Mating system of *Datura inoxia*: Association between selfing rates and herkogamy within populations (#52494)

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Mating system of *Datura inoxia*: Association between selfing rates and herkogamy within populations

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Plant mating system determines, to a great extent, the demographic and genetic properties of populations, hence their potential for adaptive evolution. Variation in plant mating system has been documented between phylogenetically related species as well between populations of a species. A common evolutionary transition, from outcrossing to selfing, is likely to occur under environmental spatial variation in the service of pollinators. Here, we studied two phenotypically (in floral traits) and genetically (in neutral molecular markers) differentiated populations of the annual, insect-pollinated, plant *Datura inoxia* in Mexico, that differ in the service of pollinators (Mapimí and Cañada Moreno). First, we determined the populations' parameters of phenotypic in herkogamy, outcrossing and selfing rates with microsatellite loci, and assessed between generation (adults and seedlings) inbreeding, and inbreeding depression. Second, we compared the relationships between parameters in each population. Results point strong differences between populations: plants in Mapimí have, on average, approach herkogamy, higher outcrossing rate ($t_m = 0.68$), lower primary selfing rate ($r = 0.35$), and lower inbreeding at equilibrium ($F_e = 0.24$) and higher inbreeding depression ($\delta = 0.25$), than the populations of Cañada. Outcrossing seems to be favored in Mapimí while selfing in Cañada. The relationship between r and F_e were negatively associated with herkogamy in Mapimí; here, progenies derived from plants with no herkogamy or reverse herkogamy had higher selfing rate and inbreeding coefficient than plants with approach herkogamy. The difference $F_e - F$ is positively related to primary selfing rate (r) only in Cañada Moreno which suggests inbreeding depression in selfing individuals and then genetic purging. In conclusion, mating system evolution may occur differentially among maternal lineages within populations of *Datura inoxia*, in which approach herkogamy favors higher outcrossing rates and low levels of inbreeding and inbreeding depression, while no herkogamy or reverse herkogamy lead to the evolution of the "selfing syndrome" following the purge of

deleterious alleles despite high inbreeding among individuals.

1   **Introduction**

2   Plant mating systems affect the demographic dynamics and genetic structure of plant

3   populations, and thus their adaptive potential (Charlesworth & Charlesworth, 1979; Eckert et al.,

4   2009). Mating systems often vary widely among populations of self-compatible species, from

5   predominant or complete selfing to complete outcrossing. Intermediate (or mixed) mating

6   systems, i.e., those that combine selfing and outcrossing strategies, are also common in nature

7   (Stebbins, 1957; Goodwillie, Kalisz & Eckert, 2005; Barrett, 2010).

8   The transition from outcrossing to selfing is one of the most frequent evolutionary shifts

9   in flowering plants (Stebbins, 1957; Barrett, 2010; Sicard & Lenhard, 2011). Evidence of this

10   phenomenon comes from phylogenetic studies (Goodwillie, 1999; Foxe et al., 2009),

11   investigating differences within and among species, and at different geographic scales (Duncan

12   & Rausher, 2013; Wright, Kalisz & Slotte, 2013).

13   Two main scenarios are thought to favor the evolution of selfing. One is the transmission

14   advantage of selfers relative to outcrossers (3:2 genomes), which would promote the expansion

15   of selfing modifiers in a population unless prevented by natural selection (Fisher, 1941). The

16   second scenario considers that under ecological conditions that are unfavorable for cross-

17   pollination (i.e., rarity or absence of potential mates and/or pollen vectors), natural selection

18   would favor reproductive assurance through selfing (Baker, 1955; Stebbins, 1957; Schoen &

19   Lloyd, 1992; Schoen, Morgan & Bataillon, 1996). Despite the potential transmission and

20   reproductive advantages, selfing as a mating system may also restrict gene flow within and

21   among populations. Consequently, selfing might increase levels of inbreeding and

22   homozygosity, and ultimately the likelihood of inbreeding depression (δ) in populations

23   (Charlesworth & Charlesworth, 1987; Charlesworth & Wright, 2001; Takebayashi & Morrell,

24 2001). Therefore, the inbreeding coefficient (F) is expected to correlate positively with the level

25 of selfing in a population. In the long term, however, theoretical models and empirical evidence

26 suggest that increasing levels of inbreeding promote selection against inbred individuals by

27 purging populations of lethal or deleterious alleles, thus reducing genetic load (Husband &

28 Schemske, 1996; Takebayashi & Delph, 2000; Crnokrak & Barrett, 2002; Morran, Parmenter &

29 Phillips, 2009). Hence, populations with a long history of inbreeding are expected to have lower

30 levels of inbreeding depression (Yahara, 1992)

31   Herkogamy, the spatial segregation of sex organs within the flower, is considered a main

32 floral trait affecting selfing rates (Webb & Lloyd, 1986). In hermaphroditic flowers, the

33 likelihood of selfing is reduced when the stigma surpasses the length of the anthers (i.e.,

34 approach herkogamy), because flower stigmas are prevented from receiving self-pollen. In

35 presence of pollinators, this also provides the opportunity for outcross pollen to first reach the

36 stigmas. In contrast, when the anthers and stigmas are placed at the same level (i.e., no -

37 herkogamy) or the stigmas are below the anthers (i.e., reverse herkogamy), selfing is likely to

38 occur, with (facilitated) or without (autonomous) pollinators (Lloyd, 1992). At the population

39 level, the relationship between herkogamy and selfing/outcrossing rates may be an indicator of

40 pollinator service reliability (Opedal, 2018). Within-population, this relationship may also be

41 explained by other causal mechanisms. For instance, stigma clogging may increase competition

42 between self- and cross-pollen in non-herkogamous flowers, favoring higher outcrossing rates in

43 herkogamous flowers (Opedal, 2018). Also, a positive correlation between approach herkogamy

44 and floral traits related to pollinator attraction, contributes to the maintenance of the relationship

45 between herkogamous flowers and an outcrossing strategy (Opedal 2018). In other species

46 herkogamy may reduce the interference between sexual functions enhancing pollen export

47   efficiency (Lloyd & Webb, 1986; Barret, 2002). Further, reverse or no herkogamy might

48   increase reproductive fitness (i.e., seed number) and thus be favored by selection, but it should

49   also positively correlate with selfing rate. Contrary, natural selection might favor/maintain

50   approach herkogamy thus increasing outcrossing rate (Motten & Stone, 2000; Elle & Hare, 2002;

51   Herlihy & Eckert, 2007).

52   Herkogamy is a complex trait determined by genetic and ecological factors (Ashman &

53   Majetic, 2006; Opedal et al., 2017). Within populations, standing genetic variation in herkogamy

54   would depend on the history of selection of herkogamy on individual lineages, and its adaptive

55   value could depend upon selection pressures exerted by pollinators −particularly when the

56   abundance of pollinators fluctuates in time− or to other selective factors. When associations

57   among selfing rate (s), inbreeding coefficient (F) and herkogamy are maintained over time, a

58   within-population dynamics among these variables may be established at the lineage level.

59   Significant associations between high outcrossing rate and approach herkogamy have been

60   reported before (Takebayashi, Wolf & Delph, 2006; de Vos et al., 2018; but see Chen et al.,

61   2009; Brys & Jacquemyn, 2012; Opedal, Armbruster & Pélabon, 2015; Toräng et al., 2017).

62   However, few studies have evaluated the association between herkogamy and outcrossing rate

63   within-populations (Epperson & Clegg, 1987; Moten & Antonovics, 1992; Carr & Fenster, 1994;

64   Karron et al., 1997; Brunet & Eckert 1998; Takebayashi & Delph, 2000; Elle & Hare, 2002;

65   Medrano, Herrera & Barret, 2005; Herlihy & Eckert, 2007) and even less have considered

66   relationships among herkogamy, inbreeding coefficient and mating system.

67   Many annual, self-fertilizing, plant species evolve in stressful environments (e.g.

68   disturbed and/or arid habitats) where pollinators' abundance is reduced and/or unpredictable

69   (Friedman & Rubin, 2015). Under high environmental variability in pollination service, daily

70 and seasonal fluctuations present different opportunities for cross- or self-fertilization

71 maintaining variation on traits that affect the plants' mating system, such as herkogamy or the

72 inbreeding history of each maternal lineage (Schoen & Lloyd, 1992; Schoen, Morgan &

73 Bataillon, 1996; Morgan & Wilson, 2005; Eckert, Samis & Dart, 2006; Barrett, 2010; Shirk &

74 Hamrick, 2014; Pannell, 2015). However, it is not clear how strong and how frequently the

75 association between mating strategies, herkogamy and inbreeding history of lineages occur

76 within populations in short-lived species. Here, we assessed the within-population mating system

77 dynamics, with replication in two populations of the annual plant *Datura inoxia* Mill. We focus

78 on the within-population level and less on among-population expectations in order to detect if

79 individuals with more herkogamous flowers show a history of less inbreeding associated to

80 lower levels of selfing. Until now, relative few studies (see review in Opedal, 2018) have

81 assessed these associations within (rather than among) populations.

82

83 **Materials and Methods**

84 *Study species and sampled populations*

85 *Datura inoxia* (Solanaceae) is a summer annual, self-compatible plant that inhabits arid and

86 semi-arid lands in Mexico and Southern USA (i.e., The Chihuahuan desert) (Barclay, 1959;

87 Lockwood, 1973). High daily fluctuation of ambient temperature is a characteristic of these

88 ecosystems. The onset of flowering of *D. inoxia* is in July and lasts through September, similar

89 to other *Datura* species (Bronstein et al., 2009). *Datura inoxia* produces large, funnel-shaped,

90 and nectar producing hermaphroditic flowers that open at dusk and remain receptive for only one

91 night. During the flowering period, individuals can display from a few up to tens of flowers each

92 night. Flower traits related to mating system such as herkogamy and flower size, vary widely

93   within and among populations (Jiménez-Lobato & Núñez-Farfán, 2012). Further, comparisons

94   between genetic differentiation at neutral loci (F_{ST}) and phenotypic differentiation (Q_{ST}) in floral

95   traits suggest adaptive evolution (Jiménez-Lobato & Núñez-Farfán, 2012). Flowers are

96   commonly visited by honeybees, which collect pollen, and hawkmoths (*Manduca sexta*, *M.*

97   *quinquemaculata* and *Hyles lineata*) which forage for nectar (Barclay, 1959; Lockwood, 1973;

98   Grant, 1983; McCall et al., 2018; V. Jiménez-Lobato, pers. Obs.).

99   Two populations of *D. inoxia* were selected to assess the relationships between

100 herkogamy, selfing rate, and inbreeding. A previous report (Jiménez-Lobato & Núñez-Farfán,

101 2012) indicated substantial within-population variation in herkogamy and flower size. The

102 Cañada de Moreno population (CM) is located in the State of Querétaro (21° 17' 43" N; 100° 31'

103 00" W) in the Mexican Bajío at 1933 m a.s.l. During the flowering period of *D. inoxia* (July to

104 September), this locality has an average temperature of 18.8 °C, with a daily range from 7.4 °C

105 to 30.8 °C, and a three-month total precipitation of 314 mm. The Mapimí population (Map) is

106 located at 1157 m a.s.l. in the Mexican Plateau in the States of Coahuila and Durango (26° 41'

107 11" N; 103° 44' 49" W). This is a more xeric environment, with a quarterly average temperature

108 of 22.8 °C (range: 12.6 °C to 32.9 °C) and a total three-month precipitation of 253 mm

109 (Meteorological Service of Mexico: http://smn.cna.gob.mx/). Collection of seed material for

110 experimental analyses was made under the permission SGPA-DGGFS-712-1596-17

111 (Subsecretaría de Gestión para la Protección Ambiental, Secretaría de Medio ambiente y

112 Recursos Naturales, Mexico).

113 *Variation in the level of herkogamy within and among populations*

114 Thirty reproductive plants were randomly selected and tagged for sampling in a 1 ha area within

115 each population. For each individual plant, 4 - 6 open flowers were randomly selected to

116   measure herkogamy. Herkogamy was calculated as the difference between pistil and stamen

117   length. Approach herkogamy was defined as the pistil surpassing the stamens in length

118   (henceforth "positive herkogamy"), whereas reverse herkogamy was the opposite trend

119   (henceforth "negative herkogamy"). Absence of herkogamy occurred when pistil and stamens

120   had equal lengths.

121   *Mating system parameters*

122   To estimate mating system parameters from each marked plant in the field, five mature fruits

123   derived from natural pollination were collected, labelled and bagged. In the laboratory, seeds of

124   each fruit were separated and germinated in a greenhouse, and seeds of each fruit within each

125   family (maternal plant) were sown in separate pots. Germination per fruit per family was

126   recorded for 30 days. To obtain an average estimate of germination rate per fruit, we recorded

127   the final number of seeds germinated in each pot. Germination percentage was $\geq 90\%$ (error SD

128   = 3.18) for all plants. Once seedlings emerged, we collected leaf tissue from young plants,

129   bagged, labelled, and stored in an ultra-freezer at -97 °C. Finally, we analyzed mating system

130   parameters in 20 seedlings from each of 30 maternal families per population (N = 600).

131   DNA was extracted from seedlings following the Miniprep protocol (Doyle & Doyle,

132   1987). Five microsatellite nuclear loci developed for *D. stramonium* (Andraca, 2009) were

133   amplified for each seedling. Further, we standardized one additional microsatellite locus for *D.*

134   *inoxia* (F8: Rw: 5´ -GGACAACATCTTTGCGACCC- 3´), in order to obtain a total of six

135   polymorphic microsatellite loci per individual plant. Primers were labelled with PET, VIC, 6-

136   FAM, and NED dyes (Applied Biosystems) (PCR protocols are described in Supplementary

137   Information).

138   Multilocus outcrossing (t_m) and selfing ($s = 1-t_m$) rates, primary selfing rate (r) and

139   inbreeding coefficient (F) were estimated for each maternal family for each population. Mating

140   system parameters (t_m and s) at the family level were calculated with MLTR 3.2 (Ritland, 2002)

141   using the Expectation-Maximization method (EM), which allows missing data and undetected

142   null alleles (Ritland & Jain, 1981). Standard errors and standard deviations were estimated by

143   bootstrapping, with 1000 replicates and re-sampling individuals at the family level. The

144   frequency of null alleles per locus, per population, was assessed using Micro-Checker v.2.2.3

145   (Cock et al., 2004). One locus (G8) did not amplify for any of the plants from CM, so analyses

146   were carried out with five loci in that population, and six loci in Map. Selfing rates (s) obtained

147   from molecular markers, after fertilization and germination, might not be completely

148   independent from inbreeding depression and thus may underestimate its true value (Lande,

149   Schemske & Schultz, 1994). Primary selfing rate (r) is a better predictor of mating system

150   because it assesses separately the magnitude of inbreeding depression. Hence, r refers to the

151   proportion of selfed progeny at the time of fertilization (Lande, Schemske & Schultz, 1994). The

152   primary selfing rate (r) was calculated for each maternal family as: $r = s / [1 - \delta + s\delta]$, where s is

153   the selfing rate obtained from microsatellite loci, and δ is the cumulative inbreeding depression

154   obtained for each population (see below).

155   The inbreeding coefficient (F) may include components of inbreeding other than just self-

156   or cross- fertilization, such as biparental inbreeding or population substructure. Here, we used F

157   as a proxy of the inbreeding history of each maternal family (i.e., adult cohort) (Latta & Ritland,

158   1994). F values were inferred for each lineage from the microsatellite loci amplified previously,

159   with GenePop v.4.2 (Rousset & Raymond, 1995; Rousset, 2008). To investigate whether

160   inbreeding depression was associated with the selfing rate and herkogamy, we calculated

161   inbreeding coefficients at equilibrium (F_e) (i.e., progeny cohort) for each lineage, assuming that

162   adult F and t_m are constant among generations (Ritland, 1990). We then related these differences

163   with selfing rate and herkogamy. Since F_e increases in relation to F due to self-fertilization,

164   larger differences between F and F_e at each maternal lineage should indicate the presence of

165   higher inbreeding depression. Once selection against inbred progeny has occurred, F_e and F will

166   be equal (Ritland, 1990; Shirk & Hamrick 2014). This estimation can be thought-out as a

167   potentially late-acting inbreeding depression in each lineage. F_e was calculated as $F_e = (1-t_m)$ /

168     $(1+t_m)$, where t_m is the outcrossing rate calculated from MLTR for each maternal family (Allard,

169   Jain & Workman. 1968; Ritland, 1990).

170

171   *Inbreeding depression*

172   To estimate inbreeding depression (δ), we collected 150 fruits from different individual

173   plants in each population. These plants did not include the maternal families previously

174   analyzed. From each fruit, we sowed ten seeds on separate pots under greenhouse conditions.

175   Average ($\pm$ S.E.) environmental variables in the greenhouse (March-September), measured by

176   HOBO sensors (Onset Computer Corporation, USA) were: temperature 27.10 ± 0.06 ºC; relative

177   humidity 42.93 ± 0.09% and light intensity $3,133.59 \pm 18.73$ Lux (Camargo, 2009). When seeds

178   germinated, one seedling per fruit was randomly chosen and grown under controlled conditions

179   until flowering. For each population, 100 individuals were randomly chosen to act as pollen

180   receptors (mothers) and 50 individuals as pollen donors (fathers). Two manual pollination

181   treatments were applied to each maternal plant: (1) cross-pollination (o), where two flowers were

182   emasculated before anthesis and hand-pollinated with pollen from one donor randomly chosen

183   from the same population; (2) self-pollination treatment (s), where two flowers of each receptor

184   plant were fertilized with self-pollen. After pollination, flowers in both treatments were bagged

185   individually with a fine nylon mesh. Since many mother plants did not produce the four flowers

186   needed for pollination treatment application, the final sample included mother plants that

187   produced at least one fruit per treatment (CM: N = 77; Map: N = 41). Two fitness components

188   per pollination treatment were evaluated in each population: seed-set mean (i.e., number of

189   seeds/ number of ovules) and seed mass. Seed mass was obtained from a random sample of 30

190   seeds per fruit using an analytical balance (Adventurer OHAUS). Allocating more resources to

191   seeds can increase quality of seeds, increasing the likelihood of successful seedlings'

192   establishment and mothers' fitness (Stanton & Young, 1994; Byers & Waller, 1999). In *D.*

193   *inoxia*, as in *D. stramonium*, under greenhouse experiments conditions, inbreeding depression on

194   seed mass has been detected even when maternal plants have been fertilized with a single pollen

195   donor (Sosensky, 2004; Jiménez-Lobato, 2013). Likely seed mass may influence germination

196   rate and seedling establishment in *Datura* species.

197   Cumulative inbreeding depression coefficient (δ) was calculated for each population as:

198    $\delta = 1 - \frac{\overline{w}_s}{\overline{w}_o}$, where $\overline{w}_s$ and $\overline{w}_o$ are the mean fitness of progenies derived from self- or cross-

199   pollination, respectively. Average fitness of self- and out-cross progenies was calculated as the

200   product of seed-set and seed mass (Schemske & Lande, 1985), and it was used to estimate the

201   primary selfing rate (*r*) at each maternal lineage. This estimation can be considered as an early-

202   acting inbreeding depression

203   **Statistical analyses**

204   Statistical analyses were implemented in R software version 4.0.2 (R Development Core Team

205   2020). To estimate phenotypic variation in herkogamy within each population, we quantified the

206 variance components within and between individuals. Using the nlme package (Pinheiro et al.,

207 2017), we fitted a linear mixed model for each population where maternal family and plants

208 nested within families were considered as random factors.

209   Statistical differences in primary selfing rates (r), inbreeding in adult cohort (F),

210 inbreeding at equilibrium (F_e) and an estimate of inbreeding depression ($F_e - F$) between

211 populations were tested by Analysis of Covariance (ANCOVA) in multcomp package (Hothorn,

212 Bretz & Westfall, 2008). Population was considered as fixed factor and herkogamy as the

213 quantitative variable.

214   Multilocus outcrossing (t_m) and primary selfing rates (r) were strongly negatively

215 correlated (CM: estimate = -1.00, $p = 0.000$, d.f. = 25; Map: estimate = -0.969, $p = 0.000$, d.f. =

216 27), hence we present here only the analyses for primary selfing rates (r). Since r is a proportion,

217 its relationship with herkogamy was analyzed with a beta regression using the betareg package

218 (Cribari-Neto & Zeiles, 2010; Douma & Weedon, 2018). This regression has been proposed for

219 modeling continuous data limited to a specific interval (0, 1) (Ferrari & Cribari-Neto, 2004). We

220 tested for cloglog, logit and log link functions and based on Akaike's criterion we selected the

221 model that best fit to the data. Estimates of β and $\emptyset$ were obtained by maximum likelihood

222 estimation. F, F_e and their differences were associated to herkogamy with a generalized linear

223 model with Gaussian distribution error (Crawley, 2013). It must be noted that F_e is a theoretical

224 prediction based on the selfing rate (r), therefore both estimators do covariate. Likewise, their

225 correlation with other variables would be very similar. Thus, to avoid redundancy, we present the

226 correlations of selfing rate with other variables.

227

228 **Results**

229 *Variation in herkogamy and mating system parameters within and among populations*

230 At CM plants were, on average, non-herkogamous but with substantial variation (mean = -4.72;

231 sd = 5.5 mm; range = 17.48 mm, from -13.83 to + 3.65 mm). In this population 20 out of 27

232 individual plants (74%) had reverse or no herkogamy and 7 (26%) exhibited approach

233 herkogamy (Fig. 1A). Herkogamy varied proportionally more among plants at Map than at CM

234 (N = 29) and on average was positive (Mean = 2.57 mm; sd = 11.15 mm; range = 44.8mm, from

235 -20 to +24.8 mm) (Fig. 1B).

236 The proportion of variance among individual plants in herkogamy was higher in Map

237 (77.32%) than in CM (50.62%), indicating higher intra-individual variation in the latter

238 population (Map: 22.68%; CM: 49.38%).

239 ANCOVA results showed that populations and herkogamy were statistically significant

240 for primary selfing rates (r), inbreeding at equilibrium (F_e) and estimation of inbreeding

241 depression ($F_e - F$) (Table S1). Inbreeding in adult cohort (F) was not different between

242 populations and was unaffected by herkogamy.

243 *Mating system estimation and inbreeding coefficient* (F)

244 The multilocus outcrossing rate (t_m) was, on average, higher in Map than in CM (0.682 vs.

245 0.294) although high variation in this parameter was detected among lineages in each population

246 (Map, 0.022 to 1; CM, 0 to 1). The distribution of t_m is skewed toward low values in CM (ca.

247 60% of mother plants), but towards high values in Map (50% of the families with $t_m > 0.8$) (Fig.

248 2A and 2B, respectively). Primary selfing rate (r) was two-fold higher in CM than in Map (0.716

249 vs. 0.353), varying from $r = 0$ to 1 and from $r = 0$ to 0.978, respectively.

250   Inbreeding coefficient in the adult cohort (F) was negative in the two populations and

251   highly variable (CM: F = -0.193, range -1 to 0.75; Map: F = -0.085, range -0.553 to 1).

252   Inbreeding coefficient at equilibrium (F_e) was higher in CM than Map (F_e = 0.626 vs. 0.238) and

253   highly variable in both populations (from 0 to 1). The difference between the F of the adult

254   cohort and the F_e of the progeny cohort was much higher in CM than in Map ($F_e - F$ = 0.819 vs.

255   0.323). The estimated magnitude of cumulative inbreeding depression, measured on greenhouse

256   conditions, was higher in Map than in CM (δ = 0.25 vs. δ = 0.09).

257   *Relationships between herkogamy and mating system parameters*

258   Correlations between primary selfing rate (r), inbreeding coefficient (F), inbreeding coefficient

259   at equilibrium (F_e) and herkogamy were only significant in the Map population (Table 1, Figs. 3

260   and 4). Primary selfing rate (r) (and hence F_e) were negatively associated with herkogamy only

261   in Map population (Table 1; Figs. 3A and 3B), indicating that individuals with reverse

262   herkogamy or without herkogamy had progenies with higher selfing rate and inbreeding

263   coefficient than plants with approach herkogamy. The best beta regression model between r and

264   herkogamy was fitted with the log link function according to the Akaike's criterion (Table S2).

265   In addition, the breeding history of each maternal lineage (F) was associated with selfing (r) in

266   the Map population only (Figs. 4A and 4B), but not with herkogamy in either of the two

267   populations (F vs. herkogamy) (Table 1; Figs. 3C and 3D). The $F_e - F$ difference was positively

268   correlated with primary selfing rate (r) only in CM (Table 1; Figs. 4C and 4D). We did not find

269   any indication of a significant correlation between $F_e - F$ and herkogamy (Table 1; Figs. 3E and

270   3F). As expected, progenies' inbreeding coefficient (F_e) was positively correlated with primary

271   selfing rates (r) in both populations (results not shown).

272

273 **Discussion**

274 In annual, short-lived, plant species, the evolution of plant mating system in association with

275 flower traits, such as herkogamy, depends on the constancy of natural selection within

276 populations according to the opportunity for cross- and self-fertilize every year (Shirk &

277 Hamrick 2014). Because herkogamy is a direct modifier of the efficiency of self- and cross-

278 pollination, it is likely to coevolve with the plants' mating system, and the different within-

279 population lineages would be expected to vary in their history of inbreeding. Here, we evaluated

280 the association of herkogamy, mating system and inbreeding history at a lineage level within two

281 populations of *Datura inoxia*, an annual/short lived species distributed in arid and semiarid

282 environments in Mexico and southern USA.

283 We found that herkogamy, selfing rates and inbreeding coefficients, as well as

284 associations among them, varied considerably between and within populations. In line with

285 expectations the Map population, where plants show pronounced approach herkogamy, had

286 higher average outcrossing rate and lower inbreeding coefficient of progenies than individual

287 plants that exhibited absence of and/or reverse herkogamy. These results are in line with the

288 hypothesis of adaptive herkogamy as a mechanism that prevents selfing in populations and avoid

289 inbreeding between individuals (Web & Lloyd 1986; Lloyd 1992). The relationship between

290 selfing/outcrossing rate and herkogamy has been found in other species of *Datura* such as *D.*

291 *stramonium* (Motten & Antonovics, 1992; Motten & Stone, 2000) and *D. wrightii* (Elle & Hare,

292 2002) as well as in other species like *Gilia achilleifolia* (Takebayashi & Morrell, 2001), *Clarkia*

293 *temblorensis* (Holtsford & Ellstrand, 2006), *Mimulus ringens* (Karron et al., 1997), *Nicotiana*

294 *glauca* (Shueller, 2004), *Aquilegia canadensis* (Herlihy & Eckert, 2007), *Gesneria citrina* (Chen

295 et al., 2009) and *Dalechampia scandens* (Opedal, Armbruster & Pelabon, 2015).

296 However, we did not detect a significant association between the inbreeding coefficient

297 of adults' cohort and herkogamy. This result suggests that the inbreeding history of each lineage

298 does not depend on herkogamy alone, and that purging of more inbred individuals and/or

299 biparental inbreeding may also come into play (Charlesworth & Charlesworth, 1987). Reports of

300 low survival rate of inbred individuals at early life, i.e., high inbreeding depression, suggests

301 reductions of genetic load in adult plants (Medrano, Herrera & Barret, 2005; Abdelaziz et al.,

302 2014). In fact, in partially selfing populations it has been observed that F in progeny cohorts

303 increase in relation to F of the parental population, and later decrease again in the adult stage

304 (Ritland 1990; Eguiarte et al., 1993). If the purge of inbred individuals occurs at Mapimí, the

305 relationship between selfing rate in relation to flower herkogamy would be related to the

306 differential contribution of lineages to the next generation, [thus] yielding evidence of natural

307 selection on herkogamy (Medrano, Herrera & Barret, 2005).

308 Although we do not know the causes of the relationship between herkogamy and mating

309 system found in this study, fluctuation of environmental factors, especially pollinator abundance,

310 as it happens at Mapimí, can contribute to the maintenance of variation in herkogamy within

311 populations. Variation in herkogamy is linked with plants' reproductive assurance when

312 pollinators are scarce, or with high outcrossing rates when abundant (Kalisz, Vogler & Hanley,

313 2004; Goodwillie, Kalisz & Eckert, 2005; Chen et al., 2009). Since outcrossing rates in Mapimí

314 are related to herkogamy, episodes of differential (or even contrasting) selection on herkogamy

315 among lineages over time may have favored either outcrossing (approach herkogamy) or selfing

316 (no herkogamy and/or reverse herkogamy). Further, phenotypic/genetic variance in herkogamy

317   could facilitate the maintenance of the mixed mating system of *D. inoxia* in Mapimí. The among

318   individual plants variance component in herkogamy strongly suggests genetic differences

319   between plants, beyond within-individual variance (i.e. heritability > 0). In this sense, our results

320   may provide evidence the herkogamy is a functional trait linked to the reliability of pollinator

321   service in the different populations (Moeller, 2006; Opedal, 2018).

322   On the other hand, the mating system of the population of Cañada de Moreno is

323   predominantly of selfing, and no association between selfing rates, herkogamy and inbreeding

324   coefficients in the adult cohort were detected at this locality. Nevertheless, like the Mapimí

325   population, there is a positive relationship between selfing rate and the F_e-F difference. These

326   results suggest inbreeding depression in selfing individuals followed by genetic purging (Ritland,

327   1990). The latter explanation is supported by the contrasting average values of inbreeding

328   depression found in the two populations of *D. inoxia*, being lower in CM than in Map.

329   Theoretical models and experimental results have demonstrated that mutations that cause strong

330   inbreeding depression can be purged from one generation to the next (Willis, 1999; Charlesworth

331   & Willis, 2009), but mutations that cause mild inbreeding depression and are rare can be

332   maintained in populations for multiple generations (Lande, Schemske & Schultz, 1994;

333   Charlesworth & Willis 2009). The expression of mutations with mild deleterious effects among

334   inbred individuals of *D. inoxia* can help to explain the differences in inbreeding coefficients

335   between progeny and adult generations and its relationship with selfing rate.

336   The magnitude of inbreeding depression has been shown to be higher in more stressful

337   than benign environments (i.e., field vs. greenhouse conditions) (Crnokrak & Roff, 1999;

338   Armbruster & Reed, 2005; Cheptou & Donohue, 2011; Fox & Reed, 2011). Both inbred and

339   outcross individuals may perform better under benign environments, and inbreeding depression

340   expresses only as limit and/stressful conditions (Angeloni, Ouborg & Leimu, 2011). Further, it

341   has been predicted that selection against deleterious mutations occurs mainly during the early life

342   history stages and lowers towards later life cycle stages (Angeloni, Ouborg & Leimu, 2011). In

343   this study the assessment of inbreeding depression under greenhouse could underestimate the

344   magnitude it can reach in natural, stressful conditions, inhabited by *Datura inoxia*. Yet, since we

345   did estimate early-acting inbreeding depression, results seem to be in agreement with expected

346   trends: CM population, a predominantly selfing population, expresses low value of inbreeding

347   depression than Map, possibly due to a faster purge of deleterious alleles.

348   Phenotypic variance in herkogamy results from genetic, developmental and

349   environmental factors (Herlihy & Eckert, 2007; Vallejo-Marín & Barrett, 2009; Camargo et al.,

350   2017). However, the evolution of mating system, linked to floral traits like herkogamy, requires

351   additive genetic variance. Additive genetic variance of corolla length and herkogamy has been

352   detected in populations of the annual *Datura stramonium* (Motten & Stone, 2000; Juárez-

353   Ramírez, 2008; Camargo et al., 2017). Further, a revision of evolvability of herkogamy measured

354   as mean-scaled additive variance, points out the high evolutionary potential of herkogamy

355   compared to male and female sexual organs or flower size (Opedal et al., 2017). Our results,

356   derived from the partition of phenotypic variance in herkogamy among individual plants and

357   random variation (within individual variation or residual term), indicate a large amount of

358   proportional variance between individuals in each population, but notably more so in Mapimí

359   (77.32%) than in Cañada de Moreno (50.62 %). Thus, there is a strong indication that individual

360   variation in average herkogamy in *D. inoxia* in Mapimí is genetically based and potentially

361   adaptive (see Jiménez-Lobato & Núñez-Farfán 2012). High within-individual variation could be

362   adaptive if, on average, high intra-individual variation is linked to higher fitness (Herrera, 2009;

363   Camargo et al., 2017). Otherwise, high intra-individual variation can be maintained in

364   environments that are highly unpredictable in pollinator's service along time. In the Cañada de

365   Moreno population, the high within-plant variation in herkogamy could limit selection on it,

366   constraining an adaptive response in this population (Falconer & MacKay, 1996; Lynch &

367   Walsh, 1998).

368   Within-individual variation in plant traits, particularly in flower characters, can be

369   developmental in origin or elicited in response to environmental variability (Herrera, 2009;

370   Camargo et al., 2017). The stability of development or homeostasis has been associated with

371   different levels of heterozygosity, being heterozygous individuals those which better buffer

372   environmental variation (Lerner, 1954). However, there is not clear consensus on this

373   hypothesis; some evidence points to the potential effect of inbreeding, with the fixation of

374   deleterious alleles and genetic drift influencing individuals' level of developmental stability

375   (Clarke, 1993). To what extent inbreeding and deleterious mutations are responsible for intra-

376   individual flower trait variation in the CM population is not known yet, but evidence suggests

377   some developmental variation in flower size and herkogamy, as shown in one highly inbred

378   population of *D. stramonium*, is linked to environmental variation (Camargo et al., 2017)

379

380   **Conclusions**

381   Associations between herkogamy, mating system and inbreeding history at a lineage level are

382   expected to occur within populations of self-compatible, hermaphroditic plant species. In *D.*

383   *inoxia*, as in other species of *Datura*, approach herkogamy is associated with higher outcrossing

384   rates and low levels of inbreeding in progeny. The results of this study show that populations of

385   *D. inoxia* are diverging in mating system characteristics with important genetic implications.

386 **Acknowledgments**

392

393 **References**

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Table 1(on next page)

Table 1

Table 1. Correlation between primary selfing rate (r), inbreeding coefficient (adult cohort: F), inbreeding coefficient at equilibrium (progeny cohort: Fe), the difference between Fe and F (Fe-F) and herkogamy in two populations of *Datura inoxia* (Cañada de Moreno, and Mapimí)*.* Parameter estimates are shown in the upper diagonal and P support values below the diagonal. Significant values are shown in bold type. Statistical models used in each test are described in the Materials and Methods section.

Cañada de Moreno	r	Herkogamy	F	Fe	Fe-F
r	-	0.023	0.307	**0.982*****	**0.739****
Herkogamy	0.016	-	-0.001	0.014	0.015
F	0.234	0.014	-	na	na
Fe	0.044	0.012	na	-	na
Fe-F	0.239	0.016	na	na	-

Mapimí					
r	-	**-0.029***	**0.572****	**0.854*****	0.283
Herkogamy	0.012	-	-0.006	**-0.009***	-0.004
F	0.154	0.005	-	na	na
Fe	0.042	0.004	na	-	na
Fe-F	0.158	0.005	na	na	-

1 * $P < 0.05$, ** $P \leq 0.005$, *** $P < 0.0005$ indicate significant association between variables.

2

Figure 1

Figure 1

Herkogamy value (average ± 1 SD) of individual plants of *Datura inoxia*. A. Cañada de Moreno and B. Mapimí. Standard deviation is the within-plant variation among flowers.
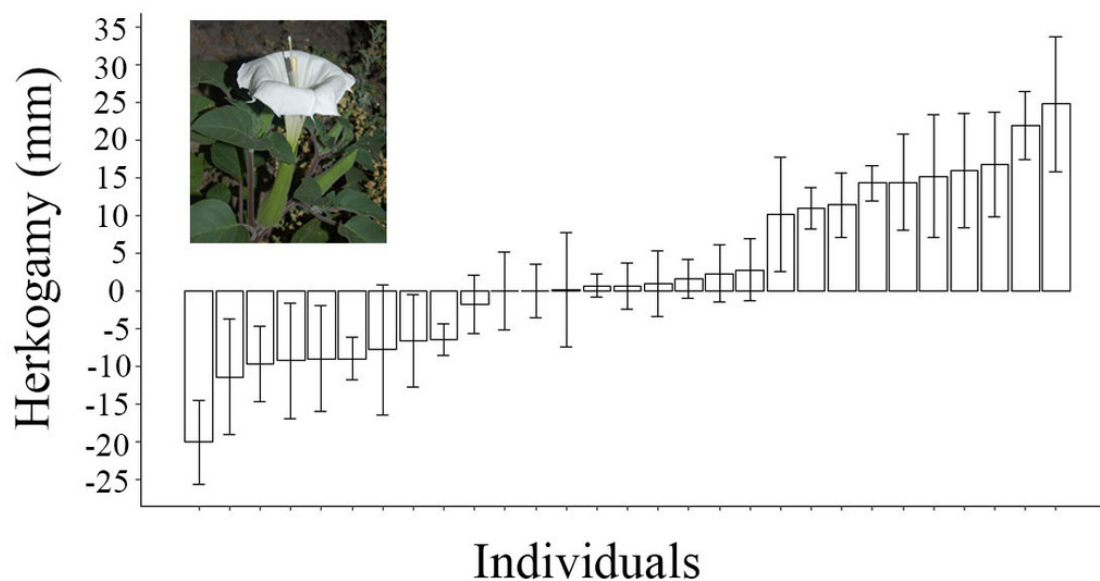
A. Cañada de Moreno

B. Mapimí

PeerJ
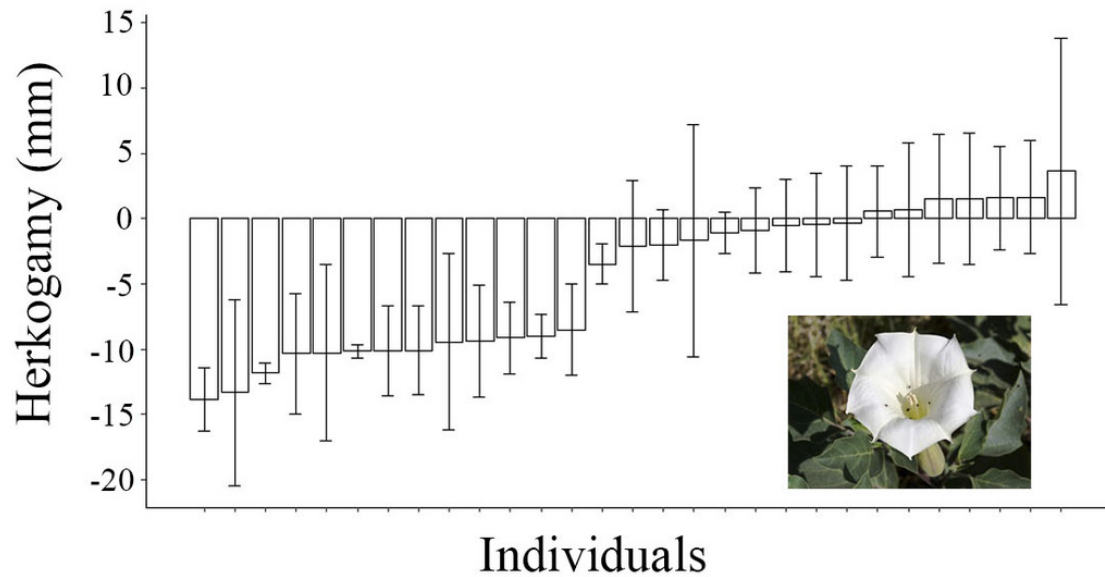
Figure 2

Figure 2

Histogram and boxplot graphs of outcrossing rate (*t*) for two populations of *Datura inoxia* in Mexico: A. Cañada de Moreno and B. Mapimí. Boxplots show quartiles and the median value for each population.
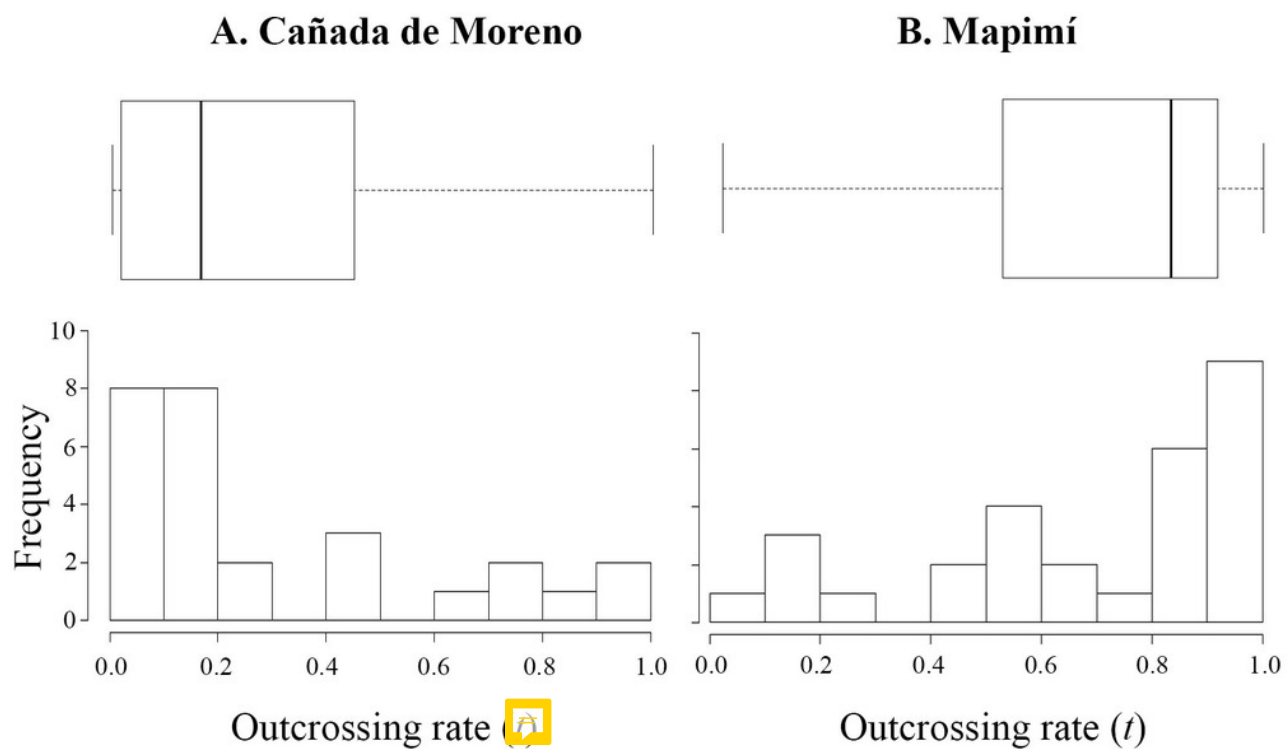
Figure 3

Figure 3

Relations between primary selfing rates (r), inbreeding coefficients estimated in adult cohort (F), and inbreeding coefficients at equilibrium (Fe) (progeny cohort), with herkogamy values in two populations of *Datura inoxia* in Mexico. Fitted model is depicted only for the significant correlation (B).
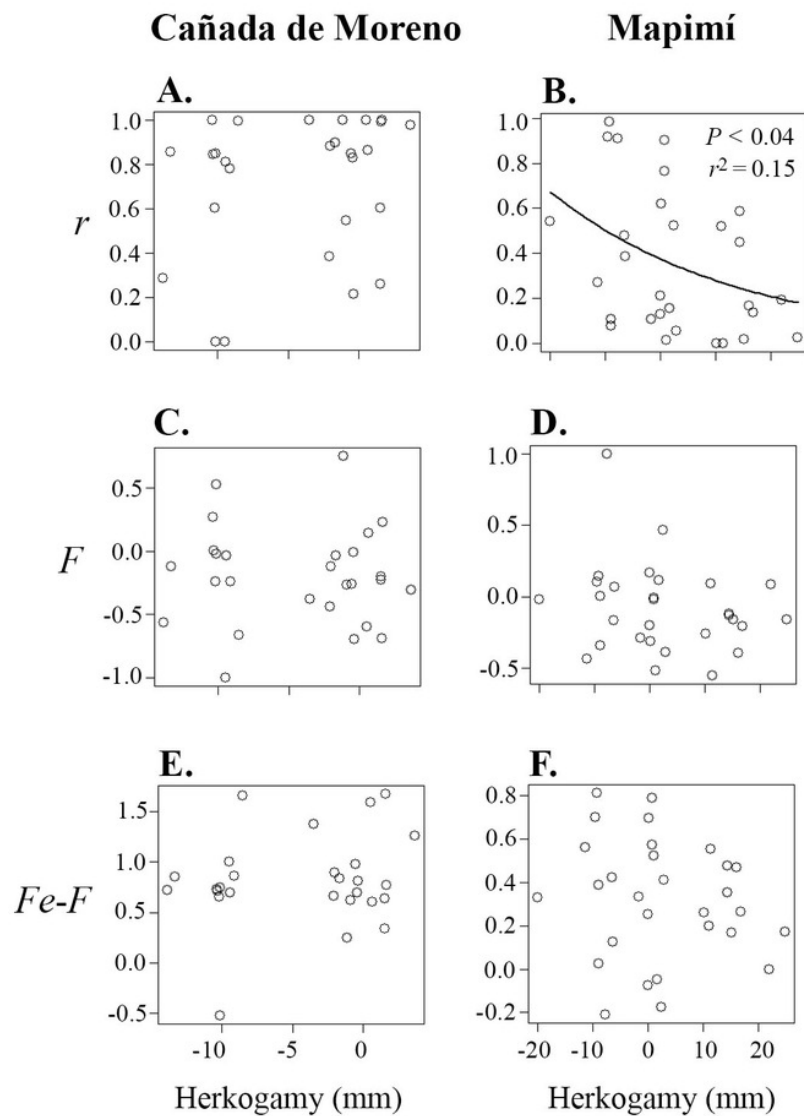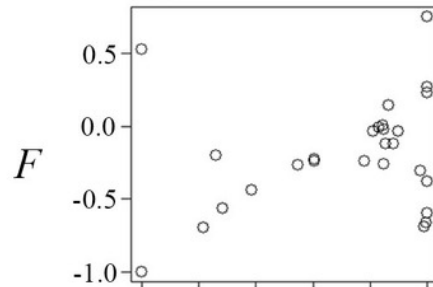
Cañada de Moreno

Mapimí

A.

B.

$P < 0.04$
$r^2 = 0.15$

r

C.

D.

F

E.

F.

Fe-F

Herkogamy (mm)

Herkogamy (mm)

Figure 4

Figure 4

Correlation between inbreeding coefficient (F), and the differences between inbreeding coefficients at equilibrium (Fe) and F (Fe-F), with primary selfing rates (r) in two populations of *Datura inoxia* in Mexico. Fitted model is depicted only for the significant correlations (B and C).
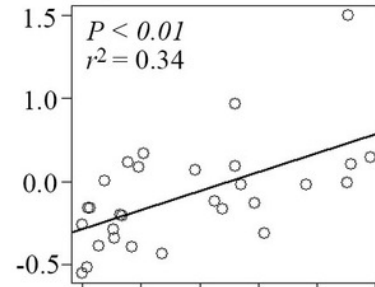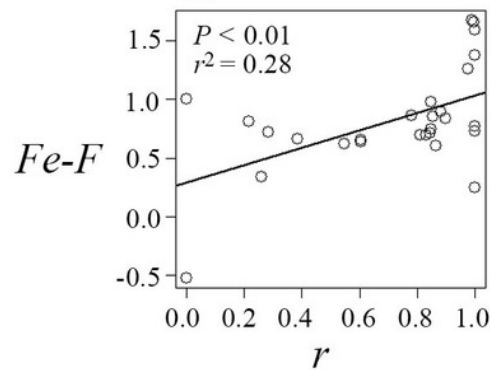
Cañada de Moreno

Mapimí

A.

F

B.

$P < 0.01$
$r^2 = 0.34$

C.

$P < 0.01$
$r^2 = 0.28$

Fe-F

D.

r

r