Carposina sasakii (Lepidoptera: Carposinidae) in its Native Range Consists of Two Sympatric Cryptic Lineages as Revealed by Mitochondrial COI Gene Sequences

J. Wang, Y. Yu, L.-L. Li, D. Guo, Y.-L. Tao, and D. Chu

1Key Laboratory for Plant Virology of Shandong Province, Plant Protection Institute, Shandong Academy of Agricultural Sciences, Jinan 250100, P. R. China
2Corresponding author; e-mail: chudong1977@hotmail.com; zbdl3@163.com
3Key Lab of Integrated Crop Pest Management of Shandong Province, College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao, 266109, P. R. China

Subject Editor: Maria Gabriela Murúa

J. Insect Sci. (2015) 15(1): 85; DOI: 10.1093/jisesa/iev063

ABSTRACT. The genetic differentiation and genetic structure of the peach fruit moth, Carposina sasakii Matsumura (Lepidoptera: Carposinidae), was investigated in China, where the moth is native. The mitochondrial cytochrome c oxidase I (COI) gene of 180 individuals from 16 collections were sequenced and analyzed. The results showed that two sympatric and cryptic mtDNA lineages existed within C. sasakii in China. The genetic differentiation has significant correlation with the geographical distance, but has no evidence for host plant associations. Our results of haplotype distribution suggest that the C. sasakii individuals can naturally move between areas, while the movement of individuals between long-distance locations may be associated with human activities such as the transport of fruit. Finally, an mitochondrial COI gene PCR-RFLP method was developed to differentiate the two cryptic mtDNA lineages within C. sasakii, which provides rapid and reliable tool for the future research of the two lineages.

Key Words: Carposina sasakii Matsumura, genetic differentiation, cryptic mtDNA lineages, mitochondrial COI gene

The peach fruit moth, Carposina sasakii Matsumura, is an important fruit pest in Korea, Japan, China, and Russia Far East (Liu et al. 1997; Kim et al. 2000). The moth larvae can damage more than 20 kinds of fruit including peach, apricot, hawthorn, apple, pear, jujube, and wild jujube (Kim et al. 2001; Kim and Lee 2002; Ishiguri and Shirai 2004; Xu and Hua 2004).

In China, C. sasakii has been recorded from 24 provinces (Liu et al. 1997). Some collections of this pest were considered host biotypes (Hua and Hua 1995) because emergence time, oviposition habitat, and damage characteristics seemed to be specific to the C. sasakii associated with particular hosts. The genetic differentiation of these purported host biotypes has been studied using esterase isozyme patterns (Hua and Hua 1995) and random amplified polymorphic DNA (RAPD) (Xu and Hua 2004). Using RAPD method, Xu and Hua (2004) documented genetic differentiation among C. sasakii collected from apple, hawthorn, peach, apricot, jujube, and wild jujube. The specimens associated with apricot differed the most markedly from other host races, and the authors suggested that it should be considered as a good species. However, the genetic differentiation of the pest throughout China has not been well studied, which is indispensable to understand the evolution of the pest in its native ranges.

The aim of this research was to investigate the genetic structure of the peach fruit moth and its relationship with geographical distance and host plants by using the mitochondrial cytochrome c oxidase I (COI) gene as a marker. The mitochondrial COI gene marker has been widely used for species identification, for determining the genetic structure and differentiation (De Barro et al. 2011). In this article, we first analyze the genetic differentiation and haplotype distribution of C. sasakii in China, the native area of the pest, using samples collected from a range of host plants. Second, we calculate the genetic diversity of different host collections. Third, the correlation between genetic distance and geographical distance was analyzed. Finally, an mitochondrial COI gene PCR-RFLP method was developed to distinguish between the lineages within C. sasakii.

Materials and Methods

Insect Samples. In the autumn of 2010, C. sasakii larvae were collected from a range of orchards in 16 main fruit-growing regions in China (Fig. 1). Collected specimens were first identified morphologically followed the description by Liu et al. (2011). In total, there were 16 collections (Table 1). The larvae were put in 95% ethanol and then stored at −20°C prior to DNA extraction.

DNA Extraction, PCR, and Mitochondrial Sequencing. DNA was extracted from a single larva using the DNAzol kit (Molecular Research Center, Inc., Cincinnati, OH) following the manufacturer’s specifications. COI fragments were amplified from 180 individuals using the primers LCO1490 (5’-GGTCAACAATATCAAAAATATTGG -3’) and HCO2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA -3’) (Folmer et al. 1994). Each PCR reaction (25 μl) contained 2 μl of template DNA, 1 unit of Taq polymerase (extracted from Thermus Aquaticus) (Biomed biotechnology Co. Ltd), 2.5 μl of MgCl2 (2.5 mmol/l), 2 μl of dNTPs (10 mmol/l), 1 μl of 20 μmol/l of each primer, and 2.5 μl of 10 × PCR buffer. PCR amplifications began with 94°C denaturation for 5 min; followed by 35 cycles of 94°C denaturation for 1 min, 52°C annealing for 1 min, and 72°C extension for 2 min; and a final 72°C extension for 10 min. The PCR product was checked by agarose gel electrophoresis. PCR should have yielded an ~710 bp fragment (Folmer et al. 1994). The PCR product was purified and then sequenced directly. The 180 sequences were aligned with Clustal W (Thompson et al. 1994) and were then checked for insertion and deletion (indel). The final 588-bp sequences (the alignment length) were used to analyze the lineage-specific restriction enzyme sites and the genetic structure of collection.DNA identification

As precise identification of young and/or old larvae of C. sasakii and similar fruits-infesting species are difficult or may be impossible practically (Sony et al. 2009; Hada and Sekine 2011), we conducted a phylogenetic analysis using the COI sequences collected in the present study and the homologous GenBank sequences of C. sasakii as well allied species available by 1 July 2012. Based on the resulting
phylogenetic tree, the haplotypes were involved in the monophyletic group of “C. sasakii” were retained and considered as C. sasakii.

**Genetic Structure Analysis.** A Median joining (MJ) network was calculated and drawn with the program NETWORK 4.5 (Bandelt et al. 1999) to investigate the possible relationships among haplotyes of C. sasakii. Genetic parameters for mitochondrial were estimated for the different collections from China using DnaSP 5.0 (Librado and Rozas 2009). The parameters included the number of polymorphic (segregating) sites ($S$), the total number of mutations ($\eta$), the average number of nucleotide differences ($K$), the number of haplotypes ($H$), haplotype diversity ($Hd$), nucleotide diversity ($\pi$), defined as the average number of pairwise nucleotide differences per site), and the nucleotide diversity with Jukes and Cantor correction ([JC]) within each collection and region.

The ARLEQUIN version 3.1 (Excoffier et al. 2005) was used for pairwise $F_{ST}$ estimation (a measure of genetic differentiation between populations) among populations, and for analysis of molecular variance (AMOVA), and pairwise haplotype distances ($P$-distance) were calculated by Mega 5.0 (Tamura et al. 2011). Gene flow within and among populations was approximated as $Nm$ [analogous to $M = (1/F_{ST} - 1)/2$] (Slatkin 1993), where $Nm$ is a measure of the extent of gene flow in an island model at equilibrium and was calculated by Dnasp 5.0 (Librado and Rozas 2009).

The correlation between $F_{ST}$ and geographical distance of all populations was estimated using the Mantel test of IBD.1.5.2 (isolation-by-distance) (Bohonak 2002). The regression was made using $F_{ST}$ against geographical distance. Pairwise geographic distances (straight-line distance) between collections were calculated by longitude and latitude.

**Identification of Lineage-Specific Restriction Enzyme Sites and Determination of Lineages Based on Mitochondrial COI Gene PCR-RFLP.** The nucleotide differences in the 37 mitochondrial COI gene haplotype sequences that were detected were analyzed to select a restriction endonuclease for distinguishing among sequences. A restriction endonuclease, BsaJI (NEB, MA) that cleaves DNA at “CCNNGG” sites was selected. All 180 individuals from the 16 collections were used to test the utility of the BsaJI-based PCR-RFLP. A 10-µl volume of PCR product was digested at 60°C for 2 h with 2 units of BsaJI. The BsaJI-digested PCR products were electrophoresed on 1.0% agarose gel and visualized by ethidium bromide staining. Based on the sizes of bands produced by BsaJI digestion, the lineage of each individual was determined.

---

**Table 1. Carposina sasakii collections from China**

| Collection code | Collection site | Host plant | Collection date | Lineages markers | Individuals of COI sequences | Individuals of COI PCR-RFLP |
|-----------------|-----------------|------------|----------------|-----------------|-------------------------------|-----------------------------|
|                 |                 |            |                | Lineage I | Lineage II | Total Lineage I | Lineage II | Total Lineage I | Lineage II |
| DL              | DaLian, LiaoNing| Apple      | 8/2010         | 12         | 10         | 2               | 12                 | 10          | 2               |
| SY              | ShenYang, LiaoNing| Apple      | 9/2010         | 6          | 4          | 2               | 6                 | 4           | 2               |
| MDJ             | MuDanJiang, HeiLongJiang| Apple      | 8/2010         | 8          | 2          | 6               | 8                 | 2           | 6               |
| XY              | XianYang, ShanXi| Apple      | 9/2010         | 8          | 0          | 8               | 8                 | 0           | 8               |
| JJ              | JinZhong, ShanXi| JuJube     | 9/2010         | 5          | 0          | 5               | 5                 | 0           | 5               |
| LS              | LiangShan, SiChuan| Apple      | 9/2010         | 5          | 0          | 5               | 5                 | 0           | 5               |
| KX              | LongKou, ShanDong| Apple      | 8/2010         | 17         | 17         | 0               | 17                 | 17          | 0               |
| NY              | NingYang, ShanDong| JuJuBeb     | 9/2010         | 11         | 11         | 0               | 11                 | 11          | 0               |
| FC              | FeiCheng, ShanDong| Peach       | 8/2010         | 19         | 18         | 1               | 19                 | 18          | 1               |
| LJ              | LaiYang, ShanDong| Apple      | 9/2010         | 18         | 8          | 10              | 18                 | 8           | 10              |
| JN              | JinNan, ShanDong| Apple      | 9/2010         | 11         | 7          | 4               | 11                 | 7           | 4               |
| TA              | TaiAn, ShanDong | JuJube     | 9/2010         | 4          | 4          | 0               | 4                 | 4           | 0               |
| YT              | YanTai, ShanDong| Apple      | 9/2010         | 16         | 16         | 0               | 16                 | 16          | 0               |
| LWP             | LaiWu, ShanDong | Apple      | 9/2010         | 13         | 0          | 13              | 13                 | 0           | 13              |
| LWZ             | LaiWu, ShanDong | JuJube     | 9/2010         | 8          | 0          | 8               | 8                 | 0           | 8               |
| LWS             | LaiWu, ShanDong | Wild juJube| 9/2010         | 19         | 0          | 19              | 19                 | 0           | 19              |

Note: all specimens were larvae and were collected in October 2010.
haplotype network (Fig. 2). One cluster (code: cluster I) included 21 haplotypes (H1, H2, H4, H6, H14–H24, and H26–H31) that were distributed in 10 of the 16 host collections; cluster II haplotypes were detected in samples from MuDanJiang (MDJ), DaLian (DL), ShenYang (SY), LaiYang (LY), JiNan (JN), TaiAn (TA), and YanTai (YT). Another cluster (code: cluster II) included 16 haplotypes (H3, H5, H25, H7–H13, H16–H23) distributed in 12 of the 16 host collections; cluster I haplotypes were included in wild jujube. The four collections labeled as LK, NY, TA, and YT contained only cluster II haplotypes, and these were designated as lineage II. The other six collections (DL, SY, MDJ, FC, LY, and JN) contained a mixture of cluster I and II haplotypes. Both cluster I and II haplotypes were found in the collections from apple, jujube, and peach, only cluster I haplotypes were detected in samples from MuDanJiang (MDJ), DaLian (DL), ShenYang (SY), LongKou (LK), NingYang (NY), FeiCheng (FC), LaiYang (LY), JiNan (JN), TaiAn (TA), and YanTai (YT). Another cluster (code: cluster II) included 16 haplotypes (H3, H5, H25, H7–H13, H16–H23) distributed in 12 of the 16 host collections; cluster II haplotypes were detected in samples from MuDanJiang (MDJ), DaLian (DL), ShenYang (SY), XianYang (XY), JinZhong (JZ), JiNan (JN), FeiCheng (FC), LaiYang (LY), LaiWu (LWP, LWZ and LWS), and LiangShan (LS).

The four collections labeled as LK, NY, TA, and YT contained only cluster I haplotypes, and these were designated as lineage I. The six collections labeled as XY, JZ, LS, LWP, LWZ, and LWS contained only cluster II haplotypes, and these were designated as lineage II. The other six collections (DL, SY, MDJ, FC, LY, and JN) contained a mixture of cluster I and II haplotypes. Both cluster I and II haplotypes were found in the collections from apple, jujube, and peach, only cluster II were included in wild jujube.

When $F_{ST}$ ranged from 0 to 0.05, the genetic differentiation is small; $F_{ST}$ ranged from 0.05 to 0.15, the genetic differentiation between populations is moderate; $F_{ST}$ ranged from 0.15 to 0.25, the genetic differentiation is very large (Weight 1978). Among the 120 pairwise $F_{ST}$ (Table 2), 85 values were higher than 0.25 and 87 values were significant, indicating that most of populations were highly differentiated from other populations. Correlation between geographic distance and genetic distance was tested by isolation-by-distance (IBD) (Fig. 3). The test showed a significant positive relationship between pairwise $F_{ST}$ values and geographic distance ($r = 0.3785; P = 0.0140$) among all of the collections.

Results of the estimates of gene flow (Table 2) revealed that TA and NY have the highest gene flow ($Nm = 24.26$), and LWS and LWZ have also the relatively high gene flow ($Nm = 15.19$). Although LS and LW
collections (WZ, LWP, LWS) have the low gene flow between them (the \( Nm \) between LS and LWF, LWP, LWS was 0.00, 0.03 and 0.04, respectively).

Geographic Distribution of Haplotypes and Genetic Diversity. Ten of the 16 collected sequences were found harboring unique haplotypes. In the MDJ, FC, LC, FY, and YT collections, 25.00, 15.79, 11.76, and 18.75% of the individuals, respectively, proved to be unique haplotypes, respectively. In the JZ, LC, LS, LWP, and LWS collections, 20.00, 25.00, 18.18, 15.79, and 15.38% of the individuals, belonged to the unique haplotypes, respectively.

The diversity indexes of LS collection and LW collection (LWZ, LWS, and LWP) were much lower than those in the other collections (Table 3). For instance, the \( Hd \) values of collections LS, LWP, and LWS were less than 0.42308, while the \( Hd \) value of the other seven collections were greater than 0.6000.

Results of AMOVA showed that there was significant genetic structure of \( C. sasakii \) among collections (Table 4), 55.96% (\( P < 0.01 \)) of the variation was among collections and 44.04% was within the collections, which indicated that a considerable portion of the variation was observed among collections.

Utility of BsaJI-Based Mitochondrial COI Gene PCR-RFLP. The restriction endonuclease BsaJI cleaves DNA at “CCNNGG”, a pattern that was present in haplotypes from lineage II but not in haplotypes of lineage I (Fig. 4). Thus, BsaJI digested the 83 mitochondrial COI gene PCR products of individuals in lineage II but did not digest the 97 mitochondrial COI gene PCR products of individuals in lineage I (Table 1; Fig. 5). These results showed that the lineage of each individual could be determined based on the sizes of bands produced by BsaJI digestion.

Discussion

Understanding the genetic differentiation of pest insects is fundamental to understanding pest biology and management. Recent studies have shown that genetically differentiated lineages can differ in adaptation to environments or in invasive ability (Scheffer and Lewis 2005; Winkler et al. 2008). For example, two genetically differentiated lineages of the copepod \( Eurytemora affinis \) in the St. Lawrence River in North America have been identified. Although the lineage that primarily occurs in the central portion of the St. Lawrence River estuary has invaded freshwater lakes, the lineage in the upstream reaches of the estuary and downstream salt marshes has not (Winkler et al. 2008). The dipteran \( Liriomyza sativae \) has at least three genetic lineages including \( sativae-A, sativae-L, \) and \( sativae-W \). However, only the \( sativae-W \) lineage was found to be invasive (Scheffer and Lewis 2005). Our analysis of mitochondrial COI gene revealed that the peach fruit moth, \( C. sasakii \), has two cryptic mtDNA lineages in China, where it is native. Until now, the possible difference in biology (or ecology, or physiology) of the \( C. sasakii \) lineages was unknown.

Our results showed the genetic differentiation has significant correlation with the geographical distance (Fig. 3), but has no evidence for host plant associations. Host plant formation is an important process studied in many species that may explain their genetic structure (Inbar et al. 2004; Ros and Breeuwer 2007). Hua and Hua (1995) studied the esterase isozymes of three host biotypes from jujube, wide jujube, and apple, respectively. They found a great similarity exist between \( C. sasakii \) in hosts of jujube and wide jujube, while the two have a great differentiation with host of apple. The other patterns of random amplified polymorphic DNA (RAPDs) (Xu and Hua 2004) suggested the existence of two host biotypes, the \( C. sasakii \) in hosts of jujube and wide jujube, while the two have a great differentiation with host of apple. The other patterns of random amplified polymorphic DNA (RAPDs) (Xu and Hua 2004) suggested the existence of two host biotypes, the \( C. sasakii \) in hosts of jujube and wide jujube, while the two have a great differentiation with host of apple. The other patterns of random amplified polymorphic DNA (RAPDs) (Xu and Hua 2004) suggested the existence of two host biotypes, the \( C. sasakii \) in hosts of jujube and wide jujube, while the two have a great differentiation with host of apple. The other patterns of random amplified polymorphic DNA (RAPDs) (Xu and Hua 2004) suggested the existence of two host biotypes, the \( C. sasakii \) in hosts of jujube and wide jujube, while the two have a great differentiation with host of apple. The other patterns of random amplified polymorphic DNA (RAPDs) (Xu and Hua 2004) suggested the existence of two host biotypes, the \( C. sasakii \) in hosts of jujube and wide jujube, while the two have a great differentiation with host of apple.
mitochondrial COI gene haplotypes can also be found on different host plant species, that means there were no closely correlation between mitochondrial divergence and host identity (Fig. 2). The distribution of unique haplotypes within different collections showed that gene flow between collections was highly variable. The data suggested individuals naturally move between areas. For example, the locations of TA, NY, and FC are close to each other, and all of the haplotypes in the TA and NY collections were found in the FC collection. Similarly, all of the haplotypes in the LWz collection were also found in the LWP and LWS collections. On the other hand, the movement of individuals between long-distance locations seems to be closely associated with human activities. For example, Hap11, a major haplotype in LW collections (representing 82.5% of all individual in the LW collections), could also be found in JZ (60.0%), FC (5.3%) and LY (50.0%) collections but not in JN, TA, and NY collections. As the map of Shandong indicates (Fig. 1), the distance among JN, TA, and NY are closer than that among JZ, FC, and LY. The movement of individuals with Hap11 among areas may be associated with human activities such as the transport of fruit.

The genetic diversity indexes of collections are variable greatly, which may be affected by the sample size. In the future, more individuals in each collection are needed to evaluate the genetic diversity. Determining whether the two cryptic mtDNA lineages differ in biological and ecological traits will require additional research, and the nuclear gene maybe important to verify this hypothesis. The information from that research should increase our understanding of _C. sasakii_ evolution and management. The current paper demonstrates that the BsaJl-based PCR-RFLP can be used to differentiate the two cryptic mtDNA lineages within _C. sasakii_. The method should be useful for the reliable and rapid monitoring of the population dynamics of the two lineages in the field.

**Acknowledgments**
This work was funded by Public Service Sectors (agriculture) Special Funding for Research (No. 201103024) and the Taishan
Mountain Scholar Constructive Engineering Foundation of Shandong, China.

References Cited
Bandelt, H. J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16: 37–48.
Bohonak, A. J. 2002. IBD (Isolation By Distance): a program for analyses of isolation by distance. Heredity 93: 153–154.
De Barro, P. J., S. S. Liu, L. M. Boykin, and A. B. Dinsdale. 2011. Bemisia tabaci: a statement of species status. Annu. Rev. Entomol. 56: 1–19.
Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (ver. 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1: 47–50.
Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.
Hada, H., and K. T. Sekine. 2011. A diagnostic multiplex polymerase chain reaction method to identify Japanese internal apple-feeding Lepidopteran pests: Grapholita molesta, Grapholita dimorpha (Lepidoptera: Tortricidae), and Carposina sasakii (Lepidoptera: Carposinidae). Appl. Entomol. Zool. 46: 287–291.
Hua, L., and B. Z. Hua. 1995. A preliminary study on the host biotypes of the peach fruit borer. Acta Phytophy. Sinica 22: 165–170.
Inbar, M., M. Wink, and D. Wool. 2004. The evolution of host plant manipulation by insects: molecular and ecological evidence from gall-forming aphids on Pistacia. Mol. Phy. Evol. 32: 504–511.
Ishiguri, Y., and Y. Shirai. 2004. Flight activity of the peach fruit moth, Carposina sasakii (Lepidoptera: Carposinidae), measured by a flight mill. Appl. Entomol. Zool. 39: 127–131.
Kim, D. S., and J. H. Lee. 2002. Egg and larval survivorship of Carposina sasakii (Lepidoptera: Carposinidae) in apple and peach and their effects on adult population dynamics in orchards. Env. Entomol. 31: 686–692.
Kim, D. S., J. H. Lee, and M. S. Yiem. 2000. Spring emergence pattern of Carposina sasakii (Lepidoptera: Carposinidae) in apple orchards in Korea and its forecasting models based on degree-days. Env. Entomol. 29: 1188–1198.
Kim, D. S., J. H. Lee, and M. S. Yiem. 2001. Temperature dependent development of Carposina sasakii (Lepidoptera: Carposinidae) and its stage emergence models. Env. Entomol. 30: 298–305.
Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.
Liu, L., H. P. Yang, F. Zhao, G. Ma, and C. S. Ma. 2011. Simplified identification system for fruit borers in northern China. Chin. J. Appl. Entomol. 48: 1896–1904.
Liu, Y. S., J. A. Cheng, and J. Y. Mou. 1997. Review of the advance of the peach fruit borer (Carposina sasakii Matsumura). J. Shandong Agric. Univ. 28: 207–214.
Ros, V. I. D., and J. A. J. Breeuwer. 2007. Spider mite (Acari: Tetranychidae) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. Exp. Appl. Acarol. 42: 239–262.
Scheffer, S. J., and M. L. Lewis. 2005. Mitochondrial phylogeography of vegetable pest Liriomyza sativae (Diptera: Agromyzidae): divergent clades and invasive populations. Ann. Entomol. Soc. Am. 98: 181–186.
Slarkin, M. 1993. Isolation by distance in equilibrium and nonequilibrium populations. Evolution 47: 264–279.
Sony, S., M. A. Alim, S. Kim, M. Kwon, D. Lee, and Y. Kim. 2009. Diagnostic molecular markers of six lepidopteran insect pests infesting apples in Korea. J. Asia Pac. Entomol. 12: 107–111.
Tamura, K., D. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739.
Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties, and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.
Weight, S. 1978. Evolution and the genetics of population variability within and among natural population. University of Chicago Press, Chicago.
Winkler, G., J. J. Dodson, and C. E. Lee. 2008. Heterogeneity within the native range: population genetic analyses of sympatric invasive and noninvasive populations of the freshwater invading copepod Eurytemora affinis. Mol. Ecol. 17: 415–430.
Xu, Q. G., and B. Z. Hua. 2004. RAPD analysis on the speciation in host races of Carposina sasakii Matsumura (Lepidoptera: Carposinidae). Acta Entomol. Sinica 47: 379–383.
Received 11 January 2015; accepted 22 May 2015.