

Flower, fruit phenology and flower traits in *Cordia boissieri* (Boraginaceae) from northeastern Mexico.

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We characterized variations in *Cordia boissieri* flowers and established if these variations occur in the same or between different blooming events. We quantified for 256 plants: highly flowering individuals (>50% flower or fruit cover), plants with fruits and plants without fruits and flowers. Flower and fruit presence were tested for a correlation with rainfall. We performed measurements of floral traits to detect variations within the population and between blooms. We determined the floral integration index. The position of the anthers with respect to the ovary was determined in 1,500 flowers. The synchronous blooms of *C. boissieri* occurred after rainfall events. Only one flowering event occurred in a drought. Most plants flowered at least twice a year. The overlapping of flowering and fruiting only occurred after rainfall. Anthesis lasted three to five days, and there were two flower morphs. Half of the plants had superior and half had inferior ovaries. Flowers had 1-4 styles; 2-9 stamens; 6.5-41.5 mm long corolla; sepals from 4.5-29.5 mm in length; a total length from 15.5-59 mm; a corolla diameter from 10.5-77 mm. The nectar guide had a diameter from 5-30.5 mm; 4-9 lobes; and 5 distinguishable nectar guide colors. The highest variation of phenotypic expression was observed between plants. We found a low floral trait integration that implies generalist flowers. *Cordia boissieri* is a new report of heterostyly in Boraginaceae and has a flowering pattern associated with rainfall that can occur up to three times a year.

1 **Flower, fruit phenology and flower traits in *Cordia boissieri* (Boraginaceae) from**
2 **northeastern Mexico**

3

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17 Short title: Floral traits in *Cordia boissieri*

19 **ABSTRACT**

20 We characterized variations in *Cordia boissieri* flowers and established if these variations occur
21 in the same or between different blooming events. We quantified for 256 plants: highly
22 flowering individuals (>50% flower or fruit cover), plants with fruits and plants without fruits
23 and flowers. Flower and fruit presence were tested for a correlation with rainfall. We performed
24 measurements of floral traits to detect variations within the population and between blooms. We
25 determined the floral integration index. The position of the anthers with respect to the ovary was
26 determined in 1,500 flowers. The synchronous blooms of *C. boissieri* occurred after rainfall
27 events. Only one flowering event occurred in a drought. Most plants flowered at least twice a
28 year. The overlapping of flowering and fruiting only occurred after rainfall. Anthesis lasted three
29 to five days, and there were two flower morphs. Half of the plants had superior and half had
30 inferior ovaries. Flowers had 1-4 styles; 2-9 stamens; 6.5-41.5 mm long corolla; sepals from 4.5-
31 29.5 mm in length; a total length from 15.5-59 mm; a corolla diameter from 10.5-77 mm. The
32 nectar guide had a diameter from 5-30.5 mm; 4-9 lobes; and 5 distinguishable nectar guide
33 colors. The highest variation of phenotypic expression was observed between plants. We found a
34 low floral trait integration that implies generalist flowers. *Cordia boissieri* is a new report of
35 heterostyly in Boraginaceae and has a flowering pattern associated with rainfall that can occur up
36 to three times a year.

37

38 **Keyword:** Anacahuita; floral dimorphism; heterostyly; Tamaulipan thornscrub.

40 INTRODUCTION

41 Flower timing is closely related to (i) environmental factors such as temperature, day length,
42 moisture (*Justiniano & Fredericksen, 2000; Yadav and Yadav, 2008; Hódar, Obeso & Zamora,*
43 *2009; Silveira et al. 2013*), soil (*Borchert, 1983*), and (ii) availability of pollen vectors (*Trapnell*
44 *& Hamrick, 2006; Stern et al., 2008; Medel & Nattero, 2009*). In plants pollinated by animals,
45 floral traits are selected to ensure sexual reproduction, because cross-fertilization increases
46 genetic variability which is advantageous to offspring (*Charlesworth & Charlesworth, 1987*).
47 Many traits are involved in attracting pollen vectors, such as flower size, shape, color and scent
48 (*Hargreaves, Harder & Johnson, 2009; Rosas–Guerrero et al., 2011*). Plants pollinated by
49 animals commonly show plastic traits, because pollinator composition and abundance vary
50 within and between reproductive seasons (*Harder & Johnson, 2005*). If floral visitors favor a
51 floral trait within a population, that could lead to a divergence in floral phenotype (*Sánchez–*
52 *Lafuente, 2002; Huang & Fenster, 2007; Brothers & Atwell, 2014*).

53 Species with floral morphology associated with specialized pollination systems
54 (zygomorphic flowers) have less variation within populations than those species with attributes
55 associated with generalized pollination systems (actinomorphic flowers in *Stebbins, 1970;*
56 *Ushimaru et al., 2007; van Kleunen et al., 2008; Rosas–Guerrero et al., 2011*). Studies in
57 phenotype selection have been mainly focused on evaluating the effect of floral attributes on
58 plant fitness; however, each flower is a complex unit with an accurate configuration for an
59 appropriate function (*Ushimaru et al., 2007; Armbruster & Muchhala, 2009; Rosas–Guerrero et*
60 *al., 2011*). This configuration can produce a diversification of flower traits with pollinators as
61 often underestimated selective agents (*Dilcher, 2000; Ushimaru et al., 2007; Rosas–Guerrero et*
62 *al. 2011*). One of these diversifications is heterostyly, a floral polymorphism in style and stamen

63 length (*Gasparino & Barros, 2009*), generally associated with an incompatibility system that
64 prevents self-fertilization. The phenomenon occurs in the same species, in two (distyly) or three
65 forms (tristyly) (*Darwin, 1877; Faivre & McDade, 2001; Gasparino & Barros, 2009*).
66 Heterostyly occurs in many families including Boraginaceae, and the dimorphism in style length
67 (*Opler et al., 1975*) was documented in Boraginaceae (*i.e. Cordia*) by Fritz Muller, when he sent
68 samples from Brazil to Charles Darwin (*Darwin, 1877*). Heterostyly has evolved at least 12
69 times in Boraginaceae (the largest number of origins in any family) (*Cohen, 2014*) and is present
70 in at least nine genera (*Naiki, 2012*). The descriptions of flower morphs we found for the species
71 of *Cordia* describe heterostyly for at least 9 of the 250 or more species (*Opler et al., 1975;*
72 *McMullen, 2012; Naiki, 2012; Canché-Collí & Canto, 2014*).

73 In *Cordia*, blooming seasonality tends to vary in relation to moisture availability:
74 northern *Cordia* species begin blooms in regions with severe drought, earlier than those from
75 areas with less water stress (*Borchert, 1996*). Anacahuita (*Cordia boissieri* A. DC.) is the state
76 flower of Nuevo Leon (a northeastern Mexican state), and its flowering has not been studied.
77 Research on other *Cordia* species (*Opler et al., 1975; de Stapf et al., 2013*) found a wide range
78 of reproductive systems, ranging from the homostylous to heterostylous and dioecious, including
79 those adapted for pollination assemblages, with both wind and animals as pollen vectors. *Cordia*
80 *alliodora* and *C. elaeagnoides*, for instance, bloom at the end of the rainy season; however *C.*
81 *alliodora* blooms were found to occur later and to last longer (*Bullock & Solis–Magallanes,*
82 *1990*). The blooms of *C. glabra* occur from August to September in a monoecious reproductive
83 system (*Justiniano & Fredericksen, 2000*). In contrast *C. multispicata* produces flowers and
84 fruits during most of the year, with peak flowering between the end of the dry season and half

85 way into the wet season (*Vieira & Da Silva, 1997*). The anthesis of this species lasts up to six
86 days.

87 Based on this background, we characterized the phenological variation in Anacahuita
88 flowers and established if this variation occurs in the same blooming process or between
89 different processes, considering the following questions: Is there a relationship between
90 flowering and fruiting with rainfall events? Is there a variation in floral traits in different
91 flowering events? Is there a variation of Anacahuita floral traits within a population? Based on
92 its flower traits, what is the floral integration index of Anacahuita? Is there heterostyly in
93 Anacahuita?

94

95 **MATERIALS AND METHODS**

96 **Study area**

97 The study was performed from October 2009 to September 2011. We worked with 75 plants
98 from a population of 256 in a Tamaulipan thornscrub fragment inside the experimental area of
99 Facultad de Ciencias Forestales (Universidad Autónoma de Nuevo León, 24° 46' 43"N 99° 31'
100 39" W) at an elevation of 370 m above sea level. The area experiences an average temperature of
101 21°C, with a maximum extreme temperature in summer and with > 40 °C and <0 °C in winter.
102 The annual rainfall average is 805 mm, and dominant soils in the area are vertisols of alluvial-
103 colluvial origin (*SPP-INEGI, 1986; Alanís-Rodríguez et al., 2008*).

104

105 **Species description**

106 Anacahuita is a native North American shrub or small tree, 5 to 8 m tall. It has simple, alternate
107 and ovate leaves from 15-20 cm in length, with a pilose-velvety surface. The flowers are trumpet

108 shaped, in groups from five to eight, white with a yellow nectar guide, up to 45 mm in length,
109 with five rounded lobes and five stamens joined at the base within the corolla tube. In addition,
110 the anthers are oblong, filiform, glabrous, and yellow-greenish; the pistil usually varies in length
111 and narrows towards the apex, ending with two stigmas. Flowering occurs throughout the year,
112 with peaks in the rainy season from late spring to early summer (*Vines, 1986; Gilman & Watson,*
113 *1993; Alvarado et al., 2004*). This species showed extensive damage, i.e., 10 cm - 40 cm below
114 the apex, after a severe frost, but it recovered because of the presence of undamaged cambium
115 and phloem tissues below the apex (*Lonard & Judd, 1985*).

116 Prospective visits were made in the studied population in order to identify and mark all
117 individuals > 1.5 m in height. Each individual was marked with a metallic label and flagging tape
118 with progressive numbers. Within this population, five plants were randomly selected to follow
119 the life of 10 flowers in each one, from bud opening to flower senescence, in order to determine
120 flower life span and avoid duplicating data during subsequent samplings.

121

122 **Blooms and rainfall**

123 We recorded the blooms of individuals within the population and the monthly amount of rainfall
124 (mm) on campus. For each synchronous bloom event we quantified highly flowering plants (as
125 seen below), plants with fruit deposition, and plants without fruits and flowers. This
126 identification was carried out through direct observation of each one of the 256 plants. Only
127 plants with abundant (i.e $>50\%$) flower or fruit cover were considered. To determine
128 relationships between rainfall and the number of flowering and fruiting plants, we performed a
129 linear regression analysis using STATS package for R software (R Core Team 2014). We used
130 the GGLOT2 package for graphics (*Wickham, 2009*).

131

132 **Flower measurements**

133 To determine phenotypic variation of flowers, we selected five flowers at random in each of the
134 four cardinal points, obtaining data from 20 flowers for each plant during each bloom. The floral
135 attributes evaluated were as follows: style type (ST); style number (SN); stamens and anthers
136 number (SAN); total length (TL; mm with a 0.05 mm accuracy); corolla length (CL; mm);
137 corolla diameter (CD; mm); nectar guide diameter (NGD; mm); number of lobes (NL); nectar
138 guide color (NGC; by direct observation of changes in patterns and color tones in the flower
139 tube) and sepal length (SL; mm), obtained through the difference of the total and corolla lengths.
140 Style type was determined in 1,500 flowers from 75 plants across flowering events.

141 Recorded data were tested for normal distribution by using the Kolmogorov–Smirnov
142 test. We performed nested ANOVAs to test for differences in floral traits among the population
143 and between different blooms ($P = 0.05$) for most quantitative variables. Kruskal-Wallis tests
144 were performed for categorical and not normally distributed variables (Zar, 2010). With this
145 analysis, we determined if flower attributes differed within the population and between different
146 rainfall events. In addition, we ran a binomial test and goodness of fit using χ^2 tests (Ríus-Díaz et
147 al., 1998) for style types and nectar guide colors to determine if there were variations in the
148 different phenotypic expressions of the studied plants.

149 We tested for a potential correlation between floral traits using Spearman's rank
150 correlation in STATS package for R (R Core Team 2014). We performed a principal component
151 analysis (PCA) by using ADE4 package for R (Dray, Dufour & Chessel, 2007), and we
152 calculated the variance of the eigenvalues extracted from PCA, in order to know the phenotypic

153 integration of flower traits (*Wagner, 1984; Cheverud, Wagner & Dow, 1989; Rosas-Guerrero et*
154 *al., 2011*).

155

156 **RESULTS**

157 **Blooms and rainfall events**

158 In this study, we collected data from 75 plants; 28 of them had at least three blooms, eight had
159 two blooms, and 39 had only one. We recorded that flowering months followed one or two
160 months of high rainfall, except in April of 2011, where we recorded a significant bloom during a
161 dry spell. *C. boissieri* blooms generally resulted after rainfall events (Fig. 1); regression analyses
162 showed a significant relationship between rainfall events and the number of plants with flowers
163 ($F_{1,22} = 8.2797$; $P = 0.008$) and fruits ($F_{1,22} = 12.9346$; $P = 0.001$). We recorded nine blooming
164 events, of which five were blooms of almost every marked individual (synchronous blooms) and
165 longer lasting. Four blooming events occurred after rainfall events. Within these nine events, we
166 observed an overlapping of blooming and fruit setting processes. In the bloom without a previous
167 rainfall, however, the flower and fruit set were observed as separate processes. The documented
168 blooms occurred as follows: one in 2009 (October), five in 2010 (March, April, June, August and
169 October) and three in 2011 (March, April and July).

170

171 **Flower trait variation**

172 We determined that anthesis (opening of floral bud to senescence) of *C. boissieri* flowers lasted
173 three to five days. We observed heterostyly on *C. boissieri* flowers, with two style types (Fig. 2):
174 an inferior style with 760 flowers (from 38 plants) and superior style with 740. The style type did
175 not vary within individuals; however, the Kruskal-Wallis test showed that there was a significant

176 variation between plants in the population ($H_{1,74} = 1499; P < 0.001$). In addition, the binomial
177 test for style types showed similar numbers of inferior style (51 %) and superior style (49%; $P =$
178 0.624). Style type and nectar guide color (NGC) did not vary within plant or within the
179 population, so for these attributes the Kruskal-Wallis test between blooms was not performed.

180 We observed 2,267 *C. boissieri* flowers and found flowers from one to four styles ($\bar{x} =$
181 $2.02 \pm 0.135 SD$), with two to nine stamens ($\bar{x} = 5 \pm 0.381 SD$) and two to nine lobes ($\bar{x} = 5.01$
182 $\pm 0.381 SD$, as seen in Fig. 3). The phenotypic expression most commonly observed was two
183 styles, five stamens and five lobes. We found five nectar guide colors (Fig. 4), in the 1,500
184 flowers from 75 plants. There were 760 flowers with yellow, 380 with yellow-orange, 260 with
185 yellow-white, 60 with yellow-white-orange and 40 with an orange-yellow nectar guide. The $\chi^2 =$
186 test for nectar guide color in flowers showed a significant variation for this attribute ($\chi^2 =$
187 1149.333; $df = 4; P < 0.001$), whereas the Kruskal-Wallis test showed a variation from plant to
188 plant ($H_{1,74} = 1499; P < 0.001$).

189 *C. boissieri* flowers ranged in length from 15.45 mm to 58.9 mm ($\bar{x} = 35.49 \pm 5.78 SD$),
190 corolla length varied from 6.45 mm to 41.1 mm ($\bar{x} = 24.08 \pm 5.26 SD$), and sepal length from
191 4.45 mm to 29.5 mm ($\bar{x} = 11.4 \pm 2.57 SD$). Corolla diameter varied from 16.3 mm to 77.0 mm (
192 $\bar{x} = 47.98 \pm 9.33 SD$), whereas nectar guide diameter ranged from 4.9 to 30.6 mm ($\bar{x} = 11.64 \pm$
193 $2.22 SD$). Nested ANOVAs for each floral attribute showed that most variations occurred
194 between plants. Total flower length ($F_{2,81} = 18.217, P < 0.001$), corolla length ($F_{2,81} = 14.532, P$
195 < 0.001), corolla diameter ($F_{2,81} = 47.316, P < 0.001$) and nectar guide diameter ($F_{2,81} = 42.335,$
196 $P < 0.001$) differed between plants but not between blooms. Sepal length varied both between
197 plants ($F_{2,81} = 5.697, P < 0.001$) and between blooms ($F_{81,1596} = 3.156, P = 0.048$).

198 There were significant variations between plants within blooms for style type ($H_{2,27} =$
199 1679.000, $P < 0.001$), style number ($H_{2,27} = 112.868$, $P < 0.001$), number of anthers ($H_{2,27} =$
200 126.521, $P < 0.001$), number of lobes ($H_{2,27} = 143.071$, $P < 0.001$) and color of nectar guide
201 ($H_{2,27} = 1679.000$, $P < 0.001$). The number of lobes also differed between blooms ($H_{2,27} =$
202 15.618, $P < 0.001$).

203

204 **Floral trait integration**

205 All floral traits measured, with the exception of style number, were inter-correlated (Table 1):
206 total length with corolla length ($\rho = 0.883$, $P < 0.001$); number of stamens with number of
207 lobes ($\rho = 0.677$, $P < 0.001$); corolla diameter with nectar guide diameter ($\rho = 0.573$, $P <$
208 0.001); and total length with corolla diameter ($\rho = 0.429$, $P < 0.001$). The floral trait
209 integration index of *C. boissieri* was 6.11%.

210

211 **DISCUSSION**

212 **Blooms and rainfall events**

213 Variation in floral phenology (including opening time and number of flowers) commonly
214 correlates with the following: temperature, moisture, and day length (*Justiniano & Fredericksen,*
215 *2000; Yadav & Yadav, 2008; Hódar, Obeso & Zamora, 2009*); edaphic factors and biotic factors
216 (Borchert, 1983) like pollinators (*Trapnell and Hamrick, 2006; Medel and Nattero, 2009*); and
217 seed dispersers and herbivores (*Mahoro, 2002; Lacey et al., 2003*). *C. boissieri* is described in
218 the literature as producing flowers and fruits all year, with two peaks of blooms in late spring and
219 early summer (*Vines, 1986; Gilman & Watson, 1993; Alvarado et al. 2004*); this may be a result
220 of a bimodal rainfall pattern (*Chapman et al., 1999*). The literature does not state if the same

221 plants produce flowers more than once a year, which we found for almost half of our studied
222 plants.

223 In our study, we observed that flowering was more abundant after rainfall, but it also
224 occurred in a dry spell. These results coincide with findings of the highest blooming events in
225 plants of an arid tropical community during the dry and warm season (*León de la Luz et al.*,
226 *1996*). In our study when there was a bloom after rainfall, the flowering and fruit set processes
227 overlapped; this maybe as a result of sufficient water to maintain both the flowering and the fruit
228 set processes. In contrast, there was no overlap of flower production and fruit set in the blooming
229 that occurred during a dry spell. *Wolfe & Burns (2001)* state that the common pattern of
230 angiosperms is of one annual production of flowers. For some plants, flowering occurs at periods
231 interrupted by non-reproductive periods, and only some species produce flowers and fruits all
232 year in almost all individuals. In the present study, many individuals of *C. boissieri* produced
233 flowers and fruits more than once per year. We recorded five synchronous blooms (in 24
234 months) in which almost all individuals produced flowers. This is consistent with findings of
235 species flowering in late spring and early summer (*Vines, 1986; Gilman & Watson, 1993;*
236 *Alvarado et al., 2004*). This flowering behavior could be caused by an overlap of blooming time
237 and activity of floral visitors, as well as significant rainfall events. The variation in time of year
238 for blooming indicates that unlike other studies (*Borcher et al., 2005*) available moisture is more
239 important than photoperiod variations in triggering flowering.

240

241 **Variation of floral phenology within the population**

242 This study showed morphological variations of flowers and the phenology of *C. boissieri*.

243 Anacahuita flowers had a lifespan of five days, and the time to anthesis of each species likely

244 varies with pollination systems; for example there are plants that have flowers that are receptive+
245 for only a few hours (*Armbruster & Muchhala, 2009*) to more than two weeks (*Steinacher &*
246 *Wagner, 2010*). *C. boissieri* flowers showed a heterostyly pattern, belonging to the group of
247 distyly flowers, coinciding with previous findings for *Cordia* (*Gibbs & Taroda, 1983;*
248 *McMullen, 2012*). Boraginaceae is within the 24 families that exhibit heterostyly, with the
249 *Cordia* species displaying distyly and tristyly (*Ganders, 1979*).

250 In our study we found that most of the variation of floral attributes measured was
251 between plants and not between blooms or within a plant, except for color (C) and style type
252 (TE) which did not vary between blooms or within a plant. These findings suggest that *C.*
253 *boissieri* has its highest variation between plants. While there was a minor variation of floral
254 attributes in *C. boissieri* between blooms, most floral phenotype variation occurred between
255 plants.

256 The observed flowers in this study allowed us to find some differences from the flower
257 description for the species. For example, in terms of styles, the general morphotype or most
258 dominant is two styles per flower (*Vines, 1986; Gilman & Watson, 1993; Alvarado et al., 2004*),
259 and we found flowers with one to four styles. Anacahuita flowers are described as having five
260 stamens and five lobes with a length of 45 mm, with flowers with a yellow nectar guide (*Vines,*
261 *1986; Gilman & Watson, 1993; Alvarado et al., 2004*); we found a flower length from 15.45 mm
262 to 58.9 mm ($\bar{x} = 35.49 \pm 5.78 SD$) and five phenotypic expressions of nectar guide color,
263 including the yellow variation cited in the literature. Other flower variables were not found in the
264 literature.

265

266 **Floral trait integration**

267 *van Kleunen (2008)* found that within a plant community zygomorphic plant species had less
268 significant variation in flower length and width than the actinomorphic species. In addition, he
269 observed two species of Boraginaceae with different types of symmetry: *Echium vulgare* as a
270 zygomorphic species and *Myostis sylvatica* as an actinomorphic species. *C. boissieri* flowers
271 belong to radially symmetrical flowers or are actinomorphic, which to several authors (*van*
272 *Kleunen, 2008; Rosas–Guerrero et al., 2011*) could be an indication of a generalist pollination
273 system, in which the plant is not primarily shaped by pollinators (*Gómez et al., 2014*). Another
274 reason to assume *C. boissieri* is a generalist is the integration degree of its traits, as suggested by
275 *Rosas–Guerrero et al. (2011)*: species with generalist pollination systems have a very low
276 integration degree of total morphology of the flower with a high covariance, as was the case in
277 our study.

278 In conclusion, the synchronous blooms of *C. boissieri* occurred during both study years
279 after heavy rainfall events, and flowering also occurred in one dry season. The overlapping of
280 flowering and fruiting events was only present after heavy rainfall. The highest variation of
281 phenotypic expression of *C. boissieri* occurred between plants and less between blooms. The
282 floral trait integration and morphology of *C. boissieri* flowers suggest that the species has a low
283 integrated flower. This could mean that the species has generalist flowers. Furthermore, *C.*
284 *boissieri* presents heterostyly.

285

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291

292 REFERENCES

293 **Alanís-Rodríguez E, Jiménez-Pérez J, Aguirre-Calderón O, Treviño-Garza E, Jurado-**
294 **Ybarra E, González-Tagle M. 2008.** Efecto del uso del suelo en la fitodiversidad del
295 matorral espinoso tamaulipeco. *Ciencia UANL* **11**:56–62. URL:
296 <http://eprints.uanl.mx/id/eprint/1827>.

297 **Alvarado M, Foroughbakhch R, Jurado E, Rocha A. 2004.** Caracterización morfológica y
298 nutricional del fruto de anacahuita (*Cordia boissieri* A. DC.) en dos localidades del
299 Noreste de México. *Phyton* **53**:85–90. URL:
300 <http://www.scielo.org.ar/pdf/phyton/v73/v73a09.pdf>.

301 **Armbruster WS, Muchhala N. 2009.** Associations between floral specialization and species
302 diversity: Cause, effect, or correlation? *Evolutionary Ecology* **23**:159–179. DOI:
303 [10.1007/s10682-008-9259-z](https://doi.org/10.1007/s10682-008-9259-z).

304 **Borchert R. 1983.** Phenology and control of flowering in tropical trees. *Biotropica* **15**:81–89.
305 URL: <http://www.jstor.org/stable/2387949>.

306 **Borchert R. 1996.** Phenology and flowering periodicity of neotropical dry forest species:
307 Evidence from herbarium collections. *Journal of Tropical Ecology* **112**:65–80. DOI:
308 <http://dx.doi.org/10.1017/S0266467400009317>.

309 **Borchert R, Renner SS, Calle Z, Navarrete D, Tye A, Gautier L, Spichiger R, Hildebrand**
310 **P. 2005.** Photoperiodic induction of synchronous flowering near the Equator. *Nature*
311 **433**:627–629. DOI: [10.1038/nature03259](https://doi.org/10.1038/nature03259).

- 312 **Brothers AN, Atwell JW. 2014.** The role of pollinator-mediated selection in the divergence of
313 floral traits between two closely related plant species. *International Journal of Plant*
314 *Science* **175**:287–295. DOI: 10.1086/673883.
- 315 **Bullock SH, Solis–Magallanes JA. 1990.** Phenology of canopy trees of a tropical deciduous
316 forest in México. *Biotropica* **22**:22–35. DOI: 10.2307/2388716.
- 317 **Canché-Collí C, Canto A. 2014.** Distylous traits in *Cordia dodecandra* and *Cordia sebestena*
318 (Boraginaceae) from the Yucatan Peninsula. *Botanical Sciences* **92**:289–297. URL:
319 <http://www.botanicalsciences.com.mx/index.php/botanicalSciences/article/view/97>.
- 320 **Chapman CA, Wrangham RW, Chapman LJ, Kennard DK, Zanne AE. 1999.** Fruit and
321 flower phenology at two sites in Kibale National Park, Uganda. *Journal of Tropical*
322 *Ecology* **15**:189–211. DOI: <http://dx.doi.org/>.
- 323 **Charlesworth D, Charlesworth B. 1987.** Inbreeding depression and its evolutionary
324 consequences. *Annual Review of Ecology and Systematics* **18**:237–268. DOI:
325 10.1146/annurev.es.18.110187.001321.
- 326 **Cheverud JM, Wagner GP, Dow MM. 1989.** Methods for the comparative analysis of
327 variation patterns. *Systematic Biology* **38**:201–213. DOI: 10.2307/2992282.
- 328 **Cohen JI. 2014.** A phylogenetic analysis of morphological and molecular characters of
329 Boraginaceae: evolutionary relationships, taxonomy, and patterns of character evolution.
330 *Cladistics* **30**:139–169. DOI: 10.1111/cla.12036.
- 331 **Gasparino EC & Barros MAVC. 2009.** Palynotaxonomy of Cordiaceae (Boraginales) from
332 São Paulo state. *Brazilian Journal of Botany* **32**:33–55. DOI: 10.1590/S0100-
333 84042009000100005

- 334 **Darwin C. 1877.** *The different forms of flowers on plants of the same species.* London: John
335 Murray.
- 336 **de Stapf MNS, dos Santos Silva TR. 2013.** Four new species of genus *Cordia* (Cordiaceae,
337 Boraginales) from Brazil. *Brittonia* **65**:191–199. DOI: 10.1007/s12228-012-9268-7.
- 338 **Dilcher D. 2000.** Toward a new synthesis: major evolutionary trends in the angiosperm fossil
339 record. *Proceedings of the National Academy of Sciences of the United States of America*
340 **97**:7030–7036. DOI: 10.1073/pnas.97.13.7030.
- 341 **Dray S, Dufour AB, Chessel D. 2007.** The ade4 package-II: Two-table and K-table methods. *R*
342 *News* **7**:47–52. URL: [http://website.dotsrc.org/pub/pub/cran/doc/Rnews/Rnews_2007-](http://website.dotsrc.org/pub/pub/cran/doc/Rnews/Rnews_2007-2.pdf#page=47)
343 [2.pdf#page=47](http://website.dotsrc.org/pub/pub/cran/doc/Rnews/Rnews_2007-2.pdf#page=47).
- 344 **Faivre AE, Mcdade LA. 2001.** Population–level variation in the expression of heterostyly in
345 three species of Rubiaceae: Does reciprocal placement of anthers and stigmas
346 characterize heterostyly? *American Journal of Botany* **88**:841–853. DOI:
347 10.2307/2657036.
- 348 **Ganders FR. 1979.** The biology of heterostyly. *New Zealand Journal Botany* **17**:607–635.
349 DOI:10.1080/0028825X.1979.10432574.
- 350 **Gilman EF, Watson DG. 1993.** *Cordia boissieri. Wild–Olive. Fact Sheet ST–181, a Series of*
351 *the Environmental Horticulture Department, Florida Cooperative Extension Service,*
352 *Institute of Food and Agricultural Sciences.* Gainesville: University of Florida.
- 353 **Gibbs PE, Taroda N. 1983.** Heterostyly in the *Cordia alliodora*-*C. trichotoma* complex in
354 Brazil. *Revista Brasileira de Botanica* **6**:1–10.

- 355 **Gómez JM, Muñoz-Pajares AJ, Abdelaziz M, Lorite J, Perfectti F. 2014.** Evolution of
356 pollination niches and floral divergence in the generalist plant *Erysimum*
357 *mediohispanicum*. *Annals of Botany* **113**:237–249. DOI: 10.1093/aob/mct186.
- 358 **Harder LD, Johnson SD. 2005.** Adaptive plasticity of floral display size in animal–pollinated
359 plants. *Proceedings of the Royal Society of London [B]* **272**:2651–2657. DOI:
360 10.1098/rspb.2005.3268.
- 361 **Hargreaves AL, Harder LD, Johnson SD. 2009.** Consumptive emasculation: The ecological
362 and evolutionary consequences of pollen theft. *Biological Reviews* **84**:259–276. DOI:
363 10.1111/j.1469-185X.2008.00074.x.
- 364 **Hódar JA, Obeso JR, Zamora R. 2009.** Cambio climático y modificación de interacciones
365 planta–animal. In: Medel R, Aizen MA, Zamora R, eds., *Ecología y evolución de*
366 *interacciones planta–animal*, Santiago de Chile: Editorial Universitaria, S. A., 287-300.
- 367 **Huang SQ, Fenster CB. 2007.** Absence of long–proboscis pollinators for long corolla–tubed
368 Himalayan *Pedicularis* species: implications for the evolution of corolla length.
369 *International Journal of Plant Science* **168**:325–331. DOI: 10.1086/510209.
- 370 **Justiniano MJ, Fredericksen TS. 2000.** Phenology of the species in Bolivian dry forests.
371 *Biotropica* **32**:276–281. DOI: 10.1111/j.1744-7429.2000.tb00470.x.
- 372 **Lacey EP, Roach DA, Herr D, Kincaid S, Perrott R. 2003.** Multigenerational effects of
373 flowering and fruiting phenology in *Plantago lanceolata*. *Ecology* **84**:2462–2475. DOI:
374 10.1890/02-0101.
- 375 **León De La Luz JL, Coria–Benet R, Cruz–Estrada M. 1996.** Fenología floral diurna de una
376 comunidad árido–tropical de Baja California Sur, México. *Acta Botanica Mexicana*
377 **35**:45–64. URL:

- 378 [http://www1.inecol.edu.mx/publicaciones/resumenes/ABM/ABM.35.1996/acta35\(45-](http://www1.inecol.edu.mx/publicaciones/resumenes/ABM/ABM.35.1996/acta35(45-)
379 [64\).pdf](http://www1.inecol.edu.mx/publicaciones/resumenes/ABM/ABM.35.1996/acta35(45-64).pdf).
- 380 **Lonard RI, Judd FW. 1985.** Effects of a severe freeze on native woody plants in the lower Rio
381 Grande Valley, Texas. *Southwestern Naturalist* **30**:397–403. DOI: 10.2307/3671272.
- 382 **Mahoro S. 2002.** Individual flowering schedule, fruit set, and flower and seed predation in
383 *Vaccinium hirtum* Thunb. (Ericaceae). *Canadian Journal of Botany* **80**:82–92. DOI:
384 10.1139/b01-136.
- 385 **McMullen CK. 2012.** Pollination of the heterostylous Galápagos native, *Cordia lutea*
386 (Boraginaceae). *Plant Systematics and Evolution* **298**:569–579. DOI: 10.1007/s00606-
387 011-0567-3.
- 388 **Medel R, Nattero J. 2009.** Selección mediada por polinizadores sobre el fenotipo floral:
389 Examinando causas y blancos de selección natural. In: Medel R, Aizen MA, Zamora R,
390 eds., *Ecología y evolución de interacciones planta–animal*, Santiago de Chile: Editorial
391 Universitaria, S. A., 77-94.
- 392 **Naiki A. 2012.** Heterostyly and the possibility of its breakdown by polyploidization. *Plant*
393 *Species Biology* **27**:23–29. DOI: 10.1111/j.1442-1984.2011.00363.x.
- 394 **Opler PA, Baker HG, Frankie GW. 1975.** Reproductive biology of some Costa Rican *Cordia*
395 species (Boraginaceae). *Biotropica* **7**:234–247. DOI: 10.2307/2989736.
- 396 **R Core Team. 2014.** R: A Language and Environment for Statistical Computing. R Foundation
397 for Statistical Computing, Vienna: R-project.
- 398 **Ríus–Díaz F, Barón–López FJ, Sánchez–Font E, Parras–Guijosa L. 1998.** *Bioestadística:*
399 *Métodos y aplicaciones. U.D. Bioestadística.* Málaga: Facultad de Medicina, Universidad
400 de Málaga., Spain.

- 401 **Rosas–Guerrero V, Quesada M, Armbruster WS, Pérez–Barrales R, Smith SD. 2011.**
402 Influence of pollination specialization and breeding system on floral integration and
403 phenotypic variation in *Ipomoea*. *Evolution* **65**:350–363. DOI: 10.1111/j.1558-
404 5646.2010.01140.x.
- 405 **Sánchez–Lafuente AM. 2002.** Floral variation in the generalist perennial herb *Paeonia broteroi*
406 (Paeoniaceae): Differences between regions with different pollinators and herbivores.
407 *American Journal of Botany* **89**:1260–1269. DOI: 10.3732/ajb.89.8.0.
- 408 **Silveira AP, Martins FR, Araújo FS. 2013.** Do vegetative and reproductive phenophases of
409 deciduous tropical species respond similarly to rainfall pulses? *Journal of Forestry*
410 *Research* **24**:643–651. DOI: 10.1007/s11676-013-0366-5.
- 411 **SPP–INEGI. 1986.** *Síntesis geográfica del estado de Nuevo León*. México, D.F.: Instituto
412 Nacional de Información Estadística y Geográfica.
- 413 **Stebbins GL. 1970.** Adaptive radiation of reproductive characteristics in angiosperms, I:
414 Pollination mechanisms. *Annual Review of Ecology and Systematics* **1**:307–326. DOI:
415 10.1146/annurev.es.01.110170.001515.
- 416 **Steinacher G, Wagner J. 2010.** Flower longevity and duration of pistil receptivity in high
417 mountain plants. *Flora* **205**:376–387. DOI: 10.1016/j.flora.2009.12.012.
- 418 **Stern K, Bidlack JE, Jansky SH. 2008.** *Introductory plant biology*, eleventh edition. Dubuque,
419 IA.: McGraw-Hill.
- 420 **Trapnell DW, Hamrick JL. 2006.** Floral display and mating patterns within populations of the
421 neotropical epiphytic orchid, *Laelia rubescens* (Orchidaceae). *American Journal of*
422 *Botany* **93**:1010–1018. DOI: 10.3732/ajb.93.7.1010.

- 423 **Ushimaru A., Watanabe T., Nakata K. 2007.** Colored floral organs influence pollinator
424 behavior and pollen transfer in *Commelina communis* (Commelinaceae). *American*
425 *Journal of Botany* **94**:249–258. DOI: 10.3732/ajb.94.2.249.
- 426 **Van Kleunen M., Meier A., Saxenhofer M., Fischer M. 2008.** Support for the predictions of
427 the pollinator-mediated stabilizing selection hypothesis. *Journal of Plant Ecology* **1**:173–
428 178. DOI: 10.1093/jpe/rtn017.
- 429 **Vieira ICG, Da Silva JMC. 1997.** Phenology, fruit set and dispersal of *Cordia multispicata*
430 Cham. an important weed shrub of abandoned pastures in eastern Amazonia. *Brazilian*
431 *Journal of Botany* **20**:51–56. DOI: 10.1590/S0100-84041997000100005.
- 432 **Vines RA. 1986.** *Trees, shrubs, and woody vines of the Southwest*. Austin: University of Texas
433 Press.
- 434 **Wagner GP. 1984.** On the eigenvalue distribution of genetic and phenotypic dispersion
435 matrices: Evidence for a nonrandom organization of quantitative character variation.
436 *Journal of Mathematical Biology* **21**:77–95. DOI: 10.1007/BF00275224.
- 437 **Wickham H. 2009.** *Ggplot2: Elegant graphics for data analysis*. New York, Springer.
- 438 **Wolfe LM, Burns JL. 2001.** A rare continual flowering strategy and its influence on offspring
439 quality in a gynodioecious plant. *American Journal of Botany* **88**:1419–1423. URL:
440 <http://www.amjbot.org/content/88/8/1419.full.pdf+html>.
- 441 **Yadav RK, Yadav AS. 2008.** Phenology of selected woody species in a tropical dry deciduous
442 forest in Rajasthan, India. *Tropical Ecology* **49**:25–34. URL:
443 http://www.tropecol.com/pdf/open/PDF_49_1/03%20Yadav.pdf.
- 444 **Zar JH. 2010.** *Biostatistical Analysis*, fifth edition. New Jersey: Prentice Hall.

445 **Figure and Table legends**

446

447 **Table 1 Spearman's rank correlation of floral traits in *Cordia boissieri* ($n = 2267$):** * < 0.05 ,

448 ** < 0.001 . ST (style type), SN (style number), SAN (stamen-anther number), TL (total length),

449 CL (corolla length), SL (style length), CD (corolla diameter), NGD (nectar guide diameter), NL

450 (number of lobes) and NGC (nectar guide color).

451

452 **Figure 1 Linear regression of the following:** A) plants with flowers, B) plants with fruits, and

453 C) plants without flower and fruits on Anacahueta trees as a function of local precipitation. The

454 rainfall considered was that from the previous month from September 2009 to August 2011.

455

456 **Figure 2 Morphs of *Cordia boissieri* flowers.** Arrows are parallel to the ovaries. S = superior

457 style, I = inferior style. The stamens are highlighted inside dotted red lines. From 15,000 flowers

458 (from 75 plants), we found 760 to have an inferior ovary (from 38 plants) and 740 to have a

459 superior ovary (from 37 plants).

460

461 **Figure 3 Example of variation on number of lobes of *Cordia boissieri* flowers.** We found

462 flowers from two to nine lobes. In pictures we show flowers with four to seven lobes.

463

464 **Figure 4 Nectar guide colors of *Cordia boissieri* flowers.** A) yellow, B) yellow-orange, C) with

465 yellow-white, D) with yellow-white-orange, E) orange-yellow, and F) the nectar guide of

466 senescent flowers turned brown.

Figure 1(on next page)

Linear regression of: A) plants with flowers, B) plants with fruits, and C) plants without flower and fruits on Anacahuita trees as a function of local precipitation.

Linear regression of: A) plants with flowers, B) plants with fruits, and C) plants without flower and fruits on Anacahuita trees as a function of local precipitation. Rainfall considered was that from the previous month from September 2009 to August 2011.

Figure 1

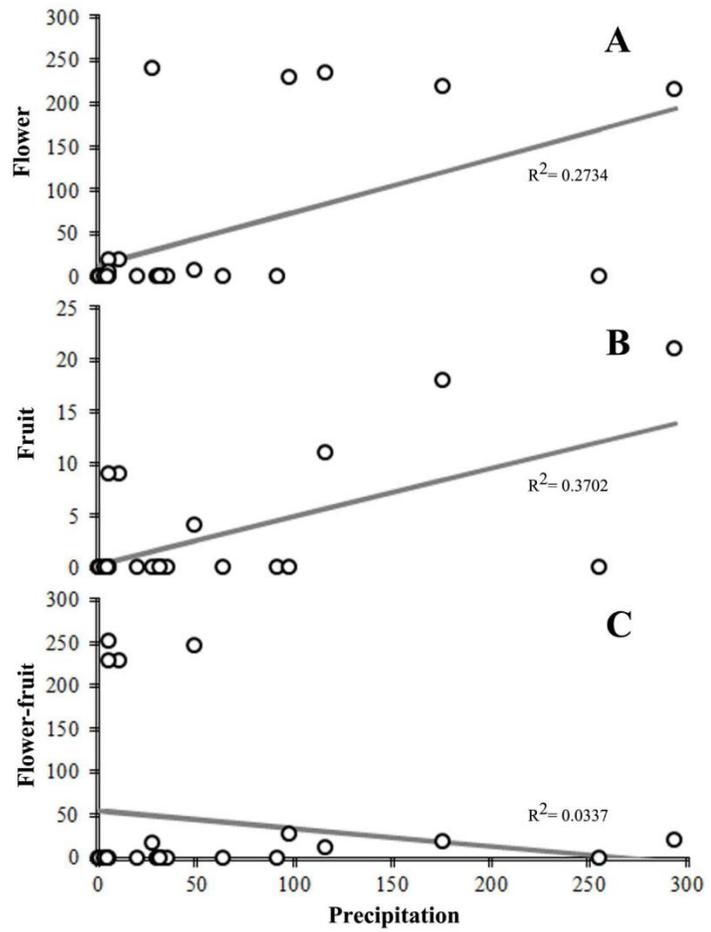


Figure 2(on next page)

Morphs of *Cordia boissieri* flowers.

Morphs of *Cordia boissieri* flowers. Arrows are parallel to the ovaries. S = superior style, I = Inferior style. The stamens are highlighted inside dotted red lines. From 1500 flowers (from 75 plants) we found 760 to have inferior ovary (from 38 plants) and 740 to have superior ovary (from 37 plants).

Figure 2

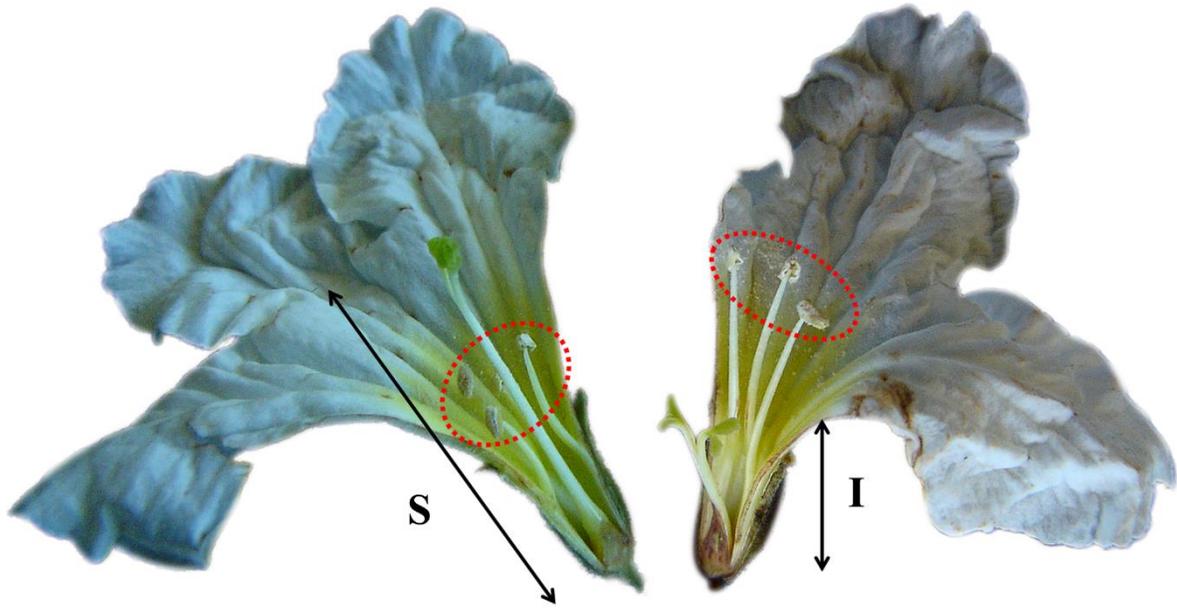


Figure 3(on next page)

Example of variation on number of lobes of *Cordia boissieri* flowers.

Example of variation on number of lobes of *Cordia boissieri* flowers. We found flowers from two to nine lobes. In pictures we show flowers with four to seven lobes.

Figure 3



Figure 4 (on next page)

Nectar guide colors of *Cordia boissieri* flowers.

Nectar guide colors of *Cordia boissieri* flowers. A) yellow, B) yellow-orange, C) with yellow-white, D) with yellow-white-orange E) orange-yellow. F) nectar guide of senescent flowers turned brown.

Figure 4



Table 1 (on next page)

Table 1 Spearman Rank Correlation of floral traits at *Cordia boissieri* (n = 2267).

Spearman Rank Correlation of floral traits of *Cordia boissieri* (n = 2267). * < 0.05, ** < 0.001. ST (style type), SN (style number), SAN (stamen-anther number), TL (total length), CL (corolla length), SL (style length), CD (corolla diameter), NGD (nectar guide diameter), NL (number of lobes) and NGC (nectar guide color).

1 **Table 1.** Spearman Rank Correlation (ρ) of floral traits at *C. boissieri* measured flowers ($n = 2267$). * < 0.05 , ** $<$
 2 0.001.

	ST	SN	SAN	TL	CL	SL	CD	NGD	NL	NGC
ST	1	0.036	0.012	-0.128**	-0.111**	-0.057**	-0.212**	-0.275**	0.029	-0.078**
SN		1	0.037	-0.046*	-0.033	-0.028	-0.041	-0.038	0.013	-0.049*
SAN			1	-0.028	-0.042*	0.032	0.062**	0.147**	0.677**	0.022
TL				1	0.883**	0.404**	0.429**	0.376**	-0.031	0.185**
CL					1	-0.014	0.347**	0.300**	-0.040	0.130**
SL						1	0.253**	0.238**	0.028	0.145**
CD							1	0.573**	0.054*	0.287**
NGD								1	0.178**	0.208**
NL									1	-0.008
NGC										1

3

4