Isolation and characterization of microsatellite loci from the European corn borer, Ostrinia nubilalis (Hübner) (Insecta: Lepidoptera: Crambidae)

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PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite loci from the European corn borer, Ostrinia nubilalis (Hübner) (Insecta: Lepidoptera: Crambidae)

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Abstract

Few useful microsatellites are available for population studies of the European corn borer, Ostrinia nubilalis (Hübner). An enrichment strategy was used to develop microsatellite markers for O. nubilalis, and over 500 positive clones were isolated. Seventy-five contained unique microsatellites, 10 of which were polymorphic with discernable polymerase chain reaction products. The 10 loci were surveyed for variability in 72 wild individuals from central Iowa. Five loci showed no deviation from Hardy–Weinberg proportions, and all were successfully cross-amplified in the related Asian corn borer, Ostrinia furnacalis. These loci represent a significant addition to microsatellites appropriate for population studies of O. nubilalis.

Keywords: European corn borer, microsatellites, molecular markers, Ostrinia furnacalis, Ostrinia nubilalis, population genetics

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Three hundred and sixty-eight *NdelI* clones and 158 *TaqI* clones (526 total) were sequenced using a Beckman-Coulter CEQ 8000 Genetic Analysis System. Of these, 305 *NdelI* clones and 131 *TaqI* clones appeared to have simple sequence repeats (SSR). Many sequences overlapped or shared a portion of the same sequences. In some sequences, flanking regions were too close to the SSRs, or the SSRs were very long. Seventy-five clones with unique SSR sequences were chosen to design microsatellite primer sets for PCR amplification. Reaction solutions for PCR consisted of 15 ng genomic DNA, 1.5 mM MgCl₂, 200 μM of each dNTP, 0.2 μM of each primer, and 0.5 U *Taq* polymerase in a total volume of 12.5 μL. PCR conditions included denaturation for 3 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C, and a final extension at 72 °C for 5 min.

Based on the results, the primers of 30 loci (23 *NdelI* clones and 7 *TaqI* clones) were labelled with Beckman-Coulter phosphoramidite fluorophores and used to test polymorphism and PCR amplification. Ten of these were polymorphic with discernable bands. Other loci showed monomorphic, multiple or nonspecific bands that made allele calling difficult. One locus (On-D1) was obtained from *TaqI* clones and the other nine loci from *NdelI* clones (Table 1).

Variability of the 10 polymorphic microsatellites was surveyed across 72 wild *O. nubilalis* individuals collected as adults in central Iowa in 2005 (Table 1). The number of alleles per locus ranged from two for On-T2 to 22 for On-D2, and expected heterozygosity values ranged from 0.327 for On-T1 to 0.934 for On-D2. All 10 loci were successfully amplified from *O. furnacalis* (Table 1). Pairwise comparison of locus pairs (45 tests) showed significant evidence of linkage disequilibrium only in one case (On-T6 vs. On-T7) based on Fisher’s method using the genotype disequilibrium option implemented in the program geniepop (Raymond & Rousset 1995).

Five of 10 loci deviated significantly from Hardy–Weinberg proportions, based on both unadjusted (*P* < 0.05) and adjusted significance thresholds (*P* = 0.005) (Table 1), with a significant deficit of heterozygotes. An exception was On-T7, which showed a significant excess of heterozygotes (*F*ₚₑₛ = −0.177 for On-T7). Analyses of these loci, except for On-T7, using micro-checker (Oosterhout et al. 2004) also indicated the presence of null alleles. However, the other five loci showed no evidence for null alleles, the preferential amplification of small alleles, or scoring error due to stuttering (Oosterhout et al. 2004). Although we caution that the remaining five markers must be examined...
carefully in future samples for null alleles and other potential problems commonly observed with microsatellites in Lepidoptera, these additional loci promise to facilitate population genetics studies of European corn borer.

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References


