

POLLINATION BIOLOGY AND GENETIC NEIGHBORHOOD SIZE IN *AGAVE OBSCURA* SCHIEDE: INSIGHTS INTO THE REPRODUCTIVE BIOLOGY OF A POPULATION FROM CENTRAL VERACRUZ

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Abstract

Background: The genus *Agave* stands out for its remarkable adaptations that benefit nectarivores and pollinivores that play crucial roles in ecosystems. The reproductive patterns and their relationship with floral visitors are unknown to many species.

Questions: What is the phenology, reproductive system, nectar production and pollinators of *A. obscura*? What is the genetic diversity, population structure, and genetic neighborhood size in the study population of *A. obscura*?

Studied species: *Agave obscura* Schiede.

Study site and dates: Toxtlaacoaya, Las Vigas de Ramírez, Veracruz, Mexico in May -June 2018.

Methods: Floral and nectar traits were analyzed. We recorded floral visitors and performed controlled crosses to evaluate the reproductive system and the role of floral visitors. We estimated the genetic structure and the genetic neighborhood size with ISSRs.

Results: *A. obscura* is a protandrous species. Floral traits suggest adaptation to nocturnal pollinators. The population shows high genetic diversity, low structure, high gene flow and a neighborhood size consistent with herbaceous plants. Although it is generalist, its pollination system shows nectarivorous bat-specialization trends. Hummingbirds, bees, bumblebees, and nocturnal moths act as secondary pollinators or nectar and pollen robbers.

Conclusions: The genetic data indicates high and widespread effective pollinator activity, while the neighborhood size is influenced by pollen and seed dispersal, the density of reproductive plants, and the pollinators behavior.

Keywords: Asparagaceae, effective population size, genetic variation, nectar-feeding bats, reproductive systems.

Resumen

Antecedentes: El género *Agave* destaca por sus notables adaptaciones que benefician a nectarívoros y polinívoros que juegan roles cruciales en los ecosistemas. Los patrones reproductivos y su relación con visitantes florales son desconocidos en muchas especies.

Preguntas: ¿Cuál es la fenología, los sistemas reproductivos, la producción de néctar y los polinizadores de *A. obscura*? ¿Cómo es la diversidad genética, estructura poblacional y el tamaño del vecindario genético en el sitio de estudio de *A. obscura*?

Especie de estudio: *Agave obscura* Schiede.

Sitio y años de estudio: Toxtlaacoaya, Las Vigas de Ramírez, Veracruz, México, en mayo-junio 2018.

Métodos: Se analizaron los rasgos florales y del néctar. Se registraron los visitantes florales, se realizaron cruces controladas para evaluar el sistema reproductivo y el rol de los visitantes florales y se estimó la estructura genética y el tamaño del vecindario genético con ISSRs.

Resultados: *A. obscura* es una especie protándrica. Los rasgos florales sugieren adaptación a los polinizadores nocturnos. La población muestra altos niveles de diversidad genética, baja estructura, alto flujo genético y un tamaño del vecindario consistente con plantas herbáceas. Aunque es generalista, su sistema de polinización muestra una tendencia a la especialización a murciélagos nectarívoros. Colibríes, abejas, abejorros y polillas nocturnas actúan como polinizadores secundarios o robadores de néctar o polen.

Conclusiones: Los datos genéticos indican una alta y ampliamente efectiva actividad de los polinizadores, mientras que el tamaño del vecindario es influenciado por la dispersión de polen y semillas, la densidad de plantas reproductivas y el comportamiento de los polinizadores.

Palabras clave: Asparagaceae, murciélagos nectarívoros, sistemas reproductivos, tamaño efectivo de la población, variación genética.

The genus *Agave* is part of the Asparagaceae family and includes plants native mainly to the arid regions of the Americas, with a total of 210 species distributed from the southern United States to Colombia and Venezuela, also present in the Caribbean. However, Mexico is the country with the greatest diversity, with 159 species (75 % of the total), of which 61 % are endemic (129 species), which is why it is considered the center of diversity of the *Agave* genus (García-Mendoza 2007, 2011, García-Herrera *et al.* 2010, García-Mendoza *et al.* 2019). Their natural distribution is related to temperature and precipitation (Eguiarte *et al.* 2021). Some agaves are well known for providing the raw material to produce alcoholic beverages and as ornamental plants. Agaves and nectar-eating bats interact forming a complex diffuse coevolutionary mosaic (Eguiarte *et al.* 2021). Therefore, to better understand this relationship, it is essential to conduct studies on the reproductive ecology and pollination of various agave species.

Pollination involves the transfer of pollen from the anthers to the stigma of the same or different flowers by biotic or abiotic factors (Kevan *et al.* 2013, Sihag 2018, Fattorini & Glover 2020). Pollination biology studies, the reproductive systems of plants, genetic transmission, and their variation in populations (Brown 1990, Kearns & Inouye 1993, Kevan *et al.* 2013, Sihag 2018), so, the knowledge of reproductive systems is important for managing forests, crops, and for developing optimal conservation strategies (Brown *et al.* 1985). Pollination, pollen, and seed dispersal studies provide important information on gene flow in populations and for estimating neighborhood size (Handel 1983, Slatkin 1985, Ellstrand 1992, Richards 2000, Ghazoul 2005, Ahee *et al.* 2015).

Effective population size (N_e) is a measure of the size of a closed panmictic population that experiences the same amount of genetic drift as the actual population of interest (Wright 1931). However, in many natural populations with a wide and apparently continuous distribution, mating probabilities depend on the distance between them (isolation by distance; Wright 1943), or on the resistance of the landscape to gene flow (isolation by resistance; McRae 2006), making it difficult to set population boundaries. These populations are internally organized into local neighborhoods of overlapping breeding individuals. To quantify N_e in isolated populations by distance, it has been proposed the concept of genetic neighborhood size (Shirk & Cushman 2014). Genetic neighborhood size is the area of a population where free and random mating between individuals occurs, related to the concept of “isolation by distance”, which refers to gene flow limited to small areas, leading to local genetic differentiation, where individuals, separated by different neighborhoods, are genetically less similar compared to those within the same neighborhood (Wright 1943). Thus, the spatial dimension of the neighborhood in plants is influenced by pollen and seed dispersal distances, pollinator movements, pollen transfer between individuals, and different foraging behaviors when different classes of pollinators visit the flowers (Crawford 1984, Young 1988, Kearns & Inouye 1993, Parra *et al.* 1993, Ghazoul 2005).

Inter-Simple Sequence Repeats (ISSRs) are molecular markers whose allelic variation is based on the presence or absence of amplified products and highly polymorphic (Pradeep *et al.* 2002, Rentaria 2007). Because it is a PCR-based marker system, the assessment of genetic diversity using ISSRs is in widespread use (Schmidt *et al.* 2025). ISSRs are also used in the identification of germplasm resources, identity of varieties/hybrids, relationships analysis, genome mapping, gene tagging and phylogenetic reconstruction (Pradeep *et al.* 2002, Zhao *et al.* 2024). ISSR is a simple and quick method that combines most of the advantages of SSR, AFLP, and RAPD markers (Pradeep *et al.* 2002), including their ease of use, low cost, and being methodologically less demanding than the others molecular markers and transferable among species (Ng & Tan 2015). Therefore, ISSR markers have been effectively used in studies of genetic diversity in natural populations (Hernández-Leal *et al.* 2024, Zhao *et al.* 2024, Schmidt *et al.* 2025).

Agave obscura Schiede (subgenus *Littaea*, Tagl; group Polycephala), an endemic species to Mexico, has small to medium-sized rosettes, and over the years form groups of numerous rosettes. *A. obscura* is not listed in any risk category of the NOM-059-SEMARNAT-2010 (SEMARNAT 2010), and no prior studies have been conducted on this species, highlighting the relevance of this work. There are reports on *A. obscura* uses for leaf fiber extraction and as a food source (García-Mendoza & Franco-Martínez 2018). In the study area, local people in the past consumed the flowers as food, preparing traditional dishes with eggs. More recently, juvenile rosettes of *A. obscura* have been observed being sold in local markets of the region during the Christmas season as ornamental decorations. Despite the ecological and economic importance of the genus *Agave*, fundamental aspects of reproductive biology and popu-

lation genetics of many species remain unexplored. *A. obscura* is an example; actually, its pollination syndrome, breeding system, and genetic structure is unknown. Research on the reproductive ecology and pollination of diverse agave populations and species continues to be critically important and necessary (Eguiarte *et al.* 2021). Reproductive traits shape the genetic patterns of the population and the pollinator behavior and seed dispersal determine the spatial scale of mating, which is quantified by Wright's neighborhood size (Kearns & Inouye 1993). Integrating pollination biology with population genetics provides a powerful framework for unravel the links between ecological processes and genetic consequences, allowing predict how reproductive strategy affects the genetic diversity. We evaluated the pollination biology and genetic neighborhood size of *A. obscura* with the following objectives: 1) to analyze the phenology and floral morphology of *A. obscura*; 2) to evaluate the production and sugar concentration of nectar; 3) to evaluate the effectiveness of its reproductive system; 4) to evaluate the effectiveness, frequency and composition of diurnal and nocturnal floral visitors; and 5) to evaluate the genetic structure, the size of the genetic neighborhood and the effective size of the neighborhood using IRRSs.

Materials and methods

Study area. The study was conducted during May -June 2018, in Toxtlacoaya, Veracruz, Mexico (19° 31' - 19° 41' N; 96° 39' - 97° 05' W; 2,426 m asl), where wild populations of *A. obscura* occur (Figure 1). The site features a temperate humid climate (mean annual temperature: 12.6 °C; average annual precipitation: 1,124.7 mm, CONAGUA 2020). Vegetation consists of pine-oak forest (*Pinus-Quercus*) dominated by *Pinus ayacahuite* C. Ehrenb. ex Schltld., *P. leiophylla* ex Schiede ex Schltld. & Cham., *P. teocote* Schltld. & Cham., *P. patula* Schltld. & Cham., *Quercus crassifolia* Bonpl., *Q. laurina* Bonpl., *Buddleja cordata* Kunth, and *Hesperocyparis benthamii* (Endl.) Bartel (Cházaro 1992). Soil is derived from < 5,000-years-old lava flow ("malpaís") originating from El Volcancillo volcanic cone (Geissert 1994).

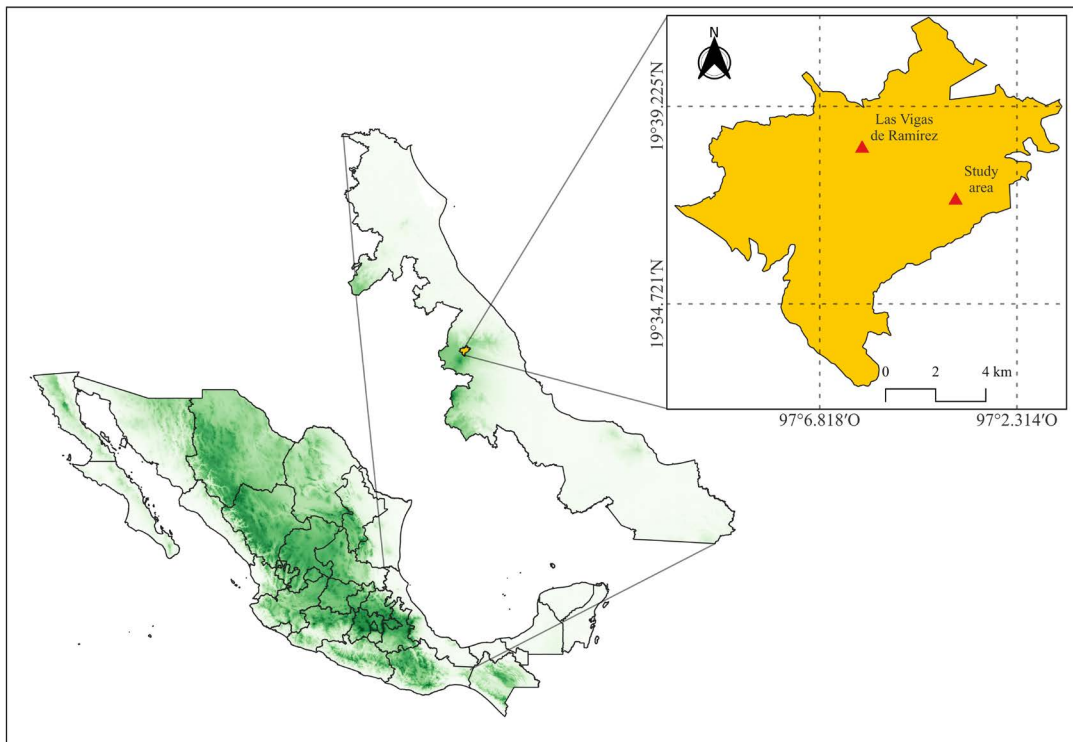


Figure 1. Study site in the municipality of Vigas de Ramírez, Veracruz, México.

Study species. *Agave obscura* Schiede (Asparagaceae: Agavoideae), a member of the Polycephala group (subgenus *Littaea*) is a medium -sized (40-60 cm tall), clustering or solitary agave endemic to Mexico's high-rainfall regions, including tropical deciduous forest, tropical savannas, and cloud forest in Tamaulipas, San Luis Potosí, Veracruz, Puebla, and Oaxaca (Cházaro 1981, Gentry 1982, Espejo-Serna 2012). Its rosettes comprise 35-65 lanceolate-acuminate leaves (green-yellow, transiently glaucous) with straight margins and small reddish-brown spines. The 2-3 m floral escape has a spike inflorescence bears dense, reddish, protandrous flowers (to 51 mm) in sequential anthesis. Fruits are dehiscent capsules containing numerous wind-dispersed seeds (fertile black and infertile white) with high germination rates (Cházaro 1981, Gentry 1982, Cuéllar-Martínez *et al.* 2024).

Phenology and floral morphology. To evaluate floral longevity and morphology, and to capture intrapopulation morphology variation with statistical robustness, we monitored 20 randomly selected *A. obscura* plants, marking five flower buds per plant (100 buds total) until senescence. Daily measurements of floral structures (ovary length and diameter, corolla length and diameter, length of the stamens and pistil, and total flower length) were recorded during anthesis using vernier caliper. We documented anther dehiscence and assessed stigma receptivity through both visual confirmation of sticky exudate (Slauson 2000, Molina-Freaner & Eguiarte 2003, Rocha *et al.* 2005) and 6 % hydrogen peroxide application on the stigma, where bubbles formation indicated active receptive (Dafni & Maués 1998). Observations continued until complete floral senescence.

Nectar production. To quantify nectar production and concentration, we randomly selected five plants with five flowers each (25 flowers total). Flowers were bagged with mesh fabric to exclude floral visitors. Nectar volume was measured every three hours using 75 µl capillary tubes (Corning®), while sugar concentration was determined with a portable refractometer 30PX (Mettler Toledo portableLab™, Columbus, Ohio). To estimate daily nectar production, an additional 25 flowers (five plants x five flowers) were randomly selected, bagged and monitored throughout anthesis. Volume of nectar and sugar concentration were recorded every 24 hr (Rocha *et al.* 2005).

Reproductive system experiments. To evaluate the reproductive system of *A. obscura*, ten plants were randomly selected, with five flowers per plant assigned to each of four treatments (50 flowers per treatment). Flowers were chosen from the mid-region of the inflorescence at the onset of anthesis. Treatments included: *Manual cross-pollination*: daily hand-pollination at 19:00 hr. using pollen from other individuals until styles withering. *Self-pollination*: Daily hand-pollinated at 19:00 hr. with pollen from the same inflorescence. *Apomixis*: flowers were emasculated (anthers removed) to prevent pollen deposition, testing for asexual reproduction (Arizaga *et al.* 2000a). *Control*: flowers were exposed to both diurnal and nocturnal floral visitors. All treated flowers were bagged with nylon mesh to exclude visitors before and after manipulations. Pollinations were performed during stigma receptivity, that is, when they clearly presented the sticky exudate.

Pollinator experiments. To evaluate the contribution of diurnal and nocturnal pollinators in *A. obscura* reproduction, ten plants were randomly selected, with five flowers per plant assigned to each of four treatments (50 flowers per treatment). Flowers were chosen from the lower/middle inflorescence sections: *Diurnal pollination*: Flowers bagged nightly (19:00-06:30 hr.) to exclude nocturnal visitors. *Nocturnal pollination*: Flowers bagged daily (06:30-19:00 hr.) to exclude diurnal visitors. *Pollinator exclusion*: flowers remained bagged throughout the flowering period to prevent all pollinator access. *Control*: flowers were marked but left unbagged, freely accessible to all pollinators.

Upon maturation before dehiscence, fruits from these treatments were collected. We quantified the fruit set and mean of fertile and infertile seed set (Slauson 2000).

Floral visitors. The floral visitation rate was quantified by video-recording four mid-flowering inflorescences for five consecutive days (06:00-08:00 hr and 20:00-22:00 hr), using an infrared-equipped digital camera (Sony DCR-SR65, Sony Corporation, Tokyo, Japan) mounted on a tripod 1 m away. A visitation event was defined as physical contact

with the inflorescence. Pollinators were identified using four mist nets (3 × 9 m) deployed near inflorescences for six days (06:00-10:00 hr and 19:00-24:00 hr) with checks every 30 minutes. Birds and bats were identified using field guides (Medellín *et al.* 2008, Arizmendi & Berlanga 2014). Pollen was collected from captured specimens with a paintbrush, preserved in 70 % ethanol within Eppendorf tubes, and later prepared for microscopic observations. Pollen grains were compared to *Agave obscura* reference samples under an optical microscope (Iroscope MOD. MG-11TF). Specimens were released after sampling at the capture site.

DNA extraction and ISSR analysis. Leaf tissue was collected from 40 *A. obscura* individuals (minimum 10 m spacing) and stored at -4 °C. DNA extraction was performed using Doyle & Doyle (1987) miniprep protocol modified by Vázquez Lobo (1996), with quality assessed via agarose gel electrophoresis and an Invitrogen™ Qubit® 3.0 fluorometer (ThermoFisher Scientific, Waltham, Massachusetts). Two high polymorphism ISSR primers (Scheinvar Gottdiener 2008) were used: 841 (GA) 8 YC and 846 (CA)8 RT. Polymerase chain reaction (PCR) amplifications was conducted under the following conditions: initial denaturation of 94 °C for 4 min. 35 cycles at 94 °C for 30 s (denaturation); 51 °C for 50 s (annealing) for primers 846, 811, and 841 (primer 857 was annealed at 53 °C); 72 °C for 2 min (extension) with a final extension of 72 °C for 5 min. The final concentrations of the PCR reagents were: 1X Buffer, 1.5 mM MgCl₂ for primers 811 and 857 (2 mM for 841 and 846), 0.2 mM dNTPs, 0.5 mM primer, 1U Taq Polymerase and 20 ng/ml of DNA. PCR products were electrophoresed in GelRed®-stained agarose gel using TAE buffer, visualized and photographed with a UVP Transilluminator UVP MultiDoc-It Imaging System (analytikjena, Jena, Germany), and read to align gel lanes and manually assign loci using ImageJ (Wayne Rasband National Institutes of Health, USA) to generate a binary presence/absence matrix (0 = band absent, 1 = band present) for analysis.

Data Analysis. The fruit and seed set percentages were analyzed using non-parametric test (Kruskal-Wallis test followed by Wilcoxon-Mann-Whitney post hoc test) due to non-normality of the transformed data. These tests evaluated the effects of pollination treatments on fruits and seeds set, as well as the influence of floral visitation rates (Hymenoptera, moths, hummingbirds and nectar-feeding bats). Nectar volume and sugar concentrations differences were assessed using Wilcoxon-Mann-Whitney test. All analyses were performed in R (R Core Team 2022).

Genetic structure was inferred using Bayesian clustering analysis in STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000). We tested $K = 1-5$ populations with a burn-in of 10,000 generations and 10,000 Markov Chain Monte Carlo (MCMC) iterations and 20 runs for each data point. The optimal K was determined using ΔK method (Evanno *et al.* 2005) via STRUCTURE Harvester, which identified the most probable number of genetic clusters (Earl & von Holdt 2012). Genetic diversity parameters were estimated, including: Observed heterozygosity (H_o), genetic diversity within population (H_s), general genetic diversity (H_p), genetic diversity between samples (D_{st}), corrected D_{st} (D_{stp}), and a measure of population differentiation (D_{est}), whose values range from 0 to 1; where values close to 1 indicate high genetic diversity and genetic differentiation, and values close to 0 represent low diversity and genetic differentiation. We quantified genetic differentiation using Nei's F -statistics (Nei 1987): the fixation index (F_{st}) and corrected F_{st} (F_{stp}) and the inbreeding coefficient (F_{is}) and we also calculated the percentage of polymorphic loci (% P) and the genetic differentiation coefficient (G_{st}). All genetic diversity parameters, Nei's F -statistic and % P were computed via the *Hierfstat* R package (Goudet *et al.* 2022) and GenAlex 6.5 (Peakall & Smouse 2012). Gene flow (Nm) was derived from G_{st} as $Nm = 0.5 (1 - G_{st}/D_{st})$ (McDermott & McDonald 1993) using POPGENE v. 1.32 (Yeh *et al.* 1999).

We estimated genetic neighborhood size using the mean squared axial parent-offspring dispersal distance (σ^2), calculated from the regression slope between genetic distances (a) on the logarithm of geographic distances (r) among individuals. The inverse of the slope of that regression (b) is an estimate of the product $4D\pi\sigma^2$, where D is population density (Rousset 2000) and was inferred using GenAlex. The mean square axial distance of the parents-offspring σ^2 was estimated as Broquet *et al.* 2006:

$$\sigma^2 = 1/b \times 4 \times D \times \pi \quad (1)$$

Population density (D) was derived by averaging counts of reproductive adults across three randomly 10 m² plots within the study area. The genetic neighborhood size was estimated as (Wright 1946):

$$N_1 = 4 \times \pi \times \sigma^2 \times D \quad (2)$$

Assuming normal dispersion distances, where σ^2 is variance of the dispersal distance between offspring and parents; D is population density and N_1 is effective population size of the neighborhood (Wright 1946, Beattie & Culver 1979).

The neighborhood area (N_a) was calculated as:

$$N_a = 4 \pi \sigma^2 \quad (3)$$

Representing the circular area where expected heterozygosity matched observed heterozygosity excluding selfing effects (Fenster *et al.* 2003).

Results

Flowering phenology and morphology. The flowering season in the study area extended from May to early August. Flowers are hermaphroditic and protandrous, with stamens (staminate phase) maturing before pistil (pistillate phase). Anther dehiscence initiated at dusk (~ 19:00 hr.), on the third day post-anthesis, while stigma receptivity began at ~ 20:00 hr. on the fifth day (Figure 2). Mean floral longevity was 5.95 ± 0.19 days (mean \pm SE), with senescence marked by tepals wilting. Mean maximum stamen length was 6.73 ± 0.10 cm (mean \pm SE), while pistil length measured 6.65 ± 0.13 cm (mean \pm SE), and total flower length was 9.02 ± 0.12 cm (mean \pm SE) (Table 1).

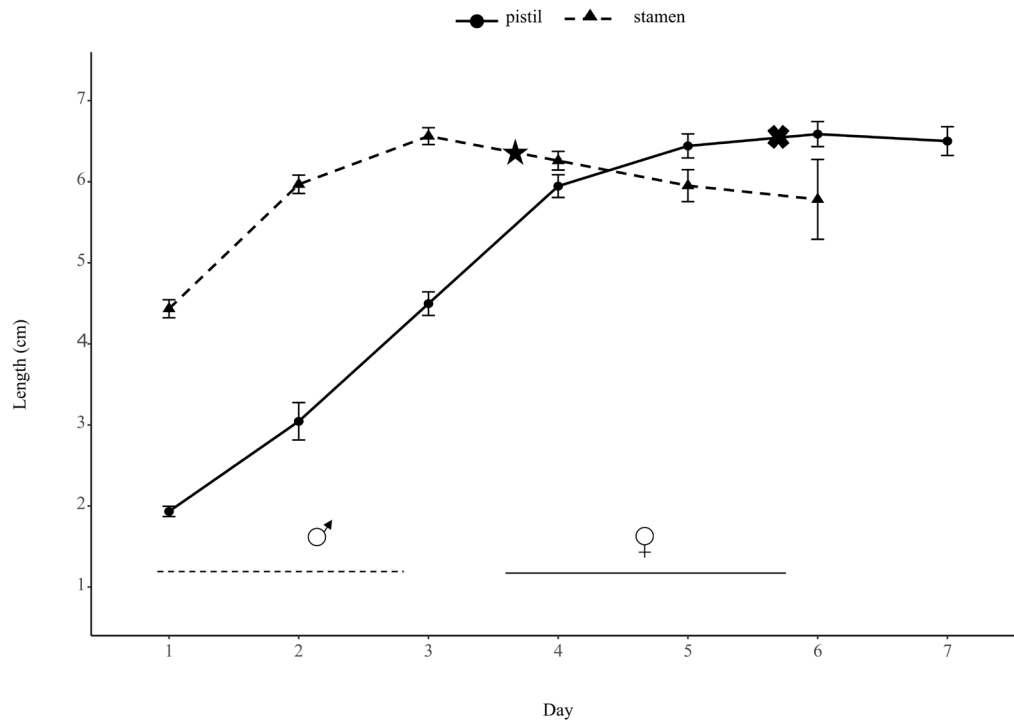


Figure 2. Development of stamen and pistil of *Agave obscura* flowers. Dotted line represents the average length of the stamens; the solid line represents pistils. The lower lines represent different phases: staminate (dotted ♂) and pistillate (continuous ♀). The symbol (★) represents the average day of anther dehiscence, and the symbol (×) represents the average day stigma receptivity; the bars correspond to the standard error (N = 100), see text.

Agave obscura pollination and genetics

Table 1. Morphological attributes of *Agave obscura* (mean \pm SE), N = 100 flowers.

Floral traits	Values
Mean ovary diameter (cm)	(0.453 \pm 0.005)
Mean ovary length (cm)	(2.110 \pm 0.028)
Mean corolla length (cm)	(1.974 \pm 0.334)
Mean corolla diameter (cm)	(3.131 \pm 0.057)
Mean maximum stamen length (cm)	(6.726 \pm 0.100)
Mean maximum pistil length (cm)	(6.659 \pm 0.135)
Mean Flower length (cm)	(9.022 \pm 0.127)
Dehiscence (Day)	(3.424 \pm 0.07)
Receptivity (Day)	(5.530 \pm 0.097)

Nectar production. Nectar volume and sugar concentration were quantified under two extraction frequencies (every 3 and 24 hr. intervals). Flowers sampled every 3-hour produced significantly greater mean total volume (223.30 μ l \pm 4.50, mean \pm SE) but lower sugar concentration (15.39 % \pm 0.36, mean \pm SE), compared to 24-hour extraction (83.71 μ l \pm 6.97, mean \pm SE volume; 18.62 % \pm 0.41 mean \pm SE concentration; Mann-Whitney *U* Test: 7,307, *P*-value < 0.05 for both). Nectar production initiated at 21:00 hr. on the first day of anthesis. Patterns production revealed peak nectar volume at 24:00 hr. (39.2 μ l \pm 3.50, mean \pm SE) and peak concentration at 03:00 hr. (17.97 % \pm 0.38, mean \pm SE), with minima for both at 15:00 hr. (10.33 μ l \pm 0.88, mean \pm SE volume; 10.35 % \pm 4.65, mean \pm SE concentration, [Figure 3A](#)). Under 24-hour sampling, maximum volume occurred on day 1 post-anthesis (132.53 μ l \pm 22.08, mean \pm SE), decreasing by day 5 (51.14 μ l \pm 14.18, mean \pm SE). Sugar concentration peaked on day 3 (19.87 % \pm 0.59, mean \pm SE) before decreasing on day 5 (16 % \pm 2.65, mean \pm SE) nectar production ceased after the fifth day ([Figure 3B](#)).

Reproductive systems. Fruit and seed production varied significantly among reproductive treatments ($\chi^2 = 13.354$, *df* = 2, *P* < 0.05). Control yielded the highest fruit set, outperforming both cross-pollination and self-pollination. Self-pollination showed the lowest reproductive success, with reduced fertile seed production per fruit ($\chi^2 = 12.819$, *df* = 2, *P* < 0.05), increased infertile seeds, and minimal fruit set ([Table 2](#)). No apomictic reproduction was detected.

Floral visitors and pollinators. *A. obscura* exhibited significantly higher fruit set under nocturnal pollination than diurnal pollination ($\chi^2 = 13.057$, *df* = 2, *P* < 0.05; [Table 3](#)). Although viable seed set did not differ significantly between pollination treatments, the control group produced the highest fertile seeds set ($\chi^2 = 12.915$, *df* = 2, *P* < 0.05). The diurnal treatment yielded more infertile seeds set, though not significantly different from nocturnal pollination, while the control showed the lowest infertile seed set.

Floral visitors. We recorded 22 hours and 51 minutes of video (11 h 53 min nocturnal, 10 h 57 min diurnal) to quantify visitation rates. Hymenoptera (*Apis mellifera*, *Bombus* sp., and other species) showed the highest visitation frequency (mean \pm SE = 32.4 \pm 5.397 visits/h; $\chi^2 = 17.243$, *df* = 3, *P* < 0.05), followed by nectarivorous bats (mean \pm SE = 23.6 \pm 6.973 visits/h). Nocturnal moths (mean \pm SE = 9.4 \pm 2.495 visits/h), and hummingbirds (mean \pm SE = 4.7 \pm 2.160 visits/h) exhibited significantly lower activity ([Figure 4](#)).

We captured 18 nectarivorous (*Leptonycteris nivalis*: n = 9; *Choeronycteris mexicana*: n = 3; *Anoura geoffroyi*: n = 6), of which, fifteen individuals carrying *A. obscura* pollen on body surfaces (snout, chest, head, and wing membranes); excluding *C. mexicana*, which carried no pollen. Eleven hummingbirds (*Lampornis clemenciae*: n = 6; *Hylocaris leucotis*: n = 5) were captured diurnally ([Figure 5](#)), none carried pollen.

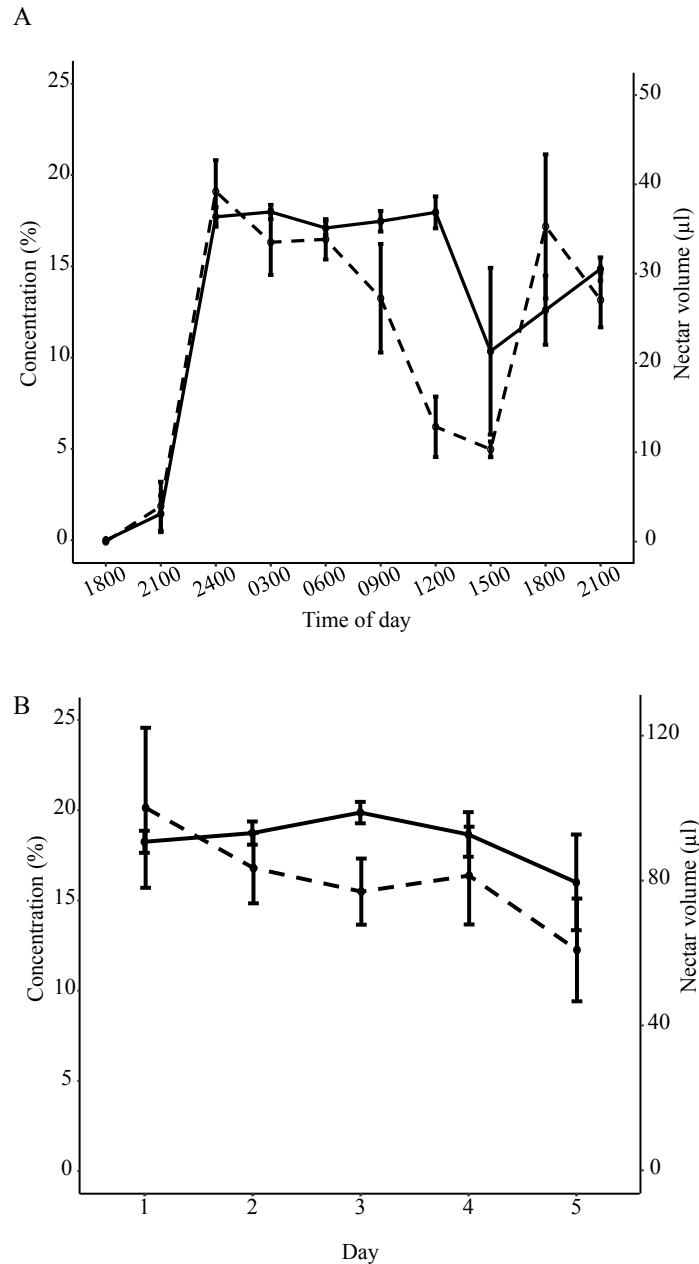


Figure 3. Nectar dynamics in *Agave obscura*. (A) Average sugar concentration (solid line) and average nectar volume (dashed line) measured every 3h (N = 40). (B) Average sugar concentration (solid line) and nectar volume (dashed line) measured every 24h (N = 25). The values are the means and bars correspond to standard error.

Genetic variation and neighborhood size. Seventeen polymorphic loci (6 from ISSR 841, 11 from ISSR 846) were identified. The most probable K value was K = 2 with 17 loci (Figure 6). The *A. obscura* population exhibited 67 % polymorphic loci, with high genetic variation ($H_o = 0.338$, $H_s = 0.336$, $H_t = 0.372$), a high number of effective migrants ($Nm = 11.995$) and low genetic structuring ($F_{st} = 0.096$). The inbreeding coefficient was negative ($F_{is} = -0.006$), indicating heterozygotes excess, and high genetic diversity (Table 4).

The regression slope of genetic distances against logarithmic geographic distances was 0.0151, indicating no significant isolation by distance (IBD) in the population, further supporting low population genetic structure. The mean density of reproductive adult *A. obscura* individuals (flowering individuals across three 10 m² quadrats) was

Table 2. Fruit and seed production in *Agave obscura* for different reproductive systems treatments. A and b indicate groups with significant differences for the number of fruits ($\chi^2 = 13.354$, $df = 2$, $P < 0.05$) and the number of viable and unviable seeds ($\chi^2 = 12.819$, $df = 2$, $P < 0.05$).

Treatments	Number of fruits	% Fruit set (mean ± SE)	% Viable seed per fruit (mean ± SE)	% Unviable seed per fruit (mean ± SE)
Cross-pollination	23	51.346 ± 7.514a	45.442 ± 2.886a	44.557 ± 2.886 a
Self-pollination	9	36.246 ± 5.928a	25.110 ± 1.752b	64.884 ± 1.752b
Control	47	83.420 ± 0.213b	41.230 ± 2.164a	48.769 ± 2.164a

Table 3. Fruit and seed production in *Agave obscura* according to different pollination treatments. The groups a and b represent significant differences in fruit number ($\chi^2 = 13.057$, $df = 2$, $P < 0.05$) and number of viable and unviable seeds ($\chi^2 = 12.195$, $df = 2$, $P < 0.05$).

Treatments	% Fruit set (mean ± SE)	% Viable seed per fruit (mean ± SE)	% Unviable seed per fruit (mean ± SE)
Diurnal pollination	35.009 ± 4.222a	20.543 ± 3.865a	69.456 ± 3.865a
Nocturnal pollination	39.005 ± 6.732a	28.469 ± 3.733a	61.530 ± 3.733a
Control	83.420 ± 4.486b	41.230 ± 2.164b	48.769 ± 2.164b

estimated at 0.33 ind/m² (ca. 1 individual every 3 m²). Using formula 1, where $b = 0.0151$ and $D = 0.33$ ind/m², the axial variance of dispersal (σ^2) was estimated at 15.96 m², suggesting a pattern of moderately restricted dispersal. From this, the neighborhood size (formula 2) was 66.22 individuals and the genetic neighborhood area (formula 3) was 200.67 m², indicating a high population density and dispersal ability. This contrast with the total sampled population of 63 adults, including only one reproductive individual (in bloom) in the sampled area.

Discussion

The nectar characteristics of *Agave obscura* attract diverse floral visitors, with nocturnal pollinators (particularly nectarivorous bats) demonstrating the highest pollination efficiency, as evidence by superior fruit and viable seed set compared to other pollination modes (Galindo-González *et al.* 2024), this aligns with findings in related *Agave* species (Molina-Freaner & Eguiarte 2003). Both diurnal and nocturnal visitors contribute significantly to reproductive success, supported by high seed germination rates (76 %) (Cuéllar-Martínez *et al.* 2024). The estimated genetic neighborhood size reflects the combined effects of anemochorous seed dispersal and efficient pollen dispersal mediated by pollinator movement.

Floral phenology and morphology. *Agave obscura* exhibits key floral traits with congeneric species (*A. striata*, *A. horrida*, *A. macroacantha*, and *A. palmeri*), particularly protandry and comparable floral longevity (Arizaga *et al.* 2000a, Slauson 2000, Molina-Freaner & Eguiarte 2003, González Pérez 2004, Rocha *et al.* 2005, Flores-Torres & Galindo-Escamilla 2017). While protandry reduces self-pollination, the temporal variation in inflorescences flowering does not completely eliminate it (Arizaga *et al.* 2000a). The floral morphology of *A. obscura*-including nocturnal anther dehiscence and stigma receptivity strongly supports bat pollination, a syndrome consistent with other members of subgenus *Littaea* (Silva-Montellano & Eguiarte 2003a, González Pérez 2004, Rocha *et al.* 2005, Flores-Torres & Galindo-Escamilla 2017, Galindo-González *et al.* 2024).

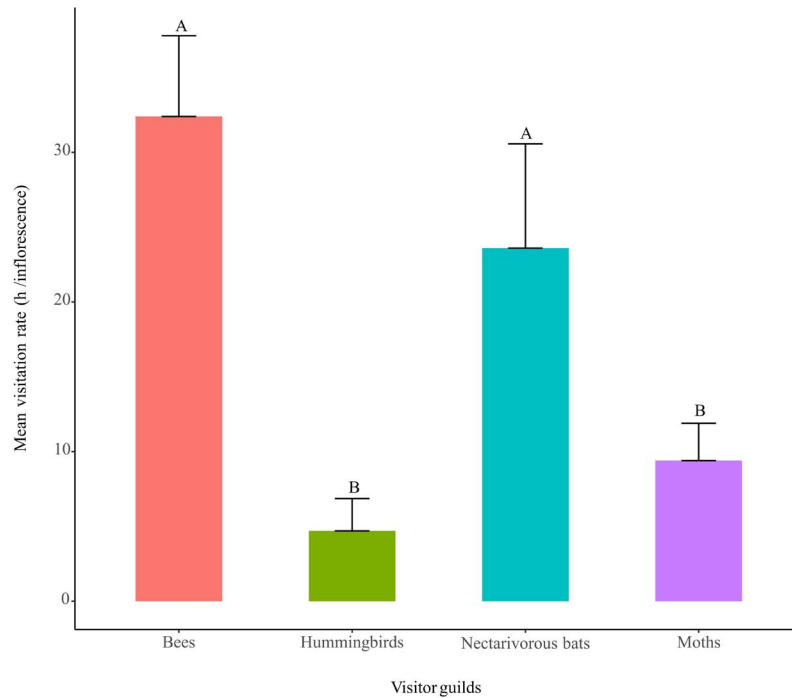


Figure 4. Rate of floral visitors per hour. The bars represent the mean frequency of visits and with standard error ($\chi^2 = 17.243$, $df = 3$, $P < 0.05$). The different labels (A, B) correspond to significance differences.

Nectar production. The nectar dynamics (volume and sugar concentration) in *A. obscura* reflect a bimodal pollination syndrome, with production patterns overlapping both nocturnal and diurnal pollinator activity periods. This is consistent with reports for *A. striata* (Rocha *et al.* 2005) and other *Agave* species, including *A. celsii* var. *albicans*, *A. difformis*, *A. xylonacantha*, *A. garciae-mendozae*, *A. horrida*, and *A. lechuguilla* (Eguiarte *et al.* 2000, González Pérez 2004, Rocha *et al.* 2005, Flores-Torres & Galindo-Escamilla 2017). The sugar concentration in *A. obscura* nectar (15-18 %) is characteristic of chiropterophilous species (Schaffer & Schaffer 1977, Slauson 2000, González Pérez 2004, Rocha *et al.* 2005, Flores-Torres & Galindo-Escamilla 2017), contrasting markedly with agaves pollinated exclusively by insects and hummingbirds (*e.g.*, *A. parviflora* and *A. toumeyana*), produce low nectar volumes (0.9-4.6 μ l) with high sugar concentrations (37-62 %) (Schaffer & Schaffer 1977). The pronounced nectar peak at 24:00 hr. and subsequent decline after 06:00 hr. further support a nocturnal adaptation, although the remaining diurnal production may facilitate pollination by secondary pollinators.

Reproductive systems and pollination experiments. *Agave obscura* aligns with the reproductive pattern observed in other *Agave* species, where cross-pollination produces significantly higher fruit and viable seeds sets than self-pollination (Arizaga *et al.* 2000a, Slauson 2000, Molina-Freaner & Eguiarte 2003, González Pérez 2004, Rocha *et al.* 2005, Flores-Torres & Galindo-Escamilla 2017). Although floral visitors, including bats and insects, may inadvertently facilitate geitonogamy during foraging (González Pérez 2004), *A. obscura* likely minimizes self-pollination through mechanisms such self-incompatibility systems, dichogamy, acropetal flowering and fruit (de Jong *et al.* 1993, Finer & Morgan 2003). Unlike most agaves, which depend heavily on nocturnal pollinators for reproductive success (Arizaga *et al.* 2000a, González Pérez 2004, Rocha *et al.* 2005, Flores-Torres & Galindo-Escamilla 2017), *A. obscura* exhibits no clear pollination preference, with both diurnal and nocturnal visitors demonstrating efficiency in fruit and seed production, a trait shared with *A. subsimplex* (Molina-Freaner & Eguiarte 2003). Although agaves exhibit inbreeding depression- characterized by reduced seed viability or germination by self-pollination (Treme *et*

al. 1995, Borbón-Palomares *et al.* 2018), *A. obscura* appears to deviate from this trend. Despite favoring cross-pollination, its self-pollinated seeds achieved higher germination rates (69 %) compared to cross-pollinated seeds (50 %) (Cuéllar-Martínez *et al.* 2024). This suggests that *A. obscura* is self-compatible and may have adapted to reproduce effectively even in scenarios with few pollinators.

Floral visitors. *Agave obscura* exhibits a generalist pollination system with chiropterophilous tendencies, a common pattern among agaves of the subgenus *Littaea* (Arizaga *et al.* 2000a, Slauson 2000, Ornelas *et al.* 2002, Molina-Freaner & Eguiarte 2003, Rocha *et al.* 2005, Magaña *et al.* 2008, Trejo-Salazar *et al.* 2015, Flores-Torres & Galindo-Escamilla 2017). While the high specialization of pollinators endangers ecosystem vulnerability (Johnson & Steinerb 2000), agaves maintain their reproductive capacity thanks to their long lifespan and vegetative propagation (Bond 1994). Their generalist pollination strategy may enhance reproductive success via supplemental visits from co-adapted pollinators, particularly in areas lacking nectarivorous bats (Rocha *et al.* 2005). In our study area, three nectarivorous bat species visited *A. obscura* flowers: *Anoura geoffroyi* and *Leptonycteris nivalis*- a known agave specialist (Moreno-Valdez *et al.* 2004, Sánchez & Medellín 2007, Burke *et al.* 2019)- serving as the primary pollinator; in contrast, *Choeronycteris mexicana*, although a frequent visitor, showed no pollen transfer (Galindo-



Figure 5. Some floral visitors. (A) *Anoura geoffroyi*, with pollen grains, (B) *Choeronycteris mexicana*, (C) *Leptonycteris nivalis*, (D) *Hylocharis leucotis*, (E) *Lampornis clemenciae*, (F) visit from *H. leucotis* and (G) bat visit to *A. obscura* captured in video.

González *et al.* 2024), probably to its long tongue (Nowak 1994), enabling nectar access without facial contact with reproductive structures (Arizaga *et al.* 2000b, Trejo-Salazar *et al.* 2015); *C. mexicana* is a generalist visitor to both columnar cacti and agaves (Arizaga *et al.* 2000b, Fleming & Holland 2018, Burke *et al.* 2019, Pérez-Hernández & Martínez-Coronel 2023), its role in *A. obscura* production warrants further study. Our results align with studies highlighting *Leptonycteris* bats-agaves coevolution. Phylogenetic evidence confirms this mutualism (Good-Avila *et al.* 2006, Gutiérrez-Guerrero *et al.* 2020), which likely drove semelparity in *Agave* via selection for bat-attractive traits like tall inflorescences, high floral density and high floral resource allocation (Eguiarte *et al.* 2021).

Secondary pollinators, including Hymenoptera and nocturnal moths, typically interact with flowers during staminate phase and often moving between them without stigmas contact, resulting in reduced pollination efficiency (González Pérez 2004, Silva-Montellano & Eguiarte 2003a, Trejo-Salazar *et al.* 2015, Clark *et al.* 2022). While some *Littaea* subgenus agaves species exhibit floral adaptations promoting entomophily (e.g., *Agave lechuguilla*; Schaffer & Schaffer 1977, Silva-Montellano & Eguiarte 2003a, Clark *et al.* 2022), bats remain the primary pollinators in most cases (Trejo-Salazar *et al.* 2015), as evidenced by the complete absence of fruit set in *Agave horrida* flowers visited by insects (Flores-Torres & Galindo-Escamilla 2017). We classified hummingbirds as secondary pollinators due to their infrequent visits and lack of *A. obscura* pollen transport, probably due to a temporal mismatch between their daytime activity and nocturnal anther dehiscence. Additionally, *Apis mellifera* removes up to 99 % of available pollen (e.g., in *Clusia arrudae* Planchon & Triana), negatively impacting native bee and wasp populations (do Carmo *et al.* 2004, Paini 2004, Cane & Tepedino 2017).

Genetic diversity and neighborhood size. The number of polymorphic loci in *A. obscura* obtained by ISSRs is comparable to other studies in agaves (Aguirre Dugua 2004, González Pérez 2004, Rocha Munive 2006, Trejo Hernández 2006, Scheinvar Gottdiener 2008, Eguiarte *et al.* 2013) and exhibits high genetic diversity (67 % of polymorphic ISSR loci). Our estimates of high genetic diversity and low structuring (Table 4) align with ranges typical for agaves using ISSRs ($H_s = 0.118 - 0.350$, F_{st} or $G_{st} = 0.063 - 0.350$) and other molecular markers ($H_s = 0.059$, $F_{st} = 0.057 - 0.39$) (Eguiarte *et al.* 2003, Silva-Montellano & Eguiarte 2003b, Aguirre Dugua 2004, González Pérez 2004, Baraza-Morales *et al.* 2006, Rocha Munive 2006, Trejo Hernández 2006, Alfaro-Rojas *et al.* 2007, Vargas-Ponce 2007, Scheinvar Gottdiener 2008, Figueredo & Nassar 2011, Félix Valdez 2014, Figueredo *et al.* 2015, Figueredo-Urbina

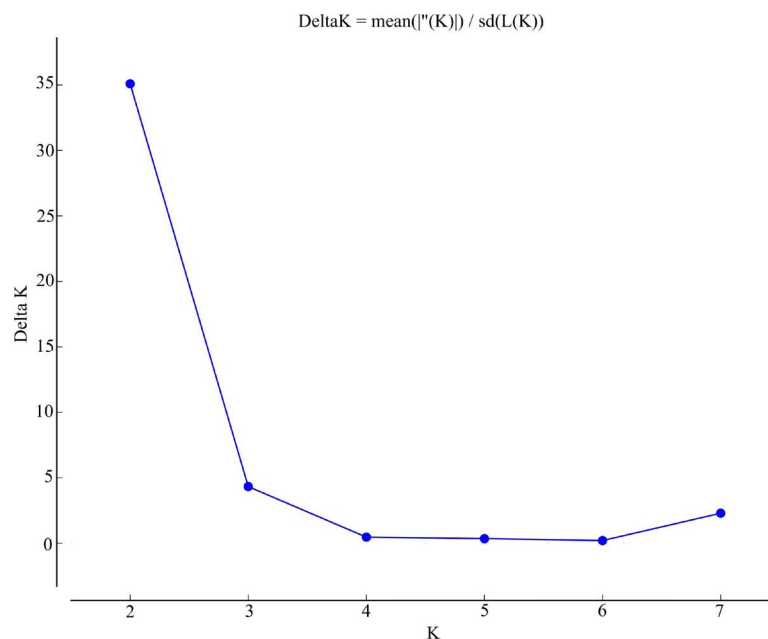


Figure 6. Estimated number of genetic groups (K) using Structure. The Delta K value was calculated with the Evanno method (Evanno *et al.* 2005).

et al. 2017, Lindsay *et al.* 2018). Agaves typically exhibit moderate genetic variation and low structure (Eguiarte *et al.* 2013), particularly in cross-pollinated species and wind-dispersed seeds (Eguiarte *et al.* 2013, Torres-Morán *et al.* 2024). These patterns reflect efficient pollinator activity (Silva-Montellano & Eguiarte 2003b, Aguirre Dugua 2004, Vargas-Ponce 2007, Scheinvar Gottdiener 2008, Figueredo & Nassar 2011). The slight heterozygote excess in *A. obscura*, suggests inbreeding depression, as reported in others *Agave* species (Silva-Montellano & Eguiarte 2003b, Figueredo & Nassar 2011, Eguiarte *et al.* 2013), this pattern is consistent with the rates of pollinator visits and the greater cross-pollination fruit set that we reported in this study, because the self-pollination rarely produces viable seeds (González Pérez 2004) or produce them in low quantities as we reported.

Neighborhood size (N_1) is an estimate of the effective population size (N_e) in distance-isolated continuous population (Wright 1969), neighborhood size and gene flow in plants are influenced by pollen and seed dispersal (Kearns & Inouye 1993, Collevatti *et al.* 2010). For *A. obscura*, we found a neighborhood area (N_a) of 200 m² and a neighborhood size (N_1) of 66 individuals, consistent with values of pollen and seed dispersal data reported for herbaceous species ($N_1 = 3-40$ individual and $N_a = 15-1,089$ m²; Eguiarte Fruns 1990, Parra *et al.* 1993, Eguiarte *et al.* 1994, Loh *et al.* 2020) and specifically for hummingbird-pollinated herbaceous plants (1-3,844 individuals; Eguiarte *et al.* 1994). These results are comparable for congeneric species, such *A. inaequidensis* ($N_1 = 36-709$; Figueredo *et al.* 2015). Notably, our N_1 value was derived from a total of 63 adult individuals, only one reproductive adult. ISSR markers have been effectively used in animal and plant studies, presenting the advantages of being cost-effective, reproducible, and technically straightforward (Eguiarte & Souza 2007). Their implementation requires basic molecular laboratory equipment, and the necessary software for data analysis is typically open-access (*e.g.*, RStudio, GenAlex). A thorough understanding of the advantages and limitations of ISSR markers, along with their proper implementation, is crucial for providing a solid justification for future, more sophisticated studies and methodologies (Ng & Tan 2015).

Table 4. Genetic diversity statistics. Percentage of polymorphic loci; Observed Heterozygosity (H_o); Average expected heterozygosity (H_e); total heterozygosity (H_t); genetic diversity between individuals (D_{st}); corrected genetic diversity (H_{sp}); corrected genetic diversity between individuals (D_{stp}); population fixation index (F_{st}); corrected population fixation index (F_{stp}); inbreeding coefficient (F_{is}); genetic differentiation coefficient (G_{st}); population differentiation (D_{est}).

% P	H_o	H_s	H_t	D_{st}	H_{sp}
67.65	0.338	0.336	0.372	0.036	0.375
D_{stp}	F_{st}	F_{stp}	F_{is}	G_{st}	D_{est}
0.038	0.096	0.102	-0.006	0.0400	0.057

Pollinator foraging behavior directly shapes pollen dispersal patterns and neighborhood size. While hummingbirds promote localized pollen flow due to territorial foraging behavior (Kearns & Inouye 1993, Parra *et al.* 1993, Loh *et al.* 2020), nectarivorous bats enable long-distance dispersal 77-144 m typically, up to 50 km like *Leptonycteris yerbabuena* can travel up to 50 km between roosting and foraging sites (Collevatti *et al.* 2010, Medellín *et al.* 2018, Diniz *et al.* 2019). Bat flight patterns are influenced by several factors, including high flowering synchrony and asynchrony of the food plants, plant population density, nectar production and foraging behavior of bats (Gribel & Hay 1993, Fuchs *et al.* 2003, Collevatti *et al.* 2010, Diniz *et al.* 2019, Loh *et al.* 2020). The elevated N_1 value observed in our study implies substantial pollen immigration from outside the sampled population.

We propose that *Agave obscura* exhibits a set of floral traits consistent with a generalist or mixed pollination systems, a recurring pattern that includes others *Agave* species (Schaffer & Schaffer 1977, 1979, Trame *et al.* 1995, Rocha *et al.* 2005, Trejo-Salazar *et al.* 2015, Flores-Torres & Galindo-Escamilla 2017) yet with a distinct tendency toward chiropterophily specialization: exposed inflorescences, reddish or greenish-white flowers, nocturnal anther dehiscence and diurnal and nocturnal nectar production, and abundant nectar characterized by low sugar concentration. This represents a conserved evolutionary strategy within the genus *Agave* that ensures both short-and long-distance gene flow while providing resilience against potential fluctuations in nectarivorous bat populations. This

reproductive strategy is reflected in the high diversity and the neighborhood size reported, which is characteristic of species with efficient and long-distance pollinators. Conversely, species like *A. parviflora* and *A. toumeyana* exhibit a distinct pattern: diurnal nectar production, low volume, and high sugar concentrations (> 37 %, Schaffer & Schaffer 1977), suggesting specialization to diurnal insect pollinators, representing an alternative extreme among agaves. In the study area, *A. obscura* faces no constraints on sexual reproduction: both cross-pollination and self-pollination ensure fruit and seed production, complemented by its high capacity for vegetative asexual reproduction and occasional recruitment of new individuals into the population. While nectarivorous bats serve as the primary pollinators, several insects-in addition to hummingbirds- act as secondary pollinators. The combined activity of diurnal and nocturnal pollinators facilitates cross-pollination, contributing to the high genetic diversity observed in *A. obscura* population. Neighborhood size is influenced by multiple factors, including reproductive adult density, pollen and seed dispersal patterns, flowering synchrony, nectar availability, and pollinator foraging behavior. The non-significant isolation-by-distance pattern, combined with the moderate axial dispersal variance and large neighborhood size reported in this study, suggests that while localized pollen and seed dispersal is frequent, long-distance gene flow events-primarily mediated by nectarivorous bats or even strong winds- prevent genetic structuring in the population. These results reinforce the strong association between *A. obscura* and its pollinators, implying a potential vulnerability to scenarios of pollination decline. Future studies integrating both population genetics and reproductive ecology in diverse *Agave* species and populations are essential to advance our understanding of their evolutionary dynamics and ecological interactions with pollinators. Such integrated approaches will not only elucidate patterns of genetic changes within populations but also reveal the bases behind these patterns, providing a scientific basis for species management and conservation strategies in face of environmental change.

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Literature cited

- Aguirre Dugua, X. 2004. *Genética de poblaciones de Agave cupreata y Agave potatorum: aportaciones para el manejo y conservación de dos especies mezcaleras*. BSc Thesis. Universidad Nacional Autónoma de México.
- Ahee JR, Van Drunen WE, Dorken ME. 2015. Analysis of pollination neighbourhood size using spatial analysis of pollen and seed production in broadleaf cattail (*Typha latifolia*). *Botany* **93**: 1-10. DOI: <https://doi.org/10.1139/cjb-2014-0169>
- Alfaro-Rojas G, Legaria-Solano JP, Rodríguez-Pérez JE. 2007. Diversidad genética en poblaciones de agaves pulqueros (*Agave* spp.) del nororiente del estado de México. *Revista Fitotecnia Mexicana* **30**: 1-12. DOI: <https://doi.org/10.35196/rfm.2007.1.1>
- Arizmendi MC, Berlanga H. 2014. *Hummingbirds of Mexico and North America*. Ciudad de México, México: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). ISBN: 9786078328031
- Arizaga S, Ezcurra E, Peters E, Ramírez AF, Vega E. 2000a. Pollination ecology of *Agave macroacantha* (Agavaceae) in a mexican tropical desert. I. Floral biology and pollination mechanisms. *American Journal of Botany* **87**: 1004-1010. DOI: <https://doi.org/10.2307/2657000>
- Arizaga S, Ezcurra E, Peters E, Ramírez AF, Vega, E. 2000b. Pollination ecology of *Agave macroacantha* (Agavaceae) in a mexican tropical desert. II. The role of pollinators. *American Journal of Botany* **87**: 1011-1017. DOI: <https://doi.org/10.2307/2657001>

- Barraza-Morales A, Sánchez-Teyer FL, Robert M, Esqueda M, Gardea-Béjar A. 2006. Variabilidad genética en *Agave angustifolia* Haw. De la sierra sonorensis, México, determinada con marcadores AFLP. *Revista Fitotecnia Mexicana* **29**: 1-8. DOI: <https://doi.org/10.35196/rfm.2006.1.1>
- Beattie AJ, Culver DC. 1979. Neighborhood size in *Viola*. *Evolution* **33**: 1226-1229. DOI: <https://doi.org/10.2307/2407481>
- Bond WJ. 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London, Series B* **344**: 83-90. DOI: <https://doi.org/10.1098/rstb.1994.0055>
- Borbón-Palomares DB, Laborin-Sivirian, F, Tinoco-Ojanguren C, Peñalba MC, Reyes-Ortega I, Molina-Freaner F. 2018. Reproductive ecology of *Agave colorata*: the importance of nectar-feeding bats and the germination consequences of self-pollination. *Plant Ecology* **219**: 927-939. DOI: <https://doi.org/10.1007/s11258-018-0847-x>
- Broquet T, Johnson CA, Petit A, Thompson I, Burel F, Fryxell JM. 2006. Dispersal and genetic structure in the American marten, *Martes americana*. *Molecular Ecology* **15**: 1689-1697. DOI: <https://doi.org/10.1111/j.1365-294X.2006.02878.x>
- Brown AHD. 1990. Genetic characterization of plant mating systems. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant Population Genetics, Breeding and Genetic Resources*. pp. 145-162. Sunderland, Massachusetts, USA: Sinauer Associates Inc.
- Brown AHD, Barret SCH, Moran GF. 1985. Mating system estimation in forest trees: models, methods and meanings. In: Gregorius HR, eds. *Population Genetics in Forestry*. pp. 32-49. New York, USA: Springer Verlag. DOI: https://doi.org/10.1007/978-3-642-48125-3_3
- Burke RA, Frey JK, Ganguli A, Stoner KE. 2019. Species distribution modelling supports “nectar corridor” hypothesis for migratory nectarivorous bats and conservation of tropical dry forest. *Diversity and Distributions* **25**: 1399-1415. DOI: <https://doi.org/10.1111/ddi.12950>
- Cane JH, Tepedino VJ. 2017. Gauging the effect of honeybee pollen collection on native bee communities. *Conservation Letters* **10**: 205-210. DOI: <https://doi.org/10.1111/conl.12263>
- Cházaro BMJ. 1981. Nota sobre la tipificación de *Agave obscura* y su confusión con *Agave xalapensis*. *Biótica* **6**: 435-446.
- Cházaro BMJ. 1992. Exploraciones botánicas en Veracruz I. Pisos altitudinales de vegetación en el centro de Veracruz y zona limítrofe de Puebla. *La Ciencia y El Hombre* **10**: 67- 116.
- Clark KB, Nabors A, Stokes D, Taylor A, Squires L, Hurtado, JB, Root S, Vanderplank S, Lombardo K, Wall M. 2022. Why is the Shaw’s agave (*Agave shawii* subsp. *shawii*) not reproducing? Investigating a rare species’ pollination and germination in a fragmented urban park. *Haseltonia* **28**: 93-105. DOI: <https://doi.org/10.2985/026.028.0109>
- Collevatti RG, Estolano R, García SF, Hay JD. 2010. Short-distance pollen dispersal and high self-pollination in a bat-pollinated neotropical tree. *Tree Genetics & Genomes* **6**: 555-564. DOI: <https://doi.org/10.1007/s11295-010-0271-4>
- CONAGUA [Comisión Nacional del Agua]. 2020. Normales climatológicas de la estación de Las Vigas de Ramírez, Veracruz, periodo 1981-2020, México. <https://smn.conagua.gob.mx> (accessed January 23, 2023).
- Crawford TJ. 1984. The estimation of neighbourhood parameters for plant populations. *Heredity* **52**: 273-283. DOI: <https://doi.org/10.1038/hdy.1984.29>
- Cuéllar-Martínez M, Galindo-González J, Andrade-Torres A. 2024. Germination of *Agave obscura* seeds: effects of storage time and crossing systems. *Plant Ecology* **225**: 189-199. DOI: <https://doi.org/10.1007/s11258-023-01389-z>
- Dafni A, Maués M. 1998. A rapid and simple procedure to determine stigma receptivity. *Sexual Plant Reproduction* **11**: 177-180. DOI: <https://doi.org/10.1007/s004970050138>
- de Jong TJ, Waser NN, Klinkhamer PGL. 1993. Geitonogamy: the neglected side of selfing. *Trends in Ecology and Evolution* **8**: 32-325. DOI: [https://doi.org/10.1016/0169-5347\(93\)90239-L](https://doi.org/10.1016/0169-5347(93)90239-L)
- Diniz UM, Lima SA, Machado ICS. 2019. Short-distance pollen dispersal by bats in an urban setting: monitoring the

- movement of a vertebrate pollinator through fluorescent dyes. *Urban Ecosystems* **22**: 281-291. DOI: <https://doi.org/10.1007/s11252-019-0825-7>
- do Carmo JH, Franceschinelli EV, Silveira FA. 2004. Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. *Biotropica* **36**: 371-376. DOI: <https://doi.org/10.1111/j.1744-7429.2004.tb00329.x>
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11-15.
- Earl DA, von Holdt BM. 2012 STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method. *Conservation Genetics Resources* **4**: 359-361. DOI: <https://doi.org/10.1007/s12686-011-9548-7>
- Eguiarte Fruns LE. 1990. Genética de poblaciones de *Astrocaryum mexicanum* Liebm. En los Tuxtlas, Veracruz. PhD Thesis. Universidad Autónoma de México.
- Eguiarte LE, Aguirre X, Rocha M, Torres C, Silva A, Valera A. 2003. Diversidad genética en dos especies mezcaleras. Informe final SNIB-CONABIO Proyecto CONABIO V038, Ciudad de México, México. <http://www.conabio.gob.mx> (accessed April 10, 2023).
- Eguiarte LE, Aguirre-Planter, E Aguirre X, Colín R, González A, Rocha M, Scheinvar E, Trejo L, Souza V. 2013. From isozymes to genomics: population genetics and conservation of *Agave* in México. *The Botanical Review* **79**: 483-506. DOI: <https://doi.org/10.1007/s12229-013-9123-x>
- Eguiarte LE, Jiménez Barrón OA, Aguirre-Planter E, Scheinvar E, Gámez N, Gasca-Pineda J, Castellanos-Morales G, Moreno-Letelier A. Souza V. 2021. Evolutionary ecology of *Agave*: distribution patterns, phylogeny, and coevolution (an homage to Howard S. Gentry). *American Journal of Botany* **108**: 216-235. DOI: <https://doi.org/10.1002/ajb2.1609>
- Eguiarte LE, Parra V, Vargas F. 1994. Biología reproductiva y tamaño efectivo en *Echeveria gibbiflora*: Un homenaje a Sewall Wright. In: Rojo A. comp, *Reserva Ecológica El Pedregal de San Ángel, Ecología, Historia Natural y Manejo*. Ciudad de México, México: Universidad Nacional Autónoma de México. Pp. 187-204.
- Eguiarte LE, Souza V. 2007. Introducción a la ecología molecular. In: Eguiarte LE, Souza V, Aguirre X, comp. *Ecología Molecular*. México: Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología, Universidad Nacional Autónoma de México, Comisión Nacional Para El Conocimiento y Uso de la Biodiversidad. pp. 1-6. ISBN: 978-968-817-839-3
- Eguiarte LE, Souza V, Silva-Montellano A. 2000. Evolución de la familia Agavaceae: filogenia, biología reproductiva y genética de poblaciones. *Boletín de la Sociedad Botánica de México* **66**: 131-150. DOI: <https://doi.org/10.17129/botsoci.1618>
- Ellstrand NC. 1992. Gene flow by pollen: implications for plant conservation genetics. *Oikos* **63**: 77-86. DOI: <https://doi.org/10.2307/3545517>
- Espejo-Serna A. 2012. El endemismo en las liliopsida mexicana. *Acta Botanica Mexicana* **100**: 195-257. DOI: <https://doi.org/10.21829/abm100.2012.36>
- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611-2620. DOI: <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Fattorini R, Glover BJ. 2020. Molecular mechanisms of pollination biology. *Annual Review* **71**: 487-515. DOI: <https://doi.org/10.1146/annurev-arplant-081519-040003>
- Félix Valdez LI. 2014. Patrones de diversidad y estructura genética de *Agave potatorum* Zucc. E implicaciones en su manejo in situ. MSc Thesis. Universidad de Guadalajara.
- Fenster CB, Vekemans X, Hardy OJ. 2003. Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculata* (Leguminosae). *Evolution* **57**: 995-1007. DOI: <https://doi.org/10.1111/j.0014-3820.2003.tb00311.x>
- Figueredo CJ, Casas A, González-Rodríguez A, Nassar JM, Colunga-GarcíaMarín P, Rocha-Ramírez V. 2015. Ge-

- netic structure of coexisting wild and managed agave population: implications for the evolution of plants under domestication. *AoB Plants* **7**: 1-14. DOI: <https://doi.org/10.1093/aobpla/plv114>
- Figueredo CJ, Nassar JM. 2011. Population genetics of *Agave cocui*: Evidence for low genetic diversity at the Southern geographic limit of genus *Agave*. *Journal of Heredity* **102**: 306-314. DOI: <https://doi.org/10.1093/jhered/esr018>
- Figueredo-Urbina CJ, Casas A, Torres-García I. 2017. Morphological and genetic divergence between *Agave inaequidens*, *A. cupreata* and the domesticated *A. hookeri*. Analysis of their evolutionary relationships. *Plos One* **12**: 1-24. DOI: <https://doi.org/10.1371/journal.pone.0187260>
- Finer MS, Morgan MT. 2003. Effects of natural rates of geitonogamy on fruit set in *Asclepias speciosa* (Apocynaceae): evidence favoring the plant's dilemma. *American Journal of Botany* **90**: 1746-1750. DOI: <https://doi.org/10.3732/ajb.90.12.1746>
- Fleming T, Holland JN. 2018. Nectar bat-plant interactions in North American deserts. *Hystrix, The Italian Journal of Mammalogy* **29**: 33-39. DOI: <https://doi.org/10.4404/hystrix-00036-2017>
- Flores-Torres A, Galindo-Escamilla A. 2017. Pollination biology of *Agave horrida* (Agavaceae) in the Chichinautzin mountain range, Central Mexico. *Botanical Sciences* **95**: 423-431. DOI: <https://doi.org/10.17129/botsci.1022>
- Fuchs EJ, Lobo JA, Quesada M. 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. *Conservation Biology* **17**: 149-157. DOI: <https://doi.org/10.1046/j.1523-1739.2003.01140.x>
- Galindo-González J, Cuéllar-Martínez M, Mac Swiney GC. 2024. *Agave obscura* nuevo registro en la dieta de los murciélagos nectarívoros *Leptonycteris nivalis*, *Choeronycteris mexicana* y *Anoura geoffroyi* en las montañas del centro de Veracruz, México. *Therya notes* **5**: 177-181. DOI: https://doi.org/10.12933/therya_notes-24-168
- García-Herrera EJ, Méndez-Gallegos SJ, Talavera-Magaña D. 2010. El género *Agave* spp. en México: principales usos de importancia socioeconómica y agroecológica. *Revista Salud Pública y Nutrición* **5**: 109-129.
- García-Mendoza A. 2007. Los agaves de México. *Ciencias* **87**: 14-23.
- García-Mendoza A. 2011. Agavaceae. *Flora del Valle de Tehuacán-Cuicatlán*. Fascículo **88**: 1-95. ISBN 978-607-02-2566-6
- García-Mendoza AJ, Franco Martínez IS. 2018. Actualización de la información de las especies y subespecies de magueyes de Oaxaca, con énfasis en las especies mezcaleras. México: Universidad Nacional Autónoma de México. Instituto de Biología. Informe final SNIB-CONABIO proyecto No. Ne012. Ciudad de México. <http://www.conabio.gob.mx> (accessed February 24, 2023).
- García-Mendoza AJ, Franco Martínez IS, Sandoval Gutiérrez D. 2019. Four new species of *Agave* (Asparagaceae, Agavoideae) from southern Mexico. *Acta Botanica Mexicana* **126**: 1-18. DOI: <https://doi.org/10.21829/abm126.2019.1461>
- Geissert KD. 1994. Cartografía, génesis y restricciones al uso de los suelos de origen volcánico de la región natural Cofre de Perote, Veracruz, México. Proyecto CONACYT No. 0796-N9110. <https://horizon.documentation.ird.fr> (accessed January 11, 2023).
- Gentry S. 1982. *Agaves of continental North America*. USA: The University of Arizona Press. DOI: <https://doi.org/10.2307/j.ctv1t4m2h4>
- Ghazoul J. 2005. Pollen and seed dispersal among dispersed plants. *Biological Reviews* **80**: 413-443. DOI: <https://doi.org/10.1017/S1464793105006731>
- González Pérez GA. 2004. Biología reproductiva y genética de poblaciones de *Agave garciae-mendozae*. BSc Thesis. Universidad Nacional Autónoma de México.
- Good-Avila SV, Souza V, Gaut BS, Eguiarte LE. 2006. Timing and rate of speciation in *Agave* (Agavaceae). *Proceedings of the National Academy of Sciences* **13**: 9124-9129. DOI: <https://doi.org/10.1073/pnas.0603312103>
- Goudet J, Jombart T, Kamvar ZN, Archer E, Hardy O. 2022. Estimation and tests of hierarchical F-Statistics. Package "Hierfstat". <https://github.com/jgx65/hierfstat> (accessed May 26, 2022).
- Gribel R, Hay JDV. 1993. Pollination ecology of *Caryocar Brasiliense* (Caryocaraceae) in Central Brazil Cerrado vegetation. *Journal of Tropical Ecology* **9**: 199-211. DOI: <https://doi.org/10.1017/S0266467400007173>

- Gutiérrez-Guerrero Y, Ibarra-Laclette E, Martínez del río C, Barrera-Redondo J, Rebollar E, Ortega J, León-Paniagua L. 2020. Genomic consequences of dietary diversification and parallel evolution due to nectarivory in leaf-nosed bats. *GigaScience* **9**: g1aa059. DOI: <https://doi.org/10.1093/gigascience/g1aa059>
- Handel SN. 1983. Pollination ecology, plant population structure, and gene flow. In: Real L, eds. *Pollination Biology*. New York, USA: Academic Press. pp. 163-211.
- Hernández-Leal E, Solano-Campos F, Azofeifa-Bolaños JB, Paniagua-Vásquez A, Hernández-Bautista A, López-Morales F, Pascual RF, Reyes-López D. 2024. Diversity and structure in single-cross intraspecific hybrids of vanilla and parental accessions. *Revista Fitotecnia Mexicana* **47**: 233-242. DOI: <https://doi.org/10.35196/rfm.2024.3.233>
- Johnson SD, Steiner K. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution* **15**: 140-143. DOI: [https://doi.org/10.1016/S0169-5347\(99\)01811-X](https://doi.org/10.1016/S0169-5347(99)01811-X)
- Kevan PG, Viana BF, Galetto L, Freitas BM, Vergara C, Meléndez-Ramírez V, Dafni A, Bernhardt P. 2013. Pollination biology: Interdisciplinarity in education from molecules to landscapes. *Biology International* **53**: 35-53.
- Kearns CA, Inouye DW. 1993. *Techniques for pollination biologist*. Colorado, USA: University Press of Colorado. ISBN: 978-0-87081-281-1
- Lindsay DL, Swift JL, Lance RF, Edwards CE. 2018. A comparison of patterns of genetic structure in two co-occurring *Agave* species (Asparagaceae) that differ in the patchiness of their geographical distributions and cultivation histories. *Botanical Journal of the Linnean Society* **186**: 361-373. DOI: <https://doi.org/10.1093/botlinnean/box099>
- Loh R, Scarano FR, Alves-Ferreira M, Salgueiro F. 2020. Fine-scale spatial genetic structure, neighbourhood size and gene dispersal in clonal plants: exploring the best possible estimates. *Botanical Journal of the Linnean Society* **192**: 760-772. DOI: <https://doi.org/10.1093/botlinnean/boz109>
- Magaña GKC, Vázquez-García JA, González GJG, Bustos OR. 2008. Visitantes flores en *Agave valenciana* Cházaro and Vázquez (Agavaceae) en Mascota, Jalisco, México. *Scientia-CUCBA* **10**: 1-6.
- McDermott JM, McDonald BA. 1993. Gene Flow in plant pathosystems. *Annual Review of Phytopathology* **31**: 353-373. DOI: <https://doi.org/10.1146/annurev.py.31.090193.002033>
- McRae BH. 2006. Isolation by resistance. *Evolution* **60**: 1551-1561.
- Medellín RA, Arita H, Sánchez O. 2008. *Identificación de los murciélagos de México. Clave de campo*. Ciudad de México, México: Instituto Ecología, Universidad Nacional Autónoma de México. ISBN 978-970-91921
- Medellín RA, Rivero M, Ibarra A, Antonio de la Torre J, González-Terrazas TP, Torres-Knoop LT, Tschapka M. 2018. Follow me: foraging distances of *Leptonycteris yerbabuena* (Cirptera: Phyllostomidae) in Sonora determined by fluorescent powder. *Journal of Mammalogy* **99**: 306-311. DOI: <https://doi.org/10.1093/jmammal/gyy016>
- Molina-Freaner F, Eguiarte LE. 2003. The pollination biology of two paniculate agaves (Agavaceae) from northwestern Mexico; Contrasting roles of bats as pollinators. *American Journal of Botany* **90**: 1016-1024. DOI: <https://doi.org/10.3732/ajb.90.7.1016>
- Moreno-Valdez A, Honeycutt RL, Grant WE. 2004. Colony dynamics of *Leptonycteris nivalis* (mexican long-nosed bat) related to flowering *Agave* in northern Mexico. *Journal of mammalogy* **85**: 453-459. DOI: <https://doi.org/10.1644/1383942>
- Nei M. 1987. *Molecular evolutionary genetics*. New York, USA: Columbia University Press. ISBN: 0-231-06320-2
- Ng WL, Tan SG. 2015. Inter-temple sequence repeat (ISSR) markers: are we doing it right? *ASM Science Journal* **9**: 30-39.
- Nowak RM. 1994. *Walker's bats of the world*. Maryland, USA: The Johns Hopkins University Press. ISBN: 0-8018-4986-1
- Ornelas JF, Ordano M, Hernández A, López JC, Mendoza L, Perroni Y. 2002. Nectar oasis produced by *Agave marmorata* Roezl. (Agavaceae) lead to spatial and temporal segregation among nectarivores in the Tehuacán Valley, México. *Journal of Arid Environments* **52**: 37-51. DOI: <https://doi.org/10.1006/jare.2002.0971>
- Paini D. 2004. Impact of the introduced honey bee (*Apis mellifera*) (Hymenoptera: Apidae) in native bees: a review. *Austral Ecology* **29**: 399-407. DOI: <https://doi.org/10.1111/j.1442-9993.2004.01376.x>
- Parra V, Vargas CF, Eguiarte LE. 1993. Reproductive biology, pollen and seed dispersal, and neighborhood size in

- the hummingbird-pollinated *Echeveria gibbiflora* (Crassulaceae). *American Journal of Botany* **80**: 153-159. DOI: <https://doi.org/10.1002/j.1537-2197.1993.tb13783.x>
- Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**: 2537-2539. DOI: <https://doi.org/10.1093/bioinformatics/bts460>
- Pérez-Hernández RB, Martínez-Coronel M. 2023. Dieta del murciélago trompudo (*Chaeronycteris mexicana*) en la Sierra de Santa Catarina. *Revista Mexicana de Mastozoología* **13**: 1-11. DOI: <https://doi.org/10.22201/ie.20074484e.2023.13.1.379>
- Pradeep RM, Sarla N, Siddiq E. 2002. Nter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* **128**: 9-17. DOI: <https://doi.org/10.1023/A:1020691618797>
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959. DOI: <https://doi.org/10.1093/genetics/155.2.945>
- R Core Team. 2022. R: A language and environment for statistical computing. R. Foundation for Statistical Computing, Vienna, Australia. <http://www.R-project.org/> (accessed May 26, 2022).
- Richards CM. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. *American Naturalist* **155**: 383-394. DOI: <https://doi.org/10.1086/303324>
- Rocha Munive MG. 2006. Ecología Evolutiva Comparada de 5 especies de *Agave*. PhD Thesis. Universidad Nacional Autónoma de México.
- Rocha M, Valera A, Eguiarte LE. 2005. Reproductive ecology of five sympatric *Agave Littaea* (Agavaceae) species in Central Mexico. *American Journal of Botany* **92**: 1330-1341. DOI: <https://doi.org/10.3732/ajb.92.8.1330>
- Rentaría AM. 2007. Marcadores moleculares. In: Eguiarte LE, Souza V, Aguirre X, comp. *Ecología Molecular*. México: Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología, Universidad Nacional Autónoma de México, Comisión Nacional Para El Conocimiento y Uso de la Biodiversidad. pp. 541-566. ISBN: 978-968-817-839-3.
- Rousset F. 2000. Genetic differentiation between individuals. *Journal of Evolutionary Biology* **13**: 58-62. DOI: <https://doi.org/10.1046/j.1420-9101.2000.00137.x>
- Sánchez R, Medellín RA. 2007. Food habits of the threatened bat *Leptonycteris nivalis* (Chiroptera: Phyllostomidae) in a mating roost in Mexico. *Journal of Natural History* **41**: 1753-1764. DOI: <https://doi.org/10.1080/00222930701483398>
- Schaffer WM, Schaffer MV. 1977. The reproductive biology of Agavaceae: I. Pollen, and nectar production in four Arizona agaves. *Southwestern Naturalist* **22**: 157-168. DOI: <https://doi.org/10.2307/3669806>
- Schaffer WM, Schaffer MV. 1979. The adaptative significance of variations in reproductive habit in the Agavaceae II: Pollinator foraging behavior and selection for increased reproductive expenditure. *Ecology* **60**: 1051-1069. DOI: <https://doi.org/10.2307/1936872>
- Scheinvar Gottdiener E. 2008. Genética de poblaciones silvestres y cultivadas de dos especies mezcaleras: *Agave cupreata* y *Agave potatorum*. MSc Thesis. Instituto de Ecología, Universidad Nacional Autónoma de México.
- Schmidt X, Barker NP, Frisby A. 2025. Assessment of the genetic diversity of the critically endangered *Aloe peglerae* Schönland (Asphodelaceae) by means of Inter-Simple sequence repeat (ISSR) markers. *South Africa Journal of Botany* **186**: 13-23. DOI: <https://doi.org/10.1016/j.sajb.2025.08.042>
- SEMARNAT [Secretaría del Medio Ambiente y Recursos Naturales]. 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección Ambiental - Especies nativas de México de flora y fauna silvestres - Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. *Diario Oficial de la Federación*. 2da Sección, 30 de diciembre de 2010.
- Shirk AJ, Cushman SA. 2014. Spatially-explicit estimation of Wright's neighborhood size in continuous populations. *Frontiers in Ecology and Evolution* **2**: 1-12. DOI: <https://doi.org/10.3389/fevo.2014.00062>
- Sihag RC. 2018. Pollination, Pollinators and pollination modes: Ecological and economic importance. In: Roubik D, eds. *The Pollination of Cultivated Plants. A Compendium for Practitioners. Vol 1*. Rome, Italy: Food and Agriculture Organization of the United Nations. pp. 21-33. ISBN: 978-92-5-130512-6

- Silva-Montellano A, Eguiarte LE. 2003a. Geographic patterns in the reproductive ecology of *Agave Lechuguilla* (Agavaceae) in the Chihuahuan desert. I. Floral characteristics, visitors, and fecundity. *American Journal of Botany* **90**: 377-387. DOI: <https://doi.org/10.3732/ajb.90.3.377>
- Silva-Montellano A, Eguiarte LE. 2003b. Geographic patterns in the reproductive ecology of *Agave Lechuguilla* (Agavaceae) in the Chihuahuan desert. II. Genetic variation, differentiation, and inbreeding estimates. *American Journal of Botany* **90**: 700-706. DOI: <https://doi.org/10.3732/ajb.90.5.700>
- Slatkin M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* **16**: 393-430. DOI: <https://doi.org/10.1146/annurev.es.16.110185.002141>
- Slauson LA. 2000. Pollination biology of two chiropterophilous Agaves in Arizona. *American Journal of Botany* **87**: 825-836. DOI: <https://doi.org/10.2307/2656890>
- Torres-Morán MI, Hormaza JI, Larranaga N. 2024. DNA analyses reveal high heterozygosity values and low-differentiation among populations of mezcal-producing *Agave durangensis*. *Genetic Resources and Crop Evolution* **71**: 3419-3431. DOI: <https://doi.org/10.1007/s10722-023-01850-y>
- Trame AC, Coddigton AJ, Paige KN. 1995. Field and genetic studies testing optimal outcrossing in *Agave schottii*, a long-lived clonal plant. *Oecologia* **104**: 93-100. DOI: <https://doi.org/10.1007/BF00365567>
- Trejo Hernández L. 2006. Genética de Poblaciones de *Agave striata* Zucc. MSc Thesis. Universidad Nacional Autónoma de México.
- Trejo-Salazar RE, Scheinvar E, Eguiarte LE. 2015. ¿Quién poliniza realmente a los agaves? Diversidad de visitantes florales en 3 especies de *Agave* (Agavoideae: Asparagaceae). *Revista Mexicana de Biodiversidad* **86**: 358-369. DOI: <https://doi.org/10.1016/j.rmb.2015.04.007>
- Vargas-Ponce O. 2007. *Diversidad y relaciones genéticas del complejo Agave angustifolia Haw. y los agaves mezcaleros del occidente de México*. PhD Thesis. Centro de Investigación Científica de Yucatán AC.
- Vázquez Lobo A. 1996. *Filogenia de hongos endófitos del género Pinus L.: Implementación de técnicas moleculares y resultados preliminares*. BSc Thesis. Universidad Nacional Autónoma de México.
- Wright S. 1931. Evolution in Mendelian Populations. *Genetics* **16**: 97-159. DOI: <https://doi.org/10.1093/genetics/16.2.97>
- Wright S. 1943. Isolation by distance. *Genetics* **28**: 114-138. DOI: <https://doi.org/10.1093/genetics/28.2.114>
- Wright S. 1946. Isolation by distance under diverse systems of mating. *Genetics* **31**: 39-59. DOI: <https://doi.org/10.1093/genetics/31.1.39>
- Wright S. 1969. *Evolution and the genetics of populations. Vol 2. The Theory of Gene Frequencies*. Chicago, USA: University of Chicago Press. ISBN: 226-91050-4
- Yeh F, Yang R, Boyle T, Ye Z, Mao J, Mao X. 1999. POPGENE, version 1.32, the user-friendly software for population genetic analysis. Molecular Biology and Biotechnology Centre. University of Alberta, Canada. <https://sites.ualberta.ca/~fyeh/popgene.html> (accessed March 13, 2023).
- Young HJ. 1988. Neighborhood size in a beetle pollinated tropical aroid: effects of low density and asynchronous flowering. *Oecologia* **76**: 461-466. DOI: <https://doi.org/10.1007/BF00377043>
- Zhao J, Wang Y, Ding W, Xu H. 2024. Microsatellite marker-based analysis of the genetic diversity and population structure of three *Arnebiae radix* in western China. *Journal of Genetic Engineering and Biotechnology* **22**: 100379. DOI: <https://doi.org/10.1016/j.jgeb.2024.100379>

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