Analysis of genetic diversity in an invasive population of Asian long-horned beetles in Ontario, Canada

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Abstract—Adult Asian long-horned beetles, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae: Lamiinae), were discovered in Ontario, Canada, in 2003 in the vicinity of a commercial warehouse. Trees were heavily scarred with signs of attack and larvae and adult beetles were common, suggesting that there had been multiple generations at the site. We amplified 16 microsatellite loci from 326 beetles to examine genetic diversity in this population. Based on Hardy−Weinberg equilibrium, 6 of 16 loci were monomorphic and 8 were not, indicating nonrandom mating. Measures of microsatellite genetic diversity and mitochondrial DNA haplotype diversity were significantly lower than those in *A. glabripennis* from China and Korea but were not significantly different from those in the New York City population. The proportion of different multilocus genotypes in the Ontario population was lower than in the populations in New York City and Linden, New Jersey. These results suggest that limited genetic diversity in the Ontario population has not hampered reproduction of this invasive insect. This genetic signature is common in other invasive species, likely because a population is founded by a few closely related individuals, or a large founding population suffers subsequent genetic bottlenecks.

Résumé—Des adultes du longicorne asiatique, *Anoplophora glabripennis* (Motschulsky) (Coleoptera : Cerambycidae : Lamiinae), ont été découverts en Ontario, Canada, en 2003 près d'un entrepôt commercial. Les arbres portaient de fortes lacérations dues à l'attaque des coléoptères et les larves et les adultes y étaient nombreux, ce qui laisse croire qu'il y avait eu plusieurs générations à ce site. Nous avons examiné la diversité génétique de la population en amplifiant 16 locus microsatellites chez 326 coléoptères. Six des 16 locus sont monomorphes et 8 locus ne suivent pas l'équilibre Hardy−Weinberg, ce qui indique que les accouplements ne sont pas aléatoires. Les mesures de diversité génétique des microsatellites et de diversité des haplotypes d'ADN mitochondrial sont significativement plus faibles que celles d'*A. glabripennis* en Chine et en Corée, mais elles ne diffèrent pas significativement des mesures de la population de la ville de New York. La proportion de génotypes différents à locus multiples est plus faible dans la population d'Ontario que dans les populations de la ville de New York et de Linden (New Jersey). Ces résultats indiquent que la diversité génétique restreinte de la population d'Ontario n'a pas nui à la reproduction de cet insecte envahissant. Une telle signature génétique est commune chez d'autres espèces envahissantes, vraisembla-blement parce que la population est fondée sur un petit nombre d'individus fortement

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apparentés ou alors parce qu'une population fondatrice de grande taille subit plus tard des goulots d'étranglement génétiques.

[Traduit par la Rédaction]

Introduction

The Asian long-horned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae: Lamiinae), is a wood-borer native to China and Korea (Cavey *et al.* 1998; Lingafelter and Hoebeke 2002). In China *A. glabripennis* is a destructive pest of man-made forests (plantations) and windrows of one to several tree species planted to shelter urban, rural, and agricultural areas from sandstorms and provide a local resource for timber and paper products (Moore and Russell 1990; Uchida *et al.* 2005). In contrast, *A. glabripennis* is rare in South Korea, occurring only in sparse populations along edges of species-rich deciduous forest stands (Williams *et al.* 2004).

Female beetles chew through bark on trunks and branches and lay eggs singly in the inner bark (Xiao 1992; Cavey *et al.* 1998). Young larvae feed at the inner bark − sapwood interface for 14–28 days (Keena 2005; Hunter *et al.* 2009), slowly destroying the vascular system and cutting off translocation (Ric *et al.* 2007). Older larvae tunnel into the heartwood, causing branch dieback, structural deterioration, and often tree mortality (Haack *et al.* 1997; Ric *et al.* 2007). Pupation takes place in the wood, where teneral adults remain for up to 7 days before chewing an exit hole and emerging (Xiao 1992; Lingafelter and Hoebeke 2002). Adult beetles feed on twigs and leaf petioles and primary leaf veins (Keena 2002; Smith *et al.* 2002; Ric *et al.* 2007). Newly eclosed females become sexually mature in 9–15 days (Keena 2002; Smith *et al.* 2002); development from egg to adult requires approximately 12–24 months. In China, *A. glabripennis* overwinter as eggs or larvae, with egg development and larval feeding resuming in the spring. Pupation occurs from May through July, with adult emergence peaking from mid-June to late July (Pan 2005). Larvae that are still young in the fall overwinter a second time,

i.e., a 2-year life cycle occurs in colder climates. Although high levels of infestation occur in weak trees grown in monoculture (Yin and Lu 2005), the beetle also attacks apparently healthy host trees (Haack *et al.* 1997). Adult males and females mated repeatedly in field (Xiao 1992) and greenhouse studies (Morewood *et al.* 2004). In the laboratory, females lay between 30 and 127 eggs, but the number varies among host tree species (Smith *et al.* 2002).

Several established populations have been discovered in the United States of America, the first in Brooklyn, New York, in 1996 (Haack *et al.* 1997). Subsequently, between 1998 and 2008, the beetle was found at other sites in New York City and elsewhere in New York State (Long Island, Prall's Island, Staten Island), New Jersey (Jersey City, Carteret, Linden), Illinois (Chicago; Poland *et al.* 1998), Massachusetts (Worchester), and California (Sacramento). This species has also been intercepted or introduced multiple times in Europe (Austria (Tomiczek *et al.* 2002), France, Germany (Hérard *et al.* 2006), the Czech Republic (Sabol 2006), and Italy (Maspero *et al.* 2007)). *Anoplophora glabripennis* is usually introduced through the transportation of solid-wood packing material from Asia to North American warehouses, from where adult beetles escape to nearby trees (Haack *et al.* 1997).

In its native range the primary hosts of *A. glabripennis* are poplars and willows (*Populus* L. and *Salix* L., Salicaceae), maples (*Acer* L., Aceraceae), and elms (*Ulmus* L., Ulmaceae), but hosts also include lindens (*Tilia* L., Tiliaceae), Russian olive and buckthorns (*Eleagnus angustifolia* L. and *Hippophae* L., Elaeagnaceae), birches (*Betula* L., Betulaceae), horse chestnuts (*Aesculus* L., Hippocastanaceae), the London planetree (*Platanus hybrida* Brot., Platanaceae), and the goldenrain tree (*Koelreuteria paniculata* Laxm., Sapindaceae) (Wang 2004). Outside its native range, hosts include maples, willows,

Fig. 1. Map of the Asian long-horned beetle (ALHB) regulated area in Vaughan and Toronto, Ontario, showing the location and number of beetles per tree that were sampled for genetic analysis.
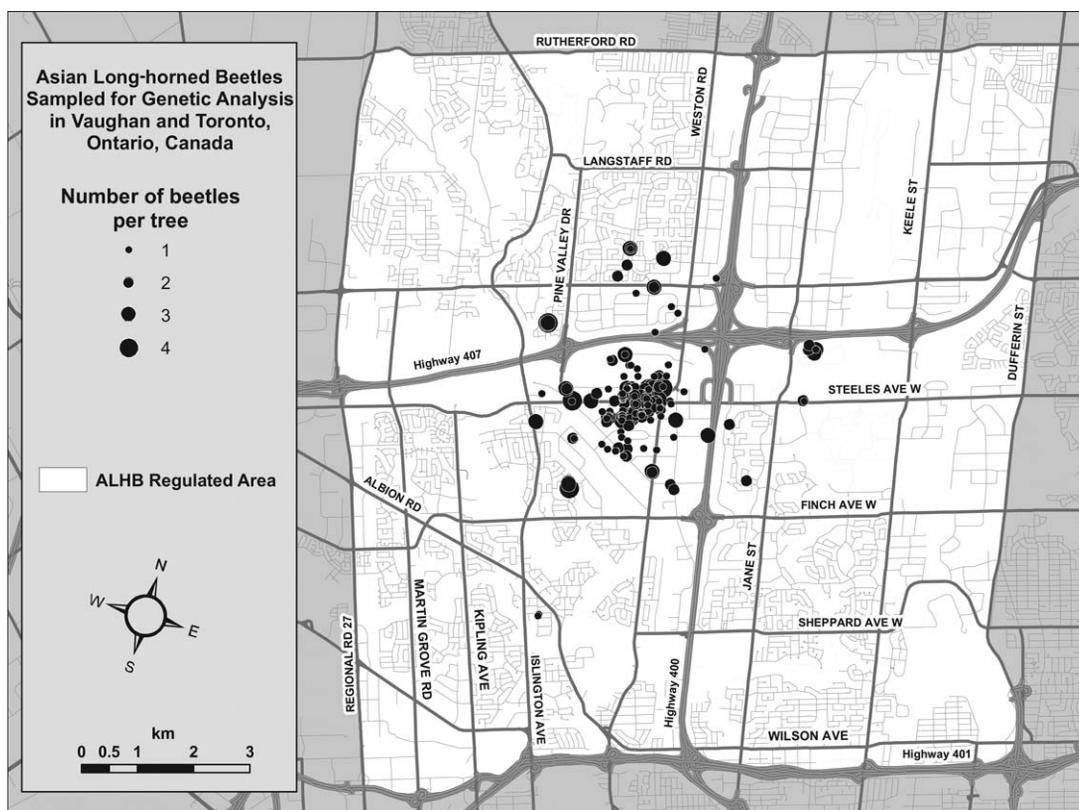
elms, birches, horse chestnuts, mountain ashes (*Sorbus* L., Rosaceae), the London planetree, and species that are not attacked in China (Nowak *et al.* 2001; Morewood *et al.* 2003; Sawyer 2003; Hajek and Kalb 2007; Ric *et al.* 2007). Various potential hosts found in the currently regulated area in Canada have not been attacked: *Prunus* L., *Crataegus* L., and *Malus* Mill. (Rosaceae) among others (Turgeon *et al.* 2007).

Anoplophora glabripennis was first intercepted in Canada in 1998 in packing material sent from China to a warehouse in Waterloo, Ontario (Sellers 2004). In Ontario, an infestation was discovered in the Greater Toronto Area in September 2003 in an industrial area on the boundary between Toronto and Vaughan, near the intersection of Weston Road and Steeles Avenue (Fig. 1). By the time it was discovered, hundreds of trees had been infested. In February 2004, a regulated area was

established to prevent the movement of infested wood from the region (Canadian Food Inspection Agency 2003). As of October 2007, approximately 27 400 host trees had been removed, including more than 600 known to be infested (North American Plant Protection Organization 2007). Additional infested trees in Toronto are being removed when found, along with any potential hosts within 200–400 m (Canadian Food Inspection Agency 2008).

Molecular markers can be used to characterize patterns of genetic variation in invasive species, revealing the signature of an expanding population and providing data about founder-population size and subsequent population bottlenecks, factors that may be important in population growth and spread (Wares *et al.* 2005; Carroll 2007; Eales *et al.* 2008; Ficetola *et al.* 2008). Additive genetic variance may increase after a population bottleneck, allowing a rapid response to

selection (Wade *et al.* 1996; Cheverud *et al.* 1999; Naciri-Graven and Goudet 2008). The projected annual economic impact of *A. glabripennis* on Canadian resources has been estimated to be over $331 million (Colautti *et al.* 2006). It is important to look for evidence of multiple founding events or population bottlenecks, as these different scenarios could influence the choice of prevention and eradication programs.

We evaluated the amount of genetic diversity in the *A. glabripennis* population in Canada and compared it with the known genetic diversity in populations of this beetle in the United States of America, China, and Korea. To estimate variation, we amplified microsatellite DNA markers consisting of tandem repetitive units 1−6 base pairs (bp) long (Oliveira *et al.* 2006). High rates of mutation (Jarne and Lagoda 1996) of these presumably neutral markers make them particularly useful for inferring current population structure and recent population history (Roderick and Navajas 2003; Schlötterer 2004). In a previous study (Carter *et al.* (2009*b*) we amplified and sequenced DNA from a subset of the *A. glabripennis* specimens from Ontario for mitochondrial DNA COI and COII genes, using species-specific primers to determine the number and source of haplotypes. Here we also consider mitochondrial DNA haplotype diversity in the Ontario *A. glabripennis* population in evaluating the history of the introduction.
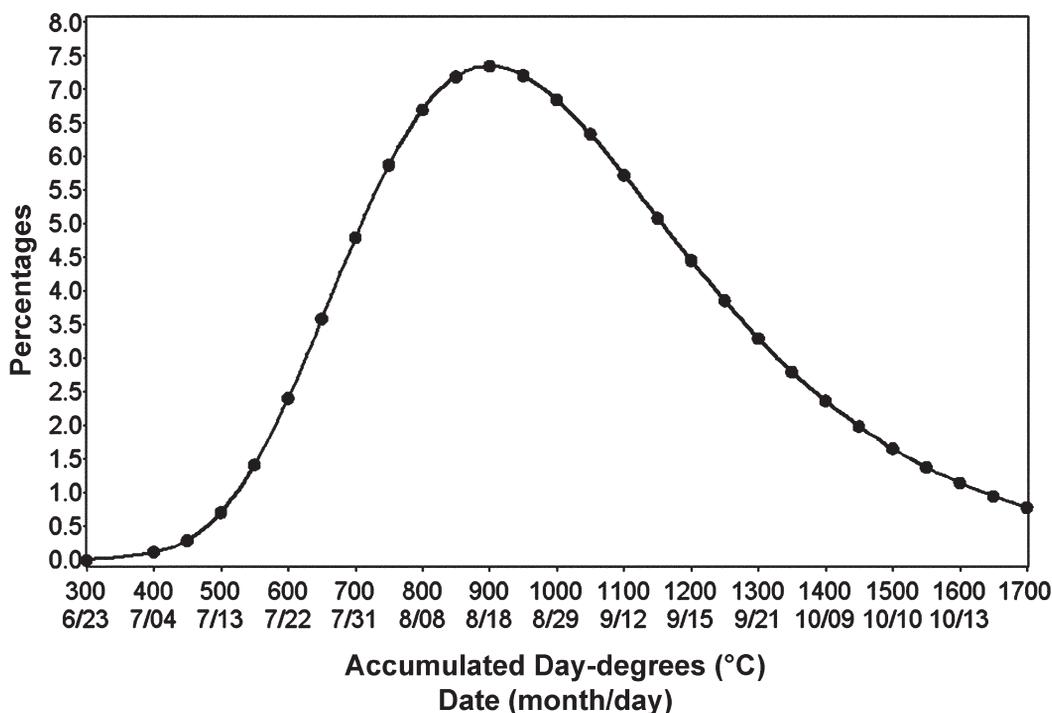
Methods and materials

Emergence period and seasonal occurrence and abundance of *A. glabripennis* in Ontario are currently unknown. However, a day-degree model predicting emergence of adult *A. glabripennis* in China and associated data were used to create an emergence model for the Ontario population, based on accumulated day-degrees: emergence rate (%) = exp(−exp(−0.004 × day-degrees + 3.5)) × 100 (Smith *et al.* 2004). The Historical Weather Database for Toronto, Ontario (1978–2008), based on data obtained from Toronto Lester B. Pearson International Airport, was used to find the mean date

corresponding to accumulated day-degrees (Fig. 2). To find the date of a specific day-degree accumulation (temperature), X, the accumulated day-degrees between (X – 5) and (X + 5) were rounded to the specific day-degree. For example, to find the corresponding mean dates for 400 day-degrees, all observations between 395 ≤ day-degree ≤ 405 for each year from 1978 to 2008 were pooled to create a new variable, "rounded-day-degree" (= 400). From the resulting data set of "rounded-day-degrees" the mean dates were calculated. Using the same method, the mean dates for rounded-day-degrees for 450−1900 were also calculated. These data were used to generate a plot of accumulated day-degrees (°C) (first x axis) *versus* percent emergence (y axis) for Toronto. A curve-smoothing function was used to generate an arc connecting all predicted points. The date corresponding to each rounded-day-degree was applied to this plot as a second x axis.

Between 26 September 2003 and 2 September 2005, live adult and larval *A. glabripennis* and eggs were collected in the regulated area (Fig. 1) and placed in 95% ethanol, and their host was recorded (Table 1). Using DNeasy tissue kits (QIAGEN), total DNA was extracted from adult thoracic muscle tissue, from a segment of the larval abdomen, or by crushing a whole egg. A subset of samples has been deposited as vouchers with the Cornell University Insect Collection (lot No. 1262).

DNA samples from 57 *A. glabripennis* were amplified and sequenced for mitochondrial DNA using six species-specific primers at optimized annealing temperatures (Carter *et al.* 2009*c*). We sequenced 1317 bp of cytochrome *c* oxidase 1, 65 bp of tRNA-leucine, and 224 bp of cytochrome *c* oxidase 2 mitochondrial DNA genes. Each PCR was run in a volume of 14 µL containing 6.7 µL of water, 1.5 µL of 10× PCR buffer (20 mmol/L Tris-HCl (pH 8.4), 500 mmol/L KCl (Invitrogen, Carlsbad, California)), 3 µL of 2.5 mmol/L dNTP, 0.75 µL of 50 mmol/L $MgCl_2$, 0.06 µL of *Taq* DNA polymerase (Invitrogen), 0.6 µL of primers at 10 mmol/L, and 20 ng of DNA. PCR products were cleaned with PCR mini-elute kits (QIAGEN).

conditions are listed in Carter *et al.* (2009*a*). Primers for an additional locus, locus 15, were GGCCTATTTTGATGCGAGTG (forward) and GGCACTACCTGCTACACAGC (reverse) fluorescently labeled with PET (Applied Biosystems) and amplified following the methodology of Carter *et al.* (2009*c*).

Diversity information was calculated in PowerMarker (Liu and Muse 1995) and included the number of alleles and genotypes and frequency of the most common (major) allele. Allelic richness was calculated in FSTAT (Goudet 1995). The observed number of alleles in a sample is highly dependent on sample size, therefore it is more accurate to estimate allelic richness (number of alleles at a locus independent of sample size). Allelic richness is estimated from *n* samples genotyped at all loci (no missing data). Also calculated were observed heterozygosity, fraction of individuals in a population with two different alleles at a locus, and expected heterozygosity (an estimate of all individuals heterozygous at a locus, calculated from allele frequencies at that locus). A lower than expected observed heterozygosity suggests a deviation from Hardy−Weinberg equilibrium due to an external factor such as migration, selection, inbreeding, or drift. To test the null hypothesis of a random union of gametes, we calculated deviations from Hardy−Weinberg equilibrium (F_{IS}; exact Hardy−Weinberg test) for each locus, and genotypic disequilibrium (Fisher's method) for each locus pair, using Genepop (Raymond and Rousset 1995). Two group comparisons of mean allelic richness and mean observed and expected heterozygosities were calculated in FSTAT (Goudet 1995) to compare data sets from China plus Korea, United States of America, and Canada. We counted the different genotypes, *i.e.*, that differ by one or more alleles across all 16 loci, in the Canadian population and in populations in the United States of America using Microsoft tools (Park 2001). With a *z* test we compared the proportions of two different groups for the number of different genotypes between New York City and Linden, New Jersey, between New York City and Toronto, and between Linden and Toronto.

To make inferences about the source of the Ontario population, we compared the multilocus genotypes of beetles from Ontario with those of reference populations from provinces of China and Korea (each province was labeled a population), using GeneClass2 (Piry *et al.* 2004). Using a resampling method (Rannala and Mountain 1997), the likelihood of assignment to a reference population was calculated for each individual. Individuals were assigned to reference populations based on the largest likelihood score. For each individual beetle, an assignment was made only if the probability of assignment was 90% with a statistical confidence of 95% (threshold 0.05). Only beetles with all 32 alleles (no missing data) were used. We repeated the assignment test for each individual beetle from Canada, with the reference data set partitioned into four clusters determined by means of a model-based clustering method for inferring population structure (*structure*; Falush *et al.* 2003).

Results

We report here the predicted seasonal phenology of adult *A. glabripennis* in Toronto (Fig. 2) and the results from two independent genetic data sets to determine the genetic diversity of the Toronto population of *A. glabripennis* and the implications of population establishment and spread.

The day-degree model predicted peak abundance of adult *A. glabripennis* in Toronto to be between approximately 850 and 950 accumulated day-degrees, coinciding with the 3rd and 4th weeks of August.

For the microsatellite markers, allele number ranged from 1 to 4, with allelic richness from 1 to 3.76 (Table 2). Of 44 pairwise comparisons for linkage disequilibrium, 5 were significant ($P \leq 0.05$ with Bonferroni correction). All other pairwise comparisons (76) involved monomorphic loci. For 9 of the 10 non-monomorphic loci, observed heterozygosity was less than expected heterozygosity (Table 2). For 8 of these 10 loci, the calculated F_{IS} value indicated a significant probability that the observed sample was not in Hardy−Weinberg equilibrium, *i.e.*, a nonrandom union of gametes.

Table 2. Number of alleles (N_A), number of genotypes (N_G), major allele frequencies (MAF), expected heterozygosity (H_E), observed heterozygosity (H_O), and allelic richness (AR) for 16 microsatellite loci in *Anoplophora glabripennis* collected in Vaughan and Toronto.

Locus	N_A*	N_G*	MAF*	H_E*	H_O*	AR[†]	F_{IS}[‡]	P[§]
alb10	1 (326)	1	1	0	0	1	—	—
alb53	2 (326)	3	0.64	0.45	0.38	2	0.156	**0.005**
alb21	1 (324)	1	1	0	0	1	—	—
alb59	1 (326)	1	1	0	0	1	—	—
alb38	3 (325)	4	0.64	0.46	0.4	2.96	0.119	**0.003**
alb77	4 (261)	4	0.98	0.34	0.01	4	1	**<0.001**
alb14	2 (303)	3	0.69	0.49	0.26	2	0.319	**<0.001**
alb30	2 (324)	3	0.62	0.47	0.44	2	0.048	0.425
alb15	1 (326)	1	1	0	0	1	—	—
alb24	1 (325)	1	1	0	0	1	—	—
alb9	4 (325)	4	0.58	0.49	0.4	3.76	0.176	**<0.001**
alb19	2 (325)	2	0.99	0.01	0	1.96	1	**0.002**
alb35	4 (325)	5	0.55	0.5	0.01	3.61	0.988	**<0.001**
alb40	1 (325)	1	1	0	0	1	—	—
alb43	2 (310)	3	0.56	0.53	0.53	2	0.019	0.823
alb44	3 (324)	6	0.47	0.62	0.18	3	0.136	**0.011**

*The total number of beetles sampled was 326. The number in parentheses is the number of beetles that amplified per locus.
[†]The minimum number of beetles sampled was 261 (locus 77).
[‡]According to Weir and Cockerham (1984).
[§]Exact Hardy–Weinberg test; values in boldface type are significantly different.

For the 230 individuals from Ontario with no missing data, there were 13 different microsatellite genotypes (allele combinations) across all 16 loci (Table 3). We refer to nonamplification of alleles at any locus as missing data. Common reasons for missing data include mutations at primer sites or PCR inhibition due to contaminants found in DNA or reagents, or DNA shearing at the amplification site. The Ontario population had fewer different genotypes relative to the number of *A. glabripennis* sampled than other invasive populations (Table 3). Pairwise comparisons of the proportions of different genotypes yielded the following results: the New York City population differed from the Toronto ($z = 16.6$, $P \leq 0.01$) and Linden ($z = 11.038$, $P \leq 0.01$) populations, and the Toronto population differed from the Linden population ($z = 3.125$, $P \leq 0.01$) (Table 3). However, allelic richness and observed and expected heterozygosities were not significantly lower in the *A. glabripennis* population from Toronto than in populations in the United States of America, but were significantly

Table 3. Numbers and proportions of different genotypes found in North American populations of *Anoplophora glabripennis*.

	No. of genotypes tested*	No. of different genotypes	Percentage[†]
United States of America			
Long Island, New York	33	33	100
New York City, New York	103	103	100*a*
Jersey City, New Jersey	23	23	100
Carteret, New Jersey	22	22	100
Linden, New Jersey	72	14	19.4*b*
Canada			
Toronto, Ontario	230	13	5.7*c*

*Only beetles with no missing genotype data were tested.
[†]The number of different genotypes as a percentage of the number tested. Values followed by a different letter show significantly different two by two group proportions (z test, $P \leq 0.01$).

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Table 4. Proportions of different mitochondrial DNA haplotypes and indices of microsatellite polymorphism in populations of *Anoplophora glabripennis* from Asia, the United States of America, and Toronto, Ontario.

Collecting locality	*H*/*N**	Percentage[†]	AR[‡]	H_O[§]	H_E[¶]
China and Korea	37/124	29.8*a*	3.28*a*	0.29*a*	0.47*a*
United States of America	11/258	0.04*b*	2.21*b*	0.15*b*	0.27*b*
Toronto	1/57	0.02*b*	2.17*b*	0.16*b*	0.27*b*

Note: Values within a category followed by a different letter are significantly different (FSTAT: $P \leq 0.05$, one-sided test).

*Number of different haplotypes (*H*) out of the total number sequenced (*N*).

[†]Proportion of different haplotypes in the population. Values followed by a different letter show significantly different two by two group proportions (*z* test, $P \leq 0.01$).

[‡]Mean allelic richness over all 16 loci.

[§]Mean observed heterozygosity over all 16 loci.

[¶]Mean expected heterozygosity over all 16 loci.

lower than in populations in China plus Korea (Table 4).

Using a threshold confidence level of 90%, 36 beetles from the Toronto population were assigned to Shanxi Province, 14 to Liaoning Province, and 2 to Hubei Province. No other beetles could be assigned with 90% confidence. When the assignment test was repeated using the four clusters of beetles from China and Korea documented in a previous *structure* analysis as reference populations (Carter *et al.* (2009*c*), 165 beetles (72%) from Toronto were assigned to a single cluster characterized by beetles from the contiguous provinces of Hebei, Inner Mongolia, Liaoning, Shanxi, and Tianjin in north-central China. Four beetles (<1%) were assigned to a cluster characterized by beetles from the contiguous provinces of Anhui, Henan, Hubei, Jangxi, and Shandong in eastern and central China.

Mitochondrial DNA sequences of 1606 bp of COI and COII genes were identical for the 57 *A. glabripennis* collected in the regulated area, *i.e.*, there was a complete absence of mitochondrial DNA sequence variation (Carter *et al.* (2009*b*). *Anoplophora glabripennis* collected from Canada exhibited less variation in mitochondrial DNA than invasive populations in the United States of America, which themselves varied far less than *A. glabripennis* populations collected in China and Korea (Table 4). A test of proportions of different haplotypes from the three collection locations showed that China plus Korea populations differ from those in the United

States of America ($z = 6.9$, $P \leq 0.01$), China plus Korea populations differ from the population in Toronto ($z = 4.1$, $P \leq 0.01$), and Toronto populations did not differ from those in the United States of America ($z = 0.51$, ns).

Discussion

Emergence of adult *A. glabripennis* peaked at similar accumulated day-degrees in Toronto (latitude 43.4°N) as in Liu Hua, Gansu Province, China (latitude 35°N; 800–900 day-degrees; Smith *et al.* 2004). This day-degree accumulation occurs in mid-August in Toronto, whereas the same day-degree accumulation occurs in mid-July in Gansu Province. This agrees with the assignment of Toronto beetle genotypes to genotypes of beetles from the cluster of more northerly provinces of China (at latitudes more similar to that of Toronto) rather than to genotypes from Jing tai, Gansu Province (latitude 37.2°N; Carter *et al.* (2009*c*).

Allelic variation is often critical for adaption to environmental changes. Genetic variation in the Ontario population of *A. glabripennis* is limited; the greatest microsatellite polymorphism observed was four alleles. This variation could be derived from a minimum sample of two individuals. The Ontario population is also fixed for a single mitochondrial DNA haplotype. Our data suggest that the Toronto population is derived from a small number of beetles, possibly one multiply inseminated female.

Polymorphism indices from tests for the 16 microsatellite markers for the Toronto population were not significantly different from similar measures for populations in the United States of America, but were significantly different from those for populations in China and Korea (Table 4). This suggests a general pattern of low genetic diversity of nuclear markers for invasive populations of *A. glabripennis*. However, the Toronto population of *A. glabripennis* exhibited reduced multilocus genotypic diversity compared with populations in the United States of America. In the Long Island, New York City, Jersey City, and Carteret populations, all individuals sampled had different microsatellite genotypes, a different pattern from that seen in Toronto, where many beetles share the same genotype. This could indicate that the Toronto population had fewer founding individuals and (or) suffered more severe population bottlenecks than did populations in the United States of America.

The mitochondrial DNA haplotype of the Toronto population has not yet been found in samples from China, Korea, or the United States of America, but has been found in two beetles collected in Neukirchen, Germany (Carter *et al.* (2009*b*). This further confirms that the Toronto population is an introduction independent of any of those in the United States of America.

Patterns similar to the limited genetic diversity we report here for *A. glabripennis* have been recorded for other invasive species (Dlugosch and Parker 2008). For example, populations of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), introduced into Europe were found to have significantly reduced variation in nuclear markers (AFLPs) compared with source populations in the United States of America, and European populations are fixed for single mitochondrial DNA haplotypes (Grapputo *et al.* 2005). Six invasive populations of cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), in North America are each characterized by a single mitochondrial DNA haplotype, in contrast to native European populations, which have more haplotypes

(Laffin *et al.* 2005). Likewise, invasive populations of red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae), in China have fewer mitochondrial DNA haplotypes for the number of beetles sampled than do potential source populations in North America (Cai *et al.* 2008). Population-genetic analyses of mitochondrial DNA sequences, microsatellite markers, and a sex-determining locus showed that a solitary bee, *Lasioglossum leucozonium* (Schrank), experienced a single but severe bottleneck during its introduction from Europe into North America (Zayed *et al.* 2007).

An alternative explanation for the reduced genetic variation in the Toronto *A. glabripennis* population is that a larger founder event was followed by one or more genetic bottlenecks resulting from intense local selection or random survival and reproduction (Nei *et al.* 1975). It is probable that *A. glabripennis* first arrived at the Ontario site in 1996 or 1997 (J. Turgeon, unpublished data). Following Sawyer (2006), we dissected all infested trees to determine the year when exit holes were created. The analyzed beetles were collected between 2003 (the year of discovery) and 2005 (when we collected hundreds of eggs, larvae, and adults). In Toronto the shortest life cycle for *A. glabripennis* is usually 1 year, which suggests that there had been seven to nine generations prior to our assessment of genetic diversity. There are no biological data to suggest that the *A. glabripennis* population in Toronto is not mating at random. It appears that *A. glabripennis* in Toronto has followed the three main steps common to successful invasions: colonization, followed by a lag phase, and then rapid population expansion (Sakai *et al.* 2001).

Our inability to assign most beetles from Toronto to a single source population (province) in China is likely due to extensive genetic admixture within Chinese populations (any single multilocus genotype can be found in many provinces). This genetic admixture is due to reforestation efforts in China, where plantations of preferred hosts of *A. glabripennis* became infested with beetles and then beetles were moved to other areas in transported cut wood (Zhang *et al.* 2000; Uchida *et al.* 2005;

Yin and Lu 2005). This was evident when we divided the reference population into four clusters, with three clusters containing beetles from more than one province in China. Using these clusters as reference, we were able to assign 165 individuals to a single cluster.

Our data show that *A. glabripennis* can multiply to outbreak proportions in urban areas even when genetic diversity is extremely low. This suggests that the best protection against future invasions of this and similar forest pests will be regulatory actions that ensure the mortality of beetles transported in solid-wood packing materials from Asia.

Acknowledgements

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