation can mediate changes in reproductive interactions, even in the absence of reproductive barriers or polyploidy. This case highlights the potential evolutionary relevance of temporal mismatches between floral traits and pollinator activity, suggesting that anthesis timing, nectar dynamics and nectar chemical composition may be as influential as morphological divergence in promoting pollinator turnover.

ACKNOWLEDGEMENTS

We are grateful to all members of the Laboratório de Ecologia da Polinização Interações (LEPI) and to Hélder Consolaro for their valuable assistance during fieldwork, Eduardo Almeida for bee identification, and Carmen Boaro for help with nectar chemical analysis. We thank Christiano Peres Coelho and Paulo E. Oliveira for kindly providing and granting permission to use the image in Fig. 1D. We are also grateful to Isabela Varassin, Leandro Freitas, Patrícia Morellato, Pietro Maruyama and Pedro Bergamo for their valuable suggestions on an early draft of the manuscript. We thank Arthur Domingos-Melo and two anonymous reviewers for their constructive comments, which helped us to markedly improve the presentation of the study, and André S. Ballarin for providing the data and plots of historical climatic trends for the studied sites. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasil) supported C.S.B., P.A.L.B. (Finance Code 001) and F.G.C. (#88881.083368/2024-01). C.S.B. also thanks the CAPES Internacionalization Programme (CAPES PrInt) for a sandwich PhD scholarship (#88887.839479/2023-00) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, #2024/02640-1) for a postdoctoral research grant. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) supported A.P.M. (#312855/2021-4) and F.W.A. (#308559/2022-3). A.P.M. also thanks the Fundação de Apoio à Pesquisa do Estado de São Paulo—FAPESP (Proc. 2022/05890-3). A.P.M., C.S.B. and F.W.A. are affiliated with the Center for Research on Biodiversity Dynamics and Climate Change (CEPID FAPESP#2021/10639-5).

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following. Video S1. Time-lapse of floral anthesis in the dwarf ecotype of pequi (*Caryocar brasiliense* subsp. *intermedium*) over two consecutive nights in the southernmost region of the Brazilian Cerrado. At the bottom of the video, temperature and time are displayed for each image, captured at 5-min intervals from 2315 h on 20 December 2020 to 0723 h on 21 December 2020. As the camera was activated by heat and movement, bee visitations were also recorded in the images. Figure S1. Historical trends and monthly variation in water balance and mean temperature. (a) Annual water balance (mm year^{-1}), (b) annual mean temperature (°C), (c) monthly water balance (mm month^{-1}) and (D) monthly mean temperature (°C) for Caldas Novas – GO (purple), Uberlândia – MG (light purple) and Botucatu – SP (green). Water balance represents the difference between precipitation and potential evapotranspiration, with positive values indicating a water surplus and negative values indicating a deficit. Mean temperatures are lower in Botucatu – SP, where minimum temperatures frequently drop below 10 °C, suggesting that cold stress (frost) adds to the ecological pressures shaping the dwarf form of *C. brasiliense* in this region. Figure S2. Long-term and monthly temperature patterns across the study regions. (a) Monthly maximum temperature (°C), (b) monthly minimum temperature (°C), (c) annual number of hot days ($T_{\max} \geq 30$ °C) and (d) annual number of cold days ($T_{\min} \leq 10$ °C) in Caldas Novas – GO (purple), Uberlândia – MG (light purple) and Botucatu – SP (green). The arboreal form of *C. brasiliense* occurs in Caldas Novas and Uberlândia, while the dwarf form occurs in Botucatu. Average temperatures are consistently lower in Botucatu, where, in addition to the natural fire regime typical of Cerrado environments, frost events also occur frequently and probably exert selective pressures on the dwarf populations. Table S1. Locations of study sites for the investigation of *C. brasiliense* ecotypes. Botucatu – SP is characterized as Cerrado ‘campo sujo’, referring to an open savanna with scattered shrubs and small trees; this site hosts dwarf morphs not only of *C. brasiliense* but also of other plant species such as *Palicourea rigida* (Rubiaceae) and *Byrsonima intermedia* (Malpighiaceae). Uberlândia – MG corresponds to Cerrado ‘*sensu stricto*’, which features a denser savanna with a continuous tree layer and a well-developed shrub stratum. Caldas Novas – GO represents Cerrado ‘*sensu lato*’, a mosaic of open savanna and forested patches with taller vegetation and higher tree density. Table S2. Flower morphometric traits of the dwarf and the arboreal forms of *C. brasiliense*. Letters after mean ± s.d. values depict multiple comparisons among ecotypes with a Tukey *post hoc* test. Subscript numbers before *F* values represent degrees of freedom. Table S3. Day and night sampling effort and floral visitation in the dwarf ecotype of *C. brasiliense.* Table S4. List of bee species recorded visiting the dwarf ecotype of *C. brasiliense*.

REFERENCES

Abrahamczyk S, **Kessler M**, **Hanley D**, ***et al.* 2017**. Pollinator adaptation and the evolution of floral nectar sugar composition. *Journal of Evolutionary Biology* **30**: 112–127. doi:10.1111/jeb.12991

Ab’Saber AN. 2000. Spaces occupied by the expansion of dry climates in South America during the Quaternary ice ages. *Revista do Instituto Geológico* **21**: 71–78. doi:10.5935/0100-929X.20000006

Aigner PA. 2001. Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? *Oikos* **95**: 177–184. doi:10.1034/j.1600-0706.2001.950121.x

Amorim FW, **Ballarin CS**, **Spicacci G**, ***et al.* 2023**. Opossums and birds facilitate the unexpected bat visitation to the ground-flowering *Scybalium fungiforme*. *Ecology* **104**: e3935. doi:10.1002/ecy.3935

Amorim FW, **Galetto L**, **Sazima M. 2013**. Beyond the pollination syndrome: nectar ecology and the role of diurnal and nocturnal pollinators in the reproductive success of *Inga sessilis* (Fabaceae). *Plant Biology* **15**: 317–327. doi:10.1111/j.1438-8677.2012.00643.x

Araujo FF, **Araújo PDCS**, **Siqueira E**, ***et al.* 2020**. Nocturnal bees exploit but do not pollinate flowers of a common bat-pollinated tree. *Arthropod-Plant Interactions* **14**: 785–797. doi:10.1007/s11829-020-09784-3

Armbruster WS. 1985. Patterns of character divergence and the evolution of reproductive ecotypes of *Dalechampia scandens* (Euphorbiaceae). *Evolution; International Journal of Organic Evolution* **39**: 733–752. doi:10.1111/j.1558-5646.1985.tb00416.x

Arroyo MT, **Cuartas-Dominguez M**, **Robles V**, ***et al.* 2022**. Pollination-associated shortening of the functional flower lifespan in an alpine species of *Alstroemeria* and the water content of flowers. *Alpine Botany* **132**: 245–256. doi:10.1007/s00035-022-00281-2

Baker HG, **Baker I. 1983**. Floral nectar sugar constituents in relation to pollinator type. In: **Jones CE**, **Little RJ.** eds. *Handbook of experimental pollination biology*. New York: Van Nostrand Reinhold Co., 117–141.

Ballarin CS, **Fontúrbel FE**, **Rech AR**, ***et al.* 2024*b***. How many animal-pollinated angiosperms are nectar-producing? *New Phytologist* **243**: 2008–2020. doi:10.1111/nph.19940

Ballarin CS, **Hachuy-Filho L**, **Doria MJW**, ***et al.* 2022**. Intra-seasonal and daily variations in nectar availability affect bee assemblage in a monodominant afforested Brazilian Cerrado. *Austral Ecology* **47**: 1315–1328. doi:10.1111/aec.13218

Ballarin CS, **Mores GJ**, **Alcarás de Goés G**, **Fidelis A**, **Cornelissen T. 2024*a***. Trends and gaps in the study of fire effects on plant–animal interactions in Brazilian ecosystems. *Austral Ecology* **49**: e13420. doi:10.1111/aec.13420

Bobrowiec PED, **Oliveira PE. 2012**. Removal effects on nectar production in bat-pollinated flowers of the Brazilian Cerrado. *Biotropica* **44**: 1–5. doi:10.1111/j.1744-7429.2011.00823.x

Bradshaw Jr HD, **Schemske DW. 2003**. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**: 176–178. doi:10.1038/nature02106

Campos FG, **Dantas MO**, **Santos JP**, **Froes SS**, **Gama JP**, **Boaro CS. 2023**. UV-B radiation in the acclimatization mechanism of *Psidium guajava* in sunlight. *Horticulturae* **9**: 1291. doi:10.3390/horticulturae9121291

Cássia-Silva C, **Oliveira RS**, **Sales LP**, ***et al.* 2022**. Acaulescence promotes speciation and shapes the distribution patterns of palms in Neotropical seasonally dry habitats. *Ecography* **2022**: e06072. doi:10.1111/ecog.06072

Castañeda-Zarate M, **Johnson SD**, **van der Niet T. 2021**. Food reward chemistry explains a novel pollinator shift and vestigialization of long floral spurs in an orchid. *Current Biology* **31**: 238–246.e7. doi:10.1016/j.cub.2020.10.024

Chiminazzo MA, **Charles-Dominique T**, **Andrade RS**, **Bombo AB**, **Fidelis A. 2025*a***. How do plants survive in the starving, burning, and hiding vegetation realms generated by novel fire regimes? *Perspectives in Plant Ecology, Evolution and Systematics* **68**: 125885. doi: 10.1016/j.ppees.2025.125885

Chiminazzo MA, **Charles-Dominique T**, **Fidelis A**, **Bombo AB**, **Jones C**, **Diggle P. 2025*b***. Plant form and height across environmental gradients: a developmental perspective on plant intraspecific variability. *Annals of Botany* mcaf130. doi: 10.1093/aob/mcaf130

Collevatti RG, **Estolano R**, **Garcia SF**, **Hay JD. 2009**. Seed abortion in the bat pollinated Neotropical tree species, *Caryocar brasiliense* (Caryocaraceae). *Botany* **87**: 1110–1115. doi:10.1139/B09-054

Collevatti RG, **Grattapaglia D**, **Hay JD. 2003**. Evidences for multiple maternal lineages of *Caryocar brasiliense* populations in the Brazilian Cerrado based on the analysis of chloroplast DNA sequences and microsatellite haplotype variation. *Molecular Ecology* **12**: 105–115. doi:10.1046/j.1365-294X.2003.01701.x

Collevatti RG, **Lima-Ribeiro MS**, **Souza-Neto AC**, **Franco AA**, **Oliveira G**, **Terribile LC. 2012**. Recovering the demographical history of a Brazilian Cerrado tree species *Caryocar brasiliense*: coupling ecological niche modeling and coalescent analyses. *Natureza & Conservação* **10**: 169–176. doi:10.4322/natcon.2012.024

de Matos Costa K, **Primo LM**, **Machado IC**, ***et al.* 2025**. Floral reward and mechanical fit of brush-type flowers to nocturnal and diurnal pollinators: an analysis of two sympatric Capparaceae species: de Matos Costa et al. *Folia Geobotanica* **60**: 1–16. doi: 10.1007/s12224-025-09465-0

De Toma A, **Carboni M**, **Bazzichetto M**, **Malavasi M**, **Cutini M. 2022**. Dynamics of dwarf shrubs in Mediterranean high-mountain ecosystems. *Journal of Vegetation Science* **33**: e13143. doi:10.1111/jvs.13143

Dias AB. 2017. Ecologia da polinização e troca de polinizadores de Caryocar brasiliense subsp. intermedium (Caryocaraceae), em área meridional do Cerrado. PhD Thesis, São Paulo State University, Brazil.

Dias CAR, **Santos Junior JE**, **Perini FA**, **Santos FR. 2017**. Biogeographic scenarios for the diversification of a widespread Neotropical species, *Glossophaga soricina* (Chiroptera: Phyllostomidae). *Systematics and Biodiversity* **15**: 440–450. doi:10.1080/14772000.2016.1271060

Diniz UM, **Domingos-Melo A**, **Machado IC. 2019**. Flowers up! The effect of floral height along the shoot axis on the fitness of bat-pollinated species. *Annals of Botany* **124**: 809–818. doi:10.1093/aob/mcz116

Dixon P. 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* **14**: 927–930. doi:10.1111/j.1654-1103.2003.tb02228.x

Doležel J, **Sgorbati S**, **Lucretti S. 1992**. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiologia Plantarum* **85**: 625–631. doi:10.1111/j.1399-3054.1992.tb04764.x

Doyle JJ, **Coate JE. 2019**. Polyploidy, the nucleotype, and novelty: the impact of genome doubling on the biology of the cell. *International Journal of Plant Sciences* **180**: 1–52. doi: 10.1086/700636

Duchen P, **Renner SS. 2010**. The evolution of *Cayaponia* (Cucurbitaceae): repeated shifts from bat to bee pollination and long-distance dispersal to Africa 2–5 million years ago. *American Journal of Botany* **97**: 1129–1141. doi:10.3732/ajb.0900385

Ebihara A, **Ishikawa H**, **Matsumoto S**, ***et al.* 2005**. Nuclear DNA, chloroplast DNA, and ploidy analysis clarified biological complexity of the *Vandenboschia radicans* complex (Hymenophyllaceae) in Japan and adjacent areas. *American Journal of Botany* **92**: 1535–1547. doi:10.3732/ajb.92.9.1535

Ehrendorfer F, **Morawetz W**, **Dawe J. 1984**. The neotropical angiosperm families Brunelliaceae and Caryocaraceae: first karyosystematical data and affinities. *Plant Systematics and Evolution* **145**: 183–191. doi:10.1007/BF00983947

Etterson JR, **Mazer SJ. 2016**. How climate change affects plants' sex lives. *Science* **353**: 32–33. doi:10.1126/science.aag1624

Etterson JR, **Toczydlowski RH**, **Winkler KJ**, **Kirschbaum JA**, **McAulay TS. 2016**. *Solidago altissima* differs with respect to ploidy frequency and clinal variation across the prairie-forest biome border in Minnesota. *American Journal of Botany* **103**: 22–32. doi:10.3732/ajb.1500146

Galetto L, **Bernardello G. 2005**. Rewards in flowers: nectar. In: **Dafni A**, **Kevan PG**, **Husband BC.** eds. *Practical pollination biology*. Cambridge: Enviroquest Ltd, 261–313.

Grant V, **Grant KA. 1965**. *Flower pollination in the Phlox family*. New York: Columbia University Press.

Gribel R, **Hay JD. 1993**. Pollination ecology of *Caryocar brasiliense* (Caryocaraceae) in central Brazil cerrado vegetation. *Journal of Tropical Ecology* **9**: 199–211. doi:10.1017/S0266467400007173

Hoffman AM, **Bushey JA**, **Ocheltree TW**, **Smith MD. 2020**. Genetic and functional variation across regional and local scales is associated with climate in a foundational prairie grass. *New Phytologist* **227**: 352–364. doi: 10.1111/nph.16547

Hoffmann WA, **Flake SW**, **Abreu RC**, **Pilon NA**, **Rossatto DR**, **Durigan G. 2019**. Rare frost events reinforce tropical savanna–forest boundaries. *The Journal of Ecology* **107**: 468–477. doi:10.1111/1365-2745.13047

Johnson SD. 2010. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 499–516. doi:10.1098/rstb.2009.0243

Johnson SD. 2025. Pollination ecotypes and the origin of plant species. *Proceedings of the Royal Society of London: Series B, Biological Sciences* **292**: 20242787. doi:10.1098/rspb.2024.2787

Johnson SD, **Harder LD**, **Barrett SCH. 2006**. Pollinator-driven speciation in plants. In: **Harder LD**, **Barrett SCH.** eds. *Ecology and evolution of flowers*. Oxford: Oxford University Press, 295–310.

Kay KM, **Anderson B. 2025**. Beyond the Grant–Stebbins model: floral adaptive landscapes and plant speciation. *Annals of Botany* **136**: 699–720. doi: 10.1093/aob/mcaf096

Klink CA, **Machado RB. 2005**. Conservation of the Brazilian cerrado. *Conservation Biology* **19**: 707–713. doi:10.1111/j.1523-1739.2005.00702.x

Knox EB, **Muasya AM**, **Muchhala N. 2008**. The predominantly South American clade of Lobeliaceae. *Systematic Botany* **33**: 462–468. doi:10.1600/036364408784571590

Kobal ROAC, **Buzato S**, **Nunes CEP**, ***et al.* 2024**. Bat or bee pollination? Floral biology of two sympatric *Cayaponia* species (Cucurbitaceae) in Southeast Brazil. *Flora* **319**: 152594. doi:10.1016/j.flora.2024.152594

Körner C. 2007. The use of 'altitude' in ecological research. *Trends in Ecology & Evolution* **22**: 569–574. doi:10.1016/j.tree.2007.09.006

Kröemer T, **Kessler M**, **Lohaus G**, **Schmidt-Lebuhn AN. 2008**. Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae. *Plant Biology* **10**: 502–511. doi:10.1111/j.1438-8677.2008.00058.x

Lenth RV. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software* **69**: 1–33. doi:10.18637/jss.v069.i01

LoPresti EF, **Van Wyk JI**, **Mola JM**, **Toll K**, **Miller TJ**, **Williams NM. 2018**. Effects of wildfire on floral display size and pollinator community reduce outcrossing rate in a plant with a mixed mating system. *American Journal of Botany* **105**: 1154–1164. doi:10.1002/ajb2.1129

Luckow M, **Hopkins HC. 1995**. A cladistic analysis of *Parkia* (Leguminosae: Mimosoideae). *American Journal of Botany* **82**: 1300–1320. doi:10.1002/j.1537-2197.1995.tb12664.x

Maglio CAFP, **Forni-Martins ER**, **Cruz ND. 1984**. Chromosome Reports in Löve, Á. (1984). Chromosome Number Reports LXXXIV. *Taxon* **33**: 536–538. doi:10.1002/j.1996-8175.1984.tb03913.x

Martén-Rodríguez S, **Fenster CB**, **Agnarsson I**, **Skog LE**, **Zimmer EA. 2010**. Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist* **188**: 403–417. doi:10.1111/j.1469-8137.2010.03330.x

Miner BG, **Sultan SE**, **Morgan SG**, **Padilla DK**, **Relyea RA. 2005**. Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution* **20**: 685–692. doi:10.1016/j.tree.2005.08.002

Moir M, **Butler H**, **Peter C**, **Dold T**, **Newman E. 2025**. A test of the Grant-Stebbins pollinator-shift model of floral evolution. *New Phytologist* **245**: 2322–2335. doi:10.1111/nph.20373

Muchhala N, **Caiza A**, **Vizuete JC**, **Thomson JD. 2009**. A generalized pollination system in the tropics: bats, birds and *Aphelandra acanthus*. *Annals of Botany* **103**: 1481–1487. doi:10.1093/aob/mcn260

Newman E, **Manning J**, **Anderson B. 2014**. Matching floral and pollinator traits through guild convergence and pollinator ecotype formation. *Annals of Botany* **113**: 373–384. doi:10.1093/aob/mct203

Oliveira-Filho AT, **Shepherd GJ**, **Martins FR**, **Stubblebine WH. 1989**. Environmental factors affecting physiognomic and floristic variation in an area of cerrado in central Brazil. *Journal of Tropical Ecology* **5**: 413–431. doi:10.1017/S0266467400003862

Paiva EAS, **Dötterl S**, **De-Paula OC**, ***et al.* 2019**. Osmophores of *Caryocar brasiliense* (Caryocaraceae): a particular structure of the androecium that releases an unusual scent. *Protoplasma* **256**: 971–981. doi:10.1007/s00709-019-01356-4

Pardee GL, **Inouye DW**, **Irwin RE. 2018**. Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wildflowers. *Global Change Biology* **24**: 848–857. doi:10.1111/gcb.13865

Pellicer J, **Leitch IJ. 2014**. The application of flow cytometry for estimating genome size and ploidy level in plants. In: **Besse P.** ed. *Molecular plant taxonomy*. New York: Springer, 279–309.

Pérez-Barrales R, **Arroyo J**, **Armbruster WS. 2007**. Differences in pollinator faunas may generate geographic differences in floral morphology and integration in *Narcissus papyraceus* (Amaryllidaceae). *Oikos* **116**: 1904–1918. doi:10.1111/j.0030-1299.2007.15994.x

Perret M, **Chautems A**, **Spichiger R**, **Kite G**, **Savolainen V. 2003**. Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear ncpGS. *American Journal of Botany* **90**: 445–460. doi:10.3732/ajb.90.3.445

Peter CI, **Johnson SD. 2014**. A pollinator shift explains floral divergence in an orchid species complex in South Africa. *Annals of Botany* **113**: 277–288. doi:10.1093/aob/mct216

Pilon NAL, **Cava MG**, **Hoffmann WA**, **Abreu RC**, **Rossatto DR**, **Durigan G. 2022**. Effects and response of the Cerrado ground-layer to frost along the canopy cover gradient. *Oecologia* **200**: 199–207. doi:10.1007/s00442-022-05259-9

Porturas LD, **Anneberg TJ**, **Curé AE**, **Wang S**, **Althoff DM**, **Segraves KA. 2019**. A meta-analysis of whole genome duplication and the effects on flowering traits in plants. *American Journal of Botany* **106**: 469–476. doi:10.1002/ajb2.1258

Potts SG, **Vulliamy B**, **Dafni A**, ***et al.* 2003**. Response of plant-pollinator communities to fire: changes in diversity, abundance and floral reward structure. *Oikos* **101**: 103–112. doi:10.1034/j.1600-0706.2003.12186.x

Prance GT, **Silva MF. 1973**. Caryocaraceae. *Flora Neotropica* **12**: 1–75. https://www.jstor.org/stable/4393688

Primo LM, **Domingos-Melo A**, **Galetto L**, **Machado IC. 2022**. Nectar secretion patterns are associated to nectar accessibility in a guild of crepuscular-nocturnal flowering plants. *Plant Ecology* **223**: 951–964. doi:10.1007/s11258-022-01250-9

Rezende L, **Suzigan J**, **Amorim FW**, **Moraes AP. 2020**. Can plant hybridization and polyploidy lead to pollinator shift? *Acta Botanica Brasilica* **34**: 229–242. doi:10.1590/0102-33062020abb0025

Rodríguez-Sánchez GT, **Pelayo RC**, **Soriano PJ**, **Knight TM. 2024**. Intraspecific variation in pollination ecology due to altitudinal environmental heterogeneity. *Ecology and Evolution* **14**: e11553. doi:10.1002/ece3.11553

Roque SQ, **Falcão LA**, **Rech AR**, ***et al.* 2023**. Reproductive biology of *Caryocar brasiliense* (Caryocaraceae) in preserved and degraded Cerrado areas in Brazil. *Botany* **101**: 357–365. doi:10.1139/cjb-2022-0132

Rosas-Guerrero V, **Aguilar R**, **Martén-Rodríguez S**, ***et al.* 2014**. A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecology Letters* **17**: 388–400. doi:10.1111/ele.12224

Sayers TD, **Johnson KL**, **Steinbauer MJ**, **Farnier K**, **Miller RE. 2021**. Divergence in floral scent and morphology, but not thermogenic traits, associated with pollinator shift in two brood-site-mimicking *Typhonium* (Araceae) species. *Annals of Botany* **128**: 261–280. doi:10.1093/aob/mcab044

Sazima M, **Buzato S**, **Sazima I. 1999**. Bat-pollinated flower assemblages and bat visitors at two Atlantic forest sites in Brazil. *Annals of Botany* **83**: 705–712. doi:10.1006/anbo.1999.0876

Schmidt-Lebuhn AN, **Schwerdtfeger M**, **Kessler M**, **Lohaus G. 2007**. Phylogenetic constraints vs. ecology in the nectar composition of Acanthaceae. *Flora* **202**: 62–69. doi:10.1016/j.flora.2006.02.005

Segura-Garcia C, **Alencar A**, **Arruda VLS**, ***et al.* 2025**. The fire regimes of the Cerrado and their changes through time. *Philosophical Transactions of the Royal Society B: Biological Sciences* **380**: 20230460. doi:10.1098/rstb.2023.0460

Silva JF, **Fariñas MR**, **Felfili JM**, **Klink CA. 2006**. Spatial heterogeneity, land use and conservation in the cerrado region of Brazil. *Journal of Biogeography* **33**: 536–548. doi:10.1111/j.1365-2699.2005.01422.x

Simon MF, **Grether R**, **de Queiroz LP**, **Skema C**, **Pennington RT**, **Hughes CE. 2009**. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences* **106**: 20359–20364. doi:10.1073/pnas.0903410106

Simon MF, **Pennington T. 2012**. Evidence for adaptation to fire regimes in the tropical savannas of the Brazilian Cerrado. *International Journal of Plant Sciences* **173**: 711–723. doi:10.1086/665973

Souza CS, **Oliveira PE**, **Rosa BB**, **Maruyama PK. 2022**. Integrating nocturnal and diurnal interactions in a Neotropical pollination network. *The Journal of Ecology* **110**: 2145–2155. doi:10.1111/1365-2745.13937

Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annual Review of Ecology and Systematics* **1**: 307–326. doi:10.1146/annurev.es.01.110170.001515

Suksom W, **Wannachai W**, **Boonchiangma S**, **Chanthai S**, **Srijaranai S. 2015**. Ion chromatographic analysis of monosaccharides and disaccharides in raw sugar. *Chromatographia* **78**: 873–879. doi:10.1007/s10337-015-2865-3

Takimoto G, **Kagawa K**, **Satow T**, **Sakamoto T. 2022**. Increased floral rewards due to local adaptation drives plant ecological speciation via learned preferences of pollinators. *The American Naturalist* **200**: 834–845. doi:10.1086/721764

Thomson JD, **Wilson P. 2008**. Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. *International Journal of Plant Sciences* **169**: 23–38. doi:10.1086/523361

Tripp EA, **Manos PS. 2008**. Is floral specialization an evolutionary dead-end? Pollination system transitions in *Ruellia* (Acanthaceae). *Evolution; International Journal of Organic Evolution* **62**: 1712–1737. doi:10.1111/j.1558-5646.2008.00398.x

Turcotte MM, **Kaufmann N**, **Wagner KL**, **Zallek TA**, **Ashman T-L. 2024**. Neopolyploidy increases stress tolerance and reduces fitness plasticity across multiple urban pollutants: support for the "general-purpose" genotype hypothesis. *Evolution Letters* **8**: 416–426. doi:10.1093/evlett/qrad072

Van de Peer Y, **Ashman T-L**, **Soltis PS**, **Soltis DE. 2021**. Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell* **33**: 11–26. doi:10.1093/plcell/koaa015

Van der Niet T, **Pirie MD**, **Shuttleworth A**, **Johnson SD**, **Midgley JJ. 2014**. Do pollinator distributions underlie the evolution of pollination ecotypes in the Cape shrub *Erica plukenetii*? *Annals of Botany* **113**: 301–316. doi:10.1093/aob/mct193

Wanderley AM, **Galetto L**, **Machado IC. 2016**. Functional decoupling between flowers and leaves in the *Ameroglossum pernambucense* complex can facilitate local adaptation across a pollinator and climatic heterogeneous landscape. *Journal of Evolutionary Biology* **29**: 528–540. doi:10.1111/jeb.12802