Mixed-vertebrate pollination traits and pollinators of *Haageocereus acranthus* (Cactaceae) in a Lomas desert ecosystem of coastal Peru

Bernardo Garcia-Simpson[1*]

Braulio A. Prado[1,2]

Juan J. Pellón[3]

Alfonso Valiente-Banuet[4]

[1]Círculo de Investigación en Taxonomía, Florística y Ecología Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad Nacional Agraria La Molina, Lima, Perú

[2]Círculo de Investigación en Mamíferos del Perú, Departamento de Biología, Facultad de Ciencias, Universidad Nacional Agraria La Molina, Lima, Perú

[3]Laboratorio de Ecología y Conservación de Vertebrados Terrestres, Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México, México

[4]Laboratorio de Ecología de Comunidades, Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México, México

Running title: Mixed-vertebrate pollination in *Haageocereus acranthus*

*For correspondence. E-mail: bernardogarcia2303@gmail.com

Keywords: Bat pollination, floral traits, hummingbird pollination, intraspecific variation, morphology, phenology.

Key message: In a Lomas desert ecosystem of coastal Peru, the columnar cactus *Haageocereus acranthus* shows traits and interactions consistent with a mixed-vertebrate pollination system (hummingbirds and bats), with no detectable differences between flower color morphotypes in either traits or interactions.

Abstract

(1) Cacti are key components of arid ecosystems and, being mostly self-incompatible, rely on animal pollination. Although bee pollination is ancestral, systems supported by birds, moths, bats, and mixed strategies have evolved. Despite the ecological uniqueness and extreme seasonality of the fog-dependent Lomas of coastal Peru, cacti pollination remains unstudied. This work examines *Haageocereus acranthus*, a characteristic columnar cactus of this ecosystem, in a population where individuals produce either white or pink-red flowers. It was hypothesized that white flowers would be associated with bat pollination and pink-red flowers with hummingbird pollination, reflected in differences in floral morphology, phenology and pollinator visitation.

(2) Year-round monitoring of the population was conducted to characterize flowering phenology. Floral morphology, daily anthesis patterns, and nectar production were quantified and compared between color morphs. Floral visitor frequency and behavior were recorded using camera traps to test for pollinator preference.

(3) Floral phenology, morphology, and nectar characteristics were broadly consistent with vertebrate pollination, showing traits associated primarily with bat pollination but also compatible with hummingbird pollination. These floral traits did not differ between color morphs. Hummingbirds were the most frequent visitors, followed by bats; yet, neither group showed preference for a specific flower color.

(4) Findings support a mixed pollination system involving both hummingbirds and bats in an ecosystem where the availability of pollinators can shift over geographic or temporal scales. This pollinator unpredictability may ensure consistent reproductive success and reduce vulnerability to the absence or decline of specific pollinator groups.

Introduction

Pollination is one of the most important mutualistic plant-animal interactions and has played a central role in angiosperm diversification (Vamosi & Vamosi 2010; Ballesteros-Mejia *et al.* 2016). These interactions reflect the evolutionary innovations of flowers in response to different ecological conditions (Armbruster 2017; Opedal 2019). In this context, species sharing pollinators have been under similar selective pressures, resulting in a convergence of floral traits known as pollination syndromes (Faegri & van der Pijl 1979; Fenster *et al.* 2004; Dellinger 2020).

Pollination syndromes have frequently been considered a principle governing plant-pollinator interactions and used to infer functional pollinator groups based on flower traits (Proctor *et al.* 1996; Fenster *et al.* 2004). However, as the understanding of pollination has expanded, increasing evidence supports that these patterns are more complex than the traditional syndromes hypothesis suggests (Ollerton *et al.* 2009; Rosas-Guerrero *et al.* 2014; Dellinger 2020). Pollinator assemblages can be highly variable across the distribution of a particular plant species (Thompson 2002), and interactions often show geographical or temporal changes (Olesen & Jordano 2002; Burkle & Alarcon 2011). Also, intraspecific variation in floral traits may contribute to deviations from consistent classic syndromes, as different morphotypes within a species distribution range can attract distinct pollinators groups (e.g., Schlumpberger *et al.* 2009; Cardona *et al.* 2020; Wenzell *et al.* 2025). These geographic patterns in interactions, along with intraspecific floral variation, can drive local adaptation, divergence in pollination strategies, and potentially lead to speciation (Kay & Sargent 2009).

Cacti are distributed across the Americas and constitute crucial components of arid and semi-arid ecosystems (Fleming *et al.* 2001; Fleming & Valiente-Banuet 2002). In addition, these are obligate out-crossers that depend on animals for pollination (Mandujano *et al.* 2009) and show adaptations mostly consistent with classic pollination syndromes (Grant & Grant 1979; Rowley 1980; Schlumpberger 2011). While bee pollination is ancestral in cacti, specialized systems for bird, moth, and bat pollination have evolved; including mixed pollination strategies (Schlumpberger 2011; Lendel 2013) (e.g., Sahley 1996;

Bustamante *et al.* 2010; Walter 2010). This diversity of strategies makes cacti an excellent group to explore and test hypotheses on pollination syndromes.

Peru is a cacti species-rich country with ~250 spp. distributed mainly in coastal and Andean ecosystems (Ulloa *et al.* 2004; Arakaki *et al.* 2006). However, although Peruvian cacti have received considerable attention at taxonomic, distributional and genetic studies (e.g., Ostolaza 1996, 2014; Arakaki *et al.* 2006, 2007, 2021; Calderón *et al.* 2007), scarce effort has been made to understand their ecological interactions (e.g., Sahley 1996; Novoa *et al.* 2005, 2022; Ceroni *et al.*, 2007).

Haageocereus acranthus (Tribe Trichocereeae) is a representative and abundant member of the genus, occurring predominantly in arid river valleys of Peru (Calderón *et al.* 2007). Flowers of *H. acranthus* are relatively large and robust, funnel- to tubular- shaped, single-opening, nocturnal, white colored and nectar-abundant. These traits have been putatively associated with bat and sphingid moth pollination by Calderón *et al.* (2007), based on classic syndromes proposal by Faegri & van der Pijl (1979). However, this species exhibits high variability in floral traits, including flower size, shape, and pigmentation ranging from white to pink-red. This variation, along with extended anthesis times (crepuscular and matutinal), suggests potential pollination by other groups, such as hummingbirds. While *Rodophis vesper* (Oasis hummingbird) and *Platalina genovensium* (Peruvian long-tongued bat) have been reported visiting *Haageocereus* flowers (Grillo & Arana 2016; Maguiña & Amanzo 2016), no further studies have analyzed these interactions in detail. Also, comparable traits occur in *Weberbauerocereus weberbaueri*, a closely related species pollinated by both bats and hummingbirds on the southern Peruvian coast (Sahley 1996).

This study examined the floral morphology, phenology, and pollinator assemblage of *Haageocereus acranthus* in a Lomas ecosystem of the Peruvian central desert coast, where the species exhibits two distinct flower colors: white and pink-red, each expressed by different individuals. We hypothesized that *H. acranthus* flowers would generally display traits associated with vertebrate pollination, particularly by bats and hummingbirds, and that these visitors would act as effective pollinators. Based on classic pollination syndromes, we further predicted intraspecific differences linked to flower color: white flowers would be more closely associated with bat pollination, whereas pink-red flowers would

show a stronger tendency toward hummingbird pollination. We anticipated that these flower types would occupy distinct regions of floral morphological space and exhibit different phenological patterns, with pink-red flowers showing a broader anthesis and nectar secretion period that extends into diurnal hours. Consequently, we also expected differences in visitation frequency between bats and hummingbirds across flower types.

Materials and Methods

Study area and species

This study was conducted in Cardal (Pachacamac, Lima, Peru) (12°11'S, 76°50'W; 215 m a.s.l.), a location with a previously documented *H. acranthus* population (Ostolaza 1996; Calderón *et al.* 2007) (Fig. 1A). This area is part of the Lomas, a unique ecosystem dependent on winter fogs, which is mainly restricted to coastal Peru and Chile, and characterized by two contrasting seasons. The wet season (May–Oct), with lower temperatures (13°C–16°C) and high relative humidity (95%–100%), promotes the growth of seasonal herbs. In contrast, during the dry season (Nov–Apr), with relative humidity ranging from 80%–90% and temperatures between 20°C–25°C, most herbs die off, leaving only perennial xerophytes (Dillon *et al.* 2011). Fieldwork was conducted from January 2022 to March 2023.

H. acranthus is a columnar cactus up to 1.5 m tall, with multiple vertical stems branching at the base and flowers often growing from areoles at the branch apex (Calderón *et al.* 2007) (Fig. 1B). Aside from observations of flowering from November to January, no detailed phenological information is available (Calderón *et al.* 2007; Maguiña & Amanzo 2016). Two flower types are present in this area: typical white flowers of *H. acranthus* subsp. *acranthus* and pink-red flowers of *H. acranthus* var. *olowinskianus* f. *rubriflorior* (Ostolaza 1996), currently a synonym of *H. acranthus* subsp. *acranthus* (Calderón *et al.* 2007; POWO 2024). Flower types are hereafter referred to as white and pink-red floral morphs.

Although *H. acranthus* dominates the site, other cacti co-occur at much lower densities, including *H. pseudomelanostele* (Werderm. & Backeb.) Backeb. and *Loxanthocereus acanthurus* (Vaupel) Backeb. The former is morphologically and phenologically similar to *H. acranthus* (Ostolaza 1996; Calderón et al. 2007), whereas the latter exhibits a hummingbird-pollination syndrome, flowering at a different time of the year (B. Garcia-Simpson, pers. obs.).

Annual phenology

We monthly monitored 30 tagged individuals throughout one year (Jan–Dec 2022). For every individual, we recorded the number of floral buds, open flowers and fruits (unripe and ripe). Unripe and ripe fruits were differentiated by coloration and firmness (firm and green for unripe, red and soft for ripe). Due to field limitations, monitoring was conducted exclusively on individuals with white flowers. However, qualitative observations from recurrent prior and subsequent visits to the study area (years 2021 to 2025; B. Garcia-Simpson, pers. obs.) suggest that the phenological pattern of the white morphotype is representative of the overall population.

Floral traits – morphology

We randomly collected 15 fully open flowers of each floral morph from different individuals. Longitudinal cut sections were photographed in the field with an iPhone XR camera (12MP, f/1.8 aperture, 26mm focal length) (Apple, California, USA) against a black background with a reference scale. Total length (TL), perianth width (PW), tube length (TuL), tube width (TuW), stigma exsertion (StiE), stamen exsertion (StaE), ovary length (OL), ovary width (OW), nectar chamber length (NL), and nectar chamber width (NW) (Fig. 2A–D; terminology modified from Nassar *et al.* 1997) were measured from photos using ImageJ v.1.54f (Schneider *et al.* 2012).

144 **Floral traits – daily phenology**

145 To characterize flower aperture and nectar production, we measured the perianth width of 23 flowers (11
146 white, 12 pink-red) from different individuals at eight stages of anthesis (15:00, 17:00, 19:00, 23:00, 03:00,
147 05:00, 07:00, 09:00) using a 0.05 mm resolution mechanical caliper (Uyustools, Hangzhou, China). Nectar
148 volume, sugar concentration, and energy content were assessed at five anthesis stages (15:00, 19:00, 23:00,
149 03:00, 06:00) using bagged flowers from different individuals. Nectar volume was measured in 34 flowers
150 (18 white, 16 pink-red), while sugar content and energy supply were evaluated from a subset of 17 flowers
151 (10 white, 7 pink-red). Nectar volume was quantified, following Ibarra-Cerdeña *et al.* (2005), by extracting
152 all possible nectar using a capillary tube inserted into the nectar chamber. Total nectar volume was
153 calculated by multiplying the column length by the specified cross-sectional area of the capillary tube. A
154 fraction of the nectar was used to determine sugar concentration with a handheld refractometer (BRIX30)
155 with automatic temperature compensation; readings were expressed as sucrose percentage following Dafni
156 (1992) and energy supply was calculated using the following formula:

157 $$J = 16.8[\left(\frac{S}{100}\right) * VD]$$

158 where J is energy in joules, S is the sugar percentage, V is nectar volume in microliters, and D is the density
159 of sucrose at the observed concentration (Dafni 1992; Ibarra-Cerdeña *et al.* 2005).

160 **Frequency and behavior of floral visitors**

161 We monitored floral visitors using two Bushnell 24MP Core Low Glow Trail Cameras (Bushnell
162 Corporation, Kansas, USA) placed 1–2.5 meters from flowers to capture interactions with vertebrate and
163 large invertebrate visitors. A total of 22 (11 white, 11 pink-red) flowers from different individuals were
164 observed over 11 non-consecutive days during the flowering season (Sep 2022–Mar 2023), totaling 378
165 observations hours (~17 hours per flower). Monitoring started in the early afternoon and continued until
166 mid-morning the following day. Based on Ibarra-Cerdeña *et al.* (2005), we recorded visitor species and

number of legitimate visits (i.e., involving contact with reproductive structures) for each flower. Additional visitors were documented through direct observation throughout anthesis.

Preliminary pollinator exclusion experiments

To evaluate how the absence of different pollinator groups influenced fruit initiation, we implemented five treatments: (i) control, with flowers left fully exposed; (ii) bat exclusion, covering flowers at night with a cylindrical metallic mesh; (iii) hummingbird exclusion, applying the same mesh only during the daytime; (iv) nocturnal exclusion, fully bagging flowers at night to prevent all nocturnal visitors; and (v) diurnal exclusion, fully bagging flowers during the daytime to block diurnal visitors. We monitored each flower over the following days to record whether it abscised or initiated ovary expansion. Due to fieldwork constraints, sample sizes were limited to 12 flowers per morph in the control treatment and six flowers per morph in each exclusion treatment. Therefore, results are presented descriptively as preliminary evidence, without formal statistical analyses.

Data analyses

All statistical analyses were conducted in R v.4.4.1 (R Core Team 2024) using RStudio (Posit Team 2024).

Intraspecific morphological variation. We evaluated the effect of flower morph (white vs pink-red) on all morphometric variables using a MANOVA (Pérez-Barrales *et al.* 2007). Variables were standardized (mean = 0, standard deviation = 1) for comparability. To avoid multicollinearity, we examined correlations among morphometric variables: total length, tube length, and nectar chamber length were highly correlated (Pearson's $r > 0.8$), therefore, we excluded tube length and nectar chamber length from the test. The analysis was performed with the *manova* function.

Modelling flower anthesis and nectar production. We fitted Generalized Linear Mixed-Effects Models (GLMMs) to examine the effects of time after midday (hours) and flower morphotype (white vs pink-red) on four response variables: (i) flower aperture, (ii) nectar volume, (iii) nectar sugar concentration,

and (iv) nectar energetic content. In the absence of specific hypotheses regarding differences among morphotypes (e.g., response magnitude or peak times), we opted for an exploratory model selection approach by generating a set of biologically plausible candidate models and selecting the best fit.

Model selection was conducted separately for each response variable. Candidate models were built using the *glmmTMB* package (Brooks *et al.* 2017), with Flower ID included as a random effect to account for individual variability. Time after midday (T) and flower morphotype (M) were included as fixed effects, testing first-, second-, and third-order orthogonal polynomial terms for time to capture potential curvilinear relationships. Fixed effects combination in candidate models included: T, T + M, T * M, T^2, T^2 + M, T^2 * M, T^3, T^3 + M, T^3 * M. When a model includes a polynomial of degree *n*, it inherently incorporates all lower-order terms (e.g., a T^3 model also includes T and T^2). Here, '+' indicates additive effects, while '*' denotes additive and interaction effects. Flower aperture and sugar content were modeled using a Gaussian distribution, while nectar volume and energetic content were modeled with a Tweedie distribution, chosen for its suitability in handling continuous data with a high proportion of zeros. Model diagnostics; presence of over/under-dispersion, outliers, and zero inflation, were assessed using the *DHARMa* package (Hartig 2022). Model selection indices were computed using *compare_performance* from the *performance* package (Lüdecke *et al.* 2021) and *model.sel* from the *MuMIn* package (Barton 2024). Final selection was based on the Akaike Information Criterion corrected for small sample sizes (AICc), with models differing by ≥ 2 AICc units considered significantly better fits (Burnham & Anderson 2002).

Frequency and behaviour of floral visitors. To visualize the temporal activity patterns of main floral visitors, we used a kernel density estimation (KDE) with the *density* function (bandwidth = 1). Densities were scaled by the total number of visits to make activity patterns visually comparable across visitor groups with differing number of visits.

To determine if the number of visits varied among floral visitors and flower types, we built Generalized Linear Mixed-effects Models (GLMMs) with a Poisson distribution (log-link). Flower ID was considered as a random effect, while visitor type (bat, hummingbird or sphingid) and flower morphotype were considered as fixed effects. We evaluated three models including effects of (i) visitor type (V), (ii)

visitor type and flower morphotype (V + M), and (iii) visitor type, flower morphotype, and their interaction (V * M). To account for differences in evaluation time among individual flowers, all models included the log-scaled evaluation time of each flower as an offset variable. Model fitting, diagnostics, and selection were performed as previously described. Based on the best-supported model, post-hoc pairwise comparisons of visitation frequencies among floral visitors were conducted using the *emmeans* package (Lenth & Lenth 2018). Pairwise differences were evaluated with the *pairs* function, applying a Tukey adjustment to account for multiple comparisons.

Results

Annual phenology

H. acranthus from Cardal displayed a single flowering peak during the year. Bud and flower production occurred primarily from January to April, followed by a sharp decline. From May to September, plants remained mostly vegetative with minimal reproductive activity. Bud production resumed in October, peaking in November, resulting in a high number of open flowers. Fruit production followed this pattern but with a slight delay due to the sequential nature of these reproductive stages. Overall, reproduction occurred during the dry season (Nov–Apr), while the vegetative phase in the humid season (May–Oct) (Fig. 3A–B; Table S1).

Floral traits – morphology

H. acranthus flowers were funnelform to tubular in shape and radially symmetric, but occasionally exhibited a certain degree of zygomorphy (Fig. 2B). The flower tube exhibited a constriction above the nectar chamber, which limits access to nectar. Perianth color varied from white and white/green to pink-red, but remained consistent within individuals (Fig. 2C–D). Overall morphometry of the two floral morphs did not differ (Table S2), as supported by the MANOVA results (Pillai's Trace = 0.221, F (8, 23) = 0.813, P = 0.598).

Floral traits – daily phenology

Unless otherwise indicated, model estimates are reported with 95% confidence intervals (mean ± 95% CI).

Flower aperture. The top-ranked model included time up to the cubic term (T, T^2, T^3) and its respective interactions with morphotype (AICc = 467.29, weight = 0.560). The second-best model additionally included the main effect of morphotype (ΔAICc = 2.31, weight = 0.176) (Table S3). The strong second-order polynomial effect confirmed the parabolic pattern in flower aperture over time, showing the expected non-linear pattern of anthesis. The interaction terms, present in the final model but with a weak effect, revealed that this parabolic relationship varied between morphs, highlighting slight differences in how aperture responds to time. Both floral morphs reached maximum aperture around 23:00 (white: 6.51 ± 0.3 cm, pink-red: 5.9 ± 0.3 cm) and differences between morphs were observed in the early stages of anthesis, where the pink-red morph showed higher estimates than the white morph, indicating a slightly earlier aperture initiation (white: 14:34, pink-red: 12:81) (Fig. 4A, Table 1A). Visually, styles and stigmas remained turgid throughout anthesis.

Nectar volume. The best model for nectar volume included time up to the third-order polynomial term but excluded interactions with morphotype and its main effect (AICc = 1025.65, weight = 0.920). The second-best model included interactions with morphotype (ΔAICc = 5.46, weight = 0.060). The main effect of morphotype was present in models of lower rank (Table S4). The second- and third-order polynomial terms together indicated a parabolic pattern in nectar volume over time, with an initial rise in late afternoon to a peak up to 93.5 ± 13.5 µL at 21:00 followed by a less steep decline, where lower quantities of nectar were available until the following morning (Fig. 4B, Table 1B).

Sugar content. The three best-ranked models showed ΔAICc < 2 among them; we selected the simpler model, which included the linear effect of time without the interaction with morphotype nor its main effect (AICc = 164.00, weight = 0.265). This model showed equivalent support compared to more complex models that included the second and third-order effect of time (AICc = 163.28, weight = 0.379), or the main effect of morphotype and its interactions (AICc = 165.08, weight = 0.154) (Table S5). The

model estimates an initial content of sugars of 23.5 ± 0.5 % at 18:30, showing a linear decline over time, reaching lower values of 15.4 ± 0.8 % at 07:40 (Fig. 4C, Table 1C).

Energy content. The best model for nectar energy content included time up to the third-order polynomial term but excluded morphotype effects (AICc = 540.06, weight = 0.738). The second-best model, which added interactions with morphotype, had less support (ΔAICc = 3.05, weight = 0.160). The main effect of morphotype was included in models of lower rank (Table S6). Energetic supply followed a similar pattern to nectar secretion, with an initial rise in late afternoon to a peak up to 529.8 ± 77.8 J at 20:30 followed by a less steep decline lasting until the following morning (Fig. 4D, Table 1D).

Frequency and behavior of floral visitors

We recorded 11 invertebrate and two vertebrate species of floral visitors; two nocturnal, five diurnal, and six active during both periods (Table 2). Nine did not contact the reproductive structures, making them unlikely pollinators due to their incompatibility with floral morphology. Two species occasionally made contact with the reproductive structures, while the remaining two consistently made effective pollinator visits, regularly contacting both reproductive structures while accessing floral resources (Table 2).

Among the small flower visitors, two ant species (Formicidae) were observed visiting flowers at night, primarily for nectar and often in large numbers (> 20 individuals). These ants continued harvesting nectar until the flowers senesced (Fig. S1A–C). One sap beetle species (*Carpophilus* sp., Nitidulidae) was found inside flowers, likely feeding on pollen or plant tissue, and was more abundant in older flowers (Fig. S1D). Three spider species (Anyphaenidae, Thomisidae, and Salticidae) were recorded on the flowers, occupying the external zones of the floral tubes and perianth, acting as predators (Fig. S1E–F; Fig. S2A–B). Two Diptera species were documented; one was abundant and found inside the floral tubes (Phoridae) (Fig. S2C), and a syrphid was observed once feeding on pollen. The common bee (*Apis mellifera*) was frequently observed collecting pollen from anthers but rarely contacting the stigma, except when gathering in larger groups (> 5 individuals) (Fig. S2D), while the metallic-green bee *Caenohalictus* sp. (Halictidae) was also recorded (Fig. S1E–F).

Regarding large flower visitors, the hummingbird *Rhodopis vesper* (Trochilidae) was the most frequent (Fig. 5A–B). Their activity started in the late afternoon (17:00) until sunset (18:30–19:00) and resumed in the early morning (05:00) until the end of anthesis (9:00–11:00) (percentage of total visits: white: 71.4%, pink-red: 81.4%). They hovered or, in occasions landed on flowers to access nectar, always contacting stamens and stigma. The Peruvian Long-tongued Bat, *Platalina genovensium* (Phyllostomidae), was the second most frequent visitor (Fig. 5C–D), active at night (20:00–03:00), peaking at 22:00 (white: 20.6%, pink-red: 16.3%). Unlike *R. vesper*, bats made single contacts to flowers while hovering but never landed on them. Visits by hawkmoths (*Manduca* sp., Sphingidae, Lepidoptera) were rare and recorded at dusk or at night (white: 7.9%, pink-red: 2.3%). They either hovered or landed on flowers, but their body not always contacted reproductive structures (illegitimate visits = 2, legitimate visits = 4) (Fig. 5E–F).

The model including only the effect of flower visitor was selected as the best-fitting model (AICc = 209.6, weight = 0.717). The additive (ΔAICc = 2.32, weight = 0.225) and multiplicative models received significantly less support (ΔAICc = 5.05, weight = 0.057) (Table S7). In consequence, we compared visitation frequencies among visitors based on the first model (Table 1E); which showed sphingids had significantly lower visitation rates compared to both *P. genovensium* (estimate = -1.20, SE = 0.465, P = 0.0262) and *R. vesper* (estimate = -2.59, SE = 0.423, $P < 0.001$). Additionally, *P. genovensium* exhibited lower visitation rates than *Rhodopis vesper* (estimate = -1.39, SE = 0.250, $P < 0.001$).

Preliminary pollinator exclusion experiments

Control flowers showed the highest fruit initiation rates (white: 41.7%; pink-red: 33.3%). Bat exclusion reduced fruit initiation in the white morph but not in the pink-red morph (white: 16.7%; pink-red: 33.3%). Hummingbird exclusion lowered fruit initiation in both morphs (white: 16.7%; pink-red: 16.7%). Nocturnal exclusion produced moderate initiation in the white morph but low initiation in the pink-red morph (white: 33.3%; pink-red: 16.7%), and diurnal exclusion resulted in the same pattern (white: 33.3%; pink-red: 16.7%). In general, fruit initiation was low across all treatments. When morphs were pooled, most

314 treatments showed a fruit initiation rate of 25%, except for the hummingbird-exclusion treatment, which
315 showed 17%. The control flowers exhibited the highest fruit initiation rate at 37.5%.

316 Discussion

317 Annual phenology

318 The Lomas formations are marked by strong seasonality in floral resources availability, with most plant
319 species reproducing during the wet season (Tovar *et al.*, 2018). In contrast, our data shows that
320 *Haageocereus acranthus* flowers in the dry season, with a single reproductive peak. Similar dry-season
321 flowering has been reported at Lachay National Reserve by Maguiña & Amanzo (2016), and Calderón *et*
322 *al.* (2007) describe flowering in January and fruiting in February. This recurring pattern across different
323 Lomas populations of *H. acranthus* suggests that rising temperatures (Servicio Nacional de Meteorología
324 e Hidrología del Perú - SENAMHI; Figure S1), and reduced humidity may act as cues for bud development,
325 although this requires further testing. Flowering during resource-limited periods positions *H. acranthus* as
326 a potential key species in Lomas ecosystems, providing an important resource for associated fauna.

327 Floral morphology, phenology, and expected pollination syndrome

328 The floral dimensions of *H. acranthus* are consistent with those of other vertebrate-pollinated cacti. Its
329 large, sturdy flowers can withstand landings and manipulations by relatively large visitors, while the broad
330 frontal area (~6.3 cm diameter) likely enhances detection by bats through echolocation, as shown for
331 *Pachycereus* (González-Terrazas *et al.*, 2016). This surface also provides ample contact for pollen transfer.
332 Each flower bears 200–400 stamens (Calderón *et al.*, 2007), indicating high pollen output and potential
333 seed set. Comparable bat-pollinated species produce 10^5–2×10^5 pollen grains per flower (Nassar *et al.*,
334 1997), with *Pilosocereus* reaching ~500 stamens, ~2000 ovules, and ~1100 seeds per fruit (Martins et al.,
335 2020). Together, these traits suggest that *H. acranthus* depends on pollinators with high pollen-carrying
336 capacities to ensure reproductive success within its short floral lifespan (< 24 h).

The floral tube is determinant in cacti plant-pollinator interactions (Schlumpberger 2011). In *H. acranthus*, an average tube length of 4.8 cm restricts nectar access to visitors with sufficiently long feeding structures, establishing a threshold for effective foraging. Some species with shorter feeding structures can still exploit the resource by inserting their head or rostrum into the funnel-shaped tube, which allows deeper access than in narrower flowers (e.g., *R. vesper*; Fig. 5B). Experimental studies further show that tube width influences foraging behavior: wider tubes extend the feeding reach of nectar-feeding bats (Winter & von Helversen 2003; Nicolay & Winter 2006), whereas narrower tubes increase hummingbird handling times (Smith *et al.* 1996). Finally, visitors with feeding structures exceeding ~6.8 cm (tube length plus stamen exsertion) may obtain nectar without contacting the reproductive organs, thereby acting as nectar robbers (e.g., sphingid moths; Fig. 5F).

Monitoring of flower phenology confirmed that *H. acranthus* flowers open only once, with peaks in aperture, nectar secretion, and energy availability occurring at night (Fig. 4A–D). Total nectar production, sugar content, and energetic supply were high, comparable to values reported for cacti pollinated by hummingbirds and bats (e.g., Rowley 1980; Grant & Grant 1980; Schlumpberger et al. 2009, Schlumpberger 2011; Albuquerque-Lima *et al.* 2023). Although flower opening varied among morphotypes (pink-red flowers opened slightly earlier than white flowers), nectar characteristics did not differ, providing insufficient evidence for phenological divergence among morphs. These characteristics suggest an association with pollination by nocturnal vertebrates or large invertebrates (Baker 1975; Medel *et al.* 2022). However, anthesis extended into the late afternoon and morning, when small amounts of nectar were available (Fig. 4B), allowing diurnal vertebrates to forage flowers and potentially contribute to pollination (Baker 1961; Miyake & Yahara 1999).

Although pink-red and white pigmentation are traditionally linked to diurnal hummingbird and nocturnal bat/sphingid pollination, respectively, our analyses did not reveal differences among white and pink-red floral morphs. Besides slight differences between flower aperture patterns, floral morphology and phenology were consistent across morphs. Overall, the observed floral traits strongly support the hypothesis

of primary adaptation to bat pollination, with characteristics also associated to hummingbird pollination. In contrast, the relatively short floral tube indicates only limited adaptation to sphingid pollination.

Empirical evidence of flower visitors and pollinators

Visitors are here grouped into three categories based on morphology, behavior, and contact with reproductive organs: (1) ants, sap beetles, flies, and arachnids, whose foraging rarely involves stigma or stamen contact and thus contribute little to no pollination; (2) bees and sphingid moths, which may occasionally transfer pollen but more often act as nectar or pollen thieves; and (3) vertebrates, whose traits align more closely with floral morphology and are therefore expected to be the most effective pollinators.

Ants, sap beetles, flies and arachnids. Ants were frequently observed in large groups on flowers, but their small size, unlikely stigma contact, and foraging behavior suggests negligible contributions to pollen transfer (Rico-Gray 1989; Fagua & Ackerman 2011; LeVan *et al.* 2014). Similarly, *Carpophilus* beetles, common in both wild and cultivated cacti, act as pollen thieves they feed on nectar, lay eggs in buds, and their larvae consume decaying flowers (Grant & Connell 1979; Miranda-Jácome *et al.* 2021). Dipterans frequently visited *H. acranthus* flowers but rarely contacted reproductive organs due to their small size, making them ineffective pollinators (Rowley 1980; McIntosh 2005; Schlumpberger *et al.*, 2009). Finally, spiders were exclusively predatory visitors, typically using flowers as hunting grounds (Su *et al.* 2020), though occasional resource use has been reported (e.g., Nelson 2023).

Bees and sphingid moths. Bees are frequent visitors due to their pollen-collecting behavior (Westerkamp 1996; Thorp 2000) and, when morphologically compatible, can be effective cross-pollinators (Armbruster *et al.* 1989; Solís-Montero & Vallejo-Marín 2017). In *H. acranthus*, however, the large flowers and exserted stigmas limit bee-stigma contact, which could reduce their effectiveness as pollinators. Although not quantified in our study, studies on tropical cacti with similar floral traits and growth habit suggest that the contribution of bees and other small insects to pollination is low or null (e.g., bees, flies, and butterflies on *Weberbauerocereus* in Sahley 1996; *A. mellifera* on *Pilosocereus* in Rivera-Marchand & Ackerman 2006; *Xylocopa grisescens* on *Pilosocereus* in Rocha *et al.* 2019; *A. mellifera* on *Cipocereus* in

Martins *et al.* 2020). In contrast, in temperate zones bees often act as effective pollinators, even in species with floral traits associated with bats or moths, reflecting more generalized pollination strategies (Valiente-Banuet et al. 1996; Fleming *et al.* 2001).

The traits of *H. acranthus* may also suggest sphingid moth pollination (e.g., white pigmentation, nocturnal anthesis, nectar rewards). However, sphingids accounted for less than 10% of visits and were observed both landing on flowers or accessing nectar without contacting the stamens or stigma. This behavior has been previously documented in moths, as it allows them to feed from the relatively short-tubed flowers of while avoiding close approaches that increase predation risk (Wasserthal 2001). By contrast, species adapted to sphingid pollination usually possess much longer floral tubes (e.g., > 10 cm in Grant & Grant 1979; > 15 cm in Silva & Sazima 1995; > 15 cm in Schlumpberger *et al.* 2009; ~24 cm in Albuquerque-Lima *et al.* 2023). Thus, the role of hawkmoths as pollinators of *H. acranthus* results unlikely.

Bats and hummingbirds. Hummingbird foraging overlapped with anthesis only during crepuscular and early morning hours, yet their visits accounted for a major proportion of total observations. The high frequency of *R. vesper* visits highlights its potential role as a pollinator, even though nectar availability at these times was often low or undetectable. This can be explained by the fact that nectar measurements involved repeated extraction throughout anthesis; therefore, morning measurements reflected nectar levels under continuous extraction rather than natural overnight accumulation and cannot rule out nectar persistence in flowers unvisited during the night.

In Cactaceae, hummingbird-pollinated flowers are typically red to orange, less robust, zygomorphic, and tubular (e.g., *Loxanthocereus*, *Borzicactus*, *Matucana* spp.) (Grant & Grant 1979; Rowley 1980; Schlumpberger 2011). Although the morphology of *H. acranthus* does not fully match this bauplan, it exhibits secondary compatibility with *R. vesper* to access nectar while ensuring contact with reproductive structures. Visitation rates did not differ between morphs, indicating that both floral color variants are equally attractive to hummingbirds. Overall, the frequent and effective visits of *R. vesper* support its role as a compatible pollinator, consistent with reports of this species visiting Peruvian cacti lacking typical ornithophilous traits (Sahley 1996; Novoa *et al.* 2022).

The synchronization of *H. acranthus* phenology with the nocturnal activity of *P. genovensium*, coinciding with peak flower aperture and nectar availability is consistent with bat pollination (e.g., Valiente-Banuet *et al.* 1996; Nassar *et al.* 1997; Ibarra-Cerdeña *et al.* 2005; Rocha *et al.* 2019; Martins *et al.* 2020; Albuquerque-Lima *et al.* 2023). Morphologically, *P. genovensium* is well-suited to exploit these flowers, as its long feeding structures match the floral tube. Observations of individuals carrying *Haageocereus* pollen (Maguiña & Amanzo 2016), along with its status as a cactus specialist (Sahley & Baraybar 1996), further support its role as a pollinator. Although bats were not the most frequent visitors, they exhibited the greatest morphological fit to the flowers, suggesting higher pollen transfer efficiency per visit compared to hummingbirds (Muchhala & Thomson 2010). Thus, *P. genovensium* may represent the most effective pollinator of *H. acranthus*, as *Leptonycteris yerbabuenae* does for several columnar cacti in North America below 22º latitude (e.g., Tremlett *et al.* 2020).

Preliminary pollination exclusion experiments in other Lomas (Lachay, northern Lima) suggest that *H. acranthus* is primarily pollinated at night (Grillo & Arana 2016). In Lachay, hummingbirds visited flowers frequently, but bat activity was slightly higher. Combined with our observations, this evidence indicates that *H. acranthus* is mainly adapted to bat pollination, though the predominance of chiropterophily may depend on the presence of *P. genovensium*. This bat species is highly dependent on cactus flowers and intact arid habitats, and its distribution is restricted to well-preserved areas that are rapidly declining in Lima (Ossa et al. 2020; Lambert 2021). Accordingly, *P. genovensium* has not been reported from urban environments, where only generalist bats persist by feeding on exotic plants (Pellón *et al.* 2021). The peri-urban setting of our study site, surrounded by towns and agriculture, contrasts with Lachay, a conserved National Protected Area (Dourojeanni 2018), likely explaining the greater prevalence of hummingbird visitation at Cardal. In addition, our preliminary exclusion experiments showed that, although fruit production been generally low, all exclusion treatments tended to produce equal or lower fruit initiation than open flowers, suggesting that both diurnal and nocturnal pollinators contribute to fruit set. Together, these patterns support that the mixed-vertebrate pollination system of *H. acranthus* may facilitate its persistence even where its ideal bat pollinators decline or are absent.

Our findings partially align with those of Sahley (1996) for *Weberbauerocereus weberbaueri* in Arequipa (southern Peru). This closely related species (Tribe Trichocereeae) shares similar morphological and phenological floral traits and is also primarily visited by *P. genovensium* and *R. vesper* (although in *W. weberbaueri* the wine-colored flowers are strongly zygomorphic, unlike in *H. acranthus)*. Sahley (1996) documented interannual shifts in pollinator dominance, with bats prevailing in one year and hummingbirds in other, a pattern linked to El Niño events that alter rainfall, vegetation, and bat abundance. We propose that *H. acranthus* may follow a similar strategy, with mixed floral traits enabling dual pollination. Such flexibility is likely advantageous in the seasonal Lomas ecosystem, where extreme climatic events like El Niño strongly influence resource availability (Ferreyra 1993; Cano *et al.* 1999). The hypothesized migratory behavior of *P. genovensium* (Sahley 1996; Sahley & Baraybar 1996; Ossa *et al.* 2020) further underscores the value of maintaining alternative pollinators: hummingbirds may provide reliable service during periods of reduced bat activity, thereby supporting reproductive success under fluctuating conditions (Muchhala & Thomson 2010). Finally, the disjunct distribution of *H. acranthus* along the Peruvian coast, with populations separated by large distances, aligns with bat-mediated pollination, as bats are highly effective long-distance pollen vectors, promoting genetic connectivity despite habitat fragmentation (Fleming *et al.* 2009).

Mixed-pollination strategies in a broader context

The most effective pollinator principle states that floral traits should evolve toward the selective optimum imposed by the pollinator contributing the most to reproductive success (Stebbins 1970), but this is not always observed. When no single guild imposes strong or consistent selection, floral phenotypes matching multiple guilds can be favored because their combined contributions exceed those provided by specialization. Such mixed strategies often emerge when main pollinator abundances, and thus their relative contributions to plant fitness, shift across space or time (Kay & Anderson, 2025).

In some cases, mixed pollination occurs even in species with clear floral syndromes. For example, *Marginatocereus marginatus* in Mexico, although exhibiting traits associated with hummingbirds,

competition with co-flowering columnar cacti reduced the reliability of individual pollinator guilds, and its dual nocturnal-diurnal anthesis allowed reliance on both bats and hummingbirds (Dar et al. 2006). In contrast, we consider it unlikely that *H. pseudomelanostele* or *L. acanthurus* generate meaningful pollination interference for *H. acranthus* at our site, as both species occur at much lower densities, and *L. acanthurus* shows little to no flowering overlap with *H. acranthus*. However, this is a possibility in several other Lomas populations of coastal Peru, where this cactus coexists with multiple hummingbird- and bat-pollinated species, often showing reproductive compatibility even across genus (Arakaki et al. 2020).

Geographic variation in pollination systems has been documented in cacti. Schlumpberger et al. (2009) found shifts from bee pollination (short, morning-opening, low-nectar flowers) sphingid pollination (long-tubed, dusk-opening, nectar-rich flowers) across populations of *Echinopsis ancistrophora* along an altitudinal gradient in Argentina. In Mexico, *Stenocereus thurberi* also showed spatial differences: northern and central populations relied mainly on bats with no pollen limitation, whereas southern populations on mixed pollinators and showed pollen limitation, likely due to temporal variation in pollinator availability (Bustamante et al. 2010). *Pachycereus pecten-aboriginum* also displayed geographic differentiation in anthesis and nectar secretion that aligns with nocturnal versus mixed nocturnal-diurnal pollination in Valiente-Banuet et al. (2004). This tendency to more generalized pollination systems has also been documented in other Pachycereeae such as *Carnegia gigantea* (Fleming et al., 1996); supporting that generalized strategies appear to be more reliable in zones where bats predictability is lower (e.g., northern Mexico versus central and Southern regions, Rojas-Martínez et al. 1999). These dynamics remain unstudied in Peruvian cacti. The mixed pollination strategy we propose for *H. acranthus* may be shaped by local factors such as habitat condition, pollinator abundance, and the presence or absence of co-flowering cacti. However, since our data comes from a single site and one sampling year, it cannot reveal if pollination mechanisms vary across space or time, or whether such variation explains possible differences among populations. Wider geographic coverage and long-term studies will be essential to address these questions.

488 **Conclusions**

489 Our study shows that *H. acranthus* exhibits floral traits aligned with nocturnal bat pollination while
490 remaining compatible with diurnal hummingbird pollination, with both likely serving as the main effective
491 pollinators. In contrast, bees and sphingids mismatched key floral traits and likely acted primarily as nectar
492 or pollen thieves. We found no differences in floral traits or visitation frequencies between color morphs,
493 providing no support for our initial hypothesis. Overall, our findings indicate a mixed vertebrate pollination
494 system involving both bats and hummingbirds. In the strongly seasonal Lomas ecosystem, where pollinator
495 availability can shift across space and time, maintaining interactions with multiple pollinator guilds may
496 enhance reproductive stability. Our results highlight the need for broader spatial and temporal studies of *H.*
497 *acranthus* reproduction.

498 ## Acknowledgements

499 We thank A. Ceroni and M. Flores (Weberbauer Herbarium-MOL) for their support in obtaining
500 funding and for their contributions during the design of the study; C. Reynel and S. Terreros (Forestry
501 Herbarium-MOLF) for receiving the botanical samples; M. Alvarado and E. Medina (Department of
502 Entomology-UNMSM) for receiving the arthropod samples and assisting with their taxonomic
503 identification. This research was funded by the 'XI Concurso de fondos de investigación para círculos de
504 investigación 2021' from La Molina National Agrarian University (Lima, Peru). This study complies with
505 legal requirements set by the Peruvian Ministry of Agriculture (MINAGRI) and the Forestry and Fauna
506 Service (SERFOR) for the collection of plant and insect specimens (RD N° D0000046-2024-MIDAGRI-
507 SERFOR-DGGSPFFS-DGSPF).

508

References

Albuquerque-Lima S., Taylor N.P., Zappi D.C., Machado I.C. (2023) Floral specialization and bat pollination in subtribe Cereinae (Cactaceae): a morphological approach. *Diversity*, **15**, 207. https://doi.org/10.3390/d15020207

Arakaki M., Ostolaza C., Cáceres F., Roque J. (2006) Cactaceae endémicas del Perú. *Revista Peruana de Biología*, **13**, 193–219.

Arakaki M., Soltis D.E., Speranza P. (2007) New chromosome counts and evidence of polyploidy in *Haageocereus* and related genera in tribe Trichocereeae and other tribes of Cactaceae. *Brittonia*, **59**, 290–297. https://doi.org/10.1663/0007-196X(2007)59[290:NCCAEO]2.0.CO;2

Arakaki M., Speranza P., Soltis P.S., Soltis D.E. (2021) Examination of reticulate evolution involving *Haageocereus* and *Espostoa*. *Haseltonia*, **27**, 102–112. https://doi.org/10.2985/026.027.0112

Armbruster W.S. (2017) The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Functional ecology*, **31**, 88–100. https://doi.org/10.1111/1365-2435.12783

Armbruster W.S., Keller S., Matsuki M., Clausen T.P. (1989) Pollination of *Dalechampia magnoliifolia* (Euphorbiaceae) by male euglossine bees. *American Journal of Botany*, **76**, 1279–1285. https://doi.org/10.1002/j.1537-2197.1989.tb15109.x

Baker H.G. (1961) The adaptation of flowering plants to nocturnal and crepuscular pollinators. *The Quarterly Review of Biology*, **36**, 64–73.

Baker H.G. (1975) Sugar Concentrations in Nectars from Hummingbird Flowers. *Biotropica*, **7**, 37–41. https://doi.org/10.2307/2989798

Ballesteros-Mejia L., Lima N.E., Lima-Ribeiro, M.S., Collevatti, R.G. (2016) Pollination mode and mating system explain patterns in genetic differentiation in neotropical plants. *PLoS One*, **11**, e0158660. https://doi.org/10.1371/journal.pone.0184674

Barton K. (2024) MuMIn: Multi-Model Inference. R package v. 1.48.4, https://cran.r-project.org/package=MuMIn

Brooks M.E., Kristensen K., Van Benthem K.J., Magnusson A., Berg C.W., Nielsen A., Skaug H.J., Mächler M., Bolker B.M. (2017) glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R journal*, **9**, 378–400. https://doi.org/10.32614/RJ-2017-066

Burkle L.A., Alarcón R. (2011) The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. *American journal of botany*, **98**, 528–538. https://doi.org/10.3732/ajb.1000391

Burnham K.P., Anderson D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer New York, New York, US: 488 pp. https://doi.org/10.1007/b97636

Bustamante E., Casas A., Búrquez A. (2010) Geographic variation in reproductive success of *Stenocereus thurberi* (Cactaceae): Effects of pollination timing and pollinator guild. *American Journal of Botany*, **97**, 2020–2030. https://doi.org/10.3732/ajb.1000071

Calderón N., Zappi D., Taylor N., Ceroni A. (2007) Taxonomy and conservation of *Haageocereus* Backeb. (Cactaceae) in Peru. *Bradleya*, **25**, 45–124. https://doi.org/10.25223/brad.n25.2007.a8

Cano A.C., Roque J., Arakaki M., Arana C., La Torre M., Llerena N., Refulio N. (1999) Diversidad florística de las Lomas de Lachay (Lima) durante el evento "El Niño 1997-98". *Revista Peruana de Biología*, **6**, 125–132.

Cardona J., Lara C., Ornelas J.F. (2020) Pollinator divergence and pollination isolation between hybrids with different floral color and morphology in two sympatric *Penstemon* species. *Scientific reports*, **10**, 8126. https://doi.org/10.1038/s41598-020-64964-8

Ceroni A., Castro-Cepero V., Teixeira V., Redolfi I. (2007) *Neoraimondia arequipensis* subsp. *roseiflora* (Werdermann & Backeberg) Ostolaza (Cactaceae): axis of the interactions in arid ecosystems. Ecología Aplicada, **6**, 155–168.

Dafni A. (1992) *Pollination Ecology: A Practical Approach*. Oxford University Press, Oxford, UK: 250 pp.

Dar S., Arizmendi M.D.C., Valiente-Banuet A. (2006) Diurnal and nocturnal pollination of *Marginatocereus marginatus* (Pachycereeae: Cactaceae) in Central Mexico. *Annals of Botany*, **97**, 423-427. https://doi.org/10.1093/aob/mcj045

Dellinger A.S. (2020) Pollination syndromes in the 21st century: where do we stand and where may we go?. *New Phytologist*, **228**, 1193–1213. https://doi.org/10.1111/nph.16793

Dourojeanni M.J. (2018) *Áreas Naturales Protegidas del Perú: El Comienzo*. Editora Grijley, Lima, Peru: 330 pp.

Faegri K., van der Pijl L. (1979) *The principles of pollination ecology*. Pergamon Press, Oxford, UK: 244 pp.

Fagua J.C., Ackerman J.D. (2011) Consequences of floral visits by ants and invasive honeybees to the hummingbird-pollinated, Caribbean cactus *Melocactus intortus*. *Plant Species Biology*, **26**, 193–204. https://doi.org/10.1111/j.1442-1984.2011.00319.x

Fenster C.B., Armbruster W.S., Wilson P., Dudash M.R., Thomson J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics*, **35**, 375–403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Ferreyra R. (1993) Registros de la vegetación en la costa peruana en relación con el fenómeno El Niño. *Bulletin de l'Institut français d'études andines*, **22**, 259–266.

Fleming T.H., Geiselman C., Kress, W.J. (2009) The evolution of bat pollination: a phylogenetic perspective. *Annals of botany*, **104**, 1017–1043 https://doi.org/10.1093/aob/mcp197

Fleming T.H., Tuttle M.D., Horner M.A. (1996) Pollination biology and the relative importance of nocturnal and diurnal pollinators in three species of Sonoran Desert columnar cacti. *The Southwestern Naturalist*, 257-269.

Fleming T.H., Valiente-Banuet A. (Eds) (2002) *Columnar cacti and their mutualists: evolution, ecology, and conservation*. University of Arizona Press, Arizona, US: 386 pp.

Fleming T.H., Sahley C.T., Holland J. N., Nason J.D., Hamrick J.L. (2001) Sonoran Desert columnar cacti and the evolution of generalized pollination systems. *Ecological Monographs*, ***71***, 511–530. https://doi.org/10.1890/0012-9615(2001)071[0511:SDCCAT]2.0.CO;2

Gonzalez-Terrazas T.P., Martel C., Milet-Pinheiro P., Ayasse M., Kalko E.K., Tschapka, M. (2016) Finding flowers in the dark: nectar-feeding bats integrate olfaction and echolocation while foraging for nectar. *Royal Society Open Science*, **3**, 160199. https://doi.org/10.1098/rsos.160199

Grillo S., Arana C. (2016) Polinizadores y visitantes florales de *Haageocereus acranthus* y *H. pseudomelanostele* (Cactaceae) en la Reserva Nacional de Lomas de Lachay, Perú. XXV Reunión Científica del Instituto de Investigación de Ciencias Biológicas "Antonio Raimondi" - Universidad Nacional Mayor de San Marcos, Lima, Peru.

Grant V., Connell W.A. (1979) The association between *Carpophilus* beetles and cactus flowers. *Plant Systematics and Evolution*, **133**, 99–102. https://doi.org/10.1007/BF00985884

Grant V., Grant K.A. (1979) The pollination spectrum in the southwestern American cactus flora. *Plant Systematics and Evolution*, **133**, 29-37. https://doi.org/10.1007/BF00985877

Hartig F. (2018) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.7. http://florianhartig.github.io/DHARMa/

Ibarra-Cerdeña C.N., Iñiguez-Dávalos L.I., Sánchez-Cordero V. (2005) Pollination ecology of *Stenocereus queretaroensis* (Cactaceae), a chiropterophilous columnar cactus, in a tropical dry forest of Mexico. *American Journal of Botany*, **92**, 503–509. https://doi.org/10.3732/ajb.92.3.503

Kay K. M., Anderson B. (2025) Beyond the Grant–Stebbins model: floral adaptive landscapes and plant speciation. *Annals of Botany*, **136**, 699-720. https://doi.org/10.1093/aob/mcaf096

Kay K.M., Sargent, R.D. (2009) The role of animal pollination in plant speciation: integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 637–656. https://doi.org/10.1146/annurev.ecolsys.110308.120310

Lambert R. (2021) Examinando la relación entre la planeación y la urbanización periférica en Lima. *Ensayo: Revista de arquitectura, urbanismo y territorio*, **2**, 71–93. https://doi.org/10.18800/ensayo.202102.004

Lendel A. (2013) *South American Cacti in time and space: studies on the diversification of the tribe Cereeae, with particular focus on subtribe Trichocereinae (Cactaceae)*. University of Zürich, Zürich, Switzerland: 214 pp.

Lenth R.V. (2018) Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, **69**, 1–33. https://doi.org/10.18637/jss.v069.i01

LeVan K.E., Hung K.L.J., McCann K.R., Ludka J.T., Holway D.A. (2014) Floral visitation by the Argentine ant reduces pollinator visitation and seed set in the coast barrel cactus, *Ferocactus viridescens*. *Oecologia*, **174**, 163–171. https://doi.org/10.1007/s00442-013-2739-z

Lüdecke D., Ben-Shachar M.S., Patil I., Waggoner P., Makowski D. (2021) performance: An R package for Assessment, Comparison and Testing of Statistical Models. *Journal of Open Source Software*, **6**. https://doi.org/10.21105/joss.03139

Maguiña R., Amanzo J. (2016) Diet and pollinator role of the long-snouted bat *Platalina genovensium* in Lomas ecosystem of Peru. *Tropical Conservation Science*, **9**, 1940082916674288. https://doi.org/10.1177/1940082916674288

Mandujano M.D.C., Carrillo-Angeles I., Martínez-Peralta C., Golubov J. (2010) Reproductive biology of Cactaceae. In: Ramawat, K. (Eds), *Desert plants: Biology and biotechnology*. Springer, Berlin, Germany: 197–230.

Martins C., Oliveira R., Aguiar L.M., Antonini Y. (2020) Pollination biology of the endangered columnar cactus *Cipocereus crassisepalus*: a case of close relationship between plant and pollinator. *Acta Botanica Brasilica*, **34**, 177–184. https://doi.org/10.1590/0102-33062019abb0219

McIntosh M.E. (2005) Pollination of two species of *Ferocactus*: interactions between cactus-specialist bees and their host plants. *Functional Ecology*, **19**, 727–734. https://www.jstor.org/stable/3599235

Medel R., López-Aliste M., Fontúrbel F.E. (2022) Hummingbird-plant interactions in Chile: An ecological review of the available evidence. *Avian Research*, **13**, 100051. https://doi.org/10.1016/j.avrs.2022.100051

Minnaar C., Anderson B., de Jager, M.L., Karron J.D. (2019) Plant–pollinator interactions along the pathway to paternity. *Annals of Botany*, **123**, 225–245. https://doi.org/10.1093/aob/mcy167

Miranda-Jácome A., Fernández-Tlapa F., Munguía-Rosas M.A. (2021) Visiting and feeding behavior of sap beetles (*Carpophilus lugubris*) in the flowers of a chiropterophilic columnar cactus (*Pilosocereus* leucocephalus). *Journal of Arid Environments*, **189**, 104482. https://doi.org/10.1016/j.jaridenv.2021.104482

Miyake T., Yahara T. (1999) Theoretical evaluation of pollen transfer by nocturnal and diurnal pollinators: when should a flower open?. *Oikos*, **86**, 233–240. https://doi.org/10.2307/3546441

Moya-Méndez N. C. (2006) *Haageocereus: Taxonomy for the Conservation of the Genus in Peru*. The Open University, Milton Keynes, UK: 164 pp.

Muchhala N., Thomson J.D. (2010) Fur versus feathers: pollen delivery by bats and hummingbirds and consequences for pollen production. *The American Naturalist*, **175**, 717–726.

Nassar J.M., Ramírez N., Linares O. (1997) Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. *American Journal of Botany*, **84**, 918–927. https://doi.org/10.2307/2446282

Nelson X.J. (2023) A road map of jumping spider behavior. *The Journal of Arachnology*, **51**, 139–154. https://doi.org/10.1636/JoA-S-22-011

Nicolay C.W., Winter Y. (2006) Performance analysis as a tool for understanding the ecological morphology of flower visiting bats. In: Zubaid A., McCracken G.F., Kunz T. H. (Eds), *Functional and Evolutionary Ecology of Bats*. Oxford University Press, Oxford, UK: 131–144.

Novoa S., Ledesma K., Linares R. (2022) Use of Camera Traps to Monitor the Fauna Associated with a Cactus Community in the Coastal Desert of Ica, Peru. *Cactus and Succulent Journal*, **94**, 28–33. https://doi.org/10.2985/015.094.0105

Novoa S., Redolfi I., Ceroni A., Arellano C. (2005) El forrajeo de la hormiga *Camponotus* sp. en los botones florales del cactus *Neoraimondia arequipensis* subsp. *roseiflora* (Werdermann & Backeberg) Ostolaza (Cactaceae). *Ecología aplicada*, **4**, 83–87.

Ollerton J., Alarcón R., Waser N.M., Price M.V., Watts S., Cranmer L., Hingston A., Peter C.I., Rotenberry J. (2009) A global test of the pollination syndrome hypothesis. *Annals of botany*, **103**, 1471–1480. https://doi.org/10.1093/aob/mcp031

Olesen J.M., Jordano P. (2002) Geographic patterns in plant–pollinator mutualistic networks. *Ecology*, **83**, 2416–2424. https://doi.org/10.1890/0012-9658(2002)083[2416:GPIPPM]2.0.CO;2

Opedal Ø. H. (2019) The evolvability of animal-pollinated flowers: towards predicting adaptation to novel pollinator communities. *New Phytologist*, 221, 1128–1135. https://doi.org/10.1111/nph.15403

Ossa G., Zamora H.T., Velazco P.M. (2020) *Platalina genovensium* (Chiroptera: Phyllostomidae). *Mammalian Species*, **52**, 105–113. https://doi.org/10.1093/mspecies/seaa008

Ostolaza C. (2014) *Todos los cactus del Perú*. Ministerio del Ambiente, Lima, Peru: 538 pp.

Ostolaza C. (1996) A closer look at the conservation status of Cacti in the vicinity of Lima, Peru. *British Cactus & Succulent Journal*, **14**, 158–174. https://www.jstor.org/stable/42793423

Pellón J.J., Mendoza J.L., Quispe-Hure O., Condo F., Williams M. (2021). Exotic cultivated plants in the diet of the nectar-feeding bat *Glossophaga soricina* (Phyllostomidae: Glossophaginae) in the city of Lima, Peru. *Acta Chiropterologica*, **23**, 107–117. https://doi.org/10.3161/15081109ACC2021.23.1.009

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

Posit team (2024) *RStudio: Integrated Development Environment for R.* Posit Software, PBC, Boston, USA.

POWO (2024) *Plants of the World Online.* Royal Botanic Gardens, Kew, London, UK.

Proctor M., Yeo P., Lack A. (1996) *The natural history of pollination*. Timber Press, Oregon, USA: 479 pp.

R Core Team (2024) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rico-Gray V. (1989) The importance of floral and circum-floral nectar to ants inhabiting dry tropical lowlands. *Biological Journal of the Linnean Society*, **38**, 173–181. https://doi.org/10.1111/j.1095-8312.1989.tb01572.x

Rivera-Marchand B., Ackerman J.D. (2006) Bat Pollination Breakdown in the Caribbean Columnar Cactus *Pilosocereus royenii*. *Biotropica*, **38**, 635–642. https://doi.org/10.1111/j.1744-7429.2006.00179.x

Rocha E.A., Domingos-Melo A., Zappi D.C., Machado I. C. (2019) Reproductive biology of columnar cacti: are bats the only protagonists in the pollination of *Pilosocereus*, a typical chiropterophilous genus? *Folia Geobotanica*, **54**, 239–256.

Rojas-Martínez A., Valiente-Banuet A., Del Coro Arizmendi M., Alcántara-Eguren A., Arita H.T. (1999) Seasonal distribution of the long-nosed bat (*Leptonycteris curasoae*) in North America: does a generalized migration pattern really exist?. *Journal of Biogeography*, **26**, 1065-1077. https://doi.org/10.1046/j.1365-2699.1999.00354.x

Rosas-Guerrero V., Aguilar R., Martén-Rodríguez S., Ashworth L., Lopezaraiza-Mikel M., Bastida J.M., Quesada M. (2014) A quantitative review of pollination syndromes: do floral traits predict effective pollinators?. *Ecology letters*, **17**, 388–400. https://doi.org/10.1111/ele.12224

Rowley G. (1980) Pollination syndromes and cactus taxonomy. *The Cactus and Succulent Journal of Great Britain*, **42**, 95–98.

765 Sahley C.T. (1996) Bat and hummingbird pollination of an autotetraploid columnar cactus,
766 *Weberbauerocereus weberbaueri* (Cactaceae). *American Journal of Botany*, **83**, 1329–1336.
767 https://doi.org/10.1002/j.1537-2197.1996.tb13918.x
768
769 Sahley C.T., Baraybar L. (1996) Natural history of the long-snouted bat, *Platalina genovensium*
770 (Phyllostomidae: Glossophaginae) in southwestern Peru. *Vida Silvestre Neotropical*, **5**, 101–109.
771
772 [dataset] Servicio Nacional de Meteorología e Hidrología del Perú - SENAMHI; 2022; Estación: VILLA
773 MARIA DEL TRIUNFO; Datos Hidrometeorológicos; https://www.senamhi.gob.pe/?p=estaciones
774
775 Schlumpberger B.O. (2011) A survey on pollination modes in cacti and a potential key innovation. In: Patiny
776 S. (Ed), *Evolution of Plant-Pollinator Relationships*. Cambridge University Press, Cambridge, UK: 301–319.
777 https://doi.org/10.1017/CBO9781139014113.011
778
779 Schlumpberger B.O., Cocucci A.A., Moré M., Sérsic A.N., Raguso R.A. (2009) Extreme variation in floral
780 characters and its consequences for pollinator attraction among populations of an Andean cactus. *Annals of*
781 *Botany*, **103**, 1489–1500. https://doi.org/10.1093/aob/mcp075
782
783 Schneider C.A., Rasband W.S., Eliceiri K.W. (2012) NIH Image to ImageJ: 25 years of image
784 analysis. *Nature methods*, **9**, 671–675. https://doi.org/10.1038/nmeth.2089
785
786 Silva W.R., Sazima M. (1995) Hawkmoth pollination in *Cereus peruvianus*, a columnar cactus from
787 southeastern Brazil. *Flora - Morphology, Distribution, Functional Ecology of Plants*, **190**, 339–343.
788 https://doi.org/10.1016/S0367-2530(17)30674-6
789
790 Smith C.E., Stevens J.T., Temeles E.J., Ewald P.W., Hebert R.J., Bonkvsky R.L. (1996) Effect of floral
791 orifice width and shape on hummingbird-flower interactions. *Oecologia*, **106**, 482–492.
792 https://doi.org/10.1007/BF00329706
793
794 Solís-Montero L., Vallejo-Marín M. (2017) Does the morphological fit between flowers and pollinators affect
795 pollen deposition? An experimental test in a buzz-pollinated species with anther dimorphism. *Ecology and*
796 *Evolution*, **7**, 2706–2715. https://doi.org/10.1002/ece3.2897
797
798 Stebbins G.L. (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination
799 mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307–326.
800

Su Q., Qi L., Zhang W., Yun Y., Zhao Y., Peng Y. (2020) Biodiversity survey of flower-visiting spiders based on literature review and field study. *Environmental Entomology*, **49**, 673–682. https://doi.org/10.1093/ee/nvaa022

Thompson J.N. (2002) *The geographic mosaic of coevolution.* University of Chicago Press, Chicago, USA: 400 pp. https://doi.org/10.7208/9780226118697

Thorp R.W. (2000) The collection of pollen by bees. In: Dafni A., Hesse M., Pacini E. (Eds), *Pollen and Pollination*. Springer, Vienna, Austria: 211–233. https://doi.org/10.1007/978-3-7091-6306-1_11

Tovar, C., Sánchez Infantas, E., Teixeira Roth, V. (2018) Plant community dynamics of Lomas fog oasis of Central Peru after the extreme precipitation caused by the 1997-98 El Niño event. *PLoS One*, **13**, e0190572. https://doi.org/10.1371/journal.pone.0190572

Tremlett C.J., Moore M., Chapman M.A., Zamora-Gutierrez V., Peh K.S.H. (2020) Pollination by bats enhances both quality and yield of a major cash crop in Mexico. Journal of Applied Ecology, **57**, 450–459. https://doi.org/10.1111/1365-2664.13545

Ulloa C., Zarucchi J.L., León B. (2004) Diez años de adiciones a la flora del Perú: 1993-2003. Arnaldoa, Trujillo, Peru: 242 pp.

Valiente-Banuet A., Molina-Freaner F., Torres A., Arizmendi M.D.C., Casas A. (2004) Geographic differentiation in the pollination system of the columnar *cactus Pachycereus pecten-aboriginum*. *American Journal of Botany*, *91*(6), 850-855. https://doi.org/10.3732/ajb.91.6.850

Valiente-Banuet A., Arizmendi M.D.C., Rojas-Martínez A., Domínguez-Canseco L. (1996) Ecological relationships between columnar cacti and nectar-feeding bats in Mexico. *Journal of Tropical Ecology*, **12**, 103–119. https://doi.org/10.1017/S0266467400009330

Vamosi J.C., Vamosi S.M. (2010) Key innovations within a geographical context in flowering plants: towards resolving Darwin's abominable mystery. *Ecology Letters*, **13**, 1270–1279. https://doi.org/10.1111/j.1461-0248.2010.01521.x

Walter H.E. (2010) Floral biology of *Echinopsis chiloensis* ssp. *chiloensis* (Cactaceae): evidence for a mixed pollination syndrome. *Flora - Morphology, Distribution, Functional Ecology of Plants*, **205**, 757–763. https://doi.org/10.1016/j.flora.2009.12.038

Westerkamp C.H. (1996) Pollen in bee-flower relations some considerations on melittophily. *Botanica Acta*, **109**, 325–332. https://doi.org/10.1111/j.1438-8677.1996.tb00580.x

Wasserthal L.T. (2001) Anpassungen bei Sphingiden zur Vermeidung von Spinnen-und Fledermausattacken. Verhandlungen Westdeustchen Entomologentag Dusseldorf, **2000**, 13–30.

Wenzell K.E., Neequaye M., Paajanen P., Hill L., Brett P., Byers K. J. (2025) Within-species floral evolution reveals convergence in adaptive walks during incipient pollinator shift. *Nature Communications*, 16, 2721. https://doi.org/10.1038/s41467-025-57639-3

Winter Y., von Helversen O. (2003) Operational tongue length in phyllostomid nectar-feeding bats. *Journal of mammalogy*, **84**, 886–896. https://doi.org/10.1644/BWG-032

Figures

Figure 1. (A) Geographic location and composition of the study area in Peru, showing the study area (SA) within the Lomas ecosystem (LE), near the Lurin River (LR), and surrounded by agricultural and semi-urban land. **(B)** Individual of *H. acranthus* in the study area.

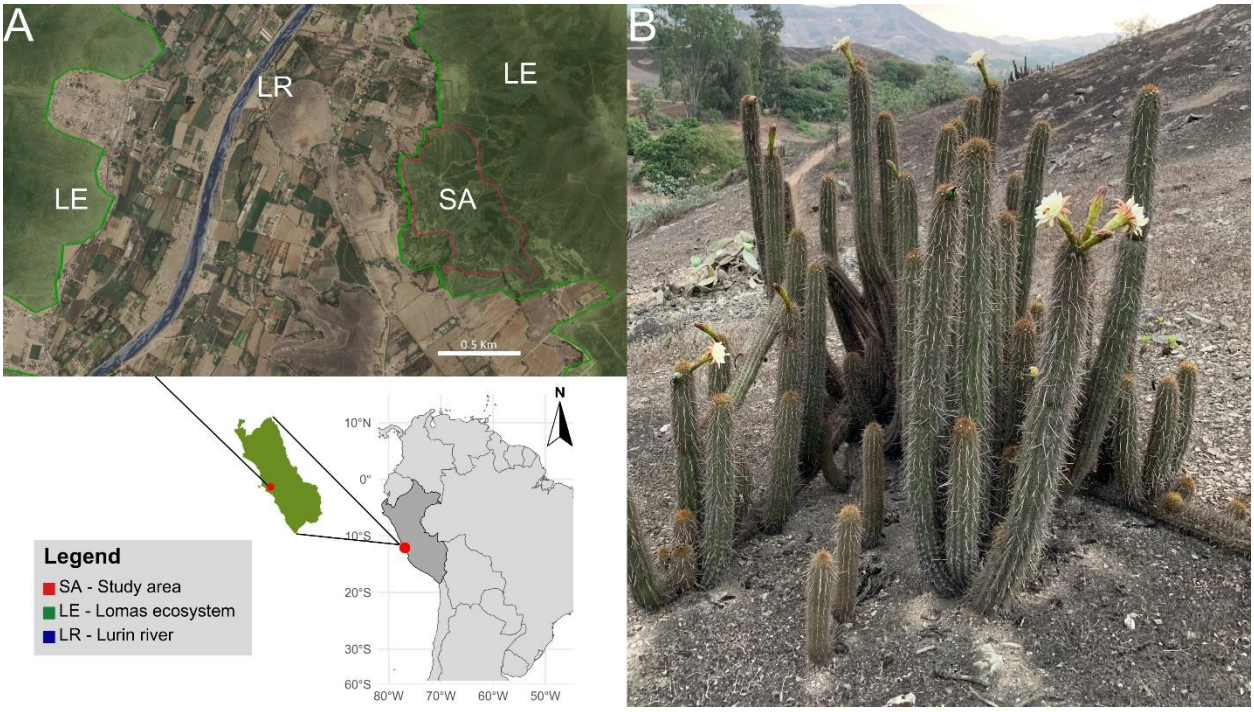
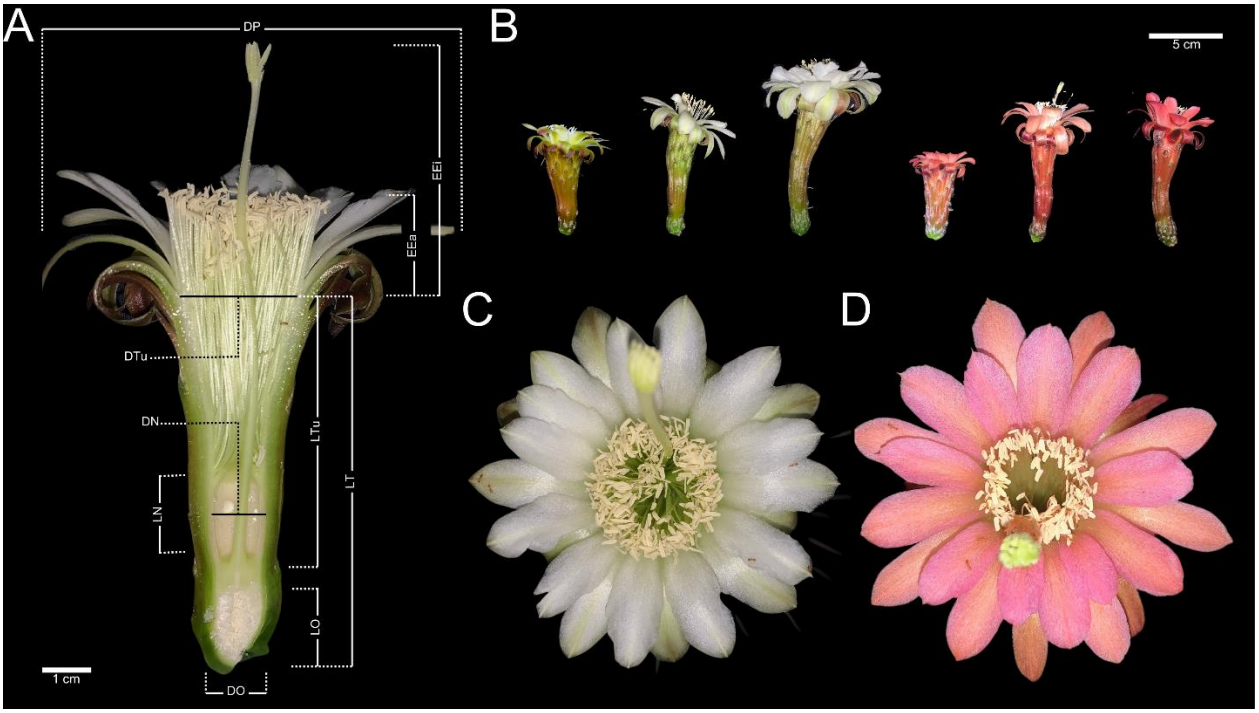
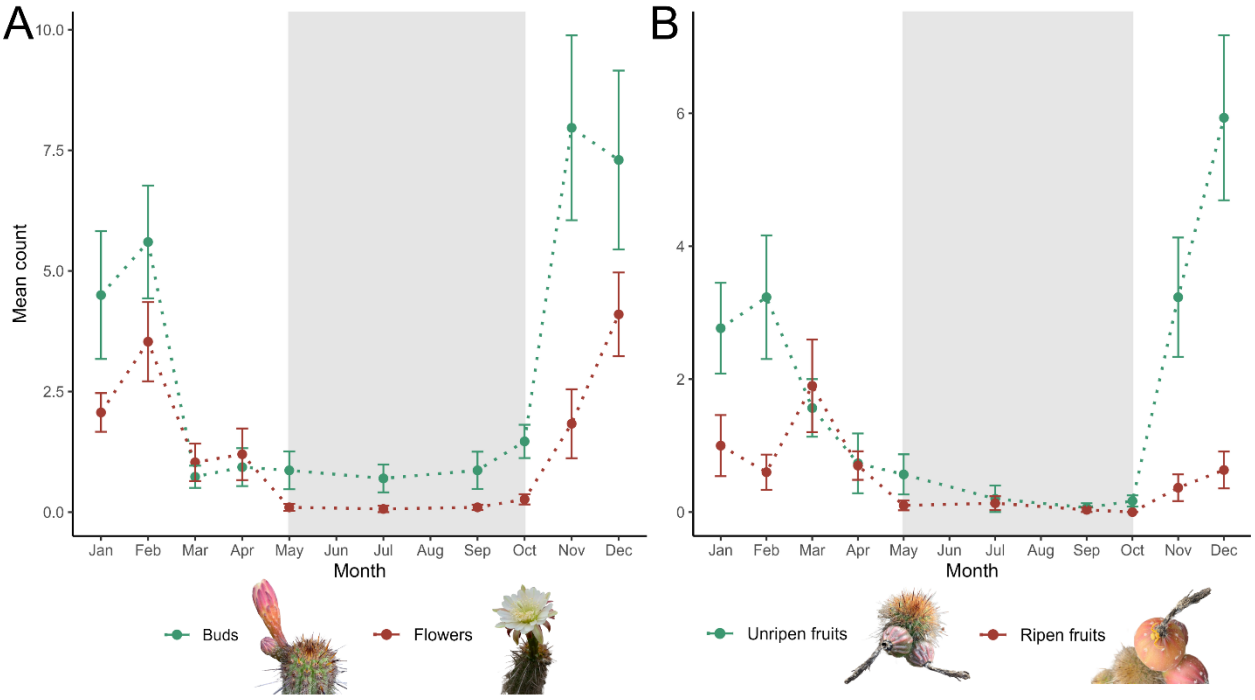


Figure 2. (A) Longitudinal cut of *H. acranthus* flowers indicating measured morphometric variables. (B) Size and shape variation in flowers of both morphotypes. Frontal view showing perianth color and reproductive structures of (C) white and (D) pink-red flower morphotypes.

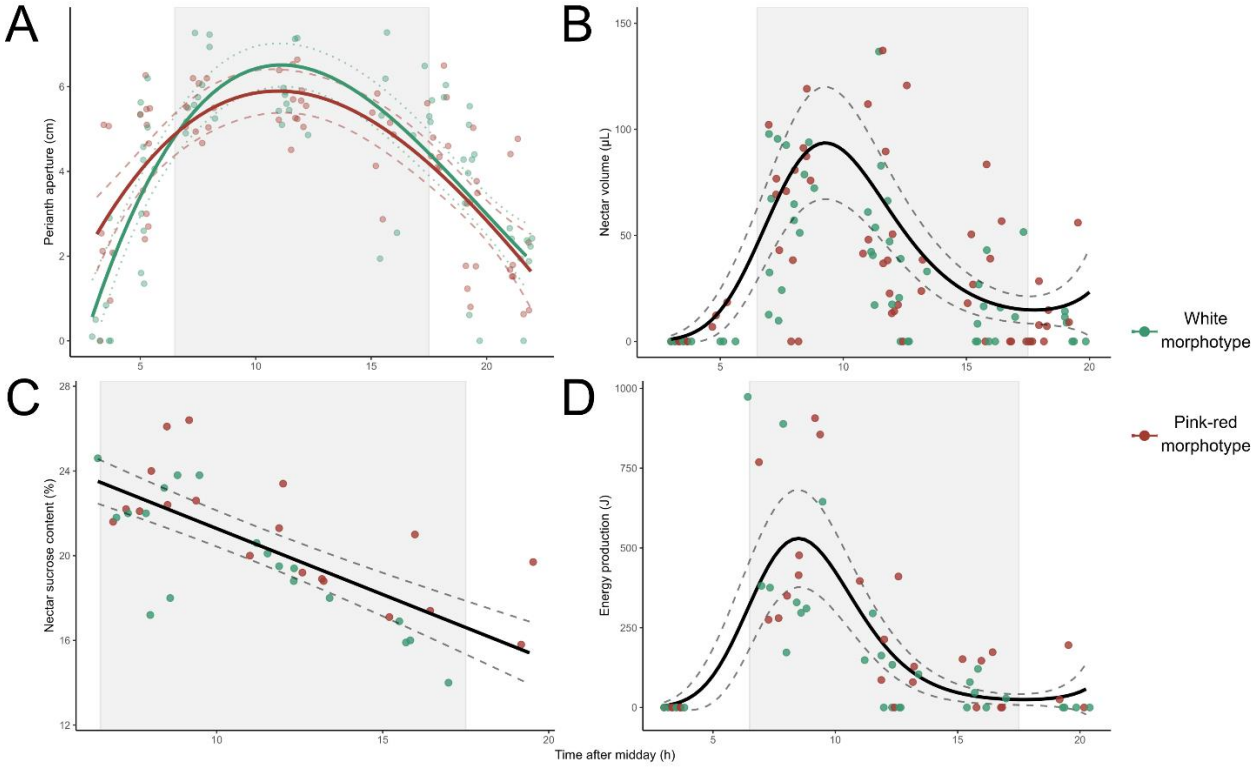


908 **Figure 3.** Monthly mean counts of (A) floral buds and flowers, and (B) unripe and ripe fruits observed
909 throughout 2022. Vertical bars indicate the standard error of the mean. Shaded gray areas correspond to the
910 humid Lomas season.



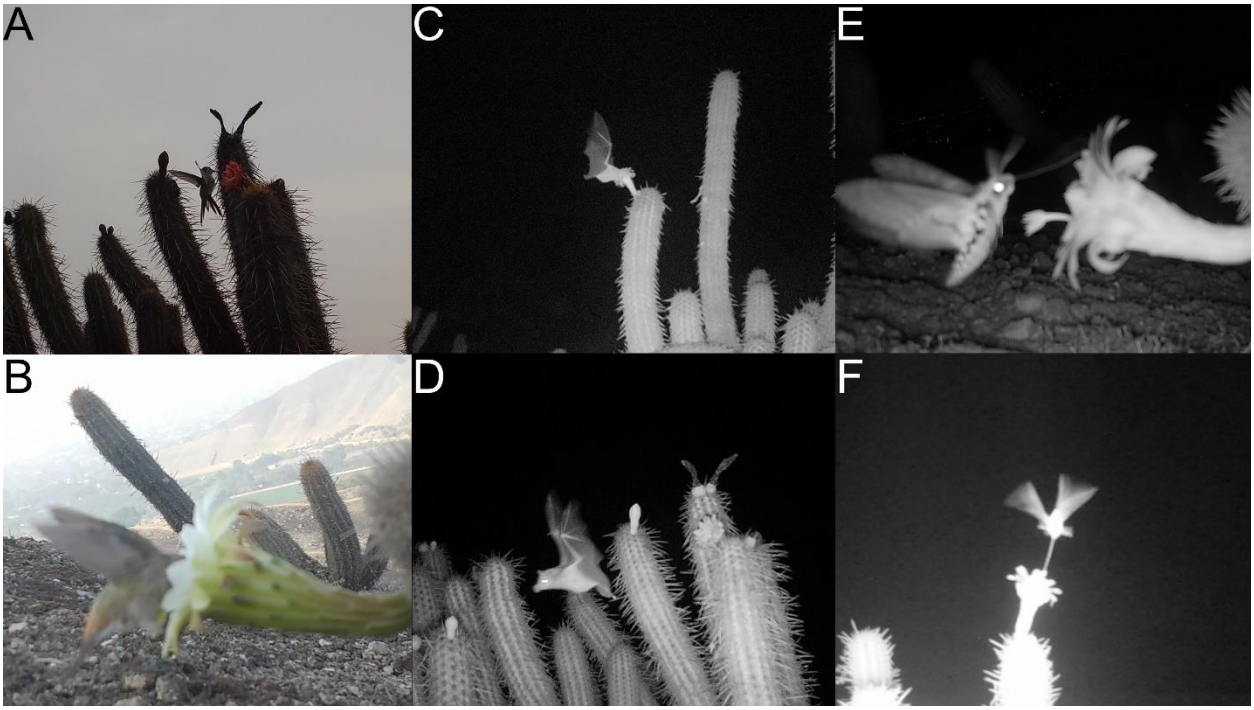
911
912
913
914
915
916
917
918
919
920
921
922
923
924

Figure 4. Temporal variation in (A) flower aperture, (B) nectar volume, (C) nectar sucrose content, and (D) nectar energy production as a function of time after midday. Solid lines represent model predictions, and dotted and dashed lines indicate 95% confidence intervals. In panel (A), the model includes the effect of morphotype, providing separate estimates and confidence intervals for each form. Shaded gray areas correspond to the nocturnal period.



941 **Figure 5.** Floral visitors of *H. acranthus*: (A–B) *Rhodopis vesper* (Trochilidae); (C–D) *Platalina*

942 *genovenisum* (Phyllostomidae); (E–F) *Manduca* sp. (Sphingidae).



943

944

945

946

947

948

949

950

951

952

953

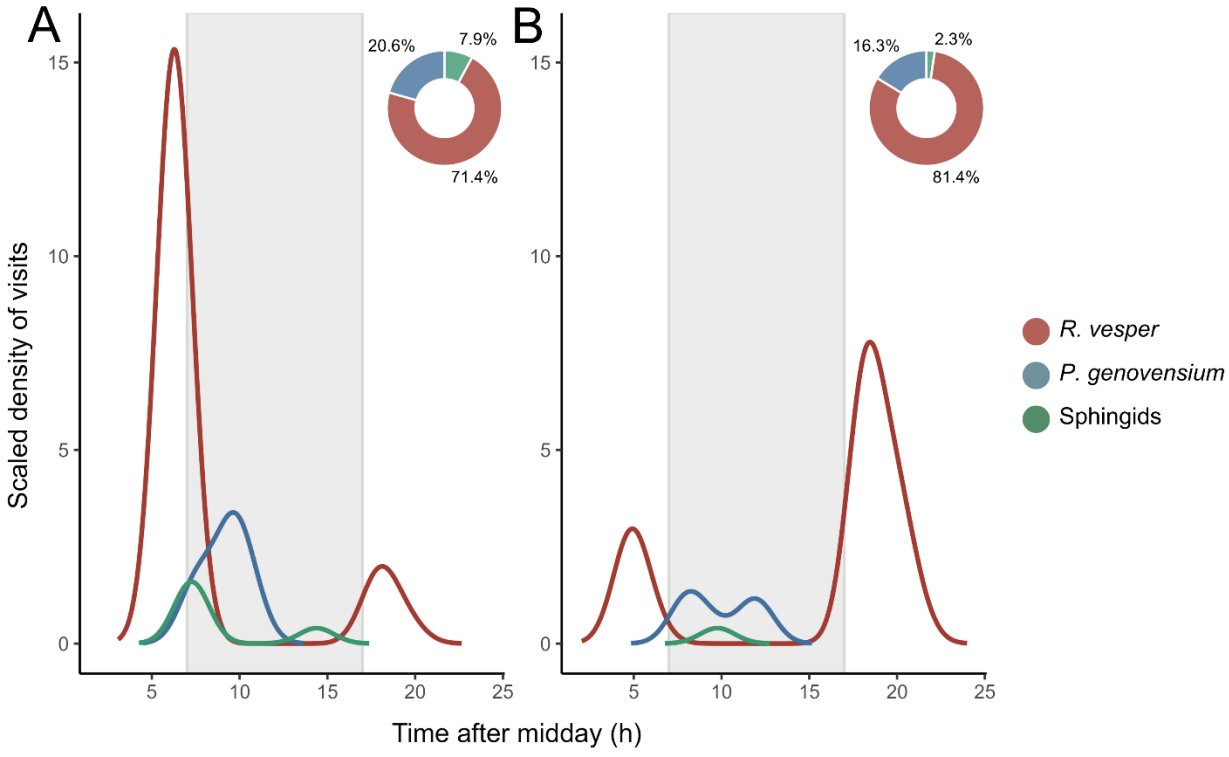
954

955

956

957

Figure 6. Scaled density of floral visits of *Rhodopis vesper*, *Platalina genovensium*, and sphingid moths over time for (A) white and (B) pink-red flower morphotypes. Ring plots indicate the percentage of total visits contributed by each visitor group for each morphotype. Shaded gray regions indicate the nocturnal period. Shaded gray areas correspond to the nocturnal period.



Tables

Table 1. Summary of the best-fitting models for each response variable: (A) flower aperture, (B) nectar volume, (C) nectar sugar concentration, (D) nectar energy content, and (E) visitation frequency. Fixed effects are expressed as estimates with their standard errors (SE), Z-values (*Z*), and p-values (*P*). Estimates are relative to white flowers for morphotype and to sphingid moths (*Manduca* sp.) for floral visitors.

Response variable	Fixed effect	Estimate	SE	*Z*	*P*
(A) Flower aperture	Intercept	4.11	0.17	24.88	<0.001
	T	-0.97	1.59	-0.61	0.540
	T^2	-18.83	1.58	-11.92	<0.001
	T^3	4.34	1.54	2.82	0.005
	T : Morph red-pink	-4.32	2.26	-1.91	0.056
	T^2 : Morph red-pink	4.89	2.24	2.18	0.029
	T^3 : Morph red-pink	-2.61	2.25	-1.16	0.247
(B) Nectar volume	Intercept	3.33	0.15	22.45	<0.001
	T	-0.11	2.05	-0.05	0.957
	T^2	-9.28	1.76	-5.29	<0.001
	T^3	8.14	1.81	4.51	<0.001
(C) Nectar sugar content	Intercept	20.39	0.43	47.27	<0.001
	T	-13.77	1.60	-8.58	<0.001
(D) Nectar energy	Intercept	4.33	0.22	19.69	<0.001
	T	-1.19	2.55	-0.47	0.639
	T^2	-7.71	2.06	-3.74	<0.001
	T^3	8.54	1.79	4.78	<0.001
(E) Visitation frequency	Intercept	-5.63	0.72	-7.83	<0.001
	Visitor *P. genovenisum*	1.20	0.47	2.59	0.01
	Visitor *R. vesper*	2.59	0.42	6.12	<0.001

´:´ indicates an interaction term. ´T´ represents time after midday (in hours).

984 **Table 2.** Summary of registered floral visitors, including visitor group, taxonomic classification, visit type,

985 floral resource utilized, and activity period.

Visitor group	Order	Family	Species	Visit type	Floral resource	Activity period
Bees	Hymenoptera					
		Apidae	*Apis mellifera*	I/L	Pollen	Diurnal
		Halictidae	-	I	Pollen	Diurnal
Ants	Hymenoptera					
		Formicidae	*Solenopsis* sp.	I	Nectar	Diurnal/Nocturnal
		Formicidae	*Linepithema* sp.	I	Nectar	Diurnal/Nocturnal
Flies	Diptera					
		Phoridae	-	I	Pollen	Diurnal
		Syrphidae	-	I	Pollen	Diurnal
Beetles	Coleoptera					
		Nitidulidae	*Carpophilus* sp.	I	Pollen, plant tissue	Diurnal/Nocturnal
Spiders	Araneae					
		Salticidae	-	I	Hunting ground	Diurnal/Nocturnal
		Anyphaenidae	-	I	Hunting ground	Diurnal/Nocturnal
		Thomisidae	-	I	Hunting ground	Diurnal/Nocturnal
Sphingids	Lepidoptera					
		Sphingidae	*Manduca* sp.	I/L	Nectar	Nocturnal
Hummingbirds	Apodiformes					
		Trochillidae	*Rhodopis vesper*	L	Nectar	Diurnal
Bats	Chiroptera					
		Phyllostomidae	*Platalina genovensium*	L	Nectar	Nocturnal

´I´ and ´L´ represent illegitimate and legitimate visits to flowers, respectively.

986

987

988

989

990

991

992

993