Analysis of Genetic Diversity and Structure of Two Clades of Aphelinus mali (Hymenoptera: Aphelinidae) in China

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ANALYSIS OF GENETIC DIVERSITY AND STRUCTURE OF TWO CLADES OF APHELINUS MALI (HYMENOPTERA: APHELINIDAE) IN CHINA

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ABSTRACT

Our prior research revealed that there are 2 mitochondrial clades of Aphelinus mali (Halde-
man) (Hymenoptera: Aphelinidae) in China, which are known as SD clade and LN clade. To furth-
er reveal their genetic characteristics and to determine the degrees of hybridization and gene flow between the 2 clades of A. mali in China, we analyzed the genetic diversities and genetic structures of 16 populations from 6 provinces (Shandong, Liaoning, Hebei, Shanxi, Xinjiang, and Yunnan) using 8 microsatellite loci. Our results showed that among the pure populations in the SD and LN clades, the greatest genetic diversities were found in the Qingdao, Shandong (QD) population and in the Dalian, Liaoning (DL) population. QD was the first population of the SD clade to be established, and DL was the first population of the LN clade to be established. In addition, genetic diversity was not substantially lower - and in some cases it was greater - in mixed-clade populations than in QD and DL. Individuals within each mitochondrial clade could not be differentiated based on microsatellite loci. Our data confirmed that the QD and DL populations, which were the first to be established in China, have served as bridgeheads for the other SD and LN populations in China. The results demonstrated that the hybridization or gene flow has occurred between the 2 mitochondrial clades.

Key Words: Aphelinus mali, microsatellite, bridgehead effect, genetic diversity, hybridization, gene flow

RESUMEN

Nuestra investigación previa reveló que hay 2 clados mitocondriales de Aphelinus mali (Haldeman) (Hymenoptera: Aphelinidae) en China, que se conocen como los clados SD y LN. Para revelar aún más sus características genéticas y determinar el grado de hibridación y el flujo de genes entre los 2 clados de A. mali en China, se analizó la diversidad y estructura genética de 16 poblaciones en 6 provincias (Shandong, Liaoning Hebei, Shanxi, Xinjiang, y Yunnan) usando 8 loci de microsatélites. Nuestros resultados mostraron que entre las poblaciones puras en los clados SD y LN, la mayor diversidad genética fue encontrada en la población de Qingdao (QD) y en la población de Dalian, Liaoning (DL). La primera población del clado SD que se estableció fue QD y DL fue la primera población del clado LN que se estableció. Además, la diversidad genética no fue sustancialmente menor, y en algunos casos fue mayor en poblaciones mixtas de los dos clados que en QD y DL. Los individuos dentro de cada clado mitocondrial no podían ser diferenciados en base a loci de microsatélites. Nuestros datos confirmaron que las poblaciones de QD y DL, que fueron los primeros en establecerse en China, han servido como una cabecera de puente para las otras poblaciones de SD y LN en China. Los resultados demostraron que la hibridación o el flujo de genes ha sucedido entre los 2 clados mitocondriales.

Palabras Clave: Aphelinus mali, microsatélites, efecto de cabecera de puente, diversidad genética, hibridación, flujo de genes

Aphelinus mali (Haldeman) (Hymenoptera: Aphelinidae), a parasitoid of the woolly apple aphid, Eriosoma lanigerum (Hausmann) (Hemiptera: Aphididae) originated from North America.
In this study, we analyzed the genetic diversity and genetic structure of 16 populations from 6 provinces (Shandong, Liaoning, Hebei, Shanxi, Xinjiang, and Yunnan) using 8 nuclear microsatellite loci. Based on these data, we wanted to reveal the genetic characteristics of the introduced populations of *A. mali* in China, and to determine the hybridization and gene flow within the 2 clades of *A. mali* in regions of China where they have been recently introduced.

Supporting material can be found online in Florida Entomologist 97(2) (June, 2014) at http://purl.fcla.edu/fcla/entomologist/browse.

### MATERIALS AND METHODS

#### Sample Collection and Species Identification

A total of 16 *E. lanigerum* populations were sampled from apple trees in 6 provinces in China during 2007, 2008, and 2012. They were placed in Petri dishes at room temperature until *A. mali* eclosed. Adult *A. mali* that emerged were examined and identified unambiguously with the aid of a microscope and were then stored in 95% ethanol at -20 °C until DNA extraction. The sampling location of each population is listed in Table 1.

#### DNA Extraction and Microsatellite Genotyping

Genomic DNA was extracted from individual female adults as described in Frohlich et al.

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<tr>
<th>Population code</th>
<th>Locations</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>Ar</th>
<th>Hs</th>
<th>Nei</th>
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<td>2.785</td>
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Mean 17 2.813 1.923 0.138 0.400 2.223 0.411 0.387

* N, sample size; Na, observed number of alleles; Ne, the effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; Ar, allelic richness; Hs, gene diversity; Nei, Nei's expected heterozygosity; Estimator of the Weir and Cockerham's fixation index (Fis).*
(1999). The DNA of *A. mali* individuals and the PCR primers were used to amplify the microsatellite loci Am13, Am14, Am19, Am27, Am34, Am35, Am36, and Am38 (Lavandero & Dominguez 2010). PCR reactions were performed as described by Lavandero & Dominguez (2010). Products were run on an ABI 3730xl DNA analyzer. Allele size was determined by comparing the mobility of the PCR products to that of the GeneScanTM 400HD size standard (Applied Biosystems).

**Genetic Diversity Analysis**

For the microsatellite data of each population, POPGENE v1.31 was used to calculate the following indices: the observed number of alleles (*Na*), the effective number of alleles (*Ne*), the expected heterozygosity (*He*), the observed heterozygosity (*Ho*), and Nei’s expected heterozygosity (*Nei*) (Yeh et al. 1997). We determined these indices for both pure and mixed populations; pure populations were those that contained only one haplotype, and mixed populations were those that contained more than one haplotype (Table S1). For mixed populations, we also determined these indices separately for each clade associated with each haplotype. The program FSTAT v2.9.3.2 was used to calculate gene diversity (*Hs*) and allelic richness (*Ar*) (Franks et al. 2010).

**Analysis of Genetic Structure and Gene Flow**

Differentiation approach based on *F*<sub>st</sub> analysis was used. GENEPOP v3.4 (Raymond & Rousset 1995) was used to calculate Weir and Cockerham’s estimator of the fixation index *F*<sub>st</sub> (Weir & Cockerham 1984). Isolation by distance (IBD) based on genetic differentiation and the logarithm of geographic distance was examined by using 10,000 permutations of the Mantel test implemented in IBDWS v3.15 (http://www.ibdws.sdsu.edu) (Jensen et al. 2005; Hasselman et al. 2013). Distances between sampling locations were calculated using the Google Earth ruler tool (Anderson & Congdon 2013). Gene flow among regions was approximated as *Nm* (analogous to *M* = (*1/F*<sub>st</sub> - 1)/2) (Slatkin 1993).

To evaluate the assignment of *A. mali* individuals to clusters, we also analyzed all of the populations and the 6 mixed populations using STRUCTURE software (Pritchard et al. 2000). For all populations analysis, we set *K* (number of clusters) from 1 to 16 with 6 iterations of this parameter set, using a burn-in of 20 000 followed by 50 000 iterations of Markov Chain Monte Carlo (MCMC). For 6 mixed populations analysis, we set *K* (number of clusters) from 1 to 6. Other parameters were similar with aforementioned. The most optimal *K* was estimated by examining the standardized second-order change of *<K* (Evanno et al. 2005). Sigmaplot 12.0 was used to graphically display the results.

**RESULTS**

**Microsatellite-Based Genetic Diversity**

Among the 6 pure populations in the SD clade (i.e., populations that were 100% Hap1) (Zhang et al. 2014), the genetic diversity was highest in the QD population, which was the first population in that clade that was introduced, and was lowest in the ZT population (Table 1). Genetic diversity was not substantially lower and in some cases was higher in the CZ (Changzhi, Shanxi), YC (Yuncheng, Shanxi), WF (Weifang, Shandong), and YT (Yantai, Shandong) mixed populations of the SD clade than in the QD population (Table 1; Table S1).

Among the 4 pure populations in the LN clade (i.e., populations that were 100% Hap2) (Zhang et al. 2014), the genetic diversity was highest in the DL population, which was the first population in that clade that was introduced (Table 1). As was the case with mixed populations in the SD clade, genetic diversity was not substantially lower and in some cases was higher in the CZ (Changzhi, Shanxi), BD (Baoding, Hebei), WF (Weifang, Shandong), and YT (Yantai, Shandong) mixed populations of the LN clade (Table 1; Table S1). Geographic distance was not correlated with ex-
pected heterozygosity ($He$) for the SD clade ($R^2 = 0.3825, F = 4.9548, df = 1, P = 0.0567$) or the LN clade ($R^2 = 0.0431, F = 0.3152, df = 1, P = 0.5920$) (Fig. 1).

Except for the YL population, all populations with small sample sizes significantly deviated from HWE. A strong inbreeding was evident ($0.424 < Fis < 1.000$), and the deviations were associated with a significant positive $Fis$ value. Null alleles were not a major factor contributing to deviations from HWE because PCR amplifications were successful for 98.1% of each locus across all populations (Table S2). We could not use software to test for null alleles because the software assumes random mating. When tested for deviation from mutation-drift equilibrium in BOTTLENECK under the IAM, 9 of 20 populations had significant heterozygosity excess (Wilcoxon test $P < 0.05$) (Table S3). However, a significant heterozygosity excess was detected in only 4 populations under the TPM model and in only one population under the SMM model (Table S3).

Population Genetic Structure and Gene Flow

When paired populations were considered, all of the 120 $F_{ST}$ values were significant (Table 2). Isolation by distance analysis revealed a significant positive correlation between genetic distance and the logarithm of geographical distance (Fig. 2).

Estimates of gene flow between paired populations (Table 2) revealed that QHD and BD had the highest gene flow ($Nm = 39.183$) and that gene flow was also relatively high ($Nm > 10$) for YC and CZ, BD and DL, QHD and DL, TA and HZ, QD and WF, and QD and YT (see population codes in Table 1). While ZT and YL had the lowest gene flow ($Nm = 0.141$), and both of these populations also had low gene flow with other populations.

According to STRUCTURE software, the genetic structure of all of the 16 populations in China was best described as consisting of 2 clusters ($K = 2$) (Fig. 3). The result demonstrated that the individuals within mitochondrial clades cannot be differentiated based on nuclear microsatellite loci.

**DISCUSSION**

Our study indicates that the nuclear genetic diversity among pure populations was highest in the initially introduced population of each clade, which was the QD population for the SD clade and the DL population for the LN clade; genetic diversity was much lower in the other pure populations (Table 1). The result suggests that the current distribution of A. mali in the introduced range is closely associated with the expansion from the 2 initially established populations, which acted as bridgehead populations, as has been shown for several invasive insects (Miller et al. 2005; Ciosi et al. 2008; Ascunce et al. 2011; Lombaert et al. 2010; Lombaert et al. 2011; Kajita et al. 2012; Yang et al. 2012). The result also confirms that the first two introduced populations, i.e., those in Qingdao, Shandong and Dalian, Liaoning, have served as the bridgeheads for the other popula-

![Fig. 1](https://bioone.org/journals/Florida-Entomologist_97(2)_June_2014)

Fig. 1. Linear regression analysis of the relationship between expected heterozygosity ($He$) and log-transformed Euclidean geographical distance from the nearest source population of Aphelinus mali in China. Data from mixed populations were separated with respect to the nearest likely source population; for example, WF data for Hap1 (indicating the SD clade and the QD source population) and Hap 2 (indicating the LN clade and the DL source population) are shown in panel A and B, respectively. (A) The regression with QD as the source population of the SD clade ($Y = -0.6526 X + 0.4943, R^2 = 0.3825, F = 4.9548, df = 1, P = 0.0567$). (B) The regression with DL as the source population of LN clade ($Y = -0.2850 X + 0.4421, R^2 = 0.0431, F = 0.3152, df = 1, P = 0.5920$). Data for $He$ in mixed populations are from Table S1, and data for pure populations are from Table 2. The Google Earth ruler tool was used to calculate the Euclidean geographical distances. Reduced Major Axis regression lines are shown.
### TABLE 2. PAIRWISE F$_{st}$ MATRIX (BELOW DIAGONAL) AND GENE FLOW (ABOVE DIAGONAL) OF 16 *APHELINUS MALI* POPULATIONS IN CHINA.

<table>
<thead>
<tr>
<th></th>
<th>CZ</th>
<th>JZ</th>
<th>YC</th>
<th>YL</th>
<th>DL</th>
<th>HLD</th>
<th>SJZ</th>
<th>BD</th>
<th>QHD</th>
<th>ZT</th>
<th>HZ</th>
<th>LC</th>
<th>TA</th>
<th>WF</th>
<th>YT</th>
<th>QD</th>
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</thead>
<tbody>
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<td>CZ</td>
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<td>1.559</td>
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<td>1.578</td>
<td>6.266</td>
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Significant values for pairwise F$_{st}$ are in bold. Gene flow was approximated as Nm (analogous to M = (1/F$_{st}$ - 1)/2).
tions in China as revealed by mitochondrial DNA markers (Zhang et al. 2014).

The initial founder effects may result in high genetic diversity near introduction points, but serial founder effects of other introduced populations may cause lower genetic diversity (Kajita et al. 2012). If the dispersal of *A. mali* is limited by distance, genetic diversity and geographical distances should be negatively correlated. However, the genetic diversity of *A. mali* was not correlated with geographical distance for either clade in China. That might be explained by anthropogenic influence on the spread of *A. mali*; the frequent transport of fruit seedlings may have facilitated the spread of the natural enemy of the woolly apple aphid. Another possibility is that the bottleneck effect during the subsequent introductions of *A. mali* was small or was mitigated by gene flow. Alien species normally experience founder or bottleneck effects, resulting in a loss of genetic diversity (Nei et al. 1975; Templeton 1980; Barton & Charlesworth 1984).

In our study, the reduction of genetic diversity within some populations might have been caused by bottlenecks (Table S3). However, most of the populations did not exhibit a heterozygosity excess (especially according to TPM or SMM models), which suggests that the bottleneck effect is a transient feature. High gene flow between populations may also obscure the genetic effects of a bottleneck (Table 2). For example, in the 4 mixed populations of the SD clade, the nuclear genetic diversity had not substantially decreased in YT and YC relative to that in QD and had even increased in WF and CZ (Fig. 1A). A similar phenomenon occurred in LN clade (Fig. 1B). Compared to the nuclear genetic diversity of the bridgehead population (DL) of the LN clade, the nuclear genetic diversity had not substantially decreased in YT and BD and had even increased in WF and CZ. In conclusion, bottleneck effects or gene flow may affect the correlation between genetic diversity and distance from the first established populations.

Hybridization or gene flow occurred between the 2 mitochondrial clades based on nuclear microsatellite loci (Fig. 3). The individuals within mitochondrial clades could not be differentiated...
based on nuclear microsatellite loci. A positive Fis index significantly different from zero (Table 1) indicates heterozygote deficiency and suggests inbreeding. This finding is consistent with the possibility of high gene flow between populations, which may obscure the genetic effects of a bottleneck. High gene flow between populations is not surprising, given the geographical proximity of the 2 release sites.

High gene flow can limit the genetic distance between nearby populations while low gene flow can increase the genetic distance between distant populations (Scott et al. 2005). Because of the high gene flow among plant species and locations, A. mali populations in Chile exhibit very low genetic differentiation (Lavandero et al. 2011). In the current study, however, Fst analyses showed that A. mali populations in China have clearly undergone genetic differentiation in the expansion range. The positive correlation between genetic and geographic distance (Fig. 2) is associated with the low flight capacity of A. mali, which limits its natural dispersal ability. With low or moderate migration, genetic differentiation between regions and a significant isolation-by-distance effect (Scott et al. 2005) occurred. Additionally, these results suggest a tendency for A. mali immigration from nearby rather than from distant populations in spite of accidental human transportation, a tendency that is consistent with the data concerning gene flow between populations (Table 2).

Invasive species have become serious problems in many regions because of their widespread and large effects on ecosystems, economies, and societies (Lockwood et al. 2007). Unraveling the dispersal pattern of invasive species is crucial for predicting and preventing additional invasions (Lockwood et al. 2007; Wilson et al. 2009). The study of the dispersal pattern of the invasive species is difficult (Chu et al. 2013), however, because the initial establishment of the invasive species can rarely be detected; the invasive species are typically not detected until a substantial time has passed after the initial introduction. Information about the further spread of “invasive” species can be gained, however, by the study of intentionally introduced biological control agents. Such agents provide an opportunity to test the effects of bridgehead populations and further spread because the timing, duration, and intensity of a demographic bottleneck are often well documented (Sakai et al. 2001; Hubbauer & Roderick 2005; Marsico et al. 2009; Franks et al. 2010).

To our knowledge, this is the first study to use the nuclear microsatellite loci to determine the hybridization and gene flow within 2 clades of A. mali in China. The genetic admixture of populations arising from different bridgeheads may help the biological control agent to adapt to the conditions in the introduced regions. The results obtained with an introduced biological control agent suggest that invasion by a species with limited dispersal ability may be facilitated by the establishment of multiple bridgehead populations.

Supplementary material for this article in Florida Entomologist 97(2) (June, 2014) is online at http://purl.fcla.edu/fcla/entomologist/browse. This material includes the following 3 tables of additional supporting information:

**TABLE S1. POPULATION-GENETICS SUMMARY STATISTICS BASED ON ASSIGNMENT OF EIGHT MICROSAT- ELLITE LOCI TO DIFFERENT CLADES IN MIXED POPULATIONS OF APHELINUS MALI IN CHINA.**

**TABLE S2. ALLELE SIZES AT EIGHT MICROSATellite LOCI SCREENED FOR APHELINUS MALI**

**TABLE S3. P VALUES FOR WITHIN-POPULATION TESTS FOR HETEROZYGOSITY EXCESS IN 16 APHELINUS MALI POPULATIONS IN CHINA.**

**ENDNOTES**

Hong-Xu Zhou and Rui-Ming Zhang contributed equally to this work. We are grateful to Dr. Gabor Lovei (Department of Agroecology, Aarhus University) and Blas Lavandero (Instituto de Biología Vegetal y Biotecnología, Laboratorio de Interacciones Insecto-Planta, Universidad de Talca, Chile) for his detailed comments on this manuscript. This work was supported by the National Natural Science Foundation (31371994), the National Key Basic Research Development Plan Project (2013CB127600), the Science and Technology Development Planning Program of Qingdao (13-1-3-108-nsh), the Special Fund for Agro-scientific Research in the Public Interest (201103026-5-2), and the Taishan Mountain Scholar Constructive Engineering Foundation of Shandong, China.

**REFERENCES CITED**


CIOSI, M., MILLER, N. J., KIM, K. S., GIORDANO, R., AND ESTOUP, A. ET AL. 2008. Invasion of Europe by the western corn rootworm, Diabrotica virgifera virgifera: multiple transatlantic introductions with


