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Spatial structure of genetic and chemical variation in native populations of the mile-a-minute weed *Mikania micrantha*

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ABSTRACT

We examined the spatial distribution and potential relationship of genetic and volatile terpenoid diversity in 13 Mexican populations of *Mikania micrantha* (Asteraceae) from the Atlantic and Pacific watersheds using six specific microsatellites. A low genetic diversity was observed in all populations ($H_E = 0.00–0.37$), which may be attributed to clonal reproduction and/or their marginal location relative to the whole species distribution in the Americas. We found a significant genetic differentiation between regions, and more genetic structure in Atlantic populations where a Mantel test also showed a pattern of isolation by distance ($r^2 = 0.478$, $P = 0.002$). In addition, we detected three genetic barriers that match geographic barriers and may be responsible for population isolation. The geographic patterns of genetic diversity were compared to those from chemical diversity but we found no correlation between the genetic and chemical distances of the populations. Our results suggest that neutral molecular markers and adaptive traits like defensive metabolites provide complementary information that may prove useful during the selection of biocontrol agents for invasive plant species.

1. Introduction

The genetic diversity of invasive plant species is recognized as one of the major drivers of their colonization success (Sakai et al., 2001). It is currently assumed that the amount, type, and organization of genetic diversity will define the populations' capacity to adapt to unfavorable conditions in the new environment (Barrett, 1992; Jones and Gomulkiewicz, 2012). In this context, genetic variation studies may help identify the evolutionary processes that cause a species to become invasive (Allendorf and Lundquist, 2003).

Furthermore, the spatial distribution of genetic diversity within and among populations can be affected by ecological factors and physical barriers that limit plant reproduction and dispersal (Loveless and Hamrick, 1984; Ward, 2006). Selection, gene flow, genetic drift and breeding system are the factors considered responsible for the distribution of genetic variation in plant populations (Ward et al., 2008). Genetic structure studies of invasive plants at large spatial scales can help identify source populations, as well as the possible introduction routes (Rijal et al., 2015; Ward, 2006).

Genetic variation can be measured using neutral or adaptive markers, and although most studies frequently employ a variety of neutral markers (AFLP, ISSR, and RAPD), the geographic structure of plant populations has also been described with chemical markers such as fatty acids (Martínez-Díaz et al., 2017; Ovando-Medina et al., 2011), terpenoids (Vieira et al., 2001) and various secondary metabolites (Martínez-Díaz et al., 2015). If the same limiting factors of dispersal and gene flow affect the spatial structure of genetic and chemical variation, a spatial correlation of these two features may occur. Such relationship has been detected in studies of commercially important species comparing neutral genetic markers and volatile terpenoids in individual plant species (Keskitalo et al., 2001; Skoula et al., 1999), plant populations (Fracaro et al., 2005; Nan et al., 2003), and cultivars (Böszörményi et al., 2009).

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However, in those studies, the assessment of the geographic structure of the chemical and genetic variation is limited or nonexistent (although see Külheim et al., 2011; for an exception). Understanding the spatial structure of genetic diversity in the native range of an invasive plant can help us identify distinct geographic regions to search for effective biological control agents adapted to particular genotypes (Gaskin et al., 2011).

In the present study, we were interested in examining the potential relationship between the neutral genetic diversity and chemical diversity in Mexican populations of *Mikania micrantha* (Asteraceae), a tropical American weed that has become a serious invasive in Southeast Asia. This species propagates by vegetative reproduction or seeds that are dispersed by wind, water, and animals (Tripathi et al., 2012); flowers are self-incompatible and insect-pollinated (Hong et al., 2007). Its native distribution includes all wet Neotropical areas from central Mexico throughout northern Argentina (Holmes, 1990). In Mexico, *M. micrantha* can be found in the tropical watersheds near the Atlantic and Pacific coasts, where it forms patchy populations of few individuals (Bravo-Monzón, pers. obs.). Volatile terpenes (Bravo-Monzón et al., 2014) and sesquiterpene lactones (Ríos et al., 2014) are the major foliar secondary metabolites of this plant. Furthermore, distinctive terpenoid phenotypes determine differential feeding in *Stolas punicea*, a *M. micrantha* specialist insect (Bravo-Monzón et al., 2016). Various microsatellite markers have been developed for this species (Hong et al., 2008; Yan et al., 2011), which have been used for describing the population genetics in the invaded range (Geng et al., 2016), but not in its native range.

The Atlantic and Pacific watersheds of Mexico are separated by numerous geographic barriers of varying magnitude from the Mexican transition zone (Morrone, 2006). We postulate that geographic barriers affecting the chemical structure in populations of *M. micrantha* will also affect their genetic structure, and we, therefore, predict: 1) a high genetic differentiation between Atlantic and Pacific populations, and 2) a positive correlation between chemical and genetic distances among populations.

2. Materials and methods

2.1. Plant material

We collected mature leaves in seven populations of *M. micrantha* along the Atlantic coast of Mexico (Dec. 2007), and six populations along the Pacific coast (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. Voucher specimens were deposited in two herbaria: 1) IE-Bajío, 196627–196632, and 2) EBUM, 20403, 20404, 22684–22699.

2.2. 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 −20 °C and centrifuged at 5000 rpm for 10 min, the supernatant was discarded. Samples were added 1 mL of CTAB buffer and incubated at 60 °C for 2 h. For the extraction, we added 500 μL of chloroform:isoamyl alcohol (24:1) and centrifuged at 13,000 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 the pellet was let stand in 800 μL of washing buffer (10 mM Tris-HCl at pH 7.5, 80% ethanol) 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.

2.3. PCR conditions

We used six microsatellite loci: Mm01, Mm05, Mm07, Mm12, Mm19, and Mm31, developed for *Mikania micrantha* (Hong et al., 2008), in three 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 (QIAGEN), 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 Mm01 and Mm07, 45.8 °C for Mm05 and Mm12 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 mixed and placed at 95 °C for 5 min to denature DNA and then 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 were revised and formatted with the software 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 (Van Oosterhout et al., 2004).

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

Population		State	Region	Latitude	Longitude	Altitude (m)	Sample size
NPA	Nuevo Padilla	Tamaulipas	Atlantic	24°05′15″N	98°52′17″W	153	6
ABA	Abasolo	Tamaulipas	Atlantic	24°03′07″N	98°22′34″W	60	15
TAM	Tampico	Tamaulipas	Atlantic	22°14′25″N	97°53′26″W	5	15
TUX	Tuxpan	Veracruz	Atlantic	20°56′44″N	97°20′31″W	8	24
ACT	Actopan	Veracruz	Atlantic	19°35′31″N	96°22′55″W	44	6
PAR	Parácuaro	Michoacán	Pacific	19°08′26″N	102°13′56″W	553	14
HUI	Huimanguillo	Tabasco	Atlantic	17°47′58″N	93°23′55″W	39	5
COY	Coyuquilla Norte	Guerrero	Pacific	17°22′48″N	101°03′08″W	209	10
WJA	Welib-Já	Chiapas	Atlantic	17°22′27″N	91°47′58″W	234	13
EPA	El Paraíso	Guerrero	Pacific	17°21′05″N	100°12′43″W	1761	15
SMC	San Miguel Chimalapa	Oaxaca	Pacific	16°42′45″N	94°44′53″W	1276	9
DCA	Dos Caminos	Oaxaca	Pacific	16°22′14″N	97°48′26″W	311	13
SAG	San Agustín	Oaxaca	Pacific	15°42′01″N	96°15′43″W	49	14

2.4. Genetic variation within populations

We used the software Genetic Data Analysis (GDA ver. 1.1) (Lewis and Zaykin, 2001) to estimate the number of private alleles (P_A), proportion of polymorphic loci (P), observed (H_O) and expected (H_E) proportion of heterozygotes as well as the genetic structure parameters F_{IS}, F_{IT}, and F_{ST} for each population. Allelic richness (*Ar*) was estimated using the rarefaction method in the software HP-Rare (Kalinowski, 2005) to account for variable population sizes.

2.5. Genetic differentiation among populations

Population genetic structure was examined by a hierarchical analysis of molecular variance (AMOVA) using the GenAlEx software (ver. 6.503) (Peakall and Smouse, 2006, 2012) with three hierarchical levels: regions (Pacific and Atlantic), populations within regions, and individuals within populations. We used the Codominant-Allelic distance option with 1000 permutations.

A comparison between Atlantic and Pacific populations for various statistics including allelic richness, observed heterozygosity, expected heterozygosity, and inbreeding coefficient within individuals was performed using the permutation test (10,000 permutations) implemented in Fstat (ver. 2.9.3.2) (Goudet, 2002).

2.6. 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 (PCoA) was performed with Nei's genetic distances among populations, and also a Mantel test with 10,000 permutations was performed to detect isolation by distance using Nei's genetic distances and geographic distances (Km) with the GenAlEx software (ver. 6.503). Possible genetic barriers were explored using the Monmonier's algorithm implemented in the Barrier 2.2 software (Manni et al., 2004).

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 an admixture 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 *K* value best fitting the data was calculated estimating the maximum value of the ΔK statistic (Evanno et al., 2005) as implemented in Structure Harvester v0.6.94 (Earl and von Holdt, 2012). Results from the STRUCTURE runs were aligned using the software CLUMPP 1.1.2b (Jakobsson and Rosenberg, 2007) and visualized with DISTRUCT 1.1 (Rosenberg, 2004).

2.7. Chemical diversity

The volatile terpenoid content was analyzed from mature leaves of flowering *M. micrantha* plants. Individual leaves were macerated for 7 days in 15 mL of hexane at 4 °C with 1 mg n-tetradecane added as internal standard. The samples were ground, filtered and concentrated under a gentle stream of nitrogen to 1 mL, from which 1 μL was analyzed in an Agilent 6890 gas chromatograph coupled to a 5973N selective mass detector under conditions reported elsewhere (Bravo-Monzón et al., 2014).

A data matrix was built with the relative concentrations of the volatile terpenoids and used to generate two measures of chemical diversity for each population: 1) the population-level diversity, which represents the within-population polymorphism and was calculated as the Shannon Index of individuals with the same chemical profile, and 2) the average individual diversity, calculated as a mean of the Shannon Index for the number of compounds and relative concentration in each individual.

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

We also constructed two Euclidean distance matrices: a) a relative terpenoid concentration matrix that reflects qualitative differences among populations, using the average proportion of terpenoids and b) an absolute terpenoid concentration matrix based on the total concentration of terpenoids that reflects quantitative differences. Both matrices were computed using the software Statistica (StatSoft, ver. 8). The relation between genetic and chemical composition was tested using Mantel tests (GenAlEx 6.503) with a matrix of Nei's genetic distances and the above chemical distance matrices.

3. Results and discussion

3.1. 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 (population COY) to 2.17 (population ABA) (Table 2). The mean allelic richness estimated with the rarefaction approach (*Ar*) was also low (1.66) and ranged from 1 (population COY) to 2.05 (population ABA). All six microsatellites were polymorphic. The average percentage of polymorphic loci per population (*P*) was 65%, varying from 0 (COY) to 83% (ABA). The number of private alleles varied from zero to three per population, for a total of 10 (Table 2). Population WJA contained the highest number of private alleles (5), followed by PAR and TAM (2 each) and EPA (1).

No differences in allelic richness (*Rs*; $P = 0.146$) or inbreeding (F_{IS}; $P = 0.924$) between Atlantic and Pacific populations were detected. Likewise, differences in the average observed and expected heterozygosity between Atlantic populations ($H_O = 0.214$, $H_E = 0.242$) and Pacific populations ($H_O = 0.153$, $H_E = 0.175$) were not significant (H_O: $P = 0.35$) (H_E: $P = 0.29$) (Table 3). Similarly, a study on populations of *Rhizophora mangle* along the Atlantic and

Table 2
Genetic population parameters for populations of *Mikania micrantha*. *A* = average number of alleles per locus, *Ar* = allelic richness, P_A = number of private alleles, *P* = proportion of polymorphic loci, H_O = observed proportion of heterozygotes, H_E = expected proportion of heterozygotes, *F* = inbreeding coefficient, (±S.E). See Table 1 for definitions of population acronyms.

Population	*A*	*Ar*	P_A	*P*	H_O	H_E	*F*
NPA	1.83	1.78	0	0.667	0.222(±0.082)	0.245(±0.088)	0.101
ABA	2.17	2.05	0	0.833	0.322(±0.078)	0.371(±0.086)	0.136
TAM	2.17	1.94	2	0.833	0.311(±0.116)	0.290(±0.113)	−0.074
TUX	2.17	1.59	0	0.833	0.125(±0.046)	0.158(±0.072)	0.214
ACT	1.83	1.78	0	0.667	0.306(±0.145)	0.295(±0.102)	−0.038
PAR	1.83	1.79	2	0.667	0.298(±0.105)	0.278(±0.103)	−0.073
HUI	1.67	1.67	0	0.500	0.233(±0.120)	0.226(±0.107)	−0.037
COY	1.00	1.00	0	0.000	0.000(±0.000)	0.000(±0.000)	0.000
WJA	2.00	1.60	5	0.833	0.090(±0.037)	0.168(±0.059)	0.475
EPA	2.16	2.02	1	0.833	0.256(±0.053)	0.300(±0.077)	0.152
SMC	1.33	1.23	0	0.333	0.019(±0.019)	0.053(±0.034)	0.667
DCA	1.67	1.36	0	0.667	0.064(±0.037)	0.086(±0.033)	0.260
SAG	2.00	1.74	0	0.833	0.179(±0.055)	0.217(±0.083)	0.184
Mean	1.83	1.66	0.77	0.654	0.186(±0.024)	0.207(±0.023)	0.105

Table 3
Comparison between Atlantic and Pacific populations of *Mikania micrantha*. R_S = allelic richness; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = inbreeding coefficient within individuals; F_{ST} = differentiation between populations.

Region	R_S	H_O	H_E	F_{IS}	F_{ST}
Atlantic	1.773	0.214	0.242	0.115	0.492**
Pacific	1.523	0.153	0.175	0.126	0.112**
	0.146	0.350	0.288	0.924	0.0003

**$P < 0.01$.

Pacific coasts of Mexico found no significant difference in three measures of genetic diversity, which was attributed to an extensive early dispersal before closure of the Central American Isthmus about 3.5 million years ago (Sandoval-Castro et al., 2014). 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. However, Micro-Checker only detected the presence of null alleles at locus Mm31 in all populations.

Our results show low levels of allelic richness and genetic diversity for 13 Mexican populations of *M. micrantha*, especially when compared to the reports for these same microsatellites in introduced populations of China (Geng et al., 2016; Hong et al., 2008). One possible explanation for this finding is that low genetic diversity in the native range is the product of predominant asexual (vegetative) reproduction; while the invasive populations are the result of multiple seed introductions. However, a study to confirm such assumption is still necessary.

Another explanation is that Mexico represents the northernmost limit of the natural distribution of *M. micrantha* (Holmes, 1990) and, according to the central-marginal model (Eckert et al., 2008), these geographically marginal populations are expected to exhibit low genetic variation, whereas populations of Central and South America should have higher genetic variation. This pattern of geographical variation in population genetic diversity is what we would expect if Mexican populations were the product of post-glacial colonization. In this scenario, populations from southern refugia would have expanded into the available northern territory by consecutive colonization events that would lead to successive founder effects and loss of genetic diversity (Hewitt, 2000). If this were the case for *M. micrantha*, the invasive populations in Asia would not likely be of Mexican origin.

3.2. Population genetic differentiation

We found a high genetic differentiation among all populations ($F_{ST} = 0.5$). The comparison between coasts showed significantly higher differentiation values in the Atlantic ($F_{ST} = 0.492$) than in the Pacific populations ($F_{ST} = 0.112$; $P = 0.0003$) which is likely a product of geographic barriers affecting the pollen and seed dispersal, thus producing genetic drift (Table 3). Despite that *M. micrantha* populations are composed of relatively few individuals, the observed mean inbreeding coefficient for all populations was low ($F_{IS} = 0.105$), a finding that is consistent with a self-incompatible species.

The AMOVA showed that a large proportion of the genetic variation occurred within populations (43%, $P < 0.001$) (Table 4). However, a substantial proportion of the variation (29%, $P < 0.001$) was found among regions, and also among populations within regions (28%, $P < 0.001$), hence confirming the presence of high genetic differentiation in *M. micrantha* at all levels.

3.3. Relationships among populations

The Atlantic and Pacific regions also vary in their genetic structure. 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)]. The cluster analysis performed with the UPGMA algorithm generated a dendrogram that divided the populations into three genetic groups: Group I contained all six Pacific populations; Group II contained six Atlantic populations while Group III only contained the WJA population from the Atlantic (Fig. 1). Although with low bootstrap support (<50%), groups I and II are quite consistent with the geographical origin of the populations.

In the PCoA, coordinate 1 explained 72.64% of the variation and coordinate 2 explained 16.56%. Both coordinates contributed to successfully separate Atlantic from Pacific populations (Fig. 2). The two northern (NPA, ABA) and the southernmost (WJA) populations from the Atlantic were further separated, while Pacific populations were more homogeneous and clustered together, thus revealing a higher genetic differentiation among the Atlantic populations.

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 from Chiapas. Some discrepancy among the results of these analyses is to be expected as they proceed in different ways. Both the UPGMA and PCoA are distance-based methods that calculate the distance between every pair of populations using a pairwise distance matrix, but the STRUCTURE analysis is a model-based method that attempts to identify the actual number of populations and probabilistically assigns the individuals to these populations (Pritchard et al., 2000).

The Mantel test found a significant correlation between geographic distance and genetic distance for Atlantic populations ($r = 0.692$; $P = 0.002$) supporting the isolation-by-distance model, but not for Pacific populations ($r = 0.023$; $P = 0.447$). The strong genetic differentiation between Atlantic and Pacific populations combined with their large geographic distance suggests they might be the product of two distinct founding events. Moreover, the pattern found in Atlantic populations is consistent with a single founding event followed by reproductive isolation among the populations of that region (Ward, 2006). Geographical barriers may also be responsible for the higher genetic differentiation of Atlantic populations. We detected three genetic barriers in the distribution

Table 4
Analysis of molecular variance (AMOVA) for six microsatellite loci in 13 *Mikania micrantha* populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	*P*
Among regions	1	78.999	0.423	29	<0.001
Among populations within regions	11	112.633	0.404	28	<0.001
Within populations	305	191.778	0.629	43	<0.001
Total	317	383.409	1.456		

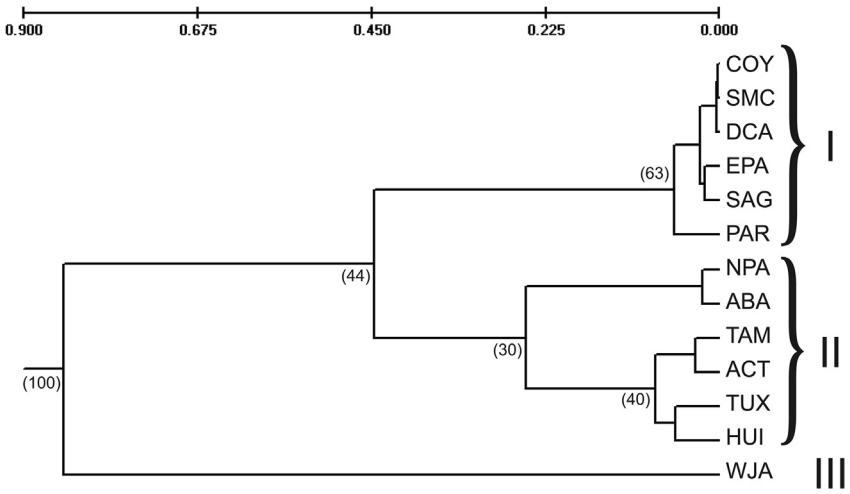

Fig. 1. UPGMA dendrogram based on Nei's (1972) genetic distances for populations of *Mikania micrantha*. Bootstrap values (1000 permutations) are indicated as a percentage at nodes. See Table 1 for definitions of population acronyms.

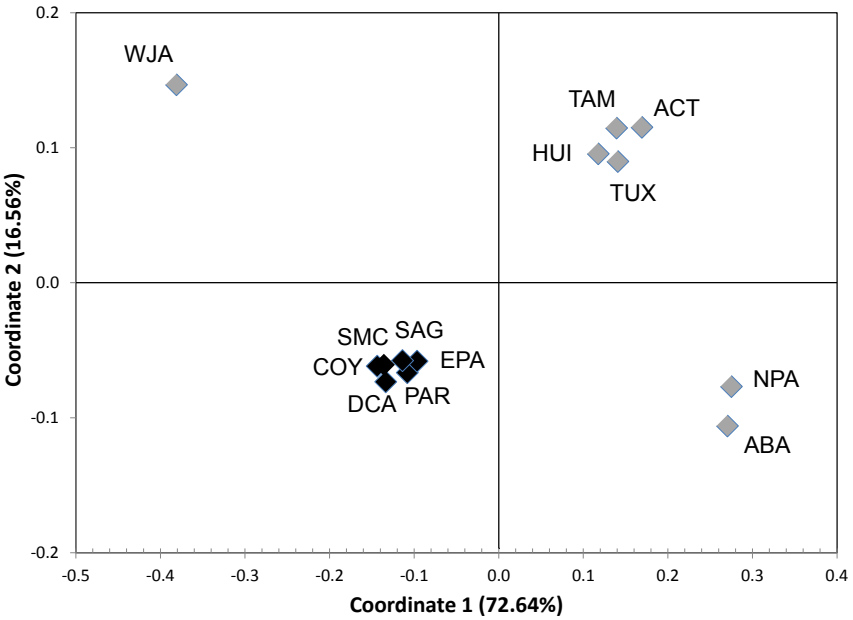

Fig. 2. Principal Coordinates Analysis for 13 populations of *Mikania micrantha*. Distribution of Atlantic (*gray*) and Pacific (*black*) in space is based on Coordinates 1 and 2. See Table 1 for population acronyms.

of *M. micrantha* using Monmonier's algorithm (Fig. 4). The barrier *a* that isolates the WJA population coincides with the Isthmus of Tehuantepec, a biogeographical barrier that reduces gene flow between east and west populations of various taxa (Gutiérrez-Rodríguez et al., 2011; Ortíz-Medrano et al., 2008; Peterson et al., 1999); the barrier *b* that separates the Pacific and Atlantic populations corresponds to several mountain chains known as the Mexican Transition Zone, a complex area that effectively isolates the two provinces: Mexican Gulf and Mexican Pacific Coast (Morrone, 2006, 2010); and the barrier *c* that isolates the northernmost Atlantic populations ABA and NPA from the other Atlantic populations, matches the small mountain range Sierra de Tamaulipas which is believed to affect the geographic distribution of two rodent species (Bradley et al., 2008).

The remarkable differentiation detected for population WJA from Chiapas with both the UPGMA and the PCoA analyses, in addition to having the principal genetic barrier, underscore its geographical isolation and hint at the possibility of there being a third group of populations distinct from those at the Atlantic and Pacific regions. However, more data from other populations in the zone is still needed to confirm such an assumption.

3.4. Genetic diversity and chemistry

A total of 25 terpenoids were identified from *M. micrantha* leaves (Table 5) and used to calculate the diversity indices of the populations (Table 6). Mantel tests between genetic and chemical composition matrices revealed no relationship between the Nei's genetic distance of the populations and their relative terpenoid concentration ($r = -0.111$, $P = 0.339$) or their total terpenoid

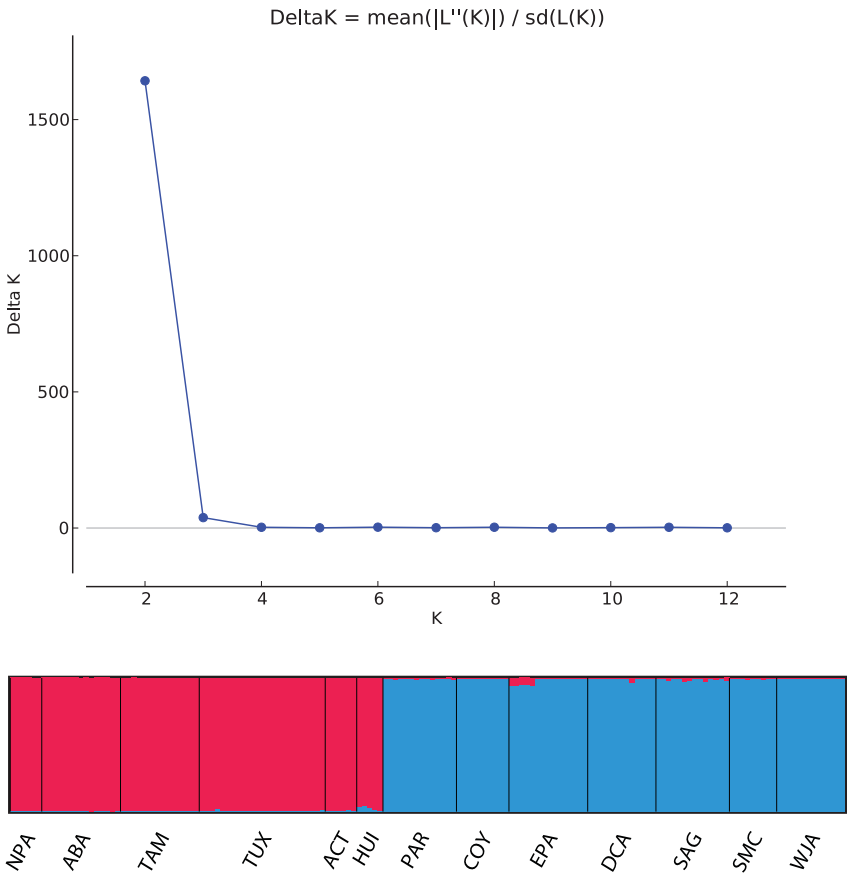

Fig. 3. Magnitude of ΔK values for each estimated number of populations (K) from the STRUCTURE analysis. The bar plot produced by DISTRUCT depicts population clustering for $K = 2$.

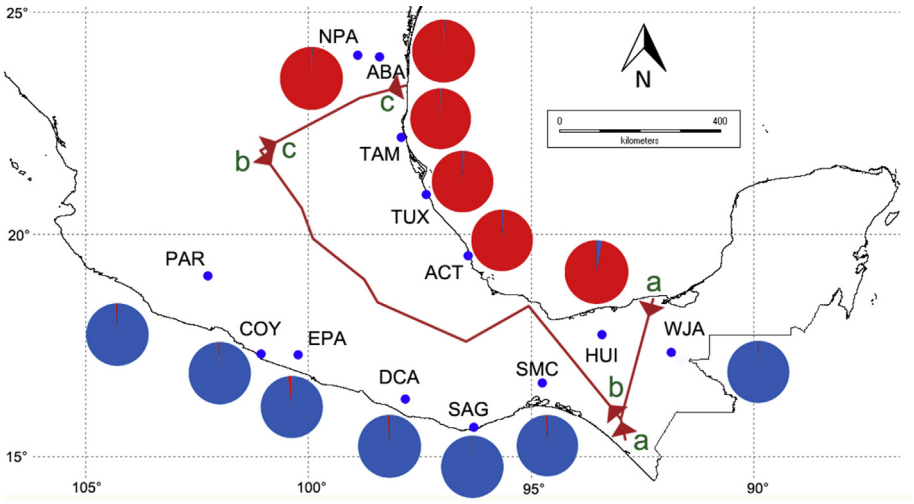

Fig. 4. Genetic barriers among populations of *M. micrantha* detected using Monmonier's algorithm and proportion in each population of the two genetic groups detected by Bayesian analysis. See Table 1 for definitions of population acronyms.

concentration ($r = 0.001$, $P = 0.605$). The multiple regression analysis also found no correlations between the chemical diversity or the terpenoid concentration or the geographic or environmental variables and allele richness ($F_{8,4} = 0.605$, $P = 0.747$, adjusted $R^2 = -0.357$).

The spatial structure of chemical variation, which comprises four barriers isolating populations of *M. micrantha* into five mosaics, coincided with the genetic structure only at the major geographic barrier separating the Atlantic and Pacific coasts (Bravo-Monzón et al., 2014), and no significant relationship between the genetic distance and chemical composition of *M. micrantha* populations was detected. The lack of association between foliar terpenoids and genetic data has been reported previously in studies with aromatic plants such as common basil (*Ocimum basilicum*)

Table 5
Identified volatile terpenoids from *Mikania micrantha* leaves.

Peak	Kovats index	Name	Concentration variation interval (%)	Average relative concentration (%)
1	952	α-Pinene	0.9–37.0	7.2
2	966	Camphene	0.0–4.2	0.7
3	990	Sabinene	0.0–9.6	2.3
4	994	β-Pinene	0.0–8.9	2.8
5	1006	β-Myrcene	0.1–3.3	1.2
6	1020	α-Phellandrene	0.2–9.9	1.9
7	1025	3-Carene	0.0–4.4	0.8
8	1031	α-Terpinene	0.0–0.8	0.1
9	1039	p-Cymene	0.1–9.5	1.7
10	1043	Limonene	0.3–21.5	7.4
11	1061	(E)-β-Ocimene	0.0–7.7	1.9
12	1342	δ-Elemene	0.0–9.0	1.1
13	1379	α-Copaene	0.0–12.8	1.2
14	1387	Modhephene	0.0–25.3	1.4
15	1390	β-Bourbonene	0.0–5.0	0.4
16	1395	β-Cubebene	0.0–7.7	0.5
17	1397	β-Elemene	0.0–37.1	1.7
18	1417	α-Gurjunene	0.0–6.9	0.9
19	1422	β-Caryophyllene	0.3–33.6	10.0
20	1455	α-Humulene	0.0–42.0	4.2
21	1481	Germacrene D	0.0–70.6	35.1
22	1485	β-Selinene	0.0–16.3	0.8
23	1497	Germacrene B	0.0–9.0	3.0
24	1506	α-Farnesene	0.0–12.1	0.9
25	1514	γ-Cadinene	0.0–8.9	1.8

Table 6
Chemical diversity indices and total terpenoid concentration of 13 *Mikania micrantha* populations.

Population	Population-level diversity	Average individual diversity	Terpenoid concentration[a]
NPA	0	2.24	15.94
ABA	1.36	2.28	8.96
TAM	1.49	2.39	5.84
TUX	1.30	2.12	8.73
ACT	0	1.96	5.26
PAR	1.10	2.63	5.89
HUI	1.04	2.45	3.89
COY	0.61	2.44	4.44
WJA	2.03	1.94	6.40
EPA	2.06	2.20	14.70
SMC	1.27	2.77	3.11
DCA	1.82	2.29	2.95
SAG	2.05	2.31	6.91

[a] mg g^{-1} dry weight.

(Labra et al., 2004), Turkish oregano (*Origanum onites*) (Tonk et al., 2010), *Thymus* spp (Rustaiee et al., 2013; Trindade et al., 2008) and the invasive *Tanacetum vulgare* (Wolf et al., 2012), however, no information on their spatial structure is available. These negative results can be attributed to the fact that neutral genetic markers are mainly affected by demographic events (i.e. gene flow, migration, dispersal) (Holderegger et al., 2006), while the terpenoid composition is an adaptive trait that changes in response to environmental variables and ecological processes. Herein lies the problem with the common practice of using neutral molecular markers as substitutes for measuring quantitative trait variation, when the use of quantitative trait loci (QTLs) would be a more suitable approach (Külheim et al., 2011). A meta-analysis conducted to test the relationship between molecular and quantitative variation revealed only a weak correlation (Reed and Frankham, 2001). Our own results with *M. micrantha* do not support the prediction that genetic and chemical compositions are associated. Therefore, despite microsatellites being a reliable molecular tool for analyzing the neutral genetic variation within and among populations, we would advise against using them as a surrogate for adaptive traits like secondary metabolites.

4. Conclusions

It has been suggested that the analysis of genetic population structure of invasive weeds in the native range may be helpful in biological control by identifying geographic regions containing genotypes similar to the invasive populations, and focusing efforts on finding locally adapted natural enemies. For example, Australian populations of the mite *Floracarus perrepae* with promising potential to control the exotic fern *Lygodium microphillum* in Florida, USA, were detected using this approach (Goolsby et al., 2006). Our results indicate that the genetic and chemical structures of populations provide different and complementary information. Hence, we recommend the inclusion of both approaches in future searches for natural enemies with potential as biocontrol agents of invasive plants.

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