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POPULATION GENETICS OF INVASIVE WEED *MIMOSA PIGRA* L. (MIMOSOIDEAE) IN THAILAND

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Abstract

Mimosa pigra L. is widely considered one of the 100 worst invasive species. Native to tropical America this species is now distributed worldwide where it causes severe impact in many regions. Population genetic data could provide important information for management of this species yet to date such study is lacking. The objectives of this effort include assessment of levels of genetic variation and genetic structure of *M. pigra* in Thailand using RAPD markers. Samples were collected from 11 populations throughout Thailand. Relatively high levels of genetic variation within populations were revealed, suggesting a lack of substantial population bottlenecks. The overall F_{ST} value of 0.271 (p<0.001) indicated considerable level of population genetic structure. UGPMA dendrogram indicate three lineages of *M. pigra* in Thailand. Principal coordinate analysis suggests that populations are not grouping according to geographic origin. Together, the results suggest that patterns of genetic variation and genetic structure are likely due to multiple introductions of *M. pigra* into Thailand.

Introduction

Invasive species cause severe negative impacts on both global biodiversity and economics (Sakai *et al*., 200; Lee, 2002; Simberloff *et al*., 2005). Native to tropical America *Mimosa pigra* L., is now distributed in tropical regions of Africa, Australia and Asia where it causes a number of ecological and economic problems. Several features enable this species to establish and eventually expand its range once it has become established. It growing very fast and matures very early; within the first year of growth *M. pigra* can set seed. This species also has a broad range of habitat preferences and environmental conditions (Walden *et al*., 2002), rendering it a highly effective and rapid invasive plant species (Sakai *et al*., 2001).

Mimosa pigra was introduced from Indonesia to Chiangmai Province, in Northern Thailand, in 1947 as a fertilizer and crop cover for tobacco plantations (Miller, 2002). It is now widespread throughout the country and causes serious problems in a number of different areas. A biological control program of the *M. pigra* was atempted in Thailand in 1980s using 7 natural enemies. Of these, 2 seed bruchid species, *Acanthoscelides quadridentatus* and *A. puniceus* have become established. However, 20 years after release, control of *M. pigra* has not been achieved due to low percentage of seed damage (Suasa-ard *et al*., 2004). Other control methods such as physical control (e.g. cutting, fire) and chemical control using several herbicides have been implemented in several areas. However, these methods also only impart temporary and localized control.

Population genetic study of introduced populations can provide important information that is frequently useful in understanding biological invasions, as well as in the management and control of invasive species (Holland, 2000; Lee, 2002; Ward *et al*., 2008). For example, genetic data can be used to infer the geographic source of introduced populations and to track and better understand distribution pathways. An understanding of levels of genetic variation may also be relevant to the resistance of introduced species to chemical or biological control agents (Sakai *et al*., 2001; Ward *et al*., 2008). In addition, population genetic data can also provide insight into the evolutionary processes involved in successful invasions (Sakai *et al*., 2001; Lavergne & Molofsky, 2007).

Despite severe and widespread impacts to local biodiversity and agriculture, population genetic data on *M. pigra* is lacking. In this study, we assessed molecular genetic variation and genetic structure of *M. pigra* in Thailand based on random amplified polymorphic DNA (RAPD) markers. Information gathered via this study will enable us to begin to understand levels of genetic variation and connectivity among populations. This information may ultimately assist in the development of effective management strategy of this invasive weed.

Materials and Methods

Sample collection, DNA extraction and RAPD-PCR assay: Eleven populations were collected throughout Thailand (Table 1 and Fig. 1). Young leaf buds were collected from each individual *M. pigra* and kept in plastic bags with silica gel to preserve each sample. Genomic DNA was extracted from dried buds using DNA extraction kits (RBC BioScience, Taiwan). Each PCR reaction (25 µL) contained 2 µL of DNA template, 0.4 µM each primer, 1.5 µM $MgCl_2$, 200 µM dNTPs and 0.5 U of *Taq* polymerase (Promega). The temperature profile was as follows, denaturing at 94º C for 5 min, followed by 40 cycles of 94º C for 1 min, annealing at 37º C for 1 min, and extension at 72º C for 2 min,with a final 7 min extension at 72ºC. PCR products were checked using 1.5% agarose gel with 0.125 mg/L Ethidium bromide. A 100 bp DNA ladder (Invitrogen) was run together with all PCR products. PCR banding patterns were visualized using GelDoc (BioDoc It System).

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Table 1. Details of sampling sites of *Mimosa pigra* and summary of RAPD band characteristics of 5 primers in 11 populations of *Mimosa pigra* in Thailand.

Population (Code)	Region	N	Latitude/ Longitude	Altitude (m)	Date	Total Number of bands	% polymorphic loci	Heterozygosity
Sawi District, Chumphon (CP)	South	12	10º14'15" N/ 99º05'41" E	16	7.05.08	58	59.2	0.187
Mueang Surat Thani District, Surat Thani (ST)	South	12	09º02'52" N/ 99º02'52" E	27	4.05.08	56	45.1	0.155
Huai Yot District, Trang (TR)	South	10	07º48'14" N/ 99º35'23" E	43	5.05.08	55	43.7	0.138
Khao Yoi District, hetchaburi (PE)	Central	3	13º20'02" N/ 99º49'47" E	20	8.05.08	43	14.1	0.085
Nong Khae District, Saraburi (SB)	Central	11	14º17'58" N/ 100º48'56" E	14	8.05.08	62	59.2	0.211
Khun Han District, Si Sa Ket (SR)	Northeast	12	14º33'16" N/ 104º22'11" E	170	17.04.08	56	57.8	0.200
Kantharawichai District, Maha Sarakham (MK)	Northeast	12	16º15'01" N/ 103º15'05" E	150	25.12.08	64	59.2	0.200
Mueang Nakhon Phanom District, Nakhon Phanom (NP)	Northeast	12	17º23'27" N/ 104º45'53" E	142	20.10.08	60	53.5	0.199
Phu Ruea District, Loei (LO)	Northeast	12	17º29'59" N/ 101º20'09" E	1153	6.04.08	66	67.6	0.242
Den Chai District, Phrae (PR)	North	12	17º58'08" N/ 103º03'50" E	157	9.02.09	59	50.7	0.186
Chom Thong District, Chiang Mai (CM)	North	12	18º29'30" N/ 98º40'39" E	295	6.02.09	54	36.6	0.114
Total		**120**				**71**	**85.9**	**0.249**

Fig. 1. Distributions of the sampling locations of *Mimosa pigra*. Details of the sampling sites were given in Table 1.

Data analysis: Banding patterns for each primer pair were scored as presence (1) or absence (0). Allele frequencies were calculated assuming Hardy-Weinberg equilibrium, according Lynch & Milligan (1994) using TFPGA 1.3 (Miller, 1997). Population genetic structure was inferred using F_{ST} and the exact test of population differentiation using the contingency table approach in TFPGA. In addition, we also conducted an analysis of molecular variance (AMOVA) to test for structure based on Euclidean distance measures (Excoffier *et al*., 1992). Populations were assigned four groups according to geographic origin, i.e. North (CM, PR), Northeast (NP, LO, MK, SR), Central (SB, PE) and South (CP, TR, ST). Significance of the fixation indices (Φ_{ST}, Φ_{SC}, Φ_{CT}) was tested using permutation of 1023 replicates. AMOVA was implemented in Arlequin 2.0 (Schneider *et al*., 2000). Principal coordinate analysis (PCO) was used to represent geographic distribution of each individual. PCO analysis based on the presence/absence matrix was performed using the program MVSP version 3.1 (Kovach Computing Service Pentraeth, UK, 1998). A UPGMA dendrogram, based on Nei's (1978) unbiased genetic distance matrix was used to illustrate genetic relationship between all 11 populations. The UPGMA was generated using TFPGA 1.3. Mantel (1967) test was used to assess the relationship between geographic (km) and Nei's unbiased (1978) genetic distance among populations in order to test and understand whether population genetic structure adheres to an isolation by distance (IBD) model of differentiation. The Mantel test was implemented in the program IBD 1.52 (Bohonak, 2002).

Results

Of the 23 primers, 5 produced clear, polymorphic and reproducible bands (Table 2). These primers amplified 71 loci of which 61 (86%) were polymorphic. Number of bands for each primer varied from 10 to 18 (Table 2). Percent of polymorphic band in each population varied from 14.1 in PE to 67.6 in LO. The mean expected heterozygosity overall was 0.249 and for each population varied from 0.085 in PE to 0.242 in LO (Table 1).

Table 2. List of primers, sequences and number of amplified band of each primer.

Primer	Primer sequence (5'→3')	Number of bands per primer	Number of polymorphic bands
OPA10	GTGATCGCAG	18	17
OPA11	CAATCGCCGT	15	15
OPN02	ACCAGGGGCA	14	13
OPN04	GACCGACCCA	14	9
OPD02	GGACCCAACC	10	7
Total		**71**	**61**

The F_{ST} value for overall population is 0.271. Genetic distance based on Nei's unbiased estimation range from 0.031 between CP and SR to 0.192 between PE and CM (Table 3). The Mantel test detected no significant relationship between genetic and geographic distance (r^2 = 0.029 p=0.118) indicated that geographic distance is not a major factor determining levels of genetic distance. Pairwise comparison of the exact test of genetic differentiation between populations revealed 21 of 55 comparisons were significant (Table 3). Two populations from the northern regions (CM and PR) were not genetically significant different from each other but they were highly differentiated from populations in other regions. Populations from the south (TR, ST, and CP) were not genetically different from each other. They are also not genetically different from two populations in the central (PE and SB) region and two populations from the northeast (SR and LO) in all comparisons. Four populations in the northeast (SR, MK, LO and NP) could be assigned into two groups based on the exact test of population differentiation. SR and LO, which were closely related to the southern populations, were genetically significantly different from MK and NP. AMOVA results (Table 4) revealed moderate levels of genetic differentiation among four geographic regions (Φ_{CT} = 0.114; P = 0.018). A large proportion (73.95%) of the genetic variation was allocated into within population component. The Φ_{ST} level (0.261 p<0.001) is consistent with F_{ST} obtained from TFPGA, indicating moderate level of genetic differentiation among populations.

The UPGMA dendrogram revealed 3 major population groups (Group I, II and III) (Fig. 2). In group I, three populations from the south (CP, TR, ST) clustered together with two populations from the northeast (SR and LO). Population PE from the central region clustered with NP from the northeast, forming group II. PR and CM from the north cluster with SB from central region and MK from northeast region forming group III. A two-dimensional plot diagram of the PCO analysis based on presence/absence of the RAPD banding patterns of 120 individuals revealed similar grouping structure found by UPGMA. The plot of the first two axes (Fig. 3) which explained 23.1% of the variation (Axis I explained 15.8%, Axis II explained 7.3%), revealed that individuals from southern populations clustered together and appear in the right side of the figure. Samples from the northern region grouped together, and fell into the left side of the plot. Individual samples from central and northeast appear in both sides of the plot, suggesting a mixture of genetically diverse populations. The PCO diagram thus indicated that although populations of *M. pigra* were separated into two major groups, some populations were mixtures of individuals from both groups.

Table 3. Pairwise comparison of Nei's (1978) genetic distance (below diagonal) and combined probabilities of the exact test of population differentiation (above diagonal) between 11 populations of *Mimosa pigra* in Thailand based on RAPD.

	CP	ST	TR	PE	SB	SR	MK	NP	LO	PR	CM
CP	-	NS	NS	NS	NS	NS	**	**	NS	***	***
ST	0.037	-	NS	NS	NS	NS	NS	***	NS	***	***
TR	0.044	0.043	-	NS	NS	NS	***	*	NS	***	***
PE	0.082	0.077	0.043	-	NS	NS	NS	NS	NS	NS	NS
SB	0.054	0.055	0.073	0.093	-	NS	NS	NS	NS	**	**
SR	0.031	0.042	0.069	0.120	0.057	-	***	***	NS	***	***
MK	0.124	0.109	0.127	0.166	0.054	0.108	-	NS	*	NS	NS
NP	0.075	0.078	0.047	0.063	0.059	0.102	0.076	-	NS	*	NS
LO	0.029	0.049	0.035	0.077	0.059	0.054	0.116	0.040	-	*	*
PR	0.142	0.160	0.133	0.192	0.064	0.139	0.047	0.090	0.106	-	NS
CM	0.131	0.148	0.105	0.164	0.070	0.166	0.081	0.068	0.091	0.032	-

*p<0.05, **p<0.01, ***p<0.001

Table 4. **AMOVA analysis of RAPD variation for 11 populations of *Mimosa pigra* populations in 4 regions (North, Northeast, Central and South) of Thailand.**

Source of variation	d.f.	Sum of squares	Variance component	% Total	Φ-statistics	*P*
Among regions	3	152.80	1.04	11.38	0.165	<0.001
Among populations within region	7	148.16	1.34	14.67	0.260	<0.001
Among individuals within population	109	736.16	6.75	73.95	0.114	0.018

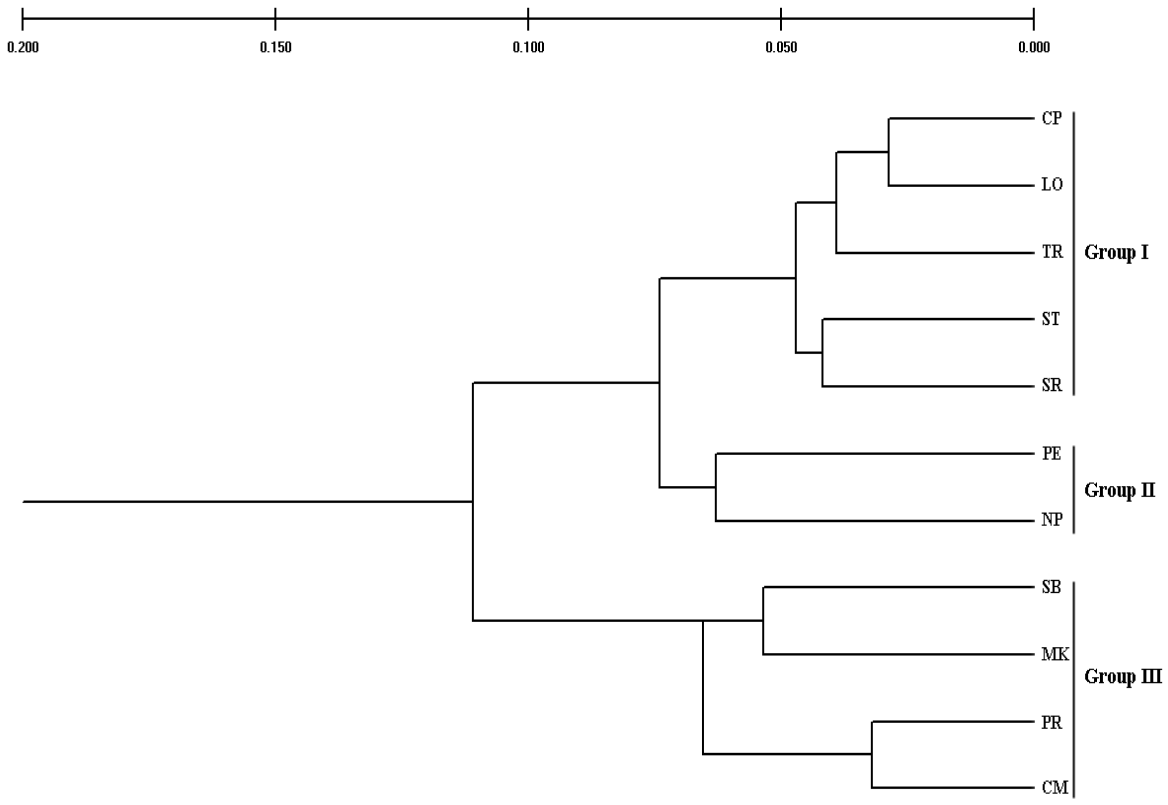

Fig. 2. UPGMA dendrogram depicting relationship between 11 populations of *Mimosa pigra* based on Nei's (1978) unbiased genetic distance.

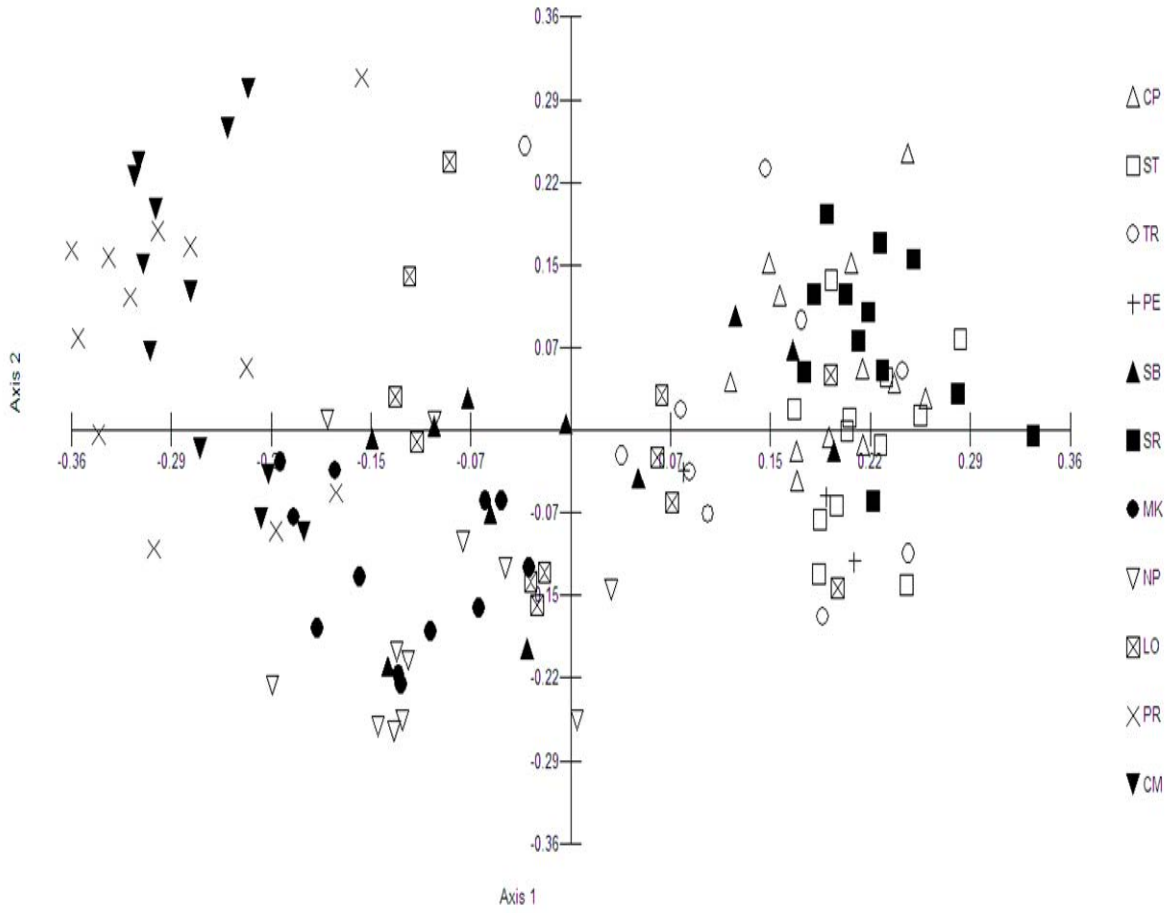

Fig. 3. Plotting diagrams of the first two principal coordinates axes based on RAPD banding patterns depicting patterns of relationship and genetic variation among 120 individuals of 11 populations of *Mimosa pigra* in Thailand.

Discussion

Genetic variation of invasive species is predicted to be lower in the introduced range than within the native populations due to population bottlenecks during the colonization (Holland, 2000). However, in the cases where there are multiple sources of introduced populations, high genetic diversity is often detected. For example, Wang *et al*., (2008) found high diversity in introduced population of weed *Mikania micrantha* in Southern China. Genton *et al*., (2005) detected high diversity in the introduced range of French ragweed *Ambrosia artemisiifolia*. Lavergne & Molofsky (2007) found high levels of genetic variation in the introduced wetland grass *Phalaris arundinacea* L., in North America.

Relatively high levels of genetic diversity were found in populations of *M. pigra* in Thailand. AMOVA revealed that a high proportion (74%) of the variation was allocated within populations. PCO results revealed that individuals from some populations were closely related to individual samples from other geographically distant populations. For example, in populations SB and LO, some samples were genetically closely related to the southern populations, whereas others were closely related to the northern populations. This suggests that samples from such populations were derived from different sources. Therefore, high levels of genetic variation detected in this study strongly suggest that the widespread invasion of Thailand by *M. pigra* is the result of multiple introductions. Another possibility that cannot be ruled out at present is the invasion of Thailand came from a single genetically diverse source. Genetic variation in invasive *M. pigra* populations may also be enhanced by the potential of long distance colonization mediated by human-related activity (e.g. seeds may be transported accidentally in sand used in construction, carried long distance distances from source to construction sites (Lonsdale *et al*., 1985)). This could increase the chance of recombination between genetically different genotypes and again maximize levels of genetic variation (Lavergne & Molofsky, 2007).

It has been reported that *M. pigra* was introduced from Indonesia to northern Thailand. (Chiang Mai Province) in 1947 (Miller, 2002) and then spread throughout the country. However, the UPGMA dendrogram revealed three genetically distant lineages of *M. pigra* in Thailand. Each lineage composed of geographically distinct populations. For example, LO and SR from the northeastern region were grouped with CP, TR and ST from the south. In contrast, MK population also from the northeast was clustered with CM and PR from the north. Because samples from other countries are not available at present, in this study thus we

were unable to trace the sources of the invasive populations. However, *M. priga* in Southeast Asia is widespread in the Lower Mekong Basin (LMB) (Triet *et al*., 2004) which connects to Thailand through river network systems. This could be a mechanism of dispersal of *M. pigra* into Thailand. Lineage I which composed of populations from the northeast (LO and SR) and from the south (CP, ST and TR) were most likely invaded from the Mekong River system because Loei province where LO samples were collected is on the border of Thailand and Lao PDR. Although the invaded from the south cannot be rule out but it is unlikely given that *M. pigra* was recently recorded in this region. Another lineage of *M. pigra* in the northeastern region (group II) was in the NP population. This population was also from the province (Nakhon Panom Province) which in the border of Thailand and Lao PDR where Mekong River is a boundary. Thus, population of *M. pigra* could be introduced into NP from the Mekong river system as in LO but from different source. Lineage III composed of populations from the north (PR and CM), northeast (MK) and central (SB) were most likely a lineage that introduced into Thailand from Indonesia about 60 years ago. Thus, the pattern of genetic structure in *M. pigra* in Thailand is most likely due to the effect of multiple introductions from genetically different populations.

In conclusion, our results indicated that genetic diversity and genetic structure of *M. pigra* in Thailand is higher than predicted for species undergoing a single introduction and a severe bottleneck. It is possible that *M. pigra* originated in Thailand via multiple genetically diverse populations. This conclusion however, is not consistent with previous reports which suggested that *M. pigra* in Thailand was introduced from single source in Indonesia and then spread throughout the country from this population. Multiple introductions play important role in determining genetic structure and diversity of this species in the introduced range. High levels of genetic variation and genetic structure detected in this study could have important implications for the management of this species.

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