UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE ECOLOGÍA
BIOLOGÍA EVOLUTIVA

Evolución de la defensa en el género *Datura*

TESIS

QUE PARA OPTAR POR EL GRADO DE:
DOCTORA EN CIENCIAS

PRESENTA:

M. en C. Eunice Kariñho Betancourt

TUTOR PRINCIPAL: Dr. Juan Núñez Farfán (Instituto de Ecología, UNAM)

COMITÉ TUTOR: Dr. Guillermo Delgado Lamas (Instituto de Química, UNAM)

Dr. Mark Earl Olson (Instituto de Biología, UNAM)

México, D. F. Agosto, 2015

UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE ECOLOGÍA
BIOLOGÍA EVOLUTIVA

Evolución de la defensa en el género *Datura*

TESIS

QUE PARA OBTENER EL GRADO DE:
DOCTORA EN CIENCIAS

PRESENTA:

M. en C. Eunice Kariñho Betancourt

TUTOR PRINCIPAL: Dr. Juan Núñez Farfán (Instituto de Ecología, UNAM)

COMITÉ TUTOR: Dr. Guillermo Delgado Lamas (Instituto de Química, UNAM)

Dr. Mark Earl Olson (Instituto de Biología, UNAM)

México, D. F. **Agosto, 2015**

UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
Presente

Me permito informar a usted, que el Subcomité de Biología Experimental y Biomedicina, en su sesión ordinaria del día 08 de junio de 2015 aprobó el jurado para la presentación de su examen para obtener el grado de **DOCTORA EN CIENCIAS**, del Posgrado en Ciencias Biológicas de la alumna **KARIÑHO BETANCOURT EUNICE** con número de cuenta **507007256** con la tesis titulada **"EVOLUCIÓN DE LA DEFENSA EN EL GÉNERO DATURA",** bajo la dirección del **DR. JUAN SERVANDO NÚÑEZ FARFAN:**

Presidente: DR. ALBERTO KEN OYAMA NAKAGAWA
Vocal: DRA. KARINA BOEGE PARÉ
Secretario: DR. GUILLERMO DELGADO LAMAS
Suplente: DR. PEDRO LUIS VALVERDE PADILLA
Suplente DR. MARK EARL OLSON

Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F. a 04 de agosto de 2015

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA

c.c.p. Expediente del (la) interesado (a).

Unidad de Posgrado • Coordinación del Posgrado en Ciencias Biológicas Edificio D, 1er. Piso, Circuito de Posgrados Cd. Universitaria
Delegación Coyoacán C.P. 04510 México, D.F. Tel. 5623 7002 http://pcbiol.posgrado.unam.mx

AGRADECIMIENTOS

Al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México (UNAM).

Al Consejo Nacional de Ciencia y Tecnología (CONACyT), y a la Dirección General de Estudios de Posgrado (DGEP) por las becas otorgadas a lo largo de cuatro años.

A los miembros del Comité Tutoral.

A mi tutor principal, el Dr. Juan Núñez Farfán, por haber conducido de manera excelente este tesis. Gracias Juan por tu gran apoyo, orientación, cariño y amistad.

Al Dr. Guillermo Delgado Lamas, por su paciencia, constante presencia y buena disposición a colaborar y aportar ideas que mejoraron y enriquecieron esta tesis.

Al Dr. Mark Olson Zunica, por su visión siempre clara y crítica, por sus valiosos comentarios y muchas clases que enriquecieron sustancialmente esta tesis y mi comprensión del estudio de la adaptación.

Al laboratorio de genética ecológica y evolución. Gracias a Vani, Pili, Mariana, LauLo, Lau(2), Rous, Marisol, Maried, Adán, Citlalli, Adriana C, Erika, Memo, Jesús, Dianita, Lulú, Adriana y Rafa, por la compañía, y por compartir ideas, pláticas, inquietudes, fiestas y muchos pasteles.

A Vani, Pili, Mariana y Laurita por la grata compañía en el cubi. Gracias por todas las reuniones, planes compartidos, por todas las carreras que corrimos, por el "comer comer" cuando se podía, por las risas y por su gran amistad.

Al Dr. Anurag Agrawal, por colaborar en el primer capítulo de la tesis y a todo el grupo de Ecología química e interacciones bióticas en Cornell por compartir ideas, en particular gracias a Jared Ali y a Sarah H.

A mi familia gracias por todo.

A mi mamá y hermana gracias por estar siempre presentes. Gracias por creer en mí e impulsarme en cada una de mis metas y sueños. Gracias por escucharme y permitirme escuchar. Gracias por vivir mis triunfos como propios y por permitirme vivir los suyos como míos. Gracias mamá por enseñarme fortaleza. Gracias hermana por enseñarme suavidad.

A César gracias por vivir la vida conmigo. Gracias por las risas, gracias por toda tu generosidad y por todas las lecciones.

A mi tía gracias por haberme enseñado que las nubes están hechas de gotas de agua.

ÍNDICE

RESUMEN

La interacción planta-herbívoro ha sido uno de los modelos más utilizados en el estudio de las interacciones bióticas y la evolución adaptativa. La teoría supone que, como resultado de la selección recíproca, plantas y herbívoros han evolucionado un conjunto de rasgos defensivos y contra-defensivos, respectivamente. Siendo este proceso (co)evolutivo el principal responsable de la diversificación de plantas y animales, y de la gran diversidad de atributos químicos (i.e. metabolitos secundarios) y físicos en los distintos linajes de plantas, observados en el presente. No obstante, identificar en qué medida los fenotipos de las plantas, son el resultado de la selección natural impuesta por sus consumidores, es uno de los grandes retos para la ecología evolutiva de la interacción planta-herbívoro. Tradicionalmente los estudios de la defensa de las plantas ya sea desde una perspectiva filogenética o poblacional, se han enfocado en un solo atributo defensivo, restringiendo además su análisis a un contexto selectivo particular. Sin embargo la evidencia empírica demuestra que la defensa de las plantas está compuesta por un complejo de atributos, cuyo valor adaptativo varía temporal y espacialmente. Desde una perspectiva experimental, este estudió combina tanto una aproximación filogenética (i.e. método comparativo) como una poblacional, para analizar la evolución adaptativa de la defensa de las plantas en contra de sus enemigos naturales, en el género *Datura*. En primer lugar, se examinó en doce especies de *Datura*, la variación intra e interespecífica en la expresión fenotípica de cinco tropano alcaloides (atropina, escopolamina, hiosciamina, solanina y tropina) y la densidad de tricomas foliares, así como la variación en la correlación entre estos caracteres en dos estados ontogenéticos (juvenil y reproductivo). Posteriormente, con el fin de determinar si el valor adaptativo/defensivo de alcaloides y tricomas de *Datura* varía en función de sus consumidores, se evaluó el desempeño de herbívoros con distinto grado de especialización y su relación con los atributos defensivos (alcaloides y tricomas) y nutricionales de *Datura* spp. Finalmente, se cuantificó a nivel intrapoblacional la magnitud y dirección de la selección natural actuando sobre los tricomas foliares, así como la correlación entre atributos de resistencia y la tolerancia al daño por herbívoros. Los resultados demuestran tres patrones generales: (1) la expresión y covariación (trade-offs y sinergismos) de los tropano alcaloides y tricomas está asociada con la ontogenia de la planta y con la variación en la comunidad de herbívoros y otros factores ambientales (aridez). (2) El valor adaptativo de las *defensas* de *Datura* varía en función del tipo y la *clase* (químico o físico) de atributo, así como del grado de especialización de los herbívoros. (3) La selección impuesta por los herbívoros actúa sobre la densidad de tricomas. Al mismo tiempo, este carácter de resistencia es evolutivamente independiente de la tolerancia, cuya correlación parece estar condicionada por la magnitud del herbivorismo (i.e. cantidad de daño foliar). Estos resultados sugieren que los tropano alcaloides y tricomas de *Datura* representan adaptaciones en respuesta a contextos selectivos complejos y cambiantes, funcionando en gran medida como defensas ante diversos herbívoros.

ABSTRACT

Plant-herbivore interaction has been one the most used models to study biotic interactions and adaptive evolution. Theory assumes that, as a result of reciprocal selection, plants and herbivores have evolved diverse defensive and counter-defensive traits. Hence, this (co)evolutionary process would be responsible for the diversification of plants and animals, and the great diversity of chemical (i.e. secondary metabolites) and physical plant attributes. However, understanding to what extent plant phenotypes are the result of natural selection imposed by consumers, it is one of the main challenges of the evolutionary ecology of plant-herbivore interactions. Traditionally studies of plant defense either from a phylogenetic or populational perspective, have focused on a specific defensive attribute, restricting their analyses to a particular selective context. Though, empirical evidence have shown that plant defense consists of a complex of attributes, whose adaptive value varies temporally and spatially. This study experimentally combines both a phylogenetic (i.e. comparative method) and a population approach to analyze the adaptive evolution of plant defense against natural enemies in the genus *Datura*. First, in 12 *Datura* species, I examined the phenotypic expression of alkaloids (atropine, scopolamine, hyoscyamine, solanine and tropina) and leaf trichome density, and the correlation among them, in two different ontogenetic stages (juvenile and reproductive). Then, in order to determine whether the adaptive value of alkaloids and trichomes of *Datura* varies according to consumers, I assessed the performance of herbivores with different degree of specialization and its relation to plant defenses (alkaloids and trichomes) and nutrients. Finally, in a natural population I quantified the magnitude and direction of natural selection acting on defensive traits and/or strategies (leaf trichomes density, total resistance and tolerance against herbivores), and the correlation among them. Results can be summarized as follows: 1) the expression and covariance (trade-offs and synergism) of tropane alkaloids and trichomes is associated to plant ontogeny, herbivore community, and other environmental factors (aridity). (2) The adaptive value of *Datura* defenses varies depending on the type and *class* of attribute, and the degree of herbivore specialization. (3) Selection imposed by herbivores acts on trichome density. At the same time, this resistance trait is evolutionary independent from tolerance, and its correlation with tolerance appears to be conditioned by the magnitude of herbivory (i.e. amount of leaf damage). Altogether suggest that tropane alkaloids and leaf trichomes of *Datura* represent adaptations in response to complex and changing selective contexts, largely function as defenses against different herbivores.

INTRODUCCIÓN GENERAL

Las plantas constituyen la fuente primaria de alimento y energía en el planeta. Organismos terrestres y acuáticos dependen de forma directa o indirecta de las plantas para sobrevivir. Existe una gran cantidad de animales que utilizan a las plantas como única fuente de alimento (i.e. herbívoros/fitófagos), dentro de los que se encuentran diversos vertebrados, nemátodos parásitos, y numerosos artrópodos. Desde la aparición de las plantas vasculares en el Devónico, hace aproximadamente 350-400 millones de años, y artrópodos, particularmente insectos, en el Carbonífero, hace aproximadamente 300 millones de años (Niklas, 1983), ambos grupos se han diversificado ampliamente en todo el planeta, presumiblemente como resultado de su interacción (Ehrlich y Raven, 1964; Strong et al., 1984; Nylin y Janz, 1999). Actualmente se estima que cerca del 50% de toda la biota está compuesta por insectos fitófagos (26%) y plantas verdes (22%) (Strong et al., 1984).

Modelo coevolutivo entre plantas y herbívoros: defensas y contra-defensas

La interacción antagonista entre las plantas y sus consumidores ha sido ampliamente documentada mediante el registro fósil (e.g., daño foliar en hojas de diversos taxa de plantas, dispersión de coprolitos, evidencia de partes bucales especializadas y contenido intestinal de los primeros ortópteros; Scott et al., 1992; Price, 2002), prácticamente desde la aparición de las primeras plantas vasculares y los artrópodos; e históricamente ha sido uno de los modelos más utilizados en el estudio de las interacciones bióticas y la (co)evolución adaptativa. Aunque ya a principios del siglo XX se sugería un proceso coevolutivo entre plantas y herbívoros (Brues, 1924), no fue sino hasta después de mediados del siglo que Ehrlich y Raven (1964) popularizaron el término estableciendo un marco conceptual para el estudio de la interacción planta-herbívoro. En función de datos ecológicos de la interacción entre plantas (hospederas) e insectos herbívoros (huéspedes), mediada por los metabolitos secundarios característicos de las plantas hospederas, los autores documentaron un patrón filogenético conservado (i.e., los herbívoros cercanamente emparentados se alimentan de plantas cercanamente emparentadas). Estas restricciones filogenéticas fueron ubicadas en un contexto teórico explícito, que después fue conocido como la hipótesis de “escape y radiación”. El modelo propuesto por Ehrlich y Raven (1964) predice la evolución de defensas por parte de las plantas hospederas y contra-defensas por parte de sus consumidores (huéspedes), en respuesta a las presiones de selección recíproca ejercidas por ambos interactuantes (Rausher, 1996; 2001).

La constancia en las interacciones bióticas entre diversos taxa de plantas y herbívoros, aparentemente mediada por la presencia de fitoquímicos (e. g., las larvas de *Danaus plexippus* se alimentan preferentemente de *Asclepias* spp. conocidas por tener cardenólidos; Malcolm y Brower, 1989; Agrawal, 2005; Agrawal y Konno, 2009), fue precisamente la observación que motivó el estudio de los metabolitos secundarios, no sólo desde una perspectiva fisiológica y bioquímica, sino además desde una ecológica y evolutiva. A pesar de la gran diversidad de fitoquímicos, por mucho tiempo se consideró que estas sustancias no nutritivas eran producto de desecho de las rutas metabólicas primarias de las plantas, al no tener una función metabólica “aparente”. Sin embargo, a mediados del siglo XX, trabajos como los realizados por el fisiólogo y entomólogo Vincent G. Dethier, el zoólogo Gottfried S. Fraenkel con su artículo *seminal* “The raison d'être of secondary plant substances”, y posteriormente (entre los 60´s y 90´s) por el botánico Jeffery Harborne, (e.g., Harborne, 1977), entre otros, demostraron que los “metabolitos secundarios” están relacionados con la aceptación de las plantas como alimento por los herbívoros (particularmente insectos), actuando como atrayentes o repelentes.

Históricamente, el rol ecológico de los metabolitos secundarios actuando como mediadores de la interacción planta-herbívoro (Apéndice A), ha tenido un papel preponderante en el desarrollo de hipótesis y modelos que han tratado de predecir los patrones de defensa de las plantas (e.g., Fenny, 1976; Rhoades y Cates, 1976; Rhoades, 1979). No obstante, a la par de los compuestos químicos, otros atributos *físicos/mecánicos* de las plantas como los tricomas foliares, o la dureza y plegabilidad de las hojas, también han sido integrados al escenario defensivo, dado su impacto negativo sobre diversos herbívoros documentado ampliamente en diversos taxa de plantas (e.g., Painter, 1958; van der Meijden et al., 1988; Karban y Baldwin, 1997, Valverde et al., 2001; Carmona et al., 2011).

Al mismo tiempo, diversas estrategias conductuales y rasgos morfológicos y fisiológicos de los herbívoros han dado cuenta de la capacidad de respuesta *contra-defensiva* de éstos a los compuestos químicos y atributos físicos de las plantas. Por ejemplo, *Pieris rapae*, un herbívoro especialista, adaptado bioquímicamente al sistema glucosinolato-mirosinasa característico de sus plantas hospederas (brassicáceas), después de la ingesta del tejido foliar, sintetiza una proteína en el intestino que evita la formación de isotiocianatos (compuestos con alta toxicidad) al reorientar la hidrólisis de glucosinolatos hacia la formación de nitrilo (Walters, 2011). Sin embargo, a pesar de la amplia evidencia experimental de las adaptaciones de los herbívoros (principalmente insectos) a sus plantas hospederas, documentada durante en las últimas décadas (e.g., Karban y Agrawal, 2002; Lankau, 2007; Rassman y Agrawal, 2011), no está claro cuándo ciertos niveles o *clases* de rasgos defensivos son más efectivos (Ali y Agrawal, 2012). Un factor clave en los modelos que predicen el desempeño y/o evolución de los herbívoros asociada a la defensa de las plantas ha sido su grado de especialización (i.e., su historia coevolutiva con las plantas). En este contexto, diversas hipótesis han predicho un efecto diferenciado e inclusive contrastante de las defensas, principalmente químicas, dependiendo de las estrategias alimenticias (polifagia *vs*. monofagia) de los herbívoros (e.g., Fenny, 1976).

Evolución correlativa de la defensa de las plantas

La concepción de que las defensas químicas y físicas son la única forma de respuesta evolutiva de las plantas a sus enemigos naturales, prevaleció cerca de 30 años después de la presentación del modelo coevolutivo de Ehrlich y Raven (1964). Dado que estos atributos, ya sea químicos o físicos, se caracterizan por reducir o evitar el ataque por parte de los enemigos naturales (Strauss y Agrawal, 1999), se definió a la *resistencia* como una estrategia de defensa. A finales de los años 80, se integró a la *tolerancia* como una forma de respuesta de las plantas ante la presión de sus consumidores (Fornoni, 2002). A diferencia de la resistencia, la tolerancia no evita el daño foliar, pero evita la reducción de la adecuación, mediante el despliegue de respuestas fisiológicas compensatorias como la activación de meristemos secundarios, o la reasignación de recursos a las partes de la planta que no han sido dañadas (Rosenthal y Kotanen, 1994; Stowe et al., 2000). Debido a que en principio se consideró que el beneficio de ambas estrategias podría resultar redundante, se propuso que ambas formas de defensa podrían funcionar como alternativas defensivas en los modelos de la defensa de las plantas que se desarrollaron a partir de los años 90 (Fineblum y Rausher, 1995; Abrahamson y Weis, 1997; Mauricio et al., 1997; Fornoni, 2002). Y esta visión, se extendió hacia las distintas *clases* de defensa (i.e. resistencia química *vs*. física), considerándose así, que cualquier par de caracteres defensivos podrían implicar una disyuntiva (trade-off univariado), dada la potencial redundancia funcional entre ambos y los costos asociados a su expresión simultánea (Cates y Orians 1975; Steward y Keeler, 1988). En consecuencia, la evolución de múltiples caracteres defensivos podría verse limitada por la asociación (correlación) negativa entre éstos.

Si bien las predicciones de la hipótesis del trade-off entre rasgos o estrategias defensivas han sido puestas a prueba de forma extensiva mediante estudios experimentales (e.g. Rehr et al., 1973; Steward y Keeler, 1988; Heil et al., 2002; Rudgers et al., 2004; Agrawal y Fishbein, 2006), éstos han provisto muy poca evidencia que respalde una asociación negativa entre los caracteres de defensa (Leimu y Koricheva, 2006). Existen al menos tres posibles explicaciones para la falta de trade-offs entre los caracteres de defensa de las plantas, observada en varios estudios: (1) la defensa de las plantas es raramente efectiva como un único carácter, y suele ser más efectiva cuando actúa de manera sinérgica (Agrawal y Fishbein, 2006). Por ello, no debe asumirse *a priori* que dos caracteres debieran correlacionarse negativamente, cuando interactúan. (2) Muchos de los caracteres tienen más de una función. Por ejemplo, además de la defensa, los tricomas foliares reflejan la luz UV y sirven de barrera contra la transpiración (Ehleringer et al., 1976). Por lo tanto, no debiera suponerse que dos caracteres involucrados en la defensa son redundantes, si se consideran diversas fuerzas selectivas bióticas y abióticas. (3) Probablemente muchos caracteres defensivos son necesarios para enfrentar un amplio rango de posibles atacantes (Coley et al., 1985; Agrawal y Fishbein, 2006; Walters, 2011).

Considerando estos argumentos, en contraste con la hipótesis del trade-off, la evolución simultánea de diferentes defensas podría ser un resultado posible (Fornoni, et al., 2004a, Núñez-Farfán et al., 2007), siempre que los beneficios de expresar múltiples caracteres sean más altos que sus costos (Ballhorn, et al., 2013 Carmona y Fornoni, 2013). De hecho, evidencia empírica ha demostrado que diversos rasgos defensivos usualmente actúan de forma complementaria (Gunasena et al. 1988, Berenbaum et al. 1991, Stapley, 1998). No obstante, la visión *univariada* de las relaciones entre caracteres ha dominado el estudio de la evolución (correlativa) de la defensa en las últimas décadas. Así, una gran cantidad de estudios ha documentado, ya sea una asociación positiva o negativa entre un sólo par de caracteres, sin considerar la asociación con otros rasgos relacionados (o no) con la defensa. Sin embargo, en los pocos estudios en los que las relaciones entre más de un par de rasgos putativos de defensa han sido analizadas, el patrón correlativo ha resultado más complejo. Por ejemplo, en un estudio filogenéticamente controlado, en el que se analizaron 35 especies de *Asclepias*, se documentaron asociaciones contrastantes entre tres compuestos fenólicos relacionados con la defensa. Se detectó una correlación negativa entre ácido caféico y flavonoides, mientras que, al mismo tiempo, se detectó una correlación positiva entre cardenólidos y flavonoides (Agrawal et al., 2009).

Aún cuando las especies de plantas puedan aparentemente defenderse a través de una única estrategia o clase de defensa (e.g., química) existen muchas formas específicas de dichas defensas (Berenbaum et al., 1986; Bennett y Wallsgrove, 1994; Becerra, 1997). Por lo tanto, es más útil considerar a la defensa de las plantas como un "paquete" o *suite* de caracteres coadaptados (*sensu* Agrawal y Fishbein, 2006) que puede incluir la calidad nutricional (e.g., proteínas), rasgos físicos (e.g., dureza de la hoja y tricomas), compuestos tóxicos (e.g., cianidas y alcaloides), fenología, capacidad de regeneración (i.e., tolerancia) y defensas indirectas (e.g., volátiles y arquitectura de la ramificación), cuyo valor adaptativo puede modificarse temporal y espacialmente. Además, estudios recientes indican que no sólo la expresión de los caracteres individuales de defensa cambia temporalmente (i.e., a través de la ontogenia), sino también la covariación entre ellos (Hjelm et al., 2000; Boege et al., 2007), sugiriendo que la evolución de la defensa podría ser mejor comprendida considerando la trayectoria ontogenética de los caracteres y no su expresión en un único estado ontogenético.

En este sentido, el reto en el estudio de la evolución correlativa de la defensa no reside solamente en la detección de asociaciones significativas entre rasgos, sino en determinar cuándo estas asociaciones son el resultado de la selección ejercida por los herbívoros u otros factores ambientales (i.e., la relación es adaptativa), descartando aquellas asociaciones que son simplemente resultado de la estocasticidad, o de restricciones fisiológicas. Para esto, es necesario examinar si ciertos factores abióticos, o comunidades de herbívoros en particular, están asociados con las estrategias o *clases* defensa de las plantas, y si su variación temporal (i.e., trayectoria ontogenética) o espacial, afecta el valor adaptativo de los rasgos asociados a la defensa.

Perspectivas del estudio de la evolución adaptativa

La evolución adaptativa puede ser estudiada mediante tres aproximaciones diferentes: (1) la poblacional (e.g. Matos et al., 2004), la filogenética (Felsenstein, 1985; Rezende y Garland, 2003), y la que parte de modelos de optimalidad (Parker y Smith, 1990). Mientras que los estudios poblacionales (intraespecíficos) permiten analizar los procesos evolutivos actuales y medir directamente la magnitud y dirección de la selección natural, los estudios filogenéticos (interespecíficos), permiten, mediante la identificación de patrones que emergen a nivel supra-específico (e.g., los clados), inferir los procesos evolutivos históricos responsables de la variación observada en el presente. Si bien ambas aproximaciones son complementarias (Grandcolas y D´Haese, 2003), la mayoría de los estudios han puesto a prueba las predicciones de la teoría clásica de la defensa, en poblacionales o en clados, pero raramente son combinadas ambas perspectivas.

A nivel poblacional, un gran número de estudios ecológicos han examinado la defensa de las plantas y demostrado el efecto de la selección natural en caracteres defensivos (e.g., Mauricio y Rausher, 1997; Shonle y Bergelson, 2000) y cómo ésta es afectada por el ambiente biótico y abiótico. Al mismo tiempo, estudios intraespecíficos en poblaciones naturales o bajo condiciones controladas han puesto a prueba el valor adaptativo de las defensas de las plantas, a través de la respuesta de los herbívoros (e.g., desempeño o sobrevivencia) ante éstas (e.g., Céspedes et al., 2000; Gassmann y Hare, 2005; Gassmann et al., 2006). En contraste, muy pocos estudios comparativos han examinado la defensa de las plantas; probablemente esta escasez se debe a limitaciones metodológicas, como el no disponer de filogenias moleculares o acceso limitado al material biológico de diversos taxa. Sin embargo, los trabajos supra-específicos han sido clave para identificar patrones comunes de las interacciones entre taxa de plantas y herbívoros (e.g., Becerra, 1997). Mediante análisis comparativos, se ha podido identificar contextos selectivos asociados a ciertas clases de defensa (i.e., evolución convergente de la defensa). Los estudios interespecíficos además, han evaluado en qué medida los herbívoros influyen en la evolución de las plantas (e.g. escalamiento de la defensa química; Apéndice B) y de manera recíproca, han permitido vislumbrar la medida en que las plantas (a través de sus defensas) han influido en sus consumidores (e.g., evolución de estrategias alimenticias de insectos fitófagos: monofagia y polifagia; Schoonhoven et al., 2005; Roslin y Salminen, 2008).

Una de las principales ventajas del enfoque comparativo para estudiar la defensa de las plantas es que revelan los mecanismos comunes de la interacción entre especies, lo que sería de otra forma imposible utilizando un sólo organismo modelo. Un triunfo de este enfoque ha sido, por ejemplo, la identificación de ácido jasmónico como regulador hormonal que activa una gama de respuestas inducidas en las plantas, tales como la producción de las enzimas oxidativas, glucosinolatos, alcaloides, tricomas, volátiles y néctar extrafloral (Agrawal, 2007). Aún no está claro sin embargo, si esta similitud constituye un conservadurismo filogenético o evolución convergente (i.e., respuesta adaptativa a ambientes selectivos posiblemente similares).

La combinación de estudios comparativos con poblacionales, permite entender mejor el funcionamiento de muchos atributos de las plantas, e interpretar de manera más acertada su rol adaptativo (de existir), y relevancia en los procesos de diversificación. En el presente estudio se combina tanto una aproximación filogenética (i.e., método comparativo) y una poblacional al analizar la evolución adaptativa de la defensa de las plantas en contra de sus enemigos naturales. Se plantean tres preguntas de investigación:

(1) ¿Es la expresión y covariación de los atributos asociados a la defensa de las plantas (i.e. alcaloides y tricomas foliares) adaptativa?
(2) ¿difiere el patrón evolutivo y/o ecológico entre *clases* de defensa (i.e., química vs. física)?

(3) ¿Son las dinámicas evolutivas inferidas a partir del enfoque comparativo similares a las que revelan los estudios temporales en las poblaciones?

Esta tesis está estructurada en tres capítulos, donde se analizan experimentalmente los patrones filogénicos y la variación intrapoblacional en la expresión fenotípica de rasgos químicos y mecánicos asociados a la defensa de las plantas, y su impacto en distintos consumidores. El modelo de estudio lo constituyen las especies del género *Datura* y sus herbívoros (Figura 1, 2).

El género *Datura* constituye un excelente modelo para el estudio de la evolución adaptativa de la defensa por (1) la gran variedad de herbívoros especialistas y generalistas (Núñez-Farfán y Dirzo, 1994; Hare y Elle, 2002; Hare et al., 2003), que ejercen distintas presiones de selección en poblaciones naturales (Shonle y Bergelson, 2000; Carmona y Fornoni, 2013).

Figura 1. *Datura* sp.

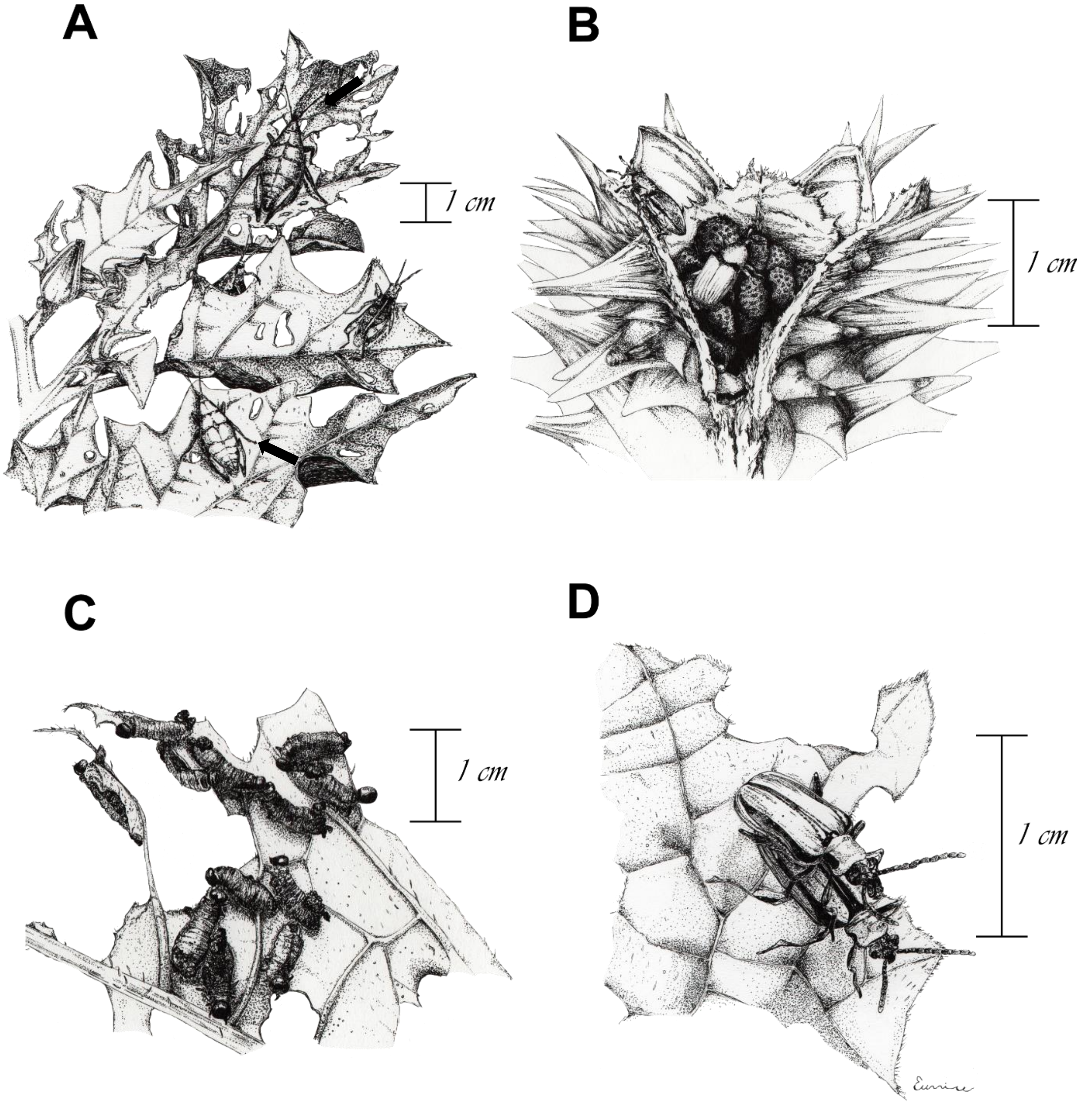


Figura 2. Herbívoros de las especies de *Datura*. A) Folívoro generalista *Sphenarium purpurascens* (Orthoptera: Phyrgomorphidae). B) Frugívoro especialista *Trichobaris soror* (Coleoptera: Curculionidae). C) Folívoro especialista *Lema trilineata daturaphila* (Coleoptera: Chrysomilidae) larva y D) adulto.

(2) La amplia gama de rasgos químicos (i.e. tropano alcaloides) y mecánicos (e.g., tricomas foliares) asociados con la defensa y su enorme variación fenotípica (Castillo et al., 2014). En particular, la hiosciamina y la escopolamina han sido fuertemente implicados como caracteres de resistencia en las *daturas* contra los herbívoros (Hsiao y Fraenkel, 1968; Wink, 1993; Shonle, 1999). Cada uno de estos rasgos ha demostrado que afecta cuantitativamente la adecuación de sus consumidores (Shonle y Bergelson, 2000), con un claro efecto tóxico (Colegate et al., 1979; Roddick, 1991; Alexander et al., 2008). Aparentemente, la resistencia proporcionada por estos compuestos químicos se produce a pesar de que muchos de los herbívoros de las *daturas* son especialistas y tienen contra-adaptaciones para “desarmar” estas defensas. No obstante, en una especie del género (*Datura stramonium*), se ha demostrado que algunos alcaloides tienen un efecto diferenciado dependiendo del grado de especialización de sus consumidores (la escopolamina actúa como fagoestimulante para el folívoro especialista *Epitrix* sp.; Shonle y Bergelson, 2000). Sin embargo, previo a este estudio, la generalidad de estas observaciones documentadas a nivel poblacional, permanecía inexplorada en el clado *Datura*. Además, como muchos de los estudios de la defensa, los realizados previamente en algunas especies de *Datura*, únicamente han estimado la variación en un sólo estado ontogenético, y muy pocos estudios empíricos en el género han incorporado al análisis, de forma simultánea, caracteres químicos y mecánicos de defensa (pero ver Castillo et al., 2014), u otros caracteres de las plantas indirectamente asociados con la defensa (e.g., nutrientes).

En el primer capítulo de esta tesis se presenta un análisis con control filogenético de la variación de múltiples rasgos químicos y mecánicos asociados a la defensa de *Datura* en contra de sus enemigos naturales. Mediante la combinación del método comparativo y análisis estadísticos paramétricos, se examina en 12 especies de *Datura* (1) la variación intra e interespecífica de cinco tropano alcaloides (atropina, escopolamina, hiosciamina, solanina y tropina) y la densidad de tricomas, en dos estados ontogenéticos diferentes (i.e. etapa juvenil *vs.* reproductiva), (2) su evolución correlativa (i.e. trade-offs o sinergismos) dentro del clado, y (3) la variación de esta correlación entre los rasgos de defensa a través de la ontogenia.

En el capítulo II se busca determinar, en primer lugar, si existen diferencias en el desempeño de dos herbívoros que difieren en su grado de especialización en *Datura*. Segundo, si el desempeño de los herbívoros, está asociado a la variación de los atributos mecánicos y químicos de las plantas, i.e., si los alcaloides y tricomas foliares, tienen un valor adaptativo (defensivo). Además, se analizó si dicho desempeño está asociado al contenido de los macronutrientes de las plantas. Mediante bioensayos y análisis comparativos y de estadística paramétrica, se contrasta (i) la expresión fenotípica de los atributos químicos y físicos de *Datura* spp., documentada en el capítulo anterior, y (ii) su calidad nutricional (i.e., contenido de nitrógeno , fósforo, carbono y agua), con el desempeño (i.e., peso ganado, alimento consumido, cantidad de hojas con daño foliar y eficiencia de crecimiento) del herbívoro especialista, el escarabajo de la papa de tres líneas *Lema trilineata daturaphila* (Coleóptera: Chrysomelidae), y el herbívoro generalista, el gusano cogollero del maíz, *Spodoptera frugiperda* (Lepidoptera: Noctuidae).

Finalmente en el capítulo III, se presenta un estudio de genética cuantitativa, para medir en una población natural de *Datura stramonium*, ubicada en el centro de México (Ticumán, Mor.), la magnitud y dirección de la selección natural (ejercida por los herbívoros) actuando sobre rasgos de defensa heredables. Además, se examina la evolución correlativa de las estrategias defensivas (i.e., tolerancia y resistencia) y de un rasgo específico de resistencia (tricomas foliares).

LITERATURA CITADA

Abrahamson WG, Weis AE. 1997. *Evolutionary ecology across three trophic levels: goldenrods, gall-makers and natural enemies*. Princeton: Princeton University Press.

Agrawal AA. 2005. Natural selection on common milkweed (*Asclepias syriaca*) by specialized insect herbivores. *Evolutionary Ecology Research* 6: 651-667.

Agrawal AA. 2007. Macroevolution of plant defense strategies *Trends in Ecology and Evolution* 22: 103-109.

Agrawal AA, Alminen JP, Fishbein M. 2009. Phylogenetic trends in phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation. *Evolution* 63: 663-373.

Agrawal AA, Fishbein M. 2006. Plant defense syndromes. *Ecology* 87: S132-S149.

Agrawal AA, Konno K. 2009 Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annual Review of Ecology, Evolution and Systematics* 40: 311-331.

Alexander J, Benford D, Cockburn A, Cravedi J, Dogliotti E, Di Domenico A, Fernandez-Cruz ML. 2008. Tropane alkaloids (from *Datura* spp.) as undesirable substances in animal feed. *European Food Safety Authority Journal* 691: 1-55.

Ali JG, Agrawal AA. 2012. Specialist versus generalist insects herbivores and plant defense. *Trends in Plant Science* 17. 293-302.

Ballhorn DJ, Godschalx AL, Kautz S. 2013. Co-variation of chemical and mechanical defenses in lima bean (*Phaseolus lunatus* L.). *Journal of Chemical Ecology* 39: 413-417.

Becerra JX. 1997. Insects on plants: macroevolutionary chemical trends in host use. *Science*276: 253-256.

Bennett RN, Wallsgrove RM. 1994. Secondary metabolites in plant defence mechanisms. *New Phytologist* 127: 617-633.

Berenbaum MR, Zangerl AR, Nitao JK. 1986. Constrains on chemical coevolution: Wild parsnip and the parsnip webworm. *Evolution* 40: 1215-1228.

Berenbaum MR, Nitao JK, Zangerl AR. 1991. Adaptive significance of furanocoumarin diversity in *Pastinaca sativa* (Apiaceae). *Journal of Chemical Ecology* 17: 207-215.

Boege K, Dirzo R, Siemens D, Brown P. 2007. Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? *Ecology Letters* 10: 177-187.

Brues CT. 1924. The specificity of food-plants in the evolution of phytophagous insects. *American Naturalist* 127-144.

Carmona D, Fornoni J. 2013. Herbivores can select for mixed defensive strategies in plants. *New Phytologist* 197: 576-585.

Carmona D, Lajeunesse MJ, Johnson MT. 2011. Plant traits that predict resistance to herbivores *Functional Ecology* 25: 358-367.

Castillo G, Cruz LL, Tapia- López R, Olmedo-Vicente E, Carmona D, Anaya-Lang AL, Fornoni J, Andraca-Gómez G, Valverde PL, Núñez-Farfán J. 2014. Selection mosaic exerted by specialist and generalist herbivores on chemical and physical defense of *Datura stramonium*. *PloS one* 9: e102478.

Cates RG, Orians GH. 1975. Sucessional status and the palatability of plants to generalized herbivores. *Ecology* 56: 410-418.

Céspedes CL, Calderón JS, Lina L, Aranda E. 2000. Growth inhibitory effects on fall armyworm *Spodoptera frugiperda* of some limonoids isolated from *Cedrela* spp. (Meliaceae). *Journal of Agricultural and Food Chemistry* 48: 1903 - 1908.

Colegate SM, Dorling PR, Huxtable CR. 1979. A spectroscopic investigation of Swainsonine: an α-12 mannosidase inhibitor isolated from *Swainsona canescens*. *Journal of Chemistry* 32: 2257- 2264.

Coley PD, Bryant JP, Chapin III FS. 1985. Resource availability and plant antiherbivore defense. *Science* 230: 895-899.

Ehleringer JR, Björkman O, Mooney HA. 1976. Leaf pubescence: effects on absorbance and photosynthesis in a desert shrub. *Science*192: 376-377

Ehrilch PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586-608.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist*125: 1-15.

Fenny P. 1976. Plant apparency and chemical defense. En Wallace JW, Mansel RL, eds. *Biochemical interaction between plants and insects*. US Springer, 1-40.

Fineblum WL, Rausher MD. 1995. Trade-off between resistance and tolerance to herbivore damage in a morning glory. *Nature* 377: 517-520.

Fornoni J. 2002. Evolución de las defensas en plantas contra enemigos naturales. Tesis Doctoral, Universidad Nacional Autónoma de México (UNAM), México.

Fornoni J, Núñez-Farfán J, Valverde PL, Rausher MD. 2004a. Evolution of mixed strategies of plant defense allocation against natural enemies. *Evolution* 58: 1685-1695.

Gassmann AJ, Hare JD. 2005. Indirect cost of a defensive trait: Variation in trichome type affects the natural enemies of herbivorous insects on *Datura wrightii*. *Oecologia* 144: 62-71.

Gassmann AJ, Levy A, Tran T, Futuyma DJ. 2006. Adaptations of an insect to a novel host plant: a phylogenetic approach. *Functional Ecology* 20: 478-485.

Grandcolas P, D´Haese C. 2003. Testing adaptation with phylogeny: How to account for phylogenetic pattern and selective value together. *Zoological Scripta* 32: 483-490.

Gunasena, GH, Vinson SB, Williams HJ, Stipanovic RD. 1988. Effects of caryophyllene, caryophyllene oxide, and their interaction with gossypol on the growth and development of *Heliothis virescens* (F.)(Lepidoptera: Noctuidae). *Journal of Economic Entomology* 81: 93-97.

Harborne JB. 1988. Introduction to Biochemical Ecology. 3th ed London: Academic Press.

Hare JD, Elle E. 2001. Geographic variation in the frequencies of trichome phenotypes of *Datura wrightii* and correlation with annual water deficit. Madroño 48: 33-37.

Hare JD, Elle E, Dam NM. 2003. Costs of glandular trichomes in *Datura wrightii*: a three-year study. *Evolution* 57: 793-805.

Heil M, Delsinne T, Hilpert A, Schürkens S, Andary C, Linsenmair KE, Sousa MS, McKey D. 2002. Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos* 99: 457-468.

Hjelm J, Person L, Christensen B. 2000. Growth, morphological variation and ontogenetic niche shifts in perch (*Perca fluviatilis*) in relation to resource availability. *Oecologia* 122: 190-199.

Hsiao TH, Fraenkel G. 1968. The role of secondary plant substances in the food specificity of the Colorado potato beetle. *Annals of the Entomological Society of America* 61: 458-503.

Karban R, Baldwin IT. 1997. *Induced Responses to Hersponse bivory.* Chicago: Chicago University Press.

Lankau RA. 2007. Specialist and generalist herbivores exert opposing selection on a chemical defense. *New Phytologist* 175: 176-184.

Leimu R, Koricheva J. 2006. Meta-analysis of trade-offs between plant tolerance and resistance to herbivores: Combining the evidence from ecological and agricultural studies. *Oikos* 112: 1-9.

Malcolm SB, Brower LP. 1989. Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. *Experientia* 45: 284-295.

Matos M, Simõtes P, Duarte A, Rego C, Avelar T, Rose MR. 2004. Convergence to a novel environment: comparative method versus experimental evolution. *Evolution* 58: 1503-1510.

Mauricio R, Rausher MD, Burdick DS. 1997. Variation in the defense strategies of plants: Are resistance and tolerance mutually exclusive? *Ecology* 78: 1301-1311.

Niklas KJ. 1983. The influence of Paleozoic ovule and cupule morphologies on wind pollination. *Evolution* 37: 968-986.

Núñez-Farfán J, Dirzo R. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: Natural selection for resistance to herbivorous insects. *Evolution* 48: 423-436.

Núñez-Farfán J, Fornoni J, Valverde PL. 2007. The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution and Systematics* 38:541-566.

Nylin S, Janz N. 1999. The ecology and evolution of host plant range: Butterflies as model group. En Olff H, Brown VK, Drent RH, eds. *Hervibores. Between plants and predators.* Oxford: Blackwell Scientific Publications, 31-54 pp.

Painter RH. 1958. Resistance of plants to insects. *Annual Review of Entomology* 3: 267-290.

Parker GA, Smith JM. 1990. Optimality theory in evolutionary biology. *Nature* 348: 27-33.

Price PW. 2002. Species interactions and the evolution of biodiversity. En Herrera CM, Pellmyr O, eds. *Plant-animal interactions: An evolutionary approach*. Oxford: Blackwell Scientific Publicatons, 3-25.

Rasmann S, Agrawal AA.2011. Evolution of specialization: a phylogenetic study of host range in the red milkweed beetle (*Tetraopes tetraophtalmus*). *American Naturalist* 6: 728-737.

Rausher MD. 1996. Genetic analysis of coevolution between plants and their natural enemies. *Trends in Genetics* 12: 212-217.

Rausher MD. 2001. Co-evolution and plant resistance to natural enemies. *Nature* 411: 857-864.

Rehr SS, Feeny PP, Janzen DH. 1973. Chemical defenses in Central American non-ant acacias. *Journal of Animal Ecology* 42: 405-416

Rezende EL, Garland T Jr. 2003. Comparaciones interespecíficas y métodos estadísticos filogenéticos. En Borzinovic ed. *Fisiología Ecológica and Evolutiva. Teoría y casos de estudio en animales*. Santiago: Ediciones Universidad Católica de Chile, 79-98

Rhoades DF. 1979. Evolution of plant chemical defense against herbivores. En: Rosenthal GA, Berenbaum MR, eds. *Herbivores: Their interaction with secondary plant metabolites*. New York: Academic Press, 3-54.

Rhoades DF, Cates RG. 1976. Toward a general theory of plant antiherbivore chemistry. En: Wallace JW, Mansel RL, eds. *Biochemical interaction between plants and insects*. US Springer, 168-213.

Roddick J. 1991. The importance of the Solanaceae in medicine and drug therapy. En: Hawkes JG, Lester RN, Nee M, Estrada RN, eds. *Solanaceae III: taxonomy, chemistry, evolution*. London: The Royal Botanic Gardens, Kew, 7-23.

Rosenthal JP, Kotanen PM. 1994. Terrestrial plant tolerance to herbivory. *Trends in Ecology and Evolution* 9: 145-148.

Roslin T, Salminen JP. 2008. Specialization pays off: contrasting effects of two types of tannins on oak specialist and generalist moth species *Oikos* 117: 1560-1568.

Rudgers JA, Strauss SY, Wendel JF. 2004. Trade-offs among anti-herbivore resistance traits: Insights from Gossypieae (Malvaceae). *American Journal of Botany* 91: 871-880.

Schoonhoven LM, Van Loon JJ, Dicke M. 2005. *Insect-plant biology*. 2nd ed Oxford: Oxford University Press.

Scott AC, Stephenson J, Cahloner W. 1992. Interaction and coevolution of plants and arthropods during the Palaeozoic and Mesozoic. *Philosophical Transactions of the Royal Society B: Biological Sciences* 335: 129-165.

Shonle I. 1999. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). PhD Thesis, University of Chicago, US, Chicago.

Shonle I, Bergelson J. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae) *Evolution* 54: 778-788.

Stapley L. 1998. The interaction of thorns and symbiotic ants as an effective defence mechanism of swollen-thorn acacias. Oecologia 115: 401-405.

Steward JL, Keeler KH. 1988. Are there trade-offs among antiherbivore defenses in Ipomoea (Convolvulaceae)? *Oikos* 53: 79-86.

Stowe KA, Marquis RJ, Hochwender CG, Simms EL. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology, Evolution and Systematics* 31: 565-595.

Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* 14: 179-185.

Strong DR, Lawton JH, Southwood TRE. 1984. *Insects on plants: community patterns and mechanisms*. Oxford: Blackwell Scientific Publications.

Valverde PL, Fornoni J, Núñez-Farfán J. 2001. Defensive role of leaf trichome in resistance to herbivorous in *Datura stramonium*. *Journal of Evolutionary Biology* 14: 424-432.

van der Meijeden E, Wijn EH, Verkaar J. 1988. Defence and regrowth: Alternative plant strategies in the struggle against herbivores. *Oikos* 51: 355-363.

Walters D. 2011. *Plant defense: Warding off attack by pathogens, herbivores and parasitic plants*. Oxford: Blackwell Scientific Publications.

Wink M. 1993. Allelochemical properties or the *raison d'être* of alkaloids. En: Cordell G, ed. *The alkaloids*. San Diego: Academic Press, 1-118 pp.

Capítulo I

LAS CORRELACIONES FILOGENÉTICAS ENTRE LOS CARACTERES QUIMICOS Y FÍSICOS DE LAS PLANTAS CAMBIAN CON LA ONTOGENIA

MANUSCRITO PUBLICADO EN *New Phytologist*

Kariñho-Betancourt E, Agrawal AA, Halitschke R, Núñez-Farfán J. (2015) Phylogenetic correlations among chemical and physical plant defenses change with ontogeny. *New Phytologist* 206: 796–806. doi: 10.1111/nph.13300

New Phytologist

Research

Phylogenetic correlations among chemical and physical plant defenses change with ontogeny

Eunice Kariñho-Betancourt[1], Anurag A. Agrawal[2], Rayko Halitschke[3] and Juan Núñez-Farfán[1]

[1]Laboratorio de Genética Ecológica y Evolución, Instituto de Ecología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, 04510 México city, México; [2]Department of Ecology and Evolutionary Biology, Cornell University, Corson Hall, Ithaca, New York, NY 14853, USA; [3]Department of Phytopathology and Applied Entomology, Justus Liebig University, Heinrich Buff Ring 26-32, 35392 Giessen, Germany

Author for correspondence:
Juan Núñez-Farfán
Tel: +52 55 56229005
Email: farfan@unam.mx

Received: *29 September 2014*
Accepted: *15 December 2014*

New Phytologist (2015)
doi: 10.1111/nph.13300

Key words: chemical ecology, comparative method, *Datura*, leaf trichome, ontogeny, plant defense, tradeoffs, tropane alkaloids.

Summary

- Theory predicts patterns of defense across taxa based on notions of tradeoffs and synergism among defensive traits when plants and herbivores coevolve. Because the expression of characters changes ontogenetically, the evolution of plant strategies may be best understood by considering multiple traits along a trajectory of plant development.
- Here we addressed the ontogenetic expression of chemical and physical defenses in 12 *Datura* species, and tested for macroevolutionary correlations between defensive traits using phylogenetic analyses. We used liquid chromatography coupled to mass spectrometry to identify the toxic tropane alkaloids of *Datura*, and also estimated leaf trichome density.
- We report three major patterns. First, we found different ontogenetic trajectories of alkaloids and leaf trichomes, with alkaloids increasing in concentration at the reproductive stage, whereas trichomes were much more variable across species. Second, the dominant alkaloids and leaf trichomes showed correlated evolution, with positive and negative associations. Third, the correlations between defensive traits changed across ontogeny, with significant relationships only occurring during the juvenile phase.
- The patterns in expression of defensive traits in the genus *Datura* are suggestive of adaptation to complex selective environments varying in space and time.

Introduction

Classical coevolutionary theory suggests that herbivores and their host plants are involved in an evolutionary 'arms race' (Ehrlich & Raven, 1964; Dawkins & Krebs, 1979), whereby reciprocal selection is thought to favor the 'escape' of plants from their herbivores by producing novel defensive traits (or by strengthening pre-existing ones; Vermeij, 1994), thus giving rise to adaptive radiation of plants. At the same time, herbivores are selected to overcome defenses and, likewise, diversify onto closely related host plants (or plants with shared chemistry) (Ehrlich & Raven, 1964; Janzen, 1980). After the introduction of Ehrlich & Raven's coevolutionary model, several hypotheses were advanced to elucidate phylogenetic patterns resulting from plant–herbivore interactions, and the mechanisms that determine variation in the expression of defensive traits (reviewed by Stamp, 2003; Agrawal, 2007).

The evolution of plant defense against natural enemies could be restricted as a result of resource limitation and functional redundancy of defense traits, leading to negative associations (i.e. tradeoffs) between defensive attributes across species (van der Meijden *et al.*, 1988; Kursar & Coley, 2003). Even in the absence of such tradeoffs, contrasting selection for different defensive traits could favor the development of one defense strategy (i.e. resistance or tolerance; Mauricio *et al.*, 1997). However, if plant defenses, like most adaptations, are composed of multiple traits that are complementary (defense syndromes; *sensu* Agrawal & Fishbein, 2006), the joint evolution of different plant defenses is a possible outcome (Fornoni *et al.*, 2004a; Núñez-Farfán *et al.*, 2007). Thus, two classes of defensive traits (e.g. chemical and physical traits) could positively covary (Ballhorn *et al.*, 2013), as long as the fitness benefits of expressing multiple defensive traits is higher than their costs. Indeed, studies in natural populations rarely provided empirical evidence for tradeoffs between defensive traits or strategies (Fineblum & Rausher, 1995; Koricheva *et al.*, 2004), but at macroevolutionary scales, fewer studies have been conducted (Becerra *et al.*, 2001; Rudgers *et al.*, 2004; Agrawal & Fishbein, 2008).

Because the environmental conditions, physiological constraints, and species interactions that plants experience during development undergo change, plant defenses may vary accordingly (Boege & Marquis, 2005; Barton & Koricheva, 2010). Although contrasting hypotheses exist as to whether plant defense should increase or decrease during development (Barton & Koricheva, 2010), it is reasonable to expect that defenses should increase at those plant stages that are more susceptible to the attack by herbivores, and in plant tissues that are most closely tied to plant fitness (McKey, 1974, 1979; Rhoades, 1979; Meldau *et al.*, 2012). Nonetheless, our understanding of tradeoffs in defense traits may be colored by a focus on a single ontogenetic stage. Indeed, recent work indicates that covariation among traits changes across ontogeny (Hjelm *et al.*, 2000; Boege *et al.*, 2007), suggesting that the evolution of plant defense strategies may be best understood by considering multiple traits along the ontogenetic trajectory. It is currently unclear as to whether common ontogenetic trajectories in the expression of chemical and physical defenses are shared by closely related species or whether there has been divergence.

Tropane alkaloids constitute one of the most distinctive groups of plant secondary metabolites (Evans, 1979; Leete, 1979; Parr *et al.*, 1990; Wink, 2003). Although they can be found in distantly related plant families (e.g. Solanaceae, Euphorbiaceae, Rhizoporaceae, Convolvulaceae; Griffin & Lin, 2000), tropane alkaloids, including tropine, scopolamine, and hyoscyamine, are best known from the genus *Datura* (Solanaceae) (Wink, 2003). Tropane alkaloids have remarkable and diverse biological activity, and have been widely used as medicinal drugs and poisons (Colegate *et al.*, 1979; Nathanson, 1984; Roddick, 1991; Alexander *et al.*, 2008). Approximately 200 tropane alkaloids are known, and all are linked to the common tropane biosynthetic pathway. As such, a negative evolutionary correlation between distinct tropane alkaloids is a possible outcome, because increased production of one compound may cause substrate limitation for the biosynthesis of another (Berenbaum *et al.*, 1986; Martens & Mithöfer, 2005). Alternatively, changes in the level of a common precursor may increase overall flux in the pathway, increasing production of both compounds (Agrawal *et al.*, 2009a). Evidence from the study of the endogenous regulation of tropane alkaloids in *Datura stramonium* indicates that feeding precursors of the tropate moiety of hyoscyamine and scopolamine have negligible to detrimental effects on hyoscyamine accumulation (Robins *et al.*, 1991). Nonetheless, such physiological experiments on a single species cannot fully predict outcomes on a macroevolutionary scale.

In addition to secondary metabolites, leaf trichomes (pubescence) have been strongly implicated in defense against herbivores. Leaf trichomes are extremely variable in shape and structure (Johnson, 1975), and although their antiherbivore function has been widely documented within and among species (e.g. Levin, 1973; Ågren & Schemske, 1993; Mauricio & Rausher, 1997; Løe *et al.*, 2007), they can also have important ecophysiological roles, reducing leaf exposure to ultraviolet light and water loss (Ehleringer *et al.*, 1976). Empirical evidence indicates that, across species, pubescence has frequently evolved in arid habitats, and it also impairs herbivore behavior and performance (Agrawal *et al.*, 2009b). Leaf pubescence has also been found to be evolutionarily associated with secondary metabolites at a single ontogenetic stage (e.g. Agrawal & Fishbein, 2008). But on a macroevolutionary scale, the relationship between trichomes and particular secondary compounds has never been assessed across ontogeny.

Thus, here we addressed the evolution of tropane alkaloids and leaf trichomes in the genus *Datura*, the effect of plant ontogenetic stage on the expression of these defensive traits, and the evolutionary correlations between traits. Specifically, we asked the following questions. Do plant chemical and physical resistance traits vary across ontogeny within a clade? Does the expression of multiple resistance traits involve tradeoffs across the phylogeny? Do phylogenetic patterns of plant resistance traits (e.g. covariation between traits) change with ontogeny?

Materials and Methods

The genus *Datura*

The genus *Datura* (Solanaceae) is a small group of annual herbs and perennial shrubs that typically grow in nitrogen-rich soils and disturbed habitats, although one species, *Datura ceratocaula*, is semiaquatic (Fig. 1). Approximately 13–14 species have been included in the genus (e.g. Avery *et al.*, 1959; Luna-Cavazos & Bye, 2011). *Datura* species are native to dry, temperate, and subtropical regions of the Americas (Barclay, 1959) and are distributed mostly in Mexico, which is considered the center of its origin (Symon & Haegi, 1991). Although *Datura ferox* occurs in Argentina, *Datura metel* in Asia, and *Datura leichardthii* in Australia, these species were probably introduced from Mexico (Geeta & Gharaibeh, 2007; Torres *et al.*, 2013). Unlike the self-incompatible sister clade *Brugmansia*, *Datura* species have a mixed mating system.

All members of the genus *Datura* are known to be rich in tropane-alkaloids (Evans, 1979). These alkaloids are synthesized in the roots, stored in vacuoles (Evans & Partridge, 1953; Conklin, 1976), and mobilized to the leaves and the reproductive structures (Conklin, 1976; Evans, 1979). The foliar alkaloid concentration in *Datura* species attains its maximum at flowering (Conklin, 1976). Numerous alkaloids have been described from the genus, with scopolamine and hyoscyamine being dominant (Parr *et al.*, 1990). These two alkaloids negatively affect a variety of herbivorous insects and vertebrate animals (Hsiao & Fraenkel, 1968; Wink, 1993; Shonle, 1999). Genetic variation and selection on alkaloid concentration has been detected in *D. stramonium* (Shonle & Bergelson, 2000). Besides alkaloids, all *Datura* species also possess leaf trichomes, another putative defensive trait against herbivores (van Dam *et al.*, 1999; Hare & Elle, 2001, 2002; Valverde *et al.*, 2001). Trichomes can be subdivided into two categories: glandular trichomes, which exude chemical substances; and nonglandular trichomes, which do not (Levin, 1973). We focused on nonglandular trichomes because they are common to all *Datura* species and have been linked to insect herbivory (Valverde *et al.*, 2001; Kariñho-Betancourt, 2009).

In natural populations of *D. stramonium* and *Datura inoxia* in central Mexico, plants are frequently attacked by specialist insects that consume leaf tissue, including *Epitrix parvula* and the three-lined potato beetle *Lema trilineata* (Coleoptera: Chrysomelidade), a generalist herbivore *Shpenarium purpurascens* (Orthoptera: Pyrgomorphidae), and a specialist seed predator, the weevil *Trichobaris soror* (Coleoptera: Curculionidae) (Núñez-Farfán & Dirzo, 1994). In natural populations of *Datura wrightii* in southern USA, besides being attacked by *L. trilineata*, plants are also consumed by the tobacco hornworm *Manduca sexta* (Lepidoptera:

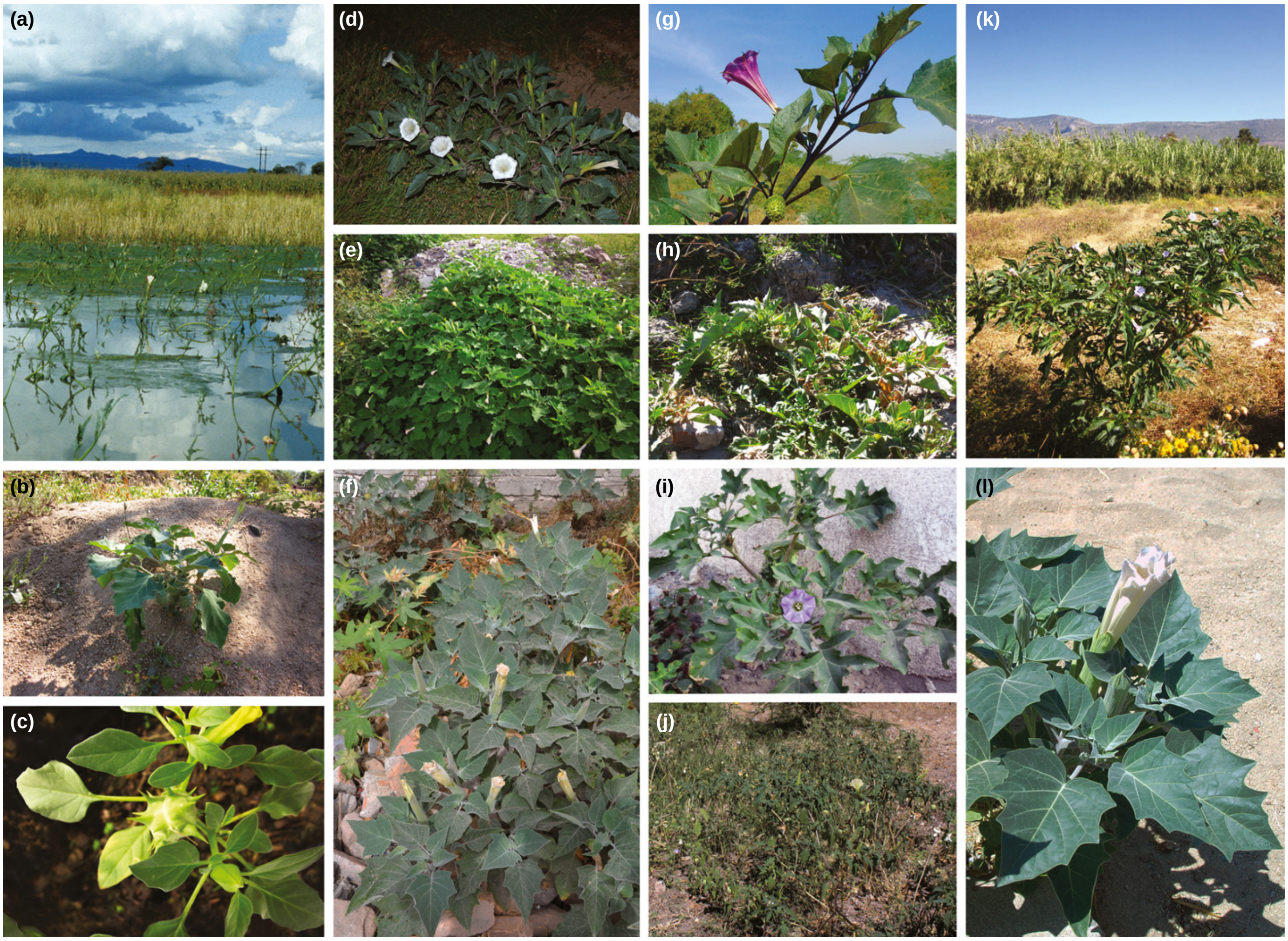


Fig. 1 Twelve of the *c.* 14 species of genus *Datura* included in this study. (a) *Datura ceratocaula*, (b) *D. discolor* (c) *D. ferox*, (d) *D. inoxia*, (e) *D. kymatocarpa*, (f) *D. lanosa,* (g) *D. metel*, (h) *D. pruinosa*, (i) *D. quercifolia,* (j) *D. reburra*, (k) *D. stramonium*, and (l) *D. wrightii.*

Sphingidae), the weevil *Trichobaris compacta* (Coleoptera: Curculionidae), and the mirid bug *Tupiocoris notatus* (Hemiptera: Miridae), among other herbivores specialized on the Solanaceae (Elle & Hare, 2000; Hare & Elle, 2002). Several studies have documented the negative impact of herbivory on plant fitness in *Datura* species (Núñez-Farfán & Dirzo, 1994; Valverde *et al.*, 2001; Hare & Elle, 2002; Fornoni *et al.*, 2004b), and variation in defensive traits, including alkaloids and trichomes, is associated with the composition of the herbivore community (Hare & Elle, 2002; Fornoni *et al.*, 2003; Valverde *et al.*, 2003; Castillo *et al.*, 2014).

Experimental design

In order to assess plant traits at two ontogenetic stages (juvenile and reproductive), replicate plants of 12 species of *Datura* (10 maternal families per species) were grown from seed in a controlled glasshouse environment (12 h, 27°C : 12 h, 20°C, day : night cycle). We followed the same protocol for each species. During the juvenile phase (hereafter time t_1), half of the plants of a given family were early-harvested (complete defoliation), whereas the other half of the plants remained undamaged (control group). Then, during flowering (hereafter time t_2), early-harvested and control plants were harvested together. A comparison of early-harvested plants at t_1 vs control plants at t_2 allowed us to determine the constitutive levels of defense at two different ontogenetic stages. Additionally, since early-harvested plants were allowed to regrow, they were compared with the defenses of control plants at t_2, allowing us to assess the extent of plant responses to defoliation. Nonetheless, because of the harvesting treatment, the ages of the leaves of early-harvested t_1 and control t_2 plants were expected to be different. Thus, to control for leaf age differences between the two harvest time points, the new leaves in the control plants produced after t_1 were marked and harvested at t_2, so that they could be compared with the regrowth leaves harvested at the same time (t_2).

Plant leaf tissue was oven-dried at 50°C (Berkov *et al.*, 2005). Tropane alkaloids are stable and this is a standard approach to preserving the tissue. Tropane alkaloids were quantified using LC-MS. Dried samples were ground and 100 mg of tissue was extracted with 1 ml extraction buffer (1% acetic acid, 40% methanol in water). Samples were homogenized in a FastPrep® -24 system (M. P. Biomedicals, Irvine, CA, USA) with 0.9 g ceramic beads (2.3 mm zirconia/silica beads; BioSpec, Bartlesville, OK, USA), centrifuged at 20 000 ***g*** for 10 min, and the supernatant was filtered through a 0.45 μm nylon membrane syringe filter before analysis. Ten microliters of the filtered extracts were injected for analysis by LC-MS (Quantum Access; Thermo Scientific, Waltham, MA, USA). Alkaloids were separated on a C18 reversed-phase column (Gemini-NX, 3 μm, 150 × 2 mm, Phenomenex, Torrance, CA, USA) with the following solvent gradient (solvent A, 0.1% formic acid in water; solvent B, 0.1% formic acid in acetonitrile): initial 5% B to 75% B at 45 min, and 45–52 min 75% B at a flow rate of 0.2 ml min^{-1}. The MS was operated in positive electrospray ionization mode with the following ionization conditions: spray voltage, 5.0 kV; capillary temperature, 315°C; sheath gas (N_2) pressure, 15 arbitrary units; auxiliary gas (N_2) pressure, 20 arbitrary units. Mass spectra were recorded in full scan mode (m/z 100 to m/z 1500). Alkaloids (tropine, hyoscyamine, scopolamine, atropine, and solanine) were identified by comparing UV spectra, retention time, and mass spectra with authentic standards (Sigma-Aldrich). Compound-specific molecular ion $[M+H]^+$ signals were used for quantification, and alkaloid concentrations were calculated using calibration curves generated with alkaloid standard dilution series. Total alkaloid concentration was obtained as the sum of the five alkaloids (μg g^{-1}).

To calculate the density of leaf trichomes, we took a random sample of 10 leaves per plant and used a plastic stencil overlay under a dissection microscope to visualize one leaf disk (22 mm^2). Trichomes were counted on the bottom (basal central area) abaxial side of each leaf disk (Valverde *et al.*, 2001).

Statistical analysis

In order to analyze the effect of ontogenetic stage and response to defoliation on the phenotypic expression of defensive traits in *Datura* species, we performed a mixed-model ANOVA, where alkaloid concentration and leaf trichome density were the response variables. Species and defoliation treatments were the random and fixed effects, respectively. Treatments included early harvest (constitutive defense at the juvenile stage), regrowth after early harvest (response to defoliation at the reproductive stage), and control group (constitutive defense at the reproductive stage). To assess constitutive trait variation among species in each ontogenetic stage, we performed a one-way ANOVA for each alkaloid and for the leaf trichomes density. Also, to visualize the relative change in the expression of a defensive trait with ontogeny (δ_{Di}), we calculated the difference in trait value at the reproductive (t_2) and juvenile (t_1) stages, weighted by the juvenile stage (t_1) as:

$$\delta_{Di} = \frac{\bar{S}_{t_2} - \bar{S}_{t_1}}{\bar{S}_{t_1}}$$

where $\bar{S}_{t_i}$ is the species average for a given stage. The ratio δ_{Di} will be positive if the trait value increases at reproduction, negative if it decreases, and zero if there is no difference between stages (see Supporting Information Table S1).

To assess phylogenetic patterns of defense against herbivores, we used a molecular phylogeny of *Datura* that includes 12 of the *c.* 14 species, constructed with chloroplast (*psb*A-*trn*H and *trn*L-*trn*F) and nuclear (ITS rRNA) datasets (Table S2). Topology was obtained by Bayesian inference and maximum likelihood (ML) analyses (Fig. 2). Correlation analysis was performed within each ontogenetic stage. Phylogenetic correlations between defensive traits of juvenile and reproductive stages were obtained using the generalized least-squares (GLS) method for calculating independent contrasts (Garland *et al.*, 1999; Pagel, 1999), implemented in the *R* statistical package using the *picante* program. For each ontogenetic stage, we estimated the phylogenetic signal (K) to determine influence of phylogenetic history on the expression of defensive traits (Bloomberg *et al.*, 2003). A value of $K < 1$ indicates departure from the Brownian model of evolution, such that species are less similar than expected based on their phylogenetic relationships; a value of $K > 1$ indicates greater similarity than expected based on the same model.

Results

Regardless of defoliation, we found that plants showed higher total alkaloid concentrations in leaves of flowering plants,

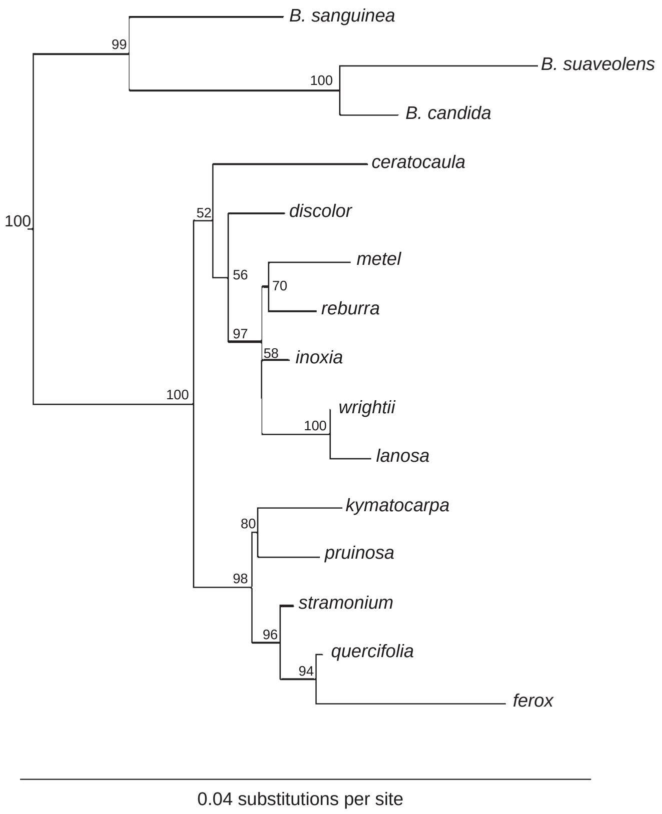


Fig. 2 Phylogram of the 12 species of *Datura* and *Brugmansia* (as outgroup) based on chloroplast (*psb*A-*trn*H and *trn*L-*trn*F regions) and nuclear (ITS rRNA) sequences. Topology was derived from Bayesian inference and maximum likelihood (ML) analyses. Branch length was optimized by ML. Numbers indicate the bootstrap values for each branch (J. Núñez-Farfán *et al.*, unpublished).

compared with similar tissues of juvenile plants. By contrast, leaf trichome density was similar between plant stages and defoliation treatments across species (Fig. 3). As defoliation had no effect on chemical or physical defensive traits, subsequent analyses of ontogenetic variation were performed only for undefoliated plants. We detected significant variation among species in all resistance traits analyzed (individual tropane alkaloids and leaf trichomes), and a significant interaction between species and ontogenetic stage for five of the seven traits (Table 1).

Ontogenetic variation of defensive traits

Total alkaloid concentration of leaves varied over 10-fold among species and ranged from 38.72 (± 35.91) to 497.52 (± 185.01) $\mu g\, g^{-1}$ DW before flowering (t_1), and over 30-fold at flowering (t_2), ranging from 101.19 (± 35.91) to 3491 (± 185.01) $\mu g\, g^{-1}$ DW. Tropine and solanine were the least abundant alkaloids across species, representing $< 4\%$ of total alkaloid concentration. By contrast, the major alkaloids, scopolamine, atropine, and hyoscyamine, together represented $> 90\%$ of total alkaloid content at both juvenile and reproductive stages. Scopolamine alone represented $> 70\%$ of the total alkaloid concentration in most *Datura* species (Fig. S1). Although all tropane alkaloids analyzed, except solanine, showed strong increases in concentration with ontogeny (Table S3), these increasing ontogenetic trajectories were not concordant across

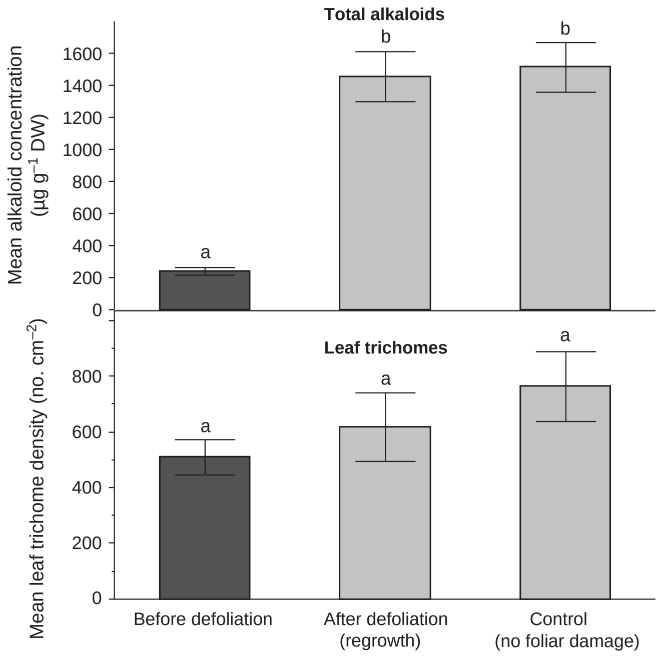


Fig. 3 Variation in chemical and physical defensive traits in response to complete defoliation. Plants were harvested at two different ontogenetic stages: before flowering (juvenile stage), during flowering (reproductive stage). Data are raw phenotypic means (± 1 SE) of 12 *Datura* species. Levels not connected by the same letter are significantly different according to Tukey's tests. A breakdown of patterns in the specific alkaloids is given in Supporting Information Table S1 and Fig. S1.

Table 1 Analysis of variance of alkaloid concentration and leaf trichome density (standardized variables) in *Datura* spp.

		Tropine		Hyoscyamine		Scopolamine		Atropine		Solanine		Total alkaloids		Leaf trichomes	
Source of variation	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species	11	22.28	**< 0.0001**	23.78	**< 0.0001**	15.55	**< 0.0001**	54.27	**< 0.0001**	49.97	**< 0.0001**	14.16	**< 0.0001**	165.06	**< 0.0001**
Ontogentic stage	1	299.92	**< 0.0001**	253.34	**< 0.0001**	127.01	**< 0.0001**	35.611	**< 0.0001**	10.43	**0.0017**	169.96	**< 0.0001**	0.38	**0.538**
Species × Os	11	1.43	0.173	4.017	**< 0.0001**	5.074	**< 0.0001**	1.28	0.244	3.045	**0.0015**	5.027	**< 0.0001**	14.167	**< 0.0001**
Error	116														
Total	139														

The species term is a random effect, whereas the ontogenetic stage (Os) is a fixed effect. A significant species × Os interaction indicates that species differed in their ontogenetic trajectory. Significant values are indicated in bold ($P < 0.05$).

the alkaloids. The major alkaloids scopolamine and hyoscyamine had the largest increases with ontogeny across the genus. By contrast, solanine and atropine had smaller ontogenetic changes (Table S1; Fig. 4). Also, only tropine increased in all *Datura* species, while scopolamine, the most abundant alkaloid, increased in concentration during flowering in eight of 12 *Datura* species, including *Datura kymatocarpa*, in which it was found in very high concentrations (Tables S1, S3). Hyoscyamine, the alkaloid with the largest ontogenetic changes (Table S1; Fig. 4), increased concentration in all but two noncoexisting *Datura* species (*Datura quercifolia* and *D. ceratocaula*, which are distributed in temperate and subtropical regions of Mexico).

Leaves of *Datura* species exhibited huge variation in trichome densities (Fig. 5), ranging from *c.* 35 trichomes cm^{-2} (*D. ferox*) to over 1800 trichomes cm^{-2} (*D. ceratocaula*) at the juvenile stage, and from *c.* 14 trichomes cm^{-2} (*D. reburra*) to over 3000 trichomes cm^{-2} (*D. ceratocaula*) at the reproductive stage (Table S3). Contrasting with the relatively consistent increases in alkaloids, leaf trichomes showed highly variable, but less pronounced (top increase *c.* two-fold), ontogenetic trajectories. At the reproductive stage, we detected both a significant increase and decrease of trichome density, in four and three species, respectively (Table S3; Fig. 4).

Based on the broad spectrum of trichome densities, we categorized species into two types: mostly glabrous (number of trichomes below average), and mostly pubescent (number of trichomes above average). In addition, to relate leaf pubescence with species habitats, we calculated an aridity index (AI; UNEP, 1992) for each *Datura* spp (see Table 2). Overall, species that coexist or occur in the same climate (e.g. arid or temperate climates) exhibit comparable leaf trichome densities (Table 2). Mostly pubescent species tend to occur in arid climates, whereas mostly glabrous species tend to occur in temperate and humid regions (Table 2).

Correlations between defensive traits

K-values indicate low phylogenetic signal for alkaloids and leaf trichomes at both juvenile and reproductive plant stages, and only atropine at the reproductive stage was significantly different from zero ($K = 0.8$, $P = 0.03$) (Table S4). The phylogenetically independent correlations did not differ substantially from raw correlations (data not shown). At the juvenile stage, we found four significant correlations (out of 20) between defensive traits (Fig. 6). Four significant correlations would be highly unlikely to occur by chance (binomial expansion test, $P = 0.01$). Total alkaloid concentration and scopolamine were each negatively correlated with leaf trichome density. Furthermore, scopolamine was negatively correlated with hyoscyamine ($r^2 = -0.68$, $P < 0.05$), while hyoscyamine was positively correlated with leaf trichome density ($r^2 = 0.61$, $P < 0.05$). However, these relationships between defensive traits changed with ontogeny: at the reproductive stage, none of these correlations was significant (Table 3).

Discussion

Ontogenetic variation of defensive traits

The phenotypic expression of defensive traits in leaves of *Datura* species was extremely variable along ontogeny, with distinctive patterns for each class of chemical and physical defense. Tropane alkaloids tended to increased concentration at the reproductive stage, but leaf trichome density showed positive and negative trajectories depending on the species. Although the increase in concentration at the reproductive stage was common for almost all alkaloids analyzed, the ontogenetic trajectories were highly variable. Scopolamine and hyoscyamine (plus atropine) exhibited the largest increases across the genus, and were dominant in leaf tissues. By contrast, tropine, although found at very low

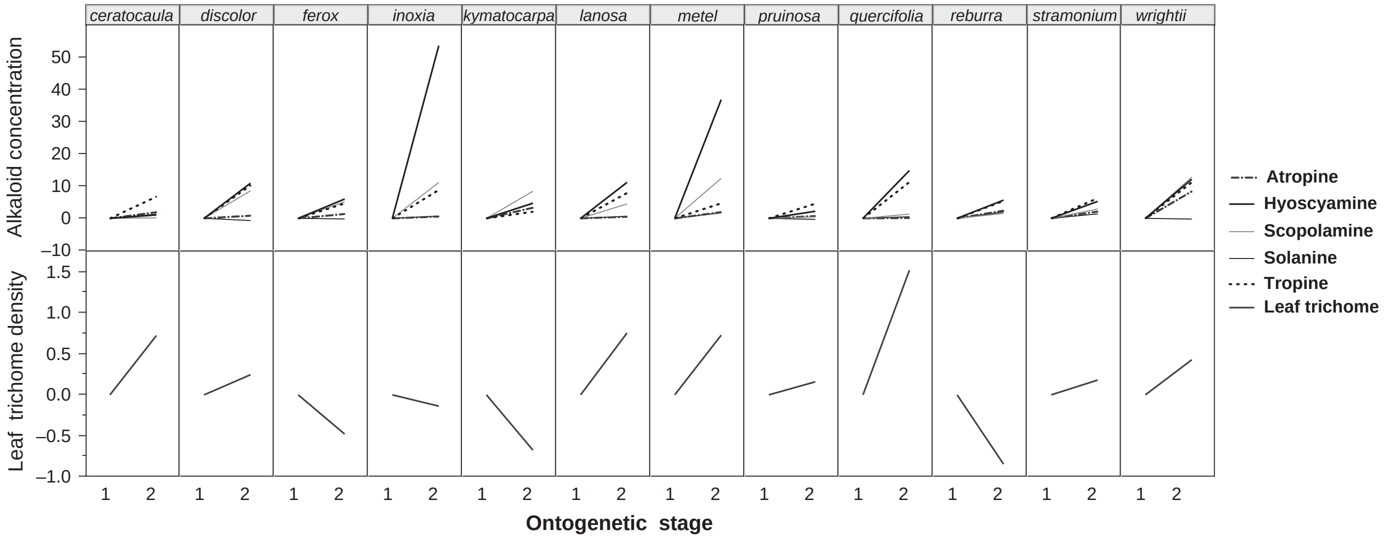


Fig. 4 Ontogenetic change of defensive traits in *Datura* spp.: 1, juvenile stage; 2, reproductive stage. For each trait, the ontogenetic trajectory ($\delta_{Di} = (\bar{S}_{t_2} - \bar{S}_{t_1})/\bar{S}_{t_1}$) is depicted. To visualize the relative variation between the two ontogenetic stages, the level of defense at the juvenile stage was set to zero units. Variation of one unit at the reproductive stage indicates either an increase or decrease of 100%.

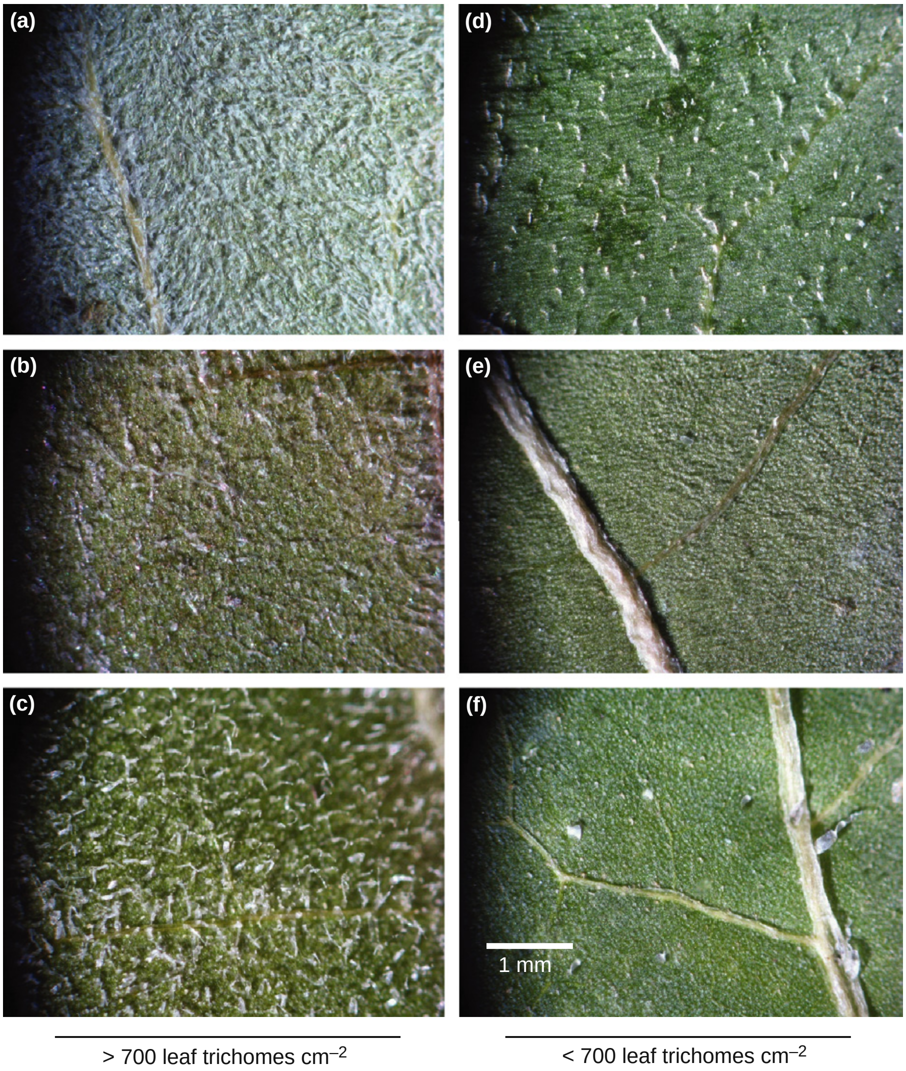


Fig. 5 Abaxial leaf surfaces of six species of *Datura*. Pubescent species: (a) *Datura ceratocaula*; (b) *D. lanosa*; and (c) *D. wrightii*; and mostly glabrous species: (d) *D. kymatocarpa*; (e) *D. reburra;* and (f) *D. ferox*. Leaves were harvested at the reproductive stage.

concentrations, was the only alkaloid that significantly increased in all *Datura* species. As tropine is the main precursor of tropane alkaloid biosynthesis (Leete, 1979), the investment in producing this alkaloid during plant development may ensure the supply for subsequent products (e.g. hyoscyamine and scopolamine). These results are in agreement with the hypothesis predicting an incremental increase of plant defense with ontogeny as a function of acquisition and allocation of resources (e.g. Bryant *et al.*, 1983; Herms and Matson, 1992). Besides the intrinsic factors that may contribute to the ontogenetic changes of alkaloids, the increase in hyoscyamine and scopolamine, known to play an important role mediating plant–herbivore interactions (e.g. Shonle & Bergelson, 2000; Castillo *et al.*, 2013), may also suggest an adaptive response to changes in the pressure exerted by herbivores during the plant's life.

Regardless of ontogeny, *Datura* species showed contrasting chemical phenotypes, with some species having high alkaloid concentrations (e.g. *D. kymatocarpa, D. inoxia*), and others very low (e.g. *D. quercifolia*) concentrations. Such phenotypic differentiation in the chemical phenotypes may reflect an association with particular species interactions. For instance, species such as *D. kymatocarpa* that showed the highest constitutive concentration of total alkaloids (at juvenile and reproductive stages) have almost no leaf damage in natural populations (E. Kariñho-Betancourt, pers. comm.). By contrast, species with a lower constitutive alkaloid concentration (e.g. *D. quercifolia*, *D. stramonium*) appear to have more leaf damage as a result of herbivory (e.g. in *D. stramonium,* the average leaf damage by herbivores ranges from 10 to 50%; Valverde *et al.*, 2001). This divergence in chemical phenotypes between closely related species was not influenced by their phylogenetic history (i.e. no phylogenetic signal), which suggests that herbivores could be driving large differences in alkaloids among species. We note that this lack of phylogenetic signal is not likely due to poor sampling, as we included > 85% of the extant *Datura* species in the analysis.

Contrasting with tropane alkaloids, leaf trichomes did not show a dominant trend across ontogeny, and their ontogenetic changes were less pronounced than for chemical defense, suggesting that physical defense is less plastic in *Datura* than alkaloid concentration. Also, leaf pubescence was associated with species' habitat.

Table 2 Species aridity index and leaf classification of *Datura* spp.

Species	Aridity index (AI)[1]	Climate	Leaf class ($\bar{x}$ leaf trichome density)
ceratocaula	0.4	Semiarid	P (3269)
wrightii	0.2	Arid	P (2208)
lanosa	0.2	Arid	P (1262)
pruinosa	0.2	Arid	P (800)
discolor	0.3	Semiarid	G (569)
inoxia	0.1	Arid	G (490)
quercifolia	0.5	Subhumid–dry	G (270)
stramonium	0.8	Humid	G (97)
metel	0.5	Subhumid–dry	G (90)
kymatocarpa	0.5	Subhumid–dry	G (43)
ferox	0.5	Subhumid–dry	G (18)
reburra	0.65	Subhumid–dry	G (14)

Species were categorized as a function of leaf trichome density (no. cm^{-2}) recorded at reproductive stage. P, mostly pubescent leaves (trichome density above average); G, mostly glabrous leaves (trichome density below average).

[1] Aridity index was calculated as $AI = P/PE$, where P is the yearly mean precipitation and PE is the yearly mean potential evapotranspiration. Data of precipitation (mm) and evapotranspiration (mm) were obtained from annual historical records (National Meteorological Service of Mexico and Argentina) of each species' population included in the study. $AI > 0.65$ indicates a humid climate, whereas $AI < 0.05$ indicates an extremely arid climate.

Regardless of the ontogenetic stage, pubescent leaves are present in species of arid habitats (e.g. *Datura lanosa, D. wrigthii, Datura pruinosa*), and glabrous leaves occur in temperate or subtropical regions (e.g. *D. stramonium, D. quercifolia, D. ferox*). However, one clear exception to this pattern is *D. ceratocaula*, which has the highest trichome density of the genus and yet occurs in temperate and humid regions of central Mexico. As *D. ceratocaula* is semiaquatic, its remarkably dense carpet of trichomes, apparently covered by wax, may be an adaptation to the aquatic habit, which presents its own set of physiological challenges.

Our results suggest that leaf pubescence may be strongly influenced by climate, but along with plant development, leaf trichomes can play a role in other ecological interactions, such as herbivory. For example, *Datura* trichomes were protective against folivore insects such as the mirid bug *T. notatus* and the three-lined potato beetle *L. trilineata* (van Dam & Hare, 1998b; Elle & Hare, 2000). In a related study on trichome evolution in the milkweeds, it was found that although trichomes pose a formidable barrier to insect feeding, even by specialist herbivores, aridity was their primary evolutionary driver in this genus as well (Agrawal *et al.*, 2009b). Accordingly, the abiotic selection for particular plant traits may intersect with biotic interactions. For trichomes and plant secondary compounds, their correlated evolution may drive the optimization of ecophysiological adaptation, coupled with differences in natural selection imposed by herbivores in different environments.

Correlations between defensive traits

Phylogenetically controlled analysis across the 12 *Datura* species revealed that major tropane alkaloids and leaf trichomes at the juvenile stage show correlated evolution. In agreement with hypotheses about tradeoffs and synergism, we found evidence of both negative and positive correlations between chemical and physical defensive traits. Negative correlations could reflect allocation costs of traits or redundancy in their ecological functions, and across related species such associations are typically interpreted as a result of either strong physiological constraint that is unbreakable over speciational timescales, or natural selection (i.e. an adaptive relationship). Although the negative association between scopolamine and hyoscyamine across the phylogeny suggests a physiological constraint for the production of alkaloids as a result of diversion of precursors (Berenbaum *et al.*, 1986; Martens & Mithöfer, 2005; Agrawal *et al.*, 2009a), this does not exclude the possibility of an adaptive role against herbivores (Roberts & Wink, 1998). Thus, it is possible that the high concentrations of scopolamine and hyoscyamine (or its racemic form, atropine) produced by *Datura* species are maintained by a tradeoff in defense against multiple herbivore species.

The positive correlation between hyoscyamine concentration and leaf trichome density suggests a synergistic interaction leading to the joint evolution of these defensive traits. The overall correlation between chemical and physical plant defense is in agreement with the defense syndromes hypothesis (Agrawal & Fishbein, 2006), suggesting that plant defenses are composed of multiple traits organized into coadapted complexes (Futuyma & Agrawal, 2009). Scopolamine concentration appears to be an alternative defense to the complex composed by leaf trichomes and hyoscyamine. Commonly covarying traits within a clade are predicted to be associated with a particular type of species interaction (Coley *et al.*, 1985; Agrawal & Fishbein, 2006). Previous studies within *Datura* spp. indicate that the guild of herbivores that feed upon *Daturas* includes specialized leaf beetles and seed weevils, as well as generalist grasshoppers among others (Núñez-Farfán & Dirzo, 1994; Hare & Elle, 2002; Hare *et al.*, 2003); scopolamine and hyoscyamine have differential effects on specialized vs generalist herbivores (scopolamine can act as a phagostimulant for the specialist flea beetle *Epitrix parvula*; Shonle & Bergelson, 2000); and generalist and specialist herbivores can exert contrasting selection on scopolamine and hyoscyamine (Shonle & Bergelson, 2000; Castillo *et al.*, 2014). Therefore, it is possible that scopolamine and the coadapted complex of hyoscyamine and leaf trichomes are being differentially selected depending on the habitat occupied by the plants and the degree of specialization of plant consumers. Accordingly, recent work indicates that across 11 *Datura* species, the generalist herbivore *Spodoptera frugiperda* performed better in species with a low concentration of alkaloids and mostly glabrous leaves, whereas the specialist herbivore *L. trilineata* performed better in species with a high concentration of alkaloids and mostly pubescent leaves (E. Kariñho-Betancourt & J. Núñez-Farfán, unpublished).

The relationships between defensive traits over ontogeny may also be related to species interactions. Host properties typically change with age (e.g. increase in size; Murdoch *et al.*, 1999), and thus dynamics of animal–plant interactions may vary accordingly. In *Datura*, even when some specialist herbivores are likely to occur in juvenile stages (e.g. *Epitrix parvula*; Núñez-Farfán &

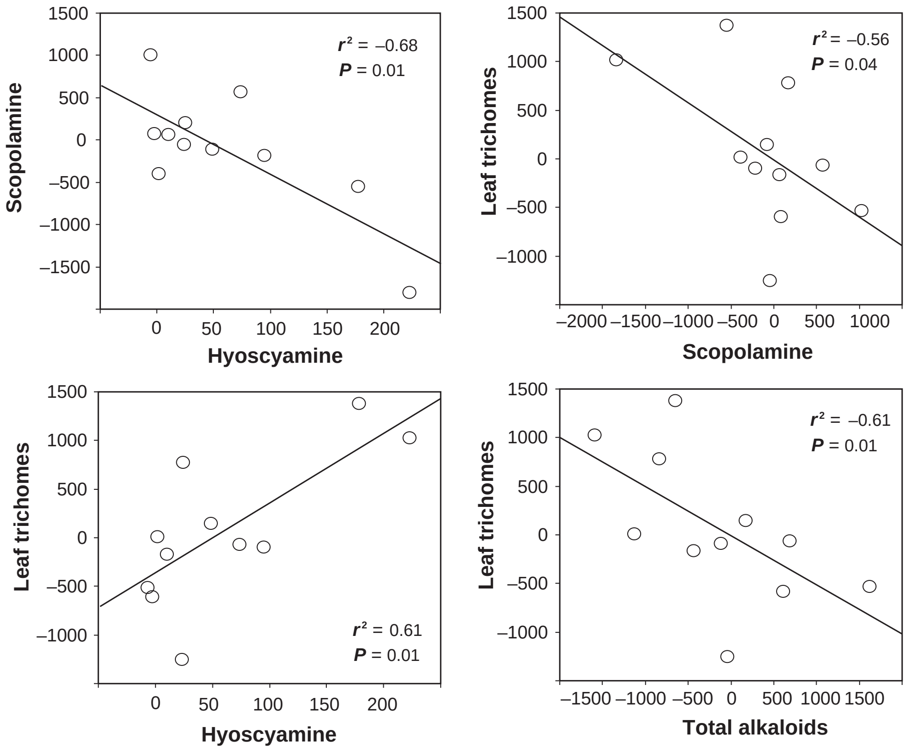


Fig. 6 Correlation between alkaloid concentration and leaf trichome density across 12 *Datura* species at the juvenile stage. Lines show the phylogenetically corrected slopes.

Table 3 Correlations between defensive traits in the genus *Datura* corrected by phylogenetic relationships (PGLs) among taxa

Defensive traits		Correlation coefficient (r^2)	
Trait 1	Trait 2	Juvenile stage	Reproductive stage
Hyoscyamine	Tropine	−0.05	0.0
Scopolamine	Tropine	0.07	0.01
Atropine	Tropine	0.35	0.26
Solanine	Tropine	−0.04	0.07
Total alkaloids	Tropine	0.31	0.38
Foliar trichomes	Tropine	−0.22	−0.09
Scopolamine	Hyoscyamine	**−0.68***	−0.05
Atropine	Hyoscyamine	−0.03	−0.07
Solanine	Hyoscyamine	0.06	−0.06
Total alkaloids	Hyoscyamine	−0.5	0.14
Foliar trichomes	Hyoscyamine	**0.61***	0.50
Atropine	Scopolamine	0.27	0.39
Solanine	Scopolamine	−0.37	−0.31
Foliar trichomes	Scopolamine	**−0.56***	−0.26
Solanine	Atropine	0.52	0.45
Total alkaloids	Atropine	0.37	0.14
Foliar trichomes	Atropine	−0.43	−0.39
Total alkaloids	Solanine	0.29	−0.09
Foliar trichomes	Solanine	−0.01	−0.03
Foliar trichomes	Total alkaloids	**−0.61***	−0.22

Plants of 12 species (and *c*. 12 replicates per species) were grown from seed in a common environment and harvested at two different ontogenetic stages: before flowering (juvenile stage) and during flowering (reproductive stage). Significant values are indicated in bold (*, $P < 0.05$).

Dirzo, 1994; Carmona & Fornoni, 2013), most are synchronized with the onset of plant reproduction (Espinosa & Fornoni, 2006). Thus, the correlation between alkaloids and trichomes only at juvenile stage, along with the increasing ontogenetic trajectory in alkaloid concentration, suggests that the change of the relationship between traits in the reproductive phase may have an adaptive value as herbivore pressures increase.

Concluding remarks

In *Datura*, the phenotypic expression of defensive traits changes during the plants' ontogeny, and differs between the classes of defense (i.e. chemical vs physical). Nevertheless, the expression of constitutive alkaloids and leaf trichomes is phylogenetically independent, hinting at an adaptive role to cope with herbivores. At the same time, leaf trichomes seem also to be related to arid habitats, thus playing an ecophysiological role in addition to defense. Both chemical and physical defenses showed correlated evolution that varies with ontogeny, thus suggesting that the breakage of this correlation may be adaptive to strengthen defense against herbivores along plant development.

Acknowledgements

We thank V. Jímenez- Lobato, R. Tapia-López, I. Camargo, and all the members of *Laboratorio de Genética Ecológica y Evolución, UNAM*, for logistic support and valuable field assistance. We thank A. Vázquez-Lobo for her help with the phylogenetic analysis of *Datura*. We also thank A. Hastings (Cornell University) for help and logistical support with the extraction of alkaloids, and M. A. Serrano-Meneses and Marjorie Weber for help with comparative analyses. We also thank G. Delgado-Lamas and M. Olson for helpful advice and comments during the study. E.K-B.

acknowledges J. Ali for housing during the alkaloid extraction, V. Jiménez-Lobato for providing some photographs of *Datura* species, and G. Castillo for helpful comments on the manuscript. All chemical analyses were conducted in Cornell University's Chemical Ecology Group's Core Facility. This research was mainly funded by a CONACyT grant (*Evolución adaptativa en Datura: Resistencia y tolerancia a los herbívoros*) to J.N-F. Grants from USA NSF-DEB 1118783 and the Templeton Foundation to A.A.A. also supported this work. This paper constitutes a partial fulfillment of the Graduate Program in Biological Sciences of the National Autonomous University of Mexico (UNAM). E.K-B. acknowledges the scholarship and financial support provided by the National Council of Science and Technology (CONACyT).

References

Agrawal AA. 2007. Macroevolution of plant defense strategies. *Trends in Ecology and Evolution* **22**: 103–109.

Agrawal AA, Alminen JP, Fishbein M. 2009a. Phylogenetic trends in phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation. *Evolution* **63**: 663–673.

Agrawal AA, Fishbein M. 2006. Plant defense syndromes. *Ecology* **87**: S132–S149.

Agrawal AA, Fishbein M. 2008. Phylogenetic escalation and decline of plant defense strategies. *Proceedings of the National Academy of Sciences, USA* **105**: 10057–10060.

Agrawal AA, Fishbein M, Jetter R, Salminen JP, Goldstein JB, Freitag AE, Sparks JP. 2009b. Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): chemistry, ecophysiology, and insect behavior. *New Phytologist* **183**: 848–867.

Ågren J, Schemske DW. 1993. The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. *American Naturalist* **141**: 338–350.

Alexander J, Benford D, Cockburn A, Cravedi J, Dogliotti E, Di Domenico A, Fernandez-Cruz ML. 2008. Tropane alkaloids (from *Datura* spp.) as undesirable substances in animal feed. *European Food Safety Authority Journal* **691**: 1–55.

Avery AG, Satina S, Rietsema J. 1959. *Blakeslee: the genus* Datura. New York, NY, USA: Ronald Press.

Ballhorn DJ, Godschalx AL, Kautz S. 2013. Co-variation of chemical and mechanical defenses in lima bean (*Phaseolus lunatus* L.). *Journal of Chemical Ecology* **39**: 413–417.

Barclay AS. 1959. *Studies in the genus* Datura *(Solanaceae). I. Taxonomy of subgenus* Datura, PhD thesis. Harvard University, Cambridge, MA, USA.

Barton KE, Koricheva J. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *American Naturalist* **175**: 481–493.

Becerra JX, Venable DL, Evans PH, Bowers WS. 2001. Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. *American Zoologist* **41**: 865–876.

Berenbaum MR, Zangerl AR, Nitao JK. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. *Evolution* **40**: 1215–1228.

Berkov S, Doncheva SP, Alexandrov K. 2005. Ontogenetic variation of the tropane alkaloids in *Datura stramonium*. *Biochemical Systematics and Ecology* **33**: 1017–1029.

Bloomberg SP, Garland T, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Boege K, Dirzo R, Siemens D, Brown P. 2007. Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? *Ecology Letters* **10**: 177–187.

Boege K, Marquis RJ. 2005. Facing herbivory as you grown up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution* **20**: 441–448.

Bryant JP, Chapin FS III, Klein DR. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **40**: 357–368.

Carmona D, Fornoni J. 2013. Herbivores can select for mixed defensive strategies in plants. *New Phytologist* **197**: 576–585.

Castillo G, Cruz LL, Hernández-Cumplido J, Oyama K, Flores-Ortiz CM, Fornoni J, Valverde PL, Núñez-Farfán J. 2013. Geographic association and temporal variation of chemical and physical defense and leaf damage in *Datura stramonium*. *Ecological Research* **28**: 663–672.

Castillo G, Cruz LL, Tapia- López R, Olmedo-Vicente E, Carmona D, Anaya-Lang AL, Fornoni J, Andraca-Gómez G, Valverde PL, Núñez-Farfán J. 2014. Selection mosaic exerted by specialist and generalist herbivores on chemical and physical defense of *Datura stramonium*. *PLoS ONE* **9**: e102478.

Colegate SM, Dorling PR, Huxtable CR. 1979. A spectroscopic investigation of Swainsonine: an α-12 mannosidase inhibitor isolated from *Swainsona canescens*. *Australian Journal of Chemistry* **32**: 2257–2264.

Coley PD, Bryant JP, Chapin FS III. 1985. Resource availability and plant antiherbivore defense. *Science* **230**: 895–899.

Conklin ME. 1976. Genetic and biochemical aspects of the development of *Datura*. In: Wolsky E, ed. *Monographs in developmental biology*. New York, NY, USA: Karger, 1–170.

van Dam NM, Hare JD. 1998b. Differences in distribution and performance of two sap-sucking herbivores on glandular and non-glandular *Datura wrightii*. *Ecological Entomology* **23**: 22–32.

van Dam NM, Hare JD, Elle E. 1999. Inheritance and distribution of trichome phenotypes in *Datura wrightii*. *Journal of Heredity* **90**: 220–227.

Dawkins R, Krebs JR. 1979. Arms races between and within species. *Proceedings of the Royal Society London* **205**: 489–511.

Ehleringer J, Björkman O, Mooney HA. 1976. Leaf pubescence-effects on absorbance and photosynthesis in a desert shrub. *Science* **192**: 376–377.

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**: 586–608.

Elle E, Hare JD. 2000. No benefit of glandular trichome production in natural populations of *Datura wrightii*? *Oecologia* **123**: 57–65.

Espinosa EG, Fornoni J. 2006. Host tolerance does not impose selection on natural enemies. *New Phytologist* **170**: 609–614.

Evans WC. 1979. Tropane alkaloids of the Solanaceae. In: Hawkes JG, Lester RN, Skelding A, eds. *The biology and taxonomy of the Solanaceae*. London, UK: Academic Press, 241–254.

Evans WC, Partridge MW. 1953. Alkaloid biogenesis. *Journal of Pharmacy and Pharmacology* **5**: 293–300.

Fineblum WL, Rausher MD. 1995. Evidence for a trade-off between resistance and tolerance to herbivore damage in a morning glory. *Nature* **377**: 517–520.

Fornoni J, Núñez-Farfán J, Valverde PL, Rausher MD. 2004a. Evolution of mixed strategies of plant defense allocation against natural enemies. *Evolution* **58**: 1685–1695.

Fornoni J, Valverde PL, Núñez-Farfán J. 2003. Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*. *Evolutionary Ecology Research* **5**: 1049–1065.

Fornoni J, Valverde PL, Núñez-Farfán J. 2004b. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. *Evolution* **58**: 1696–1704.

Futuyma DJ, Agrawal AA. 2009. Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences, USA* **106**: 18054–18061.

Garland T, Midford PE, Ives AR. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *American Zoologist* **39**: 374–388.

Geeta R, Gharaibeh W. 2007. Historical evidence of *Datura* in the Old World and implications for a first millennium transfer from the New World. *Journal of Biosciences* **32**: 1227–1244.

Griffin WJ, Lin GD. 2000. Chemotaxonomy and geographical distribution of tropane alkaloids. *Phytochemistry* **53**: 623–637.

Hare JD, Elle E. 2001. Geographic variation in the frequencies of trichome phenotypes of *Datura wrightii* and correlation with annual water deficit. *Madroño* **48**: 33–37.

Hare JD, Elle E. 2002. Variable impact of diverse insect herbivores on dimorphic *Datura wrightii*. *Ecology* **83**: 2711–2720.

Hare JD, Elle E, Dam NM. 2003. Costs of glandular trichomes in *Datura wrightii*: a three-year study. *Evolution* **57**: 793–805.

Herms D, Matson W. 1992. The dilemma of plants: to grow or to defend. *Quarterly Review of Biology* **67**: 283–335.

Hjelm J, Person L, Christensen B. 2000. Growth, morphological variation and ontogenetic niche shifts in perch (*Perca fluviatilis*) in relation to resource availability. *Oecologia* **122**: 190–199.

Hsiao TH, Fraenkel G. 1968. The role of secondary plant substances in the food specificity of the Colorado potato beetle. *Annals of the Entomological Society of America* **61**: 458–503.

Janzen DH. 1980. When is it coevolution? *Evolution* **34**: 611–612.

Johnson B. 1975. Plant pubescence. An ecological perspective. *Botanical Review* **41**: 233–258.

Kariñho-Betancourt E. 2009. *Disyuntiva evolutiva entre la resistencia y la tolerancia a los herbívoros en Datura stramonium*. MSc thesis, UNAM, Mexico City, Mexico.

Koricheva J, Nykänen H, Gianoli E. 2004. Meta-analysis of tradeoff among plant antiherbivore defenses. *American Naturalist* **163**: E64–E75.

Kursar TA, Coley PD. 2003. Convergence in defense syndromes of young leaves in tropical rainforest. *Biochemical Systematics and Ecology* **31**: 929–949.

Leete E. 1979. Biosynthesis and metabolism of the tropane alkaloids. *Planta Medica* **36**: 97–112.

Levin DA. 1973. The role of trichomes in plant defence. *Quarterly Review of Biology* **48**: 3–15.

Løe G, Toräng P, Gaudeul M, Ågren J. 2007. Trichome production and spatiotemporal variation in herbivory in the perennial herb *Arabidopsis lyrata*. *Oikos* **116**: 134–142.

Luna-Cavazos M, Bye R. 2011. Phytogeographic analysis of the genus *Datura* (Solanaceae) in continental Mexico. *Revista Mexicana de Biodiversidad* **82**: 977–988.

Martens S, Mithöfer A. 2005. Flavones and flavone synthases. *Phytochemistry* **66**: 2399–2407.

Mauricio R, Rausher MD. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* **51**: 1435–1444.

Mauricio R, Rausher MD, Burdick DS. 1997. Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* **78**: 1301–1311.

McKey D. 1974. Adaptive patterns in alkaloid physiology. *American Naturalist* **108**: 305–320.

McKey D. 1979. The distribution of secondary compounds within plants. In: Rosenthal GA, Berenbaum MR, eds. *Herbivores: their interactions with secondary plant metabolites*. San Diego, CA, USA: Academic Press, 55–133.

van der Meijden E, Wijn M, Verkaar HJ. 1988. Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos* **51**: 355–363.

Meldau S, Erb M, Baldwin IT. 2012. Defence on demand: mechanisms behind optimal defence patterns. *Annals of Botany* **110**: 1503–1514.

Murdoch WW, Briggs CM, Nisbet RM. 1999. Dynamics of consumer–resource interaction: importance of individual attributes. In: Olff H, Brown VK, Drent RH, eds. *Herbivores: between plants and predators, 1st edn*. Oxford, UK: Blackwell Science Ltd, 521–550.

Nathanson J. 1984. Caffeine and related methylwanthines: possible naturally occurring pesticides. *Science* **226**: 184–187.

Núñez-Farfán J, Dirzo R. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. *Evolution* **48**: 423–436.

Núñez-Farfán J, Fornoni J, Valverde PL. 2007. The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology Evolution and Systematics* **38**: 541–566.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Parr AJ, Payne J, Eagles J, Chapman BT, Robins RJ, Rhodes MJ. 1990. Variation in tropane alkaloid accumulation within the Solanaceae and strategies for its exploitation. *Phytochemistry* **29**: 2545–2550.

Rhoades DJ. 1979. Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH, eds. *Herbivores: their interactions with secondary plant metabolites*. New York, NY, USA: Academic Press, 1–55.

Roberts MF, Wink M. 1998. *Alkaloids: biochemistry, ecology, and medicinal applications*. New York, NY, USA: Plenum Press.

Robins RJ, Parr AJ, Bent EG, Rhodes MJ. 1991. Studies on the biosynthesis of tropane alkaloids in *Datura stramonium* L. transformed rood cultures. *Planta* **183**: 185–195.

Roddick J. 1991. The importance of the Solanaceae in medicine and drug therapy. In: Hawkes JG, Lester RN, Nee M, Estrada RN, eds. *Solanaceae III: taxonomy, chemistry, evolution*. London, UK: the Royal Botanic Gardens, Kew, 7–23.

Rudgers JA, Strauss SY, Wendel JF. 2004. Trade-offs among anti-herbivore resistance traits: insights from Gossypieae (Malvaceae). *American Journal of Botany* **91**: 871–880.

Shonle I. 1999. *Evolutionary ecology of tropane alkaloids*, PhD thesis. University of Chicago, Chicago, IL, USA.

Shonle I, Bergelson J. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution* **54**: 778–788.

Stamp N. 2003. Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* **78**: 23–55.

Symon DE, Haegi L. 1991. *Datura* (Solanaceae) is a New World genus. In: Hawkes JG, Lester RN, Nee M, Estrada RN, eds. *Solanaceae III: taxonomy, chemistry, evolution*. London, UK: The Royal Botanic Gardens, Kew, 197–210.

Torres C, Mimosa M, Galetto L. 2013. Nectar ecology of *Datura ferox* (Solanaceae): an invasive weed with nocturnal flowers in agro-ecosystems from central Argentina. *Plant Systematics and Evolution* **299**: 1433–1441.

United Nations Environment Programme (UNEP). 1992. *World atlas of desertification*. London, UK: Edward Arnold.

Valverde PL, Fornoni J, Núñez-Farfán J. 2001. Defensive role of leaf trichome in resistance to herbivorous in *Datura stramonium*. *Journal of Evolutionary Biology* **14**: 424–432.

Valverde PL, Fornoni J, Núñez-Farfán J. 2003. Evolutionary ecology of *Datura stramonium*: equal plant fitness benefits of growth and resistance against herbivory. *Journal of Evolutionary Biology* **16**: 127–137.

Vermeij GJ. 1994. The evolutionary interaction among species: selection, escalation, and coevolution. *Annual Review of Ecology and Systematics* **25**: 219–236.

Wink M. 1993. Allelochemical properties or the *raison d'être* of alkaloids. In: Cordell G, ed. *The alkaloids*. San Diego, CA, USA: Academic Press, 1–118.

Wink M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* **64**: 3–19.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Ontogenetic variation on tropane alkaloids in the genus *Datura*.

Table S1 Ontogenetic trajectory ($\delta_{Di} = (\bar{S}_{t_2} - \bar{S}_{t_1})/\bar{S}_{t_1}$) of defensive traits in *Datura* spp.

Table S2 GenBank accession number of the sequences obtained for species of *Datura* and *Brugmansia*

Table S3 Variation between ontogenetic stages in alkaloid concentration and leaf trichome density of *Datura*

Table S4 Phylogenetic signal (*K*) using chemical and mechanical defensive traits in the genus *Datura*

Table S1. Ontogenetic trajectory [$\delta_{\mathrm{D}i} = \left(\overline{\mathrm{S}}_{\mathrm{t}_2} - \overline{\mathrm{S}}_{\mathrm{t}_1}\right)/\overline{\mathrm{S}}_{\mathrm{t}_1}$] of defensive traits in *Datura* spp. One unit indicates either an increase or decrease of 100% at the reproductive stage.

Sp	**Tropine**	**Hyoscyamine**	**Scopolamine**	**Atropine**	**Solanine**	**Total alkaloids**	**Leaf trichomes**
ceratocaula	6.73	1.07	0.16	1.91	1.29	0.48	0.72
discolor	10.28	10.91	8.57	0.83	-0.66	8.1	0.24
ferox	4.66	6	5.16	1.35	-0.175	5.08	-0.47
inoxia	8.83	53.68	11.16	0.57	0.7	9.68	-0.13
kymatocarpa	2.06	4.71	8.43	3.3	4.81	6.01	-0.67
lanosa	7.89	11.2	4.51	0.5	0.65	4.64	0.75
metel	4.68	36.9	12.43	1.94	1.72	8.71	0.72
pruinosa	4.56	2.21	0.44	0.72	-0.31	0.68	0.16
quercifolia	11.3	14.87	1.31	0.13	0.5	1.61	1.52
reburra	5.4	5.67	1.52	2.39	1.95	1.67	-0.84
stramonium	6.28	5.3	2.92	2.12	1.4	2.45	0.18
wrightii	11.23	12.17	12.82	8.39	-0.22	12.92	0.42

Table S2. GenBank accession number of the sequences obtained for species of *Datura* and *Brugmansia* (see text).

Genus	**Species**	**Accession number**		
		***psb*A-*trn*H**	***trn*L-*trn*F**	**rRNA ITS**
Brugmansia	*suaveolens*	--	JX467580	JX467597
Brugmansia	*candida*	JX467616	JX467583	JX467600
Brugmansia	*sanguinea*	JX467617	JX467584	JX467601
Datura	*wrightii*	JX467618	JX467585	JX467602
Datura	*lanosa*	JX467619	JX467586	JX467603
Datura	*metel*	JX467620	JX467587	JX467604
Datura	*discolor*	JX467621	JX467588	JX467605
Datura	*reburra*	JX467622	JX467589	JX467606
Datura	*inoxia*	JX467623	JX467590	JX467607
Datura	*ceratocaula*	JX467624	JX467591	JX467608
Datura	*kymatocarpa*	JX467625	JX467592	JX467609
Datura	*pruinosa*	JX467626	JX467593	JX467610
Datura	*stramonium*	JX467627	JX467594	JX467611
Datura	*quercifolia*	JX467628	JX467595	JX467612
Datura	*ferox*	JX467629	JX467596	JX467613

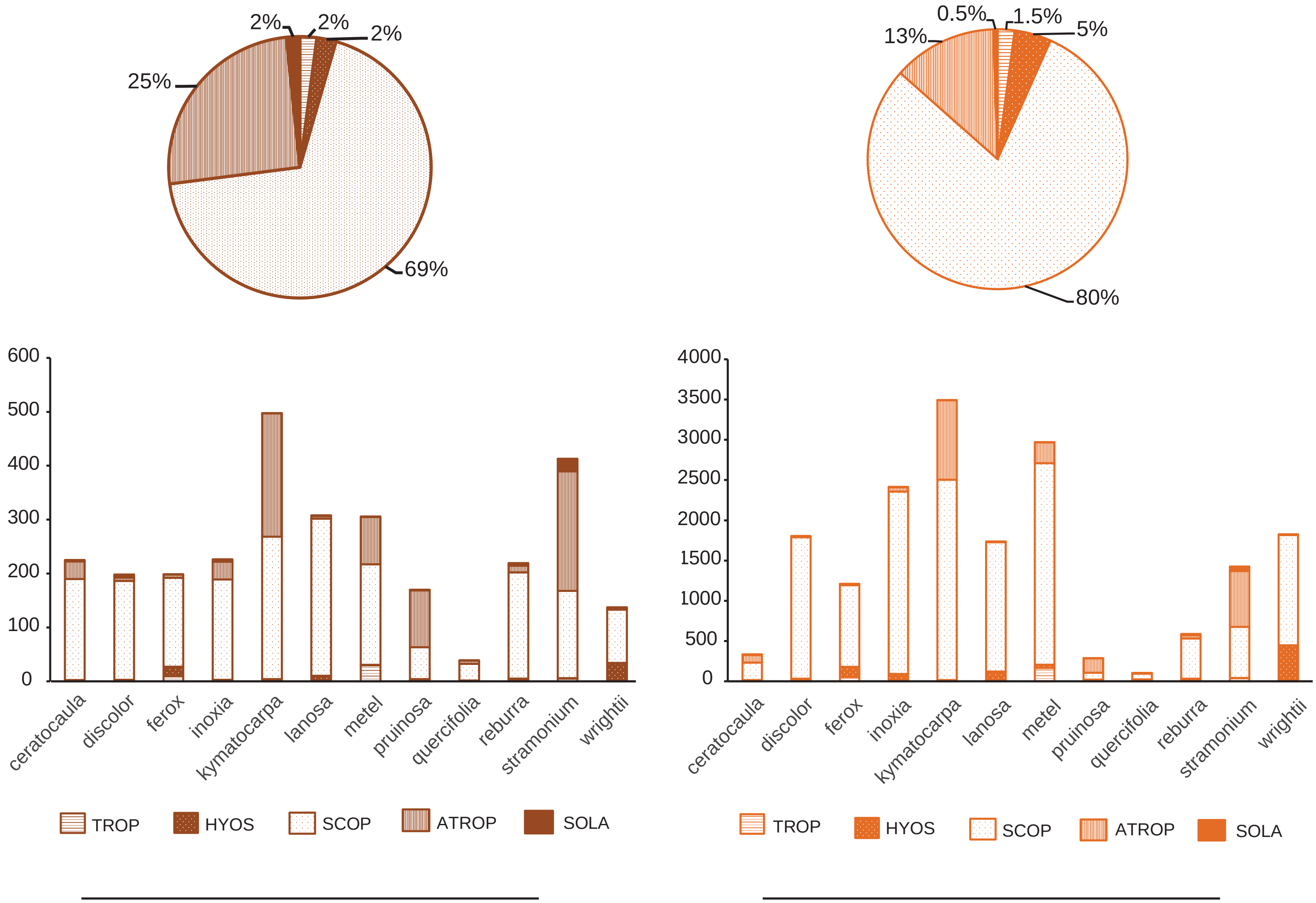
Table S3. Variation between ontogenetic stages in alkaloid concentration (µg g^{-1} dry wt) and leaf trichome density (cm^{-2}) of *Datura* spp. “1” indicates the juvenile stage (before flowering), “2” indicates the reproductive stage (during flowering). Significant values ($P< 0.05$) are indicated in bold.

	Tropine				**Hyoscyamine**			
Sp	$\bar{x}_{(1)}$± se	$\bar{x}_{(2)}$± se	***F***	***P***	$\bar{x}_{(1)}$± se	$\bar{x}_{(2)}$± se	***F***	***P***
ceratocaula	1.64(2.21)	12.7(2.21)	12.52	**0.007**	0.76(0.36)	1.58(0.36)	2.53	0.15
discolor	1.59(1.44)	17.93(1.44)	64.57	**<0.0001**	1.13(2.14)	13.58(2.14)	16.92	**0.002**
ferox	9.43(6.34)	53.35(6.34)	23.99	**0.0006**	17.83(31.83)	125.03(31.83)	5.67	**0.03**
inoxia	1.35(1.19)	13.3(1.19)	50.16	**<0.0001**	1.43(18.6)	78.19(18.6)	8.51	**0.01**
kymatocarpa	3.51(0.87)	10.77(0.87)	34.58	**0.0004**	0.56(0.26)	3.22(0.26)	50.6	**0.0001**
lanosa	1.55(3)	13.81(3)	8.3	**0.01**	8.67(20.59)	13.81(20.59)	11.13	**0.007**
metel	29.72(15.47)	168.83(15.47)	40.4	**<0.0001**	0.98(5.8)	37.41(0.98)	19.7	**0.001**
pruinosa	2.46(3.2)	13.71(3.2)	6.18	**0.03**	1.83(1.05)	5.88(1.05)	7.5	**0.02**
quercifolia	0.94(2.64)	11.58(2.64)	8.08	**0.01**	0.7(4.5)	11.14(4.5)	2.68	0.13
reburra	1.99(3.22)	12.79(3.22)	5.61	**0.03**	2.86(4.28)	19.1(4.28)	7.19	**0.02**
stramonium	5.08 (7.68)	37.04(7.01)	9.43	**0.01**	0.84(1.45)	5.30(1.32)	5.15	**0.04**
wrightii	1.58(4.66)	19.35(4.66)	12.52	**0.005**	32.43(187.22)	427.15(187.22)	7.31	**0.02**
Sp	**Scopolamine**				**Atropine**			
ceratocaula	187.61(84.19)	219.05(84.19)	0.06	0.79	32.28(18.22)	94.23(18.22)	5.77	**0.04**
discolor	183.76(328.66)	1758.68(328.66)	11.48	**0.006**	5.89(2.56)	10.82(2.56)	1.85	0.22
ferox	164.59(162.74)	1014.8(162.74)	13.64	**0.004**	6.42(3.3)	15.16(3.3)	3.5	0.09
inoxia	186.34(317.05)	2266.82(317.05)	21.52	**0.0009**	32.94(11.33)	51.97(11.33)	1.4	0.26
kymatocarpa	264.02(157.84)	2490.69(157.84)	99.5	**<0.0001**	229.26(80.77)	985.89(80.77)	43.88	**0.0002**
lanosa	291.58(290.78)	1607.95(290.78)	10.24	**0.009**	4.85(0.99)	7.29(0.99)	3.01	0.11
metel	186.28(324.6)	2502.96(324.6)	25.47	**0.0005**	87.98(28.17)	307.56(28.17)	18.38	**0.001**
pruinosa	59.05(25.45)	85.25(25.45)	0.53	0.48	105.13(45.2)	181.18(45.2)	1.41	0.26
quercifolia	30.89(29.74)	71.42(29.74)	0.92	0.36	6.03(2.54)	6.8(2.54)	0.04	0.83
reburra	197.47(141.34)	499.47(141.34)	2.28	0.16	12.03(19.66)	40.82(19.66)	1.07	0.32
stramonium	161.7(168.14)	634.01(153.5)	4.3	**0.06**	222.09(263.3)	694.3(240.4)	1.75	0.21
wrightii	99.01(286.39)	1130.75(286.39)	6.49	**0.02**	0.64(1.75)	6.04(1.75)	2.8	0.12
Sp	**Solanine**				**Total alkaloids**			
ceratocaula	2.9(1.21)	6.67(1.21)	4.84	**0.05**	225.21(93.23)	456.69(334.26)	0.68	0.43
discolor	5.7(1.15)	1.89(1.15)	5.51	**0.04**	198.09(332.01)	1802.92(332.01)	11.68	**0.006**
ferox	0.17(0.04)	0.14(0.04)	0.26	0.61	198.47(178.49)	1208.5(178.49)	16.01	**0.002**
inoxia	4.15(0.99)	7.1(0.99)	4.46	0.06	226.23(334.3)	2417.39(334.3)	21.48	**0.0009**
kymatocarpa	0.15(0.08)	0.87(0.08)	37.34	**0.0003**	497.52(185.01)	3491(185.01)	130.92	**<0.0001**
lanosa	1.12(0.33)	1.86(0.33)	2.41	0.15	307.79(313.07)	1736.79(313.07)	10.41	**0.009**
metel	0.81(0.35)	2.21(0.35)	7.8	**0.01**	305.79(363.89)	2970.22(363.89)	26.8	**0.0004**
pruinosa	1.53(0.27)	1.05(0.27)	1.6	0.23	170.01(69.18)	287.08(69.18)	1.43	0.26
quercifolia	0.153(0.06)	0.23(0.06)	0.7	0.42	38.72(35.91)	101.19(35.91)	1.51	0.25
reburra	5.17(3.63)	15.3(3.63)	3.87	0.07	219.54(144.58)	587.49(144.58)	3.23	0.1
stramonium	23.05(10.6)	55.28(9.67)	5.05	**0.05**	412.79(370.55)	1425.95(338.27)	4.07	0.07
wrightii	3.66(0.8)	2.84(0.8)	0.02	0.88	137.33(332.54)	1912.7(332.54)	14.25	**0.003**
Sp	**Leaf trichomes**							
ceratocaula	1896(530.07)	3269.73(530.07)	3.35	0.1				
discolor	457.19(63.87)	596.31(63.87)	1.54	0.24				
ferox	35.22(2.56)	18.33(2.56)	21.66	**0.0009**				
inoxia	586.84(120.6)	490.29(120.6)	0.21	0.65				
kymatocarpa	200.54(14.46)	65.09(14.46)	43.87	**0.0002**				
lanosa	718.73(95.05)	1262.59(95.05)	16.36	**0.002**				
metel	236.45(19.78)	408.79(19.78)	37.92	**0.0001**				
pruinosa	690.22(123.1)	800.07(123.1)	0.4	0.54				
quercifolia	107.27(17.06)	270.6(17.06)	45.82	**<0.0001**				
reburra	95.22(5.77)	14.62(5.77)	97.33	**<0.0001**				
stramonium	82.19(14.86)	97.04(14.86)	0.28	0.6				
wrightii	1215.22(222.7)	2208.92(222.7)	9.95	**0.01**				

Table S4. Phylogenetic signal (*K*) using chemical and physical defensive traits in the genus *Datura*. A *K* <1 indicates that species are less similar based on their phylogenetic relationships (weak phylogenetic signal). A *K*>1 indicates greater similarity based on the Brownian model of evolution (strong phylogenetic signal).

	Before flowering (juvenile stage)		**During flowering (reproductive stage)**	
Variable	***K***	***P***	***K***	***P***
Tropine	0.48	0.258	0.47	0.336
Hyoscyamine	0.47	0.40	0.6	0.087
Scopolamine	0.35	0.574	0.55	0.1
Atropine	0.49	0.365	**0.8**	**0.03**
Solanine	0.67	0.138	0.69	0.119
Total alkaloids	0.46	0.40	0.56	0.113
Leaf trichomes	0.62	0.064	0.48	0.225

Bold indicates the significant values ($P < 0.05$).



BEFORE FLOWERING (juvenile stage)

DURING FLOWERING (reproductive stage)

Figure S1. Ontogenetic variation on tropane alkaloids in the genus *Datura*.

Capítulo II

¿ESTÁ EL DESEMPEÑO DE HERBÍVOROS ESPECIALISTAS Y GENERALISTAS ASOCIADO DIFERENCIALMENTE CON LAS DEFENSAS Y NUTRIENTES DE LAS PLANTAS?

MANUSCRITO PREPARADO PARA *The American Naturalist*

IS PERFORMANCE OF SPECIALIST AND GENERALIST HERBIVORES DIFFERENTIALLY ASSOCIATED WITH PLANT DEFENSES AND NUTRIENTS?

Eunice Kariñho-Betancourt[1], Alejandra Vázquez-Lobo[2] and Juan Núñez-Farfán[1]

[1]Laboratorio de Genética Ecológica y Evolución, Instituto de Ecología, Universidad Nacional autónoma de México, Circuito Exterior, Ciudad Universitaria, 04510 México city, México.

[2]IByC, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001 Col. Chamilpa C.P. 62209, Cuernavaca, Morelos, México.

Key words: alkaloids, coevolution, herbivore performance, *Lema trilineata*, plant defense, plant nutrients.

Appendix A

ABSTRACT

As a function of the degree of specialization, herbivores are predicted to be differentially affected by either defensive or nutrient traits of plant related species, and thus perform differentially across plant taxa. However, assessing both the effects of plant defenses and nutrients on herbivore´s performance would provide a more complete view of the evolutionary dynamics of plant-herbivore interaction. We assessed the performance of the specialist herbivore *Lema trilineata daturaphila* and the generalist *Spodoptera frugiperda*, across eleven species of *Datura*, and how this performance relates to plant traits. We used phylogenetic principal components analysis, and constructed permutation models to examine the relationship of tropane alkaloids, leaf trichomes, and plant macronutrients to four components of herbivore performance. We found that across *Datura*, the specialist herbivore performed better than the generalist. Our results shown a differential effect of specific plant defenses and nutrients on herbivores, which vary as function of the performance component, and the specific defensive/nutritional trait. These contrasting pattern of herbivore performance, along with differential effects of plant defensive traits and nutrients, suggests (1) adaptive differences between herbivore insects to cope with plant toxins, and to achieve nutrient balance. And, (2) evolutionary trade-offs in plant defenses to deal with *Datura*´s consumers.

INTRODUCTION

The herbivore feeding habit of nearly 50% of all insect species described (Dethier 1954; Shoonhoven et al. 2005), include generalist (polyphagous) herbivores that feed on many different species, and specialist (monophagous) herbivores that feed on a restricted group of related host species (Strong et al. 1984; Mitter et al. 1988; Rasmann and Agrawal 2011). The fact that primary nutrients of plants are blended with non-nutritive, and even toxic compounds (i.e. phytochemicals), is one of the main challenges for phytophagous animals to overcome. Given the negative effects of such chemical compounds on plant consumers (Fraenkel 1959; Bennet and Wallsgrove 1994; Mithöfer and Boland 2012), they are regarded as plant *defensiv*e traits, and along with structural attributes of plants (e.g. leaf toughness or leaf trichomes; Harborne 1988; Fritz and Simms 1992), they have been strongly implicated in the evolution of feeding strategies (i.e. monophagy and polyphagy) of herbivorous insects (Ehrlich and Raven 1964; Shoonhoven et al. 2005), and coevolutionary dynamics of plants and herbivores (Ehrlich and Raven 1964; Fenny 1976; Rhoades y Cates 1976; Janzen 1980; Mauricio and Rausher 1997).

Classical coevolutionary theory predicts a contrasting performance of herbivores based on differences in their shared (co)evolutionary history with plants (i.e. degree of specialization). Whereas specialist herbivores are expected to be adapted to defenses of their host plant and perform better on related species (or chemically-like plant taxa), than across unrelated plant taxa, generalist herbivores are expected to lack of the adaptive mechanisms to cope with plant defenses, being thus more susceptible to their negative effects. Numerous studies have provided support for these predictions, documenting, on one hand, diverse physiological counter-measures of specialist herbivores to not only avoid defenses of their hosts (van Dam et al. 1995; Berenbaum and Zangrel 1998; Ali and Agrawal 2012), but to also use such defenses (mostly chemical) as cues for host finding or protection from predators (e.g. sequestration or fecal shields) (Ali and Agrawal 2012). And, on the other hand, studies have documented an often deterrent or toxic effect of secondary compounds on generalist herbivores (e.g. Roslin and Salminen 2008). However, some generalist insects have shown the ability to consume highly toxic host plants (Dussourd and Denno 1994; Hartmann et al. 2005). Moreover, cases where specialist herbivores are negatively affected by defense compounds have also been documented, suggesting that high levels of certain secondary metabolites are likely to be effective on herbivores despite its degree of specialization (Ali and Agrawal 2012). Hence, although abundant experimental evidence for adaptation of insects to defenses of their host plants exist (Karban and Agrawal 2002), and that defensive traits have often been used to predict performance of herbivores (e.g. Lankau 2007; Rassman and Agrawal 2011; Carmona et al. 2011), it is unclear whether certain levels, or classes, of defensive traits (physical or chemical) are more effective against specialist or generalist herbivores (Ali and Agrawal 2012).

In addition to plant defenses, nutrient balance has been considered central in the evolution of polyphagy (Kester and Smith 1984; Bernays and Minkenberg 1997). Since generalist herbivores accomplish their optimal nutrient requirements by mixing foods (e.g. Bernays and Minkenberg 1997), mismatch of insect foods to nutritional needs would increase when generalist herbivores feed from a restricted group of plants (e.g. Kester and Smith 1984). Hence, it has been predicted a differential effect of a *monospecific* diet as a function of the degree of herbivore specialization. However, based on herbivore specialization, no explicit predictions exist as to whether qualitative (e.g. presence/ absence of essential nutrients), and/or quantitative nutrition (e.g. intake requirements, and nutrient balancing of suboptimal foods), may affect specialist herbivores as well. Yet, empirical evidence have shown that not only plant defenses or nutrients, but their interplay, is key for understanding herbivore performance (Slansky 1992; Simpson and Raubenheimer 2001), and ultimately the evolutionary dynamics of plant-herbivore interactions.

In this study, we examine the performance of herbivores differing in its degree of specialization to *Datura* species, and how this relates to plants' defense and macronutrients. This is the first attempt to empirically assess, across a plant phylogeny, both the effects of diverse *classes* (chemical and physical) of defensive traits, and plant nutrients, as a function of herbivore specialization. Species of the genus *Datura* are known to produce a diverse array of highly toxic tropane alkaloids (Evans 1979). These compounds negatively affect plant consumers (e.g. Shonle 1999) by inhibiting enzymatic activity (Colegate et al. 1979; Nathanson 1984), and compete for muscarinic acetylcholine receptors (Roddick 1991). We thus employ *Datura* species to test the hypothesis that, given that generalist herbivores are typically more sensitive to plant toxins than specialist, the latter should overall performed better on *daturas* than the former. Since *Datura* exhibits great variation in traits associated to plant defense, i.e. concentration of tropane alkaloids and leaf trichome density (Kariñho-Betancourt et al. 2015), we then ask, if performance of specialist and generalist herbivores is associated with a single, or a set of chemical and/or physical defensive traits. Finally, we ask if also macronutrients of *Datura*'s leaves are associated with performance of herbivores.

This study assessed the response of herbivores to plants of *Datura* species proceeding from the same natural populations and growth under the same conditions than those from which chemical and physical defenses were previously characterized (Kariñho-Betancourt et al. 2015). Here, we combine our newly collect data of herbivore performance and plant nutrients, with the previously collected measures of plant defensive traits.

METHODS

Study system

We used *Datura* (Solanceae) as a model for studying the pattern of food consumption and growth of herbivores in relation to plant chemical and physical defenses and macronutrients. The genus *Datura* is a small group of 13 to 14 species (e.g. Avery et al. 1959; Luna-Cavazos and Bye 2011) of annual herbs and perennial shrubs mostly distributed in Mexico, which is considered its center of origin (Symon and Haegi 1991). *Datura* species are native to dry, temperate, and subtropical regions of America (Barclay 1959), and typically grow in nitrogen rich soils and disturbed habitats, although one species, *D. ceratocaula* is semiaquatic. Unlike the self-incompatible sister clade *Brugmansia*, *Datura* species have a mixed mating system.

Datura species are attacked by a wide diversity of generalist and specialist herbivores, including the specialist folivores *Epitrix parvula* (Coleoptera: Chrysomelidade), *Lema trilineata daturaphila* (Coleoptera: Chrysomelidade), *Manduca sexta* (Lepidoptera: Sphingidae), and *Tupiocoris notatus* (Hemiptera: Miridae) among others. And, specialist seed predators weevils, *Trichobaris soror* and *T. compacta* (Coleoptera: Curculionidae) (Núñez-Farfán and Dirzo 1994; Elle and Hare 2000; Hare and Elle 2002). Also, in populations of central Mexico plants are usually attacked by the generalist folivore *Shpenarium purpurascens* (Orthoptera: Pyrgomorphidae) (Núñez-Farfán and Dirzo 1994). *Datura* exhibits great variation in leaf trichomes and tropane alkaloids, within (Hare and Elle, 2002; Castillo et al. 2014), and among species (Parr et al. 1990; Kariñho-Betancourt et al. 2015), and these traits have been linked to plant resistance against natural enemies (Valverde et al. 2001; Valverde et al. 2003; Castillo et al. 2013; Kariñho-Betancourt and Núñez-Farfán 2015). Herbivores have a negative impact on plant fitness (Núñez-Farfán and Dirzo 1994; Valverde et al. 2001; Fornoni et al. 2004), and can exert contrasting selection on chemical defenses (Shonle and Bergelson 2000; Castillo et al. 2014). Also, specialist and generalist herbivores can be differentially affected by certain chemical defenses. For instance, in *Datura stramonium* the tropane alkaloid scopolamine negatively affects leaf consumption by the generalist herbivores: *Helicoverpa zea* (corn earworm), and *Popilia japonica* (Japanese beetle), and the specialist *Lema trilineata daturaphila* (Castillo et al. (2014) and at the same time acts as phagostimulant for the specialist flea beetle *Epitirix parvula* (Shonle and Bergelson 2000).

Experimental design

Our study focused on performance of chewing larvae of two herbivores differing in its degree of specialization on *Datura*: (1) the specialist herbivore, the three-lined potato beetle *L. trilineata daturaphila* (Coleoptera: Chrysomelidae) (Kogan and Goden 1970), and the generalist herbivore, the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctunidae) (fig. 1), that commonly feed on crop species (e.g. *Zea mays*), and has been usually employed to test effects of diverse chemical compounds (e.g. Céspedes et al. 2000; Gómez et al. 2009). This herbivore has also been recorded feeding upon *D. stramonium* in Central Mexico (J. N-F pers. obs. 1989).

FIGURE 1. (a) Larvae of the specialist herbivore *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae), and (b) the generalist herbivore *Spodoptera frugiperda* (Lepidoptera: Noctuidae).

Rearing of larvae

Eggs of *L. trilineata daturaphila* were collected from two natural populations of *D. stramonium* in central Mexico*,* and reared on sterilized moist soil in sealed glass jars (5 L). Larvae were fed with fresh leaf tissue of *D. stramonium* brought from naturally grown plants, stored within the jar, and replaced daily until pupation (fig. A1*a*), at 25± 1 °C, > 70% relative humidity, with a 12:12 photoperiod of light: dark. After eclosion, adults were moved to different jars (under the same conditions), and were allowed to mate during 15-20. After eggs were laid (F_1), they were collected and transferred to a different jar. We followed the same protocol to obtain F_2 larvae. *S. frugiperda* eggs from laboratory strains were established in moist filter paper in sealed Petri dishes (9 cm). Hatched larvae were reared on an artificial diet (Céspedes et al. 2000; Gómez et al. 2009) at 27± 1 °C, > 70% relative humidity, with a 12:12 photoperiod of light: dark.

Bioassays

To perform the bioassays with the specialist and generalist larvae, we employed 11 terrestrial species of *Datura*. Plants were grown in a greenhouse by sowing seeds of maternal half-sib families (natural progenies; Lawrence, 1984) into pots filled with sterilized commercial soil. Plants were daily watered and regularly rearranged to avoid positional effects. Since previous studies indicate that, in *Datura*, most specialist and generalist herbivores are synchronized with plant reproduction (Espinosa and Fornoni 2006; Carmona and Fornoni 2013), herbivores were exposed to experimental plants at the onset of flowering. We selected ten experimental plants of similar size per species to feed larvae of each *type* of herbivore (i.e., specialist, generalist). Each plant was transferred to a single pot, and for the specialist experiment plants were spaced 60 cm away from each other to avoid contact among plants, and thus potential larvae transfer while they fed.

L. trilineata daturaphila and *S. frugiperda* larvae of second-fourth instar were fed with leaf tissue of each *Datura* species. Larvae were starved 12 h before they were fed on daturas. Larvae of *L. trilineata daturaphila* were directly exposed to plants at the greenhouse. Two larvae (F_2) per plant (10 plants per species) were settled at the adaxial side of leaves of each *Datura* species. Every larva was set on a leaf located at opposite ends of the plant, and each leaf was marked with a distinctive color corresponding to each larva (larva 1: yellow; larva 2: blue) (fig. A1*b,c*). Thus, in order to register larvae damage accurately, every leaf on which each larva fed was color-marked, and feeding larvae were recorded and monitored every two hours. Since, larvae occasionally fell (mostly when feed upon pubescent leaves), a plastic stencil was overlaid on top of the pots, covering the soil to avoid the larva buried into the soil, and to facilitate its detection. Hence, whenever a larva was found *off the plant*, it was collected and placed in a non-damaged leaf of the same plant. After 48 h the experiment was concluded, and larvae and leaves were collected.

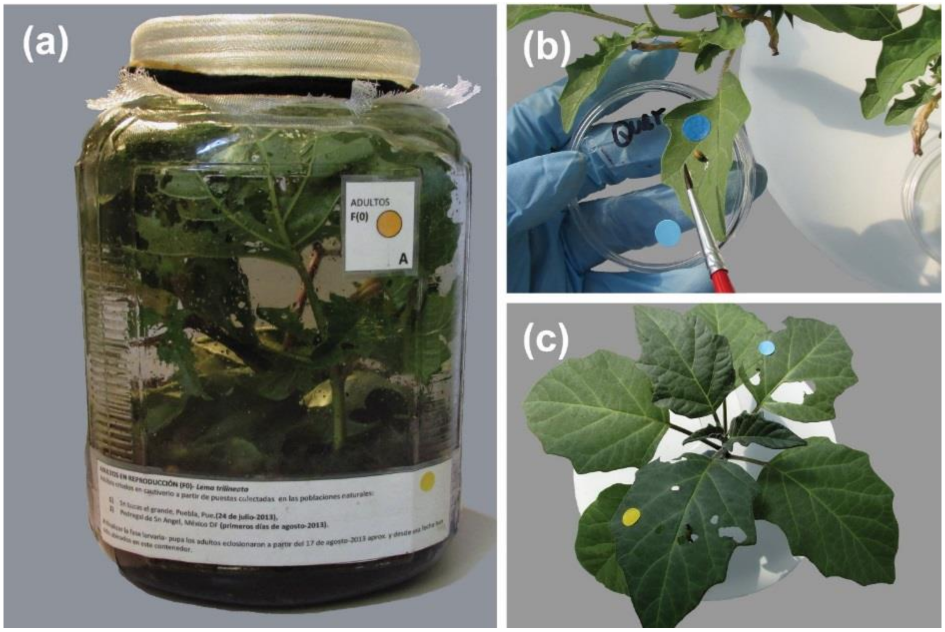


FIGURE A1. Experiment of the specialist herbivore *Lema trilineata daturaphila*. (a) Rearing of larvae, (b) setting of larvae at the abaxial side of the leaf, and (c) leaf marking and consumption by larvae.

The experimental design was modified for *S. frugiperda*, since a pilot experiment showed that larvae of this species feed and move quicker through the plant than *L. trilineata daturaphila* larvae, but not consumed a significantly different amount of food when fed directly on the plant than within a Petri dish ($t_{1,58} = 2.001$, $P = 0.512$). Thus, each *S. frugiperda* larva was fed individually in a Petri dish. As in the experiment with the specialist herbivore, two larvae were feed upon a single plant. Fresh leaf tissue from each *Datura* species was provided *ad libitum*, and regularly replaced when withered during 48 h. At the end of the experiment larvae and leaves were collected.

To measure larval growth (g) of both specialist and generalist herbivores, we weighted each larva at the start (0 h) and end (48 h) of the experiment. All larvae weights are hence expressed as fresh weight. To estimate total food /leaf tissue ingested (cm^2) by a larva during 48 h period, we measured the damaged area (A_D) of all leaves consumed per larva, by using a leaf area meter (Winfolia, Regent Instruments Inc.).

Herbivore measures

To identify different components of larval performance, we split food consumption and utilization into three components (Waldbauer 1968; Garrido et al. 2012).

A. Food consumption (cm^2): FC

B. Relative biomass increment: BI

$$\left[\frac{\text{final weight}\ (\mathrm{wt}_f)}{\text{initial weight}\ (\mathrm{wt}_i)}\right] - 1$$

C. Growth efficiency (efficiency of conversion of ingested food to body substance): GE

$$\frac{\text{biomass increment (g wwt)}}{\text{food ingested}\ (\mathrm{cm}^2)}$$

Additionally, as a measure of larva mobility during feeding, and thus an indirect indicator of the amount of energy spent, we counted the number of leaves damage by larva.

D. Number of damaged leaves: DL

Plant measures

In order to quantify macronutrients of *Datura* leaves, a sample of oven-dried and ground leaves (6 g) per species was analyzed for Nitrogen (N), Phosphorus (P), and Carbon (C) using colorimetric techniques (Reuter et al. 1986; Benton et al. 1991). Also, we recorded the wet and dry weight of leaves, to calculate water content (W) per species.

For plant defensive traits, we quantified average measures per species of five tropane alkaloids concentration (hyoscyamine, scopolamine, atropine, tropine and solanine), and leaf trichome density of 11 terrestrial species of *Datura,* harvested at the reproductive stage (Kariñho- Betancourt et al. 2015).

Statistical analysis

The variation in performance between specialist and generalist herbivores and the effect of plant species on the components of herbivore performance were assessed by a mixed model ANOVA, where (1) food consumption, (2) the number of damaged leaves, (3) the relative biomass increment, and (4) growth efficiency were the response variables. Plant species and *type* of herbivore (specialist and generalist), were the random and fixed effects, respectively.

To assess the relationship of plant traits and pattern of herbivores performance across *Datura*, we used a molecular phylogeny of the 11 *Datura* species (Table A1, fig. 2), constructed with chloroplast (*psb*A-*trn*H and *trn*L-*trn*F) and nuclear (ITS rRNA) datasets (see Kariñho-Betancourt et al. 2015). First, to examine the association of plant traits with herbivore performance we carried out a phylogenetic principal component analysis (pPCA; Revell 2009) using the *phyl.pca* function, analyzing the correlation matrix under the Brownian motion method employing the *phytools* program (Revell 2012) in R 3.02 package (R Development Core Team 2008). The phylogenetic correction in pPCA adjust the covariance/correlation matrix to remove the expected phylogenetic correlation among traits. Hence, the axes in pPCA describe the non-phylogenetic component of shape covariance and, as a result, are independent from phylogeny and orthogonal to one another (Revell 2009).The pPCA included the plant defensive and nutrient traits of *Datura* spp., and the performance components of the specialist herbivore *L. trilineata daturaphila* and the generalist *S. frugiperda*.

To identify the trait or set of traits that better explain the performance of either the specialist or generalist herbivores, we executed, for each performance component of herbivores, a permutation procedure. We split the predictor variables into defense and nutrient traits. Based on the pPCA, only most informative chemical traits [i.e. hyoscyamine (atropine), scopolamine, and total alkaloids], and leaf trichomes were included. We thus correlated plant traits with herbivore performance across *Datura* species. Phylogenetic correlations were obtained using the generalized leas-squares (GLS) method for independent contrast (Garland et al. 1999; Pagel 1999). For each group of plant traits (nutrients/defenses) we created models of all possible combination of variables (phylogenetic independent correlations) and calculated value of Akaike´s information criterion (AIC) as a measure of model fit (Burnham and Anderson 2002). The best out of all models tested to explain herbivore performance was that with the lowest AIC.

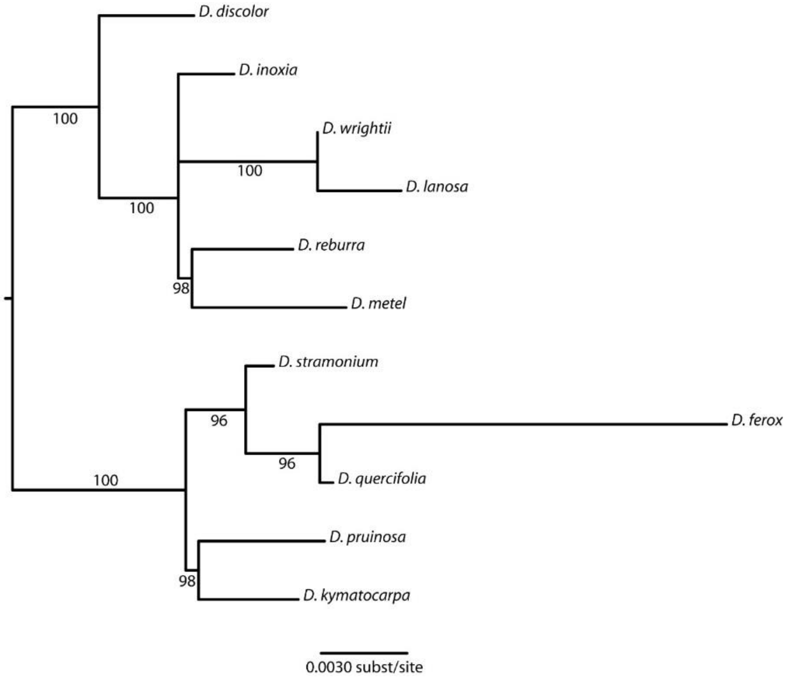


FIGURE 2. Maximum Likelihood phylogram of the eleven species of *Datura* based on chloroplast (*psb*A-trnH and *trn*L-trnF regions) and nuclear (ITS rRNA) sequences. Branch length was optimized by maximum likelihood. Numbers under branches indicate bootstrap values >70 (Núñez-Farfán et al. 2015, in prep).

RESULTS

We found significant differences in performance between the specialist herbivore *Lema trilineata daturaphila*, and the generalist *Spodoptera frugiperda,* within and among *Datura* spp. (fig. 3, A2). Among species of *Datura*, the specialist herbivore performed better in all performance components. Although *S. frugiperda* damaged on average, more leaves (fig.3).

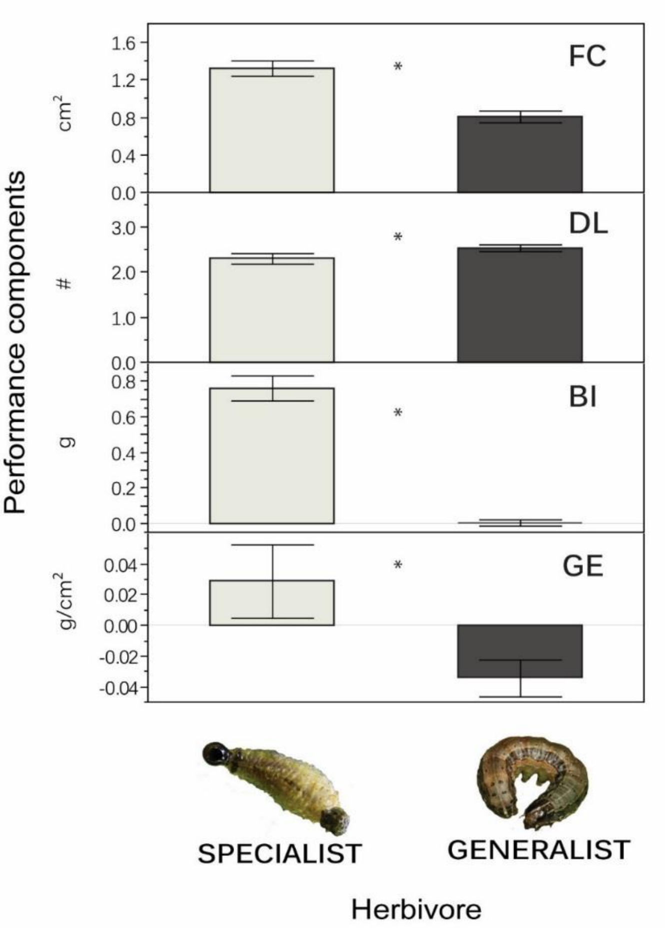


FIGURE 3. Variation in performance components of the specialist herbivore *Lema trilineata daturaphila* and the generalist *Spodoptera frugiperda*. Symbols: food consumption (FC), number of damaged leaves (DL), biomass increment (BI), and growth efficiency (GE), across eleven *Datura* spp. (*, P< 0.05).

Datura species (e.g. *Datura wrigthii*, *D. pruinosa*) in which the specialist herbivore performed better in most components (e.g. biomass increment and food consumption), the generalist did not. Likewise, *Datura* species in which the generalist performed better (e.g. *D. quercifolia*) the specialist did not (fig. A2). However, although we found a significant effect of the type of herbivore (specialist/generalist) on every performance component, the interaction of the type of herbivore between plant species (i.e. differences in herbivore performance as a function of plant species) was no significant for the growth efficiency (Table 1).

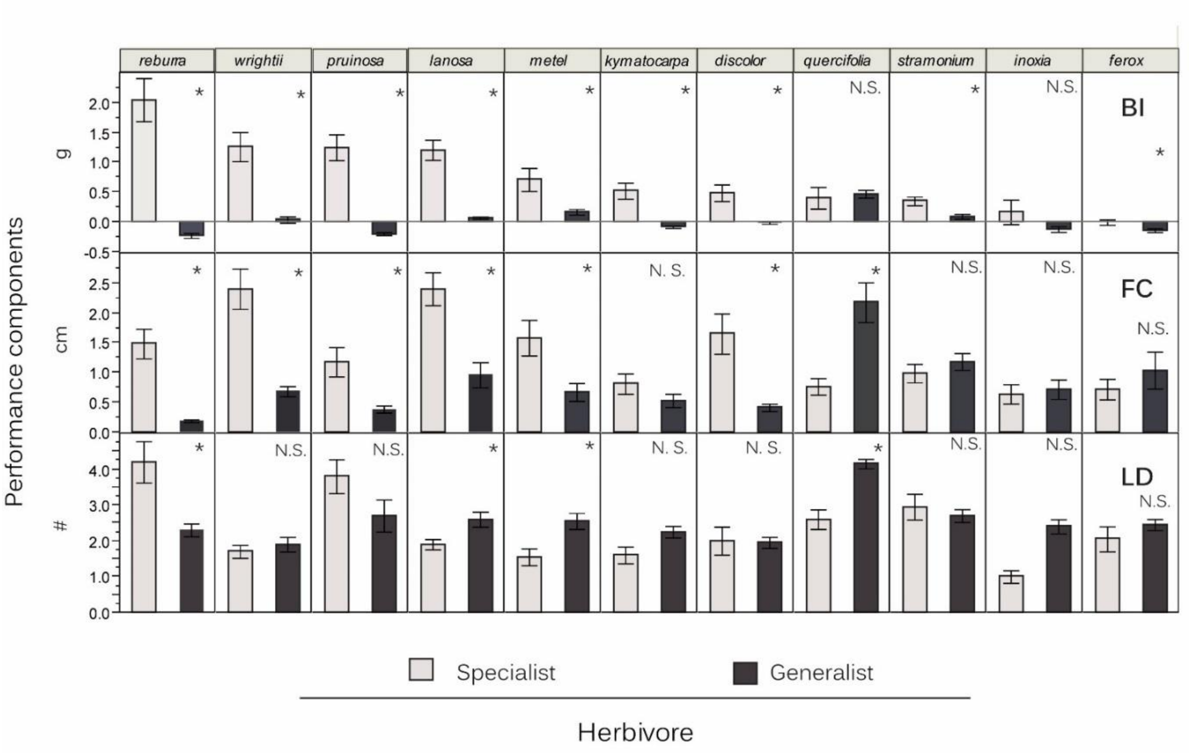


FIGURE A2. Variation of BI (biomass increment; g), FC (food consumption; cm^2), and DL (damaged leaves; number), between the specialist herbivore *Lema trilineata daturaphila* and the generalist herbivore *Spodoptera frugiperda* across *Datura* spp. (*, $P< 0.05$).

Herbivore performance and plant traits

Phylogenetic principal component analysis (pPCA) indicated that pPC1 and pPC2 accounted for 85% of the total variation of components of herbivore performance and plant attributes. In the first pPC, with greater weight (correlation coefficient > ±0.5) are defenses such as scopolamine, atropine, and total alkaloid concentration, and plant nutrition-related traits such as Nitrogen (N) and water content (W). This variables are associated to the number of damaged leaves of both the specialist and generalist herbivores, and biomass increment of the specialist. pPC2, indicates that hyoscyamine and the water content (W) had the greater loadings and were associated only to performance components of the generalist herbivore (Table 2, fig.4). The pPCA, overall indicates that all components of performance but growth efficiency of the specialist herbivore *L. trilineata daturaphila*, are positively associated with hyoscyamine and Nitrogen (N). And negatively associated with scopolamine, atropine, total alkaloid concentration, and phosphorous (P). The performance of the generalist herbivore *S. frugiperda* is positively associated to phosphorous (P), and negatively associated with hyoscyamine, and water content (W) (fig. 4).

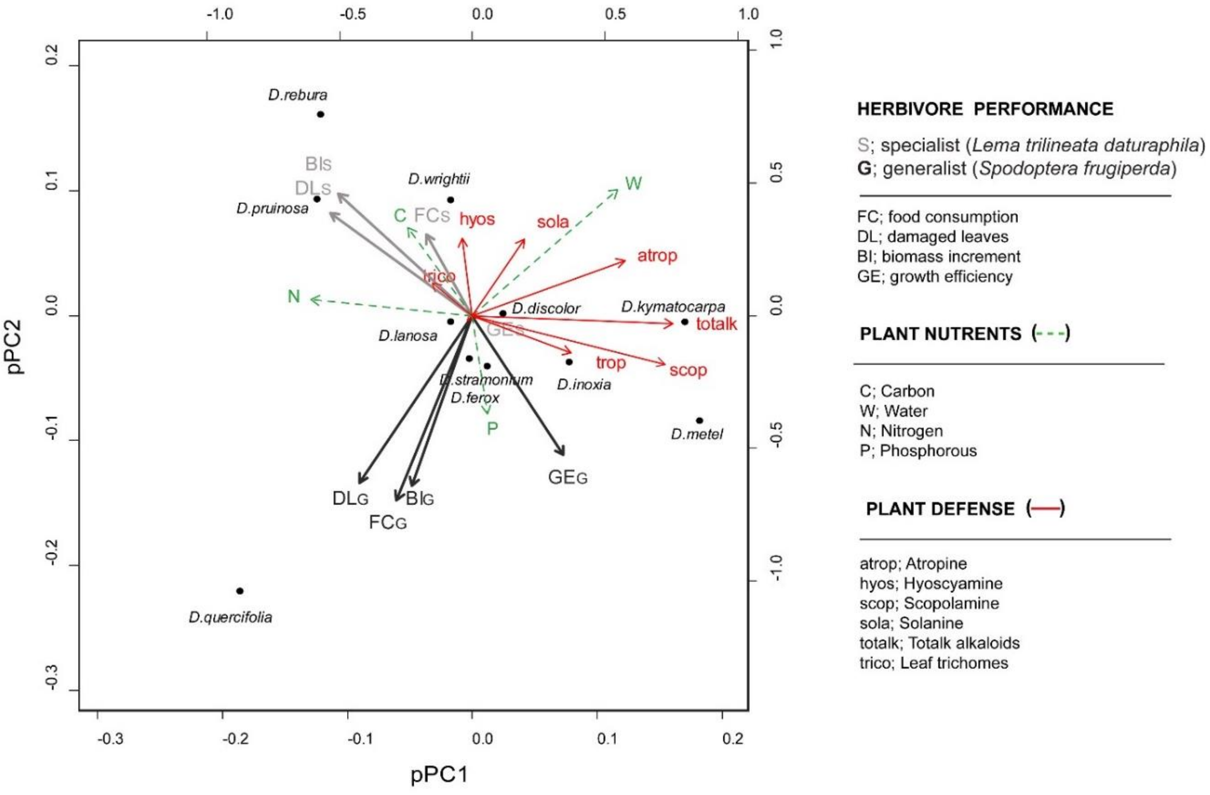


FIGURE 4. Phylogenetic principal components analysis (pPCA) of herbivore performance, plant nutrients and defenses.

Permutation method was mostly congruent with association between plant traits and herbivore performance revealed by pPCA. For plant defenses, simple models including leaf trichomes and a tropane alkaloid were the best fitted. But, only models including scopolamine were significant, indicating a negative relationship of this alkaloid with biomass increment and the number of damaged leaves by *L. trilineata daturaphila* (Table 3, A3). For plant nutrients, models including Carbon (C) were the best fitted (i.e. lower AIC value, Table A3). And, only the model for food consumption was significant, indicating a negative correlation of this performance components of the specialist herbivore and phosphorous (P). For *S. frugiperda*, models of plant defenses including leaf trichomes had the best fit. And only models for growth efficiency and number of damaged leaves that included scopolamine, leaf trichomes, and total alkaloid concentration, were significant. Suggesting these, a negative effect of those defensive traits on performance of the generalist herbivore. Nutrient models indicate that carbon (C) is negatively correlated whit growth efficiency, water content (W) is negatively correlated with the number of damaged leaves, and both plant variables are also negatively correlated with biomass increment of the generalist herbivore (Table 3, A3).

DISCUSION

We found, overall, that the specialist herbivore *Lema trilineata daturaphila*, performed better than the generalist *Spodoptera frugiperda* when feeding on plant species of *Datura*. Even when the generalist herbivore did consume a substantial amount of leaf tissue of *daturas*, its biomass increment was almost null, and the potential energy expended during feeding (number of damaged leaves) was higher to that of *L. trilineata daturaphila*, resulting in a negative growth efficiency.

Consistent with theory predicting a better performance of specialized herbivores on their host plants (e.g. Ehrlich and Raven 1964; Janzen 1980) than those non-specialized (i.e. generalist), these findings suggest adaptive differences (physiologic, metabolic and/or behavioral) between herbivore insects to cope with plant toxins, and to achieve nutrient balance (Cornell and Hawkins 2003; Thompson and Simpson 2009). Such adaptive differences between plant consumers have been predicted to be the result of a contrasting effect of (1) plant defensive traits, mostly positive effect for specialized herbivores, and negative for non-specialized (Ehrlich and Raven 1964; Futuyma and Moreno 1988), and (2) nutrients of a *monospecific* diet (negative effect for non-specialized herbivores; Bernays and Minkenberg 1997). Our results, however, showed a more complex relationship between plant traits and performance of herbivores. The effects of plant traits on performance of the specialist vs. the generalist herbivores, although differential, are not necessarily contrasting, but varying as a function of the specific plant/nutrient trait and the performance component.

Herbivore performance and plant traits

The relationship of *L. trilineata daturaphila*, and *S. frugiperda* performance, with defenses of *Datura* can be summarized as follows: (1) *Datura*´s scopolamine, total alkaloids content and leaf trichomes, can negatively affect (different) performance components of both, the specialist and generalist herbivore. Scopolamine had a negative relationship with biomass increment and the number of damaged leaves by the specialist herbivore, whereas leaf trichome density and the concentration of total alkaloids had a negative relationship to the growth efficiency and the number of damaged leaves by the generalist herbivore. (2) At the same time, as predicted by theory, certain alkaloids can also contrastingly affect specialist and generalist herbivores. The pPCA revealed a positive association of hyoscyamine with most performance components of the specialist herbivore, but a negative association with performance the generalist. These differential effects of chemical and physical defenses on different components of the performance of herbivores, and the contrasting association of one alkaloid with them, suggests that the adaptive value (efficiency) of certain defenses of *Datura* is likely related to the (co)evolutionary history shared with their herbivores (i.e. degree of specialization). Such differential effects of plant defenses on herbivores also suggest that, as documented by previous studies (e.g. within species; Lankau 2007, across clade; Kariñho-Betancourt et al. 2015), plants face a compromise (trade-off) between defend themselves against generalist herbivores while attracting coevolved specialists. The evolutionary consequences of such a trade-offs in plant defense are particularly important, possibly constraining the simultaneous evolution of different defensive traits (van der Meijden et al. 1988; Kursar and Coley 2003). On this regard, ecological and evolutionary outcomes of *Daura*-herbivore interaction, would depend on the herbivores community, hence the expected selective pressures exerted on specific defensive traits (see Castillo et al. 2015).

The specialist herbivore *L. trilineata daturaphila* showed a positive relationship with the toxic alkaloid hyoscyamine, and previous studies at intraspecific level has shown a positive relationship between atropine (the racemic form of hyoscyamine) and two specialized herbivores of *Datura*, including *L. trilineata daturaphila*; in fact, atropine concentration is selected against in populations of *D. stramonium* herbivorized by specialist herbivores but scopolamine is positively selected (Castillo et al. 2014). However, our results contrast with the phagoestimulant effect of scopolamine on specialized herbivores documented in *D. stramonium* (Shonle and Bergelson 2000). Nevertheless, our results agreed with empirical evidence either across phylogeny (Kariñho-Betancourt et al. 2015), or within *Datura* species (e.g. Shonle and Bergelson 2000; Valverde et al. 2001; Hare and Elle 2002; Castillo et al. 2014), suggesting that major alkaloids hyoscyamine (atropine) and scopolamine and leaf trichomes, play a key role mediating *Datura*-herbivore interactions, functioning either as defense for both specialized and non-specialized herbivores, or as cues only for specialist.

Although our results reveal that differences in performance of herbivore insects are related to plant defenses, macronutrients of *Datura* were also related to performance of *L. trilineata daturaphila*, and *S. frugiperda*. Like plant defenses, plant nutrients had (1) a differential effect on performance components of herbivores [e.g. the water content (W) and Carbon (C), were negatively correlated to performance components of *S. frugiperda*], and (2) a contrasting effect (of specific plant nutrient traits) on herbivores´ performance. For instance, whereas phosphorous (P) was negatively associated to almost all performance components of the specialist herbivore, it was positively associated with most generalist´s performance components. These results may suggest evolutionary differences in how herbivores balance food, and regulates growth and metabolism (Bernays and Bright 1993; Raubenheimer and Simpson 1999). And, since the generalist herbivore lost weight when feeding on *Datura*, this points to a mismatch between the food quality of *daturas* (e.g. absence of specific essential nutrients) and the nutritional requirements of the generalist herbivore. Empirical evidence has shown that suboptimal nutritional balance may increment effectiveness of plant defenses (Thompson and Simpson 2009). For instance, in the tobacco hornworm caterpillars, *Manduca sexta*, an optimal macronutrients ratio seems to modulate the toxic effects of the nicotine alkaloid (Thompson and Redak 2007). Hence, it is possible that patterns of consumption, growth, and efficiency of herbivores documented in this study in *Datura*, reflect the interaction between alkaloids, leaf trichomes, and macronutrients.

CONCLUSION

This study portrays the first experimental evidence across phylogeny of differences in performance between herbivores differing in its degree of specialization, as a function of diverse (chemical and physical) plant defenses and nutrients. We showed that the specialist herbivore *Lema trilineata daturaphila* performed better than the generalist *Spodoptera frugiperda*, across and within species. These contrasting pattern of herbivore performance, along with differential effects (at times contrasting) of plant defensive traits and nutrients, suggest (1) adaptive differences between herbivore insects to cope with plant toxins, and how they achieve nutrient balance, (2) evolutionary trade-offs in plant defenses to deal with diverse *Datura*´s consumers. And, (3) an interplay of plant nutrients and defenses to reduce nutritional balance of herbivores, and thus increase effectiveness of defenses. Patterns of herbivore performance here documented, reflect the evolutionary and ecological dynamics of *Datura*-herbivore interaction, and provide an integrative view of relationships among different components of herbivore performance and plant trait.

AKNOWLEDGMENTS

We thank Citlalli Mendoza and Lourdes Martínez for valuable assistance raring specialist larvae and helping with data collection. We thank R. Tapia-López, Adriana Pérez, Laura J. Giraldo, and Rafael Torres for logistic support and data collection, and M. A. Serrano-Meneses for his valuable advice with statistical analysis. We also thank the *Laboratorio de Fertilidad de Suelos y Química Ambiental* for helping us with nutrient quantification, and the Chemical Ecology Group´s Core Facility in Cornell University for alkaloid quantification. This research was funded by a CONACyT grant (*Evolución adaptativa en Datura: Resistencia y tolerancia a los herbívoros*) to J. N-F. Grants from USA NSF-DEB 1118783 and the Templeton Foundation also supported this work. This paper constitutes a partial fulfillment of the Graduate Program in Biological Sciences of the National Autonomous University of Mexico (UNAM). E.K-B acknowledges the scholarship and financial support provided by the National Council of Science and Technology (CONACyT).

REFERENCES

Ali, J. G., and A. A. Agrawal. 2012. Specialist versus generalist insect herbivores and plant defense. Trends in Plant Science 17. 293-302.

Avery, A. G., Satina, S., and J. Rietsema. 1959. Blakeslee: the genus *Datura*. Ronald Press, New York.

Berenbaum, M. R., and A. R. Zangrel. 1998. Chemical phenotype matching between a plant and its insect herbivore. Proceeding of the National Academy of Science 95: 13743-13748.

Barclay, A. S. 1959. Studies in the genus Datura (Solanaceae). I. Taxonomy of subgenus *Datura*, Phd thesis. Harvard University, Cambridge, MA, USA.

Bennett, R. N., and R. M. Wallsgrove. 1994. Metabolites in plant defense mechanism. New Phytologist 127: 617-633.

Benton, J.J., B. Wolf, B., and H. A. Mills. 1991. Plant analysis handbook. A practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Publishing, Athens, Georgia.

Bernays, E. A., and K. L. Bright. 1993. Mechanisms of dietary mixing in grasshoppers: A review. *Comparative Biochemistry and Physiology Part A: Physiology 104*: 125-131.

Bernays, E. A. and O. P. J. M. Minkenberg.1997. Insect herbivores: different reasons for being a generalist. Ecology 78: 1157-1169.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. New York, NY: Springer.

Carmona, D., and J. Fornoni. 2013. Herbivores can select for mixed defensive strategies in plants. New Phytologist 197: 576-585.

Carmona, D., Lajeunesse, M. J., and M. T. Johnson. 2011. Plant traits that predict resistance to herbivores. Functional Ecology 25: 358-367.

Castillo, G., Cruz, L. L., Hernández-Cumplido, J., Oyama, K., Flores-Ortiz, C. M., Fornoni, J., Valverde, P. L., and J. Núñez-Farfán. 2013. Geographic association and temporal variation of chemical and physical defense and leaf damage in *Datura stramonium*. Ecological Research 28: 663-672.

Castillo, G., Cruz, L. L., Tapia- López, R., Olmedo-Vicente, E., Carmona, D., Anaya-Lang, A. L., Fornoni, J., Andraca-Gómez, G., Valverde, P.L., and J. Núñez-Farfán. 2014. Selection mosaic exerted by specialist and generalist herbivores on chemical and physical defense of *Datura stramonium*. PloS one 9: e102478.

Céspedes C. L., Calderón J. S., Lina L., and E. Aranda. 2000. Growth inhibitory effects on fall armyworm *Spodoptera frugiperda* of some limonoids isolated from *Cedrela* spp. (Meliaceae). Journal of Agricultural and Food Chemistry 48: 1903 – 1908.

Colegate, S. M., Dorling, P. R., and C. R. Huxtable. 1979. A spectroscopic investigation of swainsonine: an α-mannosidase inhibitor isolated from *Swainsona canescens*. Australian Journal of Chemistry 32: 2257-2264.

Cornell, H. V., and B. A. Hawkins. 2003. Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. American Naturalist 161: 507-522.

Dethier, V. G. 1954. Evolution of feeding preferences in phytophagous insects. Evolution 8: 35-54

Dussourd, D. E., and R. F. Denno. 1994. Host range of generalist caterpillars: Trenching permits feeding on plants with secretory canals. Ecology 75: 69-78.

Ehrilch, P. R., and P. H. Raven. 1964. Butterflies and plants: A study in coevolution. Evolution 18: 586-608.

Elle, E., and J. D. Hare. 2000. No benefit of glandular trichome production in natural populations of *Datura wrightii*? Oecologia. 123: 57-65.

Espinosa, E. G., and J. Fornoni. 2006. Host tolerance does not impose selection on natural enemies. New Phytologist 170: 609-614

Evans, W. C. 1979. Tropane alkaloids of the Solanaceae. Pages 241-254 *in* Hawkes, J. G., Lester, R. N., and A. Skelding, eds. The biology and taxonomy of the Solanaceae Academic Press, London.

Fenny, P. P. 1976. Plant apparency and chemical defence. Pages 1-40 *in* Wallace, J. W. and R. L. Mansel, eds. Biological Interactions between plants and insects. Plenum Press.

Fornoni, J., Valverde, P. L., and J. Núñez-Farfán. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. Evolution 58: 1696–704.

Fraenkel, G. 1959. The raison d´être of secondary plant substances. Science 129: 1466-1470.

Fritz, R. S., and E. L. Simms. 1992. Plant resistance to herbivores and pathogens. University of Chicago Press.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics 19: 207-233.

Garland, T., Midford, P. E., and A. R. Ives. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. American Zoologist 39: 374-388.

Garrido, E., Andraca-Gómez G., and J. Fornoni. 2012. Local adaptation: simultaneously considering herbivores and their host plants. New Phytologist 193: 445–453.

Gómez, C. V., Martínez-Vázquez, M., and B. Esquivel. 2009. Antifeedant activity of anticopalic acid isolated from *Vitex hemsleyi.* Zeitschrift für Naturoforschung Section C-A 64: 502-508.

Harborne, J. B. 1988. Introduction to Biochemical Ecology. Academic Press 3th ed.

Hare, J. D., and E. Elle. 2002. Variable impact of diverse insect herbivores on dimorphic *Datura wrightii*. Ecology 83: 2711-2720.

Hartmann, T., Theuring, C., Beuerle, T., Bernays, E. A., and M. S. Singer. 2005. Acquisition, transformation and maintenance of plant pyrrolizidine alkaloids by the polyphagous arctiid *Grammia geneura*. Insect Biochemistry Molecular Biology 35: 1083–1099.

Janzen, D. H. 1980. When is it coevolution? Evolution 34: 611-612.

Karban, R., and A. A. Agrawal. 2002. Herbivore offense. Annual Review of Ecology and Systematics 33:641-64.

Kariñho-Betancourt, E., Agrawal, A. A., Halitschke, R. and J. Núñez-Farfán. 2015. Phylogenetic correlations among chemical and physical plant defenses change with ontogeny. New Phytologist 206: 796-806.

Kariñho-Betancourt, E. y J. Núñez-Farfán. 2015. Evolution of resistance and tolerance to herbivores: testing the trade-off hypothesis. PeerJ. doi: 10.7717/peerj.789

Kester, K. M., and C. M. Smith. 1984. Effects of diet on growth, fecundity and duration of tethered flight of *Nezara viridula*. Etomologia Experimentalis et Applicata 35: 75-81.

Kogan, M., and Goden, R. D. 1970. The biology of *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae) with notes on efficiency of food utilization by larvae. Annals of the Entomologcal Society *of America*. 63: 537-546.

Kursar, T. A., and P. D. Coley. 2003. Convergence in defense syndromes of young leaves in tropical rainforest. Biochemical Systematics and Ecology 31: 929-949.

Lankau, R. A. 2007. Specialist and generalist herbivores exert opposing selection on a chemical defense. New Phytologist 175: 176-184.

Lawrence, M. I. 1984. The genetical analysis of ecological traits. B. Shorrocks, ed. Blackwell, Oxford, Press.

Luna-Cavazos, M., and Bye, R. 2011. Phytogeographic analysis of the genus *Datura* (Solanaceae) in continental Mexico. Revista Mexicana de Biodiversidad 82: 977-988.

Mauricio R., and M. D. Rausher. 1997. Experimental manipulation of putative selective agents provides evidence from the role of natural enemies in the evolution of plant defense. Evolution 51: 1435-1444.

Mithöfer, A., and W. Boland. 2012. Plant defense against herbivores: Chemical aspects. Annual Review of Plant Biology. 63: 431-450.

Mitter, C., Farrell, B., and B. Wiegmann. 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? American Naturalist 132: 107-128.

Nathanson, J. 1984. Caffeine and related methylxanthines: possible naturally occurring pesticides. Science 226: 184-187.

Núñez-Farfán, J., and R. Dirzo. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. Evolution 48: 423-436.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401: 877-884.

Parr, A. J., Payne, J., Eagles, J., Chapman, B. T., Robins, R. J., and M. J. Rhodes. 1990. Variation in tropane alkaloid accumulation within the Solanaceae and strategies for its exploitation. Phytochemistry 29: 2545-2550.

Rasmann, S., and A. A. Agrawal. 2011. Evolution of specialization: A phylogenetic study of host range in the red milkweed beetle (*Tetraopes tetraophthalmus*). American Naturalist 177: 728-737.

Raubenheimer, D., and S. J. Simpson. 1999. Integrating nutrition: A geometrical approach. Entomologia Experimentalis et Applicata 91: 67-82.

Reuter, D. J., Robinson, J. B., Peverill, K. I., Price, G. H., and M. J. Lambert. 1986. Guidelines for collecting, handling, and analyzing plant materials. Pages 20-33 *in* Reuter, D. J., and J. B. Robinson, eds. *Plant analysis:* An interpretation manual. Inkata Press, Melbourne, Australia.

Revell, L. J. 2012. Phytools: An R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution *3*: 217-223.

Revell, L. J. 2009. Size-correction and principal components for interspecific comparative studies. Evolution 63: 3258-3268.

Rhoades D. F., and R. G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. Pages 168–213 *in* Wallace, J. W., and R. L. Mansell, eds. Recent Advances Phytochemistry. Plenum Press, New York.

Roddick, J. 1991. The importance of the Solanceae in medicine and drug therapy. Solanaceae 3: 7-23.

Roslin, T. and J. P. Salminen. 2008. Specialization pays off: Contrasting effects of two types of tannins on oak specialist and generalist moth species. Oikos 117: 1560-1568.

Schoonhoven, L.M., Van Loon, J.J., and M. Dicke. 2005. Insect-plant biology. Oxford University Press 2nd ed.

Shonle, I. 1999. Evolutionary ecology of tropane alkaloids. Ph. D. Thesis. University of Chicago, USA.

Shonle, I., and J. Bergelson. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). Evolution 54: 778-788.

Simpson, S. J., and D. Raubenheimer. 2001. The geometric analysis of nutrient-allelochemical interactions. A case study using locusts. Ecology 82: 422-439.

Slansky, F. 1992. Allelochemical-nutrient interactions in herbivore nutritional ecology. Pages 135-174 *in* Rosenthal, G. A., and Berenbaum, M. R., eds. Herbivores: Their interactions with secondary plant metabolites. Academic Pres, New York.

Strong, D. R., Lawton, and J. H. Southwood. 1984. Insects on plants: Community patterns and mechanisms. Blackwell Scientific, London.

Symon, D. E., and L. Haegi. 1991. *Datura* (Solanaceae) is a New World genus. Pages 197-210 *in* Hawkes, J. G., Lester, R. N., Nee, M., and. R. N. Estrada, eds. Solanaceae III: taxonomy, chemistry, evolution. Royal Botanic Garden Press, the Royal Botanic Gardens, Kew, London.

Thompson, S. N., and R. A. Redak. 2007 Nicotine moderates the effects of macronutrient balance on nutrient intake by parasitized *Manduca sexta* L. Journal of Comparative Physiology B 177: 375-391.

Thompson S. N., and S. J. Simpson. 2009. Nutrition. Pages 715-721 *in* Resh, V. H., and R. T. Cardé, eds. Encyclopedia of insects. Academic Press, USA.

Valverde, P. L., Fornoni, J. and J. Núñez-Farfán. 2001. Defensive role of leaf trichome in resistance to herbivorous in *Datura stramonium*. Journal of Evolutionary Biology 14: 424-432.

Valverde, P. L., Fornoni, J. and J. Núñez-Farfán. 2003. Evolutionary ecology of *Datura stramonium*: equal plant fitness benefits of growth and resistance against herbivory. Journal of Evolutionary Biology 16: 127–37.

van Dam, N. M., Vuister, L. W. Bergshoeff, C., de Vos, H., and Van der E. D. Meijden.1995. The “Raison d'être” of pyrrolizidine alkaloids in *Cynoglossum officinale*: deterrent effects against generalist herbivores. Journal of Chemical Ecology 21: 507-523.

van der Meijeden, E., Wijn, E.H., and J. Verkaar. 1988. Defence and regrowth: alternative plant strategies in the struggle against herbivores. Oikos 51: 355- 363.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Advances in insect physiology 5: 229-288.

TABLES

TABLE 1. ANOVA of herbivore performance. Performance of the generalist herbivore *Spodoptera frugiperda*, and the specialist *Lema trilineata,* was measured as food consumption (FC), number of damaged leaves (DL), biomass increment (BI), and growth efficiency (GE), across eleven *Datura* species. The plant term is a random effect, whereas the herbivore (degree of specialization) is a fixed effect. A significant plant*herbivore interaction indicates that herbivore performance varies as a function of its degree of specialization and the *Datura* species. Significant values are indicated in bold (*P* <0.05).

		FC		**DL**		**BI**		**GE**	
	DF	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Herbivore	1	33.07	**< 0.0001**	18.24	**< 0.0001**	129.14	**< 0.0001**	12.06	**0.0006**
Plant	10	6.45	**< 0.0001**	9.01	**< 0.0001**	7.16	**< 0.0001**	1.03	0.416
Plant*herbivore	10	10.27	**< 0.0001**	5.59	**< 0.0001**	10.14	**< 0.0001**	1.79	0.06
Error	416								
Total	437								

TABLE 2. Phylogenetic principal components analysis (pPCA) of herbivore performance (FC; food consumption, BI; biomass increment, GE; growth efficiency, and DL; number of damaged leaves), plant nutrients (N; Nitrogen, P; Phosphorous, C; Carbon, and W; water content) and plant defenses (trop; tropine, hyos; hoysciamine, scop; scopolamine, atrop; atropine, sola; solanine, totalk; total alkaloids, and trico; leaf trichomes).

	Lema trilineata daturaphila		***Spodoptera frugiperda***	
Plant variables	**pPC1**	**pPC2**	**pPC1**	**pPC2**
			Herbivore	**performance**
BI	**-0.630**	**0.576**	-0.284	**-0.803**
FC	-0.216	0.381	-0.357	**-0.869**
GE	-0.023	-0.023	0.429	**-0.656**
DL	**-0.670**	**0.485**	**-0.532**	**-0.785**
				Nutrients
N	**-0.761**	0.076	**-0.761**	0.076
P	0.071	-0.461	0.071	-0.461
C	-0.302	0.414	-0.302	0.414
W	0.686	**0.595**	0.686	**0.595**
				Defense
trop	0.465	-0.174	0.465	-0.174
hyos	-0.048	**0.464**	-0.048	**0.464**
scop	**0.908**	-0.228	**0.908**	-0.228
atrop	**0.718**	0.259	**0.718**	0.259
sola	0.243	0.361	0.243	0.361
totalk	**0.943**	-0.038	**0.943**	-0.038
trico	-0.188	0.154	-0.188	0.154

In bold are indicated the weightier variables.

TABLE 3. Correlation between components of herbivore performance (FC; food consumption, BI; biomass increment, DL; leaf damaged, and GE; growth efficiency) and plant traits (N; nitrogen, P; phosphorous, C; carbon, W; water, scop; scopolamine, totalk; total alkaloids, and trico; leaf trichomes) corrected by phylogenetic relationships (PGLs) of *Datura*. For each response variable Akaike´s information criterion (AIC) values, *P* of statistical model is shown.

Lema trilineata daturaphila				
Performance trait (response variable)	**Plant trait (predictor variable)**	**Correlation coefficient**	**AIC**	***P* (model)**
FC	P	-0.35*	-19.9	0.041
FC	C	-0.013	-19.9	0.041
BI	scop	-0.252*	-9.87	0.118
BI	trico	-0.079	-9.87	0.118
DL	scop	-0.166*	-20.33	0.008
DL	trico	-0.074	-20.33	0.008
Spodoptera frugiperda				
BI	C	-0.251*	-31.43	0.008
BI	W	-0.165*	-31.43	0.008
GE	N	-0.284	-48.8	0.091
GE	W	-0.133	-48.8	0.091
GE	C	-0.87*	-48.8	0.091
DL	P	0.625	-40.7	0.0009
DL	W	-0.92*	-40.7	0.0009
GE	scop	0.02	-48.96	0.05
GE	trico	-0.274*	-48.96	0.05
DL	totalk	-0.104*	-31.31	0.038
DL	trico	-0.004	-31.31	0.038

* $P< 0.05$

TABLE A1. GenBank accession number of sequences obtained for species of *Datura* (see text).

Genus	**Species**	**Accession number**		
		psb**A-*****trn*****H**	***trn*****L-*****trn*****F**	**rRNA ITS**
Datura	*wrightii*	JX467618	JX467585	JX467602
Datura	*lanosa*	JX467619	JX467586	JX467603
Datura	*metel*	JX467620	JX467587	JX467604
Datura	*discolor*	JX467621	JX467588	JX467605
Datura	*reburra*	JX467622	JX467589	JX467606
Datura	*inoxia*	JX467623	JX467590	JX467607
Datura	*kymatocarpa*	JX467625	JX467592	JX467609
Datura	*pruinosa*	JX467626	JX467593	JX467610
Datura	*stramonium*	JX467627	JX467594	JX467611
Datura	*quercifolia*	JX467628	JX467595	JX467612
Datura	*ferox*	JX467629	JX467596	JX467613

TABLE A2. Variation in food consumption (FC); cm^2, biomass increment (BI); g, damaged leaves (DL); number, and growth efficiency (GE); g/cm^2 between the specialist herbivore (S) *Lema trilineata daturaphila* and the generalist herbivore (G) *Spodoptera frugiperda* in each *Datura* spp.

	FC				**BI**			
Sp	$\bar{x}_{(S)}$ ± se	$\bar{x}_{(G)}$ ± se	***F***	***P***	$\bar{x}_{(S)}$ ± se	$\bar{x}_{(G)}$ ± se	***F***	***P***
discolor	1.64(0.24)	0.41(0.24)	3.61	**<0.0009**	0.48(0.098)	-0.01(0.098)	3.57	**0.001**
ferox	0.71(0.25)	1.03(0.25)	-0.89	0.378	-0.0003(0.038)	-0.13(0.038)	2.49	**0.017**
inoxia	0.63(0.16)	0.71(0.16)	-0.34	0.73	0.16(0.14)	-0.12(0.14)	1.36	0.18
kymatocarpa	0.8(0.14)	0.52(0.14)	1.38	0.17	0.52(0.096)	-0.083(0.096)	4.42	**< 0.0001**
lanosa	2.39(0.24)	0.95(0.24)	4.16	**0.0002**	1.2(0.12)	0.067(0.12)	6.71	**< 0.0001**
metel	1.58(0.23)	0.67(0.23)	2.73	**0.009**	0.7(0.14)	0.16(0.14)	2.73	**0.009**
pruinosa	1.17(0.17)	0.38(0.17)	3.14	**0.003**	1.24(0.15)	-0.19(0.15)	6.64	**< 0.0001**
quercifolia	0.76(0.25)	2.17(0.25)	-3.9	**0.0004**	0.4(0.13)	0.47(0.13)	-0.35	0.72
reburra	1.48(0.17)	0.18(0.17)	5.17	**< 0.0001**	2.04(0.25)	-022(0.25)	6.23	**< 0.0001**
stramonium	0.98(0.14)	1.18(0.14)	-0.93	0.35	0.34(0.05)	0.09(0.05)	3.07	**0.003**
wrightii	2.4(0.24)	0.68(0.24)	4.61	**< 0.0001**	1.26(0.17)	0.03(0.17)	4.9	**< 0.0001**
Sp	**DL**				**GE**			
discolor	2.0(0.29)	1.95(0.29)	0.12	0.9	0.26(0.18)	-0.03(0.18)	1.13	0.26
ferox	2.05(0.27)	2.45(0.27)	-1.04	0.3	-0.001(0.002)	-0.012(0.002)	3.66	**0.0008**
inoxia	1.0(0.18)	2.4(0.18)	-5.27	**< 0.0001**	-0.005(0.04)	-0.074(0.04)	1.19	0.24
kymatocarpa	1.6(0.2)	2.25(0.2)	-2.29	**0.027**	0.005(0.003)	-0.018(0.003)	5.39	**< 0.0001**
lanosa	1.9(0.17)	2.6(0.17)	-2.75	**0.009**	0.005(0.003)	0.001(0.003)	0.74	0.46
metel	1.55(0.22)	2.55(0.22)	-3.09	**0.003**	0.006(0.007)	0.02(0.007)	-1.29	0.2
pruinosa	3.8(0.46)	2.7(0.46)	1.69	0.09	0.02(0.014)	-0.059(0.014)	3.83	0.0005
quercifolia	2.6(0.21)	4.15(0.21)	-5.08	**< 0.0001**	0.011(0.005)	0.012(0.005)	-0.1	0.92
reburra	4.2(0.42)	2.3(0.42)	3.14	**0.003**	0.0006(0.075)	-0.17(0.075)	1.74	0.08
stramonium	2.95(0.28)	2.7(0.28)	0.62	0.53	0.006(0.005)	-0.001(0.005)	1.11	0.27
wrightii	1.7(0.19)	1.9(0.19)	-0.74	0.46	0.003(0.023)	-0.02(0.023)	0.97	0.33

Significant values ($P < 0.05$) are indicated in bold type.

TABLE A3. Akaike´s information criterion (AIC) values for 88 statistical models of plant nutrient (N; nitrogen, P; phosphorous, C; carbon, and W; water) and defenses (hyos; hyoscyamine, scop; scopolamine, totalk; total alkaloids, and trico; leaf trichomes)**.**

Statistical model for plant nutrients	**AIC value of "S"**	**AIC value of "G"**
		Biomass increment (BI)
N+P+C+W	-7.51	-28.15
N+C+W	-6.92	-29.97
N+P+W	-7.07	-24.41
N+C+P	-9.503	-20.36
P+C+W	-9.51	-29.71
N+P	-9.066	-19.81
N+C	-8.81	-22.32
N+W	-7.142	-26.21
P+C	-9.014	-25.66
P+W	-8.903	-21.44
C+W	**-11.496**	**-31.43**
		Food consumption (FC)
N+P+C+W	-16.21	-17.44
N+C+W	-11.8	-16.1
N+P+W	-17.32	-16.81
N+C+P	-17.91	-13.02
P+C+W	-18.09	-19.41
N+P	-19.02	-13.94
N+C	-13.17	-14.58
N+W	-13.22	-18.75
P+C	**-19.9**	-18.71
P+W	-19.29	-12.31
C+W	-13.8	**-20.75**
		Growth efficiency (GE)
N+P+C+W	-38.17	-47.21
N+C+W	-40.09	**-48.8**
N+P+W	-37.81	-42.2
N+C+P	**-40.17**	-46.6
P+C+W	-39.85	-45.3
N+P	-19.02	-43.3
N+C	-13.17	-48.38
N+W	-13.22	-43.6
P+C	-19.9	-43.62
P+W	-19.29	-46.57
C+H	-13.8	-47.06
		Damaged leaves (DL)
N+P+C+W	-9.6	-37.15
N+C+W	-11.55	-34.46
N+P+W	11.39	-39.15
N+C+P	-11.39	-26.58
P+C+W	-12.72	-38.77
N+P	-8.25	-28.58
N+C	**-13.37**	-25.43
N+W	-12.83	-36.46
P+C	-9.98	**-40.75**
P+W	-9.24	-27.26
C+W	-10.05	-36.42

Statistical model for plant defenses	AIC value of "S"	AIC value of "G"
		Biomass increment (BI)
hyos+scop+totalk+trico	-7.29	-18.3
hyos+totalk+trico	-6.31	-19.27
hyos+scop+totalk	-6.87	-18.89
hyos+scop+trico	-7.95	-18.46
scop+totalk+trico	-9.23	-19.85
hyos+scop	-8.34	-19.79
hyos+totalk	-7.96	-20.06
hyos+trico	-7.147	-20.59
scop+totalk	-8.67	-20.46
scop+trico	**-9.87**	-20.84
totalk+trico	-7.29	**-21.22**
		Food consumption (FC)
hyos+scop+totalk+trico	-11.35	-11.21
hyos+totalk+trico	-13.34	-10.91
hyos+scop+totalk	-12.22	-10.69
hyos+scop+trico	-13.35	-10.83
scop+totalk+trico	-12.69	**-13.19**
hyos+scop	-14.17	-12.63
hyos+totalk	-14.19	-12.81
hyos+trico	**-15.27**	-10.2
scop+totalk	-13.38	-10.9
scop+trico	-14.56	-11.83
totalk+trico	-14.43	-12.1
		Growth efficiency (GE)
hyos+scop+totalk+trico	-39.25	-46.6
hyos+totalk+trico	-39.74	-44.1
hyos+scop+totalk	-39.82	-48.04
hyos+scop+trico	-39.88	-41.74
scop+totalk+trico	-38.64	-47.52
hyos+scop	-40.48	-43.21
hyos+totalk	-40.47	-43.35
hyos+trico	**-41.68**	-46.105
scop+totalk	-40.12	-43.73
scop+trico	-40.06	**-48.96**
totalk+trico	-40.02	-48.11
		Damaged leaves (DL)
hyos+scop+totalk+trico	-17.79	-27.54
hyos+totalk+trico	-16.53	-29.52
hyos+scop+totalk	-15.99	-28.62
hyos+scop+trico	-18.49	-29.54
scop+totalk+trico	-17.2	-29.32
hyos+scop	-17.92	-30.58
hyos+totalk	-16.9	-31.52
hyos+trico	-11.82	-23.96
scop+totalk	-17.06	-31.27
scop+trico	**-20.33**	**-30.61**

Correlations between components of herbivore performance (food consumption; FC, biomass increment; BI, DL; leaf damaged, and GE; growth efficiency) of specialist herbivore *Lema trilineata daturaphila* (S) and generalist *Spodoptera frugiperda* (G), and plant traits were corrected by phylogenetic relationships (PGLs) of *Datura.* In bold is indicated the best model (the one with the lowest AIC) for each herbivore performance variable.

Capítulo III

EVOLUCIÓN DE LA RESITENCIA Y LA TOLERANCIA A LOS HERBÍVOROS: PONIENDO A PRUEBA LA HIPÓTESIS DEL TRADE-OFF

MANUSCRITO PUBLICADO EN *PeerJ*

Kariñho-Betancourt E, Núñez-Farfán J. (2015) Evolution of resistance and tolerance to herbivores; testing the trade-off hypothesis. *PeerJ* 3:e789 https://dx.doi.org/10.7717/peerj.789

PeerJ

Evolution of resistance and tolerance to herbivores: testing the trade-off hypothesis

Eunice Kariñho-Betancourt and Juan Núñez-Farfán

Laboratorio de Genética Ecológica y Evolución, Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México, DF, Mexico

ABSTRACT

Background. To cope with their natural enemies, plants rely on resistance and tolerance as defensive strategies. Evolution of these strategies among natural population can be constrained by the absence of genetic variation or because of the antagonistic genetic correlation (trade-off) between them. Also, since plant defenses are integrated by several traits, it has been suggested that trade-offs might occur between specific defense traits.

Methodology/Principal Findings. We experimentally assessed (1) the presence of genetic variance in tolerance, total resistance, and leaf trichome density as specific defense trait, (2) the extent of natural selection acting on plant defenses, and (3) the relationship between total resistance and leaf trichome density with tolerance to herbivory in the annual herb *Datura stramonium*. Full-sib families of *D. stramonium* were either exposed to natural herbivores (control) or protected from them by a systemic insecticide. We detected genetic variance for leaf trichome density, and directional selection acting on this character. However, we did not detect a negative significant correlation between tolerance and total resistance, or between tolerance and leaf trichome density. We argue that low levels of leaf damage by herbivores precluded the detection of a negative genetic correlation between plant defense strategies.

Conclusions/Significance. This study provides empirical evidence of the independent evolution of plant defense strategies, and a defensive role of leaf trichomes. The pattern of selection should favor individuals with high trichomes density. Also, because leaf trichome density reduces damage by herbivores and possess genetic variance in the studied population, its evolution is not constrained.

Subjects Ecology, Evolutionary Studies, Genetics
Keywords Genetic variation, Herbivory, Resistance, Leaf trichomes, Natural selection, Plant defense, Trade-offs, Tolerance

Submitted 28 November 2014
Accepted 4 February 2015
Published 3 March 2015

Corresponding author
Juan Núñez-Farfán,
farfan@unam.mx

Academic editor
Gabriele Sorci

Additional Information and Declarations can be found on page 12

DOI **10.7717/peerj.789**

INTRODUCTION

Plants rely on resistance and tolerance to avoid the negative fitness effects of damage by herbivores or pathogens (*Simms & Triplett, 1994*; *Fineblum & Rausher, 1995*; *Strauss & Agrawal, 1999*; *Núñez-Farfán, Fornoni & Valverde, 2007*). Resistant plants prevent or reduce the amount of damage through chemical secondary metabolites and physical defenses

How to cite this article Kariñho-Betancourt and Núñez-Farfán (2015), Evolution of resistance and tolerance to herbivores: testing the trade-off hypothesis. **PeerJ 3:e789; DOI 10.7717/peerj.789**

(*Ehrlich & Raven, 1964*; *Levin, 1973*; *Berenbaum, Zangerl & Nitao, 1986*; *Wink, 2003*). Tolerant plants can lessen the negative impact of herbivore damage on fitness, once it has occurred (*Rausher, 1992b*; *Stowe et al., 2000*). Unlike resistance, tolerance does not prevent herbivory but maintains fitness by eliciting compensatory physiological plant responses after damage by herbivores.

Natural selection for increased resistance to herbivores has been detected in different plant species as well as for plant traits associated to resistance (*Berenbaum, Zangerl & Nitao, 1986*; *Simms & Rausher, 1989*; *Mauricio & Rausher, 1997*; *Shonle & Bergelson, 2000*). However, investment in plant defense is thought to involve fitness costs such that optimal defense does not necessarily imply maximal investment (*Fagerström, Larsson & Tenow, 1987*; *Simms & Rausher, 1987*). Thus, potential trade-offs between different defensive traits might arise (see *Mauricio, 1998*). Furthermore, if fitness costs of herbivory can be reduced by tolerance, selection on resistance traits would be relaxed, nil, or even selected against if leaf damage could be partially or completely compensated by tolerance (*Abrahamson & Weis, 1997*; *Fineblum & Rausher, 1995*; *Mauricio, Rausher & Burdick, 1997*). Hence, the simultaneous investment in tolerance and resistance may imply a greater total cost than the possession of only one pure strategy (*van der Meijeden, Wijn & Verkaar, 1988*; *Herms & Mattson, 1992*; *Fineblum & Rausher, 1995*; *Mauricio, Rausher & Burdick, 1997*). However, if the fitness benefit of investment in tolerance and resistance is higher than its cost, the evolution of a mixed defense strategy is a possible outcome (*Fornoni et al., 2004a*; *Carmona & Fornoni, 2013*).

Nevertheless, evidence of a trade-off between plant defensive strategies is scarce, and may depend on the sort of traits involved in the defensive response. A review of literature indicates little support for a negative genetic correlation between tolerance and resistance across different plant species, and suggests that a fruitful approach is to assess the relationship between tolerance and specific plant resistance traits rather that the correlation between tolerance with total resistance (*Leimu & Koricheva, 2006*). Thus, this study aimed to determine if total resistance and a component of it (leaf trichomes) are genetically correlated with plant tolerance. We carried out an experiment to expose maternal half-sib families of the annual plant *Datura stramonium* to their natural herbivores in order to (1) assess genetic variation in plant tolerance, total resistance, and leaf trichome density, and (2) measure selection on trichome density and resistance to herbivores. Finally, (3) we estimated the genetic correlation between tolerance and total resistance and leaf trichomes.

MATERIALS AND METHODS

Study system

Datura stramonium L. (Solanaceae) is an annual herbaceous plant native to Mexico, but widely distributed worldwide. It is commonly found as ruderal in disturbed habitats (*Weaver & Warwick, 1984*; *Núñez-Farfán & Dirzo, 1994*; *Shonle & Bergelson, 2000*). In central Mexico, its leaves are consumed by specialist herbivorous insects (i.e., those that feed upon a restricted group of related plants), such as the leaf beetles *Epitirx parvula* and

Lema trilineata (Coleoptera: Chrysomelidae) (*Núñez-Farfán & Dirzo, 1994*; *Castillo et al., 2013*), and generalist herbivores (i.e., that feed upon several unrelated plant species), such as the grasshopper *Sphenarium purpurascens* (Orthoptera: Pyrgomorphidae). Also, the specialist weevil *Trichobaris soror* (Coleoptera: Curculionidae) is a seed-predator of *Datura stramonium* in populations of central Mexico (*Núñez-Farfán & Dirzo, 1994*; *Núñez-Farfán, Cabrales-Vargas & Dirzo, 1996*). Previous studies in *D. stramonium* have shown that damage caused by these insects reduces plant fitness (*Núñez-Farfán & Dirzo, 1994*; *Valverde, Fornoni & Núñez-Farfán, 2001*; *Fornoni, Valverde & Núñez-Farfán, 2004b*), and that tropane alkaloids and leaf trichomes confer resistance against its natural herbivores (*Shonle, 1999*; *Valverde, Fornoni & Núñez-Farfán, 2001*; *Kariñho-Betancourt, 2009*; *Castillo et al., 2014*). Likewise, variation among-population in such defensive traits (alkaloids and leaf trichomes) is associated with the composition of the herbivore community (*Valverde, Fornoni & Núñez-Farfán, 2003*; *Fornoni, Valverde & Núñez-Farfán, 2003*; *Castillo et al., 2013*; *Castillo et al., 2014*).

Based on previous studies with this species, we selected the Ticuman population of *D. stramonium* because genetic variance for resistance and tolerance was detected (*Fornoni, Valverde & Núñez-Farfán, 2003*), and leaf trichome density correlates with leaf damage by herbivores (*Valverde, Fornoni & Núñez-Farfán, 2001*). The vegetation in the Ticuman locality (18°47′N and 99°06′W) is a tropical dry forest at 990 m.a.s.l., with an average annual precipitation and temperature of 954.4 mm and 24 °C, respectively. Plants in this locality receive low levels of average damage by herbivores 10.9 ± 4.0%; mean ± SE; (*Valverde, Fornoni & Núñez-Farfán, 2001*; *Fornoni, Valverde & Núñez-Farfán, 2003*), compared to other populations that share the same herbivores (*Valverde, Fornoni & Núñez-Farfán, 2001*).

Experimental Design

Experimental plants were obtained in a greenhouse by sowing seeds of each of 28 maternal half-sib families (natural progenies; *Lawrence, 1982*). Once the first two true leaves appeared, plants were transplanted to an experimental plot in Ticuman under a randomized block design, and watered regularly each week.

To assess the pattern of selection on resistance characters in the presence and absence of natural herbivores (*e.gr.*, *Mauricio & Rausher, 1997*), and evaluate the cost of defensive traits, we selected a sample of 16 families and divided the progeny of each family in two groups of insecticide treatment (control and treated). We used a systemic carbofuran insecticide (FURADAN®; FMC Corporation, Philadelphia, Pennsylvania, USA). Two weeks after transplanting, we watered the experimental plants with 500 ml of a solution containing the insecticide. The same volume of water was supplied to control plants.

Plants of each family were measured for (1) plant height, (2) stem diameter, (3) number of branches, (4) number of flowers, (5) number of fruits, (6) total seeds, (7) leaf damage by herbivores, and (8) leaf trichome density.

Resistance to herbivores

To estimate total plant resistance, R_i, we randomly choose a sample (n) of 20 leaves per plant i. For each leaf we measured total (A_T) and damaged area (A_D) by using a leaf area meter (Winfolia; Regent Instruments Inc., Québec, Canada). Thus, relative resistance to herbivores of plant $i(R_i)$ is related to the proportion of leaf area damaged (D_i) as: $R_i = 1 - D_i = 1 - \left(\frac{1}{n}\sum_{i=1}^{n}\frac{A_D}{A_T}\right)$, (*Núñez-Farfán & Dirzo, 1994*). This estimate of resistance to herbivores (R_i) has been broadly related as a measure of total resistance (see *Leimu & Koricheva, 2006*).

To measure the plant's leaf trichome density, we counted the number of trichomes in three areas of 1.7 mm^2 in the abaxial side of each leaf (at the bottom, right and left edges of the leaf) using a dissection microscope (*Valverde, Fornoni & Núñez-Farfán, 2001*).

Reproductive output

We counted the total number of fruits and seeds produced by each plant (W_i) in order to obtain an estimator of maternal plant fitness. Following *Lande & Arnold (1983)* we defined relative fitness (w_i) as, $W_1 = \frac{W_i}{\overline{W}}$ where $\overline{W}$ is the average number of fruits or seeds per plant in the population. Since fitness estimated either as total fruits and total seed number were positively correlated $(r = 0.9, P < 0.0001)$, we used the estimate based on seed number for subsequent statistical analyses.

Plant tolerance

Using plants exposed to herbivores, we estimated tolerance of each family as the slope (β_i) of a linear regression between individual relative fitness (w_i) *versus* relative damage received (D_i) by herbivores (*Mauricio, Rausher & Burdick, 1997*; *Fornoni, Valverde & Núñez-Farfán, 2003*). Since tolerance benefits are expressed in the presence of damage, plants treated with insecticide were not included in this analysis.

Data analysis

Genetic variance and heritability

In order to estimate additive genetic variance, an ANOVA for each character was carried out with the family term as a random variable and the block as the fixed effect. Broad-sense heritability (h_B^2) was estimated as the ratio between twice the family component of variance (σ_f^2) divided by the total phenotypic variance (σ_p^2), since resemblance among members of a family (i.e., covariance) contains one half of additive genetic variance (*Falconer & MacKay, 1995*). Genetic correlation among characters were estimated as the correlation between family means (*Via, 1984*).

Genetic variance in tolerance was assessed by means of an ANCOVA of fitness as a function of the family, the relative damage by herbivores (the covariate), and the interaction family × relative damage. A significant family × relative damage suggests genetic variance in the reaction norms of fitness in relation to damage by herbivores (*Fornoni, Valverde & Núñez-Farfán, 2003*).

Correlation between leaf damage and resistance traits

In order to assess the relationship of leaf trichome density and damage, i.e., the defensive role of leaf trichomes, we performed a correlation analysis with plants exposed to herbivores. The analysis was conducted using the individual values of leaf trichome density, total resistance, relative leaf damage, and relative plant fitness.

Cost analysis

The cost of resistance attributes was estimated using plants that received the insecticide application by performing a linear regression of (1) total resistance and relative fitness (w_i), and (2) leaf trichome density and relative fitness (w_i). A negative slope indicates costs for the defensive traits.

Selection analysis

Natural selection on plant resistance attributes (total resistance and leaf trichomes) was estimated by a partial linear regression of fitness to detect (1) directional selection (β_i), and/or (2) non-linear selection (γ_{ij}) by means of partial quadratic regression of fitness as a function of the quadratic values of characters (*Lande & Arnold, 1983*). Selection analyses were performed on phenotypic and breeding values (*Rausher, 1992b*; *Mauricio & Mojonnier, 1997*).

Genetic correlation (trade-off) between resistance and tolerance

To assess the genetic correlation between resistance and tolerance, we performed a correlation analysis between the family average values of resistance traits (total resistance and leaf trichome density) and tolerance.

RESULTS

The amount of damage received by plants exposed to herbivores (control) was significantly higher than that received by plants in the insecticide group (Fig. 1). Although the levels of damage were low in both groups, the ANOVA indicated that plants that received the insecticide application were significantly less damaged (∼15%) than those who did not ($F_{1,135} = 5.83$, $P = 0.017$). However, in spite of the fact that plants performing better in the absence of herbivores (insecticide group), the differences in the average values of vigor, reproductive and resistance traits between the control and the insecticide group were not significant (Table 1).

Genetic variance

ANOVA detected a significant family effect of the number of flowers produced per plant and the two resistance traits estimated: total resistance ($F_{27,125} = 1.57$, $P = 0.04$) and leaf trichome density ($F_{27,125} = 3.62$, $P < 0.001$) (Table 2; Fig. 2). Heritability of leaf trichome number was high ($h^2 = 0.641$), compared to total resistance ($h^2 = 0.259$). In contrast, we failed to detect genetic variation of tolerance to damage, since the family × relative damage by herbivores was not significant ($F_{27,125} = 0.08$, n.s.).

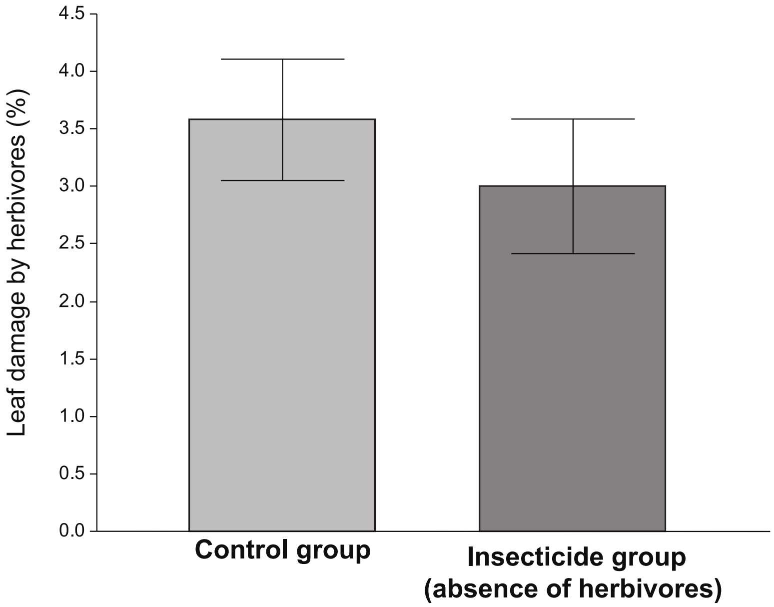


Figure 1 Percentage of leaf damage by herbivory between control and insecticide group.

Table 1 **Average values (SE) of vigor, reproductive, and defense characters of *Datura stramonium*.** The F and P values are derived from the analysis of variance for the insecticide group (I) and the replicate plants from the control group (C) (herbivores present).

Character	$\bar{x}_{(C)}$ (SE)	$\bar{x}_{(I)}$ (SE)	F	P
Plant height	5.55(0.06)	5.54(0.06)	0.008	0.926
Steam diameter	1.94(0.04)	1.97(0.05)	0.172	0.679
Branch number	1.99(0.09)	1.97(0.09)	0.043	0.836
Flower number	1.94(0.08)	1.86(0.09)	0.402	0.527
Fruit number	1.57(0.1)	1.63(0.1)	0.16	0.69
Seed number	5.85(0.21)	6.24(0.23)	1.477	0.227
Leaf trichome density	3.85(0.06)	3.83(0.07)	0.066	0.798

Correlation between leaf damage and resistance traits

We found a positive phenotypic correlation (individual values) between leaf trichome density, and relative fitness. Also, a negative correlation between leaf damage and leaf trichome density ($r = -0.17$, $P = 0.04$) was detected. However, only the genetic correlation (family values) between leaf trichome density and relative fitness was significant ($r = 0.83$, $P < 0.001$) (Table 3).

Costs

We failed to detect significant costs for any of the resistance attributes. In fact, contrary to expectations, the relationship between leaf trichomes and plant fitness was positive even in the absence of herbivores ($r = 0.92$, $P = 0.0006$).

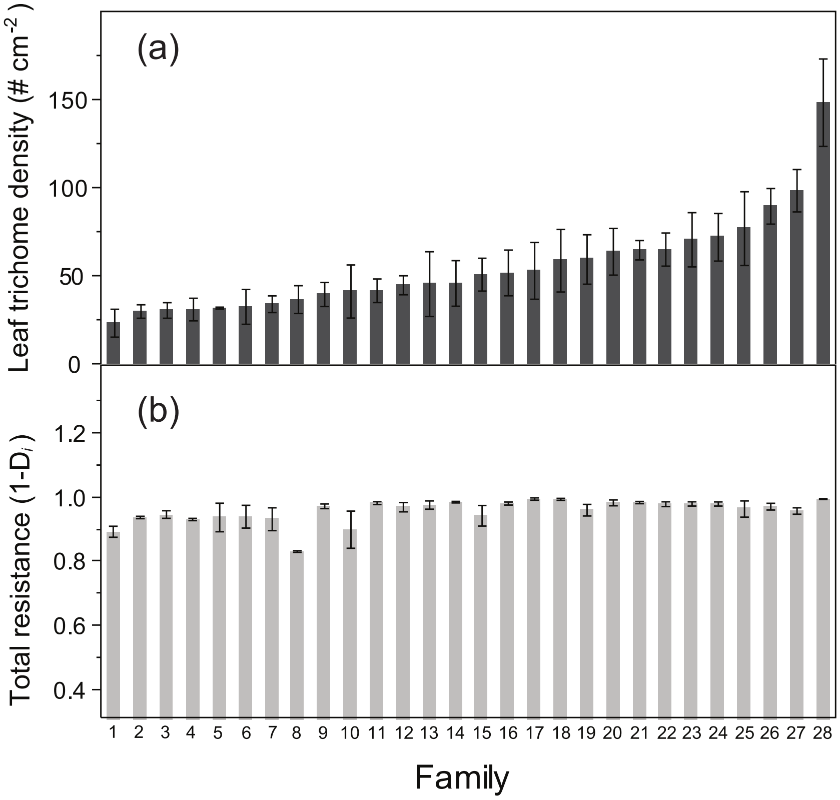


Figure 2 Average values (±SE) of (A) leaf trichome density and (B) total resistance in families of *Datura stramonium*.

Table 2 Analysis of variance of reproductive (flowers) and resistance traits (total resistance and leaf trichomes density). The family is the random effect and block is a fixed factor. A significant effect of family ($P < 0.05$) indicates that there is genetic variance. The analysis was performed with plants exposed to herbivores.

Character	Source of variation	d.f.	*F*	*P*
Flower number	Family	27	1.94	**0.01**
	Block	2	6.32	**0.002**
	Error	90		
	Total	119		
Total resistance	Family	27	1.64	**0.04**
	Block	2	0.81	0.44
	Error	96		
	Total	125		
Leaf trichome density	Family	27	3.06	<**0.0001**
	Block	2	4.62	**0.012**
	Error	96		
	Total	125		

Notes.

Values in bold indicate $P < 0.05$.

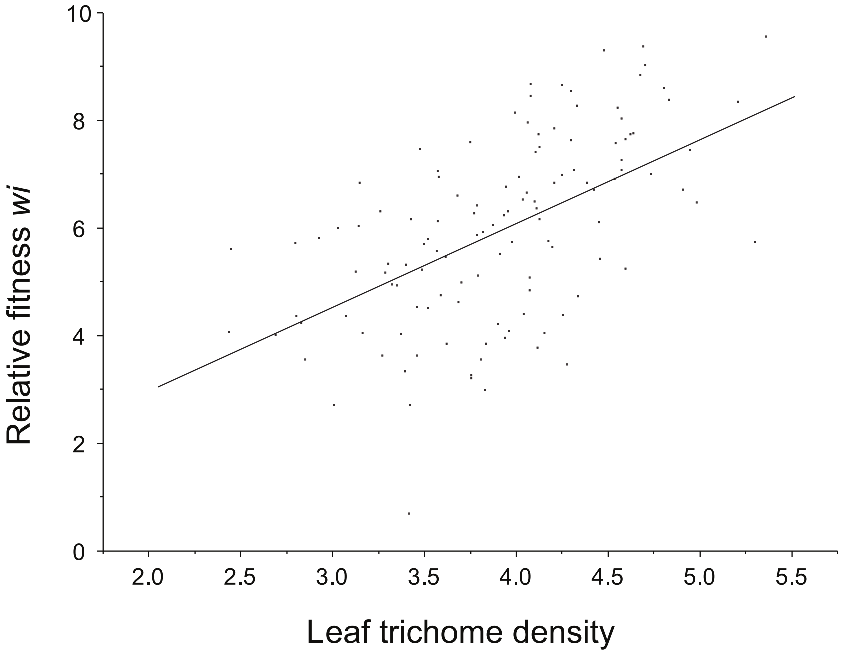


Figure 3 Relationship between leaf trichome density and relative plant fitness in the presence of herbivores.

Table 3 Phenotypic (above the diagonal), and genetic (below the diagonal) correlations between leaf damage, leaf trichome density, relative fitness (*wi*), and total resistance in *Datura stramonium* plants. The analysis was performed with plants exposed to herbivores.

	Leaf damage (relative)	Leaf trichome density	Relative fitness (wi)	Total resistance
Leaf damage (relative)	1.0	-0.179^{*}	−0.109	−1.0
Leaf trichome density	−0.29	1.0	0.562^{***}	0.18^{*}
Relative fitness (*wi*)	−0.3	0.83^{***}	1.0	0.11
Total resistance	−1.0	0.29	0.3	1.0

Notes.

* $P < 0.05$.

*** $P < 0.0001$.

Natural selection on resistance and tolerance

Positive directional selection was detected on leaf trichome density (resistance component) (Fig. 3). However, directional selection was not significant for total resistance. No non-linear selection (curvilinear) for any of the resistance attributes (leaf trichomes and total resistance) was detected (Table 4). Also, neither directional nor non-linear selection acting on tolerance was found.

Genetic correlation (trade-off) between resistance and tolerance

We did not detect the presence of a trade-off between defensive strategies (resistance and tolerance). The correlation between tolerance and the family averages of the two resistance

Table 4 Linear (β), and non-linear (γ) selection gradients on resistance traits to herbivores for *Datura stramonium* plants growing in Ticuman, Morelos.

Character	β_1	t	P	ANOVA of the multiple linear regression model	γ	t	P	ANOVA of the multiple quadratic regression model
Total resistance	0.179 (0.145)	1.23	0.22	$F = 57.27$ $P < 0.0001$ $R^2 = 0.31$	−0.002 (0.061)	−0.04	0.96	$F = 14.02$ $P < 0.0001$ $R^2 = 0.31$
Leaf trichome density	0.89 (0.117)	7.57	**<0.0001**		0.024 (0.065)	0.37	0.7	

Notes.
Values in bold indicate $P < 0.05$.

attributes analyzed was not significant (leaf trichomes density: $r = -0.217$, $P = 0.226$; total resistance: $r = 0.155$, $P = 0.431$).

DISCUSSION

Selection to increase leaf trichome was detected in the population of *D. stramonium*. This character was negatively related to damage by herbivores. However, we did not detect selection on total resistance suggesting that individual variation in resistance includes other components besides leaf trichome density (*Agrawal, 2011*). Although we found evidence of genetic variation in resistance and leaf trichome density, we failed to detect genetic variation for plant tolerance to damage. Hence, we found not support for the trade-off hypothesis between plant resistance and tolerance, or between tolerance and a specific resistance trait (leaf trichome density).

Furthermore, we did not detect fitness costs of leaf trichome density in the absence of herbivores. In fact, the effect of this trait fitness was positive even in the absence of herbivores, suggesting that such resistance trait could be correlated with other traits that were subject to selection (*Björkman & Anderson, 1990*; *Roy, Stanton & Epplely, 1999*), or that leaf trichomes may have another function besides defense. Empirical evidence has shown that, in addition to being a mechanical barrier to herbivores (*Baur, Blinder & Benz, 1991*; *Agrawal et al., 2009*), nonglandular trichomes (not-producing chemical compounds) can reduce the amount of heat on the leaf surface (*Ehleringer, Björkman & Mooney, 1976*; *Vogelmann, 1993*), thus reducing water loss through evapotranspiration. Nevertheless, our results are consistent with a defensive role, even when defense would not be the primary function of leaf trichomes.

In the present study, we did not detect genetic variance for tolerance contrasting with results reported for the same population by *Fornoni, Valverde & Núñez-Farfán (2003)*. A possible explanation is that the low levels of leaf damage recorded in this study (86% of individuals that received leaf damage, experienced less than 10% of loss) prevented the expression of differences in tolerance i.e., lack of genetic variation. Previous evidence indicates that *D. stramonium* in the same population receives, on average, more damage by herbivores (21.65% ± 0.7; mean ± SE) than the average level recorded in this study. In the Ticuman population, the detection of genetic variance occurred when the damage

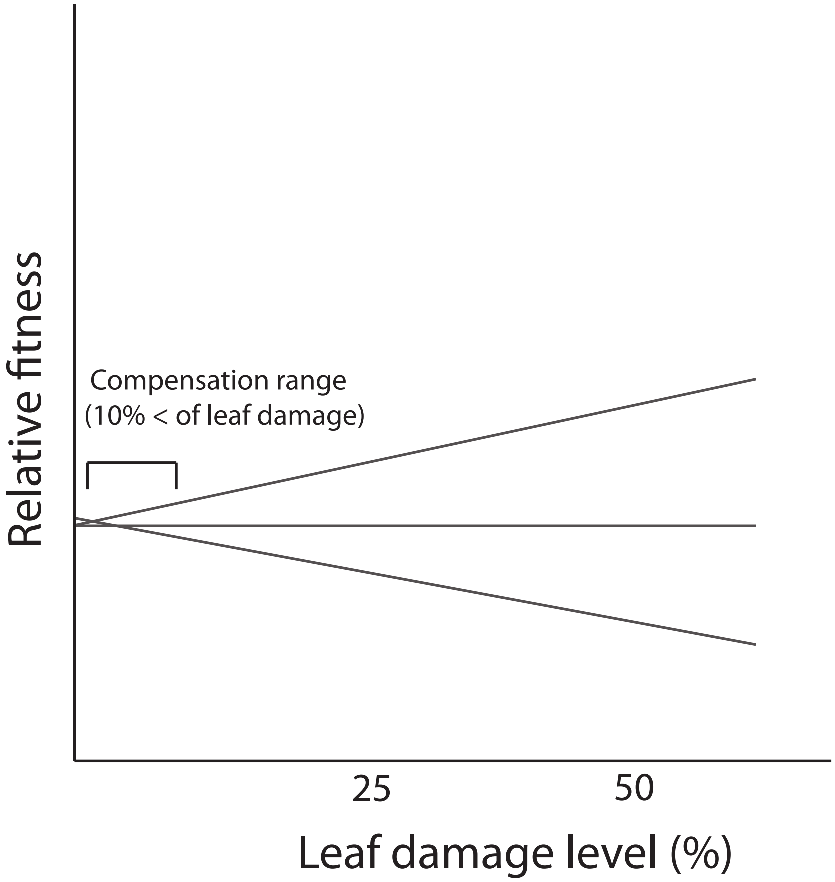


Figure 4 Fitness reaction norms of hypothetical genotypes as a function of damage by herbivores. Genetic variance in reaction norms would be detected when damage is over 10%.

surpassed 10% of total leaf area (see *Fornoni, Valverde & Núñez-Farfán, 2003*). Thus, if tolerance is a genotype's reaction norm of fitness in a damage gradient, differences in the reaction norms (i.e., G × E interaction, implying tolerance) could be detected when damage attains higher values. But at low levels of leaf damage, only part of the reaction norm is apparent in the narrow range of the damage gradient and no differences among genotypes is present (Fig. 4). This fact might possibly preclude the detection of a significant correlation between defensive strategies (or a specific resistance trait). Previous studies have shown how the correlation between resistance and tolerance may vary depending on the biotic environment (e.g., levels of leaf damage due to herbivory). For instance, *Fornoni, Valverde & Núñez-Farfán (2003)* assessed the correlation between defensive strategies by conducting a reciprocal transplants experiment between two natural populations of *D. stramonium*. They found that the detection of a trade-off between total resistance and tolerance occurred only for native plants growing in the population with the highest levels of leaf damage (i.e., Ticuman). In contrast, in the population where the average level of leaf damage was lower (i.e., Santo Domingo) no trade-off was detected, suggesting that the amount of leaf damage may restrict the detection of a significant correlation between plant defenses.

The expression of a negative correlation could also be restricted by patterns of resource acquisition and allocation to defense. Theoretically, if variation in resource allocation is greater than variation in acquisition (i.e., increasing defense costs), a negative correlation between defenses is a possible outcome (*Houle, 1991*; *Mole, 1994*). Allocation patterns depend mainly on the frequency and intensity of herbivory during the plant's life (*Basey, Jenkins & Busher, 1988*; *Boucher, 1985*; *Langenheim & Stubblebine, 1983*). The low levels of leaf damage detected in this study may indicate (besides of resistance traits acting on plant consumers) a low abundance of herbivores. This factor could reduce the variance of resource allocation to defense. Consequently, defense costs could be diminished, and plants could simultaneously allocate resources to different *classes* of defensive traits, i.e., a trade-off between defensive strategies would not be favored.

On the other hand, the trade-off hypothesis between defensive strategies is based on the assumption of redundancy. However, the defensive role of plant defense-related traits (strategies) would depend on the identity and diversity of herbivores attacking the plants. Previous studies on *D. stramonium* have shown how different herbivores could modify the selection pattern on resistance traits (*Shonle & Bergelson, 2000*; *Lankau, 2007*), or defense strategies (*Carmona & Fornoni, 2013*), and how geographic variation of the herbivore community is related to variation in the selective patterns exerted by plant consumers on chemical and physical resistance traits.

CONCLUSIONS

Altogether, our results suggest that resistance could evolve independently from plant tolerance in the Ticuman population. Leaf trichome density is a heritable resistance component and thus it has no restrictions on evolving, and the pattern of selection should favor those individuals with high levels of leaf trichomes. Even when no evidence of a trade-off between plant resistance and tolerance was found, it should not be excluded because the expression of tolerance and its correlation with resistance seems to be a function of the magnitude of damage by herbivores in this population.

ACKNOWLEDGEMENTS

We thank Jesús Vargas, Vania Jiménez-Lobato, R. Tapia-López, and the all members of the *Laboratorio de Genética Ecológica y Evolución* UNAM for logistic support and valuable field assistance. Thanks to I. Salgado Ugarte (FES Zaragoza-UNAM) for his help with statistical analyses, and to the Centro de Productos de Desarrollo Biótico (CEPROBI-IPN) for providing the experimental plot. Earlier versions of this manuscript were greatly enhanced by J. Fornoni and PL Valverde.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This paper constitutes a partial fulfillment of the Graduate Program in Biological Sciences of the National Autonomous University of Mexico (UNAM). EKB received scholarship and financial support by the National Council of Science and Technology (CONACyT). The study was funded by the CONACyT grant "Evolución Adaptativa en Datura: Resistencia y Tolerancia a los Herbívoros" to JNF. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

Graduate Program in Biological Sciences of the National Autonomous University of Mexico.

National Council of Science and Technology.

CONACyT.

Competing Interests

The authors declare they have no competing interests.

Author Contributions

- Eunice Kariñho-Betancourt conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Juan Núñez-Farfán conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.789#supplemental-information.

REFERENCES

Abrahamson WG, Weis AE. 1997. *Evolutionary ecology across three trophic levels: goldenrods, gall-makers and natural enemies*. Vol 29. Princeton: Princeton University Press.

Agrawal AA. 2011. Current trends in the evolutionary ecology of plant defence. *Functional Ecology* **25**:420–432 DOI 10.1111/j.1365-2435.2010.01796.x.

Agrawal AA, Fishbein M, Jetter R, Salminen JP, Goldstein JB, Freitag AE, Sparks JP. 2009. Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): chemistry, ecophysiology, and insect behavior. *New Phytologist* **183**:848–867 DOI 10.1111/j.1469-8137.2009.02897.x.

Basey J, Jenkins S, Busher P. 1988. Optimal central-place foraging by beavers: tree size selection in relation to defensive chemicals of quaking aspen. *Oecologia* **76**:278–282 DOI 10.1007/BF00379963.

Baur R, Blinder S, Benz G. 1991. Nonglandular leaf trichomes as short-term inducible defense of the gray alder, *Alnus incana* L, against the chrysomelid beetle, *Agelastica Alni* L. *Oecologia* **87**:219–226 DOI 10.1007/BF00325259.

Berenbaum MR, Zangerl AR, Nitao JK. 1986. Constrains on chemical coevolution: wild parsnip and the parsnip webworm. *Evolution* **40**:1215–1228 DOI 10.2307/2408949.

Björkman C, Anderson DB. 1990. Trade-off among antiherbivore defences in South American blackberry (*Rubus bogotensis*). *Oecologia* **85**:247–249 DOI 10.1007/BF00319409.

Boucher DH. 1985. *The biology of mutualism: ecology and evolution*. Oxford: Oxford University Press.

Carmona D, Fornoni J. 2013. Herbivores can select for mixed defensive strategies in plants. *New Phytologist* **197**:576–585 DOI 10.1111/nph.12023.

Castillo G, Cruz LL, Hernández-Cumplido J, Oyama K, Flores-Ortiz CM, Fornoni J, Valverde PL, Núñez-Farfán J. 2013. Geographic association and temporal variation of chemical and physical defense and leaf damage in *Datura stramonium*. *Ecological Research* **28**:663–672 DOI 10.1007/s11284-013-1059-4.

Castillo G, Cruz LL, Tapia- López R, Olmedo-Vicente E, Carmona D, Anaya-Lang AL, Fornoni J, Andraca-Gómez G, Valverde PL, Núñez-Farfán J. 2014. Selection mosaic exerted by specialist and generalist herbivores on chemical and physical defense of *Datura stramonium*. *PLoS ONE* **9**:e102478 DOI 10.1371/journal.pone.0102478.

Ehleringer JR, Björkman O, Mooney HA. 1976. Leaf pubescence: effects on absorbance and photosynthesis in a desert shrub. *Science* **192**:376–377 DOI 10.1126/science.192.4237.376.

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**:586–608 DOI 10.2307/2406212.

Fagerström T, Larsson S, Tenow O. 1987. On optimal defence in plants. *Functional Ecology* **1**:73–81 DOI 10.2307/2389708.

Falconer DS, MacKay TFC. 1995. *Introduction to quantitative genetics*. 4th edition. London: Longman London Press.

Fineblum WL, Rausher MD. 1995. Trade-off between resistance and tolerance to herbivore damage in a morning glory. *Nature* **377**:517–520 DOI 10.1038/377517a0.

Fornoni J, Núñez-Farfán J, Valverde PL, Rausher MD. 2004. Evolution of mixed strategies of plant defense allocation against natural enemies. *Evolution* **58**:1685–1695 DOI 10.1111/j.0014-3820.2004.tb00454.x.

Fornoni J, Valverde PL, Núñez-Farfán J. 2003. Quantitative genetics of plant tolerance and resistance against natural enemies in two natural populations of *Datura stramonium*. *Evolutionary Ecology Research* **5**:1049–1065.

Fornoni J, Valverde PL, Núñez-Farfán J. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. *Evolution* **58**:1696–1704 DOI 10.1111/j.0014-3820.2004.tb00455.x.

Herms DA, Mattson WJ. 1992. The dilemma of plant: to grow or defend. *The Quarterly Review of Biology* **67**:283–335 DOI 10.1086/417659.

Houle D. 1991. Genetic covariances of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* **45**:630–648 DOI 10.2307/2409916.

Kariñho-Betancourt E. 2009. Disyuntiva evolutiva entre la resistencia y la tolerancia los herbívoros en *Datura stramonium*. Masters Thesis, Universidad Nacional Autónoma de México (UNAM), Mexico.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated traits. *Evolution* **37**:1210–1226 DOI 10.2307/2408842.

Langenheim J, Stubblebine W. 1983. Variation in leaf resin composition between parent tree and progeny in *Hymenea*: implication for herbivory in the humid tropics. *Biochemical Systematics and Ecology* **11**:97–106 DOI 10.1016/0305-1978(83)90006-6.

Lankau RA. 2007. Specialist and generalist herbivores exert opposing selection on a chemical defense. *New Phytologist* **175**:176–184 DOI 10.1111/j.1469-8137.2007.02090.x.

Lawrence MJ. 1982. The genetical Analysis of ecological traits. In: Shorrocks, ed. *Evolutionary biology*. Oxford: Blackwell Scientific Publication, 27–63.

Leimu R, Koricheva J. 2006. A meta-analysis of trade-offs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos* **112**:1–9 DOI 10.1111/j.0030-1299.2006.41023.x.

Levin DA. 1973. The role of trichomes in plant defense. *Quarterly Review of Biology* **48**:3–15 DOI 10.1086/407484.

Mauricio R. 1998. Cost of resistance to natural enemies in field populations of the annual plant, Arabidopsis Thaliana. *American Naturalist* **151**:20–28 DOI 10.1086/286099.

Mauricio R, Mojonnier LE. 1997. Reducing bias in the measurement selection. *Trends in Ecology and Evolution* **12**:433–436 DOI 10.1016/S0169-5347(97)01178-6.

Mauricio R, Rausher MD. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* **51**:1435–1444 DOI 10.2307/2411196.

Mauricio R, Rausher MD, Burdick DS. 1997. Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology* **78**:1301–1311 DOI 10.1890/0012-9658(1997)078[1301:VITDSO]2.0.CO;2.

Mole S. 1994. Trade-offs and constraints in plant-herbivore defense theory: a life-history perspective. *Oikos* **71**:3–12 DOI 10.2307/3546166.

Núñez-Farfán J, Cabrales-Vargas RA, Dirzo R. 1996. Mating system consequences on resistance to herbivory and life history traits in *Datura stramonium*. *American Journal of Botany* **83**:1041–1049 DOI 10.2307/2445993.

Núñez-Farfán J, Dirzo R. 1994. Evolutionary ecology of *Datura stramonium* L. in Central México: natural selection for resistance to herbivorous insects. *Evolution* **48**:423–436 DOI 10.2307/2410102.

Núñez-Farfán J, Fornoni J, Valverde PL. 2007. The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics* **38**:541–566 DOI 10.1146/annurev.ecolsys.38.091206.095822.

Rausher MD. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* **46**:616–626 DOI 10.2307/2409632.

Roy BA, Stanton ML, Epplely SM. 1999. Effects of environmental stress on leaf hair density and consequences for selection. *Journal of Evolutionary Biology* **12**:1089–1103 DOI 10.1046/j.1420-9101.1999.00107.x.

Shonle I. 1999. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. *(Solanaceae)*. PhD Thesis, University of Chicago.

Shonle I, Bergelson J. 2000. Evolutionary ecology of the trophane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution* **54**:778–788 DOI 10.1111/j.0014-3820.2000.tb00079.x.

Simms EL, Rausher MD. 1987. Cost and benefits of plant resistance to herbivory. *American Naturalist* **130**:570–581 DOI 10.1086/284731.

Simms EL, Rausher MD. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. *Evolution* **43**:573–585 DOI 10.2307/2409060.

Simms EL, Triplett J. 1994. Costs and benefits of plant responses to disease. *Evolution* **48**:1973–1985 DOI 10.2307/2410521.

Stowe KA, Marquis RJ, Hochwender CG, Simms Roy EL. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology, Evolution and Systematics* **31**:565–595 DOI 10.1146/annurev.ecolsys.31.1.565.

Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* **14**:179–185 DOI 10.1016/S0169-5347(98)01576-6.

Valverde PL, Fornoni J, Núñez-Farfán J. 2001. Defensive role of leaf trichome in resistance to herbivorous in *Datura stramonium*. *Journal of Evolutionary Biology* **14**:424–432 DOI 10.1046/j.1420-9101.2001.00295.x.

Valverde PL, Fornoni J, Núñez-Farfán J. 2003. Evolutionary tolerance and resistance as plant defenses. *Evolutionary Ecology* **14**:491–507.

van der Meijeden E, Wijn EH, Verkaar J. 1988. Defence and regrowth: alternative plant strategies in the struggle against herbivores. *Oikos* **51**:355–363 DOI 10.2307/3565318.

Via S. 1984. Quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. *Evolution* **38**:896–905 DOI 10.2307/2408399.

Vogelmann TC. 1993. Plant-tissue optics. *Annual Review of Plant Biology* **44**:231–251 DOI 10.1146/annurev.pp.44.060193.001311.

Weaver SE, Warwick SI. 1984. The biology of Canadian Weeds. 64. *Datura stramonium* L. *Canadian. Journal of Plant Science* **64**:979–991.

Wink M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* **64**:3–19 DOI 10.1016/S0031-9422(03)00300-5.

DISCUSIÓN GENERAL

Identificar en qué medida los fenotipos de las plantas son el resultado de la selección natural impuesta por sus consumidores, i.e., son adaptaciones, es uno de los grandes retos para la ecología evolutiva de la interacción planta-herbívoro. En general, los resultados de esta tesis indican que los tricomas foliares y tropano alcaloides de *Datura* representan adaptaciones en respuesta a contextos selectivos complejos y cambiantes, funcionando en gran medida como defensas ante diversos herbívoros. En particular, los resultados indican que, (1) congruente con un rol adaptativo, la expresión y covariación de las *defensa*s está asociada con la ontogenia de la planta y con la variación en la magnitud del herbivorismo (i.e. cantidad de daño foliar) o composición de la comunidad de herbívoros (e.g., herbívoros generalistas/especialistas), y depende además, de la *clase* de carácter defensivo (i.e., químico vs. físico) o estrategia (tolerancia o resistencia) involucrada en la defensa. (2) El valor adaptativo de la *defensa* de las plantas varía en función del tipo y la *clase* (química/física) de atributo, así como del grado de especialización de los herbívoros, y factores ambientales como la aridez del hábitat. Y finalmente (3), las dinámicas evolutivas de las *defensas* de *Datura* inferidas en la filogenia son consistentes con las observaciones hechas en las poblaciones.

En el capítulo I se muestra cómo la expresión fenotípica de los tricomas foliares y tropano alcaloides (atropina, escopolamina, hiosciamina, tropina y solanina) es independiente de la filogenia (i.e. no hay señal filogenética; Bloomberg et al., 2003), y diverge entre especies de *Datura* que en las poblacionales naturales reciben niveles contrastantes de daño foliar. Al mismo tiempo, en los capítulos II y III se muestra cómo el desempeño de los herbívoros está asociado con la defensa química y física de *Datura* a través del clado, y cómo la pubescencia de las hojas reduce el daño foliar en una población natural. Estos resultados sugieren, en su conjunto, que la evolución del fenotipo químico y la pubescencia de las hojas es adaptativa (i.e., resistencia), y que responde en gran medida a la presión de selección ejercida por los herbívoros. No obstante, consistente con los modelos que predicen la evolución correlativa de las defensas (Steward y Keeler, 1988; Fineblum y Rausher, 1995; Abrahamson y Weis, 1997), los resultados obtenidos en el primer capítulo, revelan una disyuntiva (correlación filogénica negativa) en la evolución de la hiosciamina y los tricomas foliares *vs*. la escopolamina. También indican la evolución de una correlación positiva entre la hiosciamina y los tricomas foliares, sugiriendo que la defensa, en particular la resistencia como estrategia, está compuesta por un conjunto de caracteres coadaptados (Agrawal y Fishbein, 2006) que incluye atributos físicos/mecánicos de la hoja y a los principales tropano alcaloides del género *Datura*. Estas diferencias en la dinámica evolutiva de alcaloides y tricomas parecen estar relacionadas con un tipo particular de interacciones entre las *daturas* y sus consumidores.

La composición de la comunidad de herbívoros (e.g., proporción de herbívoros especialistas y generalistas) ha mostrado ser un factor central en la dinámica evolutiva y ecológica de los atributos putativos de defensa en distintos taxones de plantas (e.g., Agrawal et al., 2006), incluyendo especies de *Datura* (Shonle y Bergelson, 2000; Carmona y Fornoni, 2013; Castillo et al., 2014). A este factor se le ha atribuido entre otras cosas, la variación cuantitativa (e.g., la conservación en las poblaciones naturales de altos niveles de diversos compuestos químicos; Berenbaum et al., 1986; Bennet y Wallsgrove, 1994), y cualitativa de la defensa química (e.g., Karban y Myers, 1989; Karban y Baldwin, 1997). En *Datura stramonium*, por ejemplo, se ha documentado una alta concentración de atropina y escopolamina en poblaciones naturales, que parece variar en torno a la composición de herbívoros (Castillo et al., 2014). Así, consistente con la evidencia documentada a nivel poblacional, en el capítulo I se sugiere que las correlaciones filogenéticas entre los principales alcaloides y los tricomas foliares, podrían reflejar el efecto diferenciado de la selección ejercida por herbívoros, con distinto grado de especialización, sobre dichos atributos relacionados con la defensa de las *daturas*. Y, consistente con este planteamiento, en el capítulo II se demuestra que el desempeño contrastante entre herbívoros que difieren en su grado de especialización en *Datura*, está relacionado con la densidad de tricomas y la concentración de alcaloides, en especial de la hiosciamina y escopolamina; la hiosciamina es el único alcaloide que se asocia contrastantemente con el desempeño del herbívoro especialista y el generalista (asociación positiva con el especialista *vs*. negativa con el generalista).

A diferencia de la mayoría de estudios que han examinado el patrón de defesa en plantas empleando uno o dos rasgos defensivos (Lankau, 2007), en la presente tesis, se analizaron diversos rasgos defensivos y componentes del desempeño de los herbívoros de forma simultánea, lo que permitió dilucidar la complejidad de la relación entre los atributos de respuesta de los herbívoros y los de defensa de las plantas. Por ejemplo, se detectó un efecto negativo tanto de la escopolamina como de los tricomas foliares y la concentración de alcaloides totales (suma de los cinco alcaloides cuantificados), sobre ciertos componentes del desempeño de ambos herbívoros (no sólo del generalista), demostrando cómo, el valor adaptativo de los distintos atributos defensivos en *Datura*, varía en función del grado de especialización de sus consumidores. Un hallazgo interesante, es que el herbívoro generalista consume una cantidad substancial de tejido foliar de prácticamente todas las especies de *Datura*. Sin embargo, a diferencia del especialista, el herbívoro generalista no incrementa (e inclusive en muchos casos reduce) su biomasa, sugiriendo, en consistencia con las predicciones teóricas, una mayor sensibilidad de los herbívoros no especializados a los compuestos tóxicos de las plantas. Al mismo tiempo, estos resultados apuntan a que la eficiencia de las *defensas* de *Datura*, depende, además de la historia coevolutiva entre éstas y sus consumidores, del componente de desempeño del insecto herbívoro. Mientras ciertos atributos defensivos pueden ser efectivos afectando el incremento en biomasa o la cantidad de comida consumida, también pueden no afectar la eficiencia en crecimiento.

Si bien los resultados del capítulo II sugieren diferencias adaptativas entre los herbívoros en relación a los mecanismos (adaptativos) de desintoxicación, ya que también se observó una asociación contrastante entre el herbívoro especialista y el generalista con algunos macronutrientes (e.g., el fósforo); estos resultados apuntan a diferencias fisiológicas, metabólicas y/o conductuales de los herbívoros, para alcanzar el balance de nutrientes y regular el metabolismo. No obstante, se ha sugerido que para entender el desempeño de los herbívoros, y por tanto su éxito ecológico y evolutivo, se debe considerar no sólo las defensas o nutrientes de las plantas, sino la interacción entre de ambos (Slansky, 1992; Simpson y Raubenheimer, 2001). Por ejemplo, independientemente del grado de especialización, se ha documentado experimentalmente una relación inversamente proporcional entre la efectividad de las defensas de las plantas y el balance óptimo de nutrientes en insectos herbívoros (Thompson y Simpson, 2009). En este sentido, el análisis de atributos múltiples de la planta relacionados con la defensa, es un aspecto clave para entender cuáles factores selectivos están involucrados en la interacción planta-herbívoro y cómo éstos favorecen o restringen su dinámica ecológica y evolutiva.

Otro factor clave en el estudio de la evolución adaptativa de la defensa de las plantas, es su variación espacial y temporal. Su examen permite identificar los contextos o factores selectivos asociados con los atributos putativos de defensa. Por ejemplo, se ha demostrado que los cambios en la herbivoría y la disponibilidad de recursos durante el desarrollo de las plantas (i.e., ontogenia), promueve cambios en la funcionalidad de las estrategias defensivas (e.g., Boege et al., 2007), o de caracteres específicos de defensa (Boege y Marquis, 2005; Barton y Koricheva, 2010). En el capítulo I se muestra una gran variación en las trayectorias ontogenéticas de alcaloides y tricomas. Siendo las de los alcaloides, las que exhiben una tendencia común a incrementarse en el género, y también las de mayor magnitud. Los resultados de esta tesis indican que la ontogenia de *Datura*, afecta no sólo la expresión de alcaloides y tricomas, sino también su asociación. Ya que las correlaciones filogenéticas entre los principales alcaloides y los tricomas foliares fueron significativas sólo durante la etapa juvenil (i.e., antes de la reproducción), es probable que estos patrones ontogenéticos sean el resultado de la interacción de factores intrínsecos de la planta (e.g., adquisición y asignación de recursos; Bryant et al., 1983; Herms y Matson, 1992) y del contexto ambiental. Empero, dado que los atributos involucrados (alcaloides y tricomas) tienen una reconocida función defensiva, su variación a través del desarrollo de la planta puede estar relacionada en gran medida con la variación del ambiente biótico (i.e., herbívoros). En *Datura*, se ha documentado que la mayoría de sus herbívoros, se sincronizan con el inicio de la reproducción (Espinosa y Fornoni, 2006). Este factor puede afectar la funcionalidad de las defensas, por ejemplo diluyendo la redundancia adaptativa entre distintos rasgos defensivos durante la etapa reproductiva. Así, es posible, que tanto la variación ontogenética en la expresión fenotípica de los alcaloides y tricomas, así como en sus correlaciones, tenga un valor adaptativo en la medida en que la abundancia y tipo de herbívoros (i.e., la presión de selección) varíe temporalmente. No obstante, otros factores ambientales además de los herbívoros podrían ser responsables (al menos parcialmente), no sólo de la variación ontogenética en la expresión de los rasgos defensivos, sino también de la divergencia de los fenotipos defensivos entre las especies, observada en este estudio.

Factores climáticos como la temperatura y la precipitación han sido comúnmente relacionados con la pubescencia de las hojas (e.g., Ehleringer y Clark, 1987; Sandquist y Ehleringer, 2003). La presencia de una alta densidad de tricomas no glandulares ha sido correlacionada con hábitats abiertos, calientes y áridos. En estas condiciones los tricomas protegen a la hoja de la luz ultravioleta (UV), reducen la absorbancia de la luz solar y la pérdida de agua (Ehleringer et al., 1976). La multifuncionalidad de los tricomas foliares ha sido ampliamente documentada tanto en clados como en poblaciones. Por ejemplo, estudios interespecíficos (con control filogenético) realizados en el género *Asclepias* han demostrado que además de funcionar como barrera para el crecimiento de uno de sus herbívoros especialistas (orugas de la mariposa monarca, *Danaus plexippus*), los tricomas foliares también impiden la pérdida de agua por evapotranspiración (Agrawal y Fishbein, 2006; Agrawal et al., 2009). Los resultados de esta tesis, muestran una extraordinaria variación en la densidad de tricomas entre especies (Capítulo I). Mientras unas especies exhiben hojas glabras (baja densidad o ausencia de tricomas foliares), otras poseen hojas sumamente pubescentes, llegando a alcanzar densidades de más de 3000 tricomas cm^{-2}. Esta variación parece estar asociada con la aridez ambiental, dado que el patrón de distribución geográfica de las especies de *Datura* muestra cómo las especies pubescentes se encuentran en zonas áridas, mientras que las especies glabras tienden a distribuirse en zonas más templadas o húmedas. Al mismo tiempo, en *D. stramonium* (Capítulo III), se registró una relación positiva de la densidad de tricomas foliares con la adecuación de las plantas, aún en ausencia de los herbívoros (factor selectivo), sugiriendo que el beneficio de este atributo para las plantas va más allá de la defensa.

En el capítulo III, no sólo se documenta el rol defensivo de los tricomas foliares (reduciendo un 15% de daño foliar) y el papel de la selección ejercida por los herbívoros en incrementar su densidad, en una población natural de *D. stramonium*, sino también la ausencia de una correlación negativa (trade-off) entre los rasgos de resistencia (resistencia total y tricomas foliares) y la tolerancia. No obstante, en la misma población, estudios previos han demostrado que bajo niveles de daño foliar más alto del 10%, es posible detectar una correlación negativa entre resistencia y tolerancia. En el presente estudio, el promedio de daño foliar fue inferior al 10%. Es probable entonces que este factor condicionara la detección tanto de varianza genética en la tolerancia, como de una correlación significativa entre ésta y la resistencia. Al respecto, la evidencia acumulada a través de los tres capítulos de la tesis, apunta a que tanto tricomas foliares como alcaloides son sumamente “sensibles” al contexto biótico ambiental, en particular a la variación en la comunidad de herbívoros. Esto sugiere que el herbivorismo ha sido uno de los principales promotores de la evolución adaptativa del fenotipo químico y la pubescencia de las hojas en el género *Datura*.

En esta tesis se ha evaluado la medida en la que los herbívoros influyen en la evolución de las plantas, pero también, de manera recíproca, se ha podido vislumbrar la medida en que las plantas (a través de sus defensas) afectan a sus consumidores. La combinación de análisis filogenéticos explícitos (i.e., método comparativo), con la genética cuantitativa, permitió identificar patrones adaptativos comunes en el género *Datura*, y evaluar en qué medida la selección está actuando sobre ciertos atributos de las plantas en las poblaciones naturales. Los patrones filogenéticos aquí reportados son bastante congruentes con las observaciones hechas a nivel poblacional en algunas especies del género, y con la propia evidencia intraespecífica documentada en el capítulo III con respecto a la función defensiva de los tricomas foliares. En su conjunto, la evidencia empírica documentada aquí, apunta a que la escopolamina, hiosciamina y la pubescencia de las hojas constituyen el mecanismo defensivo común de las *daturas* para enfrentar a sus enemigos naturales. En este trabajo se puso de manifiesto la utilidad de considerar múltiples caracteres defensivos, o con influencia en la defensa (e.g., nutrientes), para dilucidar patrones ecológico-evolutivos de la defensa de las plantas, y ampliar las avenidas que permiten poner a prueba hipótesis coevolutivas, y entender la naturaleza e implicaciones de la interacción planta-herbívoro.

LITERATURA CITADA

Abrahamson WG, Weis AE. 1997. *Evolutionary ecology across three trophic levels: Goldenrods, gall-makers and natural enemies*. Princeton: Princeton University Press.

Agrawal AA, Fishbein M. 2006. Plant defense syndromes. *Ecology* 87: S132-S149.

Agrawal AA, Fishbein M, Jetter R, Salminen JP, Goldstein JB, Freitag AE, Sparks JP. 2009b. Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepia*s spp.): Chemistry, ecophysiology, and insect behavior. *New Phytologist* 183: 848-867.

Barton KE, Koricheva J. 2010. The ontogeny of plant defense and herbivory: Characterizing general patterns using meta-analysis. *American Naturalist* 175: 481-493.

Bennett RN, Wallsgrove RM. 1994. Secondary metabolites in plant defence mechanisms. *New Phytologist* 127: 617-633.

Berenbaum MR, Zangerl AR, Nitao JK. 1986. Constrains on chemical coevolution: Wild parsnip and the parsnip webworm. *Evolution* 40: 1215-1228.

Bloomberg SP, Garland T, Ives AR. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* 57: 717-745.

Boege K, Marquis RJ. 2005. Facing herbivory as you grown up: The ontogeny of resistance in plants. *Trends in Ecology and Evolution* 20: 441-448.

Boege K, Dirzo R, Siemens D, Brown P. 2007. Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? *Ecology Letters* 10: 177-187.

Bryant JP, Chapin III FS, Klein DR. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40: 357-368.

Carmona D, Fornoni J. 2013. Herbivores can select for mixed defensive strategies in plants. *New Phytologist* 197: 576-585.

Castillo G, Cruz LL, Tapia- López R, Olmedo-Vicente E, Carmona D, Anaya-Lang AL, Fornoni J, Andraca-Gómez G, Valverde PL, Núñez-Farfán J. 2014. Selection mosaic exerted by specialist and generalist herbivores on chemical and physical defense of *Datura stramonium*. *PloS one* 9: e102478.

Ehleringer J, Björkman O, Mooney HA. 1976. Leaf pubescence-effects on absorbance and photosynthesis in a desert shrub. *Science* 192: 376-377.

Ehleringer J, Clark C. 1987. Evolution and adaptation in *Encelia* (Asteraceae). In: Gottlieb LD, Jain SK, eds. *Plant evolutionary biology*. London, UK: Chapman & Hall, 221–248.

Espinosa EG, Fornoni J. 2006. Host tolerance does not impose selection on natural enemies. *New Phytologist* 170: 609-614.

Fineblum WL, Rausher MD. 1995. Trade-off between resistance and tolerance to herbivore damage in a morning glory. *Nature* 377: 517-520.

Fornoni J, Valverde PL, Núñez-Farfán J. 2003. Quantitative genetics of plant tolerance and resistance against natural enemies in two natural populations of *Datura stramonium*. *Evolutionary Ecology Research* 5: 1049-1065.

Herms D, Matson W. 1992. The dilemma of plants: To grow or to defend. *Quarterly Review of Biology* 67: 283-335.

Karban R, Baldwin IT. 1997. *Induced Responses to Herbivory*. Chicago: Chicago University Press.

Karban R, Myers JH. 1989. Induced plant responses to herbivory. *Annual Review of Ecology and Systematics* 20: 331-348.

Lankau RA. 2007. Specialist and generalist herbivores exert opposing selection on a chemical defense. *New Phytologist* 175: 176-184.

Sandquist DR, Ehleringer JR. 2003. Population- and family-level variation of brittlebush (*Encelia farinosa*, Asteraceae) pubescence: Its relation to drought and implications for selection in variable environments. *American Journal of Botany* 90: 1481–1486.

Shonle I, Bergelson J. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae) *Evolution* 54: 778-788.

Simpson SJ, Raubenheimer D. 2001. The geometric analysis of nutrient-allelochemical interactions. A case study using locusts. *Ecology* 82: 422-439.

Slansky F. 1992. Allelochemical-nutrient interactions in herbivore nutritional ecology. In *Herbivores: Their interactions with secondary plant metabolites*. Rosenthal, G. A., and Berenbaum, M. R., eds. Academic Pres, New York. 135-174 p.

Steward JL, Keeler KH. 1988. Are there trade-offs among antiherbivore defenses in Ipomoea (Convolvulaceae)? *Oikos* 53: 79-86.

Thompson SN, Simpson SJ. 2009. Nutrition. In *Encyclopedia of insects*. Resh VH, Cardé RT, eds. Academic Press, USA.

Apéndice A

FUNCIÓN DE METABLOLITOS SECUNDARIOS EN PLANTAS

Tabla A. Función de metabolitos secundarios en plantas

Tipo químico	Ejemplo	Taxón(es) vegetal(es)	Efecto	Modo de acción	Taxón(es) "blanco"	Ref
Alcaloides	**Solanina**	*Solanum demissum* (Solanaceae) "papa"	**1. Tóxico** **2. Disuasivo**	***i.*** Compite por los receptores muscarínicos de la Acetil-colina	**a.** *Empoasca fabae* (Hemiptera: Cicadellidae) "chicharrita" **b.** *Leptinotarsa decemlineata* (Coleóptera: Chrysomelidae) "escarabajo de la papa"	[1] $^{1, 2, a, b}$ [2,3] $^{1, i}$
	Escopolamina	*Datura stramonium* (Solanaceae) "toloache"	**1. Fagoestimulante** **2. Tóxico**	n. d.	**a.** *Epitrix* sp. (Coleóptera: Chrysomelidae) "pulguilla de la papa" b. *Helicoverpa zea* (Lepidóptera: Nuctunidae) "gusano del algodón"	[4]
Aminoácidos no proteicos	**Canavanina**	Leguminosas	**1. Tóxico** **2. Disuasivo**	***i.*** Es confundida con el aminoácido arginina y se incorpora a la síntesis de proteínas, con marcados efectos deletéreos.	**a.** *Caryedes brasiliensis** (Coleóptera: Bruchidae) "escarabajo bruquido" **b.** *Sternechus tuberculatus** (Coleóptera: Curculionidae) "picudo de la soya" *Poseen la capacidad de discriminar entre arginina y canavanina, reduciendo la incorporación de canavanina al sistema	[5,1]
Esteroides y Terpenos	**Gosipol**	*Gossypium* sp (Malvaceae) "algodón"	**1. Tóxico** **2. Antialimentario**	***i.*** Inhibe actividad enzimática	**a.** Artrópodos: -*Aphis gossypii* (Hemíptera: Aphididae) "pulgón del algodón"	6] $^{1, 2, i, a}$ [1]$^{2, b}$

Tabla A. Función de metabolitos secundarios en plantas (continuación)

Tipo químico	Ejemplo	Taxón(es) vegetal(es)	Efecto	Modo de acción	Taxón(es) "blanco"	Ref
Esteroides y Terpenos	**Gosipol**	*Gossypium* sp (Malvaceae) "algodón"	**1. Tóxico** **2. Antialimentario**	***i.*** Inhibe actividad enzimática	a. Artrópodos -*Lygus hesperus* (Hemíptera: Miridae) "chinche manchadora" -*Estigmene acrea* (Lepidóptera: Actiidae) "gusano peludo" b. Hongos *Verticillium dahliae* (hongo patógeno)	6] [1, 2, *i*, a] [1][2, b]
Fenoles	**Taninos**	*Quercus robur* (Fagaceae) "roble común*"*	1. **Antialimentario** **2. Tóxico** (retarda el crecimiento e incluso puede deshacer el interior de la larva)	***i.*** Alteran actividad de enzimas digestivas	a. *Operophtera brumata* (Lepidóptera: Geometridae) "palomilla de invierno"	[7]
	Flavonoides [flavonol 3-(2"-xiloxgalactosido)]	Crucíferas	**1. Atrayente y fagoestimulante**	n. d.	a. *Phyllotreta armoraciae* (Coleóptera: Chrysomelidae) "pulguilla de crucíferas"	[8]
Fitohormonas	**Ácido salicílico**	*Nicotiana* sp. (Solanaceae) "tabaco" *Brassica napus* (Brassicaceae) "canola"	1. **Inducción de resistencia** a patógenos 2. **Inducción de síntesis de defensas** (e. g. glucosinolatos)	***i.*** Inducción de proteínas (PR) y enzimas como la 2-hidroxilasa ácido benzoico.	a. Tobamovirus (TMV) "virus del mosaico del tabaco" b. Diversos artrópodos, particularmente insectos.	[1]

Tabla A. Función de metabolitos secundarios en plantas (continuación)

Tipo químico	**Ejemplo**	**Taxón(es) vegetal(es)**	**Efecto**	**Modo de acción**	**Taxón(es) “blanco”**	**Ref**
Glucósidos cardiácos (esteroides)	**Cardenólidos**	*Asclepias* sp (Apocynaceae) “hierba lechosa”	**1. Tóxico y disuasivo** (sabor amargo) **2. Atrayente**	*i.* En animales inhiben bomba de Na^+ (en células (cardiacas, vasculares y nerviosas)	**a.** Artrópodos (particularmente insectos). Se ha documento con gran amplitud el sistema Asclepias - *Danaus* sp - aves predadoras. La larva de *Danaus* sp al alimentarse, adquiere los cardenólidos que son utilizados como defensa en contra de aves predadoras. **b.** *Danaus plexippus* (Lepidóptera: Nymphalidae) ”mariposa monarca”	[10, 11][1,2,*i*,a] [12][2, b] [13][1,*i*, a]
Glucósidos cianogénicos	**Cianida** (liberada mediante cianogénesis)	*Manihot esculenta* (Euphorbiaceae) “yuca”	**1. Tóxico** (pero ver 14)	*i.* Afectan respiración celular (inhiben unión del oxígeno con la enzima citrocormo-*c*-oxidasa)	**a.** *Cyrtomenus bergi* (Hemiptera: Cydnidae) ”chinche de la viruela”	[1][1, a] [15] [*i*]
Látex	**Contenido total**	***Hoodia gordonii*** **(Apocynaceae) “hoodia”**	**1. Tóxico y disuasivo** **2. Antinutritivo** **3. Pegajoso**	n. d.	**a.** *Trichoplusia ni* (Lepidóptera: Noctuidae) “falso gusano medidor” **b.** Artrópodos, principalmente insectos	[16][1, a] [17][1,2,3 b]

n. d., no determinado

LITERATURA CITADA

1. Bennett RN, Wallsgrove RM. 1994. Metabolites in plant defense mechanism. *New Phytologist* 127: 617-633.
2. Wink M, Schmeller T, Latz-Brüning B. 1998. Modes of action of allelochemical alkaloids: Interaction with neuroreceptors, DNA and other molecular targets. *Journal Chemical Ecology* 24: 1881–1937.
3. Roddick, J. 1991. *The importance of the Solanceae in medicine and drug therapy*. Hawkes G, Lester RN, Nee M, Estrada N, eds. Royal Botanic Garden Press.
4. Shonle I, Bergelson J. 2000. Evolutionary ecology of the trophane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution* 54: 778-788.
5. Rosenthal GA, Berembaum MR. 1991. *Herbivores: Their interactions with secondary plant metabolites*. Academic Press. 2nd ed.
6. Bottger GT, Sheehan ET, Lukefahr MJ. 1964. Relation of gossypol content of cotton plants to insect resistance. *Journal of Economic Entomol*ogy 2: 283-285.
7. Feeny P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* 51: 565–581.
8. Nielsen JK, Larsen LM, Sorensen H. 1979. Host plant selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleptera: Chrysomelidae): Identification of two flavonol glycosides stimulating feeding in combination with glucosinolates. *Entomologia Experimentalis et Applicata* 26: 40-48.
9. Bartlet E, Williams IH. 1991. Factors restricting the feeding of the cabbage stem flea beetle (*Psylloides chrysocephala*). *Entomologia Experimentalis et Applicata* 60: 233-238.
10. Malcolm SB. 1991. Cardenolid-mediated interactions between plants and herbivores. En *Herbivores: Their interactions with Secondary Plant Metabolites*. Rosenthal GA, Berenbaum, MR, eds. Academic Press. 7, 251-291.
11. Agrawal AA, Petschenka G, Bingham RA, Weber MG, Rasmann S. 2012. Toxic cardenolides: Chemical ecology and coevolution of specialized plant-herbivore interactions. *New Phytologist* 194: 28-45.
12. Agrawal AA. 2005. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research*. 7: 651–667.
13. Brower LP, Edmunds M, Moffitt CM. 1975. Cardenolide content and palatability of a population of *Danaus chrysippus* butterflies from West Africa. *Journal of Entomology Series A, General Entomology* 49: 183-196.
14. Brattsten LB, Samuelian JH. Long KY, Kincaid SA, Evans CK. 1983. Cyanide as a feeding stimulant for the southern armyworm, *Spodoptera eridania*. *Ecological Entomology* 8: 125-132.
15. Mithöfer A, Boland W. 2012. Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology* 63: 431-450**.**
16. Chow JK, Akhtar Y, Ysman MB. 2005. The effect of larval experience with a complex plant latex on subsequent feeding and oviposition by the cabbage looper moth: *Trichoplusian ni* (Lepidoptera: Noctunidae). *Chemoecology* 15: 129-33.
17. Agrawal AA, Konno K. 2009. Latex: A model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annual Review of Ecology Evolution and Systematics* 40: 311–331.

Apéndice B

¿ES EL ESCALAMIENTO DE LA DEFENSA DE LAS PLANTAS UN RESULTADO MACROEVOLUTIVO COMÚN DE LAS INTERACCIONES PLANTA-HERBÍVORO?

MANUSCRITO ACEPTADO EN *New Phytologist*

Juan Núñez-Farfán and Eunice Kariñho-Betancourt. (2015)
Is escalation of plant defence a common macroevolutionary outcome of plant-herbivore interactions? *New Phytologist*

Is escalation of plant defence a common macroevolutionary outcome of plant-herbivore interactions?

"In view of these considerations, we propose a comparable pattern of adaptive radiation for each of the more or less strictly limited groups of butterflies… It is likewise probable that the elaboration of biochemical defenses has played a critical role in the radiation of those groups of plants characterized by unusual accessory metabolic products."

PR Ehrlich & PH Raven (1964)

The evolution of defence in plants against their enemies and of counter-defence and offense in the enemies has been of utmost importance to construct the theory of coevolutionary arm race (Ehrlich & Raven, 1964; Dawkins & Krebs, 1979). Selection exerted by predators on preys may give rise to escalation in the offensive weaponry from one part, and means to resist, escape, or to injure from the other (Vermeij, 1994). Escalation thus implies that traits that mediate the interaction, and incidentally others carried by linkage, increase their potency, size or quantity, and architectural and functional complexity (Ehrlich & Raven, 1964; Vermeij, 1994; Becerra *et al.,* 2009), as well as the origin of other, new defence characters. Plant chemical compounds ("secondary metabolites") are a paradigmatic example of plant defence against plants' natural enemies (herbivores, pathogens, virus, competitors); more than 200 000 compounds have been described but notably terpenoids, alkaloids and phenolics are particularly numerous (Mithöfer & Boland, 2012). But besides their number, do defensive chemical compounds show escalation in evolutionary time within plant lineages? Few studies have empirically assessed the macroevolutionary pattern of escalation in plant defence (Berenbaum & Feeny, 1981; Becerra *et al*., 2009, Agrawal *et al.*, 2009; Agrawal & Fishbein 2008) because a well-resolved phylogeny is needed in order to estimate the ancestral states of defensive traits (or other ecologically relevant traits), besides measuring defensive traits in multiple species. In this issue of *New Phytologist,* Cacho *et al*. used a phylogenetically explicit analysis to test the hypothesis of escalation in chemical defence (glucosinolates) in the genus *Streptanthus* (Brassicaceae). Species of this genus are morphologically and ecologically variable and have diversified in south-western North America where many species are edaphic specialists. Phylogenetic studies indicate that tolerance to serpentine soils has evolved between 8 to 10 times in *Streptanthus* (Cacho & Strauss 2014). The chemical defensive system against herbivores in Brassicaceae is based in the biosynthesis of glucosinolates and their hydrolyzing enzyme, a thioglucosidase myriosinase. After damage by herbivores both the glucosinolates and the enzyme come into contact to produce nitriles and isothiocyanates, toxic to insects (Mithöfer & Boland, 2012).

In the genus *Streptanthus,* total amount of glucosinolates vary independent of the phylogeny and show no evidence of escalation. However, the relative investment in aliphatic glucosinolates increases over evolutionary time, whereas branched-chain glucosinolates show de-escalation, pointing a trade-off. Further, glucosinolates diversity (Shannon index) and chemical complexity of glucosinolates also show de-escalation. Thus, results of Cacho *et al.* show, in general, a reduction of chemical diversity and complexity but escalation in the proportion of aliphatics.

Resource availability hypothesis (RAH)

Cacho *et al*. went further and using information derived from the phylogeny, chemical defence, and ecological information of contemporary habitats, particularly the type of substrate, they tested predictions of the resource availability hypothesis (Coley *et al*., 1985) in relation to plant defence. RAH predicts high investment on defence in resource-limited environments where replacement of tissue loss to herbivores is expected to be costly, and may drive edaphic specialization. As stated, many *Streptanthus* species are edaphic specialists inhabiting bare rocky soils, and serpentine soils (the latter deficient in NPK, low Ca: Mg ratios, and contain toxic elements). Thus, these ecological factors might condition the expression of chemical defence besides herbivores. Cacho *et al*. contrasted chemical defence between species from habitats that vary in nutrient composition (NO_3N, P, K), soil bareness, and soil type (serpentine *vs*. non-serpentine). Results show that total glucosinolates are not inversely related to nutrient composition as expected under RAH. However, 10 out of 14 relationships between specific glucosinolates classes and nutrient composition are negative, supporting RAH. Finally, since bare and serpentine soils are stressful environments, higher expression of chemical defence is expected. While glucosinolate richness is higher as bareness increase, total and diversity of glucosinolates did not differ between serpentine and non-serpentine soils.

Plants and herbivores diversification promoted by key innovations

The scape-and-radiate scenario proposed by Ehrlich and Raven (1964) predicts a burst of diversification after an evolutionary breakthrough, a “key innovation” (*e.g.*, a novel chemical defence on the plant side, or a counter-defence on the herbivore side). This would opens a novel evolutionary opportunity, and theory predicts that plants and herbivores should undergo rapid speciation to fill up the new adaptive zones, followed by a return to a more “normal” rate of speciation (*e.g.,* Fordyce, 2010).

Following the hypothesis of reciprocal radiation of Ehrlich and Raven (1964), much effort has been devoted to the search of key innovations (*e.g.,* Berenbaum *et al*., 1996; Wheat *et al*., 2007) that could trigger such rapid speciation. However, most examples of adaptive radiation linked to herbivore defences have not demonstrated radiation following a single key innovation, but rather a continuous process of escalation and diversification of multiple herbivore defence strategies through time (Agrawal *et al*., 2009; Becerra *et al*., 2009).

However, recent evidence gives support to the escape-radiate hypothesis. Edger *et al*. (2015) have addressed if the key innovations in the interaction between the plants in the Brassicales and butterflies in the Pierinae increased in complexity through evolutionary time and if associate with changes in diversification rates; to identify the genomic mechanisms that promoted the escalation of innovations that mediate the coevolutionary dynamics, and to test if the increase in net diversification rates is adaptive. According to Edger *et al*. (2015) the key innovation of Brassicales, glucosinolates derived from phenylalanine and chain-branched amino acids, evolved *ca*. 92 million years ago (My). A whole genome duplication (WGD) in Brassicales occurred *ca*. 77.5 My and appeared the indolic glucosinolates synthetized from tryptophan. Analyses show that escalation of glucosinolates diversity occurred both through single gene duplication and WGD, and involved the retention and neofunctionalization of regulatory and core biosynthesis genes. Colonization of Brassicales by Pierinae butterflies occurred *ca*. 68 My concordant with glucosinolate detoxification (a nitrile-specifier protein) and with diversification of Pierinae. Another important change occurred when the ancestors of the Capparaceae and Cleomaceae produced a new class of glucosinolates derived from methionine through gene duplications; today more species of Pierinae fed upon members of these families than from other Brassicales that produce only indolic glucosinolates. Finally, a WGD occurred *ca*. 32 My with the evolution of Brassicaeae, the plant family with the greatest diversity of glucosinolates within Brassicales, and coincides with its incremental diversification. Two independent lineages of Pierinae colonised Brassicaceae and increased their diversification rates. As this study shows, the use of molecular genomics to unravel the mechanism behind plant defences, insect host shifts, specialization, and speciation, has the potential to lift the veil from some of the elusive connections between micro- and macroevolution, such as how traits among the interacting species influence each other's rate of speciation (Janz, 2011). Thus, this is the best evidence available of the escape-radiate hypothesis nearly 50 year after its proposition by Ehrlich and Raven (1964).

Is plant defence escalating through evolutionary time?

As exemplified by different studies, other factors besides herbivores are responsible for the variability in plant defence at a phylogenetic scale. As Cacho *et al*. show, trade-offs between classes of glucosinolates may limit escalation of both defences. Similarly, trade-offs between defence compounds may occur between plant ontogenetic stages at the phylogenetic scale, in response to herbivore pressure (Kariñho-Betancourt *et al.,* 2015); and trade-offs between defence strategies may obscure the pattern of escalation in chemical defence, if any. For instance, Agrawal and Fishbein (2008) detected escalation in the ability of plants to regrowth after damage (tolerance), but de-escalation in resistance traits (cardenolides, trichomes, latex). Given that plants of *Asclepias* are preyed upon by specialist herbivores, the evolution of tolerance to damage can be anticipated. Yet, it is not clear that changes in tolerance impose selective pressures on herbivores; if so, coevolution and escalation based on chemical defence might reach an end.

The interaction between defence strategies, namely resistance and tolerance, at the macroevolutionary scale is an interesting topic to tackle in future studies, in the light of the potential allocation trade-off between both (Núñez-Farfán *et al.,* 2007). Until now, only the study of Agrawal and Fishbein (2008) has documented changes in regrowth in the genus *Asclepias* in the phylogeny. For the genus *Streptanthus* analysed by Cacho *et al*. this is a relevant topic to pursue, since species occupy different, poor and stressful, environments. The RAH hypothesis predicts higher investment in defence in harsh environments, but how this potential trade-off between defence strategies impairs the ability of plants to tolerate or to resist damage, across the phylogeny? Could this trade-off be broken off at the macroevolutionary level?

There are, however, other plant traits that contribute to plant defence and that have not been incorporated in the study of escalation, with one exception. These are qualitative, novel, characters that can be taken into account as an evolutionary “covariate” when testing for escalation in other quantitative defence traits. For instance, many species of *Bursera* discharge pressurized resins when damaged by herbivores. Both, the index of chemical complexity and the number of compounds increase with evolutionary time in *Bursera*, but with a lower slope in squirting *Bursera*, than in non-squirting ones (Becerra *et al*., 2009). This may obey to a relaxation of selection to produce complex and expensive mixtures of chemical compounds. Similarly, Cacho *et al*. suggest that in bare habitats, other traits may contribute to defence and be selectively favoured (*i.e*., reducing plant apparency to herbivores by being cryptic); as their results show, some environments condition the expression of chemical plant defence (*i.e*., serpentine, bare soils). Another trait in *Streptanthus*, egg mimicry, may affect offer defence by deceit and thus interact with the potential escalation of glucosinolates in evolutionary time. Characters of this kind should be considered, as rightly pointed out by Cacho *et al*., to better understand the evolution of plant defence at macroevolutionary level.

Are we looking escalation at only one dimension?

The few but relevant studies of plant defence at macroevolutionary level reveal that defence traits do not escalate simultaneously. The evolutionary trajectory of defence characters can be envisioned as a thread where individual fibres, defence traits, may escalate, de-escalate, replace others (co-opted function), be lost, or remain unchanged as relicts no longer beneficial as defence. The different trajectories of defence traits may be related to their present function and fitness benefit (and cost) in interactions with specific herbivores' traits. Thus, it is still relevant to determine the agents of selection of defence traits in order to contrast their present function with their trajectory along evolutionary time. Most studies to date have found escalation in at least one component of defence (richness, diversity, complexity, strategy). If we consider that plants produce numerous defence and offence characters —including constitutive and induced chemicals, physical defence like squirting toxins, defence by deceit, etc. — and traits to tolerate damage without reducing fitness, there is no doubt that escalation is a likely macroevolutionary outcome of the interaction between plants and herbivores.

Juan Núñez-Farfán* and Eunice Kariñho- Betancourt
Laboratorio de Genética Ecológica y Evolución,
Instituto de Ecología, Universidad Nacional Autónoma de México
Ciudad Universitaria, Coyoacán, México 04510, Distrito Federal.
*Correspondence: farfan@unam.mx
tel +52 55 5622 9005

References

1. **Agrawal AA, Fishbein M. 2008.** Phylogenetic escalation and decline of plant defense strategies. *Proceedings of the National Academy of Sciences USA* **105**: 10057-10060.
2. **Agrawal AA, Fishbein M, Halitschke R, Hastings AP, Rabosky DL, Rasmann S. 2009.** Evidence for adaptive radiation from a phylogenetic study of plant defenses. *Proceedings of the National Acadaemy of Sciences USA* 43: 18067–18072.
3. **Becerra JX, Nogeb K, Venable DL.** 2009. Macroevolutionary chemical escalation in an ancient plant– herbivore arms race. *Proceedings of the National Academy of Sciences USA* **106**: 18062–18066.
4. **Berenbaum M, Feeny P. 1981.** Toxicity of angular furanocoumarins to swallowtail butterflies: escalation in a coevolutionary arms race? *Science* **212**: 927-929.
5. **Berenbaum MR, Favret C, Schuler MA. 1996**. On defining "key innovations'' in an adaptive radiation: Cytochrome P450s and Papilionidae. *American Naturalist* **148**:S139–55.
6. **Cacho NI, Burrell AM, Pepper AE, Strauss SY.** 2014. Novel nuclear markers inform the systematics and the evolution of serpentine use in *Streptanthus* and allies (Thelypodieae, Brassicaceae). *Molecular Phylogenetics and Evolution* **72**: 71–81.

7. **Cacho NI, Kliebenstein DJ, Strauss SY.** 2015. Macroevolutionary patterns of glucosinolate defense and tests of defense-escalation and resource availability hypotheses. *New Phytologist.*
8. **Coley PD, John P. Bryant JP, Chapin III FS. 1985**. Resource availability and plant antiherbivore defense. *Science* **230**: 895-899.
9. **Dawkins R, Krebs JR**. 1979. Arms races between and within species. *Proceedings of the Royal Society of London Series B, Biological Sciences* **205**: 489-511.
10. **Edger PP, Heidel-Fischer HM, Bekaert M, Rota J, Glöckner G, Platts AE, Heckel DG, Der JP, Wafula EK, Tanga M, Hofberger JA, Smithson A, Hall JC, Blanchette M, Bureau TE, Wright SI, dePamphilis CW, Schranz ME, Barker MS, Conant GC, Wahlberg N, Vogel H, Pires CJ, Wheat CW. 2015**. The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences USA* 112: 8362–8366.
11. **Ehrlich PR, Raven PH.** 1964. Butterflies and plants: A study in coevolution. *Evolution* **18**: 586-608.
12. **Fordyce JA. 2010**. Host shifts and evolutionary radiations of butterflies. *Proceedings of the Royal Society of London. Series B* **277**: 3735-43.
13. **Janz N. 2011**. Ehrlich and Raven revisited: Mechanisms underlying codiversification of plants and enemies. *Annual Review of Ecology, Evolution, and Systematics* **42**: 71-89.

14. **Kariñho-Betancourt E, Agrawal AA, Halitschke R, Núñez-Farfán J. 2015.** Phylogenetic correlations among chemical and physical plant defenses change with ontogeny. *New Phytologist* **206**: 796–806.
15. **Mithöfer A, Boland W. 2012.** Plant defense against herbivores: Chemical aspects. *Annual Review of Plant Biology* **63**: 431-450.
16. **Núñez-Farfán J, Fornoni J, Valverde PL. 2007**. The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics* **38**: 541-566.
17. **Vermeij GJ. 1994.** The evolutionary interactions among species: Selection, escalation, and coevolution. *Annual Review of Ecology and Systematics* **25**: 219-236.
18. **Wheat CW, Vogel H, Wittstock U, Braby MF, Underwood D, Mitchell-Olds T. 2007**. The genetic basis of a plant-insect coevolutionary key innovation. *Proceedings of the National Academy of Sciences USA* **104**:20427–31.

Key words: Escalation, escape-radiate, glucosinolates, macroevolution, plant defence evolution, resource availability hypothesis.