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**ADAPTACIÓN LOCAL DEL DEPREDADOR DE SEMILLAS**

***Trichobaris soror*, UN HERBÍVORO ESPECIALISTA DE**

***Datura stramonium***

**TESIS**

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## RESUMEN

El estudio de la coevolución mediante la selección natural divergente es fundamental para entender los procesos genéticos entre localidades donde tienen lugar las interacciones ecológicas. Existen pruebas experimentales a favor y en contra de que la defensa química en las plantas coevoluciona con la herbivoría de los insectos. En esta tesis se exploró la acción de la selección natural divergente sobre los compuestos químicos secundarios de *Datura stramonium*, específicamente los alcaloides atropina y escopolamina, y su función defensiva en la coevolución con su depredador de semillas *Trichobaris soror*.

Como parte del método se siguió el protocolo propuesto por Carlos M. Herrera para estudiar la acción de la selección divergente que pudieron ejercer los agentes bióticos de selección sobre los caracteres de una especie focal entre diferentes localidades. En el primer capítulo nuestro objetivo fue explorar las posibles presiones de selección ejercidas por el depredador de semillas *T. soror* sobre los dos alcaloides más abundantes en *D. stramonium* (atropina y escopolamina) en 31 poblaciones del centro de México. En el segundo capítulo se realizó un experimento de trasplantes recíprocos en parcelas en dos localidades para probar la posible adaptación local de las plantas a nivel poblacional.

Los resultados mostrados en el primer capítulo sugieren que el depredador de semillas posiblemente ejerce presiones de selección sobre la concentración de escopolamina en las poblaciones del centro de México. Una evidencia que apoya la selección ejercida por el depredador de semillas es que la infestación disminuye conforme aumenta la concentración de escopolamina. Por otra parte, la concentración del alcaloide atropina fue mayor en poblaciones donde había humedad con respecto a los ambientes desérticos. Debido a que se encontró variación tanto en la concentración de los alcaloides como en la infestación de *T. soror* se eligieron dos poblaciones para realizar experimentos de trasplantes recíprocos.

En los resultados del segundo capítulo se comprobó que no hubo diferencias significativas en las familias genéticas respecto a la escopolamina en semillas, lo cual indicaría que existe un componente genético en la variación de la concentración de este alcaloide. En este carácter defensivo también se demostró la presencia de un patrón de selección natural y adaptación local en el tratamiento simpátrico de la planta en la localidad Teotihuacán, y en los tratamientos simpátrico y alopátrico de Morelia. Además, se sugiere adaptación local en la intensidad de la infestación del depredador de semillas y en la producción de escopolamina como defensa química de *D. stramonium*. Todos estos resultados indicarían que existe adaptación local recíproca en este sistema planta-huésped y su depredador de semillas.

## ABSTRACT

The study of co-evolution through divergent natural selection is fundamental to understanding the genetic processes between localities where ecological interactions happen. There is experimental evidence for and against that chemical defense in plants co-evolves with insect herbivory. This thesis explored the action of divergent natural selection on the defensive chemicals of *Datura stramonium*, specifically the alkaloids atropine and scopolamine, and their defensive function in co-evolution with their seed predator *Trichobaris soror*.

As part of method the protocol proposed by Carlos M. Herrera was followed to study the action of divergent selection, which could exert biotic selection agents, on the characteristics of a focal species among different localities. In the first chapter our objective was to explore the possible selection pressures exerted by the seed predator *T. soror* on the two most abundant alkaloids in *D. stramonium* (atropine and scopolamine) in 31 populations in central Mexico. In the second chapter an experiment of reciprocal transplants was carried out in plots of two localities in order to test the possible local adaptation of the plants at the population level.

The results shown in the first chapter suggest that the seed predator possibly exerts selection pressures on scopolamine concentrations in the populations of central Mexico. Evidence to support seed predator selection is that infestation decreases as scopolamine concentration increases. Furthermore, the concentration of the atropine alkaloid was higher in populations where there was humidity in comparison to desert environments. Because variation was found in both alkaloid concentration and *T. soror* infestation, two populations were chosen to perform reciprocal transplants experiments.

In the results of the second chapter it was proved that there were no significant differences in the genetic families concerning to scopolamine in seeds, which would indicate that there is a genetic component in the variation of the concentration of this alkaloid. Regarding this defensive character it was also demonstrated the presence of a pattern of natural selection and local adaptation in the sympatric treatment of the plant in the locality of Teotihuacan, and in the sympatric and allopatric treatments of Morelia. In addition, local adaptation of the intensity of seed predator infestation and scopolamine production is suggested as a chemical defense of *D. stramonium*. All these outcomes would indicate that there is local reciprocal adaptation in this plant-host system and its seed predator.

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## INTRODUCCIÓN GENERAL

El mecanismo que moldea las adaptaciones a nivel poblacional de todos los organismos vivos es la selección natural. Este mecanismo propuesto por Darwin (1859) dio un concepto sólido de una explicación comprobable y contrapuesta con la creencia de que los seres vivos son inmutables. La selección natural puede definirse como el proceso mediante el cual la variación de un carácter, cuando es benéfica en alguna proporción, puede heredarse para promover la sobrevivencia y reproducción diferencial a favor de quienes lo poseen. Los dos principios fundamentales para explicar el mecanismo por el cual actúa la selección natural son: la lucha por la existencia y la variación a nivel poblacional, ambos son procesos inherentes a los seres vivos en la naturaleza además de ser la materia sobre la cual actúa la selección natural. La lucha por la existencia fue descrita como la lucha de los seres vivos para obtener recursos. Un escenario sobre la lucha por la existencia para los organismos sésiles como las plantas podría ser la lucha por los recursos hídricos en el desierto y las espinas, que fueron seleccionadas a partir de la variación en los fenotipos de las hojas que lograron evitar la evapotranspiración del agua (Barcikowski y Nobel, 1984). Las adaptaciones son aquellas características que resultan de las presiones ejercidas por la selección natural, dan ventajas a los individuos de una población en cuanto a la sobrevivencia y reproducción y pueden heredarse de generación en generación (Futuyma, 2013). A su vez, en las interacciones ecológicas, las especies son capaces de ejercer presiones de selección entre sí y producir adaptaciones, ya sea mediante interacciones entre especies generalistas o especialistas (Dawkins y Krebs, 1979; Janzen, 1980; Price *et al.*, 1980; Herrera *et al.*, 2006).

El proceso coevolutivo puede definirse como la evolución recíproca entre especies interactuantes y que es mediada por la selección natural (Dawkins y Krebs, 1979; Janzen, 1980; Thompson, 1999; Thompson, 2005a). Para explicar la evolución conjunta de dos o más especies existen diferentes teorías, una de ellas se desarrolló por Dawkins y Krebs (1979) durante la Guerra fría y se conoce como la teoría de las “carreras armamentistas”. Esta teoría se inspiró en el hecho histórico de que las naciones más poderosas en ese entonces, Estados Unidos y la Unión Soviética (URSS), competían por implantar su modelo de gobierno mediante guerras y armamentos cada vez más poderosos en una lucha por el poder mundial (Intriligator y Brito, 1984). En ecología evolutiva, las “carreras armamentistas” son una lucha evolutiva donde las armas son las adaptaciones de los depredadores para conseguir alimento y las contra adaptaciones son las defensas de las presas para aumentar sus posibilidades de dejar

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descendencia (Dawkins y Krebs, 1979).

La teoría de las “carreras armamentistas” careció de un contexto geográfico que posteriormente fue subsanado y reemplazado por la Teoría del Mosaico Geográfico de la Coevolución (TMGC, Thompson, 2005b). La TMGC hace hincapié en que el proceso coevolutivo se desarrolla de manera dinámica entre las poblaciones de dos o más especies que interactúan entre sí (Gomulkiewicz *et al.*, 2002; Nuismer *et al.*, 2000; Thompson, 1999; Thompson, 2005a; Thompson, 2005b). La variación genética es fundamental ya que, al igual que la teoría darwiniana de evolución por selección natural, es la materia prima donde actúa la selección y en esta teoría se denomina remezcla de caracteres. La TMGC toma en cuenta la acción de fuerzas evolutivas como las mutaciones (incluso duplicaciones del genoma entero), flujo genético o deriva genética en cada una de las poblaciones en estudio (Thompson, 2005a). En esta teoría se considera que la coevolución es un proceso de cambio continuo entre las especies interactuantes ya que la acción de la selección natural depende de la expresión del *pool genético* o acervo genético, en el fenotipo de los individuos en cada población a lo largo del tiempo (Thompson, 2005a).

Para explicar el mecanismo de la coevolución, la TMGC se basa en tres supuestos principales: 1) las especies pueden definirse como colecciones de poblaciones genéticamente distintas, 2) las especies interactuantes frecuentemente difieren en sus distribuciones geográficas, 3) las interacciones entre especies difieren entre localidades (Thompson, 2005a). Bajo estos tres supuestos principales en la TMGC se predice que la selección natural es diferente entre localidades, esto se debe a las variaciones locales en el ambiente abiótico y a que la estructura genética es diferente entre las poblaciones. El mosaico geográfico coevolutivo depende de la reciprocidad de la selección entre los interactuantes (Gomulkiewicz *et al.*, 2002; Nuismer *et al.*, 2000; Thompson, 1999; Thompson, 2005a; Thompson, 2005b). Los *hot-spots* coevolutivos son localidades donde las especies interactuantes están sujetas a selección recíproca (Gomulkiewicz *et al.*, 2002; Nuismer *et al.*, 2000; Thompson, 1999; Thompson, 2005a; Thompson, 2005b). A su vez, en la TMGC se plantea que el mosaico geográfico coevolutivo se genera porque los *hot spots* están embebidos dentro de una matriz de *cold-spots* coevolutivos o poblaciones en las cuales no hay selección recíproca o dónde solamente uno de los participantes puede ejercer selección (Gomulkiewicz *et al.*, 2002; Nuismer *et al.*, 2000; Thompson, 1999; Thompson, 2005a; Thompson, 2005b).

Para estudiar los mosaicos geográficos coevolutivos es necesario conocer como es la interacción de los genotipos de una especie en diferentes ambientes (GXE), lo cual se conoce como norma de reacción ya que depende del ambiente físico donde se expresen (Gavrilets y Scheiner, 1993), y en el caso de la TMGC se analiza la expresión final de los genotipos en una interacción entre dos especies mediada por selección natural que puede expresarse como una norma de reacción (GXGXE) y da como resultado un mosaico geográfico de selección (Thompson, 2005a). La plasticidad fenotípica permite la aparición de nuevos fenotipos inducidos por el ambiente y la selección, los cuales alteran la expresión de su norma de reacción (Pigliucci *et al.*, 2006). En la interacción (GXGXE) para el genotipo de la especie 1, el genotipo de la especie 2 y el ambiente (Kingsolver *et al.*, 2007; Wendling *et al.*, 2017) la selección natural recíproca fija los fenotipos modificados que resultan de un proceso coevolutivo (Gomulkiewicz *et al.*, 2007).

La interacción entre las moscas con probóscides largas (*Moegistorhynchus longirostris*: Nemestiniidae) y las iridáceas de tubo largo (*Lapeirousia anceps*: Iridaceae) son un ejemplo de un mosaico geográfico coevolutivo, ya que en algunas poblaciones presentan selección recíproca (*hot-spots*) y en algunas existen *cold-spots* (Pauw *et al.*, 2009). La selección natural recíproca modifica la expresión de la norma de reacción de los caracteres que median la interacción, en la mosca *M. longirostris* aumenta el largo de la probóscide y en *L. anceps* se incrementa la longitud del tubo floral y la producción de néctar (Pauw *et al.*, 2009).

Los tres supuestos de la TMGC que deben probarse de manera rigurosa en este mecanismo coevolutivo son: A) las especies deben contener acervos de poblaciones genéticamente distintas, B) las especies que interactúan entre sí podrían diferir en sus distribuciones geográficas y C) las interacciones entre las especies pueden diferir en cada población, lo cual dependería de las interacciones bióticas y abióticas que posiblemente influyan en ella (Gomulkiewicz *et al.*, 2007). Además, es fundamental probar la existencia de los *hot-spots* coevolutivos, es decir, cuando la interacción entre dos especies está mediada por selección natural recíproca (Gomulkiewicz *et al.*, 2007). En caso de encontrar que la selección natural actúa sobre una de las especies el patrón correspondería solamente con la selección natural divergente. Pero incluso si se encuentra el patrón de selección predicho por los *hot-spots* y los *cold-spots* debe existir evidencia de la remezcla de caracteres, es decir, que entre las poblaciones en estudio existe flujo genético (Gomulkiewicz *et al.*, 2007; Rich *et al.*, 2008). Sin embargo, no siempre es posible probar rigurosamente todos los supuestos de la TMGC y por esa razón, a continuación, mostraremos otro enfoque para estudiar la coevolución basado en otro tipo de análisis.



Herrera y colaboradores (2006) propusieron un método para estudiar el efecto de la selección natural sobre la corola de las flores de *Lavandula latifolia* ejercida por los visitantes florales en la Sierra de Cazorla, España. Mediante este método se determinó la existencia de coevolución difusa ya que diferentes conjuntos de visitantes florales influyeron sobre los caracteres de *L. latifolia*. Específicamente, se probó el efecto de la selección difusa ejercida sobre un carácter por un conjunto de especies (Stichcombe y Rausher, 2001). El método podría utilizarse con fines de investigación sobre los caracteres que median la coevolución de una especie focal y los posibles agentes que ejercen selección sobre ella en diferentes localidades (Gómez *et al.*, 2008; Herrera *et al.*, 2006; Sobral *et al.*, 2013; Sobral *et al.*, 2015). Además, este método se diseñó para diferenciar el proceso de selección natural divergente (Herrera *et al.*, 2006; Laine, 2009) de la variación neutral fenotípica (Griswold *et al.*, 2007).

En primer lugar, debe estudiarse la materia prima sobre la cual actúa la selección natural, es decir, se demuestra que existe variación en los caracteres fenotípicos clave de la interacción entre la especie focal y las especies que pudieran fungir como agentes de selección en diferentes poblaciones (Herrera *et al.*, 2006). En segundo lugar se prueba si los caracteres de la especie focal están sujetos a la selección ejercida por las interacciones bióticas en diferentes poblaciones, de esta manera las especies que participan en la interacción se identifican como verdaderos agentes de selección (Gómez *et al.*, 2008; Sobral *et al.*, 2013; Sobral *et al.*, 2015). Posteriormente, se analiza si la selección de los caracteres de la especie focal está relacionada con la variación fenotípica de los agentes de selección a lo largo de las diferentes localidades de estudio. Por último, se determina si la variación poblacional en los caracteres de la especie focal posee bases genéticas (Herrera *et al.*, 2006).

Sobral y colaboradores (2013) retomaron este método y lo aplicaron al estudio de la selección natural divergente que ejercen los dispersores de semillas, unas aves del género *Turdus*, sobre algunos caracteres con variación intraindividual en el árbol *Crataegus monogyna*, como el tamaño de los frutos y las semillas. Las plantas son organismos modulares en los cuales incluso el mismo carácter fenotípico exhibe variación dentro del mismo individuo y esta puede influir en la adecuación de la especie en estudio (Herrera, 2009). La prueba de que la selección natural divergente actúa sobre la variación intraindividual en el tamaño del fruto es que el coeficiente de variación del tamaño del fruto correlaciona positivamente con los diferenciales de selección de la variación intraindividual del tamaño del fruto de *C. monogyna* en un gradiente latitudinal en 13 poblaciones europeas (Sobral *et al.*, 2013).

Por último, se prueba una parte fundamental de la teoría darwiniana la cual consiste en examinar si la variación en alguno o en los caracteres de los individuos que se encuentran en las poblaciones estudiadas tienen bases genéticas y son heredables. Esto puede determinarse mediante experimentos de “jardín común” y también puede aprovecharse para probar si existe adaptación local (Kawecki y Ebert, 2004). De acuerdo con la teoría de la evolución por selección natural (Darwin, 1859), la divergencia de los caracteres entre diferentes localidades es el principio en el cual se basa la teoría actual de la adaptación local, es decir, cuando “el *fitness* de la población de una especie en su localidad de origen es mayor a la expresión de su *fitness* en otras localidades” (Kawecki y Ebert, 2004). Para el estudio de la adaptación local nuevamente nos referiremos a la interacción GXGXE porque la selección puede actuar en dos o más especies interactuantes a través de varios ambientes contrastantes (Kawecki y Ebert, 2004; Kingsolver *et al.*, 2007). Los experimentos de “jardín común” se basan en estudiar poblaciones contrastantes o que se encuentren dentro de un gradiente para entender el efecto de los cambios ambientales en la expresión de la norma de reacción de los caracteres de las especies en estudio. El objetivo de la adaptación local es describir cómo las presiones selectivas producen variaciones, divergencia de caracteres y la que sería su mejor combinación de estos rasgos en una especie, lo cual se traduce en su adaptación para cada localidad (Kawecki y Ebert, 2004; Laine, 2009).

Existen dos formas experimentales de probar el patrón de adaptación local: i) al comparar el *fitness* de una especie focal en su localidad de origen con respecto a su *fitness* en otras localidades (criterio “home vs. away”), ii) al comparar el *fitness* de una especie focal en el ambiente local con los individuos de otras localidades (criterio “local vs. foreign”; Kawecki y Ebert, 2004). Además, el patrón de adaptación local en las interacciones planta huésped-insecto herbívoro puede estudiarse mediante los caracteres de defensa de las plantas y los caracteres ligados al *fitness* de los herbívoros (Garrido *et al.*, 2012; Laukkanen *et al.*, 2012).

Las plantas pueden tener diferentes atributos defensivos como las defensas físicas (por ejemplo, ceras, tricomas y espinas; Chang *et al.*, 2004; Valverde *et al.*, 2001) y químicas (por ejemplo, alcaloides, terpenoides, compuestos fenólicos y glucosinolatos; Sholne y Bergelson, 2000; Wittstock y Halkier, 2002). Con respecto a las defensas químicas de las plantas, se sabe que algunos insectos herbívoros pueden elegir entre las plantas menos tóxicas para ovipositar en ellas, ya que los insectos fitófagos especialistas poseen adaptaciones que les permiten identificarlas (Jaenike, 1990), mientras que los insectos generalistas ejercen diferentes presiones de selección y estas posiblemente sean opuestas a las que ejercen los herbívoros especialistas.

Por ejemplo la planta *Brassica nigra* produce sinigrina que es un glucosinolato atrayente del áfido especialista *Brevicoryne brassicae*, mientras que este compuesto también actúa como un deterrente del gasterópodo terrestre *Agriolimax reticulatus* (Lankau, 2007). En el caso de la hipótesis sobre la posible coevolución de las plantas y los insectos herbívoros mediada por la producción de fito-compuestos químicos secundarios y las contra adaptaciones en los insectos (Ehrlich y Raven, 1964; Ode, 2006) las pruebas no son concluyentes a favor de que este sea el principal mecanismo evolutivo en este tipo de interacciones (Agrawal y Webber, 2015; Núñez-Farfán y Kariñho-Betancourt, 2015).

El sistema de estudio en el que se enfocó esta tesis fue la planta anual *Datura stramonium* (conocida popularmente como toloache) y su depredador de semillas *Trichobaris soror* (Curculionidae). El depredador de semillas *T. soror* se distribuye principalmente en el centro de México (Barber, 1935; De-la-Mora *et al.*, 2015) y todo su ciclo de vida lo desarrolla en asociación con *D. stramonium* (Bello-Bedoy *et al.*, 2011; Borbolla, 2015; Cabrales-Vargas, 1991). El toloache también es depredado por insectos herbívoros especialistas de hojas (*Lema trilineata* y *Epitrix parvula*, Coleoptera: Chrysomelidae) y un herbívoro generalista (*Sphenarium purpurascens*) en el centro de México (Cabrales-Vargas, 1991; Castillo *et al.*, 2014; Núñez-Farfán y Dirzo, 1994). El propósito de esta tesis fue estudiar la coevolución entre la planta anual *Datura stramonium* y su depredador de semillas *Trichobaris soror*. Principalmente, uno de los objetivos fue determinar el efecto de la selección natural divergente sobre las defensas químicas de *D. stramonium* que ejerció el depredador de semillas en 31 poblaciones del centro de México, en específico sobre los alcaloides atropina y escopolamina. Por último, otro de nuestros objetivos fue probar la posible adaptación local del depredador de semillas en las dos poblaciones donde se realizaron los experimentos “home vs. away” y “local vs. foreign”, además de analizar si existió algún componente genético que sustentara el patrón de selección divergente sobre los alcaloides de la planta huésped.

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# Capítulo 1: Natural selection drives chemical resistance of *Datura stramonium*



# Natural selection drives chemical resistance of *Datura stramonium*

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## ABSTRACT

Plant resistance to herbivores involves physical and chemical plant traits that prevent or diminish damage by herbivores, and hence may promote coevolutionary arm-races between interacting species. Although *Datura stramonium*'s concentration of tropane alkaloids is under selection by leaf beetles, it is not known whether chemical defense reduces seed predation by the specialist weevil, *Trichobaris soror*, and if it is evolving by natural selection. We measured infestation by *T. soror* as well as the concentration of the plants' two main tropane alkaloids in 278 *D. stramonium* plants belonging to 31 populations in central Mexico. We assessed whether the seed predator exerted preferences on the levels of both alkaloids and whether they affect plant fitness. Results show great variation across populations in the concentration of scopolamine and atropine in both leaves and seeds of plants of *D. stramonium*, as well as in the intensity of infestation and the proportion of infested fruits by *T. soror*. The concentration of scopolamine in seeds and leaves are negatively associated across populations. We found that scopolamine concentration increases plant fitness. Our major finding was the detection of a positive relationship between the population average concentrations of scopolamine with the selection differentials of scopolamine. Such spatial variation in the direction and intensity of selection on scopolamine may represent a coevolutionary selective mosaic. Our results support the view that variation in the concentration of scopolamine among-populations of *D. stramonium* in central Mexico is being driven, in part, by selection exerted by *T. soror*, pointing an adaptive role of tropane alkaloids in this plant species.

**Subjects** Ecology, Entomology, Evolutionary Studies, Plant Science

**Keywords** *Trichobaris soror*, *Datura stramonium*, Natural selection, Plant resistance, Seed predation, Tropane alkaloids, Selection differential, Scopolamine, Atropine

## INTRODUCTION

The coevolutionary process involves reciprocal selection-adaptation between interacting species through time (*Dawkins & Krebs, 1979; Thompson, 1999; Thompson, 2005*). This

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adaptation and counter-adaptation phenomenon could result in a coevolutionary arms race, a process of offense-defense (Dawkins & Krebs, 1979). A coevolutionary relationship between plants and herbivores may generate symmetrical or asymmetrical selective pressures between interacting species. These selective pressures may be different spatially and could produce a geographic structure of interactions (Forde, Thompson & Bohannan, 2004; Gomulkiewicz et al., 2002; Nuismer, Thompson & Gomulkiewicz, 2000; Thompson, 1999; Thompson, 2005). In some locations the interacting species exert reciprocal selection pressures to one another (coevolutionary hot spots), whereas in other locations reciprocal selection is highly asymmetric (coevolutionary cold spots) (Gomulkiewicz et al., 2002; Nuismer, Thompson & Gomulkiewicz, 2000; Thompson, 1999; Thompson, 2005).

A coevolutionary arms race between herbivores and plants may be favored in specialized interactions as in the case of insects that detoxify specific compounds (Janzen, 1969; Janzen, 1973; Schoonhoven, Van Loon & Dicke, 2005). For instance, the aphid *Macrosiphum albifrons* is adapted to consume *Lupinus angustifolius* with a low content of alkaloids (Philippi et al., 2015); however, dietary specialist herbivore insects may also be adapted to tolerate secondary metabolites of their host plants without switching to other different host plants (Laukkanen et al., 2012). In *Arabidopsis thaliana*, the abundance of two aphid herbivore species is correlated to a genetic polymorphism of the plant's resistance locus. This polymorphism is under selection due to changes in population size of the two aphid species (Zast et al., 2012).

Host-parasite systems, that exert reciprocal selection pressures, offer the opportunity to assess the asymmetry in selection pressures and the potential to produce adaptation (Greischar & Koskella, 2007). Local adaptation may produce population differentiation as a by-product of natural selection (Kawecki & Ebert, 2004). This process is well illustrated by the weevil *Curculio camelliae* (Coleoptera: Curculionidae) that parasitizes the fruits of *Camellia japonica* (Theaceae) (Iseki, Sasaki & Toju, 2011; Toju, 2007; Toju, 2009; Toju & Sota, 2006). The fruits of *C. japonica* are capsules with a thick pericarp, dehiscent, with three locules and one seed per cavity (Okamoto, 1988). Females of *C. camelliae* perforate the thick pericarp with its long rostrum, modified labial cavity in insects (Resh & Cardé, 2009), and oviposit on the seeds (Toju, 2007). A successful weevil infestation, or oviposition, depends on the phenotypic match between the rostrum length and pericarp thickness. These two traits that mediate the interaction vary geographically and are under selection (Toju, 2007; Toju, 2009; Toju & Sota, 2006). Some evidence, however, indicates that these phenotypic characteristics may also vary according to abiotic factors, i.e., the latitude (Iseki, Sasaki & Toju, 2011). Furthermore, infestation by the weevil *C. camelliae* increases at higher-altitude localities and its obligated host plant decreases its resistance (Toju, 2009). In the *C. camelliae*-*C. japonica* system, natural selection acts on pericarp thickness that is a physical barrier that prevents infestation by weevils (Toju, 2007; Toju, 2009; Toju & Sota, 2006).

In the annual herb *Datura stramonium*, tropane alkaloids function either as resistance characters preventing foliar damage by herbivores and/or as phagostimulants to them (Castillo et al., 2013; Castillo et al., 2014; Shonle & Bergelson, 2000). Evidence shows that alkaloid concentration in *D. stramonium* varies across populations (Castillo et al., 2014)

and that such differentiation in chemical defense could be adaptive (Castillo *et al.*, 2015). In some populations, dietary specialist and generalist folivores select against atropine concentration, whereas scopolamine is positively selected by the dietary specialist folivore *Lema daturaphila* and by the generalist grasshopper *Sphenarium purpurascens* (Castillo *et al.*, 2014). Fruits of *D. stramonium* are parasitized by *Trichobaris soror* (Coleoptera: Curculionidae) that reduces plant fitness by consuming the seeds (Cabrales-Vargas, 1991; Cruz, 2009; De-la-Mora, Piñero & Núñez-Farfán, 2015). However, to what extent alkaloids of *D. stramonium* could affect infestation by the seed predator is not known. Here, we analyzed the relationship between tropane alkaloids produced by *D. stramonium* and infestation by the specialized seed predator *T. soror* across multiple populations in central Mexico. We aimed to determine whether *D. stramonium*'s tropane alkaloids prevent infestation by *T. soror*. Specifically, we addressed the following questions: 1. Are alkaloids resistance characters that prevent/reduce infestation by weevils; 2. Do seed predators exert natural selection upon plant's alkaloids concentration; and 3. Do variation of both alkaloid concentration and infestation by weevils across populations is correlated to the localities' environmental conditions? (*v. gr.*, Toju, 2009).

## MATERIALS AND METHODS

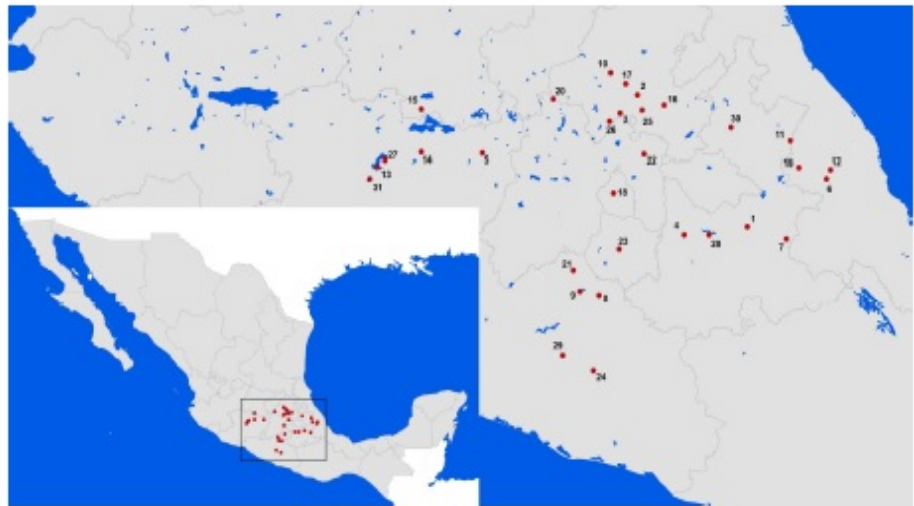
### Study system

The weevil *Trichobaris soror* (Coleoptera: Curculionidae) is intimately associated to the life cycle of *D. stramonium* (Bello-Bedoy, Cruz & Núñez-Farfán, 2011a; Borbolla, 2015; Cabrales-Vargas, 1991). *Trichobaris soror* is distributed mainly in central Mexico (Barber, 1935; De-la-Mora, Piñero & Núñez-Farfán, 2015); adult weevils feed on leaves, calyx and floral tissues of *D. stramonium*. After mating, females oviposit at the base of developing fruits. Their larvae feed exclusively on immature seeds inside the developing fruit where they build tunnels with their own feces. Larvae pupate in the fruit and sometimes are parasitized by wasps. The weevils hibernate inside the fruit of *D. stramonium* until the next rainy season (Bello-Bedoy, Cruz & Núñez-Farfán, 2011a; Borbolla, 2015; Cabrales-Vargas, 1991).

Besides the seed predator, *D. stramonium* (Solanaceae) is preyed upon by specialist leaf-beetles (*Lema trilineata* and *Epitrix parvula*, Coleoptera: Chrysomelidae). This weed species has been widely studied in relation to its resistance characters (alkaloids, leaf trichomes) against these leaf herbivores (Bello-Bedoy & Núñez-Farfán, 2011b; Cabrales-Vargas, 1991; Carmona & Fornoni, 2013; Castillo *et al.*, 2013; Castillo *et al.*, 2014; Núñez-Farfán & Dirzo, 1994; Shonle & Bergelson, 2000; Valverde, Fornoni & Núñez-Farfán, 2001). However, it is unknown if chemical defense of *D. stramonium* prevents the infestation by the weevil *T. soror*.

### Sampled populations

During the reproductive season of *D. stramonium* (September-November) in 2007, we sampled different populations across Central Mexico. We collected an average of 30 plants from 31 populations (Fig. 1, Table S1). For each plant, all mature fruits were collected and individually bagged and labeled. Before opening, the width and length of each fruit was



**Figure 1** *Datura stramonium* populations sampled in Central Mexico. The number each population corresponds to the locality given in Table S1.

measured to calculate its volume with the equation:

$$V = \left(\frac{4}{3}\right)(\pi)(\text{length})\left(\left(\frac{\text{width}}{2}\right)^2\right)$$

We estimated the number of expected seeds by multiplying the volume by 0.026 (Fornoni, Valverde & Nunez-Farfan, 2004). In the laboratory, we opened the fruits to determine the infestation, by counting the weevils. Also, for each fruit we counted the number of remaining, sound, seeds after predation.

### Seed predator's infestation measures

We measured the intensity of infestation by *T. soror* to *D. stramonium* as the number of weevils divided by the number of fruits per plant (Greischar & Koskella, 2007). Also, we measured the infestation as the proportion of infested fruits per plant.

### Alkaloid concentration

We quantified the concentration of leaf atropine and scopolamine per plant (Boros et al., 2010), by means of high-performance liquid chromatography (HPLC), following Castillo et al. (2013). We obtained the average leaf alkaloid concentration from a sample of 8–10 plants per population. In order to assess if leaf and seeds alkaloid concentration are correlated, we measured alkaloids of mature seeds in a subsample of 119 plants of 14 populations (Table S2).

### Characterization of environmental variables

In order to control for some concomitant environmental variation, the values of mean annual temperature and precipitation for the 31 studied populations of the *D. stramonium* were obtained from Worldclim (Hijmans & Graham, 2006). We characterized each population by calculating the Lang's aridity index (Rehman, 2010), as the ratio of population

mean annual precipitation and mean annual temperature. Values between 0–20 correspond to deserts, 20–40 arid habitats, 40–60 wet type steppes, 60–100 wet woodlands and 100–160 wet forests (Perry, 1986).

### Statistical analyses

We assessed the among-population variation in the intensity of infestation, the proportion of infested fruits by *T. soror*, and the concentration of atropine and scopolamine in seeds and in leaves, using generalized linear model (GzLM), assuming a gamma distribution and a log link function. Our hypothesis is that infestation by the seed predator does not vary among populations of *D. stramonium*.

The relationship between the concentration of atropine and scopolamine in both leaves and seeds was tested through generalized linear mixed models (GzLMM), with a gamma distribution, a log link function and population as a random factor. Data were obtained from individual plants from a subsample of 14 populations. In order to assess the effect of the seed predator on plant fitness, we performed a GzLMM of the number of remaining sound seeds, as estimator of fitness, as a function of the proportion of infested fruits per plant and population as a random factor. Again, we assumed a gamma distribution and a log link function. Similarly, we tested if the concentration of atropine and scopolamine in leaves increases plant individual fitness. We assumed the population  $\times$  proportion of infested fruits interaction as a random factor.

Selection differentials ( $S$ ) that account for direct and indirect selection acting on a trait were calculated through Pearson correlation coefficient for each population (Lande & Arnold, 1983). The concentration of atropine and scopolamine in seeds and leaves was standardized ( $x' = \frac{x - \mu}{\sigma}$ ). As a proxy of plant fitness we used the estimated number of seeds per plant (other examples in Kingsolver et al., 2001). The relative fitness was defined as the ratio between individual fitness and population average fitness. Selection differentials were estimated for each population by correlating alkaloid concentration standardized (atropine or scopolamine) with relative fitness (such in Sobral et al., 2013; Sobral et al., 2015).

In order to explore the effect of selection exerted by the seed predation and environmental variables on the concentrations of scopolamine and atropine in both leaves and seeds, across populations of *D. stramonium*, we performed generalized linear models (GzLM) (such in Herrera, Castellanos & Medrano, 2006; Sobral et al., 2015). In each model we included the selection differential of the corresponding alkaloid, latitude, longitude, altitude, and Lang's index of each population. We assumed a gamma distribution (log link function) for alkaloid concentration in leaves, and a Gauss inverse (identity link function) for seeds. We selected the models with lowest corrected AIC values, namely those that explain better the relationship between the variables and consider the sample size (Akaike, 1974; SPSS, 2011). Statistical analyzes were performed with SPSS v20.0 statistical package (SPSS, 2011).

## RESULTS

We detected wide variation in concentration of scopolamine and atropine in leaves (Fig. 2 and Table 1) and seeds (Fig. 3 and Table 1) across populations of *D. stramonium*. The proportion of infested fruits as well as the intensity of infestation by *T. soror* to plants of *D.*

**Table 1** Generalized linear models of the among population variation in the concentration of scopolamine and atropine in leaves and seeds, as well as the intensity of infestation (average number of weevils per fruit, per plant), and the proportion of infested fruits per plant in *Datura stramonium*.

Response variable	N	d.f.	Wald's chi-square	P	AICc
Scopolamine of leaves	278	31	684.55	<0.0001	345.19
Atropine of leaves	278	31	875.11	<0.0001	212.17
Scopolamine of seeds	119	14	13441.13	<0.0001	817.18
Atropine of seeds	119	14	13062.42	<0.0001	777.75
Intensity of infestation	859	28	835.98	<0.0001	74.73
Proportion of infested fruits	859	28	1 562.36	<0.0001	1 732.56

**Notes.**

AICc, The corrected Akaike information criterion, gives a measure of the relative quality of a statistical model, considering the sample size.

*stramonium* varied significantly among populations (Fig. 4 and Table 1). Variation in the average proportion of infested fruits ranged from zero (populations Coatepec, Huitzuco and Jalapa) up to 90% (populations Teotihuacan and Tlaxiaca; Fig. 4). The average intensity of infestation by *T. soror* varies from populations without infestation up to those with 5 weevils per fruit, per plant.

The concentration of atropine and scopolamine in leaves is positive and significantly related ( $N = 117$ ,  $Estimate = 0.289$ ,  $S.E. = 0.055$ ,  $t = 5.285$ ,  $P < 0.0001$ ,  $AICc = 306$ ), while the correlation of scopolamine in leaves and seeds is negative ( $N = 117$ ,  $Estimate = -1.29$ ,  $S.E. = 0.484$ ,  $t = -2.666$ ,  $P = 0.009$ ,  $AICc = 305.279$ ). Similarly, the concentration of scopolamine in leaves and atropine in seeds are negatively related ( $N = 117$ ,  $Estimate = -1.061$ ,  $S.E. = 0.481$ ,  $t = -2.206$ ,  $P = 0.029$ ,  $AICc = 307.407$ ).

The number of sound remaining seeds per plant showed a negative relationship with the proportion of infested fruits *T. soror* ( $N = 278$ ;  $Estimate = -0.629$ ,  $S.E. = 0.266$ ;  $t = -2.367$ ;  $P = 0.019$ ;  $AICc = 832.46$ ). We found that the number of remaining sound seeds shows a positive relationship with both scopolamine concentration in leaves ( $N = 278$ ;  $Estimate = 0.200$ ,  $S.E. = 0.076$ ;  $t = 2.639$ ;  $P = 0.009$ ;  $AICc = 886.85$ ) and seeds ( $N = 119$ ;  $Estimate = 13.56$ ,  $S.E. = 3.56$ ;  $t = 3.81$ ;  $P < 0.0001$ ;  $AICc = 403.32$ ).

A GzLM of the population average of scopolamine concentration in leaves is positively related to the selection differential ( $S$ ) of leaf scopolamine (Table 2 and Fig. 5A). The same result, although marginally significant, was detected for scopolamine in seeds. In the case atropine concentration in seeds, the GzLM indicates a positive and highly significant relationship with the Lang's aridity index; atropine concentration in leaves is marginally significant in its relationship with Lang's aridity index (Table 2 and Fig. 5B).

## DISCUSSION

Populations of *D. stramonium* vary in the concentration of alkaloids in leaves. This result is in agreement with the study of Castillo *et al.* (2013). Here, we found that populations of *D. stramonium* also vary in the concentration of alkaloids in seeds, in the intensity of infestation, as well as in the proportion of infested fruits by *T. soror*. The concentration of scopolamine in seeds and leaves is negatively associated across populations. Although such

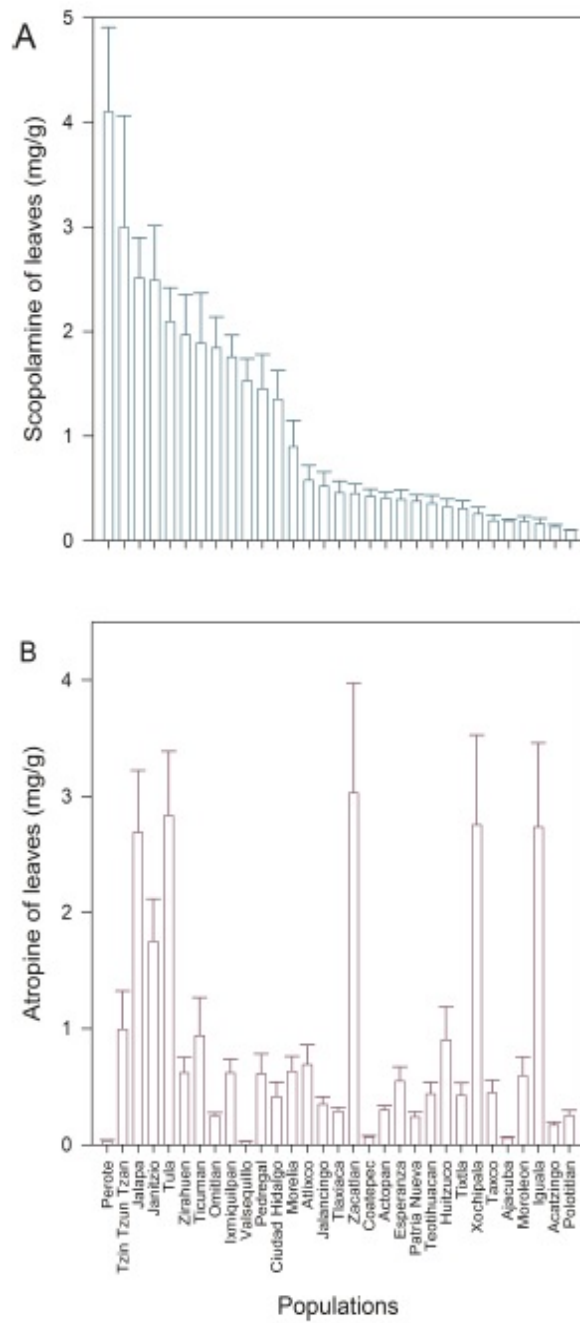


Figure 2 Average (+1 S.E.) concentration of scopolamine (A) and atropine in leaves (B), in 31 populations of *Datura stramonium* from central Mexico.

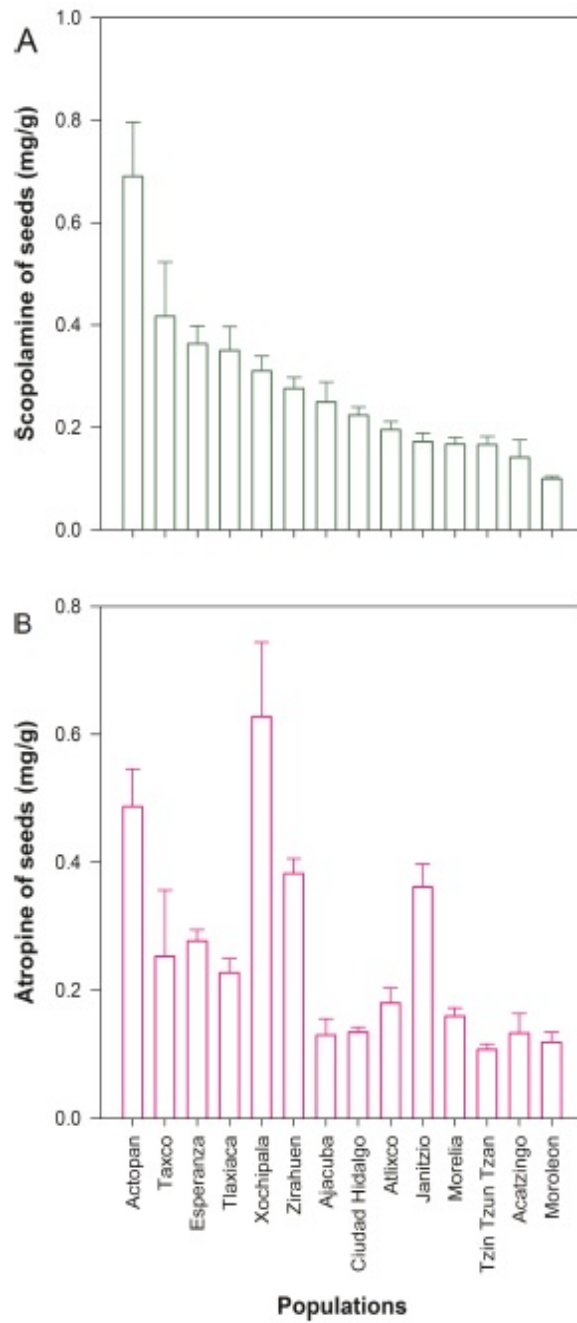


Figure 3 Average (+1 S.E.) concentration of scopolamine (A) and atropine (B) in seeds, in 14 populations of *Datura stramonium* from central Mexico.



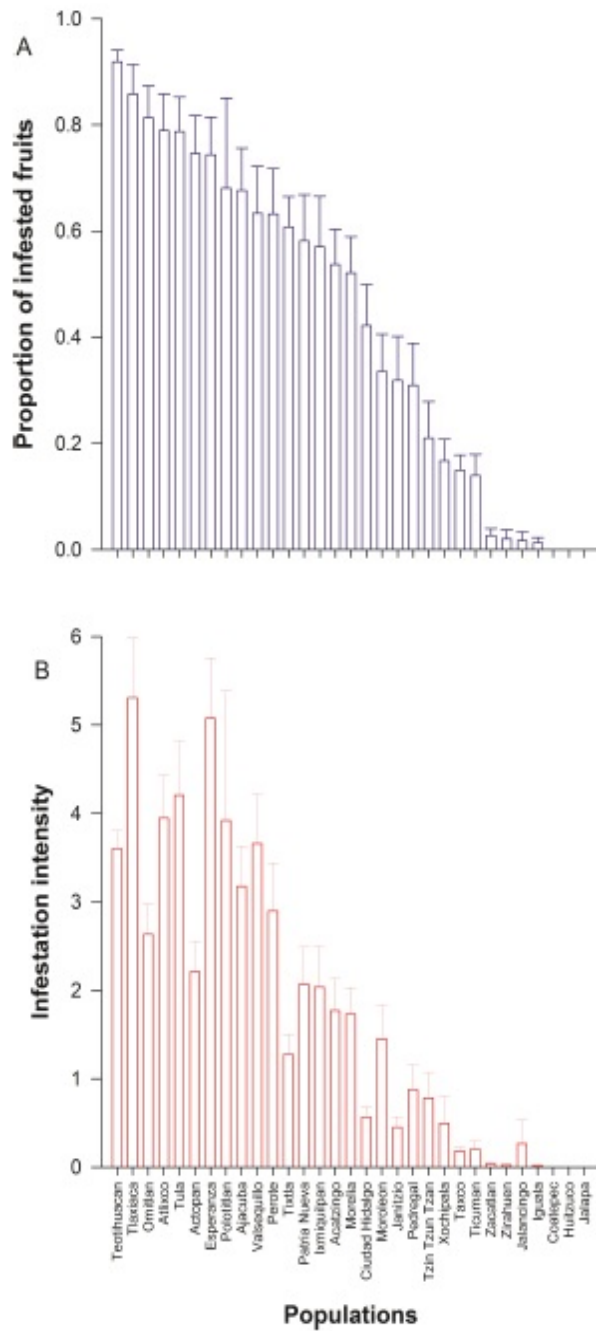


Figure 4 Average (+1 S.E.) proportion of infested fruits per plant (A) and intensity of infestation (the total number of weevils divided by the total number of fruits per plant) (B) by *Trichobaris soror*, in 31 populations of *Datura stramonium* from central Mexico.

**Table 2** Generalized linear models of population average of the concentration of scopolamine and atropine in leaves and seeds of *Datura stramonium*, as a function of the selection differential (S) for the corresponding alkaloid, and environmental variables.

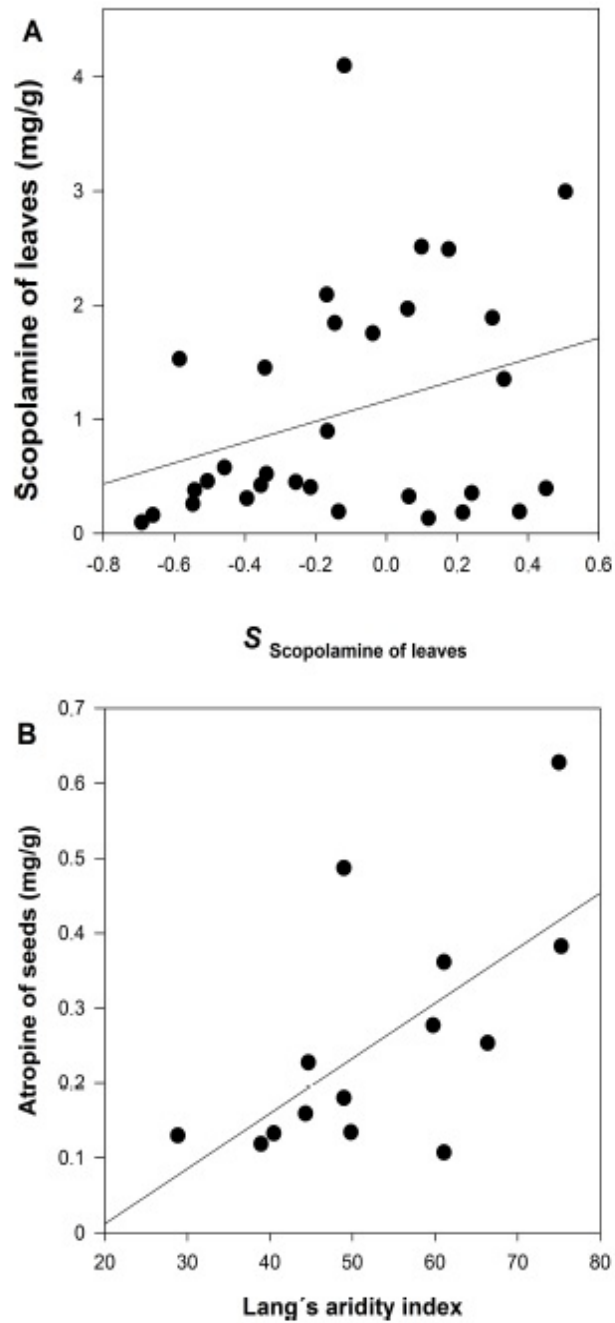
Response variable	Factors	N	d.f.	Wald's Chi-square	P	AICc
Scopolamine of leaves	Lang's aridity index	31	1	0.134	0.714	72.973
	Altitude		1	1.563	0.211	
	Latitude		1	0.552	0.458	
	Longitude		1	0.993	0.319	
	S Scopolamine		1	5.662	0.017	
Scopolamine of seeds	Lang's aridity index	14	1	3.444	0.063	121.19
	Altitude		1	2.665	0.103	
	Latitude		1	0.579	0.447	
	Longitude		1	0.912	0.339	
	S Scopolamine		1	3.006	0.083	
Atropine of leaves	Lang's aridity index	31	1	3.557	0.059	61.072
	Altitude		1	3.419	0.064	
	Latitude		1	0.004	0.95	
	Longitude		1	0.001	0.976	
	S Atropine		1	0.812	0.367	
Atropine of seeds	Lang's aridity index	14	1	11.291	0.001	122.06
	Altitude		1	0.002	0.967	
	Latitude		1	1.666	0.197	
	Longitude		1	1.288	0.256	
	S Atropine		1	0.011	0.918	

**Notes.**

AICc, The corrected Akaike information criterion, gives a measure of the relative quality of a statistical model, considering the sample size.

a pattern was not detected for atropine, the concentration of scopolamine and atropine in leaves, positively covary across populations. Remarkably, we detected that scopolamine concentration in both leaves and seeds enhances individual plant fitness. Our major finding was the detection of a positive relationship between the population average concentration of scopolamine in both leaves and seeds with the selection differentials of scopolamine. This implies that natural selection explains the among population variation in scopolamine concentration. Thus, *T. soror* is driving, at least in part, the variation in chemical defense in *D. stramonium* (Castillo et al., 2015).

A previous study has reported that scopolamine plays a role in the interaction between *D. stramonium* and its main folivore insects in central Mexico (Castillo et al., 2014). Here, we found that among-populations of *D. stramonium*, plants with higher concentration of scopolamine in leaves had a higher number of remaining sound seeds. This suggests that scopolamine acts as a defense character against *T. soror*, resulting in fewer consumed or damaged seeds. A similar trend has been found in the hemiparasitic plant *Castilleja indivisa* (Adler, 2000), where the alkaloid lupanine, obtained from its host plant, *Lupinus texensis*, reduces damage to its flowers by herbivores and increases visitation by pollinators, thus enhancing plant fitness, measured as the number of seeds.



**Figure 5** Relationships between (A) the population average of scopolamine concentration in leaves with  $S_{\text{Scopolamine of leaves}}$  ( $\rho = 0.3079$ ), and (B) the population average of atropine concentrations in seeds with the Lang's index ( $\rho = 0.6434$ ).

The fact that the concentration of scopolamine in leaves and seeds are negatively correlated, probably pointing an allocation trade-off (Kariñho-Betancourt *et al.*, 2015), this does not preclude that scopolamine concentration in leaves had a negative effect on the seed predator. Adult females and males of *T. soror* courtship, mate, and feed on *D. stramonium* leaves (J Núñez-Farfán, pers. obs., 1989). When feeding, adult weevils produce small holes on the leaf blade, and sometimes damage can be severe (Cabrales-Vargas, 1991). Thus, it is likely that *T. soror*'s females, while feeding, might "assess" the chemical resistance level of a plant (i.e., atropine/scopolamine concentration in leaves) and select which plants are suitable to oviposit. This would result in lower or null oviposition in those plants with high concentration of scopolamine in leaves. Some evidence in this line shows a close relationship between *Trichobaris* and *Datura*. For instance, *Trichobaris bridwelli* oviposits preferentially on *D. stramonium* rather than on tobacco plants (*Nicotiana tabacum*) (Cuda & Burke, 1991), since *T. bridwelli* does not tolerate the pyridine alkaloids of tobacco. On the other hand, Diezel, Kessler & Baldwin (2011) have experimentally demonstrated that *T. mucorea*, a species that burrows into the stems of *Nicotiana attenuata*, increases infestation on transgenic plants of *N. attenuata* where the chemical defenses (jasmonic acid and nicotine) were silenced.

The role of scopolamine as defense against herbivory in *D. stramonium* is supported by the findings of Castillo *et al.* (2014). In such study, they detected positive selection on scopolamine concentration in leaves in two populations of *D. stramonium*, one preyed upon by the dietary specialist *Lema daturaphila*, and the other by the dietary generalist *Sphenarium purpurascens*. However, negative selection on scopolamine was also detected in a third population of *D. stramonium* preyed upon by *L. daturaphila*. Thus, the resistance role of tropane alkaloids of *D. stramonium* varies according to the type of herbivore and the tissue they consume, as well as the environmental conditions of populations (Castillo *et al.*, 2014). Further studies are needed to determine the independent and join selective effects of folivores and seed predators on the concentration of scopolamine in seeds and leaves of *D. stramonium*.

The GzLM shows that atropine in seeds covaries positively with the Lang's index. That is to say, populations of dry environments have less atropine concentration. It has been reported that alkaloid production may be water limited, decreasing in concentration in dry environments (Baricevic *et al.*, 1999). In the interaction between *Curculio camelliae*-*Camellia japonica*, the expression of defense is also affected by the habitat's environmental conditions; infestation by the weevil was higher at high altitudes, where the fruits of *C. japonica* trees have thinner pericarps (Toju, 2009). Thus, the evolution of chemical defense against herbivores in *D. stramonium* can also be environmentally conditioned.

We found evidence of natural selection on chemical resistance of *D. stramonium* since higher scopolamine concentration increases plant fitness. The GzLM analysis revealed a positive relationship between the population average concentrations of scopolamine in both leaves and seeds with their selection differential of scopolamine. This relationship suggests that the among-population variation in chemical defense of *D. stramonium* is molded by *T. soror*. Thus, populations of *D. stramonium* vary in the direction and strength of selection on chemical defense, an expectation of the geographic mosaic of coevolution

(i.e., hot spots and cold spots; *Thompson, 2005*). Previous evidence has demonstrated that leaf herbivores exert selection pressures over physical and chemical defenses of *D. stramonium* (*Valverde, Fornoni & Núñez-Farfán, 2001; Castillo et al., 2014*). This is the first evidence that seed predators also exert a significant selective pressure on chemical defense of *D. stramonium*.

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### Competing Interests

The authors declare there are no competing interests.

### Author Contributions

- Adán Miranda-Pérez conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Guillermo Castillo and Johnattan Hernández-Cumplido performed the experiments, reviewed drafts of the paper.
- Pedro L. Valverde conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, reviewed drafts of the paper.
- María Borbolla, Laura L. Cruz and Rosalinda Tapia-López performed the experiments.
- Juan Fornoni conceived and designed the experiments, performed the experiments, reviewed drafts of the paper.
- César M. Flores-Ortiz performed the experiments, contributed reagents/materials/analysis tools.
- Juan Núñez-Farfán conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

### Data Availability

The following information was supplied regarding data availability:

The raw data has been supplied as [Data S1](#).

### Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.1898#supplemental-information>.

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**Capítulo 2: Each rooster crows  
better in their own roost:  
Reciprocal local adaptation  
among annual plant and its seed  
predator**

## **Each rooster crows better in their own roost: Reciprocal local adaptation among annual plant and its seed predator**

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## Abstract

Variable spatial selection patterns on plant defenses and their interaction with specific herbivores could lead to reciprocal local adaptation in some populations. Local adaptation implies that a population achieve higher fitness in their home site locality than in foreign localities. We aimed to assess whether chemical defense (tropane-alkaloids) of the annual plant *Datura stramonium* and the infestation traits (proportion of infested fruits and intensity of infestation) of the seed predator *Trichobaris soror* could display a local adaptation pattern. We performed an experiment with reciprocal plants transplanted in two populations of *D. stramonium* to test local adaptation. Local adaptation was tested by GzLM statistical analysis and the adaptation or maladaptation pattern was determined through the Response Ratio analysis. We found that scopolamine increased plant fitness and either reduced the infestation traits (e. g., proportion of infested fruits and a negative pattern close to the significance on the intensity of infestation exerted by the seed predator). On the scopolamine, a chemical defensive trait of *D. stramonium*, both local adaptation and natural selection were found in the home site Teotihuacán treatment sympatry and in the home site Morelia treatment sympatry and allopatry. Hence, reciprocal local adaptation was found between *D. stramonium* and their seed predator *T. soror*, in this parasite-host plant system through of the Response Ratio analysis.

**Key-words:** *Datura stramonium*, *Trichobaris soror*, seed predator, local adaptation, scopolamine, Response Ratio.

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## Introduction

Local adaptation is the evolutionary process wherein the genotypes of a species have higher fitness in their home site respect to other localities, this is because it is a phenomenon of the population differentiation due to divergent selection (Kawecki & Ebert, 2004). In a parasite-host system the expected patterns of local adaptation are: 1) that parasites tend to express local adaptation over their hosts because they may have shorter generation times (Gandon *et al.*, 1996; Kaltz & Shikoff, 1998), 2) local adaptation could be reciprocal between parasite and their host (Gandon 2002; Greischar & Koskella, 2007; Forde *et al.*, 2004; Hoeksema & Forde, 2008; Garrido *et al.*, 2012), and 3) sometimes the host could presents local adaptation without occurring maladaptation of the parasite (Lemoine *et al.*, 2012).

To assess the existence of the local adaptation pattern is required to implement some experiments. Two experimental options are available to test the hypothesis of local adaptation: i) contrasting the performance of the focal species in their home site regarding their performance in foreign localities (“home vs. away” criterion); ii) measuring the performance between the local individuals compared to that of foreign individuals within a specific locality (“local vs. foreign” criterion; Kawecki & Ebert, 2004).

In the “home vs. away” criterion, comparisons could be influenced by the environment (Kawecki & Ebert, 2004). While the criterion “local vs. foreign” tries to eliminate the influence of the environment in the experimental tests, but biotic interactions could influence the comparisons (Greischar & Koskella, 2007). The expected result between these comparisons would be that the individuals from the locality of origin where the test is carried out would have a higher performance in comparison to individuals coming from other localities, but sometimes this does not happen. Sometimes individuals from one locality perform well in their locality of origin and in other localities, which would mix up the results somewhat as we would not be able to discern whether local individuals actually performed better in their locality of origin compared to other localities (Vesakoski & Jormalainen, 2013). This phenomenon seems to be common in the literature (Hereford, 2009) and the comparison between the performance of local individuals minus the performance of foreign individuals in the local population could be the test that would allow us distinguish local adaptation from the biotic or abiotic factors that influence our findings (Hereford, 2009; Garrido *et al.*, 2012; Parachnowitsch & Lajeunesse, 2012).

The hypothesis of co-evolution between plants and the species that interact with them through secondary chemical compounds (Elrich & Raven, 1964; Mithöfer & Boland, 2012) is not

a generalization (Agrawal & Weber, 2015; Núñez-Farfán & Kariñho-Betancourt, 2015), e. g., a correlation trend was found between a higher amount plant herbivory and higher nitrogen content present in plant tissues (Agrawal & Weber, 2015). However, in some plants secondary chemical defense evolved to mitigate the selection pressure of herbivores (Berenbaum *et al.* 1986; Després *et al.* 2007) and might be an evidence of the process of local adaptation (Laine, 2009).

To demonstrate that the secondary metabolites determine the adaptation of the herbivorous insects there was performed an experiment between spider mites (*Tetranychus urticae*), plants from genus *Cucurbitaceae* and an alternative host plant (*Gossypium hirsutum*, Agrawal, 2000). In this experiment the production of cucurbitacins, secondary metabolites present in all the members of the Cucurbitaceae family, was measured and was tested the occurrence of a trade-off on fitness in different host plants. It was found that the spider mites that grew by several generations on the cotton plants preferred the favorable plants (e. g., with less secondary metabolites), whilst the individuals which were adapted to higher concentrations of cucurbitacins did not have host preferences. In this experiment was demonstrated that the feeding preference to certain host plants evolved through specific secondary metabolites.

Besides, another hypothesis states that females of herbivorous insects oviposit in suitable places (Jaenike, 1978; Jaenike, 1990; Clark *et al.*, 2011). The female's oviposition site election also may be influenced by the presence of defenses. *Lema daturaphila*, a specialist diet herbivore of *D. stramonium*, evolved to oviposit on the less defended plants (Carmona & Fornoni, 2013; Castillo *et al.*, 2014). To prove it, a greenhouse experiment was carried out where compared the amount of foliar tissue consumed through the efficiency of the folivore *L. daturaphila* and the generalist herbivore *Spodoptera frugiperda* (Kariñho-Betancourt, 2015). The measure of the adaptation was the growth of the larvae in both species (Garrido *et al.*, 2012). The larvae of *L. daturaphila* increased more their biomass than the larvae of *S. frugiperda*. When *L. daturaphila* consumed leaves with a high concentration of scopolamine and greater number of trichomes, did not increase their biomass (Kariñho-Betancourt, 2015). There is also evidence that *L. daturaphila* is locally adapted to *D. stramonium* in central Mexico (Garrido *et al.*, 2012).

In the annual herb *D. stramonium*, physical and chemical resistance traits (e. g., trichomes and tropane-alkaloids) prevents foliar damage (Castillo *et al.*, 2013; Castillo *et al.*, 2014; Kariñho-Betancourt *et al.*, 2015, Shonle & Bergelson, 2000) in addition the chemical defense could present adaptive differentiation (Castillo *et al.*, 2015). The fruits of *D. stramonium* are infested by *Trichobaris soror* (Coleoptera: Curculionidae) a specialist-diet

herbivore that reduces plant fitness by consuming their seeds (Cabrales-Vargas, 1991; Bello-Bedoy *et al.*, 2011; De-la-Mora *et al.*, 2015). Also, it is known that the chemical resistance traits in *D. stramonium* decrease the infestation in their fruits (Miranda-Pérez *et al.*, 2016).

However, it remains unclear if the chemical resistance traits of *D. stramonium* could present genetic component and enhance the process of local adaptation to its seed predator. In turn, the seed predator also may be locally adapted by means of its infestation abilities reflected as intensity of infestation or proportion of infested fruits. We aim to assess the feasible local adaptation in the parasite-host system of the weevil *T. soror* and its local host plant *D. stramonium* due to the plant resistance traits and seed predator performance traits. We will test whether i) the *T. soror* infestation is higher in the home site locality host plants than in the foreign hosts plants, ii) if *D. stramonium* shows better performance in their home site than the plants of from other localities, and iii) if this performance is linked to its defensive traits and/or by natural selection.

## Materials and methods

### Study species

The weevil *Trichobaris soror* (Coleoptera: Curculionidae) is mainly distributed in central Mexico (Barber, 1935; De-la-Mora *et al.*, 2015). All its life cycle is associated to *Datura stramonium* (Cabrales-Vargas, 1991; Figure 1). *T. soror* have sexual dimorphism, females are larger than males (Borbolla, 2015; De-la-Mora *et al.*, 2018; Figure 1). Both females and males of *T. soror* feed on leaves, their courtship and mating occur on the leaves of *D. stramonium* (J. Núñez-Farfán, *personal observation*). After mating, females oviposit at the base of the developing fruits (Cabrales-Vargas, 1991) because this behavior could “asses” the suitable place and choose the plants of *D. stramonium* with lower leaf chemical defenses (Miranda-Pérez *et al.*, 2016). Inside a developing fruit, larvae of *T. soror* feeds exclusively on immature seeds, and they shape tunnels inside the fruits with the aim of their feces and pupate in. Larvae also may be parasitized by wasps. Weevils overwinter inside the fruits of *D. stramonium* until the next rainy season (Cabrales-Vargas, 1991; Figure 1).

*D. stramonium* (Solanaceae) is an annual weed distributed from xerophytic shrublands to pine-oak forest in central Mexico, and their reproduction occurs exclusively by seeds (Weaver & Warwick, 1984; Núñez-Farfán *et al.*, 1996). In central Mexico, the specialist-diet *Lema daturaphila* and the generalist grasshopper *Sphenarium purpuracens* herbivores exert selection on defensive traits of *D. stramonium*, both physical (trichomes) and chemical (atropine and

scopolamine alkaloids) that originates a spatially variable selection pattern (Castillo *et al.*, 2014). In the same way, chemical defenses model a geographic variable selection pattern against to the infestation exerted by their seed predator *T. soror* (Miranda-Pérez *et al.*, 2016).

To test whether the degree of infestation in the home site transplants were higher than in the foreign transplants. We chose two populations of *D. stramonium* that, since previous experiments, presented contrasting chemical defense production, and we determined if they could enhance the plant fitness differentially against the degree of infestation exerted by *T. soror* by means of experimental plots.

### *Experimental design*

To test whether the parasite-host system underwent the process of local adaptation, we used samples of parental plants of two populations from central Mexico (Castillo *et al.*, 2014). The home sites were Acolman and Sanabria which since now we will name Teotihuacán (19.68 N, -98.86 W) and Morelia (19.7 N, -101.19 W), respectively.

Due *D. stramonium* is a weed with more than 90% of self-fertilization in central México (Núñez-Farfán *et al.*, 1996), the seeds (offspring) collected at field and employed in this experiment, were considered “full-siblings”. We germinated 30 families of “full siblings” (natural offspring; Lawrence, 1984) that were reciprocal transplanted (treatments: sympatry and allopatry) in two experimental plots at each population sampled. Total experimental plants can be calculated as 2 home sites X 2 treatments X 30 families X 4 individuals which in total were 480 plants. At each home site the plants were sowed under a completely randomized design with one meter spacing between them. Each plot had an area of 25 x 23 meters. This experimental design was chosen to avoid biases in each treatment (Cochran & Cox, 2001).

All the plants that survived were harvested. For each plant, all mature fruits were collected, bagged and labeled individually. Before the opening of the fruits, there was measured the width and length of each fruit to calculate its volume (Fornoni *et al.*, 2004; Miranda-Pérez *et al.*, 2016). In the laboratory, the fruits were opened to determine if were parasitized. We recorded the number of weevils and parasitoid wasps if there was any. We also registered the number of seeds. For further analysis, the remaining sound seeds were considered as the number of seeds at each fruit. We only manipulated the plants as treatments (sympatry and allopatry), and the weevils belonged to the home site of at each experimental plot.



### *Plant defensive traits*

To analyze atropine and scopolamine of seeds, alkaloids which have similar concentration in leaves (Miraldi *et al.*, 2001), we collected samples of one fruit that belonged to the first or second branching of the plants of the experiment. We got seed samples from 146 plants of the two experimental plots, and the amount of atropine and scopolamine was analyzed/quantified by means of high-performance liquid chromatography (HPLC) according to the methodology proposed in Castillo *et al.*, (2013).

### *Seeds predator performance*

We measured the intensity of infestation of the seed predator *T. soror* in the fruits of *D. stramonium* as the number of weevils per plant found divided by the number of fruits per plant (Miranda-Pérez *et al.*, 2016). Also, we measured the infestation as the proportion of infested fruits per plant (Miranda-Pérez *et al.*, 2016). The *rostrum* or oral cavity modified in weevils of the genus *Curculionidae* (Resh & Cardé, 2009) was used by the adult females of *Curculio camelliae* to drill and oviposit inside *Camellia japonica* fruits. To measure the *rostrum* length of *T. soror* weevils as a proxy of the total length of their body (since *rostrum* and the body length has a positive correlation in the weevils;  $n = 360$ ,  $r^2 = 0.411$ ,  $P < 0.0001$ ; Borbolla, 2015), we photographed 2,051 individuals collected in the fruits of *D. stramonium* with a microscope Carl Zeiss and a Digital Microscopy Camera AxioCam ICc 5 (D). We used a digital rule to measure the *rostrum* length of each weevil. The sex of the weevils was determined by sexual dimorphism, where male's *rostrum* was shorter than female's (Figures 1G and 1H, respectively).

### *Statistical analyses*

The relationship between the plant defensive traits was tested through generalized linear mixed models (GzLMM). Specifically, we assessed the relationship between the concentration of alkaloids of the seeds among themselves, the atropine was the response variable and scopolamine the covariable. In the same way, we analyzed the relationship between the seed predator performance traits through generalized linear mixed models (GzLMM), with the proportion of infested fruits as response variable and intensity of infestation as covariable. Particularly, the *rostrum* of the weevils was tested as a response variable and the covariables were the traits of infestation (*e. g.*, proportion of infested fruits and intensity of infestation) tested separately in different models.

To test whether alkaloids (atropine and scopolamine of seeds) enhance the plant fitness as defensive traits we performed a GzLMM on the remaining sound seeds as response variable and the alkaloids of seeds as covariables. The genetic component was tested on the concentration of the alkaloids by means of a nested ANOVA where the differences due to the site of origin by treatment and genetic family (within home site by treatment) were tested (Valverde *et al.*, 2003) this analysis were performed using JMP version 10 (SAS, 2012).

To avoid a pseudo-replication, because we had only one sample of alkaloid concentration per plant and several measures of *rostrum* length per fruit/plant, we estimated the average *rostrum* of the weevils per plant. Afterwards, we proved with a GzLMM whether *rostra* of the weevils as response variable could increase their length with the alkaloid concentration (atropine and scopolamine of seeds) as covariables since the hypothesis was that the alkaloids of the seeds had a negative effect on the growth of the weevils.

We also assessed the effect of the seed predator infestation on plant fitness with a GzLMM performed with the remaining sound seeds as response variable and the infestation measures in different models as covariables (*e. g.*, proportion of infested fruits and the intensity of infestation). In all the GzLMM we used the home site by treatment (sympatry and allopatry) as a random factor. In addition, all the generalized linear mixed models were performed following the AICc criterion that would explain the best relation between variables (Burnham *et al.*, 2011), and we chose the best distribution and link function. All the GzLMM analyses were performed with SPSS v25.0 (SPSS, 2019).

To test local adaptation, we performed statistical tests GzLM as in Guitián *et al.*, 2017. The local adaptation pattern of *D. stramonium* under the “home vs. away” criterion (Kawecki & Ebert, 2004) was evaluated as the performance of the alkaloids of seeds (atropine and scopolamine of seeds) where the response variable was assessed in a separate model, while the home site treatment and their interaction were fixed factors.

Local adaptation in parasites were measured in terms of the ability to infest its host, either: i) as the proportion of infested fruits, or ii) the intensity of infestation (*e. g.*, the number of parasites in the host; Refardt & Ebert, 2007; Greischar & Koskella, 2007; Miranda-Pérez *et al.*, 2016). The *rostrum* length was taken as an estimator of the performance of the weevil’s growth inside the fruits of the plant. To determine the existence of local adaptation of the seed predator *T. soror* we analyzed the performance of the weevils as the intensity of infestation, the proportion of infested fruits and the *rostrum* length under the “Local vs. foreign” criterion (Kawecki & Ebert 2004).

We compared each performance trait separately, the home site treatment and the interaction between them were fixed factors. In all the GzLM we used the lowest value of AICc, the best distribution and link function. Subsequently, if the interaction between home site and treatment were equals or less than 0.05 it suggests local adaptation. All GzLM analyses were performed with SPSS v25.0 (SPSS, 2019).

### *Response Ratio*

We assessed the Response Ratio on the traits of *D. stramonium* and *T. soror* that were significant in the home site by treatment interaction between the local adaptation tests. In the Response Ratio we obtained the logarithm of the ratio between the average sympatry treatment and the average of allopatry treatment data (Lajeunesse, 2011; Parachnowitsch & Lajeunesse, 2012). Values at the right of zero meant local adaptation, in the left were maladaptation, and on zero represented no adaptation.

### *Natural selection analyses*

Selection analyses were performed on the scopolamine for each treatment and home site separately because the individuals of *D. stramonium* expressed differences in chemical resistance.

We evaluated the strength and direction of the natural selection on the target trait (scopolamine of seeds) using the selection analyses proposed by Lande & Arnold (1983). The remaining sound seeds were the plant fitness. The concentration of scopolamine was standardized with average zero and variance one. We performed ordinary least squares (OLS; Kingsolver *et al.* 2001; Schluter & Smith, 1986). The linear selection coefficient ( $\beta_i$ ) was interpreted as directional selection. The positive values of the quadratic coefficient of the regression ( $\gamma_{ii}$ ) were interpreted as disruptive selection, while the negative values were interpreted as stabilizing selection (Lande & Arnold, 1983). All the selection analyses were performed with R 3.5.3 (R Development Core Team, 2019).

## Results

### *Scopolamine of seeds as plant defense trait and their relationship with seed predators*

We found a positive relationship between atropine and scopolamine ( $N = 146$ ,  $t = 2.433$ ;  $P = 0.016$ ,  $AICc = -1805.747$ ; Gamma distribution and identity link function). The alkaloid scopolamine of seeds enhanced the plant fitness (Table 1A). We only found differences in the treatment for atropine of the nested ANOVA (Table 2B). We did not find differences among genetic families in any alkaloid (Table 2A, 2B).

Conversely, in terms of the infestation traits (proportion of infested fruits and intensity of infestation) we found a positive relation between them ( $N = 229$ ,  $t = 13.068$ ,  $P = 0$ ,  $AICc = -67.071$ ; Gamma distribution and identity link function). We found a negative relationship between the infestation and the *rostrum* length of the weevils (proportion of infested fruits  $N = 97$ ,  $t = -2.573$ ,  $P = 0.007$ ,  $AICc = -119.709$ , Gamma distribution and log link function; intensity of infestation,  $N = 97$ ,  $t = -1.768$ ,  $P = 0.080$ ,  $AICc = -112.403$ , Gauss inverse distribution and log link function). Finally, the infestation trait measured as proportion of infested fruits showed a negative relation with scopolamine of seeds (Table 1D) and intensity of infestation indicated a negative pattern close to significance with scopolamine of seeds (Table 1E), while the *rostrum* length of the weevils did not show any relationship with the alkaloids of seeds (Table 1F).

### *Local adaptation tests*

Atropine of seeds did not exhibit any difference between treatments or home sites (Figure 2A). In contrast, the concentration of scopolamine of seeds were higher in the plants of the home site treatment allopatry Teotihuacán and home site treatment sympatry Morelia, as a result of an interaction from home site and treatment (Table 3B, Figure 2B). In the Response Ratio analysis, the scopolamine of seeds had maladaptation pattern for the home site Teotihuacán and local adaptation for the home site Morelia (Figure 4).

Concerning to the seed predator infestation (proportion of infested fruits and intensity of infestation), were found higher infestation in the plot Teotihuacán (Figure 3A, 3C). Only on the intensity of infestation was found that the interaction between home site and treatment was significant (Table 3D). The response ratio analysis on the intensity of infestation of *T. soror* showed adaptation pattern in the home site Teotihuacán and maladaptation in the home site

Morelia (Figure 4). On the *rostrum* length, we did not find differences between the plots of this experiment (Table 3E, Figure 3B).

### *Natural selection on scopolamine*

We did not estimate selection analyses on atropine of seeds because our results indicated that did not enhance plant fitness. It was detected positive natural selection on the home site Teotihuacán, treatment sympatry on the scopolamine of seeds (*Estimate* = 0.2464, *S.E.* = 0.1199, *t* = 2.054, *P* = 0.0482), but did not find any pattern in the home site Teotihuacán, treatment allopatry (*Estimate* = -0.0294, *S.E.* = 0.1132, *t* = -0.26, *P* = 0.796). Positive natural selection was detected in the home site Morelia treatments sympatry (*Estimate* = 0.2444, *S.E.* = 0.0907, *t* = 2.695, *P* = 0.0116, Figure 5B) and allopatry (Quadratic *Estimate* = -0.9028, *S.E.* = 0.3941, *t* = 2.291, *P* = 0.0427, Figure 5C) on the scopolamine of seeds. The trend of the scopolamine was the enhancement of the remaining sound seeds (Figure 5A, 5B, 5D).

### Discussion

Our fundamental finding was the reciprocal local adaptation in the parasite-host system of the seed predator *Trichobaris soror* and the host plant *Datura stramonium*. This is not the first time that is revealed a reciprocal local adaptation between *D. stramonium* and one of their herbivores (Garrido *et al.*, 2012). However, this is the first time that local adaptation was tested in the seed predator *T. soror*, since we found higher intensity of infestation on plants with lower defense. Specifically, we tested the performance of the plants from Teotihuacán (the less defended population in central Mexico) in contrast to the plants from the population Morelia, which have two times more defense (Miranda-Pérez *et al.*, 2016). The scopolamine of seeds in *D. stramonium* had natural selection concerning to the chemical defense and decremented the infestation exerted by their seed predator. This result, in addition, reinforce the hypothesis that *D. stramonium* express a spatially variable selection pattern on their chemical defenses in central Mexico (Castillo *et al.*, 2014; Miranda-Pérez *et al.*, 2016). Since the scopolamine of the seeds had a positive interaction with the number of remaining sound seeds which could indicate that this is one of the factors that increases the fitness of the plant.

We found that the interaction between the seed predator and their host plant was mediated by the plant chemical resistance traits of *D. stramonium* as expected (Ehrlich & Raven, 1964). The infestation ability of *T. soror* (proportion of infested fruits and intensity of infestation) was negatively related to the concentration of scopolamine of seeds (Table 1C, 1D). This negative relationship would be linked to the selection expressed on the scopolamine among the experimental treatments (Figure 5A, 5B and 5D). A similar pattern was found in the annual plant *Linum marginale* and the virulence of their pathogenic fungus *Melampsora lini* because the plants were selected on their plant resistance and plants with higher resistance will have a better chance of surviving as virulence increases (Thrall & Burdon, 2003).

We also found that the scopolamine of seeds, as chemical defense of *D. stramonium*, enhanced the number of remaining sound seeds since those plants with the highest concentration of scopolamine of seeds were less infested (Table 1A). Additionally, in both experimental plots the average concentration of scopolamine in seeds per home site was similar. Genetic families had no differences which suggest that scopolamine of seeds holds a genetic component that supports the possible heritability of this trait in the populations of central Mexico (Castillo *et al.*, 2015). The scopolamine of seeds was the second defensive trait of *D. stramonium* tested through an experiment with a genetic component or heritability, since the first one were trichomes (Valverde *et al.*, 2003).

In a study performed by Kariñho-Betancourt *et al.* (2015) it was found that *D. stramonium* has a low density of trichomes in comparison to *D. inoxia*, while the opposite phenomenon occurs in the alkaloid concentration, as *D. stramonium* has a higher concentration of alkaloids than *D. inoxia*. In this study we focus on the concentration of atropine and scopolamine since the former is the precursor of the latter. These differences between chemical and physical defense are due to the fact that chemical defense measured as the concentration of alkaloids, (atropine and scopolamine) had higher selection pressures than physical defense in *D. stramonium* compared to *D. inoxia* (Hirano *et al.*, 2019) and evolved as a function of resistance against generalist and specialists herbivores (Castillo *et al.*, 2014). In addition, it was found that the concentration of atropine, both in seeds and in leaves, varies depending on environmental conditions (Miranda-Pérez *et al.*, 2016).

During their coevolution with plants, insects have evolved to be able to locate their host plants for feeding and oviposition using physical or chemical cues (Rausher, 2001;

Gómez *et al.*, 2010; Wu & Baldwin, 2010). The physical and chemical defenses of *D. stramonium* (Castillo *et al.*, 2013; Fornoni *et al.*, 2004; Kariñho-Betancourt *et al.*, 20015; Shonle & Bergelson, 2000), have been well studied and are part of a spatially variable selection pattern against folivores (Castillo *et al.*, 2014) and their seed predator *T. soror* (Miranda-Pérez *et al.*, 2016). There was found no evidence that the “assess” of the oviposition place in the females of *T. soror* were influenced by the atropine of seeds (Table 1A, 1B). In the tests we detected that the plants can develop specific and differential defenses against their predators as in other species (Leimu & Koricheva, 2006; Lankau, 2007; Kalske *et al.*, 2012). Females of *Trichobaris mucorea* and *T. compacta* preferred to oviposit on *Datura wrightii*, but *T. mucorea* oviposited in *Nicotiana attenuata*. In this case females were induced to oviposit in the plants of *N. attenuata* to avoid the presence of sesquiterpenes (Lee *et al.*, 2016). Thus, females of other species of *Trichobaris* may have evolved the ability to differentiate between one or more species of host plants regarding the most convenient place to oviposit and to use efficiently the suitable available resources (Soberón, 1986; Lee *et al.*, 2016).

The fruits of *Camellia japonica* evolved in their physical defense by natural selection on the width of their pericarp to avoid the oviposition of the specialist seed predator *Curculio camelliae* (Toju, 2007). In *Brugmansia suaveolens* the concentration of the alkaloid scopolamine was negative related to the fitness of the folivore larvae *Spodoptera frugiperda* (Alves *et al.*, 2007). Also, the secondary chemicals of plants were negatively related to the infestation of the weevils from the genus *Trichobaris* and may influence their oviposition pattern (Cuda & Burke, 1991; Diezel *et al.*, 2011; Lee *et al.*, 2016; Miranda-Pérez *et al.*, 2016). Then the females would follow the expected pattern of oviposition on the “better plants” (Jaenike, 1990; Soberón, 1986), because allegedly females of *T. soror* could “sense” the concentration of scopolamine of *D. stramonium* and consequently oviposit in plants with lower chemical defenses (Miranda-Pérez *et al.* 2016).

Regarding the traits that were measured in *T. soror*, we found that the average *rostra* length had a negative relation with the infestation (proportion of infested fruits) in *D. stramonium* individuals. This negative pattern might be outcome from a dense-dependence relationship between the length of individuals and the available resources, and could be related to the survivorship. In the beetles *Ips typographus* it was found that the increasing in density of individuals produced smaller size of the eggs and mortality also was higher, which marked a limit in the size related to the mortality due to the minimum size necessary for the individuals’ survival (Anderbrant, 1990). In the case of the density of individuals that infest the host plant *Senecio* *anonymus* the density-dependence phenomenon produced the body reduction in the beetle

*Neacoryphus bicrucis*. This pattern of reduction of their body size could have consequences in the sexual selection since this change modifies the sizes of the territories that the males should monitor and increases the competition between them (McLain, 1992). Moreover, the average size of the *rostra* between plots was not significantly different because we only had the final developmental size of these individuals, therefore we were unable to determine whether the ontological size varied sufficiently and we did not appreciate any morphological changes in different stages of its ontogeny as in *Lema daturaphila*, a specialist herbivore of *D. stramonium* (Garrido *et al.*, 2012), neither the positive or negative effect of the secondary chemicals of the plants on the body of their specialist diet herbivores (Kariñho-Betancourt, 2015; Laukkanen *et al.*, 2012).

We chose to evaluate local adaptation such as the ability of the weevils to infest a plant (proportion of infested fruits and intensity of infestation) because in other organisms this ability was directly related with their performance to the next generation (Refardt & Ebert, 2007). In the plot home site Morelia, the infestation was similar in both treatments, while in the home site Teotihuacán infestation was higher in the sympatry treatment than the allopatry treatment (Figure 3A, 3C). This pattern of infestation supports, in part, the local adaptation in *T. soror* weevils which could be due to the sensitivity of females to differentiate the concentration of the scopolamine of the plants in both treatments. For example, the individuals of the generalist leaf miner *Liriomyza sativae* were adapted to infest different host plants and they did not differentiate between their secondary chemicals associated to plants as the tomato (*Lycopersicon esculentum*) and the cowpea (*Vigna unguiculata*, Via, 1984). In the case of the moth *Taumetopea pityocampa*, an herbivore of *Pinus nigra* and *P. sylvestris* (soft and thought needled, respectively) in an experiment of local adaptation it was found that the moths of the home site of the *P. nigra* population oviposited only on their host plants, while the moths from the home site of *P. sylvestris* performed equally in both host plants species (Zovi *et al.*, 2008). An explanation that matches with our infestation pattern could be that the herbivorous insects do not clearly distinguish plants with fewer defenses, when the insect grew up in a locality with high chemical defense (Fry, 1996; Parachnowitsch, 2013; Vesakoski & Jormalainen, 2013). This pattern of infestation probably has had less attention because the process of local adaptation was not reported adequately across the literature and remains elusive.



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## Tables and figures

**Table 1.** Generalized linear mixed models of the variation among populations in the remaining sound seeds and *rostrum* length of the seed predator *Trichobaris soror* regarding the scopolamine and atropine of seeds of *Datura stramonium*, and the infestation traits of the seed predator (intensity of infestation and the proportion of infested fruits).

Model	Response variable	Fixed variable	N	<i>t</i>	<i>P</i>	AICc	Distribution	Link function
A	Remaining sound seeds	Atropine of seeds	146	-1.321	0.189	338.525	Gauss inverse	Log
		Scopolamine of seeds		3.192	<b>0.002</b>			
B	Remaining sound seeds	Proportion of infested fruits	229	1.328	0.186	616.927	Gamma	Log
C	Remaining sound seeds	Intensity of infestation	229	1.687	0.093	630.424	Gamma	Log
D	Proportion of infested fruits	Atropine of seeds	146	0.030	0.976	-8.833	Gauss inverse	Identity
		Scopolamine of seeds		-2.191	<b>0.030</b>			
E	Intensity of infestation	Atropine of seeds	146	1.522	0.131	308.718	Gamma	Log
		Scopolamine of seeds		-1.223	0.224			
F	<i>Rostrum</i> length	Atropine of seeds	97	1.397	0.166	-86.433	Gauss inverse	Log
		Scopolamine of seeds		-0.262	0.794			

### Notes:

\*AICc. The corrected Akaike information criterion measure the quality of a statistical model considering the sample size.

**Table 2.** Generalized linear models of the local adaptation experiment concerning to atropine and scopolamine of seeds in the annual plant *Datura stramonium*, and the seed predator performance traits (proportion of infested fruits, intensity of infestation and *rostrum* length).

Model	Response variable	<i>df.</i>	N	Home site	<i>P</i>	Treatment	<i>P</i>	Home site by treatment [family]	<i>P</i>
A	Atropine of seeds	24	146	1.748	0.188	4.247	<b>0.041</b>	0.900	0.601
B	Scopolamine of seeds	24	146	0.027	0.868	0.032	0.858	1.173	0.279

**Notes:**

\*The *F Ratio* was provided for each source of variance (e. g., Home site, Treatment, Home site by treatment [family]).

\*\*Home sites were Morelia and Teotihuacán, and treatments were sympatry and allopatry transplants of *Datura stramonium* in the experimental plots.



**Table 3.** Generalized linear models of the local adaptation experiment concerning to atropine and scopolamine of seeds in the annual plant *Datura stramonium* and the seed predator performance traits (proportion of infested fruits, intensity of infestation and *rostrum* length).

Model	Response variable	<i>N</i>	Home site $\chi^2$	<i>P</i>	Treatment $\chi^2$	<i>P</i>	Home site X treatment $\chi^2$	<i>P</i>	<i>AICc</i>
A	Atropine of seeds	146	0.375	0.540	2.044	0.153	0.055	0.815	-1841.019
B	Scopolamine of seeds	146	0.047	0.829	0.017	0.896	6.087	<b>0.014</b>	-1144.911
C	Proportion of infested fruits	229	0.517	0.472	1.815	0.178	2.618	0.106	2.764
D	Intensity of infestation	229	0.283	0.595	0.376	0.540	3.991	<b>0.046</b>	531.350
E	<i>Rostrum</i> length	97	0.054	0.815	1.783	0.182	0.122	0.727	1371.594

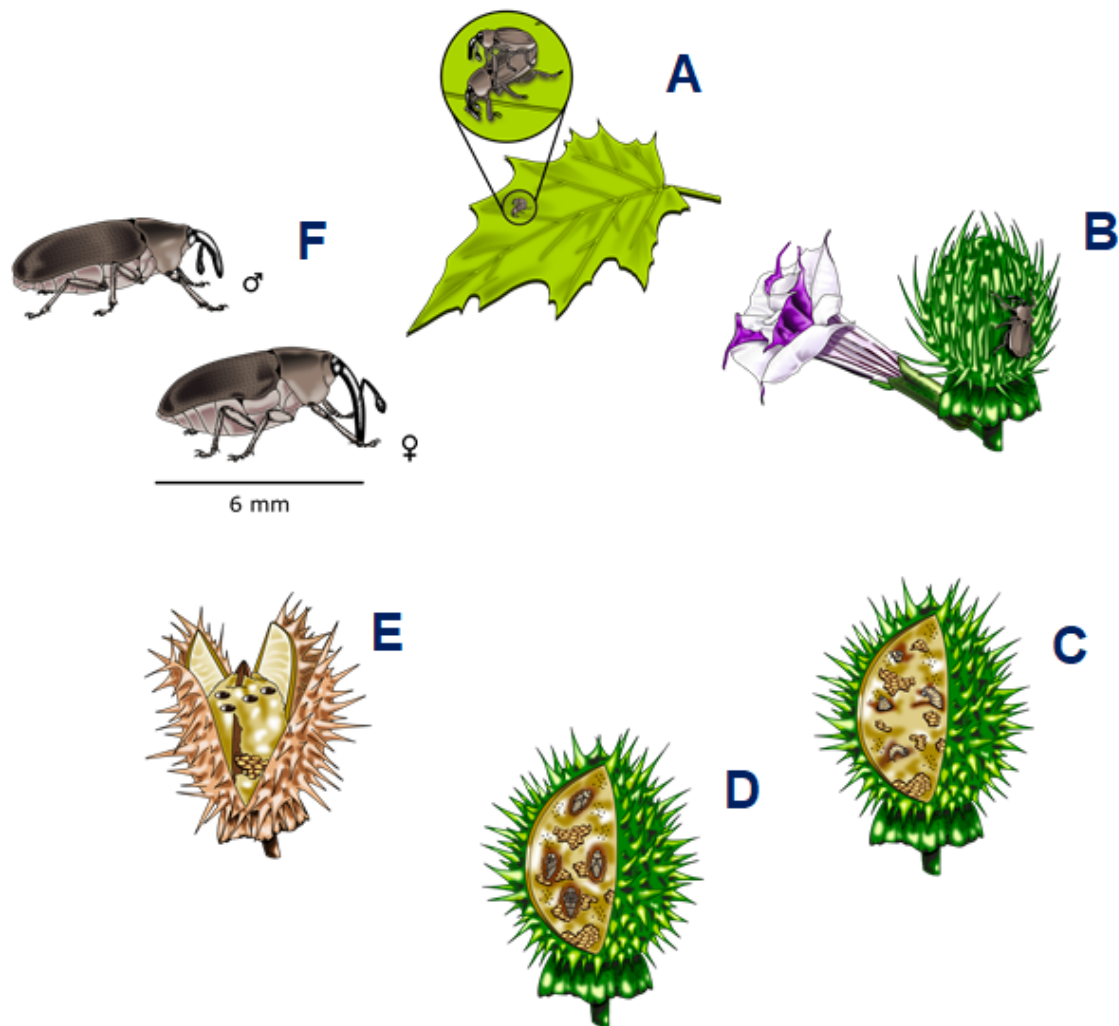
**Notes:**

\*AICc. The corrected Akaike information criterion measures the quality of a statistical model considering the sample size.

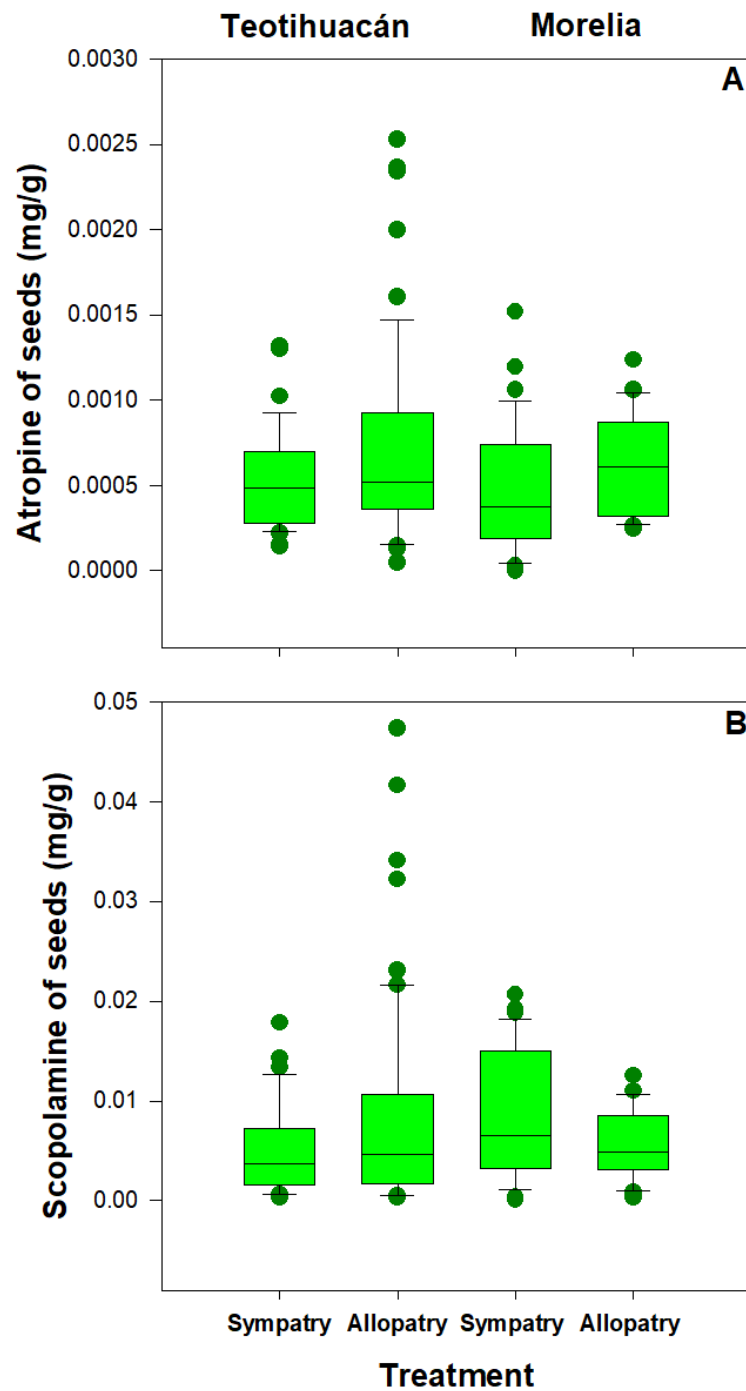
\*\*Home sites were Morelia and Teotihuacán, and treatments were sympatry and allopatry transplants of *Datura stramonium* in the experimental plots.

\*\*\*Almost all the models were adjusted to the Gamma distribution and log link function combination. In turn, the atropine of seeds was adjusted to Gauss inverse distribution and identity link function.

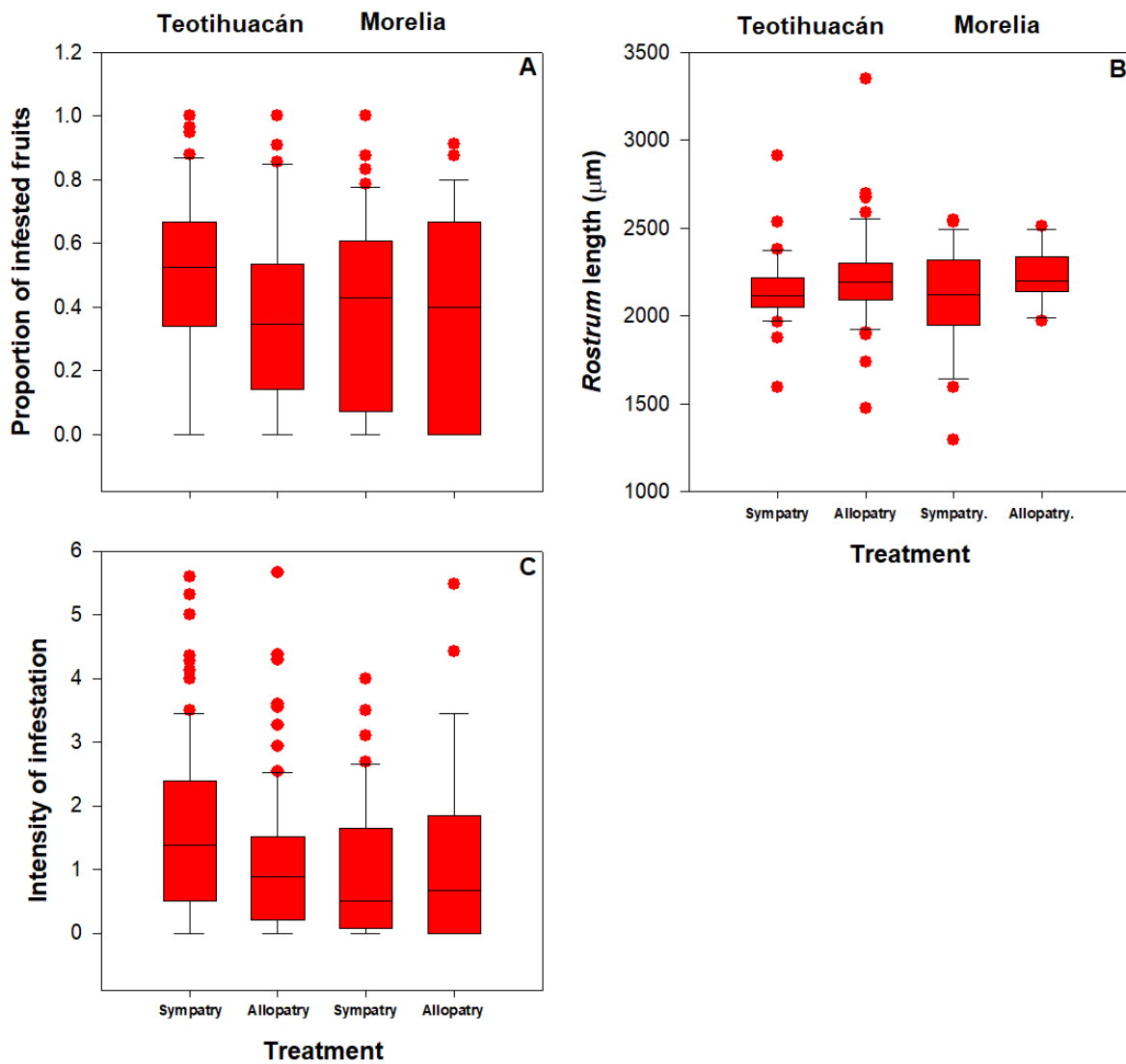
**Figure 1.** Life cycle of the seed predator *Trichobaris soror*. A) Courtship of *T. soror* adults on the leaves of *Datura stramonium*, B) Female oviposited in the developing fruit of *D. stramonium*, C) Larvae grew in the fruits, D) Pupae built tunnels (chambers) with their feces, E) A mature fruit opened and the adults of *T. soror* emerged, F) Female, G) Male of *T. soror*. Female individuals were larger than male adults.



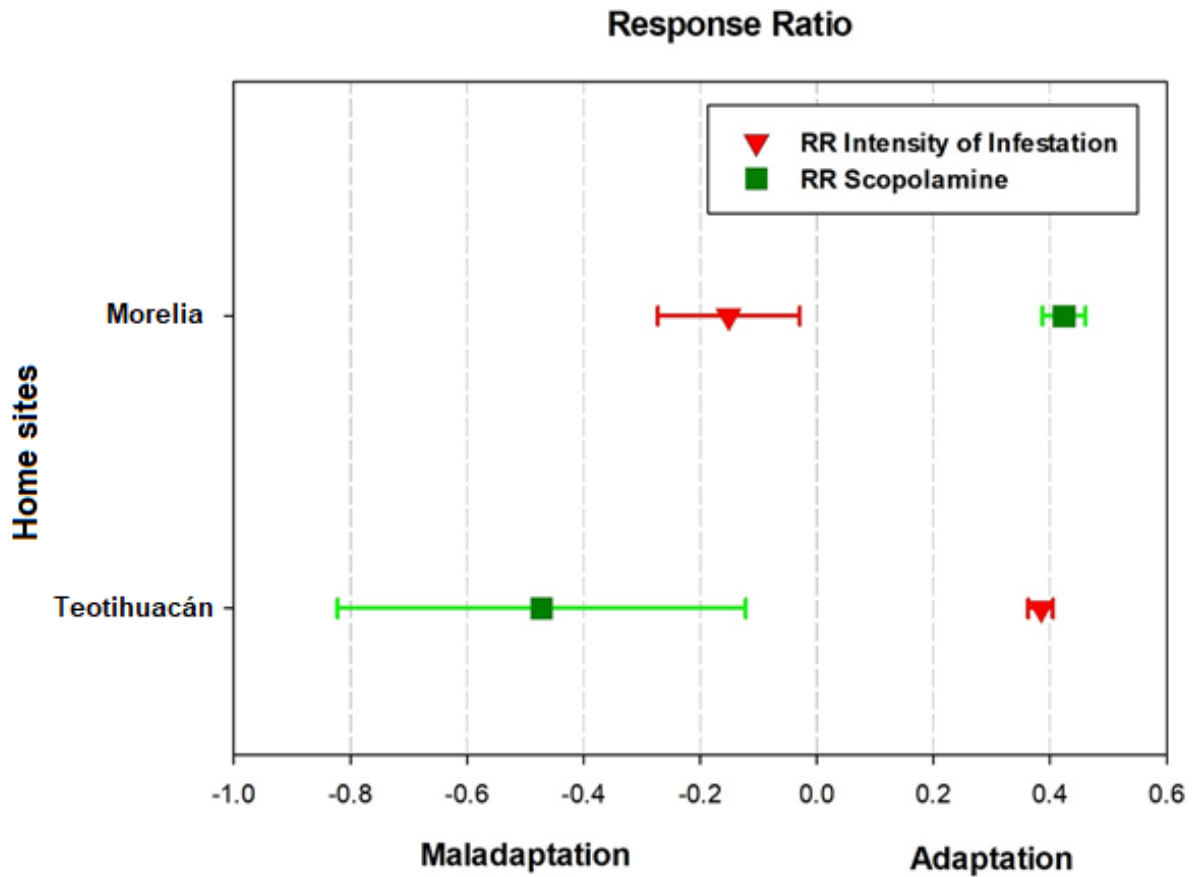
**Figure 2.** Local adaptation tests on the plant traits in the home sites Teotihuacán and Morelia. Box and whisker plots (the band inside the box is the median, outliers are plotted as individual points) represent the response variables: A) atropine of seeds, B) scopolamine of seeds. Treatments were sympatry and allopatry transplants of *Datura stramonium* in the experimental plots.



**Figure 3.** Local adaptation tests on the seed predator traits in the home sites Teotihuacán and Morelia. Box and whisker plots (the band inside the box is the median, outliers are plotted as individual points) represent the response variables: A) Proportion of infested fruits, B) *Rostrum* length of the weevils, C) Intensity of infestation. Treatments were sympatry and allopatry transplants of *Datura stramonium* in the experimental plots.



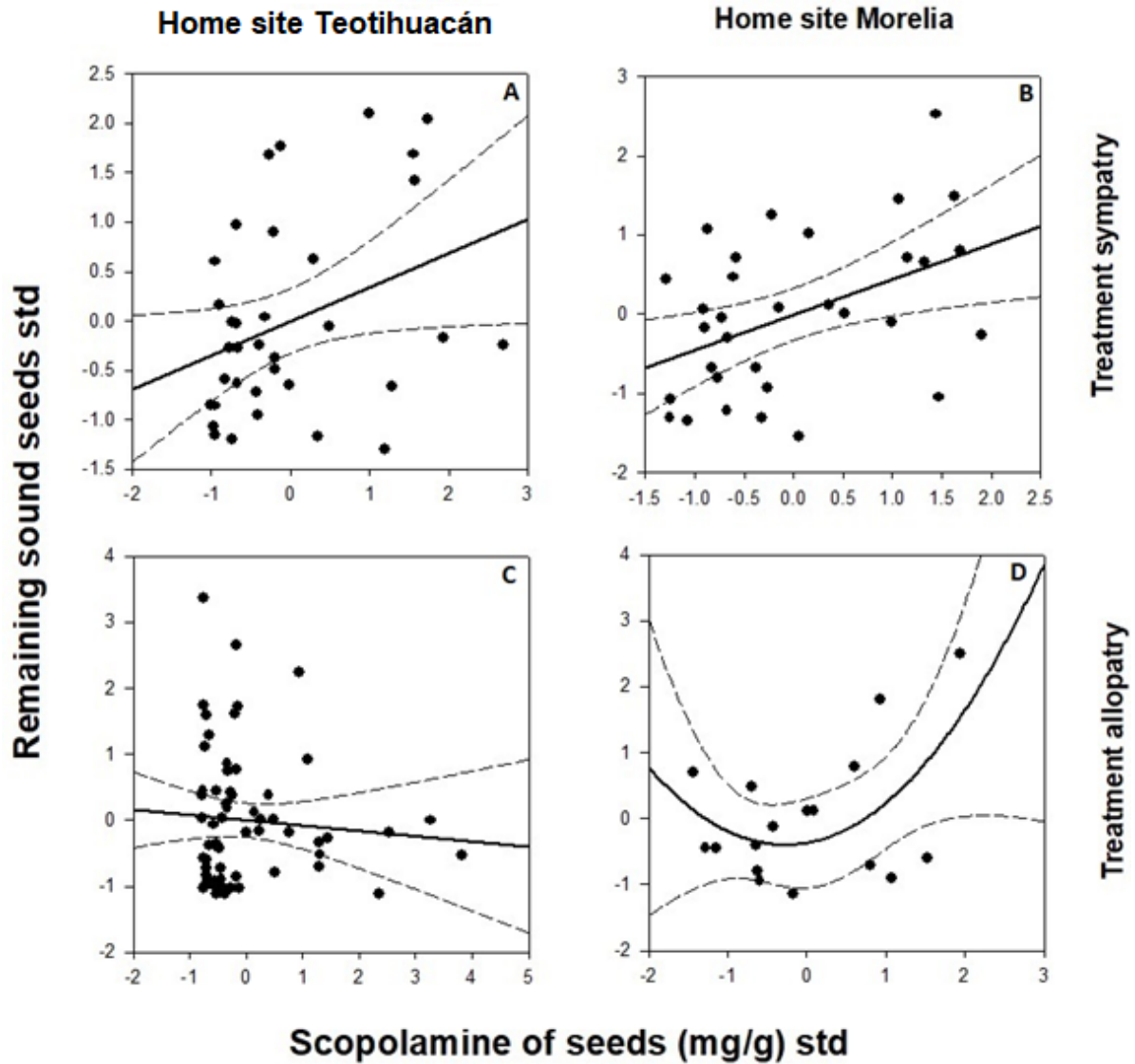
**Figure 4.** Forest plot represent the response ratio in the two home sites (Teotihuacán and Morelia) on the scopolamine of seeds of *D. stramonium* (green squares,  $\pm 1$  confidence interval of 95%) and the intensity of infestation of *T. soror* (red triangles,  $\pm 1$  confidence interval of 95%).



**Notes:**

\* Values at the right of zero meant local adaptation, in the left were maladaptation and on zero represented no adaptation.

**Figure 5.** Natural selection on scopolamine of seeds. Dotted lines indicate confidence interval at 95%. Home sites in the experiment were Morelia and Teotihuacán, treatments were sympatry and allopatry transplants of *Datura stramonium* in the experimental plots.



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## DISCUSIÓN GENERAL

De acuerdo con las hipótesis que se plantearon anteriormente, los resultados demuestran que el depredador de semillas *Trichobaris soror* ejerce presiones de selección sobre los alcaloides que funcionan como defensa química en *Datura stramonium*, ya que es un depredador que durante sus primeros estadios de vida se alimenta dentro de los frutos en desarrollo de *Datura*, es decir, es un insecto que tiene una dieta específica durante sus estadios como larva y posteriormente la cambia cuando es adulto (Mason *et al.*, 2019). La variación en la selección natural divergente puede analizarse mediante estudios sobre la variación geográfica en la selección natural y la adaptación local (Kawecki y Ebert, 2004; Laine, 2009; Blanquart *et al.*, 2013). El objetivo de esta tesis fue estudiar a los alcaloides como caracteres de defensa química en la planta huésped *Datura stramonium*, los cuales estuvieron sujetos a selección natural divergente en diferentes poblaciones debido a las presiones ejercidas por el depredador de semillas *Trichobaris soror*.

La historia evolutiva de los sistemas parásito-huésped puede dilucidarse mediante el estudio de los eventos de cladogénesis que ocurrieron en los dos linajes (Després *et al.*, 2007; Refrégier *et al.*, 2008; Gómez *et al.*, 2010) ya que las interacciones ecológicas suelen conservarse entre especies estrechamente relacionadas (Gómez *et al.*, 2010), aunque el resultado de estas sea que las especies especialistas posean menor diversidad genética que las generalistas (Laukkanen *et al.*, 2014). La coevolución mediante respuestas adaptativas recíprocas de especies que interactúan entre sí no requiere de la congruencia entre las filogenias de los insectos y sus huéspedes (Futuyma y McCafferty, 1990). Sin embargo, las plantas vasculares podrían ser la base de una “zona de adaptación”, la cual proporciona una gran diversidad de recursos en los que se especializarían los insectos (Winkler y Mitter, 2008) y esta se propagaría rápidamente en un linaje por medio del fenómeno conocido como radiación adaptativa (Schluter, 2000).

La comparación de la filogenia entre el género de gorgojos *Anthonomus* y el género de plantas *Hampea* en el centro y sur de México mostró poca congruencia, lo cual sugiere que los gorgojos se asociaron a estas plantas mediante colonizaciones y no evolucionaron en conjunto por eventos de cladogénesis (Jones, 2001). Por su parte, las especies plesiomórficas del género de gorgojos *Trichobaris* colonizaron plantas evolutivamente recientes del género *Solanum*, como *Solanum elaeagnifolium*, mientras que las especies más recientes del género *Trichobaris* parasitaron los frutos de las plantas plesiomórficas del género *Datura*. *Trichobaris soror* se

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asoció principalmente a *D. stramonium*, aunque también ha infestado a *Solanum rostratum* y *S. carolinense* (De-la-Mora *et al.*, 2018).

Aunque exista poca congruencia entre la filogenia del género *Trichobaris* con la de sus plantas huésped puede haber coevolución. En realidad la forma más común para estudiar la coevolución es mediante las interacciones ecológicas. Darwin fue el primero que reconoció las adaptaciones recíprocas entre diferentes especies (Darwin, 1862) ya que con su trabajo comprobó que entre las orquídeas y los insectos que las polinizan pueden existir adaptaciones recíprocas que favorecerían a las especies interactuantes. Por otra parte, entre las plantas y sus herbívoros pueden desarrollarse interacciones recíprocas mediadas por selección natural donde las plantas desarrollan resistencia contra los ataques de los insectos fitófagos (Züst *et al.*, 2012), incluso los grandes productores de cultivos han introducido resistencia artificial en las plantas mediante la ingeniería genética (Rausher, 2001).

En el primer capítulo se abordó la variación de la resistencia, la cual se define como la habilidad para evadir el ataque de los herbívoros (Rausher, 1996), estimada como la concentración de los alcaloides de *D. stramonium* (Sholne y Bergelson, 2000) y la intensidad de la infestación del depredador de semillas *T. soror*. Se encontró que la concentración de atropina y escopolamina de hojas en 31 poblaciones, más específicamente, en 14 poblaciones para los alcaloides en semillas y la intensidad de la infestación del depredador de semillas en 31 poblaciones del centro de México fue diferente en aquellas plantas donde hubo variación geográfica. La variación en los caracteres de defensa química de las plantas puede relacionarse con la capacidad de los insectos herbívoros para tolerar las defensas de las plantas debido a la especialización de su dieta (Barret y Heil, 2012).

En este mismo sentido, Berenbaum y Zangerl (1998), encontraron que los caracteres que median la interacción entre la polilla depredadora de semillas *Depressaria pastinacella* y la planta *Pastinaca sativa* originaron de procesos coevolutivos y presentaron variación geográfica. En cuatro poblaciones de Estados Unidos se encontraron coincidencias entre la producción de la defensa química (furanocumarinos) de *P. sativa* y la actividad metabólica del citocromo P 450 en la polilla *D. pastinacella*. En el sistema *D. pastinacella* y *P. sativa* las coincidencias entre los caracteres que median la interacción posiblemente presenten selección natural divergente entre poblaciones.

Posteriormente, se estudió si existió selección natural divergente en la defensa química de *D. stramonium* utilizando un análisis de los diferenciales de selección a nivel geográfico.



Se encontró que la variación en la concentración de escopolamina tiene una relación positiva con respecto a los diferenciales de selección de la escopolamina en 31 poblaciones del centro de México. Este resultado sugiere que el depredador de semillas dirige la selección divergente porque ejerce presiones sobre la concentración de la escopolamina en *D. stramonium*.

Aunque hubo otras características ambientales que pudieron influir en el resultado como la altitud, la aridez o la temperatura del ambiente, la señal más importante fue la depredación de semillas como fuerza de selección sobre este carácter de defensa. En un estudio sobre la planta *Erysimum mediohispanicum* se encontró que las visitas de los polinizadores en el sureste de España ejercen selección sobre la forma de la corola de las flores (Gómez *et al.*, 2008). Análisis posteriores mostraron que la corola de *E. mediohispanicum* puede dividirse en diferentes componentes principales y en este caso la relación entre los ensambles de polinizadores en las diferentes poblaciones estudiadas resultó en selección natural divergente sobre diferentes componentes principales y diferentes formas de corola (Gómez *et al.*, 2008).

Otro resultado relevante es que la concentración del alcaloide atropina está influida por la variación ambiental. Concretamente, la concentración de la atropina disminuye conforme aumenta la aridez ambiental. Este resultado también es consistente con lo que encontraron Baricevic y colaboradores (1999) donde la concentración de los alcaloides hiosciamina y escopolamina disminuyó cuando aumentaron las condiciones de aridez en el ambiente donde crece la belladona (*Atropa belladonna*). Los alcaloides (atropina y escopolamina) no presentaron variación en diferentes condiciones de luz, lo cual quiere decir que su producción no depende tanto del carbono, pero podría depender de otros compuestos como el nitrógeno (Hirano *et al.*, 2019).

En el caso de la defensa física y los tricomas como carácter defensivo de resistencia se sabe que no existe una disyuntiva o “trade-off” respecto a la capacidad de compensar el daño producido por la herbivoría mediante el crecimiento (tolerancia, Valverde *et al.*, 2003). Por otra parte, en el caso de la defensa química, las plantas que provienen de Teotihuacán son más grandes en comparación con las de Morelia (Castillo *et al.*, 2013), y a su vez las plantas de Teotihuacán producen menor concentración de alcaloides. Parecería que las plantas responden con la concentración de alcaloides debido a que entre más crecen se esperaría que sus defensas sean menores y viceversa (Herms y Matson, 1992). En el caso de *D. stramonium*, la cual es una planta de crecimiento rápido, los individuos se seleccionaron con el fin de que tuvieran una menor concentración de compuestos defensivos en comparación con las plantas de lento

crecimiento y por lo tanto presentarán una alta tasa de herbivoría (Coley *et al.*, 1985). La función de los alcaloides en *D. stramonium* es que sirvan como compuestos defensivos ya que presentan una mayor concentración cuando se les compara con *D. inoxia*, aunque esta especie contiene más tricomas lo cual compensa su capacidad de defenderse (Hirano *et al.*, 2019).

En la interacción entre *Curculio camelliae* y *Camellia japónica* en la isla Yakushima, Japón, el depredador de semillas ejerce selección natural divergente sobre la defensa de la planta, que en este caso, consiste en el grueso del pericarpio de los frutos de su planta huésped (Toju, 2009). El fruto de la camelia es una cápsula con un pericarpio grueso, dehiscente, de tres lóculos y con una semilla por cavidad (Okamoto, 1988). El grueso del pericarpio, además de experimentar presiones de selección ejercidas por el depredador de semillas es más delgado en las poblaciones con mayor altitud, es decir, la defensa de la planta huésped también presenta variación debido a la influencia ambiental (Toju, 2009).

Posteriormente, en la interacción depredador-presa entre las salamandras del género *Taricha* que desarrollaron la neurotoxina TTX como carácter de defensa, la cual puede ser letal, y las culebras del género *Thamnophis*, que desarrollaron resistencia a esta toxina mediante modificaciones en la estructura de sus canales de sodio y son capaces de ingerir salamandras adultas sin efectos adversos. La concentración de la neurotoxina TTX y la resistencia de las culebras, medida como la cantidad de TTX que es necesaria para bajar el rendimiento motor de las culebras en un 50%, varían positivamente entre poblaciones, aunque en algunas ocasiones las serpientes ganan la batalla en la carrera armamentista y son capaces de resistir la toxina que producen las salamandras (Hanifin *et al.*, 2008). Entonces, las salamandras que produjeron mayor concentración de TTX tuvieron mayores probabilidades de sobrevivir conforme aumentaron los ataques de las culebras del género *Thamnophis*. En el caso de *D. stramonium* se examinó si los caracteres de defensa se relacionan con la variación entre poblaciones de los agentes de selección. Como resultado se encontró una correlación negativa entre la concentración de escopolamina y las medidas de infestación de *T. soror*; es decir, el aumento en la concentración de escopolamina disminuye la infestación del depredador de semillas. Además, la concentración de escopolamina incrementó el número de semillas remanentes, el cual es una manera de medir el *fitness*.

En el segundo capítulo de la tesis se analizó si la selección en la defensa química de *D. stramonium* posee bases genéticas. Con el fin de probar que las normas de reacción en los caracteres de defensa de *D. stramonium* pueden mostrar adaptación local y bases genéticas, se eligieron dos localidades de *D. stramonium* con diferentes concentraciones de alcaloides.

Posteriormente, se realizó un experimento de “jardín común” donde se trasplantaron de manera recíproca las plantas y se permitió que los depredadores locales infestaran las parcelas experimentales. En dichas parcelas experimentales se encontró que la concentración de los alcaloides atropina y escopolamina no presentaron diferencias significativas entre sus familias genéticas, este resultado sugiere que las defensas químicas de *D. stramonium* cuentan con un componente genético. En el caso de la escopolamina en *D. stramonium* se sabe que la concentración posee un componente genético ya que es heredable y su variación fenotípica difiere de un patrón neutral cuando se compararon los índices  $F_{ST}$  y  $P_{ST}$  a lo largo de un gradiente de 13 poblaciones en el centro de México (Castillo *et al.*, 2015).

Los gorgojos depredadores de semillas de la especie *T. soror* que habitan en la localidad Teotihuacán están adaptados de manera local para infestar a las plantas de *D. stramonium* que son originarias de esta población y a su vez estas plantas están maladaptadas para resistir los ataques del depredador de semillas. En la localidad Teotihuacán hay menor concentración de escopolamina en las plantas locales en comparación con las plantas que provienen de Morelia, por lo tanto tienen menor defensa química y por esta razón los depredadores de semillas infestan más a las plantas locales. Como un ejemplo para explicar este proceso, se encontró que las poblaciones de escarabajos *Eucolaspis* sp. que están aisladas geográficamente difieren en sus respuestas olfativas de aquellas en las que se encontró adaptación local para alimentarse de las plantas en donde se originaron. Mediante experimentos de olfacción se demostró que los compuestos volátiles de las plantas tienen una función importante en la localización de plantas huéspedes por parte de *Eucolaspis*, pero la aceptación o rechazo de una planta huésped también puede implicar señales visuales (Doddala *et al.*, 2016).

En contraste, las plantas de *D. stramonium* cuyo lugar de origen es la localidad Morelia están adaptadas de manera local para resistir la infestación de los gorgojos de la especie *Trichobaris soror* que habitan en Morelia, mientras que este depredador de semillas no está adaptado para infestar a las plantas más tóxicas en esta localidad. En la parcela experimental de Morelia la infestación a las plantas locales es similar a la que experimentan las plantas foráneas, aunque las plantas de *D. stramonium* locales poseen mayor concentración de escopolamina que las plantas originarias de Teotihuacán. Este fenómeno se explicaría porque los individuos de *T. soror* al parecer no son capaces de distinguir las variaciones en la concentración del alcaloide escopolamina, el cual actúa como defensa química tanto en los trasplantes locales como foráneos en la localidad Morelia. Este patrón de infestación es consistente con el de los insectos

herbívoros que no pueden distinguir claramente a las plantas que poseen menor concentración de defensas químicas en parcelas experimentales donde la concentración natural de las defensas químicas es mucho mayor (Parachnowitsch, 2013; Vesakoski y Jormalainen, 2013). Además, esta conducta no descarta la existencia de adaptación local del depredador de semillas en la localidad Teotihuacán, aunque en la población Morelia no se aprecia claramente que haya un patrón de elección sobre la defensa química, pero esto sí puede visualizarse en los resultados de la prueba estadística Response Ratio. El estadístico Response Ratio se calcula mediante la fórmula  $\log_e(\bar{X}_s/\bar{X}_i)$ , donde el símbolo  $s$  se refiere al desempeño que presentan los individuos del tratamiento simpátrico y el símbolo  $i$  corresponde al desempeño de los individuos en los tratamientos alopátricos. La prueba Response Ratio se utiliza para eliminar el ruido que es ocasionado por la variación abiótica y permite reconocer claramente los caracteres de las partes interactuantes en los cuales se expresa la posible adaptación local (Parachnowitsch y Lajeunesse, 2012).

La adaptación local del depredador de semillas indica su capacidad de elección sobre los sitios de oviposición. Los gorgojos de *T. soror* tienen un patrón de oviposición donde la infestación es más intensa en las poblaciones con menor concentración de escopolamina. La selección para la escopolamina como defensa química, tiene el mismo patrón positivo en una población donde se encuentran los herbívoros generalistas de hojas (Castillo *et al.*, 2014). Este patrón positivo quiere decir que las defensas químicas en la población de Santo Domingo incrementaron el *fitness* de *Datura stramonium*. Además, la escopolamina en las plantas se relaciona de manera negativa con la infestación de los gorgojos del género *Trichobaris* (Miranda-Pérez *et al.*, 2016) e influye en sus patrones de oviposición (Cuda y Burke, 1991; Diezel *et al.*, 2011; Lee *et al.*, 2016).

Debido a que las plantas emiten ciertas señales químicas cuando sufren herbivoría en sus hojas (Wu y Baldwin, 2010), posiblemente las hembras de *T. soror* sean capaces de detectar la concentración de alcaloides cuando la planta es depredada y en consecuencia ovipositan en las plantas con menor nivel de defensa. Este patrón es consistente con la hipótesis de que los insectos herbívoros coevolucionan con los compuestos químicos secundarios de las plantas (Ehrlich y Raven, 1964). Además, este patrón de oviposición es congruente con la hipótesis de que las hembras de insectos fitófagos ovipositan en las “mejores plantas” (Jaenike, 1990; Soberón, 1986).

Como conclusión, este estudio constituye una pieza importante en la literatura disponible sobre la Ecología química y permitiría comprender las interacciones que existen entre *Datura stramonium* y su depredador de semillas *Trichobaris soror*. En el presente trabajo se realizó una fusión entre el estudio de la selección natural en poblaciones naturales y los experimentos de “jardín común” para probar el patrón de adaptación local. Nuestros resultados permiten mostrar y explicar los mecanismos sobre los cuales actúa la selección natural y las interacciones específicas subyacentes entre el depredador de semillas como un agente de selección y las defensas químicas del toloache. Consideramos que en el futuro sería importante realizar estudios adicionales que permitan responder las preguntas acerca de cómo se originaron estos patrones de adaptación local al nivel del genoma entre las especies interactuantes (Savolainen *et al.*, 2013).

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