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VARIACIÓN QUÍMICA Y GENÉTICA ENTRE LAS POBLACIONES

DE *MIKANIA MICRANTHA* H.B.K. Y SU EFECTO EN LA HERBIVORÍA

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ÍNDICE

Resumen **1**

Abstract **3**

1. Introducción General **5**

Literatura citada 8

2. Variación química y genética de las plantas dentro de sus rangos de distribución autóctonos y alóctonos **10**

Introducción 10

Variación química de las plantas invasoras 11

Metabolitos secundarios constitutivos 11

Metabolitos secundarios inducidos 12

Alelopatía 13

Variación genética de las plantas invasoras 14

Diversidad genética entre poblaciones nativas e invasoras 15

Diferenciación genética entre poblaciones nativas e invasoras 16

Conclusiones 17

Literatura citada 18

3. Diversidad química entre poblaciones de *Mikania micrantha:* estructura geográfica de mosaico y herbivoría.
Chemical diversity among populations of *Mikania micrantha*: geographic mosaic structure and herbivory **26**

Abstract 27

Introduction 27

Materials and methods 28

Field sites 28

Herbivory assessment 29

Chemical analysis 29

Data analysis 29

Results 30

Herbivory assessment 30

Chemical analysis 30

Discussion 32
Acknowledgments 34
References 34
Appendix 1 36
Appendix 2 37
Appendix 3 38
4. Estructura espacial de la diversidad genética y química en poblaciones mexicanas de *Mikania micrantha*.
Spatial structure of genetic and chemical diversity in Mexican populations of *Mikania micrantha* 39
Abstract 41
Introduction 42
Materials and methods 43
Plant material 43
DNA extraction 43
PCR conditions 44
Genetic variation within populations 44
Genetic differentiation among populations 45
Genetic structure of populations 45
Genetic diversity and chemical diversity 46
Results 46
Population genetic variation 46
Population genetic differentiation 47
Relationships among populations 47
Genetic diversity and chemistry 48
Discussion 48
Acknowledgements 49
References 50
5. Selección diferencial de hospederos en el escarabajo herbívoro *Stolas punicea* sobre fenotipos químicos de *Mikania micrantha*.
Differential host selection of the herbivore beetle *Stolas punicea* on chemical phenotypes of *Mikania micrantha* 61

Abstract 63
Introduction 64
Materials and methods 65
Plant material 65
Insects 66
Chemical analysis 66
Cafeteria experiments 66
Statistical analysis 67
Results 67
Chemical analysis 67
Preference tests 67
Discussion 68
Acknowledgements 69
References 70

6. Discusión General 74
Literatura citada 77

Resumen

Las poblaciones de plantas invasoras suelen experimentar cambios evolutivos rápidos que provocan su diferenciación química y genética de las poblaciones originales, estos cambios pueden contribuir al éxito de la invasión. Además, existe variación química y genética al interior de la distribución nativa e introducida. Sabemos que los fenotipos químicos se pueden distribuir geográficamente en las poblaciones dependiendo del grado de especialización de los herbívoros que las atacan; mientras que la composición genética de las poblaciones también puede ser afectada por los herbívoros presentes y por su ubicación dentro de la distribución. En este trabajo nos propusimos determinar la variación geográfica de la diversidad química y genética en poblaciones nativas de la maleza invasora *Mikania micrantha* (Asteraceae) y explorar su relación con el ataque de herbívoros.

Probamos las predicciones de los modelos centro-margen y mosaico geográfico de coevolución para *M. micrantha*, una planta trepadora nativa de América con una amplia distribución en las zonas tropicales de México. Analizamos la diversidad de terpenoides para 165 individuos de 14 poblaciones, y medimos la herbivoría *in situ*. Encontramos una correlación inversa significativa (r^2=0.89) entre la diversidad química de las poblaciones y su herbivoría. Los factores abióticos no explicaron la diversidad química. Aunque no encontramos aislamiento por distancia, un análisis de barreras mostró la existencia de zonas geográficas con diversidad química similar. La variación de la diversidad química en esta especie se ajusta mejor a un patrón de mosaico que al de centro-margen.

Es común que ocurra una correlación espacial entre la diversidad genética y la diversidad química porque ambas características están influenciadas por los mismos factores limitantes de dispersión y flujo génico. En este estudio nos propusimos examinar la relación entre la diversidad genética neutral (microsatélites), la diversidad química (terpenoides) y la herbivoría en poblaciones mexicanas de *Mikania micrantha*. Encontramos que las poblaciones del Atlántico tienen una mayor diversidad y mayor estructura genética que las poblaciones del

Pacífico. La mayor parte de la variación está dentro de las poblaciones (43.2%), aunque una proporción sustancial de la variación se encuentra entre los grupos (i.e. Atlántico y Pacífico). Las diferencias genéticas entre las poblaciones del Atlántico y Pacífico fueron corroboradas con un análisis de cúmulos, un análisis de coordenadas principales y un análisis Bayesiano (Structure). No encontramos correlación entre las distancias genéticas y químicas en todas las poblaciones, pero encontramos una correlación directa entre la diversidad química y el número de alelos para las poblaciones del Pacífico; y una correlación inversa entre el daño por herbívoros y el número de alelos para las poblaciones del Atlántico. Nuestros resultados sugieren que las poblaciones del Atlántico y el Pacífico han estado sujetas a procesos biogeográficos distintos, lo cual se refleja tanto en los marcadores moleculares como en los químicos.

La variación geográfica en el contenido de metabolitos secundarios de una especie vegetal puede afectar la preferencia y el desempeño de sus enemigos naturales (v.g. herbívoros y patógenos). En la última parte de este estudio utilizamos pruebas de cafetería para evaluar la preferencia del insecto herbívoro *Stolas punicea* (Coleoptera: Chrysomelidae) de dos localidades (Veracruz y Michoacán) sobre plantas de *M. micrantha* de poblaciones con distinto perfil químico. Encontramos que los escarabajos de Veracruz no mostraron preferencia y se alimentaron de manera similar de todos los fenotipos químicos ofrecidos; sin embargo, los insectos de Michoacán mostraron una mayor preferencia por las plantas de Michoacán que por las de Tabasco. Estos resultados indican que el origen geográfico de *S. punicea* juega un papel importante en la aceptación de ciertos fenotipos químicos y sugiere que ciertas poblaciones del herbívoro no serían capaces de establecerse exitosamente en algunos de éstos.

Abstract

Invasive plant populations usually experience rapid evolutive changes that lead to their chemical and genetic differentiation from the original populations, these changes can contribute to the success of the invasion. Besides, there is chemical and genetic variation within the native and introduced distributions. We know that chemical phenotypes are geographically distributed in populations according to the herbivores that attack them; while the genetic composition of the populations can also be affected by herbivores and the geographic location within the species distribution. This work aimed to determine the geographic variation on the chemical and genetic diversity of native populations of the invasive weed *Mikania micrantha* (Asteraceae) and explored their relation to herbivore attack.

We tested the predictions of the central-marginal model and geographic mosaic of coevolution hypothesis for *M. micrantha*, an invasive weed native of America with a wide distribution in the tropics of Mexico. The foliar volatile terpenoid blend was analyzed in 165 individuals of 14 populations and assessed the *in situ* herbivory. We found a significant correlation (r^2=0.89) between chemical diversity and herbivory. Abiotic factors did not explain chemical diversity. We conclude that variation in chemical diversity for this species adjusts better to a mosaic pattern rather than to the central-marginal model.

On the other hand, it is quite common to find spatial correlation between genetic diversity and chemical diversity, as both characteristics are under the influence of the same factors that restrict dispersion and genetic flow. In this study, we aimed to examine the spatial distribution and potential relationship of genetic and chemical diversity of the invasive weed *M. micrantha* in its native distribution using neutral molecular markers (microsatellites) and volatile terpenoids. We found that Atlantic populations have a higher genetic diversity and structure than Pacific populations. Most genetic variation is located within populations (43.2%), although a substantial proportion is found among regions. The differentiation between Atlantic and Pacific populations was supported consistently with a cluster analysis, a principal coordinates analysis, and a Bayesian analysis. While no correlation was

found for genetic and chemical distances, a direct correlation was found between the chemical diversity and the number of alleles in Pacific populations, and an inverse correlation between herbivore damage and number of alleles for Atlantic populations. Our results suggest that different biogeographic processes have operated in the two regions and this is reflected on the molecular and chemical markers.

Geographic variation on the secondary metabolite composition of a plant species can affect the preference and performance of its natural enemies (e.g. herbivores and pathogens). In the final part of this study we used cafeteria trials to assess the host preference of the herbivore insect *Stolas punicea* (Coleoptera: Chrysomelidae), from two provenances (Veracruz and Michoacán) on *M. micrantha* plants with different chemical profile. We found no preference for Veracruz beetles as they fed similarly on all the chemical phenotypes offered. However, Michoacán beetles showed a higher preference for plants from Michoacán than for those from Tabasco. These results indicate that the geographic origin of *S. punicea* plays an important role on the acceptance of some chemical phenotypes and suggests that some herbivore populations may not be able to successfully establish on some of them.

1

Introducción General

Los rápidos cambios evolutivos que ocurren frecuentemente en las poblaciones de plantas invasoras, provocan que se diferencien genéticamente de las poblaciones originales, y contribuyen al éxito de la invasión (Bossdorf *et al.* 2005). Este fenómeno es abordado por diversas hipótesis. Una de ellas es la hipótesis de la evolución del aumento en la habilidad competitiva (*Evolution of Increased Competitive Ability*, EICA), la cual plantea que en la ausencia de herbívoros, la selección natural favorecerá a los genotipos más competitivos y con menor asignación a la defensa química contra herbívoros (Blossey y Notzold 1995).

Adicionalmente, se ha propuesto incorporar a la hipótesis anterior la distinción del efecto de los herbívoros especialistas y generalistas. De esta manera es posible predecir que las plantas introducidas en lugares donde no hay herbívoros especialistas, pero sí herbívoros generalistas, experimentarán un decremento en su defensa (cuantitativa, costosa) en contra de los primeros y un incremento en la defensa (cualitativa, económica) contra los segundos (Joshi y Vrieling 2005).

Otra hipótesis, (*Allelopathic Advantage Against Resident Species*, AARS, también conocida como la hipótesis de armas novedosas, *Novel Weapons Hypothesis*, NWH), predice que las poblaciones de plantas invasoras que poseen compuestos químicos con propiedades alelopáticas inhibirán a las plantas de los lugares invadidos porque estos compuestos son novedosos para ellas. Por lo tanto, las poblaciones de plantas invasoras los acumularán en mayores cantidades que sus poblaciones de origen (Inderjit *et al.* 2006). Así, se pueden esperar que disminuya la asignación a la defensa en plantas invasoras únicamente cuando los metabolitos secundarios no tengan otra función para la planta como la alelopática.

Por otra parte, hay variación en la composición química y genética al interior del rango de distribución de una especie, lo cual que puede deberse a que la presión de herbivoría y la diversidad de enemigos a que están expuestas las

poblaciones son diferentes. Por ejemplo, se ha encontrado que los fenotipos químicos se distribuyen geográficamente en las poblaciones dependiendo del grado de especialización de los herbívoros que las atacan (Becerra 2007); mientras que la composición genética de las poblaciones también se ve afectada por los herbívoros presentes (Prittinen *et al.* 2006), y por su ubicación dentro del rango de distribución, siendo frecuente que las poblaciones periféricas sean menos diversas que las poblaciones centrales (Eckert *et al.* 2008).

La variación química dentro del rango de distribución y su relación con los herbívoros puede explicarse por la hipótesis del escape de enemigos naturales, la cual dice que cuando una planta entra en una nueva región, disminuye el ataque de los enemigos naturales, así como la asignación de recursos y la diversidad de metabolitos secundarios (Keane y Crawley 2002).

Una explicación alternativa es que la colonización de las áreas ubicadas en el límite del rango ocurra por parte de pocos genotipos, lo que daría como resultado un efecto fundador muy fuerte que aunado a la deriva génica y el escaso flujo génico con las poblaciones circundantes provocaría diferencias importantes en la diversidad química y genética de las poblaciones de la periferia.

En este trabajo se aborda a la planta *Mikania micrantha* Kunth (Asteraceae) como modelo de estudio. Esta especie nativa de México y del centro y sur de América, fue introducida durante la segunda mitad del siglo XX en el continente asiático (Cock *et al.* 2000). Dentro de México, los límites de distribución norte de esta especie se encuentran en los estados de Nayarit y de Tamaulipas (Villaseñor y Espinosa-García 1998), desde ahí la especie se distribuye a lo largo de las regiones tropicales del Pacífico y el Golfo de México; las poblaciones a las que consideramos “centrales” están en Chiapas, Tabasco, sureste de Veracruz y este de Oaxaca. La presencia de *M. micrantha* está asociada a sitios donde la humedad del suelo es constante como las orillas de ríos, arroyos, canales de riego y pantanos.

En su nuevo hábitat esta especie causa severos problemas en los cultivos, a la vez que desplaza a la vegetación original debido, en parte, a su rápido crecimiento, su hábito trepador (Zhang *et al.* 2004) y a las propiedades

alelopáticas de sus metabolitos secundarios, los cuales son principalmente terpenos y lactonas sesquiterpénicas (Nicollier y Thompson 1981; Ríos *et al* 2014).

Estudios de las poblaciones invasoras de *M. micrantha* reportan que la principal forma de dispersión local es por medio de propagación vegetativa, pues los fragmentos del tallo son capaces de enraizar en sus nodos (Zhang *et al.* 2004); en cambio, sus flores son auto incompatibles y la polinización se realiza por insectos (Hong *et al.* 2007). Un análisis de la variación genética en las poblaciones de China mostró que la variación es alta en comparación con otras plantas invasoras (Wang *et al.* 2008).

El propósito de este trabajo consiste en determinar la distribución geográfica de la diversidad química y genética en las poblaciones de *Mikania micrantha* y su posible relación con el ataque por herbívoros. La comparación de las poblaciones a lo largo de su rango de distribución nativo nos proporcionará información sobre la naturaleza de la colonización de la planta, su capacidad de adaptación y su susceptibilidad a herbívoros. En este caso, se plantea como hipótesis general que la variación química y genética entre poblaciones de *M. micrantha* está asociada a su origen geográfico (centro-margen y Atlántico-Pacífico), lo cual tiene efecto en su susceptibilidad a la herbivoría por insectos.

El proyecto está dividido en cuatro capítulos: en el primero hicimos una revisión de literatura sobre los cambios químicos y genéticos de las plantas invasoras en sus rangos autóctono y alóctono, en el segundo abordamos la diversidad química y la herbivoría que experimentan las poblaciones de *M. micrantha*; en el tercero nos enfocamos en la estructura genética de las poblaciones de esta especie y su correlación con la diversidad química; y en el cuarto evaluamos la preferencia de *Stolas punicea* (Boheman, 1850) (Coleoptera: Chrysomelidae) un herbívoro especialista de *M. micrantha* sobre distintos fenotipos químicos.

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2

Variación química y genética de las plantas dentro de sus rangos de distribución autóctonos y alóctonos.

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1. Introducción

Las poblaciones de plantas invasoras experimentan a menudo cambios evolutivos rápidos. Fenómenos como el efecto fundador, la deriva génica, la hibridización y la selección natural afectan la adaptación de las especies a ambientes nuevos, provocan diferenciación genética entre poblaciones nativas e introducidas y puede contribuir al éxito de la invasión (Lee 2002; Bossdorf *et al.* 2005).

En múltiples trabajos se han comparado poblaciones nativas e introducidas en aspectos como el tamaño de la población, tamaño de las plantas, fecundidad, herbivoría, habilidad competitiva, resistencia y tolerancia (ver Bossdorf *et al.* 2005).

Dos aspectos en particular pueden ofrecer información útil para entender el éxito de las especies invasoras en un nuevo ambiente. El primero está relacionado con el perfil químico de los metabolitos secundarios (MS) que intervienen en las relaciones ecológicas de la planta con sus enemigos y competidores (Anaya 2003). El segundo es el aspecto genético-evolutivo, relativamente poco explorado en plantas invasoras. Los estudios sobre la diferenciación y diversidad genética de las poblaciones nativas e invasoras resultan útiles para determinar las rutas de introducción así como los mecanismos que han participado en el éxito de la invasión (Meekins *et al.* 2001).

Aunque hay estudios que combinan métodos de biología molecular y el análisis de MS (Adams 1999, 2001; Baum *et al.* 2001; Vieira *et al.* 2001; Fico *et al.* 2003; Johnson *et al.* 2003), este tipo de enfoque que potencialmente podría aportar información sobre el papel de los MS y su evolución, apenas comienza a ser aplicado a las plantas invasoras como el caso de *Leontodon autumnalis* (Grass *et al.* 2006).

En este trabajo se realizó una revisión de los estudios que ponen a prueba las principales hipótesis que explican las variaciones químicas y genéticas de las especies invasoras en sus rangos autóctonos y alóctonos.

2. Variación química de las plantas invasoras

Se han propuesto algunas hipótesis que intentan predecir los cambios que las plantas introducidas en un nuevo sitio experimentarán en sus MS. Una de ellas es la denominada EICA (*Evolution of Increased Competitive Ability*), la cual plantea que en la ausencia de enemigos naturales, la selección favorecerá a los genotipos con mejores habilidades competitivas y baja asignación de recursos a la defensa contra sus enemigos (Blossey y Notzold 1995). Esta hipótesis predice de manera general la evolución de los rasgos defensivos en plantas, sin embargo hay pocos estudios que se hayan enfocado en el aspecto de la defensa química. Los que se han realizado hasta ahora se han dirigido a un solo compuesto o grupo de compuestos (Tabla 1).

Otra hipótesis, la NWH (*Novel Weapons Hypotesis*) postula que las especies invasoras aportan compuestos químicos únicos a las regiones donde son introducidas, lo cual les da una ventaja competitiva debido a que los nuevos vecinos no están adaptados a estos nuevos compuestos (Inderjit *et al.* 2006). La predicción que se deriva de esto, es que las plantas deberán mantener su producción de MS o incluso incrementarla en los sitios de introducción.

2.1. Metabolitos secundarios constitutivos. Este tipo de compuestos están presentes durante todo el desarrollo de la planta. Entre sus funciones reconocidas

están detener, repeler, intoxicar o alterar el desarrollo o reproducción de los herbívoros y patógenos (Anaya 2003).

Algunos trabajos apoyan a la hipótesis que predice la disminución de la defensa química en el rango introducido. Ejemplo de este caso, es el estudio sobre *Solidago gigantea* cuyas poblaciones nativas en Estados Unidos muestran una mayor variabilidad y concentración de diterpenos que poblaciones invasoras de Europa (Johnson *et al.* 2007). Otros casos son el de *Lythrum salicaria* (Willis *et al.* 1999), cuyo contenido de compuestos fenólicos fue significativamente mayor en las poblaciones nativas, y el de *Sapium sebiferum* (Siemann y Rogers 2001) cuyas poblaciones nativas tuvieron una mayor concentración de taninos. Aunque estas dos últimas especies coinciden con las predicciones de la hipótesis EICA, no se encontró diferencia en la resistencia a la herbivoría en ejemplares mantenidos en jardines comunes.

Sin embargo, el caso contrario en el que las poblaciones introducidas tienen mayor cantidad de MS también existe; un estudio con *Senecio jacobaea* encontró que las poblaciones del nuevo rango tenían una concentración total de alcaloides de pirrolizidina 90% más alta (Joshi y Vrieling 2005; Stastny *et al.* 2005). Aunque estos resultados muestran variaciones significativas en el contenido de MS, no es posible descartar que las diferencias encontradas en las poblaciones introducidas sean producto del efecto fundador más que de una selección ejercida por los herbívoros y patógenos.

2.2. Metabolitos secundarios inducidos. Este tipo de compuestos comprende a todos aquellos que son producidos por las plantas como respuesta al daño causado por un herbívoro o patógeno. De acuerdo a la teoría de la defensa óptima, la cual supone que ésta tiene un costo, el mantenimiento o incremento de compuestos de defensa inducibles se verá acompañado por una reducción en la defensa constitutiva (Zangerl y Rutledge 1996).

Los estudios que se han realizado sobre estos compuestos se enfocan principalmente en la variación de la inducibilidad entre poblaciones o en la relación que existe entre la expresión de defensa constitutiva e inducida (Tabla 1).

Para la especie *Lepidium draba*, Müller y Martens (2005) midieron tanto los glucosinolatos como la mirosinasa, una enzima encargada de su síntesis, y encontraron que esta enzima, así como el principal compuesto, el glucosinolato p-hidroxibencilo, tuvieron mayor concentración en plantas del rango invadido. Estos autores especulan que, debido al bajo costo de la defensa inducida respecto a la defensa constitutiva, en el rango introducido los compuestos inducibles, como los glucosinolatos, se encontrarán en mayor cantidad.

Algo similar ocurre con *Alliaria petiolata,* en la que tres de las cuatro poblaciones invasoras muestreadas tuvieron menores niveles constitutivos y mayores niveles de glucosinolatos inducidos que siete poblaciones nativas (Cipollini *et al.* 2005). Esto puede representar una estrategia útil en un ambiente donde la presión de selección por parte de los herbívoros es baja. Sin embargo dos compuestos en particular, el aliarinósido y la isovitexina 6´´-O-β-D-glucopiranósido, se comportaron de manera opuesta, su concentración constitutiva fue más alta en las poblaciones invasoras y su nivel de inducción fue mayor en las poblaciones nativas. En otro estudio de la misma especie, la concentración del glucosinolato sinigrina no varió significativamente entre poblaciones nativas e introducidas y las ligeras diferencias encontradas se deben posiblemente a factores ambientales que no pudieron ser totalmente controlados en los experimentos (Lewis *et al.* 2006). Eigenbrode *et al.* (2008) tampoco encontraron diferencias en las concentraciones promedio de alcaloides de pirrolizidina constitutivos o inducidos entre las poblaciones nativas e introducidas de *Cynoglossum officinale*; lo que sí encontraron fue una mayor variabilidad en las concentraciones inducidas de las poblaciones introducidas, lo cual atribuyeron a la ausencia de selección estabilizadora que generalmente ejercen los herbívoros.

2.3. Alelopatía. Una hipótesis derivada de la NWH es la AARS (*Allelopathic Advantage Against Resident Species*), la cual predice que las poblaciones invasoras estarán mejor defendidas químicamente que las poblaciones nativas. Esto debido a que las especies invasoras pueden poseer compuestos químicos que les den una ventaja competitiva o defensiva en sus nuevos hábitats. La

presión de selección a favor de la producción de éstos compuestos deberá ser mayor en los genotipos de las regiones invadidas que en los del rango autóctono (Inderjit *et al.* 2006).

Entre los casos donde se cumple esta hipótesis está *Centaurea maculosa*, una asterácea nativa de Europa cuyas poblaciones invasoras en Estados Unidos tienen niveles más altos del compuesto (-)-catequina (Bais *et al.* 2003); las concentraciones exudadas de este compuesto en el suelo son capaces de inhibir más intensamente la germinación y el crecimiento de pastos de Norte América que de pastos europeos. Algo semejante ocurre con *Phragmites australis*, un pasto cuyos genotipos reintroducidos a Estados Unidos desde Europa producen mayor cantidad de ácido gálico, que es un compuesto que induce reacciones oxidativas o incluso la muerte celular en las raíces de especies vecinas (Rudrappa *et al.* 2007).

Adicionalmente a las hipótesis sobre los MS aquí mencionadas, hay otras propuestas que aún no reciben mucha atención como la de Joshi y Vrieling (2005) que busca incluir la diferencia entre los MS dirigidos contra herbívoros generalistas y contra especialistas por separado. Según esta hipótesis, una planta invasora disminuiría su producción de compuestos contra especialistas pero aumentaría aquéllos que funcionan contra generalistas, ya que éstos seguramente se encontrarán en el nuevo rango a colonizar. El principal problema con ésta idea, es la dificultad de aplicar una clasificación tan estricta a los MS cuando su toxicidad no suele ser específica.

Los casos investigados hasta ahora muestran que la producción de MS en las plantas constituye un aspecto complejo debido a la cantidad de factores que pueden afectarlo y por las formas en que los compuestos pueden estar interactuando con otros organismos. Sin embargo su estudio es necesario ya que la química de las plantas puede explicar, al menos parcialmente, el éxito de las invasiones.

3. Variación genética de las plantas invasoras

Antiguamente la diversidad genética de una especie se medía empleando rasgos morfológicos, fisiológicos y bioquímicos. Sin embargo, debido a que los rasgos

morfológicos están sujetos a influencias ambientales, se ha puesto más énfasis en los estudios bioquímicos. Los análisis de isoenzimas se han usado desde hace décadas, sin embargo se sabe que este tipo de marcadores moleculares pueden subestimar la diversidad genética (Esselman *et al.* 1999), por lo que en la actualidad se emplean técnicas más sensibles basadas en ADN como los microsatélites, ISSR, RAPD y AFLP para estudiar la variación genética a nivel de poblaciones (Tabla 2).

Generalmente, para las poblaciones invasoras se predice que, debido al efecto fundador, sufrirán un empobrecimiento en su diversidad genética y que en ellas ocurrirán procesos evolutivos (v.g. deriva génica, cuellos de botella, selección) que aumentarán su diferenciación con las poblaciones nativas (Novak y Welfley 1997; DeWalt y Hamrick 2004).

3.1. Diversidad genética entre poblaciones nativas e invasoras. La pérdida de diversidad genética en las poblaciones invasoras se ha reportado en varios estudios (ver Tabla 2). Estas pérdidas se asumen como una consecuencia de eventos fundadores asociados a las introducciones de las plantas. Si, además, una especie es exclusivamente autógama, como *Bromus tectorum* (Novak *et al.* 1991), o tiene altas tasas de autofecundación, como *Alliaria petiolata* (Durka *et al.* 2005), no existirá entrecruzamiento con otras poblaciones introducidas y esto se reflejará en un bajo nivel de individuos heterocigotos y pérdida de diversidad. Para *Erigeron annuus* la pérdida de diversidad en las áreas invadidas es ligera, posiblemente debido a que en su área de distribución nativa la diversidad también es baja, y una muestra pequeña de individuos es capaz de representarla (Edwards *et al.* 2006). Otros estudios no encontraron diferencias en la diversidad genética entre poblaciones nativas e introducidas (Molina-Freaner y Jain 1992; Neuffer y Hurka 1999; Meekins *et al.* 2001; Maron *et al.* 2004; Milne y Abbott 2004; Genton *et al.* 2005). En algunos casos, se sospecha que el tipo de reproducción podría ser el responsable de esta ausencia de diferencia, como en *Apera spica-venti* que es predominantemente autoincompatible (Warwick *et al.* 1987); pero sobre todo se

argumenta que las poblaciones introducidas provienen de introducciones múltiples de diversos orígenes (Dlugosch y Parker 2008).

En otros estudios se ha encontrado que las poblaciones invasoras tienen una mayor diversidad genética que las poblaciones nativas (Squirrell *et al.* 2001; DeWalt y Hamrick 2004). Esto puede deberse a cuestiones como: 1) el rango de distribución nativo no fue analizado completamente, y 2) hubo múltiples introducciones de diversas fuentes lo cual incrementó la diversidad. Por lo anterior, la pérdida de diversidad en el área de distribución invadida, parece ser una excepción más que una regla al menos para las especies invasoras estudiadas hasta el momento. Aparentemente, las introducciones múltiples constituyen un fenómeno muy frecuente que permite a las poblaciones introducidas poseer una diversidad genética similar a la nativa, o incluso mayor.

3.2. Diferenciación genética entre poblaciones nativas e invasoras. En la mayoría de los estudios donde se ha medido la diferenciación genética entre poblaciones, las del rango de distribución invadido han resultado más similares entre sí, es decir, tienen una menor diferenciación que las poblaciones nativas (Warwick *et al.* 1987; Novak *et al.* 1991; Molina-Freaner y Jain 1992; Amsellem *et al.* 2000; Squirrell *et al.* 2001; DeWalt y Hamrick 2004; Genton *et al.* 2005). La baja diferenciación en las poblaciones introducidas se atribuye a que éstas se fundaron a partir de ejemplares genéticamente similares, ya sea por una sola introducción de una mezcla de propágulos seguidos de diseminación o por múltiples introducciones provenientes de orígenes con mezclas similares. Algunos casos, como el de *Ambrosia artemisiifolia* (Genton *et al.* 2005), son consistentes con un escenario de introducciones múltiples a la región invadida seguido por colonización de nuevas áreas a través de cuellos de botella secuenciales. En contraste, para otras especies no se ha detectado un cambio significativo en la diferenciación (Clegg y Allard 1972; Burdon y Brown 1986; Durka *et al.* 2005). Esto puede deberse a introducciones múltiples a lo largo del tiempo, lo cual no sería improbable para especies como *Alliaria petiolata* que tradicionalmente se ha usado como condimento y planta medicinal en su rango de distribución nativo (Durka *et*

al. 2005). Un caso especial es el de *Avena barbata*, cuyas poblaciones introducidas tomadas en conjunto no son muy diferentes a las poblaciones nativas; sin embargo en el rango introducido se aprecian claramente dos genotipos adaptados a regiones con condiciones climáticas distintas, lo cual posiblemente se ha producido debido a la selección natural (Clegg y Allard 1972).

Por otra parte, hay casos donde las poblaciones introducidas tienen una mayor diferenciación que las nativas (Glover y Barret 1987; Lee *et al.* 2004), lo cual ha sido explicado como producto tanto del efecto fundador como de la deriva génica. En particular, para *Hirschfeldia incana* el tiempo transcurrido desde la invasión parece tener un efecto en la diferenciación ya que los sitios invadidos más recientemente no mostraron diferencia con las poblaciones nativas (Lee *et al.* 2004).

Adicionalmente hay estudios donde sólo se ha medido la variación genética neutral de las poblaciones introducidas. En estos trabajos por lo general reportan altos niveles de variación y diferenciación genética (Kercher y Conner 1996; Novak y Welfley 1997; Meekins *et al.* 2001; Wang *et al.* 2008). Sin embargo, es difícil entender la variación en el rango de distribución invadido sin conocer adecuadamente las características genéticas de las poblaciones nativas.

4. Conclusiones

Resulta muy complicado derivar generalidades sobre las características químicas y genéticas de las plantas invasoras ante la diversidad de resultados encontrados. Todavía más difícil resulta el hacer predicciones que se ajusten apropiadamente a los hallazgos reportados hasta ahora. La creación de una teoría integradora sobre la dinámica de las plantas invasoras se percibe todavía como algo lejos de nuestro alcance (pero ver Catford *et al.* 2009).

Lo que puede hacerse es tomar en cuenta algunas recomendaciones para el desarrollo de futuras investigaciones. Por ejemplo: evitar incluir sólo la medición de una característica y tratar dentro de lo posible incluir varios análisis a la vez (químicos, genéticos, morfológicos y fisiológicos), de manera que los resultados

puedan ser interpretados de manera integral y puedan descubrirse las posibles relaciones entre las distintas características.

Por otra parte, es preferible que las comparaciones entre continentes se hagan con muestras grandes que incrementen la potencia del análisis estadístico y, de ser posible, que se realicen muestreos a lo largo del tiempo para detectar potenciales variaciones posteriores a la invasión. El análisis realizado solamente en el rango de distribución autóctono o solo en el alóctono produce generalmente resultados difíciles o imposibles de interpretar al momento de hacer comparaciones.

No hay que olvidar tampoco que, aun cuando el empleo de un sólo marcador molecular basado en ADN produce resultados precisos sobre las características genéticas de las poblaciones, será mejor todavía el empleo simultáneo de varios marcadores que proporcionen información complementaria sobre la estructura genética, filogeografía y forma de dispersión de las poblaciones de plantas invasoras.

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Tabla 1. Comparación del contenido de metabolitos secundarios en poblaciones nativas e invasoras.

Especie	Metabolito Secundario	Resultado	Referencia
Alliaria petiolata	Glucosinolatos	Mayor nivel constitutivo e inducibilidad en poblaciones invasoras	Cipollini *et al.* (2005)
	Sinigrina	Niveles similares en poblaciones nativas e introducidas	Lewis *et al.* (2006)
Centaurea maculosa	(±)-Catequina	Mayor cantidad de (-)-catequina en poblaciones introducidas	Bais *et al.* (2003)
Cynoglossum officinale	Alcaloides de pirrolizidina	Niveles similares en poblaciones nativas e introducidas	Eigenbrode *et al.* (2008)
Lepidium draba	Glucosinolatos	Mayor cantidad de un compuesto en poblaciones introducidas	Müller y Martens (2005)
Lytrum salicaria	Compuestos fenólicos	Mayor cantidad en poblaciones nativas	Willis *et al.* (1999)
Sapium sebiferum	Taninos	Mayor cantidad en poblaciones nativas	Siemann y Rogers (2001)
Senecio jacobaea	Alcaloides de pirrolizidina	Mayor cantidad en poblaciones introducidas	Joshi y Vrieling (2005) Stastny *et al.* (2005)
Solidago gigantea	Terpenos	Mayor cantidad de diterpenos en poblaciones nativas	Johnson *et al.* (2007)
Phragmites australis	Acido gálico	Mayor cantidad en poblaciones introducidas	Rudrappa *et al.* (2007)

Tabla 2. Comparación de la variación genética entre poblaciones nativas e introducidas. ND: no hay datos

Especie	Historia de vida	Marcador	Diversidad genética	Diferenciación entre poblaciones	Referencia
Alliaria petiolata	Bianual; autogamia	ISSR	Similar	ND	Meekins *et al.* (2001)
		Microsatélites	Menor en introducidas	Similar	Durka *et al.* (2005)
Ambrosia artemisiifolia	Anual; singamia	Microsatélites	Similar	Menor en introducidas	Genton *et al.* (2005)
Apera spica-venti	Anual; singamia	Isoenzimas	Similar	Menor en introducidas	Warwick *et al.* (1987)
Avena barbata	Anual; autogamia y asexual	Isoenzimas	Mayor en introducidas	ND	Clegg y Allard (1972) Garcia *et al.* (1989)
Bromus mollis	Anual; autogamia	Isoenzimas	Similar	ND	Brown y Marshall (1981)
Bromus tectorum	Anual; autogamia	Isoenzimas	Menor en introducidas	Menor en introducidas	Novak *et al.* (1991) Novak y Mack (1993)
Capsella bursa-pastoris	Bianual; autogamia	Isoenzimas	Menor en introducidas	ND	Neuffer y Hurka (1999)
Clidemia hirta	Perenne; mixta	Isoenzimas	Mayor en introducidas	Menor en introducidas	DeWalt *et al.* (2004)
Echium plantagineum	Anual; autogamia	Isoenzimas	Similar	ND	Burdon y Brown (1986)
Eichhornia paniculata	Anual: mixta	Isoenzimas	Menor en introducidas	Mayor en introducidas	Glover y Barret (1987)
Epipactis helleborine	Perenne; mixta	Isoenzimas cpDNA	Mayor en introducidas	Menor en introducidas	Squirrell *et al.* (2001)
Erigeron annuus	Anual; apomíctica	RAPD	Menor en introducidas	Menor en introducidas	Edwards *et al.* (2006)
Fallopia japonica var. Japonica	Perenne; mixta	RAPD	Menor en introducidas	ND	Hollingsworth y Bailey (2000)
Hirschfeldia incana	Anual, bianual y perenne; autogamia	RAPD	Similar	Similar	Lee *et al.* (2004)
Hypericum perforatum	Perenne; singamia	AFLP	Similar	ND	Maron *et al.* (2004)
Ligustrum robustum spp. *walkeri*	Perenne: singamia	RAPD	Similar	ND	Milne y Abbott (2004)
Lolium perenne	Perenne; singamia	Isoenzimas	Similar	ND	Balfourier y Charmet (1994)
Rhododendron ponticum	Perenne; singamia	AFLP	Similar	ND	Ross (2003)
Rubus alceifolius	Perenne; apomíctica	AFLP	Menor en introducidas	Menor en introducidas	Amsellem *et al.* (2000)
Senecio inaequidens	Perenne; autogamia	Isoenzimas	Similar	ND	Lafuma (2003)
		cpDNA	Menor en introducidas	ND	
Trifolium hirtum	Anual; singamia	Isoenzimas	Similar	Menor en introducidas	Molina-Freaner y Jain (1992)
Turnera ulmifolia	Perenne; singamia	Isoenzimas	Mayor en introducidas	ND	Barret y Shore (1987)

3

Diversidad química entre poblaciones de *Mikania micrantha*: estructura geográfica de mosaico y herbivoría.

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PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Chemical diversity among populations of *Mikania micrantha*: geographic mosaic structure and herbivory

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Abstract Populations of the same species vary in their secondary metabolite content. This variation has been attributed to biotic and abiotic environmental conditions as well as to historical factors. Some studies have focused on the geographic variation of chemical diversity in plant populations, but whether this structure conforms to a central–marginal model or a mosaic pattern remains unclear. Furthermore, assessing the chemical diversity of invasive plants in their native distribution facilitates the understanding of their relationships with natural enemies. We examined the geographic variation of chemical diversity in Mexican populations of the bittervine weed *Mikania micrantha* and its relationship to herbivore damage. The foliar volatile terpenoid blend was analyzed in 165 individuals of 14 populations in the Pacific and Gulf of Mexico tropical watersheds. A cluster analysis grouped individuals with similar terpenoid blends into 56 compositional types. Chemical diversity was measured using the number of compounds and their concentration within the blends for individuals, and the number and frequency of compositional types for populations. A stepwise multiple regression analysis performed with geographic, climatic, and chemical diversity variables explained herbivore damage. However, population-level chemical diversity was the only variable found to be significant ($\beta = -0.79$, $P = 0.042$) in the model ($R^2 = 0.89$). A Mantel test using Euclidean distances did not indicate any separation by geographic origin; however, four barriers were identified using Monmonier's algorithm. We conclude that variation in population-level chemical diversity follows a mosaic pattern in which geographic factors (i.e., natural barriers) have some effect and that variation is also associated with the local intensity of herbivore attack.

Keywords Terpenoid compositional types · Central–marginal · Mile-a-minute weed · Mosaic pattern · Population structure

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Introduction

As all plant secondary metabolites occur as mixtures, their variation within and among populations allows individuals to be grouped by the relative proportion of each compound found in the mixture (Langenheim 1994; Thompson et al. 2003). Chemical variability of secondary metabolites plays a major role in plant–insect relationships, determining plant susceptibility or resistance to herbivores. Examples include: pirrolizidine alkaloids in *Senecio jacobaea* and the specialist moth *Tyria jacobaeae* (Macel and Klinkhamer 2010); glucosinolates in *Brassica oleracea* and larvae of the herbivores *Pieris rapae* and *Mamestra brassicae* (Poelman et al. 2009); phenolic glycosides in *Populus tremuloides* and larvae of the moth *Lymantria dispar* (Donaldson and Lindroth 2007); and terpenoids in

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Persea americana and the gall-forming psyllid *Trioza anceps* (Torres-Gurrola et al. 2011).

The chemical variability among populations has been attributed to the effect of abiotic environmental factors such as salinity (Bourgou et al. 2010; Taarit et al. 2011), humidity (Jordán et al. 2003; Bettaieb et al. 2011), temperature, altitude, and latitude (Binns et al. 2002; Ganzera et al. 2008). Also, the variability could result from genetic drift or historical factors such as the founder effect (Wolf and Denford 1983). However, since secondary metabolites also play a key role in ecological interactions, the chemical diversity of a population may be an indicator of its relationships with the local herbivore community.

The function of secondary metabolites as defense against herbivores and pathogens is considered to be a major driver of the evolution and maintenance of chemical diversity (Ehrlich and Raven 1964; Wink 1988). Herbivores can exert differential pressures on plants depending on the plants' chemical composition (Linhart and Thompson 1995; Snyder and Linhart 1998; Macel and Klinkhamer 2010). Thus, population-level chemical diversity can be associated with the intensity of herbivore attack (Castells et al. 2005) or with herbivore specialization (Becerra 2007; Lankau 2007). There may also be discrete chemical phenotypic variation among individuals in a population; that is, a group of individuals may share a similar chemical phenotype, constituting a different compositional type from other groups within the same population (Snyder 1992; Latta et al. 2003). Therefore, the distribution of compositional types in populations could reflect the selection pressure exerted by the local herbivores (Linhart and Thompson 1999; Züst et al. 2012).

The geographic variation of biotic interactions is used by some models to predict the intraspecific variation in phenotypic diversity throughout the species' distribution. The geographic mosaic theory of coevolution proposes that each population of a plant species is under specific selection pressures from heterotrophic species, creating a mosaic of hotspots (populations with intense reciprocal interactions) and coldspots (populations with low or no reciprocal interactions) (Thompson 2001). In contrast, the central–marginal hypothesis predicts that diversity will vary throughout the species distribution following geographic gradients of environmental conditions; populations at the center will be generally more diverse than those at the border (Eckert et al. 2008). This "isolation-by-distance" pattern is commonly found in genetic diversity but remains untested in chemical diversity (Durka 1999; Lammi et al. 1999). If this pattern of diversity were confirmed, then herbivory intensity should vary along this gradient according to the "more diversity–more protection" hypothesis (Berenbaum 1985; Kubo and Hanke 1985).

Mikania micrantha Kunth (Asteraceae), commonly known as mile-a-minute weed, bittervine, climbing hemp vine, and American rope, is a weed native to tropical America that was introduced to Asia in the early twentieth century (Cock et al. 2000), where it causes severe problems with crops and native vegetation (Zhang et al. 2004). In Mexico, this species has been reported in the tropical areas along the Atlantic and Pacific watersheds, from the northern states of Tamaulipas and Nayarit to the southern states of Tabasco and Chiapas (Villaseñor and Espinosa-García 1998). Major natural enemies of *M. micrantha* in its native range include insects from the orders Thysanoptera, Hemiptera, and Coleoptera (Waterhouse 1994). The main secondary metabolites in *M. micrantha* are volatile terpenes and sesquiterpene lactones (Nicollier and Thompson 1981; Cuenca et al. 1988; Shao et al. 2005). Since these compounds may be bioactive against herbivores (Langenheim 1994; Zhang et al. 2003), the foliar terpenoid profiles in *M. micrantha* would be expected to be associated with the herbivore intensity of the populations.

Under the central–marginal hypothesis, we would expect central populations of *M. micrantha* to have (1) higher chemical diversity, (2) higher terpenoid concentration, and (3) lower herbivore damage than marginal populations. A clear correlation between chemical diversity and geographic origin would also be expected. By contrast, under the mosaic theory, no specific population structure would be expected other than spatial variation in chemical diversity and intensity of herbivory.

Assessing the variability of chemical metabolites is important in understanding plant–herbivore interactions. Specifically, understanding the population level chemical structure can facilitate the search for biological control agents by focusing on selected areas rather than the entire species distribution.

Therefore, to assess the variability of secondary metabolites in an invasive species at the population level and to determine whether it is best described by the central–marginal hypothesis or the mosaic theory, we analyzed chemical diversity and herbivory in Mexican populations of *M. micrantha* and determined their correlations with geographic and environmental variables.

Materials and methods

Field sites

We collected mature leaves and seeds in 8 populations of *M. micrantha* near the Atlantic (December 2007), and 6 populations near the Pacific (December 2009) totalling 165 individuals (Table 1). Exhaustive sampling was performed

Table 1 Location of sampled populations of *Mikania micrantha*

	Population	State	Latitude	Longitude	Altitude (masl)	Sample size
NPA	Nuevo Padilla	Tamaulipas	24º05′15′′N	98º52′17′′W	153	6
ABA	Abasolo	Tamaulipas	24º03′07′′N	98º22′34′′W	60	15
TAM	Tampico	Tamaulipas	22º14′25′′N	97º53′26′′W	5	15
TUX	Tuxpan	Veracruz	20º56′44′′N	97º20′31′′W	8	23
ACT	Actopan	Veracruz	19º35′31′′N	96º22′55′′W	44	6
PAR	Parácuaro	Michoacán	19º08′26′′N	102º13′56′′W	553	14
HUI	Huimanguillo	Tabasco	17º47′58′′N	93º23′55′′W	39	4
COY	Coyuquilla Norte	Guerrero	17º22′48′′N	101º03′08′′W	209	10
WJA	Welib-Já	Chiapas	17º22′27′′N	91º47′58′′W	234	13
EPA	El Paraíso	Guerrero	17º21′05′′N	100º12′43′′W	1,761	15
CHA	Chancalá	Chiapas	17º19′49′′N	91º41′07′′W	267	8
SMC	San Miguel Chimalapa	Oaxaca	16º42′45′′N	94º44′53′′W	1,276	9
DCA	Dos Caminos	Oaxaca	16º22′14′′N	97º48′26′′W	311	13
SAG	San Agustín	Oaxaca	15º42′01′′N	96º15′43′′W	49	14

to include all available individuals at each population. Voucher specimens were deposited at the herbaria of the Institute of Ecology-Bajío (IE-Bajío, 196627–196632) and of the Escuela de Biología de la Universidad Michoacana (EBUM, 20403, 20404, 22684–22699).

Marginal populations were located at the northern limit of the distribution (NPA, ABA, and PAR), while central populations were found towards the southeast of Mexico (WJA, CHA, and HUI), where this species is more abundant.

Herbivory assessment

We collected 100 mature leaves from all individuals in each population from which 30 leaves were randomly selected and digitized. The damaged area was quantified using the Assess software (© 2002 The American Phytopathological Society). Leaf damage was classified either as scratched (i.e., punctures caused by thrips and galleries of leaf-miners) or removed area.

Chemical analysis

For each individual, one leaf was cut longitudinally into halves; one half was used to measure dry weight and the other was macerated for at least 7 days in an amber vial with 15 mL of hexane at 4 °C. An internal standard (1 mg n-tetradecane) was added before the sample was ground with chromatographic-grade sand. Humidity was eliminated with magnesium sulfate, and the extract was concentrated to 1 mL.

From each extract, 1 μL was analyzed with an Agilent 6890 gas chromatograph equipped with a HP-ULTRA2 capillary column (25 m × 0.20 mm I.D., film thickness 0.33 mm; Agilent J&W), coupled to an Agilent 5973 N selective mass detector. Initial oven temperature was set at 50 °C and increased to 120 °C at a rate of 5 °C min^{-1}, and to 150 °C at a rate of 2 °C min^{-1}. The final temperature was maintained for 2 min. The split rate was 5:1. Injector temperature was set at 250 °C. Helium was used as carrier gas at 7.67 psi (1 psi = 6,894.76 Pa) with a 1.0 mL min^{-1} constant flow. Conditions for the mass selective detector were transfer line 280 °C, ionization source 230 °C, quadrupole 150 °C, ionization potential 69.9 eV, and scan range 35–500 *m*/*z*. Signal from the detector was processed with Environmental ChemStation software (Agilent Technologies). Retention indexes (RI) were calculated by the Kovats algorithm and an *n*-alkanes (C_8–C_{20}) series as standard. Comparison of mass spectra and RI values with those in the US National Institute of Standards and Technology (NIST) 02 library and literature allowed compound identification (Adams 2007).

Data analysis

Peak areas in all chromatograms were standardized to mg g^{-1} dry weight using the internal standard area and the dry weight of the corresponding leaf. Two matrices were constructed, one using the compound concentrations and another with the percentage values of mono- and sesquiterpenes. Terpenoids accounting for <1 % were discarded from statistical analysis.

The term "chemical diversity" can refer to different things in the scientific literature. The population-level chemical diversity reflects the within-population polymorphism and is an index calculated from the number and

frequency of compositional types in a population (Kleine and Müller 2011). Individual chemical diversity is an index calculated from the number of compounds and their relative concentrations in individuals (Becerra 2007; Torres-Gurrola et al. 2011).

We therefore estimated two measures of chemical diversity for each population. The average individual diversity was calculated as the mean of the Shannon Index for each individual in the concentration matrix, and the population-level diversity as the Shannon Index for the number of individuals of each compositional type. The number of compositional types was determined by a cluster analysis using Euclidean distances with complete linkage algorithm on the percentage matrix. Individuals with a similarity higher than 90 % were considered to have the same compositional type.

Non-parametric ANOVA, followed by post hoc means comparisons when main effects were significant, was performed using the software package Statistica 8 (Statsoft) to find differences in chemical diversity, total terpenoid concentration, and herbivore damage among populations. Comparisons between Atlantic and Pacific watersheds and between marginal and central populations were performed with a two-tailed t test. Using the percentage data matrix, we performed a principal components analysis (PCA) to identify the compounds that best explain the chemical variation among populations and a discriminant analysis (DA) to identify the compounds that classify populations (XLStat v.2009.1.02; Addinsoft).

A Mantel test with 10,000 permutations between the matrices of Euclidean and geographic distances was performed to detect isolation by distance. Geographic distances were calculated using the populations' coordinates in GenAlEx 6.41 (Peakall and Smouse 2006). To search for possible genetic barriers between populations, we applied Monmonier's algorithm to a Fisher distance matrix obtained from the DA using Barrier 2.2 software (Manni et al. 2004).

The relationships among populations were examined with a cluster analysis (Euclidean distances and UPGMA algorithm) using the average percentage of each compound. Possible relationships between herbivory, average individual diversity, population-level diversity, total terpenoid concentration, and geographic variables (latitude, longitude, altitude) as well as environmental variables [precipitation and average yearly temperature (http://www.worldclim.org)] were explored with a stepwise multiple regression analysis.

Results

Herbivory assessment

Removed leaf area (4.5 % ± 8.4; mean ± SD) was significantly higher than scratched leaf area (1.4 % ± 3.8)

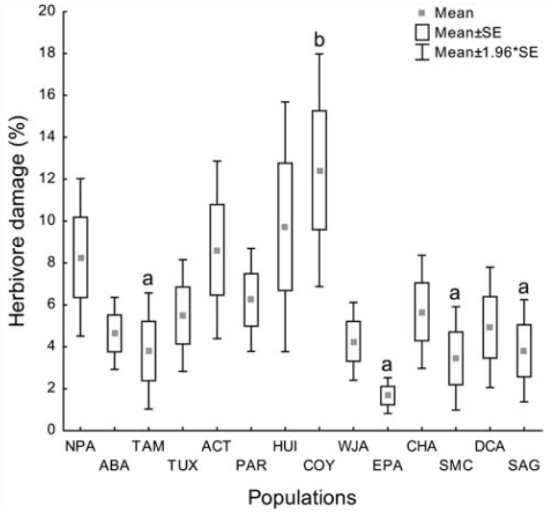

Fig. 1 Variation of total herbivore damage among Mexican populations of *Mikania micrantha.* Populations are arranged in order of decreasing latitude (*left* to *right*). See Table 1 for definitions of population acronyms

(Wilcoxon signed-rank test $T = 13\ 121$, $Z = 8.295$, $P < 0.01$; $n = 420$). Removed leaf area was statistically different only between populations HUI and EPA (see Table 1 for populations; Kruskal–Wallis test $H = 24.698$, $P = 0.024$). For total damage, some significant differences were found between populations COY-TAM (Kruskal–Wallis test, $H = 45.169$, $P = 0.0099$), COY-EPA ($P = 0.0023$), COY-SAG ($P = 0.042$), and COY-SMC ($P = 0.0031$) (Fig. 1), but there were no significant differences between Atlantic and Pacific watershed populations ($t = 0.947$, $P = 0.34$) or between marginal (NPA, ABA, PAR) and central (WJA, CHA, HUI) populations ($t = -0.035$, $P = 0.97$).

Chemical analysis

A total of 38 volatile terpenoids were isolated and identified in leaves of *M. micrantha* (Online Resource 1). Predominant compounds were monoterpenes (limonene and α-pinene), and sesquiterpenes (germacrene D and β–caryophyllene). Only six terpenoids (α-pinene, β-myrcene, α-phellandrene, p-cymene, limonene, and β-caryophyllene) were present in all individuals, although sometimes in minimal amounts.

We identified 56 compositional types by grouping individual chemical phenotypes with 10 % dissimilarity in the cluster analysis (Fig. 2). We verified that 10 % dissimilarity was a good cluster-defining criterion by comparing the chromatograms of adjacent groups and verifying that

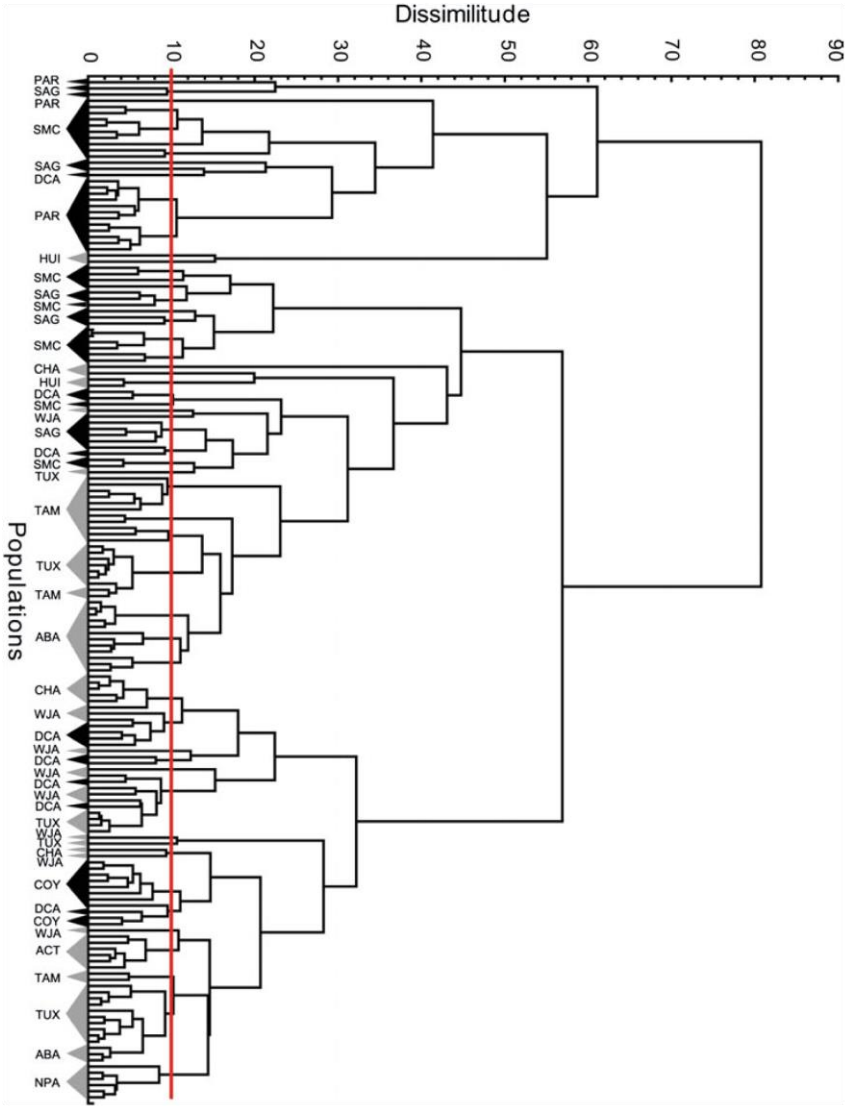

Fig. 2 Dendrogram showing dissimilarity in terpenoid composition among 165 individuals from 14 populations of *M. micrantha*. Individuals are grouped by populations. Pacific populations are colored *black*, Atlantic populations *gray*. Individuals with up to 10 % dissimilarity (indicated by the line parallel to the x-axis) were considered to have the same compositional type. For population abbreviations, see Table 1

compound proportions were actually different. Populations shared few compositional types; only 11 types were present in more than one population (Online Resource 1).

The total terpenoid concentration within populations varied significantly from 2.95 to 15.94 mg g^{-1} (Fig. 3). However, no significant difference was found between central and marginal populations ($t = 1.489$, $P = 0.14$).

Significant differences in average diversity among individuals were also found between some populations. Populations near the Atlantic Ocean had significantly lower diversity than those near the Pacific (Fig. 4). However, no difference in chemical diversity was found between central and marginal populations ($t = 0.243$, $P = 0.82$).

The PCA showed that about half (48.34 %) of the variation was explained by the first two components. Monoterpenes 3-carene and camphene have the highest relative contribution to the first component (12.19 and 12.03 %); limonene, β-myrcene, and sabinene contribute the most to the second component (20.37, 19.51, and 18.51 %, respectively) (Online Resource 1).

The DA produced two factors that effectively separated populations PAR, HUI, and SMC; the remaining populations appeared clustered in a gradient (Fig. 5). Monoterpenes sabinene and β-(E)-ocimene and sesquiterpenes α-humulene and modhephene were the most influential factors.

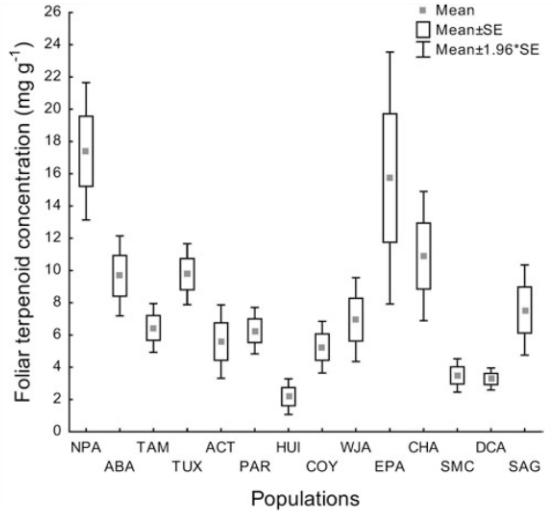

Fig. 3 Variation in foliar terpenoid concentrations among 14 populations of *M. micrantha*. For population abbreviations, see Table 1

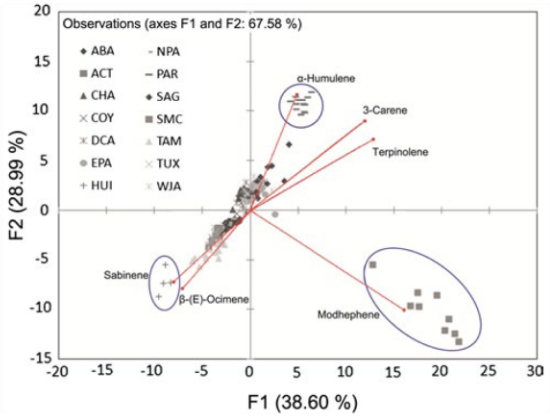

Fig. 5 Discriminant analysis of foliar terpenoids in 165 individuals from 14 populations of *M. micrantha*. Distribution in the bidimensional space based in Factors 1 and 2. Populations PAR, HUI, and SMC are clearly separated from each other and other populations in foliar terpenoid concentrations. For population abbreviations, see Table 1

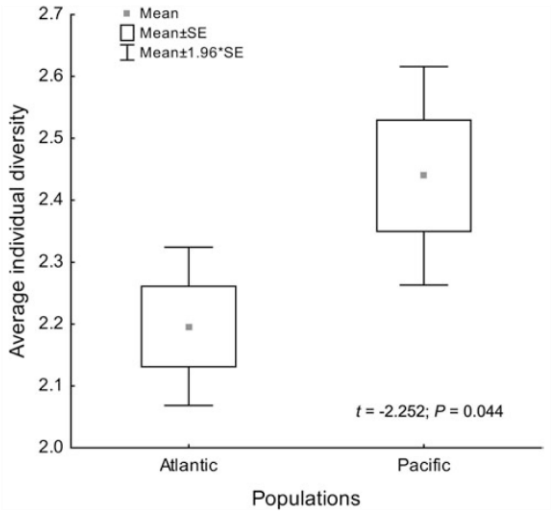

Fig. 4 Variation in individual diversity among populations of *M. micrantha* from the Atlantic and Pacific watersheds

The Mantel test showed no significant correlation between Euclidean and geographic distances ($R = 0.189$; $P = 0.13$), indicating that populations are not isolated by distance as predicted by the central–marginal hypothesis. However, Monmonier's algorithm detected two barriers that separated populations PAR and SMC, in which α-pinene is the major component. A third barrier isolated populations WJA and CHA, in which sesquiterpenes germacrene D and β-caryophyllene were dominant. A fourth barrier isolated six Atlantic watershed populations from the rest (Fig. 6).

The cluster analysis of populations separated HUI, PAR and SMC from the rest, and also found low linkage distances both for geographically close (ABA–TAM) and distant (CHA–COY) populations (Fig. 7).

The multiple regression analysis revealed an inverse relationship between the population-level diversity and total herbivory (Fig. 8). However, neither the average individual diversity nor the total terpenoid concentration was related to herbivory. None of the geographic and environmental variables was related to herbivory nor to the chemical diversity of the populations.

Discussion

According to the central–marginal hypothesis, we expected differences in herbivory intensity to be explained by gradients in geographic and climatic variables, but this was not the case. Furthermore, central populations did not experience significantly lower herbivore damage than marginal populations.

We also expected terpenoid concentration and chemical diversity to be higher in central populations, but these characteristics did not correlate with geographic distance (distant populations are not necessarily different) or with climatic variables. The fact that differences in chemical diversity were found in a comparison between Atlantic and Pacific populations, rather than between central–marginal populations, confirms that this model does not

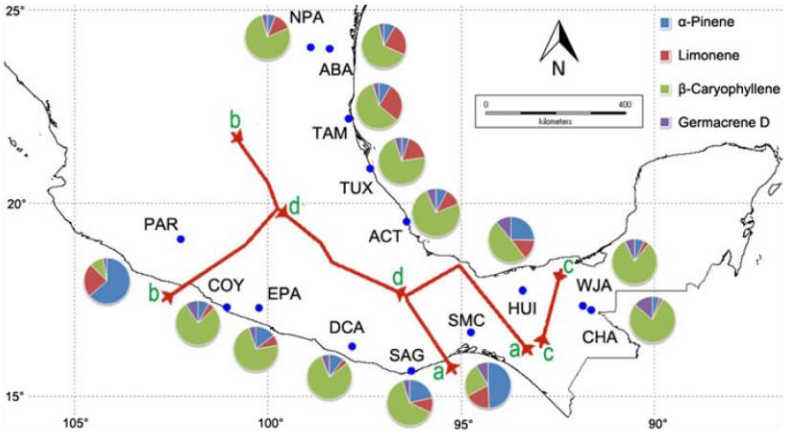

Fig. 6 Locations of sampled populations and proportions of four main terpenoids in these populations. Genetic barriers among populations found with Monmonier's algorithm are indicated by *bold lines*. For population abbreviations, see Table 1

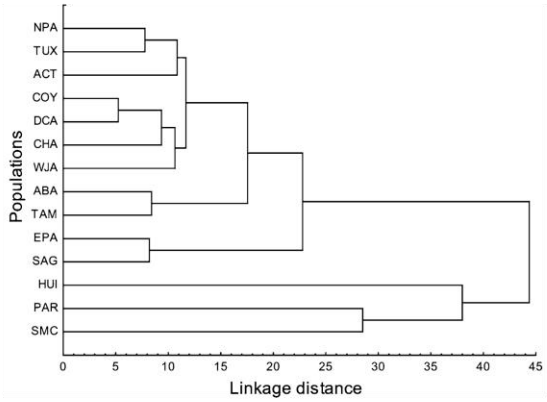

Fig. 7 Dissimilarity in average percentage of terpenoids for 14 populations of *M. micrantha*. For population abbreviations, see Table 1

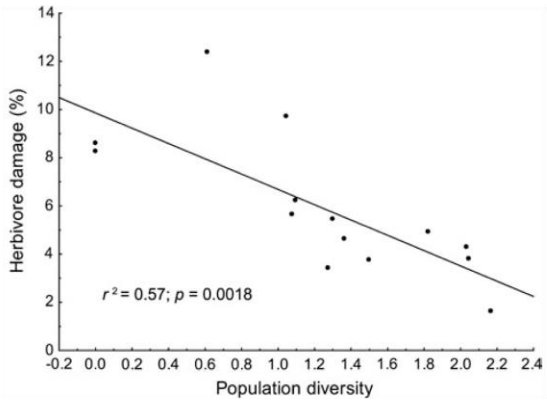

Fig. 8 Relationship between total herbivory and population chemical diversity among populations of *M. micrantha*, $n = 14$

appropriately describe the geographic structure of chemical diversity for *M. micrantha.* Instead, our results are consistent with the geographic mosaic model, in which the geographic distribution of a given species is described as a collection of populations with varying degrees of isolation characterized by their particular interactions with other species (e.g., herbivores) (Thompson 2001).

The inverse relationship between total herbivore damage and population-level chemical diversity provides confirmation of the "more diversity–more protection" hypothesis previously supported by some studies (Lindig-Cisneros et al. 2002), but not by others (Rincón-Hernández and Espinosa-García 2008). No relationship was found for individual chemical diversity, suggesting that the population-level polymorphism produces a defensive mosaic effect; that is, chemically distinct individuals within a population contribute to the heterogeneity of the population's defensive compounds, resulting in less herbivore damage (Langenheim 2003). We hypothesized that this structured variation occurs when different herbivore communities shape the population's chemical composition, as recently shown with wild *Arabidopsis thaliana* populations and their herbivores (Züst et al. 2012). Moreover, the few compositional types shared among populations, and the five areas established through Monmonier's algorithm, indicate that the geographic distribution of chemical diversity in *M. micrantha* is affected by natural barriers that create a structure of zones or regions, as expected under the mosaic model.

Populations SMC, PAR, and HUI were consistently the most chemically divergent, as they were effectively separated, as shown by discriminant analysis, cluster analysis, and genetic barriers. Barriers detected with Monmonier's algorithm match actual geographic barriers in Mexico (Fig. 6). Barrier *a,* isolating SMC, represents the mountain range Sierra Atravesada in Oaxaca; barrier *b,* isolating PAR, represents the Balsas Depression between the

Transvolcanic Belt and Sierra Madre del Sur; barrier *c*, isolating WJA and CHA, represents Montañas del Norte (Northern Mountains) in Chiapas; barrier *d*, isolating Pacific from Atlantic populations, represents Sierra Madre del Sur. Although the tropical zones of Mexico are the northernmost distribution of *M. micrantha* in the Americas, this species appears to have a discontinuous distribution towards the south due to geographic barriers in Central America (i.e., the Chiapas/Guatemala Highlands).

The geographic mosaic model for plant chemistry and herbivory has previously been tested, with varying results. The furanocoumarin phenotype in populations of *Pastinaca sativa* (Apiaceae) matched the detoxification mechanism of the webworm *Depressaria pastinacella* (Zangerl and Berenbaum 2003). However, a study that explored spatial variation in leaf chemistry and herbivory in *Vincetoxicum hirundinaria* (Asclepiadaceae) found positive and inverse correlations in some populations but not in others (Muola et al. 2010). To our knowledge, this is the first time that predictions of the two models have been tested to explain the spatial structure of chemical diversity.

This study also represents the first report of an inverse relationship between chemical diversity and herbivory in natural field populations; the relationship has previously been tested only in laboratory experiments (Feng and Isman 1995) and experimental gardens (Poelman et al. 2009; Kleine and Müller 2011; Torres-Gurrola et al. 2011). Previous studies in field populations, however, have also found associations between herbivory and individual compounds (Thoss and Byers 2006; Muola et al. 2010), groups of compounds (factors) (Keefover-Ring and Linhart 2010), or abundance of chemotypes (Züst et al. 2012).

Plant genotypic diversity is known to reduce damage from pest and pathogens, but the mechanism(s) behind this effect are rarely explored (Tooker and Frank 2012). Genotypic variation may be associated with genetically based defensive traits, which would be more difficult to overcome in populations with high diversity.

The identification of geographic zones with chemical similarity in the native distribution of *M. micrantha* could be used in biological control to help find natural enemies that can overcome the secondary metabolites produced by this species in its invaded range. Further studies are required to assess how particular compositional types of *M. micrantha* can affect both generalist and specialist herbivores.

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Appendix 1. Foliar volatile terpenoids identified in *Mikania micrantha*

Kovats index	Terpenoid	Concentration variation interval (%)	Average relative concentration (%),
944	α-Thujene	0.0 – 0.7	0.1
952	α-Pinene	0.9 – 37.0	7.0
966	Camphene	0.0 – 4.2	0.7
990	Sabinene	0.0 – 9.6	2.2
994	β-Pinene	0.0 – 8.9	2.7
1006	β-Myrcene	0.1 – 3.3	1.1
1020	α-Phellandrene	0.2 – 9.9	1.8
1025	3-Carene	0.0 – 4.4	0.7
1031	α-Terpinene	0.0 – 0.8	0.1
1039	p-Cymene	0.1 – 9.5	1.7
1043	Limonene	0.3 – 21.5	7.1
1050	(Z)-β-Ocimene	0.0 – 2.0	0.1
1061	(E)-β-Ocimene	0.0 – 7.7	1.9
1073	γ-Terpinene	0.0 – 1.0	0.2
1103	Terpinolene	0.0 – 1.0	0.2
1111	β-Linalool	0.0 – 1.0	0.2
1142	Alloocimene	0.0 – 0.2	0.03
1160	Camphor	0.0 – 0.1	0.2
1181	Borneol	0.0 – 0.8	0.1
1205	α-Terpineol	0.0 – 1.9	0.1
1298	Thymol	0.0 – 0.6	0.1
1342	δ-Elemene	0.0 – 9.0	1.1
1379	α-Copaene	0.0 – 12.8	1.3
1387	Modhephene	0.0 – 25.3	1.3
1390	β-Bourbonene	0.0 – 5.0	0.4
1395	β-Cubebene	0.0 – 33.2	0.7
1397	β-Elemene	0.0 – 37.1	1.9
1417	α-Gurjunene	0.0 – 6.9	0.9
1422	β-Caryophyllene	0.3 – 33.6	9.8
1434	γ-Elemene	0.0 – 6.7	0.6
1440	α-Guaiene	0.0 – 3.5	0.2
1455	α-Humulene	0.0 – 42.0	4.1
1458	Alloaromadendrene	0.0 – 2.1	0.2
1481	Germacrene D	0.0 – 70.6	35.6
1485	β-Selinene	0.0 – 23.7	1.0
1497	Germacrene B	0.0 – 10.5	3.1
1506	α-Farnesene	0.0 – 12.1	0.9
1514	γ-Cadinene	0.0 – 8.9	1.8

Appendix 2. Number of compositional types, chemical diversities and average herbivory.

Population	Compositional types	Average individual chemical diversity	Population chemical diversity	Average herbivory damage (%)
NPA	1	2.24	0.00	8.27
ABA	4	2.28	1.36	4.64
TAM	5	2.39	1.49	3.80
TUX	5	2.12	1.30	5.49
ACT	1	1.96	0.00	8.63
PAR	4	2.63	1.09	6.24
HUI	3	2.45	1.04	9.73
COY	2	2.44	0.61	12.43
WJA	9	1.94	2.03	4.33
EPA	10	2.20	2.17	1.67
CHA	4	2.18	1.07	5.67
SMC	4	2.77	1.27	3.45
DCA	7	2.29	1.82	4.93
SAG	9	2.31	2.05	3.81

Appendix 3. Contribution of the variables (%) to the first two Principal Components.

Compound	F1	F2
α-Pinene	11.984	0.154
Camphene	12.035	0.000
Sabinene	1.135	18.513
β-Pinene	5.778	1.048
β-Myrcene	0.333	19.510
α-Phellandrene	11.920	0.011
3-Carene	12.194	0.174
α-Terpinene	10.757	0.236
p-Cymene	11.605	0.014
Limonene	0.007	20.368
(E)-β-Ocimene	2.269	6.739
δ-Elemene	0.095	5.004
α-Copaene	0.306	3.842
Modhephene	1.350	0.063
β-Bournonene	0.027	6.357
β-Cubebene	0.318	0.154
β-Elemene	0.436	1.985
α-Gurjunene	0.033	2.360
β-Caryophyllene	0.144	3.808
α-Humulene	3.473	0.004
Germacrene D	8.846	0.600
β-Selinene	0.176	0.815
Germacrene B	2.909	4.231
α-Farnesene	0.434	3.555
γ-Cadinene	1.435	0.456

4

Estructura espacial de la diversidad genética y química en poblaciones mexicanas de *Mikania micrantha*.

Spatial structure of genetic and chemical diversity in Mexican populations of *Mikania micrantha*.

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Abstract

The genetic variability of an invasive species can affect its adaptive responses to the selective pressures in the new range. The spatial distribution of genetic diversity among populations can be a product of historical and ecological factors that limit reproduction and dispersal. On the other hand, the phenotypic variation of defense traits against herbivores, such as secondary metabolites, can be associated to the underlying genetic structure, thus producing a correlation between both characteristics. In this study, we aimed to examine the spatial distribution and potential relationship of genetic and chemical diversity of the invasive weed *Mikania micrantha* (Asteraceae) in its native distribution. For this purpose, the genetic diversity and geographic variation of 13 Mexican populations from the Atlantic and Pacific watersheds were assessed using six specific microsatellites, and compared the resulting geographic patterns of genetic and chemical diversity. We found more genetic structure and diversity in the Atlantic than in the Pacific populations. Most genetic variation is located within populations (43%), although a substantial proportion is found among regions. The differentiation between Atlantic and Pacific populations was supported consistently with a cluster analysis, a principal coordinates analysis, and a Bayesian analysis. A Mantel test showed the pattern of isolation by distance for Atlantic populations (r^2=0.478, P=0.002). While no correlation was found for genetic and chemical distances, a positive correlation was found between the chemical diversity and the number of alleles in Pacific populations, and a negative correlation between herbivore damage and number of alleles for Atlantic populations. Our results suggest that different biogeographic processes have operated in the two regions and this is reflected on the molecular and chemical markers.

Keywords

Microsatellites, population genetics, spatial structure, invasive species.

Introduction

The genetic diversity of invasive plant species is considered to be one of the major drivers of their colonization success by allowing a rapid adaptation to the new environments (Crawford and Whitney 2010; Jones and Gomulkiewicz 2012). The spatial distribution of genetic diversity within populations can be affected by historical and ecological factors that limit plant reproduction and dispersal (Loveless and Hamrick 1984).

Additionally, the spatial genetic structure can shape the phenotypic variation in herbivore defense traits, such as secondary metabolites (Andrew *et al.* 2007); but environmental variation and developmental factors (i.e. age structure and environmental heterogeneity) can also determine the spatial structure of secondary chemistry in plants (Brenes-Arguedas and Coley 2005).

Since the same limiting factors of dispersal and gene flow will affect the spatial structure of chemical and genetic variation, a spatial correlation of these two features may occur. Such relationship has been documented in studies comparing neutral genetic markers and terpenoids for individual plants (Skoula *et al.* 1999; Keskitalo *et al.* 2001), plant populations (Nan *et al.* 2003; Fracaro *et al.* 2005), and cultivars (Böszörményi *et al.* 2009).

Furthermore, intraspecific variation in chemical composition can affect the ecological interactions with other species (*e.g.* herbivores). The genetic similarity rule states that the genetic variation of a plant will affect the structure of the herbivore community and predicts that plants with similar genetic composition will have similar chemical and herbivore compositions (Bangert *et al.* 2006). Under this hypothesis, the phytochemical composition plays the role of mediator between the plant genetic composition and the herbivore community. Associations between genetic variation, defense metabolites and diversity of herbivorous insects have been found for populations of *Populus tremula* (Bernhardsson *et al.* 2013).

We were interested in investigating the relationship between neutral genetic diversity and chemical diversity in Mexican populations of *Mikania micrantha* (Asteraceae), a Tropical American weed that has become invasive in Southeast Asia. Its native distribution in Mexico comprises the tropical watersheds near the

Atlantic and Pacific coasts. Although various microsatellite markers have been developed for this species (Hong *et al.* 2008; Yan *et al.* 2011), they have not been used to evaluate the population genetics on the invaded range yet.

We postulate that the geographic barriers affecting chemical diversity will also affect the genetic diversity and increase genetic differentiation. We predict a high genetic differentiation between Atlantic and Pacific populations.

The study of the genetic variation among populations can reveal the processes that shaped its present structure. And understanding both the population structure of genetic and chemical diversity in *M. micrantha* is essential to develop successful management strategies and to identify effective biological control agents.

Materials and methods

Plant material

We collected mature leaves in seven populations of *M. micrantha* near the Atlantic (Dec-2007), and six populations near the Pacific (Dec-2009) totalizing 159 individuals (Table 1). An exhaustive sampling was performed to include all available individuals at each population. Leaves were dehydrated with silica gel and kept refrigerated until DNA extraction.

DNA extraction

Total DNA was extracted from 500 mg of leaf tissue using a previously developed protocol (Su *et al.* 1998) with minor modifications. Each sample was ground by mortar and pestle in liquid nitrogen. The resulting powder was placed in two Eppendorf tubes (1.5 mL) and extracted twice with 1 mL of acetone at -20ºC and centrifuged at 5000 rpm for 10 min, the supernatant was discarded. Samples were added 1 mL of CTAB buffer and incubated at 60ºC for 2 h. For the extraction we added 500 µL of chloroform:isoamyl alcohol (24:1) and centrifuged at 13000 rpm for 10 min. The supernatant was recovered in a new tube and DNA was precipitated with 0.6 volume of cold isopropanol. A DNA pellet was obtained by

centrifugation at 3000 rpm for 3 min; the supernatant was discarded, and let it stand in 800 µL of washing buffer for 20 min. After a final centrifugation at 1000 rpm for 10 min, the supernatant was discarded and the pellet was dried at room temperature for 15 min. The DNA was dissolved in 50 µL of deionized water.

PCR conditions

We used six microsatellite loci: Mm01, Mm05, Mm07, Mm12, Mm19 and Mm31, developed for *Mikania micrantha* (Hong *et al.* 2008), in multiplex reactions with fluorescently labeled primers. Microsatellite amplifications were performed in 5 µL reaction volume containing 10 ng DNA template, 3 µL of Multiplex PCR Master Mix, 2 µM of each primer and 1 µL of dH_2O using an Eppendorf Mastercycler® thermocycler. The temperatures for PCR amplification consisted of an initial activation step of 95°C during 15 min, followed by 35 cycles of denaturing at 95°C for 1 min, annealing at primer-specific temperatures (41.7°C for Mm01and Mm07, 45.8°C for Mm05 and Mm12 and, and 51.1°C for Mm19 and Mm31) for 1.5 min, and extension at 72°C for 1 min. After cycling, there was a final elongation step at 72°C for 5 min.

Multiplex PCR products (2 µL), 8 µL Hi-Di Formamide, and 0.3 µL GeneScan-500 LIZ (Applied Biosystems) size standard were denatured for 5 min at 95°C and analyzed by capillary electrophoresis using an ABI Prism 3100-*Avant*® (Applied Biosystems, Hitachi, Japan) automated sequencer. We used Peak Scanner™ version 1.0 (Applied Biosystems) to perform fragment analysis and final sizing. Microsatellite data was revised and formatted with the software The Excel Microsatellite Tool (Park 2001). We used Micro-Checker ver. 2.2.3 to identify the presence of null alleles at each locus per population.

Genetic variation within populations

We used the software Genetic Data Analysis (GDA ver. 1.1) (Lewis and Zaykin 2001) to estimate the average number of alleles (A), number of private alleles, proportion of polymorphic locus (P), observed (H_O) and expected (H_E) proportion of

heterozygotes as well as the parameters F_{IS}, F_{IT}, and F_{ST} for each locus and population.

Hardy-Weinberg Equilibrium deviations were detected with an exact test for individual and pairs of loci per population, and significant associations between pairs of loci were examined with a pairwise Linkage Disequilibrium test as implemented in the GDA program (chi-square test probability based on 3200 shufflings).

Genetic differentiation among populations

Population genetic structure was examined by an analysis of molecular variance (AMOVA) performed using Arlequin software (ver. 3.5), among Pacific and Atlantic populations.

Genetic structure of populations

To explore genetic similarities among *M. micrantha* populations we constructed a dendrogram using Nei's genetic distances (Nei 1972) and the UPGMA algorithm, with the Tools for Populations Genetics Analyses (TFPGA ver. 1.3) program (Miller 1997). Confidence levels for the dendrogram were calculated by bootstrapping 1000 times over the original loci.

An Analysis of Principal Coordinates was performed with Nei's genetic distances. Also a Mantel test with 10,000 permutations was performed to detect isolation by distance using genetic distances (Nei) and geographic distances (Km) with the GenAlex software (ver. 6.4) (Peakall and Smouse 2006).

Bayesian clustering was performed using the STRUCTURE (ver. 2.2) program (Pritchard *et al.* 2000) to detect the genetic structure of populations. A parameter set was defined with a burn-in length of 10^5 steps followed by 10^6 MCMC iterations, individuals were analyzed under a mixed model with correlated allelic frequencies. To obtain the most probable *K* value (number of genetic groups), values of *K* from 1 to 13 were tested, with 10 independent runs for each *K*.

The best *K* value was calculated estimating the maximum value of the ΔK statistic (Evanno *et al.* 2005).

Genetic diversity and chemical diversity

To test the relation between genetic and chemical composition, we performed a Mantel test with the genetic distances and two Euclidian distances, 1) average percentage of terpenoids, and 2) average terpenoid concentration, taken from a previous work (Bravo-Monzón *et al.* 2014).

We performed a stepwise multiple regression analysis to explore the relationships between genetic diversity, chemical diversity, total terpenoid concentration and geographic variables (latitude, longitude, altitude), as well as environmental variables (precipitation and average yearly temperature).

Results

Population genetic variation

We found a total of 29 alleles for all six loci, and an average number of alleles per locus (*A*) of 1.83, ranging from 1 (COY) to 2.17 (ABA) (Table 2). All six microsatellites were polymorphic. The average percentage of polymorphic loci per population (P) was 0.654%, varying from 0 (COY) to 0.833 % (ABA). The number of private alleles varied from one to three across loci, for a total of 10. Population WJA contained the highest number of private alleles (5), followed by PAR and TAM (2 each) and EPA (1).

The average observed and expected heterozygosity in Atlantic populations were higher (H_O = 0.230, H_E = 0.251) than in Pacific populations (H_O = 0.136, H_E = 0.156). The population with the highest H_E was ABA (0.371) from the Atlantic. For most populations, the expected heterozygosity was higher than the observed heterozygosity, which indicates an excess of homozygous individuals.

Significant Hardy-Weinberg deviations were detected for loci Mm19 in EPA, and for Mm31 in most populations except NPA, SMC, HUI and COY. Significant linkage disequilibrium was found for various loci pairs: Mm12-Mm31 (four populations), Mm07-Mm12 (four populations) and Mm07-Mm31 (three populations).

The comparison between coasts showed higher values for allelic richness, observed heterozygosity, gene diversity, F_{ST} and relatedness in the Atlantic populations than in the Pacific (Table 4).

Population genetic differentiation

The analysis of molecular variation showed that most of the variation occurred within populations (43.2%; Table 5). However, a substantial proportion of the variation (29%) was found among regions, a sign that Atlantic and Pacific populations are actually different.

Relationships among populations

The cluster analysis performed with the UPGMA algorithm generated a dendrogram that divided the populations into three genetic groups: Group I contains all six Pacific populations, Group II contains six Atlantic populations while Group III only contains the WJA population (Fig. 1). These groups are quite consistent with the geographical origin of the populations.

Nei's genetic distance (GD) for all populations presented an average value of 0.252 with a maximum value of 1.701 and a minimum value of 0.0023. Atlantic populations showed an average GD value of 0.473 [max 1.701 (NPA-WJA) – min 0.021 (ABA-NPA)]. For the Pacific populations the average GD value was 0.031 [max 0.077 (PAR-COY) – min 0.0023 (SMC-COY)].

In the principal coordinate analysis, coordinate 1 explained 72.64% and coordinate 2 explained 16.56%. Both coordinates contributed to separate Atlantic from Pacific populations; the two northern (NPA, ABA) and the southernmost (WJA) populations from the Atlantic were further separated, while Pacific populations remained clustered together (Fig. 2).

The analysis with the STRUCTURE program and the subsequent evaluation of the ΔK statistic indicated that K=2 is the most probable number of clearly differentiated genetic groups of *M. micrantha* (Fig. 3). The first group contains six Atlantic populations (NPA, ABA, TAM, TUX, ACT and HUI), the second group contains all Pacific populations plus WJA.

The isolation by distance test indicated a significant correlation between geographic distance and genetic distance for Atlantic populations (r=0.692; P=0.002), but not for Pacific populations (r=0.023; P=0.447). Furthermore the BARRIERS program detected three genetic barriers in the distribution of *M. micrantha*. The first barrier (a) isolated the WJA population, the second barrier (b) separated the Pacific and Atlantic populations, and a third barrier (c) isolated the northernmost populations ABA and NPA (Fig. 4).

Genetic diversity and chemistry

Mantel tests revealed no relation between the genetic distance of the populations and their chemical composition (r=-0.111, P=0.339) or their terpenoid concentration (r=0.001, P=0.605).

We found a positive correlation between the average individual chemical diversity and the number of alleles per population in Pacific populations (r=0.867, P=0.0258) (Fig. 5). We also found a negative correlation between the average herbivore damage and the number of alleles per population in Atlantic populations (r=0.916, P=0.0038) (Fig. 6)

Discussion

Our results show low genetic diversity for Mexican populations of *M. micrantha*, specially compared to the preliminary report for these same microsatellites in a single introduced population of China (Hong *et al.* 2008). Although it is possible for invasive populations to have a higher genetic diversity than the native populations (via multiple introductions, for example), a broader study on the invaded range is still necessary to support this claim.

Important differences were found between Atlantic and Pacific populations; various population genetic parameters revealed a higher genetic diversity in the Atlantic (Table 4). The Atlantic and Pacific regions also vary in their genetic structure. With the exception of the WJA population, all the other populations were grouped according to their origin by the UPGMA cluster analysis and the Bayesian analysis in STRUCTURE. Likewise, the principal coordinates analysis successfully

separated the populations from both regions, and revealed a higher genetic difference among the Atlantic populations. The Mantel test showed a significant correlation between the geographic distance and genetic distance in the Atlantic, supporting the isolation by distance model.

We did not find a significant relationship between the genetic distance and chemical composition of all populations, although when the two regions were analyzed separately, some noteworthy correlations were found. The lack of association between chemical and genetic data has been reported in previous studies with spices *Ocimum basilicum* (Masi *et al.* 2006), *Thymus caespititius* (Trindade *et al.* 2008) and the invasive *Tanacetum vulgare* (Wolf *et al.* 2012).

It is possible that part of the problem lies in the common practice of using neutral molecular markers as substitutes for measuring quantitative trait variation, when their correlation is weak (Reed and Frankham 2001). Although microsatellites represent a reliable molecular tool for analyzing the genetic variation within and among populations, they should not be considered as a replacement for adaptive traits like secondary metabolites.

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Table 1 Location of collection sites of *Mikania micrantha*.

	Population	State	Latitude and Longitude		Altitude (masl)	Sample size
NPA	Nuevo Padilla	Tamaulipas	24º05’15’’N	98º52’17’’W	153	6
ABA	Abasolo	Tamaulipas	24º03’07’’N	98º22’34’’W	60	15
TAM	Tampico	Tamaulipas	22º14’25’’N	97º53’26’’W	5	15
TUX	Tuxpan	Veracruz	20º56’44’’N	97º20’31’’W	8	23
ACT	Actopan	Veracruz	19º35’31’’N	96º22’55’’W	44	6
PAR	Parácuaro	Michoacán	19º08’26’’N	102º13’56’’W	553	14
HUI	Huimanguillo	Tabasco	17º47’58’’N	93º23’55’’W	39	4
COY	Coyuquilla Norte	Guerrero	17º22’48’’N	101º03’08’’W	209	10
WJA	Welib-Já	Chiapas	17º22’27’’N	91º47’58’’W	234	13
EPA	El Paraíso	Guerrero	17º21’05’’N	100º12’43’’W	1761	15
SMC	San Miguel Chimalapa	Oaxaca	16º42’45’’N	94º44’53’’W	1276	9
DCA	Dos Caminos	Oaxaca	16º22’14’’N	97º48’26’’W	311	13

Table 2 Genetic population parameters for populations of *Mikania micrantha. A* = average number of alleles per locus, *P* = proportion of polymorphic locus, H_O = Observed proportion of heterozygotes, H_E = expected proportion of heterozygotes, F_{IS} = inbreeding coefficient, (± S.D). See Table 1 for definitions of population acronyms.

Population	A	P	H_O	H_E	F_{IS}
NPA	1.833	0.667	0.222(±0.069)	0.245(±0.096)	0.101
ABA	2.167	0.833	0.322(±0.049)	0.371(±0.090)	0.136
TAM	2.167	0.833	0.311(±0.049)	0.290(±0.117)	-0.074
TUX	2.167	0.833	0.125(±0.028)	0.158(±0.074)	0.214
ACT	1.833	0.667	0.306(±0.077)	0.295(±0.111)	-0.038
PAR	1.833	0.667	0.298(±0.050)	0.278(±0.107)	-0.073
HUI	1.667	0.500	0.233(±0.078)	0.226(±0.119)	-0.037
COY	1.000	0.000	0.000(±0.000)	0.000(±0.000)	0.000
WJA	2.000	0.833	0.090(±0.032)	0.168(±0.062)	0.475
EPA	2.166	0.833	0.256(±0.046)	0.300(±0.080)	0.152
SMC	1.333	0.333	0.019(±0.018)	0.053(±0.036)	0.667
DCA	1.667	0.667	0.064(±0.028)	0.086(±0.034)	0.260
SAG	2.000	0.833	0.179(±0.042)	0.217(±0.086)	0.184
Mean	1.833	0.654	0.186	0.207	0.105

Table 3 Allele frequency-based correlation for six microsatellites of *M. micrantha* (F_{is}, F_{it}, F_{st}, H_O and H_E from Nei's -GDA).

Locus	F_{is}	F_{it}	F_{st}	H_o	H_E
Mm01	-0.081	0.498	0.536	0.176	0.335
Mm05	0.323	0.885	0.831	0.019	0.153
Mm07	-0.187	-0.075	0.095	0.270	0.250
Mm12	-0.239	0.291	0.427	0.270	0.367
Mm19	0.048	0.804	0.794	0.119	0.567
Mm31	0.486	0.666	0.349	0.258	0.746
Overall	0.120	0.560	0.500	0.186	0.403

Table 4 Comparison between Atlantic and Pacific populations of *Mikania micrantha*, R_S =allelic richness, H_O = observed heterozygosity, H_S = gene diversity, F_{IS} = inbreeding coefficient within individuals, F_{ST} = differentiation between populations, *CR* = corrected relatedness (FSTAT).

Origin	R_S	H_O	H_S	F_{IS}	F_{ST}	Relatedness	CR
Atlantic	1.773	0.214	0.242	0.115	0.492	0.635	-0.260
Pacific	1.523	0.153	0.175	0.126	0.112	0.183	-0.288
10x4 permutations	0.1457	0.3503	0.2877	0.924	0.0003	0.0006	0.9241

Table 5 Analysis of molecular variance (AMOVA) for six microsatellite loci in *Mikania micrantha* populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	*P*
Among regions	1	78.999	0.423	29.04	0.005
Among populations within regions	11	112.633	0.404	27.77	<0.0001
Within populations	305	191.778	0.629	43.19	<0.0001
Total	317	383.409	1.456		

Figure 1 UPGMA dendrogram based on Nei's (1972) genetic distances for populations of *Mikania micrantha*. See Table 1 for definitions of population acronyms.

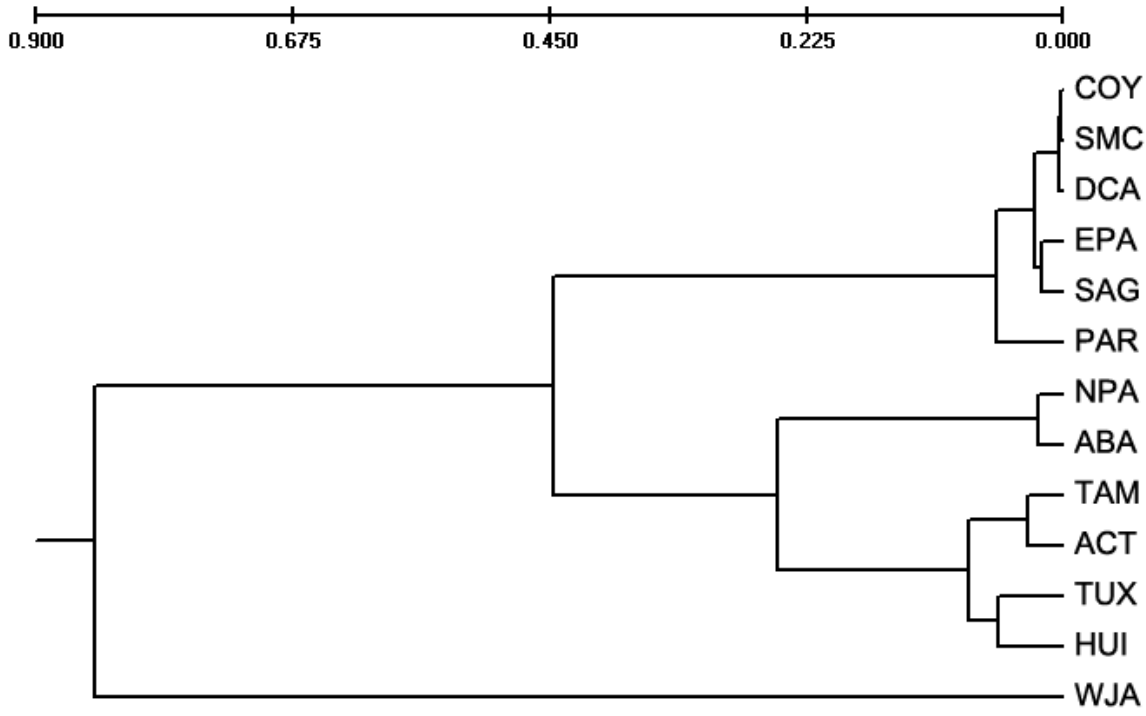


Figure 2 Principal Coordinate Analysis for 13 populations of *M. micrantha*. Distribution of Atlantic (*grey*) and Pacific (*black*) in space is based on Coordinates 1 and 2.

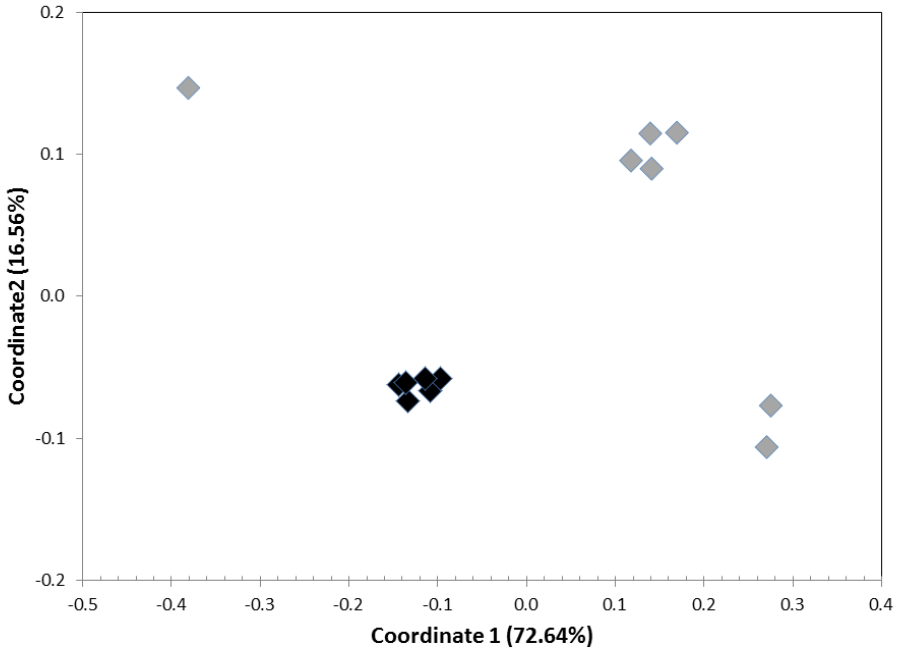


Figure 3 Magnitude of Δ*K* values for each estimated number of populations (*K*) from the structure analysis.

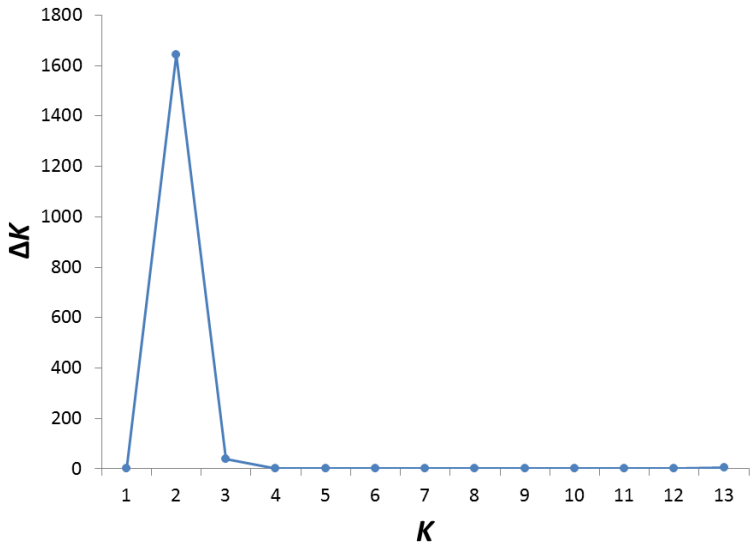


Figure 4 Genetic barriers among populations of *M. micrantha* detected using Monmonier's algorithm. See Table 1 for definitions of population acronyms.

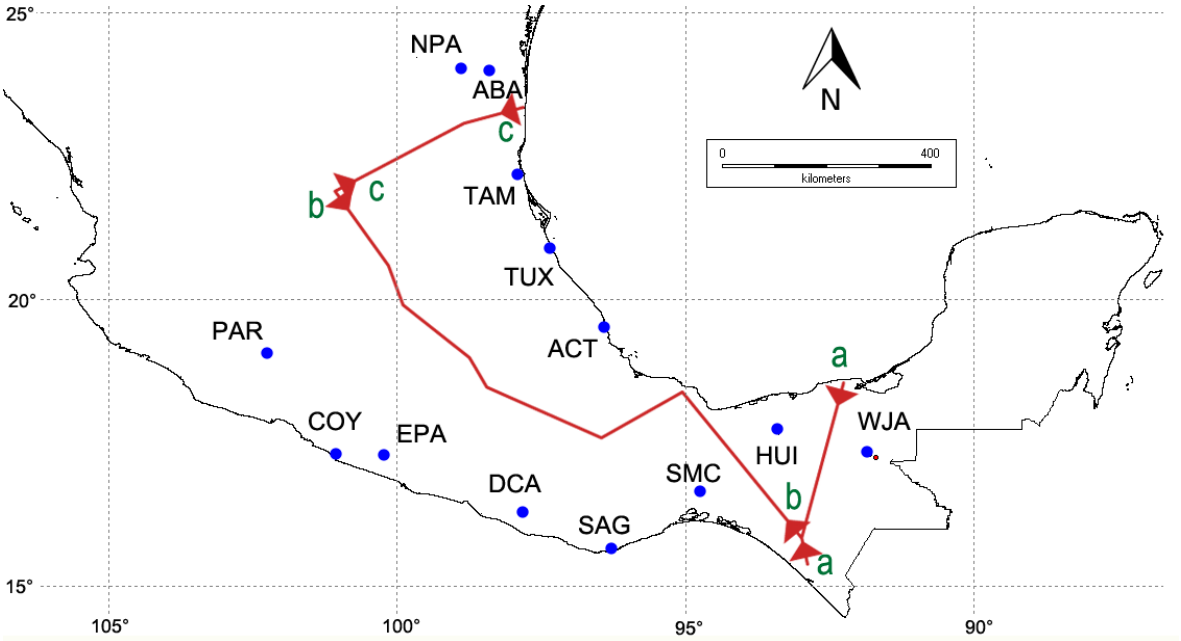


Figure 5 Relationship between herbivore damage and number of alleles among Atlantic populations of *M. micrantha*, *n* = 7.

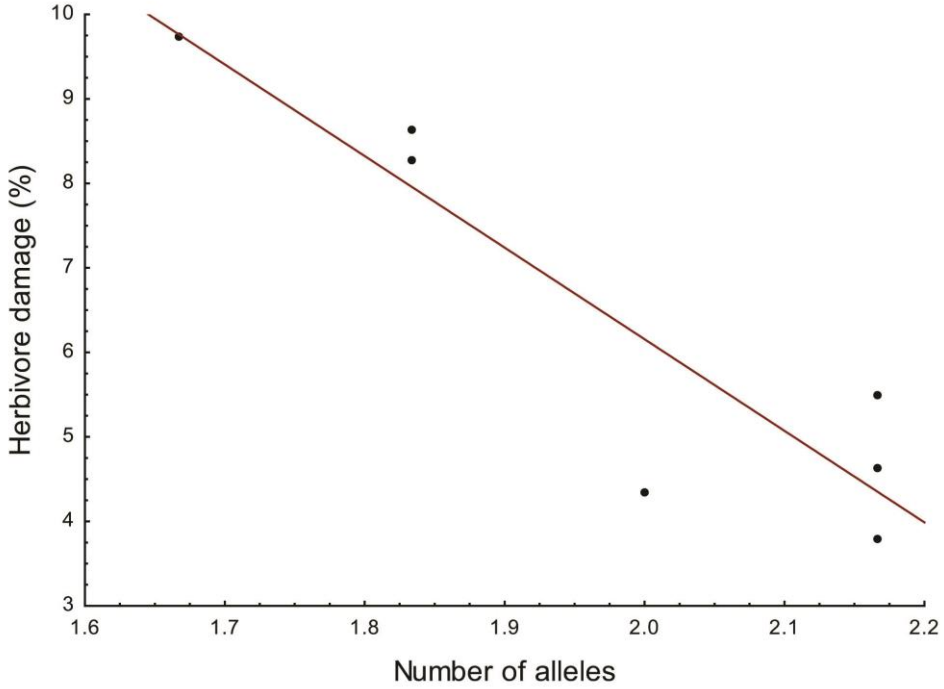
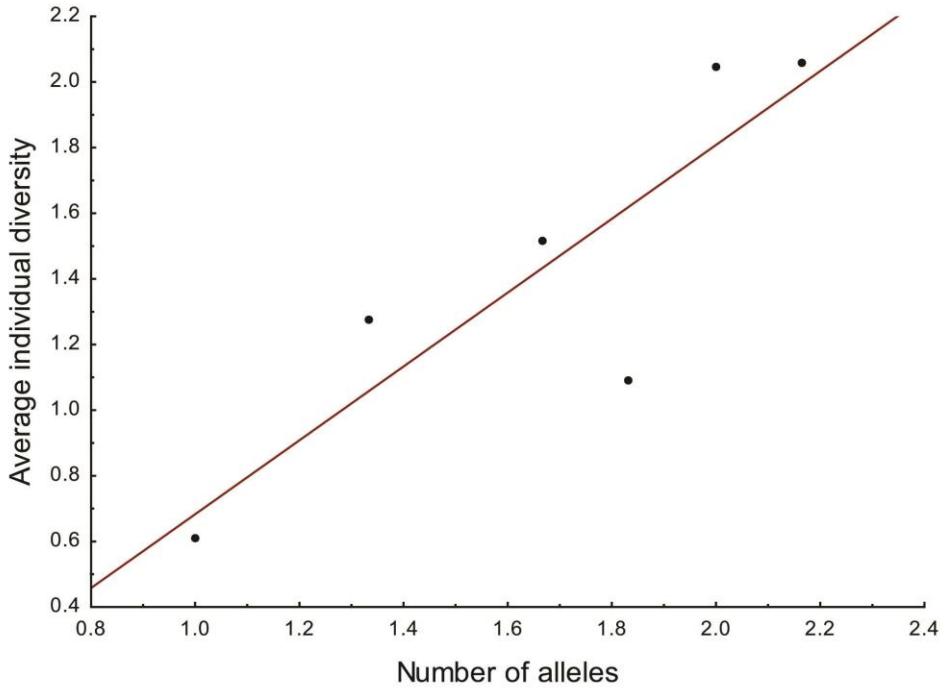


Figure 6 Relationship between average individual diversity and number of alleles among Pacific populations of *M. micrantha*, *n* = 6.



5

Selección diferencial de hospederos en el escarabajo herbívoro *Stolas punicea* sobre fenotipos químicos de *Mikania micrantha*

Differential host selection of the herbivore beetle *Stolas punicea* on chemical phenotypes of *Mikania micrantha*

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Abstract

1. Geographical variation of secondary metabolite content within a plant species can affect the preference or performance of its herbivores. Therefore, it is important to evaluate the preference of herbivores as potential biological control agents over the chemical composition of their host.
2. We used cafeteria tests to assess the preference of two provenances of the specialist herbivore *Stolas punicea* (Coleoptera; Chrysomelidae) over plants from four phytochemically differentiated geographical mosaics of the native weed *Mikania micrantha* (Asteraceae), which is invasive in tropical Asia. Female adult beetles from Michoacán and Veracruz were offered plants from different origins in four-choice arenas. The removed foliar area was quantified at the end of the assay.
3. Beetles from Veracruz showed no preferences and fed equally on all the offered chemical phenotypes; however, those from Michoacán consumed completely the plants from Michoacán and they avoided those from Tabasco.
4. These results indicate that geographic origin of *S. punicea* plays an important role on the acceptance of chemical phenotypes, and suggests that certain populations of *S. punicea* would not able to successfully establish on all *M. micrantha* chemical phenotypes.

Keywords

Host preference, geographic origin, invasive weed, Coleoptera: Chrysomelidae, insect-plant interactions.

Introduction

Variation on secondary metabolite composition among populations of the same species is usually associated with the abiotic conditions and biological interactions prevalent in the plant population locality (Züst *et al.*, 2012; Bernhardsson *et al.*, 2013). Moreover, the geographic variation on herbivory intensity produces a 'mosaic' of populations with different chemical defense levels (Zangerl and Berenbaum, 2003; Muola *et al.*, 2010). Furthermore, invasive plant species may experience quantitative and qualitative changes in their secondary metabolite content after colonizing a new range (Müller-Scharer *et al.*, 2004; Joshi and Vrieling, 2005).

Phytochemical variation can affect host detection, performance and survival of herbivores. Additionally, host preference of herbivore insects is also determined by its geographic origin and, because this preference usually remains fixed for various generations, it is suspected to be under genetic control (Thompson and Pellmyr, 1991; Kawecki and Mery, 2003). For biological control purposes, it is necessary to determine that a potential biological control agent is able to accept and establish on the various chemical phenotypes of its host.

The invasive weed species *Mikania micrantha* Kunth (Asteraceae), commonly known as mile-a-minute, is a perennial herbaceous vine native to the Neotropics that was introduced into Asia in the early 20th century, and has spread over disturbed forests and agricultural lands causing severe economic and environmental damages (Tripathi *et al.*, 2012). In Mexico, this species is found in the tropical areas along the Atlantic and Pacific watersheds, from the northern states of Tamaulipas and Nayarit to the south in Tabasco and Chiapas (Villaseñor and Espinosa-García, 1998).

The main secondary metabolites in *M. micrantha* are volatile terpenes (Bravo-Monzón *et al.*, 2014) and sesquiterpene lactones (Ríos *et al.*, 2014). Terpene profiles (also known as compositional types, chemotypes or chemical phenotypes) play a crucial role in plant defense and several of them are known to have differential repellent effects on arthropods (Langenheim, 1994). The various

chemical phenotypes are defined by the proportions of the major terpenes. A previous study on *M. micrantha*, distinguished 56 chemical phenotypes distributed into five phytochemically differentiated geographically isolated zones or 'mosaics'; the largest mosaics include considerable portions of the Mexican Pacific and Gulf of Mexico watersheds (Bravo-Monzón *et al.*, 2014).

Stolas punicea (Boheman, 1850) is a chrysomelid tortoise beetle native to Tropical America. Its known distribution comprises Mexico and Central America (Chaboo, 2002, 2003). In Mexico, *S. punicea* has been recorded in the states of Michoacán, Guerrero, Oaxaca, Puebla, Chiapas, Tabasco and Veracruz (Borowiec, 2002, 2009). Host plants in Mexico include *M. micrantha* and *M. cordifolia* (Noguera, 1988; A. Bravo-Monzón, pers. obs.). This specialist herbivore completes its life cycle on leaves and stems, frequently defoliating the plant completely (A. Bravo-Monzón, pers. obs.). Thus, we suggest that this beetle may be used as a potential control agent of *M. micrantha* in its introduced range.

This study was conducted to determine if the geographical origin of the tortoise beetle *S. punicea* affects the acceptance of *M. micrantha* plants with distinct chemical phenotype. If *S. punicea* adults are not adapted to all terpenoid profiles occurring in *M. micrantha* phytochemically differentiated mosaics, a feeding preference for some specific chemical phenotypes is expected. We predicted that beetles would show a higher preference for the plants within their own geographic mosaic because *M. micrantha* populations are highly structured in their chemical and genetic composition (Bravo-Monzón et al. submitted).

Materials and methods

Plant material

Seeds from four populations in four mosaics: Actopan, Veracruz (ACT), Huimanguillo, Tabasco (HUI), Parácuaro, Michoacán (PAR) and San Agustín, Oaxaca (SAG), were collected and germinated in a sphagnum-agrolite mix (1:1) (Cosmocel, S.A.) under similar conditions in a greenhouse. Two months-old seedlings were potted into black polyethylene bags (40 cm high and 25 cm diameter). One mature leaf of each plant was sampled for chemical analysis; those

representing the predominant chemical phenotype of the population were used in the bioassays. Voucher specimens are deposited at herbaria of the Institute of Ecology, AC (IE-BAJÍO 196632, 196628) and University of Michoacán (EBUM 20403, 22691).

Insects

Stolas punicea (Coleoptera: Chrysomelidae, Cassidinae, Stolaini) beetles were collected in Parácuaro, Michoacán in the Pacific watershed (20 adults both sexes, 5/III/13), and Catemaco, Veracruz in the Gulf of Mexico watershed (15 adults, both sexes, 8/VI/13). *Stolas* beetles on other *Mikania* populations were scarce and too few to maintain viable populations under greenhouse conditions. Specimens were identified using an online manual (Borowiec and Świętojańska, 2002) and confirmed by L. Borowiec (pers. comm.); vouchers were deposited at the National Insect Collection, UNAM; Mexico City (IBUNAM-CNIN: CO49859-CO49872). The two beetle colonies were reared on *M. micrantha* plants from their origin. Young female adults were starved for 24 h before they were used in the bioassays.

Chemical analysis

The volatile terpenoids of *M. micrantha* plants were analyzed by GC-MS, quantified using an internal standard and identified by comparison of mass spectra with the National Institute of Standards and Technology library (version 02) and literature as described elsewhere (Bravo-Monzón *et al.*, 2014).

Cafeteria experiments

A bioassay using four-choice test was performed to assess the host preference of the beetles. A single *S. punicea* young female adult was placed inside an arena containing a *M. micrantha* cutting (with a pair of fully expanded leaves with similar area) from each chemical phenotype; stems were placed in individual plastic cups containing moistened cotton, remaining alive and developing adventitious roots. The arena was enclosed in a transparent plastic dome with a piece of damp filter

paper at the bottom. Beetles were allowed to feed for 4 days. This test was repeated 24 times for each beetle population.

After the feeding period, damaged leaves were digitized and the removed area was quantified using the Assess software (© 2002 The American Phytopatological Society).

Statistical analysis

To evaluate the feeding preference of each *S. punicea* population on the chemical phenotypes of *M. micrantha*, we analyzed the differences in the removed leaf area using a Friedman ANOVA. Differences in preferences of both *S. punicea* populations were tested by Kruskal-Wallis tests.

We also explored the relationship among herbivory and total terpenoid concentration, number of compounds and chemical diversity (Shannon Index) with a stepwise multiple regression analysis. Possible relationships of herbivory and individual compound concentration were also explored. Statistical analyses were performed using Statistica 8 (StatSoft, Tulsa, OK, USA).

Results

Chemical analysis

A total of 19 compounds were isolated and identified in the plants used in these experiments. The principal terpenoids for ACT were germacrene D, limonene and β-caryophyllene; for HUI were β-selinene, β-cubebene and germacrene D; for PAR were humulene, α-farnesene and germacrene D; and for SAG were germacrene D, β-cubebene and γ-cadinene (Fig. 1).

The total terpenoid concentration varied from 0.9 mg g^{-1} (PAR) to 8.1 mg g^{-1} (SAG); the number of terpenes also varied significantly from eight (PAR) to 19 (SAG).

Preference tests

Removed leaf area by Veracruz beetles was not statistically different among plants with different chemical phenotypes (Friedman ANOVA: n=24, d.f.=3, *P*=0.826).

However, the removed area by Michoacán beetles varied significantly among chemical phenotypes (Friedman ANOVA: n=24, d.f.=3, *P*=0.034; Fig. 2). The Kruskal-Wallis test showed that HUI cuttings were significantly less damaged by beetles from Michoacán than by those from Veracruz (Kruskal-Wallis: H=11.635, *P*=0.0006).

The multiple regression analysis found no relationship between number of compounds, chemical diversity, total terpenoid concentration, and herbivore damage. No correlation was found either for herbivory and the concentration of any individual compound. Thus, the chemical mixture present in each phenotype, or non-quantified chemicals associated with the phenotype, could explain the *Stolas* differential feeding.

Discussion

The preference assays detected a geographical variation in host preference. Beetles from Veracruz were able to feed equally on all chemical phenotypes offered, while those from Michoacán preferred their original host and avoided the HUI phenotype.

A previous study on the geographical structure of chemical diversity for *M. micrantha* in Mexico (Bravo-Monzón *et al.*, 2014) found that the plant distribution was fragmented into five mosaics of different sizes, and the PAR population was geographically isolated in its own mosaic from the rest of the populations. In contrast, CAT and HUI populations belong to the largest mosaic along the Gulf of Mexico. Although the isolation for PAR would explain the apparent specialization of Michoacán beetles on the chemical phenotype of their host (while losing the ability to feed on other chemical phenotypes), our results from the Veracruz beetles indicate that geographic mosaics in *M. micrantha* are not relevant for *S. punicea*.

The differential host selection could be explained by the biogeographic studies of insects in Mexico. Track analysis of different taxa has identified generalized tracks along the Pacific Coast and Gulf of Mexico that intersect in a node near the Isthmus of Tehuantepec (Morrone and Márquez, 2001). We speculate that Veracruz beetles, being closer to this node, maintained the ability to

feed on all Mexican *M. micrantha* chemical phenotypes, but this capacity was lost on individuals from populations away from the node, because PAR beetles feed only in plants from the Pacific watershed (PAR and SAG).

The lack of preference in Veracruz beetles suggests that other herbivores or pathogens may be responsible for the existing chemical differentiation in *M. micrantha* populations, which in turn would suggest an absence of coevolution between *M. micrantha* and *S. punicea* in all but one mosaics of the plant. However, both suggestions still need to be tested in bioassays that include more chemical phenotypes and focus on the insect performance.

Concerning the potential of this beetle as a biological control agent, we conclude that *S. punicea* has a significant geographic variation in host preference which could affect its effectiveness against *M. micrantha*. Although Veracruz beetles feeding was not affected by the chemical differences of the hosts, it is likely that tortoise beetles from Michoacán would not be useful as a biocontrol agent as they would probably not be able to successfully establish and cause substantial damage on certain chemical phenotypes. In this regard, further studies are still necessary to assess the potential effects of the plant chemical phenotype on *S. punicea* fitness.

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Fig. 1 Foliar terpenoid concentration of *Mikania micrantha* plants from four locations. Terpenoid identities are: 1 = α-Thujene, 2 = α-Pinene, 3 = Sabinene, 4 = β-Pinene, 5 = β-Myrcene, 6 = Limonene, 7 = β-Ocimene, 8 = δ-Elemene, 9 = α-Copaene, 10 = β-Cubebene, 11 = β-Caryophyllene, 12 = γ-Elemene, 13 = Humulene, 14 = Alloaromadendrene, 15 =Germacrene D, 16 = β-Selinene, 17 = Germacrene B, 18 = α-Farnesene, 19 = γ-Cadinene.

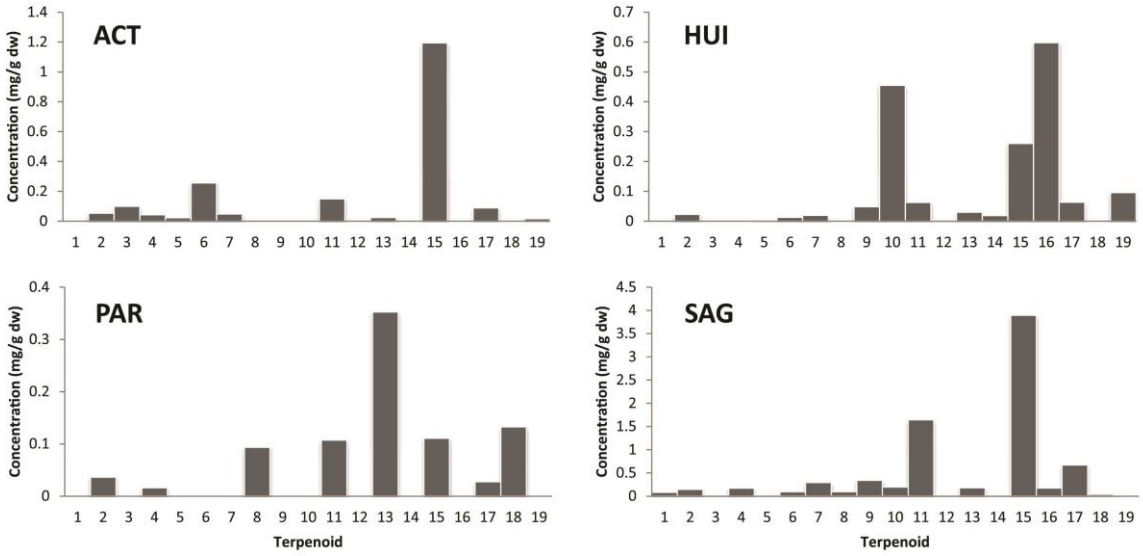
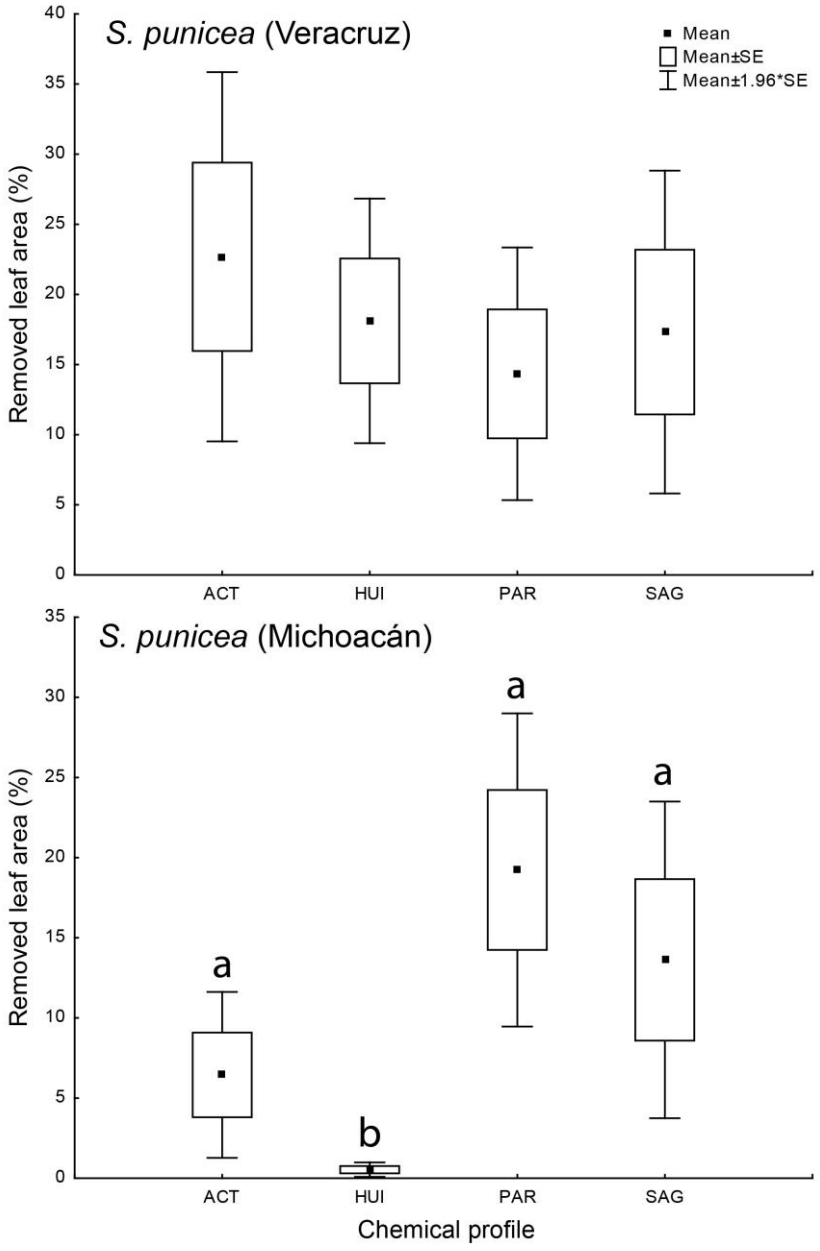


Fig. 2 Herbivory by *Stolas punicea* from two provenances on cuttings of four chemical phenotypes of *Mikania micrantha* in the four-choice tests. Different letters represent significant differences at $p<0.05$.



6

Discusión General

La capacidad de las plantas invasoras para desplazar especies nativas, cambiar la estructura y el funcionamiento de los ecosistemas las convierte en una amenaza para la biodiversidad (Vilà *et al.* 2011). El control de las invasiones es un asunto trascendental que requiere de estudios sobre las características que les permiten adaptarse a los nuevos sitios y sobrevivir al ataque de sus enemigos naturales. En este trabajo analizamos los patrones geográficos de la diversidad química y la diversidad genética en las poblaciones nativas de la maleza invasora *Mikania micrantha*, y exploramos la relevancia de estas características para los herbívoros.

En el estudio de la diversidad química pusimos a prueba las predicciones de la hipótesis centro-margen (Eckert *et al.* 2008), según la cual, tanto la concentración y diversidad de terpenoides como la intensidad del ataque de herbívoros estarían correlacionadas con los gradientes de las variables geográficas y climáticas; sin embargo, no encontramos tal correlación. Bajo esta misma hipótesis, la concentración y la diversidad de los terpenoides estarían correlacionadas con la distancia geográfica: las poblaciones lejanas serían más diferentes, pero tampoco fue el caso. En su lugar, encontramos diferencias significativas en la diversidad química entre las poblaciones del Atlántico y el Pacífico.

El hallazgo más significativo fue descubrir una relación inversa entre el daño total por herbívoros y la diversidad química poblacional, tal como lo propone la hipótesis de “más diversidad-más protección” y para la cual otros estudios han aportado evidencia contradictoria (Rincón-Hernandez y Espinosa-García 2008).

La distribución geográfica de la diversidad química de *M. micrantha* es afectada por las barreras naturales que crean la estructura de las zonas y regiones, tal como se esperaría bajo el modelo de mosaico. Evidencia de esto son los pocos tipos composicionales compartidos entre poblaciones y las cinco áreas

establecidas por el algoritmo de Monmonier, que coinciden con las barreras geográficas en México.

Los resultados obtenidos sugieren que el modelo centro-margen no es adecuado para explicar la distribución geográfica de la diversidad química de *M. micrantha*. Sin embargo, nuestros hallazgos son compatibles con el modelo del mosaico geográfico, en el cual la distribución de una especie consiste en una colección de poblaciones con grados distintos de aislamiento caracterizados por su interacción con otras especies (v.g. herbívoros, patógenos, polinizadores).

Aunque la relación entre diversidad química ha sido explorada en experimentos de laboratorio (Feng y Isman 1995) y en jardines experimentales (Poelman *et al.* 2009; Kleine y Müller 2011), nuestro estudio constituye el primer reporte de una relación inversa entre la diversidad química y la herbivoría en poblaciones naturales (Bravo-Monzón *et al.* 2014). Otros estudios en poblaciones naturales también han encontrado asociaciones entre la herbivoría y compuestos individuales (Thoss y Byers 2006), grupos de compuestos (denominados factores) (Keefover–Ring y Linhart 2010) y la abundancia de quimiotipos (Züst *et al.* 2012).

Se sabe que la diversidad fenotípica reduce el daño por plagas y patógenos (Tooker y Frank 2012), pero los mecanismos detrás de este efecto han sido poco explorados. La determinación de zonas geográficas con similitud química en la distribución nativa de *M. micrantha* puede ayudarnos a encontrar enemigos naturales que puedan contrarrestar los metabolitos secundarios producidos por esta especie en el rango de distribución invadido.

Otro aspecto que estudiamos en las poblaciones de *M. micrantha* fue la distribución espacial de su diversidad genética y su relación con la diversidad química y la herbivoría. El uso de microsatélites nucleares nos permitió determinar que las poblaciones mexicanas de *M. micrantha* poseen una baja diversidad genética, que contrasta con los reportes preliminares que existen para China (Hong *et al.* 2008). Lamentablemente, todavía no existen reportes de estudios más extensos sobre la genética de poblaciones en la región invadida que nos permitan poner en perspectiva nuestros resultados.

Entre las poblaciones del Atlántico y Pacífico encontramos diferencias significativas; las poblaciones del Atlántico tienen una mayor diversidad genética y también mayor estructura. Tanto el análisis de cúmulos, el análisis Bayesiano y el coordenadas principales coincidieron en la forma en que clasificaron a las poblaciones de acuerdo a su origen. Las poblaciones en el Atlántico también mostraron una mayor diferenciación entre ellas y dicha diferencia resultó estar estructurada geográficamente de acuerdo al modelo de aislamiento por distancia, es decir, las poblaciones más diferentes son las más distantes geográficamente.

Contrario a lo que esperábamos, no encontramos una relación significativa entre la distancia genética y la composición química de las poblaciones. Esta ausencia de relación entre la química y la genética se ha reportado anteriormente para algunas plantas con interés económico, pero solamente para una planta invasora, *Tanacetum vulgare* (Wolf *et al.* 2012).

Posiblemente esto sea una consecuencia de utilizar marcadores moleculares neutrales (i.e. microsatélites), como sustitutos de los caracteres genéticos cuantitativos. En conclusión, aunque los microsatélites representan una herramienta molecular confiable para analizar la variación genética entre y dentro de las poblaciones, no debe considerársele un remplazo de los caracteres implicados en la adaptación y sujetos a selección como los metabolitos secundarios.

Por último, exploramos la preferencia de dos poblaciones de un insecto especialista (*Stolas punicea)* sobre fenotipos químicos de *M. micrantha*. Los bioensayos nos permitieron detectar variación geográfica en la preferencia del hospedero. Los escarabajos de Veracruz se alimentaron por igual de todos los fenotipos químicos ofrecidos, mientras que los de Michoacán prefirieron a su hospedero original y evitaron alimentarse del fenotipo HUI (Tabasco). Se especula que el aislamiento del mosaico al que pertenece la población PAR (Michoacán) es la causa de la aparente especialización de los escarabajos de Michoacán en el fenotipo químico de su hospedero. Sin embargo, los resultados obtenidos de los escarabajos de Veracruz indican que los mosaicos geográficos en *M. micrantha* no representan una barrera relevante para *S. punicea*. La ausencia de preferencia en

los escarabajos de Veracruz sugiere que otros herbívoros son los responsables de la diferenciación química en las poblaciones de *M. micrantha*, lo cual a su vez indicaría que hay una ausencia de coevolución entre *M. micrantha* y *S. punicea*. Sin embargo, ambos supuestos tendrían que ser puestos a prueba con bioensayos que incluyan más fenotipos químicos y se enfoquen en el desempeño de los escarabajos.

Respecto al potencial de este insecto como agente de control biológico, podemos concluir que las poblaciones *S. punicea* varían significativamente en su preferencia de hospederos, lo cual pudiera afectar su efectividad contra *M. micrantha*. Si bien los escarabajos de Veracruz no fueron afectados por las diferencias químicas de los hospederos, es posible que los escarabajos de Michoacán no resulten útiles como agentes de control, ya que probablemente no serán capaces de establecerse exitosamente y provocar daño substancial en ciertos fenotipos químicos. En este respecto, se requieren estudios que evalúen el efecto potencial del fenotipo químico sobre el crecimiento larvario y el desarrollo de *S. punicea.*

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