Introduction

Plant mating systems have important consequences affecting, to a large extent, the demographic and genetic properties of plant populations and, in the long term, their evolutionary potential (Charlesworth & Charlesworth, 1979; Eckert et al. 2009). For the case of self-compatible plant populations, there is a wide variation in mating systems from predominant or complete selfing to complete outcrossing. However, intermediate (or mixed) mating systems, i.e., those that combine selfing and outcrossing strategies, are common in nature (Stebbins, 1957; Goodwillie, Kalisz & Eckert, 2005; Barrett, 2010).

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It is well documented that the transition from outcrossing to selfing is one of the most frequent evolutionary shifts in flowering plants (Stebbins, 1957; Barrett, 2010; Sicard & Lenhard, 2011). Evidence of this phenomenon comes from phylogenetic studies (Goodwillie, 1999; Foxe et al., 2009) that investigated inter-populational differences within species variation and at different geographic scales (Duncan & Rausher, 2013; Wright, Kalisz & Slotte, 2013). The mating system transition from outcrossing to selfing may be explained by two main hypotheses. One is the transmission advantage of selfers relative to outcrossers (3:2 genomes), that would promote the expansion of selfing modifiers in a population unless selection prevents this (Fisher, 1941). The second hypothesis considers that under unfavorable ecological conditions for cross-pollination, i.e., rarity or absence of potential mates and/or pollen vectors, natural selection would favor reproductive assurance through selfing (Baker, 1955; Stebbins, 1957; Schoen & Lloyd, 1992; Schoen, Morgan & Bataillon, 1996). Nevertheless, despite the potential advantage of selfing, it may restrict gene flow within and among populations producing high levels of inbreeding between individuals and increasing homozygosity (*Ho*), and thus the likelihood of inbreeding depression (δ) (Charlesworth & Charlesworth, 1987; Charlesworth & Wright, 2001;

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Takebayashi & Morrell, 2001). Therefore, it is expected that the inbreeding coefficient (*F*) would covariate positively with the level of selfing in a population. In the long term, however, theoretical models and empirical evidence suggest that increasing levels of inbreeding promote selection against inbred individuals by purging the populations of lethal or deleterious alleles, thus reducing genetic load (Husband & Schemske, 1996; Takebayashi & Delph, 2000; Crnokrak & Barrett, 2002; Morran, Parmenter & Phillips, 2009). Hence, it is expected that populations with a long history of inbreeding suffer from low levels of inbreeding depression.

Herkogamy, the spatial segregation of sex organs within the flower, is considered a main floral trait affecting selfing rates (Webb & Lloyd, 1986). In hermaphroditic flowers, the likelihood of selfing is reduced when the stigma surpasses the length of the anthers (i.e., approach herkogamy), because flower stigmas are prevented from receiving self-pollen; this opens the opportunity for outcross pollen to first contact stigmas instead (Lloyd & Webb, 1986; Webb & Lloyd, 1986). Contrastingly, when anthers and stigmas are placed at the same spatial level (i.e. no - herkogamy) or the stigmas are below the anthers (i.e. reverse herkogamy), autonomous or facilitated selfing is likely to occur (Lloyd, 1992). Reverse or nil herkogamy might increase reproductive fitness (*i.e.*, seed number) and be favored by selection, but it should also positively covariate with selfing rate. Natural selection, however, might oppositely favor approach herkogamy, increasing outcrossing rates (Motten & Stone, 2000; Elle & Hare, 2002; Herlihy & Eckert, 2007).

Herkogamy is determined by genetic and ecological factors (Ashman & Majetic, 2006; Oøpedal et al., 2017). Within populations, standing genetic variation in herkogamy would depend on the history of selection of individual lineages. If selective pressures exerted by the community of pollinators fluctuate in time, variation in herkogamy between lineages would be maintained, producing differences in selfing rate and variation in inbreeding among individual plants. When

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these associations are maintained through time, it would be reasonable to expect that selfing rate (*s*), inbreeding coefficient (*F*) and herkogamy coevolve at the lineage level. Significant associations between high outcrossing rate and approach herkogamy have been reported before (Takebayashi, Wolf & Delph, 2006; de Vos et al., 2018; but see Chen et al., 2009; Brys & Jacquemyn, 2012; Oøpedal, Armbruster & Pélabon, 2015; Toräng et al., 2017). However, few studies have evaluated the within-population association between herkogamy and outcrossing rate (Karron et al., 1997; Brunet & Eckert 1998; Medrano, Takebayashi & Delph, 2000; Herrera & Barret, 2005) and none with inbreeding coefficient (*F*) at a lineage level.

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Annual, self-fertilizing, plant species may evolve in heterogeneous environments (e.g. disturbed and/or arid habitats) where pollinator communities are unpredictable (Friedman & Rubin, 2015). Under high environmental heterogeneity (i.e., in pollination service), daily and seasonal fluctuations represent different opportunities for cross- or self-fertilization producing variable selection on traits that affect the plants' mating system, such as herkogamy or the inbreeding history of each maternal lineage (Schoen & Lloyd, 1992; Schoen, Morgan & Bataillon, 1996; Morgan & Wilson, 2005; Eckert, Samis & Dart, 2006; Barrett, 2010; Shirk & Hamrick, 2014; Pannell, 2015). However, it is not quite clear how strong and how frequent the association between mating strategies, herkogamy and inbreeding history of lineages occur within populations in short-lived species. Here, we assessed the extent of these associations in each of two populations of the annual plant *Datura inoxia* Mill.

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Datura inoxia (Solanaceae) is an annual self-compatible plant that inhabits arid and semi-arid lands in Mexico and Southern USA (i.e. The Chihuahuan desert). Characteristic of these ecosystems is the high daily fluctuation of ambient temperature. *Datura inoxia* produces large, nectar producing, tubular flowers that open at dusk and remain receptive one night only. Genetic differentiation at neutral loci (i.e., genetic structure) and phenotypic differentiation in floral traits related to plants' mating system have previously been documented among and within populations, suggesting adaptive evolution (Jiménez-Lobato & Núñez-Farfán, 2012). Flowers are commonly visited by honeybees and hawkmoths whose activity mainly depends on air temperature (Barclay, 1959). The wide daily range of temperature in populations generates an unpredictable pollinator activity that coupled with variation in herkogamy suggest correlated selection on herkogamy, selfing rates and inbreeding history within populations.

Materials and Methods

Species description and sampled populations

Datura inoxia Mill. (Solanaceae) is a summer annual herbaceous plant native to Mexico and southwestern USA (Barclay, 1959). It occurs in xeric environments, mainly along the Chihuahuan desert (Barclay, 1959; Lockwood, 1973). The onset of flowering of *D. inoxia* is in July and lasts up to September as others *Datura* species in this region (Bronstein et al., 2009). *Datura inoxia* produces large, funnel-shaped, hermaphroditic and self-compatible white flowers (Barclay, 1959). The flowers, that live one night only, open at dusk when both anthesis and receptivity of stigma occur. During the flowering period, individuals can display from few up to tens of flowers each night. Flower traits related to mating system, herkogamy and flower size, display broad variation within and among populations (Jiménez-Lobato & Núñez-Farfán, 2012). The species is pollinated by hawkmoths (*Manduca sexta*, *M. quinquemaculata* and *Hyles lineata*) (Grant, 1983) who forage on nectar, and by honeybees that collect pollen (Lockwood, 1973; McCall et al. 2018; V. Jiménez-Lobato, pers. Obs.). Hawkmoths visit the flowers at dusk when night temperatures reach more than 24°C (pers. Obs.). Honeybees visit the flowers in the

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afternoon, even if these are closed, and early in the morning, before flowers wilt and fall (McCall et al., 2018).

Two populations of *D. inoxia* were selected to assess the relationships between selfing rate, herkogamy and inbreeding: which?. A previous report indicates that these two populations possess large individual variation in herkogamy and flower size-related traits (Jiménez-Lobato & Núñez-Farfán, 2012). The Cañada de Moreno population (CM) is located in the State of Querétaro (21° 17’ 43” N?; 100° 31’ 00”W?) in the Mexican Bajío at 1933 m a.s.l. During the flowering period of *D. inoxia* (July to September), this locality has an average temperature of 18.8° C, with a daily range from 7.4 °C to 30.8 °C, and a three-month total precipitation of 314 mm. The Mapimí population (Map) is located at 1157 m a.s.l. in the Mexican Plateau in the States of Coahuila and Durango (26° 41’ 11” N?; 103° 44’ 49” W?). This is a more xeric environment, with a quarterly average temperature of 22.8 °C (range: 12.6 °C to 32.9 °C) and a total three-month precipitation of 253 mm. Because flight activity of hawkmoths and honeybees depends on ambient temperature, the wide fluctuation in daily temperature at both sites suggests a heterogeneous and unpredictable pollinator service along the flowering season. Collection of seed material for experimental analyses was made under the permission SGPA-DGGFS-712-1596-17 (Subsecretaría de Gestión para la Protección Ambiental, Secretaría de Medio ambiente y Recursos Naturales, Mexico).

Herkogamy level variation within populations

Thirty plants in reproductive stage were randomly selected and tagged for sampling within each population in an area of 1 ha. For each individual plant, 4-6 open flowers were selected to measure herkogamy. Herkogamy was measured as the difference between pistil and stamens length. Approach herkogamy was defined as pistil surpassing stamens in length, whereas reverse

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herkogamy was the opposite trend. Absence of herkogamy occurred when pistil and stamens had similar lengths. To estimate phenotypic variation in herkogamy for each population, we calculated the within- and between-individual variances. Individual plants were considered a random effect. The residual variance is accounted by the intra-plant variation. The significance of individuals was evaluated by a likelihood-ratio χ^2 test (LRT) between a model that includes individuals as random effect (function lme) and one that does not (LRT1; function gls) (Zuur et al., 2009). Analyses were conducted with nlme package (Pinheiro et al., 2017) in R software (R Development Core Team, 2008).

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Mating system parameters

To estimate mating system parameters from each marked plant in the field, five mature fruits derived from natural pollination were collected, labelled and bagged. In the laboratory, seeds of each fruit were separated and germinated in a greenhouse; seeds of each fruit within each family (maternal plant) were sowed separately. Germination per fruit, per family, was recorded for 30 days. To obtain an average estimate of germination rate per fruit, we recorded the final number of seeds germinated in each pot. Germination percentage was ≥ 90% for all plants. Once seedlings emerged, at least five seedlings per fruit, and 25 seedlings per maternal plant (i.e., family) were collected for further genetic analysis. We collected leaf tissue from young plants, bagged, labelled, and stored in an ultra-freezer at -97 °C. Finally, we analyzed 20 seedlings per each of 30 maternal families per population (N = 600).

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DNA of seedlings was extracted following the Miniprep protocol (Doyle & Doyle, 1987). Five microsatellite nuclear loci developed for *D. stramonium* (Andraca, 2009) were amplified for each seedling. Further, we standardized one additional microsatellite locus for *D. inoxia* (F8: Rw: 5´ -GGACAACATCTTTGCGACCC- 3´) (Supplementary Information), in order to obtain a total

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of six polymorphic microsatellite loci per individual. Primers were labelled with PET, VIC, 6-FAM, and NED dyes (Applied Biosystems) (PCR protocols are shown in Supplementary Information).

Multiloci outcrossing (t_m) and selfing (s = 1-t_m) rates, primary selfing rate (r) and inbreeding coefficient (F) were estimated for each maternal family for each population. Mating system parameters (t_m and s) at the family level were calculated with MLTR 3.2 (Ritland, 2002), using the Expectation-Maximization method (EM) that allows presence of missing data and undetected null alleles (Ritland & Jain, 1981). Standard errors and standard deviations were estimated by bootstrapping, with 1000 replicates and re-sampling individuals at family level. The frequency of null alleles per locus, per population, was assessed using Micro-Checker v.2.2.3 (Cock et al., 2004). Since one locus (G8) did not amplified for plants of Cañada de Moreno, analyses were carried out with five loci, and six loci for Mapimí. Selfing rates (s) obtained from molecular markers, after fertilization and germination, might not be completely independent from inbreeding depression and thus may underestimate its true value (Lande, Schemske & Schultz, 1994). Primary selfing rate (r) is a better predictor of mating system because it assesses separately the magnitude of inbreeding depression. Hence, r is referred to as the proportion of selfed progeny at the time of fertilization (Lande, Schemske & Schultz, 1994). The primary selfing rate (r) was calculated for each maternal family as: $r = s / [1 - \delta + s\delta]$, where s is the selfing rate obtained from microsatellite loci, and δ is the cumulative inbreeding depression obtained for each population (see below).

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The inbreeding coefficient (F) may include components of inbreeding other than mating system, such as biparental inbreeding or population substructure. Here, we use this parameter as a proxy of the inbreeding history of each maternal family (i.e., adult cohort) (Latta & Ritland, 1994). F values were inferred per lineage from the loci amplified previously with GenePop v.4.2

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(Rousset & Raymond, 1995; Rousset, 2008). To investigate whether inbreeding depression was associated with the selfing rate and herkogamy, we calculated inbreeding coefficients at equilibrium (F_e) (i.e., progeny cohort) at each lineage, assuming that adult F and t_m are constant among generations (Ritland, 1990). We then related these differences with selfing rate and herkogamy. Since F_e increases in relation to F due to self-fertilization, differences between F and F_e at each maternal lineage should indicate the presence of higher inbreeding depression. Once selection against inbred progeny occurred, F_e and F will be equal (Ritland, 1990; Shirk & Hamrick 2014). F_e was calculated as $F_e = (1-t_m) / (1+t_m)$, where t_m is the outcrossing rate calculated from MLTR for each maternal family (Ritland, 1990).

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Multiloci outcrossing (t_m) and primary selfing rates (r) were highly and inversely correlated (CM: estimated = -1.00, $p = 0.000$, d.f. = 25; Map: estimated = -0.969, $p = 0.000$, d.f. = 27), hence we present here only the analyses for primary selfing rates (r). Since r is a proportion with binomial distribution, correlation with herkogamy was calculated by a generalized linear model with a quasi-binomial distribution error (Crawly, 2013). F, F_e and their differences were associated to herkogamy and r with a generalized linear model with gaussian distribution error (Crawly, 2013). All analyses were implemented in R package (R Development Core Team 2008).

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Inbreeding depression

To estimate inbreeding depression (δ), we collected 150 fruits from different individual plants in each population, including the maternal families previously analysed. From each fruit, we sowed ten seeds on separate pots under greenhouse conditions. When seeds germinated, only one seedling per fruit was randomly chosen and grown under controlled conditions until reproduction. For each population, 100 individuals were randomly chosen to act as pollen receptors (mothers) and 50 individuals as pollen donors (fathers). Two manual pollination

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treatments were applied to each maternal receptor: (1) cross-pollination (*o*), where two flowers were emasculated before anthesis and hand-fertilized with pollen of one donor randomly chosen from the same population; (2) self-pollination (*s*), where two flowers of each receptor plant were fertilized with self-pollen. After pollination, flowers in both treatments were bagged individually with a fine nylon mesh. Since many mother plants did not produce the four flowers needed for pollination treatment application, the final sample included mother plants that produced at least one fruit per treatment (CM: N = 77; Map: N = 41). Two components of fitness per pollination treatment were evaluated in each population: seed-set mean (*i.e.* number of seeds / number of ovules) and seed mass. Seed mass was obtained from a random sample of 30 seeds per fruit using an analytical balance (Adventurer OHAUS).

Cumulative inbreeding depression coefficient (δ) was calculated for each population as: $\delta = 1 - \frac{w_s}{w_o}$, where w_s and w_o are the mean fitness of progenies derived from self- or cross-pollination, respectively. Average fitness of self- and out-cross progenies was calculated as the product of seed-set and seed mass (Schemske & Lande, 1985), and it was used to estimate the primary selfing rate (*r*) at each maternal lineage.

Results

Variation in herkogamy between populations of Datura inoxia

The average herkogamy in the Cañada de Moreno population (CM) was negative (mean = -4.72; sd = 5.5 mm) with a range of 17.48 mm (from -13.83 to +3.65 mm). In this population 20 out of 27 individual plants (74%) had reverse or nil herkogamy and 7 (26%) showed approach herkogamy (Fig. 1A). In the Mapimí (Map) population (N=29) mean herkogamy was positive

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and more variable than in Cañada de Moreno (Mean = 2.57 mm; sd = 11.15 mm; range = 44.8mm, from -20 to +24.8 mm) (Fig. 1B).

The proportion of variance in herkogamy explained by the among individual plants term was higher in Map (77.32%) than in CM (50.62%), indicating higher intra-individual variation in the latter population (Map: 22.68%; CM: 49.38%). Likelihood ratio χ^2 test (LRT) indicated a significant variation between individual plants in each population (Map: LRT = 105.04, df = 2, P <0.0001; CM: LRT = 38.56, df = 2, P <0.0001).

Mating system estimation and inbreeding coefficient (F)

The multiloci outcrossing rate (t_m) was, on average, higher in Mapimí than in Cañada de Moreno (0.682 *vs* 0.294) although high variation in this parameter was detected among lineages in each population (Mapimí, from 0.022 up to 1; Cañada, from 0 up to 1). The distribution of t_m is skewed to low values in Cañada de Moreno (ca. 60% of mother plants), but to high values in Mapimí (50% of the families with $t_m > 0.8$) (Fig. 2). Primary selfing rate (r) was more than two-fold higher in Cañada de Moreno than in Mapimí (0.716 vs. 0.353), varying from $r = 0$ to 1 and from $r = 0$ to 0.978, respectively.

Inbreeding coefficients of adult cohorts (F) were negative in the two populations and highly variable (Cañada: $F = -0.193$, from -1 to +0.75; Mapimí: $F = -0.085$, from -0.553 to +1). Inbreeding coefficients at equilibrium (F_e) were higher in Cañada than Mapimí ($F_e = 0.626$ vs. 0.238) and highly variable in both populations (from 0 to 1). The differences between F and F_e were much higher in Cañada Moreno than in Mapimí ($F_e - F = 0.819$ vs. 0.323). Cumulative inbreeding depression was higher in Mapimí than in Cañada ($\delta = 0.25$ vs. 0.09).

Relationships between mating system parameters and herkogamy

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Linear correlations between primary selfing rate (r), inbreeding coefficient (F), inbreeding coefficient at equilibrium (F_e) and herkogamy within each population were only significant in the Mapimí population (Table 1, Fig. 3). In Mapimí, r and F_e were negatively associated with herkogamy (Table 1; Fig. 3B, F), indicating that individuals with nil or reverse herkogamy had progenies with higher selfing rate and inbreeding coefficient than plants with approach herkogamy. As expected, progenies' inbreeding coefficient (F_e) is positively correlated with primarily selfing rate (r) at both populations (Table 1; Fig. 4C, D). In addition, selfing is also linked to the breeding history of each maternal lineage (F) only in Mapimí (Fig. 4A, B), but not with herkogamy in the two populations (Table 1; Fig. 3C, D). The difference $F_e – F$ is positively correlated with primary selfing rate (r) only in Cañada Moreno (Table 1; Fig. 4E). We did not find any indication of a significant correlation between $F_e – F$ and herkogamy (Table 1; Fig. 4G, H).

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Discussion

In annual, short-lived, plant species inhabiting heterogeneous environments, the evolution of plant mating system in association with flower traits, such as herkogamy, depends on the variation of natural selection within populations, and on the opportunity to cross and self-fertilize every year (Shirk & Hamrick 2014). If some associations among traits and mating strategies are favored through time, it is expected to find that different lineages within populations would vary in their history of inbreeding. In this work we evaluated the association among herkogamy, mating system and inbreeding history at a lineage level within two populations of *Datura inoxia*, an annual/short live species distributed in arid and semiarid environments in Mexico and North America.

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We found that herkogamy, selfing rates, inbreeding coefficients as well as their associations varied considerably within the two populations. In line with expectations, in one population (Mapimí), plants with more pronounced approach herkogamy showed, on average, higher outcrossing rates and less inbreeding coefficient of progenies, than those individuals that exhibited nil or reverse herkogamy. These results are in line with the hypothesis of adaptive herkogamy as a mechanism that prevents selfing in populations and avoids inbreeding between individuals (Web & Lloyd 1986; Lloyd 1992). Relationship between selfing/outcrossing rate and herkogamy has been found in other species of *Datura* such as *D. stramonium* (Motten & Antonovics, 1992; Motten & Stone, 2000) and *D. wrightii* (Elle & Hare, 2002), but also in other species like *Gilia achilleifolia* (Takebayashi & Morrell, 2001), *Clarkia temblorensis* (Holtsford & Ellstrand, 2006) and *Mimulus ringens* (Karron et al., 1997). However, we did not detect a significant association between inbreeding coefficients of adults' cohort with herkogamy. This suggests that the inbreeding history at each lineage not only depends on herkogamy; instead, purge of inbred individuals and/or biparental inbreeding may also come into play.

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Fluctuation of environmental variables, especially pollinator availability, as it occurs in Mapimí, can contribute to maintain variation in herkogamy within populations. Variation in herkogamy may be linked with plants' reproductive assurance when pollinators are scarce, or with high outcrossing rates when abundant (Kalisz, Vogler & Hanley, 2004; Goodwillie, Kalisz & Eckert, 2005; Chen et al., 2009). Since outcrossing rates in Mapimí are related to herkogamy, it is likely that episodes of differential (or even contrasting) selection on herkogamy among lineages every year, could have favored either outcrossing (approach herkogamy) or selfing (nil or reversal herkogamy). This phenotypic variance in herkogamy could explain the maintenance of the mixed mating system of *D. inoxia* in Mapimí.

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On the other hand, the mating system of the population of Cañada de Moreno is predominantly selfing, and no association between selfing rates, herkogamy and inbreeding coefficients in the adult cohort were detected. Nevertheless, like the Mapimí population, there is a positive relationship between selfing rate with inbreeding coefficients in the progenies, and with the F vs. F_e difference. These results suggest inbreeding depression in selfing individuals and then genetic purging (Ritland, 1990). This result is supported by the contrasting average value of inbreeding depression in the two populations of *D. inoxia*. Theoretical models and experimental results have demonstrated that mutations of large effect causing inbreeding depression can be purged from one to another generation (Willis, 1999; Charlesworth & Willis, 2009). However, mild in effect and rare mutations which are responsible for inbreeding depression may be maintained in populations for multiple generations (Lande, Schemske & Schultz, 1994; Charlesworth & Willis 2009). The expression of mutations with mild deleterious effect in inbred individuals of *D. inoxia*, would explain the differences of inbreeding coefficients between progeny and adult generations and the relationship with selfing rate.

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Phenotypic variance in herkogamy results from genetic (Herlihy & Eckert, 2007), developmental (Vallejo-Marín & Barrett, 2009) or environmental factors, such as soil nutrients, as demonstrated in *Datura stramonium* (Camargo et al. 2017). However, the evolution of mating system, linked to floral traits like herkogamy, requires additive genetic variance. A revision of evidence points out to the potential evolution by selection of herkogamy owing to the presence of additive genetic variance (heritability) and genetic additive variation (evolvability) (see revision in Oøpedal et al., 2017). Our results, derived from the partition of phenotypic variance in herkogamy among individual plants and random variation (within individual variation or residual term), indicate a large amount of variance between individuals in each population, but notably

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more so in Mapimí (77.32%) than in Cañada de Moreno (50.62 %). Thus, there is a strong indication that individual variation in average herkogamy in *D. inoxia* in Mapimí is genetically based and potentially adaptive (see Jiménez-Lobato & Núñez-Farfán 2012). High within-individual variation could be adaptive if, on average, high intra-individual variation is linked to higher fitness (Herrera, 2009; Camargo et al. 2017). Otherwise, high intra-individual variation can be maintained in highly heterogeneous environments (i.e., pollination likelihood by animals) through time. In Cañada de Moreno the high within-plant variation in herkogamy could limit selection on it, constraining an adaptive response in this population (Falconer & MacKay, 1996; Lynch & Walsh, 1998).

Comentado [SC9]: Population or region?

The within-individual variation in plant traits, and particularly in flowers, can originate during development or can be an elicited response to environmental variability (Herrera, 2009). The stability of development or homeostasis has been associated with different levels of heterozygosity, where heterozygous individuals are more stable than their homozygous (Lerner, 1954). However, there is not clear consensus on this hypothesis; some evidence points out to the potential effect of inbreeding, the fixation of deleterious alleles and genetic drift influencing the level of developmental stability of individuals (Clarke, 1993). To what extent inbreeding and deleterious mutations are responsible of intra-individual variation in the CM population is not known yet, but evidence suggests such kind of developmental variation as shown in one highly inbred population of *D. stramonium* (Camargo et al. 2017)

Excluído: but

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Conclusions

Association between herkogamy, mating system and inbreeding history at a lineage level is expected to occur within populations in self-compatible, hermaphroditic plant species. Similarly,

Formatado: Recuo: Primeira linha: 0 cm as environments do vary, particularly in pollination service by animals, variations in trait values and their correlations are also expected. In *D. inoxia*, as in other species of *Datura*, approach herkogamy is associated to outcrossing rates and to low levels of inbreeding in the progenies. Results of this study show that populations of *D. inoxia* are diverging in mating system characteristics with important genetic implications.

Excluído: consequences

Acknowledgments

We thank to members of the Ecological genetics and evolution lab members for field assistance, particularly to Armando López Velázquez. Thanks to Rosalinda Tapia López for lab help and to Rafael Torres and Adriana Pérez for greenhouse work assistance. Particular gratitude to Kiko Herrera´s family in Mapimí Biosphere Reserve and to Tito Galván and family in Cañada de Moreno. This study is part of the Ph, D. Thesis of V. Jiménez-Lobato.

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