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Cite this article: Ji M, Ze S, Tang S, Hu L, Li J (2021) Independent origins of populations from Dehong State, Yunnan Province, and the multiple introductions and post-introduction admixture sources of mile-a-minute (*Mikania micrantha*) in China. Weed Sci. **69**: 439–445. doi: 10.1017/wsc.2021.29

Received: 31 December 2020
Revised: 25 February 2021
Accepted: 26 March 2021
First published online: 19 April 2021

Associate Editor:
Mithila Jugulam, Kansas State University

Keywords:
Differentiation; gene flow; genetic diversity; genetic structure

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Independent origins of populations from Dehong State, Yunnan Province, and the multiple introductions and post-introduction admixture sources of mile-a-minute (*Mikania micrantha*) in China

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Abstract

Mile-a-minute (*Mikania micrantha* Kunth) is a tropical American species and has become a worldwide invasive weed. It was first introduced to mainland China in 1983 in Yingjiang City, Dehong State, Yunnan Province. To assess the origins of populations from Dehong State, Yunnan Province, the genetic structure of 427 individuals from 11 *M. micrantha* populations from Yunnan, Guangxi, Hainan, and Guangdong provinces were analyzed. A total of 28 alleles were detected in 12 nuclear microsatellite loci. Genetic diversity at the population level was relatively high. An analysis of molecular variance showed that most of the variation occurred within populations (82.73%), and only 18.27% occurred among populations. The genetic differentiation coefficient (F_{ST}) was 0.183. The estimated gene flow (N_m) from F_{ST} was 1.116. The independent origins of four populations collected from Dehong State, Yunnan Province, was determined by the unweighted pair-group method with arithmetic means clustering and STRUCTURE analysis. Three gene clusters and one admixture gene cluster were found. A Mantel test of pairwise Nei's genetic distances and pairwise geographic distances revealed no evidence for isolation by distance ($r = 0.068$, $P = 0.343$). These results suggest that the post-introduction admixture caused by multiple introductions and high gene flow might contribute to the evolutionary adaptation of *M. micrantha*. These results could provide a scientific basis for the management of invasive *M. micrantha*.

Introduction

Biological invasions cause significant damage to biodiversity and ecosystem function and are considered a major global challenge for the conservation of natural resources and biodiversity (Simberloff et al. 2013). The genetic diversity of invasive plant species is one of the major drivers of their colonization success (Sakai et al. 2001) and has attracted considerable attention (Dlugosch and Parker 2008; Dlugosch et al. 2015; Qiao et al. 2019; Smith et al. 2020; Vellend and Tomimatsu 2010; Ward 2006).

Invasive species are predicted to suffer from reductions in genetic diversity during founding events, reducing their adaptive potential (Dlugosch and Parker 2008). However, relevant studies could not show a consistent pattern of bottleneck severity within successful invasions (Bossdorf et al. 2005; Dlugosch et al. 2015; Lambrinos 2004), and a growing body of research proves that multiple introductions are common among populations of invasive species (Bossdorf et al. 2005; Dlugosch et al. 2015; Wang et al. 2012; for review, see Dlugosch and Parker 2008). For these multiply introduced species, no genetic bottleneck and isolation by distance can be inferred. Admixture between multiple source populations could benefit invasive populations through heterosis (e.g., Li et al. 2018) and, in the long term, by increasing genetic diversity and thus evolutionary potential, allowing adaptation to new environmental conditions (Li et al. 2019; Rius and Darling 2014; Schierenbeck and Ellstrand 2009; Wang et al. 2012). Therefore, understanding the genetic variation and structure of invasive plants would help to reveal their invasive origins, dispersal pattern, and evolutionary history (Dlugosch and Parker 2008; Li et al. 2019).

Mile-a-minute (*Mikania micrantha* Kunth) is a tropical American herbaceous species that has become a worldwide invasive weed (Bravo-Monzón et al. 2018; Zhang et al. 2004). It is a multibranched scrambling vine of the family Asteraceae and can reproduce easily through both sexual and vegetative mechanisms. The species is self-incompatible and spreads via wind and

insect pollination, producing a large number of small, light seeds (Hong et al. 2007). In the field, it can produce up to 20,535 to 50,297 seeds per plant (Zan et al. 2000). *Mikania micrantha* can also reproduce from stem fragments that root easily at the nodes and from vegetative ramets that arise from rosettes (Zhang et al. 2004). The basic biology, morphology, physiology, general distribution, and ecological niche modeling impacts on other plant communities, as well as natural enemies of *M. micrantha*, have been studied in detail (Banerjee et al. 2019; Zhang et al. 2004).

Various microsatellite markers have been developed for this species (Hong et al. 2010; Yan et al. 2011), and its genetic diversity, structure, and differentiation have been investigated. For example, Wang et al. (2008) tested the genetic structure of 28 *M. micrantha* populations collected in Hong Kong, Macao, and Guangdong Province in their introduced range using inter simple sequence repeat (ISSR) markers and found that during *M. micrantha* invasion, multiple introductions mitigated the loss of genetic variation associated with bottlenecks. Using microsatellite markers, we analyzed 787 samples collected in Guangdong Province, and the population genetics results suggested the importance of highways as corridors for the spread of *M. micrantha* in southern China (Geng et al. 2016). However, the sample collection localities were limited to Guangdong Province and nearby Hong Kong and Macao (Wang et al. 2012, 2016). Despite these few published genetic studies on *M. micrantha* in China, large-scale studies on the genetic background of *M. micrantha* in China are still lacking.

In Asia, the earliest record of *M. micrantha* can be dated to 1884, when it was a cultivated plant in Hong Kong (Wang et al. 2003). In mainland China, most literature advocated that the oldest record of the species is from 1984 in Yinhu, Shenzhen, Guangdong Province, which is adjacent to Hong Kong (Kong et al. 2000; Wang et al. 2003). However, Du et al. (2006) found that the first invasion of *M. micrantha* in mainland China was recorded in 1983, based on a collection by Qing Lin in Yingjiang City, Yunnan Province (specimen number 7708052, 1983-10-26). A survey among the older residents of Yunnan Province showed that the emergence of *M. micrantha* in Ruili City, Delong State, Yunnan Province, dates back to the early 1960s (Du et al. 2006). After that, *M. micrantha* was introduced into Dehong State, Yunnan Province (Du et al. 2006). Therefore, we hypothesized that the populations from Delong State, Yunnan Province, originated and developed independently. However, the answer remains unclear.

Multiple introductions are inferred among populations of *M. micrantha*, even though chloroplast simple sequence repeat (cpSSR) marker results indicated the populations were distributed only in Guangdong Province (Wang et al. 2008), while amplified fragment length polymorphism (AFLP) marker data indicated populations were distributed in Guangdong Province, Macao, and Hong Kong (Wang et al. 2012). Geng et al. (2016) found that the genetic admixtures among the roadside populations imply the occurrence of multiple population introductions during colonization, indicating that the post-introduction admixture happened during the evolution of *M. micrantha* populations. After its introduction in Guangdong Province, *M. micrantha* was introduced into Hainan and Guangxi provinces in 2003 and 2008, respectively (Wei et al. 2014; Yu and Wu 2009), and the known geographic distribution of *M. micrantha* covered Guangdong, Guangxi, Yunnan, and Hainan provinces. In this study, we sampled four populations of *M. micrantha* in Dehong State, Yunnan Province, and seven populations in Guangxi, Hainan, and Guangdong provinces. We aimed to determine the multiple introduced populations using microsatellite markers and evaluate the level of genetic admixture among *M. micrantha* populations covering Yunnan, Guangxi, Guangdong, and Hainan provinces. Our results will provide a scientific basis for the management of invasive *M. micrantha*.

Materials and Methods

Sample Collection

We collected 427 samples from 11 *M. micrantha* populations in Yunnan, Guangdong, Guangxi, and Hainan provinces (Figure 1). Basic information and the numbers of collected samples are listed in Supplementary Table S1. Fresh leaf tissue was collected and processed with silica gel as previously described (Geng et al. 2016).

DNA Extraction and Microsatellite Analysis

Total genomic DNA was extracted from the dried tissue following the manufacturer's instructions for the plant DNA extraction kit (Tsingke Biotech, Beijing, China). The quality of DNA was determined by 0.8% agarose gel electrophoresis, and the DNA was diluted to 10 ng/μl for microsatellite analysis.

Microsatellite markers were developed from the transcriptomic data of *M. micrantha* by Beijing Qingke Biotechnology Kunming Branch Company (Kunming City, Yunnan Province, China). The polymerase chain reaction (PCR) was performed in a final volume of 15 μl, containing 50 ng of genomic DNA, 1× Tsingke Master Mix (green), and 0.67 μM of each primer. The PCR reactions were performed in an ABI 2720 thermocycler (Applied Biosystems Life Technologies, Carlsbad, CA, USA) with the following cycling conditions: after 5 min at 94 C, 30 cycles were carried out for 30 s at 94 C, 30 s at 60 C, and 30 s at 72 C, with a final extension of 5 min at 72 C. Amplification products were sequenced using an ABI 3730xl DNA sequencer (Applied Biosystems Life Technologies). The band size was calculated using the software Gene Mapper 4.1 after comparison with an ABI GeneScan™ 500 LIZ™ Size Standard (Applied Biosystems Life Technologies). Twelve highly polymorphic nuclear microsatellite loci were chosen for further analysis based on the following criteria: (1) consistent amplification products, (2) reproducible fragments between replicate PCRs, and (3) no amplification in the negative control (Li and Jin 2007).

Genetic Diversity Analysis

Based on the microsatellite loci, the number of different alleles (N_a), the number of effective alleles (N_e), Shannon's information index (I), and the coefficient (F_{IS}) were estimated using GenAlEx 6.501 (Peakall and Smouse 2012). The polymorphic information content (PIC) was calculated using the Microsatellite Toolkit v. 3.1.1 (Park 2001). Expected heterozygosity (H_e) and observed heterozygosity (H_o) were estimated using POPGENE 1.32 (Yeh and Boyle 1997).

Population Genetic Structure Based on Microsatellite Loci

Nei's (1972) genetic distances among populations were calculated using GenAlEx 6.501 (Peakall and Smouse 2012). Bootstrapping of the unweighted pair-group method with arithmetic means (UPGMA) tree among populations was performed using PowerMarker v. 3.25 with 1,000 iterations. The bootstrap value was calculated using Phylip 3.68 and MEGA 5.10. The tree was constructed via FigTree v. 1.4.2.

The genetic structure of *M. micrantha* populations was assessed using Bayesian model–based clustering analysis implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000). The initial range of potential genetic clusters (K) was set from 2 to 20, with 20

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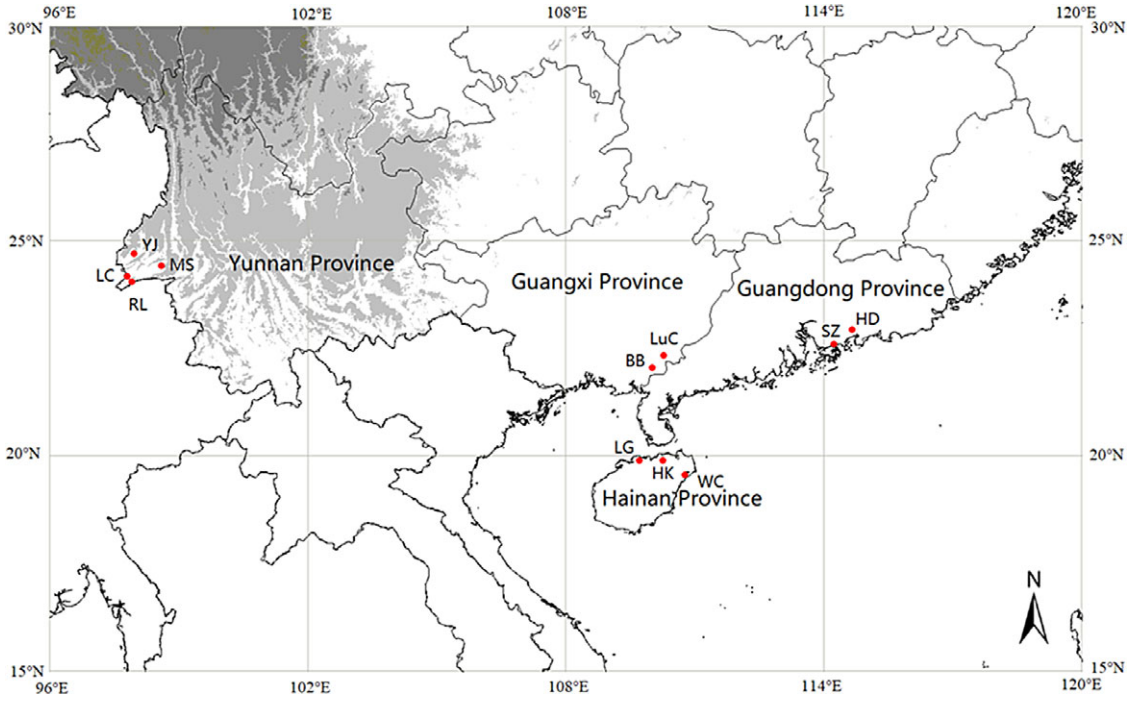

Figure 1. Geographic distribution of the 11 populations of *Mikania micrantha* sampled in this study. The abbreviations for populations are listed in Supplementary Table S1.

independent runs under the admixture model with 200,000 Markov chain Monte Carlo iterations and a 100,000 burn-in period. The most probable number of clusters (K) was selected by calculating an ad hoc statistic ΔK based on comparing the log probability of the data (LnP(D)) for each K value, as described by Evanno et al. (2005), and was implemented in the website program Structure Harvester (Earl and von Holdt 2012; http://taylor0.biology.ucla.edu/structureHarvester). The highest ΔK value was selected to determine the number of clusters.

An analysis of molecular variance (AMOVA) was conducted using GenAlEx software, and variations between and among populations, as well as the population differentiation coefficient (F_{ST}), were calculated. Gene flow (N_m) was calculated as $0.25(1 - F_{ST})/F_{ST}$, as described by Wright (1931).

Geographic distances among populations were calculated by Vincenty's formula (http://www.movable-type.co.uk/scripts/latlong-vincenty.html). Mantel tests between Nei's genetic distance and geographic distance were conducted using the GenAlEx software to estimate the effect of geographic distance on the genetic structure of the populations.

Results and Discussion

Genetic Diversity and Variation

Among the 427 individuals, 28 alleles were detected in 12 nuclear microsatellite loci (Table 1). Among the 11 populations, the polymorphic loci percentage was high and varied from 91.67% to 100%, with an average of 94.70% (Table 2). The N_e varied from 1.5287 (WC population) to 1.8693 (BB population), with an average of 1.6922; I varied from 0.4506 (WC population) to 0.6826 (BB population), with an average of 0.5549; PIC varied from 0.2359 (WC population) to 0.3579 (BB population), with an average of 0.2925; while H_o varied from 0.2935 (YJ population) to 0.4136 (BB population), with an average of 0.3314; and H_e varied from 0.2855 (WC population) to 0.4313 (BB population), with an average of 0.3541 (Table 2). The F_{IS} values varied from −0.012 to 0.227, with an average of 0.0971 (Table 2). The heterozygosity of nuclear markers is the most common metric of diversity used to estimate the intraspecific genetic diversity in plants (Dlugosch and Parker 2008). Consistent with prior studies on the genetic diversity of *M. micrantha* at the population level (Geng et al. 2016), a relatively higher level of genetic diversity was found at the population levels in this study. For example, the mean H_e was 0.3541 at the population level versus a mean H_e of 0.477 at the population level reported in a previous study by Geng et al. (2016). The genetic diversity might be primarily owing to the reproductive traits of *M. micrantha*. *Mikania micrantha* is a self-incompatible plant (Hong et al. 2007), and strong sexual reproduction helps it maintain a relatively high level of genetic diversity (Geng et al. 2016). The species produces enormous numbers of small and light seeds (1.7×10^5 m^{-2}), which are dispersed via wind (Zhang et al. 2004), although it can also reproduce vegetatively by producing shoots from stem fragments and rosettes (Zhang et al. 2004), which could reduce the genetic diversity within populations (Wang et al. 2016). Low mean rates of clonal reproduction are seen with populations sampled at intervals of at least 10 m apart (Geng et al. 2016).

Genetic Differentiation and Gene Flow

The results of the AMOVA showed that most of the variation occurred within populations (82.73%), while only 18.27% occurred among populations (Table 3). The genetic differentiation coefficient (F_{ST}) was 0.183 (Table 3), indicating a relatively low level of genetic differentiation among populations. Similar results have been reported by Geng et al. (2016), whose AMOVA results showed that 91% of the genetic variation resided within populations and 9% among populations. The self-incompatible reproduction mechanism of *M. micrantha* can effectively avoid population

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Table 1. Sequences of the 12 microsatellite primers successfully used in this study.[a]

Primer	Forward sequence (5′→3′)	Reverse sequence (5′→3′)	T_m	Product size
				—bp—
PWGJ-1	TCCTTGCCAATCGTCGAACA	GGTGCCAAGCTAACAACTCG	60	200–202
PWGJ-2	GACTCATCTGAGCTCCACCG	CACCACAATCGACGACCTGA	60	237–245
PWGJ-3	GCTTGCAATTCTAGCCGATGG	GTCTCCAAGTGATAGCCGCA	60	273–291
PWGJ-4	TCTTGGCACCGGTGTTCAAT	ACATCACTAGTTACTTGCAGCA	60	216–219
PWGJ-6	GGTGTTGGTGGAGGTAGTGG	AGCAATAGTGGCAGTGGACC	60	168–171
PWGJ-7	GGCACAGACAAGCTCTCGAT	TGAAGCTGCTGCAGGAATCA	60	188–200
PWGJ-8	GCTCATCACTGTTCCGGTCA	CCGCACTCCTCCTTACCATC	60	250–259
PWGJ-9	GGAACGAGTGAAGCTACCGA	AGCCATGCCAGATAAGCTCC	59.5	168–171
PWGJ-10	CACTACACACCACGACACGA	GATGCAGCGATCGCATCATG	60	217–224
PWGJ-12	ACAATCCACTCACTGGCACT	AGCCAGCACCATGAACATCA	59	180–182
PWGJ-13	GGACCTAGCAGTACCAAGGC	AGGCTCTCCACCACATTGTG	60	235–241
PWGJ-14	GAGCTTCGATGACGGAGGAG	TCCGATGATCTCTCCGACGA	60	215–227

[a]Abbreviations: T_m, melting temperature.

Table 2. Genetic diversity of 11 *Mikania micrantha* populations.[a]

Population	P	N_a	N_e	I	PIC	H_o	H_e	F_{IS}
	—%—							
MS	100.00	2.1667	1.6056	0.5118	0.2687	0.3091	0.3215	0.069
RL	100.00	2.2500	1.6366	0.5335	0.2808	0.3298	0.3352	0.111
LC	91.67	2.1667	1.7661	0.5905	0.3149	0.3165	0.3837	0.166
YJ	91.67	2.0833	1.6671	0.5415	0.2883	0.2935	0.3518	0.139
LuC	91.67	2.2500	1.6745	0.5330	0.2806	0.3167	0.3435	0.067
BB	100.00	2.5000	1.8693	0.6826	0.3579	0.4136	0.4313	0.057
HK	91.67	2.2500	1.6153	0.5203	0.2716	0.2946	0.3274	0.101
WC	91.67	2.0833	1.5278	0.4506	0.2359	0.3192	0.2885	-0.003
LG	100.00	2.3333	1.6827	0.5374	0.2785	0.3751	0.3356	-0.012
SZ	91.67	2.2500	1.7941	0.6065	0.3204	0.3116	0.3864	0.227
HD	91.67	2.0833	1.7746	0.5960	0.3204	0.3659	0.3906	0.146
Mean	94.70	2.2197	1.6922	0.5549	0.2925	0.3314	0.3541	0.0971
(SD)	(1.20)	(0.1251)	(0.0993)	(0.0611)	(0.0332)	(0.0375)	(0.0401)	(0.0716)

[a]Abbreviations: P, percentage of polymorphic loci (%); N_a, number of alleles at each locus; N_e, number of effective alleles; I, Shannon's information index; PIC, polymorphic information content; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{IS}, inbreeding coefficient. The abbreviations for populations are listed in Figure 1 and Supplementary Table S1.

inbreeding depression and could contribute to reducing the genetic differentiation among populations (Geng et al. 2016).

In this study, the estimated N_m from F_{ST} was 1.116, which is larger than 1.0, indicating the gene flow of *M. micrantha* was strong enough to prevent substantial differentiation owing to genetic drift (Slatkin and Barton, 1989). In a previous study, the calculated N_m value among populations collected from Guangdong Province was even higher ($N_m = 5.478$; Geng et al. 2016), which indicated that the dispersal and establishment via seed prevail over vegetative expansion in *M. micrantha* (Wang et al. 2016). The extensive gene flow, mediated by wind-dispersed seeds, is likely to be the primary factor that causes the random distribution of genetic variation of *M. micrantha* across the introduced region (Zhang et al. 2004). The strong sexual reproduction of *M. micrantha* allows for gene flow within and among populations, which helps it to overcome founder effects and tends to increase the genetic variation of populations (Geng et al. 2016). Alternatively, human-mediated long-distance dispersal events of seeds or propagules of *M. micrantha* could facilitate gene flow among distant populations (Geng et al. 2016; Wang et al. 2008).

Genetic Structure and Independent Origins of Populations from Dehong State, Yunnan Province

In the STRUCTURE analysis, the clustering level, $K = 3$, yielded the largest ΔK value (i.e., three clusters) (Figure 2). At $K = 3$, almost all individuals that belonged to the populations MS, RL, LC, and YJ from Dehong State, Yunnan Province, were grouped together in the red cluster (Figure 2; Table 4), while the LG, SZ, and LuC populations were grouped together in the green cluster, and population WC from Hainan Province was grouped in the blue cluster (Figure 2; Table 4). These results indicate that the genetic structure of *M. micrantha* populations (MS, RL, LC, and YJ) from Dehong State, Yunnan Province, is unique and distinct from that of the other populations, indicating an independent origin and development of these populations. The independent origin of populations from Dehong State, Yunnan Province, was also supported by the fact that the first specimen of *M. micrantha* was collected in 1983 by Qing Lin in Yingjiang City, Dehong State, Yunnan Province (specimen number 7708052, 1983-10-26; Du et al. 2006). In addition, 11 populations of *M. micrantha* were clustered in three groups using the UPGMA method. The MS population from Dehong State, Yunnan Province, formed a group with a single population, while the populations YJ, LC, and RL from Dehong State, Yunnan Province, clustered into the second group (Figure 3). The other seven populations from Guangxi, Guangdong, and Hainan provinces were clustered into the third group (Figure 3). Based on the UPGMA and STRUCTURE analysis results, we also deduce that the MS, YJ, LC, and RL populations from Dehong State, Yunnan Province, could have originated independently and developed.

In this study, we also found that almost all of the individuals that belong to population WC from Hainan Province were grouped in the blue cluster (Figure 2; Table 4). Yu and Wu (2009) determined that Wenchang City was the first introduction

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Table 3. Analysis of molecular variance (AMOVA) results for *Mikania micrantha* populations.[a]

Source of variance	df	SS	MS	Variance components	Percentage	P-value[b]	F_{ST}
					—%—		
Among populations	10	388.801	38.880	0.476	18.27	<0.001	0.183
Within populations	843	1,794.184	2.128	2.128	82.73	<0.001	—
Total	853	2,182.985	41.008	2.604	—	—	—

[a]Abbreviations: df, degree of freedom; SS, sum of squares; MS, expected mean squares; F_{ST}, genetic differentiation coefficient.
[b]P-value = probability of null hypothesis. Significance tests after 1,000 permutations.

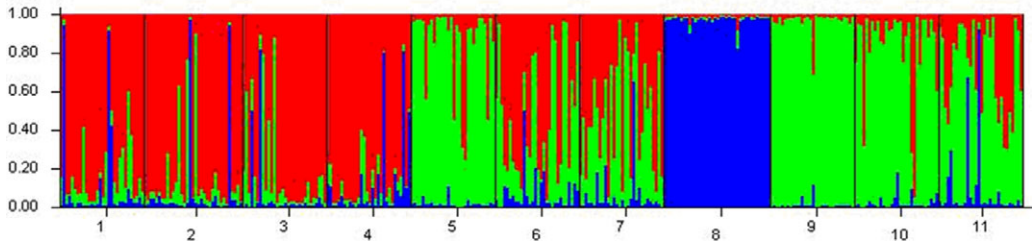

Figure 2. Bayesian assignment proportions for $K = 3$ clusters of 11 *Mikania micrantha* populations determined in the STRUCTURE software. Each vertical bar represents one individual. From 1 to 11, the populations are MS, RL, LC, YJ, LuC, BB, HK, WC, LG, SZ, and HD. The abbreviations for populations are listed in Supplementary Table S1.

Table 4. Proportion of ancestry of each *Mikania micrantha* population in three gene pools, defined via the model-based clustering method at $K = 3$.[a]

Gene clusters	MS	RL	LC	YJ	LuC	BB	HK	WC	LG	SZ	HD
Green cluster	0.117	0.111	0.144	0.061	0.841	0.403	0.429	0.012	0.955	0.811	0.639
Red cluster	0.779	0.81	0.796	0.821	0.139	0.525	0.51	0.015	0.031	0.163	0.269
Blue cluster	0.104	0.079	0.06	0.119	0.02	0.072	0.061	0.973	0.014	0.026	0.092

[a]The abbreviations for populations are listed in Figure 1 and Supplementary Table S1.

Table 5. Pairwise genetic similarity of *Mikania micrantha* populations listed above the diagonal run of dashes and Nei's genetic distance listed below the diagonal run of dashes.[a]

Pop.	MS	RL	LC	YJ	LuC	BB	HK	WC	LG	SZ	HD
MS	—	0.9783	0.9648	0.9617	0.8901	0.9504	0.8831	0.7782	0.829	0.8819	0.882
RL	0.0220	—	0.9821	0.9841	0.9097	0.9652	0.8804	0.7776	0.8035	0.8977	0.894
LC	0.0358	0.0180	—	0.9773	0.8903	0.9551	0.8753	0.7289	0.8084	0.8901	0.8788
YJ	0.0391	0.0161	0.0229	—	0.8771	0.9521	0.8651	0.7997	0.7642	0.8715	0.8702
LuC	0.1164	0.0946	0.1162	0.1312	—	0.9501	0.8726	0.7356	0.9093	0.9685	0.9739
BB	0.0509	0.0354	0.0459	0.0490	0.0512	—	0.891	0.7757	0.8778	0.9616	0.9514
HK	0.1243	0.1273	0.1332	0.1449	0.1363	0.1154	—	0.721	0.7837	0.9092	0.9164
WC	0.2507	0.2515	0.3163	0.2235	0.3071	0.2540	0.3271	—	0.6172	0.7112	0.7453
LG	0.1875	0.2188	0.2127	0.269	0.095	0.1303	0.2438	0.4826	—	0.9221	0.8754
SZ	0.1257	0.1079	0.1165	0.1375	0.0320	0.0392	0.0952	0.3408	0.0811	—	0.9773
HD	0.1256	0.1120	0.1292	0.1390	0.0265	0.0499	0.0873	0.2939	0.1331	0.0229	—

[a]The abbreviations for populations are listed in Figure 1 and Supplementary Table S1.

site in 2003 in Hainan Province. Although genetic analysis based on more populations is needed to determine the origin of the WC population of *M. micrantha*, we deduced that the WC population from Hainan Province could have originated independent of the ancestors of populations not collected in this study.

Genetic Distance and Multiple Introductions

Population pairwise relationships showed the lowest genetic distance between populations YJ and RL (0.0161) in Yunnan Province and the highest between populations WC and LG (0.4826) in Hainan Province (Table 5). We also found a lack of a clear geographic genetic structure among populations of *M. micrantha* based on the UPGMA results (Figure 3). With the exception of the four populations from Dehong State, Yunnan Province (RL, LC, MS, and YJ), that were clustered together, two closely related populations (i.e., the LG population form Hainan Province and the SZ population from Guangdong Province) are from different geographic locations, while two populations collected from the same province (i.e., the HD and SZ

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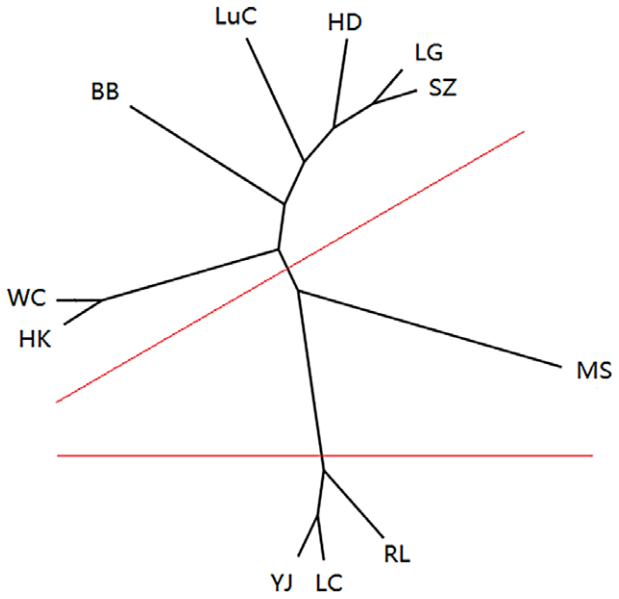

Figure 3. Unrooted unweighted pair-group method with arithmetic means (UPGMA) tree of 11 *Mikania micrantha* populations based on Nei's genetic distance. Clustering groups are artificially indicated by red lines. The abbreviations for populations are listed in Supplementary Table S1.

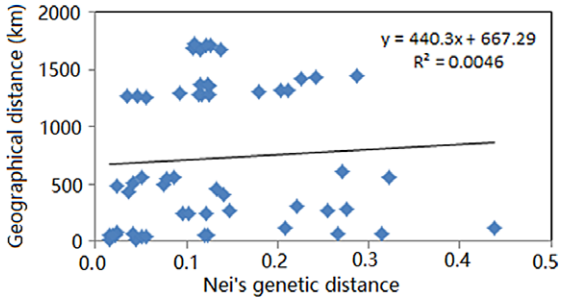

Figure 4. Mantel test of Nei's genetic distance and geographic distance of 11 *Mikania micrantha* populations. The line indicates the linear regression line between Nei's genetic distance and geographic distance.

populations from Guangdong Province) did not group together (Figure 3). In addition, a Mantel test of pairwise Nei's genetic distances and pairwise geographic distances revealed no evidence for isolation by distance ($r = 0.068$, $P = 0.343$) (Figure 4). These results indicate multiple origins of populations of *M. micrantha* in this study. Wang et al. (2016) used seven cpSSRs to investigate 28 *M. micrantha* populations and detected no evidence for "isolation by distance," indicating that multiple introductions are inferred among populations of *M. micrantha*. Multiple introductions have also been reported in *M. micrantha* populations using AFLP (Wang et al. 2012) and cpSSR (Wang et al. 2016). Multiple introductions can also contribute to the high genetic variation within populations and the low genetic differentiation among populations of *M. micrantha* by transforming among-population variation from different geographic sources into within-population variation (Geng et al. 2016; Kolbe et al. 2004; Wang et al. 2008).

Post-introduction Admixture

In this study, the STRUCTURE results also indicate that at $K = 3$, the BB population from Guangxi Province, HK population from Hainan Province, and HD population from Guangdong Province could not be assigned to a single cluster, so they were denoted by a combination of red cluster and green cluster (Figure 2; Table 3). These results indicate that the BB, HK, and HD populations could originate from the admixture of the red cluster (populations from Yunnan Province) and the green cluster (populations from Guangxi, Guangdong, and Hainan provinces). Similar mechanisms were described for this species in previous studies (Geng et al. 2016; Wang et al. 2012). Admixture creates unique opportunities for genetic interactions among previously isolated alleles and/or loci, which can dramatically affect phenotypes and fitness in admixed genotypes (Edmands 1999; Keller and Waller 2002). Compared with the lack of genetic structure and low gene flow inferred from pre-introduction admixture of common ragweed (*Ambrosia artemisiifolia* L.) (Li et al. 2019), our results imply that the post-introduction admixture caused by multiple introductions and/or high gene flow (van Boheemen et al. 2017) could be alternative mechanisms underlying the invasive success of *M. micrantha*. In the present study, it was not clear how the BB, HK, and HD populations could obtain genotypes from geographically distant red and green clusters. However, hybridization could have happened in individuals that established after long-distance migration mediated by human activities. Geng et al. (2016) found that heavy traffic on highways associated with economic development could be the main force driving the seed dispersal and resulting in the genetic admixture of roadside populations of *M. micrantha*. The post-introduction admixture may have been formed by hybridization between individuals that originated from different source populations that were carried to new sites via the heavy traffic on highways (Geng et al. 2016) and other human activities, such as trading, and established new populations.

In conclusion, we found high genetic diversity at the population level and low levels of genetic differentiation among populations of *M. micrantha* in South China. Three gene clusters and one admixture gene cluster were found. The independent origins of four populations collected from Dehong State, Yunnan Province, was determined. We conclude that post-introduction admixture, caused by multiple introductions, high gene flow, and long-distance dispersal mediated by human activities, could contribute to the evolutionary adaptation of *M. micrantha*. Further introduction and admixture should therefore be prevented.

Acknowledgments. We thank Chuanguang Zhang at the Yunnan Academy of Forestry and Grassland for kind help in sample collection. This work was financially supported by Key Research and Development Project of Yunnan Province (2018BB009). No conflicts of interest have been declared.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2021.29

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https://doi.org/10.1017/wsc.2021.29 Published online by Cambridge University Press