Isolation and cross-species characterization of polymorphic microsatellites for the orchid bee *Eulaema meriana* (Hymenoptera: Apidae: Euglossini)

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**Abstract** We describe and characterize eight polymorphic microsatellite loci for the orchid bee species *Eulaema meriana*, an abundant species and important pollinator in wet lowland forests in tropical America. We also tested the cross-species amplification of these microsatellite loci in seven other species of the genus *Eulaema*. For *E. meriana*, number of alleles per locus ranged from four to nine and expected heterozygosity ranged from 0.377 to 0.854. Seven out of the eight loci described amplified in all seven other *Eulaema* species. These microsatellite loci will be of practical use for population structure, mating system and inbreeding studies in euglossine bees.

**Keywords** *Eulaema* · Euglossine bees · Microsatellites · Diploid males

The tribe Euglossini comprises 218 species in 5 genera, including the genus *Eulaema* with 29 described species (Oliveira 2006; Nemésio 2009). Euglossine bees (Hymenoptera: Apidae), commonly known as orchid bees, are charismatic insects characterized by extremely long tongues and shiny iridescent colors (Roubik and Hanson 2004). Orchid bees are abundant in the Neotropics (López-Uribe et al. 2008) and are considered keystone species in lowland forests because of the ecological role that they play as pollinator of orchids (Dressler 1982) and many other flowering plants (Ramírez et al. 2002).

Orchid bees have recently been targets of conservation concern (Zayed 2004). There is evidence demonstrating that the species diversity of euglossine bees is negatively affected by habitat fragmentation (Brosi 2009). In addition, genetic studies using allozyme markers have shown that some orchid bee populations exhibit high frequencies of diploid males indicating high levels of inbreeding and/or low effective population size (Roubik et al. 1996; Zayed 2004; López-Uribe et al. 2007; but see Takahashi et al. 2001). However, a recent study looking for diploid males using microsatellite markers (Souza et al. 2010) found diploid males to be rare in euglossine bee populations suggesting that the high frequency of diploid males previously reported may be the result of technical flaws in the allozyme-based studies. Therefore, the development of microsatellite markers is essential for the study of population structure and conservation genetics of this group of bees. Here, we describe and characterize eight polymorphic microsatellite loci in *Eulaema meriana*, and tested these loci across seven other *Eulaema* species.

A genomic DNA library enriched for 12 microsatellite repeat motifs was created from one individual of *E. meriana* using a universal linker and ligation procedure (Hamilton et al. 1999; Grant and Bogdanowicz 2006). Transformed bacterial colonies were then screened for microsatellites through hybridization to $^{33}$P-radiolabeled oligonucleotides. More than 800 positive clones were obtained from this method and ~200 of them were sequenced with universal M13 primers that flank the cloned insert for microsatellite primer design. PCR primer
pairs were designed for 29 microsatellite loci using the software PrimerSelect (DNASTAR). Nine of these loci were tested for PCR amplification quality and variability.

For microsatellite PCR amplifications, a universal tag method with three primers was employed (Schuelke 2000). This approach allows fluorescent labeling of PCR fragments with a single dye-labeled tag used simultaneously with the unlabeled locus-specific (ULS) forward primer containing 20 additional bases at the 5′-end and the ULS reverse primer. The reverse primer was modified by adding

### Table 1

| Locus | Primer sequence (5′ → 3′) | Repeat motif | \(T_a\) | Allele size (bp) | \(N_A\) | \(H_E\) | GenBank accession no. |
|-------|-----------------|-------------|------|-----------------|------|------|------------------|
| EM8   | F: CAG CGT CGC GAT TGG TTC TAC A<br>R: TCA GCT TTG TCA CGG GCA CTG T | (GA)\(_{14}\) | 55  | 301–317 | 6  | 0.723 | GU997087 |
| EM13  | F: GGC GCA ATG ACT AAG GGA ACG<br>R: CCC ACG GGC TAA CGA TTG ATC TT | (TGC)\(_7\) | 55  | 173–185 | 4  | 0.637 | GU997088 |
| EM16  | F: AGC GCA ATT ACA TAT GCA AAA ACA<br>R: TTC GTG GGT ATC TGA GCA TTA TTT | (CAG)\(_{10}\)(CA)\(_{12}\) | 55  | 190–211 | 4  | 0.377 | GU997089 |
| EM17  | F: GGG CGA CGG CGA AGA AGA<br>R: CGT TGC GCC CGA CTT TAC A | (CTT)\(_{10}\) | 55  | 157–187 | 6  | 0.731 | GU997090 |
| EM40  | F: CGA CGC AGA CGC AGC AAC AG<br>R: CCC CGC GAC TAA ACG ACA ACA CT | (CA)\(_{10}\) | 57  | 143–164 | 7  | 0.722 | GU997091 |
| EM70  | F: GTA CCA CTG CGA GAG CGA AGA<br>R: CCA GTG GCC CGA AGT AGA AAC AGA | (AG)\(_2\)(AG)\(_3\)(AG)\(_2\)(AG)\(_4\)<br>(AG)\(_2\)(AG)\(_3\)(AG)\(_2\)(AG)\(_4\) | 55  | 280–288 | 5  | 0.596 | GU997092 |
| EM106 | F: GAC GTG GAT GAG CCG CAG AAG<br>R: TCC GAC GAT GTA CGA GCA CGA A | (AAG)\(_2\)(GAG)\(_3\)<br>(AAG)\(_2\)(GAG)\(_3\) | 55  | 261–300 | 9  | 0.731 | GU997093 |
| EM107 | F: CGA GCC CCG ACG ACG AAC<br>R: GAC CGG AAC GAG CTG GAT GAA T | (TCT)\(_2\)(TCC)(TCT)\(_2\)<br>(TCT)\(_2\)(TCC)(TCT)\(_2\) | 57  | 200–221 | 8  | 0.841 | GU997094 |

Allele size range, number of alleles (\(N_A\)) and expected heterozygosity (\(H_E\)) were calculated for the population from La Selva, Costa Rica (\(N = 40\) haploid males)

### Table 2

| Locus | Eulaema meriana \((N = 55)\) | Eulaema cingulata \((N = 15)\) | Eulaema bombiformis \((N = 10)\) | Eulaema chocoana \((N = 3)\) | Eulaema lateola \((N = 3)\) | Eulaema mocsaryi \((N = 2)\) | Eulaema nigrita \((N = 1)\) |
|-------|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|
| EM8   | 8  | 3  | 3  | 3  | 2  | 1  | 1  |
|       | (301–323) | (301–311) | (307–313) | (309–315) | (290–292) | (301) | (317) | (309) |
| EM13  | 4  | 6  | 5  | 5  | 2  | 2  | 1  | 1  |
|       | (173–185) | (161–185) | (173–188) | (197–203) | (192–198) | (173) | (164) | (182) |
| EM16  | 4  | 2  | 5  | 1  | 1  | 1  | 1  | 1  |
|       | (190–211) | (199–202) | (201–216) | (202) | (202) | (199) | (210) | (190) |
| EM17  | 7  | 2  | 5  | 1  | 1  | 1  | 1  | 1  |
|       | (157–187) | (169–184) | (178–196) | (172) | (157) | (184) | (178) | (220) |
| EM40  | 9  | 4  | 5  | 1  | 2  | 2  | 1  | 1  |
|       | (140–167) | (143–158) | (152–173) | (147) | (158–164) | (161–164) | (164) | (167) |
| EM70  | 5  | 5  | 2  | 1  | 3  | 1  | 1  | 1  |
|       | (280–288) | (284–294) | (261–294) | (288) | (225–273) | (287) | (297) | (297) |
| EM106 | 11 | 5  | 7  | 1  | 2  | 2  | 1  | 1  |
|       | (261–300) | (268–295) | (261–294) | (264) | (225–273) | (271–283) | (282) | (305) |
| EM107 | 8  | 4  | 3  | 1  | 1  | 2  | 1  | –  |
|       | (200–221) | (196–221) | (203–212) | (203) | (199) | (199–202) | (202) | –  |

Above: number of alleles per locus; below: allele range size per locus (\(N =\) number of haploid males)
a six base pair ‘pigtail’ (GTCTTCT) to the 5’-end (Brownstein et al. 1996) to facilitate genotyping by reducing stutter. PCR amplifications contained 5× GoTaq buffer pH 8.5, 2 mM MgCl₂, 0.2 mM dNTPs, 0.1 μM ULS forward primer, 0.2 μM dye-labeled tag, 0.2 μM ULS reverse primer, 1U GoTaq DNA polymerase (Promega) and 10–50 ng DNA in 20 μl total volume. PCR cycling conditions consisted of one cycle at 94°C for 30 s, 35 cycles at 94°C for 30 s, 45 s at the locus-specific annealing temperature (Table 1) and 45 s at 72°C, followed by one step of 7 min at 72°C. Cycling was carried out using a Biometra TGradient thermal cycler. Labeled PCR products were analyzed on an Applied BioSystems 3730 l DNA Analyzer using the allele size standard GeneScan-500 LIZ and called using the software PeakScanner (Applied BioSystems).

Genomic DNA was extracted from males of E. meriana (N = 55), Eulaema cingulata (N = 15), Eulaema bombyformis (N = 10), Eulaema choocoa (N = 3), Eulaema luteola (N = 2), Eulaema mocsaryi (N = 2), Eulaema nigrifacies (N = 1) and Eulaema nigrita (N = 1) (Table 1) using the QIAGEN DNeasy Tissue kit. Characterization of each locus was based on one E. meriana population (N = 40) from La Selva, Costa Rica (Table 1). All loci were checked for amplification variability in four E. meriana populations and across the other seven Eulaema species (Table 2). Due to the haploid nature of the data, tests for Hardy–Weinberg equilibrium and linkage disequilibrium were not performed. Number of alleles per locus (N_a) and expected heterozygosity (H_E) were calculated using Microsatellite Analyser (MSA) (Dieringer and Schlotterer 2003).

The number of alleles per locus for E. meriana ranged from 4 to 9 in the population from La Selva, Costa Rica (Table 1) and from 4 to 11 when including individuals from the other 3 populations analyzed (Table 2). Null alleles were only detected for the locus EM40 in one E. meriana individual. All microsatellite loci were easily genotyped in all species except for locus EM107 in E. luteola and E. nigrita. Stutter was only evident in locus EM70 for E. luteola. None of the 90 individuals analyzed showed a diploid genotype. Successful cross-species amplification of these loci shows that the microsatellite markers here described will be useful tools for future population and conservation genetic studies in E. meriana and several species of the genus Eulaema.

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