

Morphological and phenological variation of flower colour morphs in a wild population of *Opuntia streptacantha* (Cactaceae)

Gerardo Manzanarez-Villasana¹, María C. Mandujano¹

¹ Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

Corresponding author: María C. Mandujano (mcmdandujano@gmail.com, mcmdandujano@ieciologia.unam.mx)

Academic editor: Renate Wesselingh ♦ Received 5 September 2023 ♦ Accepted 28 May 2024 ♦ Published 3 July 2024

Abstract

Background and aims – *Opuntia* s.s. (Cactaceae) is one of the most diverse genera in the subfamily Opuntioideae, with approximately 220 species. The considerable morphological and anatomical diversity among these species has resulted in a remarkable adaptive plasticity, evident in both intra- and interspecific variability. Our study system is *Opuntia streptacantha*, which has two flower colour morphs: yellow and orange. The objective is to determine if there are morphological differences in the reproductive and vegetative structures between floral morphs.

Material and methods – We measured 8 cladode traits ($n = 20$ cladodes for each floral morph) and 17 flower traits ($n = 30$ flowers per morph), and reproductive phenology was recorded for both morphs to describe their phenophases ($n = 10$ individuals per morph).

Key results and conclusion – We found that floral colour morphs of *O. streptacantha* showed significant differences mostly associated with flower traits. Principal component analysis revealed seven components that explained 80% of the total variation, namely total flower length, number of stamens, distance between anther and stigma, number of pollen grains, style length, equatorial diameter of the ovarian chamber, pericarp width, and number of areole lines. Some individuals of *O. tomentosa* were classified as floral morphs of *O. streptacantha*, not having a clear separation between the species. The phenology of the floral colour morphs showed a slight lag in their peak flowering and fruiting. Very high flowering synchrony was found for each floral morph and between them. The modifications found in the flowers of *O. streptacantha* may be associated with a possible hybridization with *O. tomentosa* favouring the appearance of the two floral morphs.

Keywords

flower colour polymorphism, flower traits, flowering synchrony, cladode morphometry

INTRODUCTION

The subfamily Opuntioideae (Cactaceae) is composed of around 220–350 species (Britton and Rose 1919; Anderson 2001; Griffith and Porter 2009). Within the subfamily, the genus *Opuntia* s.s. (commonly known as prickly pears) is the most diverse with approximately 200 species (Britton and Rose 1919; Barthlott and Hunt 1993; Pinkava 2003; Porras-Flórez et al. 2017) and is of great biological, cultural, economic, and social importance

(Bravo-Hollis and Sánchez-Mejorada 1978; Aguilar et al. 2004; Reyes-Agüero et al. 2005; Mandujano and Sánchez 2017).

Prickly pears in Mexico have a wide distribution, but thrive mainly in arid and semi-arid zones, where the greatest species diversity is found. There are two significant centres of diversity: the Chihuahuan Desert zone and the central-western region (which includes the state of Mexico, Guerrero, and Jalisco) (Golubov et al. 2005; Muñoz-Urias et al. 2008). Prickly pears can also be

found, although to a lesser extent, in areas such as tropical and subtropical coniferous forests, as well as tropical and subtropical dry broadleaf and moist broadleaf forest (Esparza-Sandoval 2010; Manzanarez-Villasana et al. 2022).

Opuntia s.s. shows a marked morphological variation to the extent that its taxonomy becomes confusing (Bravo-Hollis and Sánchez-Mejorada 1978; Scheinvar 1995; Reyes-Agüero et al. 2005; Muñoz-Urias et al. 2008). Morphological variations include growth habit, stem size and pubescence, spine length, number of areoles, flower shape and colour, weight and chemical composition of the fruit, seed size, among others; and also phenological variations such as the time in which vegetative and reproductive phenophases are observed, to mention just a few (Wallace and Fairbrothers 1986; Pimienta-Barrios and Mauricio-Leguizamo 1989; Pimienta-Barrios 1994; Pimienta-Barrios and Muñoz-Urias 1995; Fordyce 2006; Muñoz-Urias et al. 2008). An example of such variation can be seen in members of the clade *Nopalea*. This clade, located in North America, presents modification in its morphology (with its androecium and gynoecium excised from the perianth segments) and in the colour of the flowers (mainly pink) (Majure et al. 2012; Majure and Puentes 2014) and thus in fitness of *Nopalea* members.

It is important to carry out taxonomic, ecological, and genetic studies on plant species with different floral colours, as they can contribute to the taxonomic and phylogenetic delimitation of morphotypes. This, in turn, could lead to the recognition of new species, subspecies, or varieties (Narbona et al. 2014). For example, in the case of *Primula vulgaris* Huds. (Primulaceae), it has been observed that most populations have yellow flowers, while colour polymorphism is present in some populations in eastern

regions. This has led to the taxonomic differentiation of the species into several subspecies (Shipunov et al. 2011).

Opuntia streptacantha Lem. is a wild species endemic to Mexico that has two floral morphs: yellow and orange, of which the yellow floral morph was the first to be described (Bravo-Hollis and Sánchez-Mejorada 1978). The objective of this work is to determine if there are morphological differences in the reproductive and vegetative structures, and phenological differences between the flower colour morphs in *O. streptacantha*.

MATERIAL AND METHODS

Study site

This study was carried out in the southern portion of the Chihuahuan desert known as Queretano-Hidalguense semi-desert, in the wilderness area protected by the Regional Botanical Garden of Cadereyta de Montes “Ing. Manuel González de Cosío”, Querétaro, Mexico. Its geographic coordinates are 20°41'15.8"N, 99°48'17.7"W, with an elevation of 2,046 m a.s.l., the vegetation type is xerophytic crassicaulous scrub. The climate is semi-dry, temperate with summer rains (Köppen climate group BS1 kw (w) modified by García 2004). The average annual temperature ranges between 12 and 19°C and the average annual precipitation is about 550 mm (Chávez-Martínez and Hernández-Magaña 2009).

Study species

Opuntia streptacantha is an arborescent or shrubby plant, up to 4 m tall, its stems (cladodes) are flattened and racket-

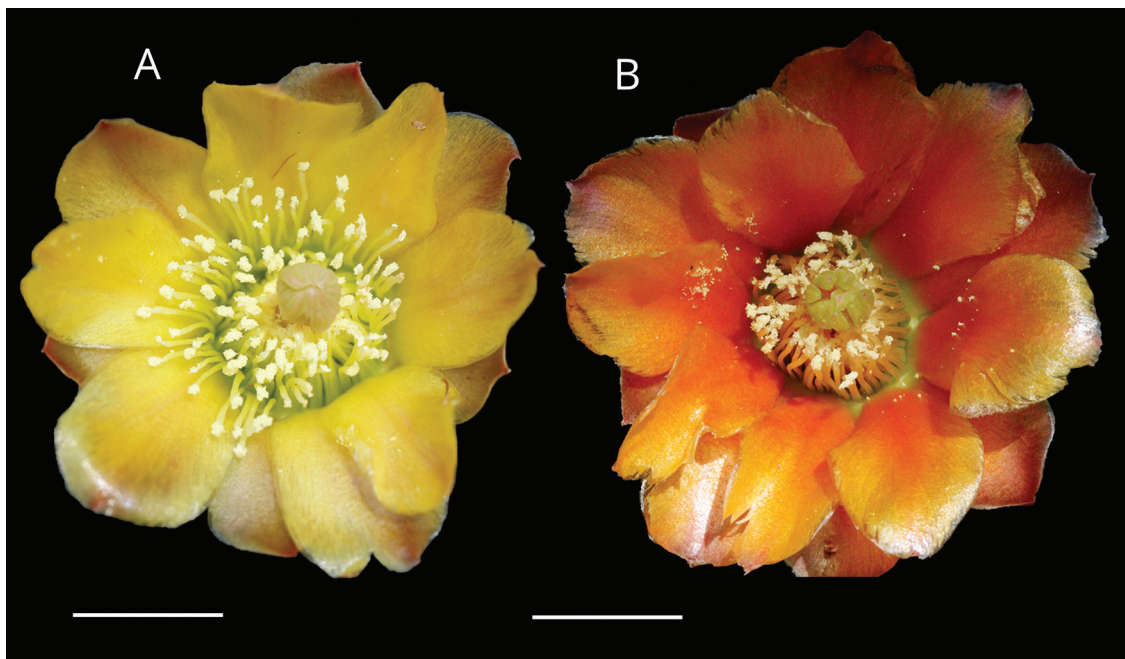


Figure 1. Floral morphs of *Opuntia streptacantha*. A. Yellow floral morph. B. Orange floral morph. Scale bars = 1 cm. Photos by Gerardo Manzanarez-Villasana.

shaped, and the flowers are yellow or orange, 5–7 cm long. The fruits are 5 cm long and 3 cm wide, globose to obovoid and usually wine-coloured when ripe. The glochids are short, the pulp is red, and the seeds are 3.8–4.5 mm long by 2.6 mm wide (Bravo-Hollis and Sánchez-Mejorada 1978; Arias et al. 2012; Galicia-Pérez et al. 2023).

This species is endemic to Mexico and is commonly known as “cardón”, “cenizo”, “chaveño”, or “nopal cardón” (Arias et al. 2012), and is distributed in the states of Aguascalientes, Mexico City, Durango, Mexico, Guanajuato, Hidalgo, Jalisco, Michoacán, Nuevo Leon, Oaxaca, Puebla, Queretaro, San Luis Potosi, Tamaulipas, Tlaxcala, and Zacatecas (Hunt et al. 2006). It grows at elevations of 1,600 to 2,400 m a.s.l. (Arias et al. 2017).

Opuntia streptacantha is generally found in xerophytic scrublands and with other co-dominant *Opuntia* species, they form a vegetation type called “nopaleras” (Arias et al. 2012). According to Bravo-Hollis and Sánchez-Mejorada (1978), it is a wild species of remarkable value because of its edible fruits and stems.

Opuntia streptacantha populations contain individuals with two different floral colour morphs: with yellow flowers (YFM) and with orange flowers (OFM) (Fig. 1). The presence of these colour morphs appears to be consistent throughout the distribution range of the species. Historically, the first description of the colour of the flowers of this species was made by Schumann (1899), who mentioned that the flowers were yellow. However, Bravo-Hollis and Sánchez-Mejorada (1978) observed that the flowers vary from yellow to orange within a population.

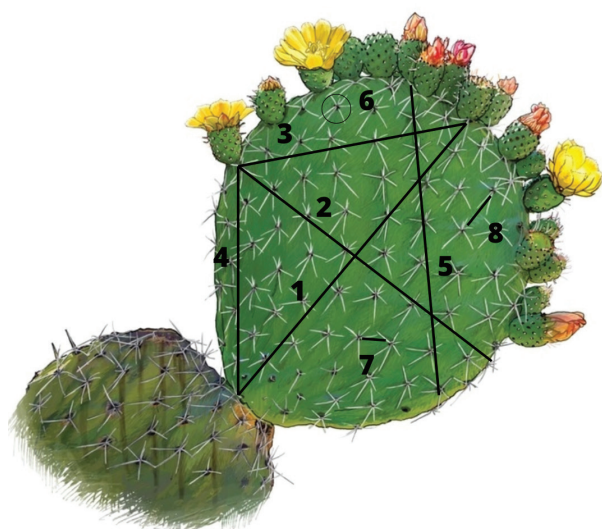


Figure 2. Morphometric variables of *Opuntia streptacantha* cladodes. 1. Cladode length (cm). 2. Cladode width (cm). 3. Maximum distance from the apical to the widest part (cm). 4. Maximum distance from the basal to the widest part (cm). 5. Number of series of areoles. 6. Areole size (mm). 7. Distances between areoles (mm). 8. Distance between lines (mm). Illustration by Rafael Ríos/CONABIO, ComenTuna et al. (2009), own modification.

Cladode and spines morphometry

Twenty mature cladodes (including those with lateral cladodes or reproductive structures such as buds, flowers, or fruits) were measured, comprising two cladodes per individual, from ten reproductive individuals of *O. streptacantha* for each floral colour morph. Similarly, 20 young cladodes (considering only lateral cladodes as young) were measured, also two per individual. The sampled individuals were checked to ensure that they had fruits, buds, or flowers, or a combination of them, and were approximately 3 meters tall. The parameters used in the work of Muñoz-Urias et al. (2008) were measured: cladode length (cm), cladode width (cm), maximum distance from the apical to the widest part (cm), maximum distance from the basal to the widest part (cm), number of series of areoles, areole size (mm), distances between areoles (mm), and distance between lines (mm) (Fig. 2). To determine differences in the spines of floral morphs, the type, colour, and number of spines on the central areole of three cladodes per individual were determined for each floral morph ($n = 10$) (López-Borja et al. 2017; Galicia-Pérez et al. 2023).

Flower morphometry

Thirty-three undamaged flowers were collected at the time of maximum flower opening and during peak flowering from different individuals of each flower colour morph of *O. streptacantha* and fixed in FAA (formaldehyde, alcohol, acetic acid) (Kiernan 2002). Following Martínez-Ramos et al. (2017), 17 morphological characters were measured to the nearest mm: corolla aperture set in FAA, perianth segment length, total flower length, pericarp length, pericarp width, distance between anther and stigma, stigma width, stigma length, style length, longest stamen length, shortest stamen length, equatorial diameter of the ovarian chamber, polar diameter of the ovarian chamber, and we counted the number of stamens, number of ovules, number of lobes, and number of pollen grains in one anther (Fig. 3).

Morphometric data of cladodes and flowers were tested for differences between floral morphs using Generalized Linear Model (GLM) with Poisson distribution for discrete counts and t-tests for continuous variables. For the spines, a paired t-test was carried out.

Fruit and seed morphometry

We collect two fruits from ten different reproductive individuals per flower colour morph ($n = 20$). We assessed fruit diameter (mm), fruit length (mm), number of spiral series, and number of seeds (López-Borja et al. 2017). A paired t-test for continuous variables and a GLM with Poisson distribution for discrete variables were used to find differences between morphs. In addition, the external colour of the fruit, colour of the pulp, colour of the glochids using the HTML colour code, and the shape

of the fruit were taken based on the classification given by Moreno (1984).

A sample of ten seeds was randomly selected from each fruit ($n = 200$ seeds per floral morph), photographed, and measured for size with length and width of each seed in mm using Adobe Photoshop CS6. A paired t-test was used to compare between morphs.

All statistical tests were performed in R v.4.2.2 (R Core Team 2022) with the packages stats v.4.2.2 (R Core Team 2022) and emmeans v.1.10.1 (Russell 2021).

Multivariate analysis

Two tests were performed to compare flower, cladode, fruit, and seed characteristics between morphs (Sokal and Sneath 1963; Cuadras 1981). The first test was a principal component analysis (PCA), to reduce the variables to those that would give us the most taxonomic information. The morphological characters of all the previously mentioned measured structures were considered, the analysis was performed in R, with the packages FactoMinerR v.2.10 (Le et al. 2008), factorextra v.1.0.7 (Kassambara and Mundt 2020), psych v.2.4.3 (Revelle 2020), and Factoshiny v.2.5 (Vaissie et al. 2020). In addition, we performed a linear discriminant analysis, including only the relevant flower and cladode characters detected by PCA. In this analysis, data from two more species were added: *Opuntia tomentosa* Salm-Dyck and *Opuntia cantabrigiensis* Lynch (Galicia-Pérez et al. 2023), as both species are found in the same study site and present a very high flowering synchrony with the floral morphs of *O. streptacantha* (Martínez-Ramos 2019; Martínez-Ramos et al. 2024), the analysis was performed in R with the lda function of the stats v.4.2.2 package (R Core Team 2022).

Reproductive phenology and flowering synchrony

Reproductive phenology (flowering and fruiting) was registered, taking monthly observations (April 2018 to March 2019) for ten individuals of each floral morph. The data were analysed with circular statistics to determine the flowering and fruiting peaks (Morellato et al. 2010), and the Rayleigh uniformity test (Zar 1999; Mendoza 2020) was calculated to identify if the distribution of the phenophases was uniform, and the non-parametric Mardia-Watson-Wheeler test (Batschelet 1981) was performed to determine differences between flowering and fruiting. Analyses were carried out in R with the circular v.0.5-0 package (Agostinelli and Lund 2022).

Two indices were evaluated to determine the flowering synchrony of the colour morphs. The Marquis (1988) index was evaluated, which considers the number of open flowers per census and the proportion that these flowers represent with respect to the total number of flowers, following the formula below:

$$S = \sum_{t=0}^n \frac{x_t}{\sum_{t=0}^n x_t} \times Pt$$

where, S is the degree of synchrony, x_t the number of open flowers per census,

$$\left(\frac{x_t}{\sum_{t=0}^n x_t} \right)$$

is the proportion of open flowers to the total number of flowers, and Pt represents the proportion of the censused individuals at flowering during time t .

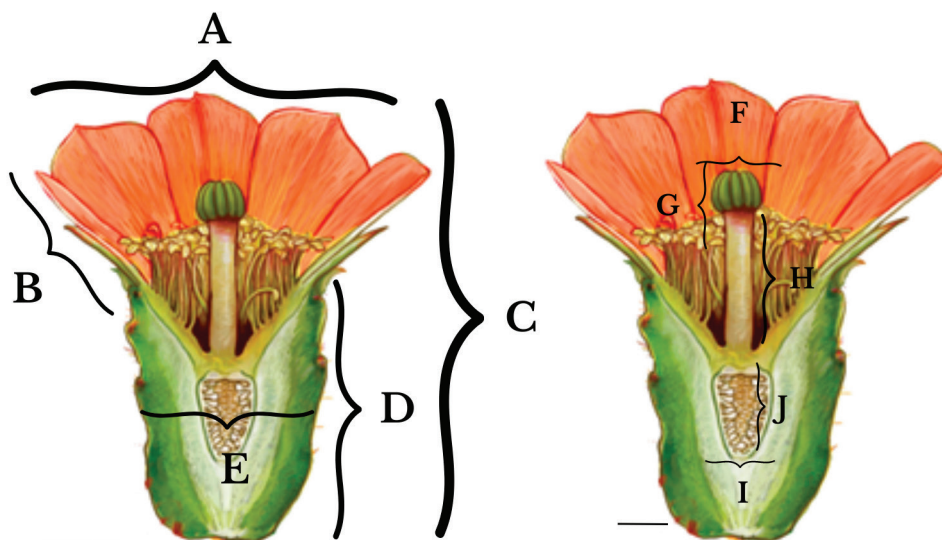


Figure 3. Morphometric variables of *Opuntia streptacantha* flowers. A. Corolla aperture set in FAA. B. Perianth segment length. C. Total flower length. D. Pericarp length. E. Pericarp width. F. Stigma width. G. Stigma length. H. Style length. I. Equatorial diameter of the ovarian chamber. J. Polar diameter of the ovarian chamber. For each morphological character, the unit of measurement was mm. Illustration by Rafael Ríos/CONABIO, ComenTuna et al. (2009), own modification. Scale bar = 5 cm.

Table 1. Mean and standard error (\pm) of cladode characteristics of both floral morphs of *Opuntia streptacantha* in Cadereyta de Montes, Querétaro, Mexico, tested with t-tests and generalized linear model with Poisson distribution. Contrasts are marked in bold type and with * ($p < 0.05$). $n = 20$ young or old cladodes per floral morph.

Cladode trait	Cladode age	Yellow floral morph	Orange floral morph	t	p
Length (cm)	Young	18.71 \pm 0.56	17.79 \pm 0.59	0.99	0.33
	Old	30.15 \pm 0.93	33.22 \pm 0.92	-2.62	0.01*
Width (cm)	Young	13.38 \pm 0.01	13.38 \pm 0.02	1	0.32
	Old	13.37 \pm 0.01	14.44 \pm 0.47	-2.23	0.03*
Areole size (mm)	Young	2.26 \pm 0.08	2.31 \pm 0.07	-0.46	0.65
	Old	3.29 \pm 0.15	3.21 \pm 0.15	0.28	0.77
Distance from the widest part to the apex (cm)	Young	10.27 \pm 0.32	9.57 \pm 0.38	1.26	0.22
	Old	17.32 \pm 0.56	18.44 \pm 0.48	-1.64	0.11
Distance from the widest part to the base (cm)	Young	11.09 \pm 0.30	10.42 \pm 0.37	1.31	0.20
	Old	16.87 \pm 0.51	18.24 \pm 0.50	-1.99	0.06
Distance between areoles (cm)	Young	17.92 \pm 0.60	18.07 \pm 0.54	0.16	0.87
	Old	29.09 \pm 1.27	30.04 \pm 1.26	-0.65	0.52
Distance between lines of areoles (cm)	Young	18.74 \pm 0.63	18.95 \pm 0.64	0.22	0.82
	Old	32.86 \pm 0.95	33.70 \pm 1.23	-0.54	0.59
				χ^2	P
Number of series of areoles	Young	8.05 \pm 0.29	8.20 \pm 0.28	0.02	0.86
	Old	8.35 \pm 0.25	9.10 \pm 0.26	0.64	0.42
Spines per areole	Old	3.63 \pm 0.49	3.93 \pm 0.52	0.36	0.55

Flowering synchrony between YFM and OFM was calculated with the index of Mahoro (2002), modified by Osada et al. (2003). For the modified version, the relative number of open flowers in each individual at an interspecific level (in this case, between YFM and OFM) is considered, following the formula below:

$$s_i = \frac{1}{2} \left(2 - \sum_{i=1}^n |y_{A,j} - y_{B,j}| \right)$$

where s_i is the degree of synchrony of species A with species B, $Y_{A,j}$ is the ratio of flowers in morph A, and $Y_{B,j}$ is the ratio of flowers in morph B.

Both indexes take values from 0 to 1, where a value close to one represents perfect synchrony and a value close to zero represents asynchrony.

RESULTS

Cladode and spines morphometry

The morphometry of old cladodes differed significantly between YFM and OFM in two variables: cladode length ($t = -2.62$, $p = 0.01$) and cladode width ($t = -2.23$, $p = 0.03$), with OFM being the largest (Table 1). No significant differences were found for young cladodes. Both flower colour morphs have ovate cladodes and two types of

spines, straight and subulate. The spines have a yellow or white colouration and the number of spines per areole in both morphs was similar (mean \pm standard error; YFM = 3.63 ± 0.49 , OFM = 3.93 ± 0.52 , $\chi^2 = 0.3569$, $p = 0.55$).

Flower morphometry

Flowers were actinomorphic in both morphs of *O. streptacantha* (Fig. 1), but we found significant differences in most of the characters (Table 2), with higher values for YFM. In contrast, the gynoecium was very similar for both morphs.

Fruit and seed morphometry

Both floral morphs had ovate fruits, a magenta pericarp with wine-coloured pulp, and opaque-golden glochids. Fruit length was the only difference, with YFM having longer fruits (Table 2). Both floral morphs have funiculate seeds, with an oval to amorphous shape and light brown colour. Significant differences in seed size (Table 2) were found between the morphs.

Multivariate analysis

Of the morphological characters, those showing significant differences between floral morphs for PCA and discriminant analysis were considered. PCA shown seven principal components that explain 80% of the total variation. The first component explains 28.37% (total

Table 2. Mean and standard error (\pm) of floral characteristics of both floral morphs of *Opuntia streptacantha* in Cadereyta de Montes, Querétaro, Mexico. Contrasts are marked in bold. *: $p < 0.05$; n.s. non significant.

Trait	Yellow floral morph (n = 33)	Orange floral morph (n = 33)	t	p
Corolla aperture set in FAA (mm)	27.87 \pm 1.57	21.95 \pm 1.01	3.16	0.0025*
Perianth segment length (mm)	27.05 \pm 0.93	22.50 \pm 0.55	4.21	0.0001*
Total flower length (mm)	58.81 \pm 2.03	50.03 \pm 1.19	3.73	0.0004*
Pericarp length (mm)	36.43 \pm 1.13	29.32 \pm 0.76	5.20	2.892e-06*
Pericarp width (mm)	20.87 \pm 0.41	24.21 \pm 0.17	-7.45	2.948e-09*
Style length (mm)	19.99 \pm 0.59	17.37 \pm 0.25	4.10	0.0001*
Stigma length (mm)	5.09 \pm 0.14	5.09 \pm 0.12	-0.01	0.98 ^{n.s.}
Stigma width (mm)	5.58 \pm 0.16	5.17 \pm 0.14	1.90	0.06 ^{n.s.}
Equatorial diameter of the ovarian chamber (mm)	4.84 \pm 0.14	5.48 \pm 0.18	-2.73	0.0081*
Polar diameter of the ovarian chamber (mm)	9.83 \pm 0.37	6.40 \pm 0.39	6.32	2.804e-08*
Longest stamen length (mm)	14.66 \pm 0.49	11.54 \pm 0.16	6.02	4.853e-07*
Shortest stamen length (mm)	8.59 \pm 0.44	6.39 \pm 0.23	4.38	6.402e-05*
Anther-stigma distance (mm)	7.88 \pm 0.41	5.86 \pm 0.28	4.06	0.0001*
			χ^2	p
Number of lobes	8.84 \pm 0.31	7.72 \pm 0.15	2.50	0.11 ^{n.s.}
Number of stamens	469.03 \pm 20.10	523.69 \pm 10.15	99.39	2.2e-16*
Number of pollen grains per anther	215.57 \pm 8.94	247.33 \pm 9.27	71.95	2.2e-16*
Number of ovules	118.06 \pm 7.36	97.66 \pm 4.73	63.72	1.436e-15*
			t	p
Fruit length (cm)	51.28 \pm 1.59	43.92 \pm 0.84	4.62	0.0001*
Fruit width (cm)	37.11 \pm 0.95	37.50 \pm 0.85	-0.38	0.71 ^{n.s.}
			χ^2	p
Number of spiral series per fruit	7.85 \pm 0.11	8.15 \pm 0.18	0.11	0.73 ^{n.s.}
Number of seeds per fruit	97.05 \pm 7.57	07.65 \pm 5.61	0.04	0.85 ^{n.s.}
			t	p
Seed length (cm)	5.10 \pm 0.04	4.55 \pm 0.04	9.71	2.2e-16*
Seed width (cm)	4.53 \pm 0.20	3.67 \pm 0.04	4.11	5.658e-05*

flower length), the second component 17.50% (number of stamens), the third component 11.01% (distance between anther and stigma), the fourth component 7.20% (number of pollen grains and style length), the fifth component 6.58% (equatorial diameter of the ovarian chamber), the sixth component 5.71% (width of the pericarpel), and the seventh component 4.05% (number of areole lines), considering only the first two components explain 45.87% of the total variation (Fig. 4; Supplementary material 1). It is important to emphasize that six of the seven components are flower morphometric variables.

Linear discriminant analysis explained 89.28% of the variation in the first two linear discriminant functions. *Opuntia cantabrigiensis* was completely separated from the other species, *O. tomentosa* was grouped with YFM, and OFM was almost separated from *O. tomentosa* but showed a small overlap with YFM (Fig. 5). The analysis was able to correctly assign 92% of the individuals

within species. YFM had the fewest correctly classified individuals (Table 3).

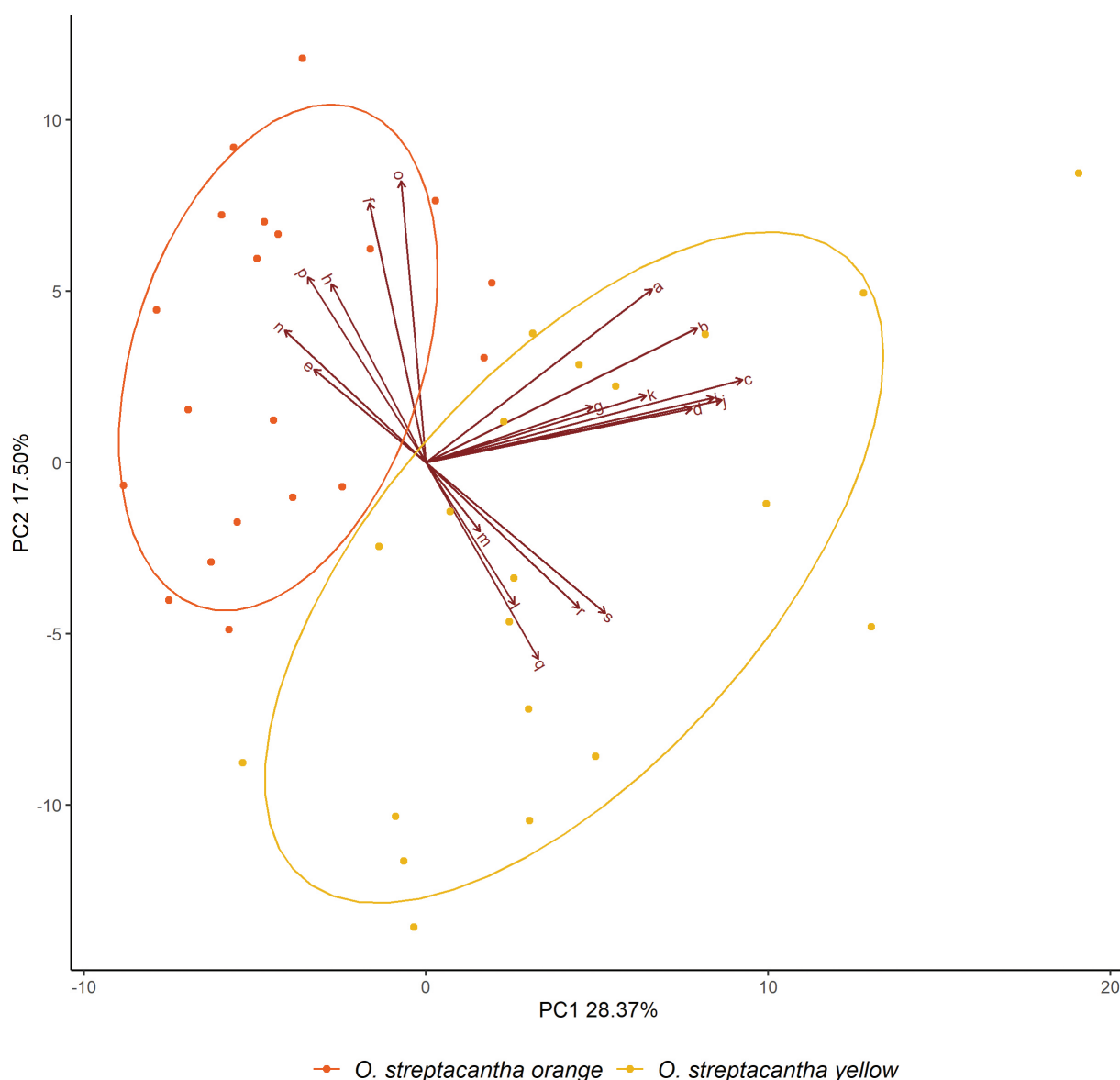
Reproductive phenology and flowering synchrony

Reproductive phenology differed between the floral morphs. Flowering for YFM was significantly ($r = 0.9566$, $p = < 0.001$) concentrated in four months, from March to June, with peak flowering in April (Fig. 6A). On the other hand, the OFM showed a significant ($r = 0.9443$, $p = < 0.001$) flowering season of five months, from February to June, with a single peak in flowering in May (Fig. 6B). The flowering patterns of the floral morphs were significantly different ($W = 43.686$, $p < 0.0001$).

Fruiting in YFM was significant ($r = 0.9023$, $p = < 0.001$) concentrated in a period of five months (June to October), with peak fruiting in July (Fig. 6C). On the other hand, in the OFM, showed a significant fruiting season ($r = 0.8749$, $p = < 0.001$), spanning seven months, from May

Table 3. Classification of the individuals (columns) based on floral morphometrics using the linear discriminant analysis.

	<i>Opuntia cantabrigiensis</i>	<i>Opuntia streptacantha</i> Orange	<i>Opuntia streptacantha</i> Yellow	<i>Opuntia tomentosa</i>	Correctly classified individuals
<i>Opuntia cantabrigiensis</i>	16	0	0	0	16
<i>Opuntia streptacantha</i> Orange	0	33	4	2	33
<i>Opuntia streptacantha</i> Yellow	0	0	26	0	26
<i>Opuntia tomentosa</i>	0	0	3	31	31
n	16	33	33	33	115 (100%) / 106 (92.17%)

**Figure 4.** PCA analysis of the variables evaluated among the floral morphs of *Opuntia streptacantha* in Cadereyta de Montes, Queretaro, Mexico. Trait: a = corolla aperture set in FAA (mm), b = perianth segment length (mm), c = total flower length (mm), d = pericarp length (mm), e = pericarp width (mm), f = cladode length (cm), g = number of ovules, h = equatorial diameter of the ovarian chamber (mm), i = polar diameter of the ovarian chamber (mm), j = longest stamen length (mm), k = shortest stamen length (mm), l = anther-stigma distance (mm), m = style length (mm), n = number of pollen grains per anther, o = number of stamens, p = cladode width (cm), q = fruit length (cm), r = seed length (cm), and s = seed width (cm).

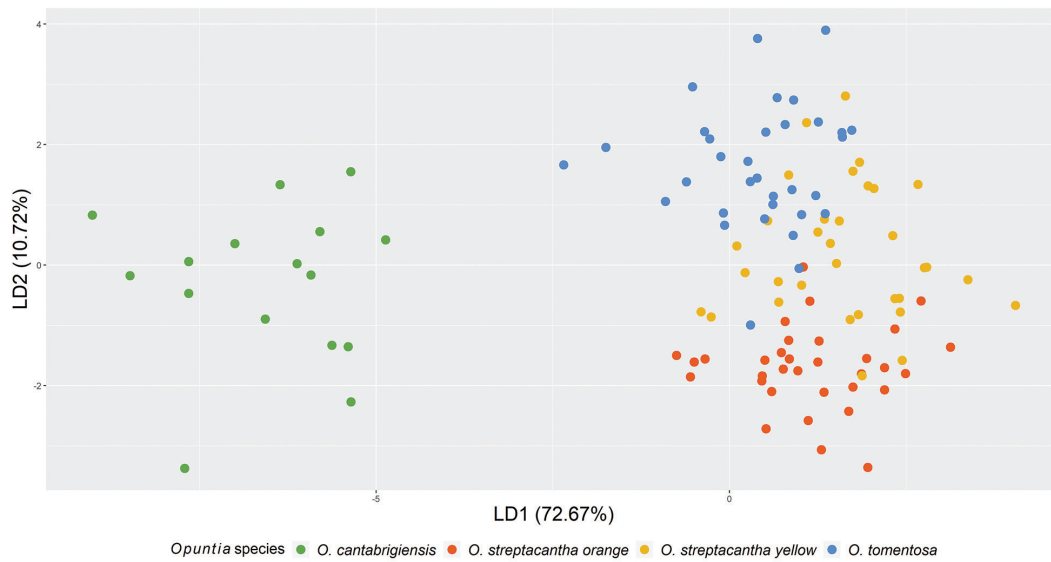


Figure 5. Linear discriminant analysis using floral morphometrics of three *Opuntia* species in Cadereyta de Montes, Queretaro, Mexico.

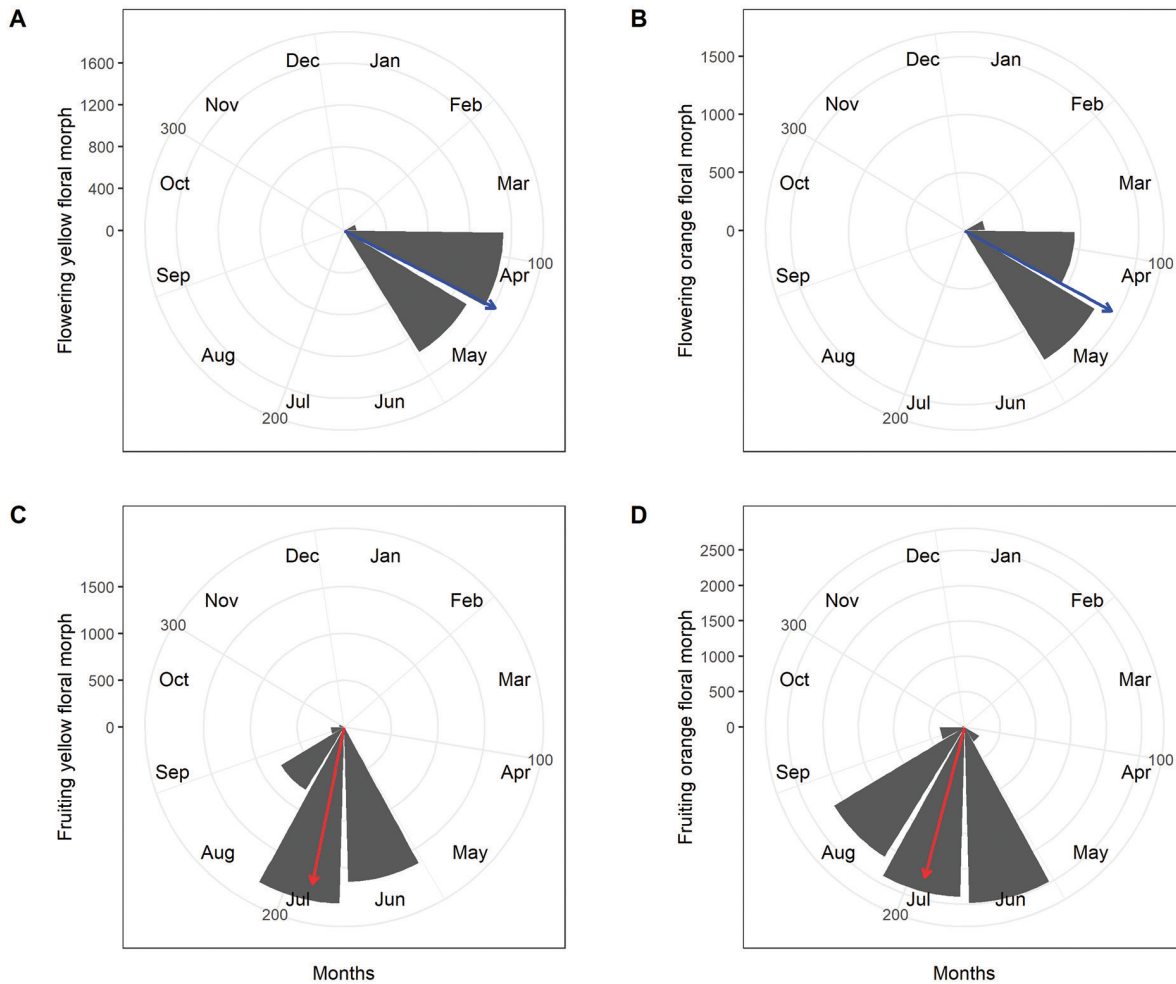


Figure 6. Rose diagram representing the months and phenology of floral morphs of *Opuntia streptacantha* in Cadereyta de Montes, Queretaro, Mexico. **A.** Flowering for the yellow floral morph. **B.** Flowering for the orange floral morph. **C.** Fruiting for the yellow floral morph. **D.** Fruiting for the orange floral morph. The blue arrow indicates the accumulation of data for flowering based on the Rayleigh uniformity test. The red arrow indicates the accumulation of data for fruiting based on the Rayleigh uniformity test.

to November, with peak fruiting in June (Fig. 6D). The fruiting patterns of the floral morphs were significantly different ($W = 206.5$, $p < 0.0001$).

According to the Marquis (1988) index, in the *O. streptacantha* population we studied, flowering synchrony is very high for both YFM ($S = 0.94$, $EE = 0.25$) and OFM ($S = 0.91$, $EE = 0.21$). For the Mahoro (2002) index modified by Osada et al. (2003), floral synchrony between YFM and OFM is also high ($S = 0.86$).

DISCUSSION

We found that the greatest morphological difference between floral colour morphs in *Opuntia streptacantha* is found for flower characteristics, both in the external part of the flower and in the reproductive structures, with the yellow-flowered morph generally being larger than the orange-flowered morph.

Although cladodes and spines are the most striking morphological characteristics in *Opuntia* (Del Castillo 1999), they showed few differences between YFM and OFM, which may reflect the fact that both floral morphs are subjected to similar environmental stress. In cacti, spines help regulate plant body temperature and also reduce photosynthetically active radiation (Majure et al. 2023).

YFM fruits are longer and have larger seeds compared to OFM; however, there is no difference in the number of seeds in each fruit. Several studies showed that seed size can vary within populations and within plants in the same species (Janzen 1977; Cavers and Steel 1984; Winn and Gross 1993; Sakai and Sakai 1996). For example, in the species *Phaseolus lunatus* L. (Fabaceae) it was found that in different regions and in the same population, there is a great variation in fruit and seed characters (Vargas et al. 2003). Another factor to consider is the reproductive success of the species, since the type of reproductive system of a flowering plant may condition in some way the production of fruits and seeds, because many depend on the efficiency of pollination (Galletto et al. 2002).

Floral morphometry studies in cacti are few, but it has been reported that there is variation in flower in some cactus species, such as *Lophophora diffusa* (Croizat) Bravo (Briseño-Sánchez 2019), where white or pink flowers have been reported, *Weberbauerocereus weberbaueri* (K.Schum. ex Vaupel) Backeb. (Sahley 1996), where it ranges from bright pink-red to white, and *Ariocarpus kotschoubeyanus* (Lem.) K.Schum. (Martínez-Peralta et al. 2014), where it ranges from white with a darker tepal line to magenta, with intermediate shades. In the case of *L. diffusa* and *A. kotschoubeyanus*, the flowers are visited by bees, while *W. weberbaueri* flowers are visited by bats and hummingbirds. However, no information is available on possible morphological differences associated with flower colour. Studies on the species *Collinsia parviflora* Douglas ex Lindl. (Scrophulariaceae) showed a positive relationship between flower size and the number of floral

visitors (Elle and Carney 2003), but another study with *Ipomoea aquatica* Forssk. (Convolvulaceae) found that floral visitors similarly visited flowers of all colours (Hassa et al. 2020).

In the linear discriminant analysis, *O. tomentosa* and *O. cantabrigiensis* were included, since they showed flower colour similarities with the floral morphs of *O. streptacantha*: *O. cantabrigiensis* has yellow flowers and *O. tomentosa* orange flowers (Galicia-Pérez et al. 2023). The analysis completely separated *O. cantabrigiensis* from *O. tomentosa*, YFM, and OFM, but the latter three were morphologically close, giving the possibility that there are some individuals with hybrid phenotypes between these species. Intermediate phenotypes are common in *Opuntia* s.s., and morphology supports this interclade hybridization (Majure et al. 2012). It is likely that the existence of YFM and OFM in *O. streptacantha* is due to a gene exchange with *O. tomentosa*, which has the greatest number of morphological and phenological similarities with YFM.

In general, the flowering peaks of YFM and OFM were unique, this agrees with the information from several studies where it is mentioned that the cacti studied so far have only one flowering peak (unimodal), although there are species that flower throughout the year and with several flowering peaks (Mandujano et al. 2010). The two flower colour morphs of *O. streptacantha* had their peak flowering in different months, this may be a strategy to ensure the reproductive success of both or may be a differential response to the environment (Fenner 1998; Matías-Palafox et al. 2017).

The floral morphs of *O. streptacantha* showed very high synchrony indices, either within the same morph or between morphs. In both cases, flowering occurred in a single period (from February to June). Rathcke and Lacey (1985) mention that simultaneous flowering between different species can be advantageous, since the flowering of one species increases the visitation rate of another species. Martínez-Ramos et al. (2024) found for the same study site as *O. tomentosa* and *O. streptacantha* (without distinguishing the morphs), that a high rate of interspecific flowering synchrony was present, adding that this could favour gene flow between these species. Matías-Palafox et al. (2017) found that during peak flowering of *Astrophytum ornatum* (DC.) Britton & Rose (Cactaceae) and *Turbincarpus horripilus* (Lem.) V. John & Ríha (Cactaceae), which cohabit the same area, both species showed synchronous flowering, bee pollination and shared floral visitors, this could result in interspecific competition or facilitation when there is a shortage of pollinators.

In conclusion, the differences between the floral morphs of *Opuntia streptacantha* extend beyond flower colour. The structure in which most of the morphological variation is found is the flower, but the differences between floral morphs are not only morphological, but also ecological since they show differences in flowering phenology. Therefore, it is important to determine

whether these floral morphs are already differentiated into another taxonomic category, further research (e.g. hand pollination to evaluate whether the flower colour morphs are sexually compatible and observations to determine floral visitors and pollen flow) will help to understand the role of flower colour polymorphism in *O. streptacantha*.

DATA AVAILABILITY STATEMENT

Data use for the statistical analyses have been deposited in Zenodo: <https://doi.org/10.5281/zenodo.11373417>

ACKNOWLEDGEMENTS

This project was funded by Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) project 221362 “Estrategias reproductivas en cactáceas, facilitación o interferencia”, the support for national research assistants’ SNI III or emeritus of CONAHCYT, 2020, institutional budget of the Instituto de Ecología, UNAM, and funding from the UNAM-DGAPA-PAPIIT Program <<IN217324>> to María del Carmen Mandujano. To the Jardín Botánico Regional de Cadereyta “Ing. Manuel González de Cosío” for granting access to the site and support during field work. Adriana Díaz-Trujillo, Salvador Arias, Martha Juana Martínez-Gordillo, Linda Mariana Martínez-Ramos, and Itzi Fragoso-Martínez for their valuable comments. Mariana Rojas Aréchiga provided logistic support for field work and processing the scientific collection permit. We thank the anonymous reviewers and the associate editor (Renate Wesselingh) for their comments and suggestions on earlier drafts of our manuscript.

REFERENCES

- Agostinelli C, Lund U (2022) R package circular: circular statistics. Version 0.5-0. <https://CRAN.R-project.org/package=circular> [accessed 07.06.2024]
- Aguilar ML, Pérez-Olvera C, Pérez-Olvera S (2004) La flora del Escudo Nacional Mexicano. Polibotánica 18: 53–73.
- Anderson EF (2001) The Cactus Family. Timber Press, Portland, Oregon, USA, 776.
- Arias S, Gama-López S, Guzmán-Cruz LU, Vázquez-Benítez B (2012) Cactaceae Juss. In: Medina-Lemos R (Ed.) Flora del Valle de Tehuacán-Cuicatlán Fascículo 95. Instituto de Biología, Universidad Nacional Autónoma de México, Mexico, 213–216.
- Arias S, Arreola H, Cházaro M, Gómez-Hinostrosa C, Hernández HM, Terrazas T (2017) *Opuntia streptacantha* (amended version of 2013 assessment). The IUCN Red List of Threatened Species 2017: e.T152896A121615882. <https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T152896A121615882.en> [accessed 12.05.2020]
- Barthlott W, Hunt DR (1993) Cactaceae. In: Kubitzki K, Rohwer JG, Bittrich V (Eds) The Families and Genera of Vascular Plants. Springer Verlag, Germany, 161–197.
- Batschelet E (1981) Circular Statistics in Biology. Academic Press, London, United Kingdom, 1–371.
- Bravo-Hollis H, Sánchez-Mejorada H (1978) Las Cactáceas de México Vol. 1. Universidad Nacional Autónoma de México, Mexico, 1–743.
- Briseño-Sánchez MI (2019) Contribución de los eventos reproductivos y el crecimiento clonal en la dinámica poblacional de *Lophophora diffusa* (Cactaceae). Master Thesis, Instituto de Ecología-UNAM, Mexico.
- Britton NL, Rose JN (1919) The Cactaceae: Descriptions and Illustrations of Plants of the Cactus Family. Vol 1. The Carnegie Institution, Washington, 248–320.
- Cavers P, Steel M (1984) Patterns of change in seed weigh over time on individual plants. The American Naturalist 124: 324–335. <https://www.jstor.org/stable/2461461> [accessed 07.06.2024]
- Chávez-Martínez RJ, Hernández-Magaña R (2009) Flora Silvestre del Jardín Botánico Regional de Cadereyta “Ing. Manuel González de Cosío”. <https://es.scribd.com/document/16178745/Flora-Silvestre-del-Jardin-Botanico-de-Cadereyta> [accessed 07.06.2024]
- ComenTuna, Red Nopal, CONABIO (2009) Nopales, Tunas y Xoconostles. Consejo Mexicano de Nopal y Tuna, A.C., Red Nopal y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Mexico, 1–9. <https://memoricamexico.gob.mx/swb/memorica/Cedula?old=TEGkNHkBsgXj28HgZ9OQ> [accessed 07.06.2024]
- Cuadras CM (1981) Métodos de Análisis Multivariante. Funibar DL, Barcelona, 1–642.
- Del Castillo RF (1999) Exploración preliminar sobre los sistemas de cruzamiento en *Opuntia*. In: Aguirre RJR, Reyes-Agüero JA (Eds) Memoria del VIII Congreso Nacional y III Internacional sobre el Conocimiento y Aprovechamiento del Nopal. Universidad Autónoma de San Luis Potosí, San Luis Potosí, 360–389.
- Elle E, Carney R (2003) Reproductive assurance varies with flowers size in *Collinsia parviflora* (Scrophulariaceae). American Journal of Botany 90: 888–896. <https://doi.org/10.3732/ajb.90.6.888>
- Esparza-Sandoval S (2010) Distribución geográfica del género *Opuntia* (Cactaceae) en México. Master Thesis, Universidad Autónoma de San Luis Potosí, Mexico.
- Fenner M (1998) The phenology of growth and reproduction in plants. Perspectives in Plant Ecology, Evolution and Systematics 1: 78–91. <https://doi.org/10.1078/1433-8319-00053>
- Fordyce J (2006) The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. The Journal of Experimental Biology 209: 2377–2383. <https://doi.org/10.1242/jeb.02271>
- Galetto L, Fioni A, Calviño A (2002) Éxito reproductivo y calidad de frutos en poblaciones del extremo sur de la distribución de *Ipomoea purpurea* (Convolvulaceae). Darwiniana 40: 1–4.
- Galicia-Pérez A, Golubov JG, Manzanarez-Villasana G, Martínez-Ramos LM, Arias S, Márquez-Guzmán J, Mandujano MC (2023) Complex taxonomy in Opuntioideae: is floral morphometry essential to identify *Opuntia* species? Botany 101(11): 485–497. <https://doi.org/10.1139/cjb-2022-0133>
- García E (2004) Modificaciones al Sistema de Clasificación Climática de Köppen. Instituto de Geografía, UNAM, Mexico, 1–90.
- Golubov J, Mandujano MC, Mandujano F (2005) Diversidad alfa y beta en *Opuntia* y *Agave*. In: Halffter G, Soberón J, Koleff P, Melic A (Eds) Sobre Diversidad Biológica: el Significado de las Diversidades Alfa, Beta y Gamma. CONABIO, Mexico, 221–230.

- Griffith PM, Porter JM (2009) Phylogeny of Opuntioideae (Cactaceae). *International Journal of Plant Sciences* 170(1): 107–116. <https://doi.org/10.1086/593048>
- Hassa P, Traiperm P, Stewart AB (2020) Pollinator visitation and female reproductive success in two floral color morphs of *Ipomoea aquatica* (Convolvulaceae). *Plant Systematics and Evolution* 306: 88. <https://doi.org/10.1007/s00606-020-01716-1>
- Hunt DR, Taylor N, Charles G (2006) *The New Cactus Lexicon*. 2 vols. DH Press, Milborne Port.
- Janzen D (1977) Variation in seed size within a crop of a Costa Rica *Mucuna andreana* (Leguminosae). *American Journal of Botany* 64: 347–349. <https://doi.org/10.1002/j.1537-2197.1977.tb15736.x>
- Kassambara A, Mundt F (2020) factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://cran.r-project.org/package=factoextra> [accessed 07.06.2024]
- Kiernan JA (2002) *Histological and Histochemical Methods: Theory and Practice*. Arnold Publisher, London, 1–571.
- Le S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* 25(1): 1–18. <https://doi.org/10.18637/jss.v025.i01>
- López-Borja E, Romo-Campos RL, Arreola-Nava HJ, Muñoz-Urías A, Loza-Cornejo S (2017) Variación morfológica en *Opuntia jaliscana* (Cactaceae). *Anales del Jardín Botánico de Madrid* 74(1): 1–11. <https://doi.org/10.3989/ajbm.2431>
- Mahoro S (2002) Individual flowering: schedule, fruit set, and flower and seed predation in *Vaccinium hirtum* Thunb. (Ericaceae). *Canadian Journal of Botany* 80: 82–92. <https://doi.org/10.1139/b01-136>
- Majure LC, Puente R (2014) Phylogenetic relationships and morphological evolution in *Opuntia* s.str. and closely related members of tribe Opuntieae. *Succulent Plant Research* 8: 9–30.
- Majure LC, Puente R, Griffith MP, Judd WS, Soltis PS, Soltis DS (2012) Phylogeny of *Opuntia* s.s (Cactaceae): clade delineation, geographic origins, and reticulate evolution. *American Journal of Botany* 99: 847–864. <https://doi.org/10.3732/ajb.1100375>
- Majure LC, Achá S, Baker MA, Puente-Martínez R, Köhler M, Fehlberg S (2023) Phylogenomics of one of the world's most intriguing groups of CAM plants, the Opuntioideae (Opuntioideae: Cactaceae): adaptation to tropical dry forests helped drive prominent morphological features in the clade. *Diversity* 15: 570. <https://doi.org/10.3390/d15040570>
- Mandujano M, Sánchez C (2017) El nopal genealógico. *Cactáceas y Suculentas Mexicanas* 62(8): 58–95.
- Mandujano MC, Carrillo-Ángeles I, Martínez-Peralta C, Golubov J (2010) Reproductive biology of Cactaceae. In: Ramawat KG (Ed.) *Desert Plants: Biology and Biotechnology*. Springer-Verlag, Berlin, Heidelberg, 197–230. https://doi.org/10.1007/978-3-642-02550-1_10
- Manzanarez-Villasana G, Morales-Martínez G, Mandujano MC (2022) Superposición de la mancha urbana en la distribución de *Opuntia* s.s de México. *Cactáceas y Suculentas Mexicanas* 67(2): 36–54.
- Marquis RJ (1988) Phenological variation in the neotropical understory shrub *Piper arieianum*: causes and consequences. *Ecology* 69(5): 1557–1565. <https://doi.org/10.2307/1941653>
- Martínez-Peralta C, Molina-Freaner F, Golubov J, Vázquez-Lobo A, Mandujano MC (2014) A comparative study of the reproductive traits and floral morphology of a genus of geophytic cacti. *International Journal of Plant Sciences* 175(6): 663–680. <https://doi.org/10.1086/676302>
- Martínez-Ramos LM (2019) Flujo polínico y su efecto en el éxito reproductivo de *Opuntia tomentosa*. Master Thesis, Instituto de Ecología-UNAM, México.
- Martínez-Ramos LM, Mejía-Rojas M, Rojas-Aréchiga M, Mandujano MC (2017) La hercogamia como indicador del sistema reproductivo de *Thelocactus leucacanthus* spp. *schmollii*. *Cactáceas y Suculentas Mexicanas* 62 (1): 13–22.
- Martínez-Ramos LM, Vázquez-Santana S, García-Franco J, Mandujano MC (2024) Is self-incompatibility a reproductive barrier for hybridization in sympatric species? *American Journal of Botany* 11(4): e16309. <https://doi.org/10.1002/ajb2.16309>
- Matías-Palafox ML, Jiménez-Sierra CL, Golubov J, Mandujano MC (2017) Reproductive ecology of the threatened “star cactus” *Astrophytum ornatum* (Cactaceae): a strategy of continuous reproduction with low success. *Botanical Sciences* 95(2): 1–14. <https://doi.org/10.17129/botsci.780>
- Mendoza I (2020) Estadística circular aplicada en la Ecología. *Ecosistemas* 29(2): 1995. <https://doi.org/10.7818/ECOS.1995>
- Morello LPC, Alberti LF, Hudson IL (2010) Applications of circular statistics in plant phenology: a case studies approach. In: Hudson IL, Keatley MR (Eds) *Phenological Research: Methods for Environmental and Climate Change Analysis*. Springer, Dordrecht, 339–359. https://doi.org/10.1007/978-90-481-3335-2_16
- Moreno N (1984) *Glosario Botánico Ilustrado*. Instituto Nacional de Investigaciones sobre Recursos Bióticos, Xalapa, Veracruz, 1–300.
- Muñoz-Urías A, Palomino-Hasbach G, Terrazas T, García-Velázquez A, Pimienta-Barrios E (2008) Variación anatómica y morfológica en especies y entre poblaciones de *Opuntia* en la porción sur del Desierto Chihuahuense. *Boletín de la Sociedad Botánica de México* 83: 1–11.
- Narbona E, Buide ML, Casimiro-Soriguer I, del Valle JC (2014) Polimorfismos de color floral: causas e implicaciones evolutivas. *Ecosistemas* 23(3): 36–47. <https://doi.org/10.7818/ECOS.2014.23-3.06>
- Osada N, Sugiura S, Kawamura K, Cho M, Takeda H (2003) Community-level flowering phenology and fruit set: comparative study of 25 woody species in a secondary forest in Japan. *Ecological Research* 18: 711–723. <https://doi.org/10.1111/j.1440-1703.2003.00590.x>
- Pimienta-Barrios E (1994) Prickly pear (*Opuntia* spp.): a valuable fruit crop for the semi-arid lands of Mexico. *Journal of Arid Environments* 28: 1–11. [https://doi.org/10.1016/S0140-1963\(05\)80016-3](https://doi.org/10.1016/S0140-1963(05)80016-3)
- Pimienta-Barrios E, Mauricio-Laguizamo R (1989) Variación en componentes del fruto maduro entre formas de nopal (*Opuntia* spp.) tunero. *Revista Fitotecnia Mexicana* 12: 183–196.
- Pimienta-Barrios E, Muñoz-Urías A (1995) Domestication of Opuntias and cultivated varieties. In: Barbera G, Inglese P, Pimienta-Barrios E (Eds) *Cultivation and Uses of Cactus Pear*. FAO, Roma, 58–61.
- Pinkava DJ (2003) Cactaceae, subfamily Opuntioideae. Vol 4. In: *Flora of North America Editorial Committee* (Eds) *Flora of North America North of Mexico*. Oxford University, New York, 102–150.
- Porras-Flórez D, Albesiano S, Arrieta-Violet L (2017) El género *Opuntia* (Opuntioideae-Cactaceae) en el departamento de Santander, Colombia. *Biota Colombiana* 18(2): 111–131. <https://doi.org/10.21068/c2017.v18n02a07>
- Rathcke B, Lacey EP (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* 16: 179–214. <https://doi.org/10.1146/annurev.es.16.110185.001143>
- R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.r-project.org> [accessed 07.06.2024]
- Revelle W (2020) psych: procedures for psychological, psychometric, and personality research. Northwestern

- University, Evanston, Illinois, USA. <https://cran.r-project.org/package=psych> [accessed 07.06.2024]
- Reyes-Agüero JA, Aguirre JR, Rogelio J, Flores JL (2005) Variación morfológica de *Opuntia* (Cactaceae) en relación con su domesticación en la altiplanicie meridional de México. *Interciencia* 30: 476–484.
- Russell VL (2021) emmeans: estimated marginal means, aka least-squares means. R package version 1.7.0. <https://cran.r-project.org/web/packages/emmeans> [accessed 07.06.2024]
- Sahley CT (1996) Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). *American Journal of Botany* 83(10): 1329–1336. <https://doi.org/10.1002/j.1537-2197.1996.tb13918.x>
- Sakai S, Sakai A (1996) Why is there variation in mean seed among plants within single populations? Test of the fertilization efficiency hypothesis. *American Journal of Botany* 83(11): 1454–1457. <https://doi.org/10.2307/2446100>
- Scheinvar L (1995) Taxonomy of utilized *Opuntias*. In: Barbera G, Inglese P, Pimienta-Barrios E (Eds) *Cultivation and Uses of Cactus Pear*. FAO, Roma, 20–27.
- Schumann K (1899) *Gesamtbeschreibung der Kakteen* (Monographia Cactacearum). J. Neumann, Neudamm, 1–832. <https://doi.org/10.5962/bhl.title.10394>
- Shipunov A, Kosenko Y, Volkova P (2011) Floral polymorphism in common primrose (*Primula vulgaris* Huds., Primulaceae) of the Northeastern Black Sea coast. *Plant Systematics and Evolution* 296: 167–178. <https://doi.org/10.1007/s00606-011-0484-5>
- Sokal RR, Sneath PHA (1963) *Principles of Numerical Taxonomy*. W.H. Freeman & co., San Francisco, London, 1–359.
- Vaissie P, Monge A, Husson F (2020) Factoshiny: perform factorial analysis from 'FactoMineR' with a shiny application. <https://cran.r-project.org/web/packages/Factoshiny/index.html> [accessed 07.06.2024]
- Vargas EM, Castro E, Macaya G, Rocha JO (2003) Variación del tamaño de frutos y semillas en 38 poblaciones silvestres de *Phaseolus lunatus* (Fabaceae) del Valle Central de Costa Rica. *Revista de Biología Tropical* 31(3–4): 707–724.
- Wallace R, Fairbrothers D (1986) Isoelectrically focussed seed proteins of populations of *Opuntia humifusa* (Raf.) Raf. (Cactaceae). *Biochemical Systematics and Ecology* 14: 365–369. [https://doi.org/10.1016/0305-1978\(86\)90020-7](https://doi.org/10.1016/0305-1978(86)90020-7)
- Winn A, Gross K (1993) Latitudinal variation in seed weight and flower number in *Prunella vulgaris*. *Oecologia* 93: 55–62. <https://doi.org/10.1007/BF00321191>
- Zar JH (1999) *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, 1–663.

SUPPLEMENTARY MATERIAL

Supplementary material 1

Principal component analysis rotation matrix and principal component significance.

<https://doi.org/10.5091/plecevo.112250.suppl1>