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Estimation of genetic divergence based on mitochondrial DNA variation for an invasive alien species, *Metcalfa pruinosa* (Say), in Korea

Deok Ho Kwon [a,1], Minyoung Kim [b,1], Hyojoong Kim [c], Yerim Lee [b], Ki-Jeong Hong [d], Si Hyeock Lee [a,b], Seunghwan Lee [a,b,*]

[a] Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea
[b] Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea
[c] Department of Biology, Kunsan National University, Daehangno 558, Gunsan, Jeonbuk 573-701, Republic of Korea
[d] Department of Plant Medical, College of Life Science and Natural Resources, Sunchon National University, 255 Joongang-ro, Sunchon 540-950, Republic of Korea

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ABSTRACT

The invasive alien species, *Metcalfa pruinosa* (Say), has been spreading in Korea since it was first recorded at Gimhae in Gyeongnam province in 2005. This species has caused damage to major forest trees and several agricultural crops both by direct plant sap feeding and by indirectly vectoring sooty mold disease. This study aimed to estimate genetic divergence based on mitochondrial DNA variation in several populations from six countries to determine the origin of the Korean population of *M. pruinosa*. A total of 23 haplotypes were observed in the analysis of nucleotide polymorphisms on mitochondrial cytochrome *c* oxidase I gene in 124 voucher specimens. Among these specimens, only two haplotypes (HAP_1 and HAP_2) were confirmed in the Korean population, which had the lowest diversities of haplotype and nucleotide (0.093 and 0.00032, respectively), whereas populations of North America and Europe exhibited a relatively higher haplotype and nucleotide diversities. Population genetic analyses revealed that the Korean population was closer to European populations than North American populations; it had the lowest F_{ST} (0.589, $p < 0.001$) and exhibited a relatively lower percentage of variation (14.6%, $p < 0.001$) in the analysis of molecular variance. Taken together, these findings suggested that the Korean population might have originated from European countries, rather than from North America.

Introduction

The frequency of invasive alien species (IAS) has dramatically increased in Korea as the volume of agricultural trades has increased over recent decades. An average of 0.9 alien species per year was introduced since 1960s, and the annual invasion rate of these alien species is steadily increasing (Hong et al., 2012).

Metcalfa pruinosa is one of the most serious IAS, which was first reported in Korea from the eastern Palaearctic region (Kim et al., 2011). The native distribution area of *M. pruinosa* is the eastern North America (Metcalf and Bruner, 1948). However, it was first reported as an invasive species in northern Italy in 1979 (Zangheri and Donadini, 1980) and has gradually spread to many neighboring European countries, such as France (Della Giustina, 1986), Slovenia (Sivic, 1991), and Greece (Drosopoulos et al., 2004), among others. Currently, *M. pruinosa* has been widely reported in over 18 European countries (Kim et al., 2011) and is considered a risk as a pest insect in several organic production areas (Strauss, 2010). In Korea, this species was first observed on persimmon trees in Gimhae in 2005 (Lee and Wilson, 2010). After four years, population outbreaks were reported in several provinces (such as Gyeonggi-do, Chungcheongbuk-do, Chungcheongnam-do, and Gyeongsangnam-do) in Korea (Kim et al., 2011). Seventy-four plant species in 41 families have been reported as host plants of *M. pruinosa*, and an area of 400 ha per year is damaged by direct feeding and indirect transmission of sooty mold disease (Kim et al., 2011). Recently, *M. pruinosa* has begun to damage economically important agricultural crops, such as ginseng, grape and pear in Korea.

It is important to know this species' origin to help reduce the likelihood of future invasions of other IAS. It is important to know this species' origin to help reduce the likelihood of future invasions of other IAS. Knowing the origin of IAS would aid the quarantine department to establish the management direction for the decision of the survey period and target host plant of putative exporting countries with high attention. It would be an essential step for the establishment of a rational IAS management that can reduce time and cost, but it is difficult to trace or collect the invading species in real time due to temporal and spatial limitation. As an alternative tracing method, population genetic

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, 151-921, Republic of Korea. Tel.: +82 2 880 4703; fax: +82 2 873 2319.
E-mail address: seung@snu.ac.kr (S. Lee).
[1] These authors contributed equally to this work.

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approaches have been widely used to track the origin of invasive species based on the principle of genetic divergence, which reflects genetic drift and gene flow (Lushai and Loxdale, 2004; Lowe and Allendorf, 2010) without using the direct or indirect observation of invading (Kim and Sappington, 2013). Many previous studies have employed molecular markers such as mitochondrial DNA (mtDNA) and microsatellite markers to determine population origins. In the United States, population genetic studies were performed to determine the long-distance dispersal of several species, *Anthonomus grandis grandis* Boheman (Kim and Sappington, 2004a, 2004b), *Diabrotica virgifera virgifera* LeConte (Kim and Sappington, 2005; Miller et al., 2005), and *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) (Kim et al., 2009). In Korea, the origin of the brown plant hopper, *Nilaparvata lugens*, was first traced using mtDNA haplotypes (a set of alleles from haploid set of chromosomes) and subsequent comparisons with several Asian populations. The major haplotypes of the Korean population were grouped with Chinese populations (Mun et al., 1999). In the case of the lantern fly, *Lycorma delicatula*, which invaded Korea in 2007, the Korean population was clustered with several Chinese populations based on a comparison of the sequence polymorphisms on NADH dehydrogenase subunit 2 and NADH dehydrogenase subunit 6 regions in mitochondrial genes (Kim et al., 2013).

In this study, we investigated to estimate the putative origin of the Korean population of *M. pruinosa* by adopting population genetics approach. The haplotypes which were determined by nucleotide polymorphism of mitochondrial cytochrome c oxidase I (mtCOI) from 124 specimens in six countries were used to estimate the genetic diversity, *F*-statistics (F_{CT}, F_{SC} and F_{ST}) enabling partitioning of genetic variation within/between subpopulations, and analysis of molecular variance (AMOVA).

Materials and methods

Sample collections and species identification

M. pruinosa specimens were collected or donated from Korea, North America and four European countries (France, Slovenia, Spain and Italy) from 2009 to 2012 (Table 1 and Supplementary Table 1). Species identification was performed based on the morphological characteristics of male genitalia. Male genitalia excised from abdomen were incubated with 10% KOH solution at 70 °C for 30 to 60 min and transferred onto a slide glass with glycerine for further observation under a microscope. The specimens observed were deposited at Seoul National University (SNU).

Table 1
Sample numbers of each population by localities of collection used in this study.

Continent	Country (abbrev.)	Locality	Number of individuals
Asia	Korea (KR)	Seoul	10
		Gyeonggi-do	12
		Chungcheongbuk-do	6
		Chungcheongnam-do	2
		Gyeongsangnam-do	9
		Jeonrabuk-do	3
North America	United States of America (USA)	Delaware	12
		Florida	5
		Maryland	9
		New Jersey	10
Europe	France (FR)	Antibes	3
		Montpellier	4
		Le Boulou	4
		Nice	3
	Italy (IT)	San Remo	4
		Savona	4
		Borghetto	4
	Slovenia (SI)	Pri hrastu	9
	Spain (ES)	Lleida	11
Total			124

Genomic DNA extraction

Genomic DNA was extracted from individual specimens using the QIAGEN DNeasy® Blood & Tissue kit (QIAGEN, Inc., Dusseldorf, Germany) according to the manufacturer's instructions with a slight modification. Briefly, the individual specimen was incubated with 180 μl of Buffer ATL and 20 μl of proteinase K for overnight at 56 °C. After spin down for 20 s, the supernatants were transferred into new tubes and mixed with 200 μl of Buffer AL. The mixtures were centrifuged using the DNA binding column at 8000 rpm. The solution was eluted and stored at −20 °C prior to PCR amplification.

PCR amplification and sequencing

Partial fragments of mitochondrial cytochrome oxidase I (mtCOI, 658 bp) were PCR-amplified with 10 μM dNTP, 5 μM of forward and reverse primers (Forward primer, GGTCAACAAATCATAAAGATATTGG; Reverse primer, AAACTTCAGGGTG-ACCAAAAAATCA), 2 μl genomic DNA, and 0.8 Unit of AmpONE™ α-Taq DNA Polymerase (GeneAll Biotechnology, Seoul, Korea) using following thermal program; 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 45 s for 35 cycles. The amplified-PCR product was confirmed using gel electrophoresis on 1.5% agarose gel. If a single PCR product was amplified, then it was directly sequenced after the purification process using the QIAquick PCR purification kit (QIAGEN). Otherwise, the amplified PCR band (approximately 658-bp) was excised from the gel and extracted using the QIAGEN gel extraction kit (QIAGEN). Sequencing was performed using an ABI prism 3730 DNA sequence analyzer (Applied Biosystems, Foster City, CA) at the NICEM sequence analysis facility (Seoul, Korea).

Data analysis

The partial mtCOI nucleotide (577 bp) was aligned using ClustalW (Larkin et al., 2007) and genetic diversity indices were calculated using DnaSP version 5.10 (Librado and Rozas, 2009). Genetic differentiation (F_{ST}) and analysis of molecular variance (AMOVA) were calculated using ARLEQUIN ver. 3.5.1.2 (Excoffier and Lischer, 2010). The phylogenetic tree was reconstructed using the FITCH and NEIGHBOR program in the PHYLIP 3.5c (Felsenstein, 1989) based on nucleotide divergence (Da) (Nei, 1987).

Results and discussion

Genetic diversities and haplotype distribution of M. pruinosa

The partial nucleotide sequence of mtCOI (577 bp) was obtained from 124 individuals from six countries (Korea, 42 specimens; North America, 36 specimens; France, 14 specimens; Italy, 12 specimens; Slovenia, 9 specimens; Spain, 11 specimens, Table 1). Detailed information on each

Table 2
Diversity indices based on mitochondrial polymorphisms in Metcalfa pruinosa from six countries.

Population	N	No. of haplotypes	No. of polymorphic sites	Haplotype diversity (± SD)	Nucleotide diversity (± SD)
KR	42	2	2	0.093 ± 0.060	0.00032 ± 0.00021
USA	36	19	40	0.951 ± 0.017	0.01560 ± 0.00209
FR	14	5	24	0.769 ± 0.08	0.01979 ± 0.00198
IT	12	2	19	0.303 ± 0.147	0.00998 ± 0.00486
SI	9	3	7	0.639 ± 0.126	0.00366 ± 0.00142
ES	11	2	19	0.182 ± 0.144	0.00599 ± 0.00473
Total	124	23	51	0.703 ± 0.044	0.01314 ± 0.00140