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 antibiotic 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—sawpwood 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
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 (Linghai) 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

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, Beijing; 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, Qiqihar; SC, Sichuan; SD, Shandong; SK, South Korea; SN, Shaanxi; SX, Shanxi; TB, Tibet; TJ, Tianjin; YN, Yunnan; ZJ, Zhejiang.
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 0.7 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 MgCl₂, 0.06 µl of TaqDNA 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), 3 µl and contained 0.75 µl of 2.5 mM dNTP, 0.75 µl of 10 mM PCR primer, and 1 µl of PCR DNA polymerase in 0.1 µl of water, 1 µl, 1 µl, 1 µl of 5× buffer (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 and Ronquist 2003) with a 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 ≥ 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 (9921: GenBank accession DR108921) are GGCCTATTTGTGGAGAT GTG (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 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 µM. 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 ≥ 3. Pairwise FST values for all pairs of populations and Nei’s average number of pairwise differences within and between popula-
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 \textit{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 \( K = \)

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**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. \textit{A. chinensis} is the outgroup.
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 \( \Delta H \), 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 \( \Delta H \), population substructure was indicated. We chose the run with the greatest likelihood at \( K \) 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 Bonferroni correction). Heterozygote deficiencies could be due to the Wahlund effect, the lumping of populations with different allele frequencies. However, other causes (e.g., null

| Haplotype no. | 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  |    |    |      |     |    |     |    |
| Haplotype 8   |    | 1  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 9   |    |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 10  | 1  | 2  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 11  |    | 1  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 12  |    |    | 4  |    |    | 2  |    |    |    |      |     |    |     |    |
| Haplotype 13  |    |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 14  |    | 1  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 15  | 1  |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 16  |    |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 17  |    | 9  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 18  |    |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 19  |    | 2  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 20  | 1  |    | 1  |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 21  |    |    | 1  |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 22  |    |    |    |    |    |    |    | 1  |    |      |     |    |     |    |
| Haplotype 23  |    | 2  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 24  |    |    | 1  |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 25  |    |    | 1  |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 26  |    |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 27  |    |    | 1  |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 28  |    |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 29  |    | 1  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 30  |    |    |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 31  | 4  | 1  | 1  |    |    | 1  |    |    |    |      |     |    |     |    |
| Haplotype 32  |    |    | 1  |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 33  |    | 2  | 1  |    |    | 1  |    |    |    |      |     |    |     |    |
| Haplotype 34  |    |    |    | 1  |    |    |    |    |    |      |     |    |     |    |
| Haplotype 35  | 5  | 4  | 1  | 1  | 10 | 5  | 6  |    |    |      |     |    |     |    |
| Haplotype 36  |    | 1  |    |    |    |    |    |    |    |      |     |    |     |    |
| Haplotype 37  |    |    |    |    |    |    |    |    |    |      |     |    |     |    |

* 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.
alleles might also contribute. Significant ($P < 0.05$ with Bonferroni’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%; $P < 0.01$) 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., Korean 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, Hebei ($n = 1$), Jiangxi ($n = 1$), and Shandong ($n = 1$) populations show ancestry predominantly from the “blue population.” Beetles from the north eastern provinces of Jilin and Heilongjiang ($n = 1$) 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 origin. 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 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 4. Combined diversity information by pop for Asian longhorned beetles genotyped at 16 microsatellite loci**

| Pop   | $n^b$ | $N_a^c$ | MAF$^d$ | $H_e^f$ | $H_o^f$ | AR$^e$ |
|-------|-------|---------|---------|---------|---------|--------|
| 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    | 0.50    | 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    |        |
| Shandong (SD) | 1     | 1.25    | 0.88    | 0.13    | 0.25    |        |
| Shandong (SD) | 1     | 1.25    | 0.88    | 0.13    | 0.25    |        |
| Shandong (SD) | 1     | 1.25    | 0.88    | 0.13    | 0.25    |        |
| Shandong (SD) | 1     | 1.25    | 0.88    | 0.13    | 0.25    |        |
| Shandong (SD) | 1     | 1.25    | 0.88    | 0.13    | 0.25    |        |
| Shandong (SD) | 1     | 1.25    | 0.88    | 0.13    | 0.25    |        |
| Shandong (SD) | 1     | 1.25    | 0.88    | 0.13    | 0.25    |        |
| 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).

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

| Pop   | AH   | GS   | HEB  | HEN  | JL   | LN   | IM   | SX   | TJ   |
|-------|------|------|------|------|------|------|------|------|------|
| GS    | 0.125$^b$ |      |      |      |      |      |      |      |      |
| HEB   | 0.114$^b$ | 0.119$^b$ |      |      |      |      |      |      |      |
| HEN   | 0.046 | 0.099$^b$ | 0.097$^b$ |      |      |      |      |      |      |
| JL    | 0.141$^b$ | 0.102$^b$ | 0.100$^b$ | 0.100 |      |      |      |      |      |
| LN    | 0.107$^b$ | 0.115$^b$ | 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    | 0.073$^b$ | 0.131$^b$ | 0.050 | 0.085 | 0.152$^b$ | 0.077$^b$ | 0.071$^b$ | 0.102 |      |
| SK    | 0.411$^b$ | 0.303$^b$ | 0.319$^b$ | 0.309$^b$ | 0.234$^b$ | 0.304$^b$ | 0.264$^b$ | 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, Shandong; TJ, Tiajin.

$^b$ Significance values, $p < 0.001$. 

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

| Pop. | Nearest city and/or county | n\(^{a}\) (DNA) | n\(^{d}\) (SSR) | North\(^{b}\) | East\(^{c}\) | 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 |
| Gansu 1 (GS) | Liu Hua/YO | 3 | 3 | 35° 57' | 103° 16' | EU914759, EU914965, EU914766 |
| Gansu 2 | Jingta | 9 | 12 | 37° 21' | 104° 03' | EU914699, EU914700, EU914701, EU914702 |
| Gansu 3 | Lanzhou/GA | 10 | 13 | 36° 19' | 103° 56' | EU914703, EU914704, EU914705, EU914706 |
| Hebei 1 (HEB) | Langfang City | 11 | 14 | 39° 30' | 116° 40' | EU914714, EU914715, EU914723, EU914724 |
| Hebei 2 | HanDan/DA | 2 | 3 | 36° 16' | 115° 06' | EU914785, EU914805 |
| Hebei 3 | HanDan/CH | 2 | 3 | 36° 26' | 114° 21' | EU914786, EU914787 |
| Hebei 4 | Chengguan/WU | 3 | 3 | 37° 36' | 116° 29' | EU914788, EU914789, EU914806 |
| Hebei 5 | ShiziZhaZhang/WJ | 2 | 3 | 38° 10' | 114° 57' | EU914790, EU914807 |
| Heilongjiang\(^{d}\) (HL) | | | | | | |
| Henan 1 (HEN) | Zhengzhou/NE | 2 | 1 | 34° 45' | 113° 38' | EU914887, EU914878 |
| 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 |
| Jiangsu\(^{d}\) (JX) | | | | | | |
| Jilin 1 (JL) | Lish | 5 | 5 | 43° 13' | 124° 19' | EU914768, EU914769, EU914770, EU914771 |
| Jilin 2 | Changchun | 0 | 2 | 45° 52' | 125° 18' | EU914772 |
| Jilin 3 | Yanji | 0 | 1 | 42° 52' | 128° 30' | |
| Liaoning 1\(^{e}\) (LN) | | | | | | |
| 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\(^{f}\) (IM) | Huhehaote | 9 | 10 | 40° 48' | 111° 38' | EU914694, EU914695, EU914696, EU914697 |
| In. Mg. 2 | Wuhai/TU | 5 | 10 | 39° 39' | 106° 47' | EU914761, EU914762, EU914763 |
| In. Mg. 3 | Hasa/AL | 2 | 3 | 40° 50' | 111° 50' | EU914789, EU914799 |
| In. Mg. 4 | | 3 | 3 | 38° 49' | 105° 41' | EU914795, EU914796, EU914797 |
| In. Mg. 5 | Bamereng/DE | 0 | 1 | 40° 37' | 107° 05' | |
| Ningxia (NX) | Qingtongxia/QI | 1 | 1 | 37° 52' | 105° 57' | EU914769 |
| Tianjin 1 (TJ) | Daitian/HA | 3 | 3 | 39° 14' | 117° 45' | EU914778, EU914779, EU914801 |
| Tianjin 2 | Chadian/HA | 3 | 3 | 39° 14' | 117° 45' | EU914780, EU914781, EU914802 |
| Tianjin 3 | Jinghai/JI | 3 | 3 | 38° 56' | 116° 54' | EU914782, EU914803 |
| Tianjin 4 | Ninghe/YU | 3 | 3 | 39° 19' | 117° 47' | EU914783, EU914784, EU914804 |
| Shandong (SD) | Guanxian/BA | 1 | 1 | 38° 18' | 112° 41' | EU914808 |
| Korea (SK) | Sokho | 17 | 11 | 38° 07' | 128° 27' | EU914690, EU914691, EU914692, EU914693 |

\(^{a}\) 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, Wuj; NE, Neihuang; LI, LingHai; AL, Alashamengzuoqi; SM, Sammenxia; 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.  
\(^{d}\) Inner Mongolia.
| Haplotype no. | Nucleotide position |
|--------------|---------------------|
| Haplotype 1   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 2   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 3   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 4   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 5   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 6   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 7   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 8   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 9   | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 10  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 11  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 12  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 13  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 14  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 15  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 16  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 17  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 18  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 19  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 20  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 21  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 22  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 23  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 24  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 25  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 26  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 27  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 28  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 29  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 30  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 31  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 32  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 33  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 34  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 35  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 36  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |
| Haplotype 37  | ATATAAATCCGAAATACCCCAAAATAATTGTACATTACATAATTCCTTGATACACTGTATGCCGCTG |