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EFECTO DE LA ENDOGAMIA SOBRE LAS INTERACCIONES ANTAGONISTAS ENTRE DATURA STRAMONIUM Y SUS ENEMIGOS NATURALES

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La hipótesis de la depresión endogámica sobre la defensa (HDED) sugiere que la resistencia y/o la tolerancia se reducirán con el incremento en la endogamia. Esta sugiere que los individuos exógamos recibirán menos daño por sus enemigos naturales y tendrán un mayor éxito reproductivo que las endógamas. Estas alteraciones en la interacción planta- enemigo natural, al afectar la expresión de la depresión endogámica, pueden afectar la dinámica del sistema de apareamiento en las plantas. En este estudio examiné los efectos de una generación de endogamia en la resistencia de la planta *Datura stramonium* sobre las interacciones contra sus herbívoros y patógenos. Esta planta es consumida por dos herbívoros y un depredador de semillas, cuyo daño reduce el éxito reproductivo. Expuse plantas de semillas de endogamia y exógamia para evaluar si las plantas endógamas eran menos resistentes y/tolerantes que las plantas exógamas. Con el aumento en la endogamia, el daño por herbívoros incrementó 6% y 16%, respectivamente. La depredación, por el contrario, incrementó 40% con la exogamia. Las plantas exógamas de *D. stramonium* fueron más tolerantes al daño en las hojas y en las semillas que las plantas endógamas. Además, encontramos variación genética en la depresión endogámica relevante para el mantenimiento de la exogamia. Los resultados de este estudio apoyan las predicciones de la HDED y sugieren que tanto los enemigos naturales como la variación genética en genes relacionados en la endogamia pueden interactuar para evitar la fijación de la autofertilización en las plantas.

ABSTRACT

The ability of plants to respond to natural enemies might depend on the availability of genetic variation for the optimal phenotypic expression of defence. Selfing can affect the distribution of genetic variability of plant fitness, resistance and tolerance to herbivores and pathogens. The hypothesis of inbreeding depression influencing plant defence predicts that inbreeding would reduce resistance and tolerance to damage by natural enemies relative to outcrossing. In field experiments entailing experimentally produced inbred and outcrossed progenies, we assessed the effects of one generation of selfing on *Datura stramonium* resistance and tolerance to three types of natural enemies, herbivores, weevils and a virus. We also examined the effect of damage on relative growth rate (RGR), flower, fruit, and seed production in inbred and outcrossed plants. Inbreeding significantly reduced plant defence to natural enemies with an increase of 6% in herbivore damage and 8 in viral infection. These results indicate inbreeding depression in total resistance. Herbivory increased 10% inbreeding depression in seed number, but viral damage caused inbred and outcrossed plants to have similar seed production. Inbreeding and outcrossing effects on fitness components were highly variable among families, implying that different types or numbers of recessive deleterious alleles segregate following inbreeding in *D. stramonium*. Although inbreeding did not equally alter all the interactions, our findings indicate that inbreeding reduced plant defence to herbivores and pathogens in *D. stramonium*.

INTRODUCCIÓN

Rafael Bello Bedoy

'To self or not to self, that is the question'

Hace más de 100 años Charles Darwin publicó los resultados de una serie de experimentos en *'The Effect of Cross- and Self-Fertilisation in the Vegetable Kingdom'* donde documentó los efectos nocivos de la endogamia en 57 especies de plantas (1876). Se creó que la mala salud de sus hijos con Emma Sedgewick, su prima en primer grado, despertó su interés para explicar si la endogamia tenía efectos negativos sobre la salud de sus hijos (Pannell, 2009). Las investigaciones de Charles Darwin acerca de la polinización y la endogamia fueron posiblemente formulados para entender sus efectos nocivos y el mantenimiento de la exogamia en la naturaleza (Darwin, 1876), las adaptaciones en las flores para promover la exogamia (Darwin, 1872) y la complejidad de los sistemas reproductivos que poseen las plantas para prevenir la auto-fertilización (1877). Sus resultados indicaron que la progenie derivada de auto-fertilización presenta desventajas, ya que las plantas endógamas de la mayoría de las especies presentaron tanto menor vigor como fertilidad que sus consanguíneos producidos por exogamia. Estos fueron los primeros estudios que combinaron la ecología de la polinización y las consecuencias de la endogamia (con sus limitaciones sobre el conocimiento sobre genética) en la evolución del sistema de apareamiento de las plantas.

"La naturaleza aborrece la auto-fertilización perpetua" fue una de las observaciones que derivaron de la larga investigación de Darwin y Knigh (1876), al encontrar que la endogamia recurrente es castigada severamente. Desde entonces se ha documentado en una amplia diversidad de organismos que los individuos que incurren en prácticas reproductivas incestuosas afectan su éxito reproductivo y el de su progenie. La irreversibilidad evolutiva de la auto-fertilización hacia la exogamia y el incremento en la tasa de extinción asociado con la auto-fertilización en angiospermas apoyan esta idea (Stebbins, 1957; Takebayashi & Morrel, 2001). Esto sugiere que esta estrategia reproductiva debería estar poco representada y que la exogamia debería de ser la regla. Sin embargo, un número considerable de angiospermas estudiadas hasta ahora se reproducen por auto-fertilización en algún grado (Goodwillie et al., 2005), lo que sugiere que tiene ventajas reproductivas y/o que la exogamia es una estrategia reproductiva sumamente lábil.

La auto-fertilización puede evolucionar fácilmente y prevalecer en las poblaciones por sus ventajas en la transmisión genética (Takebayashi & Morrell, 2001). Los individuos que se reproducen por auto-fertilización contribuyen con dos copias genéticas a la progenie derivada de endogamia y una más a través de su contribución paterna al fecundar un óvulo en otra planta. En cambio, la exogamia solo hereda dos copias genéticas a la siguiente generación, una por vía materna y otra por vía paterna (Fisher, 1941). Bajo la condición anterior, una mutación que promueva la auto-fertilización tiene una probabilidad alta de fijarse en pocas generaciones en ausencia de alguna desventaja genética, reproductiva, y/o ecológica (Maynard-Smith, 1978; Lande & Schemske, 1985).

Sin embargo, la auto-fertilización es usualmente seguida de efectos negativos que reducen el éxito reproductivo de los individuos que la practican y de su descendencia. La depresión endogámica es la reducción observada en el valor adaptativo de un carácter debido a la endogamia (Roff, 1997) y su intensidad se estima comparando el éxito reproductivo de la progenie autógena y exógena (Lande & Schemske, 1985, Charlesworth & Charlesworth, 1987). En plantas y animales se ha demostrado que la endogamia afecta diversos componentes relacionados con el éxito reproductivo (Husband & Schemske, 1996; DeRose & Roff, 1999) y se considera que la depresión endogámica son obstáculo más importante a la evolución de auto-fertilización porque reduce sus ventajas genéticas de transmisión.

La transición de la exogamia a la auto-fertilización ha evolucionado de forma paralela en familias de angiospermas (Stebbins, 1957). Estos cambios han sido asociados a ambientes desfavorables, donde las plantas experimentan limitación de polen y/o de polinizadores que faciliten el intercambio de polen (Ashman et al., 2004). Esto es porque la auto-fertilización puede garantizar la producción de semillas a pesar sus costos (*reproductive assurance*, Jain, 1976) y pueden fundar poblaciones estables con pocos individuos y favoreces el establecimiento de la auto-fertilización como una estrategia reproductiva (Baker, 1955). En contraste, la reproducción de individuos que dependen estrictamente de polinizadores y de polen de otros individuos se vería severamente afectada. Sin embargo, las ventajas de la auto-fertilización puede resultar comprometidas bajo otras condiciones, como la competencia o el daño por enemigos naturales, que reducen el éxito reproductivo (Nuñez-Farfán, 1994; Strauss & Karban, 1994; Crnokrak & Roff, 1999; Keller & Waller, 2002). Los ambientes naturales imponen retos a las plantas que pueden incrementar la expresión de los genes responsables de la depresión endogámica (West et al., 1999; Armbruster & Reed, 2005). Por lo tanto, se esperaría que la exogamia fuera más común que la endogamia en ambientes bióticos y abióticos que limitan los recursos para crecimiento y reproducción.

En particular, el estrés causado por los enemigos naturales de las plantas, como herbívoros y/o patógenos, ha llamado la atención en el estudio de la evolución de las estrategias de apareamiento. El efecto selectivo de los enemigos naturales puede afectar el establecimiento de una estrategia reproductiva (Agrawal & Lively, 2001). Levin (1975) sugirió que la recombinación o la exogamia en las plantas podrían conferir ventajas en presencia de plagas. Esto está basado en la idea de que la recombinación puede generar individuos

con combinaciones genéticas novedosas con el potencial de resistir/tolerar mejor al daño por herbívoros favoreciendo la reproducción sexual (*cf.* Williams, 1975). Mientras que la erosión en la variación genética debida a la auto-fecundación o asexualidad, junto con el impacto selectivo impuesto por los herbívoros, podría reducir el potencial evolutivo de estas poblaciones y eliminar los linajes endógamos. La exogamia ofrece ventajas individuales en términos defensivos, porque evita los costos de la depresión endogámica que reducen la resistencia y la tolerancia de su progeie, mientras que la endogamia puede incrementar la expresión de alelos negativos relacionados con ambas estrategias defensivas. Por lo tanto, los individuos endógamos pueden presentar niveles reducidos de resistencia y tolerancia a los herbívoros y patógenos en comparación los individuos exógamos.

Un enemigo natural que incremente los efectos negativos de la endogamia produciría selección en contra de la endogamia limitando su evolución (Lively & Howard, 1994). Esto sugiere que una población sexual puede ser menos susceptible a la invasión por individuos asexuales o endógamos cuando el daño por enemigos naturales interactúa con las mutaciones recesivas de efectos nocivos relacionadas con el éxito reproductivo. Esta idea no considera los efectos de las mutaciones deletéreas específicas que reducen la resistencia y/o la tolerancia al daño por enemigos naturales (Young et al., 2009). Sin embargo, la depresión endogámica en resistencia puede incrementar la desventaja reproductiva sobre éstas en la progeie de auto-fertilización. En consecuencia, el daño por herbívoros podría resultar más relevante de lo que predicen los modelos y favorecer una estrategia sexual de reproducción aún cuando el daño (i.e., infección, virulencia) sea bajo y/o cuando la tasa de mutación es menor a lo considerado por los modelos (Agrawal & Lively, 2001).

Por ejemplo, si la cantidad de daño entre la progeie endógamas y exógama difiere debido a la depresión endogámica en la resistencia y ésta se ve reflejada en un éxito reproductivo diferencial entre ellas, el daño por los enemigos naturales puede prevenir la evolución de la endogamia (Condición 1, ver arriba). Por otro lado, si la endogamia reduce la tolerancia al daño por enemigos naturales, el daño por herbívoros/patógenos puede ser relevante en la evolución de las estrategias de apareamiento aún si nivel de daño (i. e., resistencia) es igual en ambas progenies (Condición 2). En ambos casos, un incremento en la depresión endogámica asociado al daño por enemigos naturales indicaría que el efecto diferencial del daño es relevante en evolución de la sexualidad de las plantas.

La mayoría de los estudios en plantas han encontrado que la progeie endógama recibe más daño por herbívoros y patógenos en comparación a la progeie de exogamia (ver en Núñez-Farfán et al 2007; Steets et al., 2007). Aunque también se ha reportado lo contrario, lo que implica una presión que los enemigos naturales también pueden eliminar las ventajas de la exogamia (Koslow & Clay, 2007; Leimu et al., 2008). Esto cuestiona la idea de que la exogamia siempre se verá favorecida en presencia de enemigos naturales. Por lo tanto, entender bajo que condiciones bióticas y abióticas se incrementa los costos de la auto-fertilización (Armbruster & Reed 2005) y predecir la trayectoria evolutiva del sistema de apareamiento es uno de los objetivos recientes de la ecología de la depresión endogámica.

En este estudio examiné los efectos de una generación de endogamia en la resistencia de la planta *Datura stramonium* sobre las interacciones contra sus herbívoros y patógenos. Aunque esta especie se reproduce primordialmente por auto-fertilización, muestra rasgos florales que indican cierto mantenimiento de exogamia y depresión endogámica de una magnitud característica de una especie con una alta frecuencia de exogamia (Núñez-Farfán et al., 1996, Stone & Motten 2002). Además, *D. stramonium* es hospedero de dos especies de herbívoros folívoros especialistas y un depredador de semillas cuyo daño reduce el éxito reproductivo (Núñez-Farfán & Dirzo, 1994). Esta planta es un modelo de estudio idóneo para evaluar el impacto del daño por enemigos naturales en el éxito reproductivo de progenie endógama y exógama.

En primer lugar, evalué si la endogamia afecta la resistencia de la planta contra sus herbívoros especialistas por medio de una cuantificación digital del daño foliar en plantas endógamas y exógamas producidas experimentalmente en el invernadero. Un objetivo de este estudio era evaluar la presencia de depresión por endogamia en resistencia y su efecto ulterior en el éxito reproductivo. En este caso, es posible que la intensidad del daño incremente la depresión por endogamia en términos de el número de frutos, semillas por fruto y la producción individual de semillas (Condición 1; capítulo 1).

Al igual que muchas plantas, *Datura stramonium* es frecuentemente atacada por más de un herbívoro en varias de sus poblaciones y es posible que los cambios en la resistencia causados por la endogamia sean benéficos frente a un herbívoro y resulten negativos frente a otro. Esto sugiere que el daño podría ser mayor en la progenie de exogamia que en la de endogamia. Es posible que esto ocurra si: la exogamia puede romper genes co-adaptados que controlan la resistencia (Lynch 1991), o si los herbívoros son más atraídos a plantas más vigorosas, como resultan generalmente las exógamas en relación a las endógamas (Hull-Sanders & Eubanks 2005). En el capítulo 2 examiné si el grado de infestación por la especie de gorgojo *Trichobaris soror* era mayor en las plantas exógamas que en las endógamas. Las plantas exógamas de *D. stramonium* producen más frutos que las endógamas y, dado que los gorgojos de *T. soror* usan los frutos como sitios de oviposición y crecimiento, evalué 1) si el incremento la variación en la producción de frutos asociado a la endogamia afectaba la incidencia y la infestación por gorgojos en *D. stramonium* y 2) si la infestación tiene un efecto similar sobre la producción de semillas de las plantas endógamas y exógamas. Además 3) examiné si el sistema de apareamiento de *D. stramonium* tenía algún efecto sobre la biomasa del escarabajo consumidor de semillas *T. soror*.

Una de las motivaciones principales de este trabajo fue la de examinar si los enemigos naturales afectan la intensidad de la depresión por endogamia (Condición 2; ver arriba). En el capítulo 3 se presenta evidencia de un estudio experimental con progenies de endogamia y exogamia expuestas al daño natural por herbívoros, a un virus y a su depredador de semillas. Este estudio muestra la complejidad de las interacciones que enfrentan las plantas en su ambiente natural y los potenciales efectos de la endogamia sobre diferentes enemigos naturales de forma simultánea.

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Cost of inbreeding in resistance to herbivores in *Datura stramonium*

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- **Background and Aims** Experiments show that inbred progenies are frequently more damaged by herbivores than outcrossed progenies, suggesting that selfing is costly when herbivores are present and can increase the magnitude of inbreeding depression in survival and reproductive components of fitness. The present study assesses whether inbreeding increases herbivory and estimates the magnitude of inbreeding depression on reproductive components of fitness in the annual plant *Datura stramonium*.
- **Methods** Two experiments were performed under natural conditions of herbivory to assess the effect of inbreeding on plant damage in *D. stramonium*. In the first experiment, outcrossed progeny was generated using foreign pollen donors, whereas inbred progeny was produced by self-pollination. In both groups, survival, herbivore damage and reproductive components of fitness were measured. In the second experiment, inbred and outcrossed progenies were produced using only local pollen donors, and only damage by herbivores was measured.
- **Key Results** Despite yearly variation in damage caused by the same specialist herbivores, inbred progeny suffered consistently more damage than outcrossed progeny. There was a significant inbreeding depression for fruit number ($\delta = 0.3$), seed number per fruit ($\delta = 0.19$) and seed number per plant ($\delta = 0.43$). Furthermore, significant genetic variation amongst families in the magnitude of inbreeding depression was observed.
- **Discussion** The results suggest that the plant's mating system modified the pattern of herbivory by specialist insects in *D. stramonium*. Inbred plants suffer not only from the genetic cost of low vigour but also from greater damage by herbivores. The mechanism by which inbreeding reduces plant resistance to herbivores remains unknown but is an interesting area for future research.

Key words: Inbreeding depression, mating system, plant defence, total resistance, herbivores, *Datura stramonium*.

INTRODUCTION

Selfing, the ability of an individual to fertilize ovaries with its own pollen, is common in many cultivated and wild flowering plant species (Bronstein *et al.*, 2009; Karron *et al.*, 2009), and inbred progeny can often exhibit very different phenotypic characteristics from outcrossed conspecifics from the same population (Armbruster and Reed, 2005; Kouonon *et al.*, 2009; Vallejo-Marin and Barrett, 2009). Inbreeding frequently reduces individual fitness of the progeny, a phenomenon known as inbreeding depression, and there are two non-mutually exclusive genetic mechanisms whereby increased homozygosity can lower plant fitness (Charlesworth and Charlesworth, 1987). Under the dominance hypothesis, inbreeding depression arises when a progeny is homozygous for partially deleterious recessive alleles. Alternatively, the over-dominance hypothesis states that inbreeding can reduce heterozygosity, resulting in a loss of hybrid vigour (Charlesworth and Charlesworth, 1987). However, outcrossing can also have negative effects on fitness if it decouples complexes of co-adapted genes for the local environment (i.e. outbreeding depression; Maynard-Smith, 1978; Lynch, 1991). Thus, the mating system adopted by the population can be of relevance because inbreeding might be disadvantageous by lowering both the defensive capabilities of plants and their fitness components in relation to outcrossing-derived offspring.

Inbreeding can affect interactions with pollinators (Ivey and Carr, 2005; Ferrari *et al.*, 2006), mycorrhizha (Botham *et al.*, 2009) or a plant's natural enemies; inbred offspring suffer more damage by insect herbivores than outcrossed individuals (Núñez-Farfán *et al.*, 2007). This suggests that inbreeding can alter the genetic background of plant resistance to, or tolerance of, herbivore attack (Strauss and Karban, 1994; Carr and Eubanks, 2002; Hayes *et al.*, 2004; Leimu *et al.*, 2008). By limiting genetic variation, inbreeding can affect the chemical or physical qualities of plants that prevent herbivore damage, reducing further their reproductive potential. Outcrossing, by contrast, can maintain defensive traits that lower the fitness cost of herbivory (Carr and Eubanks, 2002; Ivey *et al.*, 2004; Hull-Sanders and Eubanks, 2005). Consequently, in natural ecosystems inbred and outcrossed plants should experience different rates of herbivore attack and thus vary greatly in their potential recruitment success. As seedling herbivory is one of the key environmental factors dictating plant recruitment (Hanley and Sykes, 2009), it is vital that ecologists estimate the overall cost of inbreeding depression on plant defence.

It is also thought that severe environmental filters to plant recruitment favour outcrossing because they amplify the expression of recessive deleterious alleles responsible for inbreeding depression (Armbruster and Reed, 2005); therefore,

selfing should impose higher fitness costs in the presence of herbivores if inbred offspring have a reduced anti-herbivore defence in comparison with outcrossed conspecifics. Indeed, theoretical work suggests that the interaction between selection imposed by natural enemies and inbreeding depression can prevent the adoption of selfing as a reproductive strategy (Lively and Howard, 1994). Thus, inbreeding might incur an additional cost because herbivory reduces the leaf area needed to carry out photosynthesis and can lower resource allocation to reproduction (Levri and Real, 1998).

In order to assess whether inbreeding affects damage by herbivores, inbred and outcrossed progenies in *Datura stramonium* (Solanaceae) were generated. Previous work on this species has suggested that plant traits are selected for their resistance to herbivores, and, in addition, negative effects of inbreeding on plant fitness have also been detected (Valverde *et al.*, 2001; Sosenski, 2004). Plant damage by herbivores of inbred and outcrossed progenies was measured over 2 years in field conditions where plants were exposed to natural herbivores. In addition, inbreeding depression on plant survival was estimated together with three reproductive components of fitness: fruit number, seeds per fruit and seeds per plant.

METHODS

Plant species

Datura stramonium L. is a self-compatible annual plant native to Mexico but with a worldwide distribution (Weaver and Warwick, 1984; van Kleunen *et al.*, 2007). This species produces hermaphroditic flowers with a variation in the anther–stigma distance that has been shown to influence the outcrossing rate (Motten and Antonovics, 1992; Motten and Stone, 2000). Although population outcrossing rates reported for *D. stramonium* are low (0.01–0.08; Motten and Antonovics, 1992), extensive amongst-family variation in outcrossing rates has been reported (range 0–0.80; Cuevas, 1996). Inbreeding depression has been reported as ranging from $\delta = 0.15$ for fruit production up to $\delta = 0.39$ for seed production (Núñez-Farfán *et al.*, 1996; Stone and Motten, 2002).

In central Mexico, leaves of *D. stramonium* are consumed by the specialist herbivores *Epitrix parvula* (Coleoptera: Chrysomelidae) and the three-lined potato beetle *Lema trilineata* (Coleoptera: Chrysomelidae) and by the generalist *Sphenarium purpurascens* (Orthoptera: Pyrgomorphidae) (Núñez-Farfán and Dirzo, 1994). The foliar damage caused by these herbivores imposes selection on resistance and tolerance (Núñez-Farfán and Dirzo, 1994; Valverde *et al.*, 2001; Fornoni *et al.*, 2004). Tropane alkaloids and leaf trichomes are putative components of resistance to herbivory in *D. stramonium* (Shonle and Bergelson, 2000; Valverde *et al.*, 2001), whereas leaf production is associated with the ability to tolerate damage by herbivores (Fornoni and Núñez-Farfán, 2000; Valverde *et al.*, 2003).

Experimental design

Seeds used for this experiment were collected in 1999 from a population of *D. stramonium* in the locality of Teotihuacan (State of Mexico; 19°41'N, 98°51'E) and were stored in the

laboratory until hand pollination was carried out in a greenhouse. To produce selfed and outcrossed progenies, seeds from 20 randomly chosen plants (hereafter families) were sown in plastic pots filled with a commercial soil mixture and were watered daily until germination. One seedling per family was randomly selected and transplanted individually to a 3-L plastic pot. During flowering, flowers were hand pollinated at the same position on four separate branches of each plant to minimize competition between fruits. Prior to manual pollination, flowers were emasculated. Self-pollination was accomplished by rubbing the pollen of three anthers against the stigma of the same flower. Cross-pollination was achieved by rubbing one anther of each of three different pollen donors, randomly selected, onto the stigma of a flower. Flowers were tagged and bagged after pollination. Pollen donors used for cross-pollinations were derived from a population from Ritland (MO, USA), thus ensuring that outcrossed progeny had an inbreeding coefficient $f = 0$ (Falconer and Mackay, 1997, p. 58). Fruits were collected in paper bags and tagged before they started to open.

In 2006, a second experiment was performed following the crossing protocol described above to generate inbred and outcrossed seeds; however, cross-pollinations were achieved using local pollen donors (i.e. from Teotihuacan). The aim was to assess if the differences in damage by herbivores between inbred and outcrossed progenies observed in 2004 were due to the 'introduction of new resistance alleles' from Missouri to Teotihuacan instead of inbreeding depression in plant defence.

Fieldwork

In June 2004, in an effort to obtain ten inbred seedlings and ten outcrossed seedlings for each of the 20 families ($n = 400$), seeds produced by the two pollination types were sowed in plastic trays in a greenhouse. In total, 169 inbred and 160 outcrossed seedlings from 17 families were obtained (total sample size = 329). In July 2004, the seedlings were transplanted to a 400-m² experimental plot where plants were arranged in a completely randomized design (Cochran and Cox, 1957) and spaced 1 m apart to prevent competition effects on inbreeding depression (Cheptou *et al.*, 2000).

In August 2006, inbred and outcrossed seedlings of 19 families (total sample size = 293) were planted in the same experimental plot to ensure that plants experienced similar environmental conditions, following the same procedure described above.

Herbivory

To estimate the individual proportion of damage consumed by herbivores, a random sample of 20 leaves per plant was collected, encompassing leaves along the stem and branches to prevent a biased estimation of resistance caused by possible variation in chemical defence due to leaf age. The total and damaged area of a leaf was measured using a digital image analyser (WinDias Basic; Delta-T Devices Ltd, Cambridge, UK). The intact leaf area of the damaged leaves was estimated using a regression model obtained from a sample of undamaged leaves for which lengths, widths and area were known [$Leaf\ area = 8.2080 (Leaf\ length) + 0.5704 (Leaf$

length $- 8.4861)^2 - 35.9162$; $R^2 = 0.92$, $P < 0.0001$, $n = 26$). The proportion of damage (D_i) of an individual plant was calculated as:

$$D_i = \left(\frac{1}{n} \sum_{i=1}^n \frac{A_D}{A_T} \right)$$

where A_D and A_T are the damaged and total area of a leaf, respectively, and n is the sample size of the leaves (after Núñez-Farfán and Dirzo, 1994). The inverse of the proportional damage, $R_i = 1 - D_i$, is commonly related to ‘total resistance’ to herbivores (cf. Leimu and Koricheva, 2006).

Fitness estimates

Plant survival was censused from planting through reproduction and was recorded as a binary variable (alive = 1; dead = 0). At the end of the reproductive season, all mature fruits of each plant were collected individually in paper bags. The numbers of fruits and seeds are good estimators of individual fitness in this species (Núñez-Farfán and Dirzo, 1994; Núñez-Farfán *et al.*, 1996; Stone and Motten, 2002). Survival, fruit number and seed number were estimated only in 2004.

Statistical analyses

Effects of inbreeding on herbivory. In order to compare the variation in damage between inbred and outcrossed progenies and between years (i.e. 2004 and 2006 experiments), an ANOVA was performed, including the effects of pollination type, year and their interaction.

Using the data obtained in the 2004 experiment, we assessed the effect of pollination type (fixed), family (random), and the family \times pollination type interaction (random) on plant damage by herbivores and on three reproductive components of fitness by means of a mixed-model ANOVA. In order to improve normality and meet ANOVA assumptions, plant damage was arcsine-root transformed and reproductive components of fitness were log transformed, prior to statistical analyses (Zar, 1996). All analyses were run in JMP 5.01 (SAS Institute, Cary, NC, USA).

Plant survival. Plant survival amongst families and between pollination types was analysed by means of a survival analysis, assuming the Cox proportional hazards model (Vittinghoff *et al.*, 2005).

Inbreeding depression. Inbreeding depression (δ) estimates for total resistance, fruit and seed number, and seed per fruit were computed as:

$$\delta = 1 - (\varpi_{\text{inbred}} / \varpi_{\text{outcrossed}})$$

where ϖ_{inbred} and $\varpi_{\text{outcrossed}}$ are the mean values for each character of inbred and outcrossed progenies, respectively (Charlesworth and Charlesworth, 1987).

RESULTS

Effects of inbreeding herbivory

Plants were damaged by their specialist herbivores *L. trilineata* and *E. parvula* in both experiments. The ANOVA detected a significant annual effect in average foliar damage. Average damage (D_i) was significantly higher in 2004 when pollen donors were from Missouri than in 2006 when outcrossing was performed with local pollen donors ($D_{i,2004} = 0.29$, s.e. = 0.005 vs. $D_{i,2006} = 0.22$, s.e. = 0.004; $F = 115.24$, d.f. = 1; $P < 0.0001$; $n = 483$). In both years, outcrossed progeny received lower damage than inbred progeny (2004 $D_{i,\text{inbred}} = 0.32$, $D_{i,\text{outcross}} = 0.27$ vs. 2006 $D_{i,\text{inbred}} = 0.24$, $D_{i,\text{outcross}} = 0.20$). The interaction of pollination type \times year was not significant (Fig. 1), suggesting an inbreeding depression of total resistance of a similar magnitude between years ($\delta_{2004} = 0.068$ vs. $\delta_{2006} = 0.05$; Fig. 1).

In the 2004 experiment, plant damage by herbivores was significantly affected by pollination type, whereas the effects of family and family \times pollination type were not significant (Table 1a). Similarly, the three reproductive components of fitness were significantly affected by pollination type (Table 1b–d). The family \times pollination type interaction was significant only for total seed number ($F = 1.70$, d.f. = 16; $P = 0.051$; $n = 204$; Table 1, Fig. 2), indicating the existence of genetic variation for this fitness component. The family effect was not significant for the three reproductive characters.

Plant survival

The probability of survival was 16 % lower for inbred than for outcrossed progeny (outcrossing = 0.71, inbreeding = 0.60),

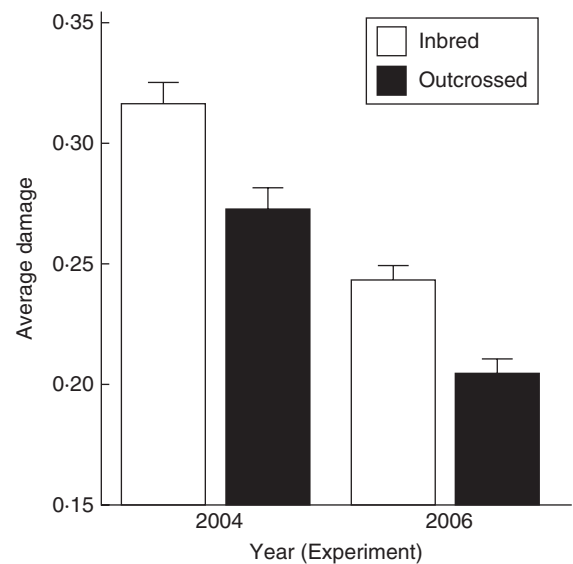


FIG. 1. Average proportion of damage (D_i) by herbivores (+ s.e.) of inbred and outcrossed progenies of *Datura stramonium* under natural conditions in the locality of Teotihuacan, Mexico. In 2004, cross-pollinations were performed using pollen donors from Missouri ($n = 189$). In 2006, cross-pollinations were performed using pollen donors from Teotihuacan. Self-pollination was performed using pollen of the same flower in a plant ($n_{\text{inbred}} = 143$, $n_{\text{outcrossed}} = 150$).

but the analysis failed to detect significant differences amongst families ($\chi^2 = 14.53$, d.f. = 16; $P = 0.55$; $n = 329$) or pollination type ($\chi^2 = 1.89$, d.f. = 1; $P = 0.16$; $n = 329$).

Inbreeding depression

As indicated by the mixed-model ANOVA, total resistance and the three reproductive components of fitness showed

TABLE 1. Mixed model analysis of variance of damage, number of fruits, seeds per fruit and total seed number per plant in relation to family, pollination type and interaction

Trait	Source of variation	d.f.	SS	F
(a) Damage 2004	Family	16	0.15	0.78 n.s.
	Pollination type	1	0.07	6.57*
	Family \times Pollination type	16	0.19	1.52 n.s.
	Error	156	1.21	
	$r = 0.33^*$			
(b) Number of fruits per plant	Family	16	1.86	1.13 n.s.
	Pollination type	1	0.83	7.91*
	Family \times Pollination type	16	2.62	1.57 n.s.
	Error	171	17.92	
	$r = 0.32^*$			
(c) Number of seeds per fruit	Family	16	0.31	1.35 n.s.
	Pollination type	1	0.44	30.58***
	Family \times Pollination type	16	0.28	1.20 n.s.
	Error	171	2.48	
	$r = 0.41^{***}$			
(d) Number of seeds per plant	Family	16	3.44	0.13 n.s.
	Pollination type	1	2.75	14.52**
	Family \times Pollination type	16	5.14	1.70 [†]
	Error	171	33.45	
	$r = 0.36^*$			

Results correspond to the experiment carried out in 2004. r and error of each model is provided. $n_{\text{inbreeding}} = 96$; $n_{\text{outcrossing}} = 109$.

[†] $P = 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant.

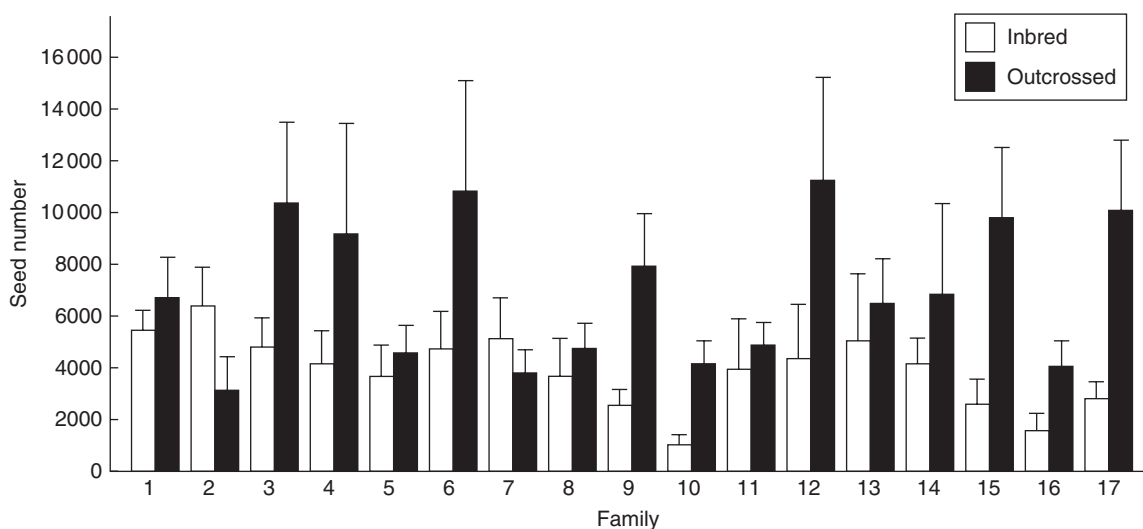


FIG. 2. Mean seed number (+ s.e.) of inbred and outcrossed progenies from 17 families of *Datura stramonium* corresponding to the experiment carried out in 2004.

inbreeding depression (Table 1), with coefficients ranging from $\delta = 0.068$ for total resistance up to $\delta = 0.43$ for total seed number per plant (Fig. 3).

DISCUSSION

Inbreeding affects the expression of plant resistance and tolerance against various natural enemies (reviewed in Núñez-Farfán *et al.*, 2007). Because resistance to herbivory has evolved to maximize individual fitness in the presence of herbivores, variation in resistance generated by inbreeding could give rise to different selective pressures on inbred and outcrossed plants. In the present study, inbred progenies were more damaged by herbivores in 2 years regardless of the origin of parental pollen donors. The reduction in leaf area was higher in inbred than in outcrossed plants, suggesting that the plant mating system altered the pattern of herbivory. Moreover, fitness components of *D. stramonium* were affected by inbreeding. With the exception of plant survival, the experiment revealed the existence of genetic variations in three reproductive components of *D. stramonium*, as indicated by the inbreeding depression coefficients. Finally, the heterogeneous effect of inbreeding on seed production amongst families suggests the existence of genetic variation in inbreeding depression, possibly caused by a different number or type of recessive deleterious alleles carried by different lineages. Altogether, the results showed that inbreeding is costly in terms of resistance to herbivores and fitness.

Inbreeding increases homozygosity at all loci and is likely to have important consequences for complex polygenic traits of plant defence, such as resistance, tolerance and vigour (Darwin, 1876; Núñez-Farfán *et al.*, 1996, 2007). Most studies to date have shown that one generation of inbreeding can reduce herbivore resistance (Núñez-Farfán *et al.*, 2007); other studies, however, have failed to detect any such effect. This contrasting evidence suggests that a negative effect of inbreeding in resistance is not a rule (Strauss and Karban, 1994; Núñez-Farfán *et al.*, 1996). Although the level of

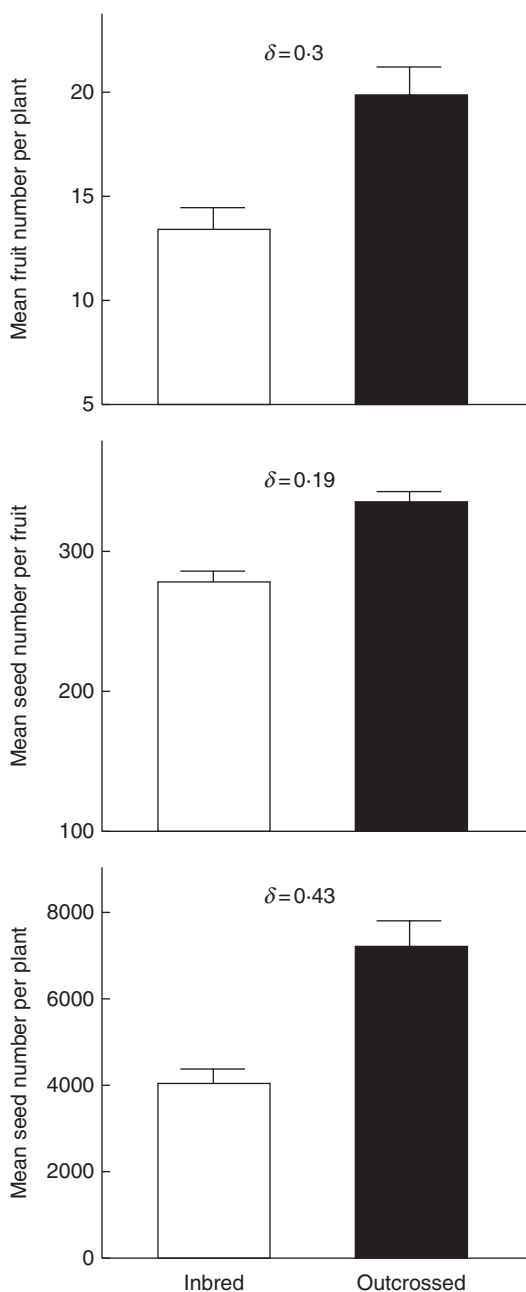


FIG. 3. Average values (+ s.e.) of the three reproductive components ($n = 204$) of inbred and outcrossed progenies of *Datura stramonium* grown in 2004 and their corresponding inbreeding depression coefficient (δ).

herbivory was variable amongst years, the present study showed that inbred plants received consistently more leaf damage than outcrossed plants in both years, suggesting a cost of selfing in ‘total resistance’ for this population. In contrast, there can be geographical variation in the cost of inbreeding for herbivory in *D. stramonium*. Núñez-Farfán *et al.* (1996) failed to detect differences in herbivory between inbred and outcrossed progenies in a different population even though plants were damaged by the same herbivore guild. As plants of both populations were subject to attack by the same herbivores, it seems possible that inbreeding depression in

resistance can be a result of a different history of inbreeding amongst populations (Carr and Eubanks, 2002) or geographical variation in the intensity of the interaction between plants and enemies (Ouborg *et al.*, 2000; Thompson, 2005).

The different levels of damage observed between inbred and outcrossed plants may be related to changes in leaf trichome density and/or tropane alkaloid concentration, putative components of defence in *D. stramonium* (Shonle and Bergelson, 2000; Valverde *et al.*, 2001; Fornoni *et al.*, 2003). Genetic variation and population differentiation in leaf trichome density has been detected in *D. stramonium* (Valverde *et al.*, 2001), and an increase in homozygosity could have affected the expression of this character in our experimental plants. Likewise, alkaloids or other secondary compounds may be altered by inbreeding, explaining the greater amount of damage on inbred plants (Kennedy and Barbour, 1992). Other studies have found that inbreeding altered the amount of plant volatile compounds in *Cucurbita pepo* (Cucurbitaceae) and *Solanum carolinense* (Solanaceae), and that these alterations were likely to modify antagonistic and mutualistic interactions with their herbivores and pollinators (Ferrari *et al.*, 2006; Delphia *et al.*, 2009b). On the other hand, inbreeding could change plant nutritional value (i.e. nitrogen content), influencing herbivores to consume more leaf area in inbred than in outcrossed plants. Previous studies suggested that herbivores might compensate for a lower nutritional quality by consuming more leaf tissue (Leimu *et al.*, 2008; Delphia *et al.*, 2009a). How inbreeding modifies the defensive or nutritional plant characters that produce differences in herbivory in *D. stramonium* remains to be determined.

In plants, stressful factors, such as competition or herbivory, can affect resource allocation to growth and reproduction (Stephenson, 1981). Inbreeding can generate offspring with reduced competitive ability or lowered resistance to natural enemies under stressful environmental conditions than under a benign environment such as a greenhouse (Armbruster and Reed, 2005). The present study detected an eight-fold increase in the magnitude of inbreeding depression in seed production compared with estimates obtained for the same population in the greenhouse (i.e. $\delta = 0.43$ in the current study vs. $\delta = 0.05$ in the greenhouse; Sosenski, 2004). Several studies have reported intense inbreeding depression coefficients in field experiments where plants can experience different types of stress, such as nutrient and water limitation or herbivory and pathogen attack, whereas greenhouse conditions minimize those stressful conditions (Armbruster and Reed, 2005). In the present study, *D. stramonium* plants were exposed to natural conditions of herbivory that commonly reduce plant fitness (Valverde *et al.*, 2001; Fornoni *et al.*, 2004), whereas plants in the greenhouse did not receive any damage. It seems that herbivory, amongst other stressing factors, intensified selfing costs by reducing the capability of allocating resources to seed production in inbred plants of *D. stramonium* (Núñez-Farfán *et al.*, 1996; Levri and Real, 1998).

Genetic variation for inbreeding depression in seed number was found here, as suggested by the different effects of inbreeding amongst families (i.e. family \times pollination type interaction). In most families, outcrossed progeny outperformed inbred progeny. However, inbred progeny of two families produced more seeds than outcrossed progeny,

suggesting a loss of the selfing cost in those lineages (i.e. Families 2 and 7). Amongst-family variation in inbreeding depression can be explained by a difference in the amount and/or type of recessive deleterious alleles that could be accumulated due to a random accumulation of mutations, which results in inbred lineages with varying fitness reduction as compared with their outcrossed relatives (Schultz and Willis, 1995). On the other hand, a variation in selfing rates can also produce a variation in inbreeding depression because highly self-fertilizing lineages may purge deleterious alleles, leading to low inbreeding, whereas outcrossing lineages can maintain deleterious alleles in the population (Byers and Waller, 1999). Seed production in *D. stramonium* depends on self-fertilization, but outcrossing still occurs within populations (Stone and Motten, 2002; van Kleunen *et al.*, 2007). Amongst-family variation in inbreeding depression in *D. stramonium* has been related to the individual anther–stigma distance (i.e. herkogamy), a heritable floral character implicated in the plant mating system, suggesting that different levels of inbreeding depression might be associated with inbreeding history (Motten and Antonovics, 1992; Motten and Stone, 2000; Stone and Motten, 2002). The present study population also had individual variations in anther–stigma distance (Sosenski, 2004), suggesting that there might be some lineages with different inbreeding histories. Thus, families that experienced low inbreeding depression may have purged some deleterious mutations, whereas outcrossing families might maintain deleterious recessive alleles in the population that were exposed by self-fertilization and resulted in strong inbreeding depression.

One caveat is that our outcrossing design could have combined resistance genes from the Teotihuacan and Missouri populations in 2004, and the relative difference in damage by herbivores between inbred and outcrossed progenies might have resulted because of the potential introduction of ‘new resistance alleles’ rather than a reduction in resistance brought about by inbreeding. However, when both progenies were generated using only local pollen donors and were exposed to herbivores (i.e. Teotihuacan in 2006), outcrossed plants were again more resistant than inbred ones. Thus, the hypothesis of the introduction of new resistance alleles has no support. In addition, the combination of genes from two distant populations could produce outbreeding depression (i.e. the negative effects of outcrossing). Because outcrossed progenies were superior to inbred progenies in defence and reproductive components, this explanation can also be ruled out. Thus, the most parsimonious explanation for the different amounts of damage found between progenies in this experiment is the existence of inbreeding depression in components of resistance or nutritional value.

This study detected costs of selfing in terms of increased herbivory and reduced seed production, suggesting that herbivores can be a factor in the evolution of the plant mating system. The finding of genetic variations in inbreeding depression for seed production suggests that this population has different types and amounts of detrimental alleles not removed by selection (Byers and Waller, 1999). Thus, variation in the cost of selfing could possibly influence the existence of a mixed plant mating system due to a low ability of ‘outcrossing lineages’ to remove mildly deleterious mutations.

The mating system altered the interaction of *D. stramonium* with its specialist herbivores given that inbred plants received more damage. However, it is necessary to disentangle the relative contribution of herbivores to inbreeding depression. Whether the differential damage by herbivores augmented the magnitude of inbreeding depression in *D. stramonium* remains to be addressed (Steets *et al.*, 2007). Yet, as pointed out by Strauss *et al.* (2002), it is necessary to measure inbreeding depression for components of defence to enhance our knowledge about how inbreeding alters the pattern of selection exerted by herbivores.

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Inbreeding alters a plant-predispersal seed predator interaction

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Abstract The effect of inbreeding on genetic diversity is expected to decrease plant defences or vigour-related traits that, in turn, can modify the pattern of attack by herbivores. The selective damage caused by herbivores can produce variable fitness costs between inbred and outcrossed progenies influencing the evolution of a species' plant mating system. By exposing inbred and outcrossed plants to natural conditions of seed predation, we assessed whether inbreeding increases weevil incidence and infestation, and how weevil seed predation affects the fitness of inbred and outcrossed progeny. To test if inbreeding affected the host's plant quality, we weighed the biomass of weevils developed in inbred and outcrossed progenies. An additional experiment was carried out to examine whether weevils preferentially attack vigorous plants regardless from the level of inbreeding. The average value of leaf size was 21% larger in outcrossed plants than in inbred plants. Likewise, weevil incidence and infestation were 13 and 40%, respectively, higher on outcrossed plants relative to their inbred counterparts. However, the relative impact of seed predation was significantly lower in outcrossed progeny than in inbred progeny. In contrast, inbreeding did not alter host plant quality and weevils developed in inbred and outcrossed plants had a similar biomass. Variations in fruit number were consistently associated with the infestation level in both experiments, whereas leaf size only predicted the number of weevils in one experiment, suggesting that fruit number is the most influential vigour-related characteristic of a weevil attack. These findings indicate that the costs of inbreeding of the interaction *D. stramonium*-*T. soror* were higher for inbred plants than for outcrossed plants. The interaction between seed predation and

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inbreeding depression could prevent the fixation of selfing as a unique reproductive strategy in *D. stramonium*.

Keywords Compensation · *Datura stramonium* · Inbreeding depression · Mating system · Plant vigour · Plant quality · Resistance · Seed predation · *Trichobaris soror* · Weevils

Introduction

Nearly 50% of flowering plants studied to date produce seeds by a mix of selfing and outcrossing, whereas the rest of species produce their offspring predominantly by one of these two mating system strategies (Goodwillie et al. 2005; but see Igic and Kohn 2006). The plant mating system influences the distribution of genetic and phenotypic variations within populations (Hamrick and Godt 1990), individual fitness (Husband and Schemske 1996) and the potential for evolutionary change (Charlesworth and Charlesworth 1987). Selfing (i.e., inbreeding), the ability of an individual to fertilize its ovules with its own pollen, confers ecological advantages when potential mates are scarce and/or when low pollinator abundance limits pollen transfer between conspecifics (Jain 1976; Lloyd 1979). However, inbreeding halves heterozygosity each generation and can produce fitness reductions by eliminating the heterozygote advantage and/or increasing the expression of recessive deleterious alleles (i.e., inbreeding depression; Charlesworth and Charlesworth 1987). Inbreeding depression in plant vigour is often expressed as declines in several life-history traits such as growth/plant size, flower or fruit production (Husband and Schemske 1996). Inbreeding depression is one of the key obstacles in the evolutionary transition from outcrossing to selfing (Lande and Schemske 1985). Therefore, it is relevant to investigate the ecological factors that influence the magnitude of inbreeding depression and its consequences in plants' mating system evolution.

It has been proposed that stressful environments could select against selfing in plants because challenging environments exacerbate the magnitude of inbreeding depression (Armbruster and Reed 2005). Herbivory is considered a common stressful factor of plants in natural and agricultural ecosystems (Kennedy and Barbour 1992). Herbivores can produce severe direct and indirect fitness reductions by attacking both the reproductive and vegetative structures of plants (Futuyma 1983). Therefore, anti-herbivore defences that have evolved in plants to reduce damage (i.e., resistance) or to ameliorate fitness losses caused by herbivory (i.e., tolerance) are expected to be under natural selection (Marquis 1992). Resistance and tolerance to herbivores are genetically based (Fornoni and Núñez-Farfán 2000; Fornoni et al. 2003), and the changes in the distribution of genetic diversity caused by inbreeding have the potential to affect their phenotypic expression, generating progenies with contrasting levels of defences to natural enemies (Núñez-Farfán et al. 2007). Thus, inbred and outcrossed progenies should experience a different selective pressure by herbivores or pathogens that could indirectly prevent the fixation of selfing as the predominant mating system strategy in a population.

Inbreeding has been found to modify the phenotypic traits related to plant vigour, thus influencing herbivore consumption (Carr and Eubanks 2002; Hull-Sanders and Eubanks 2005; Ferrari et al. 2007; Leimu et al. 2008; Delphia et al. 2009a). Several studies have reported the positive and negative effects of inbreeding in plant-natural enemy interactions, and those results are important for understanding the dynamics of damage and infections caused by herbivores and pathogens in plant populations. For instance, while inbreeding causes significant increases in herbivory rates attributed to inbreeding depression in

chemical and physical components of resistance (Carr and Eubanks 2002; Hayes et al. 2004; Hull-Sanders and Eubanks 2005; Du et al. 2008; Delphia et al. 2009a; Bello-Bedoy and Núñez-Farfán 2010a, b), outbreeding depression in resistance and/or changes in plant vigour may render outcrossed hosts more attractive for herbivores than inbred ones (Ferrari et al. 2006; Ferrari et al. 2007; Delphia et al. 2009b). Regardless of the level of inbreeding of their hosts, different herbivore species preferentially attack individuals with a high level of vigour, such as those healthy plants with large vegetative and reproductive parts in a population (Price 1991). An effect of inbreeding depression in the vegetative and/or reproductive traits of plants could modify an herbivore's choice and consequently influence the amount of damage received by the plant. Here, we hypothesized that the outcrossed plants of *D. stramonium* would be more often attacked by weevils than inbred plants would be, because insects would prefer to feed upon vigorous host plants, and the benefits of outcrossing in terms of plant vigour might disappear or even constitute a disadvantage in the presence of seed predators.

This study reports the effect of inbreeding on the interaction between the plant *Datura stramonium* and its predispersal seed predator *Trichobaris soror*. By exposing experimentally inbred and outcrossed progenies of *D. stramonium* to natural levels of seed predation, we examined the effect of inbreeding on plant vigour by using leaf size as an estimator of vegetative vigour. Further, we assessed whether inbreeding altered the level of infestation by the weevil *T. soror* and the relative impact of weevils on the seed production of inbred and outcrossed plants. In addition, we examined whether inbreeding affected the biomass of weevils developed in inbred and outcrossed progenies. Finally, we conducted an additional experiment to examine whether weevils preferentially attack vigorous plants, regardless of the level of inbreeding.

Materials and methods

Site description

The study was carried out in the region of Teotihuacan (State of Mexico; 19°41'N, 98°51'E). The native xerophytic vegetation in this zone has been subject to continuous habitat disruption by urbanization and farming, resulting in a landscape dominated by cultivars of maize (*Zea mays* L.), alfalfa (*Medicago sativa* L.) and cactus pear (*Opuntia ficus-indica* L. (Mill.) and secondary vegetation such as *Acacia* spp., *Argemone mexicana* L. and *Datura stramonium*. Annual rainfall averages 700 mm, with most precipitation occurring between June and October. This is the time when vegetation, including *D. stramonium* L., grows profusely.

Study system

Datura stramonium L. (Solanaceae) is a predominantly self-fertilizing herb that produces several flowers, fruits and seeds. An individual plant can produce up to 130 fruits and one single fruit can contain up to 1,000 seeds (L. L. Cruz, pers. obs.). Self-fertilization rates (s) estimated in two US populations of *D. stramonium* showed high values ($s = 0.9$) indicating that ca. 90% of seed production in this species is derived from self-fertilization (Motten and Antonovics 1992). However, it has been found that flowers with stigma protruding to anther level attain outcrossing rates 30–50% higher than flowers with anthers overlapping the stigma (Motten and Stone 2000; Stone and Motten 2002). Experiments

carried out under natural conditions have shown that inbreeding depression (δ) caused significant reductions in fruit number, seed number per fruit and total seed production ($\delta_{\text{fruits}} = 0.15\text{--}0.25$, $\delta_{\text{seeds}} = 0.39\text{--}43$; Núñez-Farfán et al. 1996; Stone and Motten 2002) and a significant increase in herbivore damage ($\delta_{\text{total resistance}} = 0.06$; Bello-Bedoy and Núñez-Farfán 2010a, b).

This plant species hosts various weevil species in the *Trichobaris* genus (Coleoptera: Curculionidae) that had reportedly produced important reductions in seed number (Cuda and Burke 1991; Cruz 2009). In Central Mexico, *T. soror* is known to use *D. stramonium* as its main host plant to feed and reproduce. This insect completes its life cycle from larva to adult stage inside developing fruits, suggesting a high degree of specificity to *D. stramonium* (Cabral-Vargas 1991). As adults, male and female *T. soror* consume the foliar and floral tissue of *D. stramonium* where they mate (J. Núñez-Farfán, pers. obs.). Female weevils oviposit from one up to 20 eggs across different reproductive structures, usually in floral buds, flowers and developing fruits (Cabral-Vargas 1991). Inside the fruit, developing larvae of *T. soror* feed on immature seeds until pupation. Adults burrow up the schizocarp of the capsule to exit. Previous work has estimated that one individual larva can consume up to $12 \text{ seeds} \pm \text{SE } 24$ (Cabral-Vargas 1991), but even a few larvae can damage all the seeds within a single fruit (R. Bello-Bedoy, pers. obs.). *D. stramonium* also hosts the specialists *Lema trilineata* (Coleoptera: Chrysomelidae) and *Epitrix parvula* (Coleoptera: Chrysomelidae) that feed upon leaves (Núñez-Farfán and Dirzo 1994). These insects exert selection on biochemical and physical components of defence that prevent defoliation (Shonle and Bergelson 2000; Valverde et al. 2001). In addition, leaf area is associated with the ability of plants to tolerate the defoliation (Valverde et al. 2003).

Experiment 1: Effect of experimental inbreeding on plant-weevil interaction

Experimental design

In February 2003, we conducted controlled self-pollination and cross-pollination treatments on 20 plants in a greenhouse to obtain experimental inbred and outcrossed seeds with different inbreeding coefficients (f) (outcrossed, $f = 0$; inbred $f = 0.5$). To produce inbred seeds, we self-pollinated a flower by rubbing three anthers with abundant pollen against the stigma of the same flower. To obtain outcrossed seeds, we cross-pollinated a flower by rubbing three anthers from each of three different plants against the stigmatic surface of a flower. Anthers employed in the outcrossing treatment were taken from three randomly chosen plants. At least two flowers were self-pollinated and two cross-pollinated in each of the 20 plants to produce several inbred and outcrossed seeds. Mature fruits were collected ca. 60 days after pollination treatment and the seeds of each fruit were bagged individually for further experiments. Inbred and outcrossed seeds obtained from each plant were considered a family.

In June 2004, inbred and outcrossed seeds were germinated in a greenhouse to obtain 10 inbred and 10 outcrossed seedlings per family. A number of seeds failed to germinate, resulting in a sample size of 169 inbred and 160 outcrossed plants derived from 17 families. We transplanted 3-week-old seedlings in an experimental plot in their native site and exposed them to natural conditions, where seed predation occurs yearly. Transplanted individuals were randomly arranged in a regular grid (20 m \times 20 m) and spaced 1 m apart to avoid competition effects. Plants were provided with supplemental water for 2 weeks following transplantation to prevent desiccation and then were left to natural conditions of

rainfall. Weeds growing in the plot were removed to prevent light or nutrient competition effects.

Data collection

Mortality reduced the number of plants during the experiment to 100 of inbred and 113 outcrossed plants from which we collected leaves and fruits. In October, the plants stopped producing flowers and most fruits were mature. At this time, we collected a random sample of 20 leaves from each plant to obtain estimates of average leaf size per plant by means of a digital Image Analyzer (WinDias Basic, Delta-T-Devices, Cambridge UK). We collected expanded leaves of all ages from the base of the stem to the upper part of the branches. Some plants lost several leaves and were not considered in the digital and statistical analyses of leaf size. Leaf size analysis was carried out with 85 inbred and 103 outcrossed plants.

We collected all fruits produced by a plant and bagged them individually to obtain the number of weevils and seeds per fruit. We collected fruits from 197 plants because eight plants did not produce mature fruits. *T. soror* was recorded in those plants as present if either larvae or adults were observed in the fruits.

To assess the effect of inbreeding on insect biomass, as a measure of host plant quality, we randomly collected a sample of 100 adult weevils in plastic vials, 50 insects from inbred plants and 50 from outcrossed plants. To obtain their biomass, we weighed dry insects in an analytic balance to the nearest (0.001 mg).

Experiment 1: Statistical analysis

Effect of inbreeding on plant vigour

The effect of inbreeding on vigour was assessed by a mixed-model ANOVA of leaf size. This model included the effects of family (random), pollination treatment (fixed) and family \times pollination treatment interaction (random). A significant effect of pollination treatment indicates that inbred and outcrossed plants produced leaves of a different average size. The significant effect of family suggests genetic variation for plant vigour, whereas a significant family \times pollination treatment interaction suggests that the effect of mating system on vigour varies amongst families.

Effect of inbreeding on weevil incidence, infestation and biomass

To assess the effect of inbreeding on the incidence of attack by weevils, we performed a categorical linear model, where weevil incidence was considered a binary variable (presence = 1; absence = 0; Littel et al. 1996). The effect of inbreeding on weevil infestation (i.e., weevil number) was analysed by means of a mixed-model ANCOVA. Both models included the effects of family (random), pollination treatment (fixed) and family \times pollination interaction (random). A large display of flowers and fruits can increase the level of infestation in *D. stramonium*, regardless of pollination treatment (Cabral-Vargas 1991). We included individual fruit number and average leaf size of inbred and outcrossed plants as covariates in both models to examine their potential roles in a weevil attack. Statistical analysis included plants with and without weevils.

The effect of a plant's pollination treatment on weevil biomass was evaluated by a *t* test, with pollination type as single factor. A significant effect of pollination treatment on

average weevil biomass would suggest differences in host quality between inbred and outcrossed plants.

Effect of weevils on plant seed production

In order to determine the relative cost of seed predation on inbred and outcrossed progenies, we performed an ANCOVA of seed number in relation to family (random) pollination treatment, the residuals of the number of *T. soror* (covariate) and pollination treatment \times *T. soror* residuals interaction. Prior to this analysis, we extracted the residuals from the linear regression of the number of *T. soror* per plant as a function of fruit number to control the effect of fruit number on infestation. Because the number of *T. soror* is significantly related with the number of fruits ($r = 0.72$, $P < 0.0001$), residuals were used in the analysis to avoid colinearity between independent variables. The implementation of residuals can produce an overestimation in the amount of variation explained in the dependent variable (Garcia-Berthou 2001). However, it can be a practical procedure for the purposes of a general description of the pattern of seed declines in inbred and outcrossed progenies associated with seed predators, independent of the fruit number. Residuals are a derived variable and we interpreted results as the relative rather than as the absolute effect of weevil number. A significant effect of *T. soror* would suggest that weevils affect the number of seeds. A significant interaction between pollination treatment \times *T. soror* would indicate a differential cost of seed predation between outcrossed and inbred plants. Seed number was squared root transformed to meet ANCOVA assumptions (Zar 1996).

Experiment 2: Effect of vigour on plant-weevil interaction

Experimental design

In order to test whether *T. soror* preferentially attacks vigorous plants independent of the level of inbreeding, we performed an additional experiment by using plants derived from field-collected seeds with an unknown inbreeding coefficient. In March 2004, we collected seeds of 65 plants from the population of Teotihuacan in paper bags; we considered the seeds from each single plant as a family, thus having 65 families. In June 2004, we sowed a random sample of seeds from each of 35 families randomly selected in individual plastic grids filled with commercial soil to obtain 30 seedlings per family. Only 16 families produced enough seedlings to be used in this experiment ($n = 480$). In July 2004, we transplanted 3-week old seedlings in the experimental plot 50 m apart from other experiment ensuring that plants were exposed to similar conditions of attack by *T. soror*. The plants were arranged in a regular grid following a completely randomized design (Cochran and Cox 1957). Plants were spaced 1 m from each other to prevent intraspecific competition.

Data collection

Mortality lowered the initial number of plants in this experiment from 480 to 369 plants. The mean value of plants per family was $27.11 \pm \text{SE} = 0.87$ with a range of 13–29 individuals. In October 2004, a random sample of 20 leaves per plant was collected to obtain estimates of leaf size as explained above. All fruits produced by a plant were collected and individually bagged before capsules started to open. Later, fruits were opened to register the presence of weevils and to obtain the number of insects per plant.

Experiment 2: Statistical analyses

We assessed the role of leaf size, as a vigour surrogate, on the incidence and infestation of plants of *D. stramonium* by *T. soror*. The incidence of *T. soror* as a function of family (random) and leaf size was analysed by means of a logistic ANCOVA. To assess the effect of leaf size (i.e., plant vigour) on weevil number, we performed an ANCOVA of weevil number as a function of family (random), leaf size and plant's fruit number. All statistical analyses were carried out in JMP 5.01 (SAS Institute, Cary, NC, USA).

Results

Experiment 1: Effect of experimental inbreeding on plant-weevil interaction

Effect of inbreeding on leaf size

Leaf size (i.e., vigour) was significantly affected by pollination treatment. Leaf area of outcrossed plants was 21% larger compared to inbred plants (Fig. 1a). The effects of family and family \times pollination treatment interaction were not significant (Table 1).

Effects of inbreeding on weevil incidence and infestation

Trichobaris soror was present in 83% of all experimental plants. Both weevil incidence and infestation differed between inbred and outcrossed offspring; weevil incidence and infestation were 13 and 40% higher in outcrossed progeny relative to inbred progeny (incidence: outcrossed = 89% vs. inbred = 76%; $\chi^2 = 6.54$, $df = 1$, $P = 0.01$; infestation: see Table 1; Fig. 1b). Fruit number had a significant positive effect on weevil number. In contrast, leaf size variation did not show a relationship with weevil incidence and infestation (incidence: $\chi^2 = 2.19$, $df = 1$, $P = 0.14$; infestation: see Table 1). The effects of family and family \times pollination treatment interaction on weevil infestation were not significant (Table 1).

Effect of inbreeding on weevil biomass

Plant pollination treatments did not have a significant effect on weevil biomass (t test = -1.09 , $df = 1$, $P > 0.05$, $n = 100$); weevils developed in the fruits of inbred and outcrossed progenies reached a similar average biomass (inbred = $0.0050 \text{ g} \pm \text{SE } 0.0001$, outcrossed $0.0052 \text{ g} \pm \text{SE } 0.0001$).

Effect of weevils on seed production

Outcrossing increased significantly the net seed production relative to inbred progeny (Table 2). Seed production was significantly higher in outcrossed plants than in inbred plants even after controlling for the significant effect of the relative number of weevils (Table 2). As the relative number of *T. soror* increased, the impact of seed predation was lower for outcrossed plants than for inbred plants, as indicated by the significant pollination treatment \times *T. soror* interaction (Table 2; Fig. 2). The relative cost of seed predation on seed number was greater for inbred progenies than for outcrossed progenies, suggesting a

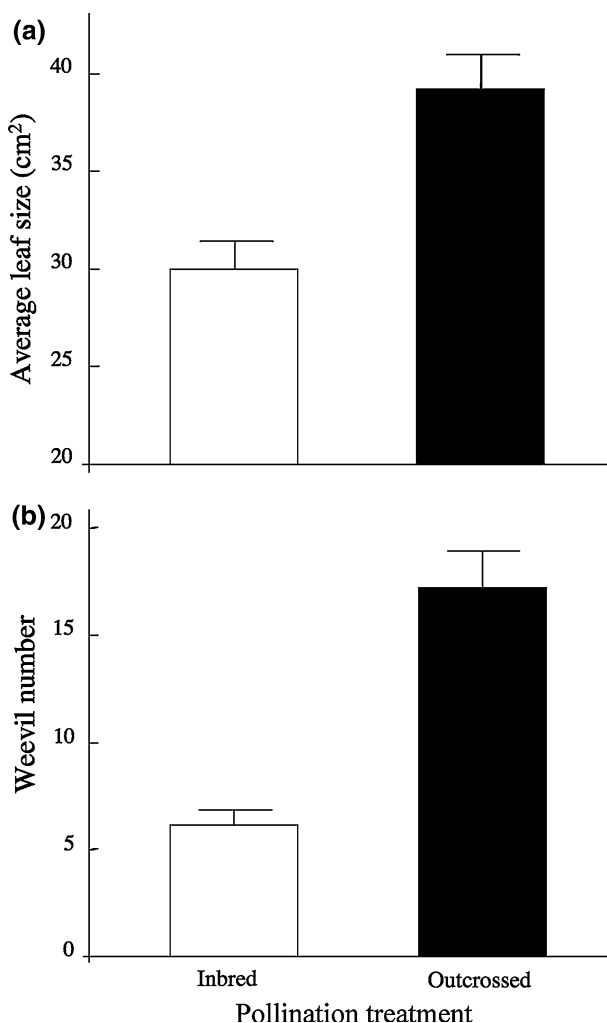


Fig. 1 **a** Difference in the average leaf size cm² between inbred and outcrossed progenies of *Datura stramonium* from the first experiment ($n = 176$). **b** Differences in the average number of weevils of *Trichobaris soror* found within the fruits of inbred and outcrossed progenies of *Datura stramonium* ($n = 197$). Error bars show one standard error of the mean in the two graphs

selective advantage of outcrossing over selfing in the presence of seed predators. Family effect was not significant (Table 2).

Experiment 2: Effect of vigour on the interaction between *D. stramonium*–*T. soror*

We detected a positive relationship between average leaf size (i.e., vigour) and the two estimators of weevil preference, suggesting that plant vigour is related to the attack of *T. soror* (Fig. 3). Leaf size showed significant relationships to both weevil incidence and infestation, i.e., weevil number (incidence: Wald test: $\chi^2 = 33.56$, $P = 0.002$; $n = 369$; infestation: $F = 16.45$, $df = 1$, $P < 0.0001$; $n = 369$; Fig. 3). A significant effect of fruit

Table 1 Mixed-model ANOVA for leaf size and mixed-model ANCOVA the number of *T. soror* in *D. stramonium* in relation to family, pollination treatment, and the family \times pollination treatment interaction

Trait	Source	df	MS	F	P
Leaf size	Family	16	359.692	1.31	0.295
	Pollination treatment	1	3,514.64	12.914	0.0022
	Family \times pollination treatment	16	273.843	1.20	0.274
<i>T. soror</i> number	Family	16	119.503	1.04	0.57
	Pollination treatment	1	1,431.09	11.28	0.003
	Family \times pollination treatment	16	131.08	1.31	0.19
	Leaf size	1	11.36	0.11	0.73
	Fruit number	1	11,250.7	113.13	<0.0001

Estimates of the whole model of leaf size: $R^2 = 0.14$, $F = 1.919$, $P = 0.004$, $n = 176$. Estimates of the whole model for *T. soror*: $R^2 = 0.57$, $F = 7.72$, $P < 0.0001$, $n = 174$

Table 2 ANCOVA for the square root of the number of seeds in relation to family, pollination treatment, *Trichobaris soror* number (residuals) and the pollination treatment \times *Trichobaris soror* interaction

Source	df	SS	F	P
Family (random)	16	629.60	1.49	0.1
Pollination treatment	1	9,248.03	21.92	<0.0001
<i>T. soror</i> number	1	2,527.50	6.09	0.01
Pollination treatment \times <i>T. soror</i>	1	4,741.22	11.24	0.0004

Whole model estimates: $R^2 = 0.16$, $F_{1,196} = 2.94$, $P < 0.0001$, $n = 197$

number was equally detected for weevil incidence and infestation (incidence: Wald test: $\chi^2 = 30.45$, $df = 1$, $P < 0.0001$; infestation: $F = 41.74$, $df = 1$, $P < 0.0001$). The effect of family was significant on weevil incidence, but not on weevil number (incidence: Wald test: $\chi^2 = 33.56$, $P < 0.0001$; infestation: $F = 1.02$, $df = 15$, $P = 0.2$).

Discussion

It has been found that inbreeding can alter the interactions between plants and their herbivores in different plant systems, and those changes can have negative results for plants most of the time (reviewed by Núñez-Farfán et al. 2007 and by Steets et al. 2007). The effect of inbreeding, however, on the performance of herbivores has rarely been assessed, thereby limiting our perception about the overall effect of plant sexual strategies on plant and herbivore interactions. The present results support the notion that the level of inbreeding of the host plant *D. stramonium* affected its interaction with the seed predator. We found a disproportionate 40% increase in the number of weevils in outcrossed plants compared with inbred plants. In contrast, the biomass of insects developed in inbred and outcrossed progeny was similar, suggesting that inbreeding did not alter host quality. This indicates that the cost of inbreeding on the interaction *D. stramonium*-*T. soror* was mainly borne by the plant. The increased weevil abundance was influenced by variations in the number of reproductive structures on inbred and outcrossed plants. However, variations in leaf size or other non-measured vigour-related traits could also modify the behaviour of

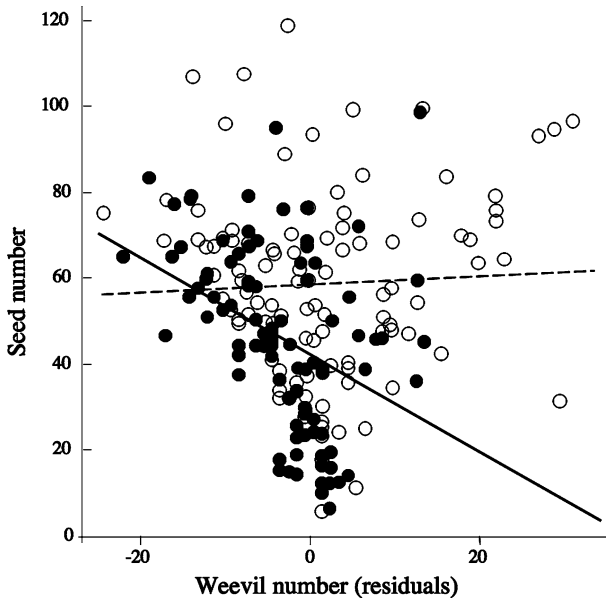


Fig. 2 Differential relationship between weevil number and the number of seeds produced by inbred (black circles, continuous line $n = 72$) and outbred progenies (open circles, dashed line $n = 105$) of *Datura stramonium* from the first experiment. Seed number is squared-root transformed and weevil number is the residuals of a regression of the number of weevils on fruit number. Slopes differ one from each other as indicated ANCOVA reported in Table 2

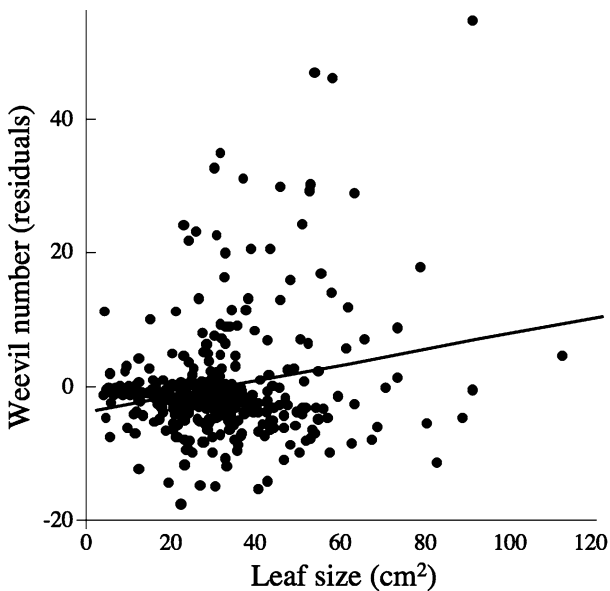


Fig. 3 Relationship between average leaf size in *Datura stramonium* and the number of weevils in plants from the second experiment examining the role of leaf size, as a vigour surrogate, on weevil infestation. Estimates of the model are shown in the figure and its significance $***P < 0.0001$

weevils. This increase in the abundance of weevils in outcrossed plants would indicate a cost of outcrossing for the plant. However, outcrossed plants were also more capable of buffering the impact of predispersal seed predation. Altogether, the results of this study suggest that the selective pressure of seed predators on inbred plants could favour the maintenance of a mixed mating system in *Datura stramonium*.

Effect on inbreeding on weevil attacks

The level of inbreeding in a plant has been found to affect the intensity and the direction (i.e., positive or negative) of its interactions with insects that feed upon different plant parts, such as nectar foragers (Ouborg et al. 2000; Ivey and Carr 2005), leaf consumers (Carr and Eubanks 2002; Hayes et al. 2004; Bello-Bedoy and Núñez-Farfán 2010a) and weevil florivores (Rohde and Ashman 2010). Experiments performed in natural conditions of herbivory have found heavier attacks on inbred plants than in outcrossed plants, suggesting a reduction in defensive components associated with inbreeding depression (Hayes et al. 2004; Stephenson et al. 2004; Bello-Bedoy and Núñez-Farfán 2010a). However, there is also evidence showing that herbivores preferred outcrossed plants over inbred ones (see Strauss and Karban 1994 and Hull-Sanders and Eubanks 2005). The latter outcome may occur if outcrossing (i.e., recombination) breaks up complex of linked loci that control characters involved in resistance to natural enemies (Koslow and Clay 2007) or if mating between individuals with different resistance level produces offspring with a low level of defence (Strauss and Karban 1994).

The high vigour of outcrossed individuals might originate a cost in the presence of herbivores with marked preferences to feed upon or to oviposit on more vigorous plants (Hull-Sanders and Eubanks 2005; Leimu et al. 2008). Variations in plant vigour have been implicated in the increased level of insect attacks observed in various species of plants, suggesting that insects become adapted to the process of plant development (Price 1991). In this study, outcrossed plants were more likely to be attacked by weevils (higher incidence) and bore more insects (higher infestation) than inbred plants. Both the incidence and infestation were significantly related to the increase in the total numbers of fruits produced by a plant, suggesting that the beneficial effects of outcrossing on plant vigour may explain the biased infestation of weevils. The results of the second experiment are in agreement with the notion that plant vigour influenced weevil infestation in *D. stramonium*. Overall, these results suggest that weevil response to *D. stramonium* was highly influenced by the effect of inbreeding on plant vigour.

The effect of leaf size on weevil number was, however, not consistent between experiments. Variations in leaf size in plants with unknown inbreeding coefficient predicted the level of infestation by weevils. However, variations in leaf size due to inbreeding failed to predict weevil attacks despite the contrasting differences in size between outcrossed and inbred plants. The female *T. soror* uses flowers and developing fruits as primary sites to lay eggs, and plants with more fruits may be more attractive hosts and influence the weevil oviposition behaviour (Cabralés-Vargas 1991). The larger flower and fruit production has been found to influence oviposition rates in other plant-predispersal seed predator systems, suggesting that the availability of flowers and fruits seemed to be a relevant attribute for female weevils when selecting among several hosts (Kudo and Whigham 1998; Marshall and Ganders 2001; Ashman et al. 2004). Although we could not rule out the potential role of leaf size on weevil infestation, it may only reveal the preference of weevils for plants with a high number of fruits that also produce bigger leaves rather than weevil preferences for plants with larger leaves.

The simultaneous effect of inbreeding on several plant traits could alter another characteristic not measured in this study, such as chemical compounds implicated in resistance to or in the attraction of weevils. Plant secondary chemistry can be used as a cue for herbivores to locate and select the host plant (Moyes and Raybould 2001), can enhance larval performance (Saastamoinen et al. 2007) or can even function as a feeding stimulant (Shonle and Bergelson 2000). There is evidence that inbreeding can alter the amount and diversity of blossom volatiles implicated in the interaction between plants and their pollinators and herbivores (Ferrari et al. 2006; Delphia et al. 2009b). Previous work in *D. stramonium* found that inbred plants were more damaged by herbivores than outcrossed plants, suggesting that inbreeding would reduce chemical or physical components of resistance in leaves of *D. stramonium* (Bello-Bedoy and Núñez-Farfán 2010a, b). The differential attack of *T. soror* on inbred and outcrossed plants found in this study contrast with the level of leaf damage previously reported (Bello-Bedoy and Núñez-Farfán 2010a). One possible explanation to these contradictory results is that an increase in chemical components of resistance to leaf consumers could have an opposite effect on weevils, attracting more weevils to plants with a higher concentration or diversity of secondary metabolites. To what extent inbreeding can change the amount and diversity of alkaloids associated with the selective behaviour of weevils and other insects is an aspect that needs further study in *D. stramonium*.

Effect of inbreeding on weevil biomass

Inbreeding can generate offspring with different chemical or nutrient quality that make their tissues less edible for insect herbivores in relation to outcrossed plants (Ferrari et al. 2006; Delphia et al. 2009b). The different growth or biomass of insects reared on inbred or outcrossed plants observed in some experiments support this idea. The evidence indicates that outcrossed plants could be more noxious for herbivores than inbred plants (Carr and Eubanks 2002; Hull-Sanders and Eubanks 2005; Leimu et al. 2008; Delphia et al. 2009a). However, some of them also showed the beneficial effect of plants' outcrossing increased plant suitability for some insect species (Strauss and Karban 1994; Carr and Eubanks 2002; Hull-Sanders and Eubanks 2005). The effect of inbreeding on plant quality was not apparent in *D. stramonium*. This study did not detect changes in the biomass of adult weevils associated with the level of inbreeding, indicating that inbred and outcrossed progenies were hosts of a similar nutritional value. Larvae of *T. soror* consume fruit and seed tissues during its development and inbreeding could have not changed the nutritional characteristics in those traits in our experimental plants. There is evidence showing no changes in seed biomass following one generation of inbreeding in this plant species (Núñez-Farfán et al. 1996), although it has been suggested that inbreeding altered the chemical traits in leaves that affected herbivore consumption (Bello-Bedoy and Núñez-Farfán 2010a). In addition, alkaloid concentration in immature seeds of *D. stramonium* is low and it increases during its maturation (Berkov et al. 2005). *T. soror* may 'escape' the toxic effects of a high-alkaloid dosage by consuming the tissues of immature fruits and seeds, however, this hypothesis remains to be explored.

Effects of weevils on seed production

Herbivores or pathogens can have a relevant role in a plant's mating system dynamics if their damage intensifies the negative effects of inbreeding depression in plants (Armbruster and Reed 2005; Núñez-Farfán et al. 2007; Steets et al. 2007; Bello-Bedoy and Núñez-Farfán

2010b). A higher fitness impact of damage by a natural enemy on inbred plants relative to outcrossed plants can prevent the establishment of selfing and/or maintain a mixed strategy of mating system in a population (Lively and Howard 1994; Agrawal and Lively 2001). Our results indicated that the ability to buffer seed predation was higher in outcrossed plants than in inbred plants, regardless of their higher level of infestation. The relative increase in the number weevils (i.e., residuals of *T. soror*) produced a higher fitness impact on inbred plants than on outcrossed plants, suggesting a higher cost for inbred plants than for outcrossed plants. Thus, an increased fitness loss by inbred progenies by seed predation may implicate selection against the adoption of complete selfing in this population of *D. stramonium*.

The difference in the capability of plants to offset seed predation observed in this study can be attributed to the higher production of reproductive structures. Outcrossed progenies bore more weevils, but also had more fruits with no damage in relation to inbred plants. This reproductive advantage in fruit and seed numbers appeared to offset the losses caused by seed predations. Evidence supporting that a higher number of reproductive structures confers the ability to offset predispersal seed predation has been reported for various plant species with different sexual systems (Brody and Mitchell 1997; Boege and Dominguez 2008). Fitness advantages are unlikely to have evolved as a mechanism to overcome the negative effects of seed predation. However, our study showed that outcrossed plants were more capable of buffering seed predation than their inbred counterparts, owing to their advantage of having greater fruit and seed production.

The mating system strategy adopted by the individuals of a population can alter the interactions with herbivores or pathogens. These enemies can, in turn, modify the mating system (Núñez-Farfán et al. 2007; Steets et al. 2007). While outcrossed progenies of *D. stramonium* had fitness costs due to seed predators, seed predation constituted a higher fitness cost for inbred progeny. Thus, seed predators, albeit indirectly, can favour the maintenance of outcrossing in this population. The effect of inbreeding on insect performance is relevant because it could modify reciprocal selection among plants and their natural enemies, and it could have implications in the coevolutionary process (Thompson 1994, 2005). However, evidence for this is very scarce in the literature. Future studies providing evidence about the effect of a plant's mating system (i.e., inbreeding or outcrossing) on their natural enemies would help us to understand the damage or infection dynamics under a mosaic of inbred and outcrossed individuals.

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The effect of inbreeding on defence against multiple enemies in *Datura stramonium*

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pathogens;
resistance;
seed predation;
tolerance;
viral infection.

Abstract

The ability of plants to respond to natural enemies might depend on the availability of genetic variation for the optimal phenotypic expression of defence. Selfing can affect the distribution of genetic variability of plant fitness, resistance and tolerance to herbivores and pathogens. The hypothesis of inbreeding depression influencing plant defence predicts that inbreeding would reduce resistance and tolerance to damage by natural enemies relative to outcrossing. In a field experiment entailing experimentally produced inbred and outcrossed progenies, we assessed the effects of one generation of selfing on *Datura stramonium* resistance and tolerance to three types of natural enemies, herbivores, weevils and a virus. We also examined the effect of damage on relative growth rate (RGR), flower, fruit, and seed production in inbred and outcrossed plants. Inbreeding significantly reduced plant defence to natural enemies with an increase of 4% in herbivore damage and 8% in viral infection. These results indicate inbreeding depression in total resistance. Herbivory increased 10% inbreeding depression in seed number, but viral damage caused inbred and outcrossed plants to have similar seed production. Inbreeding and outcrossing effects on fitness components were highly variable among families, implying that different types or numbers of recessive deleterious alleles segregate following inbreeding in *D. stramonium*. Although inbreeding did not equally alter all the interactions, our findings indicate that inbreeding reduced plant defence to herbivores and pathogens in *D. stramonium*.

Introduction

Variation in plant defence to different types of natural enemies such as herbivores or pathogens is ubiquitous in natural and managed ecosystems. This variation depends on plants' ability to resist and/or to buffer the negative impact of damage (Kennedy & Barbour, 1992). Resistance and tolerance are two defensive strategies of plants that have evolved to prevent fitness costs induced by natural enemies. Whereas resistance reduces the level of attack, damage or infection by herbivores and pathogens (Kennedy & Barbour, 1992), tolerance reduces the

fitness loss caused by a certain level of damage (Strauss & Agrawal, 1999; Heil, 2010). Understanding the contribution of sexual reproduction to genetic variation in offspring's resistance and/or tolerance to natural enemies is relevant because plant's abilities to respond rely on the genetic variation available for each defensive strategy. (Ashman, 2002; Núñez-Farfán *et al.*, 2007; Johnson *et al.*, 2009). Selfing, the ability to fertilize ovaries with own pollen, is a common mode of reproduction in plants (Eckert & Barrett, 1990; Goodwillie *et al.*, 2005), and increases homozygosity, thereby changing the distribution of genetic variation for resistance and tolerance. Therefore, the mating system can have profound effects on the offspring's defensive phenotype, altering their antagonist interactions.

Selfing can be beneficial at small population size with few available mates or in the absence of pollinators (Jain, 1976; Lloyd, 1979). However, selfing can lead to reduction

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in fitness, a phenomenon known as inbreeding depression (Hull-Sanders & Eubanks, 2005). Selfing increases homozygosity in all loci, thereby exposing the effect of recessive deleterious alleles or reducing heterozygote advantage of fitness-related traits (Charlesworth & Charlesworth, 1987). Changes in the distribution of genetic variation for resistance and tolerance caused by inbreeding can alter the distribution and impacts of herbivore or pathogen damage in a population (Núñez-Farfán *et al.*, 2007). Indeed, experimental studies have shown that inbred plants suffer more attacks by different types of natural enemies such as herbivores, fungi, bacteria and viruses (Carr & Eubanks, 2002; Hayes *et al.*, 2004; Stephenson *et al.*, 2004; Ouborg *et al.*, 2000; Bello-Bedoy & Núñez-Farfán, 2010). These studies suggest that the increased susceptibility to natural enemies associated with inbreeding is a general phenomenon in plants (hereafter, the hypothesis of inbreeding depression influencing defence). However, other studies have found no effect or positive effect of inbreeding in the same species (Núñez-Farfán *et al.*, 1996; Ouborg *et al.*, 2000; Carr *et al.*, 2003; Koslow & Clay, 2007; Leimu *et al.*, 2008), questioning the generality of the negative effect of inbreeding.

The complex effect of inbreeding on plants–natural enemies interactions has been mainly captured in experiments conducted under natural conditions of attack by natural enemies (Strauss & Karban, 1994; Stephenson *et al.*, 2004; Bello-Bedoy & Núñez-Farfán, 2010). Inbred and outcrossed plants are often attacked by several types of natural enemies that are allowed to choose from a continuum of resistant and susceptible individuals. Variation in damage and infection by natural enemies can be influenced by the level of plant resistance and by interspecific interactions between herbivores and pathogens. For instance, Stephenson *et al.* (2004) found that attack by herbivores and virus was more likely to occur on inbred plants of the wild gourd than on outcrossed, whereas the level of aphid infestation was similar in both progeny types, suggesting that inbreeding affected plant–natural enemy interactions in different ways. Ferrari *et al.* (2007) found that the increase in bacterial infection rates associated with outcrossing in *Cucurbita pepo* was influenced by the preference of its vector, the *Diabrotica* beetle, for outcrossed plants. Thus, inbreeding can directly affect the dynamics of damage or infection by natural enemies in a plant by modifying the phenotypic expression of traits mediating those interactions (Ferrari *et al.*, 2006; Delphia *et al.*, 2009). In addition, it also could give rise to indirect effects if a change in the pattern of attack by one natural enemy alters the pattern of attack of a second natural enemy.

Natural enemies are thought to play a role in the evolution of plant mating systems (Ashman, 2002). For example, foliar herbivory can reduce resource allocation to vegetative growth and reproduction (Steets *et al.*, 2007). Herbivore damage can affect the production of

outcrossed seeds by modifying floral display (Elle & Hare, 2002; Steets *et al.*, 2006a,b) and thereby interactions with pollinators (Ivey & Carr, 2005). In addition, enemies could affect the evolutionary dynamics of mating system by intensifying the magnitude of inbreeding depression (Carr & Eubanks, 2002; Ivey *et al.*, 2004). Parasites (or other natural enemies) can lead to selection for intermediate or complete outcrossing when inbreeding increases the probability of attack (i.e. reduced resistance) or reduces the capacity of hosts to maintain high fitness (i.e. tolerance; Lively & Howard, 1994; Agrawal & Lively, 2001). Experimental evidence has shown that natural enemy attacks increased the strength of inbreeding depression in some plant and animal systems, suggesting that the selective damage on inbred individuals can prevent evolution towards complete selfing in a population (Carr & Eubanks, 2002; Hayes *et al.*, 2004). However, this apparent fitness superiority of outcrossing can be reduced if the amount of damage incurred by natural enemies is higher in outcrossed than inbred progenies (Koslow & Clay, 2007; Leimu *et al.*, 2008). Thus, inbreeding depression on resistance could be more relevant than on tolerance in determining the prevalence of outcrossing in a plant population.

Datura stramonium produces most seeds by self-fertilization (81.3–91.7%; Motten & Antonovics, 1992), but the rate of production of outcrossed seeds can vary within and among populations (Motten & Antonovics, 1992; Cuevas, 1999; Motten & Stone, 2000). Selfing negatively modifies vegetative and reproductive components implicated in biotic interactions. Leaf size and fruit and seed number can show significant inbreeding depression ($\delta_{\text{leaf size}} = 0.21$, $\delta_{\text{fruit number}} = 0.15\text{--}0.3$, $\delta_{\text{seed number}} = 0.19\text{--}0.43$; Núñez-Farfán *et al.*, 1996; Stone & Motten, 2002; Bello-Bedoy & Núñez-Farfán, 2010). Inbreeding also decreases resistance to the specialist chrysomelid beetles *Lema trilineata* and *Epitrix parvula* that cause large reductions in leaf area ($\delta_{\text{resistance}} = 0.06$; Bello-Bedoy & Núñez-Farfán, 2010). In contrast, infestation rates by the weevil *Trichobaris soror* increased 40% in outcrossed progenies, although their fitness was nonetheless higher than that of inbred progenies regardless of the level of seed predation (R. Bello-Bedoy, L. Cruz & J. Núñez-Farfán, unpublished data). Thus, even though *D. stramonium* has adopted selfing as the main strategy of seed production, differential selective impacts of herbivores may counterbalance the reproductive assurance advantage of selfing.

In this study, we assessed the effect of inbreeding on the interaction between *Datura stramonium* and three types of natural enemies: two specialist folivores, a virus, and a predispersal seed predator. We exposed experimentally inbred and outcrossed progenies of *D. stramonium* in their native site to examine the effect of one generation of inbreeding under natural conditions of attack by different types of natural enemies. We addressed the following questions:

3, 4

- 1 (1) Does inbreeding simultaneously reduce resistance to
- 2 different natural enemies?
- 3 (2) Does damage by natural enemies differently affect
- 4 the fitness of inbred and outcrossed progenies?
- 5 (3) Do the effects of inbreeding differ between fitness
- 6 components and between plant families?
- 7

8 **Materials and methods**

9 **Study system**

12 *Datura stramonium* L. is an annual herb that grows in a
 13 variety of disturbed and agricultural habitats in Mexico,
 14 USA, Canada, and Europe (Weaver & Warwick, 1984;
 15 Weaver *et al.*, 1985; Valverde *et al.*, 2001; van Kleunen
 16 *et al.*, 2007; Cuevas-Arias *et al.*, 2008). In Central México,
 17 this plant is attacked by the specialist beetles *L. trilineata*
 18 and/or *E. parvula* and by a predispersal seed predator
 19 **5** *Trichobaris soror* (Núñez-Farfán & Dirzo, 1994; R. Bello-
 20 Bedoy, L. Cruz & J. Núñez-Farfán, unpublished data).
 21 The damage caused by these insects can reduce plant
 22 fitness. Trichomes and alkaloids are two defensive char-
 23 acters of *D. stramonium* that reduce leaf damage by
 24 herbivores (Shonle & Bergelson, 2000; Valverde *et al.*,
 25 2001). In turn, weevil attack is mainly influenced by
 26 variation in flowers and fruits (R. Bello-Bedoy, L. Cruz &
 27 J. Núñez-Farfán, unpublished data). In addition, *D. stra-*
 28 *monium* has been extensively used as a plant model to
 29 tests viral susceptibility under laboratory conditions
 30 (Brunt *et al.*, 1996). However, knowledge about its
 31 interactions with pathogens under natural conditions is
 32 very scarce (Sutic *et al.*, 1999). In Mexico and USA, this
 33 plant can be infected by the potyvirus *Tobacco etch virus*
 34 (TEV) that reduces growth and causes leaf and fruit
 35 **6** deformation (Blakeslee, 1921; Sutic *et al.*, 1999).

37 **Experimental design: base population**

39 The *D. stramonium* used in this experiment were collected
 40 from a population located in Teotihuacán (State of
 41 Mexico; 19°41'N, 98°51'E), for which phenotypic selec-
 42 tion, inbreeding depression to natural levels of insect
 43 damage and genetic variation for leaf trichomes have
 44 been previously reported (Valverde *et al.*, 2001; Bello-
 45 Bedoy & Núñez-Farfán, 2010). We randomly chose field-
 46 collected seeds from 30 plants of *D. stramonium* to
 47 produce outcrossed seeds, with the aim of standardizing
 48 natural levels of inbreeding (Falconer & Mackay, 1997).
 49 We considered seeds from a single plant as a family.

50 We sowed 100 field-collected seeds per family in 3-L
 51 plastic pots filled with common soil and oak and pine
 52 litter (40%, 30%, and 30%) and watered to promote
 53 seed germination. At germination we thinned, leaving
 54 one randomly chosen individual to flower, which was
 55 used as pollen recipient and donor for producing
 56 outcrossed seeds. During flowering, we cross-pollinated
 57 two flowers by rubbing three dehisced anthers with

abundant pollen on the stigmatic surface of a flower.
 Anthers were randomly taken from each of three
 different plants. Prior to hand pollination, we emas-
 culated recipient flowers to prevent autonomous self-
 pollination. After pollination, we covered the flowers
 with fine mesh bags to prevent pollen contamination.
 Mature fruits were individually collected in paper bags
 before they started to dehisce, and seeds were stored in
 the laboratory at ambient temperature.

Crossing design: inbred and outcrossed seeds

We performed controlled crosses to generate outcrossed
 and inbred seeds with an approximate inbreeding coef-
 ficient of $f = 0$ and $f = 0.5$, respectively, using the
 outcrossed base population generated earlier. We out-
 crossed by following the pollination protocol described
 earlier, and we self-pollinated two flowers on the same
 plant by rubbing the pollen of three anthers against the
 stigmatic surface of the same flower. We collected mature
 fruits in paper bags before they started to open, and the
 seeds were stored in the laboratory.

Field experiment

We sowed 100 seeds per pollination treatment from each
 of 19 families. The 456 inbred and 456 outcrossed
 seedlings we obtained were transplanted to an agricul-
 tural plot of 1800 m². We arranged the same number of
 inbred and outcrossed seedlings in a random three-block
 design with 1 m between neighbours.

We applied 250 mL of the systemic insecticide FURA-
 DAN[®] at concentration of 0.250 mL L⁻¹ every 2 weeks to **7**
 12 inbred and 12 outcrossed randomly chosen plants per
 family. The same number of inbred and outcrossed plants
 per family was supplemented with 250 mL of water
 (control group). The treatments started 1 week after
 transplantation and until leaves and fruits were har-
 vested. We applied insecticide and water to the ground at
 the base of each plant. In each block, we distributed four
 inbred and four outcrossed plants of the insecticide
 treatment and the same number of plants of the control
 group.

Effects of inbreeding on plant–natural enemy interactions

In November, a frost caused plants to stop producing
 more flowers and fruits. At this time, we collected the
 fruits of 776 plants and the leaves from 519 individuals
 because the rest had lost their leaves. We collected a
 random sample of 20 leaves per plant, including leaves
 along the stem and branches to prevent a biased
 estimation of damage (i.e. resistance) by temporal
 variation in exposure to herbivores. Total and damaged
 area was measured in each leaf by means of digital image
 analysis using WINDIAS software (Basic; Delta-T-Devices,

Cambridge, UK). Individual damage (D_i) was computed as,

$$D_i = \left(\frac{1}{n} \sum_{i=1}^n \frac{A_D}{A_T} \right),$$

where A_D and A_T are the damaged and total area of a leaf, respectively, and n is the number of leaves (Bello-Bedoy & Núñez-Farfán, 2010).

Weevil incidence and infestation

In the laboratory, we opened all fruits to record the presence and number of weevils per plant. We registered weevils as present (1) when larvae or adults were found within the fruit of a plant. If no weevils were found, we registered weevils as absent (0). In addition, we counted the larvae and adults within the fruits to obtain the number weevils per plant. Operationally, we defined weevil incidence as the probability of insect attack and weevil infestation as the number of weevils observed on a plant.

Viral infection estimates

At the end of the experiment, we noted the presence or absence leaf and flower deformation caused by virus infection in each plant. We grouped plants into three categories according to degree of damage to leaves and flowers (Stephenson *et al.*, 2004): (1) noninfected: when no viral symptoms were observed; (2) mid-infection: when viral symptoms were observed in a part of leaves and flowers; and (3) full infection: when viral symptoms were observed in all the leaves and flowers.

Components of plant fitness

In order to assess the effects of inbreeding and natural enemies on plants, we obtained individual estimates of relative growth rate (RGR_i hereafter), day to first flowering, and the number of flowers, fruits, and seeds. To estimate RGR, we measured plant height at two plant stages. We calculated individual RGR as,

$$RGR_i = \log(PS_2 - PS_1) / T_{DN},$$

where PS_1 is the first measurement carried out 1 week after transplantation and PS_2 is the second measurement carried out 40 days later; T_{DN} is the number of days elapsed between the first and the second measurement. We conducted both measures before plants started to reproduce.

The day of first flowering was the number of days from transplanting until the date of the production of the first flower. We recorded the number of flowers every second day until frost stopped further flower production. If the corolla fell off before registering, we considered developing fruits as a surrogate of flower production. Fruits were collected and counted in the laboratory.

To estimate seed number per fruit, we weighed total seed number and a sample of 50 seeds from each fruit on an analytic balance (± 0.001 g). We calculated the seed number per fruit by multiplying total seed biomass times 50 seeds and divided by the biomass of 50 seeds. We summed the seed number from each fruit to obtain total seed number per plant, the maternal fitness component. To test the accuracy of this method, we counted and weighed the seeds in a random sample of 120 fruits. A significant Pearson's correlation between seed number and total seeds biomass indicated that this method was highly accurate ($r = 0.97$, $P < 0.0001$; $n = 120$).

Statistical analysis

Effects of inbreeding on plant–natural enemy interactions

We examined the effect of inbreeding on damage caused by the three types of natural enemies independently. The continuous variables of plant damage such as herbivory (individual damage) and infestation (\log weevil number per plant) were analysed using mixed-model analyses of covariance. The nominal variables of weevil incidence (presence = 1, absence = 0), virus incidence (presence = 1, absence = 0), and virus infection level (non infection = 0, mid infection = 1, and full infection = 2) were analysed using logistic analyses of covariance (CAT-MODE; Littell *et al.*, 1996). In all models, we included the explanatory effects of family (random), degree of inbreeding (i.e. pollination treatment; fixed), and insecticide (fixed), and their interactions. The nonsignificant three-way interactions were removed from all analyses (i.e. family \times degree of inbreeding \times insecticide). We added the effect of block (fixed) in all models to control for an effect of spatial heterogeneity in the experimental plot.

To control for an effect of plant size (i.e. vigour), plant height was included as a covariate in all analyses of damage by natural enemies. Flower and fruit number have been previously associated with the abundance of weevils in *D. stramonium* (R. Bello-Bedoy, L. Cruz & J. Núñez-Farfán, unpublished data). Therefore, these data were included as covariates in their corresponding analysis.

Estimates of inbreeding depression on plant defence (δ)

We calculated the coefficient of inbreeding depression for 'total resistance' to herbivores, weevil attack, and virus infection separately. Inbreeding depression was calculated as:

$$\delta = 1 - (\bar{w}_{\text{inbred}} / \bar{w}_{\text{outcrossed}}),$$

where \bar{w}_{inbred} and $\bar{w}_{\text{outcrossed}}$ are the mean values for a given trait of inbred and outcrossed progenies, respectively (Charlesworth & Charlesworth, 1987). Positive coefficients of inbreeding depression indicate an increment in the proportion of damage attributed to inbreeding. Negative inbreeding depression coefficients indicate the reverse effect.

1 The inverse of the average proportional damage com-
 2 puted from plants in the control treatment, $R_i = 1 - D_i$,
 3 was considered as total resistance to herbivores (Leimu &
 4 Koricheva, 2006; Bello-Bedoy & Núñez-Farfán, 2010).
 5 Total resistance to viral infection was estimated as the
 6 inverse of the probability of infection (i.e. incidence) for
 7 inbred and outcrossed progeny; finally, total resistance to
 8 seed predators was estimated as the inverse of the
 9 probability of weevil attack (i.e. incidence) and also
 10 using the average values of weevil infestation in inbred
 11 and outcrossed progenies.

12 *Inbreeding and natural enemy effects on fitness* 13 *components*

14 To examine the effects of the degree of inbreeding (i.e.
 15 pollination treatment) and damage by natural enemies
 16 on fitness components of *D. stramonium*, we conducted
 17 independent mixed-model analyses of covariance
 18 (ANCOVA) for RGR, day to first flowering, flower number,
 19 fruit number and seed number. In all the models, we
 20 included the explanatory variables of family (random),
 21 degree of inbreeding (fixed), and insecticide (fixed), and
 22 their two-way interactions family \times degree of inbreeding,
 23 family \times insecticide, degree of inbreeding \times insecticide.
 24 We removed the nonsignificant three-way interactions
 25 (family \times degree of inbreeding \times insecticide) from the
 26 analyses. We included virus level and the interaction
 27 virus level \times degree of inbreeding in the statistical anal-
 28 ysis to examine the effect of viral infection on fitness
 29 components of *D. stramonium*. The effects of plant height
 30 and block were also included.

31 Because damage by larvae and adults of *Trichobaris*
 32 *soror* reduces seed number in *D. stramonium*, we included
 33 the effects of weevil number and weevil number \times
 34 degree of inbreeding interaction exclusively in the mixed
 35 model ANCOVA of seed number to examine the impact of
 36 seed predation.

37 In order to improve normality and meet ANOVA
 38 assumptions, prior to statistical analyses, we transformed
 39 weevil number, days to flowering, the number of
 40 flowers, fruits, and seeds to a logarithmic scale, whereas
 41 plant damage was square root arcsine transformed (Zar,
 42 1996). All analyses were run using JMP 5.01 software
 43 (SAS Institute, Cary, NC, USA).

44 **Results**

45 The specialists herbivores *L. trilineata* and *E. parvula*
 46 caused 20.5% reduction in leaf area in *D. stramonium*.
 47 A significant degree of inbreeding effect indicated that
 48 inbred and outcrossed plants were differentially dam-
 49 aged by herbivores (Table 1). Inbred plants suffered 4%
 50 more herbivore damage than outcrossed ones when
 51 exposed to natural level of herbivory (i.e. control plants:
 52 $D_{\text{inbred}} = 0.24 \pm \text{SE } 0.003$ vs. $D_{\text{outcrossed}} = 0.20 \pm \text{SE}$
 53 0.003 ; Table 1). This difference resulted in an inbreeding
 54 depression of $\delta = 0.05$ in total resistance to herbivores.
 55
 56
 57

No significant differences in herbivore damage were
 observed between the inbred and outcrossed progeny in
 the insecticide treatment (Table 1). Insecticide-treated
 plants suffered significantly less damage by herbivores
 than those without insecticide ($D_{\text{insecticide}} = 0.18 \pm \text{SE}$
 0.003 vs. $D_{\text{noninsecticide}} = 0.22 \pm \text{SE } 0.003$; Table 1). How-
 ever, all plants were damaged by herbivores regardless
 of insecticide and degree of inbreeding treatments.
 Plants with symptoms of viral infection suffered less
 herbivory than those plants with no signs of infection
 ($D_{\text{infected}} = 0.22$ vs. $D_{\text{noninfected}} = 0.19$; Table 1). Signifi-
 cant effects of plant height on the level of damage were
 detected, with higher plants being less damaged than
 smaller plants. The block effect was significant indicating
 spatial variation in herbivore damage. None of the
 family effects was significant (Table 1).

58 **Weevil incidence and infestation**

Trichobaris soror was present in 36% of the plants (279
 of 776). Weevil incidence (i.e. the probability of weevil
 attack) was significantly affected by several factors,
 including viral infection (Table 1). The main effects of
 family, degree of inbreeding, and insecticide did not affect
 weevil incidence though the family \times degree of inbreed-
 ing and family \times insecticide interactions were significant
 (Table 1), indicating heterogeneous effects of both
 inbreeding and insecticide among plant families. Weevil
 number significantly increased in plants with more
 flowers. The average number of weevils per plant was
 $2.4 (\pm \text{SE } 0.18)$. After controlling for the significant block
 effect, fruit number was the only variable affecting weevil
 number in *D. stramonium* (i.e. infestation; Table 1), with
 more weevils found on plants with more fruits.

61 **Viral infection**

Viral symptoms were observed in 60% of the plants (442
 of 772). Most infected leaves remained on the plant until
 harvesting, whereas infected flowers were aborted or
 fruit formation was precluded.

After controlling for the block effect, we found 8%
 higher probability of viral infection in inbred compared
 to outcrossed plants (Table 1; 0.63 for inbred progeny
 and of 0.55 for outcrossed progeny), resulting in inbreed-
 ing depression $\delta = 0.16$ in total resistance to viral
 infection. In addition, the effects of family and the
 family \times degree of inbreeding interactions were margin-
 ally significant (Table 1), suggesting the existence of
 genetic variation for viral resistance.

Significant differences were noted in the intensity of
 viral damage among families, and we found more viral
 damage in inbred than in outcrossed progenies (Table 1).
 This gave an inbreeding depression coefficient of $\delta = 0.05$
 for total resistance to viral infection. Neither insecticide
 nor family \times insecticide interaction had a significant
 effect on viral infection level.

Type of damage	Source	d.f.	χ^2 or F	P
Herbivore damage	Family	18	3.21	0.4017
	Degree of inbreeding	1	36.35	< 0.0001
	Insecticide	1	55.26	< 0.0001
	Family \times degree of inbreeding	18	0.46	0.9711
	Family \times insecticide	18	0.73	0.77
	Degree of inbreeding \times insecticide	1	2.9	0.089
	Virus infection	1	15.9	< 0.0001
	Plant height	1	6.75	< 0.0097
Weevil incidence	Block	2	23.75	< 0.0001
	Family	18	24.91	0.1273
	Degree of inbreeding	1	2.023	0.1575
	Insecticide	1	0.952	0.3291
	Family \times degree of inbreeding	18	22.82	0.0588
	Family \times insecticide	18	34.94	0.0017
	Degree of inbreeding \times insecticide	1	1.102	0.2932
	Virus infection	2	15.62	0.0001
Weevil number	Flower number	1	90.69	< 0.0001
	Block	2	52.26	< 0.0001
	Family	18	1.82	0.1783
	Degree of inbreeding	1	0.1	0.7511
	Insecticide	1	0.21	0.6489
	Family \times degree of inbreeding	18	0.85	0.6338
	Family \times insecticide	18	1.16	0.2866
	Degree of inbreeding \times insecticide	1	0.27	0.6027
Virus incidence	Virus infection	2	2.33	0.1271
	Fruit number	1	426.14	< 0.0001
	Block	2	6.617	0.0014
	Family	18	27.82	0.0647
	Degree of inbreeding	1	5.47	0.0193
	Insecticide	1	0.068	0.7932
	Family \times degree of inbreeding	18	27.6	0.0683
	Family \times insecticide	18	18.02	0.4542
Virus level	Degree of inbreeding \times insecticide	1	18.02	0.5641
	Block	2	5.64	0.0595
	Family	18	62.52	0.0041
	Degree of inbreeding	1	6.01	0.0495
	Insecticide	1	0.97	0.6139
	Family \times degree of inbreeding	18	47.84	0.0895
	Family \times insecticide	18	40	0.2971
	Degree of inbreeding \times insecticide	1	1.12	0.5717
Block	2	82.24	< 0.0001	

Table 1 Mixed Model ANCOVA of herbivory, weevil incidence and infestation, and virus incidence and infection level in *Datura stramonium* as a function of family, level of inbreeding, insecticide, virus infection, plant height and block.

Fitness components

Fitness of *D. stramonium* did not vary significantly among family or between degree of inbreeding or insecticide treatments (i.e. herbivore damage). However, the family \times degree of inbreeding interaction was significant for RGR, day to first flowering, and flower, fruit, and seed numbers (Table 2; Fig. 1a–d), suggesting genetic variation for inbreeding depression. In addition, we found negative effects of outcrossing in some families. The family \times insecticide interaction only affected the day to flowering significantly (Table 2).

The degree of inbreeding \times insecticide interaction had only a marginally significant effect on seed production, suggesting a different effect of herbivore damage

between inbred and outcrossed plants in this fitness component (Table 2). Although plants with insecticide suffered 4% less damage than those without insecticide, plants with a natural level of herbivory showed 10% higher inbreeding depression in seed number than plants in the insecticide treatment (Table 2; Fig. 2).

With the exception of day to first flowering, all fitness components were significantly affected by viral infection (Table 2). However, viral infection effects varied across fitness components. The RGR and flower number increased in the intermediate level of viral infection (Fig. 3a,b), whereas the number of fruits and seed declined as the level of viral infection increased (Fig. 3c,d). Compared to noninfected plants, seed number was up to 60% lower in fully infected plants and 33%

Table 2 ANCOVAs of fitness components of *Datura stramonium* namely, relative growth rate (RGR), flowering day, flower number (flowers), fruit number (fruits), and seed number (seeds) as function of family, level of inbreeding, insecticide, virus infection, weevil number and their interactions.

Source of variation	d.f.	Components of fitness				
		RGR log <i>F</i> value	Flowering day <i>F</i> value	Flowers <i>F</i> value	Fruits <i>F</i> value	Seeds <i>F</i> value
Family	18	1.07	1.01	1.39	1.2	0.69
Degree of inbreeding	1	0.12	0.19	0.327	0.31	0.01
Insecticide	1	0.84	1.88	0.42	1.72	0.1
Family × degree of inbreeding	18	2.48**	2.19***	1.77†	1.83†	2.07*
Family × insecticide	18	1	1.74†	0.85	1.6†	1.64†
Degree of inbreeding × insecticide	1	0.86	0.17	0.06	2.02	3.86†
Virus level	2	9.71***	0.8	4.05*	64.68***	26.67***
Virus level × degree of inbreeding	2	0.61	1.54	0.35	0.63	5.41*
Plant height	1	238.23***	432.45***	476.66***	448.86	202.01***
Block	2	11.27***	0.76	22.27***	4.12*	0.48
Weevils (0/1)	1					59.81***

Values are the *F* ratios, bold case and the sign after the *F* value indicates significances to the nearest probability ($P < 0.05^†$; $P < 0.01^*$; $P < 0.001^{**}$; $P < 0.0001^{***}$).

lower in mid-infected plants (Fig. 3d). In addition, the significant viral infection level × degree of inbreeding interaction indicates a different effect of viral infection on seed production of inbred and outcrossed progenies (Table 2; Fig. 2).

Weevils led to a significant reduction in seed production, but it was similar for inbred and outcrossed progeny as indicated by the nonsignificant weevil number × pollination type interaction (Table 2).

Discussion

Effects of inbreeding – interactions with natural enemies

Inbred progenies of *D. stramonium* suffered heavier herbivore and pathogen damage to leaves and flowers than did outcrossed progenies, though damage caused by weevils to fruits and seeds did not differ between inbreeding levels. Inbreeding increases homozygosity, also for recessive deleterious alleles, resulting in inbreeding depression (Charlesworth & Charlesworth, 1987). Here, it appears that inbreeding increases the variation in plant defence against different types of natural enemies, thereby influencing the selective pressure on homozygous and heterozygous individuals.

To date, most studies have examined the effect of inbreeding on resistance to one herbivore or pathogen (Strauss & Karban, 1994; Carr & Eubanks, 2002; Carr *et al.*, 2003; Hayes *et al.*, 2004) and evidence that inbreeding could simultaneously reduce plant resistance to various natural enemies is rare (Stephenson *et al.*, 2004). However, Stephenson *et al.* (2004) found higher levels of damage by herbivores and pathogens to inbred than outcrossed plants of wild gourd, although aphid

infestation was similar in inbred and outcrossed progenies. Their results suggest that inbred progeny suffered a simultaneous reduction in resistance to different types of natural enemies. In the present study, inbred progenies of *D. stramonium* experienced more foliar damage by herbivores and a more severe pathogen infection than did outcrossed plants. Our results suggest an overall inbreeding depression in total resistance to these enemies, supporting the hypothesis that inbreeding can simultaneously reduce defences against different natural enemies.

In addition to the direct effect of inbreeding that causes reductions in resistance to pathogen infection (Koslow & Clay, 2007), inbreeding can indirectly affect the behaviour of vector-borne pathogens, thereby altering plant exposure to pathogens diseases. Inbreeding can affect floral characters used as cues by pollinators (Ouborg *et al.*, 2000; Ivey & Carr, 2005) or biochemical signals relevant for detection of plant hosts by herbivores (Ferrari *et al.*, 2006), and these changes are likely to modify the dynamics of pathogen infection (Ouborg *et al.*, 2000; Ferrari *et al.*, 2007). For example, Ferrari *et al.* (2007) found that the increased bacterial infection observed in outcrossed plants of *Cucurbita pepo* was linked with the preference of the vector *Diabrotica* for larger plants, and not to changes in resistance to infection. Ouborg *et al.* (2000) found that inbreeding depression for petal size and nectar volume in *Silene alba* could reduce pathogen transmission, presumably because the variation generated by inbreeding altered the movement of disease vectors between plants, thereby affecting infection risk. In our experiment, all plants of *D. stramonium* showed damage by the potential virus vectors *L. trilineata* and *E. parvula*, suggesting that inbred and outcrossed plants were both exposed to viral infection. Thus, variation in

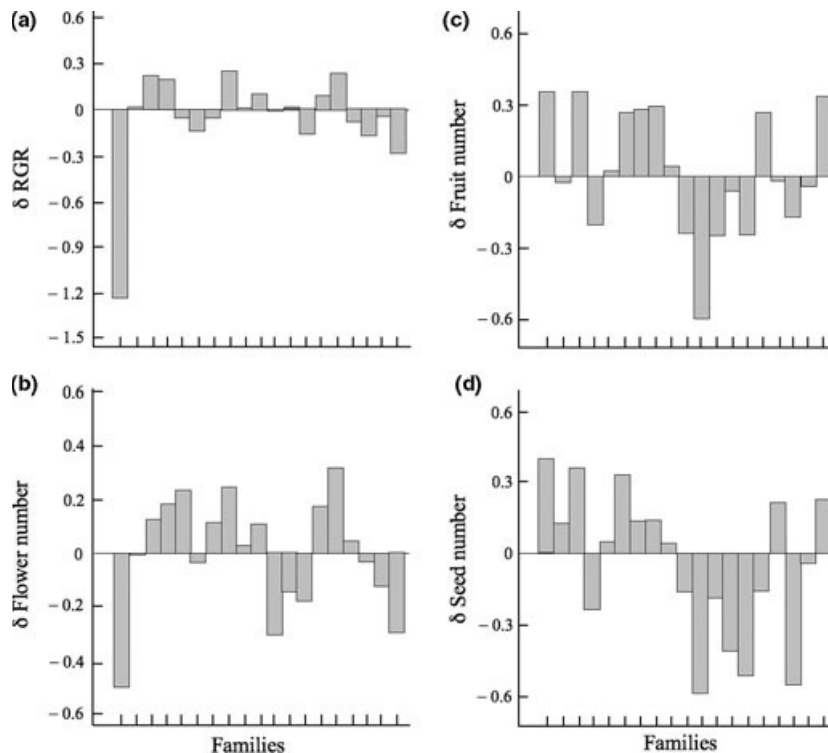


Fig. 1 Variation in mean family inbreeding depression (δ) of relative growth rate (RGR) flower number, fruit number and seed number in experimentally inbred and outcrossed progenies of *Datura stramonium*. Each bar corresponds to the inbreeding depression coefficient for each of the 19 families (1–19 from left to right). Mean family values for inbred (ϖ_{inbred}) and outcrossed progenies ($\varpi_{\text{outcrossed}}$) were used to calculate family inbreeding depression coefficients (δ_{family}) as, $\delta_{\text{family } i} = 1 - (\varpi_{\text{inbred}}/\varpi_{\text{outcrossed}})$. Numbers in the y-axis indicate the magnitude of inbreeding depression (δ) for a given character. Positive values indicate when outcrossed outperformed inbred progeny; negative values indicate the reverse.

the intensity of viral infection observed between inbred and outcrossed progenies of *D. stramonium* is most likely attributable to the direct effects of inbreeding depression on resistance.

Inbreeding seemed to alter plant–natural enemy interactions in *D. stramonium* by affecting components of total resistance. However, we found no differences in incidence and infestation by *T. soror* between inbred and outcrossed plants, indicating that the effect of inbreeding was not general for all enemies. This result contrasts with a previous study that found 40% more weevils in outcrossed than inbred progenies. The disproportionate weevil number on outcrossed plants was influenced by their greater fruit production (i.e. inbreeding depression in fruit number; R. Bello-Bedoy, L. Cruz & J. Núñez-Farfán, unpublished data), suggesting that an effect of inbreeding on reproductive components would influence infestation rates by weevils in *D. stramonium*. Our study found that weevil incidence and infestation were significantly related to the number of flowers and fruits produced by *D. stramonium*. However, this experiment did not find differences in the number of reproductive structures between inbred and outcrossed plants (i.e. no inbreeding depression), possibly explaining the similarity in the levels of attack by weevils. In both experiments, the plants were grown in their native site (i.e. Teotihuacán) in the presence of natural enemies under similar environmental conditions (R. Bello-Bedoy, L. Cruz & J. Núñez-Farfán, unpublished data). Thus, the yearly variation in infestation rates by *T. soror* could

be partially attributable to disparity in the effects of inbreeding depression on flower and fruit number in *D. stramonium*.

Effects of natural enemies on fitness components of inbred and outcrossed plants

Inbreeding can reduce plant tolerance to herbivory or pathogen infection (Ivey *et al.*, 2004). This would lead to inbreeding depression being more intense in environments where natural enemies are present compared to herbivore-free environments (Carr & Eubanks, 2002; Ivey *et al.*, 2004; Hayes *et al.*, 2004; Hull-Sanders *et al.*, 2005; but see Koslow & Clay, 2007; Leimu *et al.*, 2008). However, natural enemies could reduce or eliminate this fitness advantage if outcrossed plants suffer more damage than inbred ones (Koslow & Clay, 2007; Leimu *et al.*, 2008). It seems that the advantage of outcrossing may depend on the type of natural enemy and the severity of damage. Tolerance can be limited at certain levels of damage (Stowe *et al.*, 2000). If inbred and outcrossed plants have a different ability to tolerate specific levels of damage (Leimu *et al.*, 2008), resistance could be more relevant than tolerance in allowing plants to maximize individual fitness in the presence of several natural enemies.

It has been suggested that herbivores can exacerbate inbreeding depression in reproductive traits of *D. stramonium* (Bello-Bedoy & Núñez-Farfán, 2010). The present study supports this idea. Plants exposed to natural

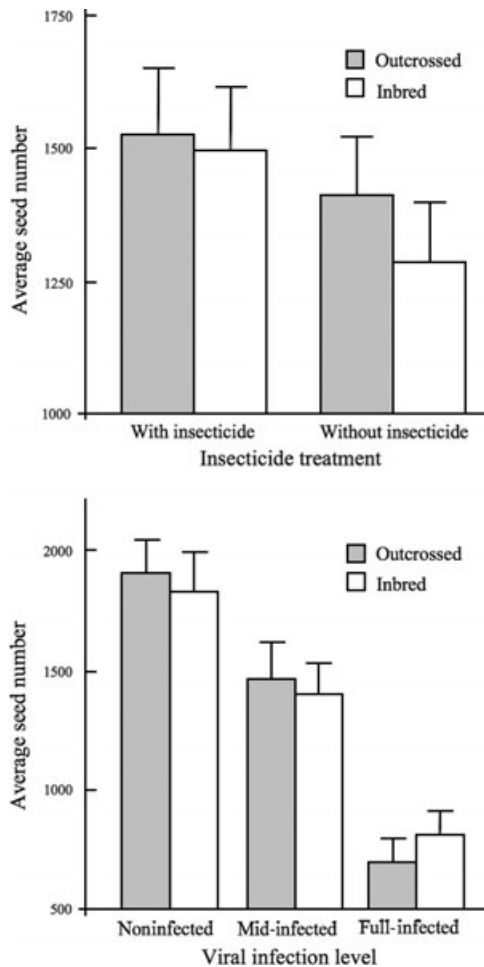


Fig. 2 Average seed number (± 1 standard error) for inbred and outcrossed progenies of *Datura stramonium* with insecticide (low damage) and without insecticide (high damage by herbivores) and average seed production of inbred and outcrossed progenies at different level of viral infection: Noninfected (0), mid-infected (1) and full-infected (2).

herbivory (i.e. the control group) suffered 10% more inbreeding depression in seed number when compared to plants with low herbivory (i.e. insecticide treated group) even though the difference in herbivory between inbred and outcrossed plants was rather low (4%). With this difference of damage, it seems unlikely that herbivores would be adequate selection agents against the adoption of complete selfing in this population (Lively & Howard, 1994). However, even a low frequency of outcrossing is expected to confer defensive advantages (Agrawal & Lively, 2001), suggesting that a mixed mating system biased towards selfing can be indirectly maintained by natural enemies. In addition, the most intense level of viral infection reduced the apparent effect of inbreeding depression, and inbred plants had a seed output slightly higher than outcrossed plants. The complex interplay of

multiple natural enemies helps to maintain a mixed mating system in *D. stramonium* (Agrawal & Lively, 2001), if selfing and outcrossing offer different benefits for facing different herbivores and/or pathogens. It is thought that a more diverse community of natural enemies should maintain high levels of genetic diversity (Fritz, 1992; Peacock *et al.*, 2001). Similarly, contrasting selective pressures on selfing and outcrossing by different herbivores or pathogens could maintain both mating system strategies in a population. Further experimental evidence is needed to support this hypothesis.

Genetic variation in inbreeding depression in fitness components

Experimental studies with *D. stramonium* have associated among-family variation in inbreeding depression for seed number with variation in herkogamy (i.e. anther – stigma distance), a floral character related to the history of inbreeding in this plant species (Motten & Stone, 2000). These studies suggest that the populations of *D. stramonium* are comprised of lineages with variable loads of recessive deleterious alleles because of differential ability for purging (Stone & Motten, 2002; Bello-Bedoy & Núñez-Farfán, 2010). Our experimental design distributed the genetic variation within the individuals of the base population, reducing the difference in inbreeding history of our lineages (Falconer & Mackay, 1997). Outcrossing treatment would not totally reduce the inbreeding coefficient to zero if cross-pollinations occurred between genetically related individuals (i.e. biparental inbreeding). Biparental inbreeding reduces the negative effects of inbreeding (Yahara, 1992), and the variable responses to self- and cross-pollination observed in this experiment may be partially attributed to differences in inbreeding history among families (Mutikainen & Delph, 1998; Goodwillie & Knight, 2006). In addition, genetic variation in inbreeding depression can be attributed to the random assortment of deleterious alleles generated by the outcrossing design (Schultz & Willis, 1995). It has been suggested that this population of *D. stramonium* maintains a significant fraction of recessive deleterious alleles for fitness (Bello-Bedoy & Núñez-Farfán, 2010). Thus, the variability in inbreeding depression can be caused by differences in the number and type of recessive deleterious alleles among the families.

Outbreeding depression could result from new combination of deleterious alleles, which reduced fitness in heterozygous individuals (i.e. negative epistasis; Fox, 2005) and/or because outcrossing broke up co-adapted gene complexes (Lynch, 1991). Individuals of *D. stramonium* self-fertilize most of the time and this might limit effective gene dispersal within and among populations. Selfing can increase the probability of linkage between gene complexes that control defence to natural enemies and/or fitness (Nordborg *et al.*, 2002). Thus, fitness decline associated with outcrossing in some families

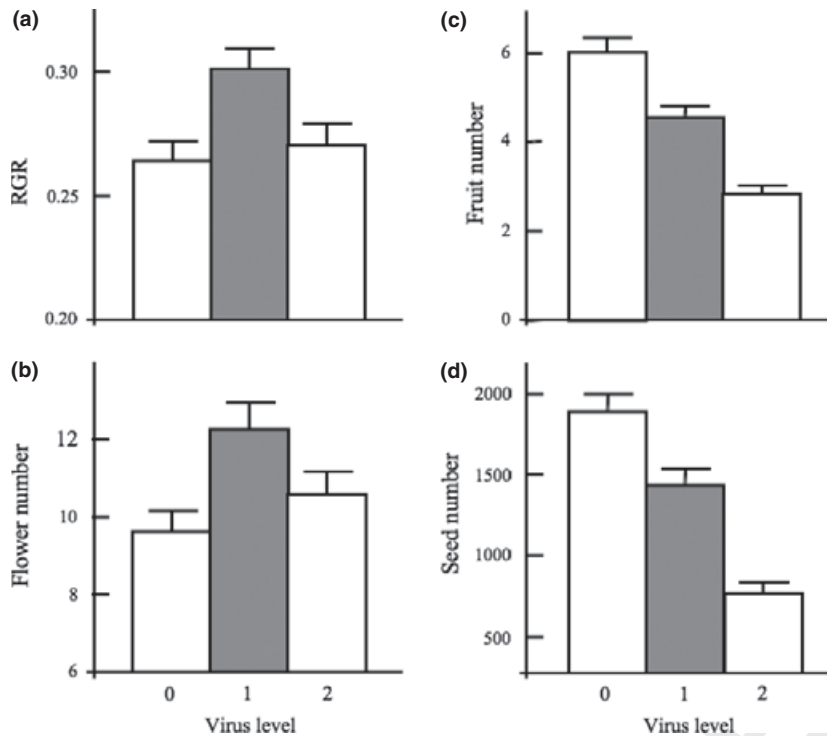


Fig. 3 Variation in the effect of viral infection on fitness components of *Datura stramonium*, relative growth rate (RGR), flower number, fruit number and seed number. Numbers on the y-axis are average values (± 1 standard error) for a given trait at different levels of viral infection: Noninfected (0), mid-infected (1) and full-infected (2).

could be explained by a disruption of extant advantageous combinations of genes in highly selfing lineages.

This study found negative effects of outcrossing using parental plants from the same population, whereas no negative effects of outcrossing were detected in plants derived from two distant populations in *D. stramonium* (Bello-Bedoy & Núñez-Farfán, 2010). An explanation is that outcrossed seeds used in this study were derived from a second generation of outcrossing, it increasing the likelihood of detecting the negative effects of recombination (F_2 generation; Lynch & Walsh, 1997). Local outcrossing has been found to reduce both resistance and fitness in other plant systems (Strauss & Karban, 1994; Waser & Price, 1994; Waser *et al.*, 2000). The negative effects of outcrossing in defensive strategies might not be rare in nature, especially in highly self-fertilizing plants (Nordborg *et al.*, 2002) and/or in plant populations growing in environments where outcrossing is limited by mate or pollinator availability, such as *D. stramonium*. Further experiments aimed at comparing whether local vs distant outcrossing could differentially disrupt local adaptation in plant defence would offer relevant information about ecological disadvantages of outcrossing in predominantly self-fertilizing species.

Inbreeding can have a similar effect on potentially linked traits, resulting in similar inbreeding depression on groups of traits (Leimu *et al.*, 2008). This study found that inbreeding depression had a similar effect on RGR and flower production and on the number of fruits and seeds (Fig. 3a–d), suggesting the existence of correlative effects of inbreeding depression on various fitness

components. At least two nonmutually exclusive mechanisms can account for the correlative effects of inbreeding in two or more traits. First, inbreeding could affect various characters simultaneously throughout the expression of detrimental recessive genes with pleiotropic effects (Thiele *et al.*, 2010). The different inbreeding depression magnitudes between fruit and seed number in *D. stramonium* reported in a previous study would suggest that different reproductive fitness components are negatively affected by specific genes (Núñez-Farfán *et al.*, 1996; Bello-Bedoy & Núñez-Farfán, 2010). Second, the genes controlling inbreeding depression in one character can affect development of another character expressed late in ontogeny, even in the absence of a genetic correlation between them. Changes in vegetative growth can later affect reproductive characters in *D. stramonium* (Weaver *et al.*, 1985; Feroni & Núñez-Farfán, 2000). Thus, an effect of inbreeding on plant growth is likely to affect the reproductive characters, such as those observed in flower, fruit and seed production in *D. stramonium*. Thus, the multiple effect of inbreeding depression could be caused by the expression of particular detrimental recessive genes and by the developmental association among characters.

Our study found that natural enemies altered the strength of inbreeding depression in *D. stramonium*. However, the magnitude and the direction varied depending on the intensity of damage caused by a particular natural enemy. The effect of natural enemies on inbreeding depression cannot be generalized and the complex nature of inbreeding on defensive strategies calls for more

sophisticated experimental designs. On the other hand, theoretical and empirical studies suggest that the selective purge of recessive deleterious mutations is relevant for the evolution of selfing (Lande & Schemske, 1985; Mutikainen & Delph, 1998; Goodwillie & Knight, 2006; Porcher *et al.*, 2009). It seems that ineffective selection on recessive deleterious mutations coupled with stressful environments can interact with exacerbate inbreeding depression in this population of *D. stramonium*. However, the increase in inbreeding depression attributable to damage by pathogens and herbivores seems too weak to prevent the fixation of selfing. Future studies might consider simultaneously assessing the role of natural levels of herbivore damage and pathogens infection on lineages with a known mutational load affecting resistance-related traits or plant fitness. Simultaneous damage by two or more natural enemies is recurrent in plant populations (Strauss & Irwin, 2004), and a contrasting effect of selfing on defensive strategies against one or the other may indirectly favour the maintenance of a mixed mating system strategy in a population.

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DISCUSIÓN

Rafael Bello-Bedoy

Se ha sugerido que uno de los beneficios ecológicos del sexo o la recombinación sobre la asexualidad es la producción de progenie genéticamente heterogénea creando un mecanismo defensivo contra parásitos que evolucionan contra-defensas rápidamente (Levin, 1975, Williams, 1975, Maynard-Smith, 1978, Bell, 1982, Lively & Howard, 1994). En una forma similar, la exogamia puede ofrecer estas ventajas al producir variación genética entre su progenie y reducir la exposición de mutaciones nocivas en individuos heterocigotos. Así, la exogamia evita los costos directos de la depresión endogámica sobre la defensa contra los enemigos naturales.

La teoría de la evolución del sistema de apareamiento predice que la intensidad de la depresión endogámica debería ser baja porque la auto-fertilización constante permite la purga de las mutaciones recesivas de efectos negativos responsables de la depresión endogámica (Lande & Schmeske 1985). También la depresión endogámica debería ser menor en estados del desarrollo que en estados tardíos porque la selección es más efectiva removiendo genes que afectan el éxito reproductivo ($\delta < 0.3$ & Husband Schmeske 1996). Estas son dos condiciones necesarias para que se fije la auto-fertilización como una estrategia de apareamiento.

Datura stramonium es una planta que produce la mayoría de sus semillas por auto-fertilización y se esperaría que la endogamia no causara costos significativos en términos de crecimiento y/o reproducción. Los resultados de este estudio están parcialmente de acuerdo con las expectativas; las etapas primordialmente tempranas – sobrevivencia, tamaño de la hoja- fueron menos afectadas por la endogamia. Sin embargo, los componentes de reproducción fueron afectados en cierto grado y la depresión endogámica fue mayor a la esperada para una especie con una tasa alta de auto-fertilización. La intensidad de depresión endogámica más alta fue para el número neto de semillas por planta ($\delta = 0.4$), pero el número de frutos y el número de semillas por fruto también mostraron reducciones significantivas, indicando que en esta especie se mantienen genes de efectos negativos relativamente ligeros que reducen el éxito reproductivo de la auto-fertilización.

Los coeficientes de depresión endogámica para el número de frutos y semillas de este estudio son similares a los que otros estudios han reportado en condiciones naturales (Núñez-Farfán et al., 1996; Stone & Motten, 2002). Sin embargo, se ha encontrado que sus costos pueden ser nulos en condiciones de invernadero (Bello-Bedoy & Núñez-Farfán 2010). Esto sugiere el daño por herbívoros pueden incrementar los efectos

negativos de la depresión endogámica y agudizar la selección en contra de los individuos homocigotos (Lively & Howard, 1994; Agrawal & Lively 2001). Al incrementar la intensidad de la depresión endogámica, el daño causado por herbívoros en las hojas y en las semillas pueden limitar fijación de la auto-fertilización como una estrategia reproductiva única en *D. stramonium*. Esto apoya dos ideas 1) que la exogamia es ventajosa en presencia de enemigos naturales porque confiere niveles defensivos más altos que la auto-fertilización (Bello-Bedoy & Núñez Farfán 2010a, b) y que los enemigos naturales son un factor que puede influenciar la evolución del sistema de apareamiento.

EFFECTO DE LA ENDOGAMIA EN LA RESISTENCIA

La evidencia de este estudio indica que la auto-fertilización causó depresión endogámica en la resistencia de la plantas a distintos tipos de enemigos naturales (Bello-Bedoy & Núñez- Farfán, 2010). La disminución de la resistencia contra enemigos naturales diferentes puede ser causada por la exposición de genes nocivos de efectos pleiotrópicos que controlan un sistema de resistencia general o porque el incremento en homocigosis afecta *loci* específicos que controlan cada sistema de resistencia.

La concentración de alcaloides y la densidad de tricomas son caracteres de la resistencia contra herbívoros en *D. stramonium* con bases genéticas (Shonle & Bergelson, 2000, Valverde *et al.*, 2001) y es muy posible que la auto-fertilización haya modificado su expresión como se ha reportado en otras plantas (Ferrari *et al.*, 2006; Delphia *et al.*, 2009). Por otro lado, las plantas pueden resistir al daño por virus mediante una cascada de respuestas que induce la síntesis y/o traslocación hormonas vegetales como los ácidos jasmónico o acetil salicílico conocida como 'sistema inmune' (SAR, Systemic Acquired Resistance). Esta inducción en la síntesis de compuestos bioquímicos reduce el daño localizado y/o la dispersión de patógeno a través de toda la planta. Ambas hipótesis sobre como la endogamia puede afectar la resistencia contra distintos enemigos naturales en *D. stramonium* son susceptibles de ser puestas a prueba.

Las plantas son frecuentemente atacadas por más de un enemigo en condiciones naturales y sería relevante entender si una comunidad más diversa de enemigos naturales puede ser factor que mantenga o acelere la adopción de un estrategia de apareamiento en una población. La poca frecuencia de estos estudios en literatura no permite predecir si la diversidad antagonística es un factor determinante (3 herbívoros, Núñez-Farfán *et al.*, 1996; 2 herbívoros, 1 patógeno, Stephenson *et al.*, 2004; 2 herbívoros, 1 consumidor de semillas, 1 patógeno, Capítulo 3) y resalta una avenida de investigación interesante para evaluar 'los dos lados de la moneda' de la interacción planta-enemigos naturales: examinar el efecto de la diversidad de enemigos naturales sobre las ventajas de la sexualidad en términos de diversidad genética de la progenie sexual (exogamia) vs asexual (endogámica) y el efecto de la estrategia de apareamiento sobre una comunidad de enemigos naturales.

El costo de la endogamia podría incrementarse en forma desproporcionada en una comunidad más grande o diversa de herbívoros si la endogamia afecta diversos componentes de la resistencia y los costos del daño por diferentes herbívoros tienen un efecto sinérgico negativo. Aunque también un daño intenso sobre ambas progenies podría eliminar la ventaja de la progenie exógama y otros factores podrían determinar la evolución del sistema de apareamiento (i.e., deriva génica).

Una reducción en vigor de igual manera puede menguar la resistencia al generar conflictos de asignación de recursos entre defensa, crecimiento y reproducción. Sin embargo, si el vigor por si mismo puede modificar interacción planta-insecto si la variación en vigor causada por la endogamia causa cambios importantes en los caracteres que median la interacción. La hipótesis defensiva del vigor predice un incremento en herbivorismo relacionado con un incremento en vigor relativo a nivel sub-individual o entre individuos en una población (Price, 1991). Esta idea supone que la variación en la tasa de herbivorismo es influenciada por diferencias en las cualidades nutricionales de las plantas que alteran el consumo de los herbívoros (Awmack & Leather, 2002). En poblaciones de plantas con sistemas de apareamiento mixto cada generación se producen individuos exógamos con un vigor superior al de sus parientes endógamos (Husband & Schemske, 1996, Darwin, 1876). En estas condiciones se predice que las plantas exógamas serán más atacadas por sus herbívoros o patógenos que las plantas endógamas y esto es relevante porque implica que una desventaja de la endogamia donde se esperaría que los enemigos naturales favorecerían la auto-fertilización.

En este estudio, las progenies exógamas produjeron 30% más frutos que las endógamas y esto aumentó la infestación por el depredador de semillas *Trichobaris soror* en *D. stramonium*. Este incremento en el ataque de *T. soror* sugiere que las plantas exógamas resultaron más atractivas para los insectos que las plantas endógamas y que su incremento en vigor representó un costo al incrementar el riesgo de depredación de semillas. Así, la ventaja del heterocigoto en relación al vigor puede aumentar sustancialmente el riesgo de daño por herbívoros, lo cual altera las predicciones esperadas bajo apareamiento mixto si los efectos de la endogamia fueran específicamente negativos.

EFECTO DE LOS ENEMIGOS NATURALES SOBRE LA DEPRESIÓN ENDOGÁMICA

Entonces, ¿Qué tan relevante es el daño por herbívoros que influye la evolución del sistema de apareamiento? Aunque la intensidad de la depresión endogámica debería ser alta para contrarrestar la ventajas automática de la auto-fertilización (Lande & Schmeske 1985), es posible que el daño por herbívoros promueva el mantenimiento de la exogamia y mantenga una estrategia mixta de sistemas de apareamiento (Lively & Howard 1994). El daño por herbívoros debería ser intenso y/o producir una reducción drástica del éxito reproductivo de la progenies endógamas en comparación a las exógamas.

En este estudio encontré que la depresión endogámica incrementó del 0.05 al 0.42 cuando las plantas fueron expuestas al daño por herbívoros en comparación al invernadero. Esta evidencia indica que la

desproporcionada desventaja de la progenie endógama en presencia de los herbívoros puede limitar la fijación de la endogamia en una población. Lo que implica que la endogamia tiene un costo en resistencia y otro reproductivo, pero además la interacción entre la disminución en resistencia y éxito reproductivo puede exacerbar sus desventajas.

Los herbívoros y los patógenos generalmente causan reducciones significativas en la biomasa de las plantas que se reflejan en una reducción en el éxito reproductivo de las plantas (Marquis, 1992). ¿Son las plantas exógamas más tolerantes al daño por enemigos naturales que las plantas endógamas? La hipótesis de la depresión endogámica que influencia la defensa sugiere que esta pregunta es central para entender el efecto de la endogamia en resistencia y la tolerancia y el papel de los enemigos naturales en la evolución del sexo en las plantas (Bello-Bedoy and Nuñez-Farfán, 2010). Esta hipótesis predice que la progenie endógama debería ser menos tolerante al daño por enemigos naturales, indicado por un incremento en depresión endogámica en plantas con daño *vs* plantas sin daño. La depresión endogámica incrementó 10% el plantas con nivel natural de daño en comparación a las plantas protegidas con insecticida, lo que indica que el daño foliar causado por los herbívoros si influyó sobre la depresión endogámica *D. stramonium* y que la endogamia redujo la capacidad de tolerar el daño por herbívoros.

La producción de frutos o semillas puede estar fuertemente condicionada por la cantidad de daño y las plantas de exogamia con un nivel de daño severo pueden exhibir una reducción intensa en producción de semillas al igual que las de endogamia. En *D. stramonium* se ha encontrado que a niveles de daño foliar mayores al 25%, la capacidad de tolerar el daño foliar es limitada (Fornoni & Núñez-Farfán, 2000). Los niveles de daño foliar observados en sus poblaciones normalmente sobrepasan este umbral de daño (Núñez-Farfán & Dirzo, 1994, Valverde *et al.*, 2001) y los estimados en estos experimentos no son la excepción (Bello-Bedoy & Núñez-Farfán, 2010; Bello-Bedoy & Núñez-Farfán 2010b). En este caso, las progenies endogamas y exógamas mostraron un decaimiento en éxito reproductivo conforme el daño por virus incrementó; incluso, la reducción en éxito reproductivo fué menor en las plantas exógamas en el nivel más severo de infección. Estos resultados apoyan la idea que las plantas exógamas pueden ser más tolerantes al daño, pero también que las ventajas de la exogamia y la tolerancia pueden ser limitadas cuando el daño es muy intenso.

La mayoría de los estudios que reportan una interacción negativa entre la endogamia y el daño por herbívoros consideran que la endogamia afectó la variación genética de la tolerancia (Núñez-Farfán et al., 2007) y asumen que se redujo algún mecanismo compensatorio relacionado con el crecimiento o la capacidad para asignar recursos. En varias poblaciones de *D. stramonium* se ha detectado variación genética en esta estrategia defensiva y se ha relacionado con la capacidad para recuperar el área foliar (Fornoni et al., 2003, Valverde et al., 2003). La depresión endogámica también puede intensificarse debido al daño por herbívoros o patógenos que exacerba la expresión negativa de mutaciones recesivas deletereas del éxito reproductivo o

caracteres relacionados a la reproducción, y no explícitamente porque la endogamia afecte la tolerancia al daño.

A pesar de que el daño foliar intensificó la depresión endogámica, no es posible diferenciar si la endogamia afectó la tolerancia o incrementó el efecto de mutaciones asociadas al éxito reproductivo. Sin embargo, es muy posible que el daño haya afectado la producción de semillas dentro de los frutos, lo que implica que su efecto pudo exacerbar la depresión endogámica a través de mutaciones relacionadas con el éxito reproductivo y que la endogamia no afecta la respuesta de las plantas contra los herbívoros.

Finalmente, la depredación de semillas causó un incremento importante en el costo de la endogamia. La relación entre el incremento en el número de depredadores y la reducción de semillas produjo un efecto significativamente negativo en las plantas de endogamia, mientras que las de exogamia amortiguaron el consumo de semillas y mantuvieron su ventaja reproductiva (Capítulo 2). Este es el primer estudio que reporta el papel potencial de la depredación de semillas como un factor que puede influenciar el sistema de apareamiento al reducir sustancialmente el éxito reproductivo de los individuos endógamos en comparación a los exógamos. Algunos estudios en especies con otros sistemas reproductivos han sugerido que la superioridad individual en flores, frutos o semillas permiten amortiguar las pérdidas provocadas por la depredación de semillas (Boege & Domínguez, 2008, Marshall & Ganders, 2001). Aparentemente este fenómeno ocurrió en nuestro estudio, las plantas exógamas produjeron más frutos con más semillas que las endógamas y esta ventaja se mantuvo a pesar que recibieron un daño desproporcionado por *T. soror*.

EFFECTO DEL SISTEMA DE APAREAMIENTO DE LAS PLANTAS SOBRE LOS INSECTOS.

La depresión endogámica que afecta los rasgos que median las interacciones entre las plantas y sus herbívoros puede alterar el comportamiento selectivo y el éxito reproductivo de sus herbívoros. Los insectos pueden responder cambios en la calidad de sus hospederos ya sea consumiendo más tejido o deponiendo más huevos en plantas menos defendidas o de mayor calidad. El nivel desproporcionado de incidencia e infestación por *T. soror* que exhibieron las plantas exógamas sugiere que éstas son más atractivas para los depredadores de semillas en comparación a las plantas endógamas. Este comportamiento selectivo del hospero ha evolucionado en muchos grupos de insectos para evitar los costos de ovipositar o consumir tejido en hospederos que afectan su fecundidad o reproducción.

En este caso no encontré diferencias en la biomasa del estadio adulto de *T. soror* asociadas a la endogamia de *D. stramonium*. Lo que indica que las plantas endógamas y exógamas son hospederos de calidad nutricional similar (ver discusión capítulo 2). Otros trabajos si reportan cambios en el desarrollo de los herbívoros cuando son alimentados con plantas con diferente nivel de endogamia (Carr & Eubanks, 2002, Hull-Sanders & Eubanks, 2005, Leimu et al., 2008, Delphia et al., 2009). Los efectos de la endogamia del hospedero sobre sus

consumidores pueden depender del tipo de insecto y del grado de especialización de la interacción (Hull-Sanders & Eubanks, 2005). *T. soror* es considerado como un especialista en *D. stramonium* y es posible que éste sea más afectado por cambios en las cualidades nutricionales de las plantas que por algún cambio en la resistencia. Un estudio previo detectó variación genética en sobrevivencia y biomasa en el herbívoro especialista *L. trilineata* que es directamente influenciada por la resistencia de *Datura stramonium* (Espinoza & Fornoni, 2006). Este contraste sugiere que la selección que impone la planta sobre sus herbívoros es distinta, que puede depender de la composición bioquímica de cada carácter intermediario entre la planta y su herbívoro (por ej. hojas vs semillas) y de la capacidad de cada insecto para amortiguar los efectos negativos de la resistencia de las plantas.

Una perspectiva interesante para futuros experimentos sería evaluar el efecto potencial de la endogamia del hospedero *D. stramonium* sobre la fertilidad (*i.e.*, número de huevos depositados) o fecundidad (adultos y larvas no desarrolladas) del depredador de semillas u otros herbívoros como *L. trilineata*. Esta planta tiene una constitución química fija que puede ejercer una presión selectiva sobre sus herbívoros (Espinoza & Fornoni, 2006) y un efecto del sistema de apareamiento a este nivel puede modificar la presión selectiva de las plantas sobre sus insectos consumidores.

La endogamia puede modificar el patrón de selección recíproco entre las plantas y sus herbívoros al generar variación fenotípica en la resistencia de las plantas. Si esto ocurre, resultaría ventajoso mantener la expresión de un sistema mixto de apareamiento porque la mezcla de progenies generaría una dinámica selectiva (relajamiento-reforzamiento) que puede mantener niveles bajos de contra-resistencia en sus enemigos. Es decir, la presencia de genotipos de plantas endógamas y de baja resistencia no impone selección y no induce un 'respuesta' en sus consumidores creando un proceso de 'relajamiento selectivo'. Por el contrario, la presencia de genotipos de plantas muy resistentes puede resultar en selección de una magnitud mayor sobre la contra-resistencia de sus consumidores creando un proceso de 'reforzamiento selectivo'. Sin embargo, la reproducción entre insectos consumidores con diferente nivel contra-resistente a la defensa de las plantas puede generar nuevas combinaciones genéticas de baja resistencia manteniendo a los insectos incapaces de alcanzar un nivel de resistencia que sobrepase a las plantas.

Este proceso ha sido analizado por Rausher (host dosage refuge hypothesis. 2001), sin embargo, este no toma en cuenta que la resistencia de las plantas puede modificarse con la endogamia y que ésta puede generar un mosaico geográfico espacial de plantas con distintos niveles defensivos. Este análisis permitiría analizar de forma empírica la host dosage refuge hypothesis y, al mismo tiempo, evaluar si el sistema de apareamiento produce una asimetría coevolutiva que retenga el proceso coevolutivo a nivel local (cold-spot *sensu* Thomson 2005) confiriendo una ventaja al sistema de apareamiento mixto en una población de plantas.

El desfase de los tiempos generacionales entre hospederos y enemigos naturales puede ocasionar que las plantas siempre estén un paso atrás en el proceso coevolutivo del tipo carreta armamentista (Thompson, 1994). Posiblemente este retraso en la respuesta de las plantas a sus insectos también este siendo causado por

la sexualidad de las plantas más que por una diferencia generacional hospedero-parásito (Bell, 1982). La prevalencia de la endogamia en un linaje tiene dos efectos clave, (1) acumula mutaciones nocivas de efectos débiles que son difíciles de eliminar por selección y, (2) además éstas pueden estar ocurriendo en los genes que controlan la resistencia contra los enemigos naturales. La auto-fertilización expone toda diversidad de mutaciones recesivas deletereas reduciendo la resistencia, este último efecto es relevante porque puede 'relajar' o disminuir la intensidad de selección que impone el hospedero sobre sus parásitos.

VARIACIÓN GENÉTICA EN LA DEPRESIÓN ENDOGÁMICA

En este estudio encontramos variación genética en la depresión endogámica en el número de semillas lo que sugiere que los individuos de *Datura stramonium* tienen diferente cantidad y/o tipo de alelos nocivos. Esta variación puede ser relevante por sí misma para explicar el mantenimiento de la exogamia en una población porque mantiene genes de efectos negativos sobre el éxito reproductivo que son expuestas por la auto-fertilización.

Los costos inmediatos de la auto-fertilización pueden eliminar una fracción importante de mutaciones deletéreas que afectan el éxito reproductivo (Lande & Schemske, 1985). Sin embargo, si la selección no su reduce su frecuencia, dichas mutaciones se pueden permanecer en las poblaciones aún en linajes con una práctica prolongada de endogamia (Byers & Waller, 1991). La variabilidad en la acumulación de mutaciones deletéreas -diferente cantidad y tipo de mutaciones- puede causar diferencias en la carga genética en distintos linajes, generando variación genética en depresión endogámica. Consecuentemente, el incremento en las mutaciones deletéreas y la ineffectividad de la selección para removerlas permite que se mantengan una estrategia de apareamiento mixta. Además, el ambiente estresante impuesto por los herbívoros o patógenos puede afectar la expresión de dichas mutaciones y acentuar de forma diferencial las desventajas de la auto-fertilización eliminando aquellos linajes incapaces de 'purgar' la carga genética.

En futuros estudios se deben de usar linajes (i.e., individuos) con una cantidad y tipo de carga genética conocida y realizar un diseño de cruces que permita predecir cuáles los efectos de la endogamia en el fenotipo de las plantas. Por ejemplo, exponer a los herbívoros plantas con mutaciones que disminuyan el tamaño de las hojas y examinar si el daño exacerban las desventajas de la endogamia al extra-limitar la asignación de recursos a la reproducción. La hercogamia, la distancia entre las anteras y el estigma, puede revelar la historia endogámica e identificar individuos endógamos en *D. stramonium* i.e. sin hercogamia y exógamos i.e. con hercogamia (Stone & Motten, 2002). La variación dentro y entre poblaciones en la expresión de este carácter y en el número de individuos endógamos y exógamos es común en *D. stramonium* y sugiere un estructuramiento no aleatorio en el sistema de apareamiento (R. Bello-Bedoy, datos no publicados). La presión selectiva de los herbívoros en poblaciones con distinta historia endogámica podría contribuir a la variación en el sistema de apareamiento a nivel regional en esta especie.

DATURA STRAMONIUM: UN MODELO PARA EL ESTUDIO DE ENFERMEDADES INFECCIOSAS EN LAS PLANTAS

Datura stramonium es una planta nativa de México e invasora de amplia distribución que crece asociado a una amplia cantidad de cultivos en México y otros países. A pesar de que esta especie es un modelo de laboratorio para el estudio de la susceptibilidad a virus, se conoce muy poco de su interacción en patógenos en condiciones naturales. Este estudio representa una de las primera evidencias de infección por patógenos en su hábitat natural y sugiere que la población de *D. stramonium* de Teotihuacan puede poseer potencial genético en resistencia contra un virus.

Un programa de investigación sobre la ecología de la infección de este virus sería relevante desde el punto de vista científico y económico para la zona. Como puntos relevantes considero: 1) la identificación del virus, estimación de la prevalencia de la infección y su potencial impacto en plantas silvestres y cultivadas 2) Asimismo, la identificación de su o sus vectores y estudiar su biología y su interacción con el virus para predecir el riesgo de infección dados por la transmisión en las planas de la zona. 3) Estudiar con más detalle el sistema *D. stramonium*–herbívoro(s)–patógeno considerando que tanto la planta y sus herbívoros *L. trilineata* y *E. parvula* pueden funcionar como reservorios del patógeno.

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