Patterns of Genetic Variation Among Populations of the Asian Longhorned Beetle (Coleoptera: Cerambycidae) in China and Korea

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Ann. Entomol. Soc. Am. 102(5): 895–905 (2009)

ABSTRACT Central to the study of invasive species is identifying source populations in their native ranges. Source populations of invasive species can provide important information about species life cycles, host use, and species-specific predators and parasites that could be deployed in a biological control program. The Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is a serious invasive pest of urban forests in North America and Europe. We analyzed mitochondrial DNA haplotype frequencies and microsatellite allele frequencies to characterize phylogeographical patterns in potential source populations of *A. glabripennis*. The data suggest that although there is evidence of genetic structure within and among populations in China, a substantial amount of genetic admixture has occurred. This admixture is probably due to recent reforestation efforts in China where beetles from previously isolated populations moved into new areas of abundant, susceptible, and stressed resources, leading to outbreak populations.

KEY WORDS *Anoplophora glabripennis*, Asian longhorned beetle, Asia, mitochondrial DNA, microsatellites

Central to the study of alien species is the identification of populations in the natural range that have been the source of invasions (Tsutsui et al. 2000). Determining source populations can inform us about traits that allow invasive species to multiply and expand their ranges (Suarez and Tsutsui 2008). Identification of source populations also motivates searches for successful biological control agents and can elucidate ways to alter the invaded environment through antibiosis and resistance effects (Sakai et al. 2001, Lieutier 2006). Polymorphic molecular markers provide the genetic tools to assess population variation, both in native source populations and in newly established alien populations (Cox 2004).

The Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is an endemic cerambycid in China and Korea (Lingafelter and Hoebeke 2002). In Asia, this beetle is a generalist attacking deciduous tree species primarily of three genera—*Populus*, *Acer*, and *Salix* (Pan 2005)—although in the United States, many other hosts are acceptable (Sawyer 2003). Adult females deposit eggs singly under the bark of the trunk and main branches of trees (Cavey et al. 1998). Upon hatching, larvae (first to third instars) feed in the inner bark–sapwood interface for 2 to 4 wk and then tunnel into the sapwood and heartwood where they feed for 1 to 2 yr, destroying the vascular tissue (Keena 2005). Newly eclosed adults stay in the tree for several days before chewing out an exit hole (Lingafelter and Hoebeke 2002). Oviposition typically peaks in July and August, with subsequent adult emergence in June to August 1 to 2 yr later. Therefore, generation time from egg to adult emergence typically ranges from 12 to 24 mo (Li and Wu 1993). Asian longhorned beetles disperse through adult flight (Smith et al. 2004); by transport along waterways; on animals or vehicles; or by being moved as eggs, larvae, or pupae in cut wood (Pan 2005).

The history of Asian longhorned beetle invasive population outbreaks is correlated with an expanding economy in China and an exponential increase in shipments of manufactured goods from China to North America and Europe (Normile 2004). Asian longhorned beetle is presumed to have been transported in solid wood packing material used to crate goods for shipping from China (Bartell and Nair 2003). In North America and Europe, Asian longhorned beetle spread has resulted in populations in metropolitan areas of Japan, New York, New Jersey, Illinois, Massachusetts, Canada, Austria, France, Germany, and Italy (Haack et al. 1996, Sellers 2004, Takahashi and Ito 2005, Hérard et al. 2006, Maspero et al. 2007, USDA–APHIS 2008). In the United States, it has been estimated that 1.2 billion city trees, worth an estimated $669 billion, are at risk of dying (Nowak et al. 2001).

To understand and combat invasive populations in North America, it is important to know the genetic structure of potential source populations of Asian

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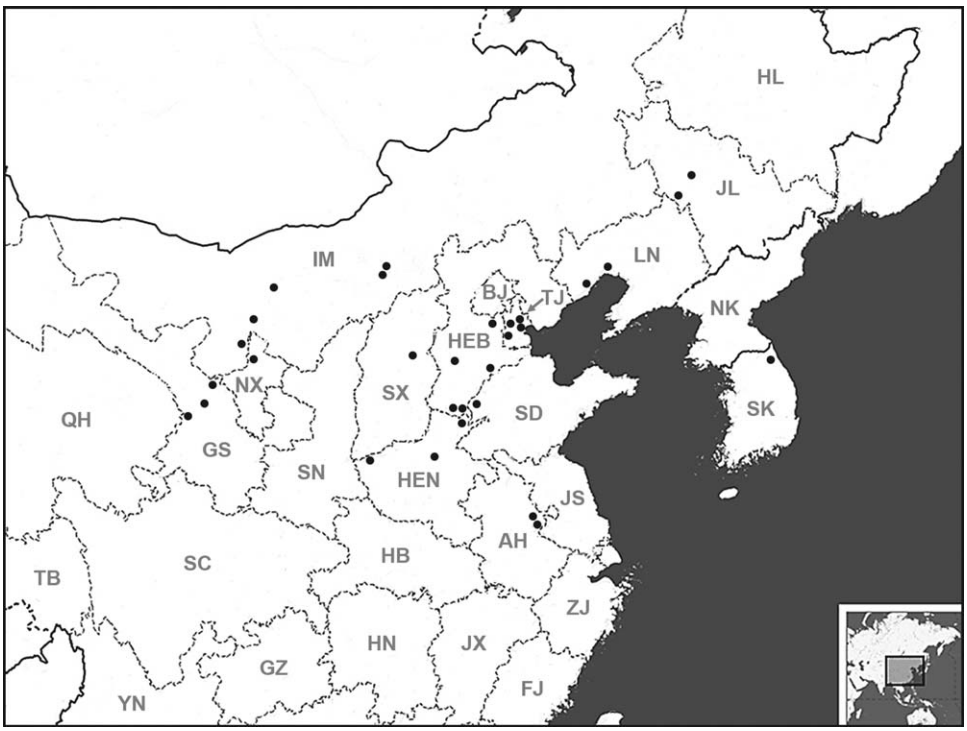

Fig. 1. Collection sites of Asian longhorned beetle in China and Korea used in this study. The map is an enlarged view of the cutout of the map of the Asian continent shown in the lower right corner. Each dot is one site and the dots follow the North and East coordinates given in Table 1. AH, Anhui; BJ, Bejing; FJ, Fujian; GS, Gansu; GZ, Guizhou; HB, Hubei; HEB, Hebei; HEN, Henan; HL, Heilongjiang; HN, Hunan; IM, Inner Mongolia; JL, Jilin; JS, Jiangsu; JX, Jiangxi; LN, Liaoning; NK, North Korea; NX, Ningxia; QH, Qinghai; SC, Sichuan; SD, Shandong; SK, South Korea; SN, Shaanxi; SX, Shanxi; TB, Tibet; TJ, Tiajin; YN, Yunnam; ZJ, Zhejiang.

longhorned beetle in Asia. A previous study characterizing populations of Asian longhorned beetle with random amplified polymorphic DNA (RAPD) markers showed six geographical populations from different provinces of China grouped into two clusters (An et al. 2004). Here, we examined microsatellite allele frequencies and mitochondrial DNA sequence data to reveal genetic population structure and to reconstruct genealogical relationships among beetles collected from multiple sites in China and one site in Korea (Roderick 2004, Wares et al. 2005). Microsatellites are presumably neutral, highly polymorphic markers that have been used to infer recent population history (Schlotterer 2004). As a maternally inherited, nonrecombining, rapidly evolving DNA marker, mitochondrial DNA has been used to characterize population structure and to produce gene genealogies and phylogenies (Ballard and Whitlock 2004).

Methods and Materials

Sample Collection. Collection sites are shown in Fig. 1, and numbers of beetles from each site used in this study are listed in Appendix A. Because only one or a small number of beetles were collected at some sites, all beetles from sites within the same province were pooled for all analyses. We chose to pool beetles by province to provide a low resolution view of geographical population structure; in all cases, except for sites in Inner Mongolia, collecting localities within a province were in proximity to each other. The majority of samples were collected from rural landscapes adjacent to agricultural fields or along dirt roads. Other sites were large shelterbelts; the Yanji site in Jilin province was urban and one Liaoning site (Ling-Hai) was a large second-growth, low-diversity forest with only a few tree species. A subset of samples is vouchered in the Cornell University Insect Collection, lot 1262.

Mitochondrial DNA Sequencing. Specimens of Asian longhorned beetle were collected alive and placed in 95% alcohol. A minority of specimens were dried and pinned. Total DNA was extracted using DNeasy tissue kits (QIAGEN, Valencia, CA). DNA was extracted from adult beetles, and immature larvae, by removing thoracic muscle tissue from adults, or a segment of the immature abdomen.

We sequenced 1,313 bp of cytochrome *c* oxidase 1, 65 bp of t-RNA-leucine, and 198 bp of cytochrome *c* oxidase 2 mitochondrial DNA genes. Polymerase chain reaction (PCR) and sequencing primers and optimized annealing temperatures are given in Table 1. Cycling was an initial denaturing at 95°C for 15 min, followed by 35 cycles of denaturing at 95°C, annealing

Table 1. Primer sequences and PCR annealing temperatures for amplification and sequencing of 1576 bp of mitochondrial DNA

Primer no.	Sequence	Annealing temp. (°C)
4S	5′-TGTAACAGCCCATGCATTCATCATAAT	58
4A	5′-GCTAAGACTACTCCTGTTAATCCTCCA ACTG	58
7S	5′-AATGATTCCCCTTATTTACAGGATTGA	56
7A	5′-CAAGAAATATAAATTAAACGTAAAGAA GGTAGAG	56
12A	5′-TAAAGCCGGTACTTCAAAATATCTATG TTCTG	57
10S	5′-CTATTGGCCTATTAGGATTTGTAGTCT GAGC	57

Table 2. Mitochondrial DNA sequence polymorphism statistics by population

Province	No. sequences	h[a]	Hd[b]	Π[c]	κ[d]	θ[e]
Anhui (AH)	11	7	0.873	0.00835	13.164	0.01018
Gansu (GS)	22	13	0.905	0.00789	12.433	0.00627
Hebei (HEB)	20	9	0.853	0.00282	4.447	0.00322
Henan (HEN)	7	3	0.524	0.00212	3.333	0.00285
Jilin (JL)	5	5	1.000	0.00698	11.000	0.00609
Korea (SK)	17	6	0.588	0.00122	1.926	0.00206
Liaoning (LN)	8	4	0.821	0.00714	11.250	0.00563
In. Mongolia (IM)	19	4	0.696	0.00561	8.842	0.00381
Tianjin (TJ)	11	2	0.182	0.00012	0.182	0.00022

[a] Haplotype number.
[b] Haplotype diversity.
[c] Nucleotide diversity.
[d] Average number of nucleotide differences.
[e] Watterson estimator (per site).

at the optimal annealing temperature, and extension at 72°C for 1 min, followed by a final elongation step of 4 min at 72°C. Each reaction was run in a volume of 14 μl and contained 6.7 μl of water, 1.5 μl of PCR 10× buffer (20 mM Tris-HCl, pH 8.4, 500 mM KCl [Invitrogen; Carlsbad, CA]), 3 μl of 2.5 mM dNTP, 0.75 μl of 50 mM $MgCl_2$, 0.06 μl of *Taq*DNA polymerase (Invitrogen), 0.6 μl of primers at 10 mM, and 20 ng of DNA template. PCR products were cleaned with PCR Mini-elute kits (QIAGEN). Each PCR product was sequenced in both directions using BigDye Chemistry (Applied Biosystems, Foster City, CA). Primer overlap was such that each nucleotide site had 2 to 6 times coverage. Sequencing reactions were run in a total volume of 5 μl with 1.88 μl of H_2O, 0.25 μl of 1 M betaine (Sigma, St. Louis, MO), 1 μl of Ready Reaction (Applied Biosystems), and 1 μl of 5× buffer (Applied Biosystems), 0.12 μM PCR primer, and 1 μl of PCR products. Sequencing reactions were cleaned with Sephadex (Sigma) and directly sequenced on an Applied Biosystems 3730 Sequencer. Sequences were assembled into contiguous arrays, edited, trimmed down to 1,576 bp without gaps, and aligned using Seqman and Megalign (Lasergene 7, DNASTAR, Madison, WI).

Beetles were grouped by province to form "populations." To construct phylogenetic trees, the DNA sequence data set was reduced to the 37 unique haplotypes that we observed. Sequence polymorphism statistics (haplotype number, haplotype diversity, nucleotide diversity, average number of nucleotide differences, and Watterson estimator) for each population with more than one haplotype were calculated using DNASP 4.0 (Table 2; Rozas et al. 2003). Bayesian analysis was performed using Mr. Bayes (Huelsenbeck et al. 2001, Ronquist and Huelsenbeck 2003) with a substitution model of GTR+I+G (general time reversible model with gamma-distributed rate variation across sites and a proportion of invariable sites). The program was run for 1,000,000 generations, sampling every 100 generations and using the default settings for priors, with a burn in of the first 25% of the total number of generations. At 1,000,000 generations the log-likelihood values stabilized and converged from the two runs, the split frequencies approached zero, and the potential scale reduction factor values were close to zero. Program runs at 3,000,000 generations gave the same results. The consensus tree was opened and annotated in TreeView (Page 1996). The tree was rooted with mitochondrial DNA sequence from a related species, *Anoplophora chinensis* (Forster). This sequence was produced using the same PCR and sequencing conditions and primers described above and in Table 1. For nine populations where $n \geq 3$ (Anhui, Gansu, Hebei, Henan, Jilin, Korea, Liaoning, Inner Mongolia, and Tianjin), population genetic structure also was inferred by an analysis of molecular variance (AMOVA; Excoffier et al. 1992) in Arlequin 3.1 (Excoffier et al. 2005).

Microsatellites. In total, 146 Asian longhorned beetles (Appendix A) were genotyped at 15 microsatellite loci with a previously published method (Carter et al. 2009). Primers for one additional locus (8921; GenBank accession DR108921) are GGCCTATTTTGATGCGAGTG (forward) and GGCACTACCTGCTACACAGC (reverse), fluorescently labeled with PET (Applied Biosystems). Amplification of this locus was accomplished using the previously published method. In brief, PCR reaction volumes were 10 μl with 20 ng of DNA in 2.0 μl, 1.7 μl of water, 1 μl of 10× PCR buffer, 2.0 μl of 5 M betaine, 0.2 μl of 10 mM dNTP mix, 0.1 μl of *Taq* polymerase in 0.1 μl, 1 μl each of the reverse specific primer and the tag sequence primers at 3.2 pmol/μl, and 1 μl of the forward specific primer at 0.8 pM. Eight standard DNA templates were run on each 96-well plate to check the accuracy of allele migration. Microsatellite data were collected and binned with Genemapper 4.0 (Applied Biosystems), and allele calling was checked by eye. Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were tested for each locus in Genepop (Raymond and Rousset 1995). Diversity information was calculated in PowerMarker (Liu and Muse 2005) and included mean genotype number, mean allele number, major allele frequency, and expected and observed heterozygosity. Allelic richness was calculated in FSTAT (Goudet 1995) for populations where $n \geq 3$. Pairwise F_{ST} values for all pairs of populations and Nei's average number of pairwise differences within and between popula-

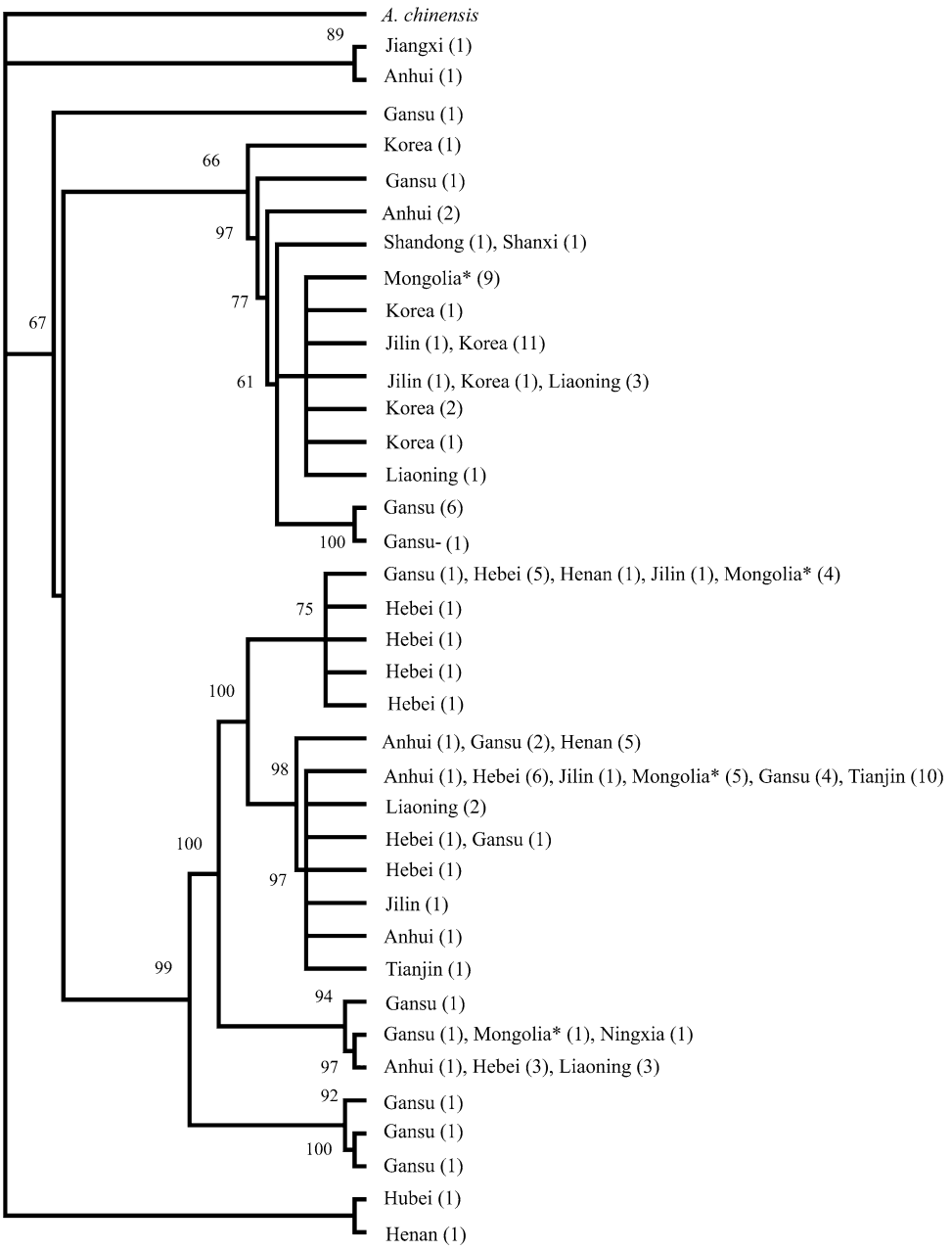

Fig. 2. Bayesian analysis consensus tree of 37 mitochondrial DNA haplotypes of Asian longhorned beetles from China and Korea. Each haplotype is a branch and is labeled with the name(s) of the collection site(s) and the number of beetles collected at those sites. Numbers adjacent to branch points are clade probability values. The asterisk (*) indicates the province of Inner Mongolia in China. *A. chinensis* is the outgroup.

tions where $n \geq 3$ were generated in Arlequin 3.1 (Nei and Li 1979, Excoffier et al. 2005), and the significance of the derived genetic distances tested with 16,000 permutations. Population genetic structure (equating province with "population") was inferred by an AMOVA in Arlequin 3.1 for 10 populations where $n \geq 3$ (Anhui, Gansu, Hebei, Henan, Jilin, Korea, Liaoning, Mongolia, Shanxi, and Tianjin).

We used a Bayesian clustering method to infer population structure without a priori characterization of groups. We implemented the program *structure* (Pritchard et al. 2000, Falush et al. 2003) to calculate the probability of assigning each individual to K hypothetical clusters, where K is initially unknown (admixture model, allele frequencies correlated). Each run was carried out for 1,000,000 generations with a burnin of 50,000 generations. This was done for $K =$

Table 3. The number of beetles by pop for the 37 mitochondrial DNA haplotypes

Haplotype no.	Pop[a]													
	SK	IM	GS	AH	JL	LN	NX	SX	TJ	HEB	HEN	JX	HB	SD
Haplotype 1				1										
Haplotype 2												1		
Haplotype 3			6											
Haplotype 4			1											
Haplotype 5			1											
Haplotype 6													1	
Haplotype 7		1	1				1							
Haplotype 8			1											
Haplotype 9						1								
Haplotype 10	2													
Haplotype 11	1													
Haplotype 12										1				
Haplotype 13				4		2				3				
Haplotype 14			1											
Haplotype 15	11				1									
Haplotype 16										1				
Haplotype 17		9												
Haplotype 18						2								
Haplotype 19										1				
Haplotype 20	1				1	3								
Haplotype 21										1				
Haplotype 22								1						1
Haplotype 23				2										
Haplotype 24			1											
Haplotype 25									1					
Haplotype 26			1							1				
Haplotype 27			1											
Haplotype 28											1			
Haplotype 29					1									
Haplotype 30										1				
Haplotype 31		4	1		1					5	1			
Haplotype 32	1													
Haplotype 33			2	1							5			
Haplotype 34			1											
Haplotype 35		5	4	1	1				10	6				
Haplotype 36	1													
Haplotype 37				1										

[a] SK, Korea; IM, Inner Mongolia; GS, Gansu; AH, Anhui; JL, Jilin; LN, Liaoning; NX, Ningxia; SX, Shanxi; TJ, Tianjin; HEB, Hebei; HEN, Henan; JX, Jiangxi; HB, Hubei; SD, Shandong.

1–10 by using 10 consecutive runs for each value of K, where K is the inferred number of genetic subpopulations or clusters. Following the method of Evanno et al. (2005), we calculated ΔK, based on the second order rate of change in the log probability of data with respect to the number of population clusters. From the value of $\Delta K = 2$, population substructure was indicated. We chose the run with the greatest likelihood at $K = 2$ to assign individuals into two subpopulations and we analyzed those groups separately in *structure* to determine the best estimate of K. We then used the *structure* admixture analysis to assign the proportion of each beetle's genotype originating from each hypothetical cluster.

Results

Mitochondrial DNA Sequence Data. Sequences were obtained from DNA of 123 beetles (Appendix A). Out of 1,576 bp of mitochondrial DNA sequence (1,313 bp of cytochrome *c* oxidase 1, 65 bp of t-RNA-leucine, and 198 bp of cytochrome *c* oxidase 2), 65 sites were variable (Appendix B). The topology of the Bayesian analysis consensus tree indicated that some clades were well supported (clade probabilities >95%) with clade probabilities for most major clades >65% (Fig. 2). Twenty-eight haplotypes were unique to a single population, and nine haplotypes were found in multiple populations (Table 3). Haplotypes from single populations (e.g., Anhui and Gansu; Table 2) occurred throughout the mitochondrial DNA tree, although clustering of haplotypes by geography was evident in a few cases. A significant (33.75%; $P < 0.01$) proportion of variation could be attributed to differences among populations. However, a greater proportion of the variation (66.25%; $P < 0.01$) was explained by haplotype diversity within populations.

Microsatellites. For most microsatellite loci, the frequency of the most common allele was greater than or equal to 0.5, and observed heterozygosity was lower than the expected heterozygosity (Table 4). We tested the data as two populations, China and Korea, for Hardy–Weinberg equilibrium and found heterozygote deficiency in 24 of 32 comparisons for the 16 loci (75%; with sequential Bonferonni correction). Heterozygote deficiencies could be due to the Wahlund effect, the lumping of populations with different allele frequencies. However, other causes (e.g., null

Table 4. Combined diversity information by pop for Asian longhorned beetles genotyped at 16 microsatellite loci

Pop[a]	n^b	$N_A{}^c$	MAF[d]	$H_E{}^e$	$H_O{}^f$	AR[g]
Anhui (AH)	13	3.13	0.60	0.50	0.31	1.47 (8)
Gansu (GS)	28	5.00	0.60	0.51	0.31	1.50 (19)
Hebei (HEB)	28	4.44	0.65	0.45	0.29	1.46 (20)
Heilongjiang (HL)	1	1.13	0.94	0.06	0.13	
Henan (HEN)	9	3.44	0.59	0.54	0.30	1.53 (7)
Hubei (HB)	1	1.13	0.94	0.06	0.13	
In. Mongolia (IM)	27	4.25	0.66	0.47	0.27	1.47 (19)
Jiangxi (JX)	1	1.19	0.91	0.09	0.19	
Jilin (JL)	9	3.00	0.69	0.41	0.24	1.44 (5)
Korea (SK)	11	2.75	0.79	0.31	0.06	1.05 (5)
Liaoning (LN)	9	3.06	0.64	0.47	0.36	1.47 (4)
Ningxia (NX)	1	1.13	0.94	0.06	0.13	
Shandong (SD)	1	1.25	0.88	0.13	0.25	
Shanxi (XS)	3	2.25	0.70	0.37	0.29	1.45 (2)
Tiajin (TJ)	12	3.50	0.64	0.46	0.35	1.44 (9)

[a] Population abbreviations are in parentheses.
[b] Number of beetles genotyped.
[c] Mean number of alleles.
[d] Mean major allele frequency.
[e] Expected heterozygosity.
[f] Observed heterozygosity.
[g] Allelic richness (number of beetles sampled).

alleles) might also contribute. Significant ($P < 0.05$ with Bonferonni's correction) linkage disequilibrium was not found for any locus pair for either data set. The AMOVA based on pairwise comparisons of F_{ST} values showed that a significant percentage of the variation (13%; $P \leq 0.01$) could be attributed to differences among populations, but that most variation (87%; $P < 0.01$) was found within populations. Pairwise F_{ST} values (Table 5) showed a number of similarities to the *structure* analysis (see below; Fig. 3), e.g., Korea beetles are very different from those found in Chinese populations.

The results from the *structure* analysis, with $K = 4$ hypothetical clusters, are shown in Fig. 3. Generally, beetles from the northwestern provinces of Gansu and Ningxia ($n = 1$) exhibit ancestry from the "yellow population." Beetles from the north central provinces of Inner Mongolia, Shanxi, Hebei, Tianjin, and Liaoning populations seem derived from the "green population". Beetles in the central provinces of Henan, Anhui, Hubei ($n = 1$), Jiangxi ($n = 1$), and Shandong ($n = 1$) populations show ancestry predominantly from the "blue population." Beetles from the north eastern provinces of Jilin and Heililongjiang ($n = 1$) and from South Korea are of "red population" ancestry.

Discussion

Differences among Asian longhorned beetle populations in mitochondrial DNA haplotype distributions and microsatellite allele frequencies are evident, but neither the mitochondrial DNA tree nor the *structure* analysis allows clear discrimination of a beetle's origins. Nine of the mitochondrial DNA haplotypes are found in multiple populations, and haplotypes from single populations are distributed across the mitochondrial DNA tree. Most populations include beetles from numerous mitochondrial DNA lineages, and many of these beetles seem to be of mixed ancestry with respect to the 16 microsatellite loci. Thus, patterns of genetic variation at our sampling sites suggest that the population structure of Asian longhorned beetle in Asia has been influenced by movement of beetles and consequent genetic admixture. In part, these patterns may reflect the considerable dispersal abilities of individual beetles (Smith et al. 2004). However, they are also consistent with what we know of the history of changes in Asian longhorned beetle distribution in China as a result of recent reforestation efforts. Our results are similar, but not identical to, the previous study using RAPD markers which found beetles from Shaanxi, Shandong, Hebei, Mongolia, and Ningxia provinces forming one group separate from Gansu province (An et al. 2004).

To combat expanding desertification, China has undertaken the largest reforestation program in the world with five major projects (Li 2004, Weilun and Wen 2005). The goals of these projects are to plant trees in belts to shelter agricultural land from the strong north-westerly winds, to control water and soil erosion, to provide firewood, timber and paper products, to decrease damage to Beijing by sandstorms, and to convert underproducing farmlands and grasslands to forests (Moore and Russell 1990). National forest cover in China has increased with plantings of plantation-style forests, whereas natural forests have declined to 30% of the total forested area (Li 2004). Although Asian longhorned beetle in China has been

Table 5. Pairwise F_{ST} values calculated from 16 microsatellite markers

Pop[a]	AH	GS	HEB	HEN	JL	LN	IM	SX	TJ
GS	0.125[b]								
HEB	0.114[b]	0.115[b]							
HEN	0.046	0.099[b]	0.097[b]						
JL	0.141[b]	0.102[b]	0.100[b]	0.100					
LN	0.107[b]	0.118[b]	0.036	0.084	0.054				
IM	0.128[b]	0.079[b]	0.028	0.095[b]	0.066	0.065			
SX	0.182	0.130	0.052	0.151	0.135	0.056	0.045		
TJ	0.075[b]	0.131[b]	0.050	0.085	0.152[b]	0.077[b]	0.071[b]	0.102	
SK	0.411[b]	0.303[b]	0.318[b]	0.369[b]	0.234[b]	0.304[b]	0.284[b]	0.431	0.418[b]

[a] AH, Anhui; GS, Gansu; HEB, Hebei; HEN, Henan; IM, Inner Mongolia; JL, Jilin; LN, Liaoning; NX, Ningxia; SK, South Korea; SX, Shanxi; TJ, Tiajin.
[b] Significance values, $p < 0.001$.

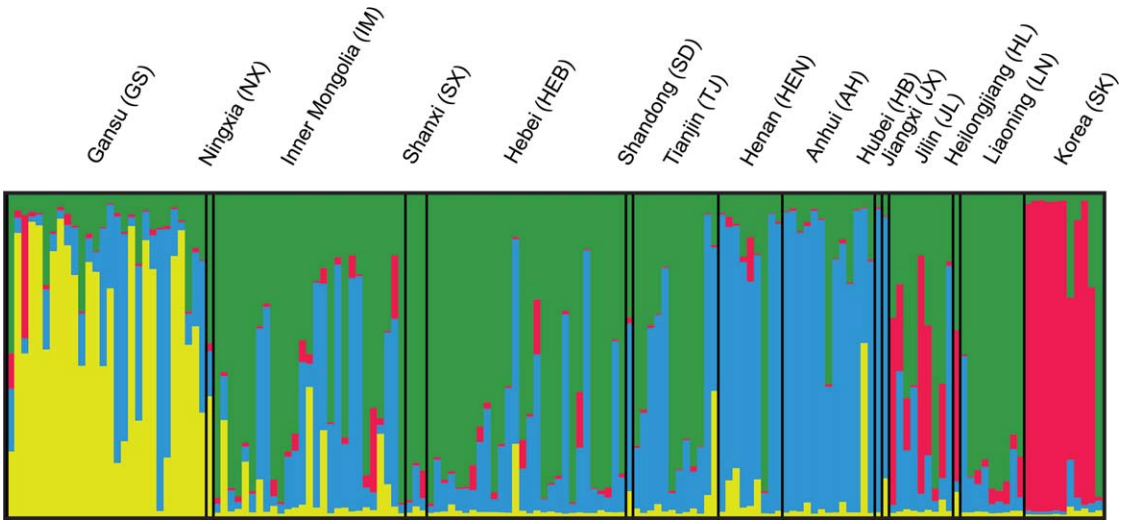

Fig. 3. Summary plot of the *structure* analysis of 16 microsatellite markers with $K = 4$ hypothetical clusters. Each individual Asian longhorned beetle is represented by a single vertical line broken into K colored segments, with lengths proportional to membership coefficients in each of the K inferred clusters. The labels above indicate the site (province of China or Korea) where beetles were collected.

known from the Qing dynasty, it was not a serious pest in China until the first outbreak populations occurred in Ningxia province in the early 1980s (Pan 2005). Initially, the Chinese planted fast-growing larch (*Larix* sp), willow (*Salix* sp.), and poplar (*Populus* sp.), in monoculture, and one outcome was insect infestations (Weilun and Wen 2005). With the advent of shelterbelt plantings of preferred host plant species, Asian longhorned beetle has spread geographically. *Populus tremula* L. was one of the tree species planted in monoculture and 15,000 ha/yr of *P. tremula* plantations have become infested with Asian longhorned beetle (including *Anoplophora nobilis* Ganglbauer) in northern China (Cao 2008). Because the decline of natural forests in China has been primarily due to tree cutting by local populations (Li 2004), and the subsequent transport of infested wood to homes and sawmills near shelterbelt areas, Asian longhorned beetle spread has been greatly facilitated. Many factors likely contributed to population growth and expansion, including few natural enemies in the newly-forested areas, poor tree growth due to environmental stresses and ineffective control, monitoring and quarantine at the local level, thereby allowing beetles to be transported great distances away from the foci of infestations (Pan 2005). Proliferation of edge habitats, as in the blocks of forests planted in the shelterbelts in China, is known to be a factor leading to an increased abundance of invasive species migrating into disturbed habitats (Didham 1997) and probably also contributed to Asian longhorned beetle outbreak status.

Asian longhorned beetle also occurs in nonoutbreak populations in Korea (Lingafelter and Hoebeke 2002, Williams et al. 2004). In Korea, Asian longhorned beetle is found at low densities and is limited primarily to native *Acer* species, on trees found at forest edges (Williams et al. 2004). An analysis of macrofossils and pollen data show a rich flora of present day dicotyledons and conifers, including *Acer* species that have had a long, continuous history on the Korean peninsula (Kong 2000). The diversity of tree species in Korean forests is suggested as one reason for the absence of outbreak populations there (Williams et al. 2004).

Thus, our results suggest that the likely source populations for Asian longhorned beetle in North America may be invasive populations themselves, with a high proportion of within population genetic variation. It is possible that among population variation, if found in historically separate populations of Asian longhorned beetle in natural forests, could have been transformed, to some extent, into within population variation through recent admixture. However, we were unable to sample the older natural forests, which would provide insight into historical population structure. It is generally assumed that the most efficient biological control agents should be selected from among those at sites that represent the geographic origin of the host species (Hufbauer and Roderick 2005). Our microsatellite data analysis revealed some geographical structure in observed patterns of variation, but many haplotypes and genotypes were found at multiple sites in eastern Asia. We suggest that it may be possible to identify source populations of alien Asian longhorned beetles from specific outbreak areas in Asia by sampling and analyzing a greater number of specimens from additional sites using more nuclear DNA markers. In addition, the considerable genetic variation revealed by our sequencing and genotyping efforts may well allow us to determine whether populations in North America and Europe have common or independent origins.

Acknowledgments

We thank the following people for contributing samples for analysis: T. Dubois, R. Haack, A. Hajek, F. Hérard, E. R. Hoebeke, M. Keena, P. Schaefer, B. Wang, and Y.-L. An. We thank J. Barnett, A. Lang, and K. Loeffler for technical assistance. This work was supported by a gift from Howard P. Milstein and Leonard Litwin and USDA specific cooperative agreement 58-1926-6-606 to R.G.H. and M.G.S.

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Received 3 February 2009; accepted 8 May 2009.

Appendix A. Collecting site information and numbers of Asian longhorned beetles analyzed for mitochondrial DNA and microsatellite markers

Pop.[a]	Nearest city and/or county[b]	n^c (DNA)	n^d (SSR)	North[e]	East[e]	Accession no.
Anhui 1 (AH)	WuHu	3	2	31° 20′	118° 21′	EU914698, EU914764, EU914767
Anhui 2	Bengbu	8	11	32° 56′	117° 22′	EU914716, EU914717, EU914718, EU914719 EU914720, EU914721, EU914722, EU914794
Gansu 1 (GS)	Liu Hua/YO	3	3	35° 57′	103° 16′	EU914759, EU914965, EU914766
Gansu 2	Jingtai	9	12	37° 21′	104° 03′	EU914699, EU914700, EU914701, EU914702 EU914703, EU914704, EU914705, EU914706 EU914707
Gansu 3	Lanzhou/GA	10	13	36° 19′	103° 56′	EU914743, EU914744, EU914745, EU914746 EU914747, EU914748, EU914749, EU914750 EU914751, EU914752
Hebei 1 (HEB)	Langfang City	11	14	39° 30′	116° 40′	EU914714, EU914715, EU914723, EU914724 EU914725, EU914737, EU914738, EU914739 EU914740, EU914741, EU914742
Hebei 2	HanDan/DA	2	3	36° 16′	115° 08′	EU914785, EU914805
Hebei 3	HanDan/CH	2	2	36° 26′	114° 21′	EU914786, EU914787
Hebei 4	Chengguan/WU	3	3	37° 36′	116° 29′	EU914788, EU914789, EU914806
Hebei 5	Shiziazhuang/WJ	2	3	38° 10′	114° 57′	EU914790, EU914807
Heilongjiang[f] (HL)		0	1			
Henan 1 (HEN)	Zhengzhou/NE	2	1	34° 45′	113° 38′	EU914687, EU914758
Henan 2	Zhengzhou/SM	3	3	35° 54′	115° 06′	EU914791, EU914792, EU914810
Henan 3	Zhengzhou/NE	2	3	34° 46′	111° 11′	EU914793, EU914811
Henan 4[f]		0	1			
Jiangxi[f] (JX)		0	1			
Jilin 1 (JL)	Lishe	5	5	43° 18′	124° 19′	EU914768, EU914769, EU914770, EU914771 EU914772
Jilin 2	Changchun	0	2	45° 52′	125° 18′	
Jilin 3	Yanji	0	1	42° 52′	129° 30′	
Liaoning 1[f] (LN)		2	2			EU914726, EU914727
Liaoning 2	LingHai	3	3	41° 09′	121° 22′	EU914773, EU914774
Liaoning 3	XingCheng	3	4	40° 36′	120° 22′	EU914775, EU914776, EU914777
In. Mg. 1[g] (IM)	Huhehaote	9	10	40° 48′	111° 38′	EU914694, EU914695, EU914696, EU914697 EU914754, EU914755, EU914761, EU914762 EU914763
In. Mg. 2	Wuhai/TU	5	10	39° 39′	106° 47′	EU914708, EU914709, EU914710, EU914711 EU914712
In. Mg. 3	Hasa/AL	2	3	40° 50′	111° 50′	EU914798, EU914799
In. Mg. 4		3	3	38° 49′	105° 41′	EU914795, EU914796, EU914797
In. Mg. 5	Bameng/DE	0	1	40° 37′	107° 08′	
Ningxia (NX)	Qingtongxia/QI	1	1	37° 52′	105° 57′	EU914760
Tianjin 1 (TJ)	Datian/HA	3	3	39° 14′	117° 48′	EU914778, EU914779, EU914801
Tianjin 2	Chadian/HA	3	3	39° 14′	117° 45′	EU914780, EU914781, EU914802
Tianjin 3	Jinghai/JI	2	3	38° 56′	116° 54′	EU914782, EU914803
Tianjin 4	Ninghe/YU	3	3	39° 19′	117° 47′	EU914783, EU914784, EU914804
Shanxi (SX)	Taiyuan/DI	1	1	38° 18′	112° 41′	EU914808
Shandong (SD)	Guanxian/BA	1	1	36° 28′	115° 26′	EU914809
Korea (SK)	Sokcho	17	11	38° 07′	128° 27′	EU914690, EU914691, EU914692, EU914693 EU914713, EU914728, EU914729, EU914730 EU914731, EU914732, EU914733, EU914734 EU914735, EU914736, EU914753, EU914756 EU914757

[a] Province followed by province abbreviations.

[b] Autonomous city or County. County abbreviations are as follows: YO, Yongjing; GA, Gao Lan; DA, Daming; CH, Chengan; WU, Wujiao; WJ, Wuji; NE, Neihuang; LI, LingHai; AL, Alashanmengzuoqi; SM, Sammnexia; TU, Tumezuoqi; DE, Dengkuo; GU, Guyuan; HA, Hangu; JI, Jinghai; YU, Yutiacun; DI, Dingxiang; BA, Balizhuang.

[c] Number of samples analyzed for mitochondrial DNA.

[d] Number of samples analyzed for microsatellite DNA.

[e] Geographic information system data in degrees (°) and feet (′).

[f] No additional locality data are available.

[g] Inner Mongolia.

Appendix B. Sequence diversity by nucleotide position for 37 mitochondrial DNA haplotypes

Haplotype no.	Nucleotide position
	_11111111111111111111
	_11111122222222233344555566677777788990000111112223344444555
	_33440223365788889993694515793454456796878233600279569013778911 2
	_12170417936007045803847121767270889105926684376265034857093874147
Haplotype	
Haplotype 1	ATATAATCGAATATCCGCCAAAATATTGATTTACCATAATTCCTTGATAACATGATATGCCGTGT
Haplotype 2	......C ......T...AG ......AA..CCGT....T..TTCAA.CTG .....
Haplotype 3	G.GC..CT...CG.TA..AGT..C..AAT.CC.T....TCCTTC.A.CTG.G.A.AG...TA.TC
Haplotype 4	..GC..CT...CG.TA..AGT..C..AAT.CC.T....TCCTTC.A.CTG.G.A.AG...TA.TC
Haplotype 5	...C.....G...AT...A.T.....AAT.CC.T..C.T..TTCAA.CTGT..A.A....TA.T.
Haplotype 6	.............T....G......AA.....T....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 7	...C.........AT.....T.G.GCAAT.CC.TT...T..TTCAA.CTG.G.A.A.C..TA.T.
Haplotype 8	......C....CG.T...AGT.....AAT.CC.T....TCCTTC.A.CTG.G.A.A....TA.T.
Haplotype 9	...C..CT......T...AGTG.C..AAT.CC.T....TCCTTC.A.CTG.G.A.AG.T.TA.TC
Haplotype 10	...C..CT......T...AGT..C..AAT.CC.T.T..TCCTTC.A.CTG.G.A.AG.T.TA.TC
Haplotype 11	...C..CT......T...AGT..C..AAT.CC.T....TCCTTC.A.CTG...A.AG.T.TA.TC
Haplotype 12	.A.CT.C......AT..T..T.G..CAATCCCGT....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 13	...C.........AT.....T.G..CAAT.CC.TT...T..TTCAA.CTG.G.A.A.C..TA.T.
Haplotype 14	...C.....G...AT...A.T.....AAT.CC.T....T..TTCAA.CTGT..A.A....TA.T.
Haplotype 15	...C..CTA.....T...AGT..C..AAT.CC.T....TCCTTC.A.CTG.G.A.AG.T.TA.TC
Haplotype 16	...C..C......AT.....T.G..CAATCCCGT....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 17	...C..CT......T...A.T..C..AAT.CC.T....TCCTTC.A.CTG.G.A.AG.T.TA.TC
Haplotype 18	...C..CTA....AT.....T....CAAT.CCGT....T..TTCAA.CTG.GCATA...TTACT.
Haplotype 19	...C..C...G..AT.....T.G..CAATCCCGT....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 20	...C..CT......T...AGT..C..AAT.CC.T....TCCTTC.A.CTG.G.A.AG.T.TA.TC
Haplotype 21	...C..C......AT.....T.G..CAATCCCGT....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 22	...C..CT......T...AGT..C..AAT.CC.T....TCCTTC.A.CTG.G.A.AG...TA.TC
Haplotype 23	.......T......T.A.AGT..C..AAT.CC.T.....CCTTC.A.CTG.G.A.AG...TA.T.
Haplotype 24	...C.........AT.....T.G..CAAT.CC.TT...T..TTCAA.CTG.G.A.A....TA.T.
Haplotype 25	...C..CT.....AT.....T....CAAT.CCGT....T..TTCAAGCTG.G.ATA...TTACT.
Haplotype 26	...C..CT.....AT.....T....CAAT.CCGT...GT..TTCAA.CTG.G.ATA...TTACT.
Haplotype 27	...C..C.......T...AG......AA..CC.T....T..TTCAA.CTGTG.A.A....TA.T.
Haplotype 28	......C.......T...AG......AA..CC.T....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 29	...C.TCT.....AT.....T....CAAT.CCGT....T..TTCAA.CTG.G.ATA...TTACT.
Haplotype 30	...C..CT.....AT.....T....CAAT.CCGT....T..TTCAA.CTG.G..TA...TTACT.
Haplotype 31	...C..C......AT.....T.G..CAATCCCGT....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 32	...C..CT......T...AGT..C..AAT.CC.T....T..TTC.A.CTG.G.A.AG.T.TA.TC
Haplotype 33	...C..C......AT.....T.G..CAAT.CCGT....T..TTCAA.CTG.G.ATA...TTACT.
Haplotype 34	...C.........AT...A.T.....AAT.CC.T....T..TTCAA.CTGTG.A.A....TA.T.
Haplotype 35	...C..CT.....AT.....T....CAAT.CCGT....T..TTCAA.CTG.G.ATA...TTACT.
Haplotype 36	...C..CTA.....T...AGT..C..AAT.CC.T....T..TTCAA.CTG.G.A.A...TTACT.
Haplotype 37	...C..CT.....AT.....T....CAAT.CCGT....T..TTCAA.CTG.G.A.A...TTACT.