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PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite markers in the newly discovered invasive fruit fly pest in Africa, *Bactrocera invadens* (Diptera: Tephritidae)

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Abstract

We describe the isolation and characterization of 11 polymorphic microsatellite loci from the recently discovered fruit fly pest, *Bactrocera invadens*. The polymorphism of these loci was tested in individual flies from two natural populations (Sri Lanka and Democratic Republic of Congo). Allele number per locus ranged from three to 15 and eight loci displayed a polymorphic information content greater than 0.5. These microsatellite loci provide useful markers for studies of population dynamics and invasion history of this pest species.

Keywords: *Bactrocera invadens*, microsatellites, polymorphism

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Asian fruit fly pests from the genus *Bactrocera* are regarded as some of the most destructive insects of fruits and vegetables worldwide (White & Elson-Harris 1992). In March 2003, three fruit fly specimens of the genus *Bactrocera* were detected in Kenya (Lux *et al*. 2003) and the species was described as *Bactrocera invadens* (Drew *et al*. 2005) in recognition of its rapid invasion of the African continent. It is feared that the geographical expansion of this pest and infestation could be more severe if no control programmes and efficient quarantine systems are implemented (Ekesi *et al*. 2006).

Microsatellite markers have been successfully applied to different invasive fruit fly species to infer evolutionary aspects underlying their invasive processes and to identify the routes of their colonization (Bonizzoni *et al*. 2004; Aketarawong *et al*. 2007). Here we report the characterization of 11 microsatellite markers in *B. invadens* and provide evidence of their usefulness for the study of genetic structure and dynamics of this species.

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Genomic DNA was extracted from *B. invadens* flies from an International Center of Insect Physiology and Ecology (ICIPE) mass-rearing strain established in 2003 from wild flies collected from rotten mangoes in Nairobi (Kenya). Following digestion of genomic DNA with *Rsa*I (Roche), fragments were ligated to two adapter oligonucleotides (Adaptor A: 5′-CTCTTGCTTACGCGTGGACTA-3′; Adaptor B: 5′-TAGTCCACGCGTAAGCAAGAGCAC-3′) and used as template in polymerase chain reaction (PCR) with 24 pmol of adaptor A as primer. PCR products were denatured and stringently hybridized to a biotinylated $(dCA)_{10}$ probe. The target fragments were captured using streptavidin magnetic particles (Promega). Captured fragments were amplified, cloned and sequenced. A total of 200 plasmid inserts were analysed on an ABI PRISM 310 Genetic Analyser (Applied Biosystems). Twenty-six clones contained microsatellite sequences. Primers were designed for 11 loci with suitable flanking sequence using Primer 3 (Rozen & Skaletsky 2000). Preliminary PCR screening performed on 18 females and 27 males from the ICIPE strain yielded single locus amplifications, and the loci were polymorphic in both sexes. This excludes any condition of sex linkage for these loci. Amplifications were performed on a PTC-100

Table 1 Characteristics of 11 microsatellite loci from *Bactrocera invadens* and their polymorphism in 54 male flies, from two wild populations

Locus	Repeat motif	Primer (5′–3′)	GenBank Accession	T_a (°C)	Allele size range (bp)	Total N_a	Sri Lanka (n = 27)			Democratic Republic of Congo (n = 27)		
							N_a	H_O	H_E	N_a	H_O	H_E
Bi1	$(CA)_9TT(CA)_2$	HEX CTCTTGACACTGGCCTCGTT R: GTATGGCCGGAGACATCAGT	EU868612	58	129–159	12	12	0.85	0.88	6	0.67	0.75
Bi2	$(CA)_7$	FAM GCACTCACTCAACCTTAGACG R: GCACTTGAATTGTGCGAAGT	EU868613	59	140–149	10	10	0.54	0.83	5	0.72	0.75
Bi3	$(TG)_5CTG$	FAM CGCGAATTTCAAGCATTTTT R: GGTCTTAAGGCCAAGCACAA	EU868614	60	130–132	3	3	0	0.14	2	0	0.07
Bi4	$(CT)_6GCT$	HEX GCACTCGCATGCTTGTAGTC R: CCGGTTTTGTGCGAAAAG	EU868615	59	123–126	4	4	0.78	0.74	4	0.48	0.70
Bi5	$(CA)_5GA(CA)_2$	HEX GCCAGTCAGTGTCTCGTCAA R: AGCGAGTTTGTTTTCGGTGA	EU868616	60	112–133	15	15	0.81	0.91	4	0.41	0.68
Bi6	$(CA)_3CC(CA)_3$	FAM GCGACAAGTTCGACACAAAA R: TACTGATTGTGCCGTGTGCT	EU868617	60	90–101	8	6	0.20	0.75*	6	0.58	0.67
Bi7	$(CA)_5A(CA)_2$	FAM CTCGCTCTTCATTCAATCCA R: CGACACGTTAAGTGGCAAAA	EU868618	58	107–118	5	5	0.56	0.49	3	0.22	0.31
Bi8	$(AC)_5AT(AC)_2$	FAM ACAAGTGCAGCAAAGACACG R: ATCACATCATGAGGCGTTCA	EU868619	61	118–138	7	6	0.59	0.57	4	0.63	0.62
Bi9	$(TG)_2TA(TG)_6$	HEX GCGCTGCTCGTAAACATCTA R: GGGCAAACACTTGGATTCAC	EU868620	61	92–108	9	8	0.74	0.75	5	0.85	0.74
Bi10	$(TG)_3AT(TG)_8$	FAM ATCGAGCAGATCACTGAGCA R: CCGCGAGTAGCAAATCTTTC	EU868621	56	142–155	11	7	0.63	0.83	7	0.63	0.71
Bi11	$(TC)_3T(TC)_3$	HEX TGGGTTCACCGTCCTTTAAT R: GCCCATAGACATCCAGGGTA	EU868622	60	140–147	3	3	0.22	0.26	2	0.19	0.17

HEX and FAM: Fluorescent dye labelled at the 5′ end of the forward primer. T_a, annealing temperature; N_a, number of alleles per locus; n, number of males tested; H_O and H_E, observed and expected heterozygosities, respectively; *deviations from Hardy–Weinberg equilibrium at $P < 0.05$ after Bonferroni correction.

thermocycler (MJR Inc.). Reactions for PCR consisted of 25 ng of genomic DNA, 1× reaction buffer, 2.5 mM $MgCl_2$, 25 μM dNTP, 0.5 U *Taq* polymerase (Invitrogen) and 5 μM of each primer, one of which was 5′ labelled with a fluorescent dye, in a total volume of 15 μL. PCR conditions were 2 min at 95 °C, 29 cycles of 30 s at 94 °C, 90 s at 56–61 °C and 90 s at 72 °C and 5 min of elongation at 72 °C. After loading on the Genetic Analyser, PCR products were analysed by the GeneScan program (Applied Biosystems). An individual was declared null (non-amplifying allele) after at least two amplification failures.

The 11 primers were tested to assess their polymorphism in two wild populations: Sri Lanka (Ranbukpitiya, 07°02′54″N, 80°30′52″E) and Democratic Republic of Congo (DRC, Kisantu Botanical Garden, 05°7′S, 15°5′E). Because only males are captured with methyl eugenol attractant, all the analyses were performed on male individuals, 27 from each population. All loci were polymorphic across the two samples (Table 1). The number of alleles per locus ranged from three to 15 and eight loci had a polymorphic information content PIC > 0.5 (Kalinowski *et al*. 2007). Genetic variability within populations was analysed using GenePop, version 3.4 (Raymond & Rousset 1995). A comparable high level of polymorphism was detected for the majority of loci in the two populations analysed, which also displayed the presence of private alleles. None of the loci displayed linkage disequilibrium (Fisher's exact test, GenePop). One case of deviation from Hardy–Weinberg equilibrium was observed at locus Bi6 after Bonferroni correction (Rice 1989) with heterozygote deficiency in the Sri Lankan population. The use of Micro-Checker, version 2.2.3 (van Oosterhout *et al*. 2004) indicates that this may be due to the presence of null alleles. This study provides the first set of microsatellite markers available for *B. invadens*. The range in repeat sequence length and diversity of these markers will allow the investigation of population structure and invasion history of this species.

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References

Aketarawong N, Bonizzoni M, Thanaphum S *et al*. (2007) Inferences on the population structure and colonization process of the invasive oriental fruit fly, *Bactrocera dorsalis* (Hendel). *Molecular Ecology*, **16**, 3522–3532.

Bonizzoni M, Guglielmino CR, Smallridge CJ, Gomulski LM, Malacrida AR, Gasperi G (2004) On the origins of medfly invasion and expansion in Australia. *Molecular Ecology*, **13**, 3845–3855.

Drew RI, Tsuruta K, White IM (2005) A new species of pest fruit fly (Diptera: Tephritidae) from Sri Lanka and Africa. *African Entomology*, **13**, 149–154.

Ekesi S, Nderitu P, Rwomushana I (2006) Field infestation, life history and demographic parameters of the fruit fly *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Bulletin of Entomological Research*, **96**, 379–386.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program Cervus accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1006.

Lux SA, Copeland RS, White IM, Manrakhan A, Billah MK (2003) A new invasive fruit fly species from the *Bactrocera dorsalis* (Hendel) group detected in East Africa. *Insect Science and its Application*, **23**, 355–361.

van Oosterhout CV, Hutchinson WF, Wills DP, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Raymond M, Rousset F (1995) GenePop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analysis tables of statistical tests. *Evolution*, **43**, 223–225.

Rozen S, Skaletsky HJ (2000) Primer 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, pp. 365–386. Humana Press, Totowa, New Jersey.

White IM, Elson-Harris MM (1992) *Fruit Flies of Economic Significance: Their Identification and Bionomics*. C.A.B International and Australian Centre for International Agricultural Research, Wallingford, UK.