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Abstract

Primer sequence and polymorphism data are presented for 13 microsatellite loci isolated from the European corn borer moth, *Ostrinia nubilalis*, as part of a project to construct a linkage map for the two pheromone strains. Experimental conditions are described for polymerase chain reaction (PCR) multiplexing, which allows genotyping in two electrophoresis runs of eight and five markers each. In a sample of 27 individuals coming from one European locality, the number of alleles per locus ranged from one to 12, and gene diversity from 0 to 0.859. Seven loci showed a deficit of heterozygotes. Eleven loci cross-amplify in the related *Ostrinia furnacalis*.

Keywords: dinucleotide microsatellites, European corn borer, Lepidoptera, moth, multiplex PCR, *Ostrinia nubilalis*, tetranucleotide microsatellite, *Zea mays*

The European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is a Palearctic polyphagous agricultural pest that was introduced to North America in the early 20th century. This extensively studied species includes different pheromone strains (Roelofs *et al*. 1987) and host races (Malausa *et al*. 2005), providing a model system for examining the genetic basis of sexual isolation and host-plant specialization. Furthermore, this species is the target of insecticide treatments and transgenic insecticidal maize, which may result in the evolution of resistance if management strategies are not based on knowledge of its population biology (Bourguet 2004). Highly polymorphic genetic markers, such as microsatellite loci, are useful tools for population genetic analyses (Goldstein & Schlötterer 1999). However, the development of microsatellite markers has been particularly difficult in Lepidoptera (Zhang 2004; Franck *et al*. 2005; Zhou *et al*. 2005; references therein) and, until recently, none were available in ECB (Dopman *et al*. 2004; Coates *et al*. 2005). Here, we present primer sequences, polymerase chain reaction (PCR) conditions and polymorphism statistics for 13 microsatellite markers.

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Microsatellite enrichment from genomic DNA is described in Dopman *et al*. (2004). Approximately 300 positive clones were sequenced and PCR primer pairs were designed for over 200 loci. In ECB, many microsatellite PCRs gave discrete yet multiple products, probably related to redundant flanking sequences across loci within microsatellite DNA families in Lepidoptera (Zhang 2004). This is a more serious deficiency when attempting to assay allelic variation in natural populations, than in building linkage maps. The 13 loci presented in this study are a combination of microsatellites that were either placed on the genetic linkage map described in Dopman *et al*. (2004), or loci that exhibited 'clean' PCRs but were not segregating in the male-informative and female-informative crosses described in that study (Table 1). In total, we present data for five of the 45 microsatellites used by Dopman *et al*. (2004) — namely D27, D104, D105, D282 and T81 — along with an additional eight loci from the same screening, but which remained unpublished so far.

Table 1 Primer sequences, PCR conditions and polymorphism statistics for 13 microsatellite loci in one European locality of *Ostrinia nubilalis*. Linkage groups (i.e. chromosome numbers) as defined by genetic mapping follow Dopman *et al*. (2004)

PCR no.	Locus	GenBank Accession no.	Primer sequences (5′–3′)	Repeat motif	μM each primer	Linkage group	Size range (bp)	*n*	No. alleles	*f*	H_O	H_E	HW test	ACB
PCR-1	D9	DQ354695	F: (6-FAM)-CTAGGAACGCCTGGGTCTTCTCATA R: ACTCGCATGTTCTTTCACAGTATCTTGTATT	AC	0.2	17	252–326	24	9	0.375	0.292	0.816	*	+ (276)
	D77	DQ354701	F: (6-FAM)-CATTCCGCCTCCTCACACTGG R: GACACCCGCGGGAAGAATAGG	AC	0.2	ND	113–120	21	4	0.500	0.381	0.595		+ (113–117)
	D104	DQ354702	F: (HEX)-AAATCGCTTCGCTGTGGCTCTT R: ACGCGTCATGGTGGCTGTGTA	AG	0.2	6	358–420	22	12	0.250	0.364	0.859	*	–
PCR-2	D27	DQ354697	F: (HEX)-CGATGGTGGTGTCGCTAGATACTAAAATA R: GAAGCCACCTAAATTCGAAATCGTTAC	AC	0.2	21	173–187	21	7	0.524	0.381	0.676	*	+ (175)
	D63	DQ354699	F: (NED)-GACCCCGAACCTGGGACTCT R: CTAGACAAATGCCTTTTAGAAATCTTCAT	AG	0.2	ND	96–111	26	5	0.327	0.692	0.735		+ (101–103)
	D105	DQ354703	F: (HEX)-AGTCGCCAGTCGCGTTCAATAATAA R: AAGGCCCAACCCCCAACATAAC	AC	0.2	2†	103–113	13	4	0.385	0.000	0.738	*	+ (103–109)
	D243	DQ354705	F: (NED)-AAGAATTTTTGAGGTTTATGTTAGTGAT R: CCAAAGGGCAAGAGGAGAGTA	AC	0.2	ND	328	23	1	1.000	0.000	0.000		+ (328)
	D282	DQ354706	F: (NED)-ACTCCGCTACTAGAGTGGGATATTGTCAG R: CAAGGAGACATTATCTTTCGCAGATTTCT	AC	0.2	4	248–274	18	4	0.500	0.167	0.652	*	–
PCR-3	D25	DQ354696	F: (HEX)-GAAGAAAACTCTCCATCGGCACTCT R: AGTCGGGGAATGGCAATCTATTAGTAAA	AG	0.2	ND	73–118	23	10	0.413	0.391	0.764	*	+ (79)
	T81	DQ354707	F: (6-FAM)-AGTGGTTTGGGTTGTGCGTTGATAG R: GCACTTTATACTCGGGCATGGGTAAT	ACTG	0.1	2†	108–179	27	12	0.333	0.704	0.838		+ (112)
	D65	DQ354700	F: (HEX)-TTGGCGCTTATTTGTTTGTGAA R: TCCGGTCGCTGCTTGAA	AC	0.2	8	255–271	27	7	0.463	0.704	0.735		+ 261
	D145	DQ354704	F: (NED)-ATTGATCGCCCATGGATAAAGTTCAT R: ATTTATTTAATTTCTACCTGCTATCACTGCTACTCA	AC	0.1	ND	161–183	26	4	0.654	0.615	0.522		+ (181–183)
	D29	DQ354698	F: (6-FAM)-CGGTCAATAGACTCGGTTCACAT R: ACATAAAATAGTTGCATACATACACAAAAAG	GT	0.2	ND	223–243	15	6	0.567	0.133	0.662	*	+ (244)

†: D105 and T81 are 50 cm apart.

n, number of individuals analysed; *f*, frequency of the most common allele; H_O, observed proportion of heterozygous individuals; H_E, heterozygosity (i.e. gene diversity; Nei 1987); HW test, Hardy–Weinberg exact test (Raymond & Rousset 1995); *, significant probability test with $\alpha = 0.05$ using the sequential Bonferroni correction; ACB, results of cross-species amplifications using two individuals of the Asian corn borer (*Ostrinia furnacalis*); –, no amplification detected; +, amplification detected (allele size range between parentheses); ND, not determined.

PCRs were performed in an MJ Research PTC-200 thermocycler. The forward primer of each pair of microsatellite DNA primers was labelled with a fluorescent dye (Table 1). Multiplex PCR amplifications were carried out for three sets of markers (PCR-1 to PCR-3; Table 1) using Multilocus Amplification Kit (QIAGEN) in a 10-μL volume containing 1 × QIAGEN multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$), 0.1–0.2 μM each primer (Table 1) and 2 μL of genomic DNA (*c.* 4 ng DNA/reaction). Cycling conditions were as follows: an initial denaturing step of 15 min at 94 °C; seven touchdown cycles starting at 94 °C (30 s), T_m = 67 °C (90 s), 72 °C (60 s), T_m was reduced by 2 °C per cycle to a temperature of 53 °C; followed by a further 25 cycles at 94 °C (30 s), 53 °C (90 s), 72 °C (60 s); and a final extension at 60 °C for 30 min.

Microsatellite loci were genotyped for 27 diapausing larvae collected in a maize field at Grignon, France (November 2004; 48°50.9′N, 01°56.2′E). DNA was extracted from the head of larvae using the Puregene DNA purification kit (Gentra Systems). PCR products were electrophoresed on an ABI PRISM 310 Genetic Analyser at 60 °C (injection: 4 s at 15 kV; migration: 27 min at 15 kV), using a 20-μL volume containing 0.2 μL of ROX-60-415-labelled internal marker size standard, 17.8 μL of formamide and 2 μL of diluted PCR products. Products from PCR-1 and -2 were combined in a first electrophoresis run with 1 μL of each PCR at dilution 1/100 and 1/50, respectively. Products of PCR-3 were analysed in a second electrophoresis run with 2 μL of PCR at dilution 1/100. Alleles were scored by length in base pairs using the GENESCAN and GENOTYPER software.

The levels of polymorphism varied widely among the 13 loci: one locus was monomorphic and for the other 12, the observed number of alleles ranged between four and 12, and gene diversity between 0.522 and 0.859 (Table 1). The exact tests for genotypic linkage disequilibrium between pairs of loci (GENEPOP 3.3 package; Raymond & Rousset 1995) gave significant results ($P < 0.05$) in five out of the 66 tests, although none remained significant after correction for multiple tests (sequential Bonferroni procedure, Rice 1989). The loci were therefore considered to be statistically unlinked. Significant deviations from Hardy–Weinberg equilibrium were detected for seven out of 12 polymorphic loci, and were likely due to null alleles. In agreement with the hypothesis of instability of microsatellite-flanking regions in Lepidoptera (Zhang 2004), a similar proportion of ECB microsatellites with deficit of heterozygote was observed in Coates *et al*. (2005), suggesting that null alleles may be frequent in this species. Cross-species amplifications were tested on two individuals from the Asian corn borer (*Ostrinia furnacalis*). This related species amplified successfully for 11 of the 13 microsatellites (Table 1). Including the set of primers developed by Coates *et al*. (2005), 23 microsatellite markers are now available for genetic analyses of *O. nubilalis*.

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