Development and Characterization of 25 Microsatellite Primers for Ilex chinensis (Aquifoliaceae)

Authors: Wen-Wen Chen, Zhen-Zhu Xiao, Xin Tong, Yu-Pei Liu, and Yuan-Yuan Li
Source: Applications in Plant Sciences, 3(10)
Published By: Botanical Society of America
URL: https://doi.org/10.3732/apps.1500057
Evergreen broadleaved forests (EBLFs) are zonal vegetation found in subtropical China. They support hyperdiverse species but have suffered from dramatic declines due to anthropogenic habitat loss and fragmentation. Less than 5% of old-growth EBLFs remain in subtropical China (Song and Chen, 2007). Thus, protecting and recovering EBLFs are crucial to sustainable ecosystem management. It has been suggested that one of the native dominant species in EBLFs, *Ilex chinensis* Sims (Aquifoliaceae), may be suitable for restoration of EBLFs. It is a dioecious evergreen tree, bearing small unisexual flowers and red globose drupes. Genetic variation provides important information for efficient management of fragmented forests and ecological restoration (Thomas et al., 2014). For this reason, it is necessary to delineate the genetic background of *I. chinensis*. In this study, 10 polymorphic microsatellites for *I. chinensis* were isolated and characterized. These can be used to evaluate the genetic diversity, genetic structure, and gene flow of this species.

**METHODS AND RESULTS**

Microsatellite loci were developed using the biotin-streptavidin capture method following the protocol reported by Liu et al. (2009) and Tong et al. (2012). Total genomic DNA was extracted from silica gel-dried leaf tissues of one individual of *I. chinensis* collected from Tiantong, Zhejiang Province, China, using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China); genetic diversity, genetic structure, and gene flow of this species.

Evergreen broadleaved forests (EBLFs) are zonal vegetation found in subtropical China. They support hyperdiverse species but have suffered from dramatic declines due to anthropogenic habitat loss and fragmentation. Less than 5% of old-growth EBLFs remain in subtropical China (Song and Chen, 2007). Thus, protecting and recovering EBLFs are crucial to sustainable ecosystem management. It has been suggested that one of the native dominant species in EBLFs, *Ilex chinensis* Sims (Aquifoliaceae), may be suitable for restoration of EBLFs. It is a dioecious evergreen tree, bearing small unisexual flowers and red globose drupes. Genetic variation provides important information for efficient management of fragmented forests and ecological restoration (Thomas et al., 2014). For this reason, it is necessary to delineate the genetic background of *I. chinensis*. In this study, 10 polymorphic microsatellites for *I. chinensis* were isolated and characterized. These can be used to evaluate the genetic diversity, genetic structure, and gene flow of this species.

**METHODS AND RESULTS**

Microsatellite loci were developed using the biotin-streptavidin capture method following the protocol reported by Liu et al. (2009) and Tong et al. (2012). Total genomic DNA was extracted from silica gel-dried leaf tissues of one individual of *I. chinensis* collected from Tiantong, Zhejiang Province, China, using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China); genetic diversity, genetic structure, and gene flow of this species.

Evergreen broadleaved forests (EBLFs) are zonal vegetation found in subtropical China. They support hyperdiverse species but have suffered from dramatic declines due to anthropogenic habitat loss and fragmentation. Less than 5% of old-growth EBLFs remain in subtropical China (Song and Chen, 2007). Thus, protecting and recovering EBLFs are crucial to sustainable ecosystem management. It has been suggested that one of the native dominant species in EBLFs, *Ilex chinensis* Sims (Aquifoliaceae), may be suitable for restoration of EBLFs. It is a dioecious evergreen tree, bearing small unisexual flowers and red globose drupes. Genetic variation provides important information for efficient management of fragmented forests and ecological restoration (Thomas et al., 2014). For this reason, it is necessary to delineate the genetic background of *I. chinensis*. In this study, 10 polymorphic microsatellites for *I. chinensis* were isolated and characterized. These can be used to evaluate the genetic diversity, genetic structure, and gene flow of this species.

**METHODS AND RESULTS**

Microsatellite loci were developed using the biotin-streptavidin capture method following the protocol reported by Liu et al. (2009) and Tong et al. (2012). Total genomic DNA was extracted from silica gel-dried leaf tissues of one individual of *I. chinensis* collected from Tiantong, Zhejiang Province, China, using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China); genetic diversity, genetic structure, and gene flow of this species.

**METHODS AND RESULTS**

Microsatellite loci were developed using the biotin-streptavidin capture method following the protocol reported by Liu et al. (2009) and Tong et al. (2012). Total genomic DNA was extracted from silica gel-dried leaf tissues of one individual of *I. chinensis* collected from Tiantong, Zhejiang Province, China, using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China); genetic diversity, genetic structure, and gene flow of this species.

Evergreen broadleaved forests (EBLFs) are zonal vegetation found in subtropical China. They support hyperdiverse species but have suffered from dramatic declines due to anthropogenic habitat loss and fragmentation. Less than 5% of old-growth EBLFs remain in subtropical China (Song and Chen, 2007). Thus, protecting and recovering EBLFs are crucial to sustainable ecosystem management. It has been suggested that one of the native dominant species in EBLFs, *Ilex chinensis* Sims (Aquifoliaceae), may be suitable for restoration of EBLFs. It is a dioecious evergreen tree, bearing small unisexual flowers and red globose drupes. Genetic variation provides important information for efficient management of fragmented forests and ecological restoration (Thomas et al., 2014). For this reason, it is necessary to delineate the genetic background of *I. chinensis*. In this study, 10 polymorphic microsatellites for *I. chinensis* were isolated and characterized. These can be used to evaluate the genetic diversity, genetic structure, and gene flow of this species.

**METHODS AND RESULTS**

Microsatellite loci were developed using the biotin-streptavidin capture method following the protocol reported by Liu et al. (2009) and Tong et al. (2012). Total genomic DNA was extracted from silica gel-dried leaf tissues of one individual of *I. chinensis* collected from Tiantong, Zhejiang Province, China, using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China); genetic diversity, genetic structure, and gene flow of this species.

Evergreen broadleaved forests (EBLFs) are zonal vegetation found in subtropical China. They support hyperdiverse species but have suffered from dramatic declines due to anthropogenic habitat loss and fragmentation. Less than 5% of old-growth EBLFs remain in subtropical China (Song and Chen, 2007). Thus, protecting and recovering EBLFs are crucial to sustainable ecosystem management. It has been suggested that one of the native dominant species in EBLFs, *Ilex chinensis* Sims (Aquifoliaceae), may be suitable for restoration of EBLFs. It is a dioecious evergreen tree, bearing small unisexual flowers and red globose drupes. Genetic variation provides important information for efficient management of fragmented forests and ecological restoration (Thomas et al., 2014). For this reason, it is necessary to delineate the genetic background of *I. chinensis*. In this study, 10 polymorphic microsatellites for *I. chinensis* were isolated and characterized. These can be used to evaluate the genetic diversity, genetic structure, and gene flow of this species.

**METHODS AND RESULTS**

Microsatellite loci were developed using the biotin-streptavidin capture method following the protocol reported by Liu et al. (2009) and Tong et al. (2012). Total genomic DNA was extracted from silica gel-dried leaf tissues of one individual of *I. chinensis* collected from Tiantong, Zhejiang Province, China, using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China); genetic diversity, genetic structure, and gene flow of this species.

Evergreen broadleaved forests (EBLFs) are zonal vegetation found in subtropical China. They support hyperdiverse species but have suffered from dramatic declines due to anthropogenic habitat loss and fragmentation. Less than 5% of old-growth EBLFs remain in subtropical China (Song and Chen, 2007). Thus, protecting and recovering EBLFs are crucial to sustainable ecosystem management. It has been suggested that one of the native dominant species in EBLFs, *Ilex chinensis* Sims (Aquifoliaceae), may be suitable for restoration of EBLFs. It is a dioecious evergreen tree, bearing small unisexual flowers and red globose drupes. Genetic variation provides important information for efficient management of fragmented forests and ecological restoration (Thomas et al., 2014). For this reason, it is necessary to delineate the genetic background of *I. chinensis*. In this study, 10 polymorphic microsatellites for *I. chinensis* were isolated and characterized. These can be used to evaluate the genetic diversity, genetic structure, and gene flow of this species.

**METHODS AND RESULTS**

Microsatellite loci were developed using the biotin-streptavidin capture method following the protocol reported by Liu et al. (2009) and Tong et al. (2012). Total genomic DNA was extracted from silica gel-dried leaf tissues of one individual of *I. chinensis* collected from Tiantong, Zhejiang Province, China, using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China); genetic diversity, genetic structure, and gene flow of this species.

Evergreen broadleaved forests (EBLFs) are zonal vegetation found in subtropical China. They support hyperdiverse species but have suffered from dramatic declines due to anthropogenic habitat loss and fragmentation. Less than 5% of old-growth EBLFs remain in subtropical China (Song and Chen, 2007). Thus, protecting and recovering EBLFs are crucial to sustainable ecosystem management. It has been suggested that one of the native dominant species in EBLFs, *Ilex chinensis* Sims (Aquifoliaceae), may be suitable for restoration of EBLFs. It is a dioecious evergreen tree, bearing small unisexual flowers and red globose drupes. Genetic variation provides important information for efficient management of fragmented forests and ecological restoration (Thomas et al., 2014). For this reason, it is necessary to delineate the genetic background of *I. chinensis*. In this study, 10 polymorphic microsatellites for *I. chinensis* were isolated and characterized. These can be used to evaluate the genetic diversity, genetic structure, and gene flow of this species.
Ten polymorphic loci were further characterized in 87 *I. chinensis* individuals sampled from the three populations mentioned above (Appendix 1). Forward primers were labeled with one of the following fluorescent dyes: HEX, ROX, or 6-FAM (Table 1). Replications of each locus in a 15-μL reaction volume containing 40 ng of template DNA, 1× PCR buffer, 1.5 mM Mg²⁺, 0.2 mM of each dNTP, 0.1 μM of each primer, and 1 unit of *Taq* DNA polymerase (Sangon Biotech) (Table 1), and 45 s at 72°C, and a final extension at 72°C for 8 min. The annealing temperatures of PCRs were different from those amplified using the Schuelke (2000) protocol, probably due to the fluorescent dye labeling the forward primers in the former. The amplification products were scanned on an ABI 3730 automated sequencer, and the alleles were called and binned using GeneMapper 4.0 software (Applied Biosystems). All 10 polymorphic primer pairs amplifying high-quality PCR products showed moderate to high levels of polymorphism across the three populations. Using the software GENEPOP v4.0 (Rousset, 2008), results showed the number of alleles per locus to vary from two to 12 with an average of 4.8. The observed and expected heterozygosities ranged from 0.0435 to 0.9032 and 0.3121 to 0.8343, respectively (Table 2). Deviations from Hardy–Weinberg proportions were found in *I. chinensis* individuals from Chun’an and Yuwang in Zhejiang Province and Shanghai Botanical Garden in Shanghai, China (see Appendix 1).

A summary of the loci is shown in the table that follows.

### Table 1. Characterization of 10 polymorphic and 15 monomorphic microsatellite loci developed in *I. chinensis*.

| Locus | Primer sequences (5’–3’) | Repeat motif | Allele size (bp) | Tₐ (°C) | Tₐ (°C) | Fluorescent dye* | Accession no. |
|-------|-------------------------|--------------|-----------------|---------|---------|----------------|--------------|
| DQ9   | F: ACTTAAGTCACCTTCTCCG | (GA)₉        | 156             | 55      | —       | ROX            | KT006006     |
|       | R: AGAAAGGTTGAGTTTGGGA | (TC)₈        | 179             | 64      | —       | ROX            | KT006007     |
| DQ20  | F: AGCAAGACCTTTGGC     | (GA)₁₀       | 234–254         | 58      | 55      | HEX            | KP325082     |
| DQ27* | F: TAGTCGCTCTGATTTG    | (GA)₇        | 170             | 58      | —       | 6-FAM          | KT006008     |
| DQ39  | F: TTCTCGTCGCTTCTCCG   | (CA)₇       | 171             | 55      | —       | 6-FAM          | KT006009     |
| DQ41  | F: TCTGGCAATGAAAGAC    | (CT)₂        | 323             | 57      | —       | HEK            | KT006010     |
| DQ43  | F: ATGCTGACGCACGCGAGG  | (TC)₁₂      | 97–109          | 58      | 56      | ROX            | KP325075     |
| DQ56* | F: AGATTAGTGAGAGCTG    | (AG)₉       | 249–261         | 55      | 51      | HEX            | KP325076     |
| DQ80* | F: AGATTAGTGGATGAGAGAG| (AG)₁₁     | 111–121         | 58      | 57.8    | 6-FAM          | KP325074     |
| DQ111 | F: TAGAAGGACGCGAGAAA   | (TC)₃,...(CA)₃ | 120             | 64      | —       | 6-FAM          | KT006011     |
| DQ137*| F: CTTGCGGCTCCTCCA    | (TC)₃       | 109–125         | 58      | 58.7    | ROX            | KP325083     |
| DQ140 | F: GAGATTAGTGAGAGCTG   | (CT)₉       | 257             | 57      | —       | HEX            | KT006012     |
| DQ141 | F: GGGTGTTGAGTTGAGAGA | (GA)₈       | 247             | 61      | —       | ROX            | KT006013     |
| DQ146*| F: CCGCATATACCAACATC  | (AG)₉       | 111–121         | 58      | 57.8    | 6-FAM          | KP325074     |
| DQ147 | F: TTCTGTAACCCTTCCCAT | (AG)₁₁      | 218             | 54      | —       | ROX            | KT006014     |
| DQ158 | F: CAAATCAGCAATGACCT   | (TC)₃       | 227             | 60.4    | —       | ROX            | KT006015     |
| DQ159 | F: GTGGCGATCAGCAGCTTAG | (GA)₆       | 173             | 58      | —       | ROX            | KT006016     |
| DQ164 | F: GTTCTGAGTTGAGCTGTC  | (CT)₁₁      | 165             | 58.7    | —       | 6-FAM          | KT006017     |
| DQ165 | F: CCCTCCGCTTGTCCGTCC | (AG)₁₁,...(AG)₃ | 118             | 58      | —       | ROX            | KT006018     |
| DQ168 | F: TTTATGCTTCTGCTTCCG  | (GA)₁₀      | 183             | 63      | —       | ROX            | KT006019     |
| DQ169*| F: ATTATCGTGACACTGCTG  | (TC)₈       | 210–236         | 60      | 57      | HEX            | KP325077     |
| DQ175 | F: CCCTCAGTACATGAGTTG  | (CT)₉       | 169             | 57.5    | —       | 6-FAM          | KT006020     |
| DQ184*| F: GCCATGAGCGAGTGGAGT  | (TC)₁₁      | 111–143         | 58.7    | 58.7    | 6-FAM          | KP325079     |
| DQ185*| F: CGCAAGTGGATCTGAGTT  | (TC)₉       | 147–171         | 58.5    | 60      | HEX            | KP325080     |
| DQ188*| F: CTAATGCAGACACCGTAC  | (CT)₁₀      | 186–224         | 57      | 56      | ROX            | KP325078     |
| DQ198*| F: ATGTTCCGAGGCTTTAACA  | (GA)₈       | 178–182         | 65      | 65      | 6-FAM          | KP325081     |

Note: *Tₐ* = annealing temperature with fluorescent dyes labeling the forward primers; *Tₐ* = annealing temperature using the genotyping protocol of Schuelke (2000).

*All values are based on the samples representing three populations located in Chun’an and Yuwang in Zhejiang Province and Shanghai Botanical Garden in Shanghai, China (see Appendix 1).

*Fluorescent dyes (i.e., HEX, ROX, and 6-FAM) used for fragment analysis.

*Polymorphic microsatellite loci.

http://www.bioone.org/loi/apps
equilibrium (HWE) and from linkage equilibrium were tested using GENEPOP v4.0 (Rousset, 2008) with sequential Bonferroni adjustment (Rice, 1989). No significant linkage disequilibrium ($P > 0.05$) was observed for each pair of loci. No locus showed significant departure from HWE in the Yuwang population. However, seven (DQ27, DQ80, DQ169, DQ184, DQ185, DQ188, and DQ198) and four loci (DQ137, DQ169, DQ185, and DQ198) significantly deviated from HWE ($P < 0.05$) in the Chun’an and Shanghai populations, respectively (Table 2).

### CONCLUSIONS

The 25 microsatellites reported here for *I. chinensis* are appropriate for studies of the population’s genetic structure. These analyses, in turn, can shed light on evolutionary forces such as the balance of mutation, gene flow, and genetic drift. Moreover, it can be expected that the genetic information of this dominant species based on these microsatellite loci may make a substantial contribution to the efficient conservation and management of EBLFs.

### APPENDIX 1

Voucher and locality information of *Ilex chinensis* samples used in this study. Voucher specimens deposited at East China Normal University.

| Voucher specimen ID | Collection locality | Geographic coordinates |
|---------------------|---------------------|------------------------|
| T19200059           | Tiantong, Zhejiang, China | 29°48'56"N, 121°47'11"E |
| Chun’an             | Zhejiang, China      | 29°30'29"N, 118°49'24"E |
| Yuwang              | Zhejiang, China      | 29°51'04"N, 121°44'16"E |
| Shanghai Botanical Garden | Shanghai, China   | 31°08'48"N, 121°26'53"E |

### LITERATURE CITED

LIU, M., M. M. SHI, M. H. LIU, AND X. Y. CHEN. 2009. Isolation and characterization of microsatellite loci in *Fagus longipetiolata* Seem. (Fagaceae). *Conservation Genetics* 10: 1981–1983.

RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.

ROUSSET, F. 2008. GENEPOP’007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

SCHULKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233–234.

SONG, Y. C., AND X. Y. CHEN. 2007. Degradation mechanism and ecological restoration of evergreen broadleaved forest ecosystem in East China. Science Press, Beijing, China.

THOMAS, E., R. JALONEN, J. LOG, D. BOSIER, L. GALLOW, S. CAVERS, S. BORDÁCS ET AL. 2014. Genetic consideration in ecosystem restoration using native tree species. *Forest Ecology and Management* 333: 66–75.

TONG, X., N. N. XU, L. LI, AND X. Y. CHEN. 2012. Development and characterization of polymorphic microsatellite markers in *Cyclobalanopsis glauca* (Fagaceae). *American Journal of Botany* 99: e120–e122.