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Short Communication

Isolation and characterization of microsatellite loci for *Rhyacionia leptotubula* (Lepidoptera: Tortricidae)

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Microsatellite as one of the most popular molecular markers provides a useful tool for population genetic structure analysis in insects. But few microsatellite loci have been developed for Lepidoptera. Here we isolated and characterized 9 polymorphic microsatellite loci in *Rhyacionia leptotubula* (Lepidoptera: Tortricidae). The number of alleles of these loci ranged from 2 to 11. Observed and expected heterozygosities ranged from 0.0000 to 0.5294 and from 0.3905 to 0.8719, respectively. Seven loci show significant Hardy-Weinberg deviations. These loci could provide insight into population genetic structure and dispersal patterns of *R. leptotubula*.

Key words: *Rhyacionia leptotubula*, microsatellite, population genetics, Lepidoptera.

INTRODUCTION

The pine tip moth *R. leptotubula* (Lepidoptera: Tortricidae) is a serious pest of *Pinus yunnanensis* and *P. armandii* (Huang, 1987). The larvae feed primarily on needles soon after hatching, and then bore into the shoots. Repeated attacks can cause severe deformation of host trees and significant long-term growth loss. Now it is distributed widely over much of Yunnan province in China. Recent survey showed that this pest can damage about 40% of the host plants in the outbreak area, and migrate with a speed of about 5 km to other regions per year (Wu et al., 2008). The increasing population of *R. leptotubula* poses an ever-increasing problem of infestations. Interestingly, we found that the distributional areas of this pest are with high mounts and deep valley as geographical barriers, and its ecological environments are in large difference. In addition, its distribution in the same occurring mountain of the altitude difference can reach 1 km (unpublished data). Such adaptations to physical barriers and diversifiable ecology may have a strong impact on the genetic structure among populations. As a basic understanding of population genetic relationships of *R. leptotubula* essential for effective management, the genetic study of this pest is critically needed. Up to data, there are no any applicable molecular markers been developed in *R. leptotubula* yet. We here report the isolation and characterization of 9 microsatellite loci for this species that will be used to population genetic analysis.

MATERIALS AND METHODS

R. leptotubula larvae were collected from the same pine tree in Huize County, Yunnan province, China. Its genomic DNA was isolated using standard phenol-chloroform extraction procedures (Sambrook et al., 1989). DNA fragments from 250-1000 bp digested with *Rsa*I (Takara) were purified from agarose gels using a QIAquick Gel Extraction Kit (Qiagen), ligated to *Rsa*21 and *Rsa*25 linkers (5`-CTCTTGCTTACGCGTGGACTA-3` and 5`-phosphate-TAGTCCACGCGTAAG CAAGAG-3`), and hybridized with the two $(CT)_{10}$ and $(GT)_{10}$ 5-biotinylated oligo-probes (Kijas et al., 1994; Franck et al., 2005). Hybridized fragments were isolated using Streptavidin Magnesphere® Paramagnetic Particles (Promega). Captured fragments were recovered by polymerase chain reaction (PCR) amplifications to increase the quantity of the resultant microsatellite-enriched eluate using *Rsa*21 as primer. Cycling conditions were 1 min at 94°C, followed by 25 cycles of 1 min at 94°C, 30 s at 55°C, and 1 min at 72°C, followed by a final extension at 72°C. PCR products with the size of 250-1000 bp were purified from 1% agarose gel, ligated into pMD18-T vector (Takara), and then transformed into competent $DH_{5\alpha}$, *Escherichia coli* (Tiangen). Resulting positive clones were randomly selected and sequenced using an ABI automated DNA sequencer model 377 (Applied

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Table 1. Microsatellite loci developed for *Rhyacionia leptotubula*.

Locus	Primer sequences (5'-3')	Repeat motif	Size (bp)[a]	Size range (bp)[b]	T_a (°C)	N_a	H_O	H_E	P	GenBank Accession no.
Rel2	F: GGGGGACATGTTTCTTTCCT R: TATTGGCAACTCATGCAAGC	$(TG)_{10}$	207	177-223	48	4	0.0000	0.3905	0.0004	JN413176
Rel6	F: GTGGTGCTAAAGGTGCAACA R: CTCGTTCGATCATGTCCTCA	$(AC)_{14}$	196	190-202	48	2	0.0000	0.5000	0.0000	JN413177
Rel8	F: CATCCTTTACGCAACCCATT R: ACATCGGTCCCGATACAAAC	$(AT)_5$ $(GT)_8$ATGG$(AT)_5$	171	165-185	48	5	0.0000	0.8061	0.0003	JN413178
Rel18	F: CGTGTGTATGGGTGAGTGTG R: GAAGGCTGTGACGAACTGGT	$(GT)_3N_8(GT)_5GG(GT)_3N_{47}$ $(TG)_4$	173	165-227	48	6	0.0000	0.5536	0.0000	JN413179
Rel24	F: CTGGTCCCTAGTGGTGGAGA R: GCGACCACAAACTCACGATA	$(AT)_3(CT)_4$	174	162-218	48	4	0.0000	0.6593	0.0000	JN413180
Rel38	F: AGGACGCCACTAAAAGTCCA R: AACAACAGGCGGTCTTATCG	$(TTG)_5T(GTT)_3$	208	181-244	48	11	0.0000	0.8580	0.0013	JN413181
Rel43	F: TTGCGGTCATTCTCTGACTG R: GTGCGTAGGGGTAAGCTCTG	$(CA)_7$	214	176-232	48	11	0.3636	0.8719	0.7258	JN413182
Rel57	F: CATGCACAAACAGACAAACA R: CAAAAGCGCGTGAGAAGTTA	$(AC)_6GCA(CACG)_7(CA)_4$	184	176-244	48	10	0.5294	0.7093	0.5027	JN413183
Rel88	F: AGCTGGGCGATATGTGGAT R: CGAAAGTGTGTGCAAGTGTG	$(AC)_9TTGC(AC)_3$	191	183-247	48	8	0.0000	0.8199	0.0000	JN413184

[a] Expected product size based on primer sequence of original clone. [b] The range of observed alleles in base pairs. T_a, annealing temperature; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P, P-value of probability test for deviation from Hardy-Weinberg equilibrium; F, forward primer sequence; R, reverse primer sequence. N_a, H_O, H_E, and P were estimated from 23 *R. leptotubula* larvae collected from one host plant.

Biosystems). Primer sets were designed using Primer 3 online for the sequences with at least 5 uninterrupted microsatellite repeat units.

To evaluate the polymorphism of the primer pairs designed, 23 individual larvae were used. PCR was performed in a 10 µl reaction containing 0.5 µl genomic DNA, 2.5 pmol of each primer, 0.2 mM of dNTP, 1×PCR buffter, and 0.2 unit Taq DNA polymerase (Takara). Thermal cycling conditions were as follows: predenaturing at 94°C for 2 min; 30 cycles at 94°C for 30 s, 30 s at the primer-specific annealing temperature (Table 1) and 72°C for 30 s; followed by a final extension step at 72°C for 10 min. PCR product mixture was analyzed on 6% denaturing polyacrylamide gels. DNA fragments were visualized by silver staining. Data analysis were performed using POPgene 1.32 to calculate the average number of alleles per locus, observed heterozygosity (H_O), expected heterozygosity (H_E), and Hardy-Weinberg equilibrium

(HWE) *P*-values (Yeh et al., 1999).

RESULTS AND DISCUSSION

One hundred positive clones were randomly selected and sequenced. Thirty-five of them have at least 5 uninterrupted microsatellite repeat units. Primer pairs designed for them were tested on 23 *R*. *leptotubula* individuals collected from one host plant. Finally, nine polymorphic microsatellites were obtained (Table 1). The number of alleles per locus ranged from 2 to 11 (average 6.5). Average observed heterozygosities (H_O) were 0.1075 (varied from 0.0000 to 0.5294). H_O of 7 homozygous loci was 0.0000. Average expected heterozygosities (H_E) were 0.6780 (varied from 0.3905 to 0.8719), and no significant LD of them was detected, suggesting a high degree of intrapopulation variation in isolated loci. Locus Rel43 and Rel57 were detected with no significant deviation from HWE. The HWE deviation of other 7 locus is probably because of the occurrence of null alleles. In addition, *R*. *leptotubula* individuals tested in this study were collected in a sampling scheme intended to look at population genetic structure at small place. Therefore, such HWE deviations could be attributed to sampling bias as all individuals from one host plant might be originated from the same one adult female. The exact reason requires further investigation. Moreover, it is well known that isolation and characterization of microsatellite markers is clearly more difficult in Lepidoptera than in most other organisms, and very few microsatellite loci have been reported for Lepidoptera (Keyghobadi et al., 2002). However, although 7 loci show HWE deviations, these primers are currently being successfully used in the samples collected from different geographic populations. Thus, these microsatellite loci described here are suitable tools for future genetic investigations in *R*. *leptotubula*.

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REFERENCES

Franck P, Guérin F, Loiseau A, Sauphanor B (2005). Isolation and characterization of microsatellite loci in the codling moth *Cydia pomonella* L. (Lepidoptera, Tortricidae). Mol. Ecol. Notes, 5(1): 99-102.

Huang FS (1987). Forest Insects of Yunnan. Yunnan Science and Technology Press, Kunming, China.

Keyghobadi N, Roland J, Strobeck C (2002). Isolation of novel microsatellite loci in the Rocky Mountain apollo butterfly, *Parnassius smintheus*. Hereditas, 136(3): 247-250.

Kijas JMH, Fowler JCS, Garbett CA, Thomas MR (1994). Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. Biotechniques, 16(4): 656-662.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York, NY.

Wu XP, Qian Y, Ma WM, Ma X, Zhang JH (2008). A preliminary study on the biology of *Rhyacionia leptotubula*. J. Southwest Forest. Coll., 28(2): 58-59.

Yeh FC, Yang RC, Boyle T (1999). PopGene (version 1.32). Microsoft Window-base Software for Population Genetic Analysis: A Quick User's Guide. University of Alberta, Center for International Forestry Research, Alberta, Canada.