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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* (Haldeman) (Hymenoptera: Aphelinidae) in China, which are known as SD clade and LN clade. To further 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 confirman 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

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Aphelinus mali (Haldeman) (Hymenoptera: Aphelinidae), a parasitoid of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) originated from North America

(Beers 2012; Lavandero and Tylianakis 2013). *Aphelinus mali* was introduced into 51 countries during the 20th century to control *E. lanigerum*, and this parasitoid successfully established populations in 42 of these countries (Zhou et al. 2010).

Aphelinus mali was introduced as an endoparasitoid of *E. lanigerum* in China during the 1940s-1960s. The first introduction involved the importation of *A. mali* from Japan to Dalian, Liaoning in 1942 (Long et al. 1960). It was reintroduced from the former Soviet Union into Qingdao, Shandong during 1953-1955 (Long et al. 1960). Since then, *A. mali* has become established in most regions of China including Shandong, Liaoning, Shanxi, Yunnan, Hebei, Henan, and Xinjiang (Zhou et al. 2010; Zhang et al. 2014). Our field survey showed that all of the mitochondrial cytochrome c oxidase subunit I (mt COI) haplotypes grouped into 2 main clades, i.e., SD and LN. The SD clade was mainly distributed in Shandong and neighboring regions while the LN clade was mainly distributed in Liaoning and neighboring regions (Zhang et al. 2014). However, the genetic diversity and genetic structure of the *A. mali* in China based on nuclear markers have not been analyzed. Such investigations may provide knowledge of the hybridization and gene flow between the 2 clades of *A. mali*, which may be helpful in the control of the *E. lanigerum* in China. Currently, microsatellite markers have been extensively used in the analysis of genetic diversity and genetic structure (e.g., Ascunce et al. 2011; Chu et al. 2013; Dickey et al. 2013; Mwacharo et al. 2013).

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.

TABLE 1. SUMMARY POPULATION GENETICS STATISTICS CALCULATED BASED ON 8 MICROSATELLITES OF 16 *APHELINUS MALI* POPULATIONS IN CHINA.

Population code	Locations	N	Na	Ne	Ho	He	Ar	Hs	Nei	Fis
CZ	Changzhi, Shanxi	39	3.875	2.566	0.263	0.585	2.832	0.590	0.578	0.553
JZ	Jinzhong, Shanxi	10	2.250	1.580	0.100	0.276	1.857	0.286	0.263	0.651
YC	Yuncheng, Shanxi	16	2.500	1.918	0.236	0.434	2.186	0.441	0.420	0.464
YL	Yili, Xinjiang	8	2.000	1.504	0.141	0.260	1.967	0.271	0.244	0.424
DL	Dalian, Liaoning	15	3.625	2.068	0.225	0.468	2.583	0.477	0.452	0.528
HLD	Huludao, Liaoning	15	2.625	1.725	0.113	0.367	2.119	0.376	0.354	0.701
SJZ	Shijiazhuang, Hebei	14	2.125	1.634	0.065	0.346	1.893	0.357	0.333	0.820
BD	Baoding, Hebei	10	2.250	1.909	0.025	0.437	2.117	0.462	0.413	0.943
QHD	Qinhuangdao, Hebei	12	2.375	1.700	0.052	0.355	2.006	0.368	0.340	0.859
ZT	Zhaotong, Yunnan	15	1.500	1.320	0.000	0.120	1.386	0.124	0.116	1.000
HZ	Heze, Shandong	15	2.250	1.548	0.008	0.288	1.861	0.298	0.278	0.972
LC	Liaocheng, Shandong	15	3.375	2.139	0.025	0.521	2.614	0.536	0.504	0.937
TA	Taian, Shandong	15	2.500	1.658	0.126	0.311	1.743	0.324	0.306	0.613
WF	Weifang, Shandong	31	4.875	2.805	0.336	0.635	3.137	0.644	0.625	0.466
YT	Yantai, Shandong	20	3.250	2.133	0.256	0.464	2.475	0.470	0.453	0.455
QD	Qingdao, Shandong	15	3.625	2.554	0.233	0.537	2.785	0.548	0.519	0.574
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).

GENEPOP v3.4 (Weir & Cockerham 1984) was used to test for linkage disequilibrium between all pairs of loci and to test for deviation from Hardy-Weinberg equilibrium (HWE) at each locus for each population; this was done with Markov chain parameters of 10,000 dememorization steps, followed by 1,000 batches of 10,000 iterations per batch (Raymond & Rousset 1995) in combination with Fisher's exact tests. The BOTTLENECK software was used to test for deviation from mutation-drift equilibrium (Cornuet & Luikart 1996). To identify the bottleneck event for each population, we investigate the occurrence of significant heterozygosity excess (which is a signature of a bottleneck) within all of the populations within clades under 3 mutational models (TPM, two-phase model of mutation; IAM, infinite alleles model; and SMM, stepwise mutation model). The TPM model was performed with the default settings of 30% variation from the IAM model and 70% from the SMM model.

A regular linear regression of the relationship between expected heterozygosity (*He*) and geographical distance from the nearest likely source population (the region of introduction mentioned in historical reports) was tested using Sigmaplot 12.0. The Google Earth ruler tool (Anderson & Congdon 2013) was used to calculate the Euclidean distance.

Analysis of Genetic Structure and Gene Flow

Differentiation approach based on F_{ST} analysis was used. GENEPOP v3.4 (Raymond & Rousset 1995) was used to calculate Weir and Cockerham's estimator of the fixation index F_{ST} (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 $M = (1/F_{ST} - 1)/2$) (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 expected 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-

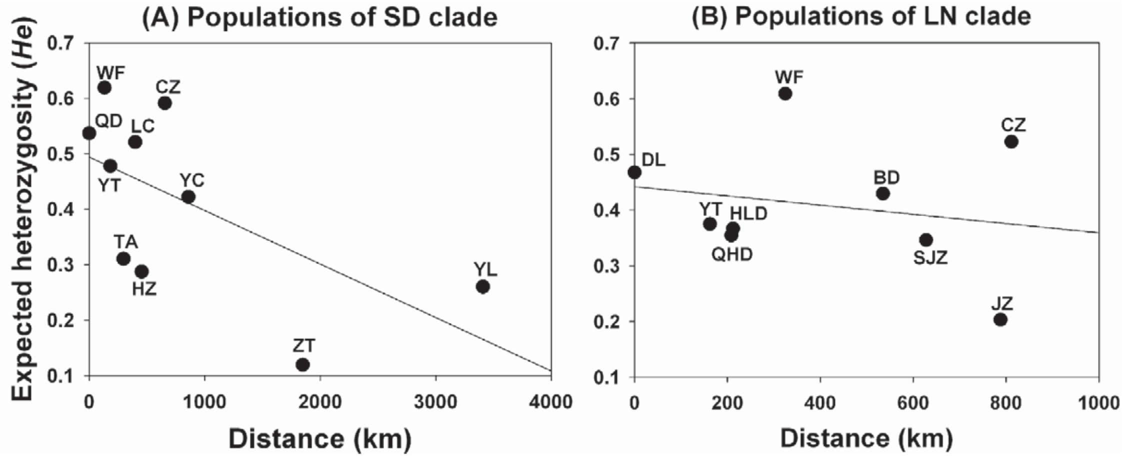

Fig. 1. Linear regression analysis of the relationship between expected heterozygosity (*He*) and log-transformed Euclidean geographical distance from the nearest source population of *Aphelinus mali* in China. Data from mixed populations were separated with respect to the nearest likely source population; 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 = -9.6526 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 = -8.2850 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.

	CZ	JZ	YC	YL	DL	HLD	SJZ	BD	QHD	ZT	HZ	LC	TA	WF	YT	QD
CZ		4.714	10.611	0.677	2.831	1.559	2.443	3.218	1.595	1.097	1.578	6.266	1.798	8.674	4.017	9.500
JZ	**0.096**		3.434	0.217	2.032	1.096	1.970	2.631	1.162	0.308	0.904	3.024	0.937	2.980	1.860	3.337
YC	**0.045**	**0.127**		0.348	1.826	1.137	1.567	2.116	1.079	0.559	0.943	3.510	1.022	3.948	2.803	3.980
YL	**0.425**	**0.698**	**0.590**		0.423	0.336	0.318	0.382	0.303	0.141	0.263	0.494	0.297	0.753	0.412	0.491
DL	**0.150**	**0.198**	**0.215**	**0.542**		1.767	2.987	20.076	14.077	0.398	0.745	1.815	0.779	3.193	1.928	3.274
HLD	**0.243**	**0.313**	**0.305**	**0.598**	**0.221**		1.062	2.071	2.743	0.293	0.463	1.248	0.504	1.702	1.034	1.515
SJZ	**0.170**	**0.202**	**0.242**	**0.611**	**0.143**	**0.320**		6.705	1.842	0.282	0.726	1.874	0.715	3.337	1.144	1.891
BD	**0.135**	**0.160**	**0.191**	**0.567**	**0.024**	**0.195**	**0.069**		39.183	0.275	0.768	2.198	0.832	3.220	1.487	2.881
QHD	**0.239**	**0.301**	**0.317**	**0.623**	**0.034**	**0.154**	**0.214**	**0.013**		0.243	0.499	1.123	0.513	1.747	1.036	1.668
ZT	**0.313**	**0.619**	**0.472**	**0.780**	**0.557**	**0.631**	**0.639**	**0.645**	**0.673**		0.397	0.691	0.449	1.019	0.765	0.707
HZ	**0.241**	**0.356**	**0.346**	**0.655**	**0.402**	**0.519**	**0.408**	**0.394**	**0.501**	**0.558**		2.818	17.616	1.660	0.873	0.948
LC	**0.074**	**0.142**	**0.125**	**0.503**	**0.216**	**0.286**	**0.211**	**0.185**	**0.308**	**0.420**	**0.151**		3.555	9.097	2.749	4.312
TA	**0.218**	**0.348**	**0.329**	**0.627**	**0.391**	**0.498**	**0.412**	**0.375**	**0.494**	**0.527**	**0.028**	**0.123**		1.814	1.078	1.067
WF	**0.055**	**0.144**	**0.112**	**0.399**	**0.135**	**0.227**	**0.130**	**0.134**	**0.223**	**0.329**	**0.232**	**0.052**	**0.216**		4.671	12.095
YT	**0.111**	**0.212**	**0.151**	**0.548**	**0.206**	**0.326**	**0.304**	**0.252**	**0.326**	**0.395**	**0.364**	**0.154**	**0.317**	**0.097**		13.585
QD	**0.050**	**0.130**	**0.112**	**0.505**	**0.133**	**0.248**	**0.209**	**0.148**	**0.231**	**0.414**	**0.345**	**0.104**	**0.319**	**0.040**	**0.036**	

Significant values for pairwise F_{ST} are in bold. Gene flow was approximated as *Nm* (analogous to $M = (1/F_{ST} - 1)/2$).

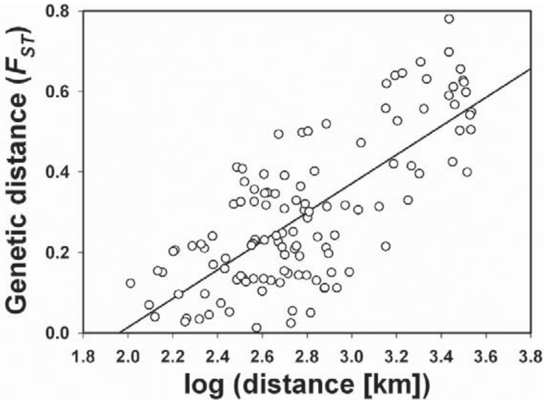

Fig. 2. Relationship between genetic distance and logarithm of geographical distance based on all possible pairs of 16 Chinese populations of *Aphelinus mali*. Google Earth ruler tool was used to calculate the Euclidean geographical distances. Reduced Major Axis regression line is shown ($Y = 0.3568 X - 0.6997$, $R = 0.7479$, $F = 149.7562$, df = 1, $P < 0.0001$).

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

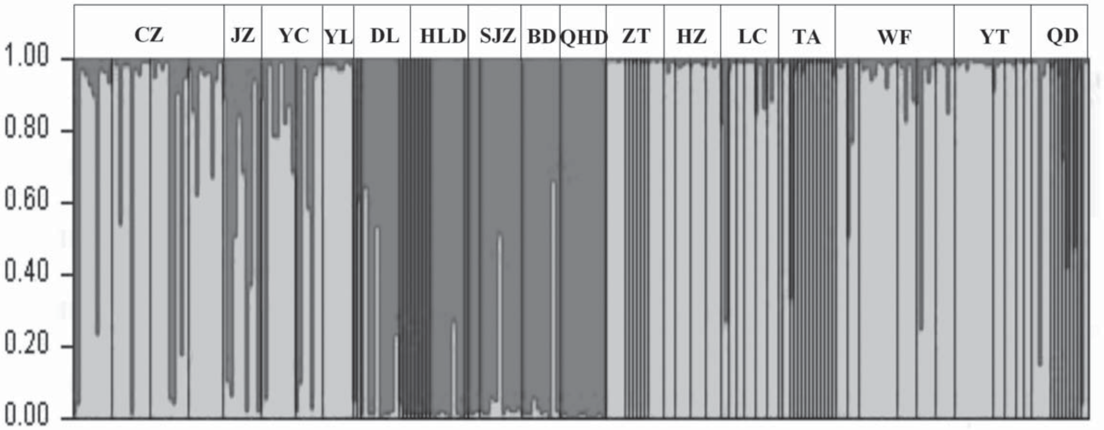

Fig. 3. Bayesian clustering analysis of 16 *Aphelinus mali* populations in China using STRUCTURE (here $K = 2$). Each individual is represented by a vertical bar displaying membership coefficients to each genetic cluster. Population codes (City, Province): CZ, Changzhi, Shanxi; JZ, Jinzhong, Shanxi; YC, Yuncheng, Shanxi; YL, Yili, Xinjiang; DL, Dalian, Liaoning; HLD, Huludao, Liaoning; SJZ, Shijiazhuang, Hebei; BD, Baoding, Hebei; QHD, Qinhuangdao, Hebei; ZT, Zhaotong, Yunnan; HZ, Heze, Shandong; LC, Liaocheng, Shandong; TA, Taian, Shandong; WF, Weifang, Shandong; YT, Yantai, Shandong; QD; and Qingdao, Shandong

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, F_{ST} 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; Hufbauer & 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 MICROSATELLITE 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

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