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Isolation and characterization of microsatellite primers for *Ceratosolen fusciceps*, the fig-pollinating wasp of *Ficus racemosa*, and amplification in two populations

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Abstract

Ten microsatellite loci were isolated and characterized for *Ceratosolen fusciceps*, the pollinating wasp of *Ficus racemosa*, based on individuals from Xishuangbanna (China) and Chiangmai (Thailand). High levels of variation were found among loci for both populations; allelic number varied from two to 18 (respectively from two to 22), expected heterozygosities ranged from 0.362 to 0.890 (respectively from 0.430 to 0.930).

Keywords: *Ceratosolen fusciceps*, coevolutionary dynamic, *Ficus racemosa*, microsatellite markers

In order to understand at what level the coevolutionary process is acting in the *Ficus*–fig wasp mutualism, we need to compare broad-scale genetic costructuring between the *Ficus* host and its specific pollinator and extend this knowledge to comparative phylogeography. Thus, we need reliable genetic markers for both partners.

Microsatellite markers have been developed for *Ficus* (Khadari *et al.* 2001; Giraldo *et al.* 2005; Zavodna *et al.* 2005; Vignes *et al.* 2006) and fig-pollinating wasps (Molbo *et al.* 2002; Zavodna *et al.* 2002; Lin *et al.* 2007). Microsatellite loci are frequently transferable between *Ficus* species (Khadari *et al.* 2001; Vignes *et al.* 2006) but not between wasp species. We report here the isolation and characterization of microsatellite loci on *Ceratosolen fusciceps*, the pollinator of *Ficus racemosa*, a widely distributed *Ficus* species (from India to Australia). Previously developed primers did not amplify *C. fusciceps* DNA and we therefore had to isolate and characterize microsatellite loci on this species.

Ceratosolen fusciceps female wasps were collected from a tree at the Xishuangbanna Botanical Tropical Garden, China (21°56′21.64″N, 101°15′14.57″E) and two trees in Chiangmai, Thailand (18°25′33.21″N, 98°40′29.43″E and 18°54′57.43″N, 98°58′16.08″E). At each site, we put several figs together in a nylon bag and waited until the emergence of wasps. The wasps were preserved in 95% ethanol. DNA was extracted from whole wasps using the Tissue Blood

Kit (QIAGEN). The isolation of microsatellite loci was performed following Vignes *et al.* (2006) except that the selection of restricted DNA containing microsatellite loci was carried out with an enriched [(CTG)_n and (TAA)_n] library as well as an enriched [(TC)_n and (TG)_n] library using the Kit Steptavidin MagneSphere Paramagnetic Particles (PROMEGA). The number of polymerase chain reaction (PCR) cycles for screening recombinant clones was reduced to 23. A total of 221 clones were screened and 201 gave a positive signal. Eighty-four clones were sequenced using an ABI PRISM 3100 DNA sequencer (Applied Biosystems). Microsatellite motifs were detected and primers were designed using the program Primer 3 (Rozen & Slatlefsky 2000). The designed primers were tested on eight individuals from Xishuangbanna and eight individuals from Chiangmai in order to detect amplification. Amplifications were carried out in 10-μL solution including 0.2 μL of each 10 μM unlabelled primer, 5 μL multiplex buffer (QIAGEN: this solution contains the *Taq* polymerase), 3.6 μL pure water and 1 μL genomic DNA. The PCR was performed using a PTC-100 thermocycler with a standardized PCR programme (15 min initial denaturation at 95 °C, 35 cycles of denaturation at 64 °C for 30 s, annealing at 57 °C for 1 min 30 s, extension at 72 °C for 60 s, and final elongation at 60 °C for 30 min). Ten pairs of primers produced a positive amplification (Table 1). To characterize these primers, we sampled other wasps in Xishuangbanna

Table 1 Characterization of 10 microsatellite loci in *Ceratosolen fusciceps*, based on individuals originating from Xishuangbanna

| Locus | Repeat motif | Forward and reverse primer sequences | Annealing temperature (°C) | N | Size range | Number of alleles | H_O/H_E | F_{IS} | GenBank Accession no. |
|-------|--|---|----------------------------|----|------------|-------------------|----------------|----------|-----------------------|
| Cer7 | (TA) ₄ (CA) ₁₃ | NED-F: GACAAGACGAAGCTATTACG R: CGGCTTATTTCATTTTAGTGC | 57 | 30 | 150–188 | 13 | 0.833/0.831 | 0.014 | FJ428272 |
| Cer32 | (GAA) ₂ GAG(GAA) ₅ | PET-F: CGGACATTCTCTATTTC R: TCTATCTATCCCCTCCATCC | 57 | 31 | 288–318 | 11 | 0.484/0.674** | 0.297 | FJ428277 |
| Cer38 | (CAG) ₆ (CAC) ₃ (CAG) ₅ CAACAT(CAG) ₇ | VIC-F: AAGCCTCGCCTAACTCAC R: AGCTTTTCGCCGTTGAC | 57 | 22 | 222–242 | 7 | 0.591/0.857*** | 0.331 | FJ428278 |
| Cer52 | (CAG) ₃ (CAACAG) ₃ (CAG) ₃ | FAM-F: ATGCTACCAACAACACCAG R: GTTAGAGGGGCTGATACGA | 57 | 31 | 148–166 | 7 | 0.548/0.776* | 0.308 | FJ428280 |
| Cer55 | (CAG) ₅ (CAA) ₄ | NED-F: CTCACCATTTACATCGAGACA R: GAGTTGCATCGAGTGGTT | 57 | 33 | 192–200 | 4 | 0.364/0.362 | 0.012 | FJ428281 |
| Cer61 | (CTA) ₂₀ | FAM-F: GTTCTCTCTGTCGTCGTAGG R: GAAGGTGTCCCGATGATA | 57 | 34 | 143–202 | 18 | 0.529/0.472 | –0.106 | FJ439558 |
| Cer62 | (AG) ₆ GG(AG) ₁₅ | VIC-F: CAGGACTCTGGGTTATTG R: AGTGTGTGGAGATCAAAGG | 57 | 34 | 206–237 | 13 | 0.824/0.890* | 0.090 | FJ439559 |
| Cer63 | (AG) ₂₀ | PET-F: GCGCAGTCATAAACAAGAAT R: CCCCGATCACTTTGCT | 57 | 34 | 107–109 | 2 | 0.853/0.880** | 0.046 | FJ439560 |
| Cer64 | (AGA) ₂₀ | PET-F: CTCGCAATTCAAAATAAAC R: AGTCGAACTATTACCGCTTC | 57 | 30 | 205–238 | 10 | 0.367/0.784*** | 0.544 | FJ439561 |
| Cer73 | (CG) ₄ CACG(CA) ₇ | NED-F: GAGCACCTTTCCGTTATTG R: AATACCAGCGAGAAATGAAG | 57 | 34 | 207–213 | 6 | 0.676/0.790** | 0.158 | FJ439562 |

Repeat motif, primer sequences, annealing temperature, number of tested individuals (N), size range, number of alleles, F_{IS} , observed (H_O) and expected (H_E) heterozygosity with significance of deviation from Hardy–Weinberg expectation ($P < 0.05 = *$, $P < 0.01 = **$, $P < 0.001 = ***$).

Table 2 Characterization of 10 loci of *Ceratosolen fusciceps*, based on individuals from Chiangmai, Thailand

| Locus | N | Size range | Number of alleles | H_O/H_E | F_{IS} |
|-------|----|------------|-------------------|----------------|----------|
| Cer7 | 32 | 150–190 | 15 | 0.656/0.884*** | 0.272 |
| Cer32 | 31 | 282–330 | 16 | 0.677/0.790 | 0.159 |
| Cer38 | 31 | 219–245 | 10 | 0.484/0.907*** | 0.479 |
| Cer52 | 32 | 141–169 | 8 | 0.750/0.821 | 0.102 |
| Cer55 | 32 | 194–203 | 4 | 0.625/0.532 | -0.160 |
| Cer61 | 32 | 149–217 | 22 | 0.438/0.430 | -0.002 |
| Cer62 | 32 | 206–250 | 20 | 0.906/0.936 | 0.048 |
| Cer63 | 32 | 107–109 | 2 | 0.719/0.930*** | 0.242 |
| Cer64 | 30 | 198–238 | 12 | 0.300/0.799*** | 0.635 |
| Cer73 | 32 | 207–212 | 6 | 0.813/0.762 | -0.051 |

by raising several female wasps per fig from four figs originating from a tree and extracted DNA with the previous protocol. PCRs were then conducted again using two mixes of F-labelled and R-unlabelled primers on these individuals ($N = 34$) (Table 1). Mix1 contained Cer7, Cer38, Cer52, Cer32, Cer55. Mix2 contained Cer61, Cer62, Cer63, Cer64 and Cer73. Two microlitres of each 100 μM F-labelled and R-unlabelled primer was mixed and complemented with pure water until 200 μL , which resulted in a solution of 1 μM mix of primers. The PCR programme remained unchanged. Genotyping was carried out using the ABI PRISM 3100 DNA sequencer and genotypes were scored using GeneMapper (ABI). All loci were polymorphic (Table 1). Only three loci were at Hardy–Weinberg equilibrium. Tests of pairwise linkage disequilibrium with Bonferroni correction showed significant values for most pairs of loci except Cer55–Cer63, Cer7–Cer64, Cer38–Cer64. The exact G -test of genotypic differentiation (Goudet *et al.* 1996) between figs showed that there was a significant differentiation ($P < 0.001$). Differentiation among figs, linkage disequilibrium for most pairs of loci, and direct observation of genotypes suggested that the individuals born within a fig were often sisters. This pattern was surprising as many females enter each fig of *F. racemosa* to oviposit (Wang *et al.* 2005). The results suggest that few of the females entering a fig effectively contribute offspring and hence brothers often mate sisters due to local mate competition conditions (Hamilton 1967). To confirm that deviations from Hardy–Weinberg resulted from individuals being inbred, while linkage disequilibrium resulted from the sampling of related individuals, we made a second sampling in Chiangmai, collecting a single female wasp per fig from a tree and thus avoiding sampling related individuals ($N = 32$). We conducted DNA extraction, PCR amplification and genotyping with the same protocols above. This sample presented slightly different allelic size ranges and a higher diversity in terms of allele number. All pairs of primers were at linkage equilibrium. Still, only six loci were at Hardy–Weinberg equilibrium (Table 2),

global F_{IS} was 0.1758 and the global test of deviation from Hardy–Weinberg equilibrium was significant ($P < 0.01$), which suggests that the individuals are indeed inbreeding. Higher diversity and lack of linkage disequilibrium are predicted to result from sampling unrelated individuals. We conclude that our loci segregated independently. We are now using these primers to investigate fine and broad-scaled genetic structure of *C. fusciceps*.

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References

- Giraldo E, Viruel MA, Lopez-Corrales M, Hormaza JI (2005) Characterisation and cross-species transferability of microsatellites in the common fig (*Ficus carica* L.). *Journal of Horticultural Science and Biotechnology*, **80**, 217–224.
- Goudet J, Raymond M, deMeeus T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.
- Hamilton WD (1967) Extraordinary sex ratios. A sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology. *Science*, **156**, 477–488.
- Khadari B, Hochu I, Santoni S, Kjellberg F (2001) Identification and characterization of microsatellite loci in the common fig (*Ficus carica* L.) and representative species of the genus *Ficus*. *Molecular Ecology Notes*, **1**, 191–193.
- Lin RC, Huang CC, Li SH (2007) Development and characterization of microsatellite markers for a fig-pollinating wasp (*Ceratosolen* sp. 1) of *Ficus septica*. *Molecular Ecology Notes*, **7**, 1360–1362.
- Molbo D, Krieger MJB, Herre EA, Keller L (2002) Species-diagnostic microsatellite loci for the fig wasp genus *Pegoscapus*. *Molecular Ecology Notes*, **2**, 440–442.
- Rozen S, Slatlefsky H (2000) Primer 3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology*, **132**, 365–386.
- Vignes H, Hossaert-McKey M, Beaune D *et al.* (2006) Development and characterization of microsatellite markers for a monoecious

Ficus species, *Ficus insipida*, and cross-species amplification among different sections of *Ficus*. *Molecular Ecology Notes*, **6**, 792–795.

Wang RW, Yang JX, Yang DR (2005) Seasonal changes in the trade-off among fig-supported wasps and viable seeds in figs and their evolutionary implications. *Journal of Integrative Plant Biology*, **47**, 144–152.

Zavodna M, Arens P, Vosman B, Van Dijk PJ, Van Damme JMM (2002) Development and characterization of microsatellite

markers for *Liporhopalum tentacularis* Grandi, the pollinator fig wasp of *Ficus montana* Blume. *Molecular Ecology Notes*, **2**, 569–571.

Zavodna M, Arens P, Van Dijk PJ, Vosman B (2005) Development and characterization of microsatellite markers for two dioecious *Ficus* species. *Molecular Ecology Notes*, **5**, 355–357.