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 and genetic variance 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 (δ = 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 nil 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 nil or reverse herkogamy leads the evolution of the selfing syndrome following the purge of deleterious alleles

despite high inbreeding among individuals.

1   **Introduction**

2   Plant mating system has important consequences affecting, to a large extent, the demographic

3   and genetic properties of plant populations and, in the long term, their evolutionary potential

4   (Charlesworth & Charlesworth, 1979; Eckert et al. 2009). For the case of self-compatible plant

5   populations, mating systems display wide variation from predominant or complete selfing to

6   complete outcrossing. However, intermediate mating systems, those that combine selfing and

7   outcrossing strategies (i.e. mixed mating systems), are common in nature (Stebbins, 1957;

8   Goodwillie, Kalisz & Eckert, 2005; Barrett, 2010).

9   It is well documented that the transition from outcrossing to selfing is one of the more

10 frequent evolutionary shifts in flowering plants (Stebbins, 1957; Barrett, 2010; Sicard &

11 Lenhard, 2011). Evidence of this evolutionary outcome spans from phylogenetic studies

12 (Goodwillie, 1999; Foxe et al., 2009), between populations within species variation, and at

13 different geographic scales (Duncan & Rausher, 2013; Wright, Kalisz & Slotte, 2013). The

14 mating system transition of outcrossing to selfing is predicted by two main hypotheses. One is

15 the transmission advantage of selfers relative to outcrossers (3:2 genomes), that would promote

16 the expansion of selfing modifiers in a population unless selection prevents this (Fisher, 1941).

17 The second hypothesis considers that under unfavorable ecological conditions for cross-

18 pollination, i.e., low abundance or absence of potential mates and/or pollen vectors, natural

19 selection would favor reproductive assurance through selfing (Baker, 1955; Stebbins, 1957;

20 Schoen & Lloyd, 1992; Schoen, Morgan & Bataillon, 1996). Nevertheless, despite the potential

21 advantage of selfing, it may restrict gene flow within and among populations producing high

22 levels of inbreeding between individuals and increasing homozygosity (Ho), and thus the

23 likelihood of inbreeding depression (δ) (Charlesworth & Charlesworth, 1987; Charlesworth &

24   Wright, 2001; Takebayashi & Morrell, 2001). For these reasons, it is expected that the

25   inbreeding coefficient (F) would covariate positively with the level of selfing. In the long term,

26   however, theoretical models and empirical evidence predict that increasing inbreeding would

27   promote selection against inbred individuals, purging the populations of lethal or deleterious

28   alleles thus reducing genetic load (Husband & Schemske, 1996; Takebayashi & Delph, 2000;

29   Crnokrak & Barrett, 2002; Morran, Parmenter & Phillips, 2009). Populations with a long history

30   of inbreeding would suffer low levels of inbreeding depression in the face of high levels of

31   inbreeding.

32   Herkogamy, the spatial segregation of sex organs within the flower, is considering a main

33   floral trait affecting selfing rate (Webb & Lloyd, 1986). In hermaphroditic flowers, the likelihood

34   of selfing is reduced when the stigma surpasses the length of the anthers (i.e. approach

35   herkogamy), preventing contact with its own pollen; this opens the opportunity for outcross

36   pollen to first contact the stigma (Lloyd & Webb, 1986; Webb & Lloyd, 1986). On the other

37   hand, when anthers and stigma are placed at the same spatial level (i.e. no - herkogamy) or the

38   stigmas are below the anthers (i.e. reverse herkogamy), autonomous or facilitated selfing is likely

39   to occur (Lloyd, 1992). Nevertheless, reverse or nil herkogamy, by increasing reproductive

40   components of plant fitness (*i.e.*, seeds), would covariate with selfing rate and be favored by

41   selection. Contrary, natural selection would increase/maintain approach herkogamy promoting

42   higher outcrossing rates (Motten & Stone, 2000; Elle & Hare, 2002; Herlihy & Eckert, 2007).

43   Herkogamy is determined by genetic and ecological factors (Ashman & Majetic, 2006;

44   Oøpedal et al., 2017). Within population, standing genetic variation in herkogamy would depend

45   on the history of selection of individual lineages. If selective pressures exerted by the community

46   of pollinators fluctuate between years, variation in herkogamy between lineages would be

47   maintained, producing differences in selfing rate and variation in inbreeding among individual

48   plants. When these associations are maintained through time, it would be reasonable to expect

49   that selfing rate (s), inbreeding coefficient (F) and herkogamy coevolve at the lineage level.

50   Significant association between high outcrossing rate and approach herkogamy have been

51   reported among populations (Takebayashi, Wolf & Delph, 2006; de Vos et al., 2018; but see

52   Chen et al., 2009; Brys & Jacquemyn, 2012; Oøpedal, Armbruster & Pélabon, 2015; Toräng et

53   al., 2017). However, few studies have evaluated the within-population association between

54   herkogamy and outcrossing rate (Karron et al., 1997; Brunet & Eckert 1998; Medrano,

55   Takebayashi & Delph, 2000; Herrera & Barret, 2005) and none with inbreeding coefficient (F) at

56   a lineage level.

57   Annual, self-fertilizing, plant species would evolve in heterogeneous environments (e.g.

58   disturbed and/or arid habitats) where pollinator communities are unpredictable (Friedman &

59   Rubin, 2015). Under high environmental heterogeneity (i.e., in pollination service), daily and

60   seasonal fluctuations represent different opportunities for cross- or self-fertilization producing

61   variable selection on traits that affect the plants' mating system, such as herkogamy or the

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

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

64   Hamrick, 2014; Pannell, 2015). However, it is not quite clear how strong and how frequent the

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

66   within populations in short-lived species. Here, we assessed the extent of these associations in

67   each of two populations of the annual plant *Datura inoxia* Mill.

68   *Datura inoxia* (Solanaceae) is an annual self-compatible plant that inhabits arid and semi-

69   arid lands in Mexico and Southern USA (i.e. The Chihuahuan desert). Characteristic of these

70   ecosystems is the high daily fluctuation of ambient temperature. *Datura inoxia* produces large,

71   nectar producing, tubular flowers that open at dusk and remain receptive one night only. Genetic

72   differentiation at neutral loci (i.e., genetic structure) and phenotypic differentiation in floral traits

73   related to plants' mating system have previously been documented among and within

74   populations, suggesting adaptive evolution (Jiménez-Lobato & Núñez-Farfán, 2012). Flowers are

75   commonly visited by honeybees and hawkmoths whose activity mainly depends on air

76   temperature (Barclay, 1959). The wide daily range of temperature in populations generates an

77   unpredictable pollinator activity that coupled with variation in herkogamy suggest correlated

78   selection on herkogamy, selfing rates and inbreeding history within populations.

79

80   **Materials and Methods**

81   *Species and sampled populations*

82   *Datura inoxia* Mill. (Solanaceae) is a summer annual herbaceous plant native to Mexico and

83   southwestern USA (Barclay, 1959). It occurs in xeric environments, mainly along the

84   Chihuahuan desert (Barclay, 1959; Lockwood, 1973). The onset of flowering of *D. inoxia* is in

85   July and lasts up to September as others *Datura* species in this region (Bronstein et al., 2009).

86   *Datura inoxia* produces large, funnel-shaped, hermaphroditic and self-compatible white flowers

87   (Barclay, 1959). The flowers, that live one night only, open at dusk when both anthesis and

88   receptivity of stigma occur. During the flowering period, individuals can display from few up to

89   tens of flowers each night. Flower traits related to mating system, herkogamy and flower size,

90   display broad variation within and among populations (Jiménez-Lobato & Núñez-Farfán, 2012).

91   The community of pollinators include hawkmoths (*Manduca sexta*, *M. quinquemaculata*, and

92   *Hyles lineata*) (Grant, 1983) who forage on nectar, and honeybees that collect pollen (Lockwood,

93   1973; McCall et al. 2018; V. Jiménez-Lobato, pers. Obs.). Hawkmoths visit flowers at dusk

94   when night temperatures reach more than 24°C (pers. Obs.), while honeybees visit the flowers in

95   the afternoon even if these are still closed, and early in the morning before flowers wilt and fall

96   (McCall et al., 2018).

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

98   rate, herkogamy and inbreeding coefficient (*F*) at the lineage level. Collection of plant material

99   (e.gr., seeds) for experimental analyses, described below, was made under the permission SGPA-

100   DGGFS-712-1596-17 (Subsecretaría de Gestión para la Protección Ambiental, Secretaría de

101   Medio ambiente y Recursos Naturales, Mexico). Previous results indicate that these two

102   populations possess ample individual variation in herkogamy and in flower size-related traits

103   (Jiménez-Lobato & Núñez-Farfán, 2012). Cañada de Moreno (CM) is located in the State of

104   Querétaro (21° 17' 43"; 100° 31' 00") in the Mexican Bajío at 1933 m a.s.l. During the

105   flowering period of *D. inoxia* (July to September), this locality has an average temperature of

106   18.8° C, with a daily range from 7.4 °C to 30.8 °C, and a three-month total precipitation of 314

107   mm. The second population, Mapimí (Map), is located at 1157 m a.s.l. in the Mexican Plateau in

108   the States of Coahuila and Durango (26° 41' 11"; 103° 44' 49"). This is a more xeric

109   environment, with a quarterly average temperature of 22.8°C (range: 12.6°C to 32.9°C) and a

110   total three-month precipitation of 253 mm. Because flight activity of hawkmoths and honeybees

111   is dependent on ambient temperature, the wide fluctuation in daily temperature at both sites

112   suggests a heterogeneous and unpredictable pollinator service along the flowering season.

113   *Herkogamy variation within populations*

114   Within each population, in an area of 1 ha, 30 reproductive plants were randomly selected and

115   tagged for sampling. In each individual plant, 4 - 6 open flowers were selected to measure

116   herkogamy. Herkogamy was measured as length of pistil – length of stamens. Approach

117   herkogamy implies that the pistil surpasses stamens length while in reverse herkogamy the

118   opposite occurs. Absence of herkogamy occurs when pistil and stamens overlap. To estimate

119   phenotypic variation in herkogamy in each population, we obtained the variance component

120   among and within individuals. Individual plants were considered a random effect. The residual

121   variance is accounted by the intra-plant variation. The significance of individuals was evaluated

122   by a likelihood-ratio χ^2 test (LRT) between a model that includes individuals as random effect

123   (function lme) and one that does not (LRT1; function gls) (Zuur et al., 2009). Analyses were

124   conducted with nlme package (Pinheiro et al., 2017) in R software (R Development Core Team,

125   2008).

126   *Mating system parameters*

127   In order to estimate mating system parameters from each marked plant in the field, five mature

128   fruits derived from natural pollination were collected, labelled and bagged. In the laboratory,

129   seeds of each fruit were separated and germinated in the glasshouse; seeds of each fruit within

130   each family (maternal plant) were sowed separately. Germination per fruit, per family, was

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

132   the final number of seeds germinated in each pot. Germination percentage was ≥ 90% for all

133   plants. Once seedlings emerged, at least five seedlings per fruit, and 25 seedlings per maternal

134   plant (i.e., family) were collected for further genetic analysis. We collected leaf tissue from

135   young plants, bagged, labelled, and stored in an ultra-freezer at -97°C. Finally, we analyzed 20

136   seedlings per each of 30 maternal families per population (N = 600).

137   Seedlings' DNA was extracted following the Miniprep protocol (Doyle & Doyle, 1987).

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

139   each seedling. Further, we standardized one additional microsatellite locus for *D. inoxia* (F8:

140   Rw: 5´ -GGACAACATCTTTGCGACCC- 3´) (Supplementary Information), in order to obtain a

141   total of six polymorphic microsatellite loci per individual. Primers were labelled with PET, VIC,

142   6-FAM, and NED dyes (Applied Biosystems) (PCR protocols are shown in Supplementary

143   Information).

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

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

146   system parameters (t_m and s) at the family level were calculated using MLTR 3.2 software

147   (Ritland, 2002), with an Expectation-Maximization method (EM) that allows missing data and

148   undetected null alleles (Ritland & Jain, 1981). Standard errors and standard deviation were

149   estimated by bootstrapping, with 1000 replicates and re-sampling individuals at family level. The

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

151   (Cock et al., 2004). Since one locus (G8) did not amplified for plants of Cañada de Moreno,

152   analyses were carried out with five loci, and six loci for Mapimí. Selfing rates (s) obtained from

153   molecular markers, after fertilization and germination, might not be completely independent

154   from inbreeding depression and thus may underestimate its true value (Lande, Schemske &

155   Schultz, 1994). Primary selfing rate (r) is a better predictor of mating system because it assesses

156   separately the magnitude of inbreeding depression. Hence, r is referred as the proportion of

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

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

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

160   obtained for each population (see below).

161   The inbreeding coefficient (F) may include components of inbreeding other than mating

162   system, such as biparental inbreeding or population substructure. Here, we use this parameter as

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

164   1994). $F's$ were inferred with GenePop v.4.2 per lineage from the loci amplified previously

165   (Rousset & Raymond, 1995; Rousset, 2008). In order to infer whether inbreeding depression is

166   associated with the selfing rate and herkogamy, we calculated inbreeding coefficients at

167   equilibrium (F_e) (i.e., progeny cohort) at each lineage, assuming that adult F and t_m are constant

168   among generations (Ritland, 1990). We then related these differences with selfing rate and

169   herkogamy. Since F_e increases in relation to F due to self-fertilization, differences between F

170   and F_e at each maternal lineage would indicate the presence of higher inbreeding depression.

171   Once selection against inbred progeny occurred, F_e and F will be equal (Ritland, 1990; Shirk &

172   Hamrick 2014). $F_e's$ were calculated as $F_e = (1-t_m) / (1+t_m)$, where t_m is the outcrossing rate

173   calculated from MLTR for each maternal family (Ritland, 1990).

174   Multiloci outcrossing (t_m) and primary selfing rates (r) are highly and inversely correlated

175   (CM: estimated = -1.00, $p = 0.000$, d.f. = 25; MAP: estimated = -0.969, $p = 0.000$, d.f. = 27),

176   hence we present the analyses only for primary selfing rates (r). Since r is a proportion with

177   binomial distribution, correlation with herkogamy was fitted by a generalized linear model with a

178   quasi-binomial distribution error (Crawly, 2013). F, F_e and their differences were associated to

179   herkogamy and r with a generalized linear model with gaussian distribution error (Crawly,

180   2013). All analyses were implemented in R package (R Development Core Team 2008).

181   *Inbreeding depression*

182   To estimate the magnitude of inbreeding depression (δ), we collected 150 fruits from different

183   individual plants in each population, including the maternal families previously analysed. From

184   each fruit, we sowed ten seeds on separated pots under greenhouse conditions. When seeds

185   germinated, only one seedling per fruit was randomly chosen and grown under controlled

186   conditions until reproduction. For each population, 100 individuals were randomly chosen to act

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

188   pollination treatments were applied to each maternal receptor. In the first treatment, cross-

189   pollination (*o*), two flowers were emasculated before anthesis and hand-fertilized with pollen of

190   one donor randomly chosen from the same population. In the second, self-pollination treatment

191   (*s*), two flowers of each receptor plant were fertilized with self-pollen. After pollination, flowers

192   of both treatments were bagged individually with a fine nylon mesh. Since many mother plants

193   did not produce the four flowers needed to apply both treatments, the final sample include

194   mother plants that produced at least one fruit per treatment (CM: N = 77, Map: N = 41). Two

195   components of fitness per pollination treatment were evaluated in each population, the mean of

196   seed-set (*i.e.* number of seeds/ number of ovules), and the seed mass. Seed mass was obtained for

197   a random sample of 30 seeds per fruit in an analytical balance (Adventurer OHAUS).

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

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

200   pollination, respectively. Average fitness of self- and out-cross progenies is the product of seed-

201   set and seed mass (Schemske & Lande, 1985). This value was used to estimate the primary

202   selfing rate (*r*) at each maternal lineage.

203

204   **Results**

205   *Variation in herkogamy between populations of Datura inoxia*

206   The population average of herkogamy in Cañada de Moreno was negative (mean = -4.72; sd =

207   5.5 mm) with a range of 17.48 mm (from -13.83 to +3.65 mm). In this population 20 out of 27

208   individual plants (74%) had reverse or nil herkogamy and 7 (26%) showed approach herkogamy

209   (Fig. 1A). In contrast in Mapimí population (N=29) mean herkogamy was positive and more

210   variable than in Cañada de Moreno (Mean = 2.57 mm, sd = 11.15 mm; range = 44.8mm (from -

211   20 to +24.8 mm) (Fig. 1B).

212   The proportion of variance in herkogamy explained by the among individual plants term

213   was higher in Mapimí (77.32%) than in Cañada de Moreno (50.62%), indicating higher intra-

214   individual variation in the latter population (Map: 22.68%; CM: 49.38%). The likelihood ratio χ^2

215   test (LRT) indicate significant variation between individual plants in each population (Map: L =

216   105.04, df = 2, P <0.0001; CM: L = 38.56, df = 2, P <0.0001).

217   *Mating system and inbreeding coefficient* (F)

218   The multi-loci outcrossing rate (t_m) was, on average, higher in Mapimí than in Cañada de

219   Moreno (0.682 *vs* 0.294, respectively) although high variation in this parameter was detected

220   among lineages in each population (Mapimí, from 0.022 up to 1; Cañada, from 0 up to 1). The

221   distribution of t_m is skewed to low values in Cañada de Moreno (ca. 60% of mother plants) but to

222   high values in Mapimí (50% of the families with t_m > 0.8) (Fig. 2). Primary selfing rate (r) was

223   more than two-fold higher in Cañada de Moreno than in Mapimí (0.716 vs. 0.353), varying from

224   r = 0 to 1 and from r = 0 to 0.978, respectively.

225   Inbreeding coefficients of adult cohorts (F) were negative in the two populations and

226   highly variable (Cañada: F = -0.193, from -1 to +0.75; Mapimí: F = -0.085, from -0.553 to +1).

227   Inbreeding coefficients at equilibrium (F_e) was higher in Cañada than Mapimí (F_e = 0.626, F_e =

228   0.238, respectively) and, again, highly variable in both populations (from 0 to 1). The differences

229   between F and F_e was much higher in Cañada Moreno ($F_e - F = 0.819$) than in Mapimí ($F_e - F =$

230   0.323). Cumulative inbreeding depression estimated the average product of seed-set and seed

231   mass was higher in Mapimí than in Cañada ($\delta = 0.25$ vs. $\delta = 0.09$, respectively).

232   *Relationships between mating system parameters and herkogamy*

233   The relationship between primary selfing rate (r), inbreeding coefficient (F), inbreeding

234   coefficient at equilibrium (F_e) and herkogamy within each population were only significant in

235   the Mapimí population (Table 1, Fig. 3). In Mapimí, r and F_e were negatively associated with

236   herkogamy (Table 1; Fig. 3B, F), indicating that individuals with nil or reverse herkogamy had

237   progenies with higher selfing rate and inbreeding coefficient than plants with approach

238   herkogamy. As was expected, progenies' inbreeding coefficient (F_e) increases positively with

239   primarily selfing rate (r) at both populations (Table 1; Fig. 4C, D). In addition, selfing is also

240   linked to the breeding history of each maternal lineage (F) only in Mapimí (Fig. 4A, B) but not

241   with herkogamy in the two populations (Table 1; Fig. 3C, D). The difference $F_e - F$ is positively

242   related to primary selfing rate (r) only in Cañada Moreno (Table 1; Fig. 4E), and statistically

243   unrelated to herkogamy in the two populations (Table 1; Fig. 4G, H).

244

245   **Discussion**

246   In annual, short-lived, plant species inhabiting heterogeneous environments, the evolution of

247   plant mating system in association with flower traits, such as herkogamy, depends on the

248   variation of natural selection within populations, and on the opportunity to cross and self-

249   fertilization every year (Shirk & Hamrick 2014). If some associations among traits and mating

250   strategies are favored through time, it is expected to find that different lineages within

251   populations would vary in their history of inbreeding. In this work we evaluated the association

252   among herkogamy, mating system and inbreeding history at a lineage level within two

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

254   environments in Mexico and North America.

255   We found that herkogamy, selfing rates, inbreeding coefficients as well as their

256   associations varied considerable within the two populations. In line with expectations, in one

257   population (Mapimí), plants with more pronounced approach herkogamy show, on average,

258   higher outcrossing rates and less inbreeding coefficient of progenies, than those individuals that

259   exhibited nil or reverse- herkogamy. These results are in line with the hypothesis of adaptive

260   herkogamy as a mechanism that prevents selfing in populations and avoiding inbreeding between

261   individuals (Web & Lloyd 1986; Lloyd 1992). Relationship between selfing/outcrossing rate and

262   herkogamy has been found in other species of *Datura* such as *D. stramonium* (Motten &

263   Antonovics, 1992; Motten & Stone, 2000) and *D. wrightii* (Elle & Hare, 2002) but also in other

264   species like *Gilia achilleifolia* (Takebayashi & Morrell, 2001), *Clarkia temblorensis* (Holtsford

265   & Ellstrand, 2006) and *Mimulus ringens* (Karron et al., 1997). However, we did not detect a

266   significant association between inbreeding coefficients of adults' cohort with herkogamy. This

267   suggests that the inbreeding history at each lineage not only depends on herkogamy; instead,

268   purge of inbred individuals and/or biparental inbreeding may also come into play.

269   Fluctuation of environmental variables but mainly pollinator availability, as it occurs in

270   Mapimí, can contribute to maintain variation in herkogamy within populations. Variation in

271   herkogamy may be linked with plants' reproductive assurance when pollinators are scarce, or

272   with high outcrossing rates when abundant (Kalisz, Vogler & Hanley, 2004; Goodwillie, Kalisz

273   & Eckert, 2005; Chen et al., 2009). Since outcrossing rates in Mapimí are related to herkogamy,

274   it is likely that episodes of differential (or even contrasting) selection on herkogamy among

275   lineages every year, could sometimes favor either outcrossing (approach herkogamy) or selfing

276   (nil or reversal herkogamy). This phenotypic variance in herkogamy could explain the

277   maintenance of the mixed mating system of *D. inoxia* in Mapimí.

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

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

280   coefficients in the adult cohort were detected. Nevertheless, like the Mapimí population, there is

281   a positive relationship between selfing rate with inbreeding coefficients in the progenies, and

282   with the F vs. F_e difference. These results suggest inbreeding depression in selfing individuals

283   and then genetic purging (Ritland, 1990). This result is supported by the contrasting average

284   value of inbreeding depression in the two populations of *D. inoxia*. Theoretical models and

285   experimental results have demonstrated that mutations of large effect causing inbreeding

286   depression can be purged from one to another generation (Willis, 1999; Charlesworth & Willis,

287   2009). However, mild in effect and rare mutations are responsible of inbreeding depression and

288   can be maintained in populations for multiple generations ((Lande, Schemske & Schultz, 1994;

289   Charlesworth & Willis 2009). The expression of mutations with mild deleterious effect in inbred

290   individuals of *D. inoxia*, would explain the differences of inbreeding coefficients between

291   progeny and adult generations and the relationship with selfing rate.

292   Phenotypic variance in herkogamy results from genetic (Herlihy & Eckert, 2007),

293   developmental (Vallejo-Marín & Barrett, 2009), and environmental factors, such as soil

294   nutrients, as demonstrated in *Datura stramonium* (Camargo et al. 2017). However, the evolution

295   of mating system, linked to floral traits like herkogamy, requires additive genetic variance. A

296   revision of evidence points out the potential evolution by selection of herkogamy owing to the

297   presence of additive genetic variance (heritability) and genetic additive variation (evolvability)

298   (see revision in Oøpedal et al., 2017). Our results, derived from the partition of phenotypic

299   variance in herkogamy amomg individual plants and random variation (within individual

300   variation or residual term), indicate a large amount of variance between individuals in each

301   population, but notably more so in Mapimí (77.32%) than in Cañada de Moreno (50.62 %).

302   Thus, there is a strong indication that individual variation in average herkogamy in *D. inoxia* in

303   Mapimí is genetically based and potentially adaptive (see Jiménez-Lobato & Núñez-Farfán

304   2012). High within-individual variation could be adaptive if, on average, high intra-individual

305   variation is linked to higher fitness (Herrera, 2009; Camargo et al. 2017). Otherwise, high intra-

306   individual variation can be maintained in highly heterogeneous environments (i.e., pollination

307   likelihood by animals) through time. In Cañada de Moreno the high within-plant variation in

308   herkogamy could limit selection on it, constraining an adaptive response in this population

309   (Falconer & MacKay, 1996; Lynch & Walsh, 1998).

310   The within-individual variation in plant traits, but particularly in flowers, can originates

311   during development or can be an elicited response to environmental variability (Herrera, 2009).

312   The stability of development or homeostasis has been associated with different levels of

313   heterozygosity, where heterozygous individuals are more stable than their homozygous (Lerner,

314   1954). However, there is not clear consensus on this hypothesis; some evidence points out the

315   potential effect of inbreeding, the fixation of deleterious alleles and genetic drift influencing the

316   level of developmental stability of individuals (Clarke, 1993). To what extent inbreeding and

317   deleterious mutations are responsible of intra-individual variation in CM is not known as yet, but

318   evidence suggests such kind of developmental variation as shown in one highly inbred

319   population of *D. stramonium* (Camargo et al. 2017)

320

Conclusions

322   Association between herkogamy, mating system and inbreeding history at a lineage level

323   is expected to occur within populations in self-compatible, hermaphroditic plant species.

324   Similarly, as environments do vary, particularly in pollination service by animals, variations in

325   trait values and their correlations are also expected. In *D. inoxia*, as in other species of *Datura*,

326   approach herkogamy is associated to outcrossing rates and to low levels of inbreeding in the

327   progenies. Results of this study show that populations of *D. inoxia* are diverging in mating

328   system with genetic consequences.

329

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336

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Table 1

Statistical results of correlation analyses among primary selfing rates (r), inbreeding coefficients (F), inbreeding coefficients at equilibrium (Fe), differences between Fe and F (Fe-F) and herkogamy (herk) values for two populations of *Datura inoxia.* The statistical models used in each test are described in the Methods section.

Variables	Estimate	Std. error	*t*-value	Estimate	Std. error	*t*-value
r vs **herk**	-0.053	0.025	-2.145*	0.062	0.056	1.112
F vs **herk**	-0.006	0.005	-1.118	-0.001	0.014	-0.096
Fe vs **herk**	-0.009	0.004	-2.194*	0.014	0.012	1.158
F vs r	0.572	0.154	3.71**	0.307	0.234	1.312
Fe vs r	0.854	0.042	20.242***	1.046	0.044	23.701***
(Fe–F) vs r	0.283	0.158	1.779	0.739	0.239	3.094**
(Fe–F) vs **herk**	-0.004	0.005	-0.821	0.015	0.016	0.959

1   * $P < 0.05$, ** $P \leq 0.005$, *** $P < 0.00005$ indicate significant association between variables.

Figure 1

Figure 1

Distribution of the herkogamy values at individual level in two populations of *Datura inoxia* in Mexico. A. Cañada de Moreno, B, Mapimí. Bars represent the standard deviation within each individual.
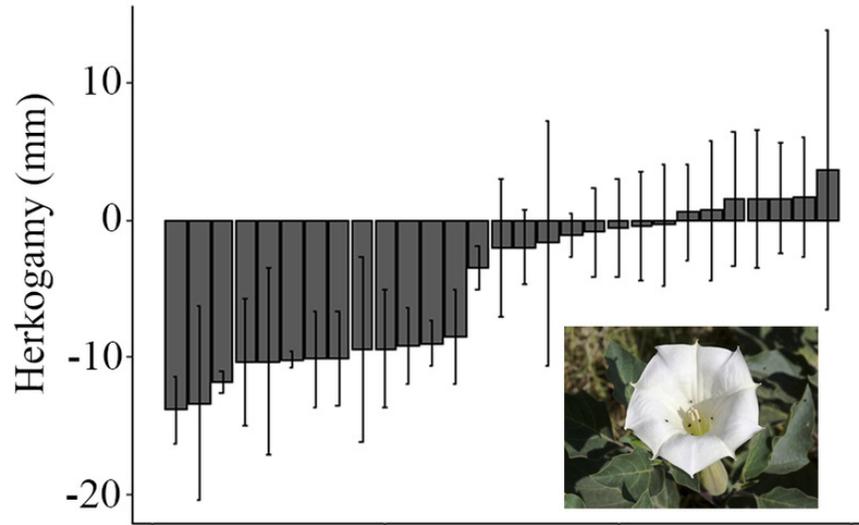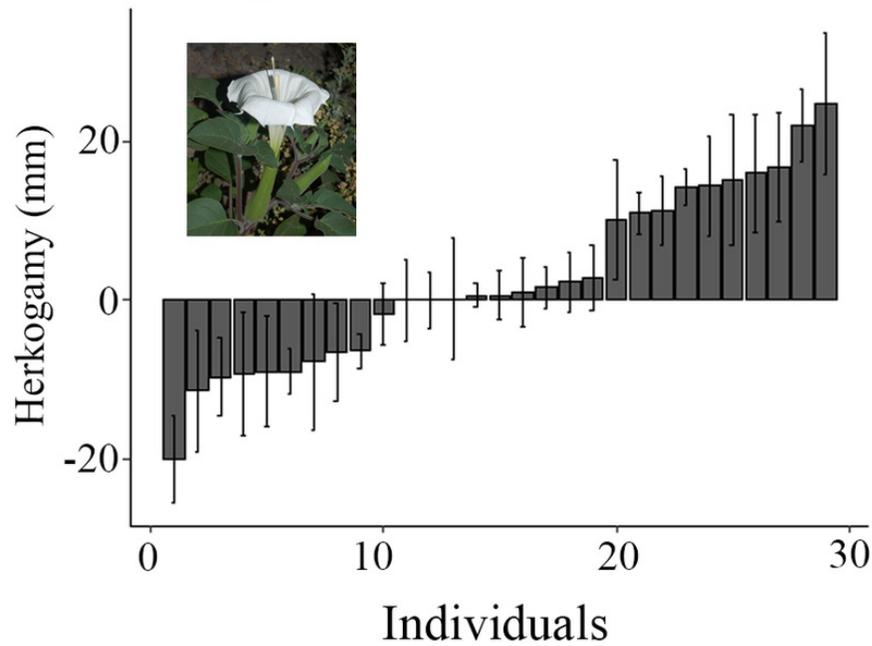
Figure 2

Figure 2

Distribution of outcrossing rates at maternal lineage level within two populations of *Datura inoxia* in Mexico. Boxplots with quartiles and the mean value are shown for each population.
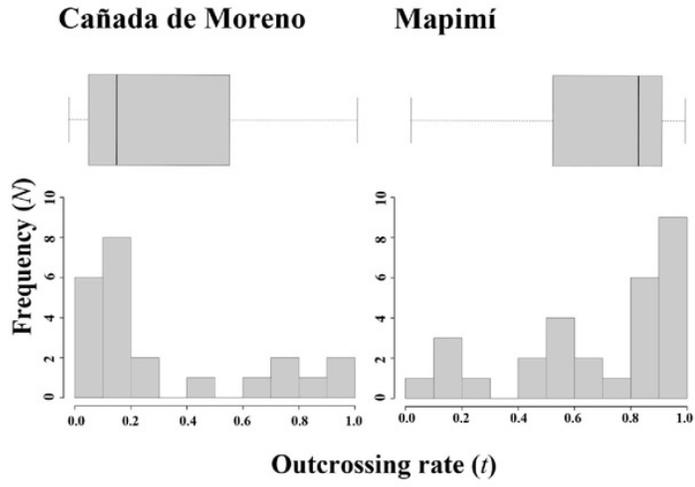
Figure 3

Figure 3

Correlations between primary selfing rates (*r*), inbreeding coefficients (*F*), inbreeding coefficients at equilibrium (*Fe*) and the differences between *Fe* and *F* (*Fe-F*) with herkogamy values in two populations of *Datura inoxia* in Mexico. *P* values are shown only for significant results. Not significant (*n.s.*).
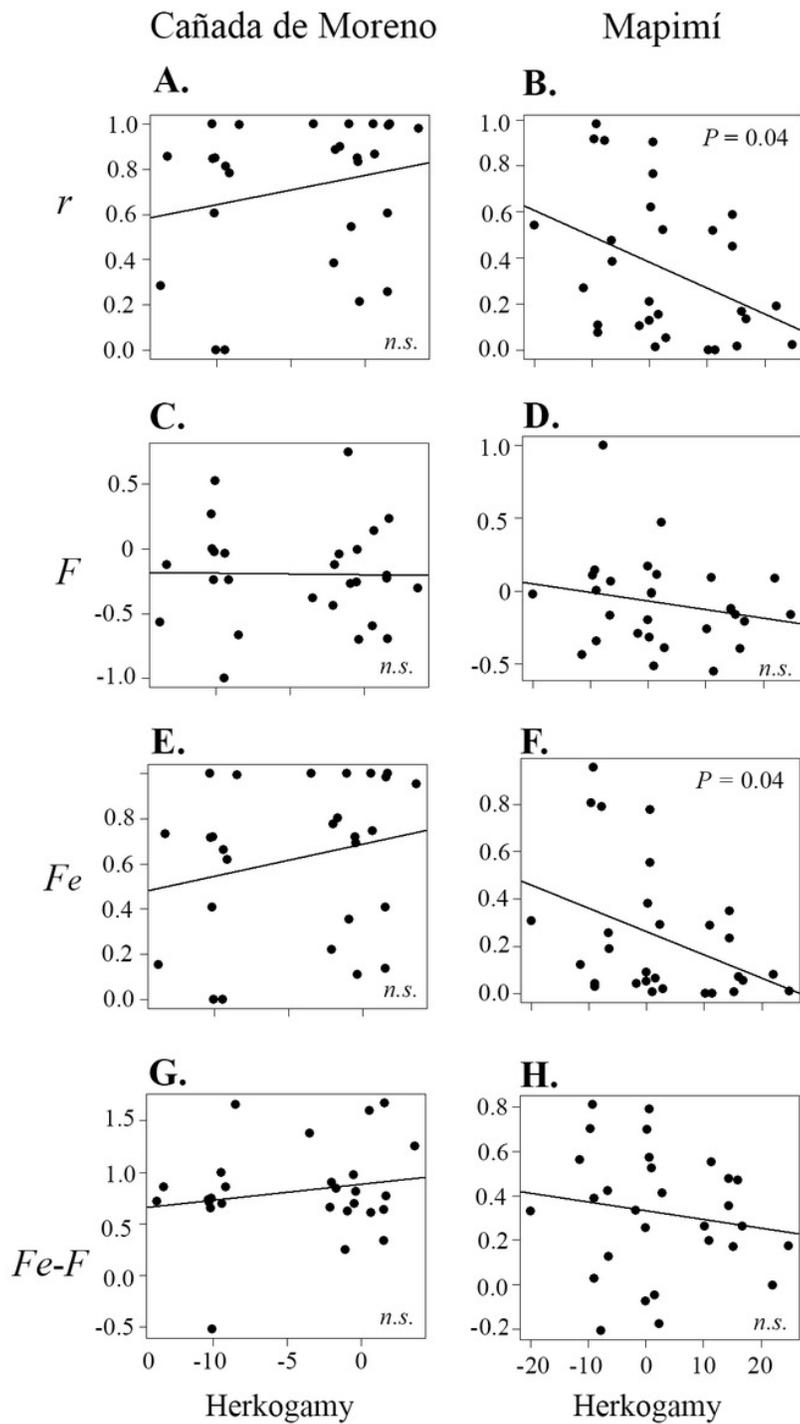
Figure 4

Figure 4

Correlations between inbreeding coefficients (*F*), inbreeding coefficients at equilibrium (*Fe*) and differences between *Fe* and *F* (*Fe-F*) with primary selfing rates (*r*) in two populations of *Datura inoxia* in Mexico. *P* values are shown only for significant results. Not significant (*n.s.*).