Molecular Ecology Notes (2007) **7**, 1120–1122 doi: 10.1111/j.1471-8286.2007.01800.x

PRIMER NOTE

Identification and characterization of 21 polymorphic microsatellite loci from the mycophagous fly *Drosophila neotestacea*

KELLY A. DYER
Institute of Evolutionary Biology, University of Edinburgh, Ashworth Laboratories, W. Mains Road, Edinburgh EH9 3JT, UK

Abstract

***Drosophila neotestacea* is a mushroom-feeding fly that is common in the temperate and boreal forests of North America. Here I describe the isolation and characterization of 21 polymorphic microsatellite loci from the *D. neotestacea* genome. In a sample of flies from Rochester, New York, the expected and observed heterozygosities ranged from 0.344 to 1.000 and from 0.384 to 0.923, respectively. Of the 21 markers, six were likely X-linked, seven showed a departure from Hardy–Weinberg equilibrium, and none were found to be in linkage disequilibrium. These loci will facilitate future ecological and population genetic studies of *D. neotestacea*.**

Keywords: microsatellites, population genetics, polymorphic marker, *Drosophila neotestacea*, mushroom-feeding

Received 6 January 2007; revision accepted 20 March 2007

Drosophila neotestacea is a member of the testacea group within the subgenus *Drosophila* (Grimaldi *et al*. 1992). *D. neotestacea* occurs throughout the temperate and boreal forests of North America, making its distribution among the broadest of the noncosmopolitan North American *Drosophila*. It is a species that is amenable to both laboratory and field studies, and some aspects of *D. neotestacea* biology that have been studied include X-chromosome meiotic drive (James & Jaenike 1990), infection by nematode parasites (Perlman & Jaenike 2003), and mating behaviour (e.g. James & Jaenike 1992). Here I describe microsatellite markers I developed for *D. neotestacea*, which are the first markers of this type available for a mycophagous *Drosophila* species.

Microsatellites were isolated from *D. neotestacea* following the subtractive hybridization protocol of Hamilton *et al*. (1999). From an inbred laboratory stock originating from Rochester, New York, genomic DNA was extracted from flies of both sexes and digested with *Sau*3a, creating a pool of fragments between 200 and 1000 base pairs. Double-stranded linkers were ligated on to both ends of each fragment, and the linked DNA was hybridized to biotinylated oligos containing one of the four target repeats: $(AC)_{15}$, $(CG)_{15}$, $(AGC)_{10}$, and $(ATC)_{10}$. From this hybridization forward, the entire procedure was completed separately for each of the target repeats. Linked enriched DNA was ligated into the plasmid Pbluescript SK+ and transformed into *Escherichia coli* XL-2 MRF′ ultracompetent cells (Stratagene). Using insert-flanking plasmid primers, inserts were amplified via colony polymerase chain reaction (PCR) and sequenced directly using BigDye chemistry (Applied Biosystems). Approximately 100 randomly chosen inserts were sequenced from each of the four hybridizations, yielding hundreds of unique microsatellites. As summarized in Ross *et al*. (2003), the most abundant motifs recovered from these *D. neotestacea* libraries were AG, AC, AAC, and AGC, and compared to other *Drosophila*, the average repeat length of microsatellites in *D. neotestacea* is relatively short.

Primers flanking microsatellite motifs were designed for 25 loci using the program OLIGO. PCRs (10 μL) were carried out using ~10 ng DNA in 1× PCR buffer, 1.5 mM $MgCl_2$, 0.4 μM of each primer, 0.2 mM of each dNTP, and 0.5 U *Taq* polymerase (Invitrogen). Thermocycler conditions for PCR were 94 °C for 2 min, 33 cycles at 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 45 min. Twenty-one of the primer sets amplified well, and among these polymorphism was assessed in one of two ways. For the first eight loci in Table 1 (Set A), I followed

Correspondence: K. A. Dyer, Fax: +44 0131650 6564; E-mail: kelly.dyer@ed.ac.uk

Table 1 Primers and characteristics of 21 microsatellite loci of *Drosophila neotestacea*

Locus (Accession no.)	Repeat motif in clone	Primer sequence (5′–3′)	Size range (bp)	*n*	N_a	H_E (H_O)
Set A						
Dn5260 (EF199819)	$(GTT)_5$	F: AATGTGCATAGTATGTAGGG R: AATGCCCTCATTGCCATC	177–184	25	5	0.535 (0.428)
Dn7002 (EF199820)	$(AC)_{11}(GC)(AC)_4$	F: CTGCCGTCCCTATAAATATAATCG R: CCACTTATGACCCACTAGACAG	116–136	27	8	0.785 (0.428†)
Dn7003 (EF199821)	$(TG)_3(CG)(TG)_8$	F: TGCGATATTCAGATTGCGAC R: CTTGCCTTGGGAATGGCGTGG	229–235	28	4	0.678 (0.285†)
Dn7014 (EF199822)	$(GT)_6$	F: CTGCTGCGTAAGTATG R: TCAACCAGACACGGACAG	260–276	28	7	0.538 (0.500)
Dn7030 (EF199824)	$(AC)_8(AA)(AC)_4(GC)(AC)_5$	F: CTCCATATCCAACGTCTCCTC R: CCTCTCCCCTTGTCGTCTAC	161–169	9	3	0.476 (0.400)
Dn7037 (EF199826)	$(CA)_{14}$	F: CCACTGTGTGTAATATTTCG R: ACTGCTAAATGAATTATGTGGG	113–140	27	14	0.945 (0.714)
Dn7041 (EF199825)	$(CT)_5(GT)_{12}(TT)(GT)_5$	F: CACACTGCACAATCTTGAGACATC R: TCCTTAATTGCTTTTCATGTAGAGTG	183–217	8	8	1.000 (0.400)
Dn8349 (EF199823)	$(GT)_6(CTGTAT)(GT)_4$	F: GGAGAGGAAGGCTATTCAC R: GGAGGTAGGTATGCCATAG	235–250	28	5	0.810 (0.857)
Set B						
Dn5261* (EF199829)	$(AC)_6(GC)(AC)_4$	F: GAAGCAACAACAAAAGCC R: AATGAGGCAAGGTCCCACTG	155–179	26	5	0.344 (0.384)
Dn5270 (EF199839)	$(AT)_6$	F: CTTCTTAGCTGCACTCTACG R: GAACACAGCTACTGACTAGG	369–453	26	16	0.941 (0.500†)
Dn6002* (EF199833)	$(CA)_{10}(TA)(CA)_2$	F: TCTAAATGCACAAATCCCAGC R: CACGACTGCGTAATACTTCACC	93–131	26	15	0.865 (0.583†)
Dn6003 (EF199834)	$(AC)_7$	F: CCCACAACCTTAAATAGAGC R: CTCTGTCTCCCACTTCCTTTAG	147–174	26	17	0.933 (0.615†)
Dn6428 (EF199838)	$(AAAT)_5$	F: CTGAACCTGAAAATGAATCC R: ATTCAATGTCAAAAATGTAGCTCAC	293–341	26	12	0.759 (0.577†)
Dn6429 (EF199835)	$(AC)_{11}$	F: CTTTGCCTCATATTTCAATCG R: CTTACCACAAAAAGTACGGTTG	242–287	26	15	0.892 (0.577†)
Dn7013 (EF199837)	$(CA)_7$	F: GTCATAGCGTAATCATGAC R: GGTCTCAACTCTTACTCGAC	223–242	26	11	0.801 (0.731)
Dn7029* (EF199828)	$(AGC)_6$	F: AGCACATGGCACAGATGTTAG R: GAAGGATACAAGAGACGTCAGC	264–279	26	6	0.684 (0.538)
Dn7040* (EF199831)	$(AC)_{13}$	F: CAAACAACAATTGCAACGTG R: GTGTGCACACATTTCCATACC	97–114	26	9	0.812 (0.769)
Dn8377* (EF199832)	$(GAT)_2(GCT)(GAT)_5$	F: TGGACAATTGTTGTGGACTG R: AACAACATCATTCGCATTCG	64–80	26	4	0.396 (0.461)
Dn8380 (EF199827)	$(CAG)_5$~$(AGC)_5$	F: CGCTCTAATTGATTTCCTTGC R: GCTGTGTTAGTTGGGAGACAG	318–364	26	9	0.584 (0.500)
Dn8385* (EF199836)	$(GAT)_6$	F: AGAGCTTTAATGTGCTGGCA R: CCCAACTGAAAGTGAATTG	323–353	26	11	0.889 (0.923)
Dn8394 (EF199830)	$(AC)_9(AT)_2(AC)_2$	F: ACTCTTCATAAAAGCGCAGCG R: GTTTTAGCTGGCATTTACGC	131–157	26	13	0.833 (0.731)

See text for description of methods for Set A and Set B loci. *Putatively X-linked locus; *n*, number of individuals genotyped; N_a, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; where †indicates a significant deficit of heterozygotes from Hardy–Weinberg equilibrium.

the protocol of Kondo *et al*. (2000) to add a fluorochrome (Applied Biosystems) to the 5′ of the reverse primer via a post-PCR labelling reaction. Each of these eight loci was amplified and surveyed from one female from up to 28 isofemale lines originating from Rochester, New York. For the last 13 primers in Table 1 (Set B), I amplified the loci using a reverse primer with a 5′ fluorochrome (Applied Biosystems). These 13 loci were amplified from 26 individuals

(13 females and 13 males) collected near Rochester, New York. All fragments were run on an ABI 3100 DNA sequencer.

Table 1 summarizes the diversity of the 21 microsatellites in the Rochester population of *D. neotestacea*. All 21 loci were polymorphic, with the number of alleles ranging from three to 17. MSA version 4.00 (Dieringer & Schlotterer 2003) was used to estimate the observed and expected heterozygosity values. For the Set A loci, if an individual had two alleles at a locus, one allele was randomly chosen to determine heterozygosity. Of the Set B loci, six of the 13 markers are putatively X-linked, i.e. the locus was always homozygous in males and heterozygous or homozygous in females; thus, for the X-linked loci heterozygosity was estimated from females only. Departure from Hardy–Weinberg equilibrium was estimated using GENEPOP 3.4 (Raymond & Rousset 1995), with significance level determined using a Bonferroni correction. Seven of the 21 loci showed a significant deficit of heterozygotes; two of these loci (Dn6002, Dn5270) were the only loci in Set B to show evidence of null alleles, as identified by the presence of null homozygous individuals. It is possible that some loci that deviate from Hardy–Weinberg equilibrium may be contained within segregating inversions, which are common in *Drosophila*. Within each set of loci I found no pairs that showed significant linkage disequilibrium using a Fisher's exact test with a Bonferroni correction. The availability of these polymorphic microsatellite markers will facilitate studies of population genetic structure, behavioural ecology, and the genetics of adaptation of ecologically important traits in *D. neotestacea* and closely related species.

Acknowledgements

I am grateful to J. Jaenike for encouragement and financial support, and to G. Campo, A. Holyoake, K. Kiesler, and M. Noor for technical advice. Funding was provided by Royal Society and NSF fellowships to KAD and NSF grants to John Jaenike.

References

Dieringer D, Schlotterer C (2003) MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, **3**, 167–169.

Grimaldi D, James AC, Jaenike J (1992) Systematics and modes of reproductive isolation in the holarctic *Drosophila testacea* species group (Diptera: Drosophiladae). *Annals of the Entomological Society of America*, **85**, 671–685.

Hamilton MB, Pincus EL, DiFiore A (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *BioTechniques*, **27**, 500–507.

James AC, Jaenike J (1990) Sex-ratio meiotic drive in *Drosophila testacea*. *Genetics*, **126**, 651–656.

James AC, Jaenike J (1992) Determinants of mating success in wild *Drosphila testacea*. *Animal Behavior*, **44**, 168–170.

Kondo H, Tahira T, Hayashi H, Oshima K, Hayashi K (2000) Microsatellite genotyping of post-PCR fluorescently labeled markers. *Biotechniques*, **29**, 868–872.

Perlman SJ, Jaenike J (2003) Evolution of multiple components of virulence in *Drosophila*–nematode associations. *Evolution*, **57**, 1543–1551.

Raymond M, Rousset F (1995) GENEPOP (Version 3.4): a population genetics software for exact tests of ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ross CL, Dyer KA, Erez T, Miller SJ, Jaenike J, Markow TA (2003) Rapid divergence of microsatellite abundance among species of *Drosophila*. *Molecular Biology and Evolution*, **20**, 1143–1157.