Morphological and phenological variation in the floral morphs of *Opuntia streptacantha* (Cactaceae)

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1   **Morphological and phenological variation in the floral morphs of *Opuntia***

2   ***streptacantha* (Cactaceae)**

3   **Running title: Floral variation in *Opuntia streptacantha***

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9   **ABSTRACT**

10   **Background and aims** – *Opuntia* s.s. (Cactaceae) is one of the most diverse genera, with

11   approximately 200 species, as species have great morphological and anatomical variation,

12   which have caused a high adaptive plasticity in the species, reflected in the intra- and

13   interspecific variability. Our study system was *Opuntia streptacantha* Lem., which has two

14   floral morphs: yellow and orange. The objective was to determine if there were

15   morphological differences in the reproductive and vegetative structures between floral

16   morphs.

17   **Material and methods** – Statistical tests were performed to determine if there were

18   differences in morphological structures (8 cladodes structures (n= 20 cladodes for each

19   floral morph) and 17 flowers structures (n= 30 flowers for each floral morph) and

20   multivariate models of principal and discriminant components. Also, reproductive

21   phenology was registered for both floral morphs to describe the phenophases of each (n=10

22   individuals for each floral morph).

23   **Key results and conclusion** – We found that floral morphs of *O. streptacantha* showed

24   significant differences mostly associated with the flowers. The principal component

25   analysis revealed seven components that explain 80% of the total variation, some

26   individuals of *O. tomentosa* were classified as floral morphs of *O. streptacantha*, not

27   having a clear separation between the species. The phenology of the floral morphs showed

28   a slight lag in their peak flowering and fruiting. Very high floral synchrony was found for

29   each floral morph and between them. The modifications found in the flowers of *O.*

30   *streptacantha* may be associated with a possible hybridization with *O. tomentosa* favoring

31   the appearance of both floral morphs.

32   **Key words:** cladodes, fruits, phenology, seeds, synchrony, spines.

INTRODUCTION

33

34 The subfamily Opuntioideae (Cactaceae) is constituted by about 220–350 species (Britton

35 and Rose 1919; Anderson 2001; Griffith and Porter 2009). Within the subfamily the genus

36 *Opuntia* s.s. (commonly known as prickly pear), is the most diverse with approximately

37 200 species (Britton and Rose 1919; Barthlott & Hunt 1993; Pinkava 2003; Porras-Flórez et

38 al. 2017) and is of great biological, cultural, economic, and social importance (Bravo-Hollis

39 and Sánchez-Mejorada 1978; Aguilar et al. 2004; Reyes-Agüero et al. 2005; Mandujano

40 and Sánchez 2017). In Mexico, prickly pears are widely distributed; however, it prospers in

41 arid and semi-arid zones, where the greatest species diversity is observed with two

42 important centers of diversity: the Chihuahuan Desert zone and the central-western region

43 (State of Mexico, Guerrero, and Jalisco) (Golubov et al. 2005; Muñoz-Urias et al. 2008),

44 and to a lesser extent in areas such as forests or jungles (Esparza-Sandoval 2010,

45 Manzanarez-Villasana et al. 2022).

46 Currently, there is evidence indicating that species richness and taxonomic complexity in

47 *Opuntia* s.s. is the result of the influence, in part, of several evolutionary, ecological events

48 and environmental factors, causing a high adaptive plasticity in the species of the genus,

49 reflected in intra- and interspecific variation (Bravo-Hollis and Sánchez-Mejorada 1978;

50 Scheinvar 1995; Reyes-Agüero et al. 2005; Muñoz-Urias et al. 2008; Arias and Flores

51 2013; Majure and Puente 2014).

52 The genus has high phenotypic plasticity. Morphological variations included growth habit,

53 stem size and pubescence, length of spines, number of areoles, shape and color of flowers,

54 weight and chemical composition of the fruit, seed size, among others; and also

55 phenological variations such as the time in which vegetative and reproductive phenophases

56 are observed, to mention a few (Wallace and Fairbrothers 1986; Pimienta-Barrios and

57 Mauricio-Leguizamo 1989; Pimienta-Barrios 1994; Pimienta-Barrios and Muñoz-Urias

58 1995; Fordyce 2006; Muñoz-Urias et al. 2008).

59 *Opuntia* s.s. shows a marked morphological variation to the extent that its taxonomy

60 becomes confusing (Bravo-Hollis and Sánchez-Mejorada 1978; Scheinvar 1995; Reyes-

61 Agüero et al. 2005; Muñoz-Urias et al. 2008). An example of this would be the species that

62 present flowers with different floral morphs of coloration, such as: *O. engelmannii* var

63   *lindheimeri* (Engelm.) B.D. Parfitt & Pinkava, where sometimes it presents yellow and

64   orange flowers on the same plant, or *Opuntia phaceacantha* Engelm. with a range of flower

65   color from yellow to pink (Majure and Puente 2014). Another important factor in the

66   evolution of the genus *Opuntia* s.s., is hybridization (Anderson 2001; Scheinvar et al.

67   2011), for which they have reported a large number of wild hybrids (Mc Leod 1975; Grant

68   and Grant 1979; Parffit 1980; Pinkava et al. 1992; Mayer et al. 2000; Griffith 2001;

69   Muñoz-Urias et al. 2008), such as those proposed as species: *Opuntia × fosbergii* C.B.

70   Wolf and *Opuntia × occidentalis* Engelm. & J.M. Bigelow. Natural hybridization in

71   *Opuntia* s.s. can generate intermediate phenotypes (Muñoz-Urias et al. 2008), which could

72   increase the degree of confusion when delimiting species.

73   Majure and Puente (2014) ponder six main reasons to explain the taxonomic problems in

74   the delimitation of *Opuntia* s.s. species, 1) A large amount of hybridization resulting in a

75   mosaic of characteristics expressed by the progeny, 2) Morphologically variable species,

76   where characters often depend on environmental variables, 3) Poor sampling of the species'

77   characters throughout its geographic range, 4) Lack of biological data such as chromosome

78   counts, 5) Deficit of detailed studies on morphology of species, and 6) Lack of

79   phylogenetic data.

80   The study of morphological variations in cacti, especially variations in floral color

81   contribute to a simple model for understanding plant adaptation (Hoballah et al. 2007;

82   Narbona et al. 2014). These studies can be approached at the inter- or intraspecific level

83   (Narbona et al. 2014). An example of this would be in members of *Nopalea*, where it was

84   observed that morphological changes in the flower were linked to changes in pollinators

85   from insects to hummingbirds (Majure et al. 2012) and thus in their fitness.

86   Due to this, it is important to carry out taxonomic and ecological studies on plant species,

87   as they will help to perform a taxonomic delimitation, which will lead to the recognition of

88   species, subspecies, or varieties (Narbona et al. 2014), for example, the cases of

89   Streptanthus glandulosus (Brassicaceae) or Primula vulgaris (Primulaceae) (Mayer et al.

90   1994; Shipunov et al. 2011).

91   Reproductive phenology is another source of variation that can be found in the individuals

92   of a population, and it can contribute to reproductive isolation. This variation spans from

93 the temporal pattern of bud formation to fruit ripening (Gordo and Sanz 2005; Yang and

94 Rudolf 2010; Ramírez-Bullón et al. 2014) and describes how those resource vary over time

95 (Mantovani et al. 2003; Ochoa-Gaona et al. 2008). Another factor to consider is floral

96 synchrony, as the number of flowers open at the same time in a population is considered a

97 strategy that allows concentrating the greatest amount of floral rewards at the same time

98 and space, guaranteeing the successful pollination of flowers (Martínez-Peralta and

99 Mandujano, 2012). The degree of floral (and fruiting) synchrony has ecological and

100 evolutionary relevance at different scales, from intra-individual flowering within a

101 population or interspecific across the landscape (Freitas and Bolmgren, 2008), high floral

102 synchrony or simultaneous flowering can be advantageous among different species

103 (Rathcke and Lacey, 1985).

104 *Opuntia streptacantha* Lem. is a wild species endemic to Mexico which has two floral

105 morphs: yellow and orange, it is important to note that the yellow floral morph was the first

106 to be described. The objective of this work was to determine if there were morphological

107 differences in the reproductive and vegetative structures, and phenological differences

108 between the floral morphs in *O. streptacantha*.

MATERIALS AND METHODS

110 **Study species**.

111 *Opuntia streptacantha* Lem., is an arborescent or shrubby plant, up to 4 m high, its stems

112 are flattened and racket-shaped (i.e., cladodes), the flowers are yellow or orange, 5−6 cm

113 long; fruits are 5 cm long and 3 cm wide, globose to obovoid and are usually wine colored

114 when ripe, the glochids are short, the pulp is red; the seeds are 3.8−4.5 mm long, by 2.6 mm

115 wide. (Bravo-Hollis and Sánchez-Mejorada 1978; Arias et al. 2012).

116 This species is endemic to Mexico and is commonly known as "cardón", "cenizo",

117 "chaveño" or "nopal cardón" (Arias et al., 2012), and is distributed in the states of

118 Aguascalientes, Mexico City, Durango, Mexico, Guanajuato, Hidalgo, Jalisco, Michoacán,

119 Nuevo Leon, Oaxaca, Puebla, Queretaro, San Luis Potosi, Tamaulipas, Tlaxcala y

120 Zacatecas (Hunt et al. 2006). In turn, it grows at elevations of 1,600 to 2,400 m.a.s.l. (Arias

121 et al. 2017).

122   *Opuntia streptacantha* is generally found in xerophytic scrublands and with other co-

123   dominant *Opuntia* species, they form a vegetation type called "nopaleras" (Arias et al.,

124   2012). According to Bravo-Hollis and Sánchez-Mejorada (1978) it is a wild species of

125   remarkable management, due to its edible fruits and stems.

126   Populations with individuals with two floral morphs: one with yellow flowers (MA) and

127   others with orange flowers (MN) (Figure. 1). Historically, the first description of the color

128   of the flowers of this species was by Schumann (1899) where he mentions that the flowers

129   are yellow, however, Bravo-Hollis and Sánchez-Mejorada (1978) noted that the flowers

130   vary from yellow to orange.

131   **Site of study**.

132   This study was carried out in the southern portion of the Chihuahuan desert known as

133   Queretano-Hidalguense semi-desert, in the wilderness area protected by the Regional

134   Botanical Garden of Cadereyta de Montes "Ing. Manuel González de Cosío", in the state of

135   Querétaro, Mexico. Its geographic coordinates are 20º41'15.8"N, 99º48'17.7"W, with an

136   altitude of 2,046 m.a.s.l., the vegetation type is xerophytic crassic scrub. The climate is

137   semi-dry, temperate with summer rains (Köppen climate group BS1 kw (w) modified by

138   Garcia (2004)). The average annual temperature ranges between 12º and 19ºC and the

139   average annual precipitation is about 550 mm (Chávez-Martínez and Hernández-Magaña

140   2009).

141   **Cladode and spines morphometry**.

142   Twenty old cladodes (considering cladodes with lateral cladodes or o with reproductive

143   structures (buds, flowers or fruit) as old) (two cladodes per individual) and twenty young

144   cladodes (considering lateral cladodes as young) (two cladodes per individual) were

145   measured from ten *O. streptacantha* reproductive individuals of both floral morphs, taking

146   as norm, that the individuals had fruits, buds or flowers, or a combination of these and a

147   height of approximately 3 m. The parameters used in the work of Muñoz-Urias et al. (2008)

148   were measured: cladode length (cm), cladode width (cm), maximum distance from the

149   apical to the widest part (cm), maximum distance from the basal to the widest part (cm),

150   number of series of areoles, areole size (mm), distances between areoles (mm) and distance

151   between lines (mm); To determine differences in the spines of floral morphs, the type,

152   color, and number of spines on the central areole of three cladodes per individual were

153   determined for each floral morph ($n=$ 10) (López-Borja et al. 2017).

154   **Flower morphometry.**

155   Thirty-three undamaged flowers at maximum opening were collected from different

156   individuals of each floral morph of *O. streptacantha* and fixed in FAA (Formaldehyde,

157   alcohol, acetic acid) (Kiernan 2002). Following Martinez-Ramos et al. (2017) seventeen

158   morphological characters of flowers were measured: corolla aperture set in FAA (mm),

159   perianth segment length (mm), total flower length (mm), pericarp height (mm), pericarp

160   width (mm), distance between anther and stigma (mm), stigma width (mm), stigma length

161   (mm), style height (mm), longest stamen height (mm), shortest stamen height (mm),

162   equatorial diameter of the ovarian chamber (mm), polar diameter of the ovarian chamber

163   (mm), number of stamens, number of ovules, number of lobes and number of pollen grains

164   in an anther.

165   Morphometric data of cladodes and flowers were tested for differences between floral

166   morphs using either *GLM* log-linear models with *Poisson* distribution for discrete counts

167   with a *t-test* for contrast and a *t-test* for continuous variables, in the case of the spines a

168   *paired t-test* was carried out, each analysis was performed in the program R version 4. 2.2.

169   (R Core Team 2022) with the stats package (R Core Team 2022) and emmeans (Russell

170   2021).

171   **Fruit and seed morphometry.**

172   We collect two fruits from ten different reproductive individuals per floral morph ($n =$20).

173   We assessed fruit diameter (mm), fruit length (mm), number of spiral series and number of

174   seeds (López-Borja et al. 2017). A *paired t-test* for continuous variables and a *GLM* with

175   *Poisson* distribution for discrete variables were performed in the R program version 4.2.2

176   (R Core Team 2022) with the stats package (R Core Team 2022) and emmeans (Russell

177   2021), to find differences between morphs. In addition, the external color of the fruit, color

178   of the pulp, color of the glochids using the HTML color code and the shape of the fruit

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

180 A sample of 10 seeds was randomly selected from each fruit (n= 200 seeds, per floral

181 morph), photographed, and measured for size with length and width of each seed (mm in

182 both cases), using Adobe Photoshop CS6. A *paired t-test* was performed in the R program

183 version 4.2.2 (R Core Team 2022) with the stats package (R Core Team 2022), to find

184 differences between morphs.

185 **Multivariate analysis**.

186 A numerical taxonomy analyses were performed to compare flower, cladode, fruit, and

187 seed characteristics between morphs (Sokal and Sneath 1963; Cuadras 1981) to identify

188 how morphological characters studied differed between morphs. The first analysis was

189 principal component analysis (PCA), to reduce the variables to those that would give us the

190 most taxonomic information. The morphological characters of all the previously mentioned

191 measured structures were considered, the analysis was performed in the R program version

192 4.2.2. (R Core Team, 2022), with the FactoMinerR (Le et al. 2008), factorextra

193 (Kassambara and Mundt 2020), psych (Revelle 2020) and Factoshiny (Vaissie et al. 2020)

194 packages. In addition, we fitted a linear discriminant analysis, which was carried out taking

195 into account the relevant flower and cladode characters in the principal components. In this

196 analysis, data from two more species were appended: *Opuntia tomentosa* and *Opuntia*

197 *cantabrigiensis* (Galicia-Pérez et al. 2023), as both species are found in the same study site

198 and present a very high floral synchrony, together with the floral morphs of *O.*

199 *streptacantha* (Martínez-Ramos 2019; pers. ob.), the analysis was performed in the R

200 program version 4.2.2 (R Core Team 2022) with the lda function of the stats package (R

201 Core Team 2022). Both multivariate analyses were run to maximize the contrasts between

202 groups (López-Borja et al. 2017).

203 **Reproductive phenology and flower synchrony.**

204 Reproductive phenology was registered for both floral morphs to describe the phenophases

205 of each (flowering and fruiting), taking monthly observations (April 2018 to March 2019)

206 of each individual (n=10 individuals for each floral morph). The data were analyzed with

207 circular statistics to determine the flowering peaks of each floral morph (Morellato et al.

208 2010), and the *Rayleigh* uniformity test (Zar 1999; Mendoza 2020) was calculated to

209 identify if the distribution of the phenophases is uniform or tends to some pattern and the

210 non-parametric Mardia-Watson-Wheeler test (Batschelet 1981) was performed to determine

211 differences between flowering and fruiting of both floral morphs. Analyses were carried out

212 in the R program version 4.2.2. (R Core Team 2022), with the *circular* package

213 (Agostinelli and Lund 2022).

214 Two indexes were evaluated to determine the floral synchrony of *O. streptacantha* of each

215 floral morph. The Marquis (1998) index was evaluated, which considers the number of

216 open flowers per census and the proportion that these flowers represent with respect to the

217 total number of flowers, following the formula below:

218
$$S = \sum_{t=0}^{n} \frac{x_t}{\sum_{t=0}^{n} x_t} \times Pt$$

219 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

220 the proportion of open flowers to the total number of flowers, and Pt represents the number

221 of individuals that flower in each census and the proportion that these represent of the total

222 number of individuals censused.

223 The floral synchrony between MA and MN was calculated with the index of Mahoro

224 (2002), modified by Osada et al. (2003). For the modified version, the relative number of

225 open flowers in each individual at an interspecific level (in this case, between MA and MN)

226 is considered, following the formula below:

227
$$s_i = \frac{1}{2}\left(2 - \sum_{i=1}^{n} |y_{i,j} - \Psi_{i,j}|\right)$$

228 Where s_i is the degree of synchrony of species A with species B, $y_{i,j}$ is the proportion of

229 open flowers of species A from ($j = 1$) until the census j, of the total number of flowers

230 open during the season, and $\Psi_{i,j}$ is the average number of open flowers of the species

231 B($y_{k,j}$).

232 Both indexes take values from 0 to 1, where a value close to one represents perfect

233 synchrony and a value close to zero represents asynchrony.

234

235   **RESULTS**

236   **Cladode and thorn morphometry**

237   Morphometry of old cladodes differed significantly between MA and MN in three

238   variables: cladode length (t= -2.62, p= 0.01) and cladode width (t= -2.23, p= 0.03), with

239   MN having the largest size measurements in these structures. (Table 1). No significant

240   differences were found for the young cladodes. floral morphs they ovate cladodes presented

241   two types of spines, straight and subulate. The spines have a yellow or white coloration and

242   the number of spines per areole in both morphs was similar (Mean ± Standard Error; MA=

243   3.6333 ± 0.4901, MN= 3.9333 ± 0.5208, x^2= 0.3569, p= 0.5502).

244   **Flower morphometry**

245   Flowers were actinomorphic in both morphs of *O. streptacantha* (Figure. 1), but we found

246   significant differences in most of the characters evaluated between (Table 2). Most

247   characters had greater values for MA (for example: total flower length, pericarp height, and

248   polar diameter of the ovarian chamber). In contrast, all similarities between floral morphs

249   were found in the gynoecium, stigma length (t= -0.0147, p= 0.9883) and width (t= 1.9016,

250   p= 0.0617).

251   **Fruit and seed morphometry**

252   Both floral morphs had ovate fruits, pericarpel color is magenta with wine-colored pulp

253   and glochids are opaque golden. Fruit length was the only difference found (t= 4.6239, p=

254   0.0002) MA having the longest fruit length (MA= 51.28 ± 1.59, MN = 43.92 ± 0.84) (Table

255   2). Significant differences in seed size (Table 2) were found in both floral morphs. Both

256   have a funiculate type seed, with an oval to amorphous shape and light brown color.

257   **Multivariate analysis**.

258   Of the morphological characters, those showing significant differences between floral

259   morphs for PCA, and discriminant analysis were considered. The PCA revealed seven

260   principal components that explain 80% of the total variation. The first component explains

261   28.37 % (total flower length), the second component 17.50% (number of stamens), the third

262   component 11.01% (distance between anther and stigma), the fourth component 7.20%

263   (pollen grains and style height), the fifth component 6.58% (equatorial diameter of the

264   ovarian chamber), the sixth component 5.71% (width of the pericarpel) and the seventh

265   component 4.05% (number of areole lines), considering only the first two components

266   explain 45.87% of the total variation. It is important to emphasize that six of the seven

267   components are flower morphometric variables.

268   Linear discriminant analysis explained 89.28% of the variation in the first two linear

269   discriminant functions. *Opuntia cantabrigiensis* was completely separated from the other

270   species, *O. tomentosa* is conglomerated with MA and MN is almost separated from *O.*

271   *tomentosa* but shows a small overlap with MA (Figure. 2). The analysis was able to

272   correctly 92% of the individuals within species. MA had the fewest correctly classified

273   individuals (Table. 3).

274   **Reproductive phenology and flower synchrony.**

275   Reproductive phenology differed between floral morphs. Flowering for MA was significant

276   in one direction ($r= 0.9566$, $p = 0.00$), covering four months (from March to June), being

277   unimodal, with peak flowering in April (Figure. 3a). On the other hand, the MN was

278   significant in one direction ($r= 0.9443$, $p =0.00$), covering five months (from February to

279   June), being unimodal and with peak flowering in May (Figure. 3b). The flowering pattern

280   of both floral morphs was different ($W= 43.686$, $p= 3.264e^{-10}$), with two months difference

281   in the flowering period of MN compared to MA, where MN has these months of difference,

282   another difference is observed in the peak of flowering, where MA is one month earlier

283   than MN.

284   Fruiting for MA was significant in one direction ($r= 0.9023$, $p= 0.00$), spanning five

285   months (June to October), being unimodal with peak fruiting in July (Figure. 3c). On the

286   other hand, in the MN, fruiting was significant in one direction ($r= 0.8749$, $p= 0.00$),

287   spanning seven months (from May to November), being unimodal, with peak fruiting in

288   June (Figure. 3d). The fruiting pattern of both floral morphs was different ($W= 206.5$, $p=$

289   $2.2e^{-16}$), with two months of difference in the period of fruiting of MN compared to MA,

290   where MN has these months of difference, another difference is observed in the peak of

291   fruiting, where the peak of flowering of MN is one month earlier than MA.

11

292   According to the Marquis (1998) index, in the *O. streptacantha* population, floral

293   synchrony in the flowering period is high for MA (S= 0.94, EE= 0.2498) and MN (S= 0.91,

294   EE= 0.2089). For the Mahoro (2002) index modified by Osada et al. (2003), floral

295   synchrony between MA and MN is high (s_i= 0.86).

296

297   **DISCUSSION**

298   The number of species that maintain morphological variations in cacti is remarkable

299   (Anderson 2001). Species varies in spines form, flower morphology, growth form, and

300   others. *Opuntia* is among the groups with important variation in morphology, flower color,

301   plant size (Bravo-Hollis and Sánchez-Mejorada 1978; Pimienta-Barrios and Muñoz-Urías

302   1995; Scheinvar 1995; Muñoz-Urias et al. 2008; López-Borja et al. 2017), which can be

303   observed in both wild and cultivated populations (Pimienta-Barrios et al. 1987). For this

304   genus, morphological characters are the main criterion for classification and separation of

305   species (Del Castillo 1999). However, only the characters of stems and plant habit are used.

306   In this study, it was found that the greatest weight of morphological variation in *O.*

307   *streptacantha* is associated with flower characteristics, both in the external part of the

308   flower and in the reproductive structures; at the same time, statistical analysis showed that

309   MA has the largest structures. A similar example can be observed in the genus *Ipomoea*

310   (Convolvulaceae), where the greatest morphological variation is found in focused on flower

311   structures, being in the species *Ipomoea purpurea* (Convolvulaceae) the most documented

312   species (Chemás-Jaramillo and Bullock 2002; Galetto et al. 2002; Carranza 2008; Rosas-

313   Guerrero et al. 2012).

314   Although cladodes and spines are the most striking morphological characteristics in

315   *Opuntia*, they showed few differences between MA and MN, which may be since both are

316   under similar environmental stresses or at similar risk of damage. Cactaceae species

317   inhabiting regions at risk of damage to apical meristems have been reported to develop a

318   dense cover of spines or a thick pubescence, or even both (Gibson and Nobel 1986). In

319   *Opuntia*, the development of a greater number of spines may be a natural response to

320   foraging by certain herbivores, and spines may be lost as the cladode age (López-Borja et

321   al. 2017).

322   MA fruits are longer and have larger seeds compared to MN; however, there is no

323   difference in the number of seeds in each fruit. Several studies showed that seed size can

324   vary within populations and within plants in the same species (Janzen, 1977; Cavers and

325   Steel 1984; Winn and Gross 1993; Sakai and Sakai 1996). For example, in the species

326   *Phaseolus lunatus* (Fabaceae) it was found that in different regions and in the same

327   population, there is a great variation in fruit and seed characters (Vargas et al. 2003).

328   Another factor to consider is the reproductive success of the species, since the type of

329   reproductive system of a flowering plant may condition in some way the production of

330   fruits and seeds, because many depend on the efficiency of pollination (Galetto et al. 2002).

331   Floral morphometry works in cacti are few, but it has been reported that there is variation in

332   the color of the flowers of some species such as in: *Lophophora diffusa* (Cactaceae)

333   (Briseño-Sánchez 2019), where white or pink flowers have been reported in individuals of

334   the population and in *Ariocarpus kotschoubeyanus* (Cactaceae) (Martínez-Peralta et al.

335   2014), where it ranges from white with darker tepal line, to magenta, existing intermediate

336   shades. In some studies of the genus *Ipomoea* showed a positive relationship between

337   flower size and floral visitors (Elle and Carney 2003), the reproductive system may

338   influence the morphological variation of individuals in the populations.

339   The use of multivariate methods helps to recognize taxonomic boundaries between a group

340   of closely related and morphologically similar taxa (López-Borja et al. 2017). In the PCA,

341   it was found that total flower length, number of stamens, distance between anther and

342   stigma, number of pollen grains, style height, equatorial diameter of the ovarian chamber,

343   pericarp width and number of areole lines were the characters with the greatest weight of

344   variation among floral morphs, with floral characters standing out above the others, which

345   emphasizes the importance of floral characteristics for the differentiation between the two

346   floral morphs.

347   In the linear discriminant analysis, *O. tomentosa* and *O. cantabrigiensis* were included,

348   since they are species that showed similarity with some of the floral morphs, for example:

349   the yellow flowers of *O. cantabrigiensis* and the orange flowers of *O. tomentosa* (personal

350   observation). The analysis completely separated *O. cantabrigiensis* from *O. tomentosa*, MA

351   and MN, suggesting that the latter three are morphologically grouped, giving the possibility

352   that there are some individuals with intermediate phenotypes between these species.

353   Linear discriminant analyses have been applied to determine the differentiation between

354   species, as in *Neobuxbaumia mezcalaensis* (Cactaceae) and *N. multiareolata* (Cactaceae)

355   where, using this type of analysis, it was possible to determine that they are independent

356   species, finding that, based on morphometric characters, they can be separated and

357   classified as different species and there is no evidence of the existence of a subspecies or

358   variety, since there were no intermediate phenotypes between the species (Arroyo-

359   Cosultchi et al. 2010). It is likely that the existence of MA and MN in *O. streptacantha* is

360   due to a gene exchange with the species that coexist in the same study site, being *O.*

361   *tomentosa* the one that morphologically and phenologically has the greatest number of

362   similarities with MA.

363   Scheinvar and Rodríguez-Fuentes (2003), proposed the subspecies *Opuntia streptacantha*

364   subsp. *aguirrana*, in the Mexican highlands, which is probably restricted to a micro-region

365   of this area, is a shrubby plant, with obovate cladodes, yellow flowers with some reddish

366   tints, obovoid to cylindrical or pyriform fruit, reddish pink, has an extremely late flowering

367   in autumn and begins to fruit in winter. The authors mention that this subspecies can be

368   recognized thanks to an active gene exchange among the other *Opuntia* species of the

369   region (*Opuntia streptacantha*, *O. hyptiacanhta*, *O. cochinera*, *O. leucotricha*, *O. robusta*,

370   *O. engelmannii*, *O. joconostle* and *O. rastrera*).

371   Morphological variation in *O. streptacantha*, could have implications for populations with

372   similar phenology (Rodríguez-Zapata 1981; López-Borja et al. 2017), due to the floral

373   visitors and pollinators shared by the species of the genus (García 1984). At the site where

374   both floral morphs of *O. streptacantha* are established, there are other species of cacti,

375   some of the genus *Opuntia*, *O. robusta*, *O. tomentosa* and *O. cantabrigiensis*. And the four

376   species coincide in some phase of the reproductive period (Cárdenas-Ramos 2019).

377   Pronounced morphological and ecological differentiation with low genetic differentiation is

378   commonly interpreted as evidence for divergent selection and adaptation to local habitats

379   (Helsen et al. 2009), for example, Hall and Willis (2006), show that there is divergent

380   selection on flowering time in two populations of *Mimulus guttatus* (Phrymaceae), which

381   contributes to local adaptation, suggesting that genetic differentiation may serve as a partial

382   reproductive isolation barrier to gene flow between populations. Nevertheless,

383   morphological, physiological, biochemical, genetic, and taxonomic research is needed to

384   increase the knowledge of the environmental effects on the diversity of the genus *Opuntia*

385   (Reyes-Agüero et al. 2005).

386   In general, the flowering peaks of MA and MN were unique, this agrees with the

387   information mentioned in several studies where it is mentioned that the cacti studied so far

388   have only one flowering peak (unimodal), although there are species that flower throughout

389   the year and with several flowering peaks (Mandujano et al. 2010). The two floral morphs

390   of *O. streptacantha* had their peak flowering in different month, this may be a strategy to

391   ensure the reproductive success of both or may be a response to the environment, factors

392   that can cause early or late flowering (Fenner 1998, Matías-Palafox et al. 2017).

393   The floral morphs of *O. streptacantha* showed very high synchrony indices, either within

394   the same morph (Marquis 1998) or between morphs (Osada et al. 2003). In both cases

395   flowering was short (from February to June), this is since in deserts or semi-deserts

396   reproductive events such as flowering and fruiting are usually limited by the availability of

397   water and the climatic conditions of these environments. Matías-Palafox et al. (2017)

398   mention that, under contrasting environments, species that present a single flowering period

399   strategy will present an increase in their reproductive success, compared to those that

400   present continuous flowering.

401   Simultaneous flowering between MA and MN can be advantageous for individuals and the

402   population. Rathcke and Lacey (1985) mention that simultaneous flowering between

403   different species can be advantageous, since the flowering of one species increases the

404   visitation rate of another species. Martínez-Ramos (2019) found for the same study site as

405   *O. tomentosa* and *O. streptacantha* (without a differentiation of morphs), that a high rate of

406   interspecific floral synchrony was present, adding that this could favor flow between these

407   species. Matías-Palafox et al. (2017) found that during peak flowering of *Astrophytum*

408   *ornatum* (Cactaceae) and *Turbinicarpus horripilus* (Cactaceae), which cohabit the same

409   area, both species share a synchrony in flowering, present a melittophilia pollination

410   syndrome and visitors in common, this could promote an interspecific competition or

411   promote a facilitation when there is a shortage of pollinators.

412   One of several reasons for maintaining flower color dimorphism is the characteristics of

413   reproductive phenophases, which involve temporal patterns of resources that will be

414   available to pollinators and seed dispersers (Elzinga et al. 2001), and in some cases

415   competition for pollinators has influenced the phenology of some wild species (Waser

416   1979; Pleasants 1980). Populations of a plant species may be subject to variations in the

417   abundance and composition of the set of floral visitors with which it interacts (Valverde et

418   al. 2014). Variation can be in space, in time (Petanidou et al. 2008), in intrinsic

419   characteristics of the plant (population size, phenotype, aggregation, etc.) or extrinsic to it

420   (local abundance of pollinators, temporal dynamics of pollinators, companion plants, etc.)

421   (Herrera 1995, Valverde et al. 2014).

422   In conclusion, the variations present in the floral morphs of *Opuntia streptacantha* extend

423   beyond flower color. The structure in which most of the morphological variation is found is

424   the flower, but these differences are not only morphological, but also ecological and floral

425   phenology. Therefore, to determine whether these floral morphs are already differentiated

426   into another taxonomic category, further genetic (e.g., chromosome number counts) and

427   ecological (e.g., floral biology) work would be necessary.

428

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714

715 Table 1. Mean and standard error ($\pm$) of cladode characteristics of both floral morphs of
716 *Opuntia streptacantha* in Cadereyta de Montes, Querétaro, Mexico. a) *t-test* y b)
717 Generalized linear model with *Poisson* distribution. Contrasts are marked with * ($p < 0.05$),
718 no difference = n.s. *n*= 20 young cladodes for each floral morph and *n*= 20 old cladodes for
719 each floral morph.

a) Cladode characteristic	Cladode age	Mean ± Standard Error of yellow floral morph	Mean ± Standard Error of orange floral morph	t	p
Length (cm)	Young	18.71 ± 0.56	17.79 ± 0.59	0.99	0.33 [n.s.]
	Old	30.15 ± 0.93	33.22 ± 0.92	-2.62	**0.01***
Width (cm)	Young	13.38 ± 0.01	13.38 ± 0.02	1	0.32
	Old	13.37 ± 0.01	14.44 ± 0.47	-2.23	**0.03***
Areole size (mm)	Young	2.26 ± 0.08	2.31 ± 0.07	-0.46	0.65 [n.s.]
	Old	3.29 ± 0.15	3.21 ± 0.15	0.28	0.77 [n.s.]
Distance from the widest part to the apex (cm)	Young	10.27 ± 0.32	9.57 ± 0.38	1.26	0.22 [n.s.]
	Old	17.32 ± 0.56	18.44 ± 0.48	-1.64	0.11 [n.s.]
Distance from the widest part to the base (cm)	Young	11.09 ± 0.30	10.42 ± 0.37	1.31	0.20 [n.s.]
	Old	16.87 ± 0.51	18.24 ± 0.50	-1.99	0.06 [n.s.]
Distance between areoles (cm)	Young	17.92 ± 0.60	18.07 ± 0.54	0.16	0.87 [n.s.]
	Old	29.09 ± 1.27	30.04 ± 1.26	-0.65	0.52 [n.s.]
Distance between lines of areoles (cm)	Young	18.74 ± 0.63	18.95 ± 0.64	0.22	0.82 [n.s.]
	Old	32.86 ± 0.95	33.70 ± 1.23	-0.54	0.59 [n.s.]
b) Cladode characteristic	Cladode age	Mean ± Standard Error of yellow floral morph	Mean ± Standard Error of orange floral morph	x^2	p
Number of series of areoles	Young	8.05 ± 0.29	8.20 ± 0.28	0.02	0.86 [n.s.]
	Old	8.35 ± 0.25	9.10 ± 0.26	0.64	0.42 [n.s.]

720

721 Table 2. Mean and standard error (±) of floral characteristics of both floral morphs of
722 *Opuntia streptacantha* in Cadereyta de Montes, Querétaro, Mexico. a), c) and e): *t-test* y b)
723 and d): Generalized linear model with *Poisson* distribution. Contrasts are marked with * (*p*
724 < 0.05), no difference = n.s. *n*= 33 orange floral morph and *n*= 33 yellow floral morph.

a) Floral characteristic	Mean ± Standard Error of yellow floral morph	Mean ± Standard Error of orange floral morph	t	p
Corolla aperture set in FAA (mm)	27.87 ± 1.57	21.95 ± 1.01	3.16	**<0.05***
Perianth segment length (mm)	27.05 ± 0.93	22.50 ± 0.55	4.21	**<0.05***
Total flower length (mm)	58.81 ± 2.03	50.03 ± 1.19	3.73	**0.0004***
Pericarp height (mm)	36.43 ± 1.13	29.32 ± 0.76	5.20	**<0.05***
Pericarp width (mm)	20.87± 0.41	24.21 ± 0.17	-7.45	**<0.05***
Style height (mm)	19.99 ± 0.59	17.37 ± 0.25	4.10	**0.0001***
Stigma length (mm)	5.09 ± 0.14	5.09 ± 0.12	-0.01	0.98[n.s.]
Stigma width (mm)	5.58 ± 0.16	5.17 ± 0.14	1.90	0.06[n.s.]
Equatorial diameter of the ovarian chamber (mm)	4.84 ± 0.14	5.48 ± 0.18	-2.73	**<0.05***
Polar diameter of the ovarian chamber (mm)	9.83 ± 0.37	6.40 ± 0.39	6.32	**<0.05***
Longest stamen height (mm)	14.66 ± 0.49	11.54 ± 0.16	6.02	**<0.05***
Shortest stamen height (mm)	8.59 ± 0.44	6.39 ± 0.23	4.38	**<0.05***
Distance between anther and stigma (mm)	7.88 ± 0.41	5.86 ± 0.28	4.06	**<0.05***
b) Floral characteristic	Mean ± Standard Error of yellow floral morph	Mean ± Standard Error of orange floral morph	x^2	p
Number of lobes	8.84 ± 0.31	7.7272 ± 0.15	2.50	0.11[n.s.]
Number of stamens	469.03 ± 20.10	523.6969 ± 10.15	99.39	**<0.05***

|				
Number of pollen grains in an anther	215.57 ± 8.94	247.3333 ± 9.27	71.95	**<0.05***
Number of ovules	118.06 ± 7.36	97.666 ± 4.73	63.72	**<0.05***
c) Fruit characteristic	**Mean ± Standard Error of yellow floral morph**	**Mean ± Standard Error of orange floral morph**	***t***	***p***
Length (cm)	51.28 ± 1.59	43.92 ± 0.84	4.62	**<0.05***
Width (cm)	37.11 ± 0.95	37.50 ± 0.85	-0.38	$0.71^{n.s.}$
d) Fruit characteristic	**Mean ± Standard Error of yellow floral morph**	**Mean ± Standard Error of orange floral morph**	***x^2***	***p***
Number of spiral series	7.85 ± 0.11	8.15 ± 0.18	0.11	0.73
Number of seeds	97.05 ± 7.57	07.65 ± 5.61	0.04	0.85
e) Seed characteristic	**Mean ± Standard Error of yellow floral morph**	**Mean ± Standard Error of orange floral morph**	***t***	***p***
Length (cm)	5.10 ± 0.04	4.55 ± 0.04	9.71	**<0.05***
Width (cm)	4.53 ± 0.20	3.67 ± 0.04	4.11	**<0.05***

725

726   Table 3. Classification of the individuals based on floral morphometrics measurements

727   using the linear discriminant analysis.

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

728

729   Figure. 1. Floral morphs of *Opuntia streptacantha*. a) Yellow floral morph. b) Orange floral

730   morph. Scale 1 cm. Photos: Gerardo Manzanarez-Villasana.
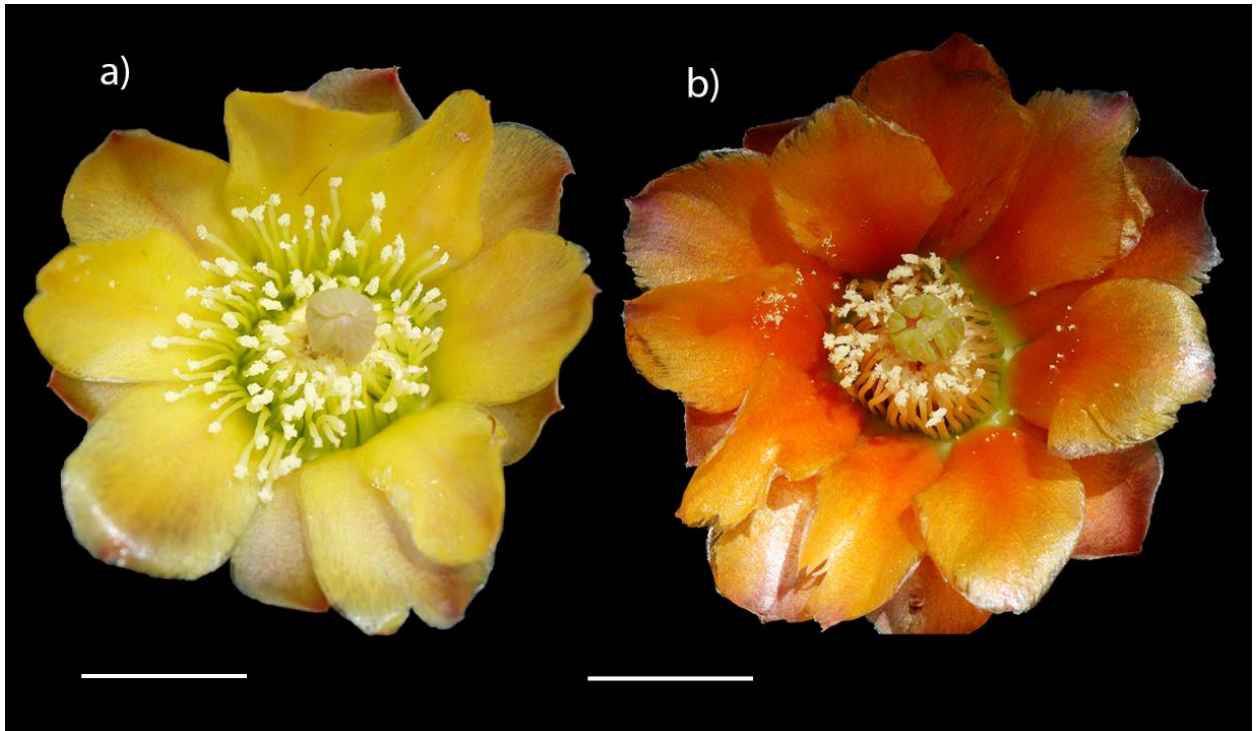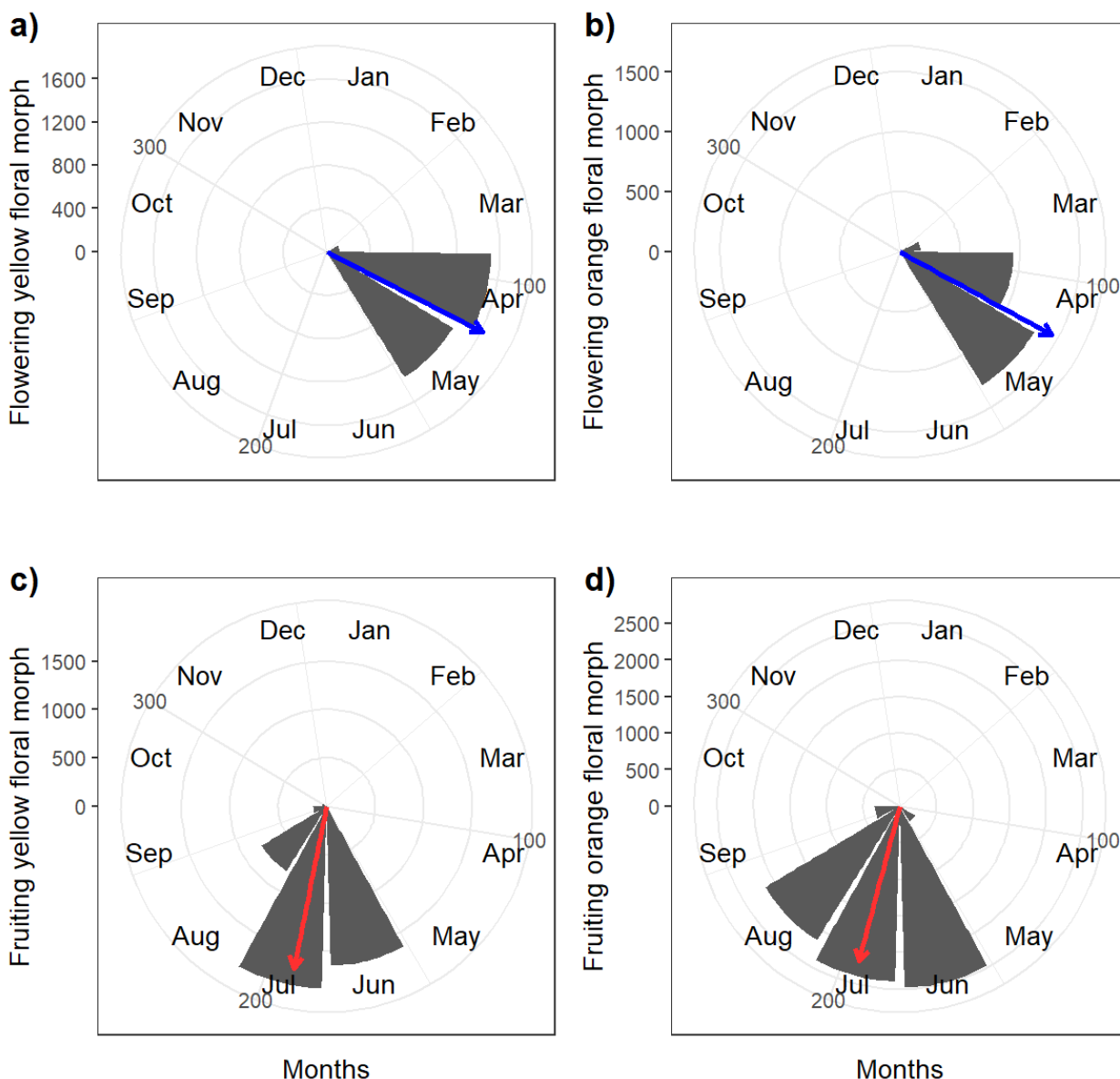

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732

733   Figure. 2. Linear discriminant analysis using floral morphometrics of three *Opuntia* species

734   in Cadereyta de Montes, Queretaro, Mexico.

735
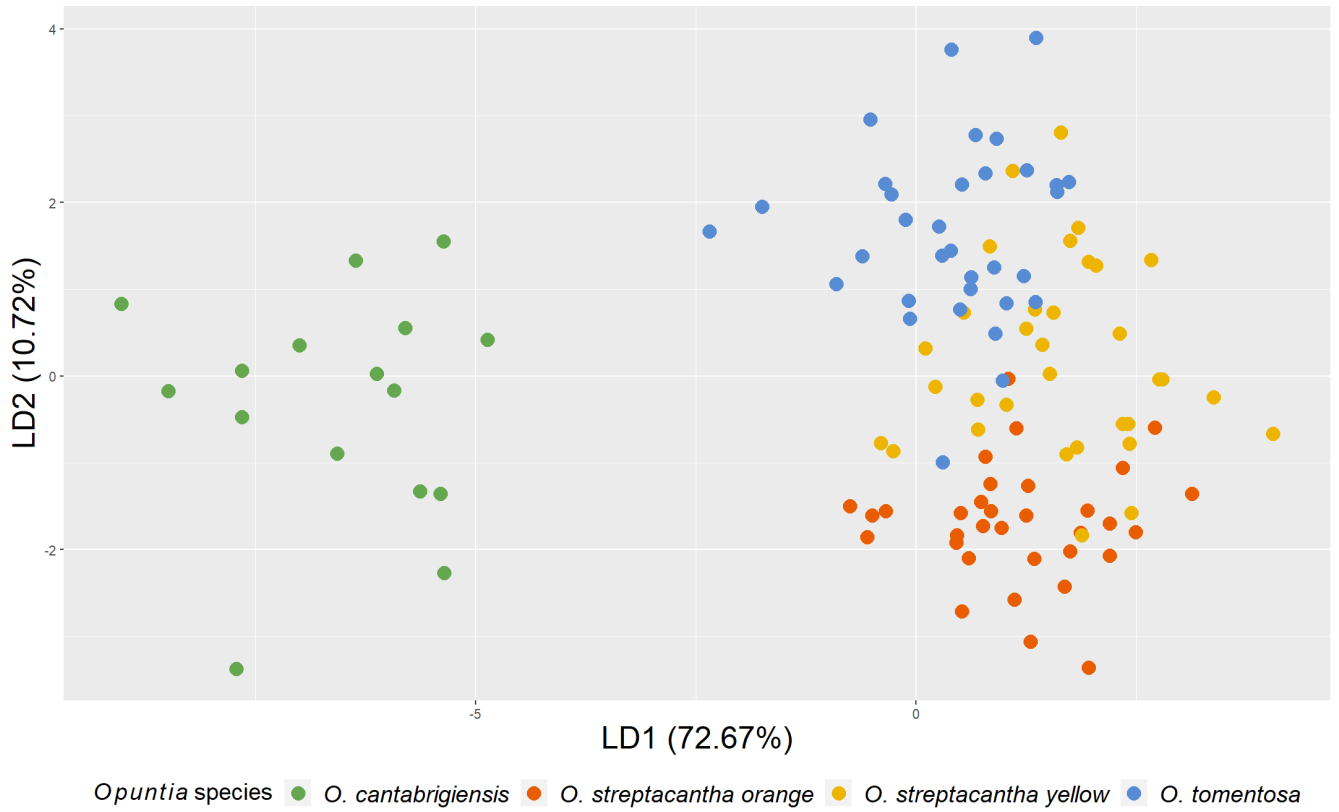
736

737   Figure. 3. Rose diagram representing the months and phenology of floral morphs of

738   *Opuntia streptacantha* in Cadereyta de Montes, Queretaro, Mexico. a) Flowering for the

739   yellow floral morph. b) Fructification for the yellow floral morph. c) Flowering for the

740   orange floral morph. d) Fructification for the orange floral morph. The blue arrow indicates

741   the accumulation of data for flowering based on the *Rayleigh* uniformity test. The red arrow

742   indicates the accumulation of data for fruiting based on the *Rayleigh* uniformity test.

743