

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

Cristian Adrian Martínez-Adriano, Enrique Jurado, Joel Flores, Humberto González-Rodríguez, Gerardo Cuéllar-Rodríguez

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.

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

Cristian A. Martínez-Adriano<sup>1</sup>, Enrique Jurado<sup>1</sup>, Joel Flores<sup>2</sup>, Humberto González-Rodríguez<sup>1</sup>  
and Gerardo Cuéllar-Rodríguez<sup>1</sup>

<sup>1</sup> Facultad de Ciencias Forestales–Universidad Autónoma de Nuevo León. Carretera Nacional  
km 145, C.P. 67700, A.P. 41 Linares, NL, México.

<sup>2</sup> Instituto Potosino de Investigación Científica y Tecnológica, División de Ciencias  
Ambientales. Camino a la Presa San José 2055, Col. Lomas 4a. Sección, CP. 78216. San Luis  
Potosí, SLP, México

**Corresponding author**

E. Jurado,  
[enrique\\_jurado@hotmail.com](mailto:enrique_jurado@hotmail.com)

Short title: Floral traits in *Cordia boissieri*

# ABSTRACT

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.

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

# INTRODUCTION

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

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

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

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

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

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

## MATERIALS AND METHODS

### Study area

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

### Species description

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

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

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

## **Blooms and rainfall**

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

# **Flower measurements**

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

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

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



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

## RESULTS

### Blooms and rainfall events

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

### Flower trait variation

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

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

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

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

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

## Floral trait integration

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

## DISCUSSION

### Blooms and rainfall events

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

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

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

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

This study showed morphological variations of flowers and the phenology of *C. boissieri*. Anacahuita flowers had a lifespan of five days, and the time to anthesis of each species likely

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

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

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

## Floral trait integration

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

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

## Acknowledgements

The authors thank J.M. López, E. Fernández, E. Zaragoza, B. Soto, D. Peñaflor, P. Hinojosa, M. Garza, D. Salas, and J.A. López for fieldwork assistance. M. Wilson edited English grammar and

style. C.A. Martínez-Adriano was the recipient of a scholarship (CONACYT 230073) during his master's studies. Funding was provided by PAICYT-UANL.

## REFERENCES

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

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

**Armbruster WS, Muchhala N. 2009.** Associations between floral specialization and species diversity: Cause, effect, or correlation? *Evolutionary Ecology* **23**:159–179. DOI: 10.1007/s10682-008-9259-z.

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

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

**Borchert R, Renner SS, Calle Z, Navarrete D, Tye A, Gautier L, Spichiger R, Hildebrand P. 2005.** Photoperiodic induction of synchronous flowering near the Equator. *Nature* **433**:627–629. DOI: 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 JL. 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.

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

- 378 [http://www1.inecol.edu.mx/publicaciones/resumeness/ABM/ABM.35.1996/acta35\(45-](http://www1.inecol.edu.mx/publicaciones/resumeness/ABM/ABM.35.1996/acta35(45-)
- 379 [64\).pdf](http://www1.inecol.edu.mx/publicaciones/resumeness/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 **Ríos-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](http://www.tropecol.com/pdf/open/PDF_49_1/03%20Yadav.pdf).
- 444 **Zar JH. 2010.** *Biostatistical Analysis*, fifth edition. New Jersey: Prentice Hall.

**Figure and Table legends**

**Table 1 Spearman's rank correlation of floral traits in *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).

**Figure 1 Linear regression of the following:** A) plants with flowers, B) plants with fruits, and C) plants without flower and fruits on Anacahuitta trees as a function of local precipitation. The rainfall considered was that from the previous month from September 2009 to August 2011.

**Figure 2 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 15,000 flowers (from 75 plants), we found 760 to have an inferior ovary (from 38 plants) and 740 to have a superior ovary (from 37 plants).

**Figure 3 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.

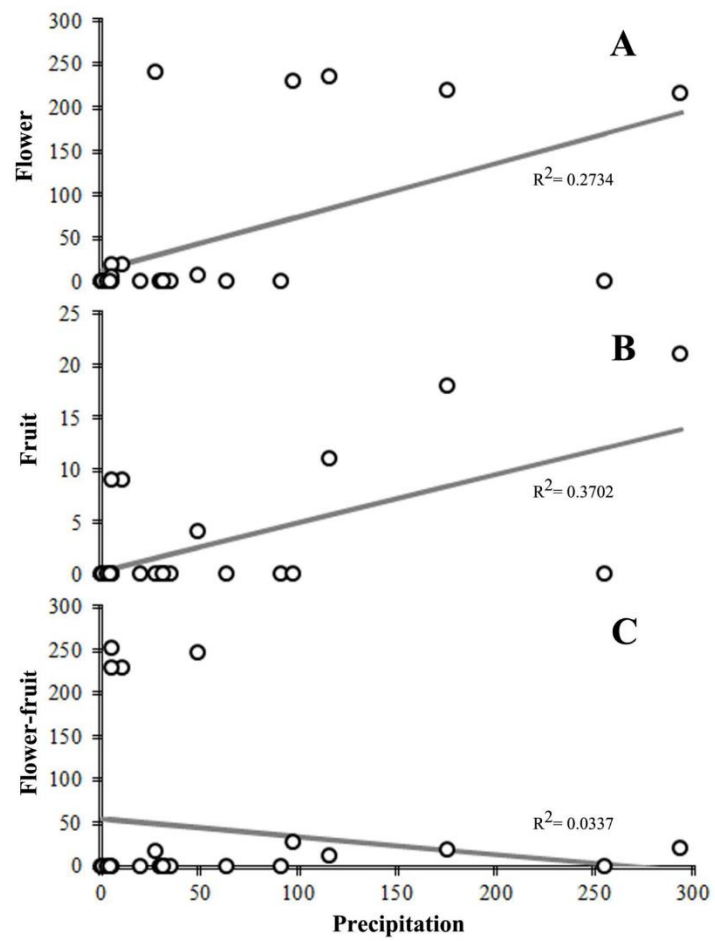
**Figure 4 Nectar guide colors of *Cordia boissieri* flowers.** A) yellow, B) yellow-orange, C) with yellow-white, D) with yellow-white-orange, E) orange-yellow, and F) the nectar guide of senescent flowers turned brown.

# **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



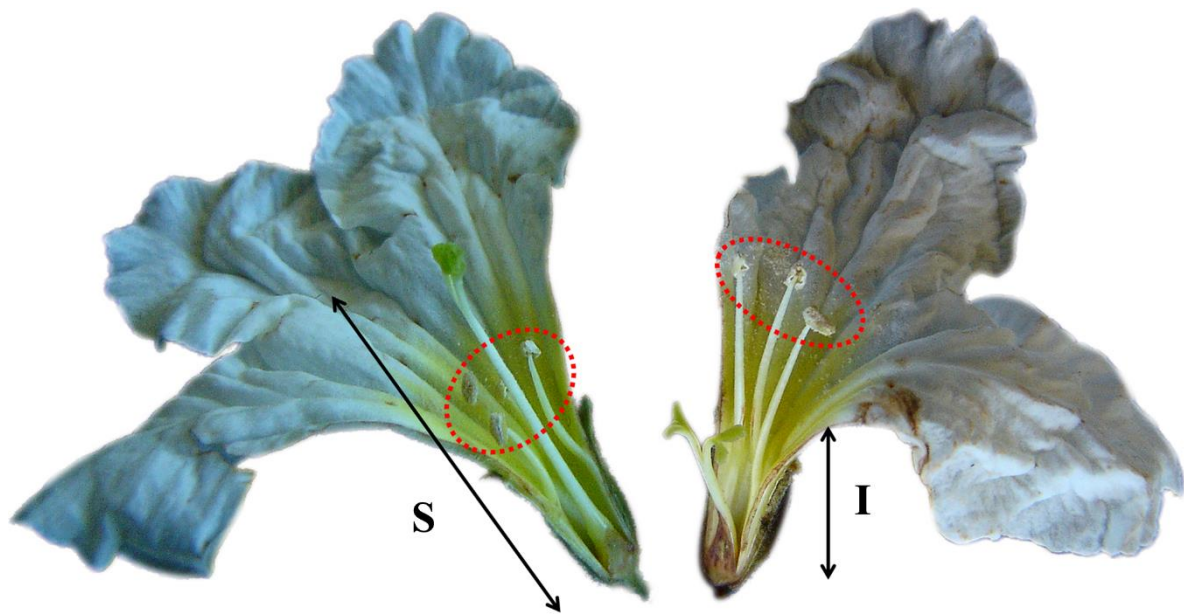


## Figure 2 (on next page)

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

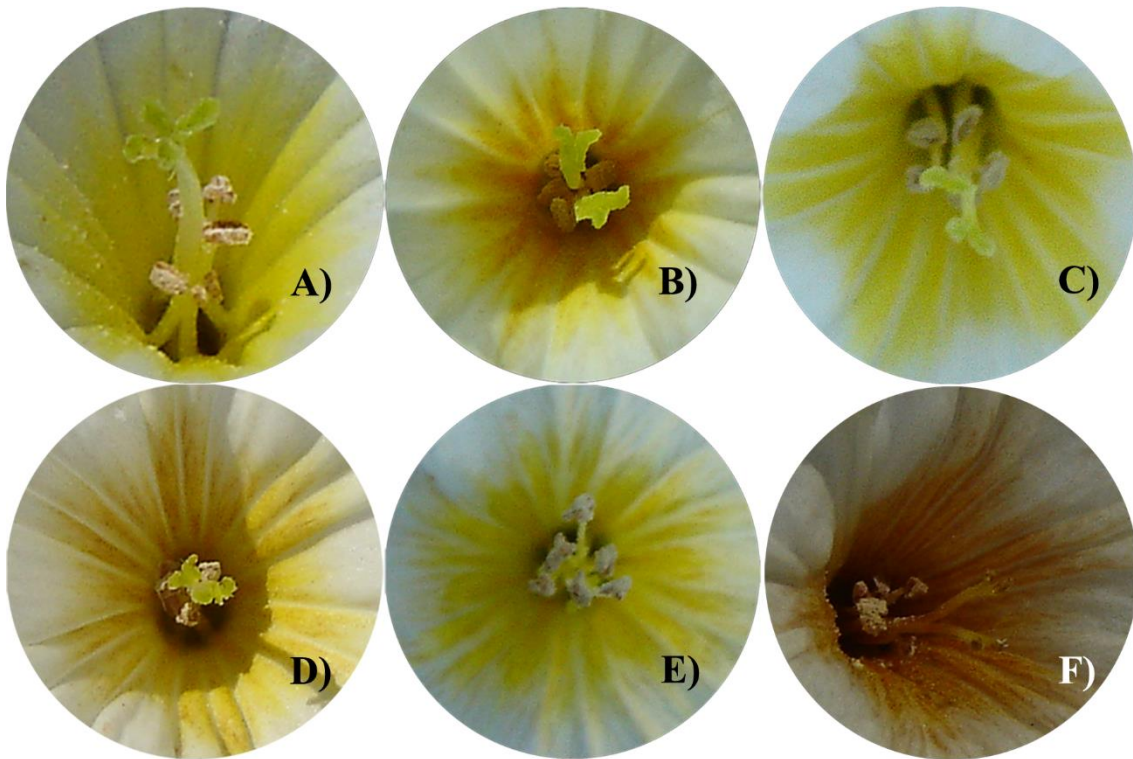


# **Figure 4**(on next page)

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).

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

|     | 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        |