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Development of SCAR Markers for the DNA-Based Detection of the Asian Long-Horned Beetle, *Anoplophora glabripennis* (Motschulsky)

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DNA markers were identified for the molecular detection of the Asian long-horned beetle (ALB), *Anoplophora glabripennis* (Mot.), based on sequence characterized amplified regions (SCARs) derived from random amplified polymorphic DNA (RAPD) fragments. A 2,740-bp DNA fragment that was present only in ALB and not in other Cerambycids was identified after screening 230 random primers in a PCR-based assay system. Three pairs of nested 22-mer oligonucleotide primers were designed on the basis of the sequence of this fragment and were used to perform diagnostic PCR. The first pair of primers (SCAR1) amplified a single 745-bp fragment of ALB DNA, but this did not differentiate ALB from other species. The other two pairs of SCAR primers (SCAR2 and SCAR3) amplified bands of 1,237- and 2,720-bp, respectively, that were capable of differentiating ALB from other closely related non-native and native Cerambycids, such as *A. chinensis* (Forster), *A. malasiaca* (Thomson), *A. nobilis* (Ganglbauer), *Monochamus scutellatus* (Say), *Plectrodera scalator* (Fab), *Saperda tridentata* (Olivier), and *Graphisurus fasciatus* (Degeer). The latter two SCAR markers could be amplified using DNA extracted from body parts of ALB such as the wing, the leg, and the antennae as well as tissues from all the developmental stages including the egg, larva, pupa, and adult. These markers were also capable of identifying ALB using the DNA extracted from frass. Our results demonstrate that the SCAR markers we have identified can be used for unambiguously identifying ALB from other closely related Cerambycids using a simple PCR procedure. Arch. Insect Biochem. Physiol. 52:193–204, 2003.

KEYWORDS: molecular identification; molecular marker; polymorphism; RAPD; Cerambycids

INTRODUCTION

The Asian long-horned Beetle (ALB), *A. glabripennis* is a wood-boring beetle that is endemic to China and the Korean peninsula where it is a serious pest of poplar. It was accidentally introduced into North America through wooden packing crates containing this pest. In North America, ALB feeds on several species of maple, such as sugar (*Acer saccharum*), Norway (*Acer platanoides*), red (*Acer rubrum*), silver (*Acer saccharinum*), and Manitoba (*Acer negundo*). They also feed on non-maple species such as the horsechestnut (*Esculus hippocastanum*), black locust (*Robinia pseudoacacia*), elm (*Ulmus americana*), birch (*Betula papyrifera*), willow (*Salix alba*), poplar (*Liriodendron tulipifera*), and green ash (*Fraxinus pennsylvanica*). Currently, there are no known control methods available for treating the infested trees. The only effective means to eliminate ALB is to remove the infested trees and destroy them by chipping and burning. At present, detection and identification of ALB-infested trees

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is based on oviposition niches, resin bleeds, and sawdust near the oviposition site. Larval activity can also be recognized by the presence of galleries under the bark and, later, tunnels in the heartwood. Wood shavings extruding from round exit holes through the bark and piles of sawdust at the base of a tree are also symptomatic. Early detection and identification of this insect and the development of a rapid treatment response are crucial for the containment of this beetle. Because many of these species are phenotypically similar, the only reliable method for unambiguously identifying ALB is the use of unique molecular markers. Currently, there are no molecular markers available for identifying ALB from other closely related cerambycid species. The objective of our study was to identify some markers that could be used to identify ALB.

Polymerase chain reaction (PCR)-based genetic markers are widely used for molecular detection, genome mapping, map-based cloning, and analysis of genetic variation in insects. These marker systems take advantage of the analyses of random amplified polymorphic DNA (RAPD) (Williams et al., 1990), simple sequence repeats (SSRs) (Tautz, 1989; Brown and Tanksley, 1996), inter simple sequence repeats-PCR (ISSR-PCR) (Zeitkeinicz et al., 1994), and amplified fragment length polymorphisms (AFLP) (Vos et al., 1995). Since the first reports of RAPD markers by Williams et al. (1990) and Welsh and McClelland (1990), this method has been widely used. RAPDs are polymorphic DNA sequences that can be amplified using PCR; the resultant products can be separated as discrete bands when subjected to electrophoresis. Decamer nucleotides are generally used as primers in a PCR system to amplify a locus of a polymorphic template DNA. Such RAPD markers are useful because the procedure is simple, fast, and requires only trace amounts of template DNA, and it does not require any prior information on the DNA sequence of the species in question. Although RAPD markers are usually dominant markers, they are sensitive to minor changes in reaction conditions during PCR amplification, which can result in irreproducible results. To improve the reliability of RAPDs and to convert them to codominant markers, Paran and Michelmore (1993) developed a technique known as sequence-characterized amplified regions (SCAR). SCARs are based on sequencing the polymorphic fragment derived from RAPD primers and designing longer primers that will specifically bind to this fragment. SCAR markers are more advantageous than RAPD markers because they usually detect only a single locus and are, therefore, more specific. Their PCR amplification is less sensitive to reaction conditions, they are more likely to be codominant markers, and are therefore reproducible.

There are many applications of SCAR markers in insect taxonomy and population biology. SCARs have been used as genetic markers to distinguish Asian gypsy moths from the established North American populations (Garner and Slavicek, 1996). SCAR markers have also been used for identifying different species and biotypes of Asian gall midge (Behura et al., 1999), a major insect pest of rice. SCAR markers were successfully used for the specific identification of the six *Trypanosoma cruzi* lineages (Sylvain Brisse et al., 2000).

The present study was undertaken to develop a reliable detection technique for the early identification of ALB where no previous knowledge of its genome is available. We have developed a relatively simple PCR based system that can be used for quickly detecting the presence of this species using trace quantities of DNA isolated from body parts at different developmental stages or even frass.

MATERIALS AND METHODS

Insects

Eight field collected Cerambycid species, Asian long-horned beetle, *Anoplophora glabripennis* (Motschulsky), Citrus long-horned beetle, *A. chinensis* (Forster), White-spotted long-horned beetle, *A. malasiaca* (Thomson), *A. nobilis* (Ganglbauer), white-spotted sawyer, *Monochamus scutellatus* (Say), Cotton wood borer, *Plectrodera scalator* (Fab), Elm borer, *Saperda tridentata* (Olivier), and *Graphisurus fasciatus* (Degeer) were used in the present study.

The four *Anoplophora* species are exotic; the other four species are native to Canada.

Genomic DNA Extraction

Whole insects (adult, pupa, larva) were ground with a mortar and pestle in liquid nitrogen. A buffer mixture containing 100 mM Tris-HCl, pH 8.0, 50 mM NaCl, 50 mM EDTA, and 1% SDS was added to the ground-up material. The mixture was then incubated at 37°C for 2 h and the proteins were removed in a series of partition procedures using phenol, phenol:chloroform:isoamyl alcohol, and chloroform: isoamyl alcohol. The DNA was precipitated with alcohol and dissolved in 1 × TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA was treated with RNase A (10 mg/ml) at 37°C for 1 h, following which it was ethanol precipitated, and dissolved in 1 × TE. DNA was also isolated from a single insect leg, wing, antenna, egg, and frass using the DNeasy Tissue kit (QIAGEN Inc. Mississauga, ON, Canada), according to the manufacturer's recommendations.

RAPD-PCR Analysis of Genomic DNAs

Genomic DNA collected separately from *A. glabripennis, A. chinensis, A. malasiaca, A. nobilis, M. scutellatus, P. scalator, S. tridentata,* and *G. fasciatus* was used as templates for RAPD amplification. Two hundred and thirty (230) different decameric Operon primers (the A to K kits from Operon Technologies, Alameda, CA) were screened against the eight Cerambycid species. Optimal amplification conditions were: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 µM of each dNTP, 100 nM primer, 20 ng genomic DNA, and 1.25 U of *Taq* polymerase (GIBCO BRL) in a final volume of 20 µl. Amplification was performed in a PTC-150 Minicycler (MJ Research, Inc., Watertown, MA), which had a set program of 45 cycles at 94°C for 1 min; 35°C for 1 min; and 72°C for 2 min. The first cycle of denaturation was carried out at 94°C for 2 min, and the last cycle of extension at 72°C was extended to 10 min. RAPD products were resolved by electrophoresis in 1.4% agarose gels. All amplification reactions were performed at least twice using different batches of DNA.

Southern Blotting

PCR products were transferred from agarose gels onto nylon membranes (Hybond N^+, Amersham Pharmacia Tech., Baie d'Urfe, Quebec) according to Sambrook et al. (1989). The RAPD fragment used as a probe was eluted from the gel and labelled with α-[^{32}P]-dATP using a Random Primer DNA labelling kit (Invitrogen Canada Inc., Burlington, ON) according to the supplier's recommendations. After hybridization, the membranes were washed under high stringency conditions (twice in 2 × SSC, 0.1% SDS at 65°C for 5 min each; twice in 0.5 × SSC, 0.1% SDS at 65°C for 15 min each and twice in 0.1 × SSC, 0.1% SDS at 65°C for 5 min) and autoradiographed. To check the copy number of the SCAR marker in the genomes, 5 µg of total genomic DNA from the eight Cerambycid species were digested for 12 h with 20 U of *Eco*R1 restriction enzyme. The DNA fragments were separated in a 0.8% agarose gel in TAE buffer at 45 V for 12 h. The procedures of transferring, hybridization, and washing were as described above.

Sequencing of PCR Products

DNA fragments were recovered from agarose gels using glassmilk. The DNA fragments were then cloned into pGEM-T Easy vector (Promega Corporation, Madison, WI) and transformed into XL-1 Blue competent cells. Sequencing grade plasmid DNA was extracted using the QIAprep Minipreps DNA kit protocol (QIAGEN), according to the manufacturer's recommendations. Sequencing of the cloned PCR products was carried out in an ALFexpress Sequencer (Pharmacia Biotech).

Diagnostic PCR

Oligonucleotide primers (22-mer) were designed and synthesized on the basis of the sequence of the *A. glabripennis*-specific RAPD-PCR fragment

and used as SCAR primers. Each pair of primers was used in 20-µl PCR reactions as follows: 2 min 94°C, 30 cycles of 15 s at 94°C, 30 s at 65°C (SCAR1 and SCAR2) or 68°C (SCAR3) and 30 s at 72°C with a final extension of 5 min at 72°C. PCR products were analysed using agarose gel electrophoresis as before.

RESULTS

RAPD-SCAR Markers of *A. glabripennis*

In order to develop a PCR-based indentification scheme for *A. glabripennis*, a total of 230 random decamer primers were screened to generate RAPDs from the 8 different Cerambycid species. One primer, OPG-04, gave rise to a polymorphic band of 2,740-bp, which was detected only in *A. glabripennis* (Fig. 1a). Nucleotide sequencing confirmed that this RAPD fragment was 2,740 bp long and had the original primer sequence (OPG-04, AGCGTGTCTG) at both ends. A BLAST Genbank database search showed that a short stretch (50–300 nt) of this fragment was similar to a core oligosaccharide biosynthetic gene cluster in the bacteria, *Salmonella enterica* (unpublished data). No other homologues were found in the Genbank. A RAPD-PCR amplified DNA blot was hybridized using a radioactively labeled full-length 2,740-bp fragment to confirm that this fragment was present only in ALB. The hybridization signal showed that this fragment was positive only for ALB (Fig. 1b). To detect cross-hybridizations as well as to check the copy number of this fragment in the genome of *A. glabripennis*, a Southern blot was performed using genomic DNA isolated from all species. Genomic DNA from the eight Cerambycid species was

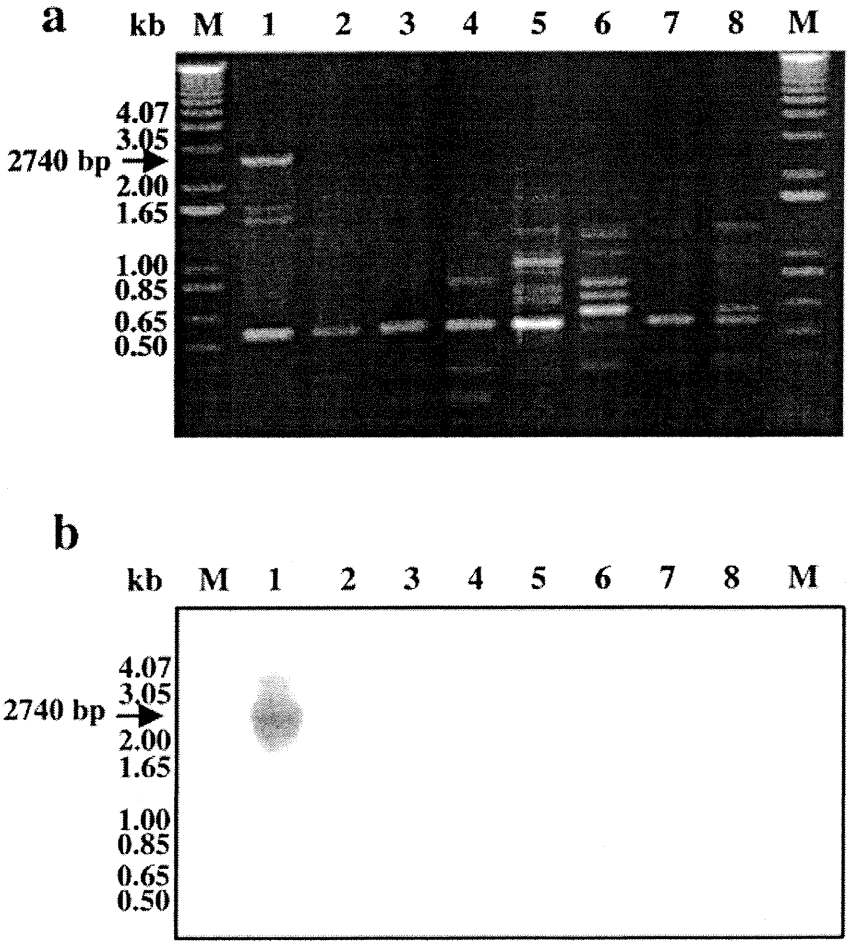

Fig. 1. RAPD analyses with primer OPG-04. **a**: RAPD profiles for eight Cerambycid species. **Lane 1**: *A. glabripennis*; **Lane 2**: *A. chinensis*; **Lane 3**: *A. malasiaca*; **Lane 4**: *A. nobilis*; **Lane 5**: *P. scalator*; **Lane 6**: *M. scutellatus*; **Lane 7**: *S. tridentata*; **Lane 8**: *G. fasciatus*. ALB-specific RAPD fragment (2,740 bp) is indicated by an arrow. **b**: Southern blot of RAPD showing the homology of a 2,740-bp fragment only in the ALB lane. The DNA in (a) was transferred to a membrane and probed with the labeled 2,740-bp PCR product. **Lane M**: Corresponds to 1 kb DNA ladder (GIBCO BRL).

digested with *Eco*R1 and probed with the labeled 2,740-bp fragment. The probe hybridized strongly as a smear in *A. glabripennis* DNA, indicating that the fragment contained multiple copies of this DNA sequence (Fig. 2a). This probe reacted weakly to a DNA fragment from all other species. For this reason, a 745-bp fragment was excluded from the 5′ end of the original ALB-specific 2,740-bp fragment and the remaining 2-kb fragment was used as probe in Southern hybridization. This probe hybridized strongly as a smear only to genomic DNA of ALB. No hybridization signal was detected with genomic DNA from the other seven species (Fig. 2b).

To develop reliable and specific markers, we followed an approach previously described by Paran and Michelmore (1993) and Agusti et al. (1999) to convert this RAPD marker into SCAR markers. Three pairs of SCAR primers (22 bp in length) were designed according to the sequence of the ALB-specific 2,740-bp fragment and used in diagnostic PCR (Fig. 3). The sequence of the 5′ forward primer (ALB-F) was the same for all three pairs of SCAR primers: 5′-GGGTTTGGGTAGTGGAGTTCAT-3′. The reverse SCAR primers were 5′-GCATAAGACCTTCT CCCATTGC-3′ (ALB-R1), 5′-CGGCGTATTCGAG TACAAAGCG-3′ (ALB-R2) and 5′-CGACCGTGCCT TTATCTTGGCA-3′ (ALB-R3). Each SCAR primer pair was used separately to amplify the DNA of the eight Cerambycidae species. The SCAR1 primers (ALB-F/ALB-R1) amplified a fragment of 745 bp in all species (data not shown), and is, therefore, not capable of differentiating ALB from other closely related species. When SCAR2 primers (ALB-F/ALB-R2) were used, a single PCR product with the expected size (1,237 bp) was detected only in the genomic DNA of *A. glabripennis* (Fig. 4). Similarly, SCAR3 primers (ALB-F/ALB-R3) resulted in amplification of a single PCR product of 2,720 bp in *A. glabripennis,* but not in other species (Fig. 4). Thus, differentiation of ALB from other closely related exotic and native species was possible with the SCAR2 and SCAR3 primers, which produced the markers of 1,237 and 2,720 bp, respectively, only in ALB and not in the other species of beetles.

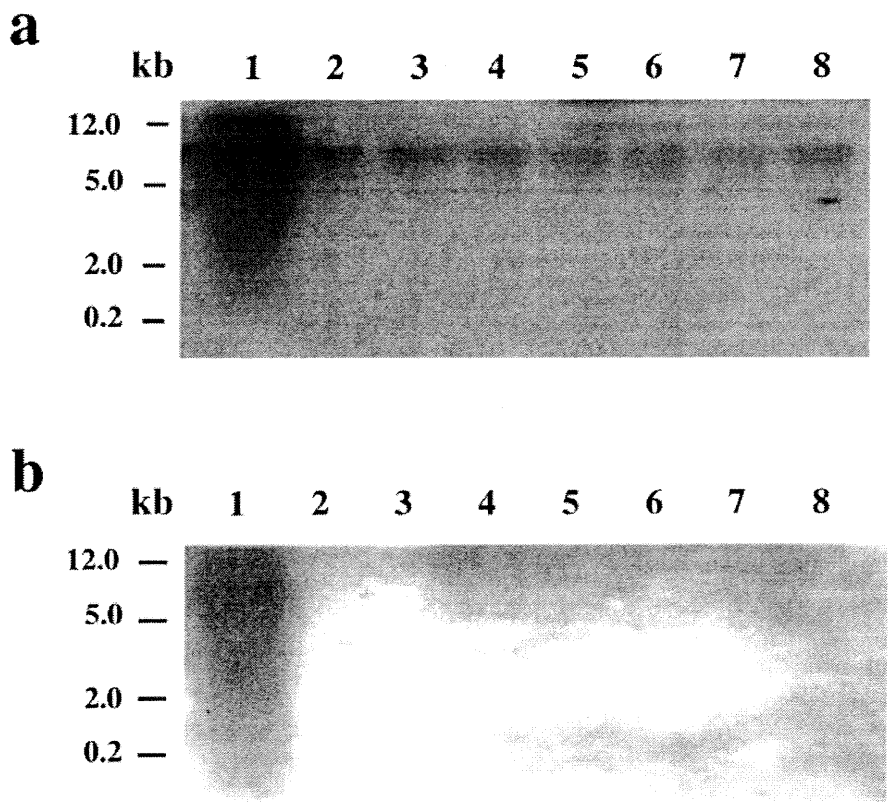

Fig. 2. Southern blot analysis of *Eco*RI-digested genomic DNAs of eight Cerambycid species hybridized with (a) 2,740-bp and (b) 2,000-bp probes. **Lane 1:** *A. glabripennis;* **Lane 2:** *A. chinensis;* **Lane 3:** *A. malasiaca;* **Lane 4:** *A. nobilis;* **Lane 5:** *P. scalator;* **Lane 6:** *M. scutellatus;* **Lane 7:** *S. tridentate;* **Lane 8:** *G. fasciatus.*

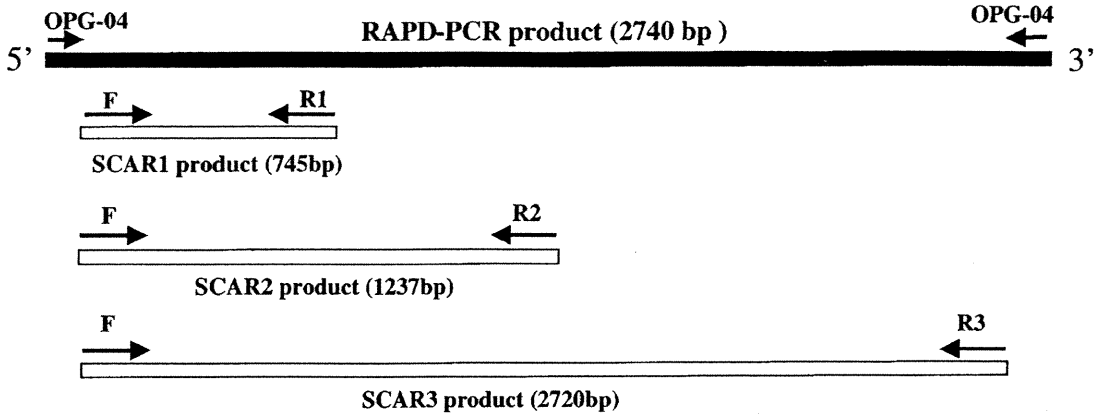

Fig. 3. Diagram of the location of the SCAR primers and markers in the OPG-04 generated 2,740-bp ALB-specific RAPD PCR product. Solid bar, RAPD fragment; open bars, SCAR products.

To check the validity of these two SCAR markers, genomic DNA samples from another 20 (10 male and 10 female) individuals of ALB were examined using the SCAR2 and SCAR3 primer pairs. The fragments of the expected sizes (1,237 and 2,720 bp) were detected in all samples (Fig. 5a,b).

Efficacy of SCAR Markers

To study the efficacy of these two pairs of SCAR primers (SCAR2 and SCAR3), we used ALB genomic DNA from different developmental stages as templates in PCR reactions. Results showed that SCAR2 yielded a single 1,237-bp PCR product in the samples of adult, pupa, larva, and egg of *A. glabripennis* (Fig. 6a). When SCAR3 was used, a single 2,720-bp PCR product was generated (Fig. 6b). We also tested these two pairs of SCAR primers on different body parts of ALB, such as wing, leg, and antenna. These two primers were capable of amplifying the expected size PCR products in all the samples (Fig. 6a,b).

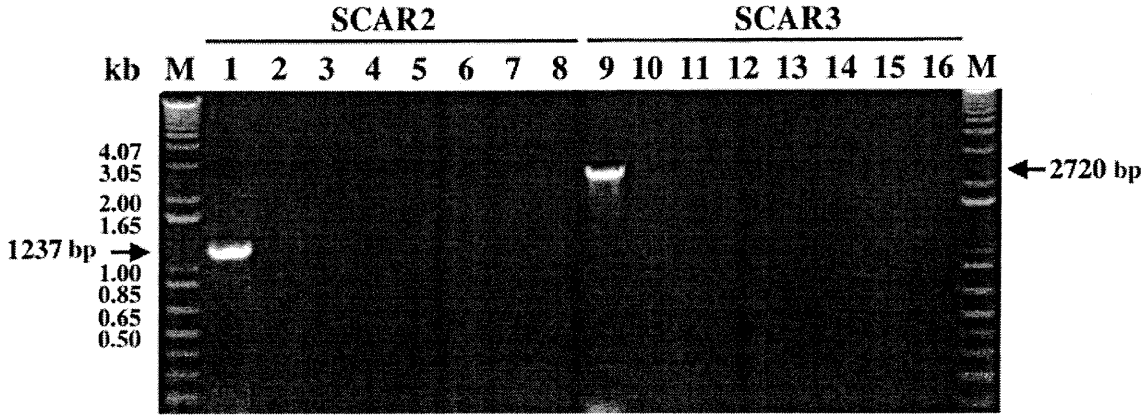

Fig. 4. Agarose gel showing the PCR products amplified from the genomic DNA of the 8 Cerambycid species using SCAR primer pairs SCAR2 and SCAR3. **Lanes 1,9:** *A. glabripennis*; **Lanes 2,10:** *A. chinensis*; **Lanes 3,11:** *A. malasiaca*; **Lanes 4,12:** *A. nobilis*; **Lanes 5,13:** P. *scalator*; **Lanes 6,14:** *M. scutellatus*; **Lanes 7,15:** *S. tridentate*; **Lanes 8,16:** *G. fasciatus*. The specific SCAR markers 1,237 and 2,720 bp are indicated by arrows. **Lane M:** Corresponds to 1 kb DNA ladder.

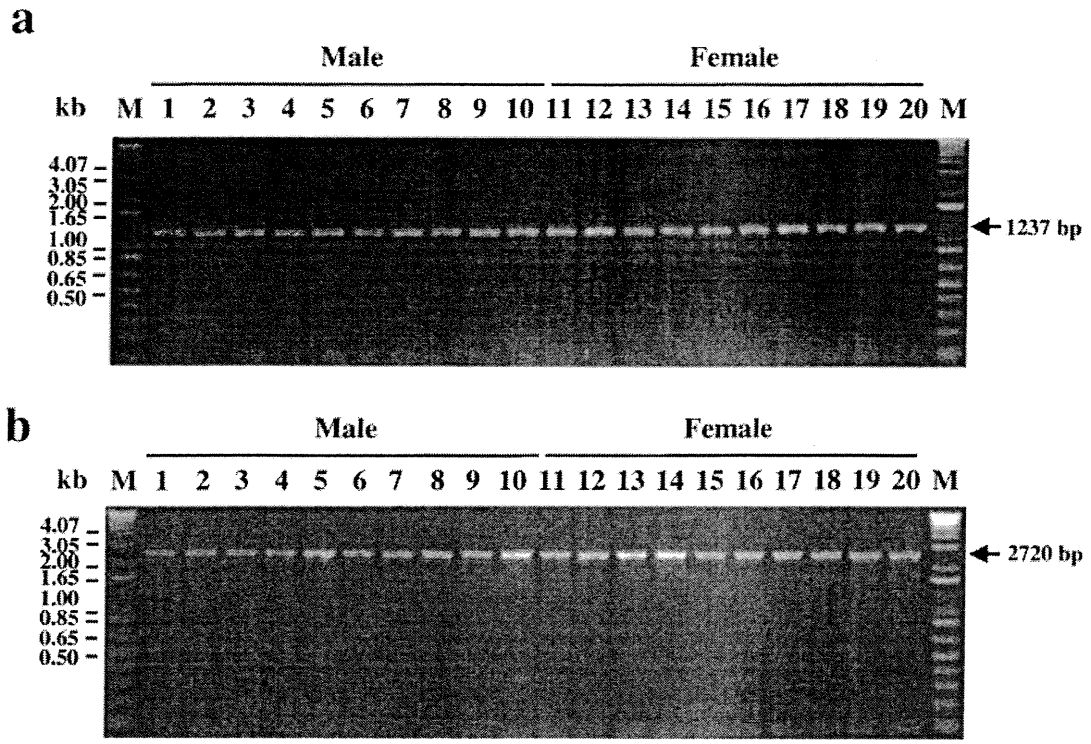

Fig. 5. SCAR markers detected in 20 individual ALB insects. The 20 ALB samples included 10 males (**Lanes 1–10**) and 10 females (**Lanes 11–20**). **a**: SCAR2 marker, 1,237 bp; **b**: SCAR3 marker, 2,720 bp. Arrows indicate the specific SCAR markers. **Lane M**: Corresponds to 1 kb DNA ladder.

Sensitivity of SCAR Markers

Sensitivity was determined by using 3 different DNA samples from both eggs and legs. DNA from a single egg and a single leg was dissolved separately in 40 µl TE and serially diluted. Dilutions of 1:6, 1:24, 1:96, 1:384, 1:1,536, and 1:6,144 gave a positive response in all samples of egg and leg DNA using the SCAR2 primers. The highest dilution detectable was 1:24,576 in one of the egg samples. Using the SCAR3 primer pair all the samples of egg and leg showed a positive response up to 1:1,536 dilutions. But in one of the egg samples, the highest dilution detectable was 1:6,144.

Detection of *A. glabripennis* Using DNA From Frass

SCAR2 and SCAR3 were tested against the PCR product of DNA isolated from five different frass samples collected from each of the 2 species, *A. glabripennis* and *M. scutellatus*, from different places. Both SCARs yielded the expected size PCR fragments in all frass samples of *A. glabripennis* but not from *M. scutellatus* (Fig. 7).

DISCUSSION

Exotic insect species introduced into North America pose a serious threat to the biodiversity and economic interest of the continent and may even alter forest ecosystems (Haack and Mastro, 1997). The Asian long-horned beetle is an important hardwood pest in China and other parts of Asia and has been recently introduced into North America (Cavey et al., 1998). The larvae attack healthy trees, stressed trees, and cut logs (Peng and Liu, 1992; Gao et al., 1993). Although adults may cause twig mortality during feeding, larvae cause most of the damage (Haack and Mastro, 1997).

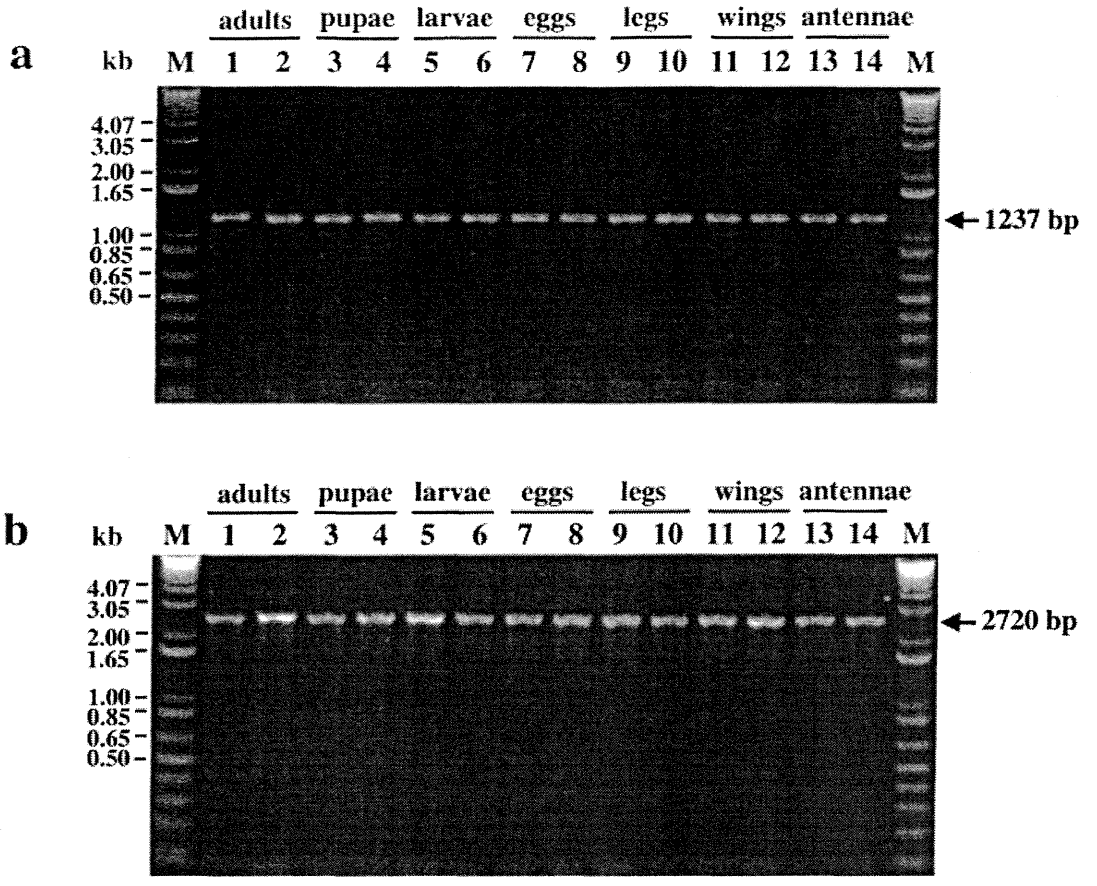

Fig. 6. Agarose gel showing SCAR markers in insects at different developmental stages and tissues of *A. glabripennis* (a) SCAR2 marker, 1,237 bp; (b) SCAR3 marker, 2,720 bp. **Lanes 1, 2**: adults; **Lanes 3, 4**: pupae; **Lanes 5, 6**: larvae; **Lanes 7, 8**: eggs; **Lanes 9, 10**: legs; **Lanes 11, 12**: wings; **Lanes 13, 14**: antennae. Arrows indicate the specific SCAR markers. **Lane M**: Corresponds to 1 kb DNA ladder.

Early detection and removal of ALB-infested material is essential for controlling the spread of this beetle. The morphological similarities between native species and ALB, especially at the larval stage, make it difficult to positively identify this species. The only reliable method for the detection and identification of this species is to use DNA markers.

In recent years, molecular DNA markers have been used extensively for identification purposes. These markers can be grouped as hybridization-based markers/non-PCR markers or PCR-based markers. In the former, DNA profiles are visualized by hybridizing the restriction enzyme digested DNA to a labeled probe, which is a DNA fragment of known origin or sequence. From a practical point of view, hybridization systems are difficult and time-consuming when analyzing a large number of samples. PCR-based systems, on the other hand, involve in vitro amplification of a particular DNA sequence or locus, with the help of specific or arbitrarily chosen oligonucleotide sequences that are used as primers. The PCR-based methods are simple and fast and require no prior knowledge of the genome of the test species. Each method has its own advantages and drawbacks with regard to ease of application, reproducibility, and detection range (Table 1).

Considerable progress has been made in the application of PCR-based methodologies for the identification of various pests and pathogens. The

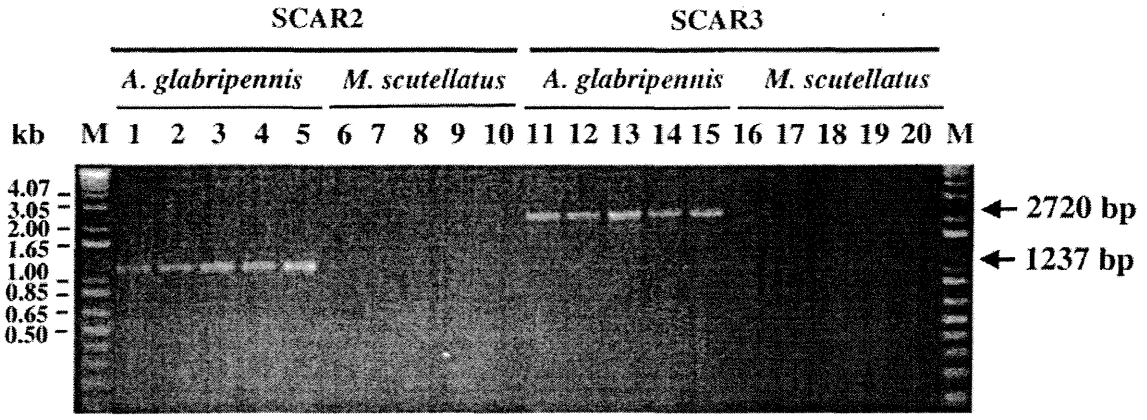

Fig. 7. Agarose gel showing PCR amplified SCAR markers from frass. **Lanes 1–10**: SCAR2. **Lanes 11–20**: SCAR3. PCR products were observed only in the ALB frass samples (Lanes 1–5 and 11–15). No PCR products were detected in the *M. scutellatus* samples (Lanes 6–10 and 16–20). Arrows indicate the specific SCAR markers. **Lane M**: Corresponds to 1 kb DNA ladder.

advances in molecular techniques have resulted in the development of various kinds of PCR-based molecular markers, such as RAPD (Williams et al., 1990), SCAR (Paran and Michelmore, 1993), and AFLP (amplified fragment length polymorphism, Vos et al., 1995). An analysis of genomic polymorphisms using RAPDs has been widely used for DNA fingerprinting, varietal identification, classification, and population genetics (Williams et al., 1990; Rollinson and Stothard, 1994; Loxdale et al., 1996). Although the RAPD technique appears to be the simplest and the most flexible method, the low annealing temperature and short primer size used for polymorphism generation may affect amplification reproducibility, and may make this technique inappropriate for diagnostic purposes. Among the molecular markers that have been developed thus far, SCAR markers appear to be one of the most reliable methods (Michelmore et al., 1991; Paran and Michelmore 1993; Nair et al., 1995, 1996). There are several advantages to the use of SCAR markers. First, SCAR markers do not require large amounts of purified DNA, in contrast to hybridization methods like restriction fragment length polymorphisms (RFLP). Second, with SCAR markers, analysis of the results is more straightforward than other PCR-based markers, such as RAPD and AFLP. SCAR markers can be revealed by a single PCR procedure, without subsequent digestion with a restriction enzyme. Third, genomic information can be obtained directly by analyzing PCR products. Consequently, SCAR markers offer the most practical method for

TABLE 1. Molecular Detection Systems Currently Being Used*

Detection system	Detection range	Reference
Non-PCR systems:		
RFLP	Species, subspecies, strain	Rademaker and de Bruijn (1997)
DNA sequencing	Family, genus, species subspecies, strain	Rademaker and de Bruijn (1997)
PCR-based systems:		
RAPD	Species, subspecies, strain	Kengne et al. (2001)
ISSR	Species, subspecies, strain	Zeitkeinicz et al. (1994); Kumar et al. (2001)
SSR	Species, subspecies, strain	Brown and Tanksley (1996)
AFLP	Species, subspecies, strain	Vos et al. (1995)
SCAR	Species, subspecies, strain	Rosa-Hermosa et al. (2001)
DAF	Species, subspecies, strain	Rademaker and de Bruijn (1997)
AP-PCR	Species, subspecies, strain	Welsh et al. (1991); Rademaker and de Bruijn (1997)
ITS	Species, subspecies	Torres et al. (2000)
mtDNA	Species, subspecies, strain	Figueroa et al. (1999)

*RFLP: restriction fragment length polymorphism; RAPD: random amplified polymorphic DNA; ISSR: inter simple sequence repeats; SSR: simple sequence repeats; AFLP: amplified fragment length polymorphism; SCAR: sequence characterized amplified region; DAF: DNA amplification fingerprinting; AP-PCR: arbitrary primed polymerase chain reaction; ITS: internal transcribed spacer; mtDNA: mitochondrial DNA.

screening numerous samples in a short time with minimal labor. They also offer the starting point for cloning any target gene uncovered by the marker sequence.

The high sensitivity of the SCAR markers together with their accuracy makes them reliable tools for the identification of ALB. The SCAR technique was first applied to the identification of the downy mildew resistance genes in lettuce (Paran and Michelmore, 1993), and more recently to the biological control agents *Gliocladium catenulatum* (Paavanen-Huhtala et al., 2000) and *Trichoderma atroviride* (Rosa-Hermosa et al., 2001). SCAR markers have been successfully developed for the detection of gut contents from the tobacco budworm, *Helicoverpa armigera* (Agusti et al., 1999) and the whitefly, *Trialeurodes vaporariorum* (Agusti et al., 2000). SCAR markers have also been used to differentiate between different strains of the Asian rice gall midge, *Orseolia oryzae* (Behura et al., 1999), and to distinguish the Asian from the North American gypsy moth, *Lymantria dispar* (Garner and Slavicek, 1996). Similarly, SCARs have been developed for tagging genes in plants that confer resistance against insects, fungi, and nematodes (Paran and Michelmore 1993; Williamson et al., 1994; Nair et al., 1995, 1996).

In the present study, an ALB-specific RAPD fragment was converted to three SCAR markers for greater reliability and reproducibility. Using the SCAR1 primer pair, a 745-bp PCR product was generated in ALB as well as all other species tested and was, therefore, deemed to be unsuitable for discriminating ALB from other species. The other two pairs of SCAR primers, SCAR2 and SCAR3, amplified bands of 1,237 and 2,720 bp, respectively, from the genomic DNA of ALB, but not from the genomic DNA of the other seven species including three invasive and four indigenous beetles. *Anophlophora* is a large genus of Asian Cerambycidae, all of which closely resemble each other. Moreover, long-horned beetles of the genus *Anophlophora* morphologically resemble the indigenous white spotted sawyer beetle, *M. scutellatus*, a common pest of conifers in North America. These phenotypic similarities between these beetles warrant a DNA-based method for unambiguous detection.

When the OPG-04 primer was used, a 2,740-bp ALB-specific RAPD fragment was amplified. However, when the SCAR1 primers were used, a 745-bp fragment was amplified, which could not distinguish ALB from other species. Furthermore, the Southern blot using the full-length 2,740-bp fragment as a probe showed that this fragment did not only hybridize to the genomic DNA of ALB but also to the genomic DNA of the other seven species (Fig. 2a). On the other hand, when SCAR2 and SCAR3 primers were used, they amplified two fragments (1,237 and 2,720 bp), that were unique to ALB (Fig. 4). When a 2,000-bp probe without the 5′ end (745 bp) of the 2,740-bp fragment was used, no hybridization was detected for the other species (Fig. 2b). This result indicates that the 5′ end (745 bp) of the fragment might be homologous to all species. The remaining region (~2,000 bp) was present only in ALB.

Any identification method for ALB should combine both sensitivity and reliability for discriminating between species. In the present work, we have shown that the SCAR primers developed can amplify the corresponding markers using a concentration as low as 1:24,576 dilution of the genomic DNA isolated from egg samples. A similar sensitivity was reported with the SCAR marker developed for *H. armigera* (Agusti et al., 1999). The accuracy rate for the SCAR markers in correctly identifying ALB samples was 100% for the 20 insects collected in North America. For the successful use of these SCAR markers, a large number of samples have to be analyzed for validation by potential users.

The PCR-based assay described here is fast, reliable, and easy to conduct in the laboratory. This PCR assay can be carried out within a day using unknown genomic DNA from any developmental stages, body parts, or frass. To implement this technique in other laboratory environments, we tested these SCAR markers (SCAR2 and SCAR3) with three different PCR machines and with three different *Taq* polymerases. The SCAR amplification profile remained the same in all the systems tested (data not shown). These SCAR markers are robust

tools for detection and identification of ALB because of their pronounced accuracy, ease of use, and cost effectiveness. They can be applied for the (1) detection of ALB in the infected regions; (2) diagnostic testing and routine screening of samples at ports of entry; (3) investigation to pinpoint their origin(s) in Asia; and (4) determination of origins from a single or multiple regions. We are currently using these SCAR markers to develop a user-friendly kit for ALB detection.

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